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MICROBIOLOGICAL QUALITY OF SHALLOW IRRIGATION WATER AT NKOLBISSON A SUB-URBAN AREA OF YAOUNDE (CAMEROON): INFLUENCE OF SOME PHYSICOCHEMICAL PROPERTIES

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ABSTRACT

In order to assess the impact of various pollution sources on the microbiological water quality mainly used for irrigation of vegetable farms and its relation with some physic-chemical parameters which can influence the bacterial growth, a study was conducted in Nkolbisson shallows in the peri-urban area of Yaounde in Cameroon. Sampling, measures of physicochemical parameters, isolation and enumeration of bacteria were performed using standard methods. Generally, physico-chemical and biological properties vary across one season to another. No significant difference was observed. However, there are significant correlations between abiotic parameters and bacterial abundance. These shallow waters host some bacteria which witness fecal contamination. Plate Count Agar showed that the abundance of these bacteria is closely related to environmental factors such as pH, Total Suspended Solid, nitrogen, phosphorus, and the electrical conductivity. These waters are unfit for use in crops irrigation, cleaning of crop products, laundry, bathing and washing of dishes.

Keywords: bacteria, irrigation, market gardeners, physico-chemical parameters, shallows, water

I. INTRODUCTION

Water, a prime natural resource and precious national asset, forms the chief constituent of the ecosystem. It has a vital role in various sectors of the economy such as agriculture, fisheries, forestry and other creative activities; but increasing population, industrialization, urbanization, agriculture are some of the factors which leads to the deterioration of the availability and quality of water.

Agricultural land use without environmental safeguards to prevent over-application of agrochemicals is causing widespread deterioration of the soil/water ecosystem as well as the underlying aquifers. The main problems associated with agriculture are Stalinization, nitrate and pesticide contamination, and erosion leading to elevated concentrations of suspended solids in rivers and streams and the siltation of impoundments. Irrigation has enlarged the land area available for crop production but the resulting Stalinization which has occurred in some areas has caused the deterioration of previously fertile soils (J. Bartram and R. Balance, 1996).

In Africa metropolis in general and particularly in Cameroon, discharge of toxic chemicals and wastewater, agriculture, long-range atmospheric transport of pollutants and contamination of water bodies with substances that promote algal growth (possibly leading to eutrophication) are some of

International Journal of Research In Earth & Environmental Sciences

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today's major causes of water quality degradation. Rapid and uncontrolled urban development and new informal settlements lead to a proliferation of individual sanitation systems consisting mostly of latrines, more or less tight septic tanks with some located along streams in a manner to empty directly the contents into these streams (Le Jallé, 1998) also lead to water quality degradation, however, these water are used for irrigation of vegetable crops.

Several studies on the microbiological quality of surface and groundwater (wells, springs) used directly for consumption (Nola et al., 2004b, Kuitcha et al., 2010) and wastewater used for irrigation of vegetable crops (Kouam Kenmogne et al., 2010) in Yaounde (Cameroon) have already been conducted. The result of the water proofed to be acidic, freshly and slightly mineralized and hosting a variety of bacterial microflora made of fecal bacteria, commensal and opportunistic pathogen bacteria, whose abundant dynamic undergoes spatial and temporal variations (Nola et al., 1998b). It has also been shown that, the settlement of these microorganisms is significantly influenced by some physicochemical factors such as dissolved gases and ions, and meteorological factors such as rainfall and sunshine (Nola et al., 2002). They also revealed that several microorganisms present in unfiltered water and surface water are retained by soil horizons and bedrock (Nola et al., 2010; Noah Ewoti et al., 2011).

The evaluation of water quality used for vegetable irrigation in Cameroon shallows, has not been systematically carried out in Cameroon. However these vegetables crops are the stable food of a number of Cameroonians and some neighboring countries, who consumed them without proper hygiene measures taken.

The objective of this research is to determine the microbiological and physicochemical water quality mainly used for crops irrigation at Nkolbisson shallows of Yaounde (Cameroon). It specifically consists of isolating and enumerating the total viable bacteria indicators of fecal contamination, determining the physicochemical parameters of this water and establishing a correlationship between bacterial and physicochemical factors

II. MATERIALS AND METHODS

2.1. Presentation of the study site

The research was conducted during the month of March to August 2013 at Nkolbisson, a suburban neighborhood in the North Eastern part of Yaounde. Analyses were performed in the Soil, Plant, Water and Fertilizer Laboratory (LASPEE) of the Institute of Agricultural Research for Development (IRAD) and at the General Biology Laboratory (Hydrobiology and Environment Unit) of the University of Yaounde I.

Nkolbisson shallow, located between 3°52' N and 11°27' E, is anthropogenic, with few people involved in market gardening. It consists of yellow lateritic soils. After prospection, 7 sampling points were identified based on the importance of the variety of vegetable crops, the frequency of water used by local populations (mainly for irrigation, laundry, dish washing and bathing) and the access (easy to ford) to it. The characteristics of different sampling points are represented on Table I.

Table I: characteristics of each sampling point

Sampling points (designation)	Geographical position		Characteristics
Abiergué 1 (A')	N 3°52 201'	E 11°29 136'	Wastewater treatment plant near the river
Abiergué 2 (B')	N 3°52 353'	E 11°27 806'	River (Laundry and car washing)
Mitotomo (C')	N 3°52 364'	E 11°27 573'	Shallow River crossing (dam)
Exutoire Lac Nkolbisson (D')	N 3°52 285'	E 11°27 343'	River (Laundry, harvested plants and accessories used to spray fungicides are washed)
Abiergué 3 (E')	N 3°52 181'	E 11°27 243'	River (Laundry, harvested plants and accessories used to spray fungicides are washed)

Abiergué 4 (F')	N 3°52 106'	E 11°27 211'	Laundry, dishwashing and car washing above the river
Mefou (G')	N 3°52 780'	E 11°29 700'	Upstream dam for drinking water

2.2. Isolation and enumeration of bacteria

Bacteria of fecal contamination (fecal coliforms (CF), total coliforms (CT) and fecal streptococci (SF)) are the bacteria specimens considered to be the best indicators of fecal contamination. Their presence in water indicates its contamination with human and/or animal feces and this water may therefore contain pathogenic microorganisms which often cause gastroenteritis (Rodier, 2009). The total flora represented by heterotrophic aerobic mesophilic bacteria (BHAM) was also counted by Nola et al., 2001 to get an idea of all the irrigation water bacteria.

2.2.1. isolation and enumeration of total and fecal coliforms

The isolation and enumeration of total and fecal coliforms were carried out by the membrane filter technique on Xylose, Lysine, Desoxycholate medium, (XLD) (Scholar). Readings were made after 24 to 48 hours incubation at 37°C for fecal coliforms and temperature of 44°C for total coliforms. The results were expressed as Colony Forming Units per 100ml of water sampled (CFU/100ml) (Marchal et al., 1991).

2.2.2. isolation and enumeration of fecal Streptococci

The evaluation of fecal Streptococci was performed on Slanetz and Bartley medium (Scholar) poured into Petri dishes by the membrane filter method. After 48 hours of incubation, colonies of purple were counted and the results expressed in CFU/100ml (Marchal et al., 1991).

2.2.3. isolation and enumeration of heterotrophic aerobic mesophilic bacteria (BHAM)

The isolation of BHAM was made after seeding on Plate Count Agar (PCA) medium (Liofilchem). After incubation at room temperature, the enumeration of all the colonies was made within 5 days (Diagnostics Pasteur, 1987).

2.3. Physicochemical parameters

The physicochemical parameters were analyzed using the Techniques developed by Rodier (2009) and APHA (2007). Table II summarizes the parameters considered, the technique, the measurements and units of measurements.

Table II: Parameters analyzed, methods of measurement, devices and units used for each parameter

Parameters	Technique	Site	Apparatus	Units
Temperature	Direct	In situ	Thermometer	°C
pH	Direct	In situ	pH-meter	u.c.
Conductivity	Direct	In situ	Conductimeter	µS/cm
Dissolved O_2	Direct	In situ	Oxymeter	% saturation
DBO5	Respirometry	Laboratory	Incubator à DBO	mg/ld'O_2
Total Suspended Solids (TSS)	Colorimetry	Laboratory	Spectrophotometer	mg/l
Dissolved CO_2	Volumetry by HCl	Laboratory	Titrimetry	mg/l
PO_4^{3-}	Colorimetry	Laboratory	Spectrophotometer	mg/l
NO_2^-, NO_3^-,	Colorimetry	Laboratory	Spectrophotometer	mg/l
NH_4^+	Colorimetry by Nessler	Laboratory	Spectrophotometer	mg/l

2.4. Data Analysis

CF/SF ratios were calculated to determine the origin of fecal pollution, (Borrego and Romero, 1982). The mean values were considered with respect to season in order to synthesize the information contained in the set of variables considered at each sampling point. Then the global comparisons were performed using Kruskal Wallis and Mann Whitney. Spearman's correlations and Principal Component Analysis (PCA) were conducted to verify the impact of physicochemical factors on microbiological parameters (Zébazé Togouet et al., 2011). The data processing was performed with Excel and SPSS 12.0 for Windows software.

III. RESULTS AND DISCUSSION

3.1. Physicochemical parameters in rainy and dry season

In both seasons, the average values of physicochemical parameters of shallows water in Nkolbisson generally vary from one sampling point to another and from one parameter to another (Table 3). In general, the average water temperature remains above 25°C. It varied between 25°C and 28°C thus, across the shallow surveyed temperatures were less than 35°C, which is considered as an indicative limiting value for water set aside for crops irrigation (FAO 2003). The pH of the water remained slightly alkaline throughout the period of study, with mean values between 7.1 CU and 7.7 CU; all the pH were within the range of several surface water used for irrigation in Africa (L. Ndiaye 2009). The electrical conductivity range between 28 and 509 µS/cm with lower values in the dry season .These slight mineralization is not able to play the role of fertilizer as sought by their use. Studies made by Ntangmo Tsafack et al., 2012 on vegetable watering water in Dschang Town, Cameroon were in the same range. It is also been observed that, high values of electrical conductivity (up to 509 µS/cm) observed in these shallows water were also obtained in Africa by Zébazé et al., 2009 (Cameroon), Souad EL BLIDI et al., 2006(Morocco) and Llewellyn J. (2005) (Canada). Average TSS ranged between 2 and 25 mg/l with highest values in the dry season, quantities which fulfill the norm of water used for irrigation. The percentage of saturated dissolved O2 remained below 97 %, while the BOD5 reached 130 mg/l without significant difference irrespective of the season. Except point C' and F' in both season and point G' in dry season, all values of BOD5 show a high pollution of water by organic compounds. The average values of orthophosphate were generally low and remained below 1 mg/l with high values in dry season. Phosphates could have come from detergents used in laundry, dishwashing, cars washings and to a lesser extent from fecal pollution fertilizer due to animal feces. This contamination shows an anthropogenic pollution (Atteia, 2005). For all the sampling points, the highest NO2- and NO3- concentrations were 1.3 and 7.9 mg/l respectively, with high values in rainy season due to emptying of contents of some septic tanks in the streams. The highest mean concentrations of NH4+ reached 13 mg/l with lower values in the dry season. Like orthophosphate, NH4+, NO2- and NO3- rarely originate from the ground although they may have an aboriginal origin in aquatic environment as a result of a more or less complete bacterial decomposition (Zébazé et al., 2011). Except ammonium, all the water sampled points have suitable nitrites and nitrates content for irrigation.

3.2. Bacteriological parameters

3.2.1. Bacterial abundance in shallow irrigation water of Nkolbisson

Figures 1 show the variation of the abundance of bacteria in shallow waters of Nkolbisson used for irrigation during the research period. It appears that the concentrations of bacteria could reach 11x105 CFU/100 ml. This value was obtained in the month of May (early rainy season). The abundance of bacteria was between 0.01x105 CFU/100 ml and 7.5x105 CFU/100 ml, 0 and 4.7x105 CFU/100ml and between 0 and 1.4 x105 CFU/100 ml for fecal coliforms, total coliforms and fecal streptococci respectively. Overall, the highest concentrations were observed during the rainy season. The bacteriological analysis during the study period showed that the abundance of bacteria considered underwent spatial and temporal variations in the waters of the shallows (Figures 1). These results are similar with those of Nola et al., 1998b, 2002 for the microbiological quality of surface and groundwater in Yaounde and Douala (Cameroon). Their studies have shown that those water host bacterial microflora consists of fecal and pathogenic bacteria and strict bacteria whose dynamics abundance undergoes spatiotemporal variation. These variation both depended on the physiological state of bacteria considered, the temperature, and the nature of the geological substratum crossed, weather factors, local physicochemical and trophic conditions, although

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some bacterial species prefer oligotrophic habitats (Nola et al., 2006). In all sampling points, the bacteria belonging to the groups of total and fecal coliforms and fecal streptococci were isolated with abundantly exceeding 105 CFU.ml-1 although these concentrations were not significantly different. Referring to WHO standards (2004), the presence of these bacteria in the waters shows a fecal contamination. These waters are thereby unfit for crop irrigation.

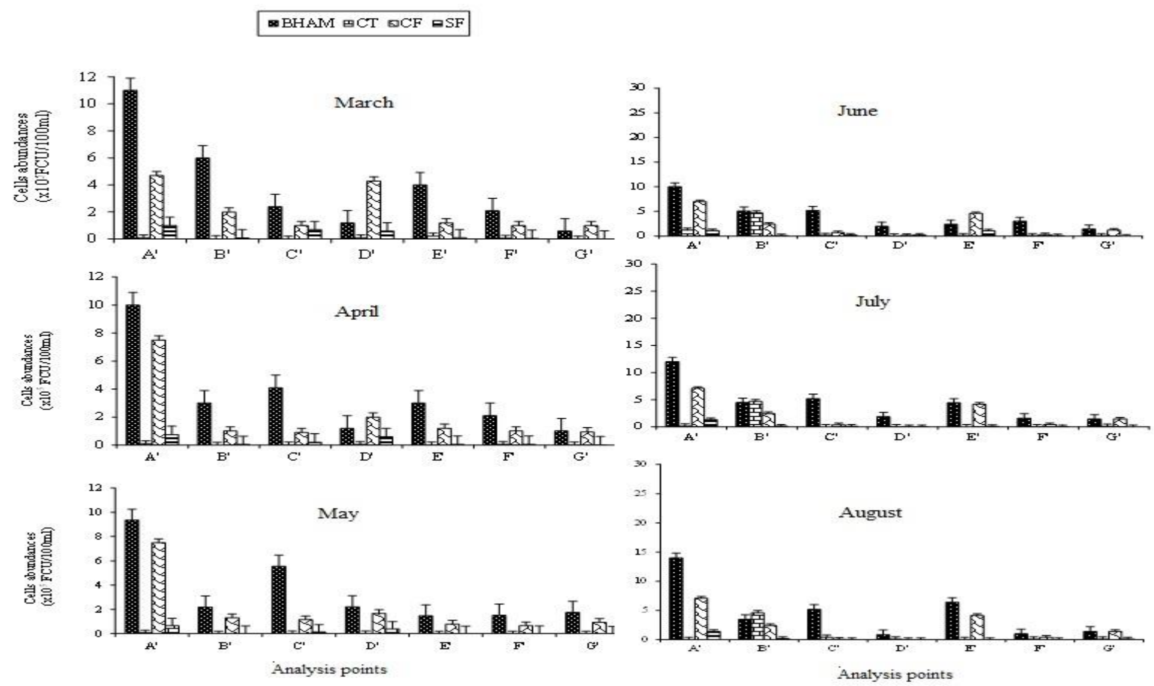

Figure 1: Abundance of bacteria in shallow waters of Nkolbisson in rainy and dry season.

3.2.2. Origin of fecal pollution in the irrigation water of the shallows in Nkolbisson

To determine the origin of fecal pollution in shallows irrigation water of Nkolbisson, CF/SF ratios were calculated with the average abundance of each season. The results are shown in Table IV. It appears that, CF/SF ratios were 10.989 and 9.803 in the rainy and dry season respectively. In both season, CF/SF ratios was greater than 4, the contamination of irrigation water from this shallows was exclusively due to human.

Table IV: Mean values of CF/SF reports in Nkolbisson shallow waters

	Rainy season	Dry season
CF/SF	10,989	9,803

FC/FS < 0.7: Mainly or entirely of animal origin; 0.7< FC/FS < 1: mixed with animal prevalence; 1< FC/FS < 2 dubious Origin; 2< FC/FS < 4 Mixed with human prevalence; FC/FS > 4 exclusively human (Borrego and Romero, 1982).

3.3. Correlation between bacterial abundance and physicochemical parameters of irrigation water of Nkolbisson shallows

The Spearman "r" correlation test was conducted to determine the degree of connection between the physicochemical and bacteriological parameters at each sampling point during the different seasons. In general, the results showed that there were significant and positive correlations between the parameters considered, depending on the sampling point, the physicochemical and bacteriological parameters considered (Table V). Note that some correlations could not be determined. This result was observed with the fecal coliform at sampling points C', D', E 'and fecal streptococci at the sampling point G'. Abiotic parameters such as temperature, concentration of nitrogen and phosphorus can have an action on cell abundance. Indeed, nitrogen compounds are affected by nitrifying and denitrifying bacteria. Nitrate reduction by denitrifying bacteria in the presence of organic compounds contained in suspended solids under anaerobic conditions, result in the production of nitrites, which are then decomposed to elemental nitrogen. Under aerobic conditions, ammonia (which may be produced during the decomposition of organic matter) is oxidized to nitrite and nitrate (Hall et al., 2002). Similarly, the ion may be subject to reduction or oxidation depending on the conditions of pH or redox potential of the water. At every moment, the metabolic potential of the microorganism is affected by the exposure time to the donor or electron acceptor molecule and, the residence time of the microorganism (Hall et al., 2002; Mailloux et al., 2003).

Table V: Spearman correlation between the physicochemical and bacteriological parameters in the irrigation water of Nkolobisson shallow

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SP	bacteria	pH	EC	Temp	TSS	O_2	BOD_5	PO_4^{3-}	NO_2^-	NO_3^-	NH_4^+
A'	BHAM	**	NS	**	**	NS	NS	**	**	NS	**
	CF	**	NS	**	**	NS	NS	**	**	NS	**
	CT	NS	**	NS	NS	**	**	NS	NS	**	NS
	SF	**	NS	**	**	NS	NS	**	**	NS	**
B'	BHAM	**	**	**	**	NS	NS	**	NS	NS	**
	CF	**	**	**	**	NS	NS	**	NS	NS	**
	CT	**	**	**	**	NS	NS	**	NS	NS	**
	SF	**	**	**	**	NS	NS	**	NS	NS	**
C'	BHAM	NS	NS	**	**	NS	NS	-	NS	NS	**
	CF	-	-	-	-	-	-	-	-	-	-
	CT	NS	NS	**	**	NS	NS	-	NS	NS	**
	SF	NS	NS	**	**	NS	NS	-	NS	NS	**
D'	BHAM	**	**	NS	NS	**	NS	NS	**	**	NS
	CF	-	-	-	-	-	-	-	-	-	-
	CT	**	**	NS	NS	**	NS	NS	**	**	NS
	SF	**	**	NS	NS	**	NS	NS	**	**	NS
E'	BHAM	NS	NS	**	**	NS	NS	-	NS	NS	**
	CF	-	-	-	-	-	-	-	-	-	-
	CT	NS	NS	**	**	NS	NS	-	NS	NS	**
	SF	NS	NS	**	**	NS	NS	-	NS	NS	**
F'	BHAM	**	NS	**	**	NS	NS	NS	NS	NS	**
	CF	**	NS	**	**	NS	NS	NS	NS	NS	**
	CT	NS	**	NS	NS	**	**	**	**	**	NS
	SF	NS	**	NS	NS	**	**	**	**	**	NS
G'	BHAM	NS	**	NS	NS	**	**	**	**	**	NS
	CF	**	NS	**	**	NS	NS	NS	NS	NS	**
	CT	**	NS	**	**	NS	NS	NS	NS	NS	**
	SF	-	-	-	-	-	-	-	-	-	-

Tailed test, ** significant correlation at P <0.01, NS: Not significant correlation;

-: Not determined correlation, df = 1; (fecal coliforms (CF), total coliforms (CT), faecal streptococci (SF), heterotrophic aerobic mesophilic bacteria (BHAM)), sampling point (SP).

3.5. Principal Component Analysis between microbiological and physicochemical parameters of irrigation water of Nkolbisson shallows

The projection of taxa on the factorial of the two components of the PCA (Figure 2) in these waters showed the formation of a core two trends. On the positive side axis coordinates of components 1 and 2, the concentrations of BHAM, total coliforms and fecal streptococci seem to be under the influence of parameters such as NO2-, DBO5, pH, electrical conductivity and NH4+. In contrast, a reduction in the abundance of fecal coliform (located on the side of negative coordinates of the axis of the component 2) is under the influence of these physicochemical parameters.

The PAC also showed here that these factors (pH, TSS, nitrogen, phosphorus, etc) are abiotic properties that actually affect the abundance of isolated bacteria (Figure 2).

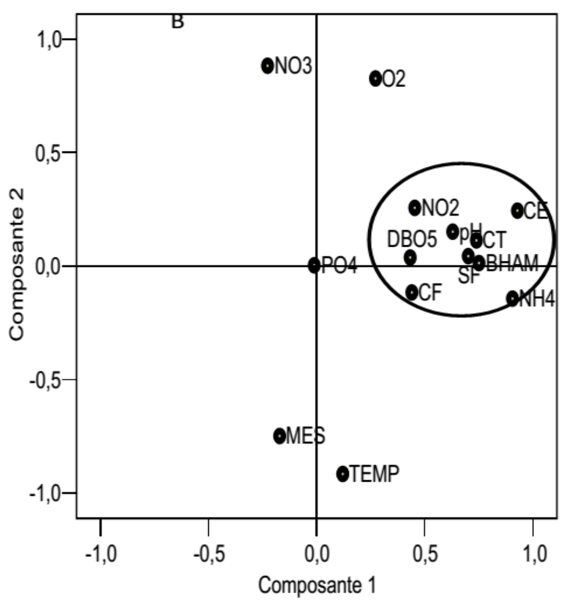

Figure 2: Principal Component Analysis between the physicochemical and microbiological of irrigation water of Nkolbisson shallows.

Legend: (fecal coliforms (CF), total coliforms (CT), fecal streptococci (SF), heterotrophic aerobic mesophilic bacteria (BHAM)).

CONCLUSION

Shallows irrigation water at Nkolbisson host microflora bacterial composed of fecal coliforms, total coliforms and fecal streptococci which indicate pathogenic bacteria. The abundance of these bacteria varied throughout from one, sampling point to another, from one season to another and from one group to another. This variation was influenced by the physicochemical properties considered ($P<0.01$). No significant difference was observed between bacterial abundances. However, PCA permit to relate or link some abiotic parameters (NO2-, DBO5, pH, electrical conductivity and NH4+) to the abundance of BHAM, fecal and total coliforms and fecal streptococci in the shallows. The waters of these shallows underwent fecal pollution of human origin and are unsuitable for use. It is highly recommended that the water be treated before using for irrigation, washing vegetables after harvest, bathing and laundry.

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Table III: Mean values of physicochemical parameters during the rainy and dry season

	A'	B'	C'	D'	E'	F'	G'	FAO 2003
				RAINY SEASON				
pH	7.3±0.08	7.6±0.08	7.5±0.08	7.6±0.06	7.6±0.09	7.1±0.06	7.2±0.09	6.5-8.5
CE(µS/cm)	509±0.63	455±0.75	211±0.66	355±0.75	348±0.75	103±0.14	32.7±0.11	<7000
T°C	25.4±0.80	25.4±0.10	25.4±0.05	25.5±0.05	25.7±0.05	25.8±0.08	26.1±0.05	<35
TSS (mg/l)	5±0.51	2±0.14	13±0.54	18±0.39	12±0.63	10±0.33	6±0.52	<30
O2 (%sat)	95.4±0.08	96.0±0.20	84.4±0.15	92.7±0.12	96.3±0.51	93.8±0.32	89.9±0.33	
BOD_5 (mg/l)	70±0.13	75±0.19	10±0.23	110±0.89	130±0.63	26±0.53	30±0.53	<30
PO_4^{3-} (mg/l)	0.01±0.01	0.06±0.01	0.01±0.01	0.01±0.01	0.01±0.01	0.03±0.01	0.06±0.01	
NO_2^- (mg/l)	0.41±0.01	1.30±0.01	0.66±0.01	0.07±0.01	0.06±0.01	0.38±0.01	0.06±0.01	<3
NO_3^- (mg/l)	6.70±0.36	6.46±0.19	7.90±0.23	6.70±0.41	6.70±0.34	6.70±0.07	6.11±0.08	<50
NH_4^+ (mg/l)	9.81±0.05	9.73±0.04	3.21±0.06	7.49±0.05	6.83±0.11	1.66±0.05	0.45±0.03	1.5
				DRY SEASON				
pH	7,5±0.14	7,7±0.07	7,5±0.09	7,3±0.09	7,5±0.07	7,2±0.07	7,3±0.09	6.5-8.5
CE(µS/cm)	477±1.70	507±1.07	202±1.89	300±2.85	345±3.24	99±2.16	28±1.49	<7000
T°C	27±0.96	28±0.94	27±0.75	27±0.58	27±0.69	28±0.50	27±0.47	<35
TSS (mg/l)	13±0.82	7±0.69	17±0.94	25±1.21	19±1.29	21±0.94	13±1.07	<30
O2 (%sat)	89±1.29	86±1.83	79±1.89	84±1.89	86±2.85	78±1.86	77±1.70	
BOD_5 (mg/l)	68±1.92	71±2.11	21±2.27	127±1.70	115±3.37	25±1.53	24±2.21	<30
PO_4^{3-} (mg/l)	0.01±0.01	0,1±0.01	0.01±0.01	0,1±0.01	0.01±0.01	0.01±.0.01	0,1±0.01	
NO_2^- (mg/l)	0,50±0.03	1,20±0.05	0,90±0.10	0.01±0.01	0.01±0.01	0,20±0.03	0.01±0.01	<3
NO_3^- (mg/l)	3,10±0.09	5,30±0.13	3,60±0.43	4,30±0.15	3,40±0.27	2,80±0.13	5,30±0.17	<50
NH_4^+ (mg/l)	11±0.81	13±1.00	7,2±0.13	9,6±0.76	8,2±0.44	5,6±0.31	2,40.33	1.5