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Anopheles Mosquito Larval Production and Association of Water Bodies with Malaria Transmission in Ife and Ilesa Areas of Osun State, Nigeria

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Abstracts: *The environmental factors influencing relative association of water bodies with malaria transmission was investigated in Ife and Ilesa areas of Osun State, Nigeria to advance strategies for malaria control at source. Randomly selected proportional number of water bodies in Ife (32) and Ilesa (23) were investigated for Anopheles mosquito larval density and physicochemical characteristics of water. Water bodies were identified with topographic maps, satellite imagery analysis and ground truthing. Laval density was estimated using the standard dip and search method and physico-chemical properties of water by the standard methods of sampling and analyses. Number of water bodies containing larvae (NWL) was equal in Ife (5) and Ilesa (5) but percentage of water bodies with larvae (PWL) was higher in Ilesa (22%) than Ife (16%). Also, NWL and PWL were each three times higher in the wet (8, 14.5%) than in dry (3, 5.5%) season. Concentrations of conductivity, chloride and calcium were significantly higher ($p< 0.05$) in Ife than in Ilesa. Temperature was significantly higher in the dry than in the wet season, while, conductivity, acidity and calcium were each higher in wet than in dry season. While these findings suggest that these physicochemical parameters are positively associated with malaria transmission, results of multiple linear regression analysis (MLRA) was not definitive. Larval production was positively and significantly associated with potassium when Ife and Ilesa data were combined and with chloride when only Ilesa data was selected. The study concluded that while these parameters may play vital roles in the larval production in water bodies, additional studies are required to elucidate their relative importance.*

Key words: *Water bodies, mosquito, Anopheles, physicochemical parameters, Ife, Ilesa, Osun State, Nigeria.*

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I. Introduction

The World Malaria Report 2012 (WHO, 2013) presents no surprises. Malaria is still endemic in 104 countries and territories worldwide, transmission is on-going in 99 countries and territories and over 3.3 billion people – half of the world's population – are at risk of infection. In 2010, active malaria were estimated to be 219 million (range: 154-289 million) and mortality over 660 000 (range: 490 000-836 000); 90% of all the cases and deaths occur in sub-Saharan Africa. Though it was reported that malaria incidence rates fell by 37% globally and 42% in the African Region between 2000 and 2015 (WHO, 2015) yet malaria is still the most important causes of death and illness particularly in sub-Saharan Africa. The trend in the declination is aftermath of concerted preventive and control efforts to reduce transmission and malaria burden through a programme of roll back malaria initiative (Nabarro and Taylor, 1998; Nabarro, 1999; Balter, 2000; Narasimhar and Attaran, 2003). The initiative increased access to effective drugs and insecticide treated nets (ITN) to reduce morbidity/mortality rates and mosquito bites, respectively. Although these efforts have proved effective, much still needs to be done to hasten reduction of malaria burden below public health importance (WHO, 2012).

In this paper, we argue for a reinvigoration of malaria and mosquito control through larval source management as an effective component of roll back malaria. This approach played vital role in the past until the use of DDT was banned worldwide. Recently, the World Health Organization advocated its reintroduction to complement adult vector mosquito control (WHO, 2013). According to the WHO, larval control, which is equivalent to tackling malaria at source, is more cost-effective and more sustainable. In a letter to the editor, Valle *et al.* (2013) agreed with this approach and contended that sustainable larval control requires knowledge of water bodies' abundance per area and relative rates of larval production per water body. That is, overall

knowledge of measures of association between larval production and the number of water bodies containing mosquito larvae identified as the vital risk factor for malaria transmission in an area. However, their report was silent on the influence of other factors especially environmental factors that could also affect the abundance of water bodies containing larvae. This was in spite of their report that high abundance of water bodies does not always translate to high abundance of water bodies containing larvae in an area. For instance, they considered two ecologically divergent areas with unequal abundance of water bodies, but containing equal number of water bodies containing mosquito larvae. They concluded that the area with greater abundance of water bodies has a negative association with the presence of mosquito larvae, hence a lower overall risk of infection, while the other with fewer number of water bodies had a positive association with mosquito larvae, hence a greater infection risk. These are strong indications that factors other than abundance of water bodies explain abundance of water bodies containing larvae, hence transmission potential in an area.

In this paper, we investigated the effects of abundance of water bodies, type, distribution, size (depth, width, etc.), season and physico-chemical characteristics of water bodies also play on larval production. This was with the view to identifying factors that are most predictive of anopheline larval production in water bodies in the study area.

II. Materials And Methods

Study Area: The study area comprises two local government areas (LGAs), two each in Ife (Ife Central and Ife East) and Ilesa (Ilesa Central and Ilesa West) in Osun State, southwestern Nigeria. Ife is located between Latitudes 07° 28′-07° 32′N; Longitudes 004° 30′E- 004° 32′E; and Ilesaon Latitudes 07° 34′N-07° 37′N; Longitudes 004° 33′E-004° 35′E (Fig. 1). Ife is about 29 Km south of Ilesa. The combined landmass of Ife LGAs is 286,438 $km^2$with a population of 355,818 (NPC, 2006). The corresponding figures for Ilesa LGAs are 135, 271 km^2 and 212, 225. Both are predominantly inhabited by the people of Yoruba ethnic group although other ethnic groups in Nigeria are proportionally represented. The climate of both areas is tropical rain forest characterized by two distinct seasons. The rainy season spans April to October and the dry season November to April. The mean annual temperature is 27˚C, mean precipitation range 1000-1250 and mean humidity range 75-100% (Ayoade, 1982; Oguntoyinbo, 1982; Ofoezie, 1999; Asaolu *et al*., 2002). The natural vegetation is the tropical rain forest containing diverse species of shrubs and tree plants. Both areas are traversed by a network of water bodies drained in Ilesa area by Osun, Sasa and Oora rivers and in Ife area by Esinmirin and Opa rivers (Owojori *et al*., 2006).

Identification of Water bodies

Satellite imageries from LandSat ETM+ (2007) of 30 m resolution and topographical maps were used to identify surface water bodies (streams, rivers, lakes, pools, gutters) in Ife and Ilesa LG areas. Fifty percent of water bodies so identified were randomly selected, validated by ground trothing and investigated in details for physicochemical characteristics of water and mosquito larval density. During the ground truthing, information was collected from each water body on location coordinates (using a GPS) and morphometric characteristics such as depth and width measured (for small informal water bodies with a tape rule) or obtained from the archives (for medium to large formal water bodies).

Physico-chemical Characterization of Water bodies

Temperature, conductivity and pH were measured *in situ* with mercury in glass thermometer, conductivity meter and pH meter respectively. Dissolved oxygen (DO) and five-day biochemical oxygen demand (BOD_5) were determined using the Winker's method (APHA *et al*., 1992; Ofoezie, 1999). Water samples for DO and BOD5 were collected in 250 ml light and dark reagent bottles respectively. The DO samples were fixed immediately in the field by an addition of manganous chloride solution and Winkler'sreagent (i.e. alkaline iodide). The BOD_5 samples were treated in like manner after five days incubation in the dark. Both DO and BOD_5 samples were titrated in the laboratory using 0.0125N sodium thiosulphate. Water samples were then taken in 2.0L plastic bottles and returned to the laboratory for analysis of other parameters. Calcium and magnesium were measured by the complexiometric titration method, potassium and sodium by the flame emission spectrophotometer, alkalinity and acidity by acid-base titration method and chloride by the Mohr titration method (Golterman *et al*., 1978; APHA *et al*., 1992).

Mosquito Larval Sampling, Identification and Estimation of Abundance

Selected water bodies were surveyed for presence and abundance of mosquito larvae using the dip estimation method (Leisnham *et al*., 2005; Varum et al., 2013; Ammar et al., 2013). Two stations per water body were selected randomly and sampled using a standard 1 L enamel dipper attached to a 1.5 m handle. Ten random dips per station were made and 10 L water sample collected was screened across a fine mesh (45 µm) to a concentrate volume of 120 ml. This was transferred to a vial and preserved in 70% alcohol solution. Larval

presence and abundance were determined in 10 ml concentrate subsample withdrawn with a 21G syringe after thorough mixing. The subsample was poured into a glass Petri dish and viewed under a dissecting microscope. The genus Anopheles was identified using standard keys (Harbach, 1985; Eugene and Ross, 1976; Rueda, 2004) and separated from other genus. The number of each Anopheles larval in each subsample was determined per liter per water body.

The number of Anopheles larval in each subsample was determined and multiplied by 12 to convert to number of Anopheles larvae per liter per water body.

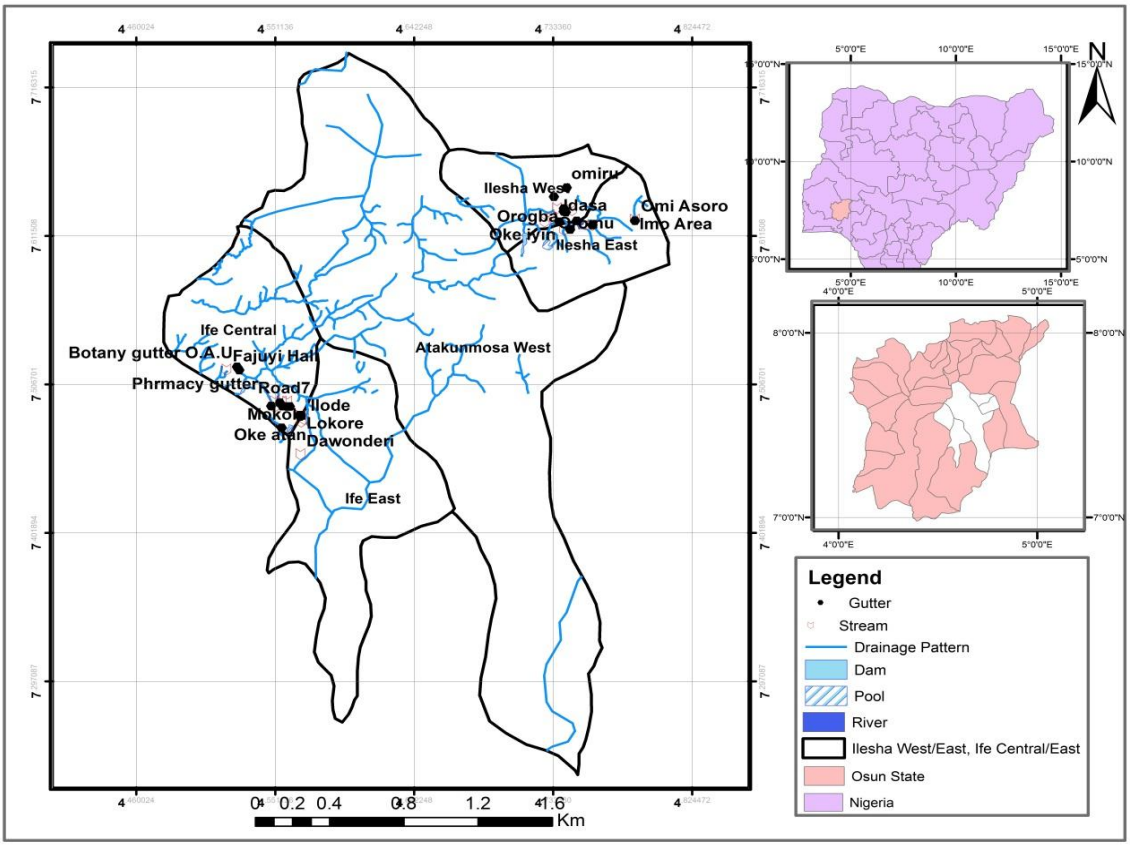

Figure I Maps of Ilesa and Ile-Ife showing Drainage Pattern and Sampling Locations respectively

Statistical Analysis

All analysis was performed on log transformed data since initial data was not normally distributed. Differences in the distribution of larvae between seasons, locations and water bodies were determined using the Oneway analysis of variance (One-way ANOVA). The relationship between larval production and the various explanatory variables (viz. abundance of water bodies, environmental factors and morphometric characteristics) was determined using the multiple linear regression analysis. The model was built on the stepwise method and $p<0.05$) set as criteria for selecting variable in the model. All analyses were performed on SPSS version 20.

III. Results

Types of water bodies in relation to Anopheles mosquito larva production

Detailed investigation was carried out on 55 of 110 water bodies (comprising 4 pools, 46 streams, 6 rivers, 4 artificial lakes and 50 gutters) identified by the preliminary desktop study analysis and subsequent ground truthing (Table 1). A total of 85 Anopheles mosquito larvae of 1381 larval collected were found both in dry and wet seasons (Table 2).

Abundance of water bodies in relation to Anopheles larva production

Larvae of *Anopheles* spp. the local vector of malaria were collected from 10 water bodies (5 streams, 2 rivers and 3 gutters) (Table 1). Streams had the highest number of water bodies containing larvae (NWL), while rivers had the highest percentage of water bodies containing larvae (PWL). Ife and Ilesa had the same number of water bodies containing Anopheles larvae (5 each) though there were more water bodies in the former (32) than

the later (23) (Table 1). Thus, PWL was higher in Ilesa (22%) than Ife (16%). Risk of transmission was also higher in the wet than in dry season. Although equal number of water bodies (55) was investigated in both seasons, the number of water bodies containing mosquito larvae was nearly three times higher in the wet (8) than in dry (3) season (Table 3). Percentage of water bodies with larvae was also nearly three times higher in the wet (14.5%) than in the dry (5.5%) season.

Physico-chemical characteristics of water bodies

The physico-chemical properties of water bodies investigated in Ife and Ilesa are presented in Tables 4 while the seasonal trends are presented in Table 5. The concentration of all parameters except width of water bodies, conductivity, chloride and calcium were comparable between Ife and Ilesa water bodies. On average, water bodies in Ilesa were significantly wider ($p< 0.05$) than those in Ife. In contrast, the concentration of conductivity, chloride and calcium was significantly higher in Ife than in Ilesa (Table 4). All measured parameters were comparable between the dry and wet seasons except temperature, conductivity, acidity and calcium (Table 5). Temperature was significantly higher in the dry than in the wet season, while the other parameters were each higher in wet than in dry season.

Multiple linear regression models

A multiple linear regression analysis revealed that larval production in the study area was positively and significantly associated with only potassium when Ife and Ilesa data were combined (Table 6). However, when Ilesa data was selected, chloride and width of water bodies became positively predictive. Conversely, no parameter was predictive of larval production when Ife data alone was selected. Generally, none of the three models (combined, Ilesa, Ife) showed a strong linear correlation between larval production and explanatory variables when only the values of the Dublin-Watson statistic was considered. The values of 1.66 and 1.71 obtained for Ilesa and combined data respectively were closer to 2 (no linear correlation) than to either 0 (strong positive correlation) or 4 (strong negative correlation). However, the high significant values of F and B in both models suggested otherwise. Thus, it is safe to conclude that the parameters in the model equations were significantly predictive of larval production in the study areas. The model equations are:

Larva = 0.201Cl +0.215Width – 3.98 …….. Ilesa

Larva= 0.032K – 0.243 ……. Combined data.

IV. Discussion

Malaria has remained intractable for hundreds of years. Africa accounts for over 80% of global malaria cases and over 90% of global malaria deaths (WHO, 2012). Nigeria and Congo Democratic Republic (DRC) account for over 50% of the African malaria burden. The abnormally high burden in Africa and in particular Nigeria was attributed to *Plasmodium falciparum* the local causative agent of malaria, and the most dangerous of the four human malaria parasites. In addition, the parasite is transmitted by *Anopheles gambiae,* the most effective malaria vector adjudged the most wide spread in Africa and the most difficult to control (WHO, 2002). These intractable problems associated with the control of adult *A. gambmiae* underscore the need for malaria control at source.

Anopheles gambiae breeds in shallow, stagnant water bodies such as ponds, marshes, swamps, floodwater, ditches and woodland pools. They also breed on shallow undulating portions of lotic water bodies such as gutters, streams and rivers (Ammar *et al*., 2012; Afolabi *et al*., 2013; Varun *et al*, 2013). At source control requires a good knowledge of the relative contributions of these habitats to the production and sustenance of mosquito larvae. The recent investigation of the importance of relative abundance and proportional number of water bodies containing larvae as an index of relative risk of malaria transmission in endemic areas is an emerging area of research that has the potential to advance malaria control at source (Valle *et al*., 2013). It raised several topical questions: (i) does relative risk of malaria transmission vary with the number of water bodies containing relevant mosquito larva in an area, (ii) does number of water bodies containing larva directly related to the abundance of water bodies in that area (iii) what factors influence the relative abundance of bodies to produce and sustain mosquito larvae. The association between abundance of water bodies and relative risk of malaria transmission has been a basis of environmental control. For a long time, disease control experts had advocated the prompt destruction of probable transmission sites as a means of reducing transmission potential. And since transmission sites are water bodies containing larva in an area and (iv) what factors affect the ability of water not too easy to identify, emphasis had been on the destruction of all water bodies where mosquito is likely to breed. This control strategy was based on a theoretical concept that abundance of water bodies in a given endemic setting is directly associated with intensity of transmission. That is, the more water bodies in a given endemic setting the higher the likelihood of transmission. But as revealed by the findings of this investigation and supported by Valle *et al.* (2013), such an association is imperfect. Relatively high abundance of water bodies does not always translate to relatively high abundance of water

bodies containing mosquito larvae neither does it lead to higher relative risk of transmission. For instance, while Ife area had more abundance of water bodies investigated than Ilesa, both areas had equal numbers of water bodies containing *Anopheles* larvae. As proved by Valle et al. (2013), Ife had a negative association with the presence of mosquito larvae and hence a lower risk of transmission than Ilesa. Conversely, Ilesa had a positive association with the presence of mosquito larvae and a higher risk of transmission. Furthermore, our results revealed unequivocally that risk of transmission varied significantly among water bodies suggesting that some water bodies are more important in malaria transmission than others. For instance, risk of transmission was highest in rivers and least in pools and lakes. This finding agreed strongly with the conclusion drawn by Afolabi *et a*l. working in another part of southwestern Nigeria and Umar (per comm.) working in northern Nigeria. Our finding also revealed a significant seasonal variation in the risk of transmission. Consistent with Afolabi *et al*. (2013) our finding revealed that transmission was significantly higher in wet than in dry season. Environmental factors analyzed to explain these trends was, however, less conclusive than expected. It was unable to conclusively point to specific physicochemical factors that significantly influenced the ability of investigated water bodies to produce and sustain mosquito larvae. Rather, different analyses pointed to different factors. For instance, while analysis of Ife and Ilesa data combining water bodies containing larvae and water bodies not containing larvae suggested that the likelihood of a water body containing larvae significantly increased with decreasing concentrations of conductivity, calcium and chloride. A similar analysis comparing water bodies containing larvae and water bodies not containing larvae but combining Ife and Ilesa data, pointed to biochemical oxygen demand as the only significant factor (Table 7). Surprisingly, none of these parameters was in the regression model equation, rather chloride was the only significant factor in Ilesa and potassium when Ife and Ilesa data were combined. These findings are as interesting as they are confusing. First, they suggest that physicochemical parameters may significantly influence the ability of water bodies to contain larva. Secondly, the conflicting results point to new opportunities for further research.

Table 1: Abundance of water bodies (number of water bodies containing Anopheles larva) in Ife and Ilesa areas of Osun State, Nigeria

Types of Water bodies	Ife	Ilesa	Total	NWL	PWL
Pool	types1	1	2	0	0
Stream	15(3)	8(2)	23(5)	5	22
River	0	3(2)	3(2)	2	67
Lake	1	1	2	0	0
Gutter	15(2)	10(1)	25(3)	3	12
Total (number of water bodies)	32	23	55(10)	10	18
Number of water bodies with larva (NWL)	5	5	10		
Percentage of water bodies with larva (PWL)	16	22	18		

Table 2: Seasonal Distribution of Anopheles mosquito larva in various water body types investigated in Ife and Ilesa areas of Osun State, Nigeria

W	Pools	Streams	Rivers	Gutters/ Drains	Total
Dry		9	29		38
Wet		11	4	32	47

Table 3: Abundance of different types of water bodies (number of water bodies containing Anopheles larva) during the dry and wet seasons of 2012

Types of Water bodies	Dry	Wet	Total	NWL	PWL
Pool	2	2	2	0	0
Stream*	23(2)	23(4)	23(6)	6	26.1
River	3(1)	3(1)	3(2)	2	67
Lake	2	2	2	0	0
Gutter	25	25(3)	25(3)	3	12
Total (number of water bodies)	55(3)	55(8)	55(11)	11	20
Number of water bodies with larva (NWL)	3	8	11		
Percentage of water bodies with larva (PWL)	5.5	14.5	20		

* Larva was found in one stream both in dry and wet seasons

Table 4: Variation in the physico-chemical properties of water bodies in Ife and Ilesa, Osun State, Nigeria

Parameter	Unit	Ife (n= 64)	Ilesa (n= 46)	P-Value
Depth	M	0.38±0.88	0.74±1.06	Ns
Breath	M	2.89±5.27	5.49±7.40	<0.05
Temperature	℃	28.08±1.45	28.06±1.56	Ns
pH		7.57±0.31	7.49±0.35	Ns
Conductivity		684.74±358.66	528.46±336.82	<0.05

DO	mg/l	2.70±2.34	2.70±1.61	Ns
BOD	mg/l	4.75±2.87	4.58±2.86	Ns
Acidity	mg/l	30.43±20.19	27.83±17.07	Ns
Alkalinity	mg/l	19.85±12.27	20.45±9.79	Ns
Chloride	mg/l	33.67±40.82	19.17±13.36	<0.05
Magnesium	mg/l	12.30±9.24	12.07±9.27	Ns
Calcium	mg/l	50.42±37.43	35.61±21.69	<0.05
Sodium	mg/l	46.17±23.88	34.73±22.38	Ns
Potassium	mg/l	35.43±25.25	27.47±21.51	Ns

Table 5: Seasonal variations in the physico-chemical properties of water bodies investigated in Ife and Ilesa, Osun State, Nigeria

Parameter	Unit	Number of samples	Dry	Wet	P-Value
Depth	M	55	0.50±0.90	0.56±1.04	Ns
Breath	M	55	3.84±6.16	4.121±6.60	Ns
Temperature	℃	55	28.44±1.76	27.71±1.05	<0.05
pH		55	7.53±0.33	7.55±0.33	Ns
Conductivity	µS/cm	55	727.59±378.92	511.19±298.82	<0.01
DO	mg/l	55	2.85±2.10	2.55±2.07	Ns
BOD	mg/l	55	5.06±3.06	4.29±2.61	Ns
Acidity	mg/l	55	33.06±18.19	25.62±19.04	<0.05
Alkalinity	mg/l	55	20.50±10.54	19.70±12.02	Ns
Chloride	mg/l	55	29.73±30.16	25.48±35.75	Ns
Magnesium	mg/l	55	12.30±8.62	12.10±9.84	Ns
Calcium	mg/l	55	30.91±23.53	57.55±34.93	<0.01
Sodium	mg/l	55	40.13±26.28	42.64±21.31	Ns
Potassium	mg/l	55	36.28±27.83	27.91±18.72	Ns

Table 6: Summary of multiple linear regression to identify environmental factors that are predictive of larval production in water bodies in Ife and Ilesa areas of Osun State, Nigeria.

Parameter	Ife	Ilesa	Combined
Dependent variable	Larva	Larva	Larva
Independent variable	Ife data	Ilesa data	All parameters
Method	Stepwise	Stepwise	Stepwise
Variable entered	None	Cl, Width	K
R		0.55	0.22
R^2		0.302; Variance explained = 30.2%	0.05; Variace explained = 5%
Durbin-Watson		1.66	1.71
F		9.32 (df: 2,45)	5.70 (df: 1, 108)
Significance of F		0.000	0.019
Coefficients			
B		Constant = -3.98; p< 0.01 Cl = 0.201; p< 0.001 Width = 0.215; p< 0.05	Constant = -0.243; p> 0.05 K = 0.032; p< 0.05
Model equation		Larva = 0.201Cl +0.215Width – 3.98	Larva= 0.032K – 0.243

Table 7: Comparative assessment of the physico-chemical properties of water bodies containing mosquito larvae and those not containing mosquito larvae

Parameter	Unit	Containing Larva (n= 11)	Not Containing Larva (n= 99)	P-Value
Depth	m	0.44±0.73	0.54±0.99	Ns
Breath	m	3.52±5.20	4.03±6.49	Ns
Temperature	℃	27.88±1.27	28.09±1.52	Ns
pH		7.64±0.37	7.53±0.32	Ns
Conductivity	µS/cm	627.03±301.11	618.54±363.59	Ns
DO	mg/l	2.67±2.46	2.70±2.02	Ns
BOD	mg/l	2.88±2.36	4.87±2.84	<0.05
Acidity	mg/l	26.38±21.03	29.67±18.75	Ns
Alkalinity	mg/l	17.52±8.16	20.39±11.54	Ns
Chloride	mg/l	35.06±20.60	26.78±34.01	Ns
Magnesium	mg/l	8.99±8.85	12.56±9.23	Ns
Calcium	mg/l	58.51±51.72	42.65±29.63	Ns
Sodium	mg/l	43.39±22.35	41.16±24.10	Ns
Potassium	mg/l	44.89±33.72	30.67±22.43	Ns

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