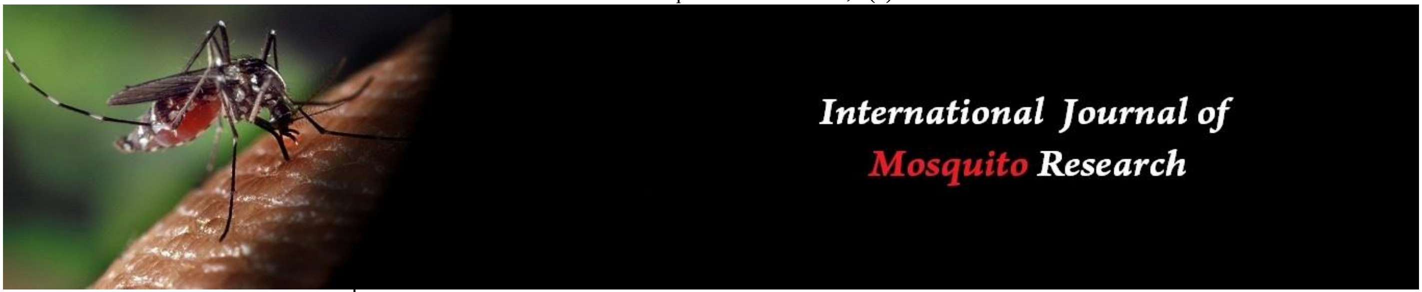

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Assessment of physico-chemical characteristics of mosquito breeding sites in Northwest Nigeria

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Abstract
Malaria and lymphatic filariasis (LF) are mosquito-borne diseases of serious public health problem in Northwest Nigeria. There is inadequate evidence on the effect of physicochemical parameters on mosquito larvae in rural and semi urban settings of Northwest Nigeria. This study aimed at assessing the effects of physicochemical parameters on *Anopheles* and *Culex* larvae in different breeding sites in Northwest Nigeria. Temperature, pH, conductivity, dissolve oxygen (DO), biological oxygen demand (BOD), chemical oxygen demand (COD), total solid (TS), total suspended solid (TSS), total dissolve solid (TDS), oil and grease, PO_4^{3-}, NO_3^{2-}, Mg^{2+}, Ca^{2+}, Fe^{2+}, Cu^{2+}, Mn^{2+}, Zn^{2+} and larval density were determined. Conductivity, BOD, Na^+ and Cl^- significantly ($P < 0.05$) influenced the larval density positively in breeding site A (residential area). But, COD and BOD significantly ($P > 0.05$) affected the larval density negatively in breeding site B (petrochemical area). It was observed that, conductivity, TS, TDS, TSS, DO, COD, BOD, Na^+, K^-, Cl^-, PO_4^- and NO_3^- significantly ($P > 0.05$) decreased the larval density in breeding site C (residential area) but, only BOD was significantly ($P < 0.05$) increased the larval density in breeding site D (agricultural area). DO, Na^+, K^-, Cl^- and NO_3^- significantly ($P > 0.05$) decreased the larval density in breeding site E (residential area) but, significantly ($P < 0.05$) increased the larval density in breeding site F (agricultural area). This study provides information on mosquito breeding sites in relation to physicochemical parameters which may be considered in larval mosquito control strategy in the Northwest Nigeria.

Keywords: *Anopheles, Culex,* temperature, pH

Introduction

Malaria is transmitted by anopheline mosquitoes, while lymphatic filariasis is transmitted by both *Culex* and *Anopheles* mosquitoes [1-3]. These mosquitoes breed in different habitats [4-5]. For instance, *Anopheles gambiae s.l* breed in small, open, sunlit, fresh water bodies, *Anopheles funestus* normally breed in water bodies with emergent vegetation such as swamps and rice fields. *Culex quinquefasciatus* breed in polluted water habitats such as pit latrines, soak pits, cesspits and open sewage systems [5, 6].

Physicochemical properties of breeding habitat influences the oviposition and development of Mosquitoes [7]. Various properties of the oviposition sites such as: temperature, turbidity, pH, concentration of ammonia, nitrite and nitrate, sulphate, phosphate, chloride, calcium, and water hardness affect mosquito larval density [8-10]. Changing these factors in breeding habitats may create conditions favorable or unfavorable for mosquito biology [11].

To control mosquitoes, a good knowledge and understanding of the breeding habitats and ecology of the target species is overemphasized [12-13]. Moreover, the knowledge of the ecological characteristics of the breeding habitats and the environmental factors affecting mosquito abundance can help in designing good vector control strategies [14]. Generally, mosquitoes breed in a wide range of habitats with different types of waters that are known to be specific for many species. The physical and chemical nature of the water probably determines the selection of the breeding sites [12]. Piyaratnea *et al.* [7] reported that breeding water quality is an important determinant of whether female mosquitoes will lay their eggs, and whether the resulting immature stages will successfully complete their development to the adult stage.

This present study was aimed at assessing the effects of physicochemical parameters on *Anopheles* and *Culex* larvae abundance in different breeding sites in a rural and semi urban setting of Northwest Nigeria.

Materials and Methods

Study Site and Collection of Larvae

Mosquito larvae were collected from gutters and puddles using classical dipping method as described by Robert *et al*. [15]. The gutters were polluted with organic materials and surrounded by residential and commercial areas and the puddles were formed by raining season water runoffs. *Culex* larvae were identified by their angular position on the surface of water and the *Anopheles* larvae were identified by their parallel position on the surface of water. The larvae were collected with a 350 ml dipper and transferred into a 5000 ml plastic container for calculating the larval density from two separate localities within the Sahel savannah region of Northwest Nigeria (Fig 1):

1. Batagarawa town, Batagarawa Local Government in the Sahel Savannah of Katsina State(12°54′17″N, 7°37′11″E), is a semi- urban area characterized by fewer of *Anopheles gambiae s.l* breeding sites;
2. Sahel Savannah of Gajerar Giwa village (12^0 95′21″N, 7^0 75′19″E) in Rimi Local Government of Katsina State, where rice and vegetables irrigation are practiced using water from Ajiwa dam, characterized by large number of *Anopheles funestus s.l* breeding sites.

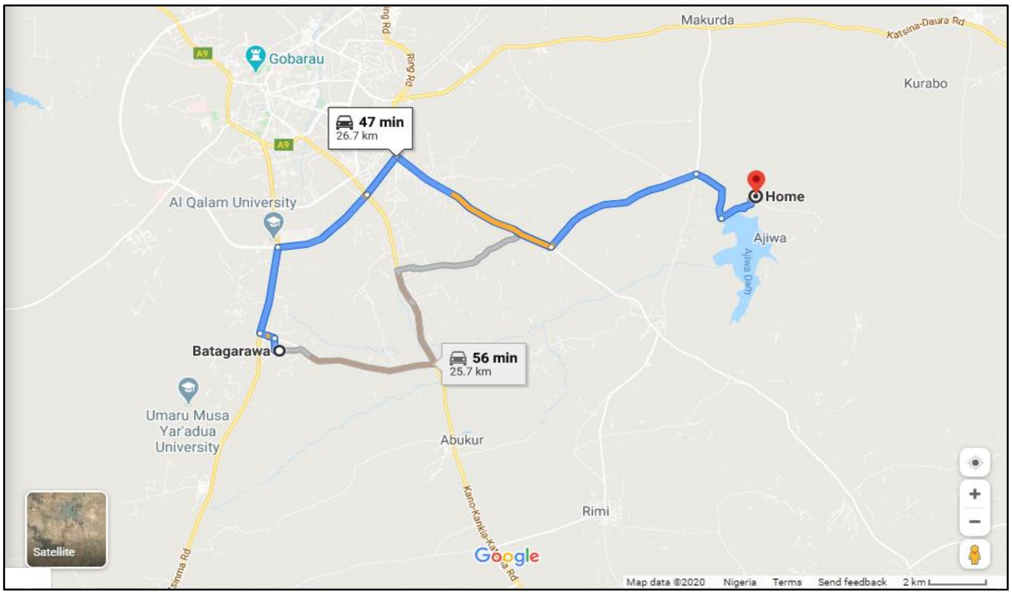

Fig 1: Adopted from Google map

Physicochemical Analysis

Conductivity, pH, temperature, and total dissolved solids were measured using COMBO pH/EC/TDS/Temperature metre (HANNA Instruments, United States). Dissolved oxygen (DO) and biological oxygen demand (BOD) were determined using a DO meter (Hach Lange, Colorado-United States) as described by Maiti, [16]. Levels of oil and grease were determined by the liquid-liquid extraction method described by Maiti, [16]. Concentration of PO_4^{3-}, NO_3^{2-}, Mg^{2+}, Ca^{2+}, Fe^{2+}, Cu^{2+}, Mn^{2+} and Zn^{2+} were determined as described by Vasu and Ramachandra [17].

Statistical Analysis

The data were analyzed using students T-test in the Statistical Package for Social Sciences (SPSS) Computer software version 20. The results were expressed as mean ± Standard Error Mean (SEM), the significant difference between the physiochemical parameters were considered at $P< 0.05$.

Results

Six different breeding sites were designated as site A, B, C, D, E & F. These sites were differentiated by the type of human related activities taking place around the mosquito breeding sites i.e. A, C & E are residential areas; B, petrochemical products are sold, processed, used and discharged; D & F are intensive agricultural areas. Breading sites A & B are of *Culex spp* and then breading sites C & D are of *Anopheles gambiae s.l* complex from Batagarawa town and there Mean± SEM of the physicochemical levels were compared at $P< 0.05$ confidence limit. However, breeding sites E & F are of *Anopheles Funestus s.l* from G/Giwa village and their Mean± SEM of the physicochemical levels were compared at $P<$ 0.05 confidence limit.

The average physicochemical parameters from the breeding sites A and B was presented in Table 1. pH, temperature, TS, TSS and TDS were not significant ($P> 0.05$) between sites, but significant differences ($P< 0.05$) were observed in conductivity, DO, BOD, COD, oil and grease, and these can be related to the larval abundance and density which was indicated to be highly significant ($P< 0.05$) between sites. However, the levels of Na^+, K^+ and Cl^- were significant ($P< 0.05$) between the sites, furthermore, concentration of PO_4^{3-}, NO_3^{2-}, Mg^{2+}, Ca^{2+}, Fe^{2+}, Cu^{2+} and Mn^{2+} were not significant ($P> 0.05$) and Zn^{2+} was not detected from the sites. However, the physicochemical parameters from the breeding sites C and D are presented in Table 2. pH, temperature, oil and grease were not significant ($P> 0.05$) between sites, but levels of TS, TSS, TDS, DO, BOD, COD and conductivity were found to be significant ($P< 0.05$) and these can be associated to the larval abundance and density in site D compared to site C. The levels of Na^+, K^+, Cl^-, PO_4^{3-} and NO_3^{2-} were significant ($P< 0.05$) between the sites. Concentration of Mg^{2+}, Ca^{2+}, Fe^{2+}, Cu^{2+} and Mn^{2+} were not significant ($P> 0.05$) and Zn^{2+} was not detected from the sites. Physicochemical parameters from the breeding sites E and F are presented in Table 3. pH, temperature, conductivity, oil and grease, conductivity, TS, TSS and TDS were not significant ($P > 0.05$) between sites, but DO, BOD and COD were significantly ($P < 0.05$) different between the sites and these can be related to the larval abundance and density in site F compared to site E. Significant ($P > 0.05$) differences in the levels of Na^+, K^+, Cl^-, PO_4^{3-} and NO_3^{2-} was observed between the sites. Therefore, concentration of Mg^{2+}, Ca^{2+}, Fe^{2+}, Cu^{2+} and Mn^{2+} were not significant ($P > 0.05$) and Zn^{2+} was not detected from the sites.

Pearson correlation between larval density of *Culex* mosquitoes and physicochemical factors from breeding sites A and B in Batagarawa town is presented in Figure 2. The result indicated highly significant correlation ($P < 0.05$) between conductivity, BOD, Na^+ and Cl^- with the larval density but, there was no significant correlation ($P > 0.05$) with the DO, COD, oil and grease and K^+ in breeding site A. However, COD and BOD decreased the larval density ($P < 0.05$) but, conductivity, DO, oil and grease, Na^+, K^- and Cl^- didn't affect the larval density in breeding site B. In breeding sites C and D, Pearson correlation between larval density of *Anopheles gambiae* and physicochemical factors is presented in Figure 3. And it was observed that, conductivity, TS, TDS, TSS, DO, COD, BOD, Na^+, K^-, Cl^-, PO_4^- and NO_3^- significantly ($P < 0.05$) affect the larval density in breeding site C but, only BOD was highly correlated ($P < 0.05$) with the larval density in breeding site D. The effect of physicochemical factors on larval density of *Anopheles funestus* is presented in Fig 4. In breeding site E DO, Na^+, K^-, Cl^- and NO_3^- were not correlated ($P > 0.05$) with the larval density. But, highly significant ($P < 0.05$) correlation with the larval density was observed in breeding site F.

Table 1: Larval density and physicochemical parameters of water from two different breeding sites of *Culex spp* in Batagarawa town

Physicochemical parameters	Breading Site A	Breading Site B
Temperature °C	34.3±4.50	38.7±3.60
P^H	7.4±0.03	7.3±0.02
Conductivity (µS/cm)	101.3±13.33	203.1±11.30
TS (mg/L)	31.2±3.03	31.2±2.50
TSS (mg/L)	5.65±0.05	4.9±0.00
TDS (mg/L)	61.5±8.66	48.2±10.10
DO (mg/L)	8.0±0.29	2.5±0.10
BOD (mg/L)	40.2±6.22	29.6±7.65
COD (mg/L)	6.5±0.33	3.3±0.17
Oil & Grease(mg/L)	1.32±0.45	7.8±2.47
Na^+ (mg/L)	70.6±11.05	6.1±1.41
K^+ (mg/L)	14.6±4.2	3.7±1.2
Ca^{2+} (mg/L)	0.3±0.03	0.1±0.03
Mg^{2+} (mg/L)	0.3±0.02	0.3± 0.02
Cl^- (mg/L	21.1±5.57	2.6±0.05
PO_4^{3-} (mg/L)	1.3±0.06	1.1±0.04
NO_3^{2-} (mg/L)	2.50±0.20	3.2±2.10
Fe^{2+} (mg/L)	0.3±0.00	0.4±0.01
Cu^{2+} (mg/L)	0.1±0.00	0.1±0.00
Zn^{2+} (mg/L)	ND	ND
Mn^{2+}(mg/L)	0.2±0.04	0.1±0.00
Culex spp Larval density per litre	65±6.10	34±2.95

TS- Total solid, TSS- Total solid suspended, TDS- Total dissolved solid, DO- Dissolve oxygen, BOD- Biological oxygen demand, COD- Chemical oxygen demand, ND-Not detected, breeding site A- residential area, breeding site B- petrochemical site. Values are expressed as Mean± SEM and significant different, ($P < 0.05$) using *T*-test.

Table 2: Larval density and physicochemical parameters of water from two different breeding sites of *Anopheles gambiae s.l* complex in Batagarawa town

Physicochemical parameters	Breading Site C	Breading Site D
Temperature °C	36.6±5.10	32.0±4.00
P^H	7.2±0.13	7.0±0.75
Conductivity (µS/cm)	81.2±4.00	151.2±4.60
TS (mg/L)	38.8±2.45	16.7±6.00
TSS (mg/L)	18.9±1.23	7.0±1.20
TDS (mg/L)	67.0±4.44	23.4±5.13
DO (mg/L)	9.3±2.00	16.0±4.80
BOD (mg/L)	23.3±7.00	48.3±8.22
COD (mg/L)	4.6±2.60	9.3±1.20
Oil & Grease(mg/L)	5.5±0.22	4.7±1.50
Na^+ (mg/L)	9.5±8.20	18.2±3.32
K^+ (mg/L)	6.4±2.4	14.5±2.30
Ca^{2+} (mg/L)	0.6±0.01	0.3±0.01

Mg^{2+} (mg/L)	0.2±0.01	0.3± 0.01
Cl^- (mg/L	4.2±4.40	14.2±3.3
PO_4^{3-} (mg/L)	2.5±0.03	7.1±0.02
NO_3^{2-} (mg/L)	3.0±1.20	11.0±2.55
Fe^{2+} (mg/L)	0.2±0.00	0.3±0.01
Cu^{2+} (mg/L)	0.0±0.00	0.1±0.00
Zn^{2+} (mg/L)	ND	ND
Mn^{2+}(mg/L)	0.30±0.01	0.1±0.00
Anopheles gambiae s.l complex Larval density per litre	20.0±5.00	46.0±7.00

TS- Total solid, TSS- Total solid suspended, TDS- Total dissolved solid, DO- Dissolve oxygen, BOD- Biological oxygen demand, COD- Chemical oxygen demand, ND-Not detected, Breeding site C- residential area, Breeding site D- agricultural site. Values are expressed as Mean± SEM and significant different, (P< 0.05) using T- test.

Table 3: Larval density and physicochemical parameters of water from two different breeding sites of *Anopheles funestus s.l* complex in Gajerar Giwa village

Physicochemical parameters	Breading Site E	Breading Site F
Temperature °C	33.5±6.12	32.0±6.00
P^H	7.4±0.55	7.2±0.11
Conductivity (µS/cm)	101.3±6.10	106.3±2.00
TS (mg/L)	37.0±5.11	38.0±5.30
TSS (mg/L)	4.55±0.11	6.2±1.00
TDS (mg/L)	22.0±3.00	23.1±2.03
DO (mg/L)	11.0±2.65	25.2±6.00
BOD (mg/L)	32.0±5.20	50.4±4.00
COD (mg/L)	6.4±3.00	13.8±2.00
Oil & Grease(mg/L)	6.8±0.04	5.4±1.00
Na^+ (mg/L)	40.8±5.00	20.6±4.23
K^+ (mg/L)	15.0±3.4	7.6±2.45
Ca^{2+} (mg/L)	0.5±0.00	0.2±0.01
Mg^{2+} (mg/L)	0.1±0.00	0.2± 0.00
Cl^- (mg/L	16.5±3.00	3.8±0.01
PO_4^{3-} (mg/L)	4.5±0.01	12.5±0.89
NO_3^{2-} (mg/L)	4.0±0.50	15.0±1.22
Fe^{2+} (mg/L)	0.1±0.00	0.2±0.01
Cu^{2+} (mg/L)	0.0±0.00	0.1±0.00
Zn^{2+} (mg/L)	ND	ND
Mn^{2+}(mg/L)	0.30±0.00	0.2±0.00
Anopheles Funestus s.l complex Larval density per litre	27.1±1.40	58.6±4.00

TS- Total solid, TSS- Total solid suspended, TDS- Total dissolved solid, DO- Dissolve oxygen, BOD- Biological oxygen demand, COD- Chemical oxygen demand, ND-Not detected, Breeding site E- residential area, Breeding site F- agricultural site. Values are expressed as Mean± SEM and significant different, (P< 0.05) using T-test.

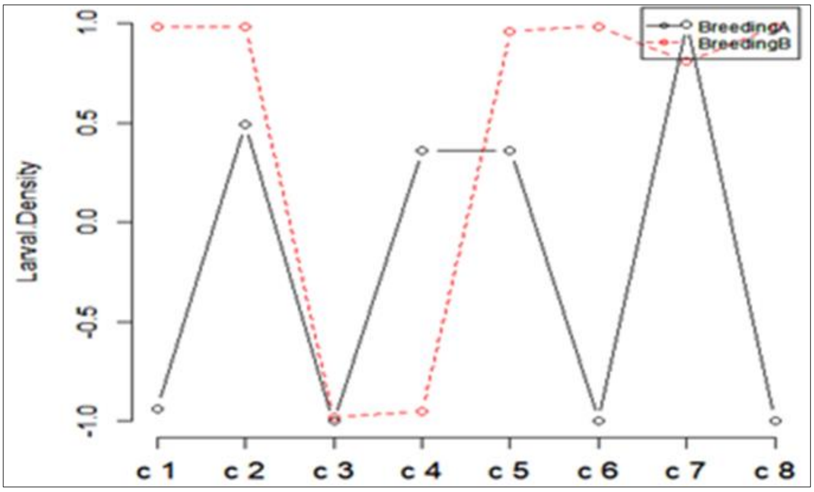

Fig 2: Pearson correlation between larval density of *Culex* mosquitoes and physicochemical factors from breeding site A & B; c1: conductivity; c2: DO; c3: BOD; c4: COD; c5: oil and grease; c6: Na^+; c7: K^+; c8; Cl^-

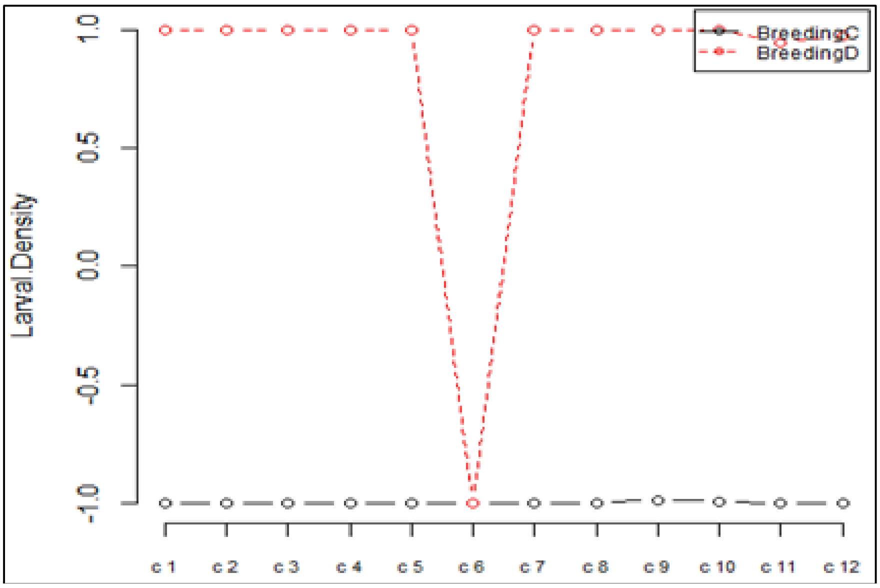

Fig 3: Pearson correlation between larval density of *Anopheles gambiae s.l* and physicochemical factors from breeding site C & D; c1: conductivity; c2: TS; c3: TSS; c4: TDS c5: DO; c6: BOD; c7: COD; c8: Na^+; c9: K^+; c10: Cl^-; c11: PO^{-4}; c12: NO^{-3}

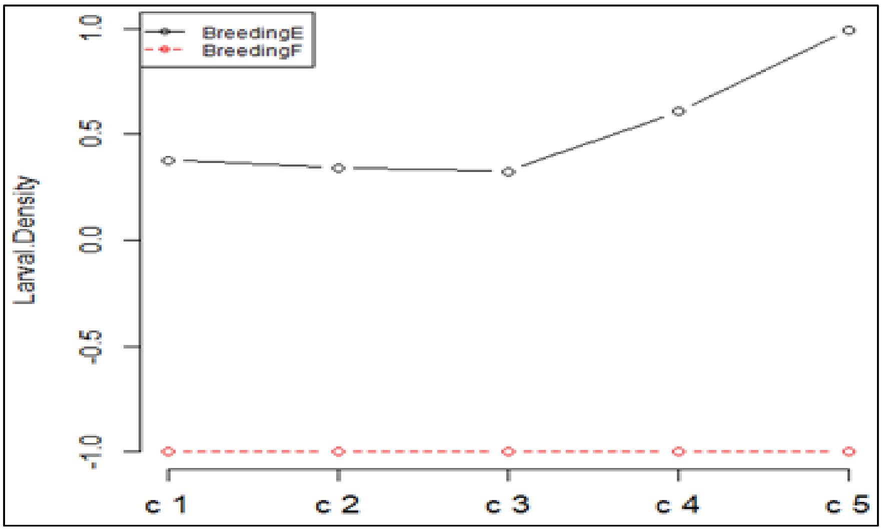

Fig 4: Pearson correlation between larval density of *Anopheles funestus s.l* and physicochemical factors from breeding site E & F; c1: DO; c2: Na^+; c3: K^+; c4: Cl^-; c5: NO^{-3}

Discussion

For any successful vector control, good knowledge of the breeding ecology of mosquitoes including, the types and preferences for larval habitats, spatial and temporal distribution of breeding sites, as well as, the physical, biological and chemical characteristics of the habitats are required [18].

In the study communities, *An. gambiae s.l*, *An. funestus* and *Cx. quinquefasciatus* were the main vectors of lymphatic filariasis [19, 2, 3] while *An. gambiae s.l*, *An. funestus* group are responsible for malaria transmission [5, 3].

This current study showed significant distribution and abundance of the *Culex* mosquito larvae between site A and B in Batagarawa town, which appear to be driven by a range of environmental factors. These environmental factors are functions of the human related activities going on around these breeding sites. Although, temperature, pH, TS, TSS and TDS were not significantly different between the sites, this could be attributed to similar human related activities going on around these breeding sites. Earlier studies from other parts of Nigeria and Africa had reported similarly as the present study example [20]. However, the level of oil and grease was higher in breeding site B which is consistent with the major types of human activities taking place. There is widespread sale, processing, use and discharge of petroleum products very close to the breeding site B and consequently, these petroleum products are inversely associated with larval density of *Culex spp*. This present study concurred with the finding of Imam and Deeni [21], although the authors observed it in *Anopheles gambiae*.

Furthermore, the levels of DO, BOD, COD and conductivity were highly significant between the breeding sites. This explained that, the levels of DO, BOD and COD are dependent on the organic content of a water body. The observed effect of DO, BOD and COD on larval density in this study contradicted with previous observations that higher aquatic organisms thrives better in water bodies with higher DO, BOD and COD than those with lower DO, BOD and COD levels [6]. This is because higher levels of organic compounds lead to increase in microbial population which depletes dissolved oxygen [22].

However, the levels of Na^+, K^+ and Cl^- were significantly different between the sites, in the context of this study; high concentrations of these parameters can be suitable to *Culex spp* [23, 24]. Nevertheless, the occurrence of high levels of Na^+, K^+, Cl^- and conductivity in *Culex* mosquitoes breeding sites are suspected to originate from the human related activities [25-27]. And this could pose a serious threat to the success of various larvicidal control interventions. Moreover, emerged adult *Culex* mosquito from such breeding sites is likely to be selected for inherent and acquired resistance to public health insecticides used for mosquito vector control even in the absence of prior exposure [28, 26, 25].

In the present study, levels of PO_4^{3-}, NO_3^{2-}, Mg^{2+}, Ca^{2+}, Fe^{2+}, Cu^{2+} and Mn^{2+} were not significantly different across the sites. And Zn^{2+} was not detected from the two sites. This could be attributed to similar human activities taken place around the breeding sites.

In this study, it is observed that, pH, temperature, oil and grease were not significant between the breeding sites. *Anopheles gambiae* larvae were associated with temperature range of 20.7 to 28.0°C and pH 5.8 (slightly acidic) to 7.8 (slightly alkaline), this is similar to study by Hanafi-Bojd *et al*. [29] who found malaria and LF vectors breeding in habitats of between 20 °C - 30 °C. It could be due to the same ecology between the breeding sites. But, higher levels of DO, BOD, COD and conductivity were observed in breeding site D which might have provided favourable environment for survival and breeding activity of the *Anopheline* species. Tadesse *et al*. [30] reported that, both anopheline and culicine larvae were positively associated with dissolved oxygen. Previous reports also indicated similar association of *Culex quinquefasciatus* and *Anopheles arabiensis* larvae with dissolved oxygen [31]. Oyewole *et al*, [32] support the idea that optimum dissolved oxygen might have contributed for survival and breeding of *Anopheles* larvae. Furthermore, the levels of TS, TSS and TDS were found to be high in breeding site C which is negatively associated with larval abundance and density. Meanwhile, the levels of Na^+, K^+, Cl^-, PO_4^{3-} and NO_3^{2-} were found to be high in breeding site D, the occurrence of high levels of such physicochemical parameters are claimed to originate from the use of agricultural and allied chemicals in farmlands located around these mosquito breeding sites [25-27]. This could cause a serious threat to the success of mosquito vector control using public health insecticides. The present study contradicts the findings of Imam and Deeni [6], which showed low levels of all the chemical environmental parameters observed, recorded the highest *An. gambiae* larval density/abundance and this was consistent with the findings of previous studies, which showed that *An. gambiae* prefers cleaner, clearer and uncontaminated breeding water [33].

Therefore, concentrations of Mg^{2+}, Ca^{2+}, Fe^{2+}, Cu^{2+} and Mn^{2+} were not significant between the breeding sites, and Zn^{2+} was not detected from the sites. This may be attributed to the nature of the soil texture and types of fertilizer used by farmers around the study locations.

This present study showed that physicochemical factors such as pH, temperature, conductivity, oil and grease, TS, TSS and TDS are statistically similar between breeding sites E and F. This could be due to similar ecology and related human activities around the breeding sites. This contradicts the finding of Basiliana *et al*. [34] which showed high levels of pH, temperature, conductivity, TS, TSS and TDS and can be negatively associated with larval abundance and density. However, the levels of Na^+, K^+, Cl^-, PO_4^{3-} and NO_3^{2-} were high in breeding site E compared to breeding site F and these can affect larval productivity of *Anopheles funestus* positively. Several studies are inconsistent with the present study [35-37]. The abundance of *Anopheles funestus* larvae in this study, from breeding site F with high levels of Na^+, K^+, Cl^-, PO_4^{3-} and NO_3^{2-}, may constitute a serious threat to the environmental management approaches to controlling malaria and LF vector abundance/density.

Therefore, concentrations of Mg^{2+}, Ca^{2+}, Fe^{2+}, Cu^{2+} and Mn^{2+} were not significant between the breeding sites, and Zn^{2+} was not detected from the sites. This could be attributed to the nature of the soil texture and types of fertilizer used by farmers around the study locations.

Conclusion

This present study highlights some of the physicochemical factors resident in different bodies of water and the impact of such factors on survival, growth, abundance and breeding activity of mosquitoes from the Northwest Nigeria. Important of this study cannot be overemphasized in constituting control strategies against malaria and LF vectors through proper environmental planning and management that will help in reducing mosquitoes breeding sites.

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