

Impact of Fluoridated Waters on the Density of Mosquito Larvae in the Municipality of Dassa-Zoume in Benin

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Received June 02, 2022; Revised July 07, 2022; Accepted July 15, 2022

Abstract Mosquitoes are sources of nuisance and vectors of pathogenic agents for humans and animals. The fight against these insects requires a very in-depth knowledge of the ecology of their places of development. This is the reason why a study on the impact of fluoride on the development of mosquitoes was carried out in the municipality of Dassa-Zoumè, in central Benin. Thus, larval habitat has been actively sought and prospected. The physicochemical and chemical (e.g.: fluorine) parameters of the water in these development sites were measured. A total of 38 larval habitats were identified and surveyed. Most larval habitats are in the immediate environment of human populations. The characterization of the substrate at the level of these development sites has informed us about the ecological requirements of larval density. Indeed, the analysis of the results obtained shows that the individual increase in fluoride ions, salinity, turbidity, suspended matter or exposure to the sun of the site favors the increase in the number of deposits of development collected. On the other hand, the opposite effect is obtained with increasing chloride ions or hydrogen potential. In addition, the combined action of fluoride ions and a few other elements inhibits the density of larvae in the stations. These results show that to limit or inhibit the density of larvae in this region, the presence of fluoride ions combined with either a high concentration of chloride ions and / or a high amount of salinity is required.

Keywords: *fluorine, fluoride, larval habitat, mosquitoes, Dassa-Zoumè*

Cite This Article: Kitidjo Victor Jacques SOSSOU, Waris Kéwouyèmi CHOUTI, Luc DJOGBENOU, Romaric AKOTON, and Carine Nelly KELOME, "Impact of Fluoridated Waters on the Density of Mosquito Larvae in the Municipality of Dassa-Zoume in Benin." *American Journal of Water Resources*, vol. 10, no. 2 (2022): 46-53. doi: 10.12691/ajwr-10-2-2.

1. Introduction

Malaria infections are a major public health problem and the main cause of morbidity and mortality, particularly among children under five and pregnant women. A global estimate of 229 million new malaria cases and around 409,000 malaria deaths were reported in 2019, with 92% of the disease burden recorded in the African regions [1]. Malaria is transmitted through the bite of female Anopheles mosquitoes, which carry the infection by protozoan parasites Plasmodium species to humans [2,3].

In most sub-Saharan African regions, the main malaria vectors are *An. gambiae* s.l., *An. funestus* s.l., *An. nili* and *An. moucheti*. In Benin, *An. gambiae* s.l. remains the major malaria vector, with variable vectorial capacities

and behaviors [4]. Immature *An. gambiae* s.l. develops in a variety of aquatic ecosystems, such as fresh, brackish waters found in rural, coastal, and urban areas, where larvae emerge into adult mosquitoes. Water quality of larval habitat is an important determinant in egg laying, for adequate growth and development from larval stages until adults [5,6,7,8]. *An. gambiae*, breeds in natural/artificial permanent and semi-permanent water bodies with floating or emerging vegetation like the edges of swamps, in weedy and grassy parts of rivers, streams, furrows, ditches and ponds with low salinity and little richness in organic matter [9]. It was reported that larval habitat characteristics could influence several life-history traits in mosquitoes, such as oviposition preferences, hatching, immature development, pupation [10]. Consequently, the habitat characteristics, especially physicochemical, could influence adult productivity, and it will have a significant impact on malaria transmission.

Potential *An. gambiae s.l.* larval habitats were randomly selected during each season where sampling was made. Larvae collections were carried out in August 2020 (dry season) and in October 2020 (rainy season). Nine (09) and twenty-nine (29) potential *An. gambiae s.l.* larval habitats were prospected (Figure 1). Mosquito larvae were collected using dipping method [13]. In each plot, 20 scoops were taken using a standard white 300 ml dipper (ladle). Mosquito larvae collected were identified morphologically using identification keys [9]. The physical parameters such as habitat positivity for *Anopheles* larvae (presence or absence of *Anopheles* larvae in the habitat), larvae count (instar stade count of *Anopheles* larvae), sun/shade (whether the water in the habitat is exposed to direct sun or it is shaded by the presence of vegetation or any other covering), were recorded on site by visual observation.

To assess the physicochemical characteristics of prospected larval habitat, water samples were aseptically collected in cleaned glass bottles boxes of 0.5 liters to avoid interference before immersing it in water. The samples were stored in coolers fitted with frozen accumulator at a temperature between 2°C and 8°C during transportation to the laboratory. Water collections in which the pre-imaginal mosquito stages were considered as larval habitat. Each larval habitat was georeferenced using a GARMIN® brand GPS and the data imported into Arc GIS version 10.1 mapping software for mapping.

2.2. Physicochemical Parameters of Breeding Water

The samples are analyzed at the water and food quality control laboratory of the National Directorate of Public Health of Benin by standardized methods and techniques. The hydrogen potential (pH) is measured in water samples using a WTW 340i pH meter. Conductivity was measured by applying alternating electric current to two electrodes immersed in a solution and measuring the resulting voltage. The differential weighing method was used for the measurement of Suspended Solids (SSD). Chloride ions are determined by the MOHR method. The analytical method used for the determination of fluorine is the SPADNS colorimetric method, based on a HACH DR/2010 spectrophotometer. Iron was not determined, but compensatory measures were taken prior to the

determination of fluorine, adding to each sample a drop of tri-ethanolamine intended to complex the iron, thus preventing it from creating interference.

2.3. Data Analysis

Excel and Rstudio software were used for data processing and graphical visualization. In addition, to highlight the links between the variables, the generalized linear model (GLM) was used. Since the target variable Y corresponding to larval density is a counting variable, Poisson regression was used as a prediction model. Thus, Poisson regression was used to establish, on the one hand, the link between fluorine content and larval density and, on the other hand, between the interaction of fluorine with each of the other parameters studied and larval density. The Stata 15 software was used to establish generalized linear models as part of this research.

3. Results and Discussion

3.1. Results

3.1.1. Typology of Larval Habitats

The types of larval development sites are presented in Table 1. The table shows that 77.78% of the deposits prospected are artificial and 22.22% are natural in dry season whereas 100% of sites are artificial in wet season. Also, 88.88% of the lodgings are sunny and 11.12% are shaded in dry season respectively compared to 62.07% and 37.93% in wet season.

Table 1. Distribution of types of larval habitat

	Site types		Sun exposure	
	Natural	Artificial	Sunny	Shaded
Dry season	22%	78%	89%	11%
Wet season	0%	100%	62%	38%

3.1.2. Global Visualization of Parameters

The spatial distribution of the physico-chemical characteristics (logarithmic scale) and the larval density (arithmetic scale) of the waters of the deposits in the dry and wet season are presented respectively in Figure 2 and Figure 3.

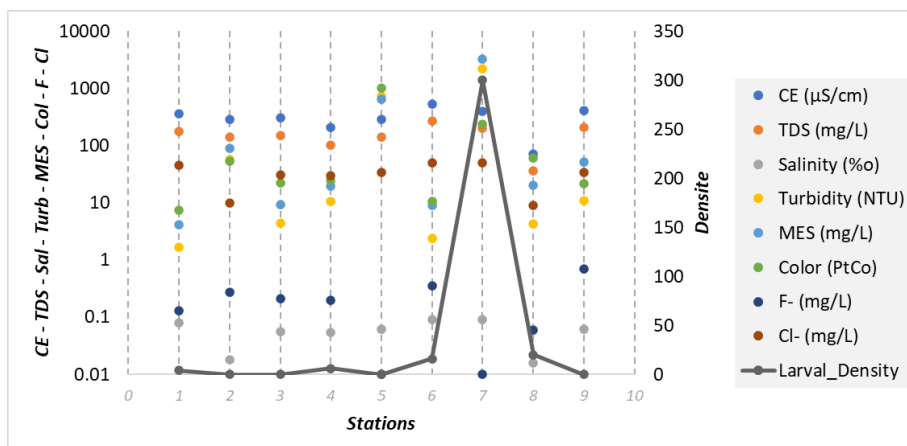


Figure 2. Spatial distribution of parameters in dry season

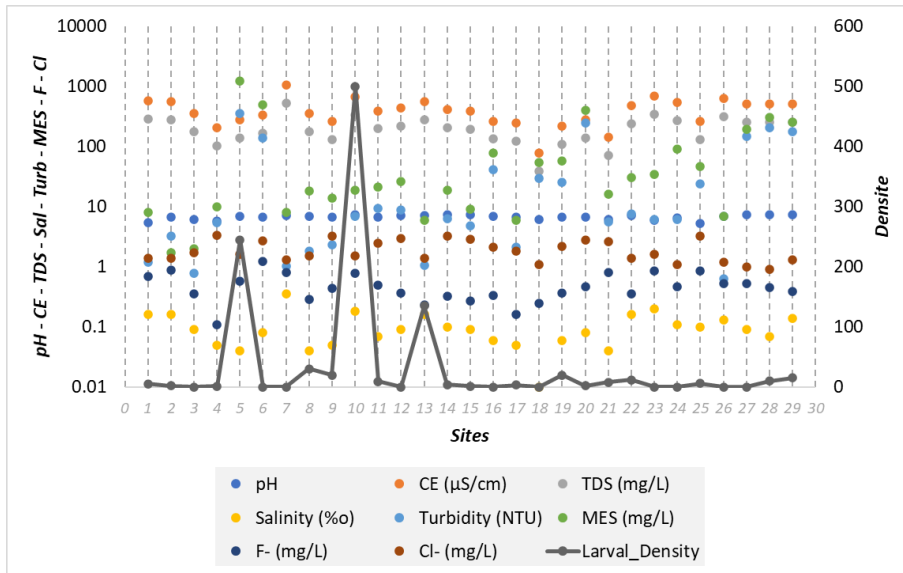


Figure 3. Spatial distribution of parameters in wet season

The spatial distribution of parameters in dry season shown in Figure 2 shows that the density values present a peak (extreme value) at station 7. This is accompanied by a low fluoride value and high values in MES and Turbidity. It should be noted that this site is heavily exposed to the sun.

The spatial distribution of parameters in wet season (Figure 3) shows that the density values have three peaks (sites 5, 10 and 13) with the extreme value in the site 10. Here, no apparent correlation emerges. Larval density peaks are obtained at sites exposed to the sun.

3.1.3. Physicochemical Characterization and Larval Density in Dry Season

The boxplots show the dispersion and the centralization of physico-chemicals parameters and larval density of sites in dry season.

The distribution of values shown in Figure 4 shows that the larval density is high when the fluoride concentration is low (<0.05 mg/L) in dry season. The values of this

boxplot are evenly distributed around the median (150 larvae).

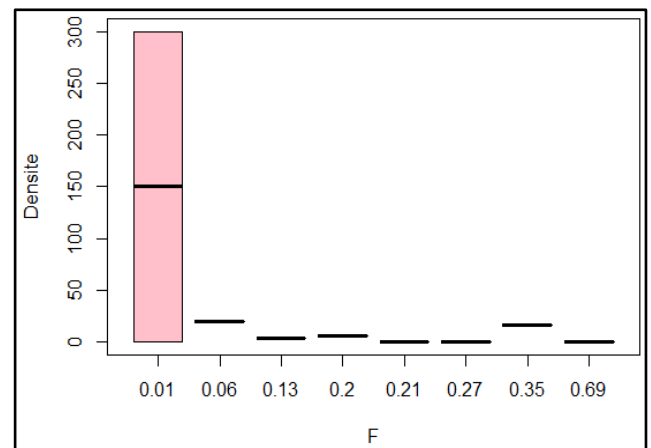


Figure 4. Distribution of fluoride (F) and larval density (Densite) in dry season

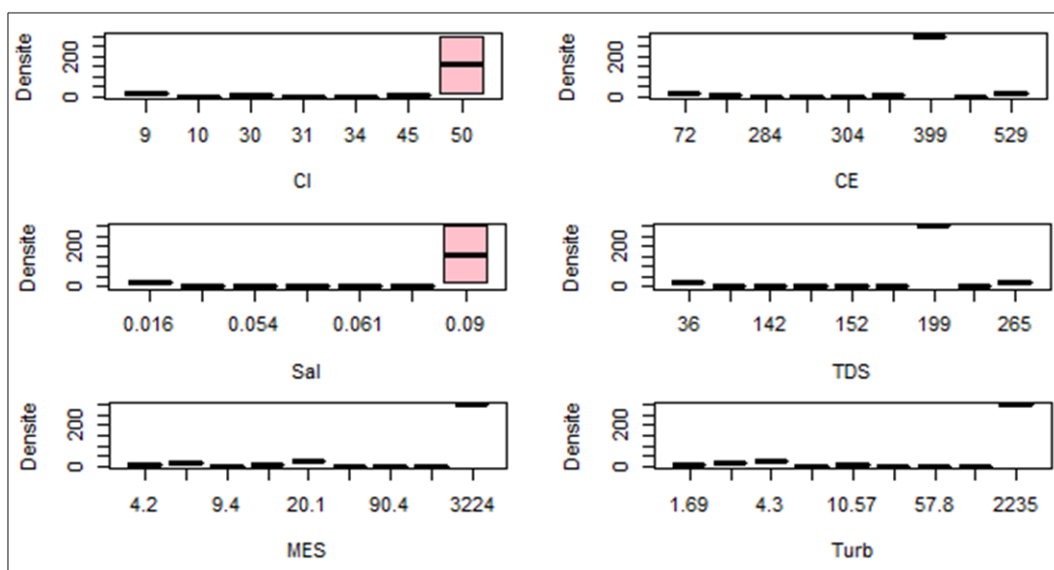


Figure 5. Distribution of Chloride (Cl), Salinity (Sal), Suspended Solids (MES), Electrical Conductivity (CE), Total Dissolved Solids (TDS), Turbidity (Turb) and larval density (Densite) in dry season

There is a similar distribution of graphs between Chloride and Salinity, Suspended Solids and Turbidity, Electrical Conductivity and total dissolved solids (Figure 5). As opposed to fluorides, the larval density is high when the values of these parameters are high in dry season.

3.1.4. Physicochemical Characterization and Larval Density in Wet Season

Are presented below the boxplots of physico-chemicals parameters and larval density of sites in wet season.

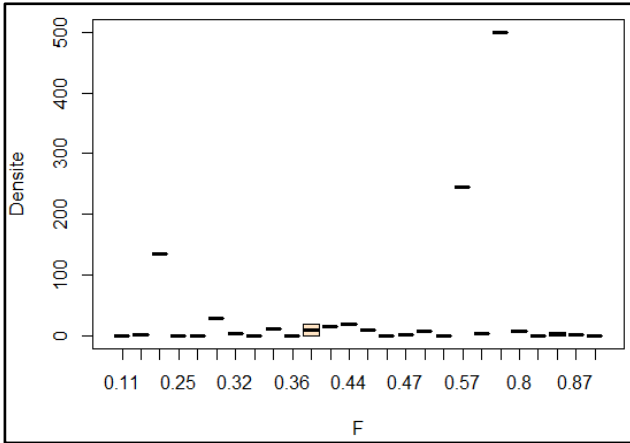


Figure 6. Distribution of fluoride (F) and larval density (Densite) in wet season

The distribution of values (Figure 6) shows that the median value of larval density is high in three points. The two highest values correspond to a relatively high fluoride concentration.

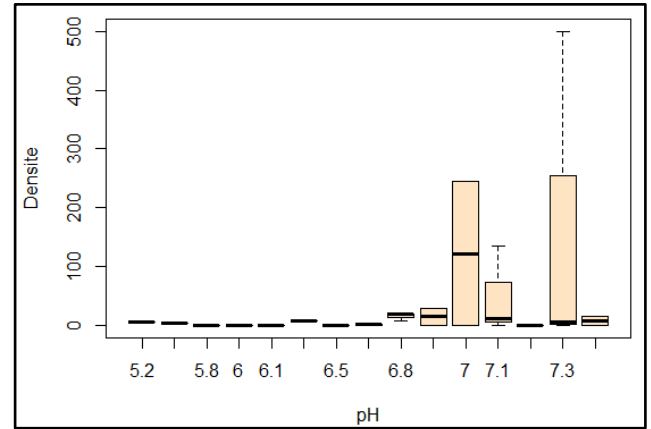


Figure 7. Distribution of hydrogen potential (pH) and larval density (Densite) in wet season

According to Figure 7, the high larval densities obtained have a high hydrogen potential. The high pH in question is around 6.8 and 7.5. Depending on the class, they tend from neutrality to basicity.

The analysis of Figure 8 does not allow us to apparently show a correlation between the different parameters. We will therefore move on to statistical analysis to better interpret the data.

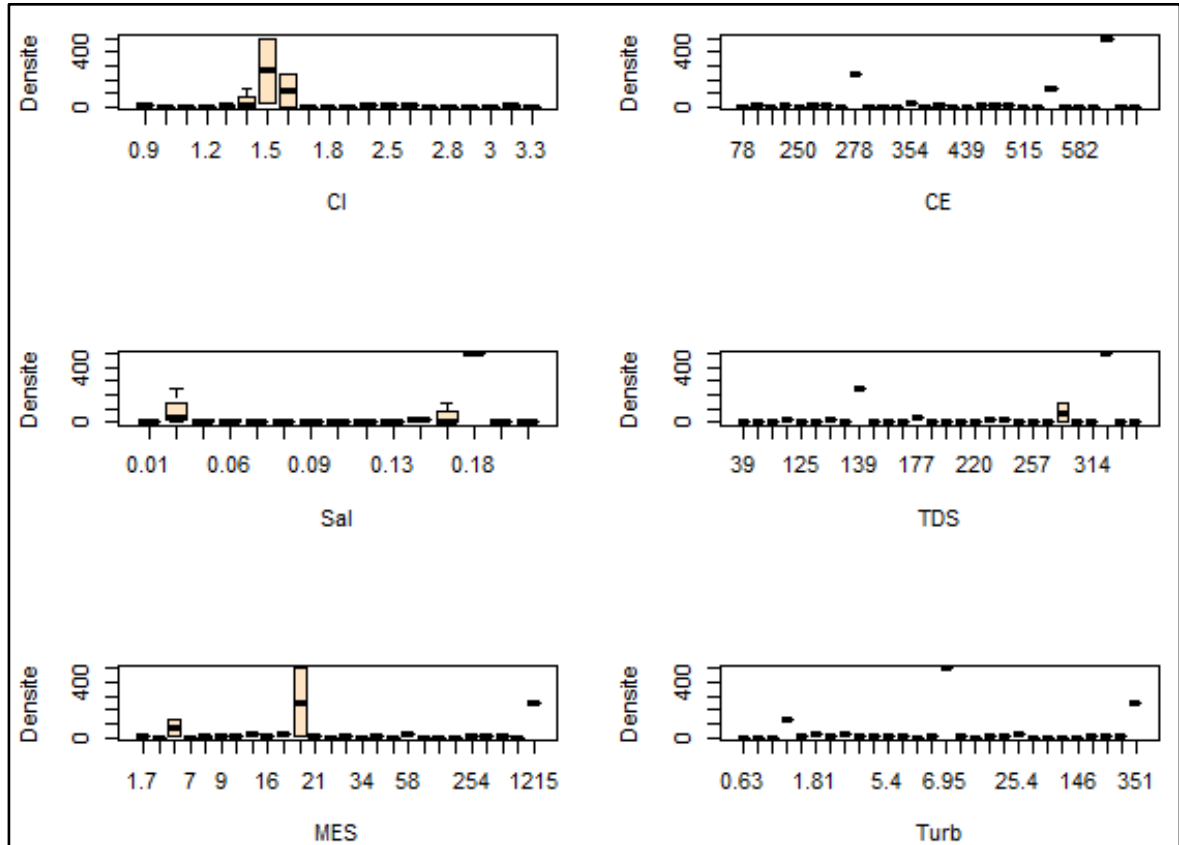


Figure 8. Distribution of Chloride (Cl), Salinity (Sal), Suspended Solids (MES), Electrical Conductivity (CE), Total Dissolved Solids (TDS), Turbidity (Turb) and larval density (Densite) in wet season

3.1.5. Impact of Physico-chemical Parameters on Larval Density

Table 2 presents the successive results of the various Generalized Linear Models (MLG) according to Poisson regression.

According to the table above the probability $\Pr(>|z|)$ is less than 5% in virtually all cases except for the chloride variable of the MLG 2 model and fluoride of the MLG 9 model. This demonstrates that the other models are significant and can be used.

- MLG 1: The logarithm of the mean number of larval densities increases by 1.34 as the amount of fluoride increases by one unit in mg / L. That is, the larval density when fluoride increases by one unit in mg / L is 3.82 (i.e.: $e^{1.34}$) times **greater** than the starting (previous) larval density. Thus, fluorides alone promote larval development.
- MLG 2: The larval density when the simultaneous fluoride * chloride interaction increases by one unit is 0.23 (i.e.: $e^{-1.47}$) times **lower** than the starting (previous) larval density. We can say with reservation from further testing that the combination of fluoride * chloride has an inhibitory influence on mosquito larvae.
- MLG 3: The larval density when the simultaneous interaction fluorides * salinity increases by one unit is $1.14 \cdot 10^{-8}$ (i.e.: $e^{-18.29}$) times **lower** than the starting (previous) larval density. We can say with reservation from further testing that the association of fluoride * salinity has a considerable inhibitory influence on mosquito larvae.
- MLG 4: The larval density when the simultaneous interaction of fluorides * electrical conductivity increases by one unit is 0.997 (i.e.: $e^{-0.0031}$) times lower than the starting (previous) larval density.

Given that $0.997 \approx 1$ we can deduce that the influence of the association fluoride * electrical conductivity on the larvae is **negligible**.

- MLG 5: The larval density when the simultaneous interaction fluorides * turbidity increases by one unit is 0.985 (i.e.: $e^{-0.015}$) times lower than the starting (previous) larval density. Given that $0.985 \approx 1$ it can be deduced that the influence of the fluoride * turbidity association on the larvae is **negligible**.
- MLG 6: The larval density when the simultaneous interaction fluorides * SS increases by one unit is 0.9 (i.e.: $e^{-0.1}$) times lower than the starting (previous) larval density. Given that $0.9 \approx 1$ it can be deduced that the influence of the fluoride * SS association on the larvae is **negligible**.
- MLG 7: The larval density when the simultaneous interaction fluorides * TDS increases by one unit is 0.99 (i.e.: $e^{-0.006}$) times lower than the starting (previous) larval density. Given that $0.99 \approx 1$ it can be deduced that the influence of the fluoride * TDS association on the larvae is **negligible**.
- MLG 8: The larval density when the simultaneous interaction of fluorides * pH increases by one unit is 3361 (i.e.: $e^{8.12}$) times **greater** than the starting (previous) larval density. We can say with reservation from further testing that the combination of fluoride * pH has a considerable favorable influence on mosquito larvae.
- MLG 9: The larval density when the simultaneous interaction fluorides * sun exposure increases by one unit is 15.8 (or $e^{2.76}$) times **greater** than the starting (previous) larval density. We can say with reservation from further testing that the combination of fluorides * sun exposure has a favorable influence on mosquito larvae.

Table 2. Generalized Linear Modeling (MLG) / Poisson Regression

Prob > khi2 = 0,000					
	Number of larvae	Estimated value	Standard error	Z statistic	Pr(> z)
MLG 1	Constant	2.825447	0.0718395	39.33	0.000***
	Fluorides	1.339357	0.1065249	12.57	0.000***
MLG 2	Constant	2.487408	0.2328246	10.68	0.000***
	Fluorides	4.566047	0.3879404	11.77	0.000***
	Chlorides	-0.0155779	0.1162371	-0.13	0.893
	Fluorides * Chloride	-1.474403	0.1949865	-7.56	0.000***
MLG 3	Constant	1.352792	0.1850875	7.31	0.000***
	Fluorides	2.849114	0.251312	11.34	0.000***
	Salinity	16.54462	1.7091	9.68	0.000***
	Fluorides*Salinity	-18.29025	2.220854	-8.24	0.000***
MLG 4	Constant	1.374002	0.2498894	5.5	0.000***
	Fluorides	2.214421	0.3294819	6.72	0.000***
	σ ($\mu\text{S/cm}$)	0.0038859	0.0005966	6.51	0.000***
	Fluorides* σ ($\mu\text{S/cm}$)	-0.0030793	0.0007692	-4	0.000***

Prob > khi2 = 0,000

	Number of larvae	Estimated value	Standard error	Z statistic	Pr(> z)
MLG 5	Constant	1.984322	0.1147689	17.29	0.000***
	Fluorides	2.364476	0.1751039	13.5	0.000***
	Turbidity	0.0119415	0.001037	11.52	0.000***
	Fluorides*Turbidity	-0.0147306	0.0017898	-8.23	0.000***
MLG 6	Constant	1.579522	0.1168863	13.51	0.000***
	Fluorides	3.006675	0.1765179	17.03	0.000***
	MES	0.007313	0.0004816	15.18	0.000***
	Fluorides*MES	-0.0999437	0.0008218	-12.1	0.000***
MLG 7	Constant	1.362026	0.2505967	5.44	0.000***
	Fluorides	2.222465	0.3304361	6.73	0.000***
	TDS	0.0078337	0.0011956	6.55	0.000***
	Fluorides*TDS	-0.0062117	0.0015413	-4.03	0.000***
MLG 8	Constant	12.05263	1.306299	9.23	0.000***
	Fluorides	-53.80648	2.60815	-20.63	0.000***
	pH	-1.449719	0.1892548	-7.66	0.000***
	Fluorides*pH	8.124514	0.373368	21.76	0.000***
MLG 9	Constant	1.05691	0.6488013	2.32	0.020**
	Fluorides	-1.743854	1.384083	-1.26	0.208
	Sun exposure	1.947208	0.6523213	2.99	0.003***
	Fluorides*Sun exposure	2.760207	1.387547	1.99	0.047**

3.2. Discussion

The larval surveys carried out in this research showed that the study area mainly has artificial larval habitat, and most are exposed to the sun. In other words, these larval habitats were created by human activities including agricultural activities, which is consistent with the observations of [14] who found that most larval habitats are artificial, as well as the results of [15] which showed the responsibility of humans in creating conditions conducive to the development and maintenance of mosquitoes through the creation of their larval habitats. Also, the proximity of mosquito development sites to homes could constitute a health and nuisance risk for the surrounding human populations, as observed by [16] in Burkina Faso.

During this study, larval production was higher in the rainy season than in the dry season. This abundance is probably linked, on the one hand, to the large number of permanent larval habitat present in the study area and, on the other hand, to the fact that the rainy season offers possibilities of choice of larval habitat for females of the different mosquito species in terms of both quality and quantity. However, this is not the case for the dry season when some breeding places favorable to the development of mosquitoes dry up or disappear; this results in a scarcity of larval habitat during this period.

Indeed, the random distribution of the results obtained illustrates that the physico-chemical and chemical elements studied individually are not sufficient to assess

the larval density of the deposits. Nevertheless, we notice that in the dry season, a high concentration of fluoride does not increase the larval density, while this is the case in the rainy season. On the other hand, it should be noted that overall salinity, total dissolved solids, suspended solids, turbidity and electrical conductivity increase proportionally with larval density, regardless of the season. As for the hydrogen potential (pH), the results clearly show that the increase in larval density is conditioned by high pH values (ranging from neutral to basic). These results agree with those obtained by [17] as well as [18] who showed the impact of the physicochemical characteristics of the larval stations, in particular the hydrogen potential, which seems to play a role in the non-proliferation of the larvae [19]. Indeed, Anopheles larvae prefer well oxygenated waters with basic pH [20]. In other words, the higher the pH increases (basic), the higher is the number of larvae in the breeding places. Likewise, the results obtained concerning the influence of the exposure of sites to the sun are those also obtained by [14] according to which the larvae of anopheles prefer fresh water, exposed to sunlight and at high temperatures. Moreover, according to the work carried out by [21], the females of *Anopheles gambiae* sl prefer to lay their eggs in sunny water collections and, according to [22] the temperature and salinity can also promote or limit the growth of larvae.

We deduce that the variation in larval density considers several physico-chemical, chemical, and environmental

parameters. However, the generalized linear model allows us to highlight the interaction between the different parameters studied. The combined action of fluoride ions and other parameters (chloride ions, salinity, conductivity, suspended solids or dissolved solids) limits or inhibits the development of larvae in stations. When fluoride ions and pH are all high or fluoride ions are rising, and the station is exposed to the sun, the larvae develop easily.

Our results showed that to limit or inhibit the density of larvae in the region, it is necessary to act mainly on pH (make the environment acidic) and sun exposure (shade larval sites). One can also act on the combined effect of fluoride and chloride, as well as fluoride and salinity. But controlling these effects will be visibly complicated and uncertain.

4. Conclusion

The results of this research reveal a heterogeneity of larval sites in terms of physicochemical and chemical parameters and environmental conditions. These sampling sites are mostly born from anthropogenic activities and are in the immediate environment of the human populations in the study area. In the light of all the results of this study, it appears that the impact of fluorine on larval density does not follow a standard chronology but also depends on other parameters and the environmental context of the environment taken simultaneously. Nevertheless, an apparent correlation between pH, shading and larval density has been demonstrated. Thus, acidic water not exposed to the sun prevents the development of larvae. Given the complexity of aquatic environments, a more appropriate study would be needed to understand the behavior of larval ecosystems to manage them effectively and contribute to the fight against malaria.

Statement of Competing Interests

The authors have no competing interests.

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