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Physicochemical Characteristics of *Aedes* Mosquito Breeding Habitats in Suburban and Urban Areas of Kinshasa, Democratic Republic of the Congo

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Background: The knowledge of key elements of the ecosystem affecting mosquito distribution and their population dynamics is essential for designing mosquito-borne disease interventions. The present study characterized the physicochemical properties of *Aedes* mosquito breeding habitats in Democratic Republic of the Congo.

Methods: A cross-sectional survey was carried out in Kinshasa, from February to April 2021. The physicochemical characteristics of the natural and artificial aquatic habitats of *Aedes* were measured using a multiparametric device.

Results: Out of 438 breeding habitats inspected, 273 (62.3%) contained mosquito larvae. The *Aedes* mosquitoes identified in 76.19% of positive breeding sites were *Aedes albopictus* (67.30%) and *Aedes aegypti* (37.98%). The median values of dissolved oxygen (DO) (1.0), turbidity (19.15), and salinity (0.115) in water breeding sites of *Aedes* were respectively 0.8, 55.0, and 0.29 in *Culex* breeding sites ($p < 0.05$). The physicochemical characteristics of the breeding habitat for *Ae. aegypti* and *Ae. albopictus* were almost identical. In urban areas, the median temperature was 29.82 while it was 29.60 in suburban areas ($p < 0.05$). Significantly, the salinity was higher in bamboo and metal containers while DO was higher in tins. After analysis using simple linear regression, total dissolved solids ($r = 0.23$; $p = 0.000$), conductivity ($r = 0.23$), salinity ($r = 0.23$), and temperature ($r = 0.13$) were associated with larval density ($p < 0.05$). In the final model ($r = 0.30$, $p = 0.01$), salinity ($r = 0.23$) and DO ($r = 0.138$) adjusted to temperature, pH, and turbidity were associated positively to larvae density.

Conclusion: The *Aedes* breeding sites and mosquito density were significantly influenced by water salinity, DO, temperature, pH, and turbidity.

Keywords: ***Aedes*****, breeding, habitat, physicochemical characteristic, Kinshasa (DRC)**

BACKGROUND

Chikungunya, Zika, yellow fever, and dengue have reemerged and emerged as an important public health challenge worldwide as well in the Democratic Republic of the Congo (DRC) (1–3). These mosquito-borne viral diseases (MBVD) are mainly transmitted by *Aedes* mosquitoes. *Aedes aegypti* and *Ae. albopictus* are known to be the major vectors of most of the MBVD (4, 5). Multiple factors ranging from climatic, environmental, ecological, and socioeconomic factors exert influence over arbovirus vectors distribution and abundance (6, 7).

Mosquito immature stages are entirely aquatic and require water for their development. Various water-holding containers ranging from small to large and natural to manmade have been reported to support *Aedes* mosquito breeding. They include tires, flower pots, plastic or metal recipients, drains, swimming pools, storm water, flooding water, bamboo sticks, and plant axils (8–10). Moreover, any standing water body represents a potential mosquito breeding site. The quality of the water might selectively favor the development of larvae and pupae of some mosquitoes species and successful development into adult mosquitoes (11. The choice of breeding sites by mosquitoes is a key element for mosquito productivity, population survival, and dynamics (12).

The physicochemical characteristics of a mosquito breeding site express a significant influence on the immature stage of the mosquito life cycle and have important implications for mosquito control (9, 13). Some of these characteristics such as pH, salinity, dissolved oxygen (DO), and total dissolved solids (TDS) have been shown to influence the density of *Ae. aegypti* and *Ae. albopictus* (14, 15). These water characteristics affect some mosquito basic features such as survival, body size, and biting behavior which are linked to mosquito vectorial capacity, hence the magnitude and intensity of disease transmission. Indeed, the deficit of food supply in the stage of immature mosquito development leads to hatching of small mosquito adult body size and decrease in longevity (16). Habitually, the small female mosquitoes are more active in host seeking and blood feeding than the larger-size females probably due to limited energy stock to support egg maturation (17). Therefore, this behavior modification exposes hosts to high mosquito biting rates with possibility of pathogen transmission (17, 18). Thus, water availability and storage procedures greatly influence mosquito immature richness and MBVD risk occurrence (19).

The majority of large cities of DRC including Kinshasa are characterized by heterogeneous levels of urbanization and unequal levels of pollution. With the geographical expansion of chikungunya and dengue and repeated yellow fever outbreaks in DRC, there is a need to improve our understanding of the vector ecological factors (20–22). The physicochemical and environmental factors in mosquito breeding sites exert an effect on the density of mosquito larvae as well as on their detoxification enzyme activities. They can present an impact on insecticide resistance occurrence (23). It has been reported that the management of the pH of favored breeding sites could serve as a tool to control mosquito, and it can also assist the choice of biopesticides to use (24). Therefore, the good knowledge on *Aedes* breeding site physicochemical properties is critical in the design of effective vector interventions (25).

Not much data are currently available on the physicochemical characteristics of *Aedes* mosquito larval habitats in DRC. The present study assessed the physicochemical characteristics of *Aedes* water breeding habitats in the urban and suburban areas of Kinshasa, DRC.

Abbreviations: MBVD, mosquito-borne viral disease; DO, dissolved oxygen; TDS, total dissolved solid; DRC, Democratic Republic of the Congo.

METHODS

Study Area and Selection of Study Sampling Sites

The study was carried out in Kinshasa, situated in the western part of DRC at latitudes 4°19′30′S and longitudes 15°19′20′E. Kinshasa is the capital province of DRC and the largest metropolis in Central Africa with a population of 12 million inhabitants. Kinshasa occupies an area of 9,965 km^2, of which only 10% represents urban areas. Kinshasa receives over 1,482 mm of rainfall annually with average temperature of 25.2°C. There are two marked seasons, a rainy season from October to the first half of May and a dry season from the second half of May to September. The topography consists of larger plains surrounded by some hills, with a significant hydrographic system. The soils are sandy and sandy-argillaceous. Kinshasa is divided into 24 municipalities (26, 27).

The study sampling sites (**Figure 1**) were chosen to reflect the wide range of different conditions within the region in terms of urbanization level, environmental parameters that are likely to promote active transmission of arboviral infections, proximity to rivers, and a history of occurrence of arboviral disease outbreaks (5, 21, 28, 29).

Study Design and Mosquito Collection

This cross-sectional survey was carried out from February to April 2021. At all study sites, various natural and artificial containers were inspected for the presence of immature stages of mosquitoes. The larval densities of each immature mosquito breeding habitats were determined.

Depending on the mosquito habitat size, larvae and pupae were collected by either dipping or pipetting or emptying the container. Water samples were collected in 350-ml plastic containers. All mosquito larvae and pupae for each habitat were counted to determine the density. The collected larvae and pupae were reared into adult. Rearing was done under ambient temperature 28°C and relative humidity 80% with a 12:12-h (light: dark) photoperiod. All hatched adult mosquitoes were identified into genera using morphological identification keys. Afterward, a pictorial key was used to identify *Aedes* mosquito into species (30).

Physicochemical Parameter Measurement

Water samples from each inspected container were collected and analyzed for the following physicochemical parameters: pH,

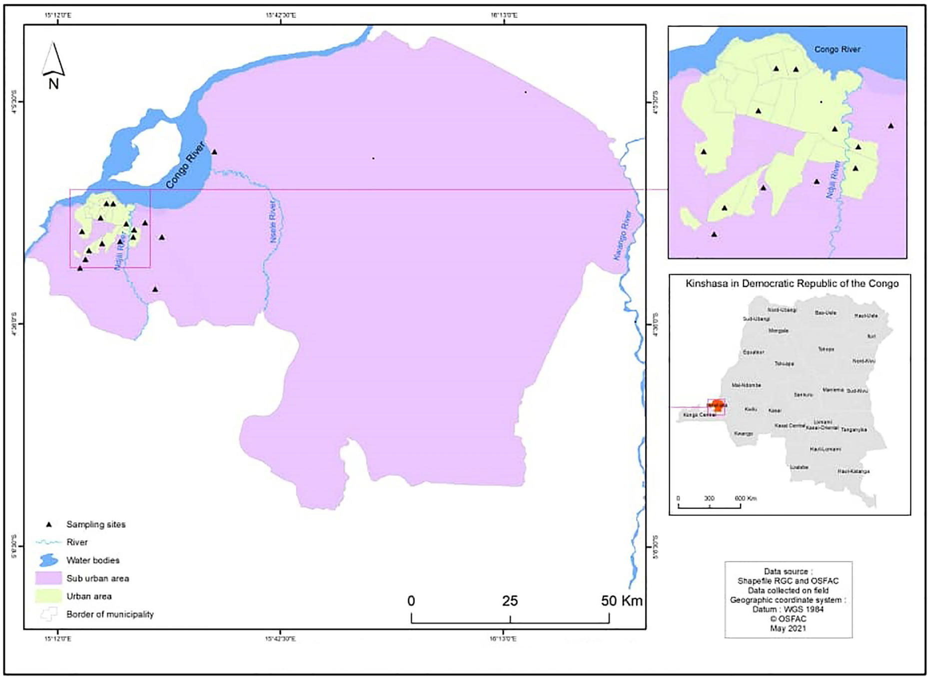

FIGURE 1 | Location of sampling areas in Kinshasa.

temperature, TDS (mg/l), DO (%), salinity (practical salinity unit), turbidity (Formazin Nephelometric Unit), and conductivity (µS/cm)using an electronic device (HANNA© HI9829). This device was regularly calibrated as per owners' manual.

Statistical Analysis

The data were entered into the Microsoft Excel 2007 spreadsheet and analyzed using GraphPad Prism version 9.1.2 for Windows (GraphPad Software, San Diego, CA, USA). Descriptive statistics were applied to summarize data. Due to non-normality of data distribution based on the Shapiro–Wilk test, the differences of the physicochemical characteristics in *Aedes*, *Anopheles*, *Culex*, and negative breeding habitats and between different *Aedes* habitats types were tested using the Kruskal–Wallis test with Dunn's multiple-comparison test as *post-hoc* test. The Mann–Whitney U test was used to compare the medians of the physicochemical characteristics between urban and suburban areas and between *Ae. aegypti* and *Ae. albopictus* breeding sites. The simple and multiple-regression linear analyses were applied to investigate the relationship between mosquito larval densities and the physicochemical factors of the breeding water. For all the statistical analysis, p value < 0.05 was considered significant.

RESULTS

A total of 438 water-breeding places were inspected. Of these, 273 contained mosquito larvae including 90 (32. 97%) tires, 56 (20.51%) plastic containers, 43 (15.82%) collections of stagnant and flooding water, 17 (6.23%) bamboo sticks, 16 (5.86%) tins, and 14 (5.13%) metal containers (**Table 1**). *Aedes* spp. were found in 208 (76.19%) breeding sites; 79 (28.94%) breeding sites were positive for *Culex* spp.; 21 (7.69%) had *Anopheles* spp.; and three (1.09%) had *Toxorhynchites* mosquitoes. The co-occurrence of *Aedes* spp. and *Culex* spp. was recorded in 23 (8.42%) breeding sites, while in seven (2.56%) breeding sites, *Aedes* cohabited with *Anopheles*. Of 208 positive *Aedes* spp. habitats, *Ae. albopictus* were found in 140 (67.30%) sites and *Ae. aegypti* in 79 (37.98%) sites, while in 23 breeding habitats, the two species of *Aedes* were found together (**Table 2**).

The overall trends of physicochemical characteristics of the positive breeding habitats were significantly different from the ones without mosquito larvae (**Table 3**). Using Dunn's multiple-comparison test as *post-hoc* test, the median of DO was significantly higher in water breeding sites of *Aedes* (1.0) compared to *Culex* mosquitoes (0.8), p = 0.0001. Respectively, the means of conductivity (228.5), TDS (112.5), turbidity (19.5), and salinity (0.115) in water breeding sites of *Aedes* were significantly lower than in *Culex* (555.0, 267.0, 55.0, and 0.29) with $p < 0.05$. The pH in the *Aedes* breeding site (6.76) was higher than in *Anopheles* (6.58) with p = 0.0002. The patterns of physicochemical characteristics favoring different mosquito occurrence are summarized in **Figure 2**.

The percentage of DO in *Aedes* breeding habitats ranged from 0.5 to 2.6. The medians of DO, pH, conductivity, salinity, TDS, and turbidity as well as temperature of *Aedes* breeding habitats are provided in **Table 4**. *Ae. aegypti* and *Ae. albopictus* were found breeding in water sites with related properties as regards

TABLE 1 | Frequency of mosquito larval habitat types in Kinshasa.

Types of breeding habitat	Positive habitats (N = 273) n (%)	Negative habitats (N = 165) n (%)
Tires	90 (32.97)	20 (12.12)
Tins/cans	16 (5.86)	2 (1.21)
Plastic container	56 (20.51)	48 (29.09)
Metal container	14 (5.13)	7 (4.24)
Bamboo	17 (6.23)	1 (0.61)
Sewage pit	13 (4.76)	17 (10.30)
Cement drain and pit	15 (5.49)	24 (14.55)
Stagnant and flooding water	49 (17.95)	31 (18.79)
Swamp	6 (2.2)	0 (0.0)
Flowerpot	3 (1.10)	9 (5.45)
Well of drink water	0 (0.0)	6 (3.64)

TABLE 2 | Frequency of breeding sites of mosquito species occurrence and co-occurrence recorded in Kinshasa.

	N = 273	%	CI 95%
Aedes spp.	208	76.19	70.69–81.12
Culex spp.	79	28.94	23.63–34.71
Anopheles spp.	21	7.69	4.82–11.52
Toxorhynchites	3	1.12	0.12–3.24
Aedes spp. and *Culex* spp. co-occurrence	23	8.42	5.42–12.37
Aedes and *Anopheles*	7	2.56	1.04–5.21
	N=208		
Aedes aegypti	91	33.33	27.77–39.27
Aedes albopictus	140	51.28	45.18–57.35
Aedes aegypti and *Ae. albopictus*	23	11.06	7.14–16.13

TABLE 3 | Comparison of the medians of the physicochemical characteristics of water from positive and negative larvae habitats of mosquito in Kinshasa.

	Larvae habitat		*p*
	Positive	Negative	
P^H	6.7	6.61	0.0001
DO	0.9	0.6	0.0001
Conductivity	298.0	440.0	0.0019
Tds	147.5	202.0	0.0036
Salinity	0.15	0.23	0.0001
Turbidity	23.5	25.5	0.2657
Temperature	29.62	28.73	0.0001

turbidity, salinity, pH, TDS, DO, conductivity, and temperature levels (**Table 5**). Compared to suburban areas, the median of conductivity in urban areas was 236 vs. 228, U = 5088, $p = 0.7118$; TDS (109.5 vs. 114.5, U = 5141, $p = 0.8059$) and salinity (0.12 vs. 0.115, U = 5084, $p = 0.7046$). The median of temperature was significantly high in urban areas (29.82) than in suburban areas 29.60, U = 4319, $p = 0.0298$ (**Table 6**). Some of these physicochemical parameters varied significantly according to the larval habitat types. A high percentage of DO was recorded in tin (1.2) and tire (1.05) and lower % DO in stagnant water (0.7) and sewage pit (0.65); U $p = 0.0014$. The salinity was high in bamboo sticks (0.31) and lower in tin (0.04), $p = 0.0001$. The trends of different physicochemical parameters of the different breeding *Aedes* habitats are summarized in **Table 7**.

In simple linear regression, the larval density in *Aedes* breeding habitats showed a positive relationship with TDS ($r_{(206)} = 0.23$; $p = 0.001$), conductivity ($r_{(206)} = 0.23$; $p = 0.001$), salinity ($r_{(206)} = 0.23$; $p = 0.001$), and temperature ($r_{(206)} = 0.138$; $p = 0.04$) (**Table 8**). In the multiple linear regression model ($r_{(202)} = 0.30$, $p = 0.002$), salinity ($r = 0.23$, $p = 0.001$) and DO = 0.138, $p = 0.047$) adjusted to temperature, pH, and turbidity were positively associated with larval density (**Table 9**).

DISCUSSION

The present study addressed the association between physicochemical properties of *Aedes* mosquito habitats in urban and suburban areas of Kinshasa. Infestation with *Ae. albopictus* and *Ae. aegypti* accounted for the over three-quarters of the breeding sites. *Ae. aegypti* and *Ae. albopictus* bred in water sites with closely related turbidity, pH, TDS, DO,

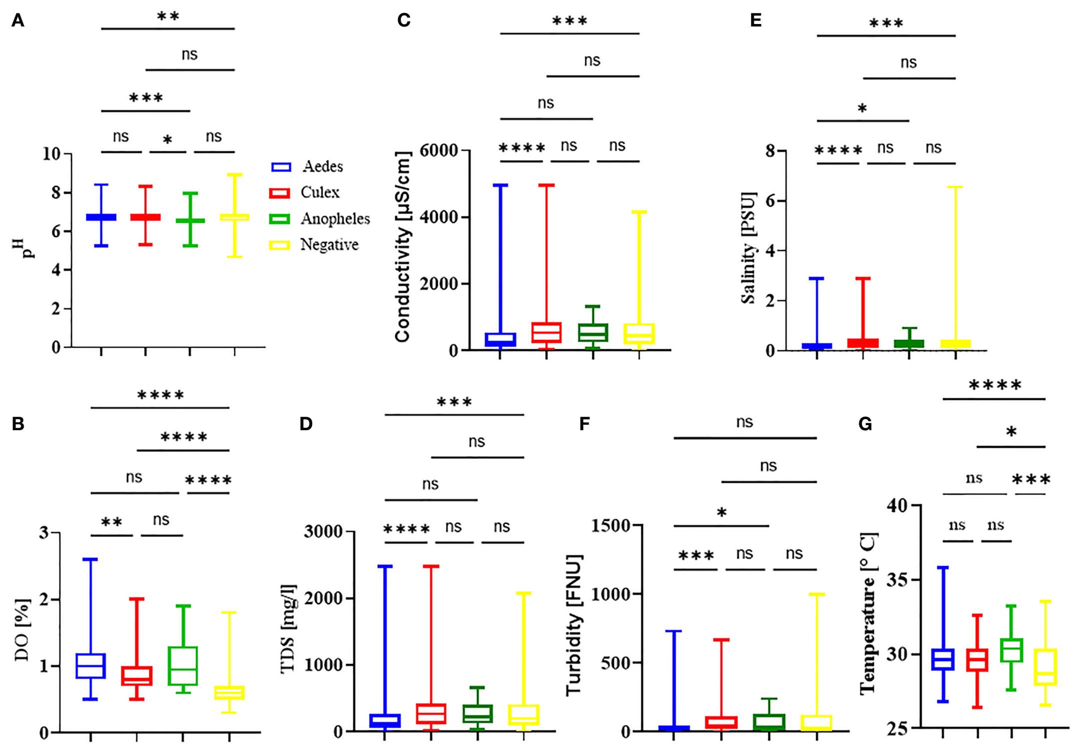

FIGURE 2 | Comparison of physicochemical characteristics of breeding habitats between *Aedes*, *Culex*, and *Anopheles* mosquito in Kinshasa. **(A)** pH, **(B)** dissolved oxygen, **(C)** conductivity, **(D)** total dissolved solids, **(E)** salinity, **(F)** turbidity, **(G)** temperature. *P < 0.05, **P ≤ 0.01, ***P ≤ 0.001, ****P ≤ 0.0001 and ns, not significant (P > 0.05).

TABLE 4 | Distribution of physicochemical characteristics of immature *Aedes* habitats in Kinshasa.

	Median	Range
P^H	6.76	5.26–8.41
Dissolved oxygen (%)	1.0	0.5–2.6
Conductivity (µS/cm)	228	13.00–4955.00
Total dissolved solid (mg/l)	112.5	9.00–2479
Salinity (PSU)	0.115	0.01–2.88
Turbidity (NTU)	19.15	0.29–731.00
Temperature (°C)	29.61	26.80–33.20

TABLE 5 | Comparison of the medians of physicochemical characteristics favoring *Ae. aegypti* and *Ae. albopictus* occurrence using the Mann–Whitney test.

	***Aedes* species**		**U**	***p***
	Ae. aegypti	***Ae. albopictus***		
P^H	6.79	6.73	5,726	0.1946
DO	1.0	1.0	5,978	0.4269
Conductivity	202.0	256.0	5,668	0.1576
Tds	102.0	128.50	5,161	0.1636
Salinity	0.10	0.13	5,796	0.2473
Turbidity	15.20	18.20	6,259	0.8230
Temperature	29.30	29.79	5,445	0.0623

conductivity, and temperature levels. However, these *Aedes* species preferred less turbid waters with moderate salinity and moderate DO. The findings of this study revealed differences in some parameters such as TDS, DO, conductivity, turbidity, and salinity according to the level of urbanization and habitant types, either natural or artificial. This is contrary to the findings from Zanzibar in Tanzania where the presence of immature *Ae. aegypti* was not linked significantly to any physicochemical characteristics of water breeding habitats (31).

Indeed, Kinshasa is characterized by uncontrolled and heterogeneous urbanization levels, poor environmental hygiene, multiple soil erosion, and recently multiple floodings (26, 27, 32). These conditions create suitable mosquito breeding habitats. The quality of water within these habitats varies in terms of their physicochemical characteristics which can promote or limit the proliferation of some *Aedes* species. The physicochemical parameters of *Ae. aegypti* and *Ae. albopictus* breeding site water were similar in regard to turbidity, pH, salinity, chloride, and phosphate levels (24).

Similar to findings from other studies, containers expressed differences in water physicochemical profile and urbanization considerably accelerates the larval development rate and survival time of adult of *Ae. albopictus*, with a positive effect on vector capacity (17). The anthropogenic *Ae. aegypti* prefers clean water found in various types of peridomestic containers while *Ae. albopictus* prefers natural containers or outdoor manmade habitats containing abundant amounts of organic debris (19). The temperature, DO, and pH of breeding site water have differences between endemic and

TABLE 6 | Comparison of the medians of physicochemical characteristics of *Aedes* spp. breeding sites according to urbanization level.

	Setting		U	p
	Urban	Suburban		
pH	6.74	6.79	5,034	0.6210
DO	1.0	1.0	4,521	0.0875
Conductivity	236.0	228.5	5,088	0.7118
Tds	109.0	114.5	5,141	0.8051
Salinity	0.12	0.115	5,084	0.7046
Turbidity	15.30	19.15	4,761	0.2574
Temperature	29.82	29.60	4,319	0.0298

TABLE 7 | Comparison of the medians of the physicochemical parameters according the type of breeding habitat of *Aedes* in Kinshasa.

	DO %	p^H	Salinity	TDS	Turbidity	Conductivity	Temperature
Bamboo sticks	0.9	6.61	0.31	277.0	71.90	599.0	30.22
Cement container	0.9	6.69	0.16	158.0	34.40	317.0	29.40
Stagnant water	0.7	6.62	0.30	288	25.30	557.0	30.60
Flower pot	1.0	6.63	0.04	31	11.0	89.0	33.01
Tin	1.2	6.87	0.04	40.0	12.75	80.5	29.58
Metal container	1.0	6.58	0.16	157	14.60	315.0	29.61
Sewage pit	0.65	7.13	0.115	113.5	85.50	226.0	29.07
Plastic container	0.9	6.78	0.1	103	14.75	206.5	29.80
Tire	1.05	6.82	0.11	109.0	17.50	217	29.30
$p < 0.05$	0.0014	0.004	0.0001	0.0001	0.07	0.0001	0.5153
KW statistic	25.12	16.0	33.9	36.65	14.18	25.18	7.22

non-endemic areas (33). The physicochemical and biological markers of mosquito larval habitats help in mapping of areas suitable for breeding and distribution for targeting surveillance and controlling activities (17, 34).

Reports from different environments show a relationship between physicochemical characteristics of immature habitats and container types. However, differences in some parameters could be linked to the nature of the container, either natural or artificial, and water source (19, 35, 36). In the current study, various natural and artificial breeding habitats of immature mosquitoes were explored and grouped according to their materials made. They exhibited a significant difference in their physicochemical properties. The concentration of TDS and the levels of turbidity and conductivity were low in tin and plastic container. The non-household locations, as well as non-disposable containers, should be targeted in the standard control activities of mosquito-borne viral diseases (37), contrary to findings from Tanzania where the presence of immature *Ae. aegypti* was not linked significantly to any physicochemical characteristics of water breeding habitats (31). In other studies, it was reported that the selection of breeding sites depends on the volume and size of the water surface and the type of material of which they are made (38.). *Aedes* mosquito prefers materials which are made from cement, metal, soil, ceramics, and plastic (36, 39.). In a study in Kolkata, India, plastic containers were reported as the most productive habitats for *Aedes* species (35). The populations of immature mosquitoes growing in ground pools are exposed to diverse factors of death, natural enemies compared to those in water-filled containers in which the growth is restricted by food availability (38).

Although in simple linear regression, larval density showed a positive relationship with the temperature, concentration of TDS, conductivity, and salinity, in multiple linear regression analysis, only salinity and DO adjusted to temperature, pH, and turbidity were associated with increasing larval density. This observation was in agreement with findings from a study in Egypt (40). It was also reported from Iran that DO presents a significant impact on larval density (41). The salinity is another parameter that can promote or limit the growth of larvae (40–43). Temperature affects the degree of evapotranspiration, and therefore, it acts on the water salinity (43). Water temperature is a crucial parameter for mosquito metabolism and development (17, 38, 44). In other studies, the breeding site characteristics of *Ae. albopictus* showed a negative correlation of the larval density with turbidity and conductivity but a positive correlation with pH and DO (40, 42, 43, 45). Water pH and turbidity in the current study seemed to decrease larval density, but the association was not significant. Similar findings have been reported from a study in Iran (44).

Limitations

Most of *Ae. aegypti and Ae. albopictus* aquatic breeding habitats are temporary, disappearing during the dry season. Therefore, it was difficult to repeat the measurement of these physicochemical parameters in the same surveyed water habitats to assess the effect of seasonality on the physicochemical characteristics of *Aedes*. Considering that the physicochemical parameters were measured only once in this study and during the raining season, it is aware to consider the probability of larval habitat physicochemical characteristics and larval density fluctuation due to seasonality. Furthermore, studies are essential to consider not only the effect of the abiotic factors (the physicochemical characteristics) on larval productivity in breeding sites but also the effect that can express the biotic factors such as larval predators, algae, and

TABLE 8 | Simple linear regression analysis of breeding habitat characteristics in relation to the larvae density into *Aedes* breeding habitats in Kinshasa.

	A	r	t	*p* < 0.05
pH	-1.159	0.029	-0.398	0.680
DO %	6.891	0.123	0.222	0.077
Conductivity	0.008	0.232	3.416	0.001
Tds	0.016	0.233	3.443	0.001
Salinity	14.129	0.234	3.449	0.001
Turbidity	0.009	0.037	0.526	0.599
Temperature	2.008	0.138	2.000	0.047

TABLE 9 | Multiple regression analysis model of breeding habitat characteristics in relation to the larval density into *Aedes* breeding habitats in Kinshasa.

	A	β	t	*p* < 0.05
pH	-1.263	-0.031	-0.457	0.648
DO %	7.703	0.138	2.003	0.047
Salinity	14.396	0.238	3.507	0.001
Turbidity	0.000	-0.001	-0.013	0.990
Temperature	1.734	0.119	1.761	0.080

Model.
$r_{(202)} = 0.300$, $D_{(5,\ 202)} = 3.99$, $p = 0.002$.

emergent plants on larval productivity in *Aedes* breeding habitats in order to consolidate the baseline information on mosquito larval habitat characteristics.

CONCLUSION

The current study showed that the salinity and percentage of DO exert influence on the distribution of *Aedes* mosquito occurrence, survival, and abundance. Other parameters such temperature, acidity or alkalinity, and turbidity indirectly influence *Aedes* mosquito proliferation. Environment factors such urbanization and the nature of breeding habitat affect these physicochemical parameters. The findings of this study can assist in designing control activities of *Aedes*-borne diseases by targeting areas suitable for breeding.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**. Further inquiries can be directed to the corresponding author.

AUTHOR CONTRIBUTIONS

KM designed the study, conducted the fieldwork, performed the statistical analysis, and prepared the manuscript for publication. RW, PM, and SIK assisted in the study design. DE, MB, and JZ participated in the fieldwork. LM and GM critically read the manuscript for publication. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fitd.2021.789273/full#supplementary-material

Additional File 1 | Dataset supporting the conclusions of study on physico-chemicals characteristics of *Aedes* breeding sites in Kinshasa.

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