

International Journal of Agriculture Extension and Social Development

Volume 7; Issue 7; June 2024; Page No. 412-419

Received: 27-04-2024
Accepted: 30-05-2024

Indexed Journal
Peer Reviewed Journal

Ecological dynamics and multidimensional analysis of phlebotomine sandflies in potentially prone communities in southeastern Nigeria

Ejiogu CC¹, Mgbeahuruike LU¹, Njoku-Tony RF¹, Onyechere CU¹, Ebe TE¹, Emereibeole EI¹, Ogbuagu DH¹, Nwachukwu JI¹, Nwachukwu IN¹, Ihejirika CC¹, Iheme PO², Ezirim KT¹ and Dike MU¹

¹Federal University of Technology, Owerri, Nigeria

²University of Agriculture and Environmental Sciences Umuagwo, Imo State, Nigeria

DOI: <https://doi.org/10.33545/26180723.2024.v7.i7f.831>

Corresponding Author: Ejiogu CC

Abstract

This study investigates the spatial and temporal dynamics of sandfly specie abundance in some selected communities in Nigeria using multidimensional approach, including entomological survey, soil analysis and Inverse Distance Weighting (IDW) spatial interpolation. The Centre for Disease Control (CDC) light and sticky paper trap method was installed three times repeatedly in a month between 6.30 am to 6.30 pm over a period of eight (8) months (August 2022 – March 2023) while Human baits was deployed once monthly. A total of 909 insects were captured to determine the spatio-temporal abundances and 525 sandflies were caught using the human baits and for estimate of the Monthly Biting Rates (MBR). The Inverse Distance Weighted (IDW) technique was deployed for spatial mapping of specie abundance. The finding reveals distinct patterns in sandfly abundance across various months, highlighting specific trends in variability and skewness. Notably, the month of August exhibits higher variability with a wider interquartile range (IQR), indicating a dynamic ecological environment. In September, a left-skewed distribution suggests consistently higher and more stable sandfly abundance. November and March present right-skewed patterns while October and December were without significant fluctuation. January and February were left-skewed and right-skewed respectively. The spatial interpolation maps generated reveals a stronger correlation between sandfly abundance and land use types; particularly during the rainy season with values of 18.6% for Umuoba and Amuzu while 26.5% for Ihube in contrast to 6.1%, 7.0% and 13.2% for Umuoba, Amuzu and Ihube in dry season respectively. This suggests a spatial pattern aligning with land use types at different location, influenced by environmental conditions and microclimate. Soil analysis results revealed that sulphate ion appeared to be associated with favoured breeding sites for these sandflies. This breeding pattern is suggestive of intense biting activities observed during the early mornings and evenings of the rainy season as against reduced biting activity in the dry season. Ihube community recorded higher specie abundance oscillating between August and October. This study underscores the value of multidimensional approach in understanding sandfly ecology, with implications for vector control and public health intervention strategies.

Keywords: Sandfly, Spatiotemporal, Umuoba, Amuzu, Ihube, leishmaniasis

Introduction

Phlebotomine sandflies, belonging to the Psychodidae family, are naturally diminutive and relevant family of the insect world. These tiny dipterans, measuring 1.25 – 2.5mm long, are known for their dual roles as vectors of disease transmission of many parasitic, bacterial and viral diseases as well as an integral component of the ecosystem saddled with the functions of nutrient cycling and ecological interaction (Faraj *et al.*, 2016; WHO, 2017) [4, 22]. These blood feasting creatures are responsible for diseases including leishmaniasis, bartonellosis and rift valley diseases of known global impact (WHO, 2017) [22].

These diseases are vector borne and account for 17% of infectious diseases with millions of deaths each year (WHO, 2017) [22], an alarming figure which calls for a major public health concern locally and globally. Understanding the ecological dynamics of these elusive insects is of paramount importance, not only from a public health perspective but also in the broader context of biodiversity conservation and

ecosystem management.

The activity of sandflies is nocturnal but some species bite during daylight. Their activity starts after sun set and ends before the sun rise with a highest peak activity after midnight. The flight pattern of sandflies is a series of short erratic hops as they are poor fliers and has a flight range up to 2 km. Morphologically, the males and females of this subgenus are easily identified from another subgenus of Phlebotomus. The females have a completely segmented spermatheca with long terminal knob, while the males of the group have a short style, five relatively long spines on the style and no tufted process on the coxite. Both sexes feed on plant juices though; the females require a blood meal in order to lay her eggs. Larval development takes 30 – 60 days in rich organically moist micronutrient (Inceboz, 2019) [10]. Sandflies normally live between 30 and 45 days and females are having longer longevity than that of males. However, their distribution and abundance are intricately linked to various environmental and climatic factors.

Research has highlighted the significance of soil composition, vegetation cover, temperature and humidity in modifying sandfly population and longevity duration (Chafika *et al.*, 2015; Ready, 2013) ^[3, 13]. Many studies have revealed that weather patterns can provide a favourable condition for the distribution of sandflies and possible transmission of leishmaniasis in endemic areas. Cutaneous leishmaniasis is known to be higher in areas with daily rainfall, maximum and minimum temperature, and humidity. These environmental factors occurred in most hyperendemic areas of cutaneous leishmaniasis, compared to the lower endemic areas (Abolfazi *et al.*, 2021) ^[1].

Globally, about 14 million people throughout Africa, Europe, Asia, and America are directly affected by the disease (WHO, 2018) ^[23]. The global burden of leishmaniasis has been stable for years, with a morbidity of 2.4 million Disability Adjusted Life Years (DALYS) and a mortality of approximately 70, 000 deaths which is obviously ranking high among infectious diseases (WHO, 2017) ^[22]. According to a report published by the world health organization (WHO) in 2019, the annual incidence of leishmaniasis has been estimated to range from 700,000 to 1.2 million in the world. This incidence is estimated to be more than 12 million cases worldwide and 1.5 to 2 million cases is added to this rate annually. 75% of these cases have been reported to be cutaneous in form and are hard to treat as there are no available vaccines yet (Seid *et al.*, 2015) ^[17]. In recent past, the risk of emergence of Neglected Tropical Diseases (NTDs) has considerably increased in Nigeria. The Nigeria Federal Ministry of Health (FMOH) recognizes nine diseases namely; Trachoma, Leishmaniasis, Trypanosomiasis, Dengue Fever, Schistosomiasis, River Blindness, Lymphatic Filariasis, Onchocerciasis and Soil Transmitted Helminthes. Of these diseases, most predominantly reported is the cutaneous leishmaniasis. Cutaneous manifestation of NTDs is closely associated with poor sanitation, migration, lack of safe water, malnutrition and poverty which are precursors to morbidity and stigmatization (FMH 2013) ^[6].

Epidemiology studies affirm that cutaneous leishmaniasis occurs mainly in the highlands of the country and is usually caused by *L.aetiopica* with an estimated 50,000 cases reported annually (Aklilu *et al.*, 2022) ^[2].

However, the Northern part of Nigeria has observed the highest burden of the Leishmaniasis disease, as 35% of the 23 local government areas in Kaduna state are either at risk or have the burden of the disease. Further studies in the northern hinterland of the country which evaluated the occurrence and monthly abundance of Phlebotomine sandflies affirms that 91.2% of these areas have more refuse dumps and 8.8% of the areas have broken sewage

tanks which may harbour sandflies of phlebotomine specie. Therefore, this study embarks on a multifaceted investigation of Phlebotomine sandflies within three communities in Imo State, Nigeria. The overall research objective is to underscore the ecological dynamics governing the distribution, abundance, and seasonal variations of these vectors within the unique ecology of these communities. To achieve this task, will require a multifaceted analytical approach including entomological survey, spatial interpolation technique and edaphic analysis to explore the complexity of sandfly ecology within their diverse habitats.

Materials and Methods

Study area

The field study, indicated in Fig x, was performed in Imo State, one of the five states in Southeast of Nigeria. Imo state is one of the 36 states of the Federal Republic of Nigeria and one of the five states that make up the South East geopolitical zone in the South Eastern part of Nigeria. The state lies within latitude 4°45'N and 7°15'N and longitude 6°50'E and 7°25'E. It occupies the area between the lower part of River Niger and the upper and middle part of Imo River from which it derives its name. Imo state is bounded on the West by Anambra State, on the East by Abia State on the South by Rivers State. The State occupies an area of about 5100km². According to the 2006 census, the population of Imo State is about 3,934,809 while the 2015 projected population is put at 5,492,993. The State has a total number of 27 Local Government Areas making up for three zones (Imo West or Orlu zone, Imo East or Okigwe zone and Imo Central or Owerri zone). There are two distinctive seasons- wet and dry seasons. The wet season last between April through October with peaks of rainfall occurring from July to September. The dry season starts from November through March. The annual rainfall vary from 1500mm to 2200 mm (Ikpeama *et al.*, 2006) ^[12] with an average annual temperature above 20°C (68.0°F) creating annual relative humidity of 75% with humidity reaching 90% in the remaining season. The vegetation is a tropical rainforest, characterized of tall trees and rich in humus soil. The study sites encompass many communities from three local government areas drawn from two zones. Nine sampling locations were selected, three from each of the selected communities. These communities were selected according to unique characteristics which cut across proximity to water bodies, sand/loamy soil and rural and peri-urban areas endemic with gardens and farms. These characteristics provide potential breeding sites for sandflies (Ullah *et al.*, 2016) ^[18].

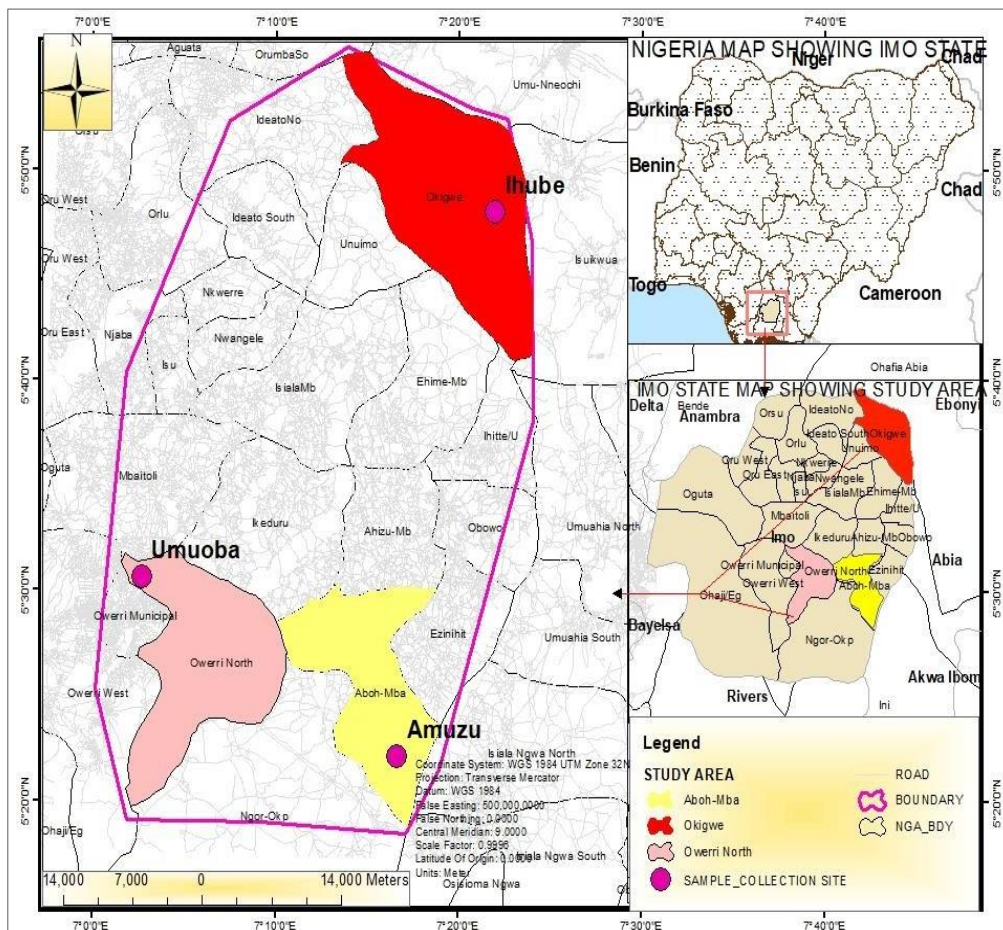


Fig 1: Imo West or Orlu zone, Imo East or Okigwe zone and Imo Central or Owerri zone

2.1 Sandfly collection and identification

To investigate the influence of ecological and climate parameters on the abundance and distribution of sandflies, a CDC light and sticky paper traps were deployed. These traps are effective for conventional method used for insect collection (Ullah *et al.*, 2016) [18]. This activity was conducted by sunrise till sunset as the flies are usually active during 6:30am and 6:30pm. The trap was suspended in farmlands, palm oil mills and livestock homes with the ice block hung beside the CDC trap. This is to cool the environment and attract sandflies. The CDC light trap with ice was used as shiny surfaces to attract the sandflies. The population size of sandflies was investigated and the genera encountered identified. The sticky paper traps were made from cardboard sheets of about 20cm by 20cm coated with castor oil and a light source suspended at about 1.5m above the ground. Both temperatures and relative humidity were measured in situ.

2.1.1 Human Bait technique

Three trained volunteers were used as human baits for the purpose of estimating the biting rate of sandflies in the study area. Simple bait involving ‘feed on host and capture’ technique conducted between the hours of 6:30am and 6:30pm once a month for a period of 8months was applied for this exercise. The caught sandflies were secured in specimen tubes and identified from other insects following the taxonomic methodology proposed by Galati *et al.* (2018) [7]. The entrapped sandflies were washed in 90% ethanol for 2-5mins to remove oil and other debris and was transferred

to specimen tubes containing 70% glycerin for preservation and proper identification.

2.2 Soil samples, collection and analysis

Soil parameters including pH, organic carbon, organic matter, moisture content, available phosphorus, nitrates and the presence of sulphate were analyzed following rigorous established Standard Operating Procedures (SOPs) throughout the entire process. Sample collection, preparation, analytical techniques and quality control were analyzed as part of the step taken to maintain consistency and precision. Each analysis was conducted using calibration equipment and verified methods in accordance with ISO - 17025.

2.3 Data analysis

Measured variables were analyzed using descriptive statistics with the aid of the SPSS-22 edition. The One-way Analysis of Variance (ANOVA) was used to determine the homogeneity in mean abundance of the insect studied and biting rates across the study locations at $p < 0.05$. The Duncan Multiple Range test was used for mean separation. The Pearson’s correlation (r) was used to determine possible relationships between the climatic variables, edaphic parameters and sandfly abundances at both $p < 0.05$ and $p < 0.01$. The student’s t-test was used to compare seasonal abundances and biting rates of the organism at the 95% confidence limit.

Inverse Distance Weighted (IDW) technique, a spatial

analyst tool in ArcGIS 10.5 was used to predict the distribution trend of sandflies across the different locations in Imo State

3. Results and Discussion

3.1 Physicochemical influences in land use types

Soil properties of three land use types in Ihube, Amuzu and Umuoba, in southeast Nigeria were analyzed and presented in Table 1. The pH of Ihube soil has a corresponding pH of 5.82 ± 0.23 , 5.61 ± 0.21 and 4.91 ± 0.23 in POM, LSH and FLA respectively (Table 1). This range of pH (4.91-5.82) of soils of Ihube area indicates slightly acidic soil reactions. Similarly, Amuzu and Umuoba communities relatively share same range of soil pH across the different land uses ranging from 4.3 ± 0.18 in FLA to 5.85 ± 0.11 in POM (Table 1), showing a very strong to strong acid reactions (Adebayo *et al.*, 2009). The status of the soil nitrate in POM across the three communities were relatively higher (23.6 ± 0.41) compared to LSH (18.8 ± 0.34) and FLA (16.0 ± 0.23) (Table 1). This variation could be attributed to soils having varying capacities to retain or release nitrate concentration. Organic carbon relatively occurred in low amounts with just slight differences in concentrations in the different land use type across the three communities (1.49 ± 1.36 – 2.11 ± 1.23). However, moderate to high amounts was observed in the increasing order of 1.52 ± 1.5 > 1.7 ± 1.28 > 2.11 ± 1.23 for FLA, LSH and POM respectively. These levels of organic matter are at variance compared to other land use types (Faraj *et al.*, 2016) [4]. The concentration of phosphorus in POM was slightly higher and in the range of 17.1 ± 0.29 to 19.9 ± 0.2 compared its concentrations in LSH (15.9 ± 0.3 to 16.44 ± 0.19) and FLA (13.67 ± 0.21 to 14.34 ± 0.29) across the three communities. According to Chude *et al.* (2012), soil available P value is low at 3–7 mg kg⁻¹, moderate at 7–20 mg kg⁻¹ and high at >20 mg kg⁻¹. The higher concentrations of available P in POM soils than in LSH and FLA, may be a function of different factors like fertilizer use, crop types and soil management as most of the P available in soil derives from the soil organic matter pool. The values of organic carbon were abysmally low as observed in POM (1.22 ± 1.34 to 1.38 ± 0.23), LSH (0.85 ± 0.58 to 0.99 ± 1.29) and FLA (0.87 ± 0.43 to 0.89) across the communities being investigated. This abysmal value in organic carbon is expected considering the observed value of organic matter reported earlier. Sulphate concentrations was highest in POM with concentrations range of 150 ± 2.26 at Amuzu, 188 ± 2.35 at Umuoba and 189 ± 2.34 at Ihube compared to moderate concentrations FLA (90.2 ± 2.3 to 92.4 ± 2.6) and low concentration in LSH (77.9 ± 2.3 to 78.5 ± 2.1). The likely reason for the elevated concentration of sulphate in POM may not be far-fetched from reasons alluded to phosphate concentrations in relation to land use types. Moisture content averagely ranged from 2.2 ± 0.41 to 4.0 ± 0.71 in land use types across the different communities investigated. Slight variations observed in content across the different land use types could be attributed to climatic factors and land use practices.

3.2 Temporal influences in specie (sandfly) abundance across the 3-communities

The boxplot in Figures 2a,b and c is used to visually represent and compare the sandfly abundance across the

three communities: Amuzu, Ihube and Umuoba. In comparison of the boxplots across the different months in Amuzu community as represented in Figure 2a observed that the median in the month of August was higher and in contrast with median values for October and March. However, a wider interquartile range (IQR) was observed for August, October and March, indicating a wider variability in sandfly abundance. The months of September, November and February, the median values remained relatively stable while the median values in the months of December and January exhibited a non-normal distribution due to the absence of prominent median, suggesting a distribution with significant skewness.

In Ihube community, as presented in Figure 2b, observed that the median values in August exhibited higher variability with a wider IQR while in September, the median value observed a left-skewed distribution. In contrast to the value trend in September, the median values of both November and March were to the right distribution trend while median values for October and December were observed to be consistent without significant fluctuations. Median values for January and February were left-skewed and right-skewed respectively.

In Umuoba community, the distribution patterns as presented in Figure 2c revealed that the median values for October, November and February were right-skewed with higher variability (wider IQR) while median values for August, September, December and March were relatively stable. In contrast, median value for January was observed to be non-normal with the absence of a prominent median trend.

The analysis of temporal distribution pattern of sandflies across Amuzu, Ihube and Umuoba, suggests the median abundance of sandflies in the months of August may be associated simultaneous increase in both rainfall and relative humidity during this period (Usman *et al.*, 2020) [19]. The increased variability suggests that the abundance of sandflies during August can vary significantly, and potentially influenced by multiple environmental factors, breeding site availability and other ecological dynamics (Fatima Zahra *et al.*, 2022) being highly sensitive to environmental conditions tend to thrive in environments with optimal moisture levels. In contrast, the month of September, November and February were characterized as relatively stable in terms of sandfly abundance. This stability might be attributed to constant environmental conditions where moderate levels of rainfall and related humidity creates a favourable but not excessive habitat for sandflies. However, the non-normal distribution of sandfly abundance in December and January, as indicated by the absence of a prominent median suggests a different ecological scenario. The erratic median values during these months could be associated with a combination of factors, including possible fluctuation of relative humidity and microclimate variability. Sandflies may encounter challenges during this period due to the reduced availability of breeding sites or less favourable conditions, leading to a more scattered distribution of abundance.

3.3 Influences in seasonal dichotomy of sandfly abundance of different land use types

In Figures 4 and 5, presents a comparative analysis of

sandfly abundance during the raining and dry seasons, shedding light on the dynamics and ecological patterns observed throughout the year. The rainy season recorded specie abundance of 18.6% for Umuoba and Amuzu while 26.5% was recorded for Ihube. These values contrasted with specie abundance of 6.1%, 7.0% and 13.2% recorded for Umuoba, Amuzu and Ihube respectively, in the dry season. This result suggests that Ihube community remained relatively a favourable habitat for prevalence of sandflies, as it recorded greater abundance during the rainy season (Fig 4). This can be attributed to the optimal environmental conditions occasioned by raining season and perceived long-term activities of herders whose cows' droppings and slaughter sites allow sandflies prevalence in the area. Farmlands at Umuoba also recorded substantial sandflies abundance due to the close proximity of the sampled sites to waterways, which provided favourable humid breeding sites for replication (Usman *et al.*, 2020) [19]. However, the dry season observed unfavourable for sandflies to thrive around Ihube farmlands (Fig 5) due to the very hot weather that suggests high temperature and reduced humidity, creating conditions that are less favourable in sustaining their breeding sites (Kasap and Alten 2013; Ikpeama *et al.*, 2018)

[13, 11].

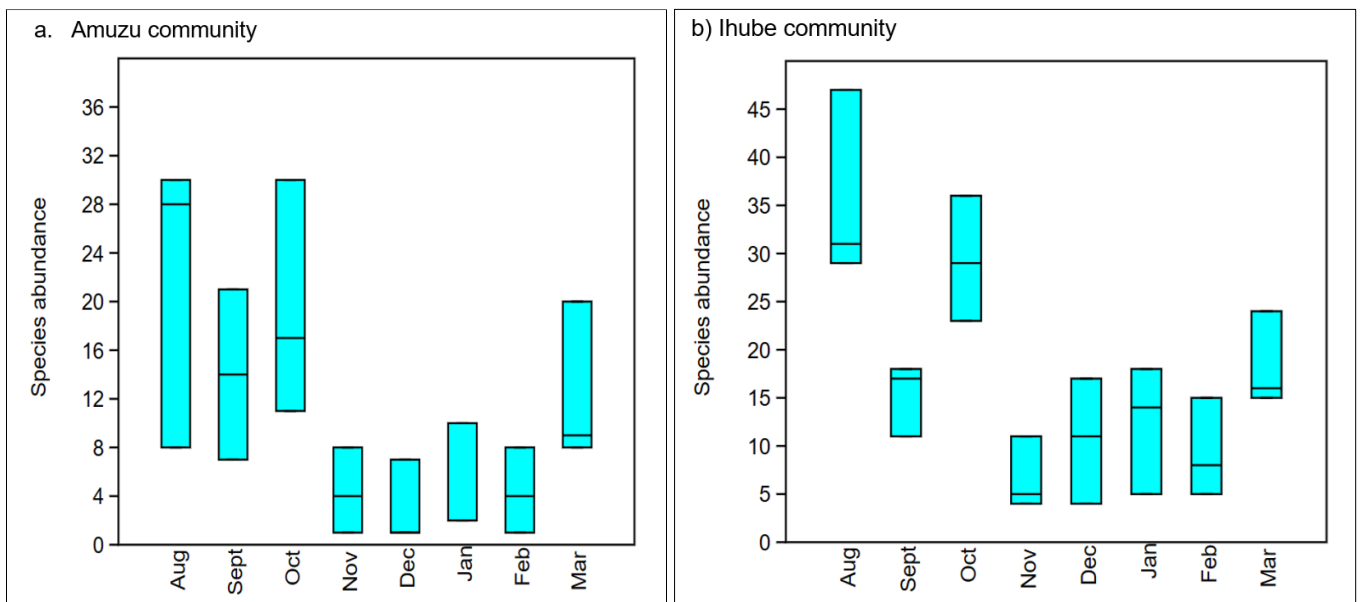
3.4 Estimated monthly biting activities of sandfly across the study locations

The presented data in Fig 3 shows hourly biting the activities of sandflies at various months across studied location. During this period, a total of 525 sandflies were captured, providing valuable insights into the seasonal dynamics of their activity. The months of August, September and October had peak values of 79, 70 and 40 for Amuzu, Ihube and Umuoba respectively compared to February and March with values of 20, 2 and 30 for Amuzu, Ihube and Umuoba respectively. However, November and January practically did not experience any viable bite as illustrated in Fig 3. The observed prevalence during the raining season aligns with expectations, given the well established link between sandfly abundance and climatic conditions favouring their proliferation. The higher prevalence in the raining season is consistent with the notion that sandflies thrive in environment characterized by increased humidity and suitable breeding conditions. The implication of this provides insight in assessing the potential risk of disease transmission in the studied area.

Table 1: Soil variables of selected land use types in three communities of Southeast

Variables	Ihube			Amuzu			Umuoba		
	POM	LSH	FLA	POM	LSH	FLA	POM	LSH	FLA
pH (H2O)	5.82±0.23	5.61±0.21	4.91±0.15	5.8±0.11	5.62±0.22	4.92±0.14	5.64±0.22	5.2±0.13	4.3±0.18
Nitrate (mg/kg)	23.6±0.41	18.8±0.34	15.9±0.30	22.9±0.25	18.5±0.29	16±0.23	22.2±0.37	18.1±0.21	16.1±0.28
Organic matter (mg/kg)	2.11±1.23	1.7±1.28	1.52±1.52	2.1±1.32	1.8±1.29	1.5±1.21	2±1.29	1.9±1.23	1.49±1.36
Phosphorus (mg/kg)	19.89±0.20	16.44±0.19	13.67±0.21	18.5±0.38	16.2±0.26	14±0.26	17.11±0.29	15.96±0.30	14.34±0.29
Organic carbon (mg/kg)	1.22±1.34	0.99±1.29	0.88±1.40	1.3±0.99	0.85±0.50	0.89±0.42	1.38±0.23	0.99±0.52	0.87±0.43
Sulphate (mg/kg)	189.73±2.34	78.45±2.18	92.4±2.62	190±2.26	78.2±2.52	91.3±2.42	188.6±2.34	77.95±2.29	90.2±2.32
Moisture cont (%)	3.2±0.56	3.4 ±0.46	3±0.52	4±0.71	4±0.51	3.8±0.40	2.4±0.48	2.8±0.40	2.2±0.41

Data were reported as means±standard errors.



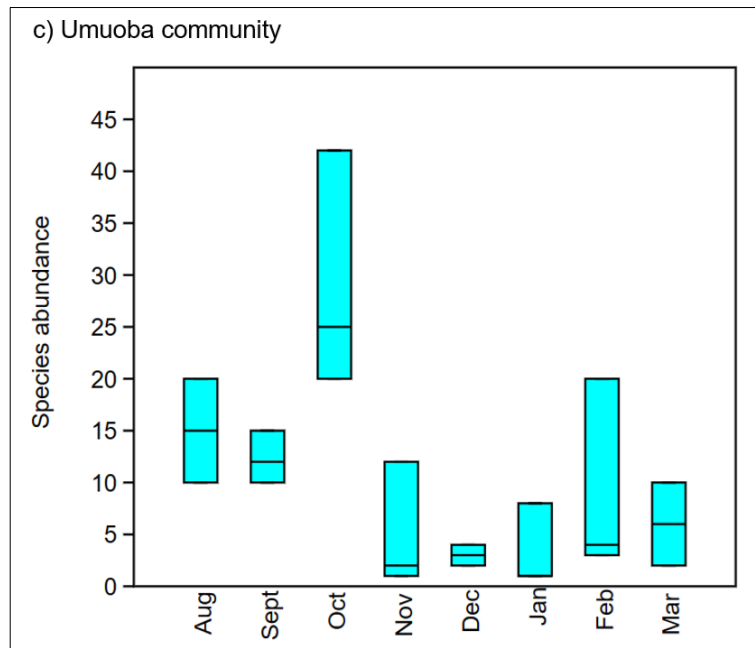


Fig 2 a, b, & c: Temporal specie (sandflies) abundance across Amuzu, Ihube and Umuoba communities

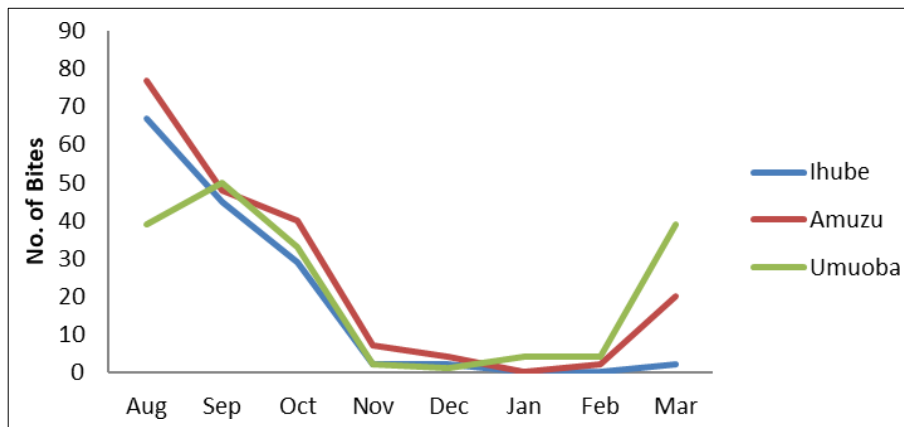


Fig 3: Average Monthly Biting Rate (MBR) across Ihube, Amuzu and Umuoba

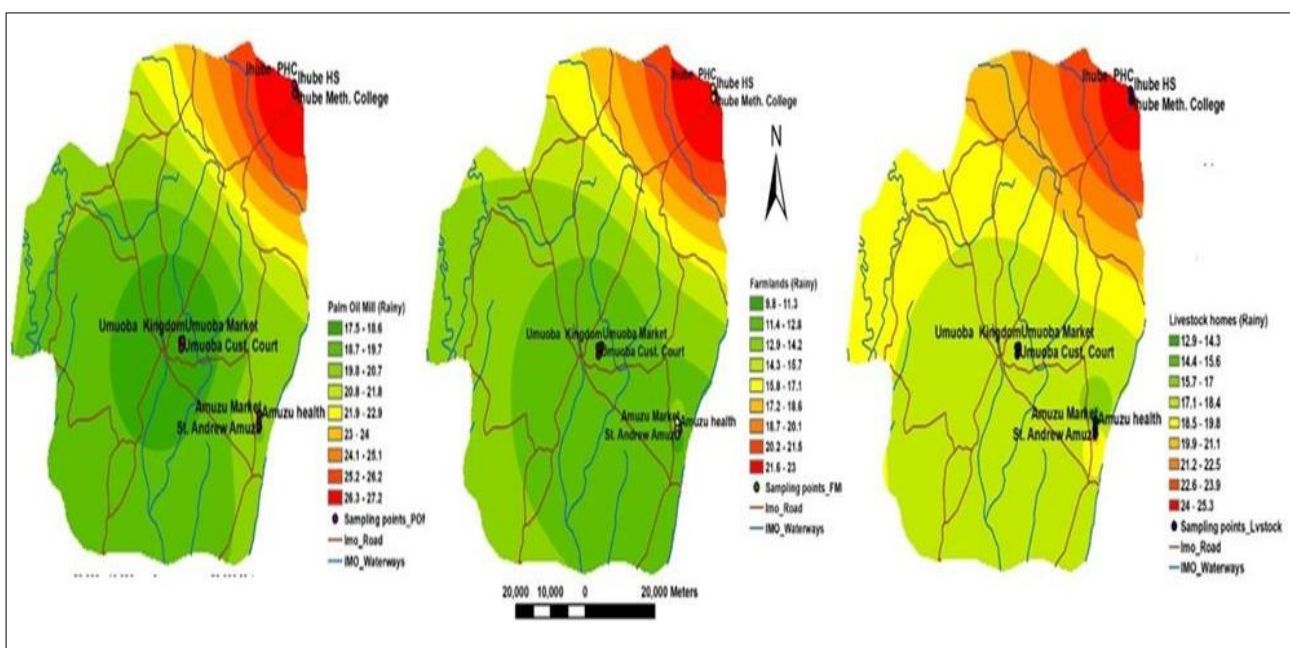


Fig 4: Average specie (sandflies) abundance of different land use types in the rainy season across the sampling locations

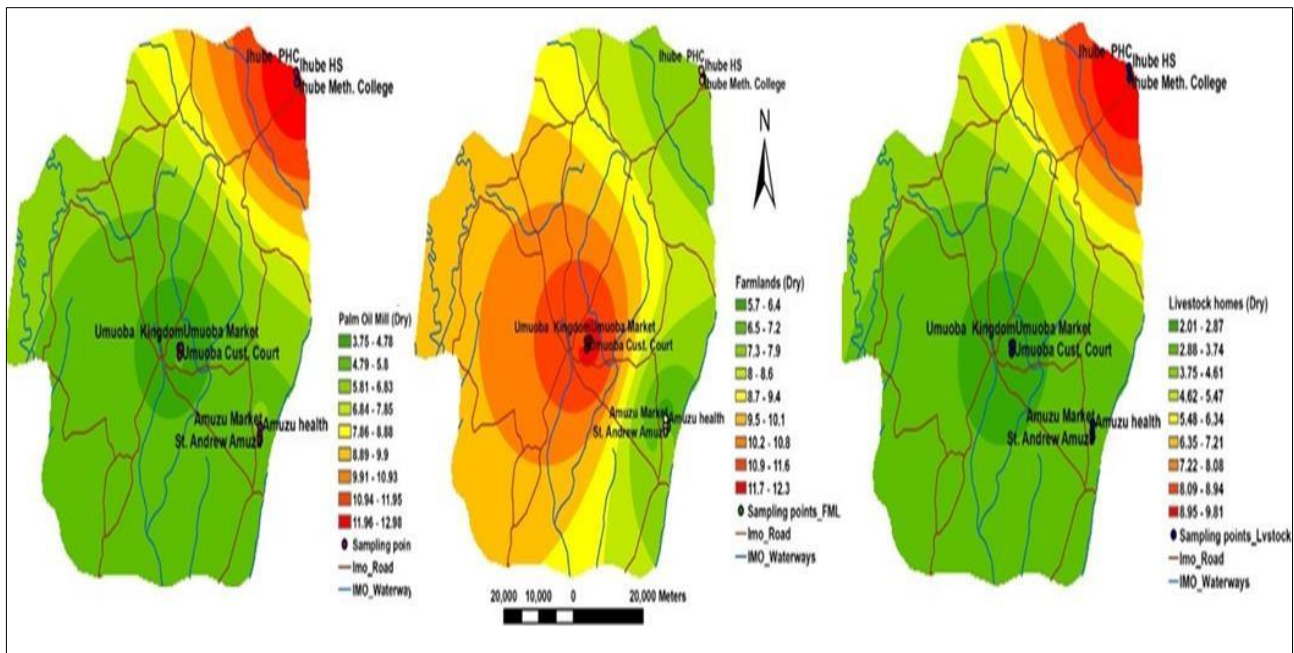


Fig 5: Average specie (sandflies) abundance of different land use types in the dry season across the sampling locations

4. Conclusion

The study has established higher prevalence of sandfly in the raining season occasioned by increased humidity and suitable breeding conditions. Therefore, the observed seasonal pattern in sandfly biting activities emphasizes the need for comprehensive strategies to manage and mitigate the potential impact on disease transmission. The study contributes valuable data to the broader understanding of the ecological dynamics of sandflies and provides a foundation for evidence-base public health intervention in the studied communities.

5. Conflict of interest

The authors declared that there is no conflict of interest regarding the publication of this manuscript

6. References

1. Abolfazi M, Salman K, Hamidreza H, Azadeh A, Shahram A, Abedin S, *et al.* An investigation of the effects of environmental factors on cutaneous leishmaniasis in the old world: A systemic review study. *Journal of Environmental Health.* 2021;36(1):117-128.
2. Aklilu E. *Phlebotomine Sandflies* (Diptera: Psychodidae) of Ethiopia. 2022.
3. Chafika F, El Balchir A, El Rhazi M, Btissan A. Distribution and bionomics of sandflies in five ecologically different cutaneous foci in Morocco. *Epidemiology.* 2015;13:1-7.
4. Faraj C, Adlaoui EB, Quahabi S, Elkohli M, Elrhazi M, Lakraa L, *et al.* Distribution and Bionomic of sandflies in fire. Ecologically different cutaneous leishmaniasis foci in Morocco; Ramon JM, Szmargd C, editors. Hindawi Publishing Corporation: Cairo, Egypt; c2016. p. 145031.
5. Talbi FZ, Fouad El-Akhal AM, Alami A, El Omari H, Najy M, Amaiaich R, *et al.* Influence of basic environmental factors on seasonal variation and

- distribution of sandflies at Ben Slimane sites in Fez city, Morocco; c2022.
6. Federal Ministry of Health Nigeria. Master Plan for Neglected Tropical Disease (NTDs). Abuja: Federal Ministry of Health. 2013-2017;142Ministry.
7. Galati AB, Galvis-Ovallos F, editors. Gaps in *Phlebotomine* distribution and their taxonomic implications. 10th International Symposium on Phlebotomine Sandflies; c2018.
8. Gonzalez MJ, Hernandez S, Martin-Martin I, Molina R. Phlebotomine sandfly survey in the focus of leishmaniasis in *infantum* infection rates and blood meal preferences; c2017.
9. El Omari H, Chahlaoui A, Talbi FZ, El Moulidi K, El Ouali Lalami A; c2020.
10. Inceboz T. Epidemiology and ecology of leishmaniasis in neglected tropical diseases. *Intechopen.* 2019;7(2):34-38.
11. Ikpeama CA, Obiajuru IOC. Bionomics of sandflies (Diptera: Psychodidae) in some remote communities in Ezinihitte Mbaise, South-Eastern Nigeria. *Biological Sciences.* 2018;2:19-28.
12. Ikpeama CA, Nwoke BEB, Anosike JC. Studies on the ecology and distribution of blackflies (Diptera: *Simuliidae*) in Imo State, Nigeria; c2006.
13. Kasap OE, Votycka J, Alten B. The distribution of the *Phlebotomus major* complex (Diptera: *Psychodidae*) in Turkey. *Acta Trop.* 2013 Sep;127(3):204-211.
14. Erguler K, Poniiki I, Lelieved J. A climate-driven and field data assimilated population dynamics model of sandflies; c2019.
15. Vivero RJ, Torres-Gutierrez C, Bajarano EE, Cadena Pena H, Estrada LG, Florez F, *et al.* Study on natural breeding sites of sandflies (Diptera: Phlebotominae) in areas of leishmania transmission in Colombia.
16. Ready PD. Biology of phlebotomine sand flies as vectors of disease agents. *Annual Review of Entomology.* 2013;58:227-250.

17. Seid A, Gadisa E, Tsegaw T, Abera A, Teshome A, Mulugeta A. Risk map for cutaneous leishmaniasis in Ethiopia based on environmental factors as revealed by geographical information systems and statistics. *Geospatial Health*. 2014;8:377-387.
18. Ullah KNH, Khan AIN, Minir SA, Wahid S. Assessing incidence patterns and risk factors for cutaneous leishmaniasis in Peshawar region, Pakistan. *Pakistan Journal of Parasitology*. 2016;102:501-506.
19. Usman M, Natala AJ, Jatau ID, Ogo NI, Balogun EO, Lawal MD, Mahmuda A. *Nigerian Journal of Parasitology*. 2020;41(1):109-113.
20. World Health Organization (WHO). WHO bi-regional consultation on the status of implementation of leishmaniasis control strategies and epidemiological in East Africa. Addis Ababa, Ethiopia; c2018 Sep 9-11.
21. World Health Organization (WHO). Leishmaniasis: Available at <http://www.who.int/news.room/fact.sheets>. (Accessed 18 September, 2022).
22. World Health Organization. Global hepatitis report 2017. World Health Organization; c2017.
23. World Health Organization. WHO report on surveillance of antibiotic consumption: 2016-2018 early implementation.