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Original Research Article

Distribution and Abundance of Sand Flies in Five Selected Communities in Fez, Morocco, and Impact of Climatic Factors

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ABSTRACT

Sand flies have been identified as mechanical vectors of the protozoan leishmania, which causes the parasitic disease leishmaniasis. The disease has become a major public health issue and is directly related to the state of the environment. The paucity of information on the sand fly inventory is limiting the aspects of the vectors' epidemiology. This study was therefore conducted to create a taxonomic inventory of sand flies from April to September 2014 and to investigate the effect of climatic factors on their distribution. Sand flies were trapped in the study area, Fez in Morocco, using sticky traps saturated with castor oil for morphological assessment and relative abundance. The trapped sand flies were identified under the microscope using the determination key established by the guide of the Moroccan Ministry of Health. The obtained data were analyzed using the Principal Component Analysis (PCA) method with the XLSTAT software. The results showed that *Phlebotomusperniciosus* was the most prevalent species (57.87%) among the 216 sand fly samples. *Phlebotomus sergenti* (22.22%), *P. papatasi* (14.82%), *and P. longicuspis* (5.09%) followed. Monitoring seasonal changes in sand fly species revealed a bimodal cycle with differing abundance peaks from one species to the next. A positive correlation was observed between temperature and the four species of *P. perniciosus*, *P. sergenti*, *P. papatasi*, and *P. longicuspis*. The findings of this study demonstrate that tolerance to the environmental parameters evaluated varies depending on the species. Monitoring the distribution of the sand flies will promote location-specific control campaigns against the vectors.

***Keywords*:** Abundance, Distribution, Leishmaniasis, *Phlebotomus* species, Sand fly.

Introduction

Sand flies are members of the phylum Arthropoda, class Insects, order Diptera, suborder Nematocera, family Psychodidae, and subfamily Phlebotominae.[1] The only proven vectors of leishmaniasis are species of the genus *Phlebotomus* in the Old World and of the genus *Lutzomyia* in the New World, although there are sand flies of other genera that bite humans.[2]Only 93 of the approximately 800 species of sand flies are proven or probable vectors of leishmaniasis. There is no clear experimental evidence that leishmaniasis is spread via the bite of hematophagous invertebrates other than sand flies (e.g., fleas or ticks).[3]

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Sand flies are primarily nocturnal and twilight active. However, they are extremely sensitive to air currents and will only emerge when the evening is calm. Their nocturnal activity is explained by the requirement for humidity and a favorable temperature. They hide in dark and secluded places throughout the day. In tropical regions, they are active all year round, but not in temperate zones, despite the recently proven Mediterranean activity.[3] The active period of adult sand flies varies with climate,[4] depending on latitude, altitude, season, and species.[5] Summer is the most active season in temperate zones. Sand flies begin to appear in May and disappear in the fall. Stormy evenings in the spring and summer in the Mediterranean basin are favorable conditions for the mass emergence of sand flies.[3] In general, the distribution of sand flies is not permanent, and climate change could modify their distribution by modifying the spread of fauna and flora.[6] The work of Kahime et al. in 2015,[7] on the relationship between sand fly species abundance and environmental parameters, such as elevation, climate, and soil texture, revealed that these factors play a significant effect on their distribution and abundance.Leishmaniasis is a parasitic disease caused by parasites of the genus *Leishmania* and transmitted by sand flies (Diptera and Psychodidae). They are emerging diseases and are directly linked to the state of the environment.[1] More so, they are represented by various clinical manifestations: anthroponotic leishmaniasis caused by *L. tropica*, zoonotic leishmaniasis caused by *L. major*, and *L. infantum*.[2] Leishmaniasis is endemic in 88 countries throughout Latin America, Africa, Asia, and Southern Europe. Almost 350 million individuals have been exposed to the risk of infestation, with a prevalence of 12 million individuals per year.[3] In Morocco, as in many Mediterranean countries, leishmaniasis is endemic and has become a major public health problem. The emergence of new leishmanial foci and the re-emergence of others is ongoing.[8] This spread could be attributed to climatic change and the geographical expansion of sand fly vectors. The Ministry of Health reported 8,846 cases of leishmaniasis in 2010, of which 8,707 were Cutaneous Leishmaniasis (CL) and 139 were Visceral Leishmaniasis (VL).[3] In 2012, 1,334 cases of leishmaniasis were recorded in Morocco during the first quarter alone.[9] Every year, there is usually the appearance of new epidemics, extending the impact of the disease. In 2011, and 2012, the Ministry of Health documented 4,426 and 2,900 cases, respectively, with 4.92 and 7.19% in the Fez-Boulmane region. At the provincial level of Moulay Yacoub, an epidemic of *L. tropica* was triggered, giving the first cases of CL with 1,600 cases.[8,10] From an epidemiological point of view, a retrospective study was carried out at the Regional Laboratory of Epidemiological Diagnosis and Environmental Hygiene in Fez during the period from 2000 to 2008. It was reported that there were 636 positive cases of CL in the city of Fez.[11] Most of the cases are from peri-urban settings, which are characterized by poor hygiene conditions, the presence of caves, and old walls that form biotopes with leishmanial risk. Furthermore, human-animal cohabitation and climate warming, particularly in recent years, are variables that support the biological development of sand flies. Except for a preliminary survey conducted in 2008 at two sites in Hafat Moulay Driss and Benslimane, in the prefecture of Fez,[11] no other study has been conducted on the inventory of sand fly vectors of leishmaniasis in the city of Fez. The present research was aimed at making a taxonomic inventory of the phlebotomine population and investigating the effect of climatic factors on their distribution.

Materials and Methods

Study area

The survey was conducted in Fez, which is located in north-central Morocco at the foot of the Pre-Rif Mountains, partly incorporating the Saïs plain and bordering the middle Atlas Mountain range. Twelve sampling locations were chosen from five communities: Ouled Tayeb, Zouagha, Ain Nokbi, Skhinate, and Ain Kadous, as depicted in Figure.1.

Sampling and identification of specimens

Sand flies were trapped using adhesive traps made from plain sheets of paper measuring 21 cm by 29.7 cm, which were saturated with castor oil. The traps were hung upright on suspended strings from dusk to dawn (i.e., from 6.00 pm to 8.00 am the following day) in the various sampling sites, namely caves, stables, and ruined houses (Figure 2). All captured sand flies were placed in properly labeled Eppendorf tubes and preserved in 75% ethanol for further assessment. The identification of species was done under the microscope using the determination key established by the guide of the Ministry of Health.[3,12] Males were identified based on the cibarial or pharyngeal armature, the shape of the penile valves, the number and position of spines on the genitalia, and the number of apical setae on the coxite. The anatomy of the spermathecae, the pharyngeal structure, and the morphology of the cibarium teeth were used to identify the females.

Statistical analysis

The data were analyzed using the Principal Component Analysis (PCA) method to determine correlations between environmental factors and species distribution and abundance, as well as sites of similarity. The PCA was performed with the XLSTAT software.

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Figure 1: Situation map of the study area in Fez, Morocco.

Results and Discussion

The specific distribution of collected sand flies

A total of 216 sand flies were captured in the city of Fez from April to September 2014, with a sex ratio of 1.7 (136/80). Four species of *Phlebotomus* flies were collected. The most dominant species was *P. perniciosus* (57.8%), followed by *P. sergenti* (22.22%) and *P. papatasi* (14.82%). *Phlebotomus longicuspis* was represented with a low abundance of 5.09% (Table 1). In 2014, an entomological investigation in Fez indicated the presence of four species of *Phlebotomus* genus sand flies at the level of five surveyed foci (Skhinate, Ain Kadous, Ouled Tayeb, Zouagha, and Ain Nokbi). A total of 213 sand flies were captured using adhesive traps. Among the four species collected, two species predominate in terms of relative abundance. These are *P. perniciosus* (57.87%) and *P. sergenti* (22.22%), followed by *P. papatasi* (14.82%) and *P. longicuspis* (5.09%). *Phlebotomus sergenti* has been identified as a vector of *L. tropica* in North African countries and in central Asia and even Morocco.[13-15]*Phlebotomusperniciosus* is considered to be one of the species capable of transmitting *L. infantum* in Mediterranean foci.[16] The difference in the number of species might be linked mostly to the bioclimate, which has already been addressed by several authors,[17] on the one hand, and to abiotic factors such as hygiene, soil nature, vegetation, andhabitat.[18-22]

Spatial distribution of collected sand flies

During this study, the sand flies were captured at five foci with varying proportions: 41% were collected in Skhinate, 26% in Ain Kadous, 24% in Ouled Tayeb, 6% in Ain Nokbi, and 3% in Zouagha. Sand flies predominated at the level of health clinics in rural Skhinate and Ouled Tayeb. They are distinguished by the presence of manure stables and various types of waste. The health center of Ain Kadous is characterized by the presence of the focus of "Hafa Moulay Driss," which is a garbage dump with caves typical for the proliferation of sand flies (Table 2). The different foci selected for this study in the city of Fez are characterized by a semi-arid Mediterranean climate and very favorable conditions for the proliferation of sand flies. This explains the presence of four species in high numbers among the five implicated in the transmission of leishmaniasis in Morocco.

Seasonal fluctuations of sand flies and the effect of climatic conditions

The activity of sand flies is influenced by environmental factors, particularly climatic factors.In a study evaluating the effect of temperature on sand fly density, the optimal temperature for sand fly activity for the genus *Phlebotomus* was found to be between 19 and 34°C. Humidity was another factor investigated. The distribution of sand flies in response to humidity variations revealed that the optimal range was between 65 and 80%. These two parameters had an impact on the distribution of sand flies captured in the city of Fez. After the indicated periods, the activity of sand flies decreased significantly (Figure 2). Monitoring of seasonal fluctuations and abundance of sand fly species study locations from April to September 2014 revealed that all species of the genus *Phlebotomus* were observed from May and remained present until September, except *P. longicuspis*, which began to surface only from July to September. The seasonal fluctuations of captured sand flies showed a bimodal cycle with different peaks of abundance from one species to another. Thus, *P. perniciosus* was found to be the most abundant with two peaks, the first was recorded in August (32°C, 70%) and the second was recorded in September (30°C, 70%). *Phlebotomussergenti*, which ranked second in abundance, had the first high in May (28°C, 50%) but the second peak in July (32°C, 70%). On the other hand, *P. papatasi* was present with a diphasic evolution in May (28°C, 75%) and July. In August (34°C, 65%), *P. longicuspis* was abundant.To determine the number of components to retain, Kaiser's criterion was adopted, which indicated that in a standardized PCA, the components whose eigenvalues are greater than 1 be retained. The table of explained variability shows that the first two components have eigenvalues greater than 1 and explain the variability of 81.81%. Therefore, these first two components were considered. Table 2 also displays the percentages of variability explained by each component and the cumulative percentages.

Table 1: Number of specimens and relative abundance of collected sand fly species

Species	Male	Female	Total	Ratio sex	Relative Abundance
P. perniciosus	80	45	125	1.77	57.87
P. sergenti	29	19	48	1.52	22.22
P. papatasi	18	14	32	1.28	14.82
P. longicuspis	9	2	11	4.5	5.09
Total	136	80	216	1.7	100

Table 2: Spatial distribution of sand flies

Site	Nature of biotope	Percentage of sand flies
Skhinate	Presence of manure stables and waste of any kind	41
Ain Kadous	Garbage dump with caves	26
Ouled Tayeb	Presence of manure stables and waste of any kind	24
Ain Nokbi	Ruined house, Waste of all kinds	6
Zouagha	Manure, waste of all kinds	3

Table 3: Contribution of component number of the total variance according to statistical analysis

Component number	Eigenvalue	Variance (%)	Cumulative %
1	4.49818	64.260	64.260
2	1.22885	17.555	81.815

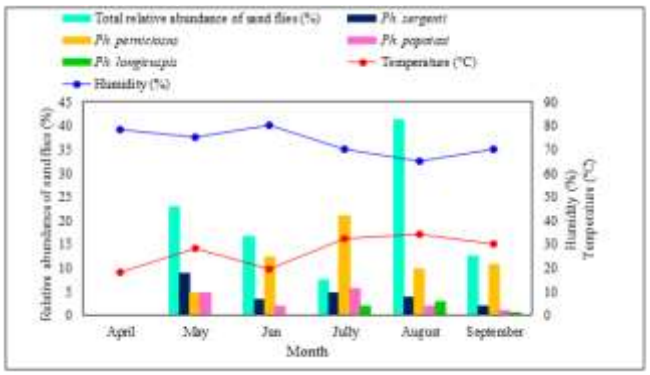

Figure 2: Representation of the relative abundance of sand flies and the monthly variation of temperature and humidity in the city of Fez, Morocco from April to September 2014.

The graphical representation of these results is shown in the eigenvalue graph (Figure 3). The parameter graph indicates the presence of certain correlations between the climatic parameters and species of sand flies. A positive correlation between the temperature and the four species (*P. perniciosus*, *P. sergenti*, *P. papatasi*, and *P. longicuspis*) was observed. In Figure 4, the abundance of species relative to the months of sampling is presented. From December through April, the absence of all species was marked by humidity. *Phlebotomus perniciosus*, *P. papatasi*, and *P. longicuspis* were most abundant in August, but *P. sergenti* peaked in July. According to the literature, sand flies are scarce

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in countries with a tropical arid desert environment and a very long and severe dry season.

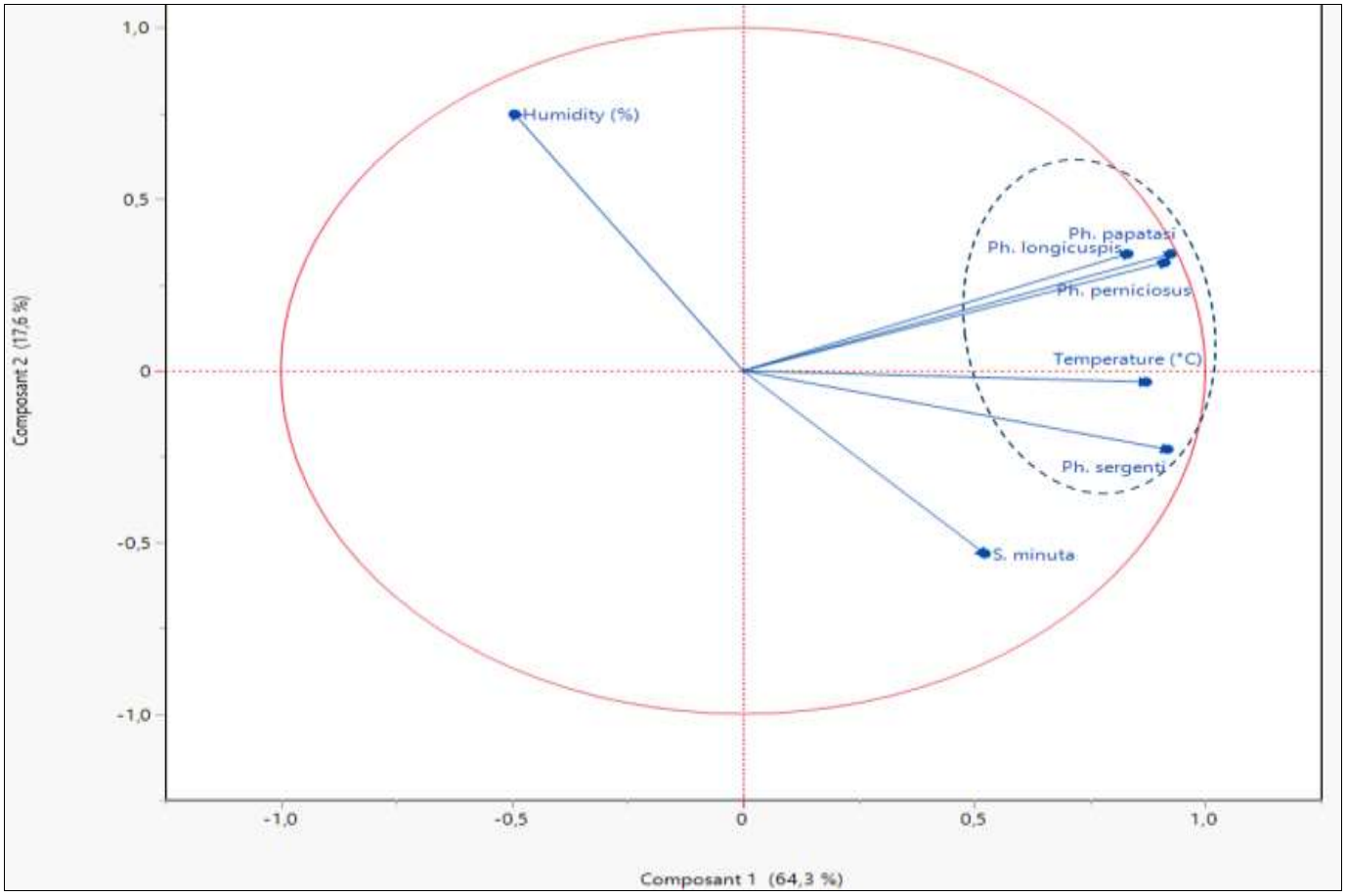

Figure 3: Representation of variables on the factorial plane

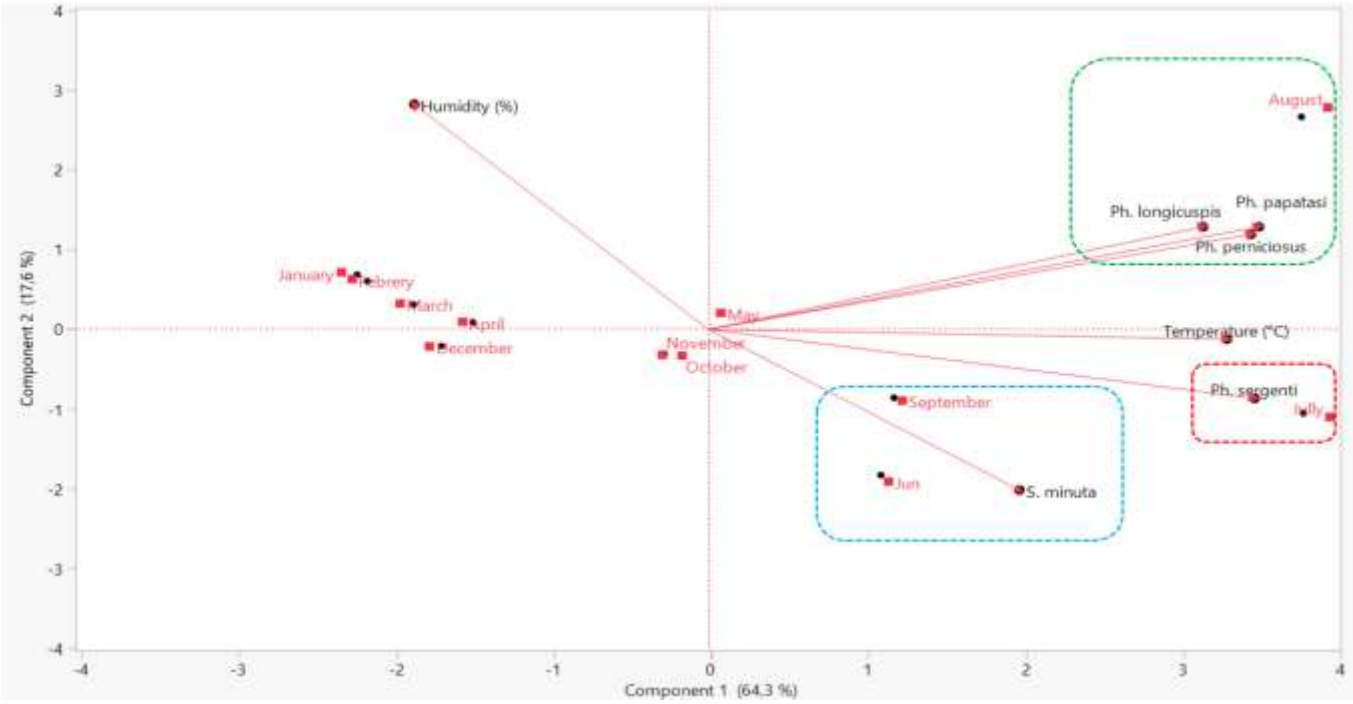

Figure 4: Representation of the variables on the factorial planes F1 and F2.

However, they are abundant throughout the year in countries with an equatorial climate with high humidity and almost constant temperatures.[5] Sand flies are distributed according to their bioclimatic affinity,[23] and life cycle. The length of the activity period and the density of these insects are largely regulated by climatic conditions,[24] yet it appears that the dynamics and activity period can fluctuate even within the same environment. Thus, in Chichaoua, Southern Morocco, with an arid climate, the activity period is short, lasting from June to November.[25] However, in Marrakech, with the same sort of climate, sand flies were active throughout the year.[26]

According to the results of the sampling carried out in different sites of Fez, the number of sand fly specimens varies depending on the month of capture. According to numerous experts, the period from May to November is characterized by the highest activity of sand flies.[17,26,27] In general, it was observed that sand fly activity begins in May and decreases significantly in September. The seasonal fluctuation of sand flies in the present study area is characterized by two peaks, the first in May with 22.68% and the second in August with 41.23%. From May to October, phlebotomine fauna activity appears in the same region of Fez Meknes, in the Aichoun locality. Sand flies were particularly active between May and October. In June and August, there were two activity peaks.[18] Generally, the distribution of sand flies is influenced by local environmental factors, such as rainfall and temperature, and biotic factors (dynamics and abundance of vertebrate hosts).[28-30] A correlation between the relative abundance of sand flies and temperature change linked to humidity at the city level of Fez was determined. Fez has a high prevalence, with an average temperature of 34°C and 65% humidity. A temperature of 18°C and a humidity of 78%, on the other hand, are limiting conditions for the evolution of sand flies. The low prevalence observed in July, even if the climatic factors are optimal, may be due to other factors (such as wind speed or the degree of environmental health during this period). Except for *P. longicuspis*, three species of the *Phlebotomus* genus exhibit biphasic variation in Fez city from May to September. *Phlebotomusperniciosus* was discovered to be the most numerous with biphasic fluctuation, with the first data collected in August (32°C, 70%) and the second in September (30°C, 70%). *Phlebotomus sergenti*, which ranked second in abundance, had the first high in May (28°C, 50%) but the second peak in July (32°C, 70%). In May (28°C, 75%), and July, *P. papatasi* was present with a diphasic evolution. *Phlebotomus longicuspis* was abundant in August (34°C, 65% humidity). A statistical investigation revealed a link between the temperature factor and the distribution of sand flies. The temperature has a positive correlation with the four species (*P. perniciosus, P. sergenti, P. papatasi, and P. longicuspis*). In the same region of Fez Meknes (ex. Fez-Boulmane), particularly in Moulay Yakoub province,[31,] and Sefrou Province,[32,33] temperature and humidity are the main parameters of the biological evolution of the disease. The phlebotomine density in Ouled Aid reaches its maximum when the temperature is between 30 and 35°C.[29]

According to the literature, male sand flies are attracted to vegetation as a source of sweet meals. Plants also act as suitable resting, breeding, and mating sites.[34] The combined effect of several environmental factors determines the distribution of vectors. In 2015, Kahime et al.,[7] demonstrated the role of soil texture as a factor influencing sand fly distribution. Sand flies are scarce or even non-existent in silty or clay-clay-silty soils, but they are abundant in sandy-textured sites. These results could be explained by the adaptation of the population to the climate and the high phlebotomine density, which depends on the climatic conditions of the study environment. Thus, any ecological studies that focus on mapping vector species should consider these variables as important parameters in their studies.

Conclusion

The findings of the present study are useful to better understand the impact of environmental factors on the dynamics of sand flies during their period of activity. Temperature and humidity, as meteorological parameters, have a limiting effect beyond certain values. The entomo-environmental study is required for the development of a control program to direct activities and evaluate its efficacy in preventing the risks of leishmaniasis.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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