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Original Research Article

Spatiotemporal Dynamics of *Phlebotomus perniciosus* (Diptera: Psychodidae) and Characterization of its Habitats Using Satellite Images in Fez City, North Central Morocco

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ABSTRACT

Leishmaniasis is a serious public and veterinary health concern. Species of the subgenus *Larroussius* are the suspected and proven vectors of the leishmaniasis visceral form. This study was aimed at investigating the spatiotemporal dynamics of *Phlebotomus perniciosus* (Diptera: Psychodidae) and characterizing its habitats using satellite images. The entomological sampling was based on a preliminary stratification of the study area, in which the sandflies were collected at 12 selected stations in Fez City using sticky paper traps. The environmental factors studied for each station were land surface temperature (LST), normalized difference vegetation index (NDVI), and soil moisture index (SMI). Correlations between the number of species of sandflies and each of the environmental factors were evaluated using Pearson's correlation. Differences were considered significant at $p < 0.05$ (95% confidence level). Two species of the subgenus *Larroussius*, which are vectors of visceral leishmaniasis, *Ph. perniciosus* (96.54%) and *Ph. longicuspis* (3.46%), were found in the collected 231 specimens. Statistical analysis of the environmental factors also demonstrated a significant positive correlation between the number of *Ph. perniciosus* and LST in all environments. However, the correlation between the isolated species and NDVI was not strong and only significant in the urban area ($r = 0.79$, $p = 0.0018$), and no significant correlation was found with the SMI. The findings of this study could help health authorities develop appropriate future vector control strategies.

Keywords: Sandfly, Visceral leishmaniasis, *Phlebotomus perniciosus*, Environmental factors, Spatiotemporal dynamics, Fez City, Morocco

Introduction

Phlebotomine sandflies (Diptera: Psychodidae) are hematophagous insects, generally active during the nocturnal and twilight hours.[1] They are often abundant near the human population and domestic animals.[2,3] These insects are the only known natural vectors of leishmaniasis caused by kinetoplast protozoa of the genus *Leishmania*.[4,5] In addition to leishmaniasis, sandflies transmit numerous viruses and pathogenic bacteria to humans and animals.[6,7] Regarding humans, leishmaniasis represents a clinical spectrum ranging from a superficial self-resolving cutaneous lesion to a visceral form that is fatal without treatment, including the highly mutilating cutaneous-mucosal form.[8] Currently, these pathologies remain a significant health problem around the world.[9] The global prevalence of leishmaniasis exceeds 10 million cases, and approximately 350 million people, or 6% of the world's population, are exposed to this infectious disease.[10]

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The World Health Organization (WHO) estimates 30,000 new cases of visceral leishmaniasis (VL) and more than one million cases of cutaneous leishmaniasis (CL) annually. Geographically, these diseases are more widespread, and cases are now reported in areas where the disease was not previously endemic.[9]

As in most Mediterranean countries, leishmaniasis represents a severe public health problem in Morocco.[11] It is the first group of vector-borne diseases, whether they are zoonotic or anthroponotic, visceral or cutaneous.[12] Leishmaniasis is among the infectious diseases that must be reported according to Ministerial Order No. 683-95 of March 31, 1995.[13,14] Zoonotic leishmaniasis, caused by *Leishmania infantum*, is rife throughout the Mediterranean region. Species of the subgenus *Larroussius*, *Phlebotomus (Larroussius) Perniciosus*, and *Phlebotomus (Larroussius) longicuspis*, are the proven and suspected vectors of visceral leishmaniasis.[15] The distribution and transmission of the disease are closely linked with the vector, ecological, and bioclimatic factors.[16,17,18]

These disease vectors and their relationship to environmental factors (such as temperature, humidity, and vegetation cover) must be explored and studied to conduct appropriate monitoring and control strategies. In this context, this research was conducted to investigate the spatiotemporal dynamics of *Phlebotomus perniciosus* (Diptera: Psychodidae) and characterize its habitats using satellite images in the city of Fez.

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Materials and Methods

Study area

The study was conducted in the North-Central of Morocco, in the region of Fez-Meknes. The city of Fez, which has an average altitude of 450 m above sea level, is located at 34° 03′ 00″ latitude and 4° 58′ 59″ longitude, which extends to the North-East end of the Saïs plain within a depression. It is surrounded on the north by the Pre-rifean units, which reach their highest point at 930 m at Jbel Zalagh. Also, Fez is bordered in the south by the tabular Middle Atlas, which peaks at 1,400 m.[19] Morocco is a North African country bordered by the North Atlantic Ocean and the Mediterranean Sea (Figure 1). The local climate of Fez is part of a Mediterranean context. Influenced by the continental climate, the city of Fez is marked by seasonal solid contrasts and apparent irregular rainfall. The average rainfall varies between 500 and 600 mm/year, and the average temperature is 17.8 ºC with a maximum of 35 ºC in July and a minimum of 9 ºC in January.[20,21] The population is approximately 1,146,088 based on the General Census of Population and Housing data.[22]

Fez comprises diverse urban landscapes, including a city center and well-developed neighborhoods with a remarkably high population density. It extends to periurban and rural peri-urban areas, characterized by houses on open terraces. Therefore, this geographical diversity is a reason to investigate further the presence of specific health issues. The city of Fez is well-known for its prevalent sandfly population, and the region hosts several high-risk sites with documented epidemiological data. In particular, caves, rodent burrows, and unmanaged waste sites present potential biotopes for leishmaniasis vectors and reservoirs, so the need for careful surveillance and preventive measures in these varied environments is mandatory to ensure public health safety.[23,24]

Sampling and experimental design

The entomological sampling plan was based on a preliminary stratification of the city of Fez. Before proceeding to the entomological collections, the city was divided into three environmental strata (urban center (Urb), periurban (P_urb), and rural-periurban (Rur)) as depicted in Figure 1. Land use percentages for each entomological sampling site were obtained from the Copernicus Global Land Service (CGLS) global land cover map for 2018.[25] Four macrohabitat environmental variables were defined: high urban cover, low urban cover, soft vegetation cover, and tall vegetation cover. With the complementary use of the Global Positioning System (GPS), field visits were made to validate ambiguous points on the ground and further refine the classification of the land cover map. Within each stratum, four sample sites were selected for a total of 12 collection sites (Figure 2). Table 1 shows the geographic coordinates and elevations of these 12 sampling sites. Concerning land use distribution, Figure 2 shows a predominance of the construction zone, which covers an area of 51.41 km^2 (46.39%), followed by periurban agriculture, with an area of 25.44 km^2 (22.96%).

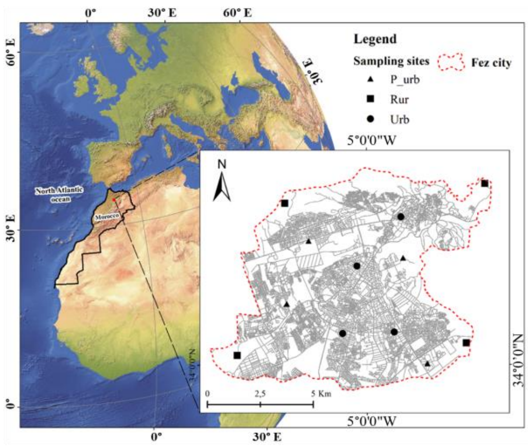

Figure 1: Geographical location of the city of Fez.

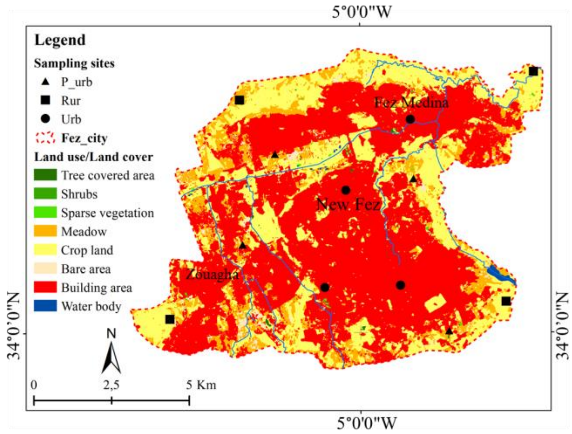

Figure 2: Land use map of the city of Fez (2018).

Table 1: Geographic coordinates and elevations of the 12 sampling sites

Sampling sites	Latitude	Longitude	Altitude (m)
Urb	34.016	-4.992	255
	34.039	-5.003	254
	34.039	-4.971	254
	34.011	-5.051	256
P_urb	34.052	-5.014	230
	34.011	-5.021	245
	34.044	-4.982	250
	33.998	-4.968	254
Rur	34.073	-4.938	210
	34.067	-5.033	232
	34.001	-5.065	255
	34.011	-4.948	252

Urb: Urban; P_urb: Periurban; Rur: Rural-periurban

Collection of sandflies

The adult specimens were collected by adhesive traps (A4: 21x29 cm) impregnated with castor oil, placed in the evening at sunset, and recovered the following day.[26] The traps were grouped in an envelope for each station by specifying the station number, the nature of the biotope, the date of the periodic visits, and the number of papers deposited and recovered. Adult sandflies were captured regularly every 15 days over the course of a year, from May 2018 to April 2019. The following locations were used for captures: animal shelters, house interiors, caves, retaining wall cracks, and rocky anfractuosity.[8]

Identification of sandfly species

The collected sandflies were placed in 70% ethanol. These specimens were then thinned in Mark André medium.[27] Identification was based on the morphology of the pharyngeal armature and spermathecae of female flies and the external genitalia of males,[28,29] using the morphological key of Morocco sandflies.[30] The morphological differentiation of the two sympatric species, *Ph. Perniciosus,* and *Ph. longicuspis,* was made according to the descriptions of Berchi *et al*., and Benallal *et al*.[31,32,33]

Collection of environmental data

Several environmental variables were used to study the spatiotemporal dynamics of potential vectors associated with VL traffic in the city of Fez. Land surface temperature (LST), normalized difference vegetation index (NDVI), and soil moisture index (SMI) were obtained from Landsat 8 OLI satellite images from the High-Resolution Imaging Spectroradiometer (30 m) during the period from April 2018 to May

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2019. These images are available at Earth Explorer (https://earthexplorer.usgs.gov/) of the United States Geological Survey (USGS). Landsat images acquired over Morocco between 10:50 and 11:00 GMT (when the emissivity is at the highest) have been selected for the LST. Furthermore, the best Landsat 8 OLI image was chosen monthly with no cloud cover or other noise. Soil temperature was assessed using a Luster Leaf Rapitest stainless steel dial soil thermometer (model 1625; Lustre Leaf Products, Inc., Woodstock, IL). Similarly, soil moisture content was measured using a Rapitest moisture meter (model 1820; Lustre Leaf Products, Inc.) to validate the LST maps for the affected months. For each measurement, the soil thermometer and soil moisture meter probe were inserted into the soil at a depth of 10 cm. Following the manufacturer's recommendations, temperature and humidity values were recorded after 3 minutes.

Soil thermometer measurements were repeated three consecutive times until a variation of less than 5% between the three straight measurements was obtained, thus enabling consideration of the potential discrepancies in the thermometers. The results were based on the average of three measurements taken once equilibrium had been reached. Concerning soil moisture, the moisture meter was calibrated by comparing its measurements with soil samples, whose moisture content was determined gravimetrically by weighing the soil before and after drying in an oven. *In situ* measurements produced results that were highly correlated with those from distant sensing as observed in Figure 3. The NDVI allows the creation of a visual representation of the relative biomass or vegetation cover. It is based on comparing two features from a set of multi-spectral data matrices: the high reflectivity of the near-infrared (NIR) channel of plant material and the absorption of chlorophyll pigments in the red (R) channel. The following equation was used to calculate the NDVI:

$$NDVI = \frac{NIR-R}{NIR+R} \quad (1)$$

Where R and NIR represent spectral reflectance measurements acquired in the visible red (R) and near-infrared (NIR) bands, respectively. This index varies between -1 and +1, with negative values corresponding to non-vegetated surfaces such as snow, water, or clouds, where the red reflectance (R) is greater than the near-infrared reflectance (NIR).

Land Surface Temperature (LST) calculation

Thermal bands from Landsat images (OLI/TIRS) were used to calculate the LST. Images captured after midnight and early morning, when the most significant emission is observed, were chosen for the LST. The LST was calculated using several factors, including solar emissivity, atmospheric transmittance, and effective atmospheric temperature. Table 2 presents the empirical values of the satellite images used to estimate the LST. The signals were captured by thermal sensors (OLI/TIRS) and converted to radiation at the sensor. Among the different methods of extracting land surface temperature from Landsat images, the technique developed by the Landsat Project Science Office in 2002 has been widely used.[34]

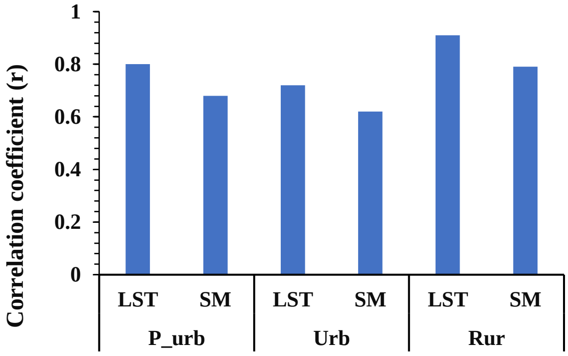

Figure 3: Correlation between the remotely sensed LST and SM with the in-situ LST and SM dataset from April 2018 to March 2019.
LST: Land surface temperature; SM: Soil moisture

Table 2: Empirical constants for Landsat 8 sensors

Constants	Landsat 8 OLI
K_1	774.8853
K_2	1321.0789
L_{max}	22.00180
L_{min}	0.10033
$Qcal_{max}$	65535
$Qcal_{min}$	1

Conversion of digital number (DN) to spectral radiance (all)

Equation (2) was used to calculate the spectral radiance (All).[35]

$$L_\lambda = \left(\frac{L_{\lambda\, max}-L_{\lambda\, min}}{Qcal_{max}-Qcal_{min}}\right) * (Qcal - Qcal_{min}) + L_{\lambda\, min} \quad (2)$$

Where $Qcal_{min} = 0$, $Qcal_{max} = 255$, Qcal is the quantized calibrated pixel value or digital number of each pixel, $L\lambda_{min}$ is the spectral radiance for the thermal band at numerical number 0, $L\lambda_{max}$ is the spectral radiance for the thermal band at numerical number 255.

Conversion of spectral radiance (Lλ) to satellite-level brightness temperatures (TB)

Once the digital numbers (DN) have been converted to reflectance, the spectral luminance of the TIRS sensor thermal bands must be converted to satellite brightness temperature (TB). The resulting equation (3) is as follows:[35]

$$TB = \frac{Kj}{\ln\left(\frac{Ki}{L_\lambda}+1\right)} \quad (3)$$

Where TB is the satellite brightness temperature (K), L_λ is the spectral radiance of the thermal band in $W.m^{-2}.sr^{-1}.mm^{-1}$, Ki and Kj are the calibration constants.

Estimation of land surface temperature (LST)

Estimating the terrestrial emissivity, which modifies the luminosity of the black body (Planck's law), is mandatory before estimating the surface temperature.[36] Therefore, changes in spectral emissivity are required, and these adjustments can be made based on the type of use and extent of ground cover in a particular area.[37] By using the values of the vegetation proportion, it is possible to calculate the emissivity of each pixel.

$$Land\ surface\ emissivity\ (\varepsilon) = 0.004 * Pv + 0.986 \quad (4)$$

Where Pv represents the proportion of vegetation, calculated using the formula below:

$$Pv = \left(\frac{NDVI-NDVI_{min}}{NDVI_{max}-NDVI_{min}}\right)^2 \quad (5)$$

Artis and Carnahan (1982) used the following equation to obtain the emissivity-corrected LST:[38]

$$LST = \frac{TB}{\{1+[(\frac{\lambda}{\rho}) ln\varepsilon]\}} \quad (6)$$

Where LST is the temperature of the earth's surface in Kelvin, k is the wavelength of the emitted radiation in meters, TB is the brightness temperature of the sensor in Kelvin, ρ=h*c/σ, σ is Boltzmann's constant (1.38×10^{-23} J/K), h is Planck's constant (6.626×10^{-34} Js), and c is the speed of light (2.998×10^{8} m/s).

Calculation of the soil moisture index (SMI)

By using the LST, the SMI was calculated according to Moawad (2012) as follows:

$$SMI = \frac{LST-LST_{min}}{LST_{max}-LST_{min}} \quad (7)$$

Where SMI is the soil moisture index, and LST_{max}, and LST_{min}, are the maximum, and a minimum of recovered LST, respectively.[39]

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Statistical analysis

Correlations between the number of *Ph. perniciosus* and each environmental factor were evaluated using Pearson's correlation, which provides information on the strength and direction of the linear relationship between the two variables. This correlation coefficient can have a value between -1 and +1. The larger the absolute value of the coefficient, the better the correlation. Differences were considered significant only if $p < 0.05$ (95% confidence level). The presence of *Ph. longicuspis* recorded in the different sites during this study was low or null; therefore, no analysis was performed for this species.

Results and Discussion

This entomological study in the city of Fez revealed the presence of two species (*Ph. perniciosus* and *Ph. longicuspis*) belonging to the genus *Phlebotomus*, which have been demonstrated to be vectors of visceral leishmaniasis caused by *L. infantum* in Mediterranean foci.[15,40,41] A total of 231 specimens were collected. The analysis of the collected species shows a high abundance of *Ph. perniciosus* (96.54%) with a modest number of *Ph. longicuspis* (3.46%). This was also reported by Talbi *et al.*, who found that *Ph. perniciosus* was the dominant species (79.52%), exceeding the cumulative abundance of the other identified species, in their study about cutaneous leishmaniasis in Aichoun, North-Central Morocco.[42] Similar findings have been reported by Ramdane *et al.*, and Berdjane-Brouk *et al.*, with a significant abundance of *Ph. perniciosus* in Algeria.[43,44] This species has a wide geographical distribution in the Mediterranean basin and North African countries.[45,46] Furthermore, this vector prefers semi-arid and humid bioclimates.[47] However, it has been captured in other bioclimatic zones.[48,49] These characteristics explain its dominance and presence in all the sites surveyed in the city of Fez. On the other hand, *Ph. longicuspis* (3.46%) was poorly represented in this study, which could be explained by the reason that the traps used were not placed in their natural habitat on the one hand and that this species is attached to bioclimatic stages other than those of the present study on the other hand.[43,15]
Previous studies on sandflies conducted in the Fez-Meknes Region revealed the presence of these two vector species in the prefecture of Meknes,[50] the province of Sefrou, and the province of Moulay Yaâcoub.[51,52] Taxonomic studies of sandflies in the city of Fez have revealed the presence of *Ph. perniciosus* and *Ph. longicuspis*.[53,26] This confirms the continuity of the developmental cycle of these vectors. The sex ratio (1.69) showed a dominance of males (62.77%) over females (37.23%). This difference could be due to the means of capture (adhesive traps) and the type of biotope surveyed. These results corroborate findings from other studies.[50,54] Based on the stomach condition of the females captured in all biotopes, non-gorged females had a higher frequency (94.19%) than gorged (2.33%) and gravid (3.49%) females (Table 3).
Geographically, the percentage of sandflies was higher in periurban sites (43.29%), which provides compatible choice habitats with the biological and ecological requirements of this species. On the other hand, it was low in the urban sites (19.48%). This result is in agreement with the one obtained in Spain by Muñoz *et al.*, who found that peri-urban residential estates provided suitable breeding habitats for *Ph. perniciosus*.[55] According to a previous observation, Abdullah *et al.* demonstrated that environmental changes caused by population growth, agricultural intensification, and developmental activities would lead to microhabitat alterations, thus affecting the distribution of sandfly populations.[56] Another study confirmed that sandflies would adapt better to human habitats.[57] This finding also agrees with that of other authors in the Middle East who highlighted the influence of land use on the distribution of two species of sandflies, the *Ph. alexandri* vector of VL, and *Ph. papatasi* vector of CL across the region.[58]
Phlebotomus perniciosus was present from April to November; the highest number was registered in June. This species also had the highest rates of occurrence during June in the periurban stratum (57.69%) and the lowest in the urban stratum (13.46%) as shown in Figure 4. The temperature of the surface of the earth during the study period varied from 4.98 to 41.7°C. The highest LST values were recorded between July and September in peri-urban areas (without vegetation) and urban regions (under construction).
The presence of *Ph. perniciosus* was associated with monthly mean LST values ranging from 16.67 to 23.73°C. According to the literature, climatic factors impact the spatiotemporal distribution of leishmaniasis disease vector species.[10,16,17,52,59,60]

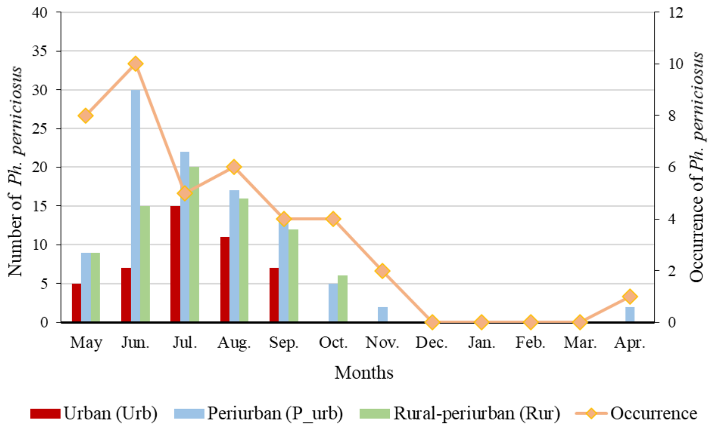

Figure 4: Monthly variations of occurrences and numbers of *Ph. perniciosus* at the level of the city of Fez for the year 2018-2019.

Table 3: Number of sandflies collected in the different sites.

Sampling sites	Species	Male		Female G		NG		Gr		Male: female ratio
		N	%	N	%	N	%	N	%	
Urban center	*Ph. perniciosus*	32	13.85	0	00	13	5.63	0	00	2.46
	Ph. longicuspis	0	00	0	00	0	00	0	00	-
Periurban	*Ph. perniciosus*	62	26.84	1	0.43	35	15.15	2	0.86	1.63
	Ph. longicuspis	0	00	0	00	0	00	0	00	-
Rural-periurban	*Ph. perniciosus*	46	19.91	1	0.43	30	12.99	1	0.43	1.44
	Ph. longicuspis	5	2.16	0	00	3	1.30	0	00	0.79
Total		145	62.77	2	0.86	81	35.07	3	1.30	1.69

Ph: *Phlebotomus*; G: Gorged; NG: Not gorged; Gr: Gravid; N: Number

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Table 4: Pearson correlation analysis of terrestrial variables and *Phlebotomus perniciosus* numbers

Variable	*Phlebotomus perniciosus*					
	Urban		Periurban		Rural-periurban	
	r	*p-value*	r	*p-value*	r	*p-value*
LST	0.6725	0.0166*	0.6597	0.0196*	0.6404	0.0249*
NDVI	0.7998	0.0018*	0.3622	0.2472	-0.3505	0.264
SMI	-0.5053	0.0938	-0.4597	0.1327	-0.2694	0.3972

LST: Land surface temperature; NDVI: Normalized difference vegetation index; SMI: Soil moisture index;
*: Significant correlations at 95% of confidence interval ($p < 0.05$).

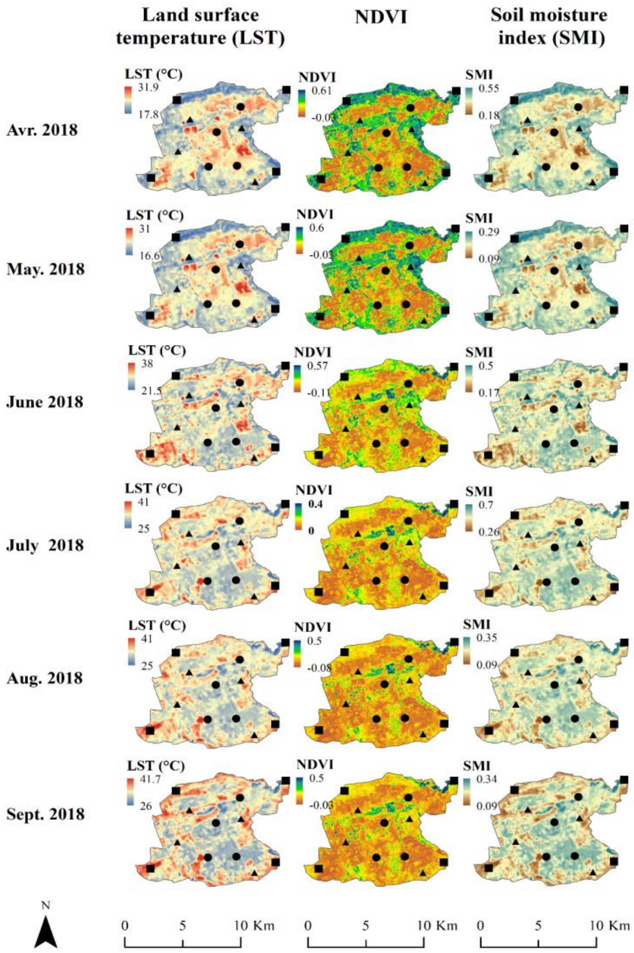

Figure 5: Spatiotemporal evolution of LST, NDVI and SMI in the city of Fez from April 2018 to September 2018.
LST: Land surface temperature; NDVI: Normalized difference vegetation index; SMI: Soil moisture index.

The analysis in the present study indicated that LST was an important factor affecting the distribution of *Ph. perniciosus* in the study area. Kesari *et al*. demonstrated that mean and maximum LST were significantly associated with the density of *Ph. argentipes* vector of kala-azar in their study of Bihar, India.[61] Abdullah *et al*. also found that LST was one of the most critical ecological determinants of disease occurrence.[56]
The lowest NDVI was observed in all dense urban areas. The maximum soil moisture index values were identical in downtown and rural-periurban areas (Figures 5 and 6). Concerning the NDVI, the number of individuals was related to the monthly average values of entomological collection sites between 0.28 and 0.19 and moderate soil moisture index values between 40 and 66%. Furthermore, it should be noted that there was a difference between the number of *Ph. perniciosus* at the different sampling sites regarding the LST, NDVI, and SMI parameters (Figures 5 and 6).

This study also revealed the importance of NDVI in the distribution of *Ph. perniciosus*, especially in urban areas. Bounoua *et al.* demonstrated that greenness, expressed as the NDVI, influences the spatial distribution of sandflies.[62] This was also reported by Boussa *et al*., who found a positive correlation between NDVI with *Ph. sergenti* densities in endemic foci of cutaneous leishmaniasis in Morocco,[63] since male sandflies are attracted to plants as a source of sweet meals. Plants also provide suitable resting, breeding, and mating sites. In the northeastern region of Brazil, NDVI has been reported as an essential risk factor for VL and has always been found to be associated with a high number of sandflies and a high incidence of human and canine VL cases.[64]
The correlations between environmental factors and the number of *Ph. perniciosus* captured in the city of Fez were examined to understand the possible impact of these variables on the dynamics of this vector species in the city of Fez (Table 4). The statistical study showed a significant positive correlation between the number of *Ph. perniciosus* and LST in all environmental strata (urban (r = 0.67, p = 0.0166), periurban (r = 0.65, p = 0.0196) and rural-periurban (r = 0.64, p = 0.0249)). This result indicates that LST directly affects abundance of this vector and spatiotemporal distribution. The correlation of this species with NDVI was strong and significant only in the urban area (r = 0.79, p = 0.0018), which was the lowest regarding vegetation cover. However, a negative and weak correlation was observed in the rural-periurban area. On the other hand, no significant correlation was found with the soil moisture index (SMI) as depicted in Table 4. These results are consistent with those obtained in other studies. Land surface temperature and the normalized difference vegetation index (obtained from satellite data) are essential in the distribution of sandflies, including *Ph. perniciosus*.[65,66] In the study area of the current investigation, SMI was not a determining factor for the presence or distribution of this potential VL vector. Determining LST, NDVI, and SMI varies occasionally depending on land surface materials. However, the increase in urbanization is rapidly changing the characteristics of surface components.[67,68]
The combination of several environmental factors could be involved in determining the distribution of sandflies. The findings of this study revealed the role and interest of some of these variables. They supported the results of previous studies regarding the importance of LST, NDVI, and land cover as crucial factors limiting the distribution of *Ph. perniciosus* in Fez City. Thus, any entomo-ecological study on mapping vector species should consider the variables of these essential parameters.
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Conclusion

The findings of this entomo-environmental study reveal the existence of two species of medical significance, with *Ph. perniciosus*, the main vector of VL. Their spatiotemporal distribution and their ecological requirements were also investigated. Environmental factors (LST and NDVI) and land cover have been found to be essential factors in the distribution of *Ph. perniciosus*. More so, SMI was not a determining factor for the presence and distribution of this vector. The findings of this study could contribute to developing future strategies for monitoring and controlling the vectors of this disease in the city of Fez.

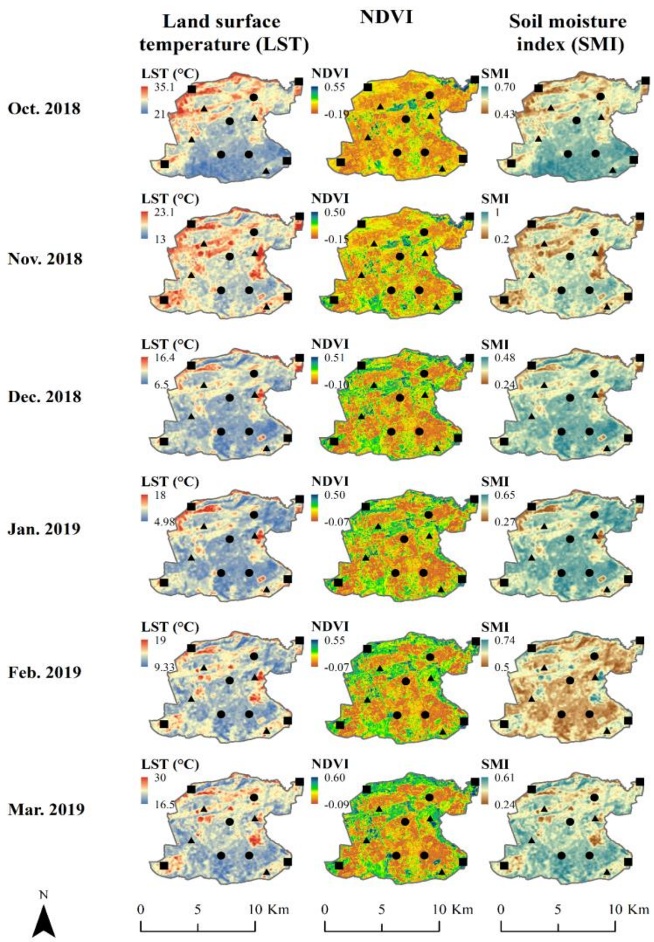

Figure 6: Spatiotemporal evolution of LST, NDVI and SMI at the level of the city of Fez during the period from October 2018 to March 2019.
LST: Land surface temperature; NDVI: Normalized difference vegetation index; SMI: Soil moisture index

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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