Soil analysis of potential breeding sites of sand flies (Diptera: Psychodidae) in Aichoun locality, central Morocco

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Résumé : Analyse du sol des sites potentiels de reproduction des phlébotomes (Diptera: Psychodidae) au niveau de la localité d'Aichoun, centre du Maroc

L'épidémiologie de la leishmaniose est liée à la nature de l'habitat et au comportement du phlébotome vecteur. Chaque espèce (Diptera: Psychodidae) possède des caractéristiques spécifiques de son gîte qui assure le développement des immatures. Compte tenu de la difficulté d'isolement des larves phlébotomiennes, la recherche d'informations sur leurs sites de reproduction est encore limitée. Cette étude, réalisée de mai à septembre 2014, recherchait l'effet des propriétés physico-chimiques du sol sur le comportement et l'abondance des phlébotomes sur leurs sites de reproduction dans la localité d'Achoun. Les phlébotomes ont été collectés à l'aide de papiers adhésifs (21x27,3 cm) enduits d'huile de ricin dans quatre stations. Le nombre total de pièges dans chaque campagne de piégeage était de 32. Des échantillons de sol ont été collectés chaque mois, puis extraits et testés. Les différents paramètres chimiques ont été étudiés : conductivité électrique, humidité équivalente, calcaire total, matières organiques, phosphore assimilable, potassium échangeable, pH, carbone organique. L'analyse statistique a été réalisée par analyse des composantes principales (ACP). Les espèces dominantes pendant cette période étaient *Ph. sergenti* et *Ph. perniciosus*. Au total, 1685 phlébotomes appartenant à cinq espèces des genres *Phlebotomus* et *Sergentomyia* ont été recueillis, à savoir *Ph. sergenti* (84,62 %), *Ph. perniciosus* (8,54 %), *Ph. papatasi* (6,17 %) *Ph. longicuspis* (0,35 %) et *S. minuta* (0,29 %). Les analyses physico-chimiques du sol des sites potentiels de reproduction des phlébotomes sur les 16 échantillons, ont montré une corrélation entre l'abondance des phlébotomes et les paramètres physico-chimiques étudiés. Les résultats obtenus dans ce travail, réalisé pour la première fois au Maroc, devraient représenter une grande contribution au programme de contrôle des phlébotomes vecteurs au niveau local et national et servir d'indicateurs pour déterminer les zones à risque de leishmaniose ainsi que pour mettre en place des stratégies efficaces de contrôle.

Sand flies
Phlebotomus sergenti
Ph. perniciosus
Ph. papatasi
Ph. longicuspis
Sergentomyia minuta
Sticky trap
Breeding preference
Soil
Electrical conductivity
Equivalent humidity
Organic matters
Assimilable phosphorus
Exchangeable potassium
pH
organic carbon
Total calcium
Leishmaniasis
Aichoun locality
Sefrou Province
Morocco
Maghreb
Northern Africa

Abstract :

The epidemiology of leishmaniasis is related with habitat and behavior of the sand fly vector. Each species of sand flies (Diptera: Psychodidae) has a specific characteristic of their sites for the development of their immatures. Information on natural breeding sites of phlebotomine sand flies is limited, due to the difficulty of isolation of immature from the soil. This study, realized from May 2014 to September 2014, aimed to determine the effect of properties of soil on behavior and abundance of sand flies in breeding habitat in Aichoun locality. Sand flies were collected using sticky papers (21 × 27, 3 cm) coated with castor oil in four stations. The total of traps in each trapping campaign is 32. Soil samples were collected each month and they were extracted and tested. The different chemical parameters have been studied: electrical conductivity, equivalent humidity, total of calcareous, organic matter, organic

Phlébotomes
Phlebotomus sergenti
Ph. perniciosus
Ph. papatasi
Ph. longicuspis
Sergentomyia minuta
Piège collant
Préférence de reproduction
Sol

carbon, assimilable phosphorus, exchangeable potassium. Statistical analysis was performed by principal component analysis (PCA). The dominant leishmaniasis vectors in Aichoun are *Ph. sergenti* and *Ph. perniciosus*. A total of 1685 sand flies were collected belonging to five species in the genera Phlebotomus and Sergentomyia, namely *Ph. sergenti* (84.62%). *Ph. perniciosus* (8.54%). *Ph. papatasi* (6.17%) *Ph. longicuspis* (0.35%) and *S. minuta* (0.29%). The chemical tests of potential breeding sites of sand flies, determined for sixteen samples, showed a correlation between the abundance of sand flies (especially the ecological requirements of larval development of the three species incriminated in transmission of the disease of cutaneous leishmaniasis) and the chemical parameters studied. The results found in this work, realized for the first time in Morocco, will be of great contribution to the control program of sand flies vectors at local and national level and as an indicators for the determination of leishmaniasis risk areas as well as to establish effective control strategies.

Conductivité électrique
Humidité équivalente
Matières organiques
Phosphore assimilable
Potassium échangeable
pH
Carbone organique
Calcium total
Leishmaniose
Aichoun
Province de Sefrou
Maroc
Maghreb
Afrique du Nord

Introduction

Cutaneous Leishmaniasis (CL) has become a serious health problem in Morocco during the last decade. The causative agents are Leishmania major, which is transmitted by *Phlebotomus* (*Phlebotomus*) *papatasi* (Scopoli), and Leishmania tropica, which is transmitted by *Phlebotomus* (*Paraphlebotomus*) *sergenti* Parrot [11]. It is endemic in many semi-arid and arid areas, especially in the center and the south of the country. In the center, it is mainly anthroponotic due to *L. tropica* and transmitted by *Ph. sergenti* Parrot [11,20]. Between 2011 and 2012, the Ministry of Health recorded, respectively, 4426 and 2990 cases in the whole country with 4.92% and 7.19% in the region of Fes-Boulemane [27]. From an epidemiological standpoint, 1242 cases of CL in the province of Sefrou were reported between 1997 and 2011 [28].

Females of sand flies are haematophagous Diptera belonging to the subfamily Phlebotominae (Psychodidae). They usually lay 30–70 eggs during a single gonotrophic cycle. The eggs require a microhabitat with high humidity in order to survive, but are not laid in water in order to hatch into larvae [2]. Normally these are mainly scavengers, feeding on organic matter (e.g. fungi, decaying leaves, animal faeces and decomposing arthropods). They also need only moist soil for development, using moisture present in cracks and crevices of soil. The larvae may acquire essential nutrient elements from soil through its decomposition. There are three types of nutrients: macronutrients, micronutrients and trace nutrients. In El Agamy, the phlebotomine sand flies breed in sheltered sites rich in organic matter, coarse sand and silt. Both species *Ph. langeroni* of the subgenus *Larroussius* and *Ph. papatasi* of the subgenus *Phlebotomus* need the same nutrients and soil conditions for their larval development [8].

The pre-imaginal ecology and breeding sites of sand flies (Diptera: Psychodidae) remain unknown for the majority of species of these medically important insects [5,14]. The scantiness of information on breeding places of *Phlebotomus* is, no doubt, largely due to the difficulty of isolating the immature stages from the soil. Among the successful efforts is that of Hanson (1961), who extracted 2219 immature sand flies, essentially from soil around the bases of trees with buttress roots in Panama [12].

In Morocco, several works have emphasized the role played by climate on the distribution of vector populations [21,22,23]. On the other hand, some of the work has focused on the habitat of sand flies. According to Boussa and Boumezzough in Morocco, *Ph. sergenti* requires a high rate of organic matter for larval development [6]. In Southern Anatolia, the increased cases of anthroponotic cutaneous leishmaniasis in is due especially to the presence of organic matter of animal origin, as the dung of cows, offering the conditions necessary for the development of eggs of sand flies [31]. This research is the first, in Morocco (Aichoun locality), to present the results of a search on potential breeding places of phlebotomines, such as: chemical and organic composition and pH of samples of soil from different microhabitats. The purpose of this study was to study the relationship between these chemical components of soil, the species composition and the existence of particular species of sand flies and their behavior in specific biotopes.

Materials and method

Trap Sites

The collections were carried out in Aichoun, a small town located in Sefrou Province, in the northwest of the Moroccan Middle Atlas (33°39'N, 04°38'W). Additionally, walls and floors of most houses in the region are made of mud leading to an ideal resting

and breeding site for sand flies. The choice of sites to activate the traps was based on the areas with characteristic *Phlebotomus* resting and breeding sites, such as soil rich in organic material, presence of poultry and farm animals, animal shelters providing shaded resting sites for adults with a high level of humidity, and dry walls with cracks and crevices where adults tend to rest [14,29].

- Station 1: Sheep pen, non-aerated, non-exposed to the sunlight, moderately salubrious, presence of sheep;
- Station 2: Stable exposed to the sunlight, continuous aeration of the site, salubrious (Hygienic activity of the inhabitants), only presence of Horse and donkey;
- Station 3: Mixed stable and hen house exposed to the sunlight, non-aerated, salubrious (presence only the chicken and sheep);
- Station 4: A winery, non-aerated, non-exposed to the sunlight, insalubrious (presence of different species of animals: donkey, cow, sheep, chicken, cat…)

Processing of soil samples

Soil samples were collected from damp spots along the perimeters of cattle sheds of Aichoun locality in 4 stations each month. The soil samples were collected from the same stations containing the traps. A shovel was used to collect samples of the top soil layer to a depth of approximately 30 cm. Four soil samples were collected from each location to produce average samples of 1 kg. It's the monthly average weight per station of soil collected. Soil samples were mixed together. The foreign materials were removed and the remaining sol was air-dried. Each soil sample was maintained in a plastic pot, covered with fabric in fine muslin and kept at room temperature. The samples were then ground by a stone masher and sieved through a wire sifter. Fine soil samples were extracted and tested. The chemical parameters (Exchangeable Potassium (EP), Assimilable Phosphorus (AP), Equivalent Humidity (EH), Electrical Conductivity (EC), Organic Carbon (OC), Total Calcareous (TC) and Organic Matter (OM)) have been studied.

Specimen collection

Collections were performed by using sticky papers (21 × 27, 3 cm) coated with castor oil in four stations. Sticky traps were used to collect sand flies from human and animal dwellings. The sticky traps were used independently to monitor populations of sand flies. They were placed in different spots and covered an area of approximately 1 m^2. Each trap was placed horizontally on a square metal frame support about 20 cm above the ground or it is shaped rolled up in the holes or between the crevices of the walls. Only the upper sides of the sticky traps were coated with castor oil because previous studies had shown that practically no fly adhered to the other side of the sticky traps (Moncaz & Warburg, unpublished).

The total of traps in each trapping campaign is 32 traps. The compound was sampled during two nights each month with 64 papers placed in four stations for a 12h dusk to-dawn period. Combined morning sand fly counts for the period yielded a density estimate, the mean number of sand flies/paper per night. Sample collection began in early May 2014 and continued until late September 2014 when sand fly activity was reduced. Trapped sand flies removed from stick papers with needles, washed with ethanol, and transferred in glass tubes containing a solution of 70° ethanol.

Identification of sand fly species

Species were identified by using specific morphologic keys. Phlebotomine specimens were identified by their morphological characteristics and identified to species level using the morphological key such as cibarium and pharynx in both male and female, spermathecae in female and the armature of genitalia in male [16]. Morphological differentiation of the two sympatric species *Ph. longicuspis* and *Ph. perniciosus* was made according to description of Berchi et al [4].

Data analysis

Data analysis is based on three characteristics of the sand fly fauna:

- The sex ratio (male/female) was also calculated for each species.
- The relative abundance: number of specimens of species (by variable)/total specimens of this species × 100.

Statistical analysis was performed by Principal component analysis (PCA) to study the possible correlation between the distribution of species and chemical parameters of soil on one hand and the similitude between the studied sites on the other hand. Principal Component Analysis (PCA) was conducted using the Unscrambler software (version 9.7).

Results

Species composition

A total count of 1685 adults phlebotomines sand flies were collected from four collection sites, with four species belonging to the genus *Phlebotomus* and one to the genus *Sergentomyia*. The relative abundance of the main species was *Ph. sergenti*

Table I

Total sand flies collected in four stations between May to September 2014
Total des phlébotomes capturés dans 4 stations entre les mois de mai et septembre 2014

Species of sand flies	Station 1 (N=303)				Station 2 (N=37)				Station 3 (N=135)				Station 4 (N=1210)			
	M	F			M	F			M	F			M	F		
		NG	G	GR		NG	G	GR		NG	G	GR		NG	G	GR
***Ph. sergenti* (84.62%)**	243	2	2	0	32	1	0	0	111	1	0	2	1000	16	7	9
***Ph. perniciosus* (8.54%)**	23	2	2	0	1	0	0	0	5	0	0	0	96	7	8	0
***Ph. papatasi* (6.17%)**	22	4	0	0	1	2	0	0	7	2	0	0	52	12	2	0
***Ph. longicuspis* (0.35%)**	0	3	0	0	0	0	0	0	1	2	0	0	0	0	0	0
***S. minuta* (0.29%)**	0	0	0	0	0	0	0	0	0	4	0	0	0	1	0	0

N: total number of sand flies; M: male; F: female; NG: non engorged female; G: engorged female; GR: gravid female

84.62% while *Ph. perniciosus* accounted for 8.54%. Other species were represented with weak abundance (*Ph. papatasi* 6.17%, *Ph. longicuspis* 0.35% and *S. minuta* 0.29%). The greatest number of specimens was collected in station 4 (Table 1). *Ph. sergenti* was the most prevalent species (61.24%), followed by *Ph. perniciosus* (6.58%), *Ph. papatasi* (3.91%) and *S. minuta* (0.05%). In the second position, the two station 1 and 3 were characterized by a high relative abundance of *Ph. sergenti* respectively with 14.65% and 6.76% in comparison with the other species. The station 2 represented a low abundance of sand flies in comparison with other biotopes with 2.17% in the total percent. Of the total sand flies caught, the males were predominant compared to the females. The physiological states of the females are given in Table 1. The sticky trap yielded about 57 of non-engorged females, 21 for engorged females and 11 for gravid females in the total.

Observations of soil morphology showed that collected soil samples ranged from loam to clay loam with medium to fine texture. The colors observed were light to dark brown with stones and other foreign materials.

Chemical characteristics were determined for all sixteen samples. Soil analysis for chemical characteristics showed a high level of phosphorus, potassium, organic carbon and organic matter for all samples. In this study, the average pH of the soil for each biotope was alkaline (Table 2).

In the present study, we associate thirteen variables to determine the correlation between the chemical parameter and the grouping of sites that show the similarity. For data processing by PCA, we used

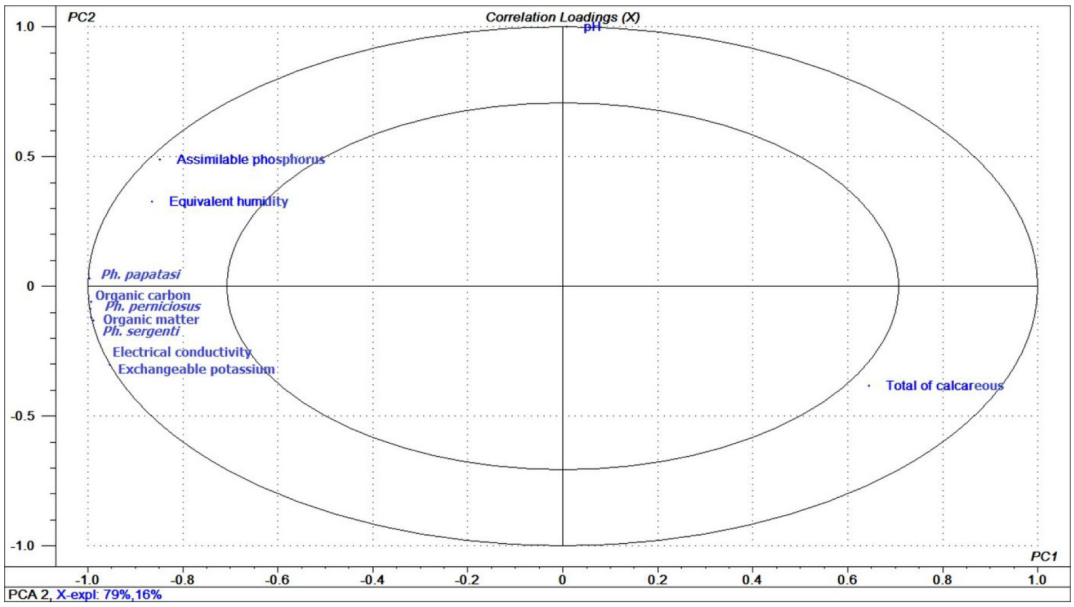

Figure 1

Representation of variables on the factorial plane F1 and F2
Représentation des variables sur le plan factoriel F1 et F2

Table II

Chemical characteristics of soil samples from station 1 in Aichoun locality during the study period from May to September
Caractéristiques chimiques des échantillons de sol de la station d'Aichoun au cours de la période d'étude de mai à septembre

	Station 1			Station 2			Station 3			Station 4		
Chemical analysis	Max.	Min.	Mean	Max.	Min.	Mean	Max.	Min.	Mean	Max.	Min.	Mean
pH	9.17	7.94	8.36	7.6	7.4	7.515	7.8	7.32	7.475	7.8	7.32	7.475
EC (mmhos/cm)	3.39	2.87	3.04	4.85	1.79	3.0125	4.12	1.31	3.0225	4.12	1.31	3.0225
EH (%)	39.15	25.67	32.12	37.15	21.9	29.27	27.09	24.54	25.72	27.09	24.54	25.72
TC (%)	49.80	36.18	41.37	73.4	41.40	58.42	49.80	38.10	44.72	49.80	38.10	44.72
OM (%)	10.10	4.07	7.70	11.1	1.64	6.21	6.56	5.08	6.06	6.56	5.08	6.06
OC (%)	5.86	2.36	4.36	6.86	0.95	3.81	3.80	2.91	3.47	3.80	2.91	3.47
AP (ppm)	413	330	381	289	43	285	345	119	248	345	119	248
EP (ppm)	7338	6310	6877	5703	769	2572	5577	876	3012	5577	876	3012

EC: electrical conductivity; EH: equivalent humidity; TC: total of calcareous; OM: organic matter; OC: organic carbon; AP: assimilable phosphorus; EP: exchangeable potassium

13 variables in which five species of sand flies were studied in the four selected sites in Aichoun locality.

In order to determine the number of components to remember, we will adopt the standard of Kaiser. At a normalized PCA, we retain the components are the eigen values greater than 1.

Table 3 shows the number components and the initial values that show the contribution of each of the components to the total variance.

The examination of the numerical results of the PCA shows that the first component explains 78.503% of the data variability while the second explains 15.624%. So, we can be satisfied to retain these two components explaining 94.127% of total data variability.

To facilitate the visualization of point clouds, they were thrown into a two-dimensional space. The percentage of inertia explained by the two axes forming a plane is 94.127% of the total variance. These two axes are considered to describe the distribution of variables and individuals on the main level. Figure 1 reveals the projection of variables in the space of factorial axes F1 and F2.

The correlation circle (Fig. 1) show that nine of eleven variables taken into account in the PCA contribute to the definition of factorial plan F1 x F2. The structurant variable positively of F1 is *Ph. sergenti, Ph. papatasi, Ph. perniciosus*, organic carbon, organic matter, assimilable P, exchangeable K, electrical conductivity and equivalent humidity. Thus, the axis F1 can be likened to an axis reflecting the parameters favorable to the proliferation of larvae of sand flies: *Ph. sergenti, Ph. papatasi* and *Ph. perniciosus*. Total calcareous, pH, are better explained by the second components F2 while *S. minuta* is badly represented by all of them. Based on the established correlation between variables and the principal components, a certain group has been identified. Principal components analysis showed that group 1 is strongly positively correlated marked by (*Ph. papatasi, Ph. sergenti* and *Ph. perniciosus*) with the electrical conductivity, organic matter and organic carbon. Group 2 is represented by the chemical parameter (assimilable phosphorus, exchangeable potassium and equivalent humidity) which is mostly positively correlated.

In order to confirm the observed correlations between the different factors influencing the behavior, the development of sand flies and the monitoring of their life cycle, we used correlation tests (Table 4).

Table III

Contribution of number components of the total variance made by PCA analysis
Contribution des composantes numériques de la variance totale effectuée par analyse ACP

Number component	Initial values		
	Eigen values	% of variance	% cumulative
1	8.63533	78.503	78.503
2	1.7186	15.624	94.127
3	0.646075	5.873	100

Table IV

Representation of the results of the PCA analysis (correlation between the different variables)
Représentation des résultats obtenus par l'analyse ACP (Corrélation entre les différents variables)

	pH	EC (%)	TL(%)	OM(%)	OC(%)	AP(ppm)	EP(ppm)	EH (%)	PS	PPE	PPA
pH	1										
EC (%)	-0,3089	1									
TL(%)	-0,3581	-0,4978	1								
OM (%)	-0,128	0,9828*	-0,582	1							
OC (%)	-0,0628	0,9645*	-0,5502	0,9944*	1						
AP(ppm)	0,4889	0,6618	-0,6029	0,7866	0,8347	1					
EP(ppm)	-0,3085	1*	-0,4974	0,9829*	0,9647*	0,6623	1				
EH (%)	0,3324	0,7267	-0,4325	0,8257	0,8789	0,9706*	0,7273	1			
PS	-0,1401	0,9821*	-0,6341	0,9961*	0,9825*	0,7616	0,9821*	0,7863	1		
PPE	-0,0963	0,9757*	-0,6283	0,9983*	0,9908*	0,7961	0,9757*	0,8212	0,9983*	1	
PPA	0,0186	0,9406*	-0,7098	0,9835*	0,9785*	0,8423	0,9407*	0,8386	0,9872*	0,9917*	1

***Correlation statistically significant. electrical conductivity (EC), total limestone (TL), organic matter (OM), organic carbon (OC), assimilable phosphorus (AP), exchangeable potassium (EP), equivalent humidity (EH), Ph. sergenti (PS), Ph. perniciosus (PPE), Ph. papatasi (PPA).**

(central Morocco) from May to September 2014 using sticky traps. According to Gebresilassie et al (2015) [10], the knowledge on the distribution, population dynamics, and behavior of sand fly vectors contributes to understanding of where, when, and how humans become infected with leishmaniasis. The literature reports that the conditions of breeding sites vary with the species of sand flies and the type of soil [5,6,15,17]. Indeed, soil pH plays an important role on behavior of sand fly and in larvae breeding habitat. In Sudan, it is confirmed that the soil containing a high quantity of clay with an alkaline pH constitutes an optimal and a characteristic biotope of leishmaniasis [13]. In our study, we found that the pH of the soil samples in different places was generally alkaline (pH>7). However, in many previous studies soil pH were reported to be 7.4, 7.2-8.5 and 7.6 in Panama, India and Thailand, respectively [19,26]. Another study performed in Sardinia from a breeding-site of *Ph. perniciosus* showed no correlation with the number of sand flies and pH from the places where samples were taken [5,9].

In Bihar state of India, the soil samples collected, from different villages with visceral leishmaniasis (VL) endemic districts, were examined for sand fly breeding in intra domestic. They found the samples to be infested with two species, among them, *Ph. papatasi* which preferred to breed in the neutral pH [25]. Naturally, according to the life cycle, females search for the optimal sites for ovipositing. The preference for breeding sites by these sand flies appeared to depend on chemical characteristic of the soil. The nature of the soil and its chemical composition has a great importance in the dipteran's matter for their food and their development [19]. In El Agamy, the phlebotomine sand flies breed in the sites rich in organic matter, coarse sand and silt with two species of *Ph. langeroni* and *Ph. papatasi* needing the same nutrients and soil conditions for their larval development [8].

In Aichoun locality, we found a relation between a high density of sand flies and high level of different chemical parameter (assimilable phosphorus, exchangeable potassium, organic carbon and organic matter, equivalent humidity). These results tell us about the behavior of sand fly and the ecological requirements of all three species: *Ph. sergenti, Ph. perniciosus* and *Ph. papatasi*. Psychodidae larvae prefer the organic material of animal origin [6]. The characteristics of the stations occupying the locality of Aichoun such dung of animals and the presence of organic material of plant origin following the abundance of vegetation around the sites offered the necessary conditions for the development of eggs of sand flies. In station 4, the presence of the three species of sand flies (*Ph. sergenti, Ph. perniciosus* and *Ph. papatasi*) can be explained mainly by the characteristic chemical of the environment (Organic carbon and Organic matter). *Ph. sergenti* soil analysis of a breeding site in Israel also shows richness in organic matter [17]. In Morocco, Ph. sergenti requires a high rate of organic matter for larval development according to Boussa and Boumezzough [6]. The disease of Anthroponotic Cutaneous Leishmaniasis in some regions is caused essentially to the presence of organic matter animal, for example in southern Anatolia [31].

In Colombia, harvested species of phlebotomine sand flies of the genus *Lutzomyia* in a wide variety of breeding sites were presented with a high density. This result is related with an abundance of organic matter and high relative humidity [30]. Some authors found that the different behavior of sand flies, are depending on the chemical and physical factors and the type of breeding places of the soil. Basimike et al concluded that the abundance of sand flies (*Phlebotomus* and *Sergentomyia*) depends to some extent on chemical and physical factors of their breeding places in the soil [3]. In burrows, no correlation was observed between these sand flies and soil chemical and physical factors. In tree bases, *Phlebotomus* present positive coefficients between calcium, manganese and moisture. Whereas the species of *Sergentomyia*, were negatively correlated with pH and positively correlated with magnesium. In another biotope, the termite mounds, *Sergentomyia* species were negatively correlated with pH and phosphorus [3].

Davies et al have reported that the principal determinant of transmission of these zoonotic diseases is probably the behavior and ecology of the sand fly vectors [7].These authors have discussed in their work how the risk of these diseases for humans also depends on factors associated with the reservoir hosts. They have also looked at the epidemiological evidence for the role of human components – such as behavioral activities or house design – which have been identified as risk factors for leishmaniasis.

In Aichoun locality, the presence of four species among the fives incriminated in the transmission of leishmaniasis in Morocco and the presence of the several factors favoring the biological development of sand flies permits the spreading of leishmaniasis. Indeed, the chemical analysis of potential breeding sites of sand flies and statistical analysis have showed a correlation between the behavior of sand flies and the chemical parameters studied (exchangeable potassium, assimilable phosphorus, equivalent humidity, electrical conductivity, organic carbon, and organic matter). This study recommends the necessity of the destruction of vectors' habitats, the improvement of human habitats, the treatment of scraps, animal droppings, dung, and the separation between animal and human dwellings. These actions and measures might be an efficient way to get rid of sand flies and consequently diminish the risks of contamination.

Conclusion

This study aimed to investigate the effect of properties of soil on abundance of sand flies in breeding habitat in Aichoun locality, central Morocco. The predominance of *Ph. sergenti* and the presence of other species of sand flies in the different stations and especially in the station 4 with high disease prevalence, is related to the presence of the chemical parameter essential for their development in the potential breeding of sand flies. These results were of great interest to analyze the blood meals and to study the host preferences of the sandfly specially Ph. sergenti in CL endemic focus. The results which were found should be taken into account to determine leishmaniasis risk areas and to establish efficient control strategies.

Conflict of Interest : The authors declare that they have no conflict of interests.

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