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Research Article

Ecological Determinants of Forest to the Abundance of *Lutzomyia longiflocosa* in Tello, Colombia

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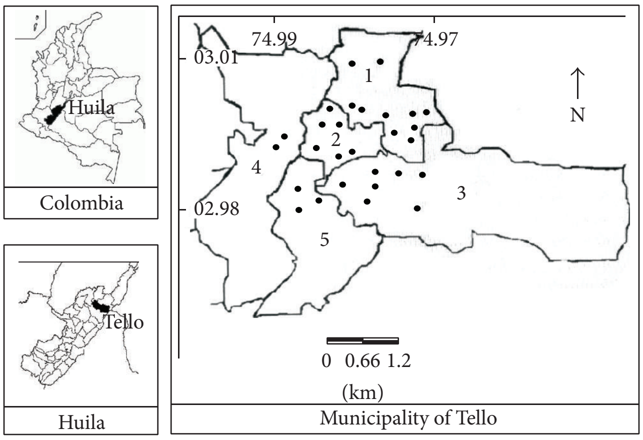
Lutzomyia longiflocosa is considered the most likely vector of cutaneous leishmaniasis in the sub-Andean region of the upper valley of the Magdalena River between 1,000 and 2,000 meters in the Department of Huila, Colombia. *L. longiflocosa* is anthropophilic, has endophagic behavior, and is especially important since its dominance in epidemics recorded in the last decade in the departments of Huila, Tolima, and the outbreak in Norte de Santander. The aim of our work is to identify ecological determinants in forest microhabitat level defining the abundance of *L. longiflocosa*. We use sampling; this was performed in 56 microhabitats of 28 forests with CDC traps for two consecutive nights from 18:00 to 06:00 hours. Each microhabitat (favorable and unfavorable) was located 10 m from the ecotone, with an approximate area of 10 m^2. Thirty-five variables were examined as potential explanatory variables which were recorded in each microhabitat. Regression models were used to identify ecological determinants. Our results confirm that there are favorable microhabitats in the forest with specific ecological determinants that define the aggregated distribution of the species and provide the conditions necessary for survival and abundance of *L. longiflocosa*.

1. Introduction

Cutaneous leishmaniasis (CL) is the clinical form recorded more frequently in Colombia with more than 95% of reported cases [1, 2]. The leishmaniasis is caused by infection of parasites to the genus *Leishmania* and is transmitted by the bite of infected female sand flies to the genus *Lutzomyia*; this disease affects skin, initially comes off as a grain, and, with the passing of more days, continues to grow in a circular form until ulcers shape [3–5]. In the sub-Andean region of Valle Alto Magdalena River between 1,000 and 2,000 meters, *Lutzomyia longiflocosa* species OMOM, Psychodidae Family [6], is considered the most likely vector for its anthropophilic behavior, blood meal inside homes or endophagic behavior [7] and especially its dominance in epidemics recorded in the last decade in the departments of Huila and Tolima and the outbreak of Norte de Santander [1, 2, 7–10]. Because of its importance in public health, there have been studies that have established aggregate species distribution and abundance of regional and local ecological determinants. Knowing the dynamics of abundance of sand fly, we can partially predict the occurrence of the disease and lower the risk and the impact on humans. Among regional determinants detected, *L. longiflocosa* has higher abundance between 1300 and 1700 m, with temperature ranges between 18°C and 19.9°C and negative association with precipitation. Locally, *L. longiflocosa* is found in forests, particularly those located in relatively inclined sites within the first four arboreal strata, protected from wind and with high percentage of litter [9]. Therefore, we propose aims to identify the ecological determinants in the forest microhabitat level favoring the abundance of *L. longiflocosa* and start the knowledge of temporal patterns vector of cutaneous leishmaniasis in the municipality of Tello, Huila, Colombia.

2. Materials and Methods

2.1. Study Area. The study was conducted in the forests of the villages of La Urraca, La Brasilia, Alto Urraca, Medio



Village:
(1) La Urraca
(2) La Brasilia
(3) Alto Urraca
(4) Medio Roblal
(5) Alto Roblal

Sample localities:
• Ecological determinants of microhabitats

Figure 1: Location of forests for ecological determinants sampled in forest microhabitats level for abundance of *Lutzomyia longiflocosa*, municipality of Tello, Colombia.

Roblal, and Alto Roblal, which recorded high prevalence of CL during the epidemic of 1993–1996, located on the western flank of the Cordillera Oriental between the 2nd and 3rd 09′ 94′ N latitude and 74° 91′ and 75° 23′ W longitude in the municipality of Tello, Huila Department (Figure 1). Most forests have a height between 20 and 35 m; 2.5 arboreal strata now reach 93% of the soil, with litter average coverage of 87% and depth of 8.4 cm [9]. The study area is classified as Rainforest Premontano bh-pM [11], with an average annual rainfall of 1346 mm and annual average temperature of 22.1°C Station: El Portal, code: 2111507, Palacio-Vegalarga, code: 2111510, and Laureles, code: 2111514 [12]. The criteria for inclusion of forests were located between 1300 and 2100 meters, not less than 0.35 hectares area (maximum recorded area was 1.2 ha) and nonintervention antropic. In each forest, taking into account ecological determinants abundance, two microhabitats located within 10 m of ecotone that are the most productive were selected [9], each with an area of 10 m^2 and a distance between them of not less than 40 m. One of them was designated as a favorable microhabitat and the other unfavorable; the favorable had three or more features described below and the unfavorable had only two or less:

(i) Presence of trees with diameter breast height (DBH) greater than 30 cm.

(ii) Trees with rough bark and roots tabloids.

(iii) Thick layer of undecomposed litter more than 5 cm deep.

(iv) High coverage, greater than 80% for plants larger than 5 m height.

(v) Wind-sheltered microhabitats.

2.2. Explanation for Abundance in the Forest Level Microhabitat Variables. Thirty-five variables were examined as potential explanatory variables; four of these had been previously identified as ecological determinants abundance of *L. longiflocosa*: (i) tree cover, (ii) litter depth, (iii) wind barrier, and (iv) distance to the nearest housing [9]. The new variables were related to coverage of shrubs, plants, and grasses; characteristics of trees as DBH, height, roots, leaves, bark type, and presence of holes in the trunk are shown in Table 1.

2.3. Trapping for Sand Flies. Sampling was performed in each microhabitat for two consecutive nights between 18:00 and 06:00 hours, using CDC light trap [13], about 1.5 m in height from ground level.

2.4. Analysis of Information. For data analysis, the STATISTIX software (version 1.0) and MATLAB 2012 are used for this purpose initially. Excel databases (version 4.0) to be exported to the software were developed. To verify data normality test Bartlett was performed [14]. Ecological determinants to detect four types of statistical analysis described below were used:

(1) To confirm whether the microhabitats designated as favorable at baseline showed higher abundance of *L. longiflocosa* than statistically calculated, an adverse descriptive statistical analysis was performed based on averages, standard deviation, standard error of the mean, maximum and minimum. Due to the variability of the data, a logarithmic transformation $[\log_{10}(x + 1)]$ was performed to normalize the data; the statistical analysis was performed by completely random models (ANOVA and MATLAB) factorial arrangements $A \times B$ where forests worked as a factor A and the microhabitat as factor B. Averages with significant differences ($P < 0.05$) underwent the unplanned Tukey test.

(2) To identify the ecological determinants favoring the abundance of *L. longiflocosa* in microhabitats, Pearson correlation coefficients were between the number of *L. longiflocosa* and explanatory variables. Likewise, a multiple linear regression model step in which the variables recorded greater contribution to the model ($P < 0.05$), until finally the determinants that most explained the abundance of *L. longiflocosa* were identified. Likewise, a model of nonlinear multiple regression type of 2nd-order polynomial to detect the determinants that most contributed to the model was performed.

(3) To determine differences in ecological determinants among the most abundant forests and not recording the presence of *L. longiflocosa* completely random analysis (ANOVA, MATLAB) and averages with significant difference ($P < 0.05$) the proof unplanned Tukey test averages were performed.

(4) To compare the four most productive microhabitats with its counterpart in the same forest, a multiple linear regression model step in which those variables

Table 1: Explanatory variables to identify ecological determinants in the forest in a microhabitat level for *Lutzomyia longiflocosa* abundance, in Tello, Colombia.

Explanatory variables	Definition	Category	Mediation level
% tree	Percentage of space between the trees in the microhabitat.	Categorical: 0: absent; 1: <15%; 2: 15%–65%; 3: >65%	Ordinal
% shrubs	Percentage of space occupied by shrubs in the microhabitat.		
% plants	Percentage of space occupied by the plants in the microhabitat.		
% grasses	Percentage of space occupied by grasses in the microhabitat.		
Tree height	Average height of dominant trees in the microhabitat.	Quantitative	Reason
Shrubs height	Average height of dominant shrubs in the microhabitat.		
Trees numbers	Number of trees that are located in the microhabitat.	Quantitative	Reason
DBH between 10 and 20 cm	Number of trees and shrubs with diameter at breast height (DBH) between 10 and 20 cm in the microhabitat.	Quantitative	Reason
DBH between 21 and 40 cm	Number of trees and shrubs with DBH between 210 and 40 cm in microhabitat.		
DBH higher than 41 cm	Number of trees and shrubs with higher DBH to 41 cm in the microhabitat.		
Total DBH	Sumatory of all DBH of all trees and shrubs in the microhabitat.		
Height between 3 m and 10 m	Number of trees with height from 3 to 10 m in the microhabitat.	Quantitative	Reason
Height between 11 m and 20 m	Number of trees with height between 11 and 20 m in the microhabitat.		
Height between 21 m and 40 m	Number of trees with height between 21 and 40 m in the microhabitat.		
More than 41 m high	Number of trees with height greater than 41 m in the microhabitat.		
Emerging root	Number of trees with roots visible above-ground and in the microhabitat.	Quantitative	Reason
Strong root	Number of trees with shallow roots with height greater than 20 cm in the microhabitat.		
Weak root	Number of trees with shallow roots with height less than 20 cm in the microhabitat.		
Tabloids roots	Number of trees with roots that are visible above-ground and in the microhabitat.		
Stilt roots	Number of trees with roots that are visible above-ground and in the microhabitat.		
Foliage between 0 and 10 m	Number of trees with leaves space taken from the shaft to the canopy from 0 to 10 m.	Quantitative	Reason
Foliage between 11 and 20 m	Number of trees with leaves space taken from the shaft to the canopy from 11 to 20 m.		
Foliage more than 21 m	Number of trees with leaves space taken from the shaft to the greatest canopy 21 m.		
Holes with radius 5 cm to 20 cm	Number of gapped trees size between 5 and 20 cm radius from the ground level up to 2 m high.	Quantitative	Reason
Holes with radius 21 cm to 40 cm	Number of gapped trees size between 21 and 40 cm radius from the ground level up to 2 m high.		
Gaps with greater than 41 cm radius	Number of trees with larger holes with a radius of 41 cm from ground level up to 2 m high.		
Smooth bark	Number of trees crusted with few layers forming flat plates in the microhabitat.	Quantitative	Reason

Table 1: Continued.

Explanatory variables	Definition	Category	Mediation level
Rough bark	Number of trees with bark layers arranged in small plates not uniform in the microhabitat.		
Bark with projections	Number of trees crusted with bumps in the microhabitat.		
Scaly bark	Number of trees crusted with overlapping structures in the form of flakes in the microhabitat.		
Fissured bark	Number of small trees with well-defined cortex and linearly spaced apart plates in the microhabitat.		
Litter depth	Inches of top soil where the litter is not decomposed in the microhabitat.	Quantitative	Reason
Wind barrier	Microhabitat located on type of relief in the form of "V."	Categorical: 0: absent. 1: present	Nominal
Housing distance	Distance to the nearest microhabitat housing.	Quantitative	Reason
Number of people	Number of people living closest to the microhabitat housing.	Quantitative reason	

were recorded that showed a greater contribution to the model ($P < 0.05$).

3. Results

3.1. Species Composition. In total, 28 forests in which 112 samples were taken and copies of *Lutzomyia* 3460 and 2519 were collected in the favorable and the unfavorable microhabitat 941, divided into 9 species, were sampled. *L. longiflocosa* was the most abundant species with 92.4% (n = 3197), followed by *L. nuneztovari* O. with 5.3% (n = 182), found with less than 1% *L. trinidadensis* N. species (n = 25), *Helcocyrtomyia* sp. (n = 21), *L. columbiana* R.V. (n = 18), *L. atroclavata* K. (n = 4), *L. pia* F.H. (n = 1), *L. dubitans* S. (n = 5), and *L. lichyi* F.A. (n = 7). Only four forests recorded more than six species and and five were negative. *L. longiflocosa* was found in 23 forests. Considering that the species *L. longiflocosa* is being considered and represented 92.4% of the collection, the results are shown only for this species.

3.2. Abundance and Distribution of Forest and Microhabitat. To the abundance of *L. longiflocosa* in the forest, 8 groups of statistically significant forests were presented; the first group was only represented by forest 14 with the highest average of 314.5 (*ee* = 158.75) specimens, in the second group was ranked forest 1 with average of 122.8 (*ee* = 54.18), the third group was formed by forests 15 and 13 with averages of 84.3 (*ee* = 41.07) and 76.3 (*ee* = 28.48), respectively, and in the remaining five groups were placed 24 forests with averages below 50 copies ($P < 0.00001$). Furthermore, significant differences in the abundance of *L. longiflocosa* by favorable and unfavorable microhabitat averaging 41.73 (*ee* = 15.54) and 15.35 (*ee* = 4.25) specimens, respectively ($P = 0.0126$), were found. Of the 23 forests in which *L. longiflocosa* was recorded in 18, abundance in the favorable microhabitat was higher than the unfavorable.

3.3. Specific Ecological Determinants in the Forest Microhabitat Level. Thirty-five explanatory variables defined in the study and nine determinants that directly explain the abundance of *L. longiflocosa* in microhabitats were identified as ecologically important. Through Pearson correlations, linear regression model step by step, ANOVA, and MATLAB model nonlinear regression were detected: the trees submit holes with radius 5 cm to 20 cm, bark scaly, foliage between 11 m and 20 m and bark rough, the latter determining favored *L. longiflocosa* abundance in favorable with respect to microhabitats of the most abundant unfavorable forests. Determining the remaining 6 was done by a single statistical analysis [15, 16]. The linear regression model explained 68.94% of the abundance of *L. longiflocosa* with the 9 detected ecological determinants, while the nonlinear regression model explained 89% with two determinants: holes with radius 5 cm to 20 cm and bark with scales as in Table 2.

4. Discussion

The strengths of this study were as follows: (i) sampling the inclusion of the 28 existing relict forests between 1300 and 2100 asl in the villages of the municipality of Tello affected by the epidemic CL, (ii) the prior identification of regional and local ecological determinants favoring the definition of the criteria for selection of favorable and unfavorable sites, (iii) CDC light trap confirming as an appropriate technique that collects abundance of *L. longiflocosa* in sub-Andean of Colombian region, (iv) the previous definition of the height at 1.5 m ground level, (v) prior definition of the location of the CDC light trap in the ecotone forest to greater abundance, and (vi) negative association with precipitation which defined the sampling month [9].

Species richness was low as was expected by the altitudinal range selected in this study and the sampling method because it is aimed at the sand fly attracted to light. However, other collection methods, such as human bait, were not used to avoid a possible transmission of the disease. CDC light tramp was used by the representative in the collection of *L. longiflocosa* in this region [9], which was demonstrated in this study, a dominance of 92.4%; *L. nuneztovari*, *L. columbiana*,

Table 2: Ecological determinants in forest microhabitats level for abundance of *Lutzomyia longiflocosa*, in Tello, Colombia.

Ecological determinants	CP between abundance of *L. longiflocosa* explanatory variables	MLRMF between abundance of *L. longiflocosa* and explanatory variables R^2: 0.6631 R tight: 0.6369	ANOVA between most abundant forest of *L. longiflocosa* and negative forests	MLRMF between microhabitats of *L. longiflocosa* more abundant forests R^2: 0.6894 R tight: 0.6672	NRM between ecological determinants detected MLEMF multiple R: 0.89 R^2: 0.8 R tight: 0.79
Holes with radius 5 cm to 20 cm	r: 0.7243***	R^2: 19.72***	F: 3.92**	Ni	R^2: 0.567***
Scaly bark	r: 0.6465***	R^2: 14.58**	F: 3.98**	Ni	R^2: 0.411**
Rough bark	r: 0.6083***	Ni	F: 4.21**	R^2: 62.68***	Ni
DBH greater than 41 cm	r: 0.5826***	Ni	Ni	Ni	Ni
Foliage between 11 m and 20 m	r: 0.4723***	R^2: 15.46**	F: 3.40**	Ni	Ni
Height between 21 m and 40 m	r: 0.4659***	Ni	Ni	Ni	Ni
Tree numbers	r: 0.4534***	Ni	Ni	Ni	Ni
Tabloids roots	r: 0.3491***	Ni	Ni	Ni	Ni
Height between 11 and 21 m	r: 0.3265***	Ni	F: 3.32**	Ni	Ni
Height between 3 and 10 m	Ni	R^2: 18.85**	F: 9.00***	Ni	Ni
Ridged bark	Ni	R^2: −15.18**	F: 23.00***	Ni	Ni
DBH between 10 and 20 m	Ni	R^2: −24.1**	F: 23.86***	Ni	Ni
DBH between 21 and 40 m	Ni	R^2: −20.05**	F: 7.75***	Ni	Ni
Holes with radius greater than 41 cm	Ni	R^2: −8.26**	Ni	Ni	Ni

CP: Pearson correlation. r: Pearson correlation coefficient (r: 0.31 and 0.6 [correlation accepted], r: 0.61 and 0.8 [good correlation]).
MLRMF: Multiple Linear Regression Model Footsteps. R^2: determination coefficient.
ANOVA-MATLAB: Analysis of Variance. F: F value.
NRML: Nonlinear Regression Model. R^2: determination coefficient.
P: probability value; ** $P < 0.01$ and $P < 0.001$; *** $P < 0.001$ and $P < 0.000$.
Ni: not identified by the statistical analysis.

and *L. pia* and five other species of other subgenres plus three more species of *verrucarum* group were captured.

The 9 specific ecological determinants in the forest microhabitat level identified for abundance of *L. longiflocosa* partially explain the aggregated distribution of this species. It was assumed that those determinants, together with regional and local ecological factors identified previously [9], generate microclimate forests which are suitable places for survival and abundance of the species, specifically (i) resting sites and places for reproduction and (ii) potential breeding sites where the life cycle develops. According to Cabanillas and Castellón [17], morphological characteristics of the bark can influence *Lutzomyia* species in the choice of resting places, as in the case of *L. umbratilis* W.F. found in bark with grooves [18]; this situation is according to Memmott who suggests that aggregate *Lutzomyia* species in forest trees distribution is not random but is due to a shortlist of trees used as daytime resting site [19].

In this study, for the rest of the *L. longiflocosa* activities it was considered that the determinants' scaly bark trees and trees with rough bark had a significant contribution as in Table 2. In like manner, possibly presenting gaps trees radio 5 cm to 20 cm contributed significantly to rest sites because studies indicate *Lutzomyia* species using this microhabitat as resting place, *L. vespertilionis* F.H. [18, 19], *L. trapidoi* F.H. captured in tree hollows below 2 m high [20], *L. shannoni* D. [18, 20–23] in holes with diameters between 40 cm and 80 cm [24], *L. spinicrassa* M.O.O.H. and *L. gomezi* N. [23] and *L. isovespertilionis* F.H. [25]. Determining the ecological determinants' roots with lower correlation tabloids possibly also provided resting sites, since according to Memmott in certain areas the *Lutzomyia* roots are aggregated in tabloids root and also have vertical zoning. The remaining determinants, number of trees, DBH greater than 41 cm, foliage between 11 m and 20 m, and trees with height between 3 m and 40 m, together with the determinants of higher correlation, were considered which remained within the microhabitat favorable microclimatic conditions [19]. Breeding sites have not been found in this study, but the obtained data suggests that they might be identical to the bedding locations for adults or very close to them as it also was reported for other *Lutzomyia* species [19]. In this study, between detected ecological determinants, the tabloids roots and holes with radius 5 cm to 20 cm provide a stable-as-possible breeding site where the immature forms are kept in microenvironment. Hanson collected *Lutzomyia* larvae in the bases (tabloids roots) of big trees [26] and Thatcher using floating collection method larvae collected *L. micropyga* M. and *L. disponeta* F.H. in hollow shaft [27].

This study is the first to identify determinants in forest ecological level microhabitat for abundance of *L. longiflocosa* complementing the findings of regional and local ecological determinants for this species, allowing microhabitats as producers to focus areas of sand fly, in synergy with parasites which favors the transmission cycle of leishmaniasis. In conclusion, there are favorable microhabitats in the forest with specific ecological determinants such as holes with radius of 5 cm to 20 cm, scaly bark, rough bark, DBH greater than 41 cm, foliage between 11 m and 20 m, height between 21 m and 40 m, tree numbers, tabloids roots, and height between 11 and 21 m, which partially explain the aggregated distribution of *Lutzomyia longiflocosa* and provide the conditions necessary for survival and abundance of this species.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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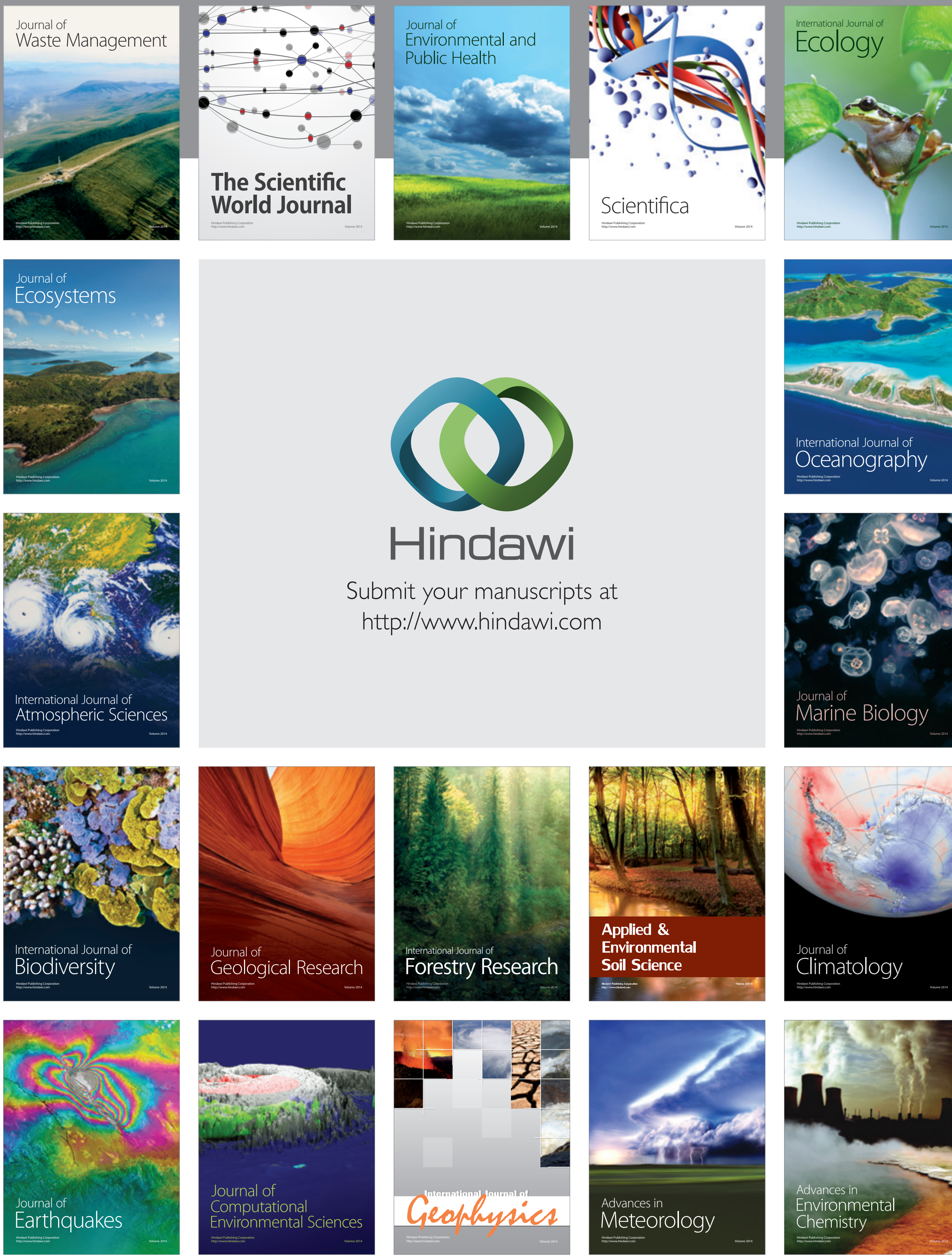
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