MONITORING OF MICROBIAL POPULATION OF THE *PÁRAMO* SOIL OF THE CHARGE ZONE OF LAKE MAPAHUIÑA IN CHIMBORAZO-ECUADOR

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Resumen

La presente investigación analiza la presencia de microorganismos en los suelos de la zona de recarga de la Laguna Mapahuiña, Ecuador. Cuenta con gran diversidad microbiana que caracteriza un ecosistema *páramo* con presencia de suelos ácidos, como otros suelos volcánicos paramunos analizados en la región. El análisis microbiano general reveló una considerable cantidad de microorganismos en la región de estudio, no se encontró correlación significativa respecto a las características físicas y químicas obtenidas. La concentración más alta (5,5 x 10^5 células / mm^3) de microalgas se presentó a orillas de la laguna Mapahuiña, en contraste con el área de bosque de pino (1,7 x 10^5 células / mm^3). El crecimiento de microalgas mostró diferencias significativas con respecto al pH, humedad relativa del suelo, y el tipo de vegetación con temperatura del suelo relativamente homogénea. Nuestro estudio es la primera investigación de la zona y presenta las bases para revelar cepas microbianas de interés biotecnológico.

Palabras claves: suelo, *páramo*, bacteriana, microalgas, recuento microbiano

Abstract

The present investigation analyzed the presence of microorganisms in soils of the recharge zone of the Lake Mapahuiña, Ecuador. The location has a large microbial diversity which is characterized by a *páramo* ecosystem presenting acidic soil types, which coincides with other paramunos volcanic soils analyzed in the region. The general microbial analysis revealed a considerable amount of microorganisms in each region of study, with no significant correlation found regarding the physical and chemical characteristics measured. For microalgae, the banks of the Lake Mapahuiña had the highest concentration (5,5 x 10^5 cells/mm^3) in contrast to the Pine forest area (1,7 x 10^5 cells/mm^3). Microalgae growth showed significant differences with respect to pH, relative soil humidity, and vegetation type with soil temperature relatively homogeneous. Our study is the first investigation of the area and presents the foundation for unearthing microbial strains of biotechnological interest.

Keywords: soil, *páramo*, bacterial, microalgae, microbial count

Introduction

The *páramo* is an ecosystem composed of grasslands combined with small remaining vestiges of native forest and in recent years an increased presence of foreign plantations. In South America it extends from Costa Rica to northern Peru and Bolivia, covering the Andes region with altitudes ranging from 3000 to over 4800 meters above sea level (1). Given its hydro-physical properties, it has a high capacity to retain water due to the andosol soils of volcanic origin with a presence of peat and a dense herbaceous vegetation cover (2). Due to the altitude at which it is located, the temperature decreases and there is a presence of fog (3). Being in the Equatorial region, the climate is stable throughout the year, although there is a marked difference between day and night, with overnight temperatures dropping significantly. Yet the *páramo* shows a great diversity of living systems such as plants, birds, amphibians, mammals and a microbiological component (4,5).

Despite the topographical variability, andosols of the *páramo* are defined by a remarkable homogeneity in their physicochemical properties (6,7). The degradation and changes in land use threaten the hydrology of the *páramo* by influencing the cycles of carbon, nitrogen, sulfur and phosphorus (2,8) , which are implicated in the activity of soil microorganisms and soil mineralization processes (9,10). Among the colonizing *páramo* soil microorganisms are the microalgae (11), which generally grow with low insolation and are exposed to extreme seasonal fluctuations in temperature UV radiation and desiccation.

They also serve as potential bio-indicators of the degree of conservation of the ecosystem (12,13). In addition to its role in regulating soil properties, microalgae are also the source of biomolecules and metabolites of great economic importance used primarily in food, medicines, fertilizers and biofuels (14–16) motivating further scientific efforts to discover new microorganisms, as in the newly identified cyanobacteria found in the *páramo* zone of Costa Rica (17). Algal activity depends on the internal cycles and partnerships between these microorganisms, interactions with organic and inorganic nutrients derived from animal or vegetable debris and exposure to surface runoff (18). Furthermore, *páramo* soil microalgae are capable of creating symbiotic associations with other soil microorganisms like bacteria, promoting the growth of plants through the production of the auxin indole-3-acetic acid (19).

In Ecuador, the *páramo* is located in the highlands of the Andes occupying an area of approximately 12,650 km2, about 5% of the territory. According to Mena (4), Ecuador is the country with the largest amount of *páramo* with respect to its land extension, yet little is known about its edaphic fauna and even less of its vast microalgae wealth. This research aims to conduct monitoring of the population of soil microorganisms, with special emphasis on microalgae, by determining whether physicochemical soil variables such as pH, moisture and temperature influence their distribution and concentration.

Materials and Methods

Area of study

This study was conducted in the recharge zone of Lake Mapahuiña (9742946 N, 747817 E) in the Sangay National Park (Chimborazo Province), which belongs to the microbasin of the Zula River, having an oval-oblong morphometry of 281,542 ha of mainly sandy loam soil. The average daily temperature is between 6-12ºC, with a daytime maximum of 15ºC and a night-time minimum of 3ºC recorded.

The annual rainfall of the zone is between 700-1000 mm . The life zones in its *páramo* grassland ecosystem include lower montane dry and wet forests which contribute to its vast ecological diversity (20, 21). The samples were collected and analyzed weekly between September and December 2013, months in which the weather conditions were suitable to attend the study site. Figure 1 outlines the various sampling areas according to land use which include: Region 1: *páramo* vegetation, with *Culcitium canescens Humb. & Bonpl*, *tristerix longebracteatus* and the endemic grass Stipa ichu; Region 2: *Azorella aretioides*

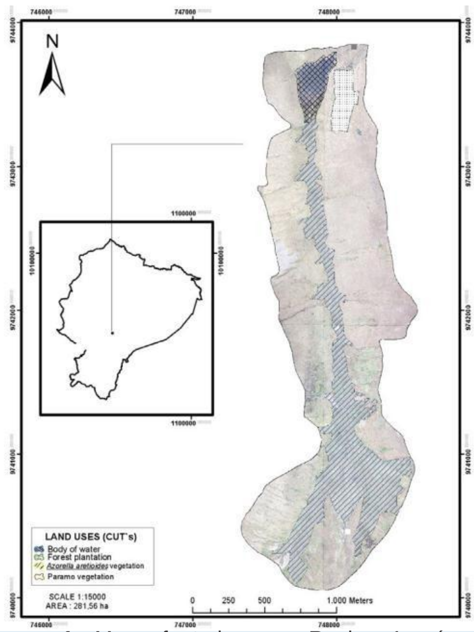

Figure 1: Map of study area: Region 1, páramo vegetation; Region 2, Azorella aretioides vegetation; Region 3, Pinus radiata forest and Region 4, Contours of lake Mapahuiña

vegetation, incorporating *Stipa ichu*, *Lachemilla orbiculata* and the grass *Calamagrostis intermedia*; Region 3: *Pinus radiata* forest and Region 4: the contours of Lake Mapahuiña, composed of volcanic rocks and *Azorella aretioides* (21).

Soil sampling

The sampling scheme was based on the surface density of land use (20) together with the aid of base mapping, accessibility and satellite imagery, placing 86 monitoring points in the recharge zone of Lake Mapahuiña (Figure 2, Table 1). The soil sampling included taking a portion of soil (200 g, depth: ~20 cm) using a plot (1 x 1 m) from the study area, which had an average altitude of 4130 meters above sea level. Sample integrity was maintained with the use of resealable plastic bags and cold storage during transportation (22) to the laboratory for analysis.

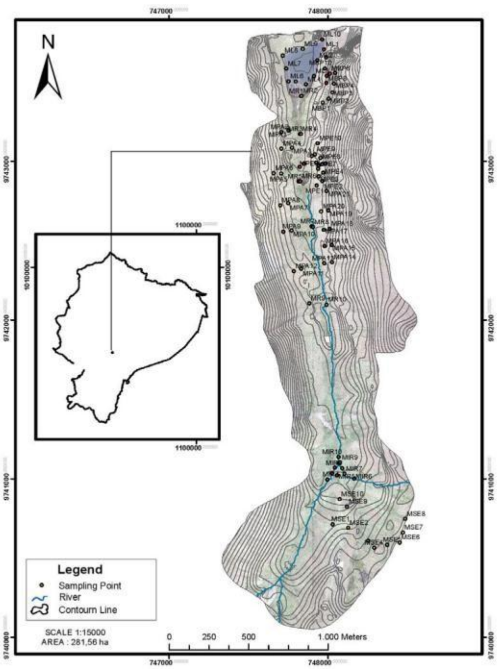

Figure 2: Sampling point distribution of the study area

Region	Surface area (ha)	Area (%)	Plots implemented†
1	205.36	72.94	47
2	62.01	22.03	24
3	5.84	2.07	5
4	7.62	2.71	10

Table 1: Amount of surface area per land use in the recharge zone of Lake Mapahuiña

†All permanent monitoring plots distributed every 50 meters unless stated; Region 1: monitoring plots distributed to account for runoff areas and convergence zones; Region 2: sample plots excluded waterlogged areas; Region 3: number of plots based on the region's area %; Estimation of plot number used a sampling error of 10% (Schlegel et al., 2011)

Soil analysis

Subsamples of surface sediment (10 g) were diluted in peptone water to achieve a stock concentration of 10^{-10} as previously described. Microalgal quantification was determined as cells/mm^3 (24) by direct microscopic counts. For the quantification of heterotrophic bacteria, a diluted sample (1 mL, 10^{-6}) was added to a Petrifilm™ aerobic bacteria plate (3M, USA) and incubated for 3 days at 26 ºC. Similarly, yeast and mould colonies (1 mL, 10^{-3}) were inoculated on PetrifilmTM yeast and mould type plates, respectively (3M, USA) and incubated for 5 days at room temperature. Microscopic colony enumeration was determined as the number of colony forming units (CFU) per gram of dry soil (CFU/g dry soil) (26).

	Region			
Characteristic†	1	2	3	4
Moisture (%)	15-80	35-90	30-40	30-85
Temperature (ºC)	5.1-13.9	7.0-11.1	6.9-10.8	10.0-16.1
pH	4.5-6.4	4.6-6.1	5.1-6.6	5.4-7.1

Table 2: Physical and chemical characteristics of soil samples from the recharge zone of Lake Mapahuiña

†Data collection: relative humidity measured with a soil hygrometer (I.C.T.S.L., Spain); pH measured with a portable pH meter (Hanna Instruments, USA); temperature recorded with a digital ground thermometer (Hanna Instruments, USA).

Statistical Analysis

Data analysis was carried out using the software packages ArcGIS Geostatistical Analyst (Esri, 10.1), which utilizes Inverse Distance Weighing (IDW) for multivariate interpolation, (27) and the statistical software InfoStat. Differences between regions for each variable were evaluated by ANOVA and Tukey"s test, with the non-parametric method of Kruskal–Wallis also employed.

Results

Results

To describe the variation of the microbial abundance present in each region of study (Table 3), descriptive statistics for natural log (ln) of CFU/ g dry soil are shown in Table 4 and illustrated graphically in Figures 3-4. The influence of pH on the growth of bacteria was not significant (Spearman r = -0.41; P <0.001), even with adequate moisture (Spearman r = 0.24; P = 0.004) and temperature (Spearman r = -0.09; P = 0.46). Similarly, the amount of mould was affected by the pH of the soil and showed a weak relationship (Spearman r = 0.06; P = 0.60), however no correlation with temperature (Spearman r = -0.15; P = 0.24) or moisture was observed (Spearman r = -0.59; P <0.001). There was no significant change in the amount of yeast observed within the soil with changes in pH (Spearman r = -0.09; P = 0.48); a weak correlation was obtained for both temperature (Spearman r = -0.13; P = 0.27) and moisture (Spearman r = -0.39; P <0.001). Similar to the other microorganisms evaluated, the relationship between microalgae and soil pH was not significant (Spearman r = 0.31, P <0.001) while a weak correlation for temperature (Spearman r = 0.45; P <0.001), and moisture (Spearman r = 0.41; P <0.001) was observed.

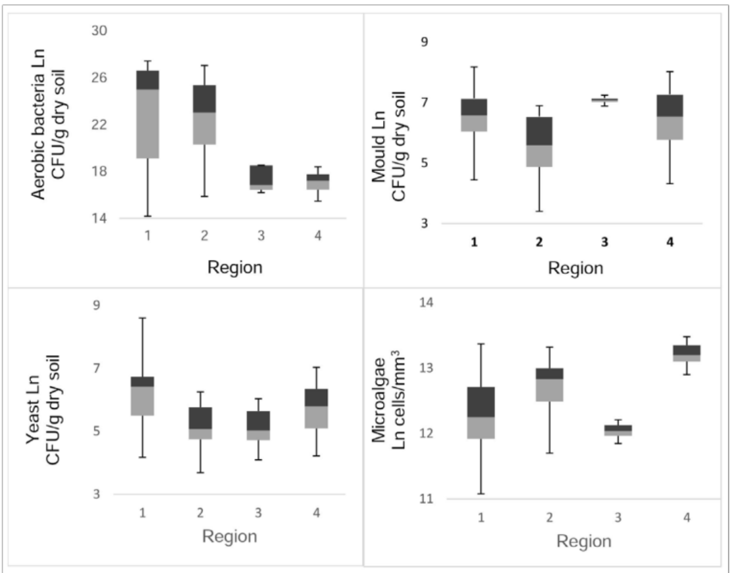

Figure 3: Box plot of data for bacteria, mould, yeast (CFU/g dry soil) and microalgae colonies (cells/mm3) established in the soil regions of the recharge zone of Lake Mapahuiña

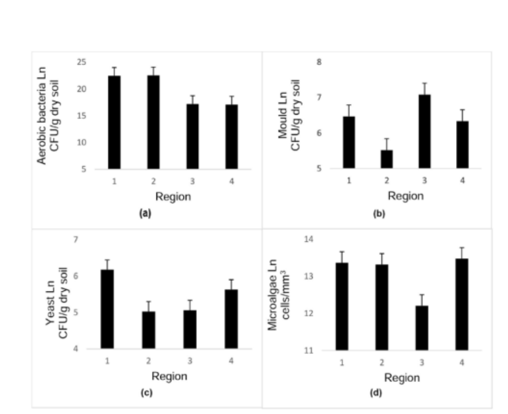

Figure 4: Number of microorganisms per soil region of the recharge zone of Lake Mapahuiña: (a) aerobic bacteria, (b) mould, (c) yeast and (d) microalgae colonies.

Region	Bacteria	Mould	Yeast	Microalgae‡
1	1.98 x 10^{11}	9.43 x 10^{2}	9.06 x 10^{2}	2.49 x 10^{5}
2	8.06 x 10^{10}	3.91 x 10^{2}	2.01 x 10^{2}	3.56 x 10^{5}
3	4.80 x 10^{7}	1.20 x 10^{3}	6.15 x 10^{2}	1.71 x 10^{5}
4	3.48 x 10^{7}	9.83 x 10^{2}	3.86 x 10^{2}	5.52 x 10^{5}

Table 3: Number of microorganisms determined for each study region†
†All values in CFU/g of dry soil; ‡cells/mm³

	Region	ln	N	Min.	Max.	SD	Var
Bacteria	1	22.99	117	14.18	27.41	4.59	20.85
	2	22.75	66	15.87	27.03	2.92	8.42
	3	17.23	9	16.20	18.53	1.00	0.89
	4	17.11	28	15.47	18.39	0.79	0.60
Mould	1	6.53	102	4.44	8.18	0.08	0.71
	2	5.60	56	3.40	6.90	0.12	0.85
	3	7.08	12	6.89	7.24	0.03	0.01
	4	6.43	30	4.32	8.03	0.20	1.12
Yeast	1	6.26	110	4.17	8.61	1.04	1.08
	2	5.08	47	3.69	6.25	0.70	0.48
	3	6.00	24	4.09	7.35	1.03	1.02
	4	5.68	30	4.22	7.04	0.80	0.62
Micro algae	1	12.27	125	11.08	13.37	0.55	0.30
	2	12.71	59	11.70	13.32	0.40	0.16
	3	12.04	15	11.85	12.21	0.11	0.01
	4	13.21	29	12.90	13.48	0.18	0.03

Table 4: Descriptive statistics† for microorganisms (Ln) for the four study regions of the recharge zone of Lake Mapahuiña
†Data generated by statistical software InfoStat; ln = natural logarithm; N = samples; Min. = minimum value recorded; Max. = maximum value recorded; SD = standard deviation; Var = variance

Discussion

Bacteria

The study was performed in a specific location amid different land uses where distinctive factors are influencing the soil bacterial communities. Throughout the recharge zone of Lake Mapahuiña the bacterial abundance was highest in Regions 1 (*páramo* vegetation) and 2 (*Azorella aretioides* vegetation), followed by Region 3 (*Pinus radiata* forest) and lastly Region 4 (Contours of Lake Mapahuiña) which produced the smallest bacterial count recorded. In particular, the availability of soil nutrients under the introduced *P. radiata* (Region 3) may be restricted by polyphenolic compounds present in pine conifers while the volcanic soil material present in Region 4 has been shown to reduce the respiration rate of soil bacteria (28). With no significant correlation with the physico-chemical characteristics of the soil and temperature and humidity generating a weak association towards microbial growth, the bacterial diversity and populations may be more dependent on the elemental availability from organic and inorganic matter (29,30) present in the rich taxonomical diversity of the plant communities in Regions 1 and 2. The distribution of bacteria in the study site was not uniform (ANOVA analysis), suggesting a connection to land use, type of vegetation in the regions (31) and several biotic factors including soil organisms and abiotic changes in nutrient supply of parent material (32).

Moulds

The results indicate significant differences (ANOVA test; $p < 0.05$) in the distribution of mould in the soil amid further evidence of the influence of different land uses on their growth as proposed by the study of Lauber (33). As shown in Figure 3, Regions 1 (*páramo* vegetation) and 4 (Contours of Lake Mapahuiña) were determined to have the greatest concentration of mould, followed by Region 2 (*Azorella aretioides* vegetation) and the lowest populations were observed in Region 3 (Pinus radiata forest). Here, the presence of pine as previously concluded, can decrease the fungal biomass in plantations containing this plant species (28).

Fungi more readily adapt to conditions of low soil moisture than bacteria (34), with the ability of these organisms to translocate water and support growth in materials or sites where there is no adequate water supply for growth. Region 1 showed a lower range in soil moisture, compared with Region 4 and 2, which had the following extent of moulds recorded.

The growths of bacteria and fungi in cold climates are affected by the soil temperature range, with optimum temperature growth below 30 °C and high temperatures decreasing their activity (35). Although a weak correlation was observed, there is not a wide range of soil temperature variability, with the highest temperature value recorded at 16 °C in Region 4 (Table 2). Accordingly, continual monitoring of temperature fluctuations in the study area may help to validate any alterations in bacteria and fungi populations, indicative of disturbances in the region.

Yeast

Yeasts were observed at lower abundance than bacteria and are unevenly distributed both in number and species type. In our study, the approximate yeast counts ranged between 5.1 to 6.3 ln CFU/g dry soil (Table 4), which is comparable to the study reported by Mestre (36) and is in good agreement with previous determinations (37). No significant correlation between pH and yeast growth was observed, in accordance with the study of di Menna (38), although Region 1 with a slightly more acidic soil pH was determined to have the highest concentration of yeast compared to the other regions.

Microalgae

As members of the soil microbial community, the importance of microalgae is its ability to contribute to the stability of the soil. A relationship between its concentration and the physicochemical characteristics of the region was not observed (Pearson $r<0.5$), concurring with previous studies on the dependence of edaphic factors for the propagation of microalgae (29,39). However the IDW analysis (Figure 5) of Region 4 shows the highest distribution of microalgae, corroborating with the existence of favorable conditions for microalgae development in close proximity to aquifers and wetland areas (29,40). Region 3 reported the smallest microalgae concentrations and together with the lowest recorded soil moisture content may be responsible for interrupting microalgae cellular processes (24). It is postulated that the observed differences in microalgae concentration and distribution is related to the various processes of colonization in *páramo* ecosystems. The difference in variability appears to be due to the study area and type of vegetation present at each region. Nevertheless, it should be emphasized that in the studied area of the recharge zone of Lake Mapahuiña in the Sangay National Park, a considerable amount of microalgae was found, having the potential of unearthing strains of interest for scientific and industrial applications.

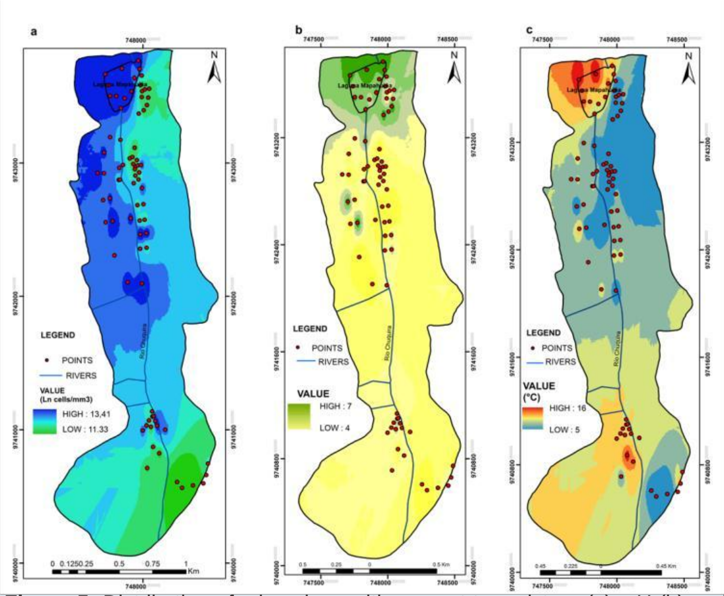

Figure 5: Distribution of microalgae with respect to moisture (a), pH (b) and temperature (c). Graphical analysis generated with ArcGIS Geostatistica Analyst.

Conclusions

The regions containing native *páramo* vegetation and on the banks of Lake Mapahuiña, the greatest wealth of microorganisms was recorded which was in contrast to the plantation of *Pinus radiata* which possibly influences the optimal growth of bacteria, microalgae and moulds. Soil pH in general demonstrates a positive correlation with bacterial growth; however, by having an acidic medium the study site may contain acidobacterial communities which counter the effect. The abundance of microorganisms varies by land use and vegetation type contained in each region. A direct dependence on physicochemical factors such as pH, temperature and humidity was not clear and further analysis of more soil edaphic factors is needed to better understand the microbial behavior. Microalgae were found throughout the study area with a higher amount recorded in the region with a body of water. Moisture at this site remains a characteristic for its optimum growth.

The recharge area of the microbasin of the river Zula, which is part of the Andean highlands, contains a significant amount of microorganisms and remains a favorable candidate to provide bacterial strains with possible biotechnological interest.

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