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Physicochemical and Bacteriological Profile of Bilanko and Ngamakala Peat Bog Soils (Republic of Congo)

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Abstract

Peatlands are unique and complex natural ecosystems that are part of the most important carbon reservoirs on our planet, home to a diversity of microorganisms responsible for fermentation, humification or peat. The aim is to understand chemical and biological indicators of peatland soils. This work aims to determine the physicochemical and bacteriological profile and lipolytic activity of soil bacteria in Bilanko peatlands. The bacterial profile with the production of lipases is carried out by classical microbiology techniques. The results show that the soils are moderately acidic with temperatures of 27.8˚C ± 0.01˚C for Bilanko and 27.1˚C ± 0.57˚C for Ngamakala. The electroconductivity (EC) varies from (9.52 ± 0.002) µS/cm to (39.01 ± 1.4) µS/cm with low turbidity of (2.04 ± 0.66) mg/L to (31.02 ± 0.84) mg/L and low ion concentrations with, however, a richness in phenolic compounds for Bilanko compared to Ngamakala. FMAT diversity ranged from $(1.71 \pm 0.88)\cdot10^4$ UFC/g to $(2.92 \pm 0.07)\cdot10^5$ UFC/g for Bilanko and $(1.30 \pm 0.73)\cdot10^4$ UFC/g to $(2.89 \pm 0.06)\cdot10^4$ UFC/g for Ngamakala. *Bacillus* loads ranged from $(5.20 \pm 1.40)\cdot10^3$ CFU/g to $(1.22 \pm 0.13)\cdot10^4$ CFU/g and from $(1.11 \pm 0.13)\cdot10^4$ CFU/g to $(9.20 \pm 2.05)\cdot10^3$ CFU/g; enterobacteria loads from $(1.40 \pm 0.76)\cdot10^3$ CFU/g to $(8.80 \pm 1.73)\cdot10^3$ CFU/g and from $(1.01 \pm 0.02)\cdot10^3$ CFU/g to $(9.20 \pm 2.05)\cdot10^3$ CFU/g; in *Pseudomonas* from 0 to $(2.30 \pm 0.53)\cdot10^2$ CFU/g and from 0 to $(8.90 \pm 2.35)\cdot10^2$ CFU/g for Bilanko and Ngamakala respectively. These results reveal a variation in bacterial similarity and distribution in the Bilanko and Ngamakala peat bogs.

Keywords

Bacteria, Lipase, Polyphenol, Soil, Peat Bog, Republic of Congo

1. Introduction

In today's environmentally conscious society, wetlands are of particular importance, and their benefits are widely recognized. The specific characteristics and constitution of these environments are sufficient criteria to justify their protection [1]. Peatlands are found in regions where precipitation exceeds the rate of evapotranspiration, resulting in water saturation. They are found in hot, cold, wet and dry climates, but predominantly in the northern hemisphere, in Asia (38.4%), North America (31.6%), Europe (12.5%) and South America (11.5%) [2] [3]. Depending on their location, peat can be permanently frozen, as in the case of palsas found in periglacial environments, but can also take the form of peat swamp forests, such as mangroves, in the tropics [4]. To date, peatlands have been found in 180 countries, covering large areas [5]. Peatlands form in depressions where water accumulates permanently, fed either by precipitation or springs. The lack of oxygen in a water-saturated environment slows down the decomposition of organic matter. There are several types of peat bog, which can be classified according to hydrology, nutrients and pH. Umbrotrophic bogs, fed by atmospheric precipitation, have low nutrient concentrations and acid pH values. Minerotrophic bogs receive water and nutrients from precipitation and local groundwater and have pH values close to neutral. They can be eutrophic, rich in assimilable nitrate and phosphate, or oligotrophic, poor in nutrients. These characteristics determine plant and microbial diversity, plant productivity, and the rate of litter decomposition [6] [7]. The characteristic species of northern temperate and boreal bogs are Bryophytes of the genus Sphagnum. In these bogs, the growth of sphagnum mosses is vital for carbon sequestration and storage, as they effectively create a moist, acidic environment that prevents, or at least slows, decomposition [8]. Furthermore, research into the role of peatlands in the global carbon cycle in the face of global warming shows that a rise in temperature leads to an increase in microbial activity, strong decomposition of organic matter, and consequently, a significant release of CO_2 through microbial respiration. It also leads to significant carbon fixation through photosynthesis [9]. Despite the discovery of peat bogs in the Republic of Congo, very few studies have been carried out on their microbiology. The results of analyses obtained by [10] on peatland soils in Likouala showed the presence of a diversity of hydrolase-producing bacteria. Similarly, those obtained by [11] using molecular biology techniques on bacteria isolated from Likouala peat bog soils revealed the presence of Bacillus, Pseudomonas and Enterobacter bacteria. However, very little information is available on chemical and, above all, biological indicators for tropical acid soils. The aim of this study was to investigate the distribution of bacteria, taking into account the physical and chemical characteristics of Bilanko and Ngamakala peat bog soils.

2. Methods

2.1. Sampling

The samples were collected in the Bilanko and Ngamakala peat bogs in the peri-

od from July to August 2022. Fifteen (15) points are randomly selected, including 8 points for the Bilanko site (point 1 to point 8) and 7 for the Ngamakala site (point 9 to point 15). At each point, three (3) samples are taken at two-week intervals. Two samples, one for bacteriological analysis and the other for physico-chemical analysis were taken from the surface 0 - 10 cm of the peat bog using a sterile spatula and transferred to sterile plastic bags for transport in a cool box. These samples are dried in the laboratory at room temperature for a week and then stored in the Cellular and Molecular Biology Laboratory of the Faculty of Science and Technology at Marien Ngouabi University for analysis.

2.2. Analysis of Physico-Chemical Parameters

Temperature, pH, electroconductivity, salinity and total dissolved solids were measured directly during sampling, using an ExStik II pH/Conductivity Meter electrode. Turbidity was measured with a measurement range of 5 to 400 units; ammonia with a measurement range of 0 to 50 mg/l; nitrite with a measurement range of 0 to 0.5 mg/l; calcium hardness with a measurement range of 0 to 500 mg/l; potassium with a measurement range of 0 to 12 mg/l; and magnesium with a measurement range of 0.005% - 2% magnesium. Turbidity, ammonia, nitrite, nitrate, potassium ion and calcium hardness were measured using a SpectroDirect/PC Spectro II_3 04/2008 spectrophotometer with wavelengths of 520 nm; 640 nm; 410 nm; 520 nm; 520 nm and 570 nm respectively.

2.3. Determination of Total Polyphenols and Flavonoids

2.3.1. Determination of Total Polyphenols

Total polyphenols were determined using a spectrophotometer. We determined the optical densities of our peat samples and compared the result with that obtained using a gallic acid standard of known concentration. The assay was carried out as follows: 0.1 mL of peat extract obtained after maceration, contained in a test tube, was followed by 0.9 mL distilled water; 0.9 mL Folin-Ciocalteu reagent (1N); then immediately 0.2 mL Na_2CO_3 solution (20%). The resulting mixture was incubated at room temperature for 40 minutes in the dark. Absorbance was then measured with a spectrophotometer at 725 nm against a methanol solution used as a blank. It should be noted that a calibration line was previously performed with gallic acid under the same conditions as the samples to be analyzed. The results obtained were expressed in mg gallic acid equivalent per 100 grams of dry matter (mgEGa/100 gMs) [12].

2.3.2. Determination of Total Flavonoids

Total flavonoids were also determined using a spectrophotometer, as follows: 250 µL of extract and 1 mL of distilled water were successively introduced into a test tube. At the initial time (0 min), 75 µL of NaNO2 solution (5%) was added, followed by 75 µL of $AlCl_3$ (10%) 5 min later. After 6 minutes, 500 µL of NaOH (1 N) and 2.5 mL of distilled water were successively added to the mixture. The absorbance of the resulting mixture was measured directly with a UV-visible spectrophotometer at 510 nm, and the results were expressed as mg rutin equivalent per

100 grams of dry matter (mgERu/100 gMs). A calibration curve was constructed using Rutin standard solutions prepared at different concentrations [12].

2.4. Microbiological Analysis

Bacterial Enumeration

10 g of peat from each sample was removed and added to 90 ml of sterile physiological water in an Erlenmeyer flask for a stock solution (SM). 1 ml of the stock solution was taken and transferred to the test tube, which already contained 9 ml of physiological liquid, and mixed to obtain a homogeneous 10 - 1 solution. This was followed by 10 - 2, 10 - 3, 10 - 4 to 10 - 5. [13] [14]. Next, 0.1 ml of each diluent was inoculated onto different culture media previously poured onto Petri dishes, followed by surface inoculation. The Petri dishes were then placed in the oven at 37˚C. Readings were taken after 24 hours. The microorganism load of the samples was determined in CFU/g using the following formula [15].

$$\text{UFC/g} = \frac{\text{Number of colonies}}{\text{Dilution factor} \times \text{Inoculated volume}}$$

1) Purification and Phenotypic Identification of Isolates

Colonies were purified on Mossel, EMB and Cetrimide. After several replicates using the streak technique, the plates were then incubated in an oven at 37˚C and observed after 24 hours. Colonies presenting an identical appearance in a Petri dish were then considered pure, stored in sterile Eppendorfs containing 800 µL of liquid LB and 200 µL of glycerol, and kept cool at 4˚C [16] [17]. Isolates were phenotypically identified by applying classical microbiology techniques, based on the investigation of a number of phenotypic characteristics (colony morphology, cell morphology, KOH and catalase production).

2) Lipolytic Enzyme Production

1 mL olive oil and 1.5 g agar were added to 100 mL distilled water, the mixture was brought to a boil and sterilized in the autoclave at 120˚C for 20 min. Once the medium had cooled (45˚C to 500˚C), it was poured and solidified. The wells were made and 50 µL of supernatant from a culture made in Erlenmeyer flasks containing 20 mL of liquid LB medium then incubated at 37˚C for 24 h and centrifuged at 600/min for 10 min was poured in. The plates were incubated at 37˚C between 24 h and 48 h. Lipid degradation is characterized by visual observation of the clear, transparent zone on the agar after reaction with Lugol [18] [19].

2.5. Analysis and Processing of Results

Microsoft Excel, GraphPad, XLSTAT, and Past were used for statistical analysis of the data linked to the graphs and other diagrams.

3. Results

3.1. Analysis of Physico-Chemical Parameters

Tables 1 and Table 2 show the results of physicochemical parameter analyses of Bilanko and Ngamakala peat bog soils.

Table 1. Physicochemical parameters of Bilanko peat bog soils.

Site	Points	pH	T (°C)	CE (µs/cm)	Turb (FAU)	TDS	Ca^{2+} (mg/l)	Mg^{2+} (mg/l)	K^+ (mg/l)	NH_4^+ (mg/l)	NO_3^- (mg/l)	NO_2^- (mg/l)	Alcalinité (mg/ $lCaCO_3$)	Salinité
Bilanko	1	6.23 ±	**26.7 ±**	18 ±	6 ± 1.33	**5.94 ±**	2.9 ± 0.26	0.83 ±	**1.4 ±**	0.02 ±	**0.12 ±**	0.03 ±	**14 ± 1.66**	10.24 ±
		0.017	**0.13**	0.66		**0.026**		0.36	**0.2**	0.006	**0.011**	0.006		1.66
	2	5.95 ±	27.1 ±	10.05 ±	**2 ± 0.66**	4.52 ±	1.37 ±	0.28 ±	0.15 ±	0.03 ±	0.45 ±	0.04 ±	3.41 ±	5.41 ±
		0.013	0.066	1.33		0.013	0.01	0.11	0.02	0.013	0.06	0.008	0.88	0.88
	3	6.40 ±	27.60 ±	24.30 ±	4.01 ±	5.01 ±	2.20 ±	0.78 ±	1.50 ±	0.05 ± 0.02	0.24 ±	**0.09 ±**	12.50 ±	13.94 ±
		0.020	0.40	2.01	0.001	1.11	0.20	0.34	0.13		0.06	**0.011**	0.639	0.64
	4	5.60 ±	26.90 ±	22.01 ±	16.01 ±	4.10 ±	1.46 ±	0.62 ±	1.30 ±	0.04 ±	0.21 ±	0.08 ±	8.54 ±	12.69 ±
		0.006	0.088	1.33	2.01	0.066	0.02	0.01	0.15	0.011	0.04	0.02	1.84	1.84
	5	**5.55 ±**	27.40 ±	12.49 ±	4.01 ±	4.50 ±	1.28 ±	**0.26 ±**	**0.14 ±**	0.03 ±	0.41 ±	0.05 ±	**3.16 ±**	6.79 ±
		0.033	0.044	0.66	0.66	0.26	0.006	**0.005**	**0.015**	0.013	0.13	0.015	**1.61**	1.61
	6	**6.44 ±**	**27.80 ±**	**26.01 ±**	18.01 ±	5.02 ±	**1.14 ±**	0.49 ±	0.17 ±	**0.01 ±**	0.38 ±	0.04 ±	5.49 ±	**14.94 ±**
		0.006	**0.001**	**0.001**	2.02	0.66	**0.02**	0.005	0.006	**0.004**	0.07	0.011	2.20	**4.20**
	7	6.31 ±	26.9 ±	24.01 ±	**18.01 ±**	**4.10 ±**	1.63 ±	0.74 ±	0.20 ±	0.05 ±	0.29 ±	**0.03 ±**	5.02 ±	13.95 ±
		0.013	0.044	2.02	**3.33**	**0.13**	0.01	0.01	0.06	0.017	0.06	**0.02**	1.98	3.98
	8	5.78 ±	27.40 ±	**9.52 ±**	7.01 ±	4.28 ±	**5.03 ±**	**1.02 ±**	0.56 ±	**0.05 ±**	**1.53 ±**	0.08 ±	12.57 ±	**5.07 ±**
		0.026	0.177	**0.002**	1.33	0.006	**0.01**	**0.015**	0.03	**0.006**	**0.13**	0.011	3.33	**1.55**

pH = hydrogen potential; T = temperature; EC = electrical conductivity; Turb = turbidity; TDS = total dissolved solids; Ca^{2+} = calcium ions; Mg^{2+} = magnesium ions; K^+=potassium ions; NH_4^+ = ammonia ion; NO_3^- = nitrite; NO_2^- = nitrate.

Table 2. Physicochemical parameters of Ngamakala peat bog soils.

Sites	Points	pH	T (°C)	CE (µs/cm)	Turb (FAU)	TDS	Ca^{2+} (mg/l)	Mg^{2+} (mg/l)	K^+ (mg/l)	NH_4^+ (mg/l)	NO_3^- (mg/l)	NO_2^- (mg/l)	Alcalinité (mg/l $CaCO_3$)	Salinité
Ngamakala	9	6.50 ± 0.054	**26.20 ± 0.02**	20.01 ± 1.45	10.01 ± 0.07	5.96 ± 0.07	2.91 ± 0.013	0.84 ± 0.01	1.20 ± 0.79	0.02 ± 0.003	4.01 ± 0.044	**0.01 ± 0.004**	**15.01 ± 1.66**	11.61 ± 1.88
	10	**5.63 ± 0.09**	26.4 ± 0.43	22.6 ± 1.6	**4 ± 0.02**	**6.01 ± 0.023**	2.43 ± 0.02	0.36 ± 0.42	1.60 ± 0.005	**0.04 ± 0.003**	3.01 ± 0.79	0.01 ± 0.0003	12 ± 1.07	13.21 ± 0.81
	11	6.10 ± 0.40	27.01 ± 0.01	25.40 ± 1.43	4.01 ± 0.06	4.62 ± 0.11	4.92 ± 0.006	1.43 ± 0.01	1.40 ± 0.01	0.03 ± 0.004	4.01 ± 0.01	0.02 ± 0.006	11.01 ± 2.01	14.81 ± 0.067
	12	5.79 ± 0.076	27.30 ± 0.02	**9.53 ± 0.36**	6.01 ± 1.60	4.28 ± 0.07	**5.06 ± 0.26**	1.04 ± 0.16	0.57 ± 0.015	**0.05 ± 0.01**	1.59 ± 0.026	**0.07 ± 0.004**	13.01 ± 0.64	**5.08 ± 0.84**
	13	**6.91 ± 0.044**	26.90 ± 0.07	**39.01 ± 1.40**	**31.01 ± 0.84**	4.60 ± 0.30	4.93 ± 0.26	**1.45 ± 0.015**	**4.39 ± 0.04**	0.04 ± 0.007	**4.39 ± 0.16**	0.06 ± 0.003	12.01 ± 3.33	**23.81 ± 1.90**
	14	6.83 ± 0.03	26.80 ± 0.09	30.30 ± 1.04	4.01 ± 0.04	**4.02 ± 0.80**	2.05 ± 0.03	**0.05 ± 0.003**	1.31 ± 0.14	0.02 ± 0.0003	4.01 ± 0.02	0.01 ± 0.002	10.01 ± 1.61	18.06 ± 0.14
	15	5.96 ± 0.033	**27.10 ± 0.57**	12.01 ± 0.84	3.01 ± 0.52	5.40 ± 1.13	**1.55 ± 0.01**	0.32 ± 0.005	**0.17 ± 0.005**	0.04 ± 0.002	**0.52 ± 0.005**	0.03 ± 0.001	**3.85 ± 0.99**	6.54 ± 2.20

pH = hydrogen potential; T = temperature; EC = electrical conductivity; Turb = turbidity; TDS = total dissolved solids; Ca^{2+} = calcium ions; Mg^{2+} = magnesium ions; K^+ = potassium ions; NH_4^+ = ammonia ion; NO_3^- = nitrite; NO_2^- = nitrate

Table 1 shows the results for the Bilanko site. Mean temperatures ranged from 26.7˚C ± 0.13˚C to 27.8˚C ± 0.001˚C, with mean pH values ranging from 5.55 ± 0.033 to 6.44 ± 0.006. Electrical conductivity ranged from (9.52 ± 0.002) µS/cm to (26.01 ± 0.001) µS/cm, with low turbidity ranging from (2.02 ± 0.66) mg/L to (18.01 ± 3.33) mg/L. The mean TDS of these samples is very low, ranging from 4.10 ± 0.026 to 5.94 ± 0.13, with a low ion concentration. The ions are respectively : Ca^{2+} from (5.03 ± 0.01) mg/L to (1.14 ± 0.02) mg/L; Mg^{2+} from (0.26 ± 0.005) mg/L to (1.02 ± 0.015) mg/L; K^{+} from (0.14 ± 0.015) mg/L to (1.4 ± 0.2) mg/L; NH_4^+ from (0.01 ± 0.004) mg/L to (0.05 ± 0.006) mg/L; NO_2^- from (0.03 ± 0.02) mg/L to (0.09 ± 0.011) mg/L and NO_3^- from (0.03 ± 0.02) mg/L to (0.09 ± 0.011) mg/L. Salinity of Bilanko bog soils has a mean value of (5.07 ± 1.55) g/L to (14.94 ± 4.20) g/L and alkalinity of (3.16 ± 1.61) mg/l$CaCO_3$ to (14.01 ± 1.66) mg/l$CaCO_3$.

Table 2 shows the results for physicochemical parameters at the Ngamakala site. Average soil temperatures range from 26.2˚C ± 0.02˚C to 27.10˚C ± 0.57˚C, with pH values from 5.63 ± 0.09 to 6.91 ± 0.044. Electrical conductivity ranged from (9.53 ± 0.36) µS/cm to (39 ± 1.4) µS/cm. Turbidity ranged from (4 ± 0.02) mg/L to (31 ± 0.84) mg/L. The mean TDS of these samples is very low, ranging from 4.02 ± 0.8 to 6.01 ± 0.023. The ion concentrations of these samples are low. The ions are respectively: Ca^{2+} from (1.55 ± 0.01) mg/L to (5.06 ± 0.26) mg/L; Mg^{2+} from (0.051 ± 0.003) mg/L to (1.45 ± 0.015) mg/L; K^{+} from (0.17 ± 0.005) mg/L to (4.39 ± 0.04) mg/L; NH_4^+ from (0.05 ± 0.01) mg/L to (0.04 ± 0.003) mg/L; NO_2^- from (0.52 ± 0.005) mg/L to (4.39 ± 0.16) mg/L and NO_3^- from (0.01 ± 0.004) mg/L to (0.07 ± 0.004) mg/L. The salinity of Ngamakala peat bog soils averaged (3.85 ± 0.99) g/L to (15 ± 1.66) g/L and alkalinity (5.08 ± 0.84) mg/l$CaCO_3$ to (23.81 ± 1.9) mg/l$CaCO_3$, (5.07 ± 1.55) g/L to (14.94 ± 4.20) g/L and alkalinity from (3.16 ± 1.61) mg/l$CaCO_3$ to (14.01 ± 1.66) mg/l$CaCO_3$.

3.2. Determination of Total Polyphenols and Flavonoids

3.2.1. Determination of Total Polyphenols and Flavonoids Using Aqueous Extracts

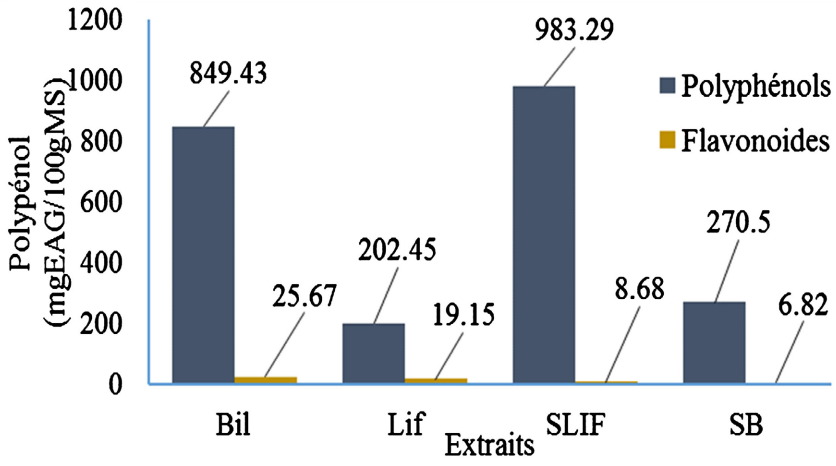

Figure 1. Total polyphenol and flavonoid contents with aqueous extracts (Bil = Bilanko soil, Lif = Ngamakala soil, SLIF = Ngamakala sphagnum, SB = Bilanko sphagnum).

Figure 1 shows the quantification of polyphenols and flavonoids in soil and sphagnum moss samples from peat bogs. It reveals a high total polyphenol content of 983.29 mgEAG/100 gMS for the SLif sample, followed by Bil with content of 849.43 mgEAG/100 gMS, and 202.45 and 270.5 mgEAG/100 Gms respectively for the LiF and SB samples. Total flavonoid levels were 25.87 and 19.15 mgEAG/100 gMS respectively for BiL and LiF, compared with 8.68 and 6.82 mgEAG/100 gMS for SLIF and SB.

3.2.2. Determination of Total Polyphenols and Flavonoids Using Hydroethanol Extracts

Figure 2 shows the total polyphenol and flavonoid contents of peat bog soils and sphagnum mosses after quantitative analysis by a UV-visible spectrophotometer. Total polyphenol and flavonoid contents are high in the Bil and LiF samples, with values of 3546.66 and 3200.86 mgEAG/100 gMS for total polyphenols and 61.98 and 61.98 mgEAG/100 gMS for flavonoids respectively.

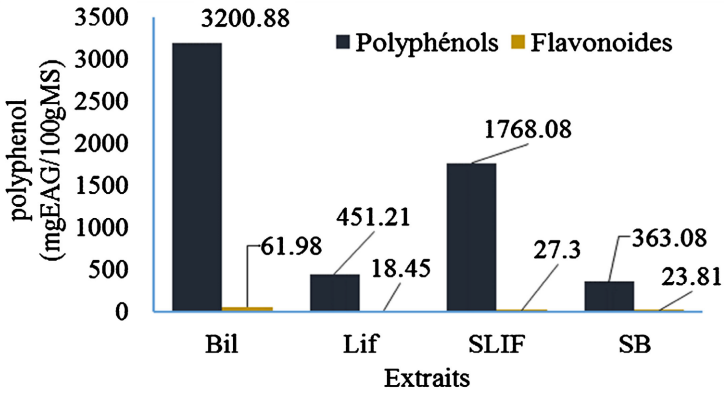

Figure 2. Total polyphenol and flavonoid contents with hydroethanol extracts (Bil = Bilanko soil, Lif = Ngamakala soil, SLIF = Ngamakala sphagnum, SB = Bilanko sphagnum).

3.3. Bacteria Count

3.3.1. Bacterial Counts at Bilanko

Table 3 shows the bacterial count in Bilanko peat bog soils. The total mesophilic flora on PCA varies from sample to sample, ranging from $(1.71 \pm 0.88)\cdot10^4$ CFU/g to $(2.92 \pm 0.07)\cdot10^5$ CFU/g; that of the Bacillus genus on Mossel ranges from $(5.20 \pm 1.40)\cdot10^3$ CFU/g to $(1.22 \pm 0.13)\cdot10^4$ CFU/g; that of enterobacteria on EMB varies from $(1.40 \pm 0.76)\cdot10^3$ CFU/g to $(8.80 \pm 1.73)\cdot10^3$ CFU/g. Finally, the isolation of bacteria of the genus *Pseudomonas* revealed concentrations ranging from 0 to $(2.30 \pm 0.53)\cdot10^2$ CFU/g.

Table 3. Bacterial counts from Bilanko samples in CFU/g.

Site	Points	Medium			
		FMAT	*Bacillus*	Entérobactéries	*Pseudomonas*
Bilanko	Point 1	$(2.92 \pm 0.07)\cdot10^5$	$(1.22 \pm 0.13)\cdot10^4$	$(1.40 \pm 0.76)\cdot10^3$	0

Continued

	Point 2	(3.90 ± 0.18)·10⁴	(9.80 ± 0.66)·10³	(5.80 ± 0.79)·10³	(1.80 ± 0.06)·10²
	Point 3	(3.90 ± 0.35)·10⁴	(8.50 ± 0.17)·10³	(8.10 ± 0.73)·10³	(1.83 ± 0.09)·10²
	Point 4	(5.70 ± .80)·10⁴	(7.90 ± 0.66)·10³	(3.90 ± 0.67)·10³	(2.20 ± 0.43)·10²
Bilanko	**Point 5**	(5.90 ±0.66)·10⁴	(9.10 ± 0.06)·10³	**(8.80 ± 1.73)·10³**	**(3.80 ± 0.6)·10²**
	Point 6	(8.10 ± 0.88)·10⁴	**(5.20 ± 1.40)·10³**	(7.20 ± 0.98)·10³	(1.50 ± 0.13)·10²
	Point 7	(3.60 ± 0.26)·10⁴	(6.80 ± 0.60)·10³	(1.80 ± 0.73)·10³	(2.30 ± 0.53)·10²
	Point 8	**(1.71 ± 0.88)·10⁴**	(7.20 ± 0.15)·10³	(4.30 ± 1.20)·10³	(1.87 ± 0.75)·10²

3.3.2. Bacterial Counts at Ngamakala

Table 4 shows the bacterial counts for the Ngamakala peat bogs. Total aerobic mesophilic flora on PCA ranged from (1.30 ± 0.73)·10⁴ to (2.89 ± 0.06)·10⁴ UFC/g. The concentration of Bacillus bacteria ranged from (1.11 ± 0.13)·10⁴ to (9.20 ± 2.05)·10³ UFC/g. Enterobacteria ranged from (1.00 ± 0.02)·10³ to (9.20 ± 2.05)·10³ CFU/g. Pseudomonas loads ranged from 0 to (8.90 ± 2.35)·10² CFU/g.

Table 4. Bacterial counts from Ngamakala samples in CFU/g.

Site	Points	Medium			
		FMAT	*Bacillus*	Entérobactéries	*Pseudomonas*
	Point 1	(2.88 ± 0.66)·10⁴	**(9.20 ± 2.05)·10³**	(9.10 ±1.98)·10³	**0**
	Point 2	(1.89 ± 0.71)·10⁴	(1.21 ± 0.09)·10³	(3.30 ±0.76)·10³	(2.60 ± 0.29)·10²
	Point 3	(2.80 ± 0.42)·10⁴	(1.35 ± 0.34)·10³	**(1.00 ± 0.02)·10³**	(7.10 ± 0.98)·10²
Ngamakala	**Point 4**	(2.09 ± 0.13)·10⁴	(6.30 ± 1.04)·10³	(6.50 ± 2.09)·10³	0
	Point 5	(2.27 ± 0.01)·10⁴	(8.90 ± 2.19)·10³	(1.40 ± 0.03)·10³	(4.60 ± 1.57)·10²
	Point 6	**(2.89 ± 0.06)·10⁴**	(7.90 ± 0.32)·10³	**(9.80 ± 3.24)·10³**	**(8.90 ± 2.35)·10²**
	Point 7	**(1.30 ± 0.73)·10⁴**	**(1.11 ± 0.13)·10⁴**	(7.20 ± 2.10)·10³	(1.20 ± 0.83)·10²

3.4. Similarity of Bacterial Loads between Points

- **Bilanko Site**

Bacterial load results for the Bilanko site are shown in the cladograms in Figure 3. In Figure 4(a), certain sampling points (4 and 5; 2 and 3; 1 and 8) show a similarity with a variable load distribution. For bacteria of the *Bacillus* genus (Figure 3(b)), there is a variable distribution and similarity of bacterial load distribution between points 7 and 8; 3 and 5, with a slight difference between points 6, 1, 2 and 4. The similarity of the enterobacteria concentration (Figure 3(c)) between the points showed that the enterobacteria load of the sampling points is variable. Points 1 and 7; 4 and 8; 3 and 5 show similar repairs in terms of bacterial load, with a slight difference at points 2 and 6. The *Pseudomonas* load (Figure 3(d)) varies from one sampling point to another. Points 3 and 2; 4 and 7, 8 and 5 show a high degree of similarity in bacterial load, with slight variations in similarity between points on the site.

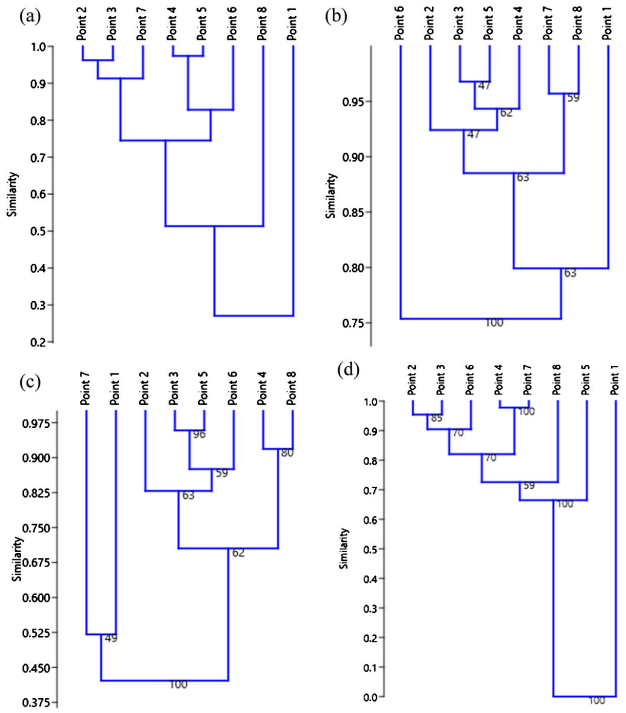

Figure 3. Dendrogram of bacterial load similarity between Bilanko site points (a = total flora concentration; b = *Bacillus* concentration; c = Enterobacteria concentration; d = *Pseudomonas* concentration).

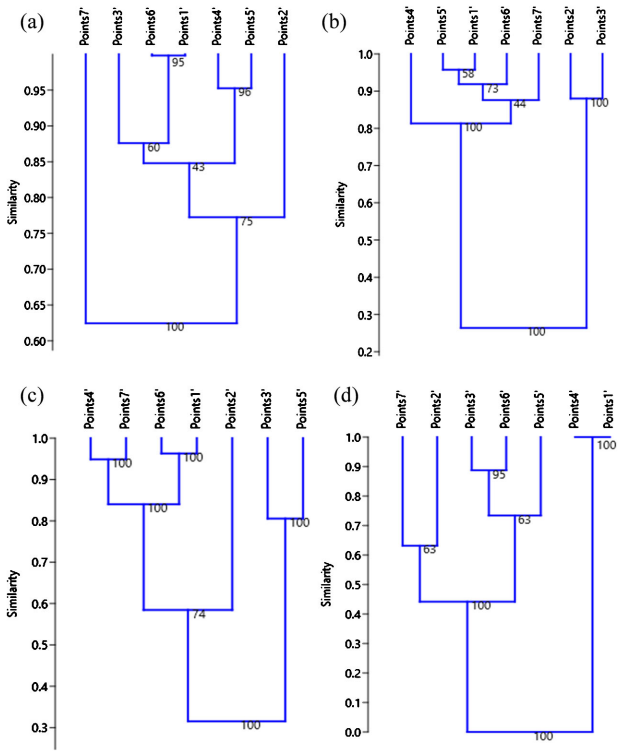

Figure 4. Dendrogram of bacterial load similarity between points at the Ngamakala site (a = total flora concentration; b = *Bacillus* concentration; c = Enterobacteria concentration; d = *Pseudomonas* concentration).

- **Ngamakala Site**

The cladograms in Figure 4 show the different bacterial concentrations at the sampling points at the Ngamakala site. The results obtained show a variable distribution of bacterial concentration at the different points of the site. Points 1' and 6'; 4' and 5' show a high degree of similarity in the distribution of total flora loads (Figure 4(a)), while points 2', 3' and 7' show a low degree of similarity. *Bacillus* concentration (Figure 4(b)) shows a variable distribution across the points. Points 1' and 5'; 2' and 3' have strong similarities in load repair compared with points 4', 6' and 7', which have a slight difference with the other points on the site. The similarity of enterobacteria concentration (Figure 4(c)) is variable. Points 4' and 7'; 6' and 1'; 3' and 5' show similar repairs in terms of bacterial load, but differ from 2'. The variation in *Pseudomonas* concentration (Figure 4(d)) varies from point to point. Points 7' and 2'; 5' and 6'; 4' and 1' show a strong similarity in load, whereas point 5' shows no similarity with the site points.

3.5. Breakdown of Physicochemical Parameters and Bacterial Loads by Sampling Point

- **Bilanko site**

The CCA in Figure 5 shows the correlation between the distribution of the bacterial community and the distribution of physicochemical parameters. The results show that Enterobacteriaceae, *Pseudomonas* and *Bacillus* are most represented in points 2, 3, 4, 5, 7 and 8, where TDS, alkalinity and temperature are high. FMAT, on the other hand, is represented at points 1 and 6. This distribution does not vary proportionally with turbidity, pH and electrical conductivity.

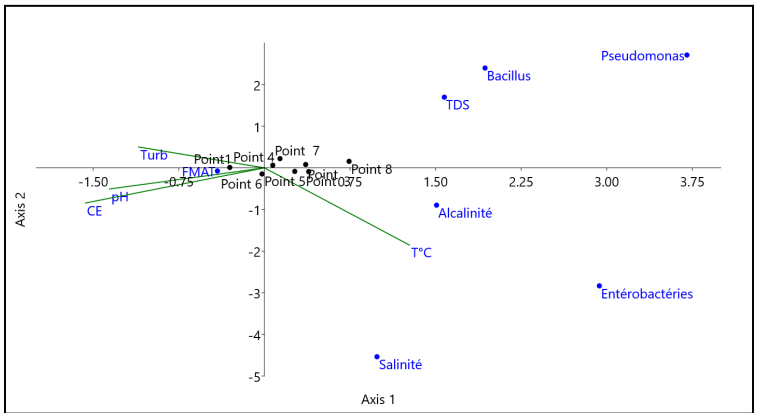

Figure 5. CCA distribution of bacteria according to the distribution of physicochemical parameters at Bilanko sampling points, taking into account two axes CCA1 and CCA2.

Figure 6 shows the CCA correlating bacterial community distribution with ion distribution at the Bilanko site. These results show that points 2, 3, 4, 5, 7 and 8 present high loads of Enterobacteriaceae, *Pseudomonas* and *Bacillus* corresponding with high concentrations of Ca^{2+}; Mg^{2+}; K^{+}; NH_4^{+} and NO_2^{-} against a low concentration of NO_3^{-}. Whereas with FMAT represented at points 1 and 6, the distribution does not vary proportionally Ca^{2+}; Mg^{2+}; K^{+}; NH_4^{+} NO_2^{-} and NO_3^{-}.

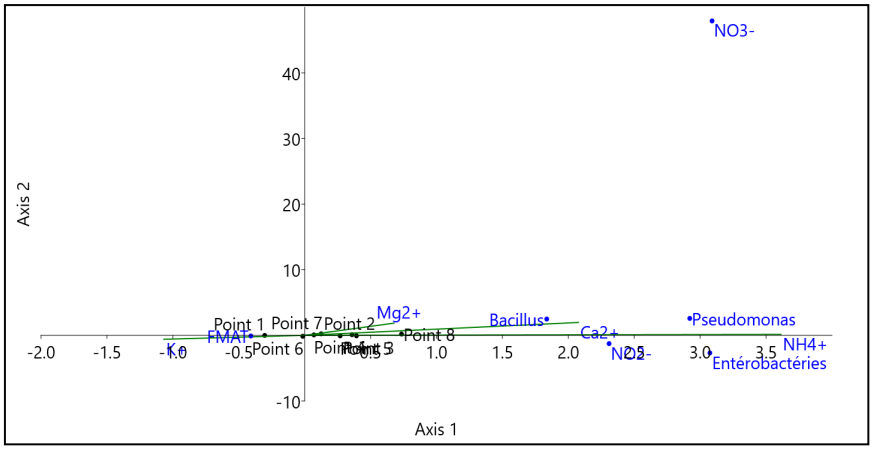

Figure 6. CCA distribution of bacteria as a function of ion distribution in Bilanko sampling points taking into account two axes CCA1 and CCA2.

- **Ngamakala Site**

At the Ngamakala site, the CCA in **Figure 7** shows that FMAT and the genus *Pseudomonas* are best represented at points 2, 3 and 5, where TDS, alkalinity, turbidity and electrical conductivity are high. On the other hand, Enterobacteriaceae and *Bacillus* are more represented at points 1, 4, 6 and 7; this distribution does not depend on temperature and pH.

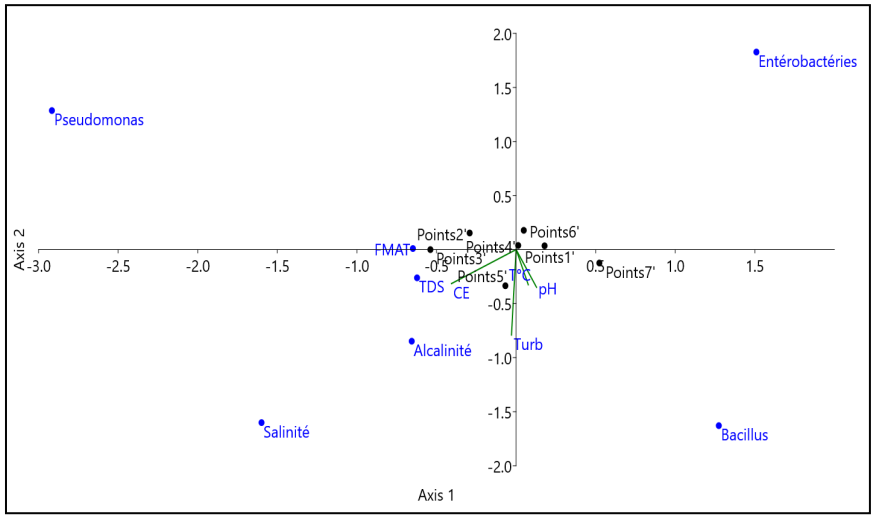

Figure 7. CCA distribution of bacteria according to the distribution of physicochemical parameters in Ngamakala sampling points, taking into account two axes CCA1 and CCA2.

Figure 8 shows the CCA correlating bacterial community distribution with ion distribution at the Ngamakala site. Points 2 and 3 are high in FMAT and Pseudomonas, but low in ions. Point 5 is rich in FMAT and Pseudomonas, with high concentrations of Ca^{2+}; Mg^{2+}; K^{+}; NO_3^- and NO_2^-. Points 1, 4, 6 and 7 show high concentrations of Enterobacteriaceae and *Bacillus*, with distributions that do not vary proportionally Ca^{2+}; Mg^{2+}; K^{+}; NH_4^+ and NO_2^- and NO_3^-.

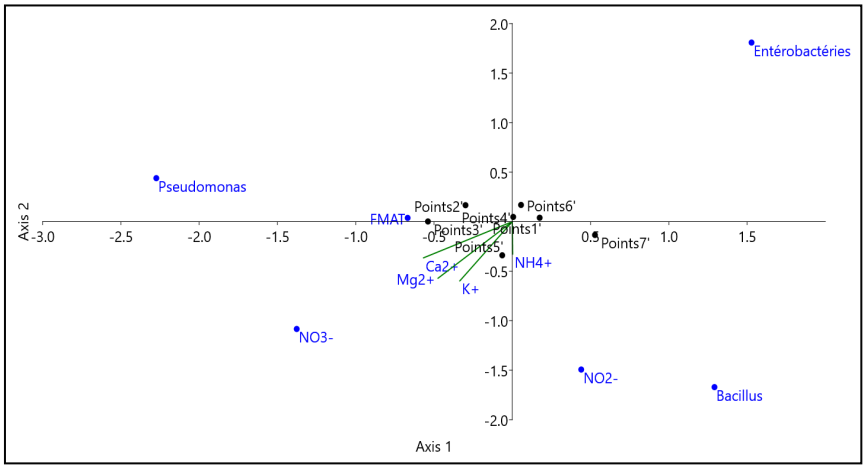

Figure 8. CCA distribution of bacteria as a function of ion distribution in Ngamakala sampling points taking into account two axes CCA1 and CCA2.

3.6. Purification of Bacterial Isolates

Table 5 shows a total of 67 isolates obtained from the 2 sites. 36 isolates from the Bilanko site: 17 bacteria from the *Bacillus* genus, *i.e.* 47.22%; 4 bacteria from the Enterobacteriaceae genus, *i.e.* 11.11%; 5 bacteria from the *Pseudomonas* genus, *i.e.* 13.89%; and 10 bacteria from an unidentified genus, *i.e.* 27.78%. 31 isolates for the Ngamakala site: 17 bacteria of the *Bacillus* genus, *i.e.* 54.81%; 3 bacteria belonging to the Enterobacteriaceae genus, *i.e.* 9.68%; 3 bacteria of the *Pseudomonas* genus, *i.e.* 9.68% and 8 bacteria of the unidentified genus, *i.e.* 25.80%.

Table 5. Distribution isolate by site.

Genus	Bilanko		Ngamakala	
	Number	%	Number	%
Bacillus	17	47.22	17	54.81
Entérobactérie	4	11.11	3	9.68
Pseudomonas	5	13.89	3	9.68
Non identifié	10	27.78	8	25.80
Total	**36**	**53.73**	**31**	**46.27**

3.7. Phenotypic Characterization of Bacterial Isolates

After purification, 67 isolates were characterized by cultural, morphological and biochemical characteristics, including 36 from the Bilanko site and 31 from the Ngamakala site.

Figure 9 shows the cultural, microscopic and biochemical characteristics of isolates from the Bilanko site. The results show that all isolates are catalase +, the majority had isolated, moderately motile, KOH-positive bacilli with circular colonies. These isolates were dominated by pink colonies, followed by yellow and white, creamy, dry and slimy.

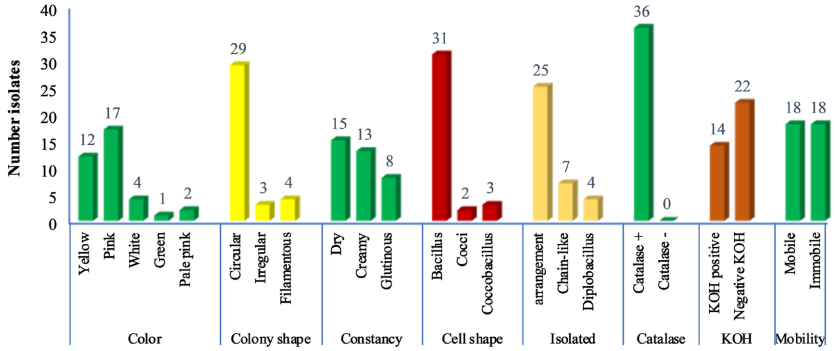

Figure 9. Phenotypic characteristics of Bilanko isolates.

Figure 10 shows the cultural, microscopic and biochemical characteristics of isolates from the Ngamakala site. All isolates are catalase +, the majority were isolated bacilli, moderately motile, KOH-negative. These isolates are dominated by the presence of pink colonies, followed by yellow, white and green; creamy, dry and slimy in appearance.

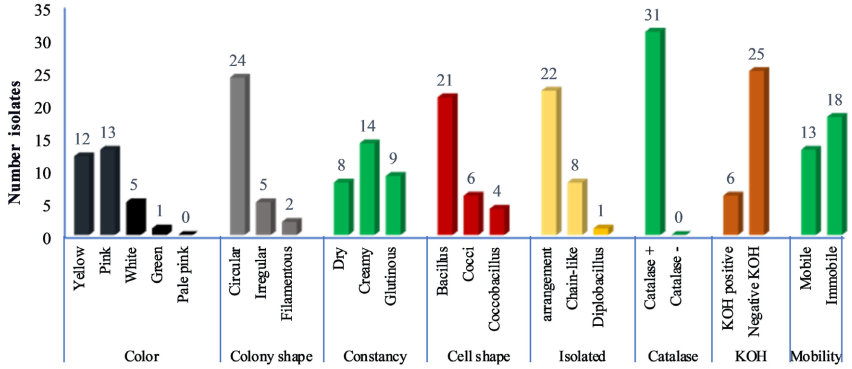

Figure 10. Phenotypic characteristics of Ngamakala isolates.

3.8. Lipolytic Activity

Figure 11 shows the light halos indicating olive oil degradation by the isolates tested. Overall, the diameter of the halos varies according to isolate and substrate.

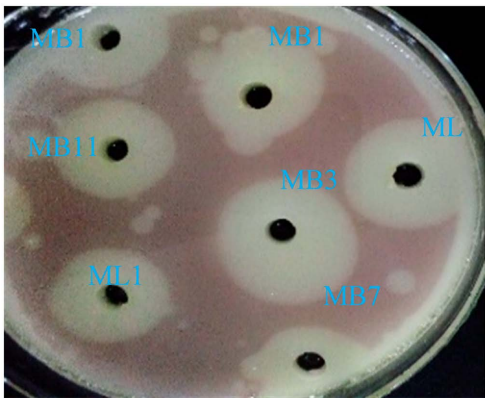

Figure 11. Demonstration of lipolytic enzyme production.

- **Lipolytic Activity of Bilanko Bacterial Isolates**

Figure 12 illustrates lipase production in Bilanko isolates, which varies from isolate to isolate. Out of 30 isolates, 22 showed the ability to produce lipase, with the largest diameter observed in isolate MB9 at 3.3 cm and the smallest in isolate MB10 at 1.5 cm.

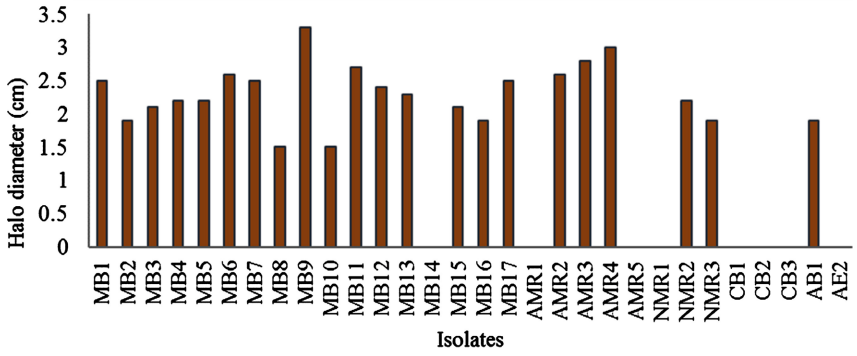

Figure 12. Lipase production profile of Bilanko bacterial isolates.

- **Lipolytic Activity in Ngamakala Isolates**

Figure 13 shows the lipase production diameters of Ngamakala isolates. Of 25 isolates, 17 showed the ability to produce lipase. Diameters varied from one isolate to another, with the highest value observed in isolate ML4 at 4 cm and the lowest in isolate ML6 at 1.3 cm.

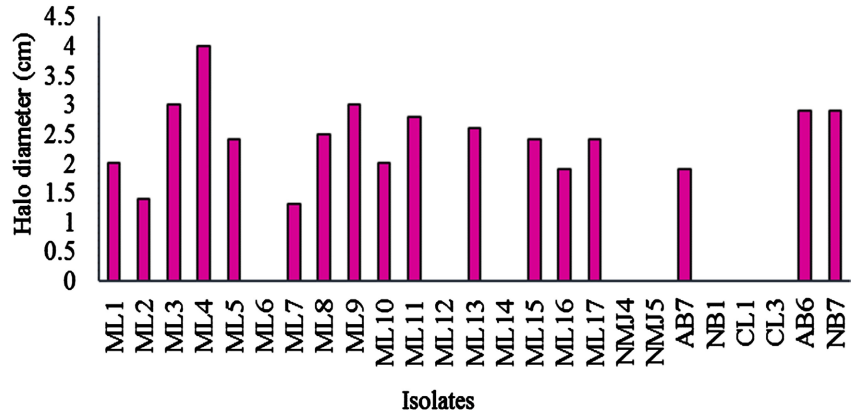

Figure 13. Lipase production profile of Ngamakala bacterial isolates.

4. Discussion

The aim of this study was to determine the lipase profile and production of bacteria isolated from the soils of the Bilanko and Ngamakala peat bogs (Republic of Congo) as a function of physic-chemical parameters. The results of the physico-chemical analyses show that the soil at the Bilanko site has an average temperature of 26.7˚C ± 0.13˚C and 27.8˚C ± 0.001˚C, while the Ngamakala site has an average temperature ranging from 26.2˚C ± 0.02˚C to 27.1˚C ± 0.57˚C. Soil temperature is a determining factor in the distribution and activity of mi-

cro-organisms, particularly for ammonification and nitrification processes. It not only affects the physiology of micro-organisms, but also influences water movement and the diffusion of gases and nutrients [20]. Temperature is a very important factor in peatlands, as the results of [9] on the role of peatlands in the global carbon cycle show that a rise in temperature leads to a significant release of CO_2 due to microbial activity and, consequently, to a high level of decomposition of organic matter. Temperature can act indirectly, interacting with humidity, greenhouse gas emissions, and modifying vegetation, microbial community structure and food webs [21].

The mean pH values obtained are moderately acidic, ranging from 5.55 ± 0.033 to 6.44 ± 0.006. At the Ngamakala site, it ranged from 5.63 ± 0.09 to 6.91 ± 0.044, and at Bilanko, from 5.55 ± 0.033 to 6.44 ± 0.006. At both sites, the pH is circumneutral. On the basis of the pH, we can assume that our sites can be considered predominantly umbroteric. These results are similar to those of [22] with tropical peat bogs in the Pastaza Marañón basin, Peruvian Amazon, at a depth of 0 - 15 and 15 - 30 cm, where it was 5.9. These pH values are close to neutral, which may determine the type of peatland, the origin of the water supply and nutrients. These characteristics determine plant and microbial diversity, plant productivity and the rate of litter decomposition [6].

Electrical conductivity is zero, ranging from (9.52 ± 0.002) to (26 ± 0.001) µS/cm at the Bilanko site and from (9.53 ± 0.36) to (39 ± 1.4) µS/cm at Ngamakala, with low turbidities of (2 ± 0.66) to (18 ± 3.33) mg/L and (4 ± 0.02) to (31 ± 0.84) mg/L at Bilanko and Ngamakala respectively. These values resulted in very low TDS averages of 4.1 to 5.94 ± 0.026 ± 0.13 at Bilanko and 4.02 ± 0.8 to 6.01 ± 0.023 at Ngamakala.

The average salinity of the Bilanko peat bog soils was (5.07 ± 1.55) at (14.94 ± 4.20) g/l, compared with (3.85 ± 0.99) at (15 ± 1.66) g/l for Ngamakala, while the alkalinity of these samples was (3.16 ± 1.61) at (14 ± 1.66) mg/lCaCO3, compared with (5.08 ± 0.84) at (23.81 ± 1.9) mg/l$CaCO_3$. These results show that the soil samples analyzed are moderately saline and not alkaline.

Ion concentrations in these samples are low. Ion concentrations at the Bilanko site ranged from (5.03 ± 0.01) to (1.14 ± 0.02) mg/l and at Ngamakala from (1.55 ± 0.01) to (5.06 ± 0.26) mg/l respectively. These ions play a role in endospore protection in the *Bacillus* and *Clostridium* genera. At Bilanko, magnesium ions ranged from (0.26 ± 0.005) to (1.02 ± 0.015) mg/l, compared with (0.051 ± 0.003) to (1.45 ± 0.015) mg/l at Ngamakala. Potassium ions at the Bilanko site ranged from (0.14 ± 0.015) to (1.4 ± 0.2) mg/L, and from (0.17 ± 0.005) to (4.39 ± 0.04) mg/L at the Ngamakala site. Potassium and magnesium are enzymatic cofactors with functions in the stability of bacterial cell structures. Organic nitrogen is a constituent of proteins, polypeptides, amino acids and urea, while inorganic nitrogen includes ammonium, nitrite and nitrate. Ammonium ions average from (0.01 ± 0.004) to (0.05 ± 0.006) mg/l; nitrites from (0.03 ± 0.02) to (0.09 ± 0.011) mg/l and nitrates from (0.03 ± 0.02) to (0.09 ± 0.011) mg/l at the

Bilanko site, compared with (0.05 ± 0.01) to (0.04 ± 0.003) mg/l at the Ngamakala site; Nitrite from (0.52 ± 0.005) to (4.39 ± 0.16) mg/l and nitrate from (0.01 ± 0.004) to (0.07 ± 0.004) mg/l. The nitrogen contained in the soil samples in our study may be of organic or mineral origin and is required for the synthesis of amino acids, nucleic acids and amino sugars [23]. During the mineralization of organic matter, saprophytes release ammonia; some of this ammonia is used directly as a source of nitrogen, while the rest is oxidized to nitrite and then nitrate by chemotrophic bacteria during nitrification. It's important to note that some bacteria can reduce nitrate to nitrogen. Thus, all three mechanisms of nitrate transformation in peatlands are possible: denitrification, assimilation by plants and reduction to ammonium [24]. Nitrogen mineralization studies have also demonstrated denitrification following aerobic incubation *in vitro* [25] or in situ [26].

Quantification of total polyphenols and flavonoids in Bilanko and Ngamakala peat bog soils and sphagnum mosses reveals a high polyphenol content of 983.29 mgEAG/100gMS for the Ngamakala SLif sample, followed by Bilanko with 849.43 mgEAG/100 gMS, and 202.45 and 270.5 mgEAG/100 gMS for the LiF and SB samples respectively. Total flavonoid contents were 25.87 and 19.15 mgEAG/100 gMS respectively for BiL and LiF samples, compared with 8.68 and 6.82 mgEAG/100 gMS for SLIF and SB. It can be seen that Bilanko peat bog soils are much richer in phenolic compounds than Ngamakala soils. These results confirm the information that peatlands possess large quantities of hydrophilic phenols that can be released by sphagnum mosses. These compounds interact not only with the environment, but also with microorganisms. Phenols can have inhibitory actions on microorganisms involved in the decomposition of organic matter [27]. These results are in line with those of Richy [28], who found significant quantities of phenolic compounds in peat samples from bogs in France and Spain.

Bacterial counts in Bilanko and Ngamakala peat bog soils vary according to site and sampling point. The bacterial load on PCA at the Bilanko site ranged from $(1.71 \pm 0.88)\cdot10^4$ to $(2.92 \pm 0.07)\cdot10^5$ CFU/g, and from $(1.30 \pm 0.73)\cdot10^4$ to $(2.89 \pm 0.06)\cdot10^4$ CFU/g at Ngamakala. These results are similar to those of Morabandza [10] with Likouala peat bogs, where bacterial loads on PCA medium had reached up to $9.50\cdot10^4$ CFU/g, while Waksman and Stevens [29] had shown bacterial loads of the order of $3.4\cdot10^5$ to $5.5\cdot10^7$ CFU/g with natural ombrotrophic peat bogs. It is important to note that these bacterial loads were obtained in Europe and the USA, where vegetation, climatic and geological conditions differ from those in Congo-Brazzaville. These factors would certainly explain the difference, as they are likely to influence the microflora and consequently microbial counts [30].

The concentration of Bacillus bacteria isolated on Mossel medium ranged from $(5.20 \pm 1.40)\cdot10^3$ to $(1.22 \pm 0.13)\cdot10^4$ CFU/g for Bilanko and from $(9.20 \pm 2.05)\cdot10^3$ to $(1.11 \pm 0.13)\cdot10^4$ CFU/g for Ngamakala. On EMB medium, the Bilanko site showed $(1.40 \pm 0.76)\cdot10^3$ to $(8.80 \pm 1.73)\cdot10^3$ CFU/g and the Nga-

makala site, $(1.00 \pm 0.02)\cdot10^3$ to $(9.20 \pm 2.05)\cdot10^3$ CFU/g. Finally, the isolation of Pseudomonas bacteria ranged from 0 to $(2.30 \pm 0.53)\cdot10^2$ and 0 to $(8.90 \pm 2.35)\cdot10^2$ CFU/g at the Bilanko and Ngamakala sites respectively. These loadings are close to those of [10] confirming the assertion of [31] and Waksman and Stevens [29], that the microbial population is abundant at the surface of peat bogs, as there is more oxygen and where carbon is readily available and decomposable. After comparison between points at each site, the results of bacterial concentrations in CFU/g at both sites show that some sampling points show a variation in similarity and variable load distribution. This can be explained by the distribution of the above-mentioned ions, which do not vary proportionally.

Morphological, biochemical and cultural characterization of bacterial isolates revealed bacterial diversity based on colony and cell shape. Isolates purified on the different media showed the presence of various Gram+ and Gram−, catalase-positive bacteria in accordance with the media used. These results show a diversity of bacteria in the composition of the bacterial community of each isolate. These results are similar to those of [10] on peat bog soils in Likouala, where the surface soils contain a variety of Gram+ and Gram− bacteria. Analysis of lipase production in isolates from Bilanko and Ngamakala revealed that, apart from isolates from sampling point 08 at each site, the majority of isolates were capable of lipase production.

5. Conclusion

In this work, we studied the profile and lipase production of bacteria in Bilanko and Ngamakala peat bog soils as a function of their physicochemical parameters. These soils were found to be moderately acidic, with temperatures of 27.8˚C ± 0.01˚C for Bilanko and 27.1˚C ± 0.57˚C for Ngamakala, and other parameters favorable to the growth of mesophilic bacteria. Electroconductivity (EC) ranged from (9.52 ± 0.002) to (39.01 ± 1.4) µS/cm, with low turbidity ranging from (2.04 ± 0.66) to (31.02 ± 0.84) mg/L and average ionic concentrations, although Bilanko was richer in phenolic compounds than Ngamakala. FMAT diversity ranged from $(1.71 \pm 0.88)\cdot10^4$ to $(2.92 \pm 0.07)\cdot10^5$ CFU/g for Bilanko and from $(1.30 \pm 0.73)\cdot10^4$ to $(2.89 \pm 0.06)\cdot10^4$ CFU/g for Ngamakala. Bacillus loads ranged from $(5.20 \pm 1.40)\cdot10^3$ to $(1.22 \pm 0.13)\cdot10^4$ CFU/g and from $(1.11 \pm 0.13)\cdot10^4$ to $(9.20 \pm 2.05)\cdot10^3$ CFU/g; enterobacteria loads from $(1.40 \pm 0.76)\cdot10^3$ to $(8.80 \pm 1.73)\cdot10^3$ CFU/g and from $(1.01 \pm 0.02)\cdot10^3$ to $(9.20 \pm 2.05)\cdot10^3$ CFU/g; in *Pseudomonas* from 0 to $(2.30 \pm 0.53)\cdot10^2$ CFU/g and from 0 to $(8.90 \pm 2.35)\cdot10^2$ CFU/g for Bilanko and Ngamakala respectively. Isolate ML4 (Ngamakala) had a lipase production diameter of 4 cm, compared with 3.3 cm for MB9 (Bilanko). These results reveal variation in the similarity and distribution of bacteria in the Bilanko and Ngamakala bogs.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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