

# Relative Importance of the Free and Fixed Bacterial Community in Three Lacustrine Ecosystems having Different Trophic Status

Raoui Sidi Mohammed, Rachiq Saad , Bouayad Kenza, Chadli Nourddine

**Abstract-** The present work consists in determining the total bacterial densities and biomasses and the relative importance of free and fixed bacterioplankton in three lacustrine environments with different trophic status: Pavin lake, oligomesotrophe, and the Villerest reservoir, eutrophe (two French ecosystems) and the dam Allal Fassi, mesotrophe (Morocco). Along the water column, the results obtained showed bacterial densities that ranged from 0.60 to  $7 \times 10^5$  cells.ml<sup>-1</sup> at Pavin Lake, from 1.01 to  $9.86 \times 10^6$  cells.ml<sup>-1</sup> in the Allal Fassi dam reservoir and from 0.70 to  $12.90 \times 10^6$  cells.ml<sup>-1</sup> in the Villerest reservoir. The average recorded bacterial biomass ranged from 0.73 to 1.92  $\mu\text{gC.l}^{-1}$  in Pavin Lake, from 10.50 to 81.10  $\mu\text{gC.l}^{-1}$  in the Allal Fassi dam and from 29.40 to 60.52  $\mu\text{gC.l}^{-1}$  in the Villerest reservoir. Seasonal variations show fluctuations in bacterial densities for the three environments. The proportion of free and fixed bacteria revealed high percentages of the fraction of free bacteria with 96.10% ; 90.50% ; 87.90% at the surface layers respectively at Pavin Lake, Villerest Reservoir and Allal Fassi Reservoir. Mean recorded values of fixed bacteria at all depths were 4.26% ; 10.35% ; 17.13% of the total bacterial density respectively in Pavin Lake, Villerest Reservoir and Allal Fassi Reservoir.

**Index Terms-** Bacterioplankton, density and biomass, free / fixed bacteria, Pavin Lake, Allal Fassi dam, Villerest Reservoir.

## I. INTRODUCTION

The bacterial community constitutes a great importance in the planctonic ecosystem [1]. It has an essential role in the composition process of the organic material. The determination of abundance and bacterial biomasses in Lacustrine environment could be one of essential parameters to understand the function of Lacustrine aquatic ecosystems [2],[3]. The degradation of the organic material in the aquatic environment is mainly due to fixed bacteria [2],[4]. In Eutrophic environment, rich in organic substances, the fraction of fixed bacteria reached a fairly high level up to 30% of the total density [2].

**Raoui Sidi Mohammed**, Higher Institute of Nursing Professions and Health Technology ISPITS Lalla Meriem, Av El Farouk, VN Meknes

**Rachiq Saad**, Laboratory of Environment and Functional Ecology, FST Fès, SMBA University (Morocco)

**Bouayadi Kenza**, Laboratory of Environment and Functional Ecology, FST Fès, SMBA university (Morocco)

**Chadli Nourddine**, Laboratory of microbiology, pharmacology, biotechnology and environment, Ain Chock Faculty of sciences, Hassan II university, Casablanca, Morocco

In addition, in oligotrophic environment the fixed bacterial cells represent only low percentages (less than 10%). The fact of fixing on a particle allows bacterial cells to find a high concentration of substrate [5] and to be less vulnerable to predation [6]. Furthermore, several authors have suggested that the fraction of fixed bacteria would be not only more active but also big in size and would be characterized by a high growth rate compared to the free bacterial fraction [7]. Other authors have highlighted that the two fractions are different from a taxonomic point of view [8]. The proportion of fixed and free cells varies from an aquatic ecosystem to another [9]. This suggests that the two bacterial communities, fixed and free, interact dynamically and the degree of similarities depend mainly on the nature of substrate.

## II. MATERIAL AND METHODS

### Pavin Lake

Oligomesotrophic Lake located in the central French massif (Puy de dome) at an altitude of 1197 m, a deep circular shape (more than 90 m) and fed from different sources (Fontaine de loup, sources de prêtres ..)

### The reservoir dam of Allal Fassi

Mesotrophic water reservoir situated in the province of Sefrou 47 Km away from Fez (Morocco) a region characterized by a temperate semi-arid climate, a maximum depth of 34 m. Sebou river the only tributary of the reservoir fed from two sources Ain Timdrin and Ain Sebou.

### The reservoir of Villerest:

Eutrophic dam reservoir located on La Loire 5 Km in the south of Roanne (France). The study site is located 12 Km upstream of the dam.

### Sampling

The samples are taken using a Van Dorn closing bottle. Samples were taken from the three lakes at the following depths : 5m ; 15m ; 30m and 50m, at the center point for Pavin Lake; 0m ; 2m ; 5m ; 10m ; 15m and 20m at the deepest point of the Allal Fassi dam reservoir at 0m ; 1m ; 2m ; 5m ; 10m and at the bottom-1m for the Villerest reservoir. After each withdrawal, the samples are immediately fixed with 37% formaldehyde (final concentration = 2%). The counts of the bacterial cells are carried out according to the method of [10]. Indeed, subsamples are stained with a fluorochrome (acridine orange or DAPI). The bacterial cells are retained after filtration under vacuum (<100 mm Hg) on a black Nucleopore polycarbonate filter and 0,2  $\mu\text{m}$  porosity. The bacterial count is made by epifluorescence microscopy (Olympus BH-2 or HBS) equipped with a 100 W HBO

lamp with an excitation light between 450 and 490 nm and a stop filter at 515 nm. The bacterial biomass, determined from biovolumes and expressed in carbon, was calculated using the conversion factor  $1 \mu\text{m}^3 = 1.21 \times 10^{-13} \text{ g.C}$ , proposed by [11]. The determination of free or fixed particle bacterial fractions was determined by direct epifluorescence observations of the filtered samples.

III. RESULTS

In this work, the results obtained concern bacterial abundances and biomasses as well as the importance of free and fixed bacteria fractions in the three lacustrine environments of different trophic status.

Table I : mean bacterial biomass ( $\mu\text{C.L}^{-1}$ )

	epilimnion	Metalimnetic	hypolimnion
Pavin	1.93	1.25	0.73
Allal Fassi	81.10	43.40	10.50
Villerest	60.52	38.10	29.40

Pavin Lake:

Bacterial abundances varied by  $0.60 \times 10^5 \text{ cells.ml}^{-1}$  at the bottom of the lake in May at  $7 \times 10^5 \text{ cells.ml}^{-1}$  to 5 m during the month of June. In average values, for all samples, bacterial densities decrease regularly with depth (Fig 1). The average biomass values are very low and fluctuated between  $0.73 \mu\text{C.L}^{-1}$  at the bottom and  $1.92 \mu\text{C.L}^{-1}$  at the surface layers (Table I).

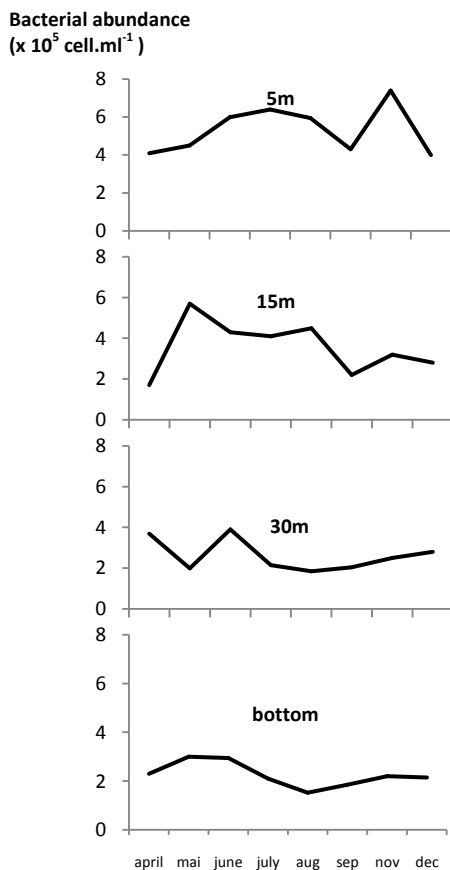


Fig 1 : Spatio-temporal variations of bacterial abundances in Pavin Lake

The average abundance of free bacteria showed a maximum at the superficial level with 97.50% of the total density. Fixed bacteria constituted a very small percentages of total bacterial abundance. Their densities vary between 2.50% in the superficial layers and 6% near the bottom of the lake (Table II). The average value of the fixed bacteria recorded for all the depths combined was 3.90% of the total bacterial density.

Table II : Percentages of free and fixed bacteria in Pavin Lake

	% free bacteria	% fixed bacteria
5 m	97.50%	2.50%
15 m	96.90%	3.10%
30 m	96.00%	4.00%
bottom	94.00%	6.00%

The dam Allal Fassi:

Spatiotemporal variations in bacterial abundances showed densities that varied by  $1.01 \times 10^6 \text{ cells.ml}^{-1}$  near the bottom in August to  $9.86 \times 10^6 \text{ cells.ml}^{-1}$  to 10m during the month of May. Abundances were important during the summer period in the superficial layers and gradually decrease with depth. (Fig 2).

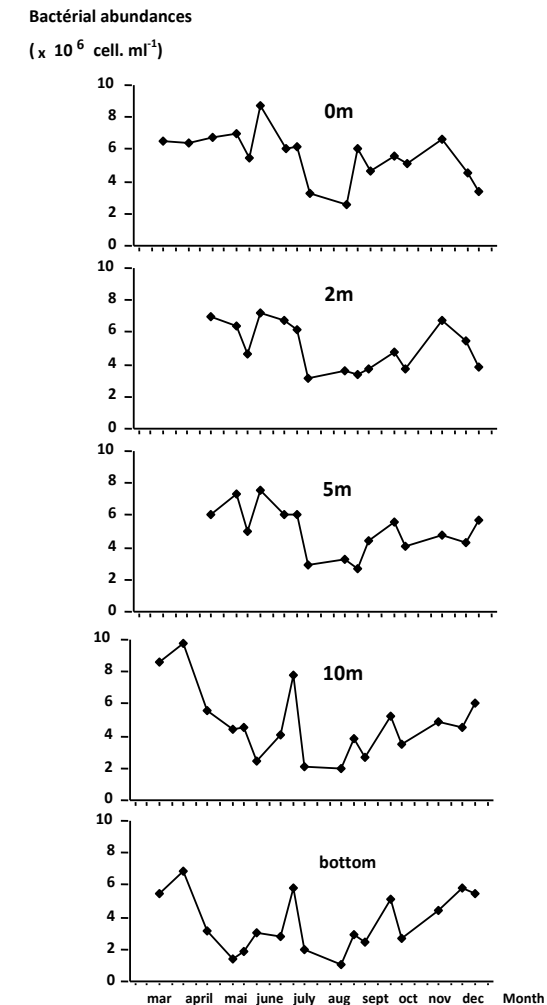


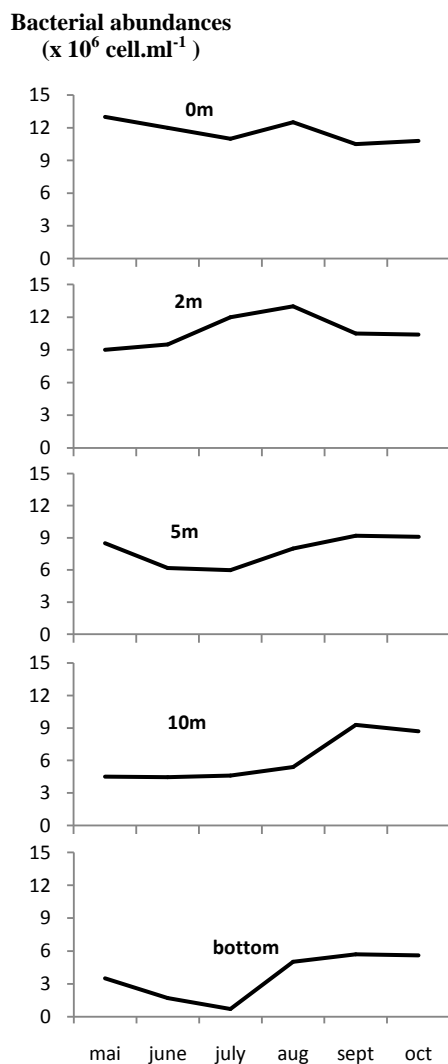
Fig 2 : Spatio-temporal variations of bacteria abundances in Allal Fassi dam

Mean values recorded by depth were  $6.30 \times 10^6$ ;  $5.20 \times 10^6$  and  $4.40 \times 10^6$  cells. ml<sup>-1</sup> respectively at 0m, 10m and near the bottom. In terms of carbon, the mean values of the bacterial biomass ranged from 10.50 µgC.l<sup>-1</sup> near the bottom of the reservoir to 81.10 µgC.l<sup>-1</sup> at the level of the epilimnion (Table I).

**Table III : Percentages of free and fixed bacteria in Allal Fassi dam**

	% free bacteria	% fixed bacteria
0 m	87.90%	12.10%
2 m	87.50%	12.50%
5 m	83.50%	16.50%
10 m	82.70%	17.30%
bottom	79.60%	20.40%

The maximum biomass of 160.70 µgC.l<sup>-1</sup> was recorded at 10m at the end of July. The average percentages of free bacteria rose a maximum of 87.90% at the 0m level and gradually decrease with depth. Fixed bacteria represented very high percentages and increased with depth. The recorded values fluctuated between 12.10% at 0m and 20.40% near bottom (Table III). The average value recorded along the water column was 17.13% of the total bacterial density.



**Fig 3 : Spatio-temporelles variations of bacterial abundances in the Villerest reservoir**

The reservoir of Villerest:

The recorded bacterial abundances varied by  $0.70 \times 10^6$  cells.ml<sup>-1</sup> near the bottom during the month of July at  $12.90 \times 10^6$  cells.ml<sup>-1</sup> at the 0m level during the month of May (Fig 3). The average value for all the samples was  $6.5 \times 10^6$  cells.ml<sup>-1</sup>. In average value of all the samples, the densities decrease regularly with the depth ( $8 \times 10^6$ ;  $6.90 \times 10^6$ ;  $4.70 \times 10^6$ ;  $3.60 \times 10^6$  cells.ml<sup>-1</sup> respectively at 0m, 2m, 5m, 10m and near the bottom). Expressed as carbon, the average biomass values for all samples fluctuated between 29.40 µgC.l<sup>-1</sup> at the bottom and 65 µgC.l<sup>-1</sup> at the upper reservoir level (Table I). The highest biomass (121 µgC.l<sup>-1</sup>) was obtained during the summer period. The fixed bacteria accounted for 9.70% of the total bacterial abundance at the upper level and at the bottom and 10.90% at 5m (Table IV). The average percentage recorded over the entire water column was 10.50% of the total bacterial density.

**Table IV : Percentages of free and fixed bacteria in the Villerest reservoir**

	% free bacteria	% fixed bacteria
0 m	90.10%	9.90%
2 m	89.30%	10.70%
5 m	89.10%	10.90%
10 m	89.20%	10.80%
bottom	90.30%	9.70%

#### IV. DISCUSSION

The bacterial density varied in the three lacustrine environments, by  $0.10 \times 10^6$  cells.ml<sup>-1</sup> in Pavin Lake at  $12.90 \times 10^6$  cells.ml<sup>-1</sup> in the Villerest reservoir. In general, the abundance of bacterial cells in aquatic environments ranges from  $10^5$  to  $10^7$  cells.ml<sup>-1</sup> [10], [12]. It is in Pavin Lake, described as meso-oligotrophic where the bacterial densities are the lowest. These densities are lower compared to those recorded in an oligotrophic mountain lake (Dayet Aoua in the Moroccan Middle Atlas) which has much higher values [13]. Globally, bacterial abundance increases with the trophic degree of the medium [14]. The low densities recorded at Pavin Lake correspond to those generally reported in pelagic aquatic ecosystems [15], whereas those recorded at the Allal Fassi dam (mesotrophic) are almost similar to those found in the Villerest Reservoir (eutrophic), and far exceed those found in Pavin Lake (oligoméotrophe). These densities generally correspond to values found in eutrophic lacustrine environments [14]. In the dam dam Allal Fassi, classified among the mesotrophic lakes [16], the bacterial densities recorded are comparable to those recorded in the eutrophic lake Aydat (France) [14]. The low densities and bacterial biomasses recorded in Pavin Lake correspond to the values found in oligotrophic media. In this lake, the fixed bacteria make up 4.29% of the microplankton and are essentially represented by free bacteria (Table V).

**Table V : Percentages of fixed bacteria in Pavin lake, Allal Fassi dam and Villerest reservoir**

	epilimnio n	metalimneti c	Hypolimnio n
Pavin	2.80 %	4.00 %	6.00 %
Allal Fassi	13.70 %	17.30 %	20.40 %
Villerest	10.55 %	10.80 %	9.70 %

Seasonal variations in bacterial density show fluctuations in bacterial densities for the three media. Various regulatory factors control the dynamics of bacterial populations in aquatic ecosystems. Among which organic and mineral resources that can come from among others phytoplankton excretion or predators [17],[18],[19], predation by heterotrophic microorganisms [20],[21], viral lysis [22], which can be more important to the effect of grazing under certain environmental conditions [23],[24]. In the end, temperature is known to be a factor that stimulates bacterial production [25],[26], and its variations may be at the origin of bacterioplankton successions [27],[25],[28].

The quantitative importance of the two free and fixed bacterial fractions is very variable in the three lacustrine environments with different trophic status. In the Pavin Lake, the fixed bacteria represent on average only 4.26% of the total bacterial abundance, which characterizes pelagic environments poor in organic matter [29]. In the Allal El Fassi dam reservoir, the high percentages of bacterial fixation (mean 17.13%) (Table 5) coincided on the one hand, with high concentrations of living particles constituted by the algal population, represented as a source of substrate support for bacteria and secondly, with the contents of suspended matter that characterize this reservoir. At the Villerest reservoir, the average fixation percentage of 10.35% (Table 5) could be related to the high phytoplankton concentrations that characterize this eutrophic reserve. However, in the Allal Fassi dam reservoir, mesotrophic, the percentages of the fixed bacteria exceed those recorded in the Villerest eutrophic reservoir. We note that each year, the Allal Fassi dam reservoir is subject to significant rainfall and summer floods, which bring huge amounts of particles as substrates for bacterioplankton. The proportion of fixed bacteria and free bacteria varies from one ecosystem to another. For example, this proportion has been estimated at a few percent in Lake Geneva [30] and up to 73% in Lake TANNING in Denmark [31],[32], which states that the concentration of detrital organic particles is a major determinant of bacterial fixation. LIND and DAVALOS [33] have shown the existence of a significant relationship between the density of fixed bacteria and the concentration of clay particles in a turbid reservoir in Texas (USA). Other studies have suggested that the role and role of fixed bacteria is important in relatively small, relatively large catchment lakes [34]. The separation between the fixed and free bacteria, allowed us to specify that the contribution of the epibacteria, in terms of abundance and activity is more important in the trophogenic zone of the lacustrine environments during

the strong photosynthetic activities. SIMON said that the abundance and production of bacteria accurately reflect changes in phytoplankton biomass [35]. The fixed bacteria seem to have, under certain conditions, a more important role than that usually attributed to them, insofar as in particular they are directly involved in the degradation of the particulate organic matter.

## V. CONCLUSION

This study, carried out in three lacustrine environments of different trophic level (Pavin Lake, Allal Fassi dam reservoir and Villerest reservoir), the densities and bacterial biomasses determined at different levels of the water column confirm the trophic level of the studied habitats with a trend towards the eutrophic level for the Allal Fassi dam reservoir, while the Pavin lake remains relatively stable on its trophic status (oligotrophic). The relative importance of free and fixed bacteria and their proportion to bacterial densities vary with ecosystems and their trophic levels. From this study, it appears that the fixed bacteria represent high values for the Allal Fassi dam reservoir and the Villerest reservoir which are classified at a higher eutrophication level compared to Pavin Lake.

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