Bacteriological study by bacterial counting on the level of the interstitial sediments of Sebou River.

Raja Guemmouh[1], Khadija Essafi[1] & Nezha Aouad[2]
[1] *Laboratoire d'Hydrobiologie et Ecologie générale. Faculté des Sciences Dhar El Mehraz B.P.1796-atlas Fès.*
[2] *Laboratoire d'Ecologie. Université Hassan II, Faculté des Sciences, Aïn Choq. Casablanca.*

The Sebou River constitutes one of the most important resources of Morocco, receives in his middle section of the daily dismissals of sloppy waters from the city of Fès (a city of Morocco). The present survey analyses the impact to the downstream of these dismissals on the quality of these interstitial waters and on the sediments. The bacterial numbering is used for the assessment of the bacterial biomass. This one shows a big spatio-temporal variability, especially as she is in narrow relation with the nature of the substratum. Within this aquiferous fluvial, the microbial activity and the bacterial numbering are bound to abundance and the distribution of the thin sediments and very ends. The temporal dynamics of the hydrologic factor is determining in the phenomena of accumulation, of retention and the dynamics of the bio-movie.

I. INTRODUCTION

The pollution of water is a deterioration that his dangerous use returns and disturb the ecosystem, it can tell to make surface and underground waters. In the aquatic environments, a lot of micro-organisms, bacteria and protozoa, of the mushrooms and algae are present, and play a considerable role in the processes of decomposition and mineralization of the organic matter [1]. The organic matter was for a long time the main pollutant of the aquatic environments. However, they are essential to the aqueous life like a food, but can become a troubling element when their quantity is too considerable. Indeed, the bacteria that consume the oxygen dissolved of the river, therefore to deprive the aqueous organizations will damage them. They come of domestic waste (garbage, excrement) agricultural or industrial (tanneries, slaughterhouses, dairies, mills of oil) that is indeed the case of the Sebou river, being one of the main resources of water to Morocco, and which receives daily, while considering his proximity of the city of Fès, a considerable volume of sloppy water. These anaerobic conditions are generally met in pore and waters of subway, what limits the diversity of fauna and flora.

II. DESCRIPTION OF THE SITES

Stations 1 and 2: Very closed one to the other, they are localized upstream on the wadi confluent Sebou with the Fez wadi, instead "the bridge Portuguese" says to roughly 12km in is North of the city of Fès. With lotic of the faces, these stations smell like the thermal influence of the spring thermal Sidi Harazem. The substratum, with dominant coarse, is composed of rollers with the sand, and mud in the side. The width is 21m, the depth varies from 15 to 96cm; speed of stream of 0.17 to 0.46 m/s. Vegetation is composed of Nerium oleander, Foeniculum sp and of thistles in side and filamentous aqueous green algae. These stations are submitted to an influence of the strong anthropozoogene by the washing of linen and wool, and the displacement of the substratum by the man.
Station 3: is Localized on the Sebou wadi, downstream the confluence with Wadi Fès, he is constantly submitted to the refusals of the city of Fès to some meters (very polluted). the substratum is black muddy very rich in organic matter (to pump very difficult that causes in the majority of the cases a replenishment of the sediments in the investigation). Euphorbiacées, Malvacées and Labiate represent the vegetation of bank.
Station 4: Localized to roughly to 18km of preceding it, difficult access, considering the crumbly marly sides. This deep river, nearly stagnant (more that a meter) and even is polluted. The blackish of the substratum very end dominates a nauseous odour, especially published on nearly all year in summer. A lot of domestic waste and float of the garbage on the surface. Width of 25m, to pass 1m of them very deeply.
Station 5: With has few km of station 4, it is characterized by has muddy substrate, sandy sediment sometimes of blackish colour. The impact of the cattle is to be announced to the level of this station, which is, used ace drenching with the herds of cows and sheep of the area. Width of 15m, depth varying of 10 to 50cm.

III. MATERIEL AND METHODS

The used method is the manual pumping, technique of Bou-rouch [2];[3];[4]. They are far from taking the specific in the space and the time. BOU and ROUCH developed a manufacture of the pump permitting to prospect the step water of very deep earth and in particular the underflows of the rivers

(Fig.3). He/it is composed of a pump of the hand joined to a rigid investigation of length of 1,2m without stain, 18mm of internal diameter and crépinée in his/her/its bottom part (36 holes of 5mm diameter localized between 4 and 17cm the distal end). The use of a head to hit makes it possible to insert it with a mass in the sediments until the wanted depth. To withdraw carried it outside in depth consists in a mixture of water of the pore, sediment and fauna that he/it accommodates. To pump inhales water and subways of the sediment. The quantity of the water mixture is pumped of the sediments, with 50cm depth, is 10 litres; whom are filtered through a net of 200µm; fauna then gathered is formulated for the determination. Gone of the sediments, the surface and interstitial waters have been brought to establish the heavy metals and the physicochemical analyses. This method is quantitative because the volume of pump to water is known precisely; he/it can be used in the immersed means and can be the returned article possible to reach major levels of the tablecloth [5]. The sediments are taken while pumping BOU-ROUCH, the finest fraction with the same fine grains is put on side to -20°c, for bacterial account. The deep transfer of the bacteria is limited on one hand by filtration, and on the other hand by adsorption.

IV. BACTERIAL ENUMERATION BY DIRECT COUNTING:

For a lot of authors [6], [7] this method remains the most convenient means for the assessment of the number of bacteria in the aquatic environment. Usually used, it especially applies to the studies carried outside on water and the sediment. Lately, it is used more and more in the induction of the bacteria on the sheets in decomposition. During our survey, the used method is in relation with the bacterial induction on the level of the sediments. We used like dye the orange acridine that has the property to bind to the bacterial nucleic acids. His/her/its passive cellular penetration has the advantage to occur in the samples arranged to formalin without all more belated evolution. The sediment very end (1g) are put in 50 ml of filtered distilled water (nucléopore of porosity 0.22 µms) passed then to the ultrasonic sounds during 3 min. to 60 Watts, 20 KHz and 50% of the active cycle (It allows a disconnecting efficient of the bacteria without too much deterioration, three samples of 1 ml each of the suspension gotten is diluted then in 4 ml of distilled water, filtered on a Millipore of the turret with pre-filters (AP20) surmounted Millipore's of the GTBP filters (porosity 0.2 µm)(Fry, 1988). These filters are put then to hatch during 15 min. in 0.5 ml of a solution orange acridness to 01 mg/ml. The complex RNA AO emits an orange fluorescence when he/it is excited with a wavelength of 460nm. After this breeding time, the filters are rinsed then with distilled water gone up between two drops of oil with immersion of the no fluorescent between blade and plate and observed under the microscope with ear fluorescence of the Leitz type DIALUX 22. The bacteria appear points or glue some oranges. To count carry away uncertainly on 40 microscopic fields chosen to cross the totality of the filter according to two perpendicular cuts.

The calculation of the number of the bacteria per G of sediment is done in the following way: N bact/g of séd. = (average Nb bact./champs x 357.82 x dilution)/(6.4 X 10-3 x 5 x mass séd.).

V. RESULTS

The results of counting appears in the following table and the histogram (Fig. 4)

dates	Station 1	Station 2	Station 3	Station 4	Station 5
June 97	0.26	0.26	0.78	0.92	0.68
July 97	0.32	0.34	1.00	1.06	0.9
Augu 97	0.36	0.4	1.34	1.14	1
Sept 97	0.14	0.16	0.18	0.24	0.68
Oct 97	0.18	0.12	0.22	0.32	1.14
Nov 97	0.2	0.24	0.24	0.22	0.68
Dec 97	0.2	0.24	0.9	0.2	0.6
Jan 98	0.1	0.1	16	20	17
Feb 98	0.1	0.1	20	125	17
Mar 98	0.12	0.13	209	172	101
Avr 98	0.25	0.15	350	357	332
May 98	0.30	0.10	159	30	101
June98	1.01	0.2	249	151	239
July 98	2.39	0.14	252	166	261
Augu 98	2.81	0.14	242	291	177

Table 1: Comptage of the bacteria by orange acridine in the interstitial sediments of the Sebou River. The results are expressed in a number of bacteria x 10^{8} / per gram of sediment.

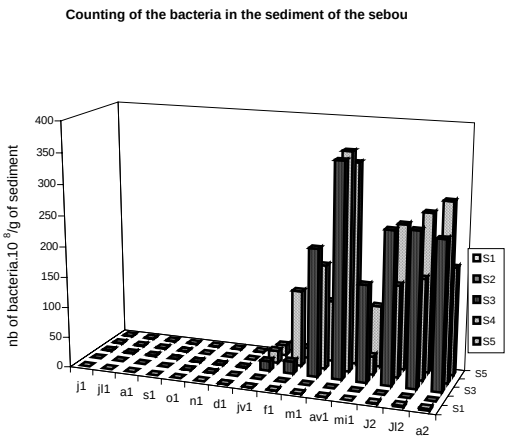

Fig. 4: Counting of the bacteria in the interstitial sediment of Sebou.

The number of bacteria announced to the level of the sediments of the station S1 and S2 is very weak compared to that noted in S3, S4 and S5. Several points can be advanced to explain these differences:

➢ **The nature of the substrate**: the stations S1 and S2, have a very coarse substrate represented by average stones, the interstitial medium is sandy, with coarse sediment, while in S3, S4 and S5, the substrate is muddy with very fine sediments. The microbial biomass and the activity are related on the abundance and the distribution of the fine sediments and very ends. The abundance of the very fine sediments involves a reduction in porosity and a containment of the medium hyporheic. It follows a reduction in the oxygen contents, biomass and bacterial activity and an adsorption of the organic compounds of the argillaceous and muddy particles. Especially in the mediums with raised hardness, this process is very effective [9]; [10]; [11]; [12]. During the period of high waters, the hydrological and trophic thermal conditions becoming unfavourable, seem to exert a scrubbing of the sediments. The biomass of the micro-organisms, and especially of the bacteria, while being affected, decreases.

➢ The situation of the stations: the S1 stations and S2 are localized on the River Sebou and receive the refusals of this river therefore only. The S3 stations, S4 and S5 are localized down-stream and receive the dismissals of the river of Fès and Sebou therefore and also a considerable volume of sloppy water of the city of Fès and his/her/its industries, in addition to surrounding agriculture. The highest securities have been observed in station 3 (to include between 0.16 and 2.52.1010/G sediment) because it is with the junction of the 2 rivers. A proliferation of the bacteria is marked very by a summit in April, with 2 falls in December and May in the time of the uprisings. Down-stream, us attends a light backward return that would be due to a self-purification because the river restores its quality progressively by the process of the self-purification. After the uprisings, us attend an enrichment of the bacterial population. This known like, the surface waters reasons of the neighbouring bring a considerable mass of bacteria that is added to the existing population.

➢ The seasons: The bacterial populations undergo of more than the variations according to the seasons. The temperatures of the summery support their multiplication, whereas the wintry temperature implies a reduction in the bacterial activity. The fall of oxygen comes with the high temperatures and indicates his use in the processes of decay of the organic matter.

The seasonal variations have direct effects on the interstitial means. Sometimes the proliferation of the bacteria persists down-stream, what probably depends on the contributions of reasons of the neighbouring in the elements of the nitrogen and the impact of livestock that come to water it.

VI. CONCLUSION

The study within the aquifer river, of the transfers of nutriments and contaminants showed that the bacterial enumeration, used for the bacterial biomass inventory, is related on the abundance and the distribution of the fine sediments and very ends. Just as the temporal dynamics of the hydrological factor is determining in the phenomena of accumulation, retention and the dynamics of the bio film. The banks being clogged, the downstream sector of the outlet (Fez river) is fed in a majority way by the tablecloth of slope whose chemical quality is strongly degraded by the agricultural activities. The high percentages of nitrates involved changes of the chemical quality of water, which had with the bacteriological activity (deoxygenating, denitrification.) The high microbial activity is in close relationship to the nature of the substrate and the seasons. The dynamics of the faunistic assemblies depends mainly on the quality of the connections of the aquifers with surface water and of the quality of subsoil waters and in particular of the oxygen contents in the medium. The dynamics of bio-film through this bacteriological study made on the interstitial sediment with very fine grains, during the period being spread out June 97 until August 98, shows that the Sebou river undergoes a strong aggression by the discharges of urban and industrial worn water coming from the town of Fès. Biological balance is broken by the additional organic matter contribution. So the bacterial activity increases, the medium consumes more oxygen and consequently, the concentrations of this parameter drop considerably, according to its capacity with O_2 to feed, there is more or less

serious disturbance of the faunistic settlement. What harms indigenous fauna involving its disappearance in an irremediable way, and the entry of epige fauna saprobionte in the aquifer, producing transformations in the operation of the watery systems. Many more recently, within the framework of the study of the river hydrosystemes, certain work concerning the surface zone was attached to describe [13] the distribution of the bacteria according to a longitudinal gradient. That made it possible to detect a gradient decreasing at the space level as one moves away from the polluting outlet, which agrees with our results relating to the hyporheic zone to describe the distribution of the bacteria on the level of the interface water underground surface-water in particular in the first centimetres inside sediments [14]; [15]. This work showed a gradient decreasing of abundance and of the bacterial activity according to depth [16], [15] the polluted water surge constitutes a trophic contribution for the bacteria saprophytes and for underground fauna (in our case, absent in this section of Sebou) and surface limnivore and detritivore (Annelids and Chironomidae) This contribution can cause an increase in the number of animals saprophages [17]. The effect of the hydrological conditions also seems to play a dominating role in the proliferation of the bacterial biomass.

---

[1] SASSON A., 1970. le rôle des micro-organismes dans la biosphère et l'avenir de la microbiologie appliquée. *Bull. Soc. Sci. Nat. Phy. Maroc.* p 8.

[2] BOU C. et ROUCH, 1967. Un nouveau champ de recherches sur la faune aquatique souterraine. *C.R. Acad. Sci. Paris,* 256: 369- 370.

[3] BOU C. 1974. Recherches sur les eaux souterraines. 25- Méthodes de récolte dans les eaux souterraines interstitielles. *Ann. Spéleol. 29(4):* 611- 619.

[4] MATHIEU J., P. MARMONIER, R. LAURENT & D. MARTIN, 1991. Récolte du matériel biologique aquatique souterrain et stratégie d'échantillonnage. *Hydrogéologie* n°3, pp 187-200, 11 fig.

[5] MARMONIER P., 1988. Biocénoses interstitielles et circulation des eaux dans les sous écoulements d'un chenal aménagé du Haut-Rhône français. *Thèse doct. Univ. Claude Bernard, Lyon,* 319 p.

[6] DALEY R.J. & J.E. HOBBIE, 1975. Direct counts of aquatic bacteria by modified epifluorescence technique. *Limnol.Oceanogr.*, 20(5): 875-882.

[8] FRY J.C., 1988. Determination of biomass. *In* B. Austin (Ed). Methods in aquatic bacteriology. John Wiley & Sons Ltd. pp 27-71

[9] DAHM C., 1981. Pathways and mechanisms for removal of dissolved organic carbon from leaf leach ate in streams. *Can. J. Fish. Aquatic. Sci*. 38: 68-76.

[10] LEICHTFRIED M., 1988. Bacterial substrates in gravel beds of a second order Alpine stream (Project Ritrodat- Lunz, Austria). *Verh. Internat. Verein. Limnol*. 24: 1921-1925.

[11] DUARTE C. M., D. F. BIRD & J. KALFF, 1988. Submerged macrophytes and sediment bacteria in the littoral zone of lake Memphremagog (Canada). *Verh. Internat. Verein. Limnol.* 23: 271-281.

[12] MEYER J. L., 1988. Benthic bacterial biomass and production in Blackwater River. *Verh. Internat. Verein. Limnol.* 23: 1832-1838.

[13] FEKHAOUI M., 1990. Recherches hydrobiologiques sur le moyen Sebou soumis au rejet de la ville de Fès ; suivi d'une macro pollution et évaluation de ses incidences sur les composantes de l'écosystème. *Thèse d'état, Fac. Sci., Rabat,* 173p.

[14] CHAFIQ M., 1995. L'interface eau souterraine/ eau superficielle comme régulateur des flux de matière organique : relation entre flux de carbone, organismes souterrains et biofilm. *Thèse doct. Univ. Claude Bernard, Lyon,* 146 p.

[15] MAUCLAIRE L., 1999. Les aquifères alluviaux : interactions entre les compartiments physique, chimique, microbiologique et faunistique. *Thèse doct. Univ. Claude Bernard, Lyon,* 160pp.

[16] MARMONIER P., VERVIER P., GIBERT J. et M.J. DOLE-OLIVIER, 1993. Biodiversity in groundwaters. TREE 8/11: 392-395.

[17] TURQUIN M.J., 1980. La pollution des eaux souterraines: incidence sur les biocénoses cavernicoles. *1er coll. Protection des eaux souterraines, Besançon.*

[18] DOLE M.J. 1983. Le domaine aquatique souterrain de la plaine alluviale du Rhône à l'Est de Lyon.1. Diversité hydrologique et biocénotique de trois stations représentatives de la dynamique fluviale. *Vie milieu, 33, (3/4) : 219- 229.*

[19] HOBBIE J.E., DALEY R.J. & S. JASPER, 1977. Use of nucleopore filters for counting bacteria by fluorescence microscopy. *Applied environm. Microbiol*. 33: 1225-1228.

[20] RTER K. & Y.FEIG, 1980. The use of DAPI for identifying and counting aquatic microflora. *Limnol.Oceanogr*.25: 943-948.

[21] BAKER J.H. & I. FARR, 1977. Origins, characterization and dynamics of suspended bacteria in two chalk streams. *Arch. Hydrobiol.*, 80 (3) : 308-326.