Wat. Sci. Tech. Vol. 24, No. 2, pp. 387–390, 1991.
Printed in Great Britain.

0273–1223/91 $0·00 + ·50

DYNAMICS OF NON-01 *VIBRIO CHOLERAE* IN EXPERIMENTAL SEWAGE STABILIZATION PONDS UNDER ARID MEDITERRANEAN CLIMATE

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ABSTRACT

The temporal dynamics of non-01 ***Vibrio cholerae*** and pollution-indicator bacteria were studied in experimental stabilization ponds under an arid Mediterranean climate after 30 months of a biweekly monitoring program. The non-01 ***Vibrio chlolerae*** MPNs showed a strong annual periodicity of the abundances for this bacterial population : low levels during the cold season, high levels during the hot season ; this seasonal cycle appeared in the inflow, in the first pond and in the final outflow. Classical pollution-indicator bacteria behaved quite differently, according to data from the literature. The sewage treatment process looked ineffective on this bacterial type : no bacterial reduction during the hot season, no significant decrease during cold season.

KEYWORDS

Stabilization pond; non-01 ***Vibrio cholerae***; pollution-indicator bacteria; arid Mediterranean climate.

INTRODUCTION

To investigate the process and efficiency of sewage treatment by stabilization ponds under an arid Mediterranean climate, a research program was carried out at Marrakech (Morocco 31°36 N, 8°02 W, alt. 471 m), from July 12th 1985 to December 23th 1987, in an experimental system.

In this report, we give our first results on the survey of non-01 ***Vibrio cholerae*** in urban wastewater and in the treatment system. There are very few published data about isolation or enumeration of ***Vibrio cholerae*** in sewage (GERICHTER et al, 1971 ; BARRETT et al, 1980 ; DANIEL and LLOYD, 1980 a; ROBERTS et al, 1982 ; URDACI-BERTRAN, 1987). There is still less information on the fate of ***Vibrio cholerae*** in sewage treatment plants (KOTT and BETZER, 1972; DANIEL and LLOYD, 1980 a, 1980 b).

To our knowledge, this is the first report on the dynamics of non-01 ***Vibrio cholerae*** in waste stabilization ponds.

The objectives of this study are mainly to determine (i) how the temporal evolution of non-01 ***Vibrio cholerae*** is related to other bacteria of sanitary interest and to environmental factors, and (ii) how much of the non-01 ***Vibrio cholerae*** is reduced along the stabilization pond system.

MATERIALS and METHODS

The experimental sewage stabilization pond system comprises two successive basins of 0.25 ha each, the bottoms of which are covered with a plastic film. The inflow is raw wastewater collected from the residential area of the town (700 000 r.), continuously coming in from a drain after a 2 km flow in the open air. The average depth in the ponds varied from 1.60 m (first basin) to 1.70 m (second basin). The total hydraulic retention time was set at about 50 days (beginning in December 1985); it was undetermined before this time (average value of about 8 days).

The monitoring program, started when sewage first filled up the two ponds, was rigorously pursued for 30 months at biweekly intervals.

Water samples were collected in sterile vials around 10 in the morning : this is the period with the highest bacterial density in sewage (BOUSSAID et al, 1987). They were analyzed within 2h after preservation in melting ice.

Inflow and sampling stations, numbered 3 and 6, are shown in Fig.1 ; they correspond respectively to the raw sewage, the first pond, and the final outflow.

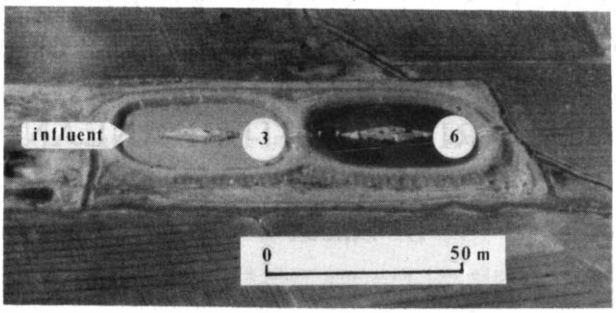

Fig. 1. Experimental sewage stabilization ponds of Marrakech (aerial photography)

For monitoring bacteria, recovery methods influence the results, especially for *Vibrio cholerae* (ROBERTS and SEIDLER, 1984; SPIRA 1984).

Series of 100, 10, 1ml, and ten to ten dilutions of water were used as inocula for the *Vibrio cholerae* MPNs with 3 tubes or flasks per dilution. Peptone Sanine Broth (1 % peptone - 3 % NaCl - pH 8,6) was used for enrichment after incubation at 37°C for 18h. The tubes or flasks showing turbidity were spread onto TCBS agar (Diagnostics Pasteur) and incubated at 37°C for 36h. The plates were examined for sucrose positive Vibrio-like colonies, and at least four typical colonies were subcultured on Nutrient Agar (Bio-Mérieux) for purification and screening tests : growth in 0 % NaCl at 42°C - oxidase reaction positive - sensitivity to the vibriostatic agent 0/129. This presumptive bacteriology was confirmed by submitting representative samples of isolated strains to speciation by Api 20 nE system. Serological analysis was carried out by using polyvalent *V. cholerae* 01 antiserum (Diagnostics Pasteur). None of the isolated strains showed agglutination. The MPN of non-01 *Vibrio cholerae* was obtained from the presumptive MPN (growth in PSB) by this confirmative scheme.

The pollution-indicator bacterial types studied simultaneously, on the same water samples, were Fecal coliforms, Fecal Streptococci, and *Pseudomonas aeruginosa* according to Baleux and Troussellier (1983).

Physical, chemical and biological environmental variables were measured concurrently with the bacterial abundances in this monitoring program. Some of them are used in this work for statistical comparison of temporal evolutions by KENDALL rank correlation : temperature (T, in °C), conductivity (C, in μ S/cm), pH, suspended solids (SS, in mg/l), transmitted radiant energy (R, in W/cm), which was obtained as the product of irradiance (E, in W/cm^2) and Secchi depth (in cm), chlorophyll a concentration (Chl a, in μg/litre) and protozoa density (Protoz, in animals/litre).

RESULTS and DISCUSSION

Figure 2 depicts temporal evolutions of non-01 *V. cholerae* MPNs in the inflow, at station 3 and at station 6, during the 30 month monitoring program. The same season cycle appears in the inflow, in the first pond and in the final outflow : low levels of *V. cholerae* MPNs during the cold season (from December to March) and high levels during the warm season (from April to November).

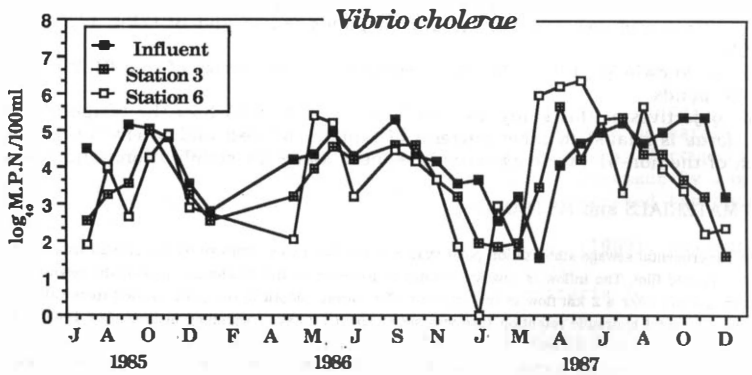

Fig. 2. Temporal evolutions of Non-01 *Vibrio cholerae*

Table 1 gives the average levels of the abundances of non-01 ***Vibrio cholerae*** (V.c.) in comparison with Fecal coliforms (F.C.), Fecal Streptococci (F.S.) and ***Pseudomonas aeruginosa*** (P.a.).

TABLE 1. Comparison of the average abundances of non-01 *Vibrio cholerae*, Fecal coliforms, Fecal Streptococci and *Pseudomonas aeruginosa*.

	WARM SEASON (April to November)			COLD SEASON (December to March)		
	Inflow	St.3	St.6	Inflow	St.3	St.6
V.c. (MPN/100 ml)*	$5.1x10^4$	$2.1x10^4$	$1.9x10^4$	$1.7x10^3$	$1.7x10^2$	$4.1x10^1$
F.C. (c.f.u./100 ml)**	$9.5x10^6$	$6.5x10^5$	$3.0x10^4$	$7.1x10^6$	$1.9x10^6$	$1.4x10^5$
F.S. (c.f.u./100 ml)**	$1.7x10^6$	$3.5x10^4$	$2.8x10^3$	$1.6x10^6$	$1.7x10^5$	$7.8x10^3$
P.a. (c.f.u./100 ml)**	$2.6x10^4$	$3.7X10^3$	$4.8x10^2$	$9.8x10^3$	$3.5x10^3$	$2.5x10^2$
* : means of 15 values **: means of 32 values				means of 4values means of 8 values		

Temporal evolutions of non-01 *V. cholerae* are positively correlated (KENDALL rank correlation) with temperature, throughout the treatment plant. The influence of temperature on the numbers of recoverable *V. cholerae* in different types of surface waters has been established in several studies (BOCKEMÜHL et al 1986 ; GARAY et al , 1985 ; SEIDLER and EVANS, 1984 ; WEST and LEE, 1982).

This could indicate control of this bacterial population by climatic factors acting through physical, chemical and biological variables. But temporal evolutions of non-01 *V. cholerae* are highly correlated (Kendall rank correlation) with several environmental factors in the first basin only (T, pH, SS, Chl.a, Protoz.) and the relations are lost in the second basin. There is no correlation with R (transmitted radiant energy) throughout the treatment plant.

In the ponds, temporal evolutions of non-01 *V. cholerae* are negatively correlated with fecal contamination indicators (Fecal coliforms and Fecal Streptococci) and not correlated with *Pseudomonas aeruginosa*. In the inflow, non-01 *V. cholerae* counts are correlated with *Pseudomonas aeruginosa*, and not with fecal contamination indicators.

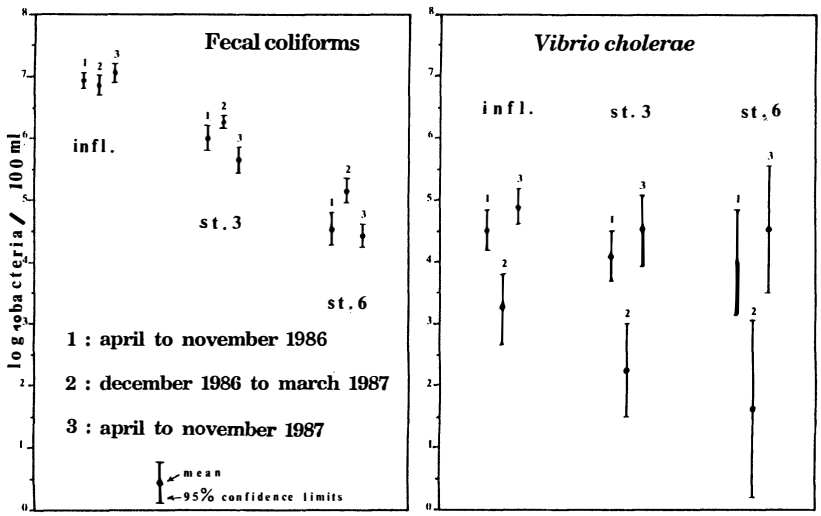

Fig. 3. Fecal coliforms and Vibrio cholerae reductions in the treatment system.

Fig. 3 shows that the treatment process is ineffective on the non-01 *V. cholerae* population : there is no bacterial reduction during the hot season, when other pollution indicators are well reduced, and there is no significant decrease during the cold season.

PERSPECTIVES

Several hypotheses must be tested with further statistical investigations and laboratory experimentations : (i) relations with other factors, (ii) considering the retention time when comparing the series inflow, station 3 and station 6, (iii) physiological differences in the population of non-01 *V. cholerae* between basin 1 and 2.

ACKNOWLEDGEMENTS

This study was financially supported by a French-Moroccan cooperation programme (A.I. 75) and a Canadian Research council grant.

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