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Removal, Species Dynamics and Antimicrobial Susceptibility of Motile Aeromonads and Faecal Bacteria during Municipal Wastewater Purification by Activated Sludges under Aride Climate

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ABSTRACT

In order to evaluate the removal efficiency of an activated sludge plant operating under aride sahelian climate in Mauritania, a 15 months period bacteriological and chemical survey was undertaken. Motile aeromonads, *Pseudomonas aeruginosa*, faecal coliforms, faecal streptococci and the total heterotrophic bacteria abundances were systematically determined at three sampling points : E (wastewater plant influent), C (oxidation pond) and S (treated effluent). The total suspended solids (TSS) and the biochemical oxygen demand (BOD), temperature and pH were also systematically measured for each sample.Two seasonal qualitative analyses for the *Aeromonas* species dynamics have been undertaken on water samples obtained from the influent (E) and the effluent of the plant (S). Results showed removal efficiency of 0,45 U log, 0,41 U log, 1,16 U log, 0,59 U log and 0,47 U log for the motile *Aeromonas* species, *Pseudomonas aeruginosa*, total heterotrophic bacteria, faecal coliforms and faecal streptococci respectively. In regard to the chemical pollution removal efficiency, the results revealed an important removal of the total suspended solids and BOD. This removal seems to occur essentially (68 %) between the oxidation channel and the clarification pond.

The species dynamics analyses showed that *A. caviae* dominated in both raw wastewater and effluent. *A. sobria* strains were slightly present (17 %) in the raw wastewater and no *A. hydrophila* strains were detected in the treated effluent in the hot period campaign. The statistical non parametric analysis revealed significant differences ($P<0.05$) between the different bacterial abundances in the inflow (E) and the outflow (S) of the treatment plant, but have showed that the bacterial removal occurred mainly during the oxidation phase and that only a slight removal (23%) were originated from the clarification phase. Antimicrobial susceptibility tests have covered two hundred and twenty four-strains of motile *Aeromonas* species and *E. coli* isolated from the influent, the oxidation pond and effluent. Fifteen of the most frequently used antibiotics in the antibiotic treatment at the national level were tested on these isolates.

The statistical analysis showed no significant differences ($P>0.05$) in the resistance patterns between influent and effluent isolates. All strains were found to be monoresistant, primarily to Vancomycin. Nearly 4.5 % of *A. hydrophila* and 3.1 % *A. caviae* were resistant to Cefoperazone, while greater than 97.1 % of *A. sobria* were found to be susceptible to this drug. The overall resistance rates to Amikacin and Chloramphenicol did not exceed 4.6 and 10.6 % respectively.

The results indicate that despite the important removal rate given by the treatment process, antimicrobial resistance incidence among pathogenic aeromonads has not been decreased and remains significant to potentially compromise the reclaim of the treated effluent in urban agricultural practices in the wastewater spreading area of Nouakchott where water reclaim permits to the urban agriculture to survive water scarcity.

KEYWORDS: Activated sludges, *Aeromonas*, species dynamics, Antibiotics, Irrigation, Polyresistance, Removal, Sanitation, Urban agriculture, Wastewater, Water reclaim.

INTRODUCTION

Municipal wastewater purification has become more important during the last decades due to increased awareness on the wastewater associated risks to both human health and the environment. Problems related to municipal wastewater have
become increasingly critical due to the growing numbers of slums that result from accelerated urbanization as a consequence of the drought in rural areas.

Mauritania is a sahelian country that is characterized by a hot and arid climate, with a permanent water shortage. Therefore, there is a great need for the reclaim of wastewater. In the capital, Nouakchott, more than 28% of the population suffers from lack of drinking water and urban agriculture begins to be highly dependent on drinking water availability. The activated sludge wastewater purification process is one of the most efficient wastewater treatment technologies (Bond et al., 1998). Nevertheless, bacteria remain present in the effluent and represent a potential risk of infection for those who come in contact with it. Furthermore, toxic effects may result from the remaining chemical pollutants including heavy metals.

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The process removal performance depends on the bacterial activity which permits rapid decomposition of the organic matters. Different removal rates have been reported for the motile *Aeromonas* species, *Pseudomonas*, the heterotrophic bacteria, the faecal coliforms and the faecal streptococci but no reliable models have been possible to establish due to the high variability of the operations and the multiplicity of the involved parameters. This study investigates the effect of the activated sludges treatment process on the bacterial survival and species dynamics through the wastewater purification process. This paper deals specifically with the ongoing characterization of the purified wastewaters of Nouakchott and the critical normative evaluation of their suitability in being continuously utilized as safe irrigation waters. This study gave for the first time an overall idea on the removal efficiency of the studied plant to play as a decision making tool since considerable quantities of micro organisms were still present in the treated effluent.

Motile aeromonads often remain in the activated sludge effluent. These species have long been reported to cause various outbreaks in humans and poikilothermic animals (Dumontet et al., 2000; Kannan et al., 2001, Mahmoud and Mohamed, 2011). These species are characterized by a dynamics that seems to play a key role in the associated infections they cause for humans. *A. caviae* has been reported to be the dominant species in the raw wastewater but no data were found in regard to any change in the species dynamics as a result of the activated sludges wastewater treatment process. In the city of Nouakchott, motile aeromonads have been identified as the source of several categories of human infections associated with raw vegetables consumption. The frequent isolation of *Aeromonas* strains in stool samples from diarrheic patients in the area indicates that these infections raise serious health concerns, calling for appropriate precaution measures and effective antibiotic therapy.

In addition, the widespread use of antibiotics in agriculture as growth promoters and therapeutic or prophylactic agents have been reported (Imziln et al., 1998). This practice could increase the risk of bacterial resistance proliferation among environmental flora, resulting in reduced effectiveness of antimicrobial chemotherapy in the event of an infection. This study investigates also the effect of the activated sludge Treatment process on the antibiotic resistance of three motile *Aeromonas* species and faecal coliforms. Antibiotic resistance profiling has been restricted to the aeromonads in regard to their recorded high infection rate in Nouakchott (Gagneux et al. 1999). In addition, *E. coli* is known as a representative faecal and opportunistic bacteria frequently associated with urinary infections. The goal of this research is to assess the health risks associated with the use of treated effluent for irrigation of vegetables in Nouakchott's urban agricultural area and to determine whether or not this practice could be seen as acceptable in terms of human health. Wastewater reclaim is undertaken in a normative context since the most frequently used standards allow restrictive irrigation of crops with purified wastewater when water quality requirements meet those of the World Health Organization (WHO) standard B category. The limit for the faecal coliforms bacteria corresponding to this standard is 10^5/100 ml, under conditions where adult farm workers are exposed to spray irrigation (Blumenthal and Mara, 2000).

METHODS AND MATERIALS

Study Area

Nouakchott (18°7 N, 15°05 W), the main urban center and capital of Mauritania, is located in the south-west of the country, along the Atlantic coast. The studied waste water treatment plant is found in the suburb of Sebkha near one of the most important urban agricultural areas in Mauritania. It became operational in 1996 and receives an average influent of 18,000 m^3/day. The facility consists of a typical activated sludge plant with primary and secondary treatment followed by retention in an oxidation channel, which is continuously oxygen-enriched by two pumps. The figure 1 shows the scheme of the process. The average inflow rate is about 37 liters / sec and the outflow rate is 32 liters / sec. The plant was initially designed for 432,000 equivalent inhabitants and is currently managed by the national office for sanitation (ONAS).

This treatment process generates significant quantities of sludge which are dried then used as fertilizer. The microbiological characterization of the dried sludge is assessed in order to assess whether or not they are suitable for be utilized as fertilizer outside the studied area.

Figure 1: Aerial view of the Nouakchott wastewater treatment plant showing the agricultural area in it neighbourhood.

Sampling

Water samples were collected in pre sterilized glass bottles and stored in cold boxes at 4°C until their processing. Three sampling points were considered for this study : E at the plant influent, C in the oxidation pond and S corresponding to the purified wastewater effluent at the end of the clarification phase. Thirty three samples were analyzed on a 2 weeks basis and for a 15 month period. Subsamples were systematically taken from each sampling point and four seasonal samples were collected between January and December and submitted to additional typing and microbiological characterization.
Between thirty and forty strains were isolated and screened from each sample. Samples were generally taken at about midday and analyzed within two hours

Bacteriological methods

Water samples were shaken gently for about five minutes, then ten-fold diluted in sterile saline solution 0.85 % (w/v of NaCl in demineralized water). Aliquots of 0.1 ml from suitable dilutions were spread onto the appropriate culture media. Plate count agar (Difco 247940), PADE agar (Imziln et al, 1997) and BD Cetrimide Pseudosel Agar (Difco 254419) were utilized as culture media respectively for the total heterotrophic bacteria, the motile aeromonads and *Pseudomonas* strains. Plates were incubated respectively at 35°C, 37°C and 42 °c. Mac Conkey agar (Difco 212123) and Sodium azide bile esculine agar (Difco 212205) was utilized as selective media for faecal coliform (44,5°c) and faecal streptococci (37°c) respectively.

Typical colonies strains from each specific group were sub cultured on trypto-casein-soja agar (TSA, Diagnostic Pasteur 64554) plates before they were submitted to species identification process. Presumptive *Aeromonas* isolates were confirmed when they were: Gram-negative rods, oxydase positive, motile, fermentative of glucose (O/F test Hugh-Leifson medium, Merck 10282), arginine dihydrolase positive (ADH, Möller), resistant to the vibriostatic agent 2,4 diamino-6,7-diisopropylpteridine phosphate (O129, 150 µg. Diagnostic Pasteur 53872). Strains corresponding to other biochemical profiles were considered as non *Aeromonas* and rejected from the antimicrobial susceptibility testing.

Aeromonas isolates were identified to the species level according to the biochemical profiles described by Popoff (1984). These biochemical tests were: fermentation of salicine, production of acetoïne (Voges-Proskauer reaction), esculin hydrolysis, production of gas from glucose, decarboxylation of lysine (Möller), fermentation of L-arabinose and production of H2S from L-cysteine. API 20 NE strips (bioMerieux 20 050) were alternatively utilized for this purpose. Atypical *Aeromonas* strains were also rejected from the additional typing. Coliforms strains screening were undertaken through the IMViC tests (Indole, Methyl red, Voges-Proskauer and Simmon's Citrates) and *Pseudomonas aeruginosa* strains were confirmed when they were Gram-negative rods, oxydase and catalase positive, motile, oxidative of glucose (O/F test Hugh-Leifson medium, Merck 10282).

Antibiotic susceptibility testing

Antimicrobial susceptibility were tested only for the *Aeromonas* strains and was determined using the standard diffusion method of Kirby Bauer (Bauer et al., 1966). Isolates were checked for purity, sub cultured in brain heart broth tubes, grown on TSA plates, and then inoculated by spreading on Mueller-Hinton agar (Mueller Hinton 2, bioMérieux 51861). Antibiotic concentrations (μg/ml) were : Ticracillin 75 μg; Sulphamides 200 μg; Imipenem 10 μg; Colistin 50 μg; Vankomycin 30 μg ; Piperacillin 100 μg; Cefoperazone 30 μg; Cefsulodin 30 μg ; Fosfomycin 50 μg; Amikacin 30 μg; Oxacillin 1 μg; Tobramycin 10 μg; Ampicillin 10 μg; Erythromycin 15 μg and Chloramphenicol 30 μg. All chemicals were from bioMérieux (Marcy, France) and were of an analytical grade.

Chemical methods

The biochemical oxygen demand (BOD) and the total suspended solids (TSS) were measured according to the procedures of the American Public Health Association (APHA, 1998). The total organic carbon (TOC) was determined according to the standard measurement procedure for the wastewater (ISO 8245:2000). Conductivity, pH and wastewater temperature were monitored by a digital probe, Hanna instruments (HI 9146).

Data analysis

Bacterial abundances were expressed in a 10-basis logarithmic scale and quantified as logarithmic unit (U log). The antibiotic resistance index (ARI) was calculated according to Hinton and Linton (1983) using the following formula: ARI = x/ny, where *x* represents the number of resistant determinants in a population *y*, and *n* represents the number of antibiotics tested. The comparison of the antibiotic resistance index (ARI) was performed by the Wilcoxon Signed rank non parametric statistical test and the StatView software was utilized for the statistical comparison for the all data.

RESULTS

The spatiotemporal evolution of the total aerobic heterotrophic bacteria within the total15-months period is shown in the figure 2. The curve demonstrates clearly expressed seasonal evolution trends when the abundances decreased very considerably during the cold months of the year. The average abundances in terms of colony forming units (CFU) numbers of the aerobic heterotrophic bacteria by millilitre of the wastewater were 7,3 U log and 6.15 U log respectively for the raw wastewater and the effluent and the overall removal efficiency percentage corresponding to this population group was 91,2 %.

The evolution of the *Aeromonas* species strains population abundances is shown in the figure 3. The abundance averages of this group were 3.69, 3.36 and 3.26 U log respectively in the raw wastewater (E), the oxidation pond (C) and the treated effluent (S). The removal efficiency rates corresponding to these counts evolution within the plant were 0.32 U log between sampling points E and C and 0.13 U log between sampling points C and S. The overall performance is 0.45 U log (87,53 %). The temporal evolution of this group showed fluctuant trends but no clear seasonal evolutions have been revealed through the direct examination of the evolution curves. In regard to the species dynamics, the results indicate that *A. caviae* strains have been found to dominate in both raw (58 %) and the treated effluent (63 %) for the two climate periods. In addition, the presence of *A. sobria* and the *A . hydrophila* strains in the raw wastewater was significantly different for the two periods. The *A. hydrophila* strains have been found considerably in the cold period as shown in the figures 4 and 5. The results obtained in regard to the faecal coliforms abundances monitoring are given in the figure 6. Seasonal evolution seems to be most clear in the case of this group with a maximum abundance recorded at 5.23 U log and a minimum at 3.33 U log in the raw wastewater.The faecal streptococci, the global abundances averages were 3.52 and 3.05 U log CFU/ml respectively for the two sampling points E and S. Evolution within the 15-months period survey is shown in figure 7 and did not shown clear trends of spatial distribution.

Pseudomonas aeruginosa abundances evolution is represented in the figure 8 with an average of 2.77 and 2.36 U log for the raw wastewater and the treated effluent respectively. Abundances showed slight increasing trends during the hot period. However, the removal efficiency reached for the *Pseudomonas aeruginosa* strains (0.32) has been found to occur mainly during the oxidation phase.
Results regarding the biochemical oxygen demand(BOD) are shown in figure 9. The averages obtained for the complete

period of the survey, were 184 mg/l in raw wastewater, 91 mg/ml in the oxidation pond (C) and 35 mg/l in the effluent at the exit of the clarification phase (S). The results related to the chemical load did not reveal seasonal evolution for both the biochemical oxygen demand and the total suspended solids (figure 10). The average load of the total suspended solids were 51 mg/l and 34 mg/l between the sampling points E and C and 19 mg/l at the exit of the plant, corresponding to a total removal efficiency of 62 %.

The antimicrobial susceptibility tests have targeted 224 bacterial strains, including 151 *Aeromonas* and 73 *E. coli*. The specific composition and origin of the 224 tested bacterial isolates is given in table 1. The *Aeromonas* population consisted of 35 *A. hydrophila*, 64 *A. caviae*, 44 *A. sobria* and 8 atypical *Aeromonas* isolates. 57 strains representing 37.8 % of the entire aeromonads population originated from raw wastewater, while 94 strains representing 62.2 % were obtained from the plant effluent.

All of the 73 coliforms strains were formally identified as *E. coli*, of which, 35 (47.9 %) were isolated from raw wastewater and 38 (52.1 %) were isolated from the effluent. The results related to the antimicrobial susceptibility to the fifteen tested antibiotics are given in the Table 2. The antibiotic resistance levels are expressed in percentages of the whole population and as antibiotic resistance index numbers. Analysis regarding the three *Aeromonas* species polyresistance patterns in relation to the origin is shown in the Table 3. Data were calculated for the combinations of resistance from 2 to 7 antibiotics.

Figure 2 : spatiotemporal evolution of total aerobic bacteria spp abundances

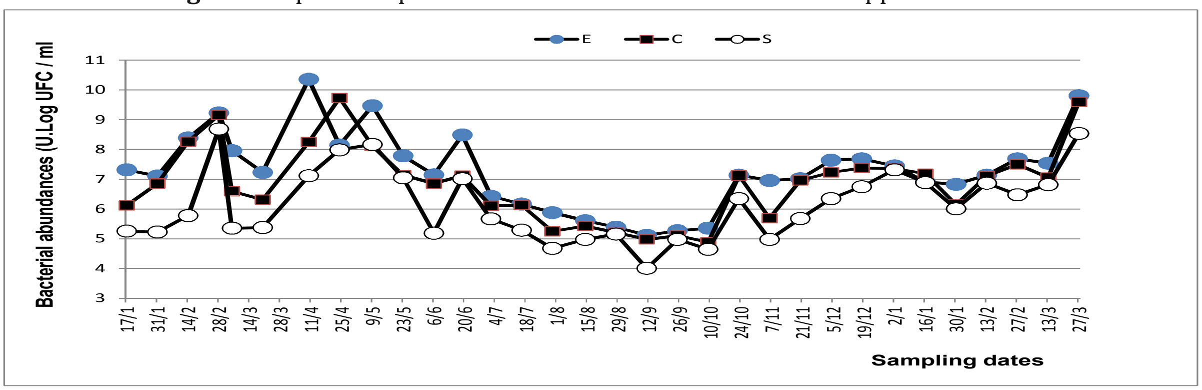

Figure 3 : spatiotemporal evolution of Aeromonas spp abundances

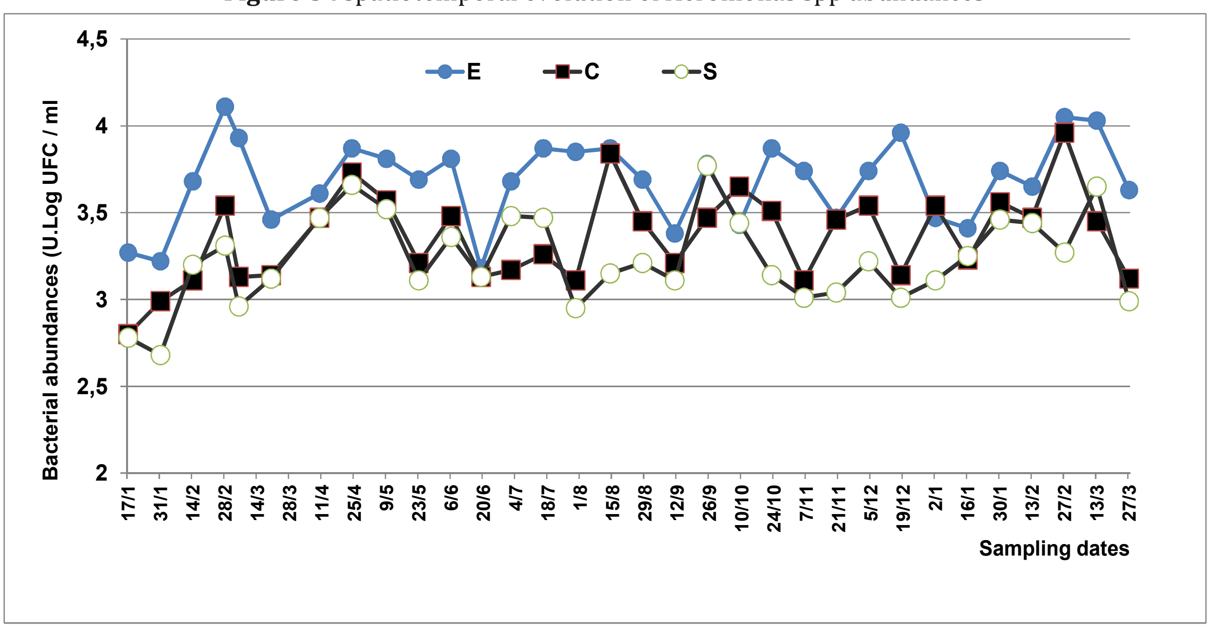

Figure 4 : Species dynamics of motile aeromonads during the hot period

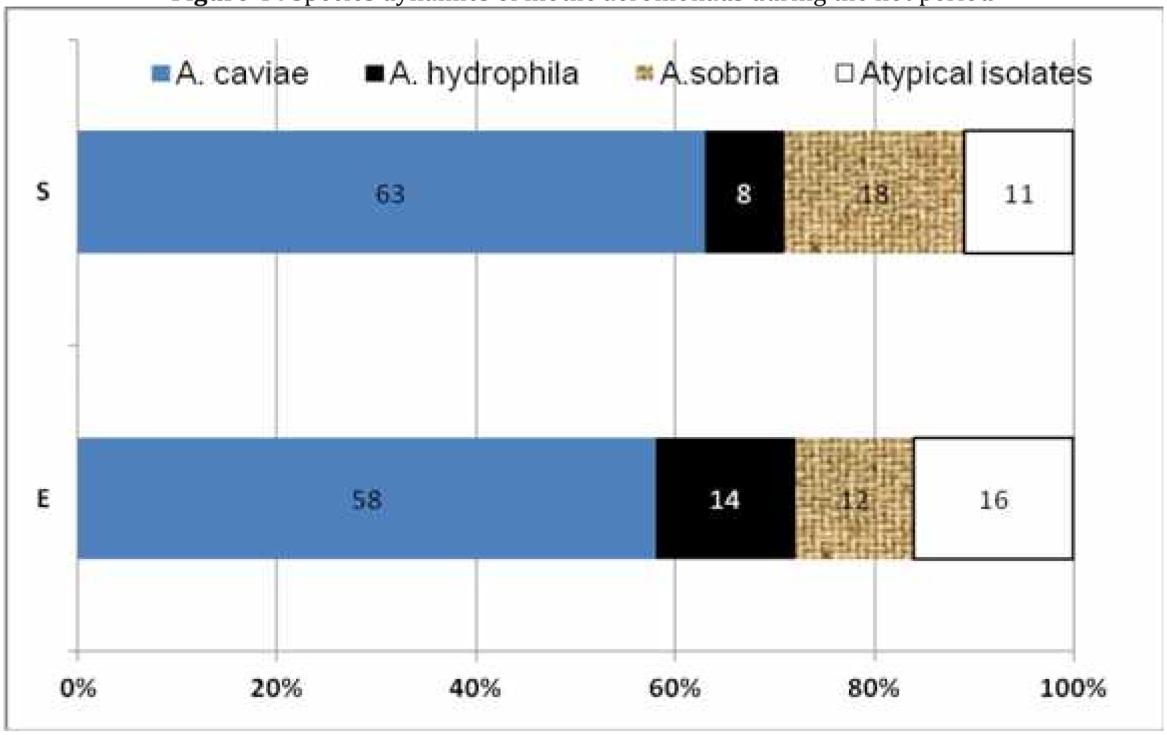

Figure 5: Species dynamics of motile aeromonads during the cold period

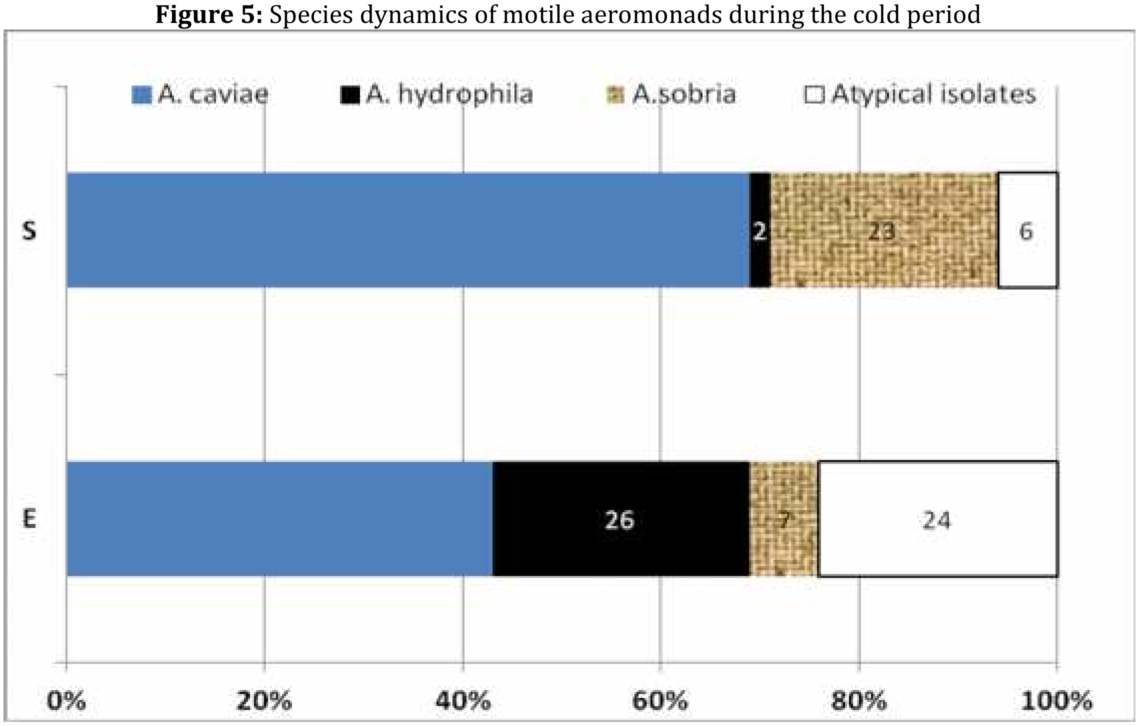

Figure 6 : spatiotemporal evolution of Faecal coliforms abundances

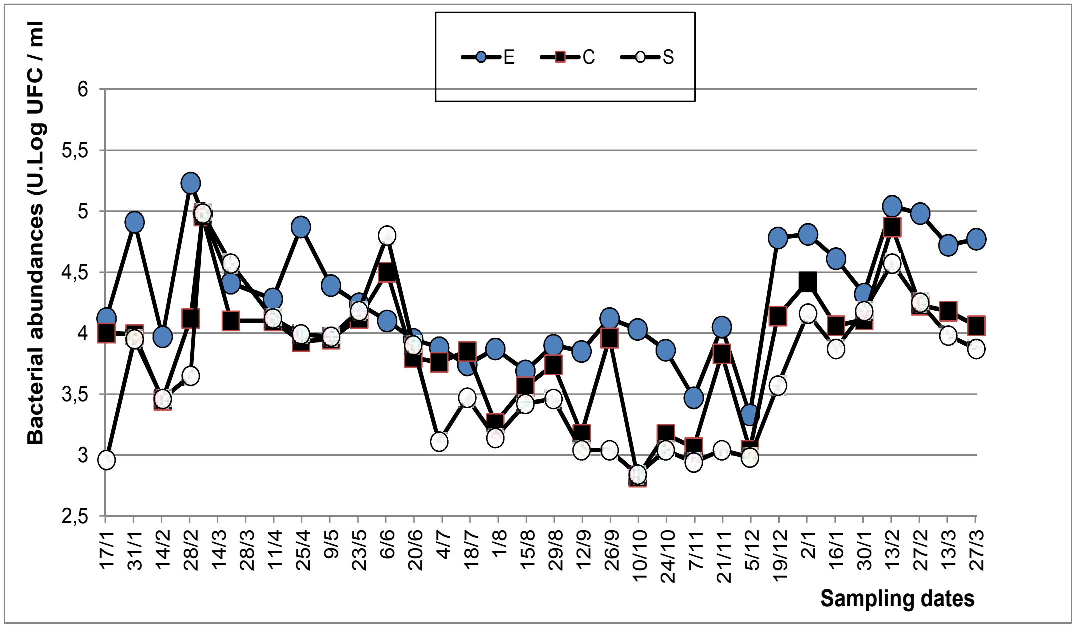

Figure 7 : spatiotemporal evolution of faecal streptococci abundances

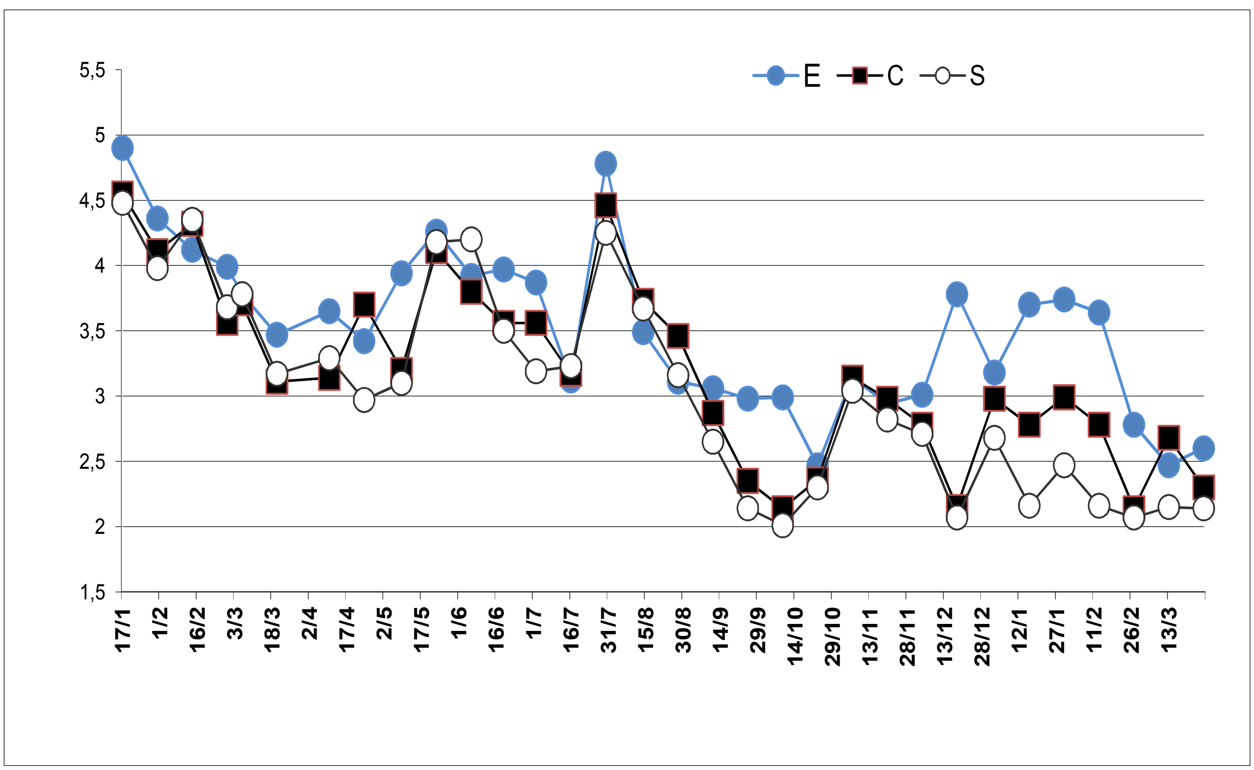

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Figure 8 : spatiotemporal evolution of Pseudomonas spp abundances

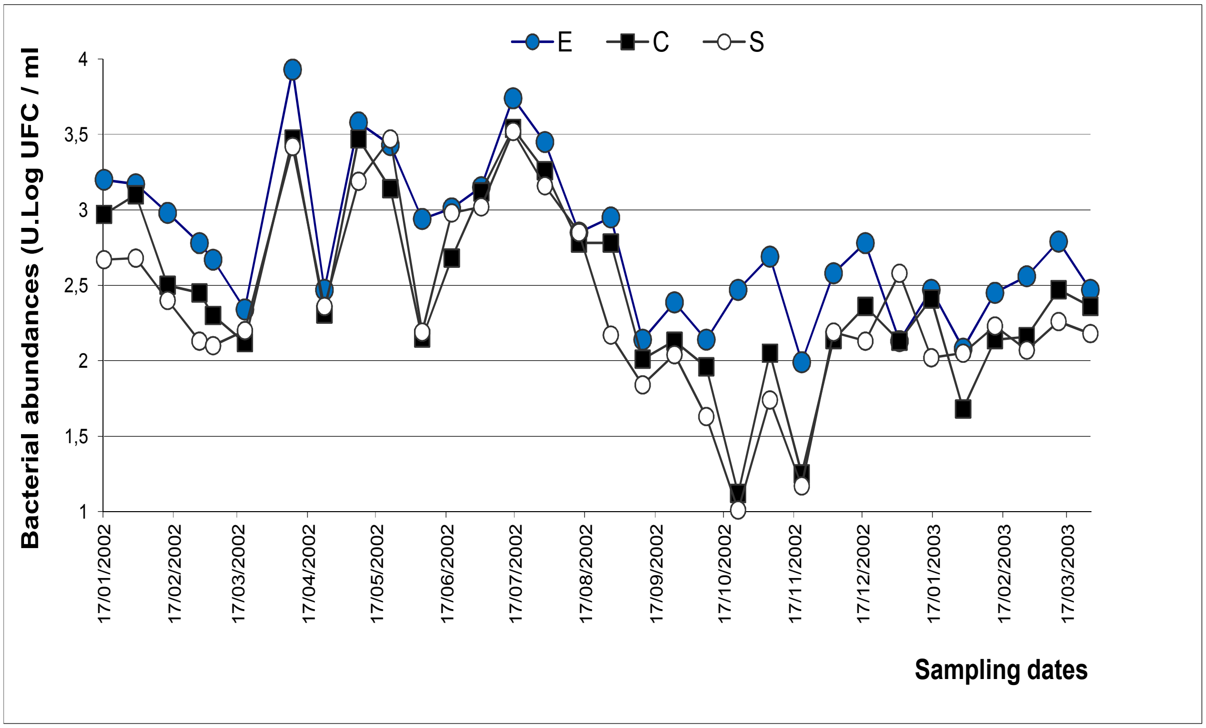
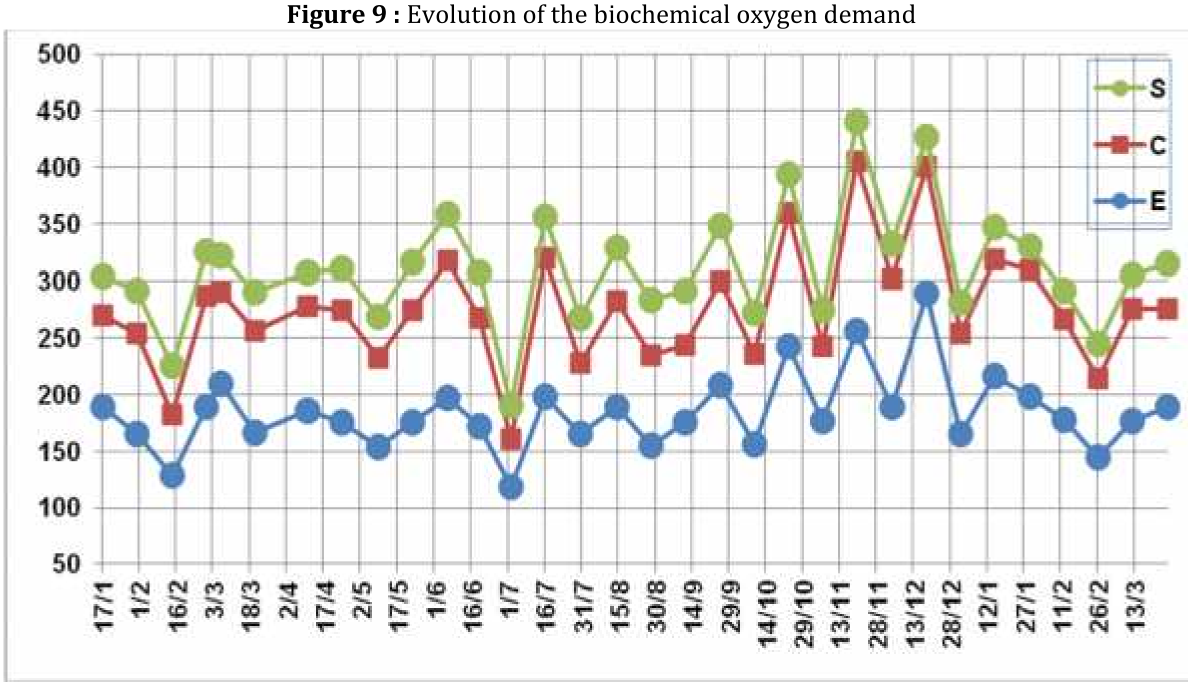
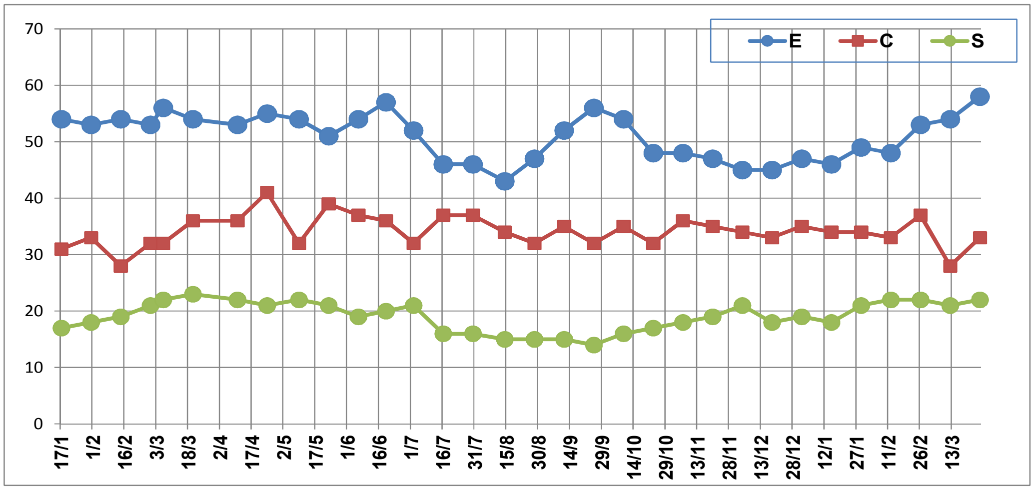


Figure 9 : Evolution of the biochemical oxygen demand



Sampling dates

Figure 10 : Evolution of the total suspended solids



Sampling dates

Table 1 : Specific composition and origins of the Aeromonas and E. coli isolates

Species	Influent	Effluent	Total
Aeromonas caviae	**23**	**41**	**64**
Aeromonas sobria	**11**	**33**	**44**
Aeromonas hydrophila	**17**	**18**	**35**
Atypical isolates	**06**	**02**	**08**
Escherichia coli	**35**	**38**	**73**
Total	**92**	**132**	**224**

Table 2 : Percentages of monoresistance and antibiotic resistance index among Aeromonas spp. and E. coli isolates

Antibiotic	*Aeromonas caviae*		*Aeromonas hydrophila*		*Aeromonas sobria*		*Atypical isolates*		*Escherichia coli*	
	Num	%	Num	%	Num	%	Num	%	Num	%
Amikacin	03	4.7	01	2.3	02	5.7	01	12.5	02	2.7
Chloramphenicol	06	9.4	04	9.1	02	5.7	04	50	02	2.7
Ticracillin	12	18.8	08	18.2	06	17.1	04	50	06	8.2
Cefsulodin	02	3.1	02	4.5	02	5.7	01	12.5	67	91.8
Erythromycin	09	14.1	04	9.1	03	8.6	05	62.5	04	5.5
Fosfomycin	01	1.6	01	2.3	02	5.7	00	00	02	2.7
Cefoperazone	02	3.1	02	4.5	01	2.9	02	25	09	12.3
Sulphamides	00	00	01	2.3	00	00	00	00	01	1.4
Vancomycin	64	100	44	100	33	94.3	07	87.5	02	2.7
Colistin	01	01.6	00	00	00	00	00	00	00	00
Piperacillin	14	21.9	07	15.9	09	25.7	08	100	00	00
Imipenem	06	09.4	02	4.5	04	11.4	06	00	08	11
Oxacillin	17	26.6	05	11.4	14	40	03	37.5	09	12.3
Tobramycin	02	03.1	01	2.3	03	8.6	02	25	06	8.2

Ampicillin	**64**	**100**	**44**	**100**	**35**	**100**	**08**	**100**	**42**	**57.5**
ARI*	**0.2115**		**0.1909**		**0.2210**		**0,2104**		**0.3750**	

*** ARI : antibiotic resistance index**

Table 3: Percentages of polyresistance among motile Aeromonas in relation to the origin

	Influent		**Effluent**	
	Number	**%**	**Number**	**%**
Number of strains resistant to 2 ATB	**57**	**100**	**94**	**100**
Number of strains resistant to 3 ATB	**36**	**63.2**	**54**	**57.4**
Number of strains resistant to 4 ATB	**21**	**36.8**	**20**	**21.3**
Number of strains resistant to 5 ATB	**13**	**22.8**	**18**	**19.1**
Number of strains resistant to 6 ATB	**02**	**03.5**	**05**	**05.3**
Number of strains resistant to 7 ATB	**01**	**01.8**	**00**	**00**

DISCUSSIONS AND CONCLUSIONS

The average quantities of the aerobic heterotrophic bacteria in the raw wastewater (E), the oxidation pond (C), and the exit of the treatment plant (S) were 7,3 U.Log, 6,88 U.Log and 6,15 U log respectively. Removal efficiency percentages corresponding to the aerobic heterotrophic bacteria was 91,2 %. The survey has recorded average removal rates, showed an average of 0.68 logarithmic unit (61.2 %) for the *E. coli* population. The faecal coliforms seems to follow a seasonal evolution trend. The abundances of these bio indicators decrease considerably during the hot period. A similar seasonal evolution has been found to exist for the faecal streptococci population.

The bacteriological 15-months survey undertaken at the plant, has recorded average removal rates, showed only an average of 0.39 logarithmic unit (U. Log) removal (33.3 %) for *Aeromonas* species.

This research has shown that *A. caviae* strains dominated in both raw sewage (sampling point E) and in activated sludge plant effluent (sampling point S). The domination trends of *A. caviae* species in the wastewater activated sludges purification system agrees the findings of Martone-Rocha et al. (2010) who have reported domination of *A. caviae* strains in both the inflow and the outflow of an activated sludge wastewater treatment plant. Overall results of the monitoring showed that counts of motile aeromonads were found to be 3,69 U log in the raw wastewater, 3,36 U log in the oxidation pond and 3,23 U.Log in the effluent. In regard to the faecal streptococci, the overall averages for the three considered sampling points were 3,52 U.Log, 3,21 U.Log and 3,05 U.Log respectively. Removal efficiency percentages corresponding to the faecal streptococci was 41,8 %. Our results suggest that the activated sludges treatment under sahelian climate gives lower removal rates for the motile *Aeromonas* species in comparison to the performance of such purification plants when they are operating under ordinary climatic conditions.

The present study provided valuable information regarding the impact of the municipal wastewater purification (using the activated sludge process under sahelian climate conditions) on the incidence of antimicrobial susceptibility among aeromonads and *E.coli*. The results showed that the antibiotic resistance levels remain significant in the effluent of the plant and could lead to a probable proliferation throughout the bacterial population, seriously compromising the use of reclaimed effluent for irrigational purposes.

faecal coliforms distributions showed seasonal cycles, the amplitude of which increased at distances further from the wastewater source, so that in the last pond there was an inversion of the *Aeromonas spp*. cycle in comparison with that of faecal coliforms. The analysis of the *Aeromonas* species population structure showed that, regardless of the season, *Aeromonas caviae* was the dominant species at the pond system inflow. However at the outflow the *Aeromonas spp*. population was dominated by *A. caviae* in winter, whereas *Aeromonas sobria* was the dominant species in the treated effluent from spring to fall. Most activated sludge upsets and loss of process control are caused by one of several microbiological problems which include poor floc formation, pin floc, dispersed growth, filamentous and slime bulking, filamentous foaming, zoogloeal bulking, nitrification and denitrification problems and toxicity.

However, toxicity problems were expected to be among the problems affecting the studied plant. This effect is probably engraved by the small size of the whole system. Such functional problems are most frequent in small communities compared to larger cities, due to the lack of dilution of toxic releases in small systems. Examples of toxicity events were the washing of cement or lime trucks to a manhole, dumping of congealed diesel fuel to the sewer system, and overload of small systems with septage (which contains a high amount of organic acids and sulphides which can be toxic).

The predominate type of bacteria present can be determined by the nature of the organic substances in the wastewater, the mode of operation of the plant, and the environmental conditions present for the organisms in the process.

On the basis of the results obtained from the current study, we conclude that the activated sludges treatment of municipal wastewater gives slightly less performance under hot conditions in comparison to such removal rates obtained under mediterranean climatic conditions. However, the strains of the species *A. caviae* dominate the motile *Aeromonas* population in both raw wastewater and purified effluent and the activated sludges process did not select any species among the motile Aeromonas species.

The evolution of the aeromonads is characterized by a slight higher abundance during the hot period. a significant correlation has been found to exist between the removal efficiency in terms of organic chemical loads and total heterotrophic bacteria; The bacteriological quality of the purified wastewater, in terms of faecal coliforms abundances does not satisfy the requirements of WHO guideline B category. Consequently, the crop irrigation presently practiced must be avoided until requirements are met. The results of the present study are consistent with other studies (Kannan et al.,2001) and in agreement with many reports which suggest that *A. caviae* is the dominating species in the human faeces and consequently, in raw municipal wastewater. It has been found elsewhere that *A. caviae* abundances correlate with those of faecal coliforms (Araujo et al., 1991). In addition, many authors have reported that the stabilization ponds select *A. sobria* strains, leading to its domination in the effluent (Monfort and Baleux, 1990 ; Stecchini and Domenis, 1994).

Despite the relevance of these overall removal rates, this could not be considered as satisfactory, given the risk associated with the consumption of the crops produced in the spreading area and the occurrence of a significant antibiotic resistance. Nevertheless, the faecal coliforms loads in the effluent (<1000 CFU /100 ml) do not meet the requirements of WHO wastewater category A, henceforth prohibiting irrigation of the various crops for human consumption. These records correspond to the removal rates of 60,21% and 50,54 % for the oxidation pond and the clarification phase respectively, showing that the main removal is obtained during the oxidation phase in the process.

Furthermore, these results indicate that the removal of organic chemical loads proceeded primarily, as expected, from the oxidation pond process. The removal rate of the total suspended solids appears to be occurring during the clarification phase (66 %), while a moderate removal of suspended solids (34 %) occurs during the primary treatment of the raw wastewater owing to the additional dissolution of the suspended particles during the intensive mechanical aeration in the oxidation pond. Therefore, data indicated that aeromonads bacterial population is removed primarily in the oxidation pond (0,32 U log) corresponding to 71,11 % of the overall removal efficiency for this population.

The *Aeromonas* abundances did not show significant seasonal evolution pattern despite the observation of the slight "pik" which has been found in the cold period. However, the *Aeromonas* abundances when submitted to the Wilcoxon signed rank non parametrical test, showed statistically significant differences between the influent and effluent. The bacterial removal seems to be occurring essentially between the sampling points E and C, indicating that the removal occurs mainly at the oxidation pond. These results agree with different reports who investigate the removal efficiency of activated sludges in removing *Aeromonas* species from raw municipal wastewaters. A slight difference seems to exist in the studied case and could be due to the plant operation mode, the raw wastewater pollution load and to climatic conditions of the area. The hydraulic parameters seem to be not completely under control and this usually leads to considerable fluctuation in the retention time and consequently affects the removal efficiency. Moreover, failure has been occasionally associated with the sludge by-pass leading to a considerable release of sludges through the purified wastewater.

Several reports have mentioned the efficiency of different wastewater treatment processes in the removal of motile aeromonads. Among these, stabilization ponds have been reported as very satisfactory in the removal of motile aeromonads. Unfortunately, there are no results about the activated sludges operating under sahelian climate and we expect that different climatic conditions and raw wastewater profile in the sahelian zone did not improve the removal efficiency. However, the *Aeromonas* species did not showed a significant seasonal evolution trend. This evolution was marked by a decreasing during the hot period. We expect that evolution could be in relation to the increasingly consumed water volumes during the hot periods. Moreover, the selection of the *A. sobria* strains which has been reported for stabilization ponds seems to be inexistent in the activated sludges process.

The evolution of the faecal coliforms group abundances seems to follow a significant decreasing during the hot period, but the removal rates did not reveal important differences along the 15-months survey. Since the water consumption usually increases during the hot period, important water volumes are expected to cause a dilution effect which may be responsible for the low abundances encountered during this period. The removal efficiency obtained for the faecal coliforms was 0.59 U log corresponding to 86 %. This study has concluded that the reuse of the treated effluent in the neighbourhood of the plant still associated with significant health risks for humans and recommend to undertake an in depth microbiological and epidemiological assessment to allow to mitigate theses risks. Results corresponding to the antimicrobial susceptibility of the isolates to the fifteen tested antibiotics are shown in Table 2.

Due to the fact that the isolation medium already contained Ampicillin, absolute resistance to this drug was expected to be confirmed and therefore it was not practical to consider these resistance levels, although Figueira et al. (2011) have reported high to absolute rates of Ampicillin resistance among motile *Aeromonas* species. Genghesh et al. (2001) who studied the antibiotic susceptibility of aeromonads in untreated wells water found absolute resistance rates for Ampicillin. The high rate of Ampicillin resistance among *Aeromonas* strains has been confirmed by other reports (Rippey and Cabelli, 1979 ; Ansary et al., 1992) and has thus led to the frequent utilization of Ampicillin as a selective agent in the most common *Aeromonas* culture media (Rogol et al, 1979; Palumbo et al, 1985; Havelaar et al, 1987; Imziln et al., 1997; Vila et al., 2003). Furthermore, Lauria (1996) who compared resistance to Ampicillin among two *Aeromonas* populations isolated with and without Ampicillin as a selective agent, found that the resistance to Ampicillin was at least 62.5 % among the total tested population.

In addition, our research indicated that 148 of the 151 (98.01) % of *Aeromonas* isolates exhibited resistance to Vancomycin, which confirmed the results of Iversen et al. (2002) and Vandan et al. (2011) who reported only 3 % of *A. hydrophila* strains sensitive to this drug. The recorded resistance rates to Vancomycin could confirm the reliability of using this drug as a selective agent in several media like Vancomycin Ampicillin Blood Agar of Koehler and Ashdown (1993). Resistance rate to Cefsulodin was 4.6 % (7 strains). This resistance level is supporting the results of Alonso et al. (1996), who suggested that Cefsulodin may be a useful selective agent for *Aeromonas spp*. growth when faecal coliform isolation is targeted and high levels of background flora are expected.

However, incidence of resistance to Imipenem, Sulphamides, Cefoperazone and Fosfomycin were respectively 7.9, 0.7, 4 and 2.6 % while appearing to be intermediate for the different *Aeromonas* species. The resistance recorded for the total *E. coli* population showed an absolute susceptibility to Colistin and Piperacillin. The highest resistance rate corresponds to Cefsulodin (91.8%) with 67 resistant isolates. The resistance levels discovered for Ampicillin and Chloramphenicol were respectively 42 and 2,7%. Theses results agree with those of Hassani et al. (1999), who have reported 44 and 9% respectively for Ampicillin and Chloramphenicol resistance among a wastewater originated faecal coliform population. In regard to the antibiotic resistance variability between the three *Aeromonas* species, the results did not reveal significant differences. The comparison of the susceptibility rates of [2]the three *Aeromonas* species and *E. coli* to the fifteen antibiotics is shown in the table 2. Slight differences can be observed. The obtained ARI values for *A. caviae*, *A. hydrophila*, *A. sobria,* the atypical *Aeromonas* isolates and *E. coli* were 0.2115, 0.1909, 0.2210, 0,2104 and 0.3750 respectively.

Nevertheless, *A. hydrophila* strains appear to be the most sensitive with an ARI of 0.1909. In many reports, it has been suggested that the species *A. sobria* is the most susceptible due to its high sensitivity to Cephalotin (Janda and Motyl, 1985). According to these results, Cephalotin resistance has been proposed as a potential marker for the *A. sobria* species. Meanwhile, the Wilcoxon's signed rank non parametric test does not reveal significant statistically differences ($P > 0.05$) between the values of ARI obtained for the three species. Comparison of the resistance rates obtained from the influent and effluent originated strains revealed negligible statistically significant differences between the two sampled populations. The overall ARI values calculated for the fifteen antibiotics tested on the influent and the effluent originating populations were 0.2082 and 0.2071 respectively. Although the results showed the existence of a slight variability for some antibiotics, they did not show any significant differences in the antimicrobial susceptibility behaviour at the inflow and the outflow of the wastewater purification plant.

The analysis related to the three *Aeromonas* species polyresistance patterns is shown in the table 3. In regard to the influent population, 100 % of the isolates were found to be resistant to at least two antibiotics. The combination was Vancomycin and Ampicillin. The percentages of aeromonads possessing only this double resistance were 100 % at both the influent and the effluent, while 36 strains (63,2 %) at the influent and 54 strains (57,4 %) at the effluent were found to possess a maximum triple resistance pattern. This antibiotic resistance profile consisted of Ampicillin, Vancomycin and one of the following drugs: Oxacillin, Erythromycin, Piperacillin, Cefoperazone or Ticracillin. Quadruple resistance among aeromonads existed in only 21 isolates (36.8 %) from the influent and 20 (21.3%) from the effluent. Simultaneous resistance toward six antibiotics was recorded in only 2 strains from the influent and 5 isolates from the effluent. The combination involved Ampicillin, Vancomycin, Piperacillin, Oxacillin, Ticracillin and Erythromycin. No isolates were found to develop multiple resistances to seven antibiotics.

The Wilcoxon's signed ranks did not reveal significant differences between the percentages of polyresistance at the influent and effluent of the plant. Our results showed that the antibiotic resistance rates obtained for the process surviving bacteria remain at levels of those existing in raw wastewater. In other cases, the resistance levels have increased between up-stream and down-stream sampling points in urban effluent (Goni-Uriza et al., 2000). Nevertheless, the incidence of resistance may increase considerably among the effluent population since it has been suggested that this resistance is essentially transferable plasmid mediated (Adams et al., 1998). The overall results of this study concludes that the reclaim of the purified wastewater remains associated with significant risks for human health and therefore recommend to end the irrigation with wastewaters unless a scientific assessment is undertaken.

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