The Journal of Microbiology (2011) Vol. 49, No. 2, pp. 224-232
DOI 10.1007/s12275-011-0227-x

Seasonal Abundance and Distribution of *Vibrio* Species in the Treated Effluents of Wastewater Treatment Facilities in Suburban and Urban Communities of Eastern Cape Province, South Africa

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(Received June 21, 2010 / Accepted December 25, 2010)

We assessed the seasonal abundance and distribution of *Vibrio* species as well as some selected environmental parameters in the treated effluents of two wastewater treatment plants (WWTP), one each located in a suburban and urban community of Eastern Cape Province, South Africa. *Vibrio* population density ranged from 2.1×10^1 to 4.36×10^4 CFU/ml in the suburban community and from 2.80×10^1 to 1.80×10^5 CFU/ml in the urban community. *Vibrio* species associated with 180 μm, 60 μm, and 20 μm plankton sizes were observed at densities of 0-1.36×10^3 CFU/ml, 0-8.40×10^2 CFU/ml, and 0-6.80×10^2 CFU/ml, respectively at the suburban community's WWTP. In the urban community, observed densities of culturable *Vibrio* were 0-2.80×10^2 CFU/ml (180 μm), 0-6.60×10^2 CFU/ml (60 μm), and 0-1.80×10^3 CFU/ml (20 μm). The abundance of free-living *Vibrio* species ranged from 0 to 1.0×10^2 and 1.0×10^3 CFU/ml in the suburban and urban communities' WWTPs, respectively. Molecular confirmation of the presumptive *Vibrio* isolates revealed the presence of *V. fluvialis* (41.38%), *V. vulnificus* (34.48%), and *V. parahaemolyticus* (24.14%) in the suburban community effluents. In the urban community molecular confirmation revealed that the same species were present at slightly different percentages, *V. fluvialis* (40%), *V. vulnificus* (36%), and *V. parahaemolyticus* (24%). There was no significant correlation between *Vibrio* abundance and season, either as free-living or plankton-associated entities, but *Vibrio* species abundance was positively correlated with temperature (r=0.565; p<0.01), salinity, and dissolved oxygen (p<0.05). Turbidity and pH showed significant seasonal variation (p<0.05) across the seasons in both locations. This study underscores the potential of WWTPs to be sources of *Vibrio* pathogens in the watershed of suburban and urban communities in South Africa.

Keywords: environmental parameters, public health, *Vibrio* pathogens, treated effluents

Vibrio species are halophilic, Gram-negative bacteria that occur naturally in aquatic environments and are transmitted to humans primarily through consumption of contaminated water or raw or mishandled seafood (Oliver and Kaper, 2001; CDC, 2004). *Vibrio* infections can result in gastroenteritis, causing bloody diarrhea, or even septicemia in individuals with underlying chronic illness (CDC, 2004). A number of *Vibrio* infection outbreaks have been reported in many developed and developing countries, igniting interest in these pathogens and their ecology (DePola *et al*., 2003; Parveen *et al*., 2008).

Although they require a host for growth, *Vibrio* species are common in natural waters, such as streams, rivers, and lakes that have been impacted by inefficiently treated effluents discharge from wastewater treatment plants, runoff from agriculture lands, and/or direct contamination with feces of wild birds and animals (Hänninen *et al*., 2003; National Department of Health, 2003). Once *Vibrio* species are introduced into the environment, their survival or persistence depends on many factors, such as oxygen content, the presence of nutrients, temperature, and pH (DePola *et al*., 2003; Parveen *et al*., 2008). Previous studies have documented a strong relationship between *Vibrio* abundance and environmental conditions such as salinity, temperature, and attachment to planktonic organisms (Tamplin *et al*., 1990; Heidelberg *et al*., 2002; Maugeri *et al*., 2004) which have also been implicated in municipal wastewaters (Ahmadi *et al*., 2005; Chindah *et al*., 2007; Mukhopadhyay *et al*., 2007). Additionally, *Vibrio* species may enter a viable but non-culturable state in which organisms are not able to grow in culture but remain metabolically active, as first described by Rollins and Colwell (1986). This state is considered by some to play a role in prolonging *Vibrio* species survival in the environment (Hutchison *et al*., 2005) and appears to be important in the winter persistence of *Vibrio* populations, which could be a public health concern (Maugeri *et al*., 2004).

Vibrio pathogens are distributed throughout the world, and their reported densities in the environment and treated effluents vary greatly with season, location, sample type, and the analytical methods employed (Cook *et al*., 2002; Ristori *et al*., 2007; Martinez-Urtaza *et al*., 2008). Most *Vibrio* cases in the world occur in the summer, and there are declines in the fall and winter (Skirrow and Blaser, 1992; CDC, 2004). It has been suggested that changes in food handling, water supply, and consumption in the summer months may lead to this predictable seasonal change (Skirrow and Blaser, 1992).

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The seasonal cycles of *V. parahaemolyticus* in sediment, water, and plankton in the Chesapeake Bay of the United States, where it can be detected in sediment during the winter months, were first reported by Kaneko and Colwell (Kaneko and Colwell, 1973, 1975). In the Pacific Northwest, *V. parahaemolyticus* was detectable only during the summer months, when water temperatures range from 15-22°C (Kelly and Stroh, 1988; Kaysner *et al*., 1990). Duan and Su (2005), reported that 15% of oyster samples, 20% of seawater samples, and 48% of sediment samples collected bi-weekly from two Oregon oyster-growing areas during a one year period were positive for *V. parahaemolyticus.* Densities of *V. parahaemolyticus* in both the Northeast and Northwest United States were found to be positively correlated with water temperature, even though higher densities of *V. parahaemolyticus* varied considerably at optimal temperatures, and links between density and other environmental factors remain uncertain (Watkin and Cabelli, 1985; Phillips *et al*., 2007). Turbidity and pH levels have been hypothesized to correlate with *Vibrio* densities (Watkin and Cabelli, 1985; Huq and Colwell, 1996; Lobitz *et al*., 2000).

Over the past few years, the region encompassing South Africa has been plagued by outbreaks of *Vibrio*-related waterborne infections that are suspected to be linked to inefficiently treated effluents discharge from wastewater treatment facilities. In this paper, we report the seasonal abundance of *Vibrio* species as well as their relationship with several other environmental parameters in the treated effluents of two wastewater treatment plants in the Eastern Cape Province of South Africa.

Materials and Methods

Sampling sites

The wastewater treatment plants used in this study are located in Dimbaza [suburban community] and East London [urban community] (geographical coordinates 32°51′28″S, 27°35′29″E and 33°02′07″S, 28°16′17″E, respectively) in the Eastern Cape Province of South Africa. The Dimbaza plant is designed to treat an average dry weather flow of 7,000 m^3/day and an average wet weather flow of 21,000 m^3/day, and it receives domestic sewage, industrial wastewater, and run-off. It discharges treated effluents into a stream that empties into the Tembisa River. The East London wastewater treatment plant has a higher average daily inflow of 36,200 m^3/day because it services more industries and a higher population of residents. It discharges treated effluents into the Indian Ocean between Nahoon and Eastern Beach at Bats Cave. Both treatment facilities use activated sludge systems.

Sample treatments

Wastewater treated effluent samples were collected monthly between August 2007 and July 2008. All samples were collected aseptically using sterile 1 L Nalgene bottles containing 0.5 ml of sterile concentrated sodium thiosulphate solution, giving a final concentration of 100 mg/L, and transported on ice to the laboratory for analysis. Samples were stored at 4°C until analyses were completed. All samples were processed within 24 h of collection.

As described by Alam *et al.* (2006a), wastewater samples (duplicate 1 L samples) were filtered successively through 180 μm, 60 μm, and 20 μm nylon nets (Millipore Corp., USA), sequentially arranged in that order into sterilized collection bottles. The water that flowed through the 20 μm pore-size nylon net was collected in sterilized containers for free-living *Vibrio* cell analysis. After filtration, each of the nylon nets and their contents were resuspended in 25 ml physiological-buffered saline containing 12.5 g of sterile 0.1 mm glass beads (Biospec Products Inc., USA) and allowed to stand for 2 min and thereafter homogenized for 10 min in a glass homogenizer at 3,000×g at ambient temperature to dislodge the attached bacteria. Thereafter, the samples were allowed to settle, and the supernatant was used for direct plating of plankton-associated *Vibrio*. This analysis was carried out in triplicate for each sample.

Physicochemical analyses

All field meters and equipments were checked and calibrated according to manufacturer specification. The pH, temperature, salinity, and dissolved oxygen (DO) of the samples were determined onsite using a multiparameter ion specific meter (Hanna instruments, version HI9828). Turbidity was measured onsite using a microprocessor turbidimeter (HACH Company, model 2100P). Free residual chlorine was estimated using a multi-parameter ion-specific meter (Hanna BDH-laboratory). All analyses were carried out in triplicate.

Estimation of total Vibrio species densities

For direct plate count analyses of plankton-free samples, the samples were serially diluted and aliquots pour-plated in triplicates using thiosulphate-citrate-bile-salts-sucrose (TCBS) agar and incubated at 37°C for 24 h (Seeley and Vandermark, 1981). For the plankton-associated samples, the abundance of *Vibrio* spp. was obtained by inoculating aliquots of 0.5 μl supernatant onto thiosulfate-citrate-bile salt-sucrose (TCBS) agar and incubating at 37°C for 24 h, in accordance with Alam *et al.* (2006a). Yellow and green colonies were considered presumptive *Vibrio* colonies and counted as described in Alam *et al.* (2006b).

Isolation and biochemical identification of *Vibrio* species

Aliquots of the plankton-free and plankton-associated samples were

Table 1. Primers used in this study

Target species	Primer name	Primer sequences (5′→3′)	Target gene	Amplicon size (bp)	Reference
All *Vibrio* spp.	*V.* 16S-700F	5′-CGG TGA AAT GCG TAG AGA T-3′	16S rRNA	663	Kwok (2002)
	V. 16S-1325R	5′-TTA CTA GCG ATT CCG AGT TC-3′			
V. parahaemolyticus	*Vp. flaE*-79F	5′-GCA GCT GAT CAA AAC GTT GAG T-3′	*flaE*	897	Tarr *et al.* (2007)
	Vp. flaE-934R	5′-ATT ATC GAT CGT GCC ACT CAC-3′			
V. vulnificus	*Vv. hsp*-326F	5′-GTC TTA AAG CGG TTG CTG C-3′	*hsp60*	410	Wong and Chow (2002)
	Vv. hsp-697R	5′-CGC TTC AAG TGC TGG TAG AAG-3′			
V. fluvialis	*Vf- toxR* F	5′-GAC CAG GGC TTT GAG GTG GAC GAC- 3′	*toxR*	217	Osorio and Klose (2000),
	Vf- toxR R	5′-AGG ATA CGG CAC TTG AGT AAG ACTC-3′			Chakraborty *et al*. (2006)

inoculated into alkaline peptone water (APW) and incubated aerobically at 37°C for 18-24 h (Jiang, 2001; Jiang and Fu, 2001; Choopun *et al*., 2002). Turbid cultures were streaked onto TCBS agar incubated at 37°C for 24 h (Jiang, 2001; Jiang and Fu, 2001; Choopun *et al*., 2002). Five to ten isolated colonies per plate were randomly picked from each sample and subsequently purified on fresh TCBS agar plates. The pure isolates were subjected to Gram staining and an oxidase test. Only Gram-negative, oxidase-positive isolates were selected for biochemical identification using an API 20 NE kit. The strips were read, and the final identification was determined using API lab plus software (bioMérieux, France).

Molecular identification

Polymerase chain reaction (PCR) was used to confirm the identities of *Vibrio* species using the species-specific primers described in Table 1. Deoxyribonucleic acid (DNA) extraction and PCR were carried out as described by Maugeri *et al.* (2006), with slight modifications. Single colonies of presumptive *Vibrio* strains grown overnight at 37°C on TCBS agar plates were picked, suspended in 200 µl of filtered distilled water, and bacterial cells were collected by centrifugation at 11,000×g for 10 min at 4°C. The pellet was suspended in 100 µl of filtered, autoclaved water and boiled for 10 min. The cell lysates (10 µl) were used as a template in the PCR assays immediately after extraction or following storage at -80°C. The thermal cycling profile for *V. vulnificus* and *V. parahaemolyticus* was as follows: 15 min denaturation at 93°C followed by 35 cycles at 92°C for 40 sec, 57°C for 1 min, and 72°C for 1.5 min, followed by a final extension at 72°C for 7 min. The amplified products were held at 4°C after completion of the cycles. For *V. fluvialis*, the amplification conditions were: initial denaturation at 94°C for 5 min followed by 30 cycles of denaturation at 94°C for 40 sec, annealation at 65°C for 40 sec, and extension at 72°C for 1 min. The PCR products were electrophoresed in 1.5% agarose containing 0.5 mg/L ethidium bromide for 1 h at 100 V and then visualized using a UV transilluminator.

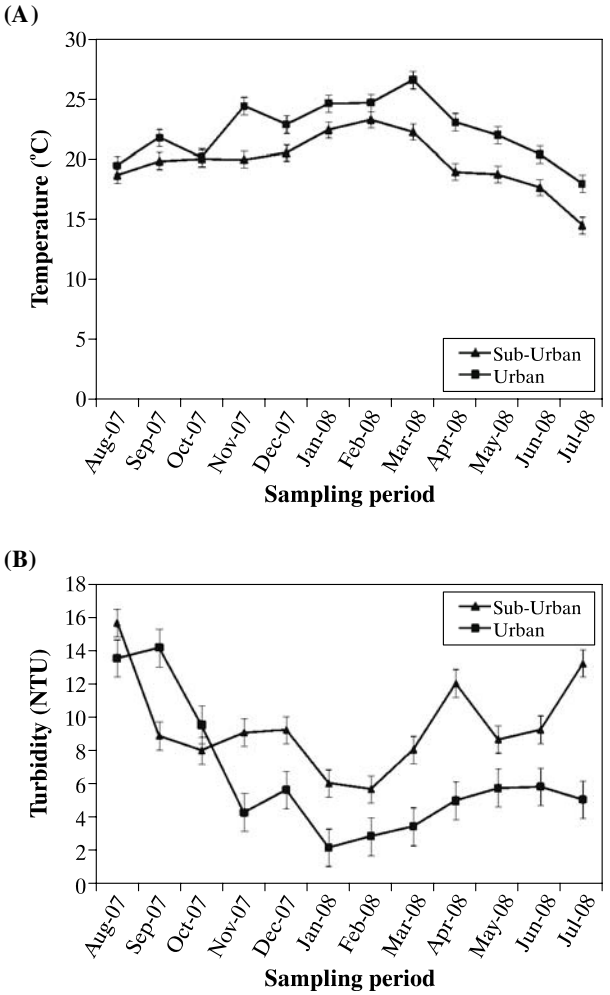

Fig. 1. Profiles of (A) temperature and (B) turbidity of the effluents from the suburban and urban wastewater treatment plants.

Statistical analysis

Total *Vibrio* species counts were analyzed by regressing the mean $\log_{10}$ density of replicate samples against environmental factors (temperature, turbidity, pH, salinity, and DO). For seasonal analyses, summer was considered to be November to March, autumn was April to May, winter was June to August, and spring was September to October, in accordance with the South African Weather Service. The significance of differences in *Vibrio* species abundance and environmental parameters between the two sampling sites were tested using a one-way analysis of variance (ANOVA). All statistical analyses were performed using Statistical Analysis System, Version 8 (SAS Institute, USA).

Results and Discussion

Effects of environmental parameters on the abundance and distribution of *Vibrio* species

Total *Vibrio* species counts in the treated effluents were compared with environmental parameters. Overall, the temperature of the effluents ranged from 14.4°C (July 2008) to 26.5°C (March 2008). The temperature of the treated effluents from the suburban treatment plant ranged from 14.5-23.3°C, while the temperature from the urban treatment plant ranged from 17.8 to 26.5°C (Fig. 1A). Regression analysis indicated a significant positive association between *Vibrio* abundance and temperature in suburban samples ($p<0.001$; Table 2A). The abundance of *Vibrio* species in urban samples was seasonal and also significantly positively associated with temperature ($r=0.48$, $p<0.001$; Table 2A). The abundance of *Vibrio* species appeared to be generally affected by environmental variables, although on some occasions, it appeared to fluctuate independently of temperature. The correlation between temperature and total *Vibrio* counts in this study corroborates other research that has reported similar trends for the Atlantic, Pacific, and Gulf Coasts (DePaola *et al*., 2003; Hännien *et al*., 2003), as well as in Japan and Germany (Duan and Su, 2005; Lhafi and Kuhne, 2007).

The turbidities of the treated effluents ranged from 2.16 NTU (January 2008) to 17.5 NTU (August 2008) (Fig. 1B), and varied significantly ($p<0.05$) with season at both study locations. *Vibrio* abundance counts were significantly correlated with turbidity ($p<0.0001$; Table 2A). Turbidity was higher at the suburban location than the urban one, and analysis indicated a significant positive association between turbidity and *Vibrio* density at the suburban site ($p<0.0001$). Lower and less variable turbidity levels at the urban site may have

Table 2A. Linear regression analysis of *Vibrio* abundance versus environmental variables

		Linear regression				
	Variables	R^2	*F*-values	*P*-values	Coefficient	Pr > F
Urban	Temperature	0.549	58.391	<0.00001	0.987	<0.00001
	pH	0.485	11.325	<0.00001	0.768	<0.00001
	Turbidity	0.572	14.973	<0.00001	0.659	<0.00001
	Salinity	0.66 3	2.961	<0.0007	0.990	<0.00001
	Dissolved oxygen	0.737	8.655	<0.02	0.543	<0.00001
	Chlorine residual	-0.325	7.286	<0.001	-0.817	0.002
Attached *Vibrio*	$\log_{10}$ 180 μm	0.468	25.451	<0.0005	1.105	<0.00001
	$\log_{10}$ 60 μm	0.572	18.765	<0.00001	1.021	<0.00001
	$\log_{10}$ 20 μm	0.615	12.873	<0.001	0.670	<0.005
	$\log_{10}$ free-living *Vibrio*	0.448	23.492	<0.0007	0.288	<0.00001
	Total *Vibrio* counts	0.482	20.64	<0.0002	1.15	<0.00001
Sub-urban	Temperature	0.456	33.898	<0.002	0.884	0.006
	pH	0.784	8.327	<0.0001	0.562	<0.00001
	Turbidity	0.726	29.535	<0.0001	0.365	0.005
	Salinity	0.482	2.733	0.003	0.512	<0.0001
	Dissolved oxygen	0.116	2.372	0.044	-0.358	<0.0001
	Chlorine residual	-0.655	17.151	0.001	-0.278	0.006
Attached *Vibrio*	$\log_{10}$ 180 μm	0.595	33.961	<0.00001	0.112	<0.0001
	$\log_{10}$ 60 μm	0.676	16.572	<0.05	0.782	0.005
	$\log_{10}$ 20 μm	0.967	22.498	<0.0007	0.525	0.0001
	$\log_{10}$ free-living *Vibrio*	0.448	17.542	<0.0005	0.517	0.0001
	Total *Vibrio* counts	0.783	19.19	<0.0001	0.94	<0.00001

Table 2B. Correlation coefficients of environmental variables from an urban wastewater treatment facility

Variables	Temperature	pH	Turbidity	Salinity	Dissolved oxygen	Chlorine residual
Temperature	1.00					
pH	0.697[a]	1.00				
Turbidity	-0.537[a]	-0.397a	1.00			
Salinity	-0.044[ns]	0.349b	-0.171b	1.00		
Dissolved oxygen	-0.560[a]	-0.180ns	0.537a	0.074ns	1.00	
Chlorine residual	0.271[ns]	0.026ns	-0.356b	-0.062ns	-0.290ns	1.00

[a] Correlation is significant at $p<0.01$ level (2-tailed);
[b] Correlation is significant at $p<0.05$ level (2-tailed); ns=not significant

Table 2C. Correlation coefficients of environmental variables from an urban wastewater treatment facility

Variables	Temperature	pH	Turbidity	Salinity	Dissolved oxygen	Chlorine residual
Temperature	1.00					
pH	0.447a	1.00				
Turbidity	-0.360b	0.462a	1.00			
Salinity	-0.035ns	0.407a	0.417a	1.00		
Dissolved oxygen	-0.067ns	0.200ns	-0.039ns	0.071ns	1.00	
Chlorine residual	-0.124ns	-0.449a	-0.181ns	0.177ns	0.014ns	1.00

[a] Correlation is significant at p<0.01 level (2-tailed);
[b] Correlation is significant at p<0.05 level (2-tailed); ns=not significant

obscured its effects. An increase in turbidity was associated with increasing *Vibrio* abundance, which has also been reported in other recent studies (Zimmerman *et al*., 2007). This is consistent with observations obtained by Watkins and Cabelli (1985), which found *V. parahaemolyticus* abundance in water to be strongly correlated with turbidity during the summer, when water temperatures were relatively constant. Watkins and Cabelli (1985) hypothesized that higher nutrient levels associated with more turbid and polluted waters may have stimulated *V. parahaemolyticus* growth.

The pH values of the treated effluents ranged from 6.63 (July 2008) to 7.74 (August 2007) and varied significantly with season ($p<0.05$). The mean annual pH of the treated effluents was 7.04 and 7.14 for suburban and urban sites, respectively (Fig. 2). There was marginal evidence of an association between *Vibrio* abundance and pH. There were significant

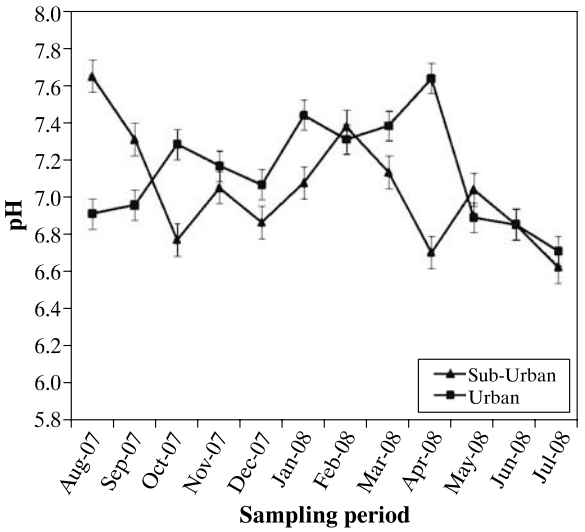

Fig. 2. Profiles of pH of the effluents from the suburban and urban wastewater treatment plants.

positive correlations between pH and temperature (r=0.697; P<0.01) and pH and salinity (r=0.349; p<0.05) (Table 2B). The salinity of the treated effluents ranged from 0.11 psu (January 2008) to 0.82 psu (April 2008) (Fig. 3A). The mean annual salinity of the treated effluents was 0.13 psu and 0.42 psu at suburban and urban site, respectively. The mean salinity at the suburban location, which had the lowest incidence of *Vibrio* species, was found to be significantly lower than that of the urban community. Salinity was found to have a significantly negative correlation with turbidity (r=-0.171; p<0.05; Table 2B). There was a significant correlation between salinity and *Vibrio* density at the urban site, and the opposite trend observed at the suburban plant is likely a consequence of the lower and narrower range of salinity observed there. Some previous studies have indicated that *V. parahaemolyticus* densities decrease as salinity increases (DePaola *et al.*, 1990, 2003; Anonymous, 2005). However, in the present study, the density of *Vibrio* species was generally positively associated with increasing salinity. Thus depending on the range of salinities encountered, some studies (Cook *et al*., 2002; Duan and Su, 2005) have found a significant direct relationship between salinity and the abundance of *V. parahaemolyticus*, but other studies (DePaola *et al*., 1990; Ristori *et al*., 2007) have reported otherwise. These different findings are likely a consequence of the differences in the range of salinities and sample sizes of the studies. In general, a wide range (5.6-34 ppt) of salinities was observed in the Cook *et al.* (2002) study compared to other studies, where no significant salinity effect was identified.

The DO concentration varied significantly (p<0.0001) with season and ranged between 2.38 mg/L (March 2008) and 6.78 mg/L (May 2008) (Fig. 3B), with annual means of 5.01 mg/L and 4.45 mg/L for the suburban and urban sites, respectively. It was significantly positively associated with *Vibrio* abundance (p<0.05), and it correlated inversely with the effluent's temperature at the urban site (r=-0.560; p<0.01; Table 2B). Watkins and Cabelli (1985) reported that *V. parahaemolyticus* levels in water were strongly correlated with DO, which agrees with our findings. However, their study was restricted to the summer season when water temperatures were relatively constant and the importance of other environmental parameters was likely increased.

A correlation matrix of the various environmental parameters for the urban location is given in Table 2B. There was significant positive correlation between pH and temperature (r=0.697; p<0.01) and pH and salinity (r=0.349; p<0.05). While temperature was negatively correlated with turbidity and DO (r=-0.537 and r=-0.560, p<0.01, respectively), turbidity was negatively correlated with chloride residual (r= -0.356; p<0.05). A correlation matrix of the environmental parameters for the suburban location is given in Table 2C. There was significant positive correlation between pH and temperature (r=0.447; p<0.01) and significant negative correlation between pH and chloride residual (r=-0.449; p<0.05). Temperature was negatively correlated with turbidity (r=-0.360, p<0.05).

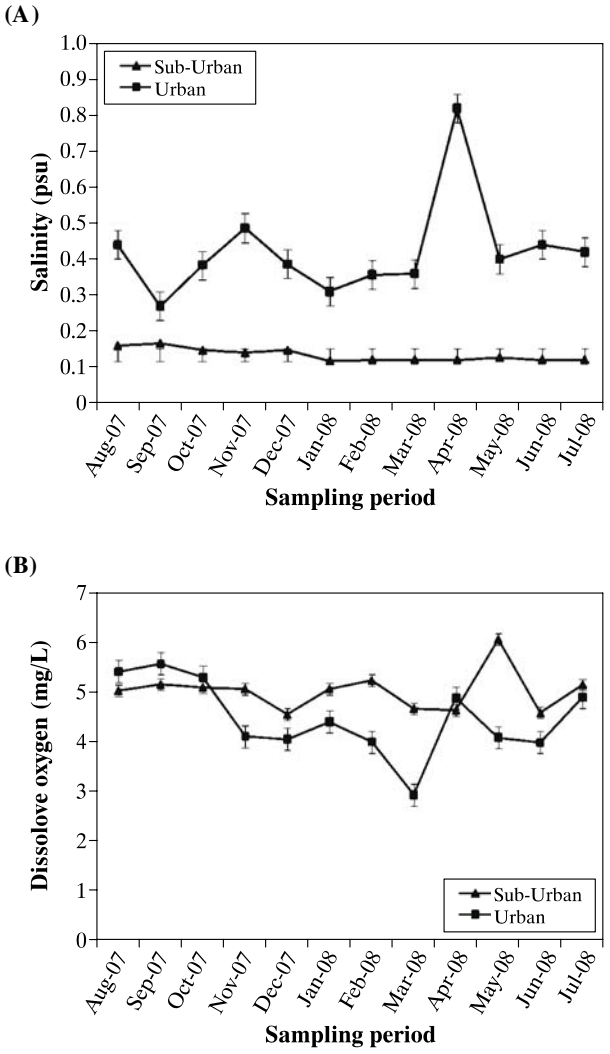

Fig. 3. Profiles of (A) salinity and (B) dissolved oxygen of the effluents from the suburban and urban wastewater treatment plants.

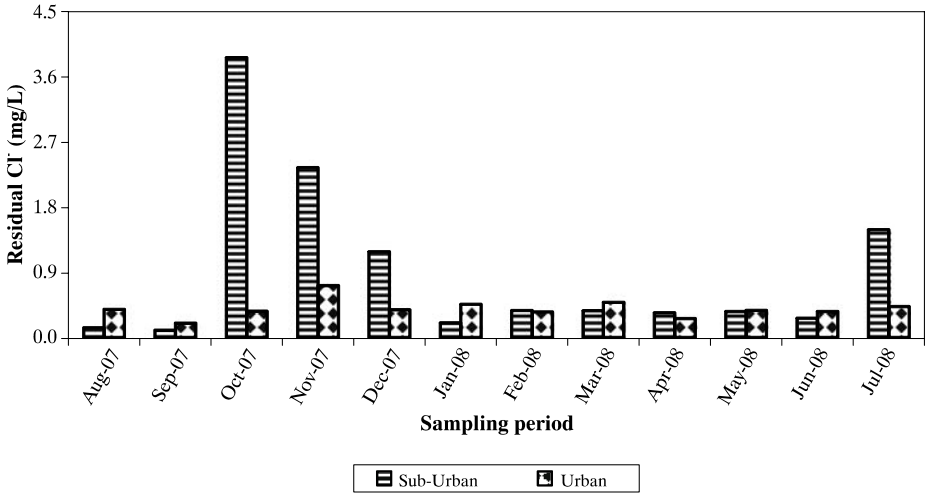

Fig. 4. Concentrations of free chlorine residual (mg/L) in the effluents from suburban and urban wastewater treatment plants.

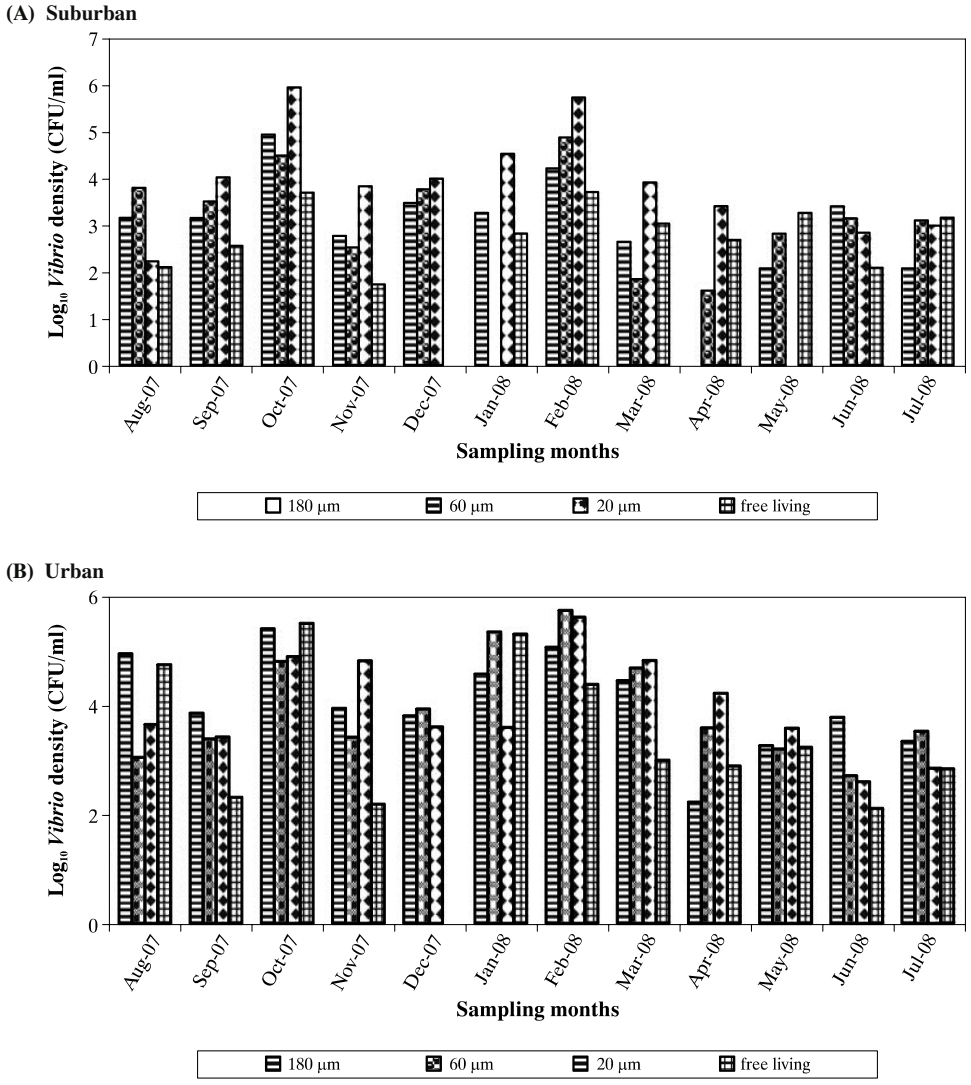

Fig. 5. Densities of *Vibrio* species in the effluents from wastewater treatment plants in suburban (A) and urban communities (B).

Table 3. Prevalence of *Vibrio* pathogens in effluents from wastewater treatment plants in suburban and urban communities

Target *Vibrio* species	Sub-urban site					Urban site				
	Plankton associated				Total frequency (%)	Plankton associated				Total frequency (%)
	180 μm	60 μm	20 μm	Free-living		180 μm	60 μm	20 μm	Free-living	
V. fluvialis	6/14 (42.86%)	7/15 (46.67%)	5/15 (33.33%)	6/14 (42.86%)	24/58 (41.38%)	5/12 (41.67%)	4/13 (30.76%)	5/11 (45.45%)	6/14 (42.85%)	20/50 (40%)
V. vulnificus	4/14 (28.57%)	5/15 (33.33%)	6/15 (40%)	5/14 (35.71%)	20/58 (34.48%)	3/12 (25%)	6/13 (46.15%)	4/11 (36.36%)	5/14 (35.71%)	18/50 (36%)
V. parahaemolyticus	4/14 (28.57%)	3/15 (20%)	4/15 (26.67%)	3/14 (21.43%)	14/58 (24.14%)	4/12 (33.33%)	3/13 (23.07%)	2/11 (18.18%)	3/14 (21.43%)	12/50 (24%)

The chlorine residual of samples from treated effluents from the suburban treatment plant ranged between 0.07 and 3.85 mg/L, and those from the plant located in the urban community ranged from 0.19-0.71 mg/L. These variations in chlorine residual are significantly ($p<0.001$) across the seasons and between the treatment plants, with the low concentration of 0.07 mg/L observed in the month of September up to a high concentration of 3.85 mg/L observed in the month of October (Fig. 4). Most South African wastewater treatment plants disinfect wastewater by chlorination prior to discharge into receiving waterbodies. The intention of this practice is to eliminate pathogens from effluents. To do this, residual chlorine is maintained at sufficient levels in contact with the microbial community in the chlorination tank. Currently, there is no recommended standard for residual chlorine concentration for wastewater effluents in South Africa. Current domestic water supply standards recommend a residual chlorine concentration range of 0.3 to 0.6 mg/L to be ideal and a range of 0.6-0.8 mg/L to be good, resulting in an insignificant risk of health effects (Mooijiman *et al*., 2001). Based on these recommendations, the free residual chlorine in the effluents studied complied with regulatory standards, but failed to eliminate the *Vibrio* species (Fig. 4). Even when there was an overdose of residual chlorine at the suburban site in October and November 2007, high densities of *Vibrio* pathogens were observed. Other studies (Obi *et al*., 2007, 2008) have reported varied chlorine residual concentrations at other South African wastewater treatment plants, suggesting that many do not comply with stipulated standards.

Seasonal abundance and distribution of *Vibrio* species

There were similar seasonal trends in *Vibrio* abundance at the two sampling sites (Figs. 5A and B). *Vibrio* counts ranged from 2.1×10^1 to 4.36×10^4 CFU/ml at the suburban site and from 2.80×10^1 to 1.80×10^5 CFU/ml at the urban site, and these variations were not significant. At the suburban site, the lowest count was observed during the winter of May 2008, while the highest count was observed during the summer month of October 2007. At the urban site, the lowest *Vibrio* density was observed during the winter month of June 2008, while the highest was observed during the summer month of February 2008. Abundance of free-living *Vibrio* species varied between 0 and 8.0×10^2 CFU/ml at the suburban site and 0 and 1.52×10^3 CFU/ml at the urban site. *Vibrio* species associated with 180 μm, 60 μm, and 20 μm plankton sizes were observed at densities of 0-1.36×10^3 CFU/ml, 0-8.40×10^2 CFU/ml and 0-6.80×10^2 CFU/ml, respectively, at the suburban site. At the urban location, species associated with the same plankton sizes were observed at densities of 0-2.80×10^2 CFU/ml, 0-6.60×10^2 CFU/ml, and 0-1.80×10^3 CFU/ml, respectively. There was no significant correlation between either free-living or plankton-associated *Vibrio* abundance and season. The counts at both sites followed a seasonal trend, with higher *Vibrio* counts in the warmer months.

Of a total of 66 suburban and 60 urban isolated presumptive *Vibrio*, 31 suburban and 23 urban were identified as *Vibrio* species by the API 20 NE system. The PCR assay confirmed that, of the isolates, 58 suburban and 50 urban belonged to the *Vibrio* genus. Detection of the target *Vibrio* species by PCR revealed the presence of 41.38% *V. fluvialis*, 34.48% *V. vulnificus*, and 24.14% *V. parahaemolyticus* in the suburban effluents. In the urban effluents, PCR revealed 40 % *V. fluvialis*, 36% *V. vulnificus*, and 24% *V. parahaemolyticus*. *V. fluvialis* was the most predominant species isolated from both sites. The results of species identification from both methods were pooled to determine the total occurrence of the target species (Table 3).

In this present study, *Vibrio* species were isolated more frequently and in larger densities during the summer months, which is consistent with the trends for clinical cases of *Vibrio* infections (Parveen *et al*., 2008), but this does not correspond with the low survival rates of *Vibrio* reported *in vitro* at summer temperatures (Zimmerman *et al*., 2007). Some previous studies have shown higher levels of *V. parahaemolyticus* in oysters (plankton) during warmer months and lower levels during cooler months (Cook *et al*., 2002; DePola *et al*., 2003; Duan and Su, 2005). In the South African region, the highest numbers of clinical cases of *Vibrio*-related infections occur in the summer months (National Department of Health, 2003), when precipitation tends to be high. These results differ from the results of other environmental studies, where the detection of *Vibrio* peaked in late autumn and winter months (DePola *et al*., 2003).

In this study, we observed that *V. fluvialis* was the most predominant species in both sampling locations. This is alarming given that *V. fluvialis* is an emerging strain that has been shown to produce enterotoxin and clinical symptoms of gastroenteritis similar to those of *V. cholerae* O1 and non-O1 strains (Kothary *et al*., 2003). In addition, there has been a recent characterization of an enterotoxigenic El Tor-like haemolysin in *V fluvialis,* which represents one of the virulence factors of *V. cholerae* (Kothary *et al*., 2003).

Conclusion

These results show that there are temporal and spatial varia-

tions in the densities of *Vibrio* species in suburban and urban communities in the Eastern Cape Province of South Africa. Our study suggests that most of the pathogenic *Vibrio* strains isolated from treated effluents possess potential virulence traits. These data should be valuable for an assessment of human health risk due to consumption of water from watersheds impacted by inadequately treated effluents.

Acknowledgements

We are grateful to the National Research Foundation (NRF) of South Africa for financial support (Grant Ref: FA20060424 00043).

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