

Water quality and ecological status of the Alcantara River estuary (Italy)

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SUMMARY

The Alcantara River estuary was studied to obtain the first data on both water quality and ecological status of the only River Park in Sicily (Italy). Water samples were seasonally collected from three selected stations in the estuarine area and from one station at the mouth of the estuary in the marine coastal zone.

Picoplankton and picophytoplankton counts were among the highest observed in estuarine environments and in other coastal waters of the Mediterranean Sea. The contribution of picophytoplankton to total picoplankton showed greater variations in freshwater (from 0.53% to 8.6%) than in coastal waters (from 1.8% to 4.3%). Picophytoplankton abundance increased in Alcantara waters in winter.

Vibrio and *Aeromonas* spp. abundances ranged from $\times 10^2$ to $\times 10^3$ CFU 100 ml⁻¹ and from $\times 10^3$ to $\times 10^5$ CFU 100 ml⁻¹, respectively. *Vibrio* and *Aeromonas* spp. counts were independent from the fecal contamination level.

Bacterial identification of the isolates revealed the presence of pathogenic and potentially pathogenic *Vibrio* and *Aeromonas* spp. for humans and animals.

KEY WORDS: *Aeromonas* spp., *Vibrio* spp., Picoplankton, Picophytoplankton, Water pollution

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INTRODUCTION

Anthropic activities strongly influence the natural status of almost all aquatic ecosystems. The input of large amounts of nutrients, pollutants and urban wastewater discharges can alter the quality of the recipient lakes, streams, rivers and coastal marine ecosystems.

From an ecological point of view, it is well known that aquatic microbial communities are useful indicators of ecological status because microorganisms are able to quickly respond to environmental changes by modifying their community

structure and composition. Among microorganisms, picoplanktonic cells (between 0.2 and 2 μm in diameter) (Sieburth *et al.*, 1975) are abundant in different aquatic environments and represent an important component of the pelagic food web (Bell and Kalf, 2001). Phototrophic picoplankton, or picophytoplankton, contributes at least 10% to the total global aquatic net primary productivity (Waterbury, 1979; Raven, 1998; Callieri and Stockner, 2002). Several factors, including light, temperature, inorganic nutrients availability, and river flow, may influence the estuarine picophytoplankton distribution, biomass and growth, and the relative abundances of each picophytoplankton group.

Urban sewage often represents the main source of microbial contamination of waters, which often determines a great limitation for human health in recreational activities and the utilization of economic resources. From the sanitary standpoint, the presence of pathogenic microorganisms in

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the aquatic environment is related to their ability to resist and survive to different natural factors of stress, such as low temperature, low or high pH, high salinity, and scarce organic matter (Caruso *et al.*, 2000).

The microbiological quality of waters in many European countries is currently established by evaluation of *Escherichia coli* and enterococci levels. However, some enteric bacterial pathogens have different survivability compared with fecal bacterial indicators in aquatic environments (Rozen and Belkin, 2001; Winfield and Groisman, 2003). Therefore, *E. coli* and enterococci do not always appear sufficient to assess the hygienic quality of waters.

Vibrio and *Aeromonas* spp. are saprophytic bacteria, normal inhabitants of marine and estuarine waters. In general, vibrios tolerate a wide range of salinity and tend to be more common in warm water, when temperature exceeds 17°C (Wright *et al.*, 1996). *Vibrio cholerae*, *Vibrio parahaemolyticus*, and *Vibrio vulnificus* account for the majority of *Vibrio* infections in humans (Farmer *et al.*, 2003). An increasing number of facultative pathogenic strains, *Vibrio alginolyticus*, *Vibrio fluvialis*, *Vibrio mimicus*, *Vibrio metschnikovii*, *Vibrio hollisae*, and *Vibrio harveyi* have been also recognized as human pathogens related to water and seafood enteric pathologies and wound infections (Chakraborty *et al.*, 1997; Oliver and Kaper, 1997; Thompson *et al.*, 2004). The presence of pathogenic *Vibrio* spp. in estuarine and coastal waters of the Mediterranean Sea has been previously reported (Baffone *et al.*, 2001; Barbieri *et al.*, 1999; Dumontet *et al.*, 2000; Gugliandolo *et al.*, 2005; 2008; Hervio-Heath *et al.*, 2002; Maugeri *et al.*, 1994; 2000; 2004; 2006; Masini *et al.*, 2007).

Aeromonas strains have been isolated from both oligotrophic marine waters and coastal nutrient rich environments (Araujo *et al.*, 1990; 1991). Since their abundance is strictly related to the trophic status of water, *Aeromonas* could be considered useful indicators of water quality (Rippey and Cabelli, 1989). Members of the *Aeromonas* genus are recognized as pathogen for humans (Janda, 2001) and animals, including amphibians, reptiles and fish (Austin and Austin, 1997). Strains of *Aeromonas hydrophila*, *Aeromonas caviae*, *Aeromonas sobria*, *Aeromonas veronii*, and *Aeromonas salmonicida* have been isolated from

coastal waters in Italy (Fiorentini *et al.*, 1998; Sechi *et al.*, 2002; Maugeri *et al.*, 2004; Gugliandolo *et al.*, 2008).

Because of its independence on bacterial fecal indicators, the presence and distribution of pathogenic *Vibrio* and *Aeromonas* provide important information for control of water quality.

To study the ecological status of the Alcantara River estuary, and the receiving marine coastal area, our investigations were carried out to evaluate the seasonal and spatial distribution of:

- 1) pico- and picophyto-plankton, as important components of the aquatic food web;
- 2) *Vibrio* and *Aeromonas*, considered useful indicators of the trophic status of waters;
- 3) pathogenic species of *Vibrio* and *Aeromonas*, as indicators of the sanitary status of waters, and
- 4) *Escherichia coli* and enterococci, as indicators of fecal pollution.

MATERIALS AND METHODS

Study area and sample collection

The Alcantara River is one of the most important rivers in Sicily and is part of the only River Park in Sicily. The river basin covers an area of about 573 km². Its waters flow between the Etna volcano in the South and the southern spurs of Nebrodi and Peloritani mountains in the North, reaching the Ionian Sea after about 52 km.

Water samples were seasonally collected (from October 2006 to July 2007) from three selected stations in the estuarine area where the river receives the effluents of Calatabiano and Giardini wastewater plants (1, 2 and 3) and one station (4) at the mouth of estuary in the marine coastal zone (Figure 1).

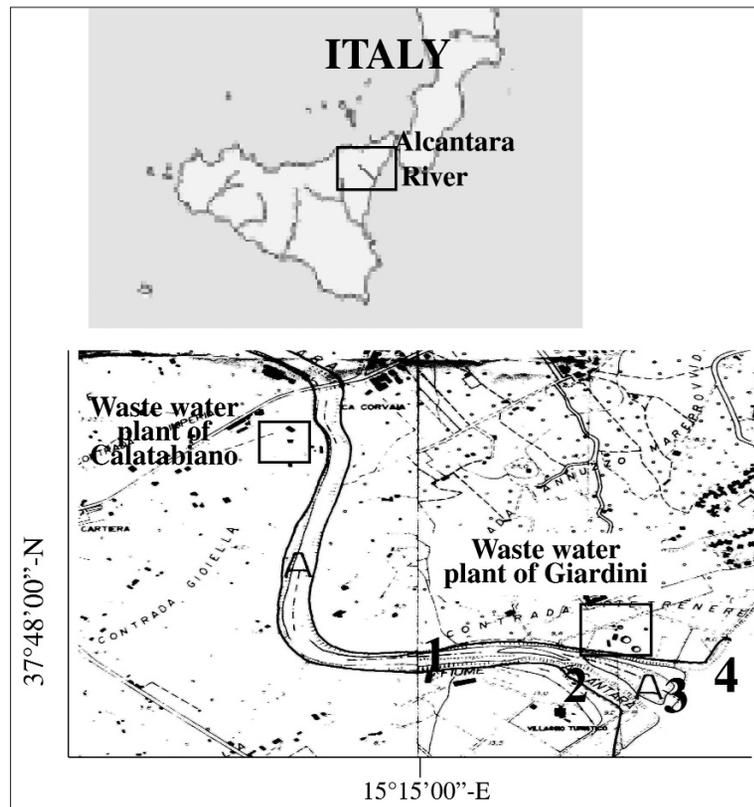
Environmental parameters

Temperature, dissolved oxygen, pH, and salinity were measured by a portable multiparametric probe (Idromar IM 201, Inc. Austin, USA). The concentration of total phosphorus and dissolved nitrogen were evaluated according to the methods of Strickland and Parsons (1972).

Evaluation of pico- and picophyto-plankton abundances

Direct counts of picoplanktonic cells (between

FIGURE 1 - The Alcantara estuarine area and location of sampling stations (1, 2, 3 and 4).



0.2 and 2 μm in size) (TP) were carried out according to the procedures described by Maugeri *et al.* (1990). Water samples were pre-filtered through a 2- μm pore-size membrane filter and concentrated onto 0.2- μm pore-size Nuclepore black polycarbonate filter. Picoplanktonic cells were stained with 4,6-diamidino-2-phenylindole (DAPI) fluorochrome and counted under the 1000x magnification, by epifluorescence microscopy (Olympus BX-60M). To estimate the phototrophic picoplankton, or picophytoplankton (PP), prefiltered water samples were fixed in glutaraldehyde (1%) and microscopically counted according to Maugeri *et al.* (1990). Picophytoplanktonic cells were distinguished as either phycoerythrin-phycoyanin rich cyanobacteria (Cyan) (yellow-orange fluorescent cells) or chlorophyll-dominant eukaryotes (Euka) (red or green fluorescent cells).

Enumeration, isolation, and identification of *Vibrio* spp.

To enumerate total vibrios, 1 and 10 ml of each water sample, filtered through a 0.2 μm mem-

brane (Millipore Corp., Bedford, MA, USA) were directly inoculated onto plates of Thiosulphate Citrate Bile Salts Sucrose medium (TCBS; Oxoid, Unipath, Basingstoke, UK), incubated at 37°C for 24 h. The number of *Vibrio* spp. was expressed as CFU per 100 ml. After counting, colonies were isolated and identified at genus and species level (Maugeri *et al.*, 2004). Isolates were maintained on trypticase soy agar with 1% NaCl.

To isolate *V. vulnificus*, 1 and 10 ml of each sample were inoculated onto plates of VVM selective medium (Cerdà-Cuéllar *et al.*, 2000) incubated at 37°C for 18-24 h. Yellow colonies (cellobiose fermenting) were considered presumptive *V. vulnificus* strains.

To search for *V. cholerae*, 250 ml of each water sample were concentrated onto 0.2- μm -pore membrane filter and inoculated in alkaline peptone water. After incubation at 30°C for 18 h (Choopun *et al.*, 2002), turbid cultures were streaked onto plates of TCBS agar incubated at 37°C for 24 h. Hemolytic activity was tested on Wagatsuma agar (Sakazaki, 1973). To confirm the phenotypic identification of *V. cholerae*, *V.*

parahaemolyticus, and *V. vulnificus* isolates, a PCR method was performed on DNA extracted from single colonies as previously reported (Gugliandolo *et al.*, 2005). *V. parahaemolyticus* strains were tested for the gene encoding the thermostable direct haemolysin (*tdh*), associated with *V. parahaemolyticus*-related illnesses (Bej *et al.*, 1999).

O serotyping of confirmed *V. cholerae* was performed by slide agglutination with commercial anti-*V. cholerae* O1 and anti-*V. cholerae* O139 antisera (Biogenetics Diagnostics, Padua, Italy).

Enumeration, isolation and identification of Aeromonas spp.

To evaluate total *Aeromonas* spp. abundances, 1, 10 and 100 µl of each sample were inoculated both onto MacConkey agar (Oxoid), incubated at 30°C for 24 h. Colonies that were cytochrome oxidase positive were considered presumptively *Aeromonas* spp. The isolates were studied for their biochemical properties by using the API 20E system (bio-Mérieux, Marcy l'Etoile, France) and identified according to Abbott *et al.* (2003). Hemolytic activity was tested on Wagatsuma agar (Sakazaki, 1973).

Evaluation of fecal indicators

Escherichia coli - Aliquots (1, 10, and 100 ml) of each sample, concentrated onto 0.2-µm pore-size membrane filters (Millipore, Bedford, MA, USA), were inoculated onto plates of Tryptone, Bile salts, X-glucuronide medium (Oxoid). After incubation at 44°C for 24 h, blue colonies were counted and results were expressed as *E. coli* CFU per 100 ml.

Enterococci - Membrane filters (0.2 µm pore-size)

were used to concentrate aliquots (1, 10, and 100 ml) of each sample and transferred onto Slanetz and Bartley plates (Oxoid). After incubation at 37°C for 48 h, filters with reddish brown colonies were transferred for 2 h onto Bile Esculin Agar (Oxoid) plates at 37°C. Colonies with black halo were considered positive for esculine hydrolysis and after counting they were expressed as enterococci CFU per 100 ml.

RESULTS

Environmental parameters

Temperature, oxygen concentration, pH, and salinity of Alcantara waters are given in Table 1. Higher temperatures were registered in July. Oxygen concentration ranged from 5.22 ml l⁻¹ (station 4) in July to 8.9 ml l⁻¹ (station 1) both in October and May. The pH values were higher in stations 1, 2, and 3 than in station 4 over the period examined. Salinity in stations 1, 2, and 3 had typical freshwater values (0.5–0.65 ppt) while in station 4, it had brackish characteristics (26.18–34.90 ppt).

Concentration of nitrate in freshwater ranged from 1.23 (May) to 2.59 mg l⁻¹ (October) and that of total phosphorous from 0.07 (May) to 0.24 mg l⁻¹ (October).

Distribution of pico- and picophyto-plankton abundances

Picoplanktonic abundance in freshwater ranged from 2.33×10⁶ (station 2, May) to 1.00×10⁷ cells ml⁻¹ (station 1, July) (Figure 2). At the mouth of the estuary (station 4), picoplankton ranged from 2.91×10⁶ (May) to 9.21×10⁶ cells ml⁻¹ (July).

TABLE 1 - Physico-chemical characteristics of Alcantara River estuary (stations 1, 2, 3 and 4) from October 2006 to July 2007.

Station	T°C				O2 (ml l ⁻¹)				pH				Salinity (ppt)			
	Oct	Jan	May	July	Oct	Jan	May	July	Oct	Jan	May	July	Oct	Jan	May	July
1	16.00	13.00	18.00	19.50	8.90	7.62	8.90	8.70	8.53	8.32	8.55	8.59	0.65	0.50	0.50	0.60
2	18.00	14.00	19.00	20.00	7.55	7.25	8.70	8.23	8.58	8.30	8.48	8.50	0.64	0.50	0.50	0.50
3	18.00	14.00	19.00	20.00	7.45	7.79	8.40	8.14	8.57	8.33	8.45	8.55	0.56	0.50	0.50	0.50
4	20.00	16.00	19.00	22.00	6.60	7.96	8.30	5.22	8.20	7.93	8.44	8.08	28.00	34.90	26.18	28.00

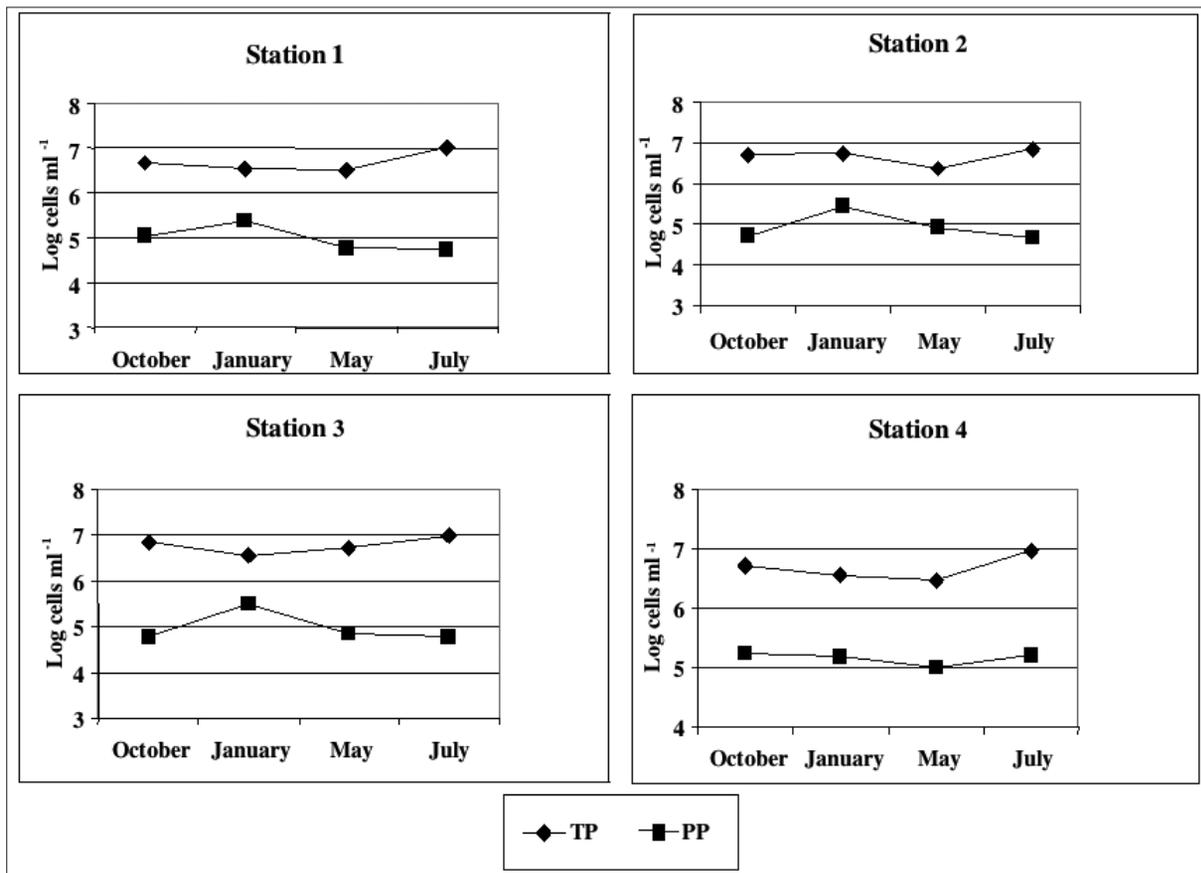


FIGURE 2 - Temporal variation of total pico- (TP) and picophyto-plankton (PP) abundances in Alcantara River estuary (stations 1, 2, 3 and 4) from October 2006 to July 2007.

Picophytoplanktonic cell abundance in freshwater varied between 5.07×10^4 cells ml^{-1} (station 2, October) and 3.07×10^5 cells ml^{-1} (station 3, January) and in station 4 ranged from 9.96×10^4 (May) to 1.72×10^5 cells ml^{-1} (October) (Figure 2). Cyanobacterial densities in freshwater ranged from 2.13×10^4 cells ml^{-1} in July (stations 1 and 2) to 2.78×10^5 cells ml^{-1} in January (station 3). Their contribution to total picophytoplankton varied from 40.0% (station 1, July) to 96.59% (station 1, May) (Figure 3). An opposite temporal trend was observed in station 4, where cyanobacterial densities varied from 8.36×10^4 (53.25% of PP, January) to 1.48×10^5 (89.25% of PP, July) cells ml^{-1} . Eukaryotes in river varied between 2.00×10^3 cells ml^{-1} (station 1, May) and 5.96×10^4 cells ml^{-1} (station 1, October) and their contribution to total picophytoplankton ranged from 3.41% in May to 60.0% in July. In station 4, eukaryotes ranged from 1.42×10^4 (May) to 7.74×10^4 cells ml^{-1}

(October) and their percentage of total PP varied from 10.75 (July) to 46.43% (January).

Vibrios, aeromonads, and fecal bacteria

The abundance of *Vibrio* spp. in the upper stations ranged from 5.25×10^2 (station 1, January) to 7.30×10^3 CFU 100 ml^{-1} (station 1, October) (Figure 4). *Vibrios* abundance in station 4 ranged from 2.75×10^2 (January) to 8.5×10^3 CFU 100 ml^{-1} (October).

Aeromonas spp. numbers were on average higher than those of *vibrios* in all samples. They varied from 1.0×10^3 CFU 100 ml^{-1} (station 1, January) to 2.0×10^5 CFU 100 ml^{-1} (station 3, January) (Figure 4). In station 4, *aeromonads* ranged from 1.0×10^3 (July) to 1.10×10^5 CFU 100 ml^{-1} (October).

Fecal indicators were always present in all samples (Figure 4). *E. coli* densities in freshwater ranged from 10 CFU 100 ml^{-1} (station 1, May) to

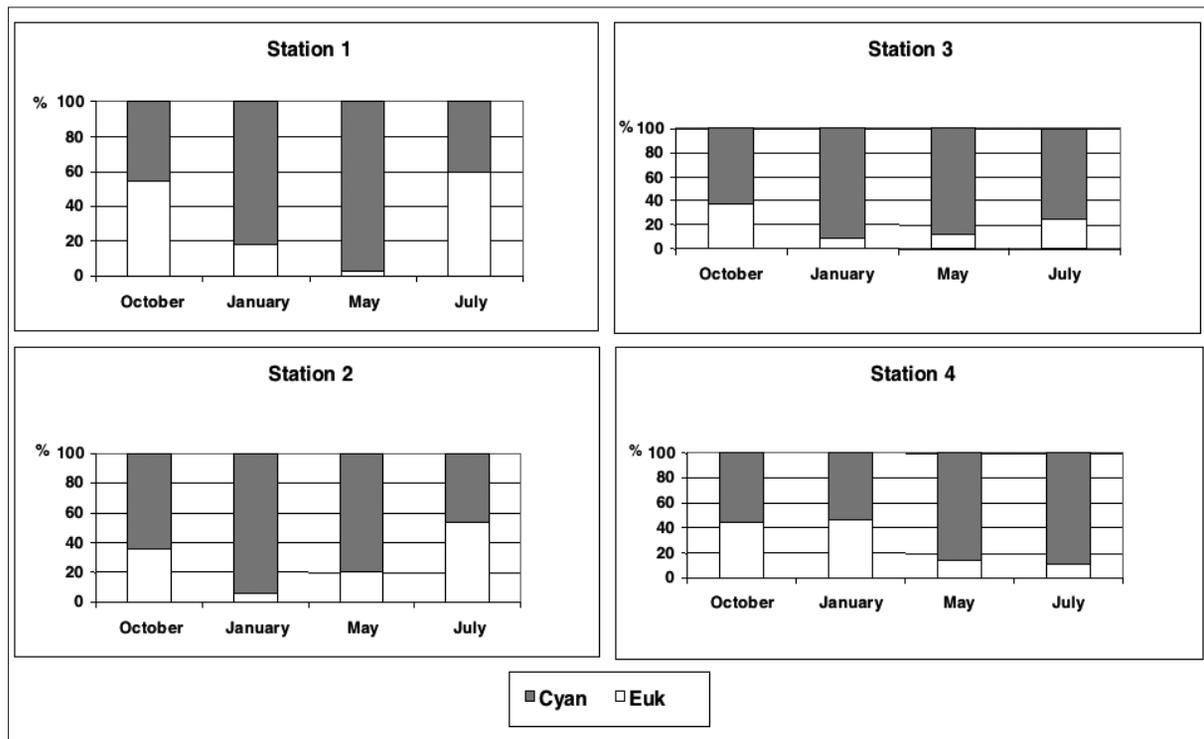


FIGURE 3 - Contribution of cyanobacteria (Cyan) and eukaryotes (Euk) to total picophytoplankton in the Alcantara River estuary (stations 1, 2, 3 and 4) from October 2006 to July 2007.

4.8×10^3 CFU 100 ml⁻¹ (station 1, October). *E. coli* counts in station 4 were lower than those registered in the upper stations and varied between 10 (January) and 5.45×10^2 CFU 100 ml⁻¹ (October).

Enterococci densities ranged from 1.25×10^2 CFU 100 ml⁻¹ (station 1, October) to 7.0×10^3 CFU 100 ml⁻¹ (station 1, May) and in station 4 varied from 80 (October) to 7.0×10^3 CFU 100 ml⁻¹ (May). Different from those of *E. coli*, the counts of enterococci did not show any decrease in brackish water.

A lack of correlation was observed between *Vibrio* and *Aeromonas* abundances and fecal indicators.

Distribution of *Vibrio* and *Aeromonas* isolates

A total of 114 strains were isolated in this study, 21.05% from station 1, 22.81% from station 2, 27.19% from station 3, and 28.95% from station 4. Of the total isolates, 35.96% (41/114) was identified as *Vibrio* spp., 41.23% (47/114) as *Aeromonas* spp., and the remaining 22.81% (26/114) was ascribed to other genera.

V. alginolyticus (18/41) was the predominant

species followed by *V. fluvialis* (4/41), *V. parahaemolyticus* (3/41), *V. cholerae* (3/41), *V. mimicus* (3/41), *V. vulnificus* (2/41), and *V. metschnikovii* (1). The remaining (7/41) strains were grouped as *Vibrio* spp.

No potentially pathogenic *Vibrio* strain was isolated from station 4 in January, or from other freshwater samples in July (Table 2). In January, three strains of *V. alginolyticus*, one strain of *V. parahaemolyticus* and one of *V. vulnificus*, were isolated from freshwater.

None of the isolates of *V. parahaemolyticus* showed hemolytic activity on Wagatsuma agar, nor were they positive for the *tdh* gene. The two *V. vulnificus* strains were indole positive, ADH-, LDC+, and ODC-. Their phenotypic identification was confirmed by PCR analysis of 16S rRNA. Two strains of *V. cholerae* were recovered from seawater in October and July and from freshwater in May. The PCR-confirmed *V. cholerae* isolates were serotyped as *V. cholerae* non-O1/non-O139. Most of *V. fluvialis* strains (3/4) were recovered from stations 1, 2, and 3 in May.

Among aeromonads, *A. hydrophila* (8) and *A. cavi-*

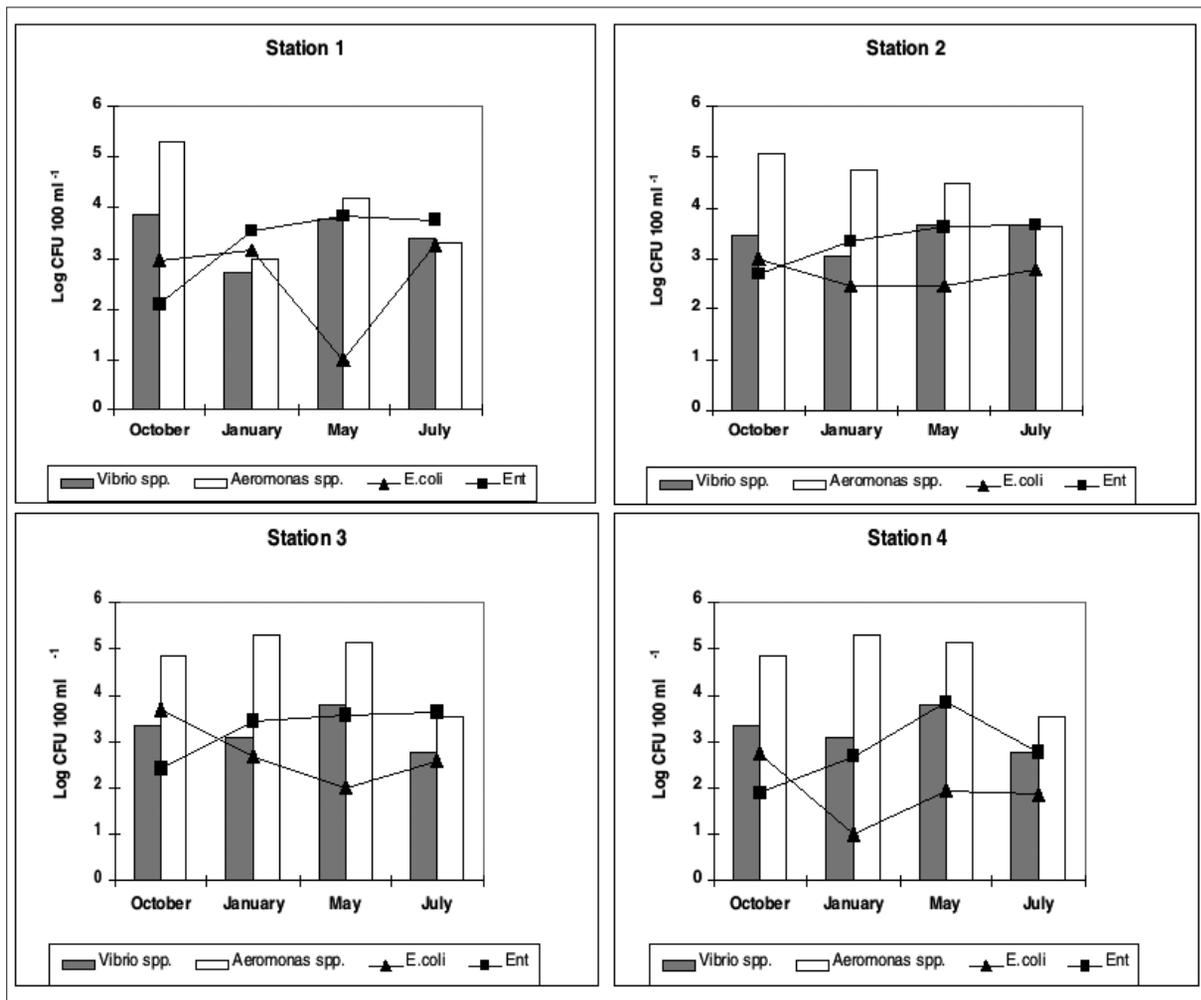


FIGURE 4 - Distribution of total *Vibrio* and *Aeromonas* spp. in comparison with *E. coli* and enterococci (expressed as Log CFU 100 ml⁻¹) in Alcantara River (stations 1, 2, 3 and 4) from October 2006 to July 2007.

ae (7) were the most abundant strains, followed by *A. sobria* (4), *A. salmonicida* (3), *A. veronii* (2), and *A. trota* (2). The remaining strains were grouped as *Aeromonas* spp.

Four *A. hydrophila* isolated in July, two from station 2 and two from station 4, showed hemolytic activity on Wagatsuma agar. Only one strain of *A. caviae* and one of *A. sobria*, isolated in May from station 4 and from station 3 respectively, showed hemolytic activity.

DISCUSSION

The microbial quality of Alcantara estuarine waters appears to be strongly influenced by the

contributions of domestic and agricultural waste, discharge of effluents from wastewater plants, and several focal sources. When compared with European microbiological standards for bathing waters, abundances of enterococci, which are considered the best indicators of contamination of surface and marine waters (WHO, 2003), were always higher than the recommended guideline limits (<100 enterococci 100 ml⁻¹), except in station 4 in October. In agreement with the data reported by the local control service (ARPA Sicilia), fecal contamination is at levels that might prevent important recreational purposes and also the utilization of water for agricultural use.

The abundance and composition of the aquatic microbial community of Alcantara estuarine zone

TABLE 2 - *Vibrio* and *Aeromonas* strains (and their relative abundance) isolated from Alcantara River estuary (stations 1, 2, 3 and 4) from October 2006 to July 2007.

Station	October 2006	January 2007	May 2007	July 2007
1	<i>V. alginolyticus</i> (2) <i>A. sobria</i> (1)	<i>V. alginolyticus</i> (1) <i>A. salmonicida</i> (1) <i>A. trota</i> (1)	<i>V. alginolyticus</i> (1) <i>V. metschnikovii</i> (1) <i>A. caviae</i> (1)	
2		<i>V. alginolyticus</i> (1) <i>V. parahaemolyticus</i> (1) <i>A. caviae</i> (1) <i>A. hydrophila</i> (1) <i>A. sobria</i> (1) <i>A. veronii</i> (1)	<i>V. alginolyticus</i> (2) <i>V. fluvialis</i> (2) <i>V. mimicus</i> (1)	<i>A. hydrophila</i> (3)
3	<i>V. alginolyticus</i> (2) <i>A. sobria</i> (1) <i>A. veronii</i> (1)	<i>V. alginolyticus</i> (1) <i>V. vulnificus</i> (1)	<i>V. alginolyticus</i> (3) <i>V. fluvialis</i> (1) <i>V. cholerae</i> (1) <i>A. hydrophila</i> (1) <i>A. salmonicida</i> (1) <i>A. sobria</i> (1)	<i>A. caviae</i> (1)
4	<i>V. alginolyticus</i> (3) <i>V. cholerae</i> (1) <i>A. hydrophila</i> (2) <i>A. trota</i> (1)	<i>A. caviae</i> (1) <i>A. salmonicida</i> (1)	<i>V. alginolyticus</i> (1) <i>V. vulnificus</i> (1) <i>V. parahaemolyticus</i> (1) <i>V. fluvialis</i> (1) <i>A. caviae</i> (2)	<i>V. alginolyticus</i> (1) <i>V. cholerae</i> (1) <i>V. mimicus</i> (2) <i>V. parahaemolyticus</i> (1) <i>A. caviae</i> (1) <i>A. hydrophila</i> (1)

appeared to be influenced by the effluents discharging into the river. Microbial abundance, referred to picoplankton and to its phototrophic component (or picophytoplankton), was among the highest recorded in other estuarine environments (Trousselier *et al.*, 2004) and in marine zones of the Mediterranean Sea (Maugeri *et al.*, 1992; Vanucci *et al.*, 1994; Acosta Pomar *et al.*, 1998), indicating an eutrophicated status for this estuary. Picoplanktonic cells abundance increased in the Alcantara estuary with temperature, while a different spatial and an opposite temporal trend was observed for picophytoplankton. The contribution of picophytoplankton to total picoplankton showed greater variations in freshwater (from 0.53% to 8.6%) than in coastal water (from 1.8% to 4.3%), reaching the highest values in January, when fecal contamination increased and low salinity values, pH, and oxygen concentration were observed. The highest variation was observed in the upper station (station 1) in the river, influenced by the discharge of the Calatabiano wastewater plant. Picophytoplankton was mainly composed of cyanobacteria both in fresh and brackish waters. In Alcantara waters,

cyanobacteria outnumbered eukaryotes in January, while the ratio of eukaryotes to cyanobacteria increased in July. Since the composition of picophytoplanktonic groups in freshwater showed an opposite temporal pattern from brackish water, we might assume that changes displayed by the picophytoplankton in freshwater may be attributed to a combination of salinity variations and nutrient supply, rather than to the seasonal difference in temperature. Consequently, not only the abundance but also the composition of picophytoplankton can be considered a useful indicator of the eutrophic status of water.

Over the examined period, a high abundance of aeromonads was recovered, which outnumbered vibrios and fecal bacterial indicators. The increase in aeromonads abundance in January, when non-treated effluents of wastewater plants discharged into the river, indicated a modification of the trophic status of the estuarine waters. Among aeromonads, *A. hydrophila*, *A. sobria*, and *A. caviae* were the most abundant in the Alcantara estuarine waters. *A. sobria* was isolated only from freshwater samples in all months except in July. The presence of *A. hydrophila* and *A. caviae* is

indicative of the trophic status in water and may be considered a better indicator of fecal pollution (Monfort and Baleux, 1990; Araujo *et al.*, 1991). *A. hydrophila* and *A. caviae* isolated in July, and *A. sobria* isolated in May, possessed hemolytic activity. This finding allows us to hypothesize that seasonal factors, mainly temperature, may influence the maintenance of factors of the virulence.

The recovery from Alcantara waters of *A. salmonicida*, a pathogen for cultivated and wild fish species, may also be considered a useful indicator of water pollution (Pavan *et al.*, 2000; Sechi *et al.*, 2002).

Bacterial identification of *Vibrio* isolates revealed the presence of potentially pathogenic strains for humans and animals in the Alcantara River estuary (*V. alginolyticus*, *V. cholerae* non-O1/non-O139, *V. fluvialis*, *V. metschnikovii*, *V. parahaemolyticus*, *V. vulnificus*). Although the abundance of total vibrios did not show any correlation with temperature, a marked seasonality in vibrios composition of water samples was observed, confirming that distribution of pathogenic *Vibrio* spp. in aquatic environments is greatly influenced by temperature (Maugeri *et al.*, 2004). However, in estuarine and coastal environments, physical and chemical characteristics may change considerably, both spatially and temporally, and consequently may influence the ecology of these bacteria. It is known that among vibrios, *V. alginolyticus*, *V. cholerae*, *V. fluvialis*, *V. parahaemolyticus*, and *V. vulnificus* can adapt themselves to adverse conditions, e.g. organic matter limitation and unsuitable temperature, by means of different survival strategies, such as by adhering to different substrata and planktonic organisms (Tamplin *et al.*, 1990; Maugeri *et al.*, 2004; 2006; Gugliandolo *et al.*, 2005; 2008).

The most abundant species isolated from all waters was *V. alginolyticus*, which is considered a pathogenic *Vibrio* species, particularly of wounds and ear infections following exposure to seawater (Farmer *et al.*, 2003). This species showed higher salinity adaptation than the other vibrios, and especially *V. fluvialis*, which was mainly recovered from freshwater. *V. parahaemolyticus* and *V. vulnificus* were sporadically isolated from waters. *V. vulnificus* is an etiologic agent in severe human infection generally acquired through wounds or by the consumption of contaminated seafood. *V. cholerae* and *V. mimicus*, the less salt-

tolerant vibrios, were recovered in the estuarine waters when water temperature was $>18^{\circ}\text{C}$. *V. cholerae* isolates belonged to non-O1/non-O139 serogroups, which are recognized as normal components of aquatic microflora (Sechi *et al.*, 2000). However, most *V. cholerae* environmental isolates may possess various virulence factors and pathogenicity-associated genes that may circulate among *Vibrio* spp. (Sechi *et al.*, 2000).

The detection of pathogenic and potentially pathogenic strains of *Vibrio* and *Aeromonas*, which may be considered a potential risk for health in estuarine and coastal environment, supports the proposal that microbial quality study of waters should include a specific search for these bacteria. Their presence in the Alcantara estuarine waters not only represents a major health concern, but also might prevent the utilization of these waters for important economic resources, such as fishing, aquaculture and mussel farming. The Alcantara River, as part of the only River Park in Sicily, is a water body that requires reaching at least "sufficient" water quality status by 2008 and "good" by 2016 (60/2000/EU). The water quality and the ecological status of the Alcantara River, apart from being revealed by the routine microbiological analyses, should also be monitored by the quantification of all aquatic microorganisms and by the relevant abundance of species that are considered as emerging pathogens for humans and animals.

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