Chapter 6
The distribution of *Aeromonas* species and *A.hydrophila* in water, sediment and fish samples collected from four different sampling stations of River Amaravathy, South India was investigated for a period of two years (June 1998 to May 2000). Numerical taxonomy was carried out for the identification of *Aeromonas* species. Antibiotic resistance of strains of *A.hydrophila* (isolated from water, sediment and fish) was studied against fifteen antibiotics. Haemolytic and proteolytic activity was carried out for all the *A.hydrophila* strains and the selected *A.hydrophila* strains were examined for the production of protease enzyme. Susceptibility of *A.hydrophila* strains to residual chlorine under different concentrations with various time intervals was studied.

Dissolved oxygen ranged between 3.9 (station 2) to 6.0 mg. L\(^{-1}\) (stations 2 and 4). Sodium concentration varied from 12.1 (station 1) to 18.8 mg. L\(^{-1}\) (station 4) and calcium from 10.9 (station 1) to 24.8 mg. L\(^{-1}\) (station 3). The lowest concentration of potassium was 4.8 mg. L\(^{-1}\) in station 2 and the highest concentration was 8.0 mg. L\(^{-1}\) in station 4 and total phosphate ranged between 4.0 - 7.2 mg. L\(^{-1}\) in stations 2 and 4 respectively.

Maximum THB population (5.37 log cfu mL\(^{-1}\)) was encountered in station 4. The population of *Aeromonas* species was between 3 and 4. 37 log cfu mL\(^{-1}\) and *A.hydrophila* population occurred between 3 to 3.9 log cfu mL\(^{-1}\). *Aeromonas* spp and *A.hydrophila* were at maximum numbers in station 3 with 4.37 and 3.9 log cfu mL\(^{-1}\) respectively.
The maximum occurrence of THB was recorded during premonsoon in stations 1 and 3 with 37.3 and 38.5 log cfu mL\(^{-1}\) respectively. Whereas in stations 3 and 4 it was in different season. *Aeromonas* species and *A.hydrophila* showed its highest occurrence during postmonsoon in stations 2 and 3.

In water sample, significance was recorded at 1 % level between *Aeromonas* spp. - *A. hydrophila* in stations 1, 2 and 3. In the case of station 3, both *Aeromonas* species and *A.hydrophila* showed 1% significance with dissolved oxygen. But none of the parameters in station 4 showed significant relation.

In sediment samples, the pH varied from 6.8 to 7.5 in all the stations and calcium in station 2 was recorded with 22.6 mg. g\(^{-1}\) and the calcium content was from 17.0 to 22.6 mg. g\(^{-1}\) in stations 1 and 2 respectively. The concentration of sodium was found to be lower in station 1 with 24.8 mg. g\(^{-1}\) and the highest (30.4 mg. g\(^{-1}\)) in station 4 and total phosphate was found to be higher in station 4 with 7.0 mg. g\(^{-1}\)

In sediment samples, the highest THB population (5.36 log cfu g\(^{-1}\)) was recorded in station 2. Maximum *Aeromonas* species was noticed in station 1 with 3.84 log cfu g\(^{-1}\) and *A.hydrophila* was found to be more in both station 1 and 3 with 3.6 log cfu g\(^{-1}\). The occurrence of *Aeromonas* species in all the station was between 3 to 3.84 log cfu g\(^{-1}\) and the population of *A.hydrophila* was recorded between 3 to 3.6 log cfu g\(^{-1}\).

The highest incidence of both *Aeromonas* species and *A.hydrophila* in all the sampling stations was found in premonsoon and the distribution of THB varied from station to station.
Significant correlation was recorded between *Aeromonas* spp. and *A. hydrophila* at 5 % level in the sediment samples of stations 1 and 4 and at 5% in station 3. In station 3 significant correlation between *Aeromonas* spp., *A. hydrophila* and pH was noticed at 5 % level and in station 2 none of the parameters showed significant relation.

A total of 262 fishes were analysed for the incidence and 51.1 % of the fishes were found to be associated with *A. hydrophila*. All the fishes analysed during October 1998 and December 1999 were found to carry *A. hydrophila*. The highest incidence (65.7 %) was recorded during the monsoon of the year 1999 -2000.

About 58.3 % of *Sarotherodon mossambicus* carried *A. hydrophila* followed by *Catla catla* (54.7 %). Among the three body parts (body surface, gill and intestine) analysed, intestine showed maximum (50.8 %) incidence to *A. hydrophila*.

Numerical taxonomy using UPGMA analysis showed six different phenons belonging to *A. hydrophila*, *A. sobria*, unidentified *Aeromonas* species, *A.jandaei*, *A.caviae* and *A. enchleia*. In all the samples *A. hydrophila* was the dominant species.

All the strains isolated from water sample showed resistance towards bacitracin, kanamycin, methicillin and novobiocin. Totally 24 different patterns of multiple antibiotic resistance (MAR) was exhibited by *A. hydrophila* strains. About 29.3 % of the strains produced MAR frequency between 0.51 - 0.60.

In sediment samples all the strains exposed to bacitracin, methicillin and novobiocin showed resistance. A total of 34 antibiotic resistance patterns were noticed and all the strains showed MAR frequency above 0.50 level.
One hundred and fifty *A. hydrophila* strains isolated from fish samples showed resistance towards bacitracin, methicillin and novobiocin. Maximum of 82 different patterns were noticed and all the strains produced MAR frequency above 0.40 level.

About 62 %, 68 %, and 50 % of *A. hydrophila* strains isolated from water, fish and sediment samples were able to produce haemolysin. *A. hydrophila* strains extended proteolytic activity and the maximum protease enzyme production (152 µg mL⁻¹) was noticed in *A. hydrophila* strain isolated from fish intestine.

The study on the susceptibility of *A. hydrophila* strains towards chlorine (commercial bleach) showed that the only strain that is survived in 0.45 ppm was Ahw3 with 0.3 log cfu mL⁻¹. The effective concentration to kill 2.03 log cfu mL⁻¹ was found to be 0.5 ppm after exposing to 150 minutes.

CONCLUSION

The occurrence of *A. hydrophila* with multiple antibiotic resistance, haemolysin and protease enzyme production in water, sediment and fish samples was evidenced in this study. The present investigation gives basic information about the emergence of *Aeromonas* species in River Amaravathy and also it alarms that the normal recommended concentration (0.2 ppm) of chlorine is insufficient to control this organism. So further steps has to be taken for the frequent monitoring of the river water by considering the well being of the people who are depending on the river for their day to day activities.