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Population Dynamics and Seasonal Variation of Bacterial System Utilizing Single Carbon from River Cooum and River Adyar, Chennai, Tamilnadu, India

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A B S T R A C T

Though the single carbon utilizing bacterial system, Methylotrophs are rich source of biological compounds and good source of bio-remeidation, their diversity in river system have not paid much attention. In this study, the population of methylotrophs in two main water ways, river Coovum and river Adyar of Chennai, fourth cosmopolitan city, of India was carried out. The samples were collected for 12 months from four different sites every fortnight and grouped into four seasons. The sampling sites are either ecologically sensitive or severely affected by contamination. The methylotrophic densities were found high in river Coum 5.9 to 6.7 Log CFU mL^{-1} than Adyar river 5.5 to 5.9 Log CFU mL^{-1} and also fluctuated significantly over time which showed a clear seasonal variation. The bacterial count also varied depending upon the nature of site of sample collection with gradual decrease from upstream to downstream. Cluster analysis (similarity) based on the average values of methylotrophic counts explained the site-wise and month-wise relationship individually.

Introduction

Study of bacterial community is complicated by the dilution, survival and re-suspension of sediment bound groups, which are all affected by continuous and often violent environmental fluctuations (Martinez-Urtaza *et al.*, 2004). Sewer outflows and runoffs from farms and urban areas are reported to carry a wide diversity of microbial communities to the coastal regions through rivers (Pruss, 1998). There are numerous reports on the diversity of different groups of bacteria with different potential traits.

However, diversity and population analysis of methylotrophs are very much limited. These are the bacterial group with the ability to grow at the expense of reduced carbon compounds containing one or more carbon atoms with no carbon-carbon bonds as sole source of energy and have been known since the late 19th century (Patt *et al.*, 1976). They also represent promising organisms in biotechnology for the conversion of one-carbon (C1) substrates to value-added products such as single cell proteins,

vitamins, aminoacids, biopolymers, enzymes, products of biotransformation and also for their role in carbon-cycling, bioremediation and in replacing petrochemical-based chemical processing in future (Chistoserdova *et al.,* 2003; Schradar *et al.,* 2009).Members of this group possess great metabolic versatility with the ability to scavenge trace amounts of single carbon, nitrogen and resistance to a certain degree of desiccation which contributes their survival in the hostile environments (Anthony and Williams, 2003). Faria and Lokabharathi (2006) reported the marine and estuarine methylotrophs abundance in the samples of Dona Paula beach, Goa, India. Active methylotrophs present in the sediments of Lonar Lake, India, was reported earlier (Antony *et al.,* 2010). There are few reports that depict the diversity of methylotrophs especially in phyllosphere of rice, cotton, maize and sunflower plants (Balachandar *et al.,* 2008; Raja *et al.,* 2008;). However, diversity of methylotrophs in river eco-systems as a whole remains unexplored. Chennai (Madras), the capital of Tamil Nadu state, the fourth largest metropolitan city in India is located on the southeast Coramandel coast of Bay of Bengal is traversed by four waterways namely River Cooum, River Adyar, Buckingham Canal and Otteri Nullah. Of the rivers, the major ones are the River Cooum, runs in the centre of the city and the Adyar River, meandering through the south of Chennai. The waterways of Chennai are not perennial but receive flood discharge and surplus water released from the reservoirs during the monsoon season. These waterways constitute an important environmental component for absorption of wastewater, recharge of groundwater aquifers and also for maintaining the aesthetic quality. Both Cooum and Adyar Rivers are heavily polluted with effluents and waste from domestic and other sources. They serve as elongated lagoons for reception of wastewater from a wide range of domestic, commercial and industrial enterprises. The total drainage area of the waterways basin in Chennai city has been estimated to be 83 Km^2. The state government periodically removes silt and pollution from the Adyar River, which is much less polluted than the River Cooum. As a consequence of pollution, these Rivers carry large diverse microbial populations (Shanmugam *et al.,* 2007). Therefore the present study was undertaken for the first time to study the methylotrophic bacterial count from rivers, Cooum and Adyar River (two main water ways) in Chennai, Tamil Nadu, India in response to the seasonal variation.

Materials and Methods

Site of Sample Collection

Water samples were collected for 12 months along the Cooum and Adyar Rivers, Chennai.

Cooum River water: The River rises due to the bifurcation of Nallar River at Kesavaram anikat near Arakkonum. From Arakkonam, it takes its own course and passes through the suburbs and small towns in the west of Chennai Tamil Nadu, India. It enters Chennai near Thiruverkkadu and meets sea near marina beach. This carries predominately the seweage generated from the settlements of suburban regions of Chennai. Water samples were collected from 4 sites namely Choolaimedu (MLW), Thousand lights (MQW), Chithadripet (MIW) and Chepauk (MNW).

Adyar River water: The River rises in the Chembarambakkam tank, flows through the city of Chennai, Tamil Nadu, India for a distance of 14 km, before it joins the Bay of Bengal. Urban pollution, mainly contributed

by waste water disposal within the city limits and industrial effluents enters the Adyar River through several outlets and empties into the Bay of Bengal. Water samples were collected from 4 sites along the river which includes Saidapet (MDW), Kotturpuram (MOW), Adyar (MHW) and Fore shore estate (MFW).

A map showing the sites of sampling spots along Cooum and Adyar Rivers in Chennai and different forests in various districts of Tamil Nadu, India were presented in Pic.1.

Collection of Water Samples

A total of 96 water samples were collected from the each four sampling sites along Cooum and Adyar Rivers, Chennai, Tamil Nadu, India, every fortnight from June to May. Water samples for microbiological examination were collected using the standard procedure, one meter away from the shore at a depth of a few centimeters using 50 mL sterile bottles for each sample. Samples were placed in ice and transported to the laboratory and processed on the same day of collection. pH and temperature of the water samples were recorded at the collection site using a digital pH meter Eco Scan (Eu-tech instruments, Singapore) and mercury centigrade thermometer.

Preparation of Samples and Culture Conditions

Enumeration and isolation of methylotrophs were carried out in specific Methanol Mineral Salts (MMS) medium containing 0.5% (*v/v*) methanol as carbon source. Water samples were serially diluted upto 5 folds in sterile MMS media and evenly spread plated. The plates were incubated at room temperature 30±2℃ upto 15 days. The necessary control plates for MMS medium with and without methanol were maintained. Following incubation, colony-forming units (CFU) were counted and the results were recorded as Log CFU mL^{-1}.

Statistical Analysis

Unless otherwise mentioned, all the results were evaluated by analysis of variance (ANOVA) and means were compared by applying Tukey's test using SPSS 14.0 statistical package. The level of statistical significance was accepted as $P<0.05$.

Results and Discussion

The study period of enumeration (June to May) was grouped into three seasons *viz.*, June, July, August and September as South West (SW) monsoon, October, November, December and January as North East (NE) monsoon and February, March, April and May as Post monsoon (PM). The average methylotropic counts for different periods such as South West (SW) monsoon, North East (NE) monsoon and Post monsoon (PM) of River Cooum and Adyar of Chennai are depicted in Fig. 1a & 1b. The methylotrophic population ranged from 5.7 - 6.5 Log CFU mL^{-1} during the SW monsoon, 5.5 - 6.1 Log CFU mL^{-1} during the NE monsoon and 6.6 - 7.4 Log CFU mL^{-1} during the PM in Cooum River, Chennai. In Adyar River, the methylotrophs ranged from 5.4 – 6.1 Log CFU mL^{-1}, 5.3 – 5.7 Log CFU mL^{-1} and 6.0 - 6.3 Log CFU mL^{-1} during the SW, NE and PM respectively.

The results revealed that the Cooum River harbored significantly higher number of methylotrophic bacteria ranging from 5.9 to 6.7 Log CFU mL^{-1} than Adyar River with 5.5 to 5.9 Log CFU mL^{-1} (P value < 0.005) (Fig. 2).Among the four sites along Cooum River, site 1 (Choolaimedu - MLW) had the highest average level of 6.7 Log CFU mL^{-1} methylotrophs followed by site 2 (Thousand

lights - MQW) with 6.3 Log CFU mL^{-1}, site 3 (Chithadripet - MIW) with 6.1 Log CFU mL^{-1} and the least level was observed in site 4 (MNW) with 5.9 Log CFU mL^{-1} irrespective of the seasons. In Adyar river, highest count was observed in site 2 (MOW) with 6.2 Log CFU mL^{-1} followed by site 1 and 3 (saidapet - MDW with 5.9 and adyar - MHW with 5.7 Log CFU mL^{-1}). The least was found in site 4 (fore shore - MFW) with 5.5 Log CFU mL^{-1}.

Cluster analysis (similarity) was carried out based on the average values of methylotrophic counts to understand the site-wise and month-wise relationship individually. Data grouped according to the sites of the Cooum and Adyar rivers formed three clusters (Fig. 3). Cluster 1 comprised site 1 (Saidapet, MDW) of River Adyar. Site 1 of Cooum (Choolaimedu, MLW) and site 2 of Adyar (Kotturpuram, MOW) Rivers formed cluster 2. Cluster 3 was formed by sites 3 and 4 of both the river (Chithadripet, MIW; Chepauk, MNW; Adyar, MHW; Fore shore estate, MFW).

The dendrogram for similarity of month-wise methylotrophic counts of Cooum river showed three clusters (Fig. 4). Cluster 1 was formed by the month May. Months of South west monsoon and North East monsoon occupied second cluster. Months namely February, March and April formed cluster 3. Clustering of methylotrophic counts in Adyar River generated a dendrogram, grouping the 12 months into two clusters (Fig. 5). Cluster 1 was further divided into two clusters: South west monsoon (June - September) and North East monsoon (October - January). Cluster 2 consisted of months February - March also includes the month of May.

The analytical data of the river water such as pH and temperature are summarized in Table 1. The pH values throughout the study ranged from 6.0 to 7.8 and 6.0 to 8.8 in the sites of Cooum and Adyar Rivers. The pH was high during post monsoon period which ranged from 6.5 to 9.0 in Cooum and 7.0 to 8.8 in Adyar Rivers. During SW and NE monsoon period, the water samples of Cooum River had pH ranged from 6.0 to 7.5. The pH of Adyar River ranged from 6.0 to 7.8 during SW and NE monsoon period. During the study period, the water temperature ranged from 20℃ to 35℃ in Cooum and Adyar rivers with maximum in post monsoon period (30℃ to 35℃) and minimum in SW and NE monsoon period (20℃ to 28℃).

The rivers carried higher methylotrophic count during post monsoon and lower numbers during the SW and NE monsoon. Anand *et al.* (2006a) reported higher bacterial count (total bacterial count) in dry seasons and the lowest in rainy months in the water samples of River Yamuna in Delhi. The results of the present study suggest the trend of Neumann *et al.* (1972) who reported that in the tropical countries the seasonal cycle of the micro organism can be correlated with the lowest numbers in rainy months, and the highest numbers in dry season.

Similarly Sood *et al* (2008) reported higher total viable count in summer than rainy season from the water samples of Gangetic river system of Uttarakhand (India). However, the reason for this could be due to the reduced flow of water content in the river during post monsoon months (March, April, May and June) which would have supported the bacterial survival when compared to other seasons (Anand *et al.*, 2006b). From this study, it is also evident that the significant reduction of bacterial count in River Cooum and Adyar during NE monsoon may be due to the flushing effect of rain, while comparatively high count during the SW monsoon months may be due to receiving of surface runoffs.

The River Cooum harbored significantly higher methylotophic population than the Adyar River. In general it has been found that the waters of the Cooum River are grossly contaminated by microbial flora, than the Adyar River which could be related to the impact of human activities including land drainage, dumping of enormous quantities of sewage, discharge of untreated municipal wastewater and industrial effluents and natural changes (Ezeronye and Ubalua, 2005). Moreover, the state government periodically removes silt and pollution from the Adyar River, which is much less polluted than the River Coovum (Shanmugam *et al.,* 2006). The population of resident bacteria in the river basins also differs greatly with respect to the degree of the impact of human activities on them. The variation in the count depending upon the nature of site of sample collection with gradual decrease from upstream to downstream can be discussed with the increased anthropogenic activities and social-cultural activities at different sites of river stretches as reported by Kumarasamy *et al.* (2009).

Cluster analysis is a helpful tool for organizing a particular set of data from various points into clusters or groups and determining the relationship between the various points (McGarial *et al.,* 2000). Hence the sampling sites in both the rivers and the sampling months were grouped in accordance with the methylotrophic count using cluster analysis. Clustering of sampling sites formed four clusters, the upstream site (MDW) of Adyar river formed cluster I as it has relatively lesser methylotrophic count than other sites. The most probable explation could be that the saidapet (MDW) site of Adyar River was widely used for the laundry purposes and readily contaminated with detergents which might have inhibited the growth and could be responsible for the decreased count. Venugopal *et al*. (2008) reported the effect of laudering in the quality of Adyar river water. Further, the effect of detergents over the microflora in the River Nile water was well defined by Issa and Ismail (1994). In support, Salton (1953) has reported that a Gram-negative bacterium that contains appreciable amounts of lipid gets disaggregated due to the exposure of detergents. Sampling site MLW (Cooum River) at the upstream end formed second cluster and this site was observed with relatively higher methylotrophic count during the study period. The reason may be due to the prevailed highest domestic human activities in the stretch of the river around this site including the discharge of domestic waste, sewage outfalls and absence of sanitation facilities. Irrespective of the rivers, sites MQW (Cooum River), MIW Cooum River), MOW (Adyar River) and MHW (Adyar River) at the intermediate regions of the river stretch were grouped together as cluster III while the sites MNW (Cooum River) and MFW (Adyar River) at the downstream stretch were found closer to form cluster IV. The results of the cluster analysis suggested that the variation in the total count of methylotrophs in the sampling sites might also be dependent on the order of magnitude, greater at the freshward than at the seaward end, which could be attributed to the tidal movements and dilution of bacterial count due to variations in the continual confluence of freshwater and coastal water at the downstream (Neill, 2004). According to Neal (1972), the bacteria entering the estuary from the river and the coastal ocean are mixed by tidal actions which were then washed out of the estuary by incoming coastal marine water. Moreover it has been reported that the bacterial population in a river depends on the function of initial loading and the disappearance rate, which, in turn, is a

function of the time or the distance of travel from the source and of other factors including temperature, salinity, and light intensity (Eleria and Vogel, 2005). These factors might have contributed, independently or in combination, to the levels of methylotrophic bacterial counts that were observed at these sampling sites of the two rivers.

Fig.1a Seasonal Variations of Methylotrophic Bacterial Counts in Cooum River

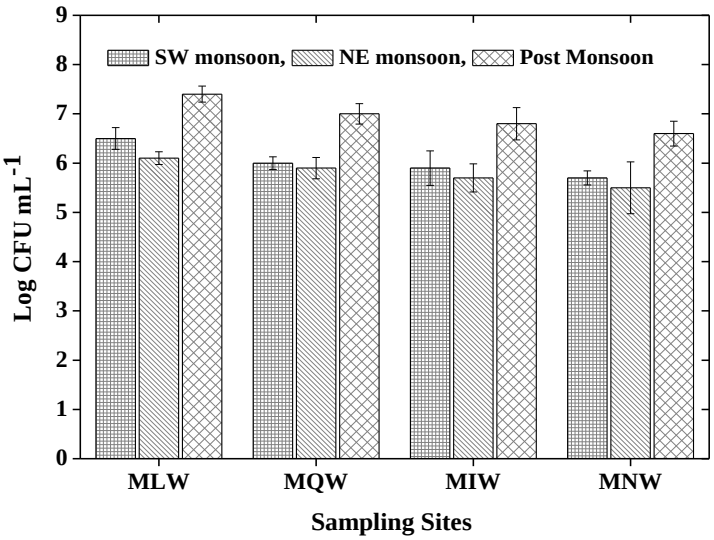
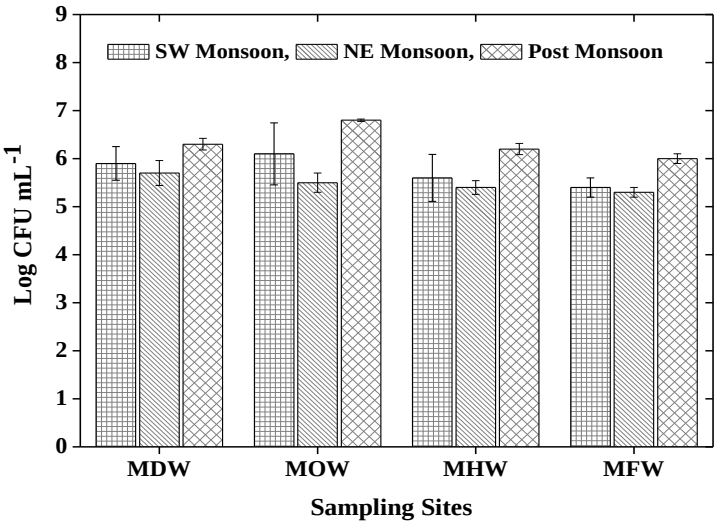


Fig.1b Seasonal Variations of Methylotrophic Bacterial Count in Adyar River

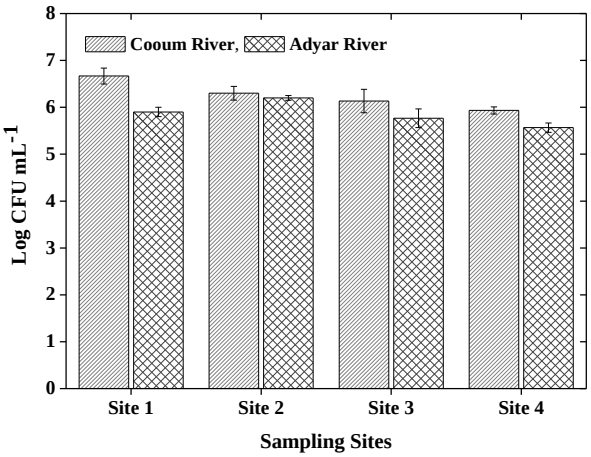


SW - South West monsoon (June, July, August and September); NE - North East monsoon (October, November, December and January); Post monsoon (February, March, April and May)

Water samples collected every month for 12 months were serially diluted and plated on MMS medium containing 0.5% methanol as carbon source and incubated at room temperature 30±2^{0}C.

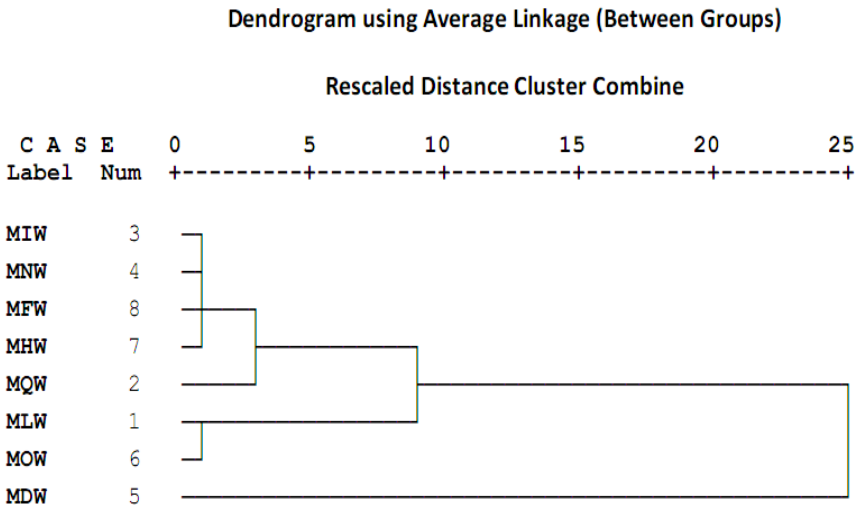
Values represent the mean±S.D. of four determinations.

Fig.2 Overall Methylotrophic Bacterial Counts in the Sampling Sites of Cooum River and Adyar River



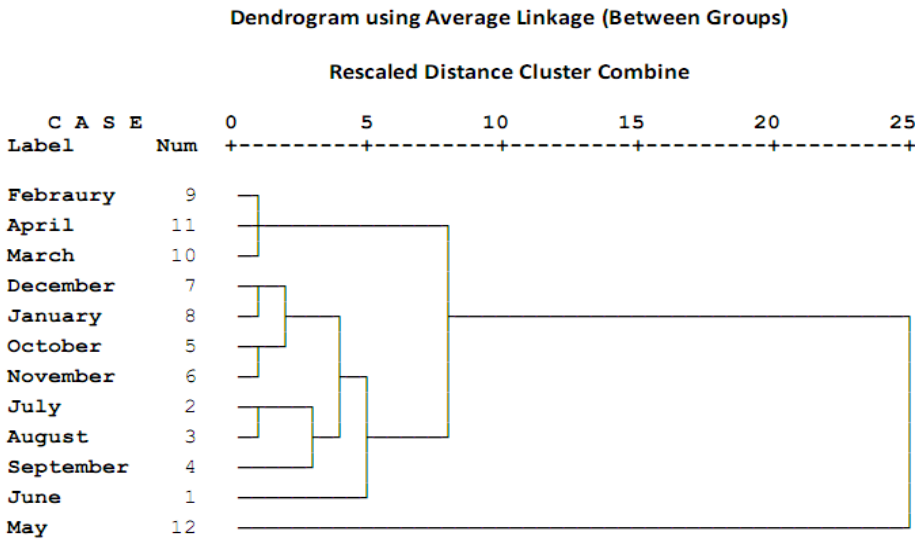
Cooum River: Site 1 - Choolaimedu (MLW); Site 2 - Thousand lights (MQW); Site 3 - Chithadripet (MIW); Site 4 - Chepauk (MNW)
Adyar River: Site 1 - Saidapet (MDW); Site 2 - Kotturpuram (MOW); Site 3 - Adyar (MHW); Site 4 - Fore shore estate (MFW)
Results represent mean ± SD.

Fig.3 Dendogram Showing the Relationship of Methylotrophic Counts among the Sampling Sites of Cooum River and Adyar River



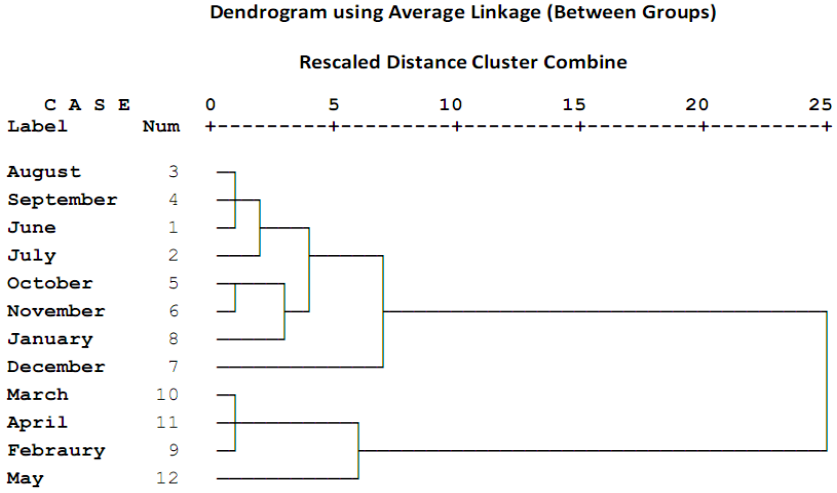
Cooum River: Site 1 - Choolaimedu (MLW); Site 2 - Thousand lights (MQW); Site 3 - Chithadripet (MIW); Site 4 - Chepauk (MNW)
Adyar River: Site 1 – Saidapet (MDW); Site 2 - Kotturpuram (MOW); Site 3 - Adyar (MHW); Site 4 - Fore shore estate (MFW)
Clusters formed between the sampling sites of the Cooum and Adyar Rivers of Chennai based on the methylotrophic counts in the water samples to understand the relationship among the stations.
Dendrogram was derived from Pearson correlation.

Fig.4 Dendogram Showing the Relationship among the Months in Methylotrophic Counts of Cooum River



Clusters based on the methylotrophic counts collected between the months in the water samples collected from Cooum River of Chennai.
SW - South West monsoon (June - September); NE - North East monsoon (October - January); Post monsoon (February - May).
Dendrogram was derived from Pearson correlation.

Fig.5 Dendogram Showing the Relationship among the Months in Methylotrophic Counts of Adyar River



Clusters based on the methylotrophic counts collected between the months in the water samples collected from Adyar River of Chennai.
SW - South West monsoon (June - September); NE - North East monsoon (October - January); Post monsoon (February - May).
Dendrogram was derived from Pearson correlation.

Pic.1 Map Showing the Sites of Sampling Spots Along Cooum and Adyar Rivers in Chennai, Tamil Nadu, India

* Stars represent the sites of sample collection.
Water samples were collected from each four sites along Cooum and Adyar Rivers, Chennai every fortnight for 12 months and were enumerated for methylotrophic count by serial dilution method.

Clustering of methylotrophic count based on sampling months showed the seasonal effect on the methylotrophs population. In both the rivers, irrespective of their nature, the months were grouped as two main clusters except in Cooum River where the count in the month of May 08' formed a separate cluster which may be due to transition period of methylotrophic count from one season to another. Post monsoon have formed the Cluster I, which could be characterized as the effect of reduced water flow in the river due to absence of rainfall. It has also been reported that these rivers serves as a conveyor of storm water from the city's sewage drain network which becomes stagant during post monsoon with rich organic matter (Shanmugam *et al.*, 2006). In contrast, the methylotrophic population during the SW monsoon (June, July, August and September) and NE monsoon (October, November, December and January) formed the 2nd cluster indicating the effect of influence of rain which tend to push the

river waters toward seaward direction and also the soil runoffs during this season. In both the rivers Cluster I recorded the maximum methylotrophic population when compared to cluster II. This shift in bacterial count from cluster I to another may be due to the effect of monsoon. Castillo (2000) has reported the influence on hydrologic seasonality in rivers of differing chemistry which varied across years of contrasting rainfall patterns. Besides, influence of key factors such as light, salinity, rainfall, available nutrients and environmental pollutants on the bacterial growth and abundance in water bodies has also been reported earlier (Pernthaler *et al.,* 1998; Solo-Gabriele *et al.,* 2000; Bezuidenhout *et al.,* 2002). This indicated that the population of methylotrophs over a point of 1 year resembles two periods in water samples of River Cooum and Adyar.

The pH of river water in all the stations was found to be slightly acidic during monsoon period but changed to slight alkaline condition during post monsoon. The observed variation in pH could be attributed to the negligible terrestrial runoff, precipitation and also absence of fresh water discharge caused the change of pH within a very narrow limit. The surface water temperature during the present study varied with current reversal. Similar variation of temperature has been reported earlier (Venugopal *et al.,* 2008). However, in the water samples methylotrophic density does not seem to be correlated with temperature. This finding suggests that temperature is not the only factor driving differences in seasonal variation in bacterial count but also fluctuations in other factors such as substrate and nutrient availability (Castillo *et al.,* 2004).

The results indicate that Cooum and Adyar Rivers in Chennai are rich source of methylotrophic bacteria which in turn could be a source of various compounds. Further studies on these sites including study of various microbial biodiversity and evaluation of biotechnological potential of the isolated strains with exploration of functional aspects would add value for their use in large scale application.

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