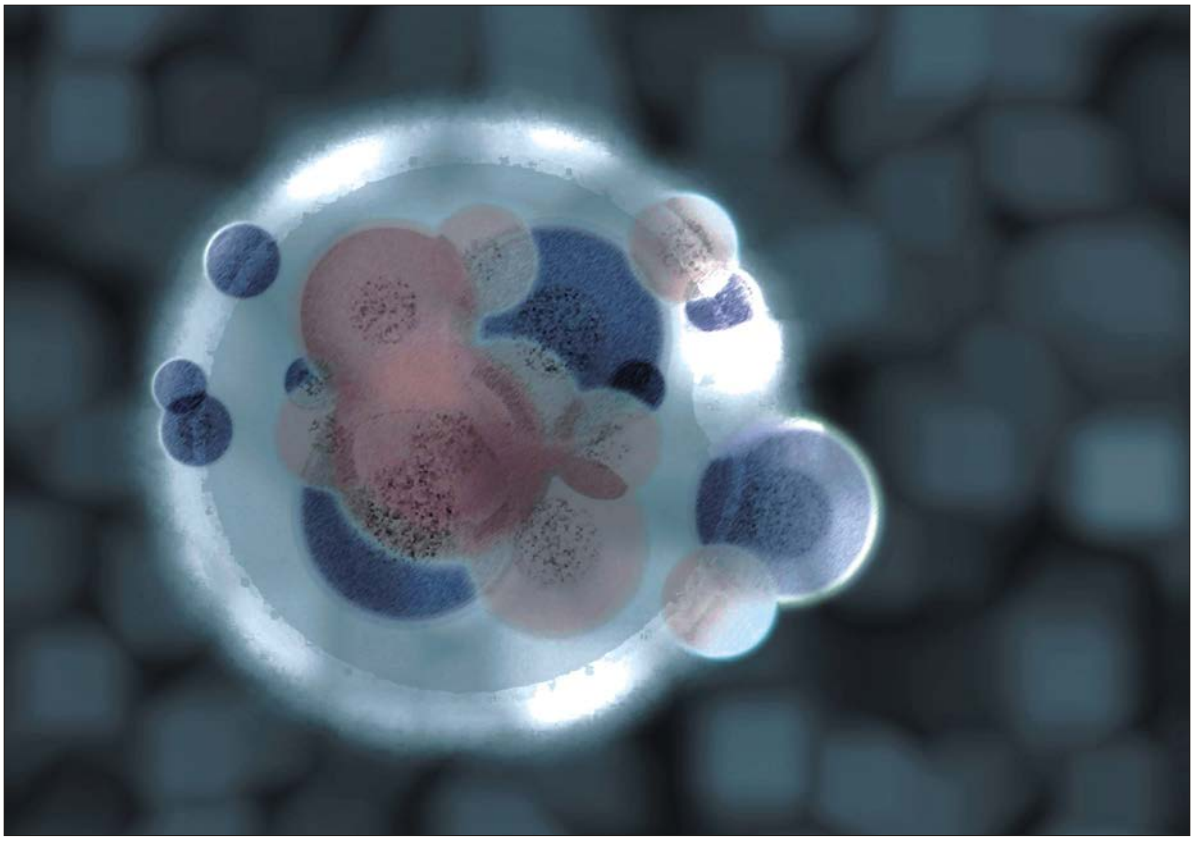

# Journal of Biological Sciences

ISSN 1727-3048

science alert

ANSInet
an open access publisher
http://ansinet.com

Journal of Biological Sciences 9 (7): 772-777, 2009
ISSN 1727-3048


# Allocation and Abundance of Protozoa among Soil Aggregates

C.R. Shalinimol, R. Arunachalam and G. Annadurai
Environmental Nanotechnology Division, Sri Paramakalyani Centre for Environmental Sciences,
Manonmaniam Sundaranar University, Alwarkurichi-627 412, Tamil Nadu, India

**Abstract:** In this present investigation, soil samples were collected from two different paddy fields such as Kollemcode (Tamil Nadu) and Pappanancode (Kerala) using PVC pipes. The physico-chemical characteristics of the soil sample were analyzed. Organic content, nutrient content and numbers of ciliates were high in upper segments of soil samples were recorded. The ciliates were higher amount in wet field paddy soil than flooded field paddy soil. The ciliates such as *Microthorax* sp., *Vorticella* sp., *Oxytricha* sp. and *Euplotes* sp. were identified in both field samples. In enriched culture, *Microthorax* sp., *Vorticella* sp., amoebae and some small flagellates were also observed. It was shown that the distribution of protozoan cells was not even but greatly conglomerative among soil aggregates. On the basis of data generated from this study could be exploited for wastewater treatment studies.

**Key words:** Soil aggregates, protozoa ciliates, paddy soil, distribution, wastewater treatment

## INTRODUCTION

An artificial ecosystem of biological wastewater treatment plant (Fried *et al*., 2000), consisting of abiotic and biotic components interacting. The abiotic components are represented by the plant and the sewage, whereas the biotic components comprise the decomposers (bacteria and fungi) that take energy for their growth from the dissolved organic matter and the oxygen in incoming wastewater and by protozoa and metazoa microfauna grazing on the decomposers (Madoni *et al*., 1993). The microfauna present in the aeration tank of an activated-sludge plant include protozoa (flagellates, sarcodines and ciliates) and metazoa (rotifers, nematodes, tardigrades, gastrotrichs and oligochaetes) specimens (Ginoris *et al*., 2007). Even though the bacteria are generally prevalent in the aeration tank, high concentrations of protozoa in the tank normally indicates a good performance. The faunal species distribution and abundance have been pointed out as indicators of the water quality of the effluent issuing from an activated-sludge plant, providing a useful mechanism to evaluate and assess its performance. Several authors have already investigated the importance and role of the protozoa and metazoa community in the purification process of activated-sludge plants (Curds, 1982). The correlation between the plant performance or operational conditions and the abundance of certain species has also been an object of study (Fried *et al*., 2000; Madoni, 2000; Madoni *et al*., 1993, 1996; Salvado and Gracia, 1993; Nicolau *et al*., 2001, 2005), which led to the development of a number of methodologies based on protozoa populations structure to assess the activated sludge plant performance (Ginoris *et al*., 2007). Thereby, the present study was aimed to determine the physico-chemical conditions, distribution and abundance of protozoa presence flooded and wet paddy soil.

## MATERIALS AND METHODS

**Sample collection:** The present investigation was performed during the period of four months (November 2007 to February 2008). Soil samples were collected from two different paddy fields, one at Kollemcode (Kanyakumari District, Tamil Nadu) and another at Pappanancode (Trivandrum district, Kerala) on November, 2007. The soil samples were collected by the help of PVC pipes (2 cm in diameter). The pipe was inserted into the soil and the sample was recovered. Five representative soil samples were collected from wet and flooded paddy fields. The sample was brought to the laboratory in PVC pipes. Soil sample column was pushed out with the help of sterilized glass rod. The length of soil sample column was measured by centimeter scale. The soil column was cut into 1 cm segments and numbered subsequently. The weight of the segments was recorded. The identical segments (segments of same level) from replicate samples pooled together and suspended in 100 mL distilled water in a 250 mL conical flask.

**Corresponding Author:** G. Annadurai, Environmental Nanotechnology Division,
Sri Paramakalyani Centre for Environmental Sciences, Manonmaniam Sundaranar University,
Alwarkurichi-627 412, Tamil Nadu, India Tel: +91-4634-283406

**Physico-chemical analysis of soil sample**

**pH:** The paddy field soil sample was subjected to physico-chemical analysis. One gram of sample was dispensed in 10 mL of distilled water, it was mixed uniformly and pH of the solution was recorded with a pH meter (Systronics).

**Salinity:** One gram of sample was dispensed in 10 mL of distilled water, it was mixed uniformly. Salinity and conductivity of the solution were recorded with a REDOX meter.

**Organic carbon content:** The detection of soil organic carbon was based on the Wet Oxidation method (Adams, 1934). In brief, oxidisable matter in the soil was oxidized by 1 N $K_2Cr_2O_7$ solution. The reaction was assisted by the heat generated when two volumes of $H_2SO_4$ were mixed with one volume of the dichromate. The remaining dichromate was titrated with $FeSO_4$. The titre was inversely related to the amount of carbon presenting the soil sample.

The samples were air dried ground and passed through 0.42 mm sieve. One gram of soil sample from each segment was used. Ten milliliter of 1 N $K_2Cr_2O_7$ was added accurately and the flask was swirled gently to disperse the soil in the solution. A stream of 20 mL of concentrated $H_2SO_4$ was added directly into the suspension. Immediately the flask was swirled until the soil and the reagents were mixed. A 200°C thermometer was inserted and heated while swirling the flask and the contents on hot plates or over a gas burner and gauze until the temperature reached up to 135°C (approximately 1/2 min). Then it was set aside on an asbestos sheet to cool slowly in a fume cupboard. Two blanks (without soil) must be run in the same way to standardize the $FeSO_4$ solution. The contents were cooled for 20 to 30 min and diluted up to 200 mL with deionized water and proceed with $FeSO_4$ titration using the ferroin indicator.

**Ferroin titration:** To the above mixture 3/4 drops of ferroin indicator was added and titrated against 0.4 N $FeSO_4$. Before the end point was approached, the pale green solution will turn to dark green. At that point, ferrous sulphate was added drop by drop until the color changes sharply from blue green to reddish grey, which was the end point. If the end point was over shot, 0.5 or 1.0 mL of 1 N $K_2Cr_2O_7$ was added and reapproach the end point drop by drop.

$$\text{Organic carbon (Y)} = \frac{0.003 \times N \times 10\ mL\langle 1 - T/S\rangle \times 100}{ODW}$$

where, N indicated Normality of $K_2Cr_2O_7$ solution, T is volume of $FeSO_4$ used in sample titration (mL), S is volume of $FeSO_4$ used in blank titration (mL) and ODW is oven dry sample weight (g).

**Total phosphorus content:** The soil phosphorus content was detected by the method of Pierzynski (2000) with slight modification. In brief, poly phosphates were converted to the orthophosphate form by a sulphuric acid digestion and organic phosphorus was converted to orthophosphate by a persulphate digestion. When the resulting solution was injected on to the manifold, the orthophosphate ion ($PO_4^{3-}$) reacts with ammonium molybdate and antimony potassium tartrate under acidic conditions to form a complex. This complex was reduced with ascorbic acid to form a blue complex that absorbs light at 880 nm. The absorbance is proportional to the concentration of total phosphorus in the sample. Two gram of soil sample was weighed and mixed with 25 mL of distilled water. To that 5 mL of concentrated $HNO_3$ and 1 mL of concentrated $H_2SO_4$ were added. It was taken in Kjeldahl flask and digested the mixture up to 1 mL. The mixture was cooled and filtered by using Whatman No. 1 filter paper taken in 50 mL standard flask. One drop of phenolphthalein indicator was added. Sodium hydroxide solution was added as much to produce a faint pink color and made up to 50 mL of combined reagent (50 mL 5 N $H_2SO_4$), 5 mL potassium antomonyl tartrate, 15 mL ammonium molybdate solution, 30 mL ascorbic acid solution).

**Total nitrogen content (Kjeldahl method):** The soil nitrogen content was detected by using the Kjeldahl standard method. In the presence of $H_2SO_4$, potassium sulphate ($K_2SO_4$), cupric sulphate catalysts, amino nitrogen of many organic materials was converted to ammonium. After addition of base, the ammonia was distilled from an alkaline medium and absorbed in boric or sulphuric acid. The ammonia might be determined calorimetrically, by ammonia selective electrode, or by titration with a standard mineral acid.

Two gram of soil sample was weighed and mixed it with 50 mL of digestion reagent (134 g $K_2SO_4$, 7.38 g $CuSO_4$, 800 mL distilled water, 134 mL con. $H_2SO_4$ and diluted to made 1000 mL solution). The mixture was digested up to 20 mL and then it was filtered with Whatmann No. 1 filter paper. The reading was obtained by using ion analyzer.

**Microbiological analysis of protozoa:** The total numbers of protozoa present in each soil segment samples were counted. Known quantity (nearly 40 g) of each segments of soil sample was suspended in 100 mL of distilled water.

The sample was mixed umformLy and 1 mL of the sample was transferred to a counting chamber. The slide was observed under 4X, 10X and 40X objectives of optical microscope (Nikon). The total ciliates and number

of different kinds were counted separately. The count was repeated 5 times and average number of ciliates was calculated from which the total number of ciliates in original soil sample in each segment was calculated as number of ciliates per gram of sample.

**Identification of protozoa:** Individual ciliates were observed under a high power microscope (Leica Dm 2500). The specimen was observed under bright field, dark field and phase contrast objectives. Dimensions of ciliates were also noted. This information was compared with identification key of Patterson and Hedley (1992) and the ciliates were identified.

**Enrichment of protozoa:** The protozoa present in the natural soil sample were enriched by inoculating in hay infusion broth.

**Hay infusion broth:** The paddy straw (hay) was cut down into small pieces and washed thoroughly. Then the hay with some rice grains were boiled with distilled water. The juice (broth) was filtered with any sterile cloths or sieves and it was taken in conical flasks and again sterilized for 1 h. It was cooled thoroughly and used for the inoculation purpose.

**Sample inoculation:** Five milliliter of the diluted soil sample was inoculated into the 100 mL of the hay infusion broth. Mixed thoroughly and incubated at room temperature for one week. Different kinds of ciliates were counted separately. The ciliates were counted as per above mentioned methods.

**Bacterial counting:** Bacteria present in the soil sample were counted by staining with (SGI) SYBER Green I and subjected to fluorescent microscopic observation (Leica Dm 2500). Counting was done by conventional plating method. The plating of the sample was done in $R_2A$ media.

## RESULTS

**Physico-chemical characteristics of the paddy soil sample:** The physico-chemical characteristics of the soil samples collected from wet and flooded paddy fields have shown (Table 1) that the upper soil layer (segment 1) have higher organic (1.29%), total phosphorus (0.105 mg $g^{-1}$) and total nitrogen (3.3 mg $g^{-1}$) content. Table 1 shows, the weight, pH, salinity and nutrient content of six paddy soil samples.

**Analysis of protozoa:** Microscopic observation of protozoa in wet and flooded paddy field soil samples indicated that ciliates were the dominant group in both samples. Amoebae and flagellates were also present in low numbers.

In paddy soil samples, four different types of ciliates such as *Microthorax* sp., *Vorticella* sp., *Oxytricha* sp. and *Euplotes* sp., were identified (Fig. 1A-D). The size range of different ciliates was shown in Table 2. Among the different ciliates found in the present study, *Vorticella* sp., was sessile (attached to stalk), while *Microthorax* sp., *Oxytricha* sp. and *Euplotes* sp., were motile. Among these species *Oxytricha* sp., was the largest while *Vorticella* sp., was the smallest. *Oxytricha* sp., was a filter feeding ciliate protozoa which was reported from benthic environments (Balczon and Pratt, 1996). *Euplotes* sp., was also a benthic ciliate that feeds on particles. It has been reported from both freshwater as well as marine environments (Carter, 1972; Wen *et al*., 2005).

**Quantitative analysis of ciliates:** Quantitative analysis of ciliates in each segments indicated a variation in their number. Ciliates were more abundant in the upper soil segments. The total numbers of ciliates present in 1 g of the soil segments were shown in Fig. 3.

A comparative analysis of ciliates in wet and flooded paddy field soil sample have shown that more number of ciliates seen in wet samples. The differential counts of the four types of ciliates present in both samples were shown in Table 2. It could be seen that the flooded paddy soil contains less number of the four ciliates. *Vortecella* sp. and *Euplotes* sp., was the dominant ciliates in both samples, where as *Oxytricha* sp., represented a small population.

Table 1: Physicochemical characteristics of soil sample from flooded and wet paddy fields. (The average values of the segments 1, 2, 3, 4, 5 and 6 represent samples from 1 and 2 cm deep, respectively from the surface)

| Segment | | Weight | | pH | | Salinity | | Organic carbon content of flooded | Total phosphorus content of flooded soil | Total nitrogen content of flooded soil |
|---|---|---|---|---|---|---|---|---|---|---|
| No. | Segments | Wet soil | Flooded soil | Wet soil | Flooded soil | Wet soil | Flooded soil | soil (%) | (mg $g^{-1}$) | |
| 1 | 5 samples | 42.2 | 44.9 | 6.45 | 6.43 | 0.0 | 0.0 | 1.29 | 0.105 | 3.34 |
| 2 | 5 samples | 41.6 | 45.3 | 5.79 | 6.30 | 0.0 | 0.0 | 0.84 | 0.091 | 2.52 |
| 3 | 5 samples | 43.5 | 44.6 | 5.76 | 6.26 | 0.0 | 0.0 | 0.80 | 0.073 | 1.49 |
| 4 | 5 samples | 43.2 | 45.1 | 5.57 | 6.25 | 0.0 | 0.0 | 0.66 | 0.062 | 0.41 |
| 5 | 5 samples | 42.1 | 46.2 | 5.53 | 6.14 | 0.0 | 0.0 | 0.64 | 0.056 | 0.36 |
| 6 | Only two samples | 19.6 | 20.3 | 5.51 | 6.00 | 0.0 | 0.0 | 0.57 | 0.032 | 0.35 |

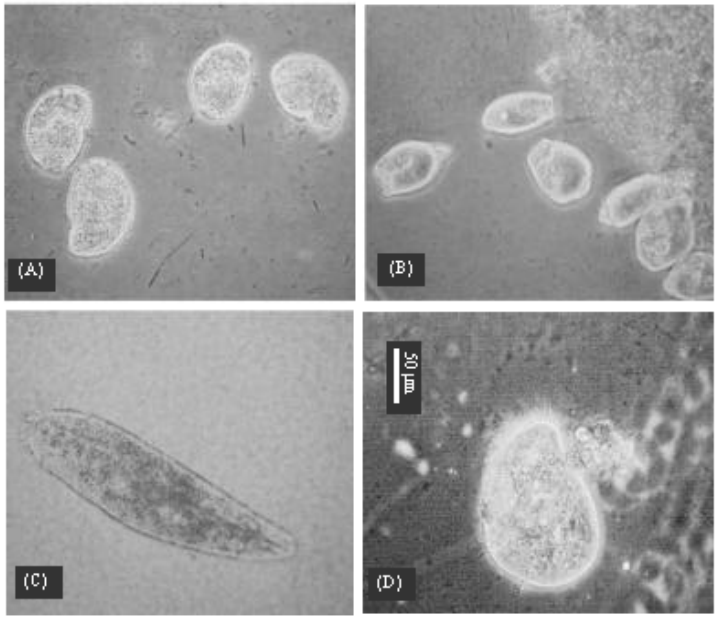


Fig. 1: Four types of protozoa ciliates were observed in paddy soil samples by microscopically with suitable magnification. (A) *Microthorax* sp., (B) *Vorticella* sp., (C) *Oxytricha* sp. and (D) *Euplotes* sp.

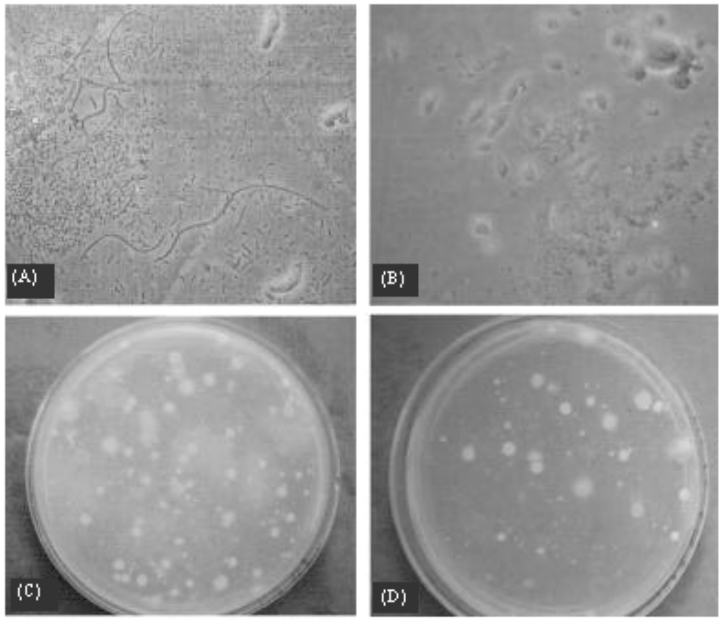


Fig. 2: Protozoa and microbial colonies were observed in hay infusion broth. (A) Amoebae (B) Flagellate (C) Bacterial colonies formed in $R_2A$ agar-upper segment paddy soil and (D) Bacterial colonies formed in R $\frac{2}{A}$ agar-lower segment paddy soil

**Enriched culture of protozoa:** The enriched cultures of protozoa in hay infusion broth was shown the growth of only two ciliates, *Microthorax* sp. and *Vorticella* sp., meanwhile, few flagellates and amoebae were observed in the incubated broth (Fig. 2A, B). The results are summarized in Table 3.

Table 2: The quantitative data and size range of identified ciliates present in wet and flooded paddy soil sample

| | | Segments | | | | | | Length range | Breath range |
|---|---|---|---|---|---|---|---|---|---|
| Ciliate sp. (g) | Type of soil | 1 | 2 | 3 | 4 | 5 | 6 | (μm) | |
| *Microthorax* sp. | Wet soill | 21 | 7 | 6 | 7 | 6 | 4 | 25 to 45 | 5 to 15 |
| | Flooded soil | 14 | 11 | 8 | 7 | 3 | 2 | | |
| *Oxytricha* sp. | Wet soil | 13 | 9 | 5 | 4 | 2 | 0 | 125 to 280 | 35 to 120 |
| | Flooded soil | 7 | 5 | 6 | 5 | 1 | 1 | | |
| *Vorticella* sp. | Wet soil | 27 | 16 | 9 | 8 | 8 | 2 | 35 to 65 | 10 to 30 |
| | Flooded soil | 11 | 7 | 5 | 4 | 4 | 3 | | |
| *Euplotes* sp. | Wet soil | 25 | 23 | 10 | 8 | 6 | 4 | 30 to 100 | 10 to 55 |
| | Flooded soil | 7 | 6 | 8 | 6 | 2 | 2 | | |

Table 3: The protozoa present in soil samples inoculated hay infusion broth

| Segments | Wet soil | Flooded soil |
|---|---|---|
| 1 | Some small flagellates, amoebae and *Microthorax* sp. | *Microthorax* sp. only |
| 2 | *Microthorax* sp. only | *Microthorax* sp. only |
| 3 | *Microthorax* sp. only | Both *Microthorax* sp. and *Vorticella* sp. |
| 4 | Both *Microthorax* sp. and *Vorticella* sp. | Both *Microthorax* sp. and *Vorticella* sp. |
| 5 | Both *Microthorax* sp. and *Vorticella* sp. | *Vorticella* sp. only |
| 6 | Both *Microthorax* sp. and *Vorticella* sp. | *Vorticella* sp. only |

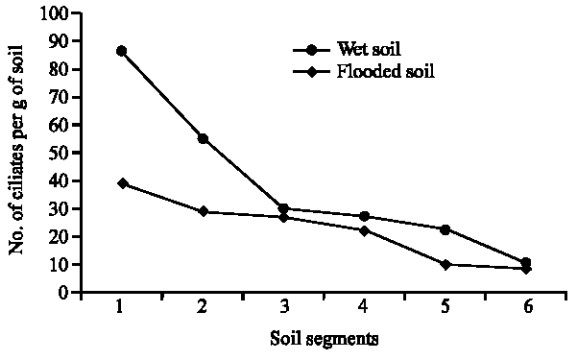


Fig. 3: Ciliates present in each segment of wet and flooded paddy soil samples

**Bacterial count:** The number of bacterial colonies present in lower segments (deep layer) of the soil was less in number when compared with the upper segment of the soil. The image of bacteria from paddy field soil was shown in Fig. 2C and D.

## DISCUSSION

Yoshinaga *et al.* (2003) were reported that total nitrogen and total phosphorus decreases gradually with depth. The pH of wet and flooded soil was in slightly acidic range of 5.5 to 6.4. Furthermore salinity was totally nil in both the samples. Table 1 shows, the weight, pH, salinity and nutrient content of six paddy soil samples. The physico-chemical conditions strongly determine the presence and abundance of organisms including micro-metazoa, protozoa, bacteria and archaea in soil and sediments. These microorganisms on the other hand help in the cycling of elements including carbon, nitrogen and phosphorus. The higher nitrogen content of the paddy soil was characterized by anaerobic condition. Therefore there could be change in the microbial profile during wet to flooded stages. The shift in the population structure of different microorganisms influences the cycling of nutrients (nitrogen and phosphorus) as well as other elements. Flooded rice field was became a model system to study soil microbial ecology.

Foissner (1997) was reported 10 different types of ciliates in anoxic soil. However, Schwarz and Frenzel (2003) were found 23 taxa of anoxic ciliates in paddy soil. In the present study, in paddy soil samples, less number of ciliates such as *Microthorax* sp., *Vorticella* sp., *Oxytricha* sp. and *Euplotes* sp., were identified. It was shown that the abundance and distribution of protozoan cells was not even but greatly conglomerative among soil aggregates. The presence of ciliates in a soil could be varied widely due to the variation in physico-chemical characters of soil.

A comparative analysis of ciliates in wet and flooded paddy field soil sample have shown that more number of ciliates seen in wet samples. Schwarz and Frenzel (2003) were reported a decline in ciliate population size after flooding in a paddy field and also the presence of anaerobic ciliates like *Metopus* sp., *Plagiacampa pentadactyla* in flooded paddy soils. The ciliates in rice fields had to adapt to dual limitations: water and oxygen during the dry and vegetative (cultivation) periods, respectively. Fenchel and Finlay (1995) reported an over all reduced protozoa in flooded paddy soil due to short food chains in anoxic environments.

The more number of protozoa was present in the upper part of the paddy field soil because the presence of higher amount of organic and nutrient content. It appears that individual soils, depending on type, climate and nutrient have been typical carrying capacities for both bacteria and protozoa. The free-living protozoan play an important role in maintaining the ecological balance in

nature as many of them feed on fungi and bacteria. Some protozoan, including *Microthorax* sp., *Vorticella* sp., *Oxytricha* sp. and *Euplotes* sp., helped in the purification of polluted water as they utilize the inorganic nitrates and phosphates for their own metabolism (Sullia and Shantharam, 1998). It has been proved that in addition to grazing fauna (higher tropic organisms), microbial communities also plays significant role in waste water treatment system. The basic scientific information about the abundance and distribution of protozoa in natural environments will complement waste water treatment process development studies. The optimal environmental conditions required for maintaining protozoa in bioreactor for waste water treatment system could be understood from studying them in natural habitat.

## CONCLUSION

Therefore, in conclusion, more number of protozoa was present in the upper part of the paddy field soil because the presence of higher amount of organic and nutrient content and the data generated from this study could be exploited for waste water treatment studies. However, more samples, protozoan populations and communities to investigate protozoan life, competition and predation in paddy fields which will be a model system for the study of soil microbial ecology.

## ACKNOWLEDGMENT

Authors thanks Manonmaniam Sundaranar University, Alwarkurichi for provided necessary facilities.

## REFERENCES

Adams, J.E., 1934. Determination of total carbon in soils by the wet oxidation method. Ind. Eng. Chem. Anal., 6: 277-279.

Balczon, J.M. and J.R. Pratt, 1996. The functional responses of two benthic algivorous ciliated protozoa with differing feeding strategies. Microbial. Ecol., 31: 209-224.

Carter, H.P., 1972. Infraciliature of eleven species of the genus Euplotes. Trans. Am. Soc., 91: 466-492.

Curds, C.R., 1982. The ecology and role of protozoa in aerobic sewage treatment processes. Ann. Rev. Microbiol., 36: 27-28.

Fenchel, T. and B.J. Finlay, 1995. Ecology and evolution in anoxic worlds. Oxford University Press, United Kingdom, ISBN: 0-19854838-9, pp: 276.

Foissner, W., 1997. Protozoa as bioindicators in agro ecosystems with emphasis on farming practices, biocides and biodiversity. Agric. Ecosyst. Environ., 62: 93-103.

Fried, J., G. Mayr and H. Berger, 2000. Monitoring protozoa and metazoa biofilm communities for assessing wastewater quality impact and reactor up-scaling effect. Water Sci. Technol., 41: 309-316.

Ginoris, Y.P., A.L. Amaral, A. Nicolau, M.A.Z. Coelho and E.C. Ferreira, 2007. Development of an image analysis procedure for identifying protozoa and metazoan typical of activated sludge system. Water Res., 41: 2581-2589.

Madoni, P., D. Davoli and M. Chierici, 1993. Comparative analysis of the activated sludge microfauna in several sewage treatment works. Water Res., 27: 1485-1491.

Madoni, P., D. Davoli, G. Gorbi and L. Vescovi, 1996. Toxic effect of heavy metals on the activated sludge protozoam community. Water. Res., 30: 135-141.

Madoni, P., 2000. The allocation of the ciliate Drepanomonas revoluta to its correct functional group in evaluating the sludge biotic index. Eur. J. Protistol., 36: 465-471.

Nicolau, A., N. Dias, M. Mota and N. Lima, 2001. Trends on the use of protozoa in the assessment of wastewater treatment. Res. Microbiol., 152: 621-630.

Nicolau, N., M.J. Martins, M. Mota and N. Lima, 2005. Effect of copper in the protistan community of activated sludge. Chemosphere, 58: 605-614.

Patterson, D.J. and S. Hedley, 1992. Free Living Fresh Water Protozoa: A Color Guide. Aylesbury: Wolfe Publishing Ltd., London, ISBN: 0723409919, pp: 48-95.

Pierzynski, G.M., 2000. Methods of Phosphorus Analysis for Soils, Sediments, Residuals and Waters. Southern Cooperative Series Bulletin, No. 396, SERA-IEG 17, North Carolina State University, USA., ISBN: 1-58161-396-2.

Salvado, H. and M.P. Gracia, 1993. Determination of organic loading rate of activated sludge plants based on protozoan analysis. Water Res., 27: 891-895.

Schwarz, M.V.J. and P. Frenzel, 2003. Population dynamics and ecology of ciliates (Protozoa, ciliophora) in an anoxic rice field soil. Biol Fertil. Soils, 38: 245-252.

Sullia, S.B. and S. Shantharam, 1998. General Microbiology. Oxford and IBM Publishing Co., Pvt., New Delhi, ISBN: 81822053471 pp: 84-85.

Wen, Z., M. Zheng, X. Xu, X. Liu, G. Guo and Z. He, 2005. Biological and ecological features of saline lakes in northern Tibet, China. Hydrobiologia, 541: 189-203.

Yoshinaga, I., Y.W. Feng, T. Hitomi, E. Shiratani and H. Hasebe, 2003. Nitrogen removal functions of paddy field in a circular irrigation system. 7th Int. Conf. Diffuse Pollut. Basin Manage., 4: 43-48.