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Fresh water Micro algal Diversity of Noyyal River at Tamil Nadu State, India.

Mohanapriya K R and Geetharamani D*

Department of Microbiology, Dr.N.G.P Arts and Science college, Coimbatore-641048, India
*Corresponding author email: geethavinoth@gmail.com

Abstract

Microalgae are the most widespread microorganism in freshwater environments and they have numerous functions in environment. Microalgae play a vital role in the nutrient recycling in the environment. Water pollution due to industrialisation has led to the extinction of some species. At the same time due to eutrophication, some microalgal species overgrow and form algal bloom. Microalgal biodiversity studies not only serve as indicator of water quality but may also help to tap the biotechnological potential of these organisms. This paper deals with isolation and identification of microalgae from freshwater samples collected from different regions of Noyyal River. Micro algal diversity was evaluated by light microscopy and culturing techniques. In total 35 green algae (chlorophytes), 10 blue green algae (cyanophytes) and 4 brown algae (bacillariophytes) were isolated and described.

Keywords: Fresh water microalgae, Noyyal River, chlorophytes, cyanophytes, bacillariophytes.

INTRODUCTION

The biodiversity of microalgae is enormous and they represent an almost untapped resource. It has been estimated that about 200,000-800,000 species exist of which about 35,000 species are described (Cheng and Ogden 2011). But only a few hundred of which are cultured in laboratories at present (Richmond 2004). Among the few cultured in laboratories only a handful of them have been characterized and assessed in detail for their economic potential.

. Microalgae, are the major O_2 producer and they have been ascertained as promising and commercially important in the food industry and aquaculture, as a natural source of high-value products such as fatty acids, carotenoids, steroids etc (Cardozo *et al.,* 2007; Olmos Soto *et al.,* 2002).The problems such as soaring of oil prices, diminishing world oil reserves and the environmental deterioration affiliated with fossil fuels consumption have renewed the interest in using microalgae as an alternative sources for fuel production (Saha *et al.,* 2003; Illavarasi *et al*., 2011).

Microalgal research is expanding rapidly worldwide and is attracting some major companies to explore it. A wide number of green algae strains have the potential to grow in mass culture and can accumulate large quantities of lipids under nutrient stress. *Chlorella* sp., exhibits rapid growth can be cultured on a large scale. Cost efficiency, easy cultivation, similar glycosylation patterns with higher plants and biological safety makes microalgae one of the potential candidate for the production of valuable products from them (Wang *et al*., 2005).

The objective of the present study was to isolate freshwater microalgae from Noyyal river starting from Perur, Coimbatore district (Latitude: 10° 58’06″ N and Longitude: 76° 55’41″) to Orathupalayam dam Tirupur district (Latitude: 11°06′31″N and Longitute77°32′23″E) and Athupalayam dam, Karur district. The microalgal diversity of these areas is unexplored. Several methods of isolation and purification methods were adopted and identification was done mainly based on morphological characteristics.

MATERIALS AND METHODS:

Study area:

The **Noyyal River** rises from the Vellingiri hills in the Western Ghats in Tamil Nadu, South-eastern India and drains into the Kaveri River. The river's basin is 180 km long and 25 km wide and covers a total area of 3,500 km^2 (Figure

1).The Noyyal river basin covers a total area of 3510 km2 and is located between north latitude 10056’ and 11019’ and east longitude 76041’ and 77056’.

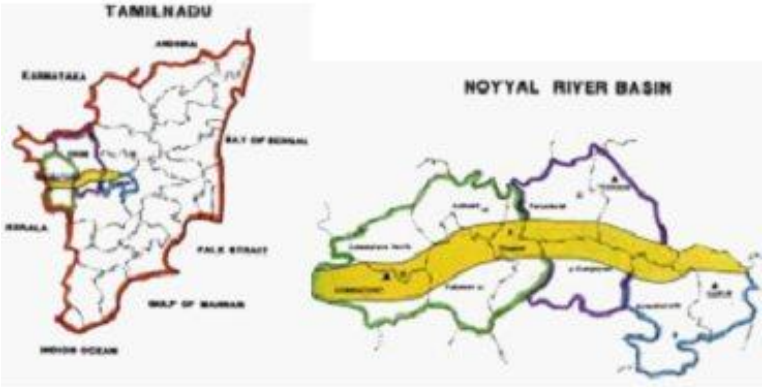

Figure 1. Loaction map of Noyyal River

Sampling site in Noyyal River:

Samples were mainly collected from tributaries of Noyyal River located at different areas in Coimbatore like Perur, Ukkadam, Sulur, and Irugur and also from two dams of Noyyal River (Orathuppalayam and Aathupalayam dam) , Tirupur district. Coimbatore big lake is situated near to Ukkadam bus-stand. This tank is located north of the River Noyyal. Latitude: 10° 59.103’ N and Longitude: 76° 56.959’ E. Perur Big Lake is situated in the Perur Village close to Sundakkamuthur village. Latitude: 10° 58’06″ N and Longitude: 76° 55’41″ E. Sulur lake is located near NH 81 Trichy road. Latitude:11º01’40”N and Longitude 77º07’20”E. Irugur lake is located near Irugur byepass to Ravathur link road. Latitude 11º01’15” and Longitude77º05’00”. Orathupalayam dam is located near Chennimalai, Tirupur district. Latitude: 11°06′31″N and Longitute77°32′23″E. These areas are seasonal in nature, usually containing water for about 5-6 months per year from June to December (Figure 2).

Figure 2. Sampling sites

Collection of samples

Samples were collected from four different areas in different locations in the month of October 2012 to February 2014. Algal samples were collected in a sterile 50 ml plastic bottles.

Isolation of microalgae

The collected samples were initially inoculated into five different 100 ml conical flask containing 50ml of BG 11 medium, BBM medium, Walne's medium, Chu 13 medium and BB medium. After inoculation the flasks were incubated at 24+/-2^{o}C under 37.5 µmol^{-1}m^{-1}sec^{-1}intensity with 16:8h photoperiod for 15 days (Dayananda *et al*., 2010).

Method of isolation and maintenance of microalgae in axenic cultures are based on serial dilution culture technique and agar plate method as described by Gopinathan (1996). The mixed Microalgal cultures were then serially diluted using respective culture medium where growth was observed. After serial dilution the flasks were maintained at the above said condition. The contents of the serially diluted flasks were inoculated into agar plates incorporated with 10mg/l of antibiotic for final purification process. The pure cultures thus obtained were sub-cultured and maintained.

IDENTIFICATION OF MICROALGAE

The isolated microalgae were observed microscopically using light microscope and identified (Desikachary 1959; Philipose 1967 and David *et al*., 2011).

RESULT AND DISCUSSION

Algae are distributed in almost all types of habitat. Algae are chlorophyll bearing thallophytes. The thalli show great variation in organisation. They include motile unicellular forms, motile colonial forms, palmelloid, filamentous, heterotrichous, siphonaceous, uniaxial and multiaxial. The cell constituting the thalli is basically of two kinds prokaryotic and eukaryotic. The cell in all members of algae remain surrounded by a cellulose cell wall which encloses the protoplast (Maniakm *et al*., 2012) simply, the algae have been classified into 11 classes. They are chlorophyceae, xanthophyceae, chrysophyceae, bacillariophyceae, rhodophyceae, cyanophyceae, pheaophyceae, chyrptophyceae, chloromonodinae, euglinophyceae and dinophyceae. In the present study, chlorophycaea (green algae), cyanophyceae (blue green algae) and bacillarophyceae (diatoms) the primary producers forming the base of the autotrophic food chain were identified.

Cyanophyta (Figure3)

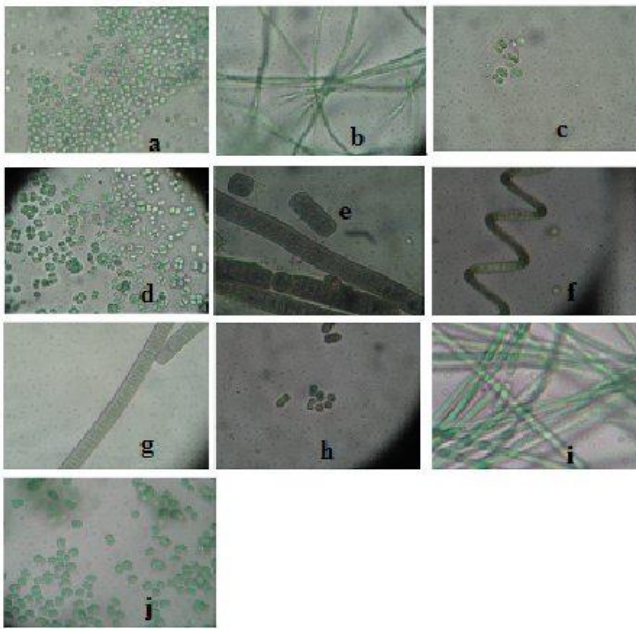

Figure 3. Cyanophyta

Fig 3a Aphanocapsa holsatica

Young cells were more or less spherical, but later became irregular, lobate, were upto 300 µm across with densely arranged cells in colourless, clearly visible, mucilage, colonies were pale greyish blue-green in color.

Fig 3b, 3i Phormidium corium

Colony was spread out, leathery, and blue green in color. Trichomes were thickly interwined. Cross walls were neither narrowed nor adjacent granules were seen, end cells were rounded or shortly conical no calyptras were observed.

Fig3c Chroococcus

Cells were initially found single, spherical, club or pear shaped, later formed groups of 2-4 cells or short rows, which eventually gave rise to a 2- layered colony with a thick, layered sheath.

Fig3d,3j Gloeocapsa alpine

Colonies were formed with 2-8 spherical cells which were frequently aggregated into macroscopic irregular blackish mass. Cells of sub colony lamellate were with the blue mucilage.

Fig3e Lyngbya martensiana

Long, flexuous filaments usually forming blue green tufts were observed. Cells were wide and cross walls were not narrowed, and possessed small granules on either side; The end cells were rounded and calyptras was absent. A thick, colourless, sheath rough at the outer surface was present.

Fig3f Spirulina maior

The trichome was 1-2 µm wide and the helix was 2.5-4µm wide. The coils of helix were regular and 2.7-5µm apart. Trichomes showed motility.

Fig3g Oscillatoria limosa

The isolate formed dark blue-green to brown mats. Trichome was more or less straight, blue-green, brown or olive green in color. Cells were long, one-sixth to one third as long as wide, cross walls were slightly narrowed, with adjacent granules. The end cell was rounded, with slightly thickened membrane.

Fig3h Aphanotheca pallid

Colony was microscopic with no characteristic shape, and cells were denser towards the exterior, especially in larger colonies. The Main mass of mucilage was colourless, but cells near the surface usually possessed a lamellate yellowish brown sheath. Cells were long oval to cylindrical.

Chlorophyta (Figure 4 and 5)

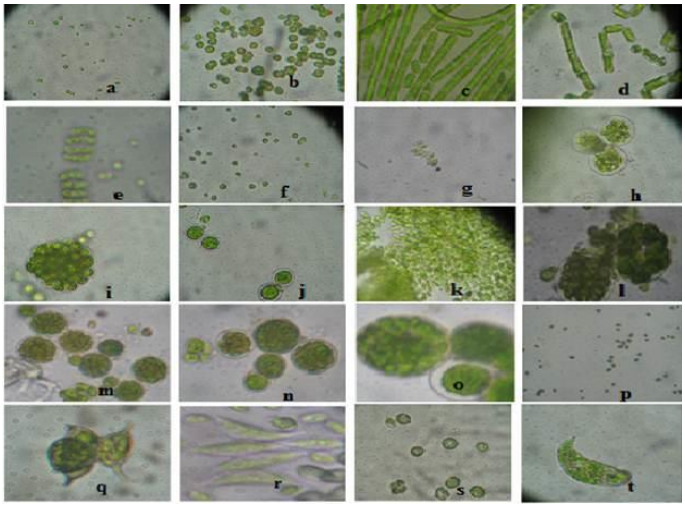

Figure 4. Chlorophyta

Fig 4a Chlorella vulgaris

Cells were wide, spherical or almost spherical; chloroplast broadly band- shaped or cup-shaped. The pyrenoids were spherical to broadly ellipsoidal and were usually surrounded by starch grains. The autospores were spherical, and in each sporangium, 2, 4 or 8(-16) autospores were seen.

Fig 4b Botryococcus braunii

Colonies were green to brown, consisting of irregularly arranged cells protruding or embedded within the periphery of tough, folded, wrinkled mucilage and numerous oil globules were observed. Cells were oval, each with an apical cap and completely embedded in the periphery of the mucilaginous masses.

Fig 4d Klebsormidium rivulare

The filaments are long and cells are cylindrical. Chloroplast covering 70% of cell circumference. H- shaped pieces produced at cross walls between adjacent cells in some filaments.

Fig 4d Klebsormidium dissectum

The Filaments were mostly short, straight or hooked. The filaments broke into single cells or short fragments (4-15 cells). The cells were cylindrical and became barrel-shaped in old filaments with 7-10 µm width. The cells were covered with chloroplast covering 50%-70% of cell circumference. The walls were smooth and thin and thickened in ageing barrel-shaped cells, H-shaped pieces were frequently formed.

Fig 4e Scenesdesmus

Coenobia of 2, 4 or 8 cells in a single linear or alternate row were seen. Cells were long, ovoid, ellipsoid or cylindrical, the outer wall was slightly convex, with apices broadly rounded, without ornamentation.

Fig 4g Desmodesmes

The Coenobia were flat, straight or slightly curved, with cells in one row, usually 2, 4, or sometimes 8-celled, more rarely 16 celled, joined laterally and lying parallel to each other. The cells were elongated or cylindrical, ovoid, ellipsoidal to ovoid, with rounded apices. The Chloroplast was parietal with single pyrenoid, and autospores released by fracture of lateral cell wall was seen.

Fig 4h Botryosphaerella

Colonies were spherical or grape like, consisting of cells embedded along the pheriphery of mucilaginous masses or united by irregular fused mucilaginous strands. The cells were spherical or broadly ovoid, containing starch and oil droplets. Chloroplast was parietal, cup shaped without pyrenoids. Autospores were found to be produced in each cell.

Fig 4i Chlorococcum

Cells were wide, spherical, rarely ovoid. The walls thickened with age. Chloroplast had a hollow sphere with a lateral pore. The pyrenoid was eccentrically positioned, with several small starch grains. Oval cylindrical zoospores were present.

Fig 4j Botrydium

The aerial thallus is coenocytic with pear shaped or spherical branched and with a colorless rhizoidal system. Chloroplast was parietal, numerous and disc shaped.

Fig 4k Dicytochloris

Cells were wide, spherical, sometimes protruding on one side. Chloroplast was net like in older cells with starch. The zoospores were ovoid with an eyespot opposite to flagella and the aplanospore was released by gelatinization of mother cell.

Fig 4l Chlamydomonas

Cells were ellipsoid, wide with a small conical papilla. The chloroplast was cup shaped, slilghtly irregular, with a small lateral pyrenoid. The eye spot was apical.

Fig 4m Chaetopeltis

Circular and mucilaginous, a single or occasionally a multilayered disc of cells radiating from a common center was observed. The cells were round and angular. The chloroplast has a single pyrenoid. Contractile vacuoles were present.

Fig 4n Paulschulzia

Colonies were spherical or broadly ellipsoidal. Larger colonies cells were often in sub groups of 2-6 surrounded by an inner mucilage investment. Cells were spherical in diameter and thin walled. Cup shaped occasionally lobed Chloroplast was seen.

Fig 4o Chlorococcum

Cells were spherical to ovoid. The chloroplast was found with a hollow sphere, sometimes having a lateral spore in which are often 2 contractile vacuoles were present. Pyrenoid was eccentric in position. Zoospores and aplanospores were formed by successive division of content of sporangial mother cell.

Fig 4p Pseudodidymocystis

Coenobia of two broadly oval cells, each cell connected by a inner walls were seen. Apices were broadly rounded. Chloroplast was parietal and each with pyrenoid. Reproduction was by formation of autospores in mother cell.

Fig 4q Tetradesmus

Coenobia atre 2-4 celled, the cells were in contact by long axis, each cell was spherical in dorsal or ventral view forming a square and without the mucilaginous envelope. Cells were spindle shaped or cylindrical. Chloroplast was parietal and with a single pyrenoid. Asexual reproduction was by formation of 2-4 autospores which are released by fracture of mother cell.

Fig 4r Acutodesmes

Coenobia of 2,4 or 8 were linearly arranged in 1 or 2 rows. Cells were long and broadly spindle shaped, tapering to slightly extended apices, The inner cells were straight, and the marginal cells were slightly curved but only in sub apical part.

Fig 4s Oocystis

Cells were single or contained 2, 4, 8 densely packed and tetrahedrally arranged cells. Cells were broadly ovoid or ellipsoid. The apices were broadly rounded and usually without polar wall thickenings. 1-4 Chloroplast were seen in each cell with a pyrenoid.

Fig 4t Euglena

Cells were long widely spindle shaped or cylindrical to spindle shaped. The anterior end was slightly narrowed and round at apex and the posterior end was tapering to a long tail piece. The pellicle was spirally striated. Numerous disc shaped chloroplasts were seen located towards the posterior end, without pyrenoids. Euglenoid movement was seen.

Fig 5a Cruciginella

Coenobia were rectangular or rhomboidal, 4-celled to many hundreds of cells and were irregularly arranged. The cells were oval and convex on one side than the other. The wall was slightly thickened at the apical end.

Fig 5b Botryococcus braunii

The colonies were same as fig 2b but isolated from different water sample**.** Colonies were green to brown, consisting of irregularly arranged cells protruding or embedded within the periphery of tough, folded, wrinkled mucilage containing numerous oil globules. Cells were oval, each with an apical cap and completely embedded in the periphery of the mucilaginous masses.

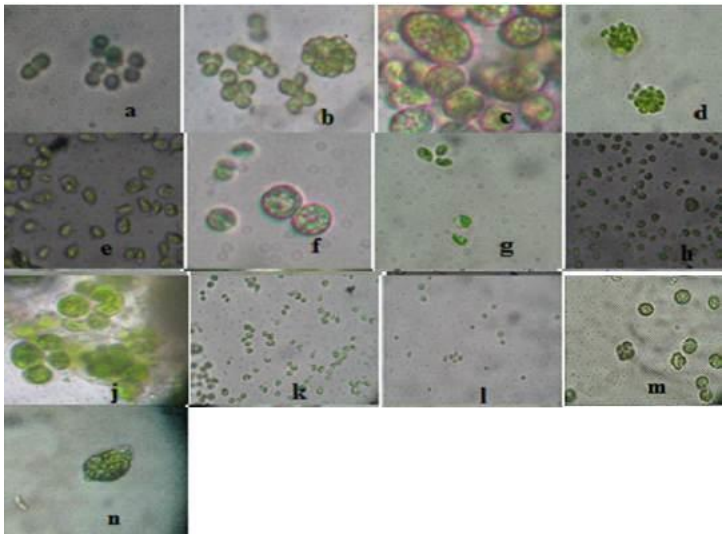

Figure 5. Chlorophyta

Fig 5c Characiochloris

Cells were wide and elongated. The apex was bluntly rounded and showed a nipple shaped terminal wall thickening.

Fig 5d Chlamydomonas actinochloris

Cells were ellipsoid, wide with a small conical papilla. The chloroplast was cup shaped, even or slightly irregular, with a small lateral pyrenoid and an apical eye spot.

Fig 5e Coenocystis

Microscopic colonies consisting of 4 or 8 celled groups within an ellipsoid to almost spherical or irregular shaped mucilaginous envelope were seen. Cells were ovoid with rounded apices. Large colonies were fragmented into smaller one.

Fig 5f Coelastropsis

Cells were spherical or broadly ovoid and consisted of 2-4,8 or 16 spherical or broadly ellipsoidal cells. Adjacent cells were connected by very short proturbans.

Fig 5g Oocystis

Cells were ovoid to spindle shaped, smooth walled and the acute apices sometimes showed a nipple-like thickening of wall.

Fig 5j Chlorococcum

Cells were same as fig 2o but isolated from different sample**.** Cells were wide , spherical, rarely ovoid, walls thickened with age. The chloroplast had a hollow sphere with a lateral pore and eccentrically positioned pyrenoid, usually associated with several small starch grains was also seen. Cylindrical or oval cylindrical zoospores with papilla possessing contractile vacuoles and an eye spot were also observed.

Fig 5 h, k, l Chlorella

The isolates 3h, 3k and 3l were identified to be *Chlorella*, but isolated from different water samples. Cells were wide, spherical or almost spherical; chloroplast were broadly band- shaped or cup-shaped, pyrenoid was spherical to broadly ellipsoid and usually surrounded by 2-4 starch grains. 2, 4 or 8(-16) spherical autospores were found in each sporangium and were released by breaking of wall into 2-4 parts.

Fig 5m Desmococcus

Cells were single spherical or angular in shape and are found in indefinite clusters and often formed 2,3, or 4 celled cuboidal packets.

Fig 5n Kentrosphaera gibberosa

Cells are spherical, ellipsoid and irregularly shaped. Walls are thick and stratified. Chloroplast are present with single pyrenoid.

Diatoms (Figure 6)

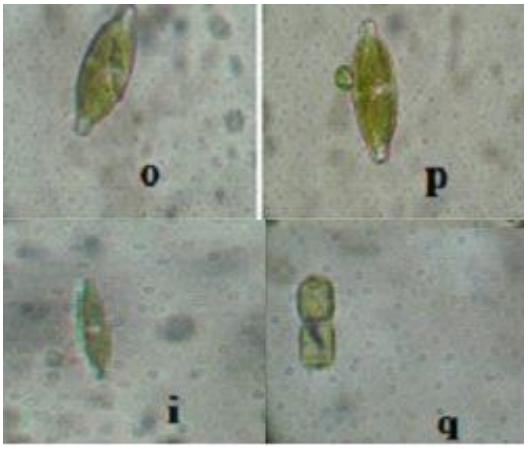

Figure 6 Diatoms

Fig 6i, o, p Navicula

6 i, o and p were isolated from three different water samples**.** The Valves were linear elliptical with convex margins and blunt apices. Cells were irregular and the axial area was narrow near the apices. The central area was lanceolate, with a thickened and distinct central nodule (Otu et al., 2011).

Fig 6 q Achnanthes coarctata

Heterovalvar frustules were seen. The central area was distinct with transverse asymmetric fascia. The proximal raphe ends were straight and slightly expanded.

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