International Journal of Agriculture Sciences
ISSN: 0975-3710 & E-ISSN: 0975-9107, Volume 12, Issue 12, 2020, pp.-9957-9961.
Available online at https://www.bioinfopublication.org/jouarchive.php?opt=&jouid=BPJ0000217

Research Article

PERIPHYTON COMMUNITIES IN LOW SALINE GROUNDWATER BASED CEMENT AND EARTHEN CULTURE SYSTEMS OF *PENAEUS VANNAMEI*

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Received: June 01, 2020; Revised: June 18, 2020; Accepted: June 19, 2020; Published: June 30, 2020

Abstract: Experimental study was conducted to assess on the impact of bamboo substrate in *Penaeus vannamei* culture in cement and earthen system upon the taxonomic diversity of periphyton in low saline ground water (5 gL^{-1}) salinity. This study was conducted for a period of 90 days in 15 tonnes outdoor circular cement tanks and 3000m^2 earthen ponds using Split bamboo (4 cm width) as substrate. The taxonomic community reported from the cement tanks of this study was from 9 genera representing 4 groups of Bacillariophyceae (4), Chlorophyceae (2), Cyanophyceae (2) and Euglenophyceae (1). In earthen system the taxonomic community of periphyton was belonging to 10 genera of phytoperiphyton from 4 groups of Bacillariophyceae (4), Chlorophyceae (3), Cyanophyceae (2) and Dinoflagellate (1). Zooperiphyton was represented from 1 group with 1 genus in the earthen system. The overall taxonomic community recorded in inland low saline water were comparatively lower than those reported from fresh and marine waters reported in other studies.

Keywords: *Periphyton, Substrate, Vannamei shrimp*

Citation: K.S.Vijay Amirtharaj, *et al*., (2020) Periphyton Communities in Low Saline Groundwater based Cement and Earthen Culture Systems of *Penaeus vannamei*. International Journal of Agriculture Sciences, ISSN: 0975-3710 & E-ISSN: 0975-9107, Volume 12, Issue 12, pp.- 9957-9961.

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Academic Editor / Reviewer: C. K. Saxena, S. M. Zofair, Dr.C.Anand, Anindita Paul

Introduction

Substrate based aquaculture is gaining importance in recent years due to its eco-friendly approach by serving as natural food source and through maintaining good water quality in the aquatic system. The growth and development of complex living organisms over the substrate is commonly referred to as Periphyton. The active complex of periphyton comprise of autotrophic and heterotrophic microorganisms, mucopolysachraides and dead particles [1]. In an aquatic ecosystem periphyton is responsible for nutrient cycling and biological productivity [2]. Growth of periphyton on a substrate in an aquatic system, besides improving the water quality by nutrient utilization it also enhances the productivity of the pond and serves as source of natural food to the aquatic system [3-6]. Periphyton community is found to exhibit some important relationship with the aquatic ecosystem, like the composition of periphytic community in the surface of the aquatic system is found to be colonized by cynaobacteria, heterotrophic bacteria, virus, protists and fungi. whereas the bottom of the aquatic system is dominated by heterotrophic bacteria. The growth, productivity and metabolism of periphyton community is determined by the internal recycling of nutrients. Therefore, the periphytic community is found to be more productive due to the conservation, retention and utilization of externally captured nutrients [7]. In the modern aquaculture systems around 50-60% of the production cost is incurred towards supplementary feeding. Substrates in an aquaculture system supports growth of periphyton which provides addition food for the culture organisms [8] thus reducing the need for supplementary feed and lowering the feed cost.

Studies on production performance of culture systems with substrates has proved with better production compared to the culture systems without substrates [9]. The community of algae are very diverse and cosmopolitan in nature, there are approximately around 26,000 algal species defined under 24 classes [10]. Among this based on the nature of its attachment to substrate throughout or partially during its life cycle they are regarded as periphyton. In freshwater system different authors have studied on the composition of algae in periphyton from different substrates. Gangadhar and Keshavanth [11] studied on the periphyton growth potential in different degradable and non-degradable substrates in cement tanks manured with poultry waste has reported 26 genera of planktons from substrates and 24 genera from water. Studies conducted on swine waste water with bamboo substrate by Tippayadara *et al* [12] has recorded phytoplankton belonging to 8 genera and zooplankton from 5 genera. The assemblage of periphyton community was assessed by Pandey *et al* [13] on different natural and synthetic substrates and found phytoplankton to be dominated only by 3 major groups of Bacillariophyceae, Chlorophyceae and Cyanophyceae. Planktonic communities from four different substrates like, paddy straw, sugarcane bagasse, plastic sheet and tile in 500 Litre fibre reinforced plastic (FRP) tanks were studied by Bharti *et al*. [14]. The phytoplankton communities recorded were from group Bacillariophyceae (7 genera), Chlorophyceae (10 genera), Cyanophyceae (2 genera) and Euglenophyceae (1 genus). Off all groups of phytoplankton Chlorophyceae dominated the group.

Mohapatra *et al* [15] conducted an laboratory experiment to observe the growth and diversity of periphytic algae in four types of plastic sheets, such as polyethylene (PE), polypropylene (PP), fiber reinforced plastic (FRP) and acrylic placed inside the glass aquaria filled with fertilized freshwater and observed significant difference in the quantity of periphyton in FRP compared to other plastic sheets. Tortolero *et al*., [16] studied on the development of periphyton in natural (macrophyte, Pistia stratiotes) and artificial substrates. From natural substrates 28 genera of microalgae were documented from Chlorophyta, Heterokontophyta, Cyanophyta and Euglenophyta. Whereas 20 genera of algae were observed from artificial substrate belonging to Chlorophyta, Cyanophyta, Euglenophyta and Heterokontophyta. The study conducted by Simson *et al*., [17] has recorded and identified a total of 39 genera of periphytic community. The experimental study conducted by Helena Khatoon *et al* [18] in marine shrimp ponds on periphyton growth using five different substrates has revealed that all the substrates in the marine shrimp ponds were colonized by 19 different periphytic algae, mainly diatoms (8 genera), green algae (7 genera) and blue-green algae (4 genera) during the first week of the study period.
The present study was conducted with an aim to assess the biodiversity of periphyton in low saline groundwater-based cement and earthen *Penaeus vannamei* culture systems.

Materials and Methods

Cement tank system

The experimental study on cement tank system was conducted for a period of 90 days using split bamboo substrates at the Department of Aquaculture, Fisheries College and Research Institute, Thoothukudi, Tamil Nadu, India. This experiment was conducted in 13-ton outdoor circular cement tanks in duplicate using $5gL^{-1}$ ground saline water. The split bamboos substrates of 4 cm width were tied into strings using two ropes on top and bottom of the substrate material and provided with weight in the bottom to keep the substrate immersed in the cement tank.

Earthen pond system

This experimental study was conducted for a period of 90 days using split bamboo substrates at Palani Kumar Aqua Farm, Devakottai, Tamil Nadu, India. This experiment was conducted in 3000 m2 pond system using $5gL^{-1}$ ground saline water. The split bamboos substrates of 4 cm width were tied into 20 m strings using 20 split bamboos. Two ropes were used on top and bottom for fabrication of the substrate.

Penaeus vannamei post larvae stocking and sampling

P.vannamei seeds were purchased from CAA approved commercial hatchery (Dolphin Hatchery) located in Melamundhal, Ramnad District, Tamil Nadu, India. The shrimp seeds were purchased from hatchery after reducing the salinity and acclimatizing the shrimp seeds to 5 gL^{-1} salinity for direct stocking into the experimental cement tanks and earthen pond system. *P.vannamei* post larvae (PL 12) of average weight 0.015 g were stocked in treatment and control tanks at a density of 60 m^{-2}.

Periphyton and water quality parameters sampling and analysis

Periphyton sampling was done every 10 days once and water quality parameters were analysed on daily (pH, Dissolved Oxygen and Temperature) and weekly basis (Salinity, Alkalinity, Hardness, Ammonia-Nitrogen, Nitrate-Nitrogen, Nitrite-Nitrogen, Calcium and Magnesium) both in cement and earthen pond systems
Periphyton samples were collected from split bamboo substrates collected from cement and earthen system were carefully scrapped using a scalpel blade from 2 x 2 cm2 surface area. The bamboo substrate used for sample collection from both the system were marked and placed inside the systems to avoid repetition of substrate during next sampling. The periphyton samples collected were re-suspended in 50 ml distilled water and stored in plastic vials with addition of 5% formalin for later analysis of the samples. Stereoscopic binocular microscope was used for periphyton counting and identification was done according to Azim *et al* [19] by taking 1 ml of preserved periphyton sample into Sedge Wick Rafter counting cell and the periphyton cell counting is calculated as per the formula.

Number of periphyton cells/Sq.cm =(P x C / S) / x 100
Where, P is the number of periphyton counted in 10 units, C volume of final concentrate of the sample (ml) and S is the area of the scrapped surface [20].
Genus level identification of periphyton was done according to the keys by Jayashree *et al* [21].

Statistical Analysis

The substrate wise plankton taxonomic classification was analysed using Kruskal Wallies non parametric one-way ANOVA and day wise plankton taxa were analysed using one-way ANOVA using SPSS 25.0 statistical analysis software.

Results

Taxonomic composition of periphyton in cement tank system

Periphyton community recorded on the treatment tanks with split bamboo poles as substrate comprised of 4 groups representing Bacillariophyceae, Chlorophyceae, Cyanophyceae and Euglenophyceae. Bacillariophyceae was represented by 4 genera, 2 genera each was represented by Chlorophyceae and Cyanophyceae and 1 genus from Euglenophyceae. Among the group Bacillariophyceae, Achnanthes genera was observed with a mean value of 5050 ± 1586 cells/ml and with a minimum and maximum values of 2415 cells/ml and 7365 cells/ml. In case of Cyclotella, Nitzschia and Navicula the mean value, minimum and maximum values observed were, 1762 ± 582 cells/ml, 856 cells/ml and 2559 cells/ml, 4949 ± 1504 cells/ml, 2361 cells/ml and 7251 cells/ml and 1600 ± 582 cells/ml, 952 cells/ml and 2364 cells/ml respectively. The mean values recorded for 2 genera Chlorophyceae, Ankistrodesmus and Scenedesmus were, 6291 ± 2270 cells/ml and 2712 ± 1068 cells/ml respectively and the respective minimum and maximum values observed for these two genera were 3565 cells/ml and 10564 cells/ml and 1152 cells/ml and 4625 cells/ml. From Cyanophyceae among the two genera Anabena was found with a mean value of 1754 ± 582 cells/ml and with minimum and maximum values of 848 cells/ml and 2548 cells/ml, whereas Microcystis was observed with a mean value of 2759 ± 1000 cells/ml, with minimum and maximum values of 995 cells/ml and 3994 cells/ml. The mean value, minimum and maximum values recorded respectively for the one genus represented (Euglena) from the group Euglenophyceae was 7919 ± 2685 cells/ml, 4521 cells/ml and 12984 cells/ml [Table-1].

Taxonomic composition of periphyton in earthen pond system

The periphyton community recorded from the earthen pond collected spit bamboo poles as substrate comprised of 4 groups representing Bacillariophyceae, Chlorophyceae, Dinoflagellates, Cyanophyceae and Rotifers. Bacillariophyceae was represented by 4 genera, 3 genera have been represented from Chlorophyceae, Dinoflagellate was represented by 1 genus, Cyanophyceae was represented by 2 genera and zooplankton was represented by rotifers. Among the group Bacillariophyceae, Achnanthes genera was observed with a mean value of 4007 ± 1472 cells/ml and with a minimum and maximum values of 2129 cells/ml and 6178 cells/ml. The mean value, minimum and maximum values recorded for Cyclotella, Nitzschia and Navicula was, 4131 ± 922 cells/ml, 2875 cells/ml and 5531 cells/ml, 7526 ± 2451 cells/ml, 4325 cells/ml and 11246 cells/ml and 5530 ± 1009 cells/ml, 4145 cells/ml and 6943 cells/ml respectively. The mean values recorded for 3 genera of Chlorophyceae, Ankistrodesmus, Scenedesmus and Chlorella were, 4612 ± 993 cells/ml, 4535 ± 576 cells/ml and 4090 ± 1048 cells/ml respectively and the respective minimum and maximum values observed for these three genera were 3310 cells/ml and 6127 cells/ml, 3684 cells/ml and 5364 cells/ml and 2891 cells/ml and 5791 cells/ml. Procentrum genus from Dinoflagellate has recorded a mean value of 4668 ± 1062 cells/ml, with a minimum and maximum values of 3245 cells/ml and 6132 cells/ml. In Cyanophyceae among the two genera documented Anabena was found with a mean value of 7185 ± 982 cells/ml and with a minimum and maximum values of 5245 cells/ml and 8426 cells/ml, whereas Oscillatoria was observed with a mean value of 5459 ± 641 cells/ml, with minimum and maximum values of 4581 cells/ml and 6354 cells/ml. The mean value, minimum and maximum values recorded respectively for rotifer under zooplankton was 3289 ± 1164 cells/ml, 2154 and 4651 cells/ml [Table-2].

Table-1 *Taxonomic composition of periphyton in split bamboo substrate collected from cement system*

Periphyton	Sampling Days								
Days	10	20	30	40	50	60	70	80	90
Bacillariophyceae (Cells/ml)									
Achnanthes	2455	3301	3916	4577	5224	5628	6179	6879	7300
Cyclotella	875	969	1308	1692	1919	1987	2177	2430	2508
Nitzschia	2378	3339	4168	4370	5042	5425	6076	6638	7108
Navicula	958	989	1304	1487	1671	1820	1902	1973	2307
Chlorophyceae (Cells/ml)									
Ankistrodesmus	3595	3915	4322	5027	6202	6704	7698	8729	10433
Scenedesmus	1198	1605	1811	2211	2692	3183	3399	3826	4488
Cyanophyceae (Cells/ml)									
Anabena	861	963	1302	1680	1912	1987	2166	2422	2496
Microcystis	1004	1548	2161	2664	2845	3195	3609	3846	3970
Euglenophyceae (Cells/ml)									
Euglena	4578	5286	5701	6087	7754	8677	9685	10900	12611

Table-2 *Taxonomic composition of periphyton from split bamboo substrates collected from earthen system*

Periphyton	Sampling Days								
Days	10	20	30	40	50	60	70	80	90
Bacillariophyceae (Cells/ml)									
Achnanthes	2,129	2,325	2,698	3,478	3,852	4,624	5,126	5,627	6,178
Cyclotella	2,875	3,162	3,352	3,725	4,125	4,512	4,759	5,142	5,531
Nitzschia	4,325	4,981	5,426	6524	7256	8214	9412	10351	11246
Navicula	4,145	4,351	4,625	5243	5687	5864	6231	6681	6943
Chlorophyceae (Cells/ml)									
Ankistrodesmus	3,310	3,587	3,895	4125	4451	4897	5249	5873	6127
Scenedesmus	3,684	3,857	4,121	4374	4562	4754	4975	5127	5364
Chlorella	2,891	3,058	3,251	3456	3875	4253	4891	5346	5791
Dinoflagellate (Cells/ml)									
Procentrum	3,245	3,568	3,794	4112	4658	4957	5624	5927	6132
Cyanophyceae (Cells/ml)									
Anabena	5,245	6,451	6,684	6972	7253	7628	7851	8157	8426
Oscillatoria	4,581	4,752	4,931	5148	5462	5781	5978	6147	6354
Zooplankton (Cells/ml)									
Rotifers	2,154	2358	2518	2874	3241	3657	3891	4258	4651

Table-3 *ANOVA comparison of genus obtained from cement and earthen tanks*

Source of Variation	SS	df	MS	F	P-value	F crit
Achnanthes^{C} *Achnanthes*^{E}	125212837	2	62606418	38.826532	3.00E-08	3.40282
Cyclotella^{C} *Cyclotella*^{E}	25248802	1	25248802	41.707383	7.90E-06	4.49399
Nitzschia^{C} *Nitzschia*^{E}	29884181	1	29884181	7.1125821	0.01687	4.49399
Navicula^{C} *Navicula*^{E}	69468649	1	69468649	113.09578	1.16E-08	4.49399
Ankistrodesmus^{C} *Ankistrodesmus*^{E}	12679809	1	12679809	3.9359352	0.064694	4.49399
Scenedesmus^{C} *Scenedesmus*^{E}	14954069	1	14954069	19.400304	0.000443	4.49399
Anabena^{C} *Anabena*^{E}	132741787	1	132741787	200.26555	1.82E-10	4.49399

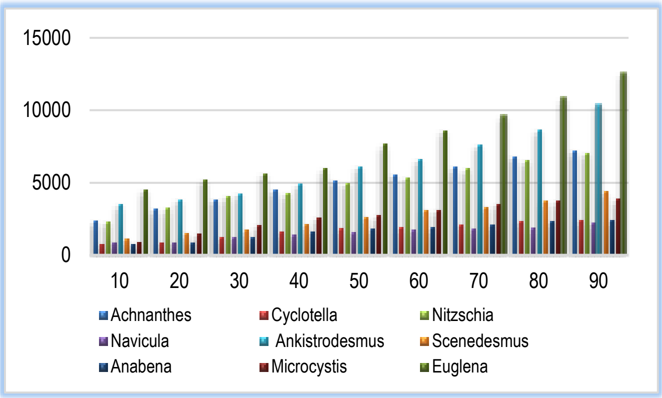

Fig-1 Day wise distribution of periphyton in cement culture systems

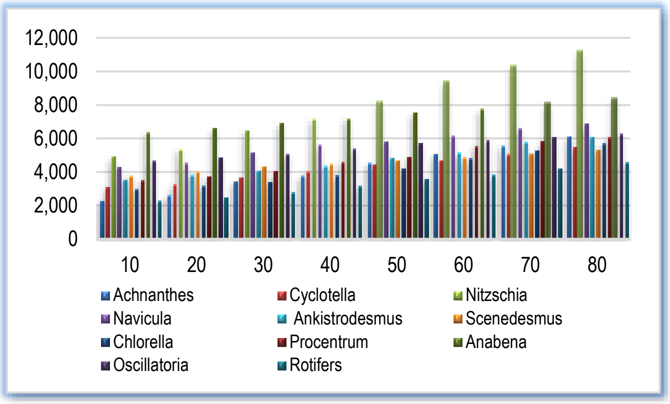

Fig-2 Day wise distribution of periphyton in earthen culture systems

Comparison of periphyton composition between cement and earthen system

The diversity of periphyton documented from the bamboo substrates collected from cement and earthen vannamei culture systems were observed with genera in earthen system. The earthen system has documented with 4 genera from Bacillariophyceae, 3 genera from Chlorophyceae, 1 genus from Dinoflagellate, 2 genera from Cyanophyceae and 1 genus from zooplankton. Earthen system periphyton has been represented with 10 genera of phytoperiphyton and 1 genus from zooplankton. Whereas in cement system the periphyton diversity was recorded with 10 genera of phytoperiphyton and zooperiphyton was not documented. In cement system Euglena dominated the periphyton population but it was not observed in the split bamboo substrate from earthen system. ANOVA statistical comparison made between genus obtained from vannamei culture cement and earthen system has shown significant difference in values (P<0.05) with higher values documented in earthen system [Table-3].

C-Cement tank, E-Earthen pond

The periphytic community from the cement tanks were observed to increase with time and there was significant difference observed (P<0.05) in the periphyton growth with increase in time [Fig-1]. The growth of Euglena and Microcystis were observed as distinct genus compared to that in the earthen system and occurrence of zooperiphyton was not recorded in cement system.

In earthen pond vannamei culture system the periphytic community from the split bamboo substrate were observed to increase with time and there was significant difference observed (P<0.05) in the growth of periphyton with increase in time [Fig-2]. Compared to cement system the diversity of periphytic group in earthen system were high and zooperiphyton (Rotifer) has also been documented in earthen system.

Discussion

The growth of periphytic biomass on a substrate dependent highly on different parameters like availability of nutrients, type of substrate, temperature and photoperiod. Summer with higher temperatures and longer days with greater incidence and intensity of light that influenced in periphytic biomass [22]. Periphyton growth has shown short range of variation throughout the experiments conducted by Rafel *et al* [23] due to low nutrient attribution and availability in the water column, with specific reference to phosphorus. In freshwater ecosystems phosphorous is the key component which regulates the growth of the autotrophic biomass [24], as it was reported by McCormick *et al* [25] that phosphorus to be the prime factor affecting structure and function of wetland periphyton. The sub optimal levels of N:P ratio and low nutrient content is responsible for the lower yield of micro algae in saline ground water [26]. Phosphate is highly essential for the cell growth of algae, the precipitation of phosphate to calcium phosphate may be a factor influencing slow growth of algae [27, [28]. The results from the present investigation on the growth of periphyton in low saline ground water has reported less diversity in both cement and earthen systems are in accordance with the impact of low nutrient condition interference in the growth and proliferation of periphyton.

Experimental study on growth of periphyton using 4 different plastic sheets by Bikas *et al* [29] has concluded with 38 different types of periphytic algae belonging to Chlorophyceae (4), Cyanophyceae (11), Bacillariophyceae (15) and Desmidiaceae (8) which is to a acceptance level with the present investigation in terms of groups recorded but not in line with the number of genera, maximum number of periphytic algae documented in the present study was 11 genera in earthen system and 9 genera in cement system. Studies done across the South Nation River watershed, Canada by Rebecca *et al* [30] is in accordance with the present study which has reported with periphyton community representing with 60.9% Bacillariophyceae, 28.1% Chlorophyceae, 6.9% Cryptophyceae and 4.1% Euglenophyceae. Kalpajit *et al*. [31] has found 28 genera of periphyton from group Bacillariophyceae, Chlorophyceae, Cyanophyceae and Euglenophyceae, higher periphytic community was recorded in bamboo substrate is in acceptance with results reported from the cement system. Keshavanath *et al* [32] has reported zooperiphyton like chironomids, ostracods, nematomorphs, oligochaetes, rotifers and calanoids in bamboo substrates. Among these zooperiphyton chironomids dominated the population. During this trial Hydra, Ephemeroptera and Trichoptera were also documented. Zooperiphyton population observed in bamboo substrate was 2 and 3 folds higher than that documented in the ambay and leucaena substrates respectively. Phytoperiphyton population recorded from the substrates belonged mainly to the families of Bacillariophyceae, Chlorophyceae, Cyanophyceae and Chrysophyceae. The periphytic community represented from the earthen system of this present investigation is in acceptance with the above report.

Conclusion

The present experimental study was conducted to assess on the growth and diversity of periphyton using bamboo substrates in low saline water in two different systems of cement tanks and earthen ponds. This is a primary attempt to report on the impact of natural soil pond bottom and cement bottom condition on growth and diversity of periphyton in low saline ground water condition. The results from the present investigation has evidently proved that the taxonomic diversity of periphyton community collected from the substrates from cement tank system was less in terms of group and in number compared to that documented from the earthen system. This study also reports lower periphyton diversity in both cement and earthen system compared to those reported by other others in fresh and marine water and this may due to the impact of variation in the nutrient composition in low saline water. Further repeated studies are required in low saline condition with addition of supplementary minerals at different composition levels for further validation on the composition of the periphytic taxonomic periphytic community and its diversity.

Application of Research

This present study results reported on the periphyton community based from cement and earthen system of vannamei culture condition will ensure entrepreneurs to take up vannamei farming in low saline ground water with periphyton as additional food source. Enhanced production with low production cost and effective utilization of unsuitable (for Agriculture) low saline ground water for aquaculture is the ultimate objective.

Research category: Aquaculture production

Acknowledgement / Funding: Authors are thankful to Department of Aquaculture, Fisheries College and Research Institute, Thoothukudi, 628008, Tamil Nadu Dr.J.Jayalalithaa Fisheries University, Nagapattinam, 611002, Tamil Nadu, India

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Research project name or number: PhD Thesis

Author Contributions: All authors equally contributed

Author statement: All authors read, reviewed, agreed and approved the final manuscript. Note-All authors agreed that- Written informed consent was obtained from all participants prior to publish / enrolment

Study area / Sample Collection: Department of Aquaculture, Fisheries College and Research Institute, Thoothukudi, 628008, Tamil Nadu, India

Species Name: *Penaeus vannamei*

Conflict of Interest: None declared

Ethical approval: Ethical approval taken from Fisheries College and Research Institute, Thoothukudi, 628008, Tamil Nadu Dr.J.Jayalalithaa Fisheries University, Nagapattinam, 611002, Tamil Nadu, India

Ethical Committee Approval Number: Nil

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