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RESEARCH ARTICLE

Seismic Analysis of RC Frame Structure with and without Masonry Infill Walls.

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ABSTRACT

Masonry infills are normally considered as non-structural elements and their stiffness contributions are generally ignored in practice, such an approach can lead to an unsafe design. The masonry infill walls though constructed as secondary elements behaves as a constituent part of the structural system and determine the overall behaviour of the structure especially when it is subjected to seismic loads. In this paper seismic analysis has been performed using Equivalent Lateral Force Method for different reinforced concrete (RC) frame building models that include bare frame, infilled frame and open first storey frame. The results of bare frame, infilled frame and open first storey frame are discussed and conclusions are made. In modelling the masonry infill panels the Equivalent diagonal Strut method is used and the software ETABS is used for the analysis of all the frame models.

Key words: Masonry infill, RC frame, Equivalent Diagonal Strut, Equivalent Lateral Force Method and Environmental Impacts

INTRODUCTION

Reinforced concrete (RC) frame buildings with masonry infill walls have been widely constructed for commercial, industrial and multi storey residential uses in seismic regions. Masonry infill typically consists of bricks or concrete blocks constructed between beams and columns of a reinforced concrete frame. The masonry infill panels are generally not considered in the design process and treated as architectural (non-structural) components. Nevertheless, the presence of masonry infill walls has a significant impact on the seismic response of a reinforced concrete frame building, increasing structural strength and stiffness (relative to a bare frame) [1]. Properly designed infills can increase the overall strength, lateral resistance and energy dissipation of the structure. An infill wall reduces the lateral deflections and bending moments in the frame, thereby decreasing the probability of collapse. Hence, accounting for the infills in the analysis and design leads to slender frame members, reducing the overall cost of the structural system. The total base shear experienced by a building during an earthquake is dependent on its

time period. The seismic force distribution is dependent on the stiffness and mass of the building along the height. The structural contribution of infill wall results into stiffer structure thereby reducing the storey drifts (lateral displacement at floor level). This improved performance makes the structural design more realistic to consider infill walls as a structural element in the earthquake resistant design of structures.

V.K.R.Kodur, M.A.Erki and J.H.P.Quenneville [2] considered a three storey RC frame building models for the analysis. These RC frames were analyzed for three cases i) Bare frame ii) Infilled frame iii) Infilled frame with openings. Based on the analysis results they found that Base shear of infilled frame is more than infilled frame with openings and bare frame. Time period of infilled frame is less as compare to infilled frame with openings and bare frame. The natural frequency of infilled frame is more as compare to infilled frame with openings and bare frame. Mehmet Metin Kose [3] studied the different RC frame models that were bare frame, frame without open first storey and frame with open first storey. Based on the results obtained from different computer models, it was found that the number of floors (height of building) was the primary parameter affecting the fundamental period of building. The fundamental period of frame without open first storey is less than frame with open first storey and bare frame.

Jaswant N.Arlekar, Sudhir K.Jain and C.V.R.Murty [4] analyzed the different building models that include building with masonry infill walls in all the storey and building with no walls in the first storey and bare frame building model. Static and dynamic analysis of building models were performed using software ETABS. It was seen that the natural period of the building by ETABS analysis do not tally with the natural period obtained from the empirical expression of the code IS 1893-1984. The natural period of infilled frame is less as compare to soft first storey frame and bare frame building models. Also from the analysis they concluded that RC frame building with soft storey perform poorly during strong earthquake shaking. The drift and the strength demands in the first storey column are very large for building with soft first storey.P.M. Pardhan, P.L. Pardhan, and R.K. Maske [5] highlighted the need of knowledge on partial infilled frames and the composite action and also summarize the findings till date done by various researches on the behaviour of partial infilled frames under lateral load. The infill contributes in the stiffening of the frame and it was reported that the infills can increase the stiffness of the frame 4 to 20 times (referring to number of literature).

B.Srinivas and B.K.Raghu Prasad [6] discussed the effect of masonry infill walls on dynamic behaviour of structure. A five storey RC masonry infilled frame, soft first storey frame and bare frame models were selected and designed according to IS 1893 code provisions. Equivalent diagonal strut approach was used for modelling the masonry infill panels. Non linear static and non linear dynamic analysis was performed to study the response behavior of the building. The results shown that the presence of infill reduces the lateral deflection and increases the overall strength of the structure. The storey drift decreases due to the presence of masonry infill walls in the infilled frame but the storey drift of the soft storey is significantly large. These effects however not found to be significant in bare frame model.

Mulgund G.V studied the seismic performance of bare frame and frames with various arrangements of masonry infill and nonlinear static pushover analysis has been used to determine the earthquake response of the structure using ETABS software [7]. P.G.Asteris studied the influence of brick masonry panel on the behaviour of infilled frames subjected to in-plane loading using the method of contact points for the analysis [8]. Diptesh Das and C.V.R.Murty studied the effect of brick infills on seismic performance of RC buildings using non-linear pushover analysis [9].

MATERIALS AND METHODS

A study is undertaken which involves seismic analysis of RC frame buildings with different models that include bare frame, infilled frame and open first storey frame. The parameters such as base shear, time period, natural frequency,

storey drift and bending moments are studied. The software ETABS is used for the analysis of the entire frame models [10].

Analysing the data

Following data is used in the analysis of the RC frame building models

- Type of frame: Special RC moment resisting frame fixed at the base
- Seismic zone: III
- Number of storey: Ten
- Floor height: 3.5 m
- Depth of Slab: 150 mm
- Size of beam: (230 × 450) mm
- Size of column: (230 × 600) mm
- Spacing between frames: 5 m along both directions
- Live load on floor: 3 KN/m^2
- Floor finish: 0.6 KN/m^2
- Terrace water proofing: 1.5 KN/m^2
- Materials: M 20 concrete, Fe 415 steel and Brick infill
- Thickness of infill wall: 230 mm
- Density of concrete: 25 KN/m^3
- Density of infill: 20 KN/m^3
- Type of soil: Medium
- Response spectra: As per IS 1893(Part-1):2002
- Damping of structure: 5 percent

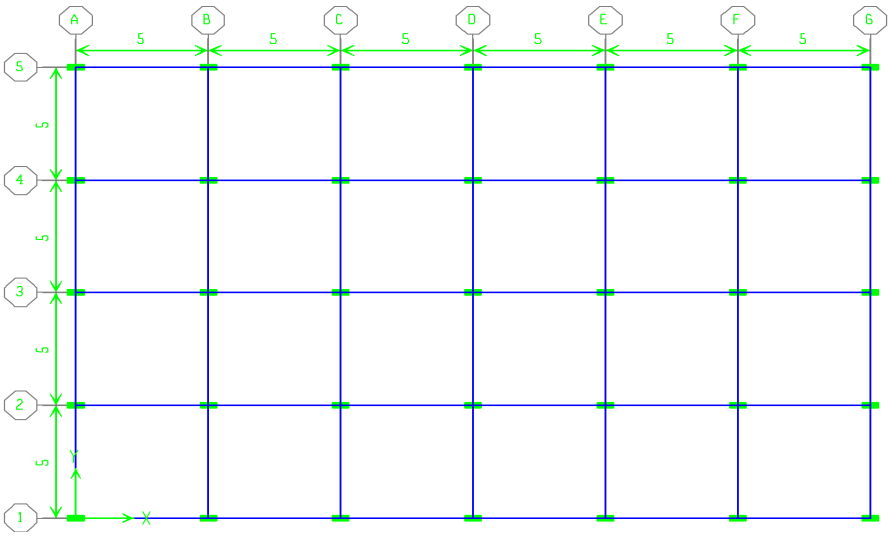

Fig.1. Building Plan (ETABS model)

Modelling of Infill Wall

Equivalent Diagonal Strut Method is used for modelling the infill wall. In this method the infill wall is idealized as diagonal strut and the frame is modelled as beam or truss element. Frame analysis techniques are used for the elastic analysis. The idealization is based on the assumption that there is no bond between frame and infill.
The width of the diagonal strut is given as

$$w = 0.175\,(\lambda' h)^{-0.4} d'$$

Where

$$\text{Contact length parameter } (\lambda') = 4\sqrt{\frac{E_i\, t \sin(2\theta)}{4 E_f I_c h'}}$$

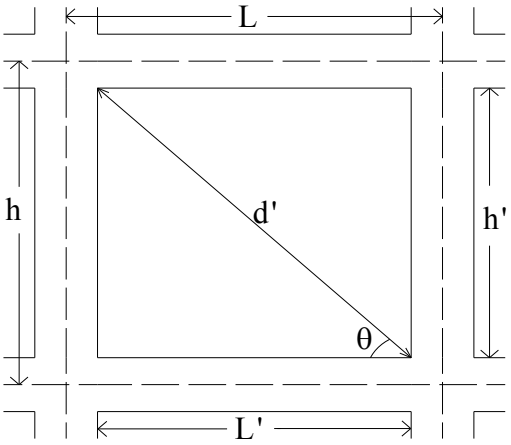

Fig.2. Diagonal strut modelling of infill panel

E_i = modulus of elasticity of infill material
E_f = modulus of elasticity of frame material
L = beam length between centre lines of columns
L' = length of infill wall
h = column height between centre lines of beams
h' = height of infill wall
I_c = moment of inertia of column
t = thickness of infill wall
d' = diagonal length of strut
θ = angle between diagonal of infill wall and the horizontal in radian

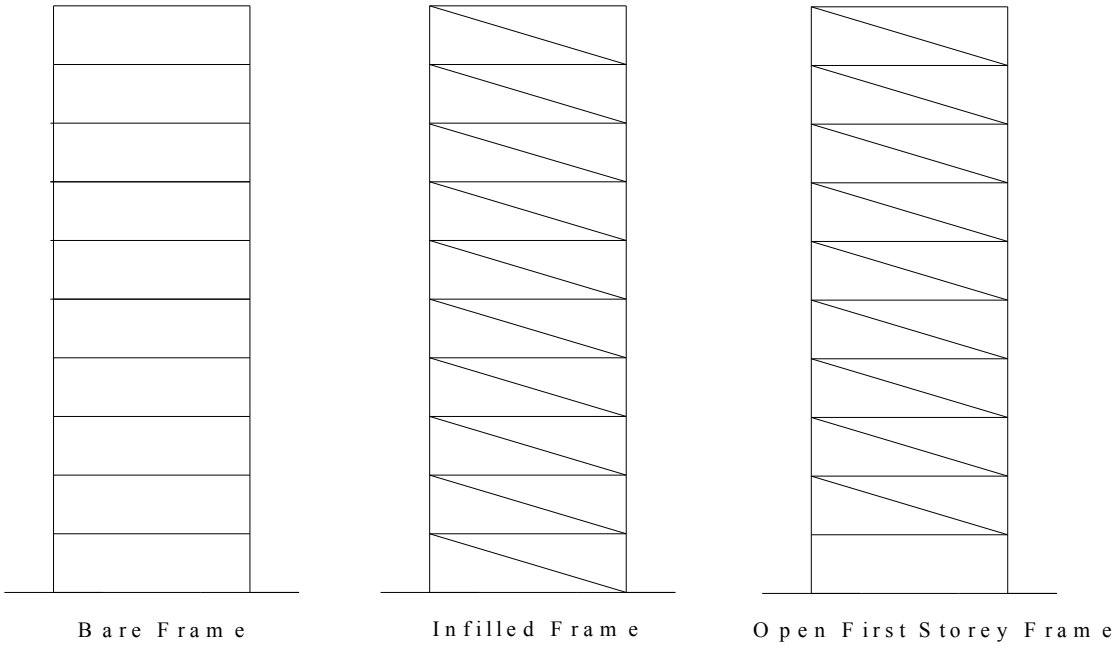

Fig.3. Different frame building models

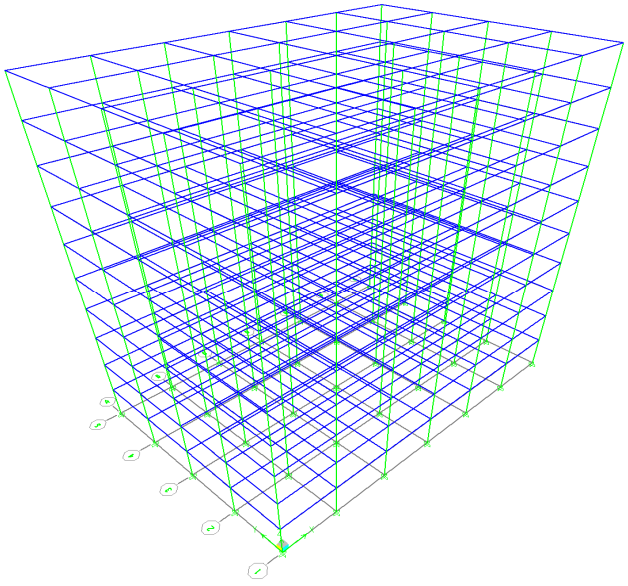

Fig.4. 3D Bare frame building model

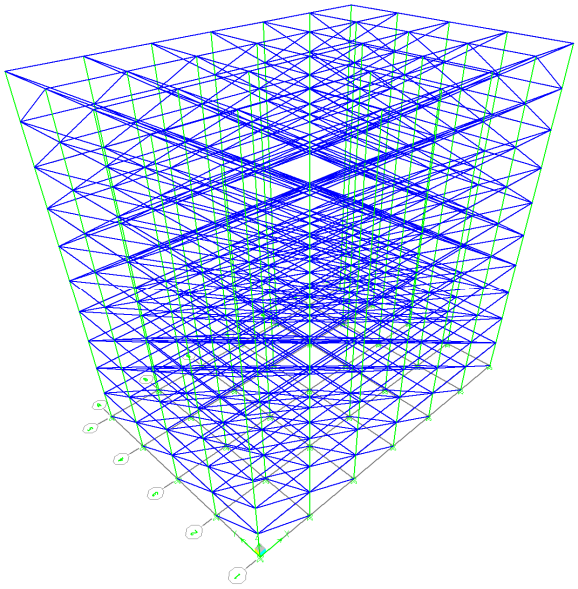

Fig.5. 3D Infilled frame building model

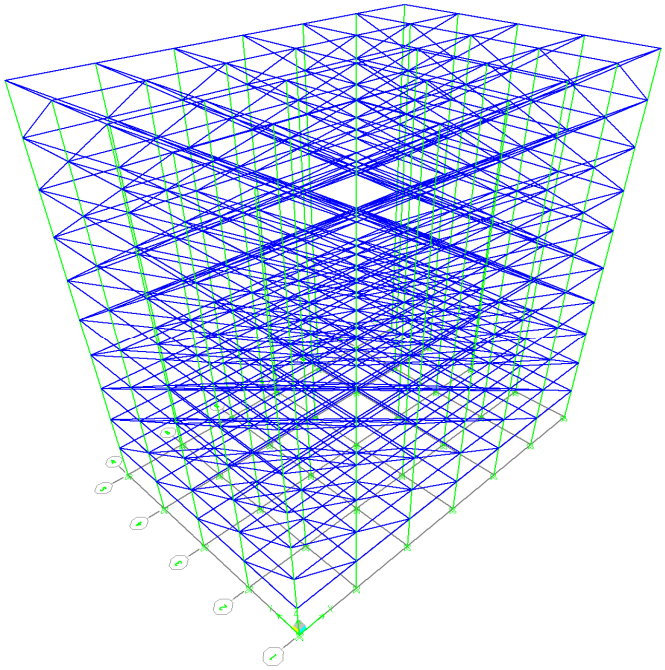

Fig.6. 3D Open first storey frame building model

RESULTS AND DISCUSSION

The seismic analysis of all the frame models that includes bare frame, infilled frame and open first storey frame has been done by using software ETABS and the results are shown below. The parameters which are to be studied are time period, natural frequency, base shear and storey drift.

Table.1. Dynamic characteristics of all the frames

Parameter	Bare frame	Infilled frame	Open first storey frame
T_x (sec)	2.812	0.554	0.930
T_y (sec)	4.375	0.693	1.840
ω_x (H_z)	2.234	11.341	6.756
ω_y (H_z)	1.435	9.066	3.414
V_{Bx} (KN)	673	3412	2034
V_{By} (KN)	473	2726	1038

Where

T_x – Time period in X direction in seconds
T_y – Time period in Y direction in seconds
ω_x – Natural frequency in X direction in Hz
ω_y – Natural frequency in Y direction in Hz
V_{Bx} – Base shear in X direction in KN
V_{By} – Base shear in Y direction in KN

Table.2. Storey drift in X direction

Storey/Storey Drift (mm)	Bare Frame	Infilled Frame	Open First Storey Frame
10	0.314	0.188	0.115
9	0.523	0.218	0.132
8	0.715	0.236	0.143
7	0.867	0.243	0.147
6	0.976	0.240	0.147
5	1.050	0.235	0.144
4	1.091	0.229	0.139
3	1.095	0.222	0.132
2	1.014	0.226	0.156
1	0.587	0.245	1.210

Table.3. Storey drifts in Y direction

Storey/Storey Drift (mm)	Bare Frame	Infilled Frame	Open First Storey Frame
10	0.437	0.243	0.093
9	0.817	0.267	0.102
8	1.129	0.281	0.107
7	1.366	0.285	0.108
6	1.536	0.281	0.106
5	1.650	0.267	0.101

4	1.721	0.248	0.095
3	1.751	0.224	0.086
2	1.745	0.205	0.085
1	1.385	0.198	2.740

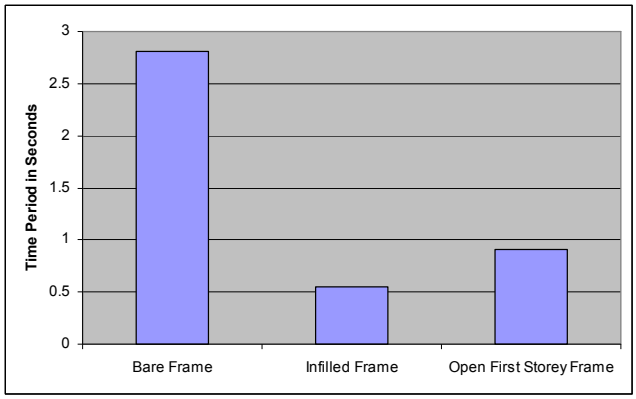

Fig.7. Graph showing time period in X direction

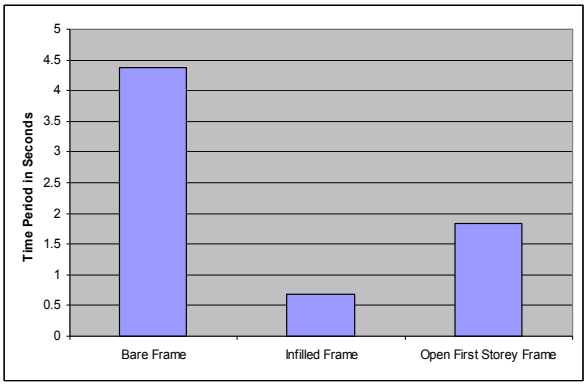

Fig.8. Graph showing time period in Y direction

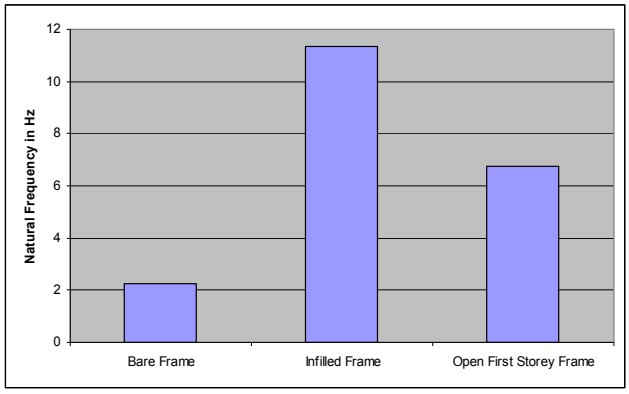

Fig.9. Graph showing natural frequency X direction

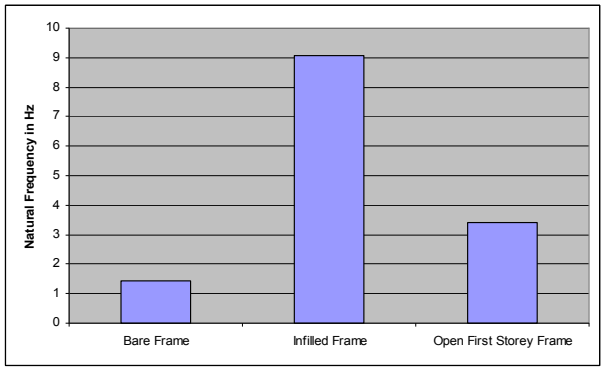

Fig.10. Graph showing natural frequency in Y direction

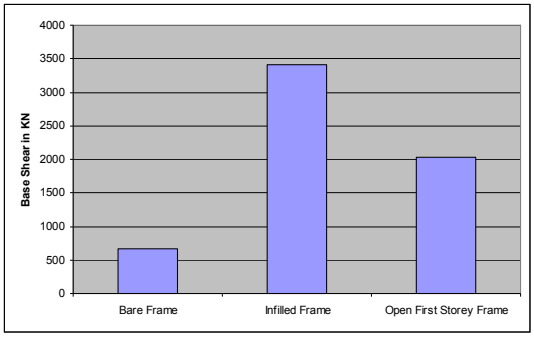

Fig.11. Graph showing base shear in X direction

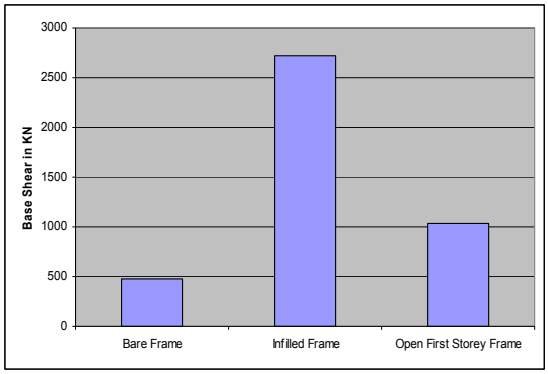

Fig.12. Graph showing base shear in Y direction

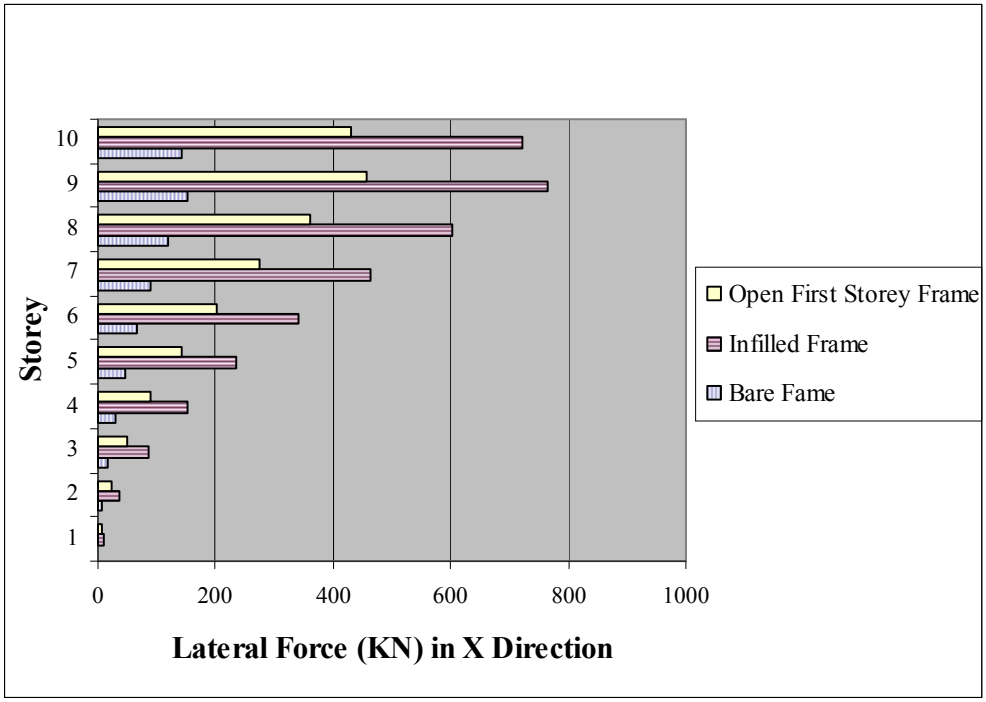

Fig.13. Graph showing lateral force in X Direction

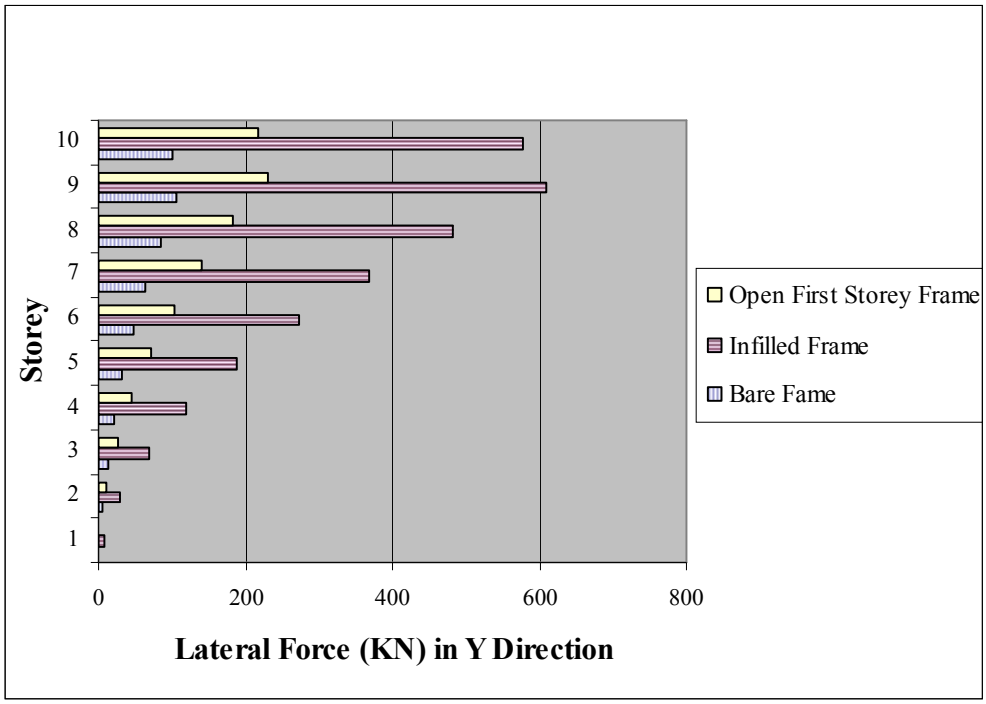

Fig.14. Graph showing lateral force in Y Direction

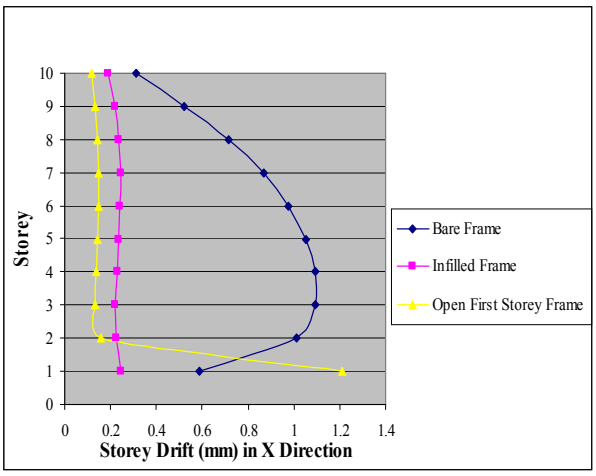

Fig.15. Graph showing storey drift in X Direction

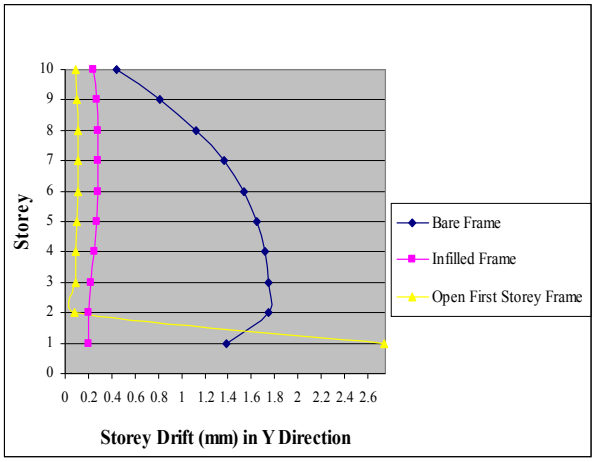

Fig.16. Graph showing storey drift in Y Direction

In this paper ten storey RC frame building models are studied that includes bare frame, infilled frame and open first storey frame. The parameters which are studied are time period, natural frequency, base shear and storey drift.

I) Comparison between bare frame and infilled frame: There is a considerable difference in the base shear and hence the lateral forces of bare frame and infilled frame. Also considerable difference is observed in the time period, natural frequency and storey drift of bare frame and infilled frame. The base shear of infilled frame is more than bare frame and hence there will be a considerably difference in the lateral force along the height of the building. The time period of infilled frame is shortened because of increased stiffness of the structure. The natural frequency of the infilled frame increases due to the decrease in time period. The storey drift of bare frame is more than infilled frame.

II) Comparison between infilled frame and open first storey frame:
i) Base shear: The base shear of infilled frame is more than open first storey frame.
ii) Time period: The time period of infilled frame is less than open first storey frame.
iii) Natural frequency: The natural frequency of infilled frame is more than open first storey frame.
iv) Storey drift: The storey drift of first storey of open first storey frame is very large than the upper storeys, this is because of the absence of infill walls in the first storey. However in the infilled frame the storey drift of first storey is less because of the presence of infill wall in the first storey (ground storey).

CONCLUSION

In this paper seismic analysis of RC frame models has been studied that includes bare frame, infilled frame, and open first storey frame. From the seismic analysis of RC frames following conclusions are drawn, \

1) The seismic analysis of RC frames should be done by considering the infill walls in the analysis. For modelling the infill wall the equivalent diagonal strut method can be effectively used.
2) Infilled frames should be preferred in seismic regions than the open first storey frame, because the storey drift of first storey of open first storey frame is very large than the upper storeys, this may probably cause the collapse of structure.
3) The presence of infill wall can affect the seismic behaviour of frame structure to large extent, and the infill wall increases the strength and stiffness of the structure.
4) The seismic analysis of RC (Bare frame) structure leads to under estimation of base shear. Therefore other response quantities such as time period, natural frequency, and storey drift are not significant. The underestimation of base shear may lead to the collapse of structure during earthquake shaking. Therefore it is important to consider the infill walls in the seismic analysis of structure.

In case of an open first storey frame structure, the storey drift is very large than the upper storeys, which may cause the collapse of structure during strong earthquake shaking. Therefore the infilled frame structures will be the better option to prefer in the seismic regions.

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RESEARCH ARTICLE

Effect of Leaf Litter Waste in Vermicompost.

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ABSTRACT

The study performed to evaluate the potential of epigeic earthworm *Eudrilus eugeniae* to transform waste leaf litter and waste cattle manure into a more useful i.e., vermicompost.Vermicomposting caused significant increased in available macro nutrients such as nitrogen 14.9% in leaf litter vermicompost and 1.62% in cattle manure vermicompost; Potassium 1.69% in leaf litter vermicompost and 0.56% in cattle manures vermicompost; phosphorus 1.46% and 1.11% in leaf litter vermicompost and cattle manure compost respectively. Reduction in carbon nitrogen ratio 0.33% and 2.76% in leaf litter vermicompost and cattle manure vermicompost respectively.

Key words: Leaf litter waste, *Eudrilus eugeniae*, Cattle manure Vermicomposting

INTRODUCTION

Scientific investigations have established the viability of using earthworms as a treatment technique for numerous waste streams besides producing organic fertilizers. vermicompost is considered as an excellent product since it is homogenous, has desirable aesthetics, has reduced level of contaminates, has plant growth hormones, higher level of soil enzymes, greater microbial population and tends to hold more nutrients over a longer period without adversely impacting the environment.

The degradable organic matter from these wastes when dumped in open undergoes either aerobic or anaerobic degradation. These un-engineered dumpsites permit fine organic matter to become mixed with percolating water to form leachate. The potential for this leachate to pollute adjoining water and soil is high. India where a lot of solid organic waste is available in different sectors with no dearth of manpower, the environmentally acceptable vermicomposting technology using earthworms can very well be adopted for converting waste into wealth [1].

Considerable work has been carried out on vermicomposting of various organic materials and it has been established that epigeic forms of earth-worms can hasten the composting process to a significant extent, with production of a better quality of composts as compared with those prepared through traditional methods. The viability of using earthworms as a treatment or management technique for numerous organic waste streams has been investigated by a number of workers [2]. Similarly a number of industrial wastes have been vermicompost and turned into nutrient rich manure.Being rich in macro and micro nutrients, the vermicompost has been found ideal organic manure enhancing biomass production of a number of crops. The importance of vermicompost in agriculture, horticulture, waste management and soil conservation has been reviewed by many workers [3].

MATERIALS AND METHODS

In this study, wastes such as leaf litter and cattle manure were utilized as the raw material for composting. The cow dung substrate (C) used was dry cow dung in the form of powder and the leaves substrate (L) used was in dried and powder form. They were mixed with soil separately and set aside a period for stabilize. The earthworms *Eudrilus eugeniae* was used to compost the waste materials.The nutrition quality such as Nitrogen, Phosphorus and Potassium by the method of Jackson [4] and carbon nitrogen ratio of end products i.e. vermicompost were analyzed.

RESULTS AND DISCUSSION

The nutritional values of the two vermicompost were analyzed and the results are presented in the figure. The highest Nitrogen (N) content was recorded in the vermicompost collected from Leaf litter wastes and the values are 14.9%. N value was found as 1.62%, in cattle manure wastes (Fig. No:1). Some workers have reported higher content of nitrogen, phosphorus and potassium and micronutrients in vermicompost [5]. Increase in nitrogen content in *P. excavates* worked vermicompost of sugarcane trash and cowdung substrate as compared to controls was reported [6].

In present study, the phosphorus (P) content was recorded in the vermicompost collected from Leaf litter wastes and cattle manure wastes, the values are 1.46% and 1.11% in respectively. It has been established that higher amount of phosphorus is found in test experiment than control (soil and Cow dung) experiment using earthworm species [7].The highest potassium (K) content 1.69% was recorded in the vermicompost collected from Leaf litter wastes and 0.56% in cattle manure wastes. It has been reported that higher content of potassium found in a vermicompost. The increase in nitrogen, phosphorus and potassium in the vermicompost confirms the enhanced mineralization of these elements due to enhanced microbial and enzyme activity in the guts of worms [8].The lowest carbon and nitrogen ratio was calculated as 0.33% in Leaf litter vermicompost followed by 2.76% in cattle manure vermicompost. Vermicompost with *Eisenia foetida* of crop residues and cattle dung resulted in significant reduction in C: N ratio and increase in N [9].

The nutrient status of vermicompost produced with different organic waste is; organic carbon 9.15 to 17.98 %, total nitrogen 0.5 to 1.5 %, available phosphorus 0.1 to 0.3 % and available potassium 0.15 [10]. Tajbakhsh *et al*, [11] study the performed to evaluate the potential of epigeic earthworms *Eisenia foetida* and *Eisenia andrei* to transform spent mushroom compost into a more useful product in i.e., vermicompost. Vermicomposting caused significant reduction in C:N ratio (56%) and increased in available macro and micronutrients such as K (68%) P by 3-fold and N 1.37-fold, compared to those of the initial substrate.The surrounding soils and reported their results [12]. The vermicasts have been reported with a higher Base Exchange capacity and are rich in total organic matter, phosphorus potassium and calcium with a reduced electrical conductivity, large increase in oxidation potential and significant.

CONCLUSION

Vermicomposting technology involves harnessing earthworms as versatile natural bioreactors playing a vital role in the decomposition of organic matter, maintaining soil fertility and in bringing out efficient nutrient recycling and enhanced plants' growth. A variety of organic solid wastes, domestic, animal, agro-industrial, human wastes etc can be vermicomposted. The value of vermicompost is further enhanced as it has simultaneously other benefits [13]. It is demonstrated that vermicomposting could be considered as an alternate technology for recycling and environmentally safe management of which generate in abundance as residues using epigeic earthworms.

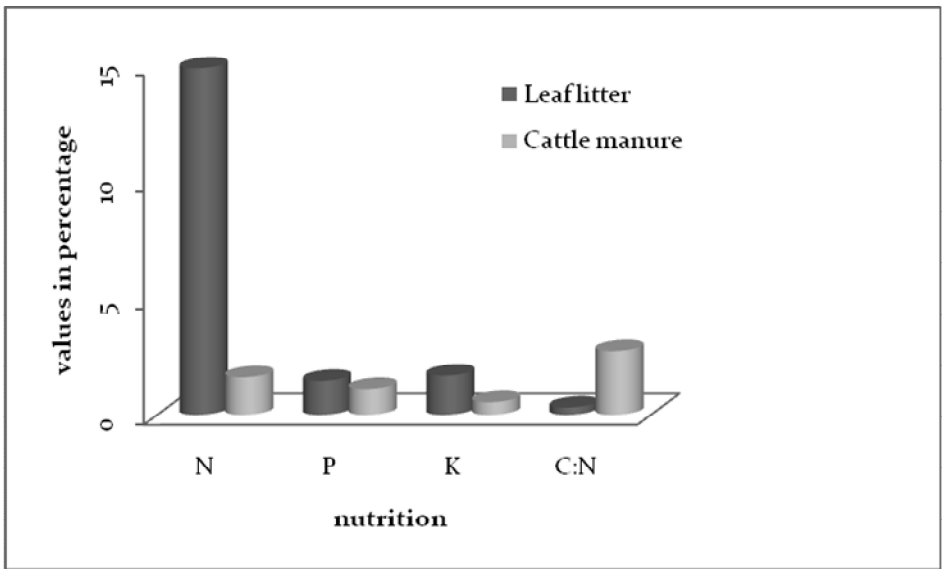

Fig 1: Nutrition values in leaf litter and cattle manure vermicompost

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RESEARCH ARTICLE

Isolation and Characterization of Polyhydroxyalkanoates (PHAs) Producing Bacteria from Sago Industrial Wastewater.

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ABSTRACT

Polyhydroxyalkanoates (PHA's) are biological polyesters that are produced by a wide variety of bacteria as an intracellular storage material of carbon and energy. Recently PHA has attracted considerable industrial attention because they are biodegradable and in many cases, their physico and mechanical properties compare favorably with synthetic polymers and are ecofriendly. For a developing country like India, use of cheap waste effluent production of PHA is quite attractive and interesting. In our study The Nile blue A staining was done to screen for PHAs producing bacteria followed by nucleotide sequencing which analysis further confirmed the bacterial strain, and finally these isolates were then subjected to identification and quantification of P(3HB-co-3HV) co polymer using GC-MS. These results demonstrate that the environmental bacterial stains were able to accumulate the PHA which was confirmed by Nile blue A staining method, and also GC-MS was used to quantify by the PHA produced by the strains were isolated from sago waste effluent. Five different forms were screened in which *Bacillus sp* was selected for further studies, i.e. for PHA production using different carbon and nitrogen sources. The paper will elucidate the primary screening of PHA production and characterization in the isolated strain *Bacillus sp.*

Key words: Polyhydroxyalkanoates, Bacillus spp., Nile blue A staining, P(3HB-co-3HV)

INTRODUCTION

PHAs are a polymer of hydroxyalkanoates and accumulate as intracellular biomass under specific unfavorable nutrient condition or during starvation as a reserve carbon and energy source [1]. PHAs have a wide range of agriculture, marine and medical application [3]. They are considered as good substitute for petroleum derived synthetic plastics because of their similar material properties of synthetic plastics and complete biodegradability after disposal. The main advantage of this type of polymers is that since they are of biological origin, they degrade naturally and completely to carbon dioxide and water under natural environment by the enzymatic activities of microbes. Microorganisms are able to accumulate various types of PHAs in the form of homopolymer, copolymer and polyester blends[2]. The best PHAs producing species are able to grow fast by utilizing the cheap carbon source and produce high PHAs. The PHAs production is usually a two stage process. The first stage is initial balanced growth phase and in this stage high protein biomass is produced. The second stage is nutrient limitation phase in which the number of cells remain constant but cell size increase because accumulation of PHAs[23]. The condition for bacterial PHAs production can be met in soil, due to its heterogeneous nature. Sago wastewater originated from the pressing of cassava roots and is considered to be a harmful residue to the environment due to its organic material load and high level of cyanide. It also contains 50% of carbohydrate, minerals and metals in trace amount and less than 1% of phosphorus and sulfur[9]. Excess carbon with less nitrogen in wastewater creates stress to bacteria that ultimately enhances PHAs accumulation. The objective of the present study was to investigate the presence of PHAs producing microorganisms from the sago industrial wastewater samples.

MATERIALS AND METHODS

Isolation of PHA – producing microorganisms from sago wastewater

Sago wastewater samples were collected from Sago industry located in Namakkal District, Tamil Nadu, India, samples were collected in sterile container, immediately transferred to the laboratory in icebox and stored in a refrigerator at 4 °C until analysis. Wastewater was serially diluted, whereby each dilution was spread on nutrient agar plates, and the plates were incubated at 37°C for 24 hours. Bacterial strains isolated from these environments were screened for PHA production. Mineral salt medium (MSM) was used for the production of PHA[14]. pH of the media was neutralized and was sterilized before use.

Cell cultivation

Identification bacterial strains was first grown in nutrient broth for 24 h in 250 ml Erlenmeyer flask and it was incubated at 37°C with shaking. After 24 h, cells were then harvested by centrifugation at 8000 rpm for 12 min and washed aseptically with sterile distilled water and were resuspened into 1L of MSM medium. The carbon source is glucose (10g/ml) and incubated at 37 °C for 48 h. After incubation, cells were harvested by centrifugation at 8000 rpm for 12 min, Cells was washed with sterile distilled water and recentrifuged similarly. Cell pellets was used for further studies.

Nile blue A Staining

Heat fixed bacterial smear was stained with 1% aqueous solution of Nile blue A at 55°C for 10 min. The slide was washed with tap water to remove excess stain and then washed with 8% aqueous acetic acid for 1 min .The staining smear was again washed with water and blot dried. Prior to observation, the slide was remoistened with a drop of water and coverslip was placed on the smear [22].The slides were viewed in fluorescence microscopy at wavelength of 480 nm.

Extraction of DNA for sequence analysis

Bacterial cells were harvested and washed twice with 1ml of phosphate buffered saline (PBS). Extraction of DNA was carried out [15].For amplification of 16s rRNA gene by polymer chain reaction (PCR). The 16s rRNA was amplified using with bacterial universal primers f24 and f25 as previously [17].Purified DNA obtained from PCR was then sequenced by using a LICOR IR2 4200 DNA sequencer (LI-COR, Lincoln, NE, USA) according to manufacturer's instructions. The sequence of 16S rRNA gene was compared with the 16S rRNA gene sequences available in the NCBI public data-bases.

Extraction and purification of PHA

PHA was extracted from dried cells material with chloroform at 100 ˚C for 3 h. The chloroform solution was filtered to remove any cellular debris and it was concentrated by rotary evaporator. PHA polymer was precipitated from the chloroform solution by adding chilled methanol (1:10) drop wise. The methanolchloroform mixture was then centrifuged at 5000 rpm .Then the polymer was dissolved in chloroform and was again precipitated in methanol to obtained highly purified polymer [18]. Finally, it was dried in (Bucchi, Switzerland) room temperature and weight of the polymer was measured. The polymer content was calculated as the percentage of PHAs in the cell upon drying. Residual cell mass (RCM) was defined as cell dry weight (CDW) minus the PHAs concentration.

Preparation and analysis of PHAs

To determine the polymer content of the cells and its composition, the precipitated PHAs was subjected to methanolysis. Approximately 5mg of precipitate was subjected to methanolysis (3h at 100 ˚C) with a solution consisting of 1.7 ml of methanol, 0.3 ml of 98% sulfuric acid and 2ml of chloroform [11]. After phase separation and two washes with water, the organic phase (bottom) was dried with anhydrous sodium sulphate. Separation of methylesters was performed using a Shimadzu GC-MS (QP-2010) with splitless injection.The GC-MS was operated with an interface temperature of 270ºC and an ion source temperature of 230ºC. The mass spectrometer was used with silica capailary column (30m x 0.32 mm, thinness 0.25µm, J & W scientific, folsom, CA, USA). Helium with a maximum purity 99.999 % was used as the carrier gas at a flow rate of 2.25 ml/min. The gas chromatography was equipped with split/splitless injector port operated mode at a colum temperature of 70ºC using an auto sampler AOC-20; containing 10µl syringe the GC oven temperature was programmed as follows; initial temperature of 50 ºC for 1 min, from 50 ºC to 220 ºC at a rate of 30 ºC/min and finally to 320 ºC at a rate of 20 ºC/min. The mass spectrometer was operated in the positive ion electron impact EI mode using ionization energy of 70 eV and an emission current of 60µA. Full scan data were obtained with a mass range of m/z 35-500. Scanning interval and SIM sampling rate were 0.5 and 0.2 sec, respectively. The mass selective detector was operated in selected ion monitoring (SIM) mode. The initial structural assignment of the methylesters analyzed based on their retention times compared to those of authentic standards.

RESULTS

PHA producing bacterial strains that was isolated from sago wastewater were investigated for its PHA producing ability by staining and then further confirmed through GC-MS. The strains were stained based on the production capacity is tested by using Nile blue A staining method its observed in florescence microscopy at wavelength of 480 nm followed by stain identification and it was evident that the belongs to the genus *Bacillus* and closely related to *B. cereus* stain. We can further confirm that *Bacillus cereus* EBS 31strain is producing PHA (Genbank accession Number is GU 131271). The carbon source was always supplied in excess to allow maximum accumulation of PHB. However under nonoptimized condition, the total accumulation of PHB in *Bacillus cereus* EBS 31 does not exceed 20% (wt/wt) of the cell dry weight when compared to other organism, such as *A .eutrophus* which has been reported to

accumulated up to 90% (wt/wt) of the biomass production [8]. Further confirmation was done using GC-MS to identify and quantify PHA production. From shank-flask cultivation, up to 20% of P(3HB-co-3HV) copolymer was accumulated at 48 h under controlled culture condition. This polyhydroxyalkanoates accumulation was studied under the interactive condition of N- deficiency in presence of glucose as the substrate. The cassation of P(3HB) accumulation in *A. eutrophus* was a result from a physical space limitation[10]. This is probably not the case for *P. pseudoflava* and studies should be done to optimize the growth and PHB accumulation as well as to identify and modify the control mechanism that limits accumulation in organism that produced little PHB[5].

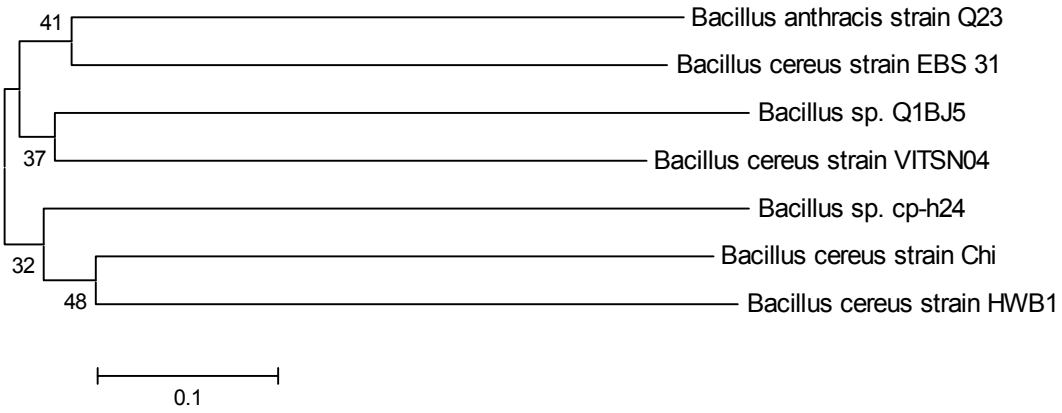

Fig. 1.Phylogentic tree based on 16s rRNA gene sequence for *Bacillus cereus* EBS 31

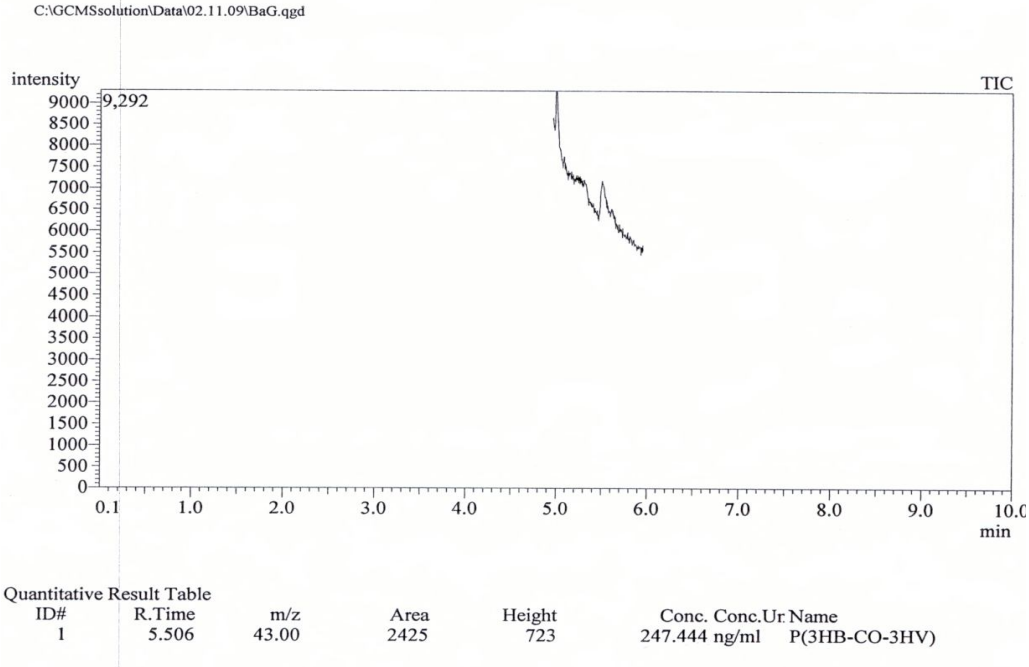

Fig. 2. GC-MS chromatogram of P(3HB- *co*-3HV) from *B. cereus* EBS 31

DISCUSSION

Gram-positive bacteria have another potential advantage in terms of raw materials for PHA production. The gram-positive genera *corynebacterium, Nocardia,* and *Rhodococcus* are capable of naturally synthesizing the commercially important copolymer P(3HB-co-3HV) from abundant and inexpensive carbon sources such as glucose[21]. In contrast, gram-negative bacteria need expensive structurally related substrate such as propionic acid, valeric acid or other fatty acids with an odd number of carbon atoms to produce 3HV units [20] hence, gram-positive producers could considerable reduce the production cost [16]. The best characterization gram-positive bacterial group and the first PHA producer from the genus *Bacillus* was identified as *Bacillus megaterium* in 1926[12]. The best characterized gram-positive bacterial group and the first PHA producer from the genus Bacillus was identified as *Bacillus megaterium,* till date many species of PHA copolymers from inexpensive and structurally unrelated carbon sources. Bacillus sp.88D isolated from municipal sewage treatment plant is able to produced P(3HB-co-3HV) from glucose as a sole carbon source [13] *Bacillus cereus* SPV is able to use fructose, sucrose and gluconate to produce P(3HB-co-4HB) and (3HB-co-3HV-co-4HB) [20]. *Bacillus sp*. INT005 isolated from gas field soil produces P(3HB-co-3HHx) from glucose [19]. The diversity of PHA product in the genus *Bacillus* is presumed to be due to class IV PHA synthase, broad monomer specificity [21]. This result indicates that the genus *Bacillus* could be used for the industrial production of PHA copolymer, which prompted us to isolated new copolymer producers with excellent cell growth and polymer accumulation.

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RESEARCH ARTICLE

Investigation of Marine Actinomycetes from Karankadu, Ramanathapuram District, South East Coast of TamilNadu, India.

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ABSTRACT

A total number of 121 colonies were isolated by plating method in the Mangrove soils of Karankadu in Ramanathapuram district, southeast coast of India. The soil characteristics such as pH 7.02 to 7.56, electrical conductivity 0.16 to 0.23 dSm^{-1}, cation exchange capacity 20.5 to 26.3 c.mol $proton^{+}$/kg, organic carbon 0.23 to 0.36%, organic matter 0.46 to 0.72%, available nitrogen 102.4 to 110.5(Kg / ac), available phosphorus 3.25 to 4.75 (Kg / ac), available potassium 132 to 135(Kg / ac), available zinc 1.23 to 1.25 ppm, available copper 1.09 to 1.45 ppm, available iron 8.65 to 9.64 ppm, available manganese 3.15 to 3.56 ppm, calcium 12.6 to 15.6 (C. Mole $Proton^{+}$ / kg), magnesium 10.3 to 11.2 (C. Mole $Proton^{+}$ / kg), sodium 2.19 to 2.57 (C. Mole $Proton^{+}$ / kg) and potassium 0.23 to 0.26 (C. Mole $Proton^{+}$ / kg) were also showed variation during different seasons. The investigation was carried out by collections and examination of sediment samples during January 2011 – December 2011, at seasonal intervals.

Keywords: Actinomycetes, Marine mangroves, Physico-chemical character.

INTRODUCTION

Mangroves represent one of the most productive ecosystems in tropical environment and are characterized by efficient turnover of nutrients [13]. Mangroves are highly productive ecosystem next to the coral reefs and provide energy to marine habitats through production and decomposition of plant detritus [1,18]. Actinomycetes are the most economically and biotechnologically valuable prokaryotes. They are responsible for the production of about half of the discovered bioactive secondary metabolites [3]. Because of the excellent track record of actinomycetes in this regard, a significant amount of effort has been focused on the successful isolation of actinomycetes from marine sources for drug screening programs in the past fifty years. Recently the rate of discovery of new compounds from

terrestrial actinomycetes has decreased, where as the rate of re – isolation of known compound has increased [9].Actinomycetes population has been identified as one of the major group of soil population which may vary with the soil types [25]. When compared to terrestrial actinomycetes, very little work has been conducted on marine actinomycetes, as marine environmental conditions are extremely different from terrestrial ones.

MATERIALS AND METHODS

Description of sampling site and sample collection

Soil sample were collected in each location seasonally for a period of one year (January – December, 2011) from Karankadu (Lat. 9^0 36′ N long. 78^0 83′ E) representing different location found in Palk Strait region of South East Coast of India. Sediment samples were collected with the help of mini sediment samples [22] at random between 5 – 15cm depth of each location into sterile plastic bags and brought to the laboratory in a pre sterile sip – lap plastic bag individually and stored in an ice box for the isolation of Actinomycetes.

Isolation of Actinomycetes

Isolation and enumeration of actinomycetes were performed by sediment dilution plate technique, [38] using starch casein agar medium. The antifungal (50 μg / ml cyclohexamide) and antibacterial (20 μg / ml of tetracycline) were added to medium after sterilization and prior to the pouring of the agar medium [7]. After attaining a powdery growth, the colonies were counted and recorded. Statistical analysis was carried out to find out the level of significance.

Physico – chemical analysis of soil

Moisture content was estimated by finding the weight difference of known quantity of soil before and after drying in a hot air oven at 60°C for 6 hours. Soil samples after removing the debris were suspended in distilled water (1:2 w/v) and allowed to settle down the sand particles. The pH of the suspension was read using pH meter (Systronics, India), to find out the soil pH.Electrical conductivity of soil was determined in the filtrate of the water extract using conductivity bridge as described by Jackson (1973)[10], Cation exchange capacity (CEC) of the soil was determined by using 1 N ammonium acetate solution as described by Jackson (1973)[10].

Organic carbon content was determined by adopting chromic acid wet digestion method as described by Walkley and Black (1934)[37]; available nitrogen was estimated by alkaline permanganate method as described by Subbiah and Asija (1956)[29] and available phosphorus by Brayl method as described by Bray and Kutz (1945)[4]. Available potassium was extracted from soil with neutral 1 N ammonium acetate (1:5) and the potassium content in the extract was determined by using flame photometer [28]. Calcium (Neutral 1 N NH_4 OAC extractable 1:5) was extracted with neutral 1 N ammonium acetate and the available calcium in the extract was determined by versenate method (Jackson, 1973)[10].

Available micronutrients such as Zn, Cu and Mn were determined in the diethylene triamine pentaacetic extract of soil using Perkin-Elmer (model 2280) Atomic Absorption Spectrophotometer [17].Other nutrients such as magnesium, sodium and available iron were analysed following the method of Barnes (1959)[2] and Muthuvel and Udayasoorian (1999)[19].

RESULTS AND DISCUSSION

In the present investigation, the species diversity of the Actinomycetes revealed with the existence of 23 species. When compare to Ravikumar *et al*., (2011)[26] the maximum of Actinomycetes were present in the depth of 10-20 cm. A few reports are available on Actinomycetes isolated from marine sediments [27,6,35]. A great majority of them were also reported from mangrove of Karankadu [25]. Totally 71 species were isolated by Vijayakumar *et al*.,(2004)[35] in the mangrove, seashore and saltpan soils of Point Calimere, east coast of India, Ramesh and Mathivanan (2009)[24] isolated 208 species of Actinomycetes from marine sediments of Bay of Bengal, India and Vijayakumar *et al.* (2010)[34] were isolated 30 Actinomycetes from Muthupet mangrove of Tamilnadu, India. In the present study (number of species), minimum of 12 species were recorded in the soils collected during monsoon season in 2011. The maximum of 19 species were recorded in the soils collected during premonsoon season in 2011 (Table 1). The trend of species composition with bulk number of *Streptomyces* species were reported from the Nahoon beach in the Eastern Cape Province of South Africa by Ogunmwonyi *et al.,* (2010)[21]. Actinomycetes especially streptomyces, have been reported from the marine sub habitats of marine sediments [31,36]marine soil [23,36] and also from almost all parts of the world. Also, the dominant Actinomycetes especially streptomyces in soils has been reported by many workers [11,23].

Actinomycetes population density also showed variations in different seasons. The mean density was range from 0.33 to 1.33 x 10^2 CFU/g (Table 1). Percentage contribution of the individual species to the total Actinomycetes population showed variation. *Streptomyces* sp (BPM20) contributed the maximum percentage (8.26 %) followed by *Nocardiopsis* sp (BPM13), *Saccharopolyspora* sp (BPM14), *Streptomyces* sp (BPM21), *Micropolyspora* sp (BPM09), *Actino bispora* sp (BPM05) and *Streptomyces* sp (BPM17)(6.61 % each), *Streptomyces* sp (BPM18), *Saccharopolyspora* sp (BPM15), *Microtetraspora* sp (BPM08), *Actinosynnema* sp (BPM02) and *Actinomadura* sp (BPM01) (4.13 % each), *Streptoverticillium* sp (BPM22), *Saccharopolyspora* sp (BPM16), *Glycomyces* sp (BPM07), *Actino polyspora* sp (BPM04) and *Actino bispora* sp (BPM03) (3.30 % each) and *Micromonospora* sp (BPM10), *Microtetraspora* sp (BPM11), *Nocardiopsis* sp (BPM12), *Saccharothrix* sp (BPM23), *Streptomyces* sp (BPM19) and *Catellospora* sp (BPM06)(2.47 % each) (Table 1).

During the entire period of study all the soil samples were analysed in alkaline nature. It was in the range from 7.02 to 7.56 during premonsoon in 2011and postmonsoon season in 2011(Table 2). Alkaline condition has been explained as the characteristic feature of marine soil [20]. Marine habitats such as coastal and brackish environs [30]and sand dunes [33]were also reported as alkaline conditions as reported in the present study. Electrical conductivity value is an indirect measure for the salinity. In the present study, it was recorded in the range from 0.16 to 0.23 dSm^{-1}(Table 2). This was comparatively lower than the marine and brackish water sediments of Madras coast [30] and mangroves of Andaman [5]. This may be due to the washing of freshwater by the heavy flow through the distributaries of the river Cauvery, which traverse along the coast, and monsoonal rainfall. Likewise, CEC in the soils was also low which was in the range from 20.5 to 26.3 c.mol $proton^+$/kg (Table 2), when compared to the salt affected soils of Elhussinia plane, Sharkia Governorate, Egypt, that was in the range of 31 to 54.8 c.mol proton +/kg,[8]); and 189 and 275 mmol kg^{-1} in the soil samples of Andamans[5].

Organic Carbon content was considered to be the factor responsible to influence the population of any of the heterotrophic microorganisms. It showed variations in the range from 0.23 to 0.36 per cent in all the sampling stations (Table 2).These values were comparatively less than that of the river sediments [15] and various biotopes in the Muthupettai mangroves [12] Nitrogen, an important nutrient for the growth of plants, is fluctuated from 0.011 to 0.022% during different seasons (Table 2), which is also less than other marine environs. Available potassium was in the range from 132 to 135 (Kg / ac) (Table 2) in the soils which was less than the range reported from Shenzhen (1.6 per cent) [32] Fujian (2.07%)[16], Hain mangroves (0.42 – 1.19%)[15]and mangroves of Andamans (0.81 – 125%) [5].Correlation analysis made for various physico-chemical parameters and Actinomycetes population. The negative correlation were observed between available Manganese and total number of colonies ($r = 0.994$; $P < 0.01$), Sodium and total number of colonies ($r = -0.981$; $P < 0.05$) (Table 3).

Soils of bio shield plantation with *Casuarina* along the coastal are also supporting fungal diversity, but it varies in terms of species diversity and population density from mangroves, which is one of the major vegetation along the coastal, area. Like wise the soil character also revealing less nutrient content than that of mangrove soils. But the species composition is comparable with the mangroves and other coastal habitats, with the dominance of Actinomycetes.

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Table :1. Number of Colonies, Mean Density (CFU/g) and Percentage Contribution of Actinomycetes Recorded During Different Season from Marine Mangroves, Karankadu.

S. No	Name of the organisms	Postmonsoon		Summer		Premonsoon		Monsoon		Total No. of colonies	% contribution
		TNC	MD	TNC	MD	TNC	MD	TNC	MD		
1.	*Actinomadura* sp (BPM01)	4	1.33	-	-	1	0.33	-	-	5	4.13
2.	*Actinosynnema* sp (BPM02)	2	0.66	-	-	3	1	-	-	5	4.13
3.	*Actino bispora* sp (BPM03)	1	0.33	1	0.33	-	-	2	0.66	4	3.30
4.	*Actino polyspora* sp (BPM04)	1	0.33	2	0.66	-	-	1	0.33	4	3.30
5.	*Actino bispora* sp (BPM05)	4	1.33	-	-	3	1	1	0.33	8	6.61
6.	*Catellospora* sp (BPM06)	-	-	1	0.33	2	0.66	-	-	3	2.47
7.	*Glycomyces* sp (BPM07)	1	0.33	-	-	1	0.33	2	0.66	4	3.30
8.	*Microtetraspora* sp (BPM08)	2	0.66	-	-	3	1	-	-	5	4.13
9.	*Micropolyspora* sp (BPM09)	3	1	2	0.66	3	1	-	-	8	6.61
10.	*Micromonospora* sp (BPM10)	2	0.66	1	0.33	-	-	-	-	3	2.47
11.	*Microtetraspora* sp (BPM11)	-	-	1	0.33	2	0.66	-	-	3	2.47
12.	*Nocardiopsis* sp (BPM12)	-	-	-	-	2	0.66	1	0.33	3	2.47
13.	*Nocardiopsis* sp (BPM13)	-	-	1	0.33	5	1.66	2	0.66	8	6.61
14.	*Saccharopolyspora* sp (BPM14)	3	1	1	0.33	4	1.33	-	-	8	6.61
15.	*Saccharopolyspora* sp (BPM15)	2	0.66	2	0.66	-	-	1	0.33	5	4.13
16.	*Saccharopolyspora* sp (BPM16)	2	0.66	1	0.33	1	0.33	-	-	4	3.30
17.	*Streptomyces* sp (BPM17)	2	0.66	3	1	2	0.66	1	0.33	8	6.61
18.	*Streptomyces* sp (BPM18)	1	0.33	1	0.33	1	0.33	2	0.66	5	4.13
19.	*Streptomyces* sp (BPM19)	-	-	-	-	3	1	-	-	3	2.47
20.	*Streptomyces* sp (BPM20)	3	1	2	0.66	4	1.33	1	0.33	10	8.26
21.	*Streptomyces* sp (BPM21)	3	1	2	0.66	1	0.33	2	0.66	8	6.61
22.	*Streptoverticillium* sp (BPM22)	2	0.66	-	-	2	0.66	-	-	4	3.30
23.	*Saccharothrix* sp (BPM23)	-	-	-	-	2	0.66	1	0.33	3	2.47
	Total	**38**	**12.6**	**21**	**6.94**	**45**	**14.93**	**17**	**5.61**	**121**	

TNC – Total number of colonies, MD – Mean density

Table:2 Physico-chemical parameter analysis of Marine Mangroves from Karankadu.

S. No	Name of the parameter	Sample Details			
		Post Monsoon	**Summer**	**Pre Monsoon**	**Monsoon**
1.	pH	7.56	7.52	7.02	7.12
2.	Electrical conductivity (dsm^{-1})	0.23	0.21	0.16	0.21
3.	Organic Carbon (%)	0.23	0.36	0.29	0.31
4.	Organic Matter (%)	0.46	0.72	0.58	0.62
5.	Available Nitrogen (Kg / ac)	110.5	106.3	102.4	106.3
6.	Available Phosphorus (Kg / ac)	4.50	4.75	3.75	3.25
7.	Available Potassium (Kg / ac)	165	155	132	148
8.	Available Zinc (ppm)	1.23	1.20	1.21	1.25
9.	Available Copper (ppm)	1.20	1.09	1.45	1.36
10.	Available Iron (ppm)	9.64	8.79	8.65	9.62
11.	Available Manganese (ppm)	3.21	3.56	3.15	3.63
12.	Cat ion Exchange Capacity (C. Mole $Proton^{+}$ / kg)	21.6	20.5	21.8	26.3
Exchangeable Bases (C. Mole $Proton^{+}$ / kg)					
13.	Calcium	12.6	13.2	15.6	13.2
14.	Magnesium	10.3	10.8	11.2	10.3
15.	Sodium	2.36	2.54	2.19	2.57
16.	Potassium	0.23	0.25	0.26	0.24

Table : 3. The correlation coefficient between the physico-chemical characters and total number of colonies in Karankadu.

	pH	EC	OC	OM	AN	AP	K	AZ	AC	AI	AM	CEC	C	M	S	P	TNC
pH	1																
EC	0.786	1															
OC	-0.089	-0.202	1														
OM	-0.089	-0.202	10.000**	1													
AN	0.800	0.949	-0.476	-0.476	1												
AP	0.878	0.395	0.051	0.051	0.447	1											
K	0.909	0.965*	-0.263	-0.263	0.967*	0.602	1										
AZ	-0.243	0.390	-0.412	-0.412	0.365	-0.669	0.184	1									
AC	-0.938	-0.711	-0.260	-0.260	-0.625	-0.854	-0.801	0.350	1								
AI	0.237	0.751	-0.539	-0.539	0.763	-0.237	0.618	0.880	-0.069	1							
AM	0.073	0.383	0.705	0.705	0.075	-0.163	0.197	0.274	-0.338	0.218	1						
CEC	-0.550	0.081	-0.028	-0.028	-0.042	-0.876	-0.173	0.888	0.515	0.593	0.484	1					
C	-0.763	-0.994**	0.105	0.105	-0.910	-0.368	-0.942	-0.390	0.724	-0.730	-0.479	-0.120	1				
M	-0.414	-0.884	0.348	0.348	-0.836	0.069	-0.755	-0.776	0.309	-0.962*	-0.402	-0.507	0.879	1			
S	0.332	0.642	0.533	0.533	0.369	0.017	0.482	0.328	-0.531	0.406	0.954*	0.402	-0.720	-0.607	1		
P	-0.573	-0.908	0.552	0.552	-0.948	-0.141	-0.856	-0.640	0.385	-0.928	-0.133	-0.262	0.873	0.948	-0.395	1	
TNC	-0.160	-0.485	-0.637	-0.637	-0.188	0.115	-0.303	-0.317	0.400	-0.305	-0.994**	-0.477	0.575	0.494	-0.981*	0.241	1

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RESEARCH ARTICLE

Growth and Reproduction Rate of *Eisenia fetida* (Savigny) During Vermicomposting of Banana Agro Wastes.

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ABSTRACT

The goal of this work was to investigate the potential of banana-agro wastes mixed with cow dung to influence the growth and reproduction rate of *E. fetida* during vermicomposting. Banana-agro wastes (BW) with Cow dung (CD) mixture were taken in different ratio namely T_1, T_2, T_3, T_4 and T_5. The maximum growth and reproduction rate (6.08 ± 0.05 mg wt. worm^{-1} day^{-1} and 4.93 ± 0.4 cocoons/worm) were observed in T_4 (0.40 kg BW + 0.60 kg CD) and the minimum (4.31 ± 0.04 mg wt. worm^{-1} day^{-1} and 1.06 ± 0.1 cocoons/worm) were observed in T_1 (1 kg BW alone). The earthworm morality was higher (39.2 ± 3.2) in treatment which is containing 1 kg BW alone (T_1) and the minimum (9.6 ± 1.3) mortality rate was observed in T_4. Hence, the present study revealed that greater proportion of BW in feed mixtures significantly affected the growth and reproduction of *E. fetida* during vermicomposting. This study also clearly indicates that BW could be potentially useful as raw substrate in vermicomposting if mixed with cow dung in appropriate quantities.

Key words: Vermicomposting, *Eisenia fetida,* Banana-agro wastes, Cocoons, Reproduction.

INTRODUCTION

Banana is an important food crop of the world which is cultivated over an area of more than four million hectares and its annual production is more than seventy million tonnes. India is one of the leading producers of Banana. Particularly, Tamilnadu is having first rank in the banana production. After the harvesting of fruits, the whole plants are left in the field which takes several months for their natural degradation. Earthworms are employed to convert the organic waste materials into vermicompost, excellent organic manure [1]. Several epigeics (*Eudrilus eugeniae,*

Eisenia fetida, Perionyx excavatus) have been identified as a potential candidates to decompose organic waste materials [6].Manivannan *et al.* (2004) has studied the growth, reproduction and life cycle of *E. eugeniae* cultured in sugar industry waste. Many authors have studied the life cycle of the composting earthworm species *E. fetida* [4,5,7].Earthworms have been used in the vermiconversion of urban, industrial and agro-industrial wastes to produced biofertilizers [2,3]. It is well established that a large number of organic wastes can be ingested by earthworms and egested as peat like materials termed as vermicompost.Traditionally vermicompost has been generated with animal manure as the substrate and has been recognized as a good soil conditioner and fertilizer [8]. In recent years, other organic substrates have also been vermicomposted and the products have been found to be as good as the manure based vermicompost [4]. *E. fetida* seems to be the must promising species for vermicomposting under tropical conditions and is extremely prolific for use in vermiculture and very easy to handle and to harvest. The objective of the present study is to determine the best mixture combinations of banana agro waste and cow dung that would support the maximum production of cocoons, rate of hatchlings and growth in epigeic worm, *E. fetida*.

MATERIALS AND METHODS

E. fetida, banana waste (BW) and cow dung (CD)

Healthy clitellate specimen of *E. fetida* used in the experiment were collected from stock culture maintained in the laboratory, Department of Zoology and Biotechnology, A.V.V.M. Sri Pushpam College (Autonomous), Poondi, Thanjavur, India. The banana agro waste (dried leaves and pseudostem) were collected from local farm following harvesting. Fresh CD was procured from an intensively live stocked farm at Poondi, Thanjavur. The BW was cut into pieces of 2-3 cm for the present study before mixing with CD.

Experimental setup

Five feed mixtures having different ratios of BW and CD, including BW alone was established. One kg of feed mixtures (on dry weight basis) was put in each circular plastic container (Vol 10L, diameter 40 cm, depth 12 cm). The composition of the BW and CD in different treatments is given below.

Treatment 1 (T_1) : 1.00 kg BW
Treatment 2 (T_2) : 0.80 kg BW + 0.20 kg CD
Treatment 3 (T_3) : 0.60 kg BW + 0.40 kg CD
Treatment 4 (T_4) : 0.40 kg BW + 0.60 kg CD
Treatment 5 (T_5) : 0.20 kg BW + 0.80 kg CD

The moisture content of the feed in each treatment was maintained at 60-80 per cent periodic sprinkling of adequate qualities of water. All the containers were kept in darkness under identical ambient room temperature (24-28°C). The experiments were replicated thrice for each treatment. At the end of the experiment (after 60 days), the substrate material in each treatment was turned out. The earthworms, hatchlings and cocoons were separated from the feed by hand sorting, after which they were counted and weighed after washing with water and dried by paper towels.

Statistical analysis

All the reported data are the arithmetic means of three replicates. Two-way analysis of variance (ANOVA) was done to determine any significant difference among the treatments analysed during vermicomposting at 0.05 per cent level of significance.

RESULTS AND DISCUSSION

Growth rate of *E. fetida*

The growth rate of *E. fetida* cultured in banana waste (T_1) and different ratios of banana waste (BW) and cow dung (CD) feed mixture (T_2-T_5) for a maximum period of 90 days is presented in Table 1. Changes in growth of *E. fetida* reared in different ratios of BW + CD substrate are also graphically presented in Fig.1-3. *E. fetida* recorded higher biomass in T_4 treatment when compared to all the other treatments. On the day 90, *E. fetida* registered 666.36 ± 11.35 mg; 686.52 ± 10.65 mg; 787.96 ± 12.47 mg; 841.68 ± 9.05 mg and 837.43 ± 11.01 mg respectively in T_1, T_2, T_3, T_4 and T_5 treatments. Among the five treatments, the net individual weight gain of *E. fetida* were in the following order: BW + CW 4:6 ratio (T_4) > BW + CD 2:8 ratio (T_5) > BW + CD 6:4 ratio (T_3) > BW + CD 8:2 ratio (T_2) > BW alone (T_1). In the present study, the maximum net individual weight (547.47 ± 4.7 mg) and the maximum mean growth rate (6.08 ± 0.05 mg wt. worm^{-1} day^{-1}) of *E. fetida* were observed in T_4 treatment followed by T_5, T_3, T_2 and T_1. Statistically, the growth rate of *E. fetida* showed significant difference among the different treatments (T_1-T_5). However, *E. fetida* did not show any significant difference among T_5, T_4 and T_3 treatments. Suthar (2009) found that the growth of earthworms depends on the quality of the available food and adequate temperature and moisture. Murchie (1960) experimentally proved the existence of a significant relationship between weight increase and substrate type, which may reasonably be attributed to nutritional quality of the substrate.

In the present study, there was a consistent pattern in worm growth tend to be decreased with increasing BW concentration in treatments, excepting T_5, T_4 and T_3. The decreasing composting efficiency in treatments with higher contents of BW was due to changed chemical environment in the substrate, which possibly affected the earthworms potential and survival rate. Since, organic matter content plays an important role in decomposition process due to its direct relation with microbial population and their mineralization activities.

Reproduction rate of *E. fetida*

The total number of cocoons after 90 days in different treatments has been represented in Table 2 and Fig.4-7. The maximum number of cocoons (98.64 ± 8.5) were observed in T_4 treatment and minimum (21.2 ± 1.3) were in T_1 treatment. A comparison of the data revealed that the cocoon numbers and hatchling production was not statistically different among the treatments T_5-T_3, inferring that upto 40 per cent addition of CD in BW can support effective reproduction rate in earthworm. *E. fetida* showed significant differences among T_1 and T_2 treatments in reproduction rate. Similarly, the maximum reproduction rate was registered by *E. fetida* in T_4 treatment (4.93 ± 0.4) and minimum in T_1 (1.06 ± 0.1). The maximum number of hatchings production by *E. fetida* was recorded in T_4 (67.4 ± 5.4) followed by T_5 (67.3 ± 2.7), T_3 (63.2 ± 6.7), T_2 (16.7 ± 0.2) and T_1 (14.3 ± 2.1) treatments. The maximum mortality (Percentage of initial population) was recorded in T_1 treatment (39.2 ± 3.2) followed by T_2, T_3, T_4 and T_5 treatments. Julka *et al.* (2009) reported that the reproductive potential of earthworm was influenced by the quality and availability of food. Tripathi and Bharawaj (2004) have studied the growth rate, rate of maturation, cocoon production, the hatching success cocoons under controlled laboratory conditions. Garg and Kaushik (2005)[5] found that *E. fetida* showed that more mortality in beddings, which contained lower amounts of organic supplements in textile mill sludge vermibeds. The results clearly suggest that mixing of cow dung in waste not only support earthworm growth and reproduction, but at the same time also lowers the risk of earthworm mortality during the process of vermicomposting. In the present study, the reasons for the enhanced cocoon production and hatchability rate of earthworms seems to be due to rich nutrient content found in BW + CD mixture, microbial population activity and the enhanced water holding capacity which enable the BW + CD mixture to maintain good and ideal moisture. From these observations it is recommended that the banana wastes could be better vermicomposted by *E. fetida* and could be used for vermiculture practices.

Table:1. Biomass production by *E. fetida* in different treatments of banana agro wastes and cow dung mixture (mean ± S.E.M., n=6)

Treatments	Mean initial individual weight (mg)	Maximum individual weight achieved (mg)	Net individual weight gained (mg)	Growth rate (mg wt. worm^{-1} day^{-1})
T1	278.21 ± 4.37	666.36 ± 11.35^{a}	388.15 ± 6.3^{a}	4.31 ± 0.04^{a}
T2	254.14 ± 7.16	686.52 ± 10.65^{a}	432.38 ± 4.2^{a}	4.80 ± 0.07^{a}
T3	266.80 ± 4.28	787.96 ± 12.47^{b}	521.16 ± 8.2^{b}	5.81 ± 0.09^{b}
T4	294.21 ± 4.35	841.68 ± 9.05^{c}	547.47 ± 4.7^{b}	6.08 ± 0.05^{b}
T5	291.13 ± 7.21	837.43 ± 11.01^{c}	546.30 ± 3.8^{b}	6.07 ± 0.04^{b}

T1: BW alone; T2: BW + CD (80:20); T3: BW + CD (60:40); T4: BW + CD (40:60); T5: BW + CD (20:80)
Mean value followed by different alphabets is statistically different (ANOVA; Duncan's multiple- ranged test, ($p < 0.05$)

Table:2. Reproduction rate and mortality of *E. fetida* in different treatments of banana agro wastes and cow dung mixture (mean ± S.E.M., n=6)

Treatments	Total cocoons obtained at the end	Reproduction rate (cocoons/worm)	Total no. of hatchlings	Total mortality during experimentation (%)
T1	21.2 ± 1.3^{a}	1.06 ± 0.1^{a}	14.3 ± 2.1^{a}	39.2 ± 3.2^{d}
T2	29.51 ± 3.2^{a}	1.51 ± 0.6^{a}	16.7 ± 0.2^{a}	25.7 ± 4.3^{c}
T3	85.61 ± 9.3^{b}	4.28 ± 0.5^{b}	63.2 ± 6.7^{b}	13.1 ± 4.6^{b}
T4	98.64 ± 8.5^{b}	4.93 ± 0.4^{b}	67.4 ± 5.4^{b}	10.1 ± 2.1^{b}
T5	97.53 ± 6.9^{b}	4.87 ± 0.3^{b}	67.3 ± 2.7^{b}	9.6 ± 1.3^{b}

T1: BW alone; T2: BW + CD (80:20); T3: BW + CD (60:40); T4: BW + CD (40:60); T5: BW + CD (20:80)
Mean value followed by different alphabets is statistically different (ANOVA; Duncan's multiple- ranged test, ($p < 0.05$)

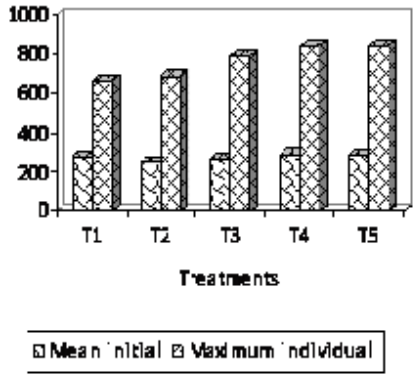

Fig.1. Showing the mean initial and maximum individual weight achieved by *E. fetida.*

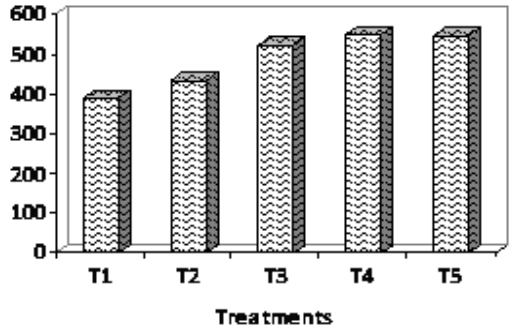

Fig.2. Showing the net individual weight gained by *E. fetida.*

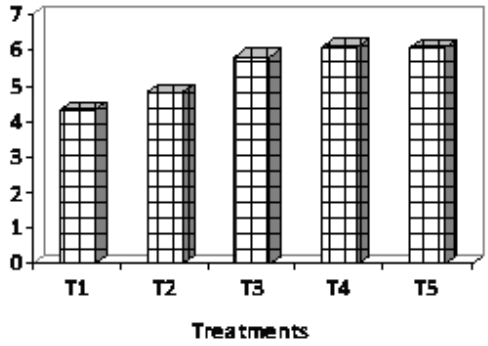

Fig.3. Showing the growth rate of two earthworms.

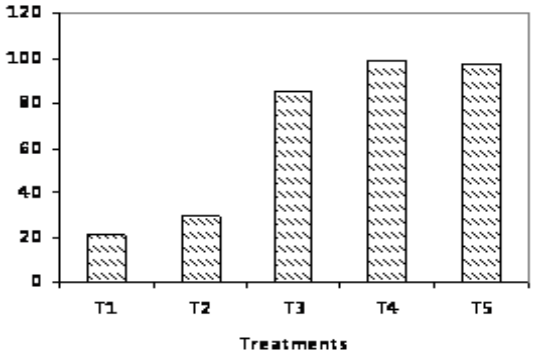

Fig.4. Showing the cocoon production by two earthworms.

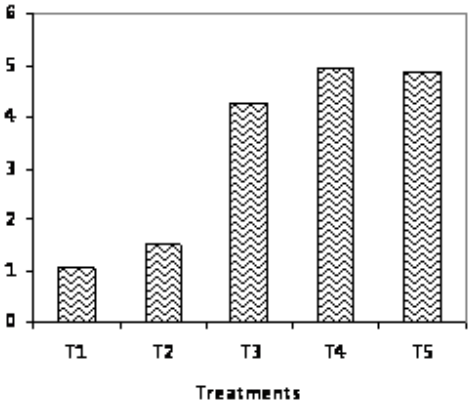

Fig.5. Showing the reproduction rate of *E. fetida.*

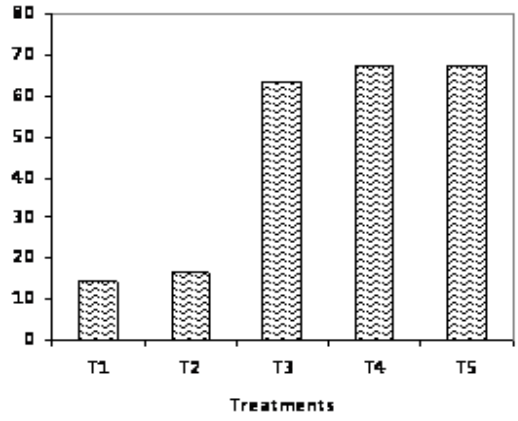

Fig.6. Showing the total number of hatchings produced by *E. fetida.*

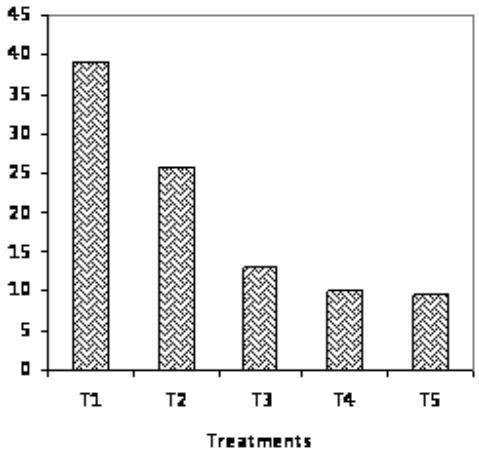

Fig.7. Showing the total mortality rate of *E. fetida*.

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RESEARCH ARTICLE

Masspropagation of *Spilanthes calva* L.through Nodal Explants.

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ABSTRACT

An efficient protocol was devised for rapid propagation of *Spilanthes calva* from nodal explants. MS medium containing different concentration of BAP (4.44 – 17.76 ◎M/l) and Kn (4.65 – 18.6 ◎M/l) in combination with NAA (0.27 ◎M/l) was tested for their efficiency in micropropagation. Proliferation of multiple shoots was very high in BAP (13.32 ◎M/l) followed by Kn (18.60 ◎M/l).The well developed shoots were rooted on MS medium supplemented with IBA (9.80 ◎M/l). All the regenerated shoots were successfully established in natural environment.

Keywords: *Spilanthes calva*, Masspropagation, Micropropagation, Nodal explants

INTRODUCTION

Spilanthes calva L. commonly known as toothache plant is an important medicinal plant of the family *Asteraceae*. It occurs in the tropical areas and subtropical parts of the world. The genus comprises around 60 species [1] spread throughout Mexico and Central Africa, Cuba, Curacau, India and Tanzamia [2] In India the plants have been growing in the northern and southern hills and plateaus. There are five species of *Spilanthes* occurring in India [3]. *S. calva* have shown anti-inflammatory, antibacterial and antifungal properties. Traditionally, this plant is also used in treatment of toothache, throat infection and gum diseases [4][5][6][7][8]With the worldwide increasing demand for plant derived medicines, there has been a concomitant increase in the demand for raw material. However, the increasingly human and livestock populations have affected the status of wild plants, particularly those used in herbal medicine [9]. Plant tissue culture is a useful tool for the conservation and rapid propagation of medicinally important and endangered plants [10][11][12]. *Spilanthes calva* is one such plant to be conserved through *in vitro* techniques. There are few reports available on regeneration of *Spilanthes acmella* from leaf explants [13], axillary bud [14] and cell layer [15]. To the best of our knowledge, no report is available on *in vitro* propagation of *Spilanthes calva* L. The present study was carried out to investigate the possibility of micropropagation of *S. calva* that could be conserved for future.

MATERIALS AND METHODS

Nodal explants of *S. calva* L.were collected from the healthy mother plants of natural population. The explants were washed with running tap water for 30 minutes to remove the surface adherents. Surface sterilization was carried out by an initial dip in 70% alcohol for few seconds followed by a treatment with 0.1% (w/v) $HgCl_2$ for 3 minutes and thoroughly rinsed several times in sterile distilled water. Explants were cultured on MS [16] medium supplemented with different concentration of BAP (4.44 – 17.76 ⊚M/l) and Kn (4.65 – 18.60 ⊚M/l) in combination with a stable concentration of NAA (2.69 µM/l). The pH of the medium was adjusted to 5.6 prior to autoclaving at 121°C for 15 minutes. The regenerated shoots were transferred to a rooting medium containing IBA (4.90 – 14.70 ⊚M/l). The rooted plantlets were acclimatized and established in the field.

RESULTS AND DISCUSSION

Nodal explants were capable of directly developing multiple shoots on MS medium containing different Cytokinins (BAP & Kn) Multiple shoots initiated from nodal explants in both Cytokinins within 15 days after inoculation. Initially two shoots developed from the nodal explants (Fig a), later the number of shoots increased to 49 at 13.32 µM/l concentration of BAP. On the other hand in Kn, a maximum of 26 shoots were obtained at 18.6 µM/l concentration (Table 1 and Fig b).Sharon Marie (2000) [17] reported that the nodal explants were preferred over meristem to produce large number of genetically identical clones in *Bixa ovellana* L.The potential of shoot proliferation from nodal explants of *S. calva* depended on plant growth regulators.Pawar PK *et al* (2002)[18] reported that BAP and Kn individually and in combination induced a higher frequency of adventitious shoots from leaf explants of *Solanum xanthocarpum*. In the present investigation, the relative effectiveness of BAP and Kn varied for *in vitro* multiplication of shoots from nodes. BAP was found be the ideal Cytokinin for shoot proliferation in *S. calva* than Kn which was less effective. Similar observation was made by [19].The elongated shoots were harvested periodically and transferred to a rooting medium (MS) fortified with different concentrations (4.90 – 14.70 µM/l) of IBA. Roots initiated from the base of shoot after 10 days of incubation. Of the various concentrations of IBA, 9.80 µM/l was found to be suitable for root induction (Table 2 and Fig C). Similar effect of IBA was reported in *Datura metel* [20] and *Hybanthus enneaspermus* [21].The regenerated plantlets were transferred to plastic cups containing sterilized vermiculite and nourished with half strength MS liquid medium for 10 days. Later they were transferred to polythene bags containing a soil mixture of red and sand (1:1) (Fig d). They were supplied with tap water for a few days and successfully established in the field. The protocol described in this paper would facilitate the rapid *in vitro* propagation of this important medicinal plant.

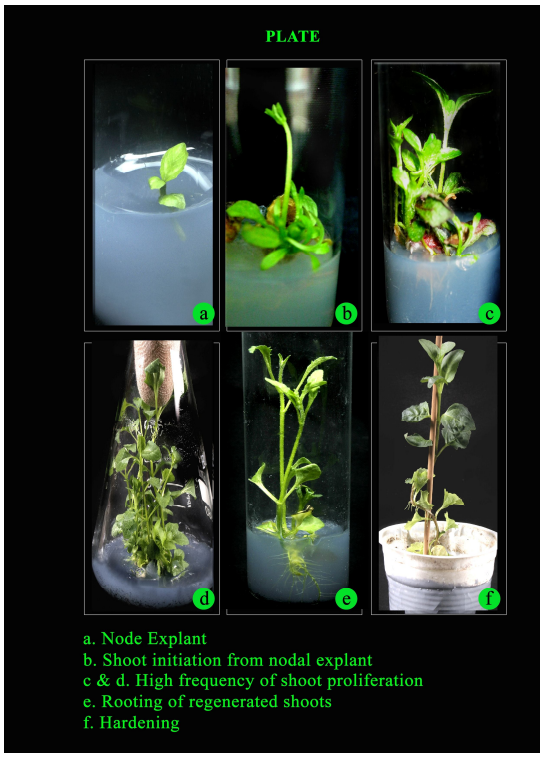

Table 1: Effect of cytokinin on multiple shoot proliferation from nodal explants of *Spilanthes calva* L.

Plant growth regulators			Percentage of shoot proliferation	Shoot elongation (cm)	No. of shoots/explant (Mean±SD)
BAP	Kn	NAA			
4.44		2.69	59.8	4.0	24.0±2.94
8.88		2.69	70.0	4.2	35.0±2.45
13.32		2.69	88.4	6.8	49.0±2.0
17.76		2.69	66.2	5.4	30.0±2.90
	4.65	2.69	48.6	3.8	21.3±2.49
	9.30	2.69	58.8	4.6	23.0±2.16
	13.95	2.69	68.4	5.2	35.3±1.88
	18.60	2.69	70.2	5.8	37.6±1.69

Table 2: Effect of IBA on root induction from regenerated shootlets.

Plant growth regulators - IBA (⚜M)	Percentage of root induction	Root length (cm)	No. of roots/explan Mean±SD
4.90	56.2	3.4	3.33±1.25
9.80	75.4	5.8	10.0±1.63
14.70	64.8	3.2	5.6±1.24

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RESEARCH ARTICLE

Laboratory Culture of Marine Cyclopoid Copepod *Oithona rigida* Giesbrecht.

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ABSTRACT

It is proved that the failure of quality seed production for commercial fish occurs due to the lack of nutritionally balanced live feeds. Hence, the developing nutritionally superior live feed is timely required one. It is well accepted that the marine copepods play an important role in the first feeding of fin and shellfish larvae owing to their nutritionally superiority especially in HUFA, PUFA for the need of fish larvae. Furthermore, copepod has many life stages including 6 nauplii, 6 copepodite including adult, so larvae with different mouth sizes are benefitted in size and nutritionally profile. Hence the present attempt was made to culture the marine cyclopoid copepod *Oithona rigida.* in laboratory scale with the controlled water quality parameters viz., temperature, salinity, pH and dissolved oxygen in the ranges of 26-34^{o} C; 26-35‰; 7.5-8.5; 5.0-7.5 ml/l respectively at low cost technique fed with mixed marine microalgae. Present culture system produces 44,871.02 L^{-1} that including nauplii, copepodite and adults for 35 days culture period.

Keywords: Marine copepod, *Oithona rigida* Giesbrecht., Microalgae, Temperature, Salinity.

INTRODUCTION

The major problem in Indian aquaculture system is lack of good quality seed and cost-effective and balanced feed in hatchery and farming level. Successful culture of commercially important aquatic species essentially depends on the initial feed for its larvae and juvenile [1]. Nowadays, in fish and shrimp hatcheries *Artemia* and rotifer being used as initial feed for larval rearing. Unfortunately the traditional live feeds such as *Artemia* and rotifer are fail to prove in nutritional efficiency and must be enriched with fatty acids to improve their nutritional quality before being feed to

marine fish larvae. Moreover, most of the marine fin fish larvae mouth size was smaller than *Artemia*. Hence it needs to be identified the alternative live feed which have different size ranges [2]. Marine copepods are fulfilling nutritional related issues and it provides several stages and sizes (6 nauplii, 6 copepodite including adult). When copepod passes through the gut of fish larvae, it can be digested more quickly compared to *Artemia* sp. which can leads to uptake more prey in fish larvae [3].

Marine copepods are considered as precious one in aquaculture [4]. Copepods make up over 70 percent of animals in ocean. The collection of copepod from wild is a time consuming job and there are lot of other contamination of zooplankters also possible. To avoid these problems, copepod culture should be standardized for sustainable aquaculture. Therefore, the present study was aimed to culture the copepod *Oithona rigida* at laboratory scale level.

MATERIALS AND METHODS

Collection and identification of copepod

The zooplankton samples were collected from the Muthupet lagoon by using standard plankton net with 158μm mesh. The collected samples were immediately transported to the laboratory by providing with vigorous aeration using battery aerator. In the laboratory, the zooplankton sample was thoroughly rinsed and graded through the superimposed sieves to reduce the concentration. From the diluted samples, *Oithona rigida* was identified under the microscope using the key of [5 -7].

Copepod culture

The 280 individuals of female and male cyclopoid copepod *Oithona rigida* were isolated and stocked in 80 ltr. of seawater in 100 litres FRP tank at outdoor. The stock cuture of *O.rigida* was maintained at indoor condition in 200 ml glass beakers. The water quality parameters such as temperature, salinity, pH and dissolved oxygen were maintained in the range of: 26-34^{o} C; 26-35‰; 7.5-8.5; 5.0-7.5 ml/l respectively during culture period and fed with a daily ration of mixed Microalgae viz., *Chlorella marina, Nannochloropsis* sp, *Isochrysis galbana, Dunaliella salina* and *Tetraselmis* sp, in the concentration of 30,000 cells/ml.The cultures were harvested at every 12 days by gentle siphoning and photoactic separation using halogen light. Finally the adult gravid female copepods were used to restart stock culture. Water quality parameters such as temperature, salinity, pH, Dissolved oxygen and the population density of nauplii, copepodite and adults of *Oithona rigida* were observed daily. Utmost care has been taken during the time of copepod culture to avoid contamination with rotifers and ciliates.

Microalgal culture

The marine microalgae *Chlorella marina, Nannochloropsis sp, Isochrysis galbana, Dunaliella salina* and *Tetraselmis* sp. strain was obtained from the Central Institute of Brackishwater Aquaculture (CIBA), ICAR, Chennai. The indoor algal stock culture was maintained in special air conditioning room to feed copepod. Stock cultures were kept in 1 and 2 liters culture flasks, 5 and 15 liters plastic containers. The seawater was filtered by using filter bag (1 micron), the filtered seawater was sterilized by using autoclave and after cooling water was transferred to the culture flask. Culture flasks are plugged with cotton or covered by aluminum foil. All vessels are used for algal culture was sterilized properly and dried in an oven before use. The Conway's medium was used for indoor culture.

RESULTS

Culture of copepod *O.rigida*

The favorable result on the total density of *Oithona rigida* was obtained at the temperature of 26-34° C, salinity 26-35 ‰ and food concentration 30,000 cells/ ml using mixed microalgae. Over 12 days culture, the culture system produced an average of 2126.54 nauplii L^{-1}, 1016.58 copepodids L^{-1} and 624.89 adults L^{-1} on the 12th day. For the entire 35 days culture period, the total mean production was 44,871.02 L^{-1}, comprising 19,026.54 nauplii, 14,256.89 copepodids and 11,589.64 adults L^{-1}. The detailed result on population density of different stages of copepod *O.rigida* was shown in Fig. 1. The maximum mean density of *O. rigida* was recorded as 4524.9 nauplii L^{-1}, 2900.32 copepodids L^{-1} and 1906.23 adults L^{-1} on 10, 12 and 12th day(s) of culture respectively (Fig. 2).

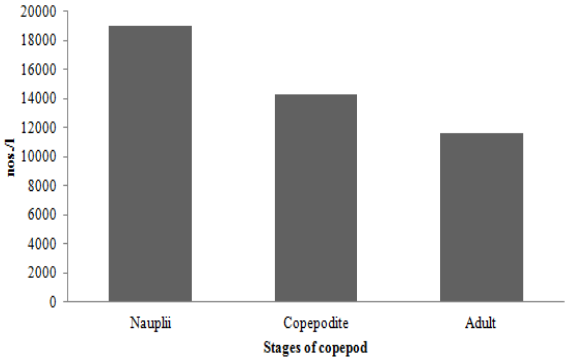

Fig. 1 Total density of *O.rigida*

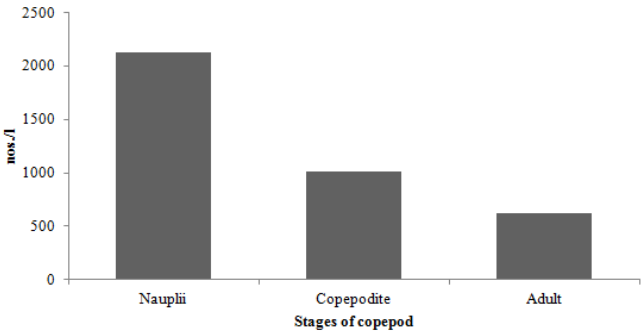

Fig. 2 Maximum density *of O. rigida*

DISCUSSION

In the present attempt only 280 adult copepods were used to start the culture. Nauplii and copepodite were not used due to problem in separation. Total density of *O. rigida* was reported as 44,871.02 L^{-1} within 35 days culture period, this is relatively higher than the earlier reports of [8,9].Authors reported 41,603 org. /l in two months culture period. The average production of nauplii and copepodids of our study was similar to [10] in 12 days of culture. The production rate of copepod, *O.rigida* exceeded than those reported for *Acartia* sp. by [11]. The recorded density of *O.rigida* was moderately higher than that of earlier worker of [12] in the same species. The variation obtained in the present study might be due to the different environmental conditions maintained during the culture period [13] and diverse algal feed used [14-18]. In our study, the maximum density of 44,871.02 L^{-1} was yielded in 35 days culture period which is dissimilar to [12] who achieved the maximum density of 33,867 ind./l on same species for 2 months culture period.

It is understood that the temperature and salinity are the two most important environmental parameters affecting the seasonal and spatial distribution of marine copepods in the wild [19]. Temperature and salinity play a major key role to maintain the growth, survival and reproduction in captive condition too. The present study indicated that the presently provided temperature (26-34°C) is found to be suitable to yield maximum density for *O.rigida*. It is accepted that the egg production and hatching rate of copepod are normally lower at low temperatures [20]. Chinnery and Williams [21] have found that the nauplii of *A. discaudata, A. clausi, A. tonsa* and *A. bifilosa* had improved survival and developed faster as temperature increased from 5 to 20 °C. Similarly the egg production, hatching rate, development and survival was diminishing as temperature rises beyond a certain level [12]. The finding of the present study was agreed by earlier workers [12, 19] who stated that the temperature between 26-34°C survival and production rate is extremely high.

Salinity is another important environmental variable that can influence copepod production and nauplii survival [19].Estuarine copepods can tolerate different ranges from 5-30‰. *Oithona rigida* can also survive in the salinity ranged between 5 and 40‰. However, the ultimate salinity to yield maximum population of *O.rigid*a was found to report 26-35‰. Tackx and Polk [22] have found that within the salinity range of 9-35 psu, the *Gladioferens imparipes* was surviving whereas the maximum density was obtained at an intermediate range of 18–27 psu. However, Chinnery and Williams [21] have found that the egg hatching rates of four calanoid species, *A. discaudata, A. clausi, A. tonsa* and *A. bifilosa,* were not significantly affected by salinity between 15.5 and 33.3 psu, and they remained relatively high at all salinities. The variations in salinity also affect the growth and development time, with lower salinity the copepods showing the slower development. In the present study *O.rigida* was able to survive in low and high salinities, but the growth and production was obtained more in the salinity between 26 and 34‰. This result indicated that the salinity also has strong contact on the growth and production of any copepods.

Along with other water quality parameters, the algal food also played an important and major role in copepod culture system. Presently observed maximum density in *O.rigida* could be influenced by food. In the present study, mixed algal feed gives a good and healthy result at the concentration of 30,000 cells/ ml. In the present experiment, the successful rearing of copepod, *O.rigida* has been accomplished by providing mixed microalgae with high concentration, which results the higher density. This is in agreement with earlier works of [23] and [24]. In response to being supplied with high concentration of algal food, the copepods had the highest survival rate. But in low food concentration survival is comparatively low because of the food scarcity [10]. In some cases, the over feeding of microalgae may be results the high pH which can reduce the population of copepods when the copepods uptake of food (Personnel communication).

The cyclopoid copepod *O.rigida* can tolerate at different ranges of salinity and temperature. While in low temperature they cannot show any development and metabolism, it clearly has a less pronounced effect on survival than salinity [21]. In the present study, maximum density of *O.rigida* performed in maximum salinity and generally decreased with reduced salinity. The present study concluded that the economically practicable and more numbers of copepod, *O.rigida* can be possibly produced if it reared under optimal water quality and food conditions. Due to fact that the above *O.rigida* can be considered as a better candidate species for mass propagation and further use as live-feed for the mass rearing of fish larvae.

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RESEARCH ARTICLE

Short Term Investigation on Vertical Distribution of Physico-Chemical and Phytoplankton Biomass in Pambanar Estuary, Southeast Coast of India.

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ABSTRACT

The present investigation deals with the perpendicular (Surface, Middle and Bottom) variations on the physico-chemical and chlorophyll 'a' concentration of neritic (Palk Bay) and estuarine (Pambanar) waters of Muthukuda, Southeast coast of India. In this study we have examined the physico-chemical parameters such as water temperature, salinity, pH, and inorganic nutrients *viz.,* nitrate, nitrite, phosphate ammonia and reactive silicate concentration for a period of four months (January-April 2012). The study inferred that the water temperature, salinity, pH, total hardness and calcium hardness was varied from 26.9-31.0^{o}C, 25-32‰, 7.05-7.86, 51.5-56.8 mg/l and 43.7-53.3 mg/l respectively. The concentration of inorganic nutrients such as nitrate (0.02 to 2.73µmol/l), nitrite (0.02-2.12µmol/l), ammonia (0.06 and 13.92µmol/l), phosphate (0.05 to 8.56µmol/l) and reactive silicate (0.06 to 25.45µmol/l) were found to be varied in respect to depth of water. Chlorophyll 'a' concentration was noticed in the range between 0.001 and 0.062µg/l. The present study obviously indicated that the physico-chemical and phytoplankton biomass (chlorophyll 'a') varied autonomously in subject to the depth of coastal system.

Keywords: Physico-chemical, Chlorophyll 'a', Palk Bay, Pambanar estuary, Muthukuda.

INTRODUCTION

Phytoplankton forms the vital source of energy in the coastal and marine environment. They initiate the marine food chain, by serving as food to primary consumers, which include zooplankton, shellfish, finfish and others [1,2].The pelagic algal communities make an important contribution to the smooth functioning of coastal ecosystem. The

plankton in mangrove habitats contributes from 20 to 50% total fish productivity [3]. Fertility and healthiness of mangrove environment is reflected through productivity of the phytoplankton and zooplankton as primary and secondary producers. Plankton is very sensitive to the environment alteration which leads to the change in the plankton communities in terms of tolerance, abundance, diversity and dominance in the habitat. Therefore, observation on plankton population may serve as a reliable tool for biomonitoring studies to assess the pollution status of aquatic bodies [4]. The study of plankton as an index of water quality with respect to industrial, municipal and domestic pollution [5,6].The biomass distribution and species composition of phytoplankton have important effects on carbon fixation rates and on transfer of energy in food webs. Studies on the abundance, distribution and composition of phytoplankton communities are, therefore, a fundamental contribution to our understanding of the structure and function of estuarine ecosystems.Phytoplankton is multispecies communities, which are highly multifaceted in terms of their diversity and dynamics. Succession shifts in phytoplankton community structure are mainly due to changes in environmental variables such as nutrients and other physico-chemical variables which influence the distribution and abundance of plankton communities in estuaries [7-9].The planktonic study is very useful tool for the assessment of water quality and fishery potential of any ecosystem [10]. Species composition and seasonal variation in phytoplankton abundance has been studied in other regions of Indian coastal waters [11-18]; However studies on phytoplankton biomass in the Palk Bay mangrove belt of Muthukuda, Pudukkottai district of Tamil Nadu state is absent. Hence, the present short term study was undertaken to investigate the phytoplankton biomass in Muthukkuda region for the period of four months from January- April 2012 at different depths.

MATERIALS AND METHODS

The sampling site (Muthukuda) is located along the Palk Bay in the Pudukkottai district of Tamil Nadu, Southeast coast of India (Lat. 9.8^{o} N and Long. 79.1^{o}E). Water samples were collected from two stations viz. Station-1 (Palk Bay) and Station-2 (Pambanar estuary) for the period of four months from January to April 2012 in different depths viz. surface, middle and bottom using 5 liter capacity Niskin water sampler for the estimation of physico-chemical and chlorophyll 'a' concentrations. The water temperature was measured using a standard centigrade thermometer. Salinity was estimated with the help of hand refractometer (ERMA, Japan).The pH was measured using an ELICO grip pH meter. For the nutrient analysis, surface water samples were collected in polyethylene bottles and kept immediately in an icebox and transported to the laboratory for analysis. Then the water samples were filtered using a Millipore filtering systems and analysed for inorganic nitrate, nitrite, phosphate, reactive silicate and ammonia adopting the standard procedures described by [19,20]. For the estimation of water hardness, the water sample was buffered to pH 10.1 and indicator was added to the buffered sample. The indicator, when added to a solution containing Ca and Mg ions, turns red. The EDTA, the titrant and complexes with Mg and Ca cations removing them from association with the indicator. When all the Mg and Ca are complexed with EDTA, the indicator turns blue. For chlorophyll 'a' estimation, the five liters of water samples were collected, from these 250 ml of water were filtered using the Millipore filtering unit through a membrane filter (47 mm or 0.47 µm pore size). The pigments were extracted from the concentrated algal sample in an aqueous solution of acetone. Chlorophyll '*a*' concentration was determined spectrophotometrically by measuring the absorbance (optical density-OD) of the extract at various wavelengths. The resulting solution absorbance measurements were then applied to a standard equation adopted by [21].

RESULTS AND DISCUSSION

The detailed findings of depth wise variations on the physico-chemical Parameters of Muthukuda coastal waters were shown in Fig.2. The water temperature was varied from 26.9 to 31.0^{o}C. Usually, coastal water temperature is influenced by the intensity of solar radiation, evaporation, freshwater influx and cooling and mix up with ebb and flow from adjoining neritic waters [22]. Presently, water temperature was low during the month of January at both the stations because of strong land sea breeze and rain and recorded high value during summer (April, 2012) could be attributed to high solar radiation [23-27]. Hydrogen ion concentration (pH) of the study area remained alkaline

throughout the study period at both the stations. The maximum pH of 7.86 was noticed at surface water of station 2 during the month of April, 2012 while minimum of 7.05 was recorded at both surface and middle layer of station 1 in February, 2012. Generally variation in pH is endorsed to factors like removal of CO_2 by photosynthesis during bicarbonate degradation, dilution of seawater by freshwater influx, low primary productivity, reduction of salinity and temperature, and decomposition of organic matter [28,29]). The recorded high summer pH might be due to the influence of seawater penetration and high biological activity [30]) and due to the occurrence of high photosynthetic activity [18,31].

The recorded salinity was varied from 25 to 32‰. The higher salinity (32‰) was obtained during the month of April, 2012 at surface waters of station 1 whereas least salinity (25‰) was recorded during the month of January, 2012 at bottom waters of station 2. The recorded maximum values could be attributed to high rate of evaporation besides high temperature as agreed by earlier workers [32,18,27]. The salinity of the study area was found to be lower during the month of January, 2012 (post monsoon season) owing to low evaporation rate due to low temperature besides cloudy sky and more freshwater discharges from catchment areas after monsoonal rainfall as agreed by [27]. The total hardness over the four months sampling period shows a variation from 51.5 to 56.8 mg/l. This hardness of water is due to the chlorides and sulfates of calcium and magnesium in the water. The recorded calcium hardness was varied between 43.7 and 53.3 mg/l. The monthly values of magnesium and calcium content in estuarine and adjacent neritic water differ from one another. In the present study it has been clear that the total hardness and calcium and magnesium hardness reported to be higher at station 2 (Pambanar estuary) than that of station 1 (Palk Bay) could be attributed to discharges of calcium and magnesium ions from the catchment areas like aquaculture and agriculture farming systems where lime and pesticides being used in an objective for better production. Total hardness is mainly due the presence of ions like calcium (Ca^{++}) and magnesium (Mg^{++}) [33].

The behaviour of nutrients in estuarine waters may be non-conservative, as they are either utilized biologically in productive estuarine areas or are removed a biologically in certain other areas due to various physico-chemical processes. Elements like nitrogen, phosphorus and silicon play a major role in the marine eco-systems as they make up the basic nutrients required by primary producers. These elements occur in the form of nitrates (NO_3-N), nitrites (NO_2-N), phosphates (PO_4-P), ammonia (NH_3-N) and silicates (Si_2O_3-Si).The nitrate-nitrogen content of four months study reported in the range between 0.02 and 2.73µmol/l (Fig. 1) with an average of 0.68µmol/l in the water column (Table 1). Monthly observations thus showed high values of nitrate at the station-1 than station-2. Generally the nitrate concentration was found to be more at bottom water than surface and middle depth. The maximum nitrate concentration (2.73µmol/l) was observed during the month of January in bottom water at station-1. The present result clearly indicates that the nitrate level increases due to mangrove litter fall decomposition and terrestrial run-off [24]. Another promising way of nitrate entry in to coastal ecosystem is by oxidation of ammonia and nitrite [34]. The low values (0.02µmol/l) recorded during April might be due to its utilization by phytoplankton as evidenced by high chlorophyll content recorded and also due to the neritic water control, which contained insignificant amount of nitrate [35,23,30].

The monthly variation of nitrite-nitrogen observed in the range between 0.02 and 2.12µmol/l with an average of 0.42µmol/l (Fig. 1). The nitrite content was found higher at station-2 (2.86µmol/l) than station-1 (0.87µmol/l). The high values indicated that the external additions from the mangrove litter fall might increases the nitrite concentration at station 2.The nitrite concentration was noticed to be high at bottom water than that of surface and medium water.The above cited nitrite values recorded high during the month of January could be due to the increased phytoplankton excretion, oxidation of ammonia and reduction of nitrate and by recycling of nitrogen and bacterial decomposition of planktonic detritus present in the environment [36,30]. Further, the denitrification and air-sea interaction exchange of chemicals are also responsible for these increased values [37,38].Nutrients are considered as one of the most important parameters in the mangrove environment influencing growth, reproduction and metabolic activities of living being. Distribution of nutrients is mainly based on the season, tidal conditions and freshwater flow from land source [22]. In the present study, the inorganic phosphate was obtained in the range from 0.05 to 8.56µmol/l at both the stations includes three depths during January to April, 2012. The lowest value (0.05µmol/l) was record in summer

season (April, 2012) at surface water in station-1 attributed to the limited flow of freshwater, high salinity and utilization of phosphate by phytoplankton [25,34]. High concentration of inorganic phosphate (8.56µmol/l) was noticed during post monsoon season (March, 2012) might be possibly due to intrusion of freshwater into the creek and coastal sea which increased the level of phosphate [39].The recorded NH_3-N concentration was ranged between 0.06 and 13.92µmol/l with an average of 2.08µmol/l. The maximum concentration (13.92µmol/l) of ammonia was recorded at station-2 during January and March, 2012 in both surface and bottom water sample owing to freshwater runoff and organic matter decomposition and lowest (0.06 µmol/l) ammonia recorded at surface water sample during the month of January. Ammonia content was comparatively higher in bottom water than surface and middle water. Mostly, the station-2 showed higher ammonia compared to station-1. This clearly indicated that mineralization of ammonia from the mangrove litter fall and an organic matter oxidation of dead plant and animal mater associated with mangrove vegetation along the banks of Pambanar estuary. The silicate content was higher than that of the other nutrients like NO_3, NO_2 and PO_4 [27] and this present investigation observed high silicate values (25.45µmol/l) in February at bottom water samples. It may be due to heavy inflow of post monsoonal (end of rainy season) freshwater derived from land drainage carrying silicate leached out from rocks and agriculture lands.

Moreover, due to the turbulent nature of water, the silicate from the bottom sediment might have been exchanged with overlying water in this mangrove environment [34]. Besides this, the dissolution of particulate silicon carried by the river, the removal of silicates by adsorption and co-precipitation of soluble silicate silicon with humic compounds and iron [34]. The low silicate concentration (0.06µmol/l) was recorded at station 1 during April in surface water sample may be attributed to uptake of silicate by phytoplankton for their biological activity [40,41]. The overall trend indicated that the maximum value of silicate was noticed at station-1 might be due to upwelling and down welling process, indicating a continuous circulation of silicate by wave action in the open sea compared to estuarine region of Muthukuda. Chlorophyll 'a' is considered as an index of biological productivity. Based on the chlorophyll 'a' concentration the productivity of a estuarine and neritic waters can be estimated. The chlorophyll 'a' observed in two stations for four months was ranged from 0.001 to 0.062µg/l (Fig. 1)**.** The maximum chlorophyll 'a' concentration (0.619µg/l) was recorded at station-2 in surface water sample during the month of April, 2012 whereas the lowest chlorophyll 'a' (0.018µg/l) concentration was found in February at station-1. The chlorophyll 'a' were found to be high at station-2 compared to station-1 might be due to presence of more phytoplankton owing to rich organic matter supported by mangrove vegetation located along the banks of station 2 (Pambanar estuary). In the present study the chlorophyll 'a' concentration were reported to high during April, 2012 (summer season) and at surface water whereas the low chlorophyll 'a' value have been noticed during February , 2012 at bottom water. The chlorophyll 'a' concentration of both the stations shows strong positive correlation with salinity while negatively correlated with temperature and nutrients in respect to depths (Tables 2 & 3). The presently recorded high summer and surface chlorophyll 'a' might be due to high light intensity, clear water condition as opined by [17].

CONCLUSION

The present short term investigation on depth-wise distribution of physico-chemical and chlorophyll 'a' concentration of Muthukuda coastal waters concluded that the Pambanar estuary and adjacent neritic waters (Palk Bay) can considered as one of the productive coastal area for phytoplankton productivity. Further it is also evident that the rich mangrove vegetation along with seagrass meadows distributed in the area can supports the biogeochemistry of the study area. Further detailed long term studies are required for understanding of nutrient dynamics and primary and secondary productivity status of this coastal water for the continuous monitoring and assessment.

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Table1.Mean values of depth-wise physico-chemical and chlorophyll 'a' concentrations of Muthukuda coastal waters

	Water line	Temperature (°C)	pH	Salinity (‰)	Hardness (mg/l)		Phosphate (µmol/l)	Nitrate (µmol/l)	Nitrite (µmol/l)	Ammonia (µmol/l)	Silicate (µmol/l)	Chlorophyll 'a' (µg/l)
					Calcium	Total						
Station - 1	Surface	29.00±1.41	7.40±0.33	28.50±2.38	48.13±3.36	54.46±0.62	0.78±1.24	0.80±1.28	0.09±0.06	0.35±0.44	05.30±6.17	1.32±1.20
	Middle	28.73±1.52	7.4±0.28	28.25±2.22	47.48±4.56	53.68±0.98	2.25±4.21	0.11±0.12	0.23±0.16	1.19±2.09	06.96±4.80	0.82±0.71
	Bottom	28.18±1.37	7.41±0.16	28.75±1.50	48.93±2.90	54.82±1.27	2.68±2.90	1.02±1.17	0.38±0.41	1.76±3.04	10.22±11.6	0.56±0.43
Station - 2	Surface	29.78±0.26	7.78±0.22	30.0±1.41	49.98±1.09	54.60±1.50	0.20±0.17	0.67±0.62	0.11±0.09	0.98±0.82	5.89±6.47	1.74±2.50
	Middle	29.05±0.67	7.76±0.17	29.5±1.73	49.28±1.86	53.80±2.35	1.32±2.28	0.30±0.11	0.30±0.41	1.75±3.12	5.68±3.41	0.88±0.45
	Bottom	28.75±0.13	7.63±0.17	28.0±2.16	50.93±1.51	54.33±0.97	1.18±1.34	0.81±0.95	1.07±1.19	3.95±6.67	9.12±8.19	0.62±0.14

± - Values are mean +4 months in each parameter (January-April, 2012)

Table 2. Correlation matrix for different water quality parameters in different depths at Muthukuda mangrove waters at station-1

Depth	Parameters	Temperature	pH	Salinity	Calcium Hardness	Total Hardness	Phosphate	Nitrate	Nitrite	Ammonia	Silicate	Chlorophyll 'a'
Surface	Temperature	1.00										
	pH	-0.39	1.00									
	Salinity	-0.87*	0.80**	1.00								
	Calcium Hardness	-0.19	0.98**	0.65**	1.00							
	Total Hardness	0.71**	0.37	-0.27	0.55**	1.00						
	Phosphate	0.98**	-0.19	-0.75*	0.01	0.84**	1.00					
	Nitrate	0.95**	-0.07	-0.66*	0.14	0.90**	0.99**	1.00				
	Nitrite	-0.34	-0.73*	-0.18	-0.86*	-0.90*	-0.52*	-0.62*	1.00			
	Ammonia	-0.31	-0.76*	-0.21	-0.88*	-0.89*	-0.49*	-0.60**	0.98**	1.00		
	Silicate	0.02	-0.93*	-0.52*	-0.99*	-0.68*	-0.18	-0.30	0.93**	0.94**	1.00	
	Chlorophyll 'a'	-0.47*	1.00	0.85**	0.96**	0.28	-0.28	-0.16	-0.67*	-0.69*	-0.89*	1.00
Middle	Temperature	1.00										
	pH	0.16	1.00									
	Salinity	-0.92*	0.23	1.00								
	Calcium Hardness	-0.10	0.97**	0.47	1.00							
	Total Hardness	0.46	0.95**	-0.09	0.83**	1.00						
	Phosphate	0.99**	0.27	-0.88*	0.01	0.56**	1.00					
	Nitrate	0.98**	0.37	-0.82*	0.11	0.64**	0.99**	1.00				
	Nitrite	0.99**	0.13	-0.94*	-0.13	0.43	0.99**	0.97**	1.00			
	Ammonia	0.99**	0.26	-0.88*	0.01	0.56**	0.99**	0.99**	0.99**	1.00		
	Silicate	0.40	-0.84*	-0.72*	-0.95*	-0.62*	0.30	0.20	0.43	0.30	1.00	
	Chlorophyll 'a'	-0.42*	0.83**	0.73**	0.95**	0.61**	-0.31	-0.22	-0.45*	-0.32	-1.00	1.00
Bottom	Temperature	1.00										
	pH	-0.70*	1.00									
	Salinity	-0.65*	-0.09	1.00								
	Calcium Hardness	-0.01	-0.71*	0.76**	1.00							
	Total Hardness	0.56**	-0.98*	0.26	0.82**	1.00						
	Phosphate	0.99**	-0.79*	-0.54*	0.14	0.68**	1.00					
	Nitrate	0.54**	0.23	-0.99*	-0.85*	-0.40	0.41	1.00				
	Nitrite	0.97**	-0.84*	-0.46	0.23	0.74**	0.99**	0.33	1.00			
	Ammonia	0.99**	-0.79*	-0.54*	0.13	0.67**	0.99**	0.42	1.00	1.00		
	Silicate	0.20	0.57**	-0.87*	-0.98*	-0.70*	0.05	0.93**	-0.04	0.06	1.00	
	Chlorophyll 'a'	-0.15	-0.61*	0.84**	0.99**	0.74**	0.00	-0.91*	0.09	-0.01	-0.98*	1.00

**Strong positive correlation is significant at the p<0.01 level, * Strong negative correlation is significant at the p<0.01 level

Table 3. Correlation matrix for different water quality parameters in different depths at Muthukuda mangrove waters at station-2

Depth	Parameters	Temperature	pH	Salinity	Calcium Hardness	Total Hardness	Phosphate	Nitrate	Nitrite	Ammonia	Silicate	Chlorophyll 'a'
Surface	Temperature	1.00										
	pH	-0.35	1.00									
	Salinity	-0.50*	0.99**	1.00								
	Calcium Hardness	-0.27	1.00	0.97**	1.00							
	Total Hardness	0.61**	-0.95*	-0.99*	-0.93*	1.00						
	Phosphate	0.50	0.64**	0.50**	0.70**	-0.38	1.00					
	Nitrate	0.73**	0.38	0.22	0.46	-0.09	0.96**	1.00				
	Nitrite	0.38	0.74**	0.61**	0.79**	-0.50*	0.99**	0.91**	1.00			
	Ammonia	0.96**	-0.08	-0.25	0.01	0.37	0.72**	0.89**	0.62**	1.00		
	Silicate	0.55**	-0.97*	-0.99*	-0.95*	0.99**	-0.45	-0.16	-0.56*	0.31	1.00	
	Chlorophyll 'a'	-0.99*	0.40	0.55**	0.32	-0.66*	-0.45	-0.69*	-0.33	-0.94*	-0.60*	1.00
Middle	Temperature	1.00										
	pH	-0.92*	1.00									
	Salinity	-0.92*	0.99**	1.00								
	Calcium Hardness	0.19	0.22	0.22	1.00							
	Total Hardness	-0.03	-0.37	-0.37	-0.99*	1.00						
	Phosphate	-0.40	0.00	0.00	-0.98*	0.93**	1.00					
	Nitrate	0.32	-0.67*	-0.67*	-0.87*	0.94**	0.74**	1.00				
	Nitrite	0.83**	-0.54*	-0.54*	0.70**	-0.58*	-0.84*	-0.26	1.00			
	Ammonia	0.79**	-0.48	-0.48	0.75**	-0.64*	-0.88*	-0.33	0.99**	1.00		
	Silicate	0.62**	-0.88*	-0.88*	-0.65*	0.76**	0.47	0.94**	0.08	0.01	1.00	
	Chlorophyll 'a'	-0.17	0.55**	0.55**	0.94**	-0.98*	-0.84*	-0.99*	0.41	0.47	-0.88*	1.00
Bottom	Temperature	1.00										
	pH	-0.76*	1.00									
	Salinity	-0.98*	0.63**	1.00								
	Calcium Hardness	-0.31	0.85**	0.12	1.00							
	Total Hardness	-0.99*	0.68**	0.99**	0.19	1.00						
	Phosphate	0.75**	-0.14	-0.86*	0.40	-0.82*	1.00					
	Nitrate	0.62**	0.03	-0.76*	0.56**	-0.71*	0.98**	1.00				
	Nitrite	0.75**	-0.15	-0.86*	0.40	-0.82*	0.99**	0.98**	1.00			
	Ammonia	0.80**	-0.22	-0.90*	0.33	-0.86*	0.99**	0.97**	0.99**	1.00		
	Silicate	0.78**	-0.99*	-0.65*	-0.83*	-0.70*	0.17	0.00	0.18	0.25	1.00	
	Chlorophyll 'a'	0.31	0.38	-0.49	0.81**	-0.42	0.86**	0.94**	0.86**	0.82**	-0.35	1.00

**Strong positive correlation is significant at the p<0.01 level, * Strong negative correlation is significant at the p<0.01 level

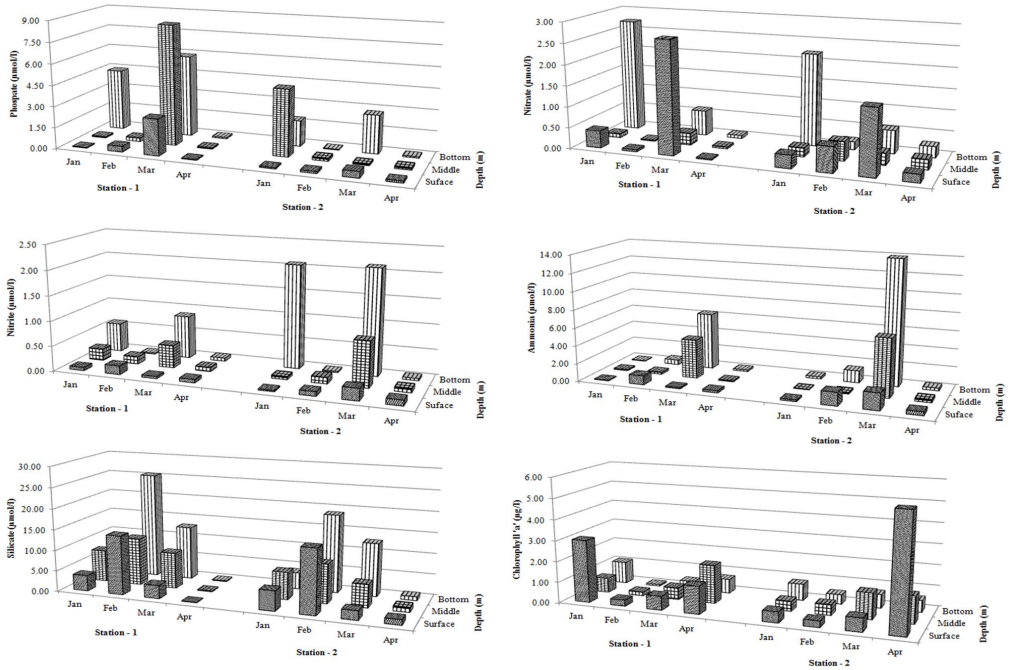

Fig. 1. Depth-wise distribution of inorganic nutrients and chlorophyll 'a' concentrations of Muthukuda coastal waters

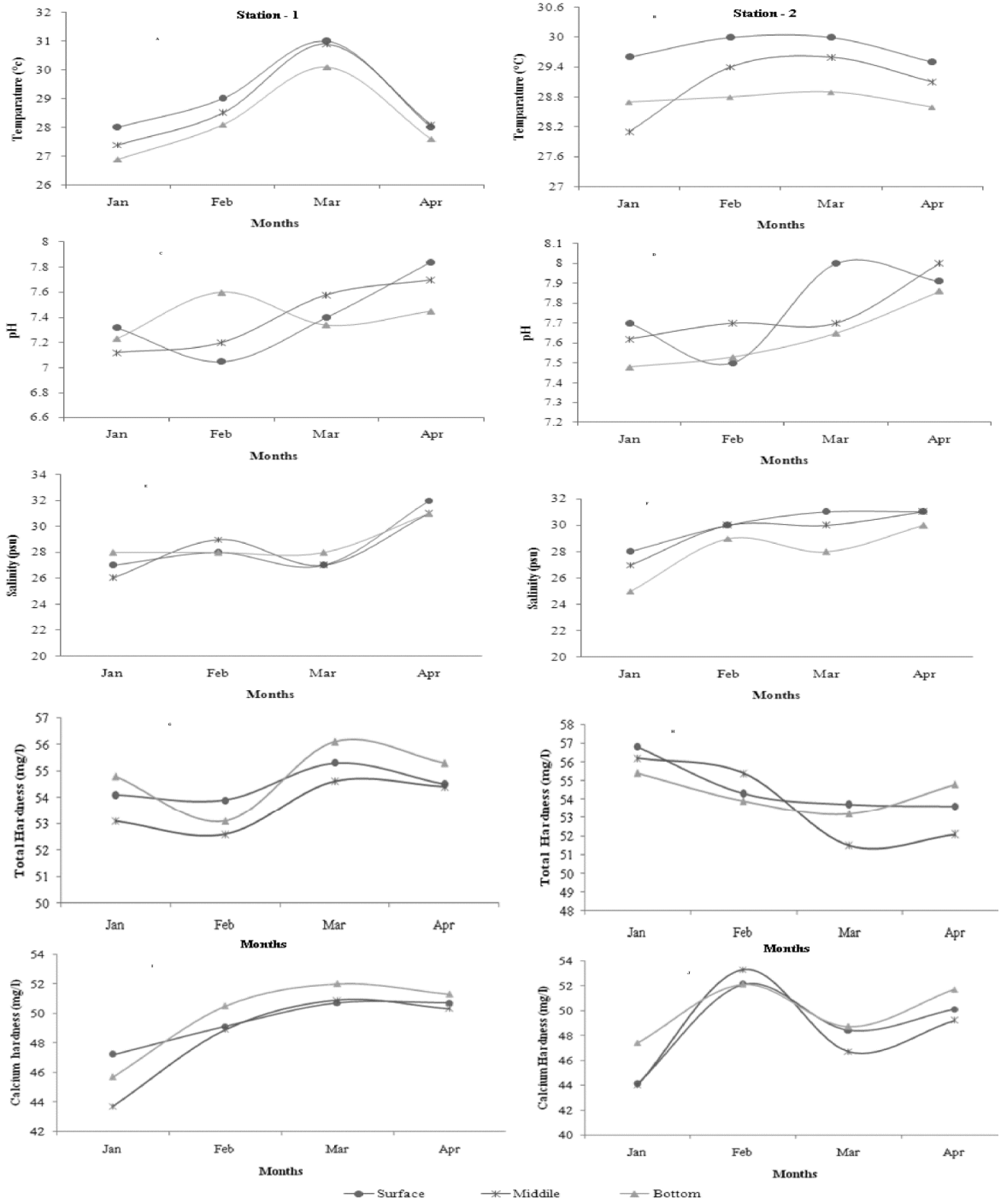

Fig. 2. Depth-wise distribution of physico-chemical parameters of Muthukuda coastal waters

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Books and other Monographs

4. Personal author(s): Ringsven MK, Bond D. Gerontology and leadership skills for nurses. 2nd ed. Albany (NY): Delmar Publishers; 1996.
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