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Impact of Flood on Litter Heterotrophic Bacteria and its Enzyme Production from Ayiramthengu Mangrove Ecosystem of Kerala Coast, India

Mintu Ann Varghese, Anit M. Thomas, R. Sunil Kumar

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ABSTRACT

The present study deals with the isolation of heterotrophic bacteria from leaf litter surface from three sampling stations of Ayiramthengu mangrove before and after the major flood of Kerala, occurred in July and August, 2018. Fifteen bacterial genera were isolated totally. Station 3 noted highest bacterial count during pre-flood (74.5×10^4 cfu/g) and post flood (81.5×10^4 cfu/g) study. Extracellular enzymes such as amylase, protease, cellulase, lipase and phosphatase were screened with different bacteria. Highest phosphatase producers and lipase producers (53% each) found in pre-flood analysis while it was increased during the time of post flood. Highest phosphatase index (1.83) and cellulase index (2.97) was found in pre-flood study but lipase index (2.92) and amylase index (2.31) were seen during the post flood period. Hence the mangrove ecosystem offers great potential bacterial diversity for future.

Keywords Mangrove, Flood, Litter, Total heterotrophic bacteria, Enzymes.

INTRODUCTION

Flooding is considered as the most harmful natural disaster that causes substantial damage to people as well as infrastructure all around the world (Liu *et al.* 2016). It was determined that flood in Kerala during August 2018 was a "calamity of severe kind", affecting 23 million people (Nowfal and Sarath 2018). Since the Great Flood of 1924, this was the greatest flood on record (Agarwal 2018). The cumulative rainfall in Kerala state during the time of flood was recorded as 2346.3 mm, which is 42% more than the normal monsoon season (Jaya Divakaran *et al.* 2019).

Flooding is an important environmental factor for mangrove ecosystem which subject to tidal flushing. Mangroves are considered as the most productive and significant ecosystems of the planet. Mangrove ecosystem consists of numerous microbial populations. Microbes play various roles in its ecosystem including decomposition and biogeochemical cycles (Bani *et al.* 2018). As the flooding results in alterations in mangrove ecosystem, it is essential to study how these changes will affect the mangrove ecosystem and its

Dr Mintu Ann Varghese*[1], Dr R Sunil Kumar[3]
[3]Associate Professor,
Research Department of Zoology, Catholicate College, Affiliated to Mahatma Gandhi University, Pathanamthitta 689645, Kerala, India
Dr Anit M. Thomas[2]
[2]Assistant Professor,
Department of Zoology, Baselius College, Affiliated to Mahatma Gandhi University, Kottayam 686001, Kerala, India
Email: mintuvar@gmail.com
*Corresponding author

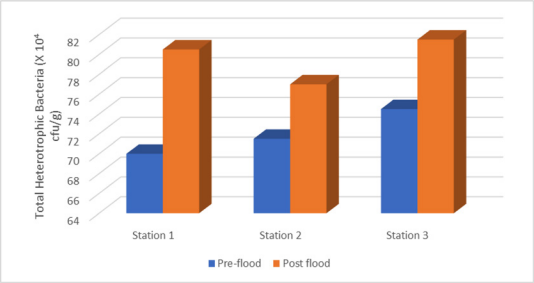

Fig. 1. Total heterotrophic bacterial population during pre-flood and post flood period.

functions. However, microbial communities respond frequently to flooding (Randle-Boggis *et al.* 2018).

Mangrove leaves consist of various group of elements including carbon, nitrogen and energy which actively take part in the various biogeochemical cycling with the help of microorganisms (Nordhaus and walf 2007). The decomposition process in mangrove forest begins with the colonization of microorganisms on to the fallen leaves from mangrove plant (Kathiresan *et al.* 2011). Litter degradation is a multifaceted process in which degradation is initiated and aided by various microbes that colonizes litter surface and break it down into simple organic compounds (Van der Heijden *et al.* 2016). Several aspects like mangrove species, season, and its location in the intertidal zone in turn affects the process of degradation (Mfilinge *et al.* 2002). This process of degradation was done with the secretion of several enzymes by the bacteria (Kristensen *et al.* 2017). The objective of this work was to determine the effect of flood on the litter bacterial populations of the Ayiramthengu mangrove ecosystem.

MATERIALS AND METHODS

Ayiramthengu mangrove ecosystem (lat. 9° 07' 30"- 9° 07' 40" N and long. 76° 28' 40"- 76° 28' 50" E) was the study area, which was divided into three sampling stations. Station 1 lie close to the land area, Station 2 situated in the middle of the mangrove and Station 3 was the area adjoining the estuary. Pre-flood sampling was done during May and June, 2018 whereas post flood sampling was done during July and August, 2018.

Biofilm from decomposing litter was collected using sterile blade from three sampling stations and aseptically transferred into sterile bottles. Surface contaminants were removed using sterilized sea water. Samples were immediately preserved at -20°C and analyzed in the laboratory. The estimation of Total Heterotrophic Bacteria (THB) was done by standard plate method using Zobell Marine Agar (Hi Media). The plates were incubated for 48 h at 28±2° C and number of colony forming units (cfu) per gram wet weight were calculated. Colonies were isolated and purified on saline nutrient agar. All the identification procedure was done by following Bergey's Manual of Determinative Bacteriology (Holt *et al.* 2000) up to generic level.

Bacterial isolates were tested for various enzymes like phosphatase, lipase, cellulase and amylase (Gerhardt *et al.* 1981). Enzymatic Index was calculated for each investigated enzyme. It was expressed by the relationship between the average diameter of the degradation halo and the average diameter of the colony growth (Soares Junior *et al.* 2013). One- way ANOVA was done using SPSS to find significance of total heterotrophic bacteria population for pre-flood and post flood analysis. Also, it was used to understand the relation of average enzymatic index value for pre-flood and post flood study.

RESULTS AND DISCUSSION

The total heterotrophic bacterial study indicated that an increase in bacterial population was found during post flood (Fig.1). Station 3 noted highest bacterial count both for pre-flood (74.5×10^4 cfu/g) and post flood (81.5×10^4 cfu/g) study. Statistical analysis using one way ANOVA indicated that pre-flood bacterial population showed no significant difference between different stations ($F = 0.217$, $p > 0.05$) whereas post flood bacterial population recorded significant difference between different stations ($F = 11.167$, $p < 0.05$). Randle-Boggis *et al.*(2018) stated that increased frequency of flood has a great impact on microbial communities and their potential functions.

A total of fifteen genera were isolated during the study and grouped into the following genus *Pseudomonas* sp., *Acinetobacter* sp., *Alteromonas* sp.,

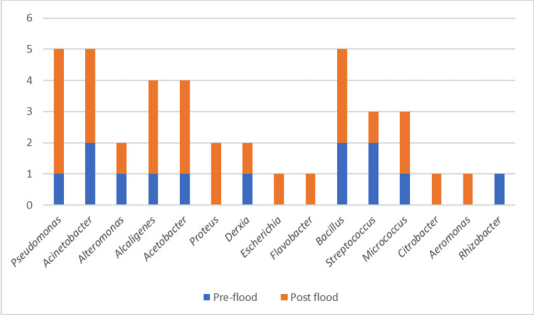

Fig. 2. Distribution of bacterial genera during pre-flood and post flood period.

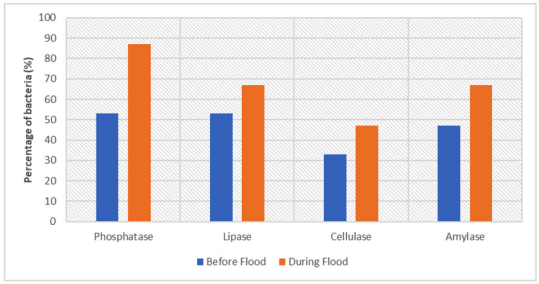

Fig. 3. Percentage composition of bacterial enzyme production during pre-flood and post flood study.

Alcaligenes sp., *Acetobacter* sp., *Proteus* sp., *Derxia* sp., *Escherichia* sp., *Flavobacterium* sp., *Bacillus* sp., *Streptococcus* sp., *Micrococcus* sp., *Aeromonas* sp., *Rhizobacter* sp. and *Citrobacter* sp. (Fig. 2). Certain bacterial genera including *Pseudomonas, Acinetobacter, Escherichia, Flavobacterium, Bacillus* and *Micrococcus* were isolated from decomposing mangrove leaf litter (Ogbonna 2011). Rajendran and *Kathiresan* (2007) reported *Flavobacterium, Pseudomonas, Acinetobacter* and *Azotobacter* from Pichavaram mangrove litter. The presence of *Escherichia* during the time of flood might be due to the human wastes from the nearby settlement that gets interacted with the mangrove ecosystem. *Pseudomonas, Derxia* and *Azotobacter* were involved in nitrogen fixation in mangrove whereas *Bacillus* and *Alcaligenes* participated in phosphate solublization (Sahoo and Dhal 2009, Zhang *et al*. 2008, Behera *et al.* 2017). *Bacillus, Micrococcus, Alteromonas, Escherichia, Alcaligenes, Proteus, Citrobacter, Acetobacter, Streptococcus* and *Aeromonas* were reported from different mangroves (Grisi Lima de and Liri 2010, Essien et al. 2013, Castro *et al*. 2014, Ambeng *et al.* 2019). Most of the bacterial population increased during the time of flood and could be observed from post flood analysis. This may be due to the entry of flood water from the nearby area into the mangrove ecosystem.

The isolates that could produce at least one of the four evaluated enzymes were considered separately before and during the flood (Fig. 3). The percentage composition of pre-flood bacterial enzyme study indicated that 53% were phosphatase producers, 53% were lipase producers, 33% were cellulase producers and 47% were amylase producers. While the percentage composition of post flood bacterial enzyme study suggested that the isolates that could produce four studied enzymes increased. Thus, 87% for phosphatase, 67% for lipase, 47% for cellulase and 67% for amylase enzyme were observed during the time of post flood. This increase in number of isolates capable of producing enzymes might be due to the flood. Saha (2018) stated that different environmental stressors influence the regulation of mangrove bacterial enzyme production, that can alter the physico-chemical characteristics by mangrove ecosystem. The change in physico-chemical factors as well as energy source during the time flood forces more bacterial isolates to produce enzymes.

The average enzymatic index of pre-flood and post flood bacteria was evaluated for phosphatase, lipase, cellulase and amylase enzymes (Fig. 4). On comparing the average enzymatic index it was noticed that highest phosphatase index (1.83) and cellulase index (2.97) was found during pre-flood study. But

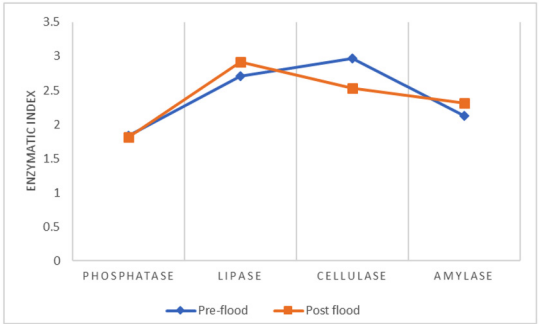

Fig. 4. The average enzymatic index during pre-flood and post flood study.

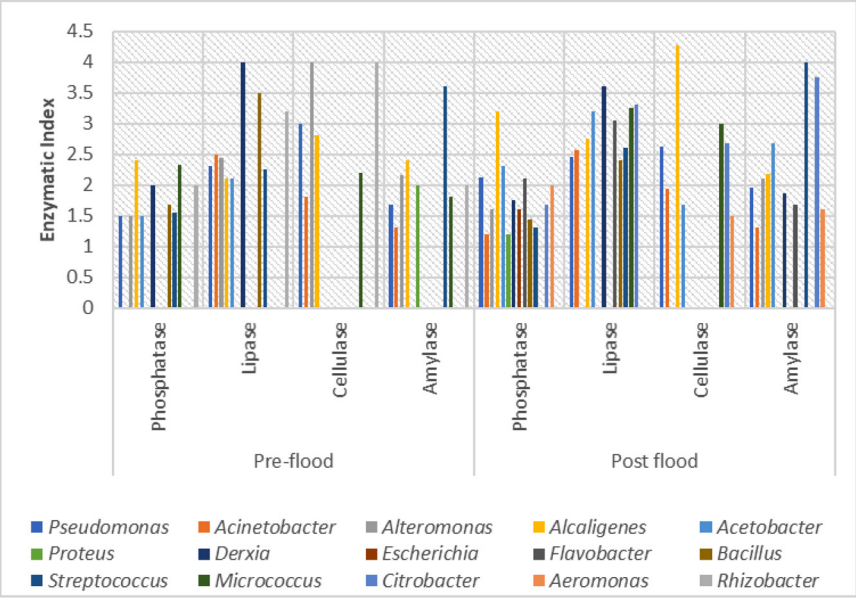

Fig. 5. Average enzymatic index during pre-flood and post flood period.

highest lipase index (2.92) and amylase index (2.31) were reported during the post flood analysis. One way ANOVA study indicated that there is no significant difference between the pre-flood and post flood average enzymatic index value ($F = 0.02$, $p > 0.05$). Based on the value of enzymatic index, certain enzymes were classified into four groups as no enzyme activity with value 1, low activity with value 2, medium activity with value 3 and high activity with value 4 (Lechuga *et al.* 2016).

Bacterial isolates were studied for enzyme production and its average index was represented (Fig. 5). The result revealed that enzyme production differs among different bacteria and the types of enzymes produced. Out of the isolated genera of bacteria, *Alcaligenes* sp. exhibited highest phosphatase index (2.4) and cellulase index (2.8), *Bacillus* sp. noted highest lipase index (3.5) and *Streptococcus* sp. showed highest amylase index (3.6) during the pre-flood study. While during the time of post flood, *Alcaligenes* sp. displayed highest phosphatase index (3.2) and cellulase index (4.28), *Aeromonas* sp. showed highest lipase index (3.3) and *Citrobacter* sp. displayed highest amylase index (3.75). The pre-flood and post flood enzymatic index value was almost similar.

CONCLUSION

In the present study a change in bacterial communities subjected to flood was found. This change had an impact on the bacterial enzyme production by increasing a greater number of isolates to produce enzymes. But this does not have much influence on its enzymatic index so that the average enzymatic index noted during pre-flood and post flood period was not much different but in a range. Also, it gave an idea about the importance of flood that triggers many bacteria to produce enzymes. Thus, the new insight of bacterial enzyme production from mangrove could find commercial applications.

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