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Flood induced changes in the diversity of yeasts from the mangroves of Valanthakadu, Kerala, India

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This is the first report on the influence of the devastating August 2018 floods of Kerala on yeast communities associated with the mangrove ecosystem of Valanthakadu, 'Kochi's oxygen parlour'. Samples were collected for the study from six stations in Valanthakadu during three different periods with regard to floods *i.e.* April – May 2018: Pre flood (PRF), September – October 2018: Just after floods (JAF), and March 2019: Post floods (POF) and plated onto Malt-yeast-glucose-peptone agar for isolation and enumeration. Morphologically distinct colonies were picked, clustered on biochemical basis, representative strains identified on molecular basis or using API 20 C AUX strip. On enumeration, the average colony counts of yeasts in the sediments were found to be greater than in water samples. Out of 89 morphologically distinct yeast strains isolated, the majority were non-pigmented (73 %) and belonged to the phyla Ascomycetes (58 %). Six different yeast genera: *Candida* spp., *Cryptococcus* spp., *Hortaea* sp., *Rhodotorula* spp., *Rhodosporidiobolus* sp., and *Trichosporon* sp. were recorded in this study. *C. tropicalis* was noted to be not only the predominant species but also resilient to the effect of floods. Flood was found to influence the diversity of yeasts, with the introduction of certain genera (*Hortaea wernekii* (first report)) and the loss of certain others (*C. gatti*). Analysis of diversity indices evidently revealed the influence of floods, with an increase in yeast diversity just after floods (1.62) when compared to other periods of sampling. A change in the hydrolytic profile of isolates was also noticed in relation to floods. This study clearly indicated that floods do affect the diversity of yeast communities and their function in the ecosystems, at least temporarily. Nevertheless, the long-term impacts remain unknown and deserve further studies.

[**Keywords**: Black yeast, *Candida tropicalis*, Floods, Hydrolytic enzymes, Mangroves, Yeast Diversity]

Introduction

The frequency and intensity of natural disasters are on rise across the world. The state of Kerala in South India too has been experiencing a rise in natural calamities in the last few years and the state faced one of the worst floods in nearly 100 years in August 2018. Floods are reported to affect soil structure and fertility, reducing nutrient availability and initiating primary succession processes in the case of strong disturbances[1]. Natural calamities like floods are also identified to impact the fragile balance of the land and disturb the microbial communities[2]. Though the effect of the 2018 floods on mankind and on assets has been evaluated to an extent; however, the effect of floods on microbial communities has not been well investigated. It is important to understand these effects on microbes, especially yeasts as they play a vital role in ecosystem functioning, maintenance, and services.

To investigate the possible effect of floods on yeast communities, the pristine mangroves of Valanthakadu were chosen. It is a small island, aptly described as the 'Kochi's oxygen parlour', the nearest wilderness island of the bustling financial capital of Kerala, Kochi. This Island has an area of about 256 acres and lies in close proximity to the city of Kochi. It has still no bridge or road connectivity to the mainland. This pristine island has a boundary wall made of mangroves. Fungi occupy the second position in the floral diversity of mangroves. Yeasts are fungi that reproduce vegetatively mainly by budding or fission and do not enclose their eventual sexual states inside fruiting bodies[3]. Although traditionally studied as a single group, they are divided among the Phyla Ascomycota and Basidiomycota. The mangrove ecosystem provides several microhabitats that harbour yeast communities[4]. Yeasts in these ecosystems play an important role in the food web, as they supply food

for some marine invertebrates together with zooplankton[5], and they are also essential in decomposition and nutrient cycling, biodegradation of xenobiotics and even serve as parasites in some cases. Yeast diversity in the ecosystem is highly affected by a variety of biotic and abiotic factors[6]. Therefore, it is likely that natural calamities of a very large scale like that of 2018 floods would affect yeast communities even in the most resilient ecosystems like mangroves. This study is highly relevant as very little is known about the diversity of yeasts from mangroves and how natural disasters such as floods can affect yeast communities in the mangroves. This paper makes an attempt to build baseline data in this regard by analysing the yeast diversity during the different periods of floods.

Materials and Methods

Sampling site

Sediment and water samples were collected from Valanthakad mangroves (9°55'24" N latitude and 76°20'1.23" E longitude). The sampling site was divided into 6 stations each 50 m apart (Fig. 1). The dominant mangrove trees found in Valanthakadu were *Rhizophora* spp. and *Avicennia* spp.

Fig. 1 — Map of Valanthakadu mangrove

Sample collection

Sampling was done during 3 different periods in relation to floods *i.e.* April – May 2018: Pre flood (PRF), September – October 2018: Just after floods (JAF), and March 2019: Post floods (POF). After removing the surface litter, approximately 5 – 10 g of sediment was aseptically collected using a hand corer. The sub-surface water samples were collected in sterile sampling bottles. The collected samples were brought to the laboratory in an icebox. Samples on reaching the laboratory were immediately processed or stored in a fridge at 4 °C.

Isolation and enumeration of marine yeast

Sediment and water samples were serially diluted and 0.1 ml and 0.5 ml of the sample spread plated onto Malt-yeast-glucose-peptone agar (Wickerham's agar - Malt extract - 3 g, Yeast extract - 3 g, Peptone - 5 g, Glucose - 10 g, Agar - 20g, Seawater (35 ppt) - 1000 ml, and Chloramphenicol - 200 mg/l) for isolation and enumeration as per Kutty & Philip[7] with slight modifications. The plates were incubated at 28 °C for 2 – 3 days and colony counts were made. Morphologically distinct colonies were picked, purified by quadrant streaking, and maintained on Malt Extract agar slants for further study.

Effect of temperature on growth

The effect of temperature on the growth of the isolates was determined by inoculating yeast into Malt Extract broth, incubating the tubes at different temperatures (4 °C, 11 °C, 29 °C, 37 °C), and measuring OD_{580} of broth after 48 h of incubation.

Identification of yeast strains

The isolates were identified as per Barnett *et al.*[8]. This identification was done on the basis of the microscopic appearance of the cell, the mode of reproduction, and certain biochemical characteristics. Isolates were clustered on of basis of these morphological and biochemical characters using PRIMER 5 software. The identity of representative strains (randomly selected) from each cluster was ascertained. The representative strains VA244, VA113, and VA252 were identified using API 20 C AUX strips whereas VA93, VA94, VA240, VA242, VA245, and VA247 were identified on a molecular basis.

Metabolic identification using API 20 C AUX system of selected isolates

The API 20 C AUX strip (consisting of 20 cupules containing dehydrated substrates) enabled the performance of 19 assimilation tests used for species-level identification. This test was performed as per the manufacturer's protocol (bioMérieux® SA). The reactions were read by comparing them to growth controls and identification was obtained by referring to the analytical profile index.

Molecular identification of the selected marine yeast isolates

Representative isolates were identified by sequencing the ITS region. For this, the genomic DNA was extracted using Nucleo Spin® Plant II Kit (Macherey-Nagel) as per the manufacturer's protocol. The ITS region was amplified using the primers ITS-1 &ITS-4 (Forward ITS 1-5' TCC GTA GGT GAA CCT GCG G 3' and Reverse ITS 4- 5' TCCTCC GCT TAT TGA TAT GC 3')[9]. The amplification was carried out in a PCR thermal cycler (GeneAmp PCR System 9700, Applied Biosystems) which involved initial denaturation at 98 °C for 30 sec followed by 40 cycles of (98 °C for 5 sec, 60 °C for 10 sec, and 72 °C for 15 sec) and a final extension at 72 °C for 60 sec. Sequencing was done at Rajiv Gandhi Centre for Biotechnology, Thiruvananthapuram using the BigDye Terminator v3.1 Cycle sequencing Kit (Applied Biosystems, USA) following manufacturer protocol. The ITS gene sequence obtained was analyzed using the Basic Logical Alignment Search Tool (BLAST) online (https://blast.ncbi.nlm.nih.gov/) to determine the degree of similarity with the closest phylogenetic affiliate in the database. The sequences obtained have been deposited in the NCBI database.

Statistical analysis

The Shannon-Weiner diversity, Peilous evenness, and Species richness were analyzed using PRIMER 5[9]. The diversity index provides a good measure of the community composition.

Screening for hydrolytic enzyme production

Nutrient agar medium supplemented with casein (2 %), gelatin (2 %), starch (1 %), and tributyrin (1 %) were prepared for the detection of caseinase, gelatinase, amylase and lipase activity, respectively[7]. Plates were spot inoculated and incubated at 28±2 °C for 2 to 3 days. The presence of a clearance zone indicated a positive result. In the case of amylase, plates were flooded with Gram's iodine solution (Iodine 1 g and Potassium iodide 2 g in 300 ml distilled water) and for detecting gelatinase activity gelatin agar plates were flooded with mercuric chloride solution (15 %). The presence of clearance zones was noted.

Results and Discussion

The present study has helped to generate baseline data on the diversity of marine yeasts from the mangroves of Valanthakad Island during different periods of 2018 Kerala floods.

The pH and temperature values were recorded from the sampling sites at the time of sample collection. pH of the sediments varied between 6.4 – 7. There was no significant variation in temperature during collection; it was around 28 °C.

Enumeration of yeast colonies

On enumeration, the average colony counts of yeasts in the sediment were found to be greater than in water samples. The colony counts of yeasts in the sediment ranged between 18.6 – 22.8×10^2 CFU/gm and in the water samples between 44 – 80×10^1 CFU/ml. A distinct temporal variation in the population density of yeasts in the sediment, as well as water samples, was observed in this study. The cultivable yeasts numbers were highest in the post-flood samples (both sediment & water) when compared to other sampling periods (Table 1). This increase in numbers could be due to the introduction of allochthonous species along with flood waters.

Growth of isolates increased with increase in incubation temperature from 4 – 28 °C. However, it was noted that most isolates (76 %) obtained during the Pre-flood sampling exhibited greater growth at 37 °C; whereas, those obtained after floods (both JAF and POF) had greater growth at 28 °C (Table 2).

Identification and diversity

A total of 89 yeast strains were isolated from 6 sites in Valanthakad. Maximum macroscopically distinct yeast isolates were obtained during the post-flood sampling (34) followed by the sampling just

Table 1 — Population density of yeasts during different periods of sampling

Samples	PRF	JAF	POF
Sediment	18.60×10^2 CFU/gm	20.8×10^2 CFU/gm	22.8×10^2 CFU/gm
Water	44×10^1 CFU/ml	76×10^1 CFU/ml	80×10^1 CFU/ml

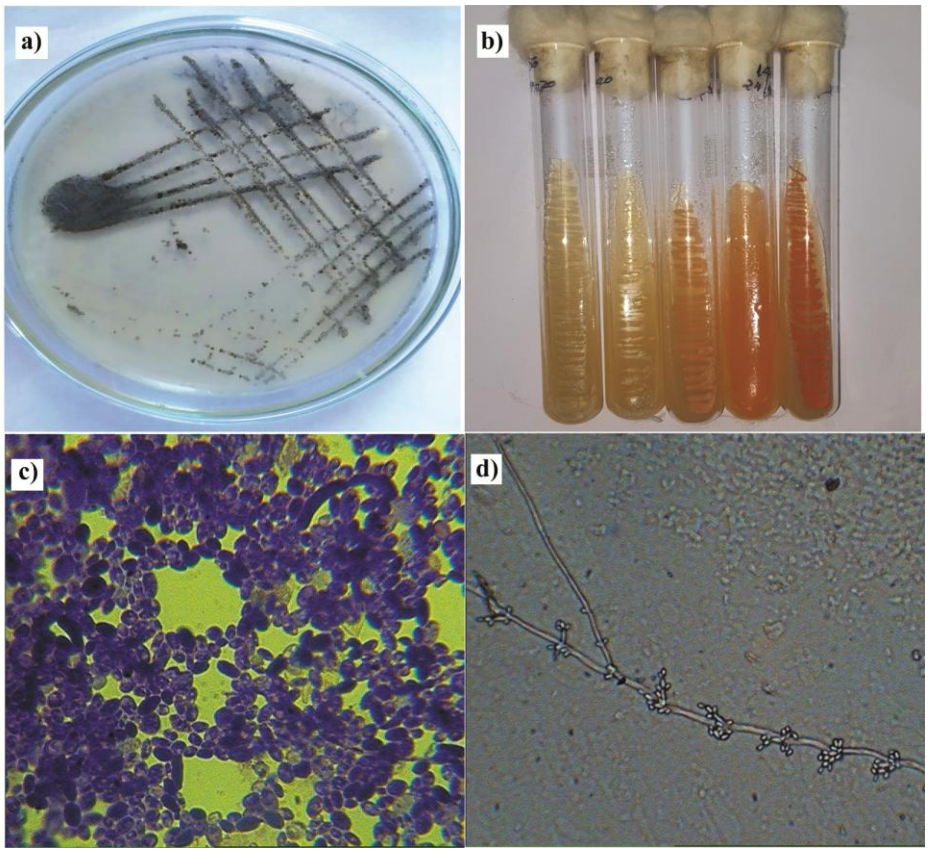

Fig. 2 — Macroscopic and microscopic appearance of isolates: a) Black yeast on agar slants, b) Red-yellow pigmented isolates on Malt extract agar, c) Microscopic appearance grams-stained cells, and d) Pseudo hyphae

Table 2 — Growth of isolates at different temperatures

Different periods of flood	Temperature			
	4 °C	11 °C	28 °C	37 °C
	Percentage of isolates			
PRF	0 %	10 %	14 %	76 %
JAF	0 %	3 %	89 %	8 %
POF	0 %	1 %	93 %	6 %

after floods (30) and least during the pre-flood sampling (25). Among these 89 isolates, only 27 % were pigmented. Their microscopic appearances and their ability to form pseudo hyphae were noted (Fig. 2).

The isolates were clustered based on their morphological and biochemical characteristics. The representative strains VA244, VA113, and VA252 were identified using API 20 C AUX strips; whereas, VA93, VA94, VA240, VA242, VA245, and VA274 were identified on a molecular basis. The results of API & molecular identification have been summarized in Table 3. Based on these results it was found that the yeast isolates from the mangrove island of Valanthakad belonged to 6 different genera

Table 3 — Identification details of yeast strains

Molecular identification

Culture no.	Genbank accession no	Identity
VA93	OK 353778.1	*Candida tropicalis*
VA94	OK 353779.1	*Candida tropicalis*
VA240	OK 353780.1	*Rhodosporidiobolus* sp.
VA242	OK3533781.1	*Rhodotorula paludigena*
VA274	OK 353783.1	*Rhodotorula mucilaginosa*
VA350	MT 461155	*Hortea werneckii*

API identification

Culture no.	Identity
VA113	*Trichosporon mucoides*
VA244	*Cryptococcus gatti*
VA252	*Cryptococcus* sp.

namely, *Candida* spp., *Cryptococcus* spp., *Hortaea* sp., *Rhodotorula* spp., *Rhodosporidiobolus* sp., and *Trichosporon* sp. (Fig. 3). *Candida tropicalis* (51 %) was the predominant species and their highest numbers were obtained during post-flood (67 %) sampling. *Rhodotorula* (16 %) was the second most predominant genera and their highest numbers were recorded just after the flood (28 %). Earlier studies also reported that *Candida* sp. is the dominant species in the coastal waters that are in close proximity to urbanized regions where waters are highly polluted[1]. In estuaries, *Candida* and *Rhodotorula* are generally dominant genera[10]. The results obtained in the present study are in conformity with this observation. During the pre-flood sampling, the number of isolates of *Trichosporon*, *Cryptococcus*, and *Rhodotorula* obtained was the same (20 %). The species distribution during different periods of sampling is given in Figure 4.

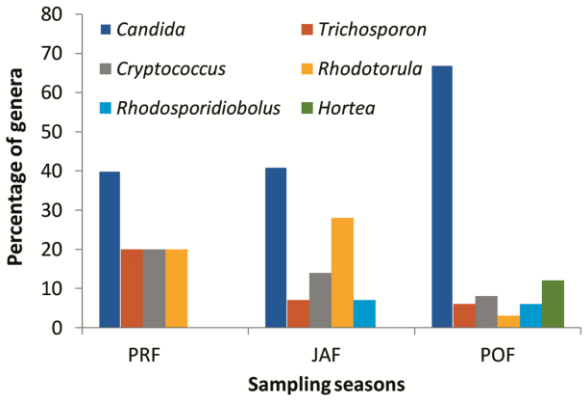

Fig. 3 — Generic composition of yeasts from the mangroves of Valanthakad during three different study periods

In this study, it was found that the majority of the isolates belonged to the Ascomycetes yeast (58 %) and the rest (42 %) belonged to Basidiomycetes. It has been reported that Ascomycetes yeasts are in general more abundantly found than Basidiomycetous yeasts in the marine environments[7,11].

The pigmented strains isolated in this study belonged to *Rhodotorula* spp., *Rhodosporidiobolus* sp., and *Hortaea werneckii*, whereas the non-pigmented strains belonged to the genera *Cryptococcus* spp., *Trichosporon* sp., and *Candida* spp. Apart from red yeast, *Hortaea werneckii* commonly referred to as black yeast was also isolated in the current study. They were observed only during the post-flood sampling. This is the first report of these strains from the mangroves of Kerala. However, they have been previously reported from the mangroves of Goa[12].

Flooding can cause a long-term modification in the unique mangrove ecosystem, sometimes marked by the emergence of novel microbial communities or even the loss of certain species[2]. In this study black

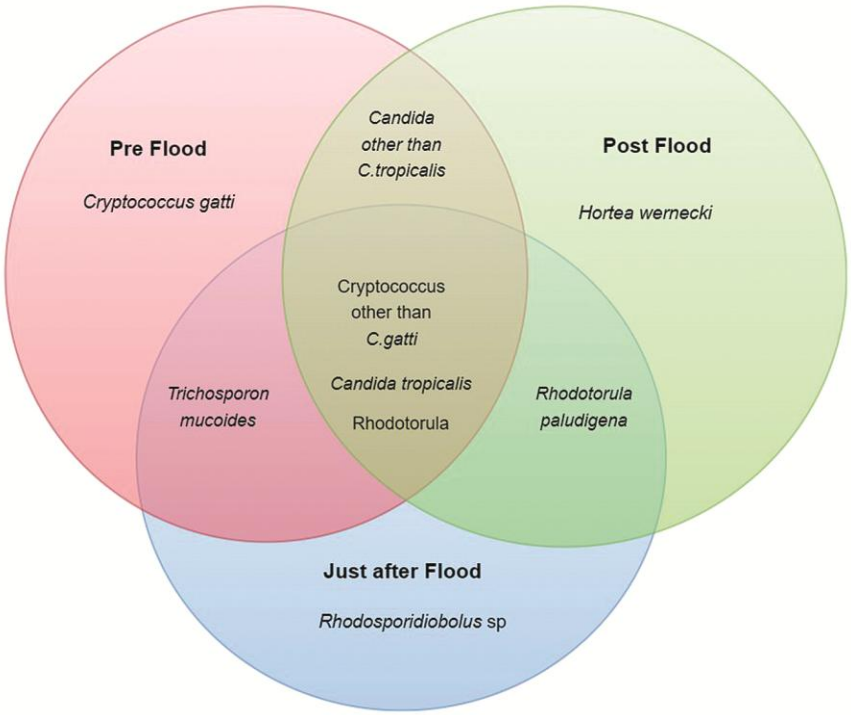

Fig. 4 — Distribution of yeast species during sampling periods

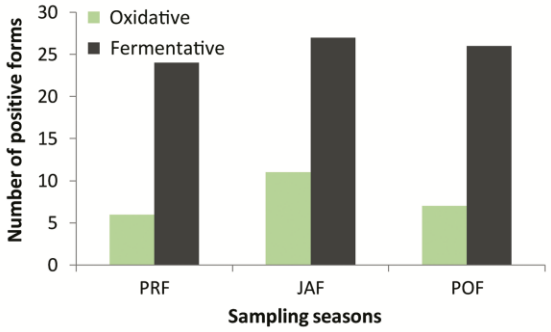

Fig. 5 — Percentage of oxidative and fermentative forms

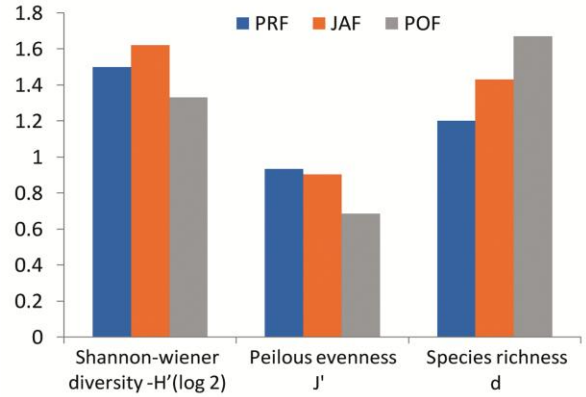

Fig. 6 — Diversity indices of yeast isolates from various flood periods

yeasts, *Hortaea werneckii* (4 nos.) were isolated only during our post-flood period. *H. werneckii* are generally found in hypersaline environments and have not been reported from Kerala so far. This could be a novel strain introduced as a result of floods and which later established itself in an altered environment facilitating its isolation in Post flood sampling. Another observation was that the *Cryptococcus* species, *C. gatti* which was isolated during pre-floods was missing after floods from these sites. *Rhodosporidiobolus* sp. was isolated only in sampling conducted immediately after floods, indicating it was introduced into the mangrove ecosystem by flood waters. It is highly likely that changes in abiotic factors brought about by the floods were responsible for altering the yeast communities in Valanthakadu mangroves. *Candida tropicalis* was isolated during all three samplings conducted and their numbers increased after the floods. This species of yeasts was found to be highly resilient and adapted to tolerate changes brought about by floods.

As a part of biochemical characterization, the oxidative/fermentative potentials of the isolates were determined. Among the 89 isolates, only 24 isolates were oxidative (27.3 %) and the rest of the cultures were fermentative (72.7 %; Fig. 5). Fermentative forms dominated oxidative forms during all the periods of sampling. Yeasts found in aquatic environments are generally oxidative or weakly fermentative according to the nature of water[7]. The strictly aerobic yeasts in clean water can be shifted to fermentative yeasts in polluted waters indicating pollution in this area. However, an increase in the number of oxidative forms was noted immediately after floods which could be due to the flushing action of flood waters creating a cleaner environment.

Diversity index gives an idea about the way in which individuals in an ecological community are distributed among species. Diversity indices revealed that Shannon-wiener diversity (H'(log2)) was found to be maximum during JAF (1.62) and least during POF (1.33) sampling. This could be due to the increase in the introduction of allochthonous groups into the mangrove environment by flood waters. The Species richness (d) was greatest during POF with a value of 1.67 and least during PRF (1.2). Evenness (J') was found to be maximum during PRF (0.93) and least during POF (0.68) (Fig. 6). A high evenness value (Peilou's evenness J') in the pre-flood sampling could be an indication of a stable community and a low evenness value after floods indicates a disturbed system.

Hydrolytic profile

Extracellular enzyme production pattern was studied over three different periods Pre-flood, Just after foods and Post floods. All the isolates were producers of one or more hydrolytic enzymes. It was interesting to note that all the isolates were lipase positive regardless of the period of isolation. This indicated the presence of lipid matter in the sampling region[13]. Paskevicus[14] too noted that almost all the yeast strains produce lipase. Caseinase activity was exhibited by strains isolated only during POF sampling. Out of the 89 isolates, 76 % were urease and 55 % were nitrate positive. No gelatinase activity was observed among yeasts isolated during JAF (Fig. 7). An overall change in the hydrolytic profile of the isolates was noted after the floods. The post-flood cultures were found to produce more hydrolytic enzymes; this could be a consequence of the input of detritus in sediments after floods. All these observations point to the influence of floods on the hydrolytic potential of yeasts in the Valanthakadu mangroves.

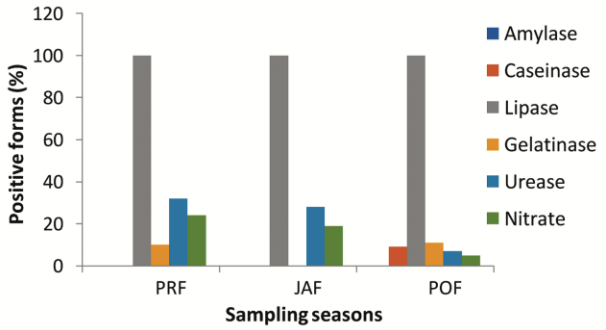

Fig. 7 — Percentage of hydrolytic enzymes produced by marine yeasts isolated during different flood periods

Conclusion

The findings of the study indicate that the 2018 floods of Kerala did disturb the diversity, density and stability of yeast communities of Valanthakadu mangroves with the introduction of new species, loss of certain others, and gaining dominance of some species in an altered environment. The hydrolytic potentials of the yeast isolates were also influenced by the floods. However, some yeast genera exhibited high resistance and resilience to natural disasters. Future studies should investigate the effect of these changes in yeast communities on the functioning of the ecosystem. The long-term impacts of the changes remain unknown and hence invite further studies.

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Conflict of Interest

There is no conflict of interest.

Ethical Statement

The authors declare that this article does not contain any studies involving human participants or animals performed by any of the authors.

Author Contributions

Study conception and design: KM; design of experiment: SNK; specifically performing the experiments, or data/evidence collection: NN; analysis and interpretation of results: KAN & KM; draft manuscript preparation: KAN. KM, SNK & VSA reviewed the results and approved the final version of the manuscript.

References

1 Walker L R, *The biology of disturbed habitats*, (Oxford University Press), 2012, pp. 360. DOI: https://doi.org/10.1093/acprof:oso/9780199575299.001.0001

2 Bhat G S & Bindiya E S, *Metagenomic analysis of soil microbial diversity in post flood Mangroves*, Kerala Biodiversity Board, 2019.

3 Kutty S N, *Marine Yeasts From the Slope Sediments of Arabian Sea and Bay of Bengal*, Ph.D. thesis, Cochin University of Science and Technology, India, Kochi, 2009.

4 Chi Z M, Liu T T, Chi Z, Liu G L & Wang Z P, Occurrence and Diversity of Yeasts in the Mangrove Ecosystems in Fujian, Guangdong and Hainan Provinces of China, *Indian J Microbiol*, 52 (3) (2012) 346–353. https://doi.org/10.1007/s12088-012-0251-5

5 Meyers S P, Ahearn D G, Alexander S K & Cook W L, *Pichia spartinae*, dominant yeast of the Spartina salt marsh, *Dev Ind Microbiol*, 16 (1975) 262-267.

6 Brandão L R, Libkind D, Vaz A B M, Espírito Santo L C, Moliné M, *et al*., Yeasts from an oligotrophic lake in Patagonia (Argentina): Diversity, distribution and synthesis of photoprotective compounds and extracellular enzymes, *FEMS Microbiol Ecol*, 76 (1) (2011) 1–13. https://doi.org/10.1111/j.1574-6941.2010.01030.x

7 Kutty S N & Philip R, Marine yeasts - A review, *Yeast*, 25 (7) (2008) 465–483. https://doi.org/10.1002/yea.1599

8 Barnett J A, Payne R W & Yarrow D, *Yeasts: Characteristics and identification*, (Cambridge University Press, New York), 1990, pp. 1002.

9 White T J, Bruns T, Lee S & Taylor J, *Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics*, *PCR Protocols: A guide to methods and applications*, (Academic Press, Inc., San Diego, California), 1990, pp. 315-324.

10 Kandasamy K, Alikunhi N M & Subramanian M, Yeasts in marine and estuarine environments, *J Yeast Fungal Res*, 3 (6) (2012) 74-82. DOI: 10.5897/JYFR12.003

11 Adams G O, Tawari-Fufeyin P & Okoro S E, Bioremediation, biostimulation and bioaugmentation: a review, *Int J Environ Bioremed Biot*, 3 (2015) 28–39.

12 Nazareth S & Gonsalves V, Occurrence of the halophilic black yeast *Hortaea werneckii* from brackish waters of mangroves of, *Kavaka*, 39 (2015) 23–26.

13 Pothayi V & Devasia S C, A study on the distribution and hydrolytic enzyme potential of yeasts in the mangrove sediments of Northern Kerala, *Indian J Microbiol Res*, 7 (2020) 161-167.

14 Paskevicius A, Lipase activity of yeasts and yeast-like fungi functioning under natural conditions, *Biologija*, 4 (2001) 16-18.