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Impact of Anthropogenic Activities on Genetic Variability of Yeasts inhabiting Mangrove sediments of Dar es Salaam, Tanzania

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Abstract

The objective of the study was to investigate changes in natural yeast populations in mangrove sediments as a result of human (anthropogenic activities) disturbance. DNA techniques in form of Random amplified polymorphic DNA (RAPD) - PCR was employed in this study to assess the genetic variability of yeasts isolated from a natural sediment (non impacted) known as Ras Dege and another mangrove ecosystem which has been impacted (polluted with sewage runoffs) known as Mtoni Kijichi both along the coast of Dar es Salaam. A total of 25 morphologically different yeasts from the two mangrove sites were isolated and their genetic variability was investigated. The RAPD-PCR profiles did reveal more yeast genotypes (higher variability) in Ras Dege area compared to those at Mtoni Kijichi. Implying that, the anthropogenic activities have modified and reduced the diversity of mangrove yeasts to fewer genotypes. The results of this study points out to the negative impacts of dumping of untreated waste water in mangrove ecosystems.

Keywords: anthropogenic, activities, mangrove, yeasts, genetic, variability.

1. Introduction

Mangroves are coastal wetland forests established at intertidal zones of estuaries, deltas, creeks, lagoons, marshes and mudflats of tropical and subtropical latitudes (Ananda & Sridhar, 2004). Approximately one fourth of the world's coastline is dominated by mangroves that are distributed in 112 countries and cover about 180,000 km^2 of the globe's surface in subtropical and tropical regions (Latha & Mitra, 1998). Among marine ecosystems, mangroves constitute the second most important ecosystem in productivity. Mangrove forests are believed to be an important sink of suspended sediments (Kathiresan & Bingham, 2001). In these forests, mangrove trees catch sediment by their complex aerial root structure, thus functioning as land builder (Holguin *et al*., 2001). They also generate considerable amount of detritus such as leaf litter and woody debris hence constitute an ideal environment that support or harbor diverse groups of marine animals, plants and microorganisms that are widely acknowledged to be important elements in coastal ecosystems in the tropics (Holguin *et al*., 2001). Mangroves preserve water quality and reduce pollution by filtering suspended materials and by assimilating dissolved nutrients, stabilize sediments and protect the shoreline from erosion.

In mangrove sediment communities, substantial fungal populations exist as part of the vast microbial diversity involved in detritus processing (Abdel-Wahab, 2005). Marine fungi (which are those that grow and sporulate exclusively in a marine or estuarine habitat) are major decomposers of woody and herbaceous substrata in marine ecosystems, where they also degrade dead animals (Kohlmeyer *et al*., 1996a). Marine fungi are the primary degraders of lignin, cellulose and other plant components in mangroves as they can synthesize all the necessary enzymes (Singh & Steinke, 1992; Bremer, 1995). Marine fungi play an important role in the complex microbial mediated nutrient cycling processes and biodegradation of xenobiotics such as petroleum and its derivatives (De Araujo *et al*., 1995).

Yeasts are fungi that predominantly exist as unicellular organisms and at present there are about 1500 recognized yeast species which are distributed between the ascomycetes and the basidiomycetes (Kurtzman & Fell, 2005; Botha, 2011).Yeasts play a role in maintenance of soil and sediment structure and aggregate formation. Also, Yeasts participate in soil nutrient cycles and mineralization processes. On the other hand, Yeasts serve as a nutrient source for a diversity of soil predators and they have potential as plant growth promoters and soil conditioners (Yurkov *et al*., 2012). However, among the marine microbiota in East Africa, it is only bacteria that have been investigated and reported (Lyimo, 1999; Machiwa, 1999; Marshal, 1994 & Mgaya *et al*., 2004), leaving the potential of East African fungal diversity unfamiliar.

Anthropogenic or human impact on environment includes impacts on biotic and abiotic environments (Sahney *et al*., 2010). Due to the growing rate of urbanization in many tropical coastal areas, there continues to be an increasing concern on the impact of anthropogenic activities to mangrove forests (De Wolf *et al*., 2001; Mwevura *et al*., 2002; Gulis & Subekropp, 2003; Mgaya *et al*., 2004; Kruitwagen *et al*., 2006). During recent years, Tanzania has experienced a rapid increase in urbanization and industrialization, particularly

in the coastal area of Dar es Salaam. The mangroves ecosystems along the coast of Dar es Salaam are subject to a number of sources of pollution such as urban sewage, industrial effluents and agricultural chemicals which reach the mangroves via rivers and via direct disposal (Mgaya *et al*., 2004).

Dar es Salaam region of Tanzania has several mangrove stands which include Kunduchi, Selander Bridge, Mbweni, Ras Dege, Msimbazi, Mtoni Kijichi and Mji mwema (Semesi, 1998). Sewage which is water-carried waste, in solution or suspension, has long been reported to be dumped along the coast of Dar es Salaam (Machiwa, 1999). Furthermore, a wide range of sewage impacts on marine Tanzanian coastal communities has been reported (Machiwa, 1998; Gulis & Subekropp. 2003; Kruitwagen *et al*., 2006).

In previous studies the sediments of the Mtoni estuary (in Dar es Salaam) were found to be polluted with polychlorinated hydrocarbons, petroleum hydrocarbons and organochlorines pesticides (Machiwa, 1998; Mwevura *et al*., 2002). Many of these environmental pollutants which are released into the waters of the Dar es Salaam coastal areas pass through mangrove forests before reaching the Indian Ocean coast. The mangroves and associated biota in mangrove forests, which are within the city (Mtoni Kijichi), are therefore heavily impacted by anthropogenic activities (Mremi & Machiwa, 2003) compared with Ras Dege mangrove forests which are located a distance away from the city and hence far from human settlements (Mtanga & Machiwa, 2007).

Elsewhere in the world, pollution has been implicated in the modification, increases or reduction of genetic diversity in various organisms in mangrove coastal ecosystems (Latha & Mitra. 2004; Kokare *et al*., 2004; Limtong *et al*., 2007). Due to continued human interference and depletion of mangrove habitats, dangers of losing some precious fungal resources cannot be ruled out, as many are habitats/ host-specific (Latha & Mitra. 2004).

Previous pollution studies on the coast of Tanzania have focused on the chemical analysis of environmental pollutants and their effect on higher organisms (Mremi & Machiwa, 2003; Mtanga & Machiwa, 2007; Rumisha *et al*., 2012), leaving behind a dearth of information on the effects of these pollutants on mangrove sediments microorganisms. This study therefore endeavored to isolate yeasts from both sewage impacted and non impacted mangrove sediments along the coast of Dar es Salaam and carried out a randomly amplified polymorphic DNA – PCR analysis to reveal changes in the numbers of genotypes in the yeast populations caused by the pollution.

2. Materials and Methods

2.1 The Study Sites

The two mangrove forest ecosystems (Mtoni Kijichi and Ras Dege) were selected for the intended study owing to their features; one constantly receiving sewage contamination due to proximity to human settlements and the other far from any notable pollution.

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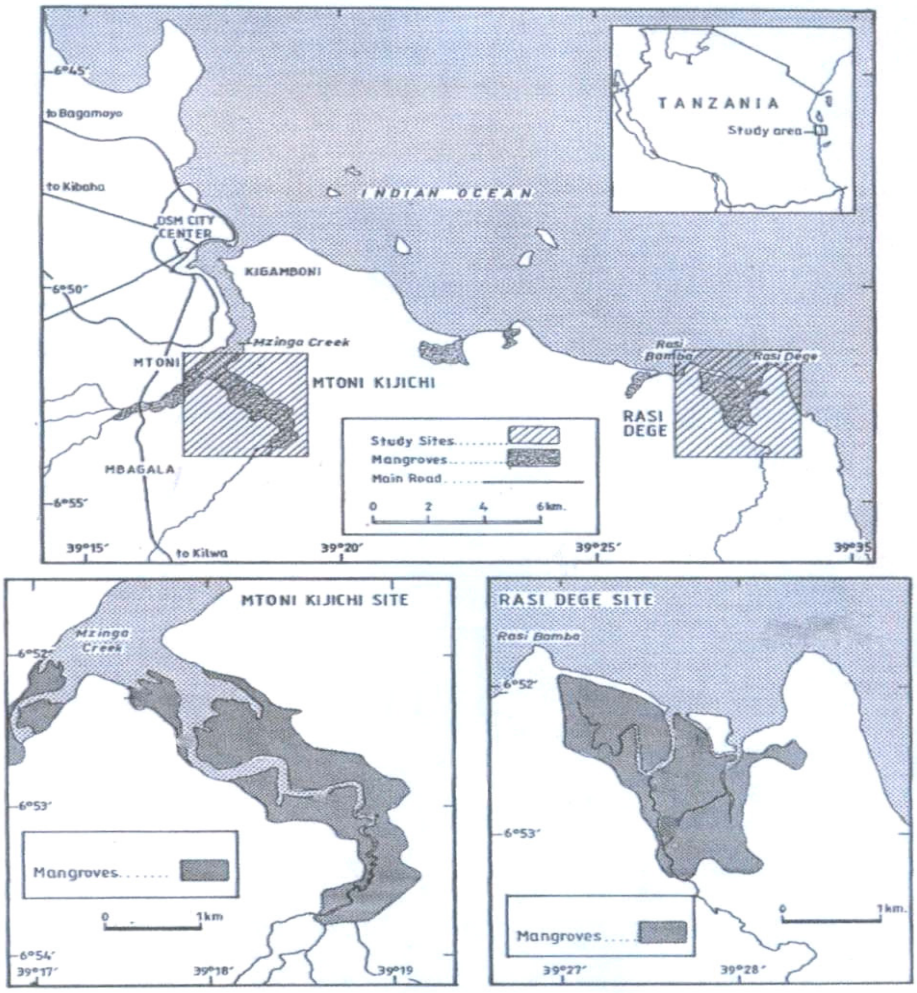

Figure 1. Map of Dar es Salaam Coast showing the study sites (Source: Abbu, 2006)

2.1.1 Mtoni Kijichi Mangrove Site

This site is located 15 km south of Dar es Salaam city centre. The mangrove forest in Mtoni Kijichi is estimated to cover 378.4 hectares. Two small permanent creeks namely Kizinga and Mzinga along with incoming rivers which are polluted with sewage run through this forest. Some streams of fresh water and seepage that may also be contaminated with sewage from nearby settlements also enter these forests (Mgaya *et al*., 2004).

2.1.2 Ras Dege Mangrove Site

Ras Dege is located along the coast approximately 60 km south of Dar es Salaam city center. This site lies at the mouth of Mbalaganje River and is estimated to cover about 245 hectares (Semesi, 1991). Ras Dege is considered as non sewage-impacted area as is very far from human settlements with exception of one small recreational hotel (Mbamba bay hotel) and thus experiencing very little impact from anthropogenic activities.

2.2 Research Methodology

2.2.1 The Sediment Sampling for Yeast Isolation

Mangrove sediments were randomly selected from Ras Dege mangrove ecosystems and Mtoni Kijichi.

2.2.2 Yeasts Isolation Methodology

Aquatic Yeast Broth (AYB) was made by the following composition; Glucose 1%, $(NH_4)_2SO_4$ 0.5%, yeast extract 0.5%, NaCl 1% and NaH_2PO_4 0.2 %. Malt Extract Agar – MEA - hyperosmotic medium was prepared according to Sosovele, 2008 and Groenewald *et al*., 2011, with the following composition; malt extract 5%, saccharose 3% and NaCl 8%. 200 µl of sediment sample were directly inoculated into either 25 ml Aquatic Yeast Broth (AYB) or onto an agar plate of the same media (Kurtzman & Robnett, 1998: Sosovele, 2008; Groenewald *et al*., 2011). The incubation of AYB broth flasks started in the field and in the laboratory, they were incubated overnight on a shaking incubator set at 200 rpm at 30^{o}C. The broth was then aseptically streaked on the Aquatic Yeast Broth (AYA) plates and incubated for two days. The agar plates which were directly inoculated with the sediment were also incubated at 30^{o}C for up to two days. In case there was no growth observed after this incubation period, plates were incubated for a further two – three days. Emergent colonies were purified by the serial transfer technique. All media were made selective for fungi by including the antibiotic Ampicillin (0.02 %) after sterilization to minimize bacterial growth as successfully done in a previous study.

The pure cultures were maintained on AYA slants (Glucose 1%, (NH4)2SO4 0.5%, yeast extract 0.5%, NaCl 1%, NaH2PO4 0.2 % and 2% agar). The isolates were stored at 4^{o}C after sub culturing after every fortnight.

2.2.3 DNA Isolation and DNA Amplification (PCR)

Genomic DNA (gDNAs) isolation from all yeast isolates was done as described previously (De Barros Lopez et al., (1998). The quantity of DNA was estimated by spectrophotrometric measurement of absorbance at 260 nm following a standard protocol (Sambrook et al., 1989). The concentration of DNA obtained in this study ranged from 50 ng - 100 ng. The Random Amplification of Polymorphic DNA (RAPD) PCR was done using 50 ng gDNA as template.

The primer sequence used for the random amplifications was; Mg I (5'-CGA CTG CAG T-'3) as used previously by Liu et al., 1996. RAPD PCR reactions were performed in 50 µl volume, with 1 µl (50-100 ng) of each genomic DNA template, 5 µl reaction buffer, 2.5 mM MgCl2, 0.2 mM each dNTPs (dATP, dGTP, dCTP, and dTTP), 0.8 pmol/µl of primer, and 1.25 units per 50 µl of Taq DNA Polymerase. The PCR conditions included initial denaturation step for 4 minutes at 94oC. Then, 40 cycles of denaturation for 1 minute at 94^{o}C, primer annealing for 60 seconds at 36^{o}C, extension for 2 minutes at 72^{o}C and final extension for 5 minutes at 72^{o}C. After PCR reactions, the tubes were maintained at 4^{o}C until further analysis. The RAPD products were then resolved by electrophoresis in 1.2% agarose gels stained with ethidium bromide (0.5 µg/ml) and TBE buffer (0.5%) for 30 minutes following a standard protocol.

3. Results

A total of 25 morphologically different yeasts from mangrove sediments of selected sites of Dar es Salaam (Ras Dege and Mtoni Kijichi) were isolated. 12 yeasts were isolated from Ras Dege sediments and 13 from Mtoni Kijichi sediments.

Table 1.List of yeast isolates from Ras Dege and Mtoni Kijichi and their code names

S/N	**Code**	**Origin of an isolate**	S/N	**Code**	**Origin of an isolate**
1	RY1	Isolate from Ras Dege	1	MtY2	Isolate from Mtoni Kijichi
2	RY2	Isolate from Ras Dege	2	MtY3	Isolate from Mtoni Kijichi
3	RY3	Isolate from Ras Dege	3	MtY4	Isolate from Mtoni Kijichi
4	RY4	Isolate from Ras Dege	4	MtY5	Isolate from Mtoni Kijichi
5	RY5	Isolate from Ras Dege	5	MtY6	Isolate from Mtoni Kijichi
6	RY6	Isolate from Ras Dege	6	MtY7	Isolate from Mtoni Kijichi
7	RY7	Isolate from Ras Dege	7	MtY8	Isolate from Mtoni Kijichi
8	RY8	Isolate from Ras Dege	8	MtY9	Isolate from Mtoni Kijichi
9	RY9	Isolate from Ras Dege	9	MtY10	Isolate from Mtoni Kijichi
10	RY10	Isolate from Ras Dege	10	MtY11	Isolate from Mtoni Kijichi
11	RY11	Isolate from Ras Dege	12	MtY12	Isolate from Mtoni Kijichi
12	RY12	Isolate from Ras Dege	12	MtY13	Isolate from Mtoni Kijichi
13	MtY1	Isolate from Mtoni Kijichi			

3.1 Yeast Cells Morphology

From isolated single colonies of yeasts, microscopic examination at 400X magnification was used to arrive at a total number of 25 morphologically different yeasts. Thirteen yeast isolates were isolated from random, representative sites in the polluted mangrove sediment ecosystem (Mtoni Kijichi), and the other twelve yeast isolates were isolated from non polluted mangrove sediment ecosystem (Ras Dege).

3.2 RAPD-PCR

RAPD-PCR analysis of the yeast isolates from Ras Dege and Mtoni Kijichi gave distinctive patterns that permitted a clear differentiation of the considered isolates. Only Mg1 primer was used for RAPD analysis in this study.

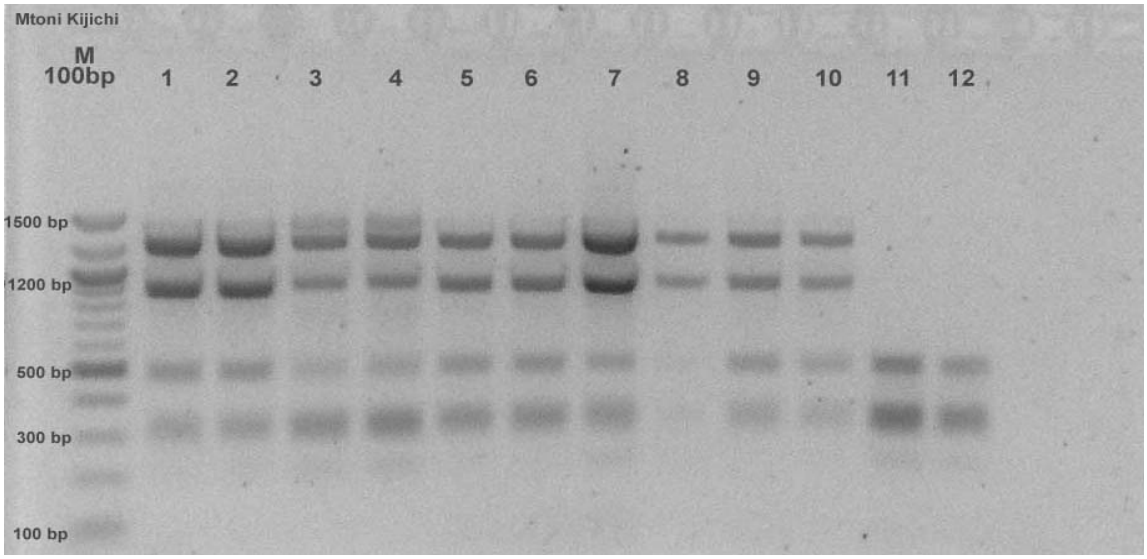

Figure 2. Results of RAPD PCR of Mtoni Kijichi (MtY) 12 yeast isolates using Mg 1 primer. Lanes 1: 100 bp markers. Lane 2 - 13: DMtY1 – 13

The results of the RAPD-PCR analysis of 12 yeast isolates from Mtoni Kijichi (a polluted ecosystem) produced two types of banding patterns (Figure 3). One type of banding patterns comprised 4 PCR bands each (Lane 2 – 11) and the other type had only two PCR bands each (lane 12 – 13). The PCR products ranged from 300 bp – 1400 bp in size as summarized in Table 2.

Table 2. Mtoni Kijichi2 Yeast Genotypes (RAPD-PCR profiles)

Genotype	Number of yeast isolate	Number of PCR bands produced	PCR bands size range	Yeast isolates lane/s no on gel photo
1	10	4	300bp – 1400bp	2 - 11
2	2	2	300bp – 500bp	12 - 13

The results of the RAPD-PCR analysis of 13 yeast isolates from Ras Dege (a non polluted ecosystem) are shown in figure 4.8. Various banding PCR banding patterns were observed.

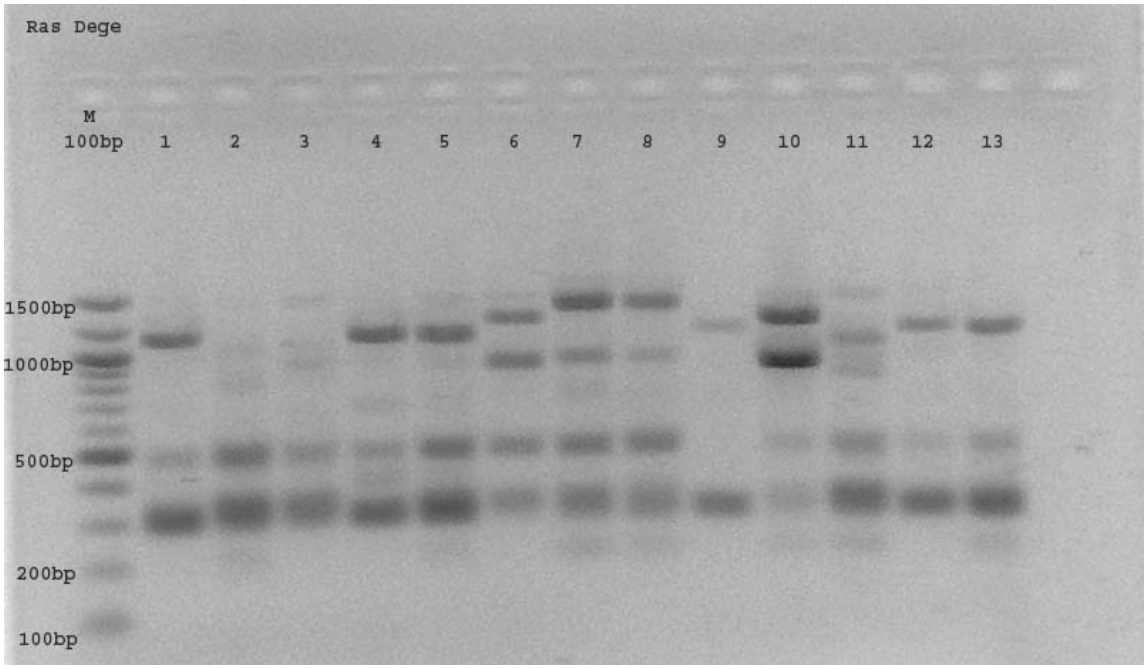

Figure 3. Results of RAPD PCR of Ras Dege (RDY) yeast isolates using Mg 1 primer. Lanes 1: 100 bp markers. Lane 2 - 13: DRDY1 – 12

5 yeast isolates produced 3 PCR bands each of the size ranging from 300 bp – 1200 bp (Lane 2, 5, 6, 13 and 14), 2 yeast isolates produced 2 PCR bands of sizes 300 bp and 500 bp (lane 3 & 4), 1 yeast isolate produced 4 PCR bands each (lane 7) with their sizes ranging from 300 bp – 1300 bp, 1 yeast isolate produces 2 PCR bands each and their sizes ranged from 300 bp – 1000 bp (lane 10), 2 yeast isolates produced 4 PCR bands each with their sizes ranging from 300 bp – 1500 bp (lane 8 & 9), 1 yeast isolate produced 2 bands with their sizes ranging from 1000 bp – 1200 bp (lane 11) and 1 yeast isolate produced 3 bands which had sizes ranging from 300 bp – 1000 bp as summarized in Table 3 below.

Table 3. Ras Dege Yeasts Genotypes (RAPD-PCR profiles)

Genotypes	No. Of Yeast Isolate	No. Of PCR Bands Produced	PCR Bands Size Range	Yeast Isolates Lane/S No On Gel Photo
1	5	3	300 Bp – 1200 Bp	2, 5, 6. 13 & 14
2	2	2	300 Bp – 500 Bp	3 & 4
3	1	4	300 Bp – 1300 Bp	7
4	2	4	300 Bp – 1500 Bp	8 & 9
5	1	3	300 Bp – 1000 Bp	10
6	1	2	300 Bp -1200 Bp	11
7	1	3	300 Bp – 1000 Bp	12

4. Discussion

In this study 25 yeast isolates from two distant mangrove sediments (more than 40 km apart) were used to study the impact of anthropogenic activities on genetic variability of yeasts.

The yeast isolation methodology based on selective media and streaking for single colonies was standard and has been used by various other studies (Kurtzman & Robnet, 1998; Sosovele, 2008; Groenewald *et al*., 2011). However, this study may have been limited to the culturable yeast populations, and a culture independent analysis of the yeast community DNA may have revealed more information on the genotypes of the members of the yeast communities in the sediments as done in some other studies (Iacumin *et al*., 2009; Oguntoyinbo, 2012).

However, the results of this study as summarized in Tables 2 and 3 clearly indicate the impact of sewage pollution with only 2 genotypes of yeasts persisting in the sewage impacted mangrove sediments of Mtoni Kijichi whereas 7 genotypes were revealed in the non sewage impacted mangroves sediments of Ras Dege. The few genotypes that still survive the pollution are most likely including *Candida* yeasts which have been reported in other studies to dominate yeast populations in sediments impacted by human wastes (Kennish, 2001; Loureiro *et al*., 2005; Vogel *et al*., 2007: Kutty & Phillip, 2008).

The higher number and variation of yeast genotypes observed in the non polluted sediments is in agreement with previous report of yeasts from similar ecosystems whereby various genera such as *Candida, Trichosporon, Rhodotolura, Cryptococcus, Debaryomyces and Pichia* were reported (Loureiro *et al*., 2005).

As mangroves are characterized by high population densities of microbes and other types of living organisms, these organisms tend to be highly vulnerable to human activities in coastal watersheds and adjoining embayment (Kennish, 2001). Other anticipated high priority problems are excessive nutrient and sewage inputs to mangroves which tend to modify the genetic variability of microbes and other living organisms in a particular environment (Gulis & Subekropp. 2003; Kruitwagen *et al*., 2006). This appears to be the case of Mtoni Kijichi mangroves sediments which are receiving domestic sewage from nearby settlements (Mgaya *et al.,* 2004),

The technique used in this study, RAPD PCR, is a simple analysis involving a single PCR step following extraction of DNA, It is therefore a rapid methodology for studying genetic characteristics that has been successfully used before (Pinto *et al*, 2004) and is recommended for similar studies.

Furthermore, it is being recommended that culture independent community DNA approach be used to analyze microbial communities in sediments the rDNA is recommended for the identification of modified yeast genotypes.

As the results of this study have clearly showed negative impacts of sewage pollution to natural ecosystems, a recommendation is being made to introduce strategies which may minimize and mitigate future pollution impacts. These may include mandatory liquid waste management systems including treatment before being discharged through the coastline into the sea. Further studies are also being proposed to be carried out within the Tanzanian mangroves for the purpose of gaining more knowledge of the fungal diversity and to step up the conservation of the mangroves which plays a vital role in the survival of the marine

ecosystems.

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References

Abbu, A. (2006). Assessment of feacal Bacteria contamination in sewage and non-sewage impacted mangrove ecosystems along coast of Dar es Salaam. MSc Dissertation (Environment Science) University of Dar es Salaam.

Abdel-Wahab A. M. (2005). Diversity of marine fungi from Egyptian Red Sea mangroves. *Botanica Marina*, *48,* 348-355.

Ananda K & Sridhar R. K. (2004). Diversity of Filamentous fungi on decomposing leaf and wood litter of mangrove forests in the southwest coast of India. *Current science*, *87*(10), 1431-1437.

Botha, A. (2011). The importance and ecology of yeasts in soil. *Soil Biology & Biochemistry*, 43: 1e8.

Bremer, G. B. (1995). Lower marine fungi and decay of mangrove leaf litter. *Hydrobiologia*, *295,* 89-95.

Couto, B. M. M., van der Vossen, J. M., Hofstra, H., & Huis in't Veld, J. H. J. (1994). RAPD analysis: a rapid technique for differentiation of spoilage yeasts. *International Journal of Food Microbiology*, *24,* 249-260.

De Araujo, F. V., Soares, C. A., Hagler, A. N., & Mendonca-Hagler, L. C. (1995). Ascomycetous Yeast communities of marine invertebrates in a South East Brazilian mangrove Ecosystem. *Antonie van Leeuwenhoek*, *68*(2), 91-99.

De Barros, L. M., Soden, A., Martens, A. L., Henschke, P. A., & Langridge, P. (1998). Differentiation: a species identification of yeasts using PCR. *International Journal of Systematic Bacteriology*, *48*(1), 279-286.

De Wolf, H., Ulomi, S. A., Backeljay, T., Pratap, H. B., & Blust, R. (2001). Heavy metal levels in the sediments of four Dar es Salaam mangroves; accumulation in, and effect on the morphology of periwinkle, *littoraria scarbra* (Mollusca: Gastropoda). *Environmental International*, *26,* 243-249.

Groenewald, M., Robert, V., & Smith, M. (2011). The value of the D1/D2 and internal transcribed spacers (ITS) domains for the identification of yeast species belonging to the genus *Yamadazyma*. *Persoonia*, *26,* 40-46.

Gulis, V., & Subekropp, K. (2003). Leaf litter decomposition and microbial activity in

nutrient-enriched and unaltered reaches of a headwater stream. *Freshwater Biology*, *48,* 123-134.

Holguin, G., Vazquez, P., & Bashan, Y. (2001). The role of sediment microorganisms in the productivity, conservation, and rehabilitation of mangrove ecosystems. *Biology and fertility of soils*, *33*, 265-278.

Iacumin, L., Cecchini, F., Manzano, M., Osualdini, M., Boscolo, D., Orlic, S., Comi, G. (2009). Description of the microflora of sourdoughs by culture-dependent and culture-independent methods. *Food microbiology*, *26*(2), 12-135. http://dx.doi.org/10.1016/j.ijfoodmicro. 2008.07.017

Kathiresan, K., & Bingham, L. (2001). Biology of Mangroves and Mangrove Ecosystems. *Advances in marine Biology*, *40,* 81-25.

Kennish, J. M. (2001). Environmental threats and environmental future of estuaries. *Environmental conservation*, *29,* 78-107.

Kohlmeyer, J., Volkmann-Kohlmeyer, B & Eriksson, O. E. (1996a). Fungi on *Juncus roemerianus*. New marine and terrestrial ascomycetes. *Mycological Research*, 100, 393-404.

Kokare, C. R., Mahadik, K. R., Kadam, S. S., & Chopade, B. A. (2004). Isolation, characterization antimicrobial activity of marine *halophilic actinopolyspora species* AHI from the west coast of India. *Current science*, *86*(4).

Kruitwagen, G., Hecht, T., Pratap, B. H., & Wendelaar-Bonga, S. (2005). Changes in morphology and growth of the mudskipper (*Periophthalmus argentlineatus*) associated with coastal pollution. *Marine Biology*, *149,* 201-211.

Kurtzman, C. P., & Fell, J. W. (2005). Yeast systematics and phylogeny – implications of molecular identifications methods for studies in ecology. In: Rosa CA and Peter G, Editors. The Yeast Handbook. Germany: Springer-Verlag berlin Herdelberg, P.11-30.

Kurtzman, C. P., & Robnett, C. (1998). Identification and phylogeny of ascomycetous Yeasts; from analysis of nuclear large subunit (26S) ribosomal DNA partial Sequences. *Ant Van Leeuwen*, 73, 331-371.

Kutty, S. N., & Philip, R. (2008). Marine yeasts - a review. *Yeast*, (www.interscience.wiley.com) DOI: 10.1002/yea.1599

Latha, R., & Mitra, S. (1998). Mangrove fungi in India. *Current Science*, *86,* 12.

Limtong, S., Yongmanitchau, W., Kawasaki, H., & Seki, T. (2007). *Candida thaimueangensis* sp.nov, an anamorphic yeast species from estuarine water in a mangrove forest in Thailand. *International Journal of systematic and Evolutionary Microbiolgy*, *57,* 650-653.

Liu, D., Coloe, S., Jones, L. S., & Pederson, J. B. (1996). Genetic speciation of *Candida* isolates by arbitrarily primed polymerase chain reaction. *FEMS Microbiology Letters*, *145,* 23-26.

Loureiro, S. T., de Queiroz Cavalcanti, M. A., Neves, R. P., & de Oliveira Passavante, J. Z. (2005). Yeasts isolated from sand and sea water in beaches of Olinda, Pernambuco, Brazil. *Brazilian Journal of Microbiology*, *36*, 333-337.

Lyimo, T. J. (1999). Methanogenesis and Sulphate Reduction in Tanzanian Mangrove Sediments, Ph. D. Thesis (Applied Microbiology) University of Dar es Salaam.

Machiwa, J. F. (1998). Distribution and remineralization of organic carbon in sediments of a mangrove stand partly contaminated with sewage waste. *Ambio*, *27*(8), 740-744.

Machiwa, J. F. (1999). Lateral fluxes of organic carbon in a mangrove forest partly contaminated with sewage waste. *Mangroves and salt marshes*, *3,* 95-104.

Marshal, N. (1994). Mangrove conservation in relation to overall environmental considerations. *Hydrobiologia*, *285,* 303-309.

Mgaya, Y., Machiwa, J. F., Lugomela, C., Lyimo, T. J., Muzuka, A., Shaghude, Y., Mvungi, A., Mapunda, B., & Mwanuzi, F. (2004). *Environmental Studies of Mtoni Mangrove Ecosystem, Dar es Salaam*, Final report submitted to the faculty.

Mremi, S. D., & Machiwa, J. F. (2003). Heavy metal contamination of mangrove sediments and the associated biota in Dar es Salaam, Tanzania. *Tanzania Journal of Science*, *29,* 61-76.

Mtanga, A., & Machiwa, J. F. (2007). Heavy metal pollution levels in water and Oysters, Saccostrea cucullata, from Mzinga creek and Ras Dege mangrove ecosystems, Tanzania. *African Journal of aquatic science*, *32*(3), 235-244.

Mwevura, H., Othman, O. C. & Mhehe, G. L. (2002). Organochlorine pesticide residues in sediments and biota from the coastal area of Dar es Salaam city, Tanzania. *Marine pollution bulletin*, *45,* 262-267.

Oguntoyinbo, F. A. (2012). Culture-independent analysis for determination of yeast diversity during solid substrate fermentation of grated cassava for gari production. *World Journal of Microbiology and Biotechnology*, ***27***(10), 2461-2465. http://dx.doi.org/10.1007/s11274-011-0703-9.

Pinto, M. P., Resende, A. M., Koga-Ito, Y. C., & Tendler, M. (2004). Genetic Variability Analysis among clinical Candida species isolates using Random Amplified Polymorphic DNA. *Memórias do Instituto Oswaldo Cruz*, *99*(2), 147-152.

Rumisha**,** C., Elskens**,** M., Leermakers, M., & Kochzius**,** M. (2012). Trace metal pollution and its influence on the community structure of soft bottom mollusks in intertidal areas of the Dar es Salaam coast, Tanzania. *Marine pollution Bulletin*, *64*(3), 521-531. http://dx.doi.org/10.1016/j.marpolbul.2011.12.025.

Sahney, S., Benton, M. J., & Ferry, P. A. (2010). Links between global taxonomic diversity, ecological diversity and the expansion of vertebrates on land. *Biology Letters*, *6*(4), 544-547. http://dx.doi.org/10.1016/j.marpolbul.2011.12.025

Semesi, A. K. (1991). Management Plan for the Mangrove Ecosystem of Mainland Tanzania.

Volumes 1-10. Ministry of Tourism, Natural Resources and Environment, Forest and Beekeeping Division, Dar es Salaam.

Singh, N., & Steinke, T. (1992). Colonization of decomposing leaves of *Bruguier a gymnorrhiza* (Rhizophoraceae) by fungi, and *In vitro* cellulolytic activity of the isolates, Egypt. *Limnologica*, *24,* 525-529.

Sosovele, M. E. (2008). Genetic variability and phylogeny of yeasts from sediments of selected mangrove ecosystems of coastal regions of Tanzania. MSc thesis; University of Dar es Salaam.

Vogel, C., Rogerson, A., Schatz, S., Laubach, H., Tallman, A., & Fell, J. (2007). Prevalence of yeasts in beach sand at three bathing beaches in South Florida. *Water research*, *41*(9), 1915-1920. http://dx.doi.org/10.1016/j.watres.2007.02.010

Yurkov, A. M., Kemler, M., & Begerow, D. (2012). Assessment of yeast diversity in soils under different management regimes. *Fungal ecology*, *5,* 24-35. http://dx.doi.org/10.1016/j.funeco.2011.07.004