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Colonization Pattern of *Rhodotorula* sp. in Polluted Tilapia Fish Aquaria and the Risk of *Rhodotorula* Caused Infection

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Authors' contributions

This work was carried out in collaboration between all authors. Authors AIS and DVA designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript and managed literature searches. Authors AIS, AMO and TMO managed the analyses of the study and literature searches. All authors read and approved the final manuscript.

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ABSTRACT

In this study, the trend of colonization of e-waste soil polluted fish aquaria by *Rhodotorula sp* was monitored. The aquaria containing the specie *Oreochromis niloticus* were polluted separately with different quantities of soil from e-waste dumpsite and the soil without e-waste. The soil sample from e-waste dumpsite differs from soil without e-waste in all of the parameters determined. Higher organic contents (17.60%), moisture content (3.86%), organic carbon (10.17%) and higher value of organic nitrogen (0.35%) were recorded. Four species of fungi were isolated from soil of e-waste dumpsite while two species of fungi were isolated from soil without e-waste. *Rhodotorula* presence in the aquaria was only observed in the first and second week of the research. The highest isolation was from the aquarium polluted with 75 g of soil without e-waste (34 isolates) at week one while the lowest was from the control aquarium (15 isolates) also at week one. It was also observed that plates and week where *Rhodotorula* sp population was high, the populations of other fungi were lower. Most of the other fungi isolated within the two weeks period of *Rhodotorula* colonization were inversely proportional to the population of *Rhodotorula* sp. The pH values and the biochemical

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oxygen demand were significantly affected by the pollutant. The momentary colonization of the aquaria by *Rhodotorula* sp, posed health risk to both the living organisms in the aquaria and human having contact with the aquaria while the antagonistic effect on other fungi could lead to imbalance in the fungi community in the aquaria.

Keywords: Polluted aquaria; e-waste; Rhodotorula sp; colonization; infection.

ABBREVIATIONS

A1 – polluted with 25 g of e-waste soil; A2 – polluted with 50 g of e-waste soil; A3 - polluted with 75 g of e-waste soil; B1 – polluted with 25 g of soil without e-waste; B2 – polluted with 50 g of soil without e-waste; B3 - polluted with 75 g of soil without e-waste; WK – week.

1. INTRODUCTION

Rhodotorula species are ubiquitous, saprophytic yeasts that can be recovered from many environmental sources. *Rhodotorula*, are common environmental yeast that are found in air, soil, lakes, ocean water, milk, fruit juice including sites with unfavourable conditions. Studies also, have reported the occurrence of *Rhodotorula* species in marine waters polluted by household waste [1]. *Rhodotorula* species are part of the Basidiomycota phylum that colonises plants, humans, and other mammals. *Rhodotorula* produces pink to red colonies and blastoconidia that are unicellular lacking pseudohyphae and hyphae. *Rhodotorula mucilaginosa, Rhodotorula glutinis,* and *Rhodotorula minuta* were known to cause disease in humans [1]. *Rhodotorula* spp have been recognized as emerging yeast pathogens in humans. Although the consumption of food contaminated with yeast may not have a direct role in causing opportunistic infections, there is growing concern that food may be an underestimated source of environmental pathogens [2]. In addition, environmental monitoring of yeasts in specific areas of two tertiary local hospitals, revealed the presence of *Rhodotorula* species in a substantial amount of air samples [3]. As a direct consequence of the wide exposure to *Rhodotorula* in the hospital environment, patients who have a depressed immune system can develop Rhodotorulosis, causing a variety of systemic infections. *Rhodotorula* spp have been isolated from stool samples, indicating that these yeasts can survive in the extreme conditions of the gastrointestinal tract, and it is still uncertain whether *Rhodotorula* is capable of passing from the gastrointestinal tract into the bloodstream [4]. There were reports of an outbreak of skin infections in chickens and a report in lung infection in sheep, both caused by *Rhodotorula mucilaginosa* [1]. *Rhodotorula* was reported as the causative agent of epididymitis, skin lesions in a sea lion and dermatitis in a cat that had crusted lesions and mastitis [5-7]. This fungus can also be found in pools where sea animals were kept in captivity [8]. Also *Rhodotorula* in humans previously considered non pathogenic, have emerged as opportunistic pathogens with the ability to colonises and infect susceptible patients.

E-waste contains valuable metals (copper, platinum group) as well as potential environmental contaminants, especially lead, mercury, nickel, selenium, cadmium, polybrominateddiphenyl ethers (PBDEs) and polychlorinated biphenyls (PCBs). Most e-waste is disposed in landfills, effective reprocessing technology, which recovers the valuable materials with minimal environmental impact, is expensive. Some reprocessing initially results in extreme localized contamination followed by migration of the contaminants into receiving waters and food chains [9,10].

Fishes, among aquatic species, are the inhabitants that cannot escape from the detrimental effects of pollution. This is because of their very intimate contact with water that carries the pollutants in solution or suspension [11]. Fish play an important role in the nutrition of man and therefore, there is need to monitor the water quality for fish culturing and the water from which fish is harvested in the natural environment. The location of e-waste dumpsite and the burning of the e-waste on this dumpsite which is not far from Lagos lagoon (Lagos State, Nigeria) can lead to the pollution of the environments (land and water), which can promote the growth of diverse fungi including *Rhodotorula,* which poses health risk to fishes, other aquatic animals and the human inhabitants relying on such water for either domestic or agricultural purposes. The results in this research

were geared towards raising public awareness on the danger of water pollution, in this case improper disposal of e-waste which has substances that can affect aquatic life and promote the proliferation of disease causing microbes such as *Rhodotorula* in aquatic environment.

2. MATERIALS AND METHODS

2.1 Collection of Soil Samples

Two different soil samples (soil from e-waste dumpsite and soil without e-waste), were collected from e-waste dumpsite and distance away from the e-waste dumpsite both at Alaba International Market, Lagos State, Nigeria. The samples were collected in sterile containers and taken for physiochemical and microbiological analysis [12,13].

2.2 Set up and Pollution of Aquaria

Seven aquaria in triplicates, each containing six juvenile tilapia fish, were polluted with three different quantities of the e-waste soil sample and soil without e-waste (25 g, 50 g and 75 g for both soil samples) in the ratio of 25 L: 25 g, 25 L: 50 g, 25 L : 75 g of water to soil samples after acclimatization of the fishes for six weeks and the seventh aquarium is the control (aquaria without any pollution). The aquaria were monitored weekly for five weeks for physiochemical parameters; pH, dissolved oxygen, biochemical oxygen demand while the microbiological analyses were; isolation and identification of yeast in the polluted tilapia aquaria.

2.3 Fungi Identification

Visible observation and microscope at low power magnification were used to determine the parameters such as colony colour, characteristics of the submerged hyphae rhizoid, spiral or regular and characteristic shape of mature fruiting bodies were all observed. Microscopic examination of fungi involved transferring a small piece of mycelium free of medium using a sterile inoculating loop unto a clean glass slide containing a drop of cotton blue-in-lactophenol. The mycelium was then spread properly. The preparation was covered with a clean grease free cover slip and observed under medium power (x100). The observations made were used in identifying the fungi species [14].

2.4 *Rhodoturula* Identification

The identification of *Rhodoturula* was based on morphological, cultural, physiological and biochemical characteristics [15].

2.5 Determination of Physicochemical Properties of the Soil Samples

The pH was measured using pH meter standardized at pH 7.0 using appropriate buffers [16]. Biochemical oxygen demand was determined using the method described by [16]. The moisture content of each soil sample was determined by drying 10 g of the soil in an oven at 80℃ until a constant weight was reached and the percentage moisture content was calculated. The organic carbon content was determined using the Walkey-Black wet oxidation method as described by [13]. Available phosphorus, exchangeable bases (magnesium, calcium, sodium and potassium ion) concentration were determined using standard methods [18]. Total nitrogen was measured using the kjeldahl, digestion method [19].

3. RESULTS

Tables 1 and 2 show the physiochemical characteristics of the soil samples while Tables 3 and 16 show the microbiological outcomes.

4. DISCUSSION

Articulospora inflata, Zoopage nitospora, Varicosporium elodeae, Penicillium italicum, Candida sp *Aspergillus niger, Aspergillus flavus, Aspergillus repens, Rhizopus stolonifer* and *Mucor mucedo* were the other fungi species isolated alongside *Rhodoturula* sp, from the soil samples and the polluted aquaria (Tables 3 - 4). The populations of these isolates on cultured plates were inversely proportional to the population of *Rhodoturula* sp on the same culture plates and in the same week (Tables 5 - 14). This could be as a result of antagonistic activities of *Rhodoturula* sp. *Rhodotorula* had been known for antagonistic activities against other fungi [20], [21]. This could be attributed to the production of antifungal substances or metabolites, which antagonized or suppressed the growth and proliferation of other fungi. They can therefore be used as biological control in the control of infections caused by these other fungi [22]. *Rhodoturula* sp was not isolated from the soil samples (Table 3) but was isolated from the aquaria therefore the aquaria were colonized by

Rhodoturula sp. This colonization, could be due to its ability to adapt to different environmental conditions and use wide range of food substances as nutrient and energy source (Tables 1 - 2) [23,4,1,24]. Some of the tilapia fish died (19% of the tilapia fish died) at the initial stage this also could have resulted in the *Rhodoturula* colonization of the aquaria. Since it is also a saprophytic fungi; it can utilized dead organic matter for its cellular division and energy source [1]. The colonization pattern was transitory, observable only in the first two weeks of the research (Table 5). Additional nitrogen would have been added to the aquaria resulting from the pollutant that had nitrogen content (Table 1) and also possibly from the fish metabolites. Although, *Rhodoturula* sp had been known to scavenge nitrogen, but at the end of the first two weeks when probably nitrogen in the aquaria would have been lower, *Rhodotorula* scavenging ability should have been observed if it were continuously isolated from the aquaria. *Rhodotorula*'s presence (colonization) in the aquaria was more for its saprophytic activities rather than the other reasons (such as nitrogen scavenging and biosoption activities), since it was absence in subsequent weeks when there were no more death of the fishes. [25] reported *Rhodotorula* has having ability to scavenge nitrogenous compounds from its environment remarkably well, growing even in air that has been carefully cleaned of any fixed nitrogen contaminants. In such conditions, the nitrogen content of the dry weight of *Rhodotorula* can drop as low as 1%, compared to around 14% for most bacteria growing in normal conditions. [26] documented the use of *Rhodotorula mucilaginosa* in the biosorption of heavy metals. This was in line with the earlier findings of [27], who documented that fungi of metal contaminated soil have high level of metal tolerance and biosorption properties.

Table 1. Physicochemical properties of soil samples from e-waste dumpsite

Soil samples	pH	Moisture content (%)	Organic matter (%)	Organic carbon (%)	Organic nitrogen (%)
A	7.90	3.86	17.60	10.17	0.35
B	8.70	2.24	5.00	2.89	0.21

Key: A- soil from e-waste dumpsite, B- soil without e-waste

Table 2. Mineral content of soil samples from e-waste dumpsite

Soil samples	Sodium (mg/kg)	Potassium (mg/kg)	Calcium (mg/kg)	Magnesium (mg/kg)	Phosphorus (mg/kg)
A	24.40	33.30	182.00	34.00	146.65
B	31.40	32.90	245.00	29.70	160.00

Key: A- soil from e-waste dumpsite, B- soil without e-waste

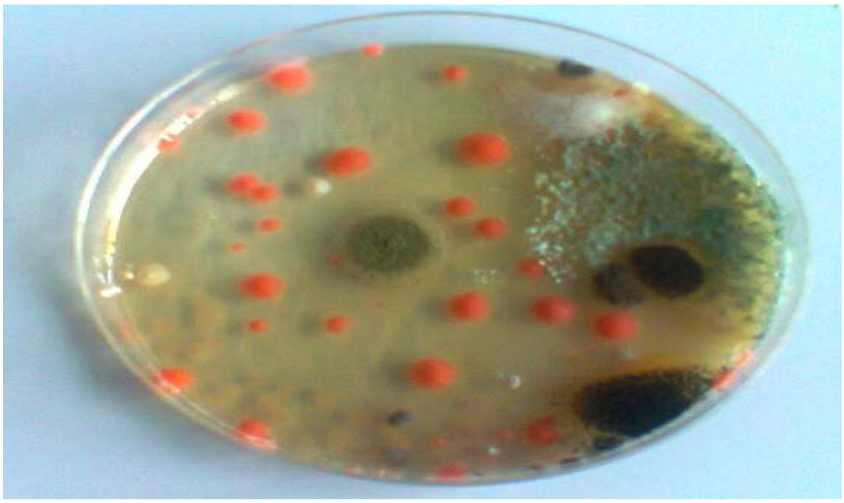

Fig. 1. Mixed culture of fungi - *Rhodotorula* spp growing with other fungi

Table 3. Isolated fungi from the e-waste soil and the soil without e-waste

Isolates	E-waste soil	Soil without e-waste
Candida sp	+	+
Zoopage nitospora	+	-
Articulospora inflata	+	+
Varicosporium elodeae	+	-

Key: + = present, - = absent

Table 4. Probable fungal isolates from tilapia control aquaria and tilapia aquaria polluted with sample A and B

Isolate	Control	Sample B	Sample A
Penicillium italicum	+	+	+
Articulospora inflata	+	+	+
Aspergillus niger	+	+	+
Rhizopus stolonifer	+	+	+
Aspergillus flavus	+	+	+
Mucor mucedo	+	+	+
Zoopage nitospora	+	+	+
Varicosporium elodeae	+	+	+
Rhodoturula sp	-	+	+
Aspergillus repens	-	+	+

Key: Sample A – soil from e-waste dumpsite, sample B – soil without e-waste, + = present and - = absent

Fig. 2. *Rhodotorula* spp growing on Potato dextrose agar plate

Table 5. Occurrence of *Rhodoturula* sp in polluted tilapia fish aquaria

Samples	WK1	WK2	WK3	WK4	WK5
Control	15	13	-	-	-
A1	24	25	-	-	-
A2	29	8	-	-	-
A3	20	10	-	-	-
B1	31	8	-	-	-
B2	30	25	-	-	-
B3	34	9	-	-	-
Total	183	98	-	-	-

Table 6. Occurrence of *Penicillum italicum* in polluted tilapia fish aquaria

Samples	WK1	WK2	WK3	WK4	WK5
Control	-	2	4	5	1
A1	-	1	5	-	-
A2	-	-	1	8	-
A3	-	1	7	1	-
B1	-	1	3	-	-
B2	-	1	9	2	-
B3	-	-	15	-	-
Total	-	6	44	16	1

Table 7. Occurrence of *Articulospora inflata* in polluted tilapia fish aquaria

Samples	WK1	WK2	WK3	WK4	WK5
Control	2	3	6	3	3
A1	1	2	4	2	2
A2	2	1	5	2	3
A3	-	3	3	3	2
B1	-	2	3	2	1
B2	-	2	4	2	3
B3	-	3	3	2	2
Total	5	16	28	16	16

Table 8. Occurrence of *Aspergillus niger* in polluted tilapia fish aquaria

Samples	WK1	WK2	WK3	WK4	WK5
Control	-	-	-	1	-
A1	1	1	-	-	1
A2	1	1	1	-	-
A3	5	1	1	-	-
B1	6	1	-	-	1
B2	9	-	1	-	-
B3	9	2	-	-	-
Total	31	6	3	1	2

Table 9. Occurrence of *Rhizopus stolonifer* in polluted tilapia fish aquaria

Samples	WK1	WK2	WK3	WK4	WK5
Control	-	1	-	1	-
A1	5	2	1	-	-
A2	1	-	1	1	-
A3	1	1	1	-	-
B1	2	-	5	-	-
B2	1	-	-	-	-
B3	1	1	-	-	-
Total	11	5	8	2	-

Table 10. Occurrence of *Aspergillus repens* in polluted tilapia fish aquaria

Samples	WK1	WK2	WK3	WK4	WK5
Control	-	-	-	-	-
A1	-	1	-	1	-
A2	-	-	-	1	5
A3	-	4	-	-	-
B1	-	-	-	1	2
B2	-	-	-	3	-
B3	-	-	-	-	2
Total	-	5	-	6	9

Colonization of the aquaria by *Rhodotorula* can leads to *Rhodotorula* infections in aquatic animals. There have been reports of skin infections in both terrestrial and aquatic animals (chickens, sea animals). It can also cause lung infections and otitis in sheep and cattle [23,1]. Human interaction with such *Rhodotorula* colonized environment can lead to any of the *Rhodotorula* caused infections or fungemia especially in susceptible individuals.

Table 11. Occurrence of *Aspergillus flavus* in polluted tilapia fish aquaria

Samples	WK1	WK2	WK3	WK4	WK5
Control	-	1	3	1	9
A1	-	-	5	-	2
A2	-	-	2	8	2
A3	-	2	7	1	1
B1	-	-	4	4	2
B2	-	-	4	6	2
B3	-	-	6	-	2
Total	-	3	31	20	20

Table 12. Occurrence of *Mucor mucedo* in polluted tilapia fish aquaria

Samples	WK1	WK2	WK3	WK4	WK5
Control	-	3	-	-	-
A1	-	-	-	-	1
A2	-	-	-	-	-
A3	-	-	-	-	-
B1	-	-	3	-	-
B2	-	-	-	-	-
B3	-	1	-	-	1
Total	-	4	3	-	2

Table 13. Occurrence of *Zoopage nitospora* in polluted tilapia fish aquaria

Samples	WK1	WK2	WK3	WK4	WK5
Control	-	-	-	1	1
A1	-	-	-	-	2
A2	-	-	-	-	4
A3	-	-	-	-	1
B1	-	-	-	1	-
B2	-	-	-	1	4
B3	-	-	-	-	-
Total	-	-	-	3	12

The colonization of the aquaria by some of the other fungi (such as *Aspergillus* spp, *Mucor mucedo*, *Penicillum italicum, Rhizopus stolonifer*), might not be of wholesome benefit to the fishes and human. Some are known to produce inhibitory substances such as toxin or toxic metabolites (aflatoxins, cyclopiazonic acid, ochratoxins, kojic acid), which might affect or inhibited the growth of the other microorganisms in the aquaria, leading to microbial imbalance in the aquaria. These inhibitory substances might as well affect the fish and human as they come in

Table 14. Occurrence of *Varicosporium elodeae* in polluted tilapia fish aquaria

Samples	WK1	WK2	WK3	WK4	WK5
Control	-	-	3	-	1
A1	-	-	2	-	-
A2	-	-	2	-	2
A3	-	-	2	-	2
B1	-	-	2	-	2
B2	-	-	2	-	1
B3	-	-	-	-	-
Total	-	-	13	-	8

Table 15. Weekly pH of tilapia aquaria

Samples	WK1	WK2	WK3	WK4	WK5
Control	6.60^{a}±0.00	7.15^{ab}±0.05	6.80^{a}±0.05	7.20^{ab}±0.05	7.40^{bc}±0.10
A1	6.70^{ab}±0.00	7.25^{bc}±0.05	7.00^{b}±0.00	7.00^{a}±0.00	7.70^{d}±0.10
A2	7.05^{c}±0.00	7.00^{a}±0.10	7.00^{b}±0.00	7.20^{ab}±0.05	7.30^{abc}±0.05
A3	6.80^{b}±0.10	7.40^{bc}±0.10	7.00^{b}±0.00	7.30^{b}±0.10	7.15^{ab}±0.05
B1	7.00^{c}±0.00	7.40^{bc}±0.00	7.20^{c}±0.10	7.20^{ab}±0.10	7.11^{a}±0.11
B2	6.70^{ab}±0.00	7.40^{bc}±0.10	7.05^{cb}±0.05	7.30^{b}±0.00	7.48^{cd}±0.08
B3	6.70^{ab}±0.00	7.50^{c}±0.00	7.10^{bc}±0.00	7.30^{b}±0.00	7.55^{cd}±0.05

Values are presented as Mean ±S.E (n=3). Means with the same superscript letter(s) along the same column are not significantly different (P>0.05). Means in the same column are the comparison between the different groups

Table 16. Weekly biochemical oxygen demand of tilapia aquaria (mg/l)

Samples	WK1	WK2	WK3	WK4	WK5
Control	0.83^{a}±0.12	3.63^{b}±0.08	3.05^{b}±0.05	3.13^{a}±0.11	3.50^{a}±0.20
A1	2.30^{b}±0.04	3.72^{b}±0.07	3.47^{c}±0.02	3.82^{b}±0.04	3.78^{ab}±0.13
A2	0.85^{a}±0.04	5.80^{d}±0.12	4.16^{e}±0.06	4.78^{e}±0.13	4.79^{d}±0.10
A3	0.82^{a}±0.02	1.67^{a}±0.16	2.58^{a}±0.16	3.16^{a}±0.12	4.21^{bc}±0.20
B1	3.76^{d}±0.20	3.58^{b}±0.03	3.88^{d}±0.05	4.30^{cd}±0.24	4.36^{cd}±0.13
B2	4.42^{e}±0.05	1.70^{a}±0.18	3.53^{c}±0.08	3.92^{bc}±0.06	3.71^{a}±0.07
B3	3.42^{c}±0.06	4.57^{c}±0.20	4.28^{e}±0.05	4.65^{de}±0.03	4.66^{cd}±0.05

Values are presented as Mean ±S.E (n=3). Means with the same superscript letter(s) along the same column are not significantly different (P>0.05). Means in the same column are the comparison between the different groups

contact with them. Fungi had been known to produce toxins (mycotoxin) which are harmful to man. Some of the resultant harmful effects in human are; vomiting, coma, convulsions, malaise, abdominal discomfort, liver cell necrosis even death [28,24]. Ochratoxin-A was reported to cause major abnormalities in eggs of zebra fish leading to high mortality in hatchlings. Immunological changes, cancerous tumours, reduced feed consumption, pale gills, kidney abnormalities, gastric gland damage, anaemia (low red blood cell counts), poor growth and feed efficiency were other effects of mycotoxin in fishes [29,30].

The pH values (Table 15), in all the aquaria tended towards neutrality with no particular trend. The fluctuations in pH values could have been due to the different fermentative activities of the microorganisms present [31].

The significant differences observed in the BOD (Table 16) could be due to the differences in the quantity of organic matter present (from the soil samples) in the aquaria alongside differences in microbial activities going on in these aquaria.

5. CONCLUSION

In conclusion, *Rhodotorula* yeasts observed in this research had an evanescent colonization of the aquaria and also buttress its ubiquitous nature. This posed antagonistic effect on the fungal biota in the aquaria and posed health risk to both the fish and human interacting with the aquaria. Hence a need to monitor fungal biota of

aquatic environment especially where fish are cultured or where fishing activities are taking place to prevent imbalance fungi community in the aquatic environment and fungemia or *Rhodotorula* caused infections. Considering the fact that *Rhodotorula* are ubiquitous, saprophytic and opportunistic pathogenic fungus, its isolation in this research calls for caution in disposing e-waste and waste generally that encourage unhealthy fungi colonization of an aquatic environment.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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