Fungal Diversity In Water And Mangrove Oyster (*Crassostrea Gasar*), Woji/Trans-Amadi Creek, Port Harcourt, Nigeria

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Abstract: **The fungal diversity in water and mangrove oyster (*Crassostrea gasar*),Woji/Trans-Amadi Creek, Port Harcourt, Nigeria was studied for six months between January and June,2017 covering both dry and wet seasons. Water and mangrove oysters (*Crassostrea gasar*) samples were collected from three stations and analyzed for bacteria using standard method. The data obtained were subjected to SPSS software version 20 for descriptive and inferential statistics using one-way analysis of variance and Duncan multiple range test. It was observed that a total of the 8 species of fungi, *Aspergillus niger, Aspergillus fumigatus, Penicillium species, Fusarium sp, Physanium cinerium, Candida sp, Aspergillus vasicular, and Pennisetum cemenberti* with different frequencies were isolated from the isolates. The diversity indices (Margalef, Menhinick, Shannon diversity, Shannon Wiener, Eveness/ Equitability and Simpson dominance) were identified/calculated. The values of Margalef, Menhinick and Shannon diversity indices were consistently higher in station 1 than the other stations in the order Station 1> Station 2> Station 3. Shannon Wiener values showed that the water and oyster tissues are heavily polluted. It was therefore recommended that anthropogenic activities going on in the area should be regulated.**

Key words: **Fungal diversity, Mangrove oyster, Woji/Trans-Amadi Creek, Port Harcourt**

I. INTRODUCTION

Due to increased anthropogenic activities resulting from urbanization in the past decade there has been increased introduction of organic and inorganic wastes into the aquatic environments (Otene and Alfred-Ockiya, 2019). This therefore, caused increased loads and diversity of species of microbial organisms especially fungi in the aquatic environment and biota. It has been observed that the diversity of fungi and other microbes found in water are also associated with the biota therein.

Nontokozo *et.al* (2017) opined that there is a global growing interest in the occurrence and diversity of fungi and their secondary metabolites in the aquatic ecosystems especially with respect to their roles in water quality and human health. The safety of seafood consumption by humans among other things depends largely on the diversity and loads of fungi and other microbes present in the food.

According to WHO (1997) water that can be considered safe and fit for human consumption is that which cannot cause any significant health hazard when consumed and that which has microbial, chemical and physical characteristics that meet the World Health organization guidelines.

Oyster is a bivalve mollusks considered as a major source of aquatic or seafood and economic resource among the inhabitants of Niger Delta. The presence of certain species of fungi (especially the toxic species) in water tends to infect the aquatic animals including those consumed by man (Pascho *et.al*, Paxton, 2000). Various fish species including the mollusks have been severally and previously reported to have the disease called Epizootic Ulcerative Syndrome (EUS) a most dangerous disease in the aquatic ecosystems, on the African continents reported to be caused by the fungi, *Aphanomyces invadans* (Huchzemeyer *et.al*, 2010). Mastan (2015) reported the two fungal genera, *Saprolegni* and *Achyla* to be responsible for infections which affected the fish species mainly *Channa, Heterioprestis, Mystus and Labeo*. Eli *et.al* (2011) reviewed various fungal infections in African fish to include Saprolegniasis, Dermal Mycoses, Brachiomyces infections, systemic mycoses and Dermocystidium. According to Jalees *et.al* (2012) the fungal species responsible for infecting fish organs include *Aspergillus species, Penicillium specie, Rhizopus specie*, Blastomyces species and Alternaria.

Similarly, humans may be directly exposed to fungal toxins either through ingestion of contaminated water and sea food (fish, prawn, sprirulina etc) or by ingestion of plant products (vegetables and fruits). Gottlich *et.al* (2002) reported that harmful aquatic fungal species, *Aspergillus fumigatus* was isolated from patients suffering from immunosuppressant infections and organ failure which was confirmed by comparing the genotypes of the species and that of a micro-organizms isolated from the patients (Pereira *et.al*; 2010 Gonclaves *et.al*, 2006).

Woji/Trans Amadi creek has been considered to be an essential water body, considering the fact that it is a source of livelihood for the inhabitants. The Niger Delta aquatic environment has been widely studied but very few microbiological (fungal) studies have been carried out on the brackish water creek with respect to diversity. This paper is therefore, designed to investigate the fungal load and diversity of water and mangrove oyster of Woji/Trans-Amadi creek.

II. MATERIALS AND METHODS

Study Area

The study area with respect to stations falls within latitudes 4°48'44.285" N and longitudes 7°3'2.182" E from station one using the WGS-84 and 4°49'21.376" N, 7°2'45.861"E. Three sampling stations were selected within the study area with a distance of 500m apart. The stations were chosen based on ecological settings and human activities in the area. The stations include Oginigba, Okujagu and Azubie along Trans-Amadi axis (Figure 1).

These sites receive a lot of effluents from the ongoing anthropogenic activities. The river channel widened as it crosses the railway bridge at the Woji area. It maintained this broad width, though at a small divergent angle through to Slaughter Bridge (Trans-Amadi area), then into the Amadi Creek behind the Port Harcourt Zoo and finally joined the Bonny River which is a trajectory of the Atlantic Ocean.

Samples Collection and Preparation

Water samples were collected based on standard limnological method in triplicate for six months from the three stations in sterile containers and transported to the laboratory in an ice pack the same day where microbiological analysis were carried out immediately. Oyster samples were randomly cut with a sharp knife on the roots of mangrove at the river bank collected by hand picking at river bank intertidal zone (sediment) from the various sampling points through the help of the fishers in the areas and transported to the laboratory where they were sorted, rinsed, processed and kept in refrigerator for further analysis.

Isolation and identification of fungi

Ten-fold serial dilution of the sampled water and oyster tissues were prepared aseptically in sterile physiological saline up to 10^{-5} and 0.1 ml aliquot of each dilution was inoculated on dried nutrient agar and Sabouraud dextrose agar (Oxiod Limited, Wade Road, Basingstoke Hampshire United Kingdom) plates, in triplicate using the spread plate technique for enumeration of and fungi count. The nutrient agar plates were incubated at 35°C for 24 h under aerobic condition, while the Sabouraud plates were incubated at room temperature for five days. Plates containing 30-300 colonies were selected and counted. The number of colony forming units per ml (cfu/ml) was calculated by multiplying the number of colonies per dilution factor. Identification of the fungi was done by macroscopic observation of the growth morphology followed by microscopy after staining with lactophenol cotton blue (Abolude *et al*, 2013; Willoughby, 1994). Cfu/ml = number of colonies per ml plated/total dilution factor (Onions *et al*., 1981).

Statistical Analysis

SPSS software version 20 was used to carry out the statistical analysis of the fungi samples. One-way analysis of variance was carried out at P = 0.05 and ANOVA test was used to determine source of the observed differences.

Calculation of Bio-indices

These indices were used to obtain estimation of species diversity, species richness and species evenness.

1. Species richness (R1 and R2) obtained using the equation

R1 = (Margalef, 1958) $\frac{S-1}{N}$

R2 = (Menhinick, 1964) $\frac{S}{\sqrt{\Sigma ini}}$

Where,

R = Index of species richness

S = Total number of species

N = Total number of individuals

Ln= Natural logarithm

3. Shannon and Wiener (1949) and Simpson (1949) diversity index values were obtained by using the following equation:

(Shannon's index)

(Shannon's index) $-\sum_n \left(\frac{ni}{N} - log2\,(\frac{ni}{N}) \right)$

(Simpson index) $\frac{\Sigma ini\,(ni-1)}{N\,(N-1)}$

4. Species evenness was determined using the following expression.

Shannon's equitability (EH) was calculated with the equation:

$\frac{\Sigma i(\frac{ni}{N} - \ln(\frac{ni}{N}))}{\ln N}$

Equitability assumes a value between 0 and 1 being complete evenness.

5. Dominance index is used to characterize most conspicuous and abundant species with its relative importance related to degree of influence it has on ecosystem components.

Dominance index = $1-(\frac{\Sigma ini\,(ni-1)}{N\,(N-1)})$

6. The Berger – Parker Dominance Index is a simple measure of the numerical importance of the most abundance species.

Berger – Parker Dominance Index = $\frac{nmax)}{N}$

Where

nmax= maximum number of organisms

N= Total number of individuals

III. RESULTS

Table 1 showed the spatial and temporal values of fungi in the study area with the fungal loads in oyster tissues consistently higher than that of water. The highest fungal load ($3.40x10^4$cfu/100g) was observed in January and May in stations 1 and 2 respectively. Fungal count/load of oyster tissues was significantly higher in station 2 than both stations 1 and 2 at $p<0.05$ (Fig.2).The fungal load in water was significantly higher in the wet season (5035 ± 9765.50cfu/ml) than the dry season (2900 ± 741.6cfu/ml) at $p<0.05$. The fungal load in the Oyster was also significantly higher in the wet season (49333.33 ± 642cfu/100g) than the dry season (20988.89 ± 11108cfu/100g) at $p<0.05$ (Fig.3).

Table 1. Monthly and Spatial Values of Fungi in Water and Oyster in the Study Area

Month	Station	Fungi in Water (Fw)	Fungi in Oyster (Foys)
January	1	$2.10x10^3$	$2.30x10^4$
	2	$4.00x10^3$	$3.40x10^4$
	3	$2.60x10^3$	$3.20x10^3$
	Total	$8.60X10^3$	$6.02X10^4$
February	1	$2.20x10^3$	$2.50x10^4$
	2	$4.10x10^3$	$3.30x10^4$
	3	3.10x103	2.30x104
	Total	$9.40X10^3$	$8.10X10^4$
March	1	$2.30x10^3$	1.80x104
	2	$3.10x10^3$	$2.60x10^4$
	3	$2.60x10^3$	$3.70x10^3$
		$8.00X10^3$	$4.77X10^4$
April	1	$1.80x10^3$	$2.30x10^4$
	2	$2.40x10^3$	$3.20x10^4$
	3	$2.20x10^2$	$2.10x10^4$
	Total	$4.42X10^3$	$7.60X10^4$
May	1	$2.80x10^3$	$3.40x10^4$
	2	$2.80x10^3$	$3.40x10^4$
	3	$1.40x10^3$	$2.10x10^4$
		$7.00x10^3$	$8.90x10^4$
June	1	$2.00x10^3$	$3.00x10^4$
	2	$3.10x10^4$	$2.20x10^5$
	3	$2.10x10^3$	$3.10x10^4$
	Total	$3.50X10^4$	$2.80X10^5$

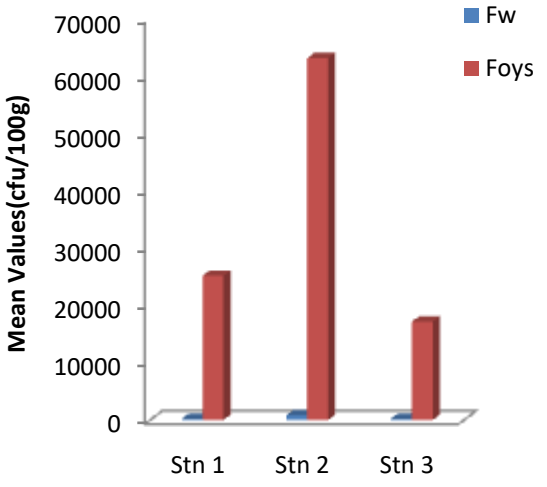

Fig.2. Spatial Mean Values of Fungi in Water (cfu/ml) and Oyster Tissues (cfu/100g) in the Study Area

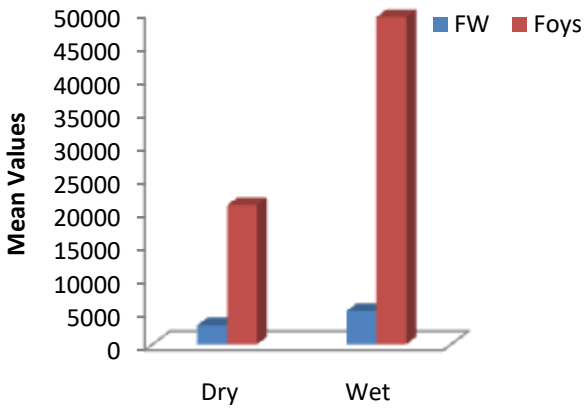

Fig.3. Seasonal Mean Values of Fungi in Water (cfu/ml) and Oyster Tissues (cfu/100g) in the Study Area

Table 2 showed the various species and frequency of fungi in both water and oyster tissues. The result of this study showed that a total of 8 species of fungi with different frequencies were isolated from the isolates which include *Aspergillus niger, Aspergillus fumigatus, Penicillium species, Fusarium sp, Physanium cinerium, Candida sp, Aspergillus vasicular, and Pennisetum cemenberti.*

Candida species, Aspergillus niger and *Aspergillus fumigates* had the highest frequencies in water in station 1 respectively. They were also consistently present across the 3 stations in the study area except *A. fumigatus*.

Table 2. Spatial Frequency Distribution of Fungal Species in Water and Oyster Tissues

S/N	Fungal Species	Stn 1		Stn 2		Stn 3	
		Water	Oyster	Water	Oyster	Water	Oyster
1	*Penicllium spp.*	+	+	+	+	+	+
2	*Aspergilllus niger*	+	+	–	+	+	+
3	*Fusarium sp*	-	+	+	+	–	+
4	*Aspergillus fumigates*	–	+	+	+	+	+
5	*Aspergillus vascular*	+	+	+	+	–	–
6	*Candida sp*	+	+	+	+	–	–
7	*Pennicilliu m cemenberti*	+	+	+	+	+	+
8	*Physarium cinerium*	+	+	–	+	+	+

Key: +=Present, - = Absent

Tables 3 and 4 showed the biodiversity indices of fungi in water and oyster tissues respectively. The Margalef index of water ranged between 0.913 and 1.155 with the mean value of 1.070±0.14 while that of oyster tissues varies between 1.095 and 1.517 with the mean value of 1.3730±0.24. The highest Margalef indices (1.155, 1.517) were observed in stations 1 while the lowest (0.913, 1.095) were observed in stations 3 for water and oyster respectively.

Menihick index for water ranged between 2.389 and 2.883 with the mean value of 2.713±0.28 while that of oyster tissues ranged between 2.809 and 3.724 with the overall mean of 3.415±0.53. The values for both water and oyster tissues were highest in station 1 but lowest in station 3 respectively.

Shannon diversity index for water varies between 1.538 and 1.727 with the overall mean value of 1.660±0.90 while that of oyster tissues varies between 1.782 and 2.066 with the mean value of 1.970±0.16. The highest Shannon diversity index for water and oyster tissues were observed in stations 1 and 2 respectively while the lowest values for both were observed in stations 3 respectively.

Shannon Wienner index for water ranged from 0.670 to 0.787 with the overall mean value of 0.738±0.56 while that of oyster tissues ranged from 0.771 to 0.897 with the overall mean value of 0.855±0.07. The values were highest in stations 2 but lowest in stations 3

Evenness index for water ranged from 0.947 to 0.968 with the mean value of 0.960±0.110 while that of oyster ranged from 0.991 to 0.995 with the overall mean value of 0.993±0.003.Values were highest in stations 3 but lowest in stations 2 and 1 for water and oyster tissues respectively.

Simpson dominance index for water ranged between 0.189 and 0.200 with the mean value of 0.201±0.02 while that of oyster tissues ranged between 0.128 and 0.170 with the mean value of 0.143±0.02 (Table 3 and 4)

Table 3 Biodiversity Indices of Fungi in Water

Indices/Station	1	2	3	Mean	Mini Max
Margalef (d)	1.155	1.141	0.913	1.070±0.14	0.913-1.116
Menhinick (d_1)	2.883	2.866	2.389	2.713±0.28	2.389-2.883
Shannon Dversity (H^1)	1.727	1.696	1.538	1.660±0.90	1.558-1.727
Shannon Wiener (H)	0.750	0.787	0.677	0.738±0.56	0.670-0.787
Eveness/Equitability (E)	0.964	0.947	0.968	0.960±0.11	0.947-0.968
Simpson Dominance (C)	0.189	0.195	0.220	0.201±0.02	0.189-0.200

Table 4 Biodiversity Indices of Fungi in Oyster Tissue

Indices/Station	1	2	3	Mean	Mini Max
Margalef (d)	1.517	1.507	1.095	1.3730±0.24	1.095-1.517
Menhinick (d_1)	3.724	3.712	2.809	3.415±0.53	2.809-3.724
Shannon Diversity (H^1)	2.061	2.066	1.782	1.970±0.16	1.782-2.066

Shannon Wiener (H)	0.895	0.897	0.774	0.855±0.07	0.771-0.897
Eveness/Equitability (E)	0.991	0.993	0.995	0.993±0.00	0.991-0.995
Simpson Dominance (C)	0.130	0.128	0.170	0.143±0.02	0.128-0.170

IV. DISCUSSION

The variation and biodiversity of the isolated fungi from different geographical locations show different factors that affect the growth and distribution of fungi which include soil pH, moisture content, salinity, organic carbon, nitrogen sulfur and potassium (Sharma and Raju, 2013; Yu *et al*., 2007). In this study, *Aspergillus* predominated the fungal species, followed by *Penicillium* and *Cladosporium species*. Some toxigenic species of the genus *Aspergillus* and *Penicillium* were also isolated from the samples. Occurrence of other mycotoxins and aflatoxins at high levels in a particular food raises concerns about its safety. Aflatoxins, especially aflatoxin B, are recognized in both humans and animal as responsible for toxic signs and lesions, reduced growth, immune suppression and liver cancer (Osweiler,2010; Turner *et al*..,2003). The species of fungi observed in this study is in line with the finding of Abolude *et al* (2012) who reported *Aspergillus species, Penicillium species* etc from eggs and broodstock of *Clarias gariepinus* in Zaria.

The higher fungal load/count observed in water and oyster tissues especially in station 2 in this study could be attributed to high influx of allochthonous material in the area. This therefore confirms the assertion by Graca, (2006) and Ikpesu *et al* (2017) that areas with high fungal load usually receive allochthonous material. Similarly, the higher fungal load in oyster tissues than water observed in this study is in line with the finding of Scorezynska *et al* (2012) which was attributed to the assertion that bivalve mollusks of genius *Crassostrea thizophorae* usually have high microbial load owing to the fact that they build great communities both in estuary edges and stick to the substrates where there are numerous microbes.

The significantly higher fungal load observed in water and oyster at $p < 0.05$ in the wet season than the dry season in this study could be attributed to high level of nutrients resulting from decomposition of allochthonous material in the area. This could also be attributed to the assertion by Venugopal (2010) that the dynamics for fungal community may be attributed generally to abiotic variables and nature of substrate.

The eight species of fungi observed in this study is in line with the nine species belonging to six genera isolated and reported by Kortee *et al* (2017) but in disagreement with the four species reported by Walaa *et al* (2016). The high fungal load reported in this study in the months of January and May could be attributed to high availability of nutrient due to high allochthonous material. The consistency in presence of *Candida species, Aspergillus niger* and *A. Fumigatus* in all the stations could be attributed to similarity in environmental factors.

Otene *et al* (2002) opined that diversity index is a quantitative measure reflecting how many different types of species (fungi in this case) in a data set and simultaneously taken into account how evenly the basic entities (such an individual's) and distributed among types. According to Otene *et al* (2020) in Kocatas (1992), Menhinick and Margalef indices attempt to estimate species richness but at the same time independent of the sample size.The consistent higher values of all indices except evenness index in oyster tissues than water in this study could be due to more number of species in oyster tissues than water as shown by Ejiko and Otene (2019) and Ravera (2001).

The fungi species isolated from both oyster tissues and water in this study could be of public health significance because of the possible acute deleterious effects they may cause leading to oyster mortality and morbidity in humans that might consume them. Kelley *et al* (2003) opined that mycotoxins and other metabolites can be produced by fungi in water which could be extremely diluted thereby reducing its toxicity but according to Paterson and Lima (2005) consumption of mycotoxin in small amount over a long period of time could be hazardous to human health. De.Hoog *et al* (2000) attributed kidney and liver disorder, allergies sinusitis, burns, otitis media and increased risk of invasive infections to the presence of *Aspergillus species* in water and fish. Pal (2012) reported the spoilage fungi such as *Apergillus Candida, Cryptococcus* and *Rhodotonula* from fresh and spoiled fish and other sea foods.

In general the observed high microbial load (especially fungi) in oyster tissues in this study could be attributed to pollution of the environment in which the oysters were caught. Shannon wiener index takes into account the number of individuals as well as taxa which varies from 0 for communities with numerous taxa but with few individual. It has normal values ranging from 0 to 4. Otene *et al* (2020) as contained in Williams and Doris (1968) opined that index greater than 3 indicates clean, 1-3 are characterised by moderate pollution while value less than 1 are characterized as heavily polluted. Considering the Shannon Wiener index of this study and the classification above the oyster and the water are said to be heavily polluted since the values are less than 1 across the 3 stations. The observed higher Simpson dominance index in station 2 for both oyster tissues and water could be attributed to the assertion by Whitaka (1965) that Simpson index is higher where community is dominated by less number of species and when the dominance is shared by large number of species.

V. CONCLUSION

The species of fungi isolated in this study especially *Aspergillum* and *penicillium* among others in both oysters and water could be of public health significance due to their possible deleterious effects. These fungal species and others

isolated in this study are known to be major source of mycotoxins in food especially seafood.

VI. RECOMMENDATION

There is therefore the need for public awareness campaign on the potential health implications associated with consumption of fungal contaminated seafood to avoid further risk of contamination

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