

Evaluation of Fungal Diversity in Water and Sediment, Agba-Ndele Segment, Sombriero River, Rivers State

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Review Article

Volume 8 Issue 1 Received Date: October 30, 2023 Published Date: January 05, 2024 DOI: 10.23880/ijoac-16000289

Abstract

This study was carried out between September and December, 2022 in Sombriero river to assess the water and sediment quality using fungal load and diversity. Water and sediment samples collected were analysed in the laboratory for fungi using standard method of APHA and the obtained data were subjected to descriptive and inferential statistics. The results showed that Total fungi in water (Tfw) and sediment (TFs) ranged from 1.0x101 to 1.4x102cfu/ml and 1.0x101 to 1.7x102cfu/g with the overall mean values of 114.37±35.397 cfu/ml and 168.44±358.999 cfu/g respectively. Spatial mean values of TFw and TFs varies significantly at p<0.05 with station 3 higher than all other stations. TFw and TFs values exhibited non-seasonality and seasonality respectively at p<0.05. The total of 5 and 6 fungal species observed in the water and sediment samples included *Aspergillus niger, Rhizopus oligospora, Pennicilium species, Mucoccus caris* and *Micrococcus caris* with *Fusarium solani* absent in water. The values of diversity indices varied spatially with Simpson's dominance index (D) of fungi in water ranging between 0.2503 and 0.6428 while that of sediment ranged from 0.255 to 0.609. Based on the fungal load, diversity index and some pathogenic species the water is therefore under stress/threat. There is need to regulate the anthropogenic activities in the area to avoid further discharge of wastes capable of causing further pollution of the area.

Keywords: Evaluation; Fungal Diversity; Water and Sediment; Agba-Ndele Segment; Sombriero River; Rivers State

Abbreviations: TFW: Total Fungi in Water; TFS: Total Fungi in Sediment; RBC: Rose-Bengal Chloramphenicol; THF: Total Heterotrophic Fungi; ANOVA: Analysis of Variance; DMRT: Duncan Multiple Range Test; WHO: World Health Organisation; EPA: Environmental Protection Agency.

Introduction

The global increase in loads and diversity of microbial organisms such as fungi especially in the aquatic ecosystem including biota is attributed to increased anthropogenic activities causing indiscriminate discharge of organic and inorganic wastes into the aquatic environment [1-3]. Microbes play diverse roles in biotechnology among which is bio-remediation referred to as use of biota in the cleanup of polluted environment [4]. Fungi are known to exhibit diverse growth pattern such as secretion of extracellular enzymes and invasive mode of growth. It has been noted that the release of petroleum and its related products artificially or naturally into the environments endangers aquatic and terrestrial life forms by causing devegetation, contamination of portable water sources, fall in reproduction of biota due to disruption in food chain and death of biota inhabiting polluted environment [5].

The globally grown interest in the occurrence and diversity of fungi and their secondary metabolites in the aquatic ecosystems is attributed to their roles in water quality and human health [1]. According to WHO [6] as reported by Otene BB, *et al.* [3] water could be considered safe and fit for human consumption when it cannot cause any significant health hazard when consumed and that which has microbial, chemical and physical properties that meet the World Health Organization Guidelines.

The presence of certain fungi species (toxic) in water tends to infect the aquatic animals including those consumed by man as opined by Otene BB, et al. [3] and Jalees MM, et al. [7] opined that fungi species such as Aspergillus, Pencillium, Rhizopus, Blastomyces and Aiternaria are responsible for infecting fish organs. Agba-Ndele river which is a segment of Sombricro river has been considered as an essential water body considering the activities going on in the area and the fact that iit is a source of livelihood for the inhabitants. Oil exploration and other anthropogenic activities are ongoing in the area. Several researches have been carried out in this area but to the best of my knowledge only few works on fungal diversity has been done in this river. This paper is therefore aimed at investigating the fungal load and diversity in water and sediment so as to ascertain its health/pollution status.

Materials and Method

Study Area

This research was carried out in Agba-Ndele river, a segment of Sombriero river which is a mangrove intertidal wetland and a thicky populated municipal environment. The study was carried out at the middle reaches of Sombreiro River at Agba-Ndele, Rivers State, Nigeria with the coordinate to be 4.8976° E and 6.6990° N. The water is a tidal fresh water part of the Sombreiro river which serves as a boundary between Agba-Ndele in Emohua Local Government Area and Abua, Rivers State.

Sample Collection

Four sampling stations were selected within the study area with a distance of 500m apart considering ecological setting and human activities. Water samples were collected from the respective stations for four months in ragolis bottles as described in APHA,(2012) in Otene *et al.*,(1). Sediment samples were collected also from the bottom of water using Eckman grab as described in APHA. Samples were transported to the laboratory for further analysis where they were sorted, rinsed, processed and kept in refrigerator.

Isolation and Identification of Fungi

Each of 1.0g and 1.0ml of sediment and water samples were aseptically diluted in nutrient broth followed by 0.1ml aliquots of each 10 fold serially diluted samples transferred into triplicate plates of Rose-Bengal Chloramphenicol (RBC) agar and Bushnell-Hass (mineral salt) agar supplemented with 0.05% (v/v) streptomycin. Total heterotrophic fungal counts (THF) were obtained from the innoculated RBC agar after incubation for 24hrs at 37°C. Fungal colonies were subcultured on nutrient agar plates to obtain pure culture and stored on agar slants in the refrigerator at 4°C. Isolates were identified using morphological features Chessbrough, et al. followed by microscopy after staining with lactophenol collon blue [8].

Analysis of Data

A PAST Software Version 4 and Statistical packages for Social Sciences (SPSS) version 25 were used in carrying out the statistical analysis of the fungi data for descriptive and inferential statistics at P<0.05. The Analysis of variance (ANOVA) was determined while the spatial means were subjected to Duncan Multiple Range Test (DMRT) for differences at p<0.05.

Calculation of Bio-Indices

The fungal diversity indices such as Simpson's dominance index (D), Simpson index of diversity (1-D), Simpson's reciprocal index (1/D), Shannon diversity index (H'), evenness index (E1), Brillouin (HB), Menhinick's Index of Species Abundance, Margalef 's index of species richness (S), equitability (J), fisher alpha and Berger-parker (d) were estimated for each sampling point using the appropriate formulae as in Otene *et al.*,[1].

Fungal species richness in the sites was evaluated using two indices; menhinick's and margalef's indices.

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

Species richness (R1 and R2) obtained using the equations

$$R1 = \frac{S-1}{N} = S - 1/LogeN$$
$$R2 = \frac{S}{\sqrt{\Sigma ini}}$$

Where,

R = Index of species richness S = Total number of species N = Total number of individuals Ln= Natural logarithm

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

$$(Shannon's index) = -\sum_{n} \left(\frac{ni}{N} - \log 2 \left(\frac{ni}{N} \right) \right)$$

Simpson's Diversity Index (D)

This is a measure of diversity used to quantify the biodiversity of a habitat. It takes into account the number of species present as well as abundance of each species. It measures the probability that two individuals randomly selected from a sample will belong to the same species (or some category other than species).

$$(Simpson index) = \frac{\sum ini(ni-1)}{N(N-1)}$$

Where ni = the number of individuals in the ith species N = the total number of individuals S = the total number of species The value of D ranges between 0 and 1.

Simpson's indices of diversity (1-D) and reciprocal (1/D) are obtained from Simpson's dominance index by subtracting dominance index from 1 and dividing 1 by dominance index respectively.

$$(Dominance index) = 1 - \left(\frac{\sum ini(ni-1)}{N(N-1)}\right)$$

Species evenness index (E) was determined using the following expression:

$$H^{I}/LogeS$$
 (Pielou, 1966)

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

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

Species Equitability or Evenness (J) Jaccard

This is a measure of how evenly the individuals are distributed among the species present in a sample. It ranges between 0 and 1, the maximum value. One represents a situation where individuals are spread evenly among the

species present Jaccard (1912). It was calculated as follows:

- 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.
- The Berger Parker Dominance Index is a simple measure of the numerical importance of the most abundant species.

Berger – Parker Dominance Index =
$$\frac{(n \max)}{N}$$

Where

nmax= maximum number of organisms N= Total number of individuals

Fisher's alpha is a diversity index, defined implicitly by the formula:

$$S = a * Loge(1 + n/a)$$

Where S is number of species, n is number of individuals and a is the Fisher's alpha.

The Berger–Parker index equals the maximum pi value in the dataset or sampling station, i.e. the proportional abundance of the most abundant species.

Where pi = ni/N as has been earlier expressed.

Results

Total fungi in water (Tfw) ranged from 1.0x101 (Station 4 October) to 1.4x102 (Station 3 September) with the overall mean value of 114.37±35.397 cfu/ml while Total fungi in the sediment (Tfs) ranged from 1.0x101 (Station 1 November) to 1.7x102 (Station 3 December) with the overall mean value of 168.44±358.999 cfu/ml (Tables 1 & 2). This showed that the fungi load of sediment is higher than that of the water. The spatial mean value of Tfw was highest in station 3 (142.50±34.034 cfu/ml) but lowest in station 4 (95±57.45cfu/g) while TFs was highest in station 3 (470.00±686.73 cfu/ml) but lowest in station 1 (35±50.00 cfu/ml). Spatial mean values of TFw and TFs varies significantly with station 3 higher than all other stations at p<0.05 (Table 3). The monthly values of TFw and TFs were uniform throughout the period of study except in the Months of November and October respectively (Figure 1). TFw values exhibited non-seasonality though with higher wet season value than the dry season while TFs values exhibited seasonality with higher dry season than wet season values at p<0.05 (Figure 2). The range and mean values of fungi in water exceeded the permissible limits of WHO/FAO and EPA.

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S/No	Isolate Parameters	Mean	Mini	Max	WHO/FAO	EPA
1	Tfw	114.37±35.397	10	190	10 ²	10 ²
2	Tfs	168.44±358.999	10	1500		

Table 1: Overall Mean Value for Fungi 1solates in the Area.

Month	Station	Season	TFw	TFs
	1	А	1x10 ²	1x10 ¹
Cont	2	А	1.2x10 ²	1.0x10 ¹
Sept.	3	А	1.4x10 ²	1.0X10 ²
	4	А	1.3X10 ²	1.0X10 ²
	1	А	1.0x10 ²	1.0x10 ²
Oat	2	А	1.2x10 ²	1.1x10 ¹
UCL	3	А	1.1x10 ²	1.2x10 ²
	4	А	1.3x10 ¹	0.9x10 ²
	1	В	B 1.1x10 ²	
Nov	2	В	1.2x10 ²	1.2x10 ²
NOV	3	В	1.3x10 ²	1.5x10 ²
	4	В	1.0x10 ¹	1.0x10 ²
	1	В	1.0x10 ²	1.0x10 ¹
Dec	2	В	1.1x10 ²	1.0x10 ₂
Dec.	3	В	1.9x10 ²	1.7x10 ²
	4	В	1.1x10 ¹	1.3x10 ²

Key: Number of Month September to December, Number of Station 1 to 4, Season A = Wet Season (September to October) B = Dry Season (November to December). Key: TFW= Total fungi in water, TFS= Total Fungi in sediment **Table 2:** Monthly and Spatial Value of Fungi in the Study Area.

S/No	Parameters Stn 1		Stn 2	Stn 3	Stn 4	
1	Tfw	102.50±0.50°	$117.50 \pm 5.00^{\mathrm{b}}$	142.50±34.034ª	95±57.45°	
2	Tfs	35±50.00°	55.50±51.965°	470.00±686.73ª	105.25±17.23 ^b	

Table 3: Spatial Mean Value Fungi Isolate.





Table 4 showed the macroscopic and microscopic characteristics of fungi in the study area. A total of 5 and 6 fungal species were observed in the water and sediment samples respectively which include *Aspergillus niger*,

Rhizopus oligospora, Pennicilium species, Fusarium solani, Mucoccus caris and *Micrococcus caris* with *Fusarium solani* absent in water.

S/N	Macroscopic Characteristics	Microscopic	Water	Sediment	Suspected Fungi
1	Dark brown colony with dense growth grow to cover plate	Conidia head are radiated, conidiosphore is unbranched, no rhizoid, hyphae is seplate.	+ve	+ve	Aspergillus niger
2	Pale brownish grey colour and fast growing whitish colony seen	Black pigmentation, sporangim	+ve	+ve	Rhizopus oligospora
3	Green pigmentation with white black ground powdery surface in shape with elevated centre see.	Conifiophore is septate, erect, and	+ve	+ve	Penicillium spp
4	White colony with mass rapid growth covering the surface	Short cresent shaped conidiophores microcomidia hyphae septate	-ve	+ve	Fusarium solani
5	Cotton wool like aerial mycelia first grey in colour later become darker	Non-seplate mycelia bears sporangio scattered over the mycelia. Sporangio are erect and branched	+ve	+ve	Mucoccus caris
6	Lemon yellow apron seen with pigments	Gradnules on the surface of the conidia are many.	+ve	+ve	Microccuss caris

 Table 4: Macroscopic and Microscopic Characteristics of Fungi Isolates.

Tables 5 & 6 showed the diversity indices of fungi in water and sediment. Simpson's dominance index (D) for fungi in water ranged between 0.2503 (Station 2) and 0.6428 (Station 4) with the mean value of 0.351 while that of sediment ranged from 0.255 (Station 4) to 0.609 (Station 1) with the mean value of 0.387. Simpson's index of diversity (_I-D) of fungi in water ranged between 0.3572 (Station 4) and 0.7497 (Station 2) with the mean value of 0.691 (Station 1) to 0.7499 (Station 4) with the mean value of 0.613. Shannon index for

water ranged between 0.7369 (Station 4) to 1.386 (Station 2) with the mean value of 1.219 while that of sediment ranged between 0.794 (Station 1) and 1.376 (Station 4) with the mean value of 1.130. Evenness index for water was between 0.5224 (Station 4) and 0.9993 (Station 3) with the mean value of 0.875 while that of sediment was between 0.553 (Station 1) and 0.990 (Station 4) with the mean value of 0.798. Brillouin index for fungi in water and sediment are as in the table below.

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Indiana	1	2	2	4	Maan	Danga
Indices	1	2	3	4	Mean	Range
Dominance_D	0.2504	0.2503	0.2607	0.6428	0.351	0.2503-0.6428
Simpson_1-D	0.7496	0.7497	0.7393	0.3572	0.649	0.3572-0.7497
Shannon_H	1.385	1.386	1.366	0.7369	1.219	0.7369-1.386
Evenness_e^H/S	0.9991	0.9993	0.9796	0.5224	0.875	0.5224-0.9993
Brillouin	1.363	1.366	1.349	0.6985	1.194	0.6985-1.366
Menhinick	0.1975	0.1845	0.1675	0.3123	0.216	0.1675-0.3123
Margalef	0.4987	0.4876	0.4728	0.5883	0.512	0.4728-0.5883
Equitability_J	0.9994	0.9995	0.9851	0.5316	0.879	0.5316-0.9995
Fisher_alpha	0.615	0.6002	0.5805	0.74	0.634	0.5805-0.740
Berger-Parker	0.2683	0.2553	0.3333	0.7927	0.412	0.2683-0.7927
Chao-1	4	4	4	4		

Table 5: Diversity Indices for Fungi in Water.

Indices	1	2	3	4	Mean	Range
Dominance_D	0.6095	0.4239	0.2599	0.2551	0.387	0.255-0.609
Simpson_1-D	0.3905	0.5761	0.7401	0.7449	0.613	0.391-0.7449
Shannon_H	0.7937	0.9851	1.366	1.376	1.13	0.794-1.376
Evenness_e^H/S	0.5529	0.6695	0.9801	0.9902	0.798	0.553-0.990
Brillouin	0.7468	0.9558	1.349	1.355	1.102	0.7468-1.349
Menhinick	0.3508	0.2577	0.1721	0.1952	0.244	0.1721-0.351
Margalef	0.6163	0.547	0.4768	0.4967	0.534	0.4768-0.616
Equitability_J	0.5726	0.7106	0.9855	0.9929	0.816	0.5726-0.993
Fisher_alpha	0.7812	0.6813	0.5859	0.6123	0.665	0.5859-0.7812
Berger-Parker	0.7692	0.4979	0.3148	0.3095	0.473	0.3095-0.7692
Chao-1	4	4	4	4		

Table 6: Table Diversity Indices of Fungi in Sediment.

Discussion

Reports from different researchers Shah JA, *et al.* [9] and Otene BB, *et al.* [1] revealed species in the natural community respond differently to the varying environmental conditions whether biotic or abiotic which causes diverse niche organizations and consequently diverse communities. The observed mean values and ranges in this study are less than the finding of Otene BB, *et al.* [3] in Trans-Amadi/Woji Creek. This range is also lower than the ranges (0.1x104cfu/100ml to 4.6x104cfu (100ml) and 0.4x104cfu/100g to 42.5x104cfu/100g for water and sediment respectively reported by Doi SA, *et al.* [10] from Araca Bay in Sao Sebastiao. This value is also lower than the range (5.2x105cfu/g and 9.7x105cfu/g) reported by Pupin B, *et al.* [11] from a swamp on Cardoso Island.

The spatial and temporal variations in fungal population observed in this study could be attributed to some biotic factors and anthropogenic activities in the respective areas. This confirms the assertion by Sharma MS, et al. [12] that variation and biodiversity of fungal population could be caused by soil pH, moisture content, salinity, organic carbon, nitrogen, sulphur and potassium. The higher fungal load in station 3 in both water and sediment in this study could be attributed to high level of anthropogenic activities in the area. This therefore, corresponds with the assertion that certain physical and chemical aspects of the environment including temperature and pH are important for the survival adaptability and growth of microbes such as fungi [13]. Similarly, Wong MKM, et al. [14] opined that temperature affects the distribution and diversity of fungi and exist more in the tropic than temperate waters. The observed uniform

fungal load in most of the months could be due to uniform rainfall and decomposition during the period. The observed non-seasonality in fungal value in this study could be attributed to uniformity in anthropogenic activities during the wet and dry season. The observed higher fungal load in the wet than the dry season in this study is in tandem with the finding of Otene BB, et al. [1] (though without seasonality) in Trans-Amadi/Woji Creek which was attributed to high level of nutrient resulting from decomposition of allochthonous materials in the area. This could also be attributed to the assertion by Venugopal P, et al. [15] that the dynamic for fungal community may be attributed generally to abiotic variables and nature of substrate in the area. The exceedance of the permissible limits of WHO/FAO by the fungal load in this study is a clear indication that the area/water is polluted and therefore unsafe for human consumption. WHO [6] opined that water can be considered safe and fit for human consumption if it cannot cause any significant health hazard when consumed and that it has microbial, chemical and physical properties that meet the World Health Organization Guidelines.

The observed 5 to 6 fungal species in water and sediment respectively in this study is contrary to the eight (8) species reported by Otene BB, *et al.* [1] from Trans-Amadi/Woji Creek and nine (9) species reported by Kortee NK, *et al.* [16]. However, this result is in line with the four (4) species reported by Ibrahim WH, *et al.* [17]. The species of fungi such as *Aspergillus niger, Rhizopu species, Penicillium species* etc observed in this study is similar to those reported by Abolude DS, *et al.* [18] in Zaria. According to Kelly MG, *et al.* [19] mycotoxins and other metabolites can be produced by fungi in water which could be extremely diluted thereby reducing its toxicity. Consumption of mycotoxins in small amount over a long period of time could cause hazardous health effect to man [20].

Hoog D, *et al.* [21] and Otene BB, *et al.* [22] reported that diversity index is a qualitative measure that reflects how many different species in a data set and simultaneously taken into account how evenly the basic entities (such as individual are distributed among these types. Chiu GS, *et al.* [23] opined that biological diversity, abundance, tolerance and composition are community metrics frequently used inadequate environment to assess ecosystem health.

In this study, there has been no much variation among most of the diversity indices used across the stations and could be attributed to similarity in anthropogenic activities in the respective sampling stations. Simpson dominance index in this study which were highest in Station 4 and 1 for fungi in water and sediment respectively clearly satisfied the assertion by Otene BB, *et al.* [22] in Whittaka RH [24] that Simpson diversity index is usually higher where community is dominated by less number of species and when the dominance is shared by large number of species.

Considering the values of Shannon Wiener index in the respective media, the water and sediment are said to be moderately and heavily polluted. This is in line with the William JL, et al. [25] classification that values of the index greater than 3 indicated clean water values, range of 1 to 3 is moderately polluted while that less than 1 (< 1) is heavily polluted. The consistent higher values of Shannon Wenner index in water across the Stations 1-3 in this study confirmed the assertion by Davies OA, et al. [26] that they are indicators of environmental pollution. Shannon Wenner index in this study is less than the value (3.90) reported by Antai EE, et al. [27] in great Kwa rivers, Cross River State which was attributed to difference in environmental factors. The Evenness/Equitability value showed that there is evenness in fungal distribution in water across the stations except Station 4 unlike in the sediment where there is inconsistency and unevenness in species distribution except in Stations 3 and 4. The non-evenness in species distribution in water (Station 4) and sediment (Station 3 and 4) could be attributed to stress resulting from more anthropogenic activities in the areas. The consistent fluctuation in Margalef and Menhinick indices in this study (for water and sediment)) could be attributed to fluctuation in fungal population/species across the stations in both water and sediment.

The species of fungi such as *Aspergillus niger*, *Penicillium species*, *Mucor species* etc isolated from the study area have been reported to be causative agents of asthma, hypersensitivity, pneumonia and pulmonary mycosis [28,29]. Other diseases known to be caused by these fungi are Aspergillosis by *Aspergillus species* [30,31]. Ariyo AB, *et al.* [32] and Sokolo RS, *et al.* [33] reported some of these fungi to be waste degraders in aquatic environment [34].

Conclusion and Recommendation

The Agba-Ndele segment of Sombriero river is under threat/stress considering the species of fungi isolated in this study and the fact that the fungal load of the water exceeded the permissible limits (10²cfu/ml) set by World Health Organisation (WHO), Food and Agriculture Organisation and Environmental Protection Agency (EPA) guidelines. The observed/calculated bio-indices values in this study especially Shannon wiener index which ranged between 0.7369-1.386 showed moderate pollution since it is between the range of 1-3. There is therefore the need to regulate the anthropogenic activities in the area to avoid further discharge of wastes capable of causing further pollution of the area [35,36].

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