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Physicochemical Properties and Identification of Fungi from Reservoir Water in Benin City

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Abstract

The Earth's surface is predominantly covered by water, with fresh water comprising a mere 3%. Given its vital importance to all known living organisms, understanding water quality parameters and microbial populations in reservoirs is crucial. This study aimed to assess the physicochemical parameters and identify fungi in reservoir water across various communities in Benin City, Edo State. Water samples were collected from different locations in Benin City, Edo State. The samples were then taken to the laboratory and cultured on potato dextrose agar using the pour plate technique. The pure culture of fungal isolates were identified phenotypically. The antifungal susceptibility profiles of isolates were determined. The results for physiochemical analysis of the reservoir water sample showed that the pH values were within the range of 6.63 - 7.59, electrical conductivity ranged from 13 - 159 µS/cm. The temperature range gotten from this study is 25.3 – 29.4 ºC. Total suspended solid varied from 0.59 – 0.98 g/l. Biochemical oxygen demand ranged from 0.02 – 0.07. The alkalinity ranged from 0.12 to 0.5 g/l. Manganese was present and it ranged from 0.01 to 0.15 mg/l. The total fungal count was shown a ranged from 0.025 x 10^3 - 2.3 x 10^3 cfu/ml. The identified fungal isolates from the various samples of water were Penicilium chrysogenum, Yeast, Rhizopus stolonifera, Aspergillus flavus and Aspergilus niger. The isolate with the lowest percentage distribution was Penicilium chrysogenum, asides Penicilium chrysogenum the rest of the isolates were found in all the sampled locations. The pathogenicity result showed all isolates were positive to Gamma Haemolysis and Lipase production. All isolates showed susceptibility to Nystatin but showed varying resistance to Vericonazole with penicillin showing the highest susceptibility. Recent discoveries on fungi requiring special attention include the presence of opportunistic and emerging pathogens in water sources.

1.0.Introduction

Water is a vital resource for all known living organisms, as there is no life without it [1]. The Earth’s surface is 71% water and fresh water comprises 3% of the total amount water on the Earth’s surface [2]. This small percentage of fresh water plays an essential role in human life. There are many sources of water pollution, but the most notable source is microbial contamination. Sewage o

r untreated wastewater releases a large amount of dissolved organic material, suspended materials, and harmful microbiological organisms [3].

Fungi are ubiquitous, heterotrophic organisms present in oceans, fresh water and drinking water. They can be divided based on the ability to colonize different environments into three groups: as mesophilic fungi, generalists and specialists [4]. Mesophilic species inhabit niches with moderate physicochemical parameters, while generalists grow under changing life conditions, but with growth optimum under moderate conditions. Specialists inhabit extreme habitats and are unable to grow under moderate conditions [4]. Ecologically, fungi are saprophytes, degrading organic matter, with some species acting also as parasites or symbionts [5]. Due to their diverse life cycle, ability to form large hyphal networks and produce spores, or growing as single yeast-cells [5]. In the last 30 years, the presence of a high variety of fungi was reported from European water, including surface, ground and tap water intended for human consumption [6].

Fungi are one of the most important biological sources of water contamination. These fungi may originate from the soil. Several studies have been conducted to detect the presence of fungi in water. Muhsin *et al.* [7] isolated several fungi from well water, including *Alternaria* sp., *Aspergillus* sp., *Trichoderma viride, Penicillium* sp., *Fusarium oxysporum*, and *Cladosporium* sp. Also, Rajanaika *et al*. isolated Pythium, *Allomyces, Rhizopus, Achyla, Alternaria, Aspergillus*, *Saprolegnia*, *Chaetominm, Cladosporium*, *Fusarium, Trichoderma*, and *Penicillium* from the Tunga River in India. Awad and Kraume [8] found 60 species of fungi belonging to 27 genera in wastewater samples, including 8.8% *Geotrichum*, 75% *Penicillium*, 65.7% yeasts, and 55.5% *Trichoderma* [8].

Fungi growing in biofilms inside taps and in tap water affect the taste and odour, interfering with the chlorination process, due to the release of a large scale of products known as secondary metabolites. These may be very diverse and specific for different fungal species [9]. While the role of secondary metabolites in the ecology of fungi is to defend their habitat, and suppress the growth of competitors [10], some of them are toxic to animals, and may present a risk for human health in higher concentrations or under prolonged time of exposure [11]. Not only secondary metabolites, but also fungal cell wall components and the fungal load itself may contribute to the emergence of allergies and other opportunistic and systemic infections, mainly in immunocompromised individuals [12]. Although in the last few decades fungi are becoming frequently recognized as causative agents of respiratory, mucosal, rhinocerebral, cutaneous and subcutaneous infections [12].

2.0. Materials and Methods

2.1. Study Area and Collection of Water Samples

This study was conducted in Benin City, the capital of Edo State in Nigeria, which is situated in the country's south-south geopolitical zone and has a total area of roughly 500 square kilometers. Benin City is bordered by the latitudes 6° 06' N and 6° 30' N and the longitudes 5° 30' E and 5° 45' E. The City is surrounded by a sedimentary deposit that consists of a top layer of reddish clayey sand that covers extremely porous fresh water-bearing loose sands, as well as thin local clay and shale that are thought to have originated in braided streams. It is commonly thought to be highly porous, permeable and abundant in water production [13].

Water samples were randomly collected from different districts within Benin City. The groundwater samples comprise of seven boreholes and two well water. Samples were collected into 250 ml sterile

sampling bottles observing aseptic procedures and immediately transported to the laboratory for analysis.

2.2. Physico-chemical tests (Water Quality Test)

The evaluation of water qulity involves the aassessment of various physic-chemical parameters that can provide information about its suitability for different purposes such as drinking, recreational activities or industrial use. Several equipment and processes are used to measure and analyze these parameters. Different physicochemical parameters amenable to water quality assessment, namely, pH, temperature, salinity, dissolved salts measured as electrical conductivity, total suspended solid, essential elements and their corresponding compounds (nitrates, phosphates, sulphate), dissolved oxygen, biological oxygen demand and carbon-oxygen demand [14, 15].

2.3. Enumeration and Identification of Fungi *s*pecies

The mycoflora were isolated by pour plate method. After collection of the water specimen, 1ml each of water samples was dissolved in 9ml of sterile water respectively, which served as stock cultures under aseptic conditions. Using a sterile pipette, 1 ml of the stock water sample was inoculated into the first test tube which gave a dilution factor of 10^{-1} and 10^{-2} 10^{-3} 10^{-4}. One ml suspension of 10^{-3} 10^{-4} were transferred into sterile petri plates and 10-15 ml of autoclaved but luck warm Potato Dextrose agar (supplemented with chloramphenicol) was added to the plates. The plates were incubated for 3–7 days at 25°C [6]. Colony forming unit per ml (CFU/ml) of fungal isolates were counted and pure cultures were identified. Fungi growing on the agar plates were identified based on macroscopic and microscopic morphological features as described by Pitt and Hocking [16] and.[17].

2.4. Antibiotics Susceptibility Test

The antibiotic sensitivity testing was carried out to determine the susceptibility of the isolated fungi to various antifungal agents. The test was carried out using the disc diffusion method as described by Bauer *et al.*, [18] on Mueller-Hinton agar and was interpreted according to the guidelines of the Clinical and Laboratory Standards Institute [19]. The antibiotics used for isolates are Nystatin and Vericonazole.

2.5. Test for Pathogenicity

The isolated fungi were screened for protease, haemolysin and lipase production. Protease test is a test that measures the activity of protease enzymes. Proteases are enzymes that break down proteins into smaller polypeptides or single amino acid. Samples were inoculated on Trypton soy agar and 10% Tween 80 was added as a supplement. The presence of a clear zone around the colonies shows a positive result indicating protease production [20].

Haemolysin production was detected using the method described by Martinez-Martinez *et al*., [21]. All the fungal isolates were grown on 5% sheep blood agar at 37^0C for 24hr. The presence of a clear zone around the colonies was taken as positive for haemolysin production.

The isolated fungi were screened for the production of lipase using spirit blue agar containing lipase reagent as described by Marshall [22]. The lipolytic activities of the isolates were then compared by measuring the width of the areas of clearing or area of deep colour around the colonies.

3.0. Results

The physiochemical analysis of the reservoir water sample is presented on table 1. The pH observed in this study falls within the range of 6.63 – 7.59, electrical Conductivity exhibited differences across the different locations and then ranged from 13 to 159 µS/cm. The temperature ranged from 25.3 – 29.4^{0}C. Total suspended solid varied from 0.59 – 0.98 g/l. Biochemical oxygen demand ranged significantly among the sampled reservoir water from 0.02 – 0.07. The alkalinity ranged from 0.12 to 0.5 g/l. Manganese was present in the reservoir water and the concentration ranged from 0.01 to 0.15 mg/l. Table 2 shows the total fungal count of the water samples, and it ranged from 0.025 x 10^3cfu/ml to 2.3 x 10^3cfu/ml. The highest fungal count was observed in reservoir water site C, having a value of 2.3 x 10^3cfu/ml while the lowest fungal count was from reservoir water site A, having a count value of 0.025 x 10^3cfu/ml.

The percentage distribution of the fungal isolates is presented in Figure 1. The fungal isolated from the various samples of water were *Penicilium chrysogenum* with smooth and grey-greenish colonies, with septate hyphae and branched spores, Yeast was cream, smooth, oval shape, convex elevated colonies, *Rhizopus stolonifera* was white-brown colonies, non septate and broad hyphae, *Aspergillus flavus* yellow-green colonies and septate hyphae *and Aspergilus niger* black colonies and septate hyphae. The percentage distribution ranged from 71.4% to 100%. The isolate with the lowest percentage distribution was *Penicilium chrysogenum (*71.4%). Table 3 shows the Antibiotics susceptibility pattern of fungal isolates from Reservoir Water. The fungal isolates were resistance to Vericonazole. The isolates *Penicilium chrysogenum, Yeast, Aspergillus flavus and Aspergilus niger* were susceptible to Nystatin, except *rhizopus stolonifer* which showed resistance. *Penicilium chrysogenum* showed the highest zone of inhibition with a value of 27 mm. The pathogenicity test of the isolates is shown in Table 4. The isolates *Yeast, Rhizopus stolonifera, Aspergillus flavus and Aspergilus niger* showed their ability to produce lipase and gamma heamolytic activity on blood agar, and *Penicilium chrysogenum* was negative to protease production.

3.1. DISCUSSION

The results from this study showed that reservoir water has high fungal contaminants. Ground water is probably the most common source of fungal contamination. Due to high adaptability at a physiological level, fungi may colonize environments with extreme growth conditions, one of them being also oligotrophic water systems [23].

The ideal pH for good water is 6.5 - 9.5, while an acceptable range is 5.5 - 10.0. The pH found in this study falls within the range of 6.63 – 7.59 as observed which is within the WHO limit of 6.5 to 8.5. This finding aligns with the conclusions of Stone and Thomforde [24]. The Electrical Conductivity exhibited differences across different locations, spanning from 0.13 to 15.6 mS/cm (WHO limit for 1000ms/cm). In contrast, the findings of Akhionbare *et al.* [25] displayed elevated conductivity values for natural water in Okada. The alkalinity of water sampled in this study was within the range of 0.12 – 1.83. The WHO limit for alkalinity is < 50. Alkaline conditions can harm acid-loving microorganisms

The temperature range in this study is 25.3 – 29.4^{0}C. The WHO limit for temperature is < 35^{0}C. Total dissolved solid varied from 0.59 – 0.98 g/l. The highest value being 0.98 g/l and the least value being 0.59 mg/l. Ground water contamination and contamination with soil have been reported to

increase total dissolved solids [26, 27]. This may have been responsible for the variation from location to location of the reservoir water in this study. COD indirectly affects microorganisms by indicating the presence of organic pollutants. High COD levels suggest the presence of organic compounds that microorganisms can potentially break down. However, excessively high COD may indicate the presence of toxic substances or a high organic load that can overwhelm microbial populations. The COD in this study ranged from 0.31 to 0.56 which is within the WHO limit of 10.

Biochemical oxygen demand varied significantly among the sampled reservoir water 0.02 – 0.07. The highest value was 0.07 and least was 0.02 mg/l. These are all below FEPA (Federal Environmental Protection Agency of Nigeria) standard [28]. The FEPA limit is 30 mg/l and the WHO limit is 10mg/l. This directly tells that the reservoir water is not polluted and may have no negative impact on human health if consumed. However, permissible limit as reported by APHA [29] is 4 mg/l. This highest value from this study is significantly lower than this bench mark. Accumulation of low BOD results in organisms being stressed, suffocated and death [29]. Elevated BOD levels can lead to an increase in the activity of microbial decomposers microorganisms as they work to break down the excess organic material. However, this can deplete dissolved oxygen levels, potentially harming fish and other aerobic organisms. Nitrate is an essential nutrient but also a good indicator of contamination from natural and human activities. Levels above 45 mg/l are considered harmful to aquatic organisms. The nitrates ranged from 0.54 mg/L to 0.95 mg/L in the present findings. The average nitrates were found to be extremely low as compared to the tolerable limits in the entire reservoir. When NO_3 level is above 40 mg/L, it leads to "methaemoglobinaemia" also called blue -baby disease [30]. Microbes involved in nitrogen cycling (*Nitrosomonas, Nitrobacter*) can thrive in the presence of nitrates, helping to convert toxic ammonia into less harmful nitrate. Water hardness refers to the concentration of calcium and magnesium. As calcium and magnesium bond with carbonates and bicarbonates, alkalinity and water hardness are closely interrelated and produce similar measured levels. The hardness of water is not a pollution parameter but indicates water quality. Waters are often categorized according to degrees of hardness as follows: 0 – 75 mg/L = soft 75 – 150 mg/L= moderately hard 150 – 300 mg/L= hard and Above 300 mg/L= very hard. In the present investigation, total hardness level varied from 1.05 to 1.99 mg/L and included under soft category Kiran *et al.* [30]. The water softness maybe attributed to the rainy season as this study was conducted during the rainy seasons. The fungal species isolated from the various samples of water were *Penicilium notalatum, Yeast, Rhizopus stolonifera, Aspergillus flavus* and *Aspergilus niger*. These isolates are similar to that observed from the work of Muhsin *et al*. [7] who isolated several fungi from well water, including *Alternaria alternate Aspergillus sp., Trichoderma viride, Penicillium sp., Fusarium oxysporum, and Cladosporium sp.* Moreover, Rajanaika *et al.* [31] also isolated *Pythium, Allomyces, Rhizopus, Achyla, Alternaria, Aspergillus, Saprolegnia, Chaetominm, Cladosporium, Fusarium, Trichoderma,* and *Penicillium* from the Tunga River in India. Kraume and Awad, [32] in their study found 60 species of fungi belonging to 27 genera in wastewater samples, including 8.8% *Geotrichum,* 75% *Penicillium*, 65.7% yeasts, and 55.5% *Trichoderma*.

The reason for the presence of *Aspergillus* sp, Yeast, and *Rhizopus* during the study period may be attributed to the fact that these fungi are soil fungi that may have contaminated the water from the ground since these reservoirs are mostly dug underground. This is supported by several studies showing the presence of soil fungi in water sources [33]. Another reason for the abundant presence of *Aspergillus* may be attributed to the fact that this fungus is able to produce large numbers of asexual breeding units, it is able to survive in different environments, and it secretes enzymes that

enable it to benefit from different food sources [34]. *Rhizopus* is a widespread fungus that may exist in water. *Candida*, which is a genus of pathogenic fungi, was also isolated, indicating the serious public health impact of water from these wells if it was used for drinking or domestic purposes. The pathogenicity of the isolates showed that, all isolates showed Gamma Haemolysis and were all positive to Lipase, also, all the isolates except *Penicilium chrysogenum* were positive for protease production. All isolates showed susceptibility to Nystatin but showed varying resistance to Vericonazole with penicillin showing the highest susceptibility.

Table .1: The Physiochemical Analysis of Reservoir Water Samples from different Locations in Benin City.

Parameter	BHS1	BHS2	BHS3	BHS4	BHS5	BHS6	BHS7	WWS1	WWS2	WHO
pH	7.59±0.15	6.71±0.55	6.66±0.25	6.33±0.25	6.72±0.12	6.18±0.35	6.69±0.55	6.06±0.23	6.63±0.35	6.5-8.5
Tempt. (oC)	25.3±1.50	28.30±1.50	25.3±1.50	26.30±2.15	29.40±1.90	28.3±2.15	26.30±2.50	29.30±2.00	25.30±1.85	< 35
EC (µS/cm)	46.00±3.50	37.00±3.90	48.00±3.00	33.00±3.11	159.00±8.11	26.00±1.25	13.00±2.00	110.00±10.00	16.00±1.55	1000
Turb (NTU)	0.63±0.15	0.27±0.04	1.83±0.25	1.11±0.19	0.90±0.04	0.74±0.04	0.21±0.12	0.84±0.06	1.45±0.7	5
TSS	0.78±0.25	0.98±0.25	0.90±0.02	0.59±0.14	0.65±0.02	0.72±0.03	0.69±0.24	0.87±0.04	0.23±0.02	< 10
Alkalinity	0.21±0.01	0.41±0.11	0.43±0.03	0.40±0.05	0.50±0.05	0.24±0.01	0.12±0.00	0.20±0.00	0.12±0.01	<50
Hardness	1.99±0.22	2.15±0.15	2.67±0.95	2.95±0.23	1.05±0.00	2.50±0.05	2.57±0.09	1.66±0.09	2.54±0.32	100-500
Phosphate	0.12±0.01	0.56±0.04	1.84±0.85	1.99±0.35	0.09±0.00	0.11±0.01	0.10±0.00	0.14±0.00	1.53±0.07	5
Nitrate	0.67±0.01	0.95±0.05	1.05±0.15	1.50±0.05	0.57±0.25	0.66±0.23	0.54±0.03	0.50±0.01	1.12±0.09	40-50
Sulphate	0.75±0.05	0.82±0.03	0.91±0.09	0.79±0.00	1.00±0.00	0.12±0.05	0.13±0.02	0.15±0.05	0.53±0.10	60
BOD	0.02±0.01	0.01±0.00	0.02±0.00	0.03±0.50	0.07±0.00	0.02±0.04	0.03±0.00	0.03±0.00	0.02±0.00	10
COD	0.41±0.05	0.56±0.05	0.50±0.01	0.26±0.05	0.31±0.00	0.36±0.01	0.34±0.04	0.48±0.06	0.02±0.00	10

Key: BHS1: Borehole Water Site 1, BHS2: Borehole Water Site 2, BHS3: Borehole Water Site 3, BHS4: Borehole Water Site 4, BHS5: Borehole Water Site 5, BHS6: Borehole Water Site 6, BHS7: Borehole Water Site 7, WWS1: Well Site 1, WWS2: Well Water Site 2, WHO: World Health Organisation

Table 2: Total Fungal Count (cfu/ml x 10^3) of Water Samples.

Sample location	Fungal count (CFU x10^3)
RWSA	0.06
RWSB	1.00
RWSC	2.30
RWSD	0.26
RWSE	0.025
RWSF	0.285
RWSG	0.19

Key: RWSA=Reservoir Water site A, RWSB=Reservoir Water site B, RWSC=Reservoir Water site C, RWSD=Reservoir Water site D, RWSE=Reservoir Water site E, RWSF=Reservoir Water site F, RWSG=Reservoir Water site G

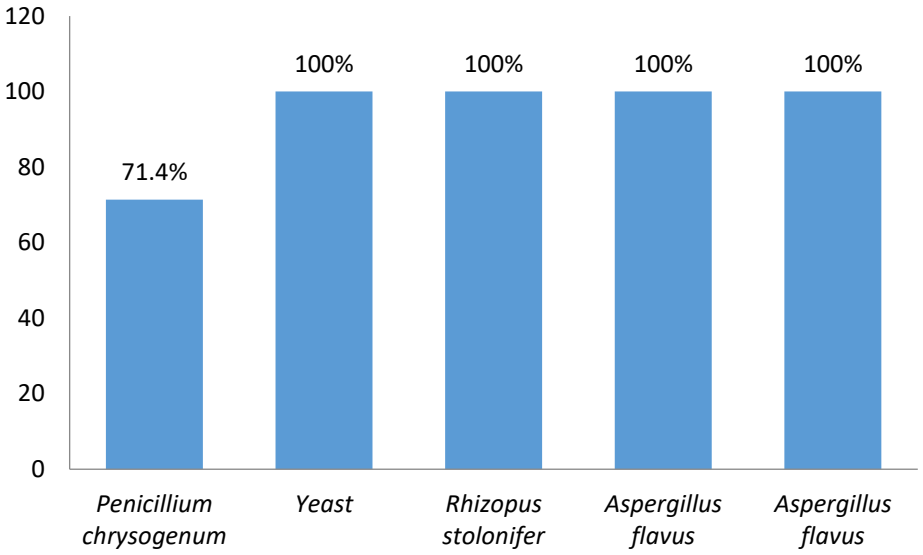

Figure 1: Percentage distribution of the fungal isolates in the water samples

Table 3: Antibiotics Susceptibility of Fungal Isolates from Reservoir Water.

Isolates	**Zone of inhibition (mm)**	
	Nysiatin	**Vericonazole**
Penicilium chrysogenum	S(27)	R(0)
Yeast	S(18)	R(0)
Rhizopus stolonifer	R(10)	R(0)
Aspergilus flavus	S(22)	R(0)
Aspergilus niger	S(22)	R(0)

Key; 0-11 mm= Resistant (R), 11-12 mm= Intermediate (I), ≥13= Susceptible (S)

Table 4: Pathogenicity test of the isolates from reservoir water samples of different locations in Benin City.

Isolates	**Lipase**	**Haemolysin**	**Protease**
Penicilium chrysogenum	+	ϒ	-
Yeast	+	ϒ	+

Rhizopus stolonifer	+	ϒ	+
Aspergilus flavus	+	ϒ	+
Aspergilus niger	+	ϒ	+

KEY; + = Positive, - = Negative, ϒ= Gamma

4.0. Conclusion

The physicochemical analysis revealed that most of the parameters like pH, electrical conductivity, temperature, total suspended solids, biochemical oxygen demand, alkalinity, and manganese levels were within the permissible limits set by regulatory bodies such as the World Health Organization (WHO) and the Federal Environmental Protection Agency of Nigeria (FEPA). This suggests that the reservoir water quality is generally suitable for various purposes. However, the presence of fungal contaminants in the reservoir water samples is a cause for concern. The total fungal counts ranged from 0.025 x 103 to 2.3 x 103 CFU/ml, indicating varying levels of fungal contamination across the sampling sites. The identified fungal isolates included Penicillium chrysogenum, yeasts, Rhizopus stolonifera, Aspergillus flavus, and Aspergillus niger. These fungi are commonly found in soil and water sources, and some species like Aspergillus and Candida are known opportunistic pathogens with potential public health implications. The pathogenicity tests revealed that most isolates exhibited gamma hemolysis and lipase production, while all isolates except Penicillium chrysogenum were positive for protease production. These findings indicate the potential pathogenic nature of the isolated fungi.
The antibiotic susceptibility testing showed that all isolates were susceptible to the antifungal agent nystatin, while varying degrees of resistance were observed against voriconazole.

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