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Isolation and identification Fungi associated with fish pond water within Maiduguri metropolitan Council, Borno State, Nigeria.

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ABSTRACT

Microbiological analysis for fungi in water samples collected from fish ponds in Maiduguri Metropolitan council was carried out. The pH of the water samples was measured using pH meter .All the fish pond water samples are slightly acidic with the exception of water from Dr, Idowu's fish pond which was neutral. The water samples of dilution factor 10^{-3} and 10^{-5} folds was cultured in the laboratory using potato dextrose agar (PDA) and Malt extract agar(MEA) for isolation of fungal pathogens. According to the standard method for examination of water and waste water. The Fungi isolated from the water Samples include: Aspergillus flavus, A. niger, A. terreus, A. penicilloides Alternaria alternata, Rhizopus stolonifer, Scopulariopsis brevicaulis, Saccharomyces cerevisiae and Zygosaccharomyces bailii. Aspergillus was the most prevalent and 50% of the water samples contained Aspergillus.

KEYWORDS:
Fungi; Fish water; and fishpond

INTRODUCTION

Fish farming is the principal form of aquaculture while other methods may fall under mariculture. Fish farming involves raising fish commercially in tanks, enclosures, usually for food.

A facility that releases juvenile fish in the wild for recreational fishing or to supplement a species of natural numbers is generally referred to as fish hatchery. The most common fish species raised by fish farmers are salmon, carp, tilapia, catfish and cod (Brown, 2001).

A pond may be regarded as an earthen vessel for collecting and holding water. A fish pond is therefore such an artificial impoundment filled and is a depression on land, by digging flat land or enlarging a natural depression which can be drained. Therefore easy drainage is one of the main characteristics of a pond. The overall aim in fish pond culture is to achieve maximum fish production and this will depend on the management techniques employed. Ponds are likely to be of use to the farmer who is not restricted by the availability of water or space.

Small ponds have the advantage of relatively inexpensive construction, low

running cost and natural production of food for fish (Mclarnay *et al*., 1972).

Fish farming is a common occupation of people living in the coastal areas and along major river banks in Nigeria. Fish has a high source of protein. The flesh is consumed when prepared as one form of delicacy or the other while various brands of oils are extracted from flesh and fluids of fish (Eyo, 1993).

Estuaries are unique in many respects, their calm, nutrient rich water, serves as nurseries for juvenile forms of many commercially important fish and invertebrates (Prescott *et al.*, 2008). Studies have revealed that the microbial flora of caught fish and other aquatic specimens is largely a reflection of the microbial quality of the water where they have harvested. (Pelczar *et al.*, 1986). Fungi were originally found as part of the plant kingdom and grouped to the kingdom protista, but in 1969, Whittaker argues that there multicellular nature meant they should be in their own kingdom. Fungi cells are different from the cells of plant not only by absence of chloroplast but forming a cell wall that posse's chitin instead of cellulose(Sylvia, 2001). The longer evolutionary theory of fungi is rich in examples of convergent and divergent that is to say many structure evolved independently (converged) while other structures became dissimilar (diverged) overtime (Foster *et al*., 2004).

The body of vegetative structure of a fungus is called thallus (singular) thalli (plural) which varies in complexity and size ranging from singled-celled microscopic puff balls and mushrooms and the fungal cell is usually encased in a cell wall of chitin(Griffin, 1996).

Some species are pathogenic multicellular and yeast like forms in their host but when growing as saprophytes in soil or on laboratory media they have a filamentous mold form parasitic fungi causes diseases in plants, human and other animals and aquatic organisms(Pelczar *et al*., 1986).

Fungi are a group of organisms called heterotrophs that require living or death matter for growth and reproduction. Unlike plants they are incapable of manufacturing their own nutrient by photosynthesis. The fungal spore is like seed which is resistant to heat, drying disinfectants and the natural defense system of aquatic organisms and fishes in water due to this they are capable of causing diseases in fish (Ruth., *et al* 1996). Other examples of aquatic fungi include those living in hydrothermal areas of the ocean (Vapoutic *et al*., 2008).

Generally, water is vital for life and life processes, and its significance covers domestic, agricultural and industrial uses. Water for most uses involves its bulk property of nutrition, hydration, cooling, and carrier, as applicable in domestic, agricultural, industrial and recreational activities (Udoma, 2005).

Water is chemical substance with the chemical formula H_2o. Its molecule contains one oxygen and two atoms of hydrogen connected by covalent bonds. Water is a liquid at ambient condition but it often co-exists on earth with its solid state (ice) and gaseous state (water vapour or steam).

Water covers 70.9% of the earth's surface and is vital for all living forms of life on earth; it is found everywhere, in oceans and other large water bodies with 1.6% of water below ground in aquifers and 0.001% in air as vapour clouds formed of solids and precipitation. Ocean holds 97% of surface water. Glaciers and polar ice caps 2.4% and other land surface water such as rivers, lakes, and ponds. 0.6%. A very small portion of earth water is contained within biological bodies and manufacture of products.

Water as a microbial habitat depends on a number of physical factors such as temperature, pH, light intensity, turbidity and one of the most important of these is dissolved oxygen level of the water All microbes are aquatic even those that live on land, prokaryotes, protists and most fungi require at least a thin film of liquid for replication.

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Marine and fresh water environments have varied surface areas and volumes, they are found in locations as diverse as in the human body, in beverages and the usual place one would expect rivers, lakes, oceans and ponds (Prescott *et al*., 2008).

Marine water environment usually comprises of the water bodies found on oceans, sea(s) and estuaries. The marine water environment consists of 3 major zones (habitat), the neritic zone, the pelagic zone and the benthic zone. Sources of water include groundwater (underground) and surface water. Because surface water and shallow wells often receives run off water, they frequently are polluted with various waste including animal excrement and human sewage which may contain pathogens (disease causing organisms). According to the world health organization (WHO) world health report 2002, contaminated water is responsible for death of over 3 million people per year and 90% of whom are children and most death of aquatic organisms(Richard, 2005).

USES OF WATER

Agricultural purposes-: used in fish farming and irrigation purposes on irrigation land, Used for domestic purposes like drinking, cooking, washing, bathing and industrial process of goods production.

Water is used as scientific standards because many biochemical reactions take place in the presence of water i.e. in aqueous solution, Recreational activities (sports i.e. swimming),Water is used in heat transfers fluids in diverse heat exchange and as an agent of fire extinguishing, Used in chemical reaction environment as a solvent or reactant for many chemicals and less commonly use as solute or catalyst.

The fish pond water serve as an avenue for the cultivation of fish for consumption(food), source of income and the fish pond may be of significance in terms of study and exploration of new knowledge and in the process may be contaminated with microorganisms from various sources. Therefore, this study was aimed at isolation and identification of fungi associated with in fish pond water.

MATERIALS AND METHODS

STUDY AREA

the study was carried out in Borno State situated in the Northeastern part of Nigeria lies on latitude 10^0Nand 13^0E.itr occupies the greater part of Chad Basin in the Northeastern part of the country and shares international border with the republic of Niger to the North, Chad to the Northeast and Cameroun to the east. Most important to the country is the strategic location as gate-way in the neighbouring state of Adamawa to the South, Yobe to the west, Kano to the Northwest and Gombe to the Southwest. The state has an area of 69,435square kilometres about 7.69% of the total land area of the country.

SAMPLE COLLECTION.

Using a sterile container, water samples were collected from various fish ponds within Maiduguri Metropolitan Council and the sample were named based on the area collected from.

1. Sample M_1 was collected from Giwa fish pond.
2. Sample M_2 was collected from Giwa fish pond.
3. ALIR 3 was collected from Aliram fish pond.
4. ALIR 4 was collected from Aliram fish pond.
5. Sample 5 was collected from Mammy fish pond.
6. Sample 6 was collected from an Earth fish pond.

SAMPLE PROCESSING

pH: The pH of the fish pond water samples was measured to know the alkalinity, acidity or neutrality of the various fish pond water samples.

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SERIAL DILUTION

10ml of sterile distilled water was pipette into each test tube (5 test tubes). 1 ml of fish pond water sample was added to the first tube and stirred. 1ml was drawn out from the first test tube to the second test tube and gradually to the fifth test tube. 1ml from the fifth test tube was discarded and this was done to all the fish pond water samples.

STERILIZATION OF EQUIPMENT AND WORK BENCH

Glass wares (bottles and test tubes) were washed with detergent and allowed to dry and then autoclaved at 121^0c for 15minutes to achieve sterilization. The work bench was disinfected using 70% alcohol.

INOCULATION

10^{-3} and 10^{-5} folds of dilution of the fish pond water samples was inoculated on the surface of the prepared potato dextrose agar and malt extract agar and examine for growth.

MACROSCOPY

This was carried out by observing the morphological characteristics like size, shape growth and colour of the plate.

MICROSCOPY

The identification of fungi and fungus like organisms involve the observation of morphological features such as shape, size of hyphae, shape of sporangia, conidia, conidiophores and spores.

Using a flamed inoculating needle, the edge of each colony is picked and slides of the different colonies are made, a drop of lacto phenol cotton blue stain is added to the slides and covered with cover slip and examine under the microscope using x100 and x400 magnification starting from third day of the culture. The fungi and fungus like organisms were identified using the works of Bedenek (1972) Batko (1975), Dick(1990) and Pystina (1998).

RESULTS

pH values of the samples

SAMPLE	PH VALUE	INFERENCE
1. Sample Giwa fish pond (G_1).	6.83	Slightly Acidic
2. Sample Giwa fish pond (G_2).	6.86	Slightly Acidic
3. Aliram fish pond (ALIR3).	6.57	Slightly Acidic
4. Aliram fish pond (ALIR4).	6.44	Slightly Acidic
5. Dr. Idowu pond.	7.05	Neutral
6. Earth fish pond.	6.81	Slightly Acidic

MACROSCOPIC EXAMINATION AFTER 24 HOURS AT 28^0c

SAMPLE S/NO	SAMPLE NAME	DILUTION WATER	OBSERVATION
1.	Sample Giwa fish pond water (A)(G_1).	10^{-3} 10^{-5}	No growth was observed.
2.	Sample Giwa fish pond water (B)(G_2).	10^{-3} 10^{-5}	No growth was observed.
3.	Aliram fish pond water (ALIR3).	10^{-3} 10^{-5}	No growth was observed.

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4.	Aliram fish pond water (ALIR4).	10^{-3} 10^{-5}	No growth was observed.
5.	Mammy fish pond water.	10^{-3} 10^{-5}	No growth was observed.
6.	Earth fish pond water.	10^{-3} 10^{-5}	No growth was observed.

The organisms below shows the result of fungi isolated and identified from the fish pond water samples.

SAMPLE S/NO	SAMPLE NAME	MACROSCOPIC OBSERVATION	MICROSCOPIC OBSERVATION	FUNGI IDENTIFIED
1.	Sample Giwa fish pond (G_1 &G_2).	Cluster of cells, mucoid appearance with whitish colouration on plates.	Cells are round and ovoid in shape that reproduces by cells.	*Saccharomyces cerevisiae*
2.	Sample Giwa fish pond (G_2).	Whitish colouration, mucoid appearance on surface of plates.	Cells are globose to oval-kidney shaped.	*Zygosaccharomyces bailii*
3.	Aliram fish pond water (ALIR3).	Dense yellow-green becoming dark yellow-green on plates.	Conidia globose to subglobose, conidiophores hyaline coarsely, Roughened with vesicles globose to subglobose and conidial heads radiate and later splitting into loose column.	*Aspergillus flavus*
	Aliram fish pond water (ALIR3).	Dense felt dark green colouration on plate.	Conidial ellipsoidal to barrel shaped and conidial head radiate when young. Conidiophore stalks hyaline smooth-walled.	*Aspergillus penicilloides*
	Aliram fish pond water (ALIR3).	Dense felt yellow-brown colouration on surface plate.	Conidia are globose to ellipsoidal, conidiophores, hyaline, smooth walled and conidial heads compact.	*Aspergillus terreus*
4.	Aliram	Whitish colonies	Non-septate mycelia	*Rhizopus stolonifer*

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	fish pond water (ALIR4).	becoming grayish-brown due to brownish sporangiophore and brown black sporangia	produce stolons and rhizoids. Both rhizoids and sporangiophore arise from the node. Sporangiophore bears columella.	
		Whitish becoming brownish rose more or less funiclose at first becoming powdery with prominent central tuft. Reverse side in cream to brownish shades on plate.	Conidia are globose to ovoid with distinctly truncated base and once or twice branched.	*Scopulariopsis brevicaulis*
5.	Mammy fish pond water.	Compact white or yellow basalt felt with a dense layer of dark brown to black colouration on the surface of plate.	Conidial heads radiate tending to split into loose column with age, vesicles are globose to subglobose. Conidiophores stipes smooth walled.	*Aspergillus niger*
6.	Earth fish pond water.	Black or olivaceous black or grayish colour appears on surface of plate.	Conidia in long branched chains, conidiophores are septate with ovoid to ellipsoidal shape conidia.	*Alternaria alternata*
	Earth fish pond water.	Whitish colonies becoming grayish-brown due to brownish sporangiophore and brown black sporangia.	Non-septate mycelia produce stolons and rhizoids, both rhizoids and sporangiophores arise from the node. Sporangiophore bears columella.	*Rhizopus stolonifer*
	Earth fish pond water.	Dense yellow-green becoming dark yellow green on surface of plates.	Conidia globose to subglobose, conidiophores, hyaline, coarsely roughened with vesicles globose to subglobose and conidial heads radiate and later split into loose columns.	*Aspergillus flavus*

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DISCUSSION

The result showed that there was no fish pond water sample that was free from fungi, an indication that the entire fish pond water samples were contaminated by fungi. The contamination could have arisen from different sources which include air, source of water and fish feeds could have been responsible for the introduction of these organisms into the fish pond.

This study identified the prevalence of these fungi species in the sample: *Aspergillus spp, Scopulariopsis brevicaulis, Rhizopus stolonifer, Saccharomyces cerevisiae, and Zygosaccharomyces bailii and Alternaria alternata*.

The isolation of *A*spergillus spp, *R*hizopus spp in this study is in agreement with the work of (Adebayo-Tayo *et al*., 2006) that identified them as mycoflora in decreasing sequential order in market bush mango. The presence of *A. flavus* in these samples might probably makes the consumption of a fish hazardous to man according to the findings of (Akande and Tobor, 1992) which was also identified in the study. The works of (Clucas and Ward, 1996) also identified, *A. niger, A. flavus* which pose potential health hazard to its numerous consumers which was also isolated in this study. The isolation of *A. niger A. terreus, A. flavus* as contaminants of fish and fish water is in agreement with similar findings earlier by (Philips and Wallbridge, 1977) in Zambia.

Isolation of moulds belonging to the following genera *Aspergillus spp, Rhizopus spp* and *Scopulariopsis brevicaulis* in this study agreed with the findings reported by Abdel Alim (1992) and Khalil (1993) which was also identified the same organism from fish.

The yeast *Saccharomyces cerevisiae* is one cell organism which produces alcohol from the fermentation and break of sugar to alcohol. Yeast is an available feed ingredient for fish feed process besides it is easy to find (Tacon, 1994). In nature yeast are found in all habitats (Schlegel, 1993).

The isolation of the *Saccharomyces cerevisiae* (yeast) in the study is in agreement with the findings of (Tacon, 1994) that most yeast has been used as aqua feeds. The isolation of yeast from the study is in agreement with the findings of (Spinelli *et al.,* 1979) and (Mahnken *et al*., 1980), that yeast are used for supplementation based diets with deficient amino acids was shown to have beneficial effect on fish growth.

Studies reveal that the microbial flora of caught fish and other aquatic specimen is largely a reflection of the microbial quality of the water where they are harvested (Pelczar *et al*., 1986) In most cases fresh fish flora mainly are bacteria, Fungi are absolutely absent in fresh fish except a few yeast which have been shown to play no role in spoilage (Wood, 1940).However this does not mean they cannot produce disease, they can be considered as opportunistic fungi (Refai, 1987) as many of them posses virulence factors which enable them to cause disease (Refai *et al*., 2004).

Of the six fish pond water sample studied the Aliram fish pond (ALIR3) was found to be the most contaminated with the highest number of fungi. It had more fungi species than any of the other samples studied.

PH of the water sample studied shows slightly acidic in the entire fish pond water sample with the exception of Mammy fish pond which had a neutral pH.

CONCLUSION

It is important to state that majority of the fungal isolates obtained are of veterinary and medical importance.

Moreover there are other fungi that have been implicated in fish diseases some of the genera involved *Aspergillus spp*

(Salem et'al; 1989) mycotic infections of fishes by oomycetes are widespread in fresh water and represent the most important fungal group affecting wild and cultured fishes.

Most fish pond waters are contaminated by micro organisms which may consequently affect the fishes and quality of fish produced .Efforts should be made by fish farmers to prevent contamination of fish ponds as much as possible.

Many of the fungi that affect fishes are considered opportunistic, attacking fish when they are stressed or immunocompromised because of unfavorable condition or secondary to bacterial or viral infections, (Roberts, 1989) and (Quiniou *et al*., 1998).

Fungal infections in fish often are indicative of a more serious problem, some of the fungal infections or diseases in fish are Saprolegniasis, Branchiomycosis and icthyophonus disease which can be controlled by good management, good water quality, good nutrition and proper handling of the fish pond.

RECOMMENDATION

The tanks, raceways and aquaria must be disinfected; ponds should be treated with quicklime (Calcium oxide).Overcrowding and (quarantine) isolation of infected fishes should be employed in a fish pond farming when there is disease or outbreak of an infection. Though this research work did not study the bacteria, viruses and parasites of the fish pond water, so it is recommended that further research should be done on bacteria associated with fish pond water and their implication on the fish or the immediate consumer(s).

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