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## Funagal flora of sediment, pond water, fishfeed and fish from the fish culture pond of the michael okpara university of agriculture, umudike

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### ABSTRACT

Samples of sediment, fishfeed, pond water and tilapia (*Oerochromis niloticus*) from the Michael Okpara University fish culture pond were examined for fungal quality. The samples were collected using sterile bottles for the sediment, pond water, and fish feed samples, and a bucket for collecting the fish sample. The samples were homogenized and cultured on Potato Dextrose Agar. Results revealed that the fungal load of fishfeed was higher than that of the sediment with pond water having the lowest fungal load. The isolated fungi were *Aspergillus niger*, *Cladosporium herbarum*, *Fusarium* (Hyalophragmia), *Penicillium notatum*, Yeast (*Mycoderma*), *Rhizopus nigricans* and *Mucor*. Some precautionary measures were recommended such as regulating and inspecting the water quality of the pond to avoid spread of pathogens, removing an infected fish to an isolation tank which serves as a hospital for immediate treatment, application of medications, adequate stocking and fertilization of the pond.

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### INTRODUCTION

Fish culture has been on the increase in order to meet the growing demand for fish. The demand for fish took an upward surge when livestock production took a downward trend. In recent times, the high cost of animal feeds and feeding stuff has caused a depression in animal protein supply. The only option for adequate animal supply has been the fishery sector<sup>[12]</sup>. The awareness of aquaculture had led to several studies on the microbiology of the culture system.

Fishes have been observed to be associated with various diseases such as viral, bacterial, mycotic and protozoal diseases caused by different microorganisms such as viruses, bacteria, fungi and protozoa<sup>[15]</sup>.

Different sets of workers in the past have been able

to isolate some fungal flora associated with the culture systems. These fungi are the genera *Ichthyosporidium*, *Saprolegna*, *Achyla* and *Branchiomyces* have been regularly reported in cultured fish<sup>[13,16]</sup>. A different set of workers<sup>[7]</sup> (Srivastava, 1982) have isolated *Aphanomyces*, *Dictyuchus*, *Fusarium*, *Pythium*, *Leptomitius* in addition to *Saprolegna*, *Brachyomyces* and *Achyla* from fresh water fish culture in India. Similar organisms have been discovered by Kenneth and Austin<sup>[6]</sup>. There is also great need to detect these organisms and eliminate them from the pond<sup>[11]</sup>. Therefore, this work was undertaken to establish the fungal flora of samples of sediment, pond water, fishfeed and fish, tilapia (*Oerochromis niloticus*) from Umudike fish culture pond. Also, to determine the fungal load of the samples. Lastly, detect their relationship with fish diseases.

## MATERIALS AND METHODS

### Source of samples

The test samples pondwater, sediment, fishfeed and fish, tilapia (*Oerochromis niloticus*) were collected from the fish culture pond of the Michael Okpara University of Agriculture, Umudike. The feed was in the form of pellets. Pond water, sediment and feed samples were collected and stored using sterile glass containers. The fish samples were collected using a bucket.

### Sample preparation

Before the samples were subjected to analysis, each of them was prepared depending on their respective nature. The body part of the fish was cut into bits with flamed scalpel (with longhandle) and forceps. The cut bits were homogenized in a sterilized blender to obtain a pulpy (slurry) sample used for the analysis. Other samples; pond water and sediment were used directly.

### Determination of fungal flora

To determine the fungal flora of each test sample, the isolates from such samples were characterized and identified accordingly. Characterization of the isolates was based on the colony features and microscopic examination. Each colony of fungi on potato Dextrose Agar was examined closely for the features, which included the extent of growth, presence of mycelia, nature of mycelia, colony and others. In addition, plate culture technique<sup>[14]</sup> was used to examine the full features of some fungi without distortions.

Molten agar was used to make ridges on a sterile grease free microscope slide. When covered with a cover slip, an enclosure was inoculated. The slide culture was carefully transferred to a sterile petri dish containing two short sterile glass rods. As rails on which the slide rested, a sterile cotton wool which was made wet with sterile distilled water was left inside the plate culture to provide the moist atmosphere needed for good growth of the test fungi. After incubating the plate for 2-5 days, the slide culture was brought out and examined directly under the microscope. The features were recorded.

In addition to the above tests, mounts of each fungal isolate were made in lactophenol cotton blue (dye), examined microscopically and the features were re-

TABLE 1: Total fungal counts of water, sediment, fish feed and fish

Sample	1 <sup>st</sup>	2 <sup>nd</sup>	3 <sup>rd</sup>	Mean×10 <sup>4</sup>	Total count
Fish (cfu/g)	5	5	4	4.67×10 <sup>4</sup>	4.67×10 <sup>4</sup> ×10 <sup>-1</sup> =4.67×10 <sup>3</sup>
Sediment (cfu/ml)	8	6	8	7.33×10 <sup>4</sup>	7.33×10 <sup>4</sup> ×10 <sup>-1</sup> =7.33×10 <sup>3</sup>
Pond water (cfu/ml)	2	3	3	2.67×10 <sup>4</sup>	2.67×10 <sup>4</sup> ×10 <sup>-1</sup> =2.67×10 <sup>3</sup>
Fishfeed (cfu/g)	16	14	14	14.67×10 <sup>4</sup>	14.67×10 <sup>4</sup> ×10 <sup>-1</sup> =1.47×10 <sup>4</sup>

N=Mean number of colonies counted; D=Dilution factor (10<sup>4</sup>); V=volume of sample (10); I/V=(One tenth of the volume of sample used) = 1/10 = 0.1

corded. Information which included the presence and nature of mycelia and hyphae (septate or non-septate), branching of vertical hyphae, nature of sporangiophore or conidiophores were recorded.

## RESULTS AND DISCUSSION

It has been established that the fish culture pond situated at the Michael Okpara University of Agriculture, Umudike was contaminated as expected. The presence of fungal pathogens isolated from samples such as sediment, pond water, fish feed and fish determined using different characterization methods revealed that the fish culture pond is actually contaminated.

The morphological, cultural and microscopic examination of fungal isolates showed that these fungi: *Aspergillus niger*, *Cladosporium herbarum*, *Fusarium (Hyalophragtniae)*, *Penicillium notatum*, *Mycoderma*, *Rhizopus nigricans* and *Mucor* were present in the culture pond. Relevant result has been obtained in the past<sup>[7,6,5]</sup>.

The fungal counts of the different samples were shown in TABLE 1. The results revealed that the fishfeed had higher fungal counts compared to the sediment, fish and pond water. This could have indicated that the fishfeed may be a source of contamination of sediment, fish and pond water<sup>[11,3]</sup>. Similar observations have been reported for bacteria<sup>[8,10]</sup>. Also the higher fungal count in the sediment than in the water could be an indication that the sediment contains more valuable nutrients than the water. This may not be surprising as the bulk of the nutrients added either as fish feed or manure settled at the pond sediment where they are decomposed by heterotrophic microorganisms of wide specificity<sup>[9]</sup>. The

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**TABLE 2 : Incidence (frequency) of the occurrence of fungal isolates from pond water, sediment, fishfeed and fish**

Fungal species	Pond water	Sediment	Fish	Fish feed
<i>Aspergillus niger</i>	+	-	+	+
<i>Cladosporium herbarum</i>	-	+	-	+
<i>Fusarium (hyalophragmial)</i>	-	-	-	+
<i>Penicillium notatum</i>	+	+	+	+
Yeast ( <i>Mycoderma</i> )	+	+	+	+
<i>Rhizopus nigricans</i>	-	+	+	-
<i>Mucor</i>	+	+	-	+
No. of Genera isolated	4	5	4	6

+ =present; - =absent

isolation of higher population of fungi from the fish, tilapia (*Oerochromis niloticus*) than the water may be as a result of several domestic wastes which are added to pond system for fertilization. The pond water containing fewer fungi might be showing that it does not contain many nutrients so the organisms mostly dwell bountifully in areas where there is much food. Observations that are similar to the pond contributing more or less to the infection of the fish have been documented<sup>[4,2]</sup>.

TABLE 2 showed that the highest occurrence of fungal isolates were obtained from the fish feed. The high number and diversity of isolates from the fish feed showed that it was the principal source of these fungal species in the culture system. Among the fungi isolated, it was only *Rhizopus nigricans* that was not present in the fish feed.

The isolation of yeast (*Mycoderma*) from all the four samples could confirm it as a normal flora of the culture system. The occurrence of *Penicillium notatum* in all the four samples also showed it as one of the pathogens of the pond.

### Recommendation

The pond is a type of fresh water where fish is cultured. Many aquacultures exist nowadays due to the high economic yield of fish.

Fungal fish disease with overcrowding, handling, accumulation of metabolic waste products and organic matter in the water<sup>[3]</sup>.

Different fungal floras have been isolated from samples collected at the Michael Okpara University of Agriculture fish pond showing that the pond water is contaminated.

Therefore, to limit the infection of the pond, water quality, which is an important aspect in aquaculture sys-

tem, should be well regulated. The pond should be regularly inspected for any infection to avoid spread of pathogens.

Fishes get ill with various diseases from time to time, therefore they need to be taken care of because they cannot stroll to the doctors<sup>[1]</sup>. As long as a reasonable approach to maintaining a pond has been adopted, the fish should enjoy good health all the time.

Fish should be checked on regular basis and if a fish is identified as being ill, it should be removed from the pond to an isolation tank immediately, which serves as a hospital<sup>[1]</sup>. The ailment of the fish should be diagnosed, treating the tank and the pond accordingly.

Medications are also recommended. Some medications used for cure have their after effect that is to say that they are detrimental in the long term and some can destroy our beneficial organisms<sup>[17]</sup>. Instructions should be read carefully before use. Under treating the water can be just as bad as over-treating the water. Overstocking and over-fertilization of ponds by aquaculturists who crave for high yield should be stopped for the maintenance of optimum growth condition<sup>[11]</sup>.

### CONCLUSION

Based on the microbial analysis of samples obtained from the Michael Okpara University of Agriculture, Umudike fish culture pond, it was established that the pond was contaminated. The highest occurrence of fungal isolates were obtained from the fish feed showing that it is the main source of these fungal species in the culture system. On the other hand, the sediment sample with its high fungal load showed that the sediment was a potential source of reinfection for cultured fish.

This high fungi must have come from deposited feed, organic manure and other sources while pond water had the lowest fungal species showing that it contributed little to the infection of cultured fish.

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**REFERENCES**

- [1] C.K.Brunett; Environmental microbiology, **54**, 3021-3026 (2004).
- [2] F.Chain, T.Goddy; 'Diseases of Pond water', Biverlly Press Inc, 120-122 (2003).
- [3] J.C.Fox; 'Laboratory Animal medicine', Academic press, 77-798 (2000).
- [4] J.M.Grizzle; 'Anatomy and Histology of Tilapia Fish', Auburn Printing Co.USA, 160-162 (1988).
- [5] M.N.Jasper; 'Fungal Fish diseases', Wilson and Sons, New York, 132-135 (2000).
- [6] G.C.Kenneth, E.Austin; 'Principal diseases of fish', University printing press, Michigan, 317-318 (2001).
- [7] R.D.Khulb; Mykosan, 26, 273-275 (1988).
- [8] G.C.Okpokwasili, A.M.Alapiki; Journal of Aquaculture in the tropics, **5**, 87-90 (1990).
- [9] G.C.Okpokwasili, P.N.Ekeke; Tropical freshwater biology, **5**, 67-83 (1996).
- [10] G.C.Okpokwasili, Oro.Obah; Journal of aquaculture in the tropics, **6**, 157- 172 (1991).
- [11] G.C.Okpokwasili, J.N.Ogbulie, N.P.Okpokwasili; Journal of Aquaculture in the Tropics, **13(4)**, 269-276 (1998).
- [12] J.A.O.Oronsaye; African Biosciences Network, A.B.N, Senegal, 1-11 (1991).
- [13] I.Paperna; Parasite Infections and Diseases of Fish in Africa, CIFA Tech., 7-21 (1980).
- [14] M.I.Pelczar, E.S.C.Chan; Black.Dot.Inc.USA, 297-309 (1977).
- [15] R.J.Roberts; 'Fish pathology', 2<sup>nd</sup> edition Balliere Tindall, London, 130-134 (1989).
- [16] R.J.Roberts, C.Sommerville; 'Disease of Tilapias in: The Biology and Culture of Tilapias', R.S.V. Pullin, R.R.Lowe-McConnel (eds.); Management, Manila, Philippines, 247-263 (1982).
- [17] K.Wolf; 'Fish Viruses and Fish Viral Diseases', Cornell University Press, London, 204-206 (1988).