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Heavy Metals and Total Microbial Load in Industrial Wastewater Treated with *Moringa* Seed: Implication on *Clarias lazera* Fish Farming

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Abstract:
Fish farming is gaining more attention in Nigeria; Clarias sp. can be reared in any type of water including waste water. Fish especially Clarias lazera is a very important source of protein and nutritional value of unsaturated fatty matter, in developing countries. Bioaccumulation of heavy metals and rapid loss of quality that might occur very rapidly after catch, especially in hot climates and tropical areas are a huge source of concern. The aim of this study was determination of the heavy metals and total microbial load in waste water treated with Moringa seed and used in the rearing of Clarias lazera. Standard procedures were used for the analyses. Atomic Absorption Spectrophotometer was used for the determination of heavy metals. Nickel was below detection limit in all water samples. Chromium and nickel was below detection limit in gills and flesh of the fish samples. In the gills cadmium ranged between 0.01 and 0.08mg/l and reduced to 0.02mg/l in the flesh in treated water. Lead and chromium increased in waste water and treated water. Moringa seed was able to remove the following microorganisms from the wastewater E. coli, Staphylococcus aureus, Salmonella spp., Shigella spp., Vibrio spp., Cladosporum spp., Bacillus spp. But two new microorganisms were found in the fish samples which were Aeromonas spp., Clostridium lockleadii. The microbial count was reduced in the fish samples reared in moringa treated water as compared to the count in waste water: total Bacteria count by 1.6 x 107 Cfu/ml, Fungal count 0.5 x 105 Cfu/ml, Salmonella Count 0.9 x 105 Cfu/ml, Vibrio Count by 1.4 x 104 Cfu/ml and Total Coliform Count by 3.3 x 101 Cfu/ml. The implication was that Moringa seed can be used in remediating microbial contaminated industrial wastewater but not very active in remediating water polluted by heavy metals as it may not be able to remove lead and chromium from the sample.

Keywords: *Clarias lazera, Moringa, wastewater, microbial load, heavy metals*

1. Introduction

Fish is widely accepted as a rich source of protein and Omega A. In Nigeria *Clarias* species is a delicacy in most of the cultures. Fish farming is also gaining more ground and the species can survive even in extreme conditions. Fish can respond to environmental changes and *Clarias* species had been confirmed to have ability to bioaccumulate heavy metals that are dissolved in their aquatic environment, measuring the concentration in their whole bodies have consistently been used as a bio-indicator of the pollution of their aquatic environment (Adegbola *et al.,* 2012). One major source of human exposure to metal is through food web, causing acute and chronic effect to human. The unnecessary quantity of these metals in edible things is linked with various disorders, particularly with cardiovascular, nervous, kidney, as well as bone disorders; encourage tumor and mutations at greater amounts in animals (Degraeve, 2010; Groten and Vanbladeren, 2005). They are accountable for declining immunological defenses, growth delay, reduced psychosocial abilities, incapacities related with Malnutrition and greater occurrence of upper gastrointestinal cancer degrees (Iyengar and Nair, 2006). Excessive intake of Nickel leads to hypoglycemia, asthma, headache and epidemiological symptoms like cancer or nasal cavity and lungs. Lead toxicity includes colic, constipation and anemia. Higher contact with Cadmium may result in lung disorders like bronchiolitis, emphysema and alveolitis.

Microbial pollution is the pollution that is caused by the tiny small organism that is called microorganisms; some of which are not dangerous while some of the organisms like bacteria, viruses that can lead to serious diseases and cause death. The source of microbial pollution is often inadequately treated human sewage or runoff from animal husbandry facilities into streams or lakes; in addition, some microbial pollution can increase in drinking water distribution systems. Other factors influencing microbial levels, includes wild

animals which are reservoir for bacteria or protozoa that can infect humans, variations in turbidity can affect bacterial densities, and also algal blooms may increase bacterial abundance. Bacteria, including the nonpathogenic and pathogenic, are usually present in small numbers in most fish and in normal situation seldom cause any problem as the fish possess adequate immune system capable of fending off infections (Shawn, 1997).

Wastewater is any water that has been adversely affected in quality by anthropogenic influence. Wastewater can originate from a combination of domestic, industrial, commercial, or agricultural activities, surface runoff, and from sewer infiltration. Wastewater treatment is the process of taking wastewater and making it suitable to discharge back into the environment (Brown, 2009). Scarcity with an increased demand for water has necessitated the reuse of effluent in agricultural practices, especially in developing countries (Marshall *et al.,* 2010). The inability of different waste water methods to remove trace metals completely from treated wastewater has been a source of concern because of the health risk associated with contamination of water which ultimately enters the food chain (Fytianos *et al.,* 2010).

Bioaccumulation of heavy metals and microbial activities especially in wastewater had generated great concern in the environment. Therefore, the aims of this study were determination of the heavy metal and microbial load in waste water treated with *Moringa* seed and used in the rearing of *Clarias lazera.*

2. Material and Methods

2.1. Sample and Sampling Procedure

Borehole water from a private residence and wastewater was collected from a stream that effluent from a food industry in Eleyele Area, Ibadan empty into were collected into a sterilized container and transported to the laboratory for analysis. For the wastewater treatment 85 g of *moringa* seed were soaked into the waste water for 12 hours. Fish samples that were four weeks old for this study were obtained from a local - made collapsible plastics pond in a residential area in Eleyele, Ibadan during the month of June, 2015. The samples were identified at the Fishery Department, University of Ibadan.

Thirty *Clarias lazera* were randomly selected from the pool of fishes reared in a clean container with borehole water, and the container was covered with a net for the fish protection. At four weeks the fishes were distributed into three treatment groups: group A were introduced into wastewater. Group B were introduced into another container containing waste water treated with *moringa* seed. Group C were introduced into another container with clean borehole water this was used as control. The fish samples were reared for two weeks in each treatment groups.

2.2. Sample Preparation

After two weeks of treatment, fish from each group were slaughtered and cleaned; the gills and flesh were taken into foil papers and were further processed in the laboratory.

2.3. Digestion of Water Sample

10ml each of concentrated H_2SO_4 and HNO_3 was added to 5ml of each water samples, and it was transported into a digestion flask, and was digesting for 30 minutes. The digestate was allowed to cool, and was filtered into a standard volumetric flask (100ml) and made up to mark with distilled water, and then it was transported into a clean sample bottle. Chromium, Nickel, Cadmium, and Lead were analyzed using Atomic Absorption Spectrophotometer.

2.4. Digestion of Fish Sample

Fish gills and flesh were dried in an electric oven at 100^o-105^oC until constant weight. The samples were pulverized using a clean mortar and pestle to produce its powdered form. A homogenized 2g of each grounded fish were weighed using an analytical balance. The samples were transferred into digestion flask and 10mls each of concentrated $HNO_3$and H_2SO_4 was added. The samples were digested for one hour, until a clear solution was obtained, after the clear solution, then it was allowed to cool and it was filtered into 100ml standard volumetric flask and made to mark with distilled water. Chromium, Cadmium, Lead and Nickel were analyzed using Atomic Absorption Spectrophotometer.

2.5. Total Microbial Analysis of Water Samples Procedure

2.5.1. Isolation of Vibrio Species

The thiosulphate Citrate bile salt agar (TCBS) was poured onto sterilized petri-dishes. On solidification, 0.1ml of each pond water sample were previously enriched in alkaline peptone water and was transferred unto the dried agar plate in duplicate using 1ml pipette and was spread evenly with a sterile hockey stick. The sample was incubated at 35^oC for 24 - 48 hours. After incubation vibrio colonies were enumerated for vibrio count and for identification using biochemical reactions.

2.5.2. Enumeration of Total Coliform

The most probable number (MPN) was adopted in the determination of the total coliform bacteria using MacConkey broth the five tubes techniques. All the positive tubes from the MPN tubes were sub-cultured on an EMB agar plates in duplicated and was incubated at 35^oC for 24 hours.

2.5.3. Isolation of Salmonella/Shigella

The salmonella/shigella agar (SSA) was prepared according to the manufacturer's instructions.0.1ml aliquot of each water sample was transferred into the surface of dried sterilized SSA plate. The plates were inoculated in triplicated and it was incubated at 37^{o}C for 24 – 48 hours. Thereafter, pure cultures were obtained by sub-culturing onto freshly prepared SSA plates and pure colonies were identified using biochemical reactions.

2.5.4. Isolation of Total Bacteria and Fungi

Ten-fold of serial dilution of the water samples were prepared aseptically in sterile physiological water up to 10^{-5} and 0.1ml aliquot of each dilution was inoculated on a dried nutrient agar, and sabouraud dextrose agar plates, in triplicates, the spread plate techniques for enumeration of total bacteria count and fungi were used respectively. The nutrient agar plates were incubated at 35^{o}Cfor 24 hours under aerobic condition, white the sabouraud plates were incubated at room temperature for five days. The plates containing 30 - 300 bacterial 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. The fungi isolates were identified using lacto-phenol cotton blue stain, while the bacteria isolates were identified using series of biochemical reactions. Cfu/ml = number of colonies per ml plated/total dilution factor.

3. Results and Discussion

Results for Heavy metals were reported in tables1 and 2 below:

Sample	Cr	Cd	Pb	Ni
Borehole water	BDL	0.01	0.17	BDL
Waste water	0.21	0.07	0.44	BDL
Moringa Treated Water	0.81	BDL	0.66	BDL

Table 1: Concentration of heavy metals in water used for rearing Clarias lazera (mg/l)
Key: BDL- Below Detection Level

Sample	GILLS				FLESH			
	Cr	Cd	Pb	Ni	Cr	Cd	Pb	Ni
Borehole water	BDL	0.01	0.67	BDL	BDL	0.06	BDL	BDL
Waste water	BDL	0.03	1.14	BDL	BDL	BDL	0.18	BDL
Moringa Treated Water	BDL	0.08	4.16	BDL	BDL	0.02	0.27	BDL

Table 2: Concentration of heavy metals in gills and flesh of Clarias lazera reared in different water (mg/g)
Key: BDL- Below Detection Level

From tables 1 and 2 chromium and nickel were not detected in both the gills and flesh of *Clarias lazera* reared in different water. The maximum tolerance limit by WHO is 0.05mg/l. The maximum tolerance limit by W.H.O., 1993 for chromium and nickel is 0.05mg/l. Chromium and nickel metal ion were observed to be below maximum permissible limit set by W.H.O. Cadmium and lead increased from 0.03 – 0.08 mg/g and 1.14 to 4.16mg/g in gills of *Clarias lazera* reared in wastewater and *Moringa* treated water respectively. While cadmium was not detected in flesh of fish in wastewater but was detected at 0.02mg/g in *Moringa* treated water. The maximum tolerance limit by WHO for cadmium are 0.003mg/g in fish and 0.005mg/l in water. Cadmium metal ion was observed to be above maximum permissible limit set by W.H.O. in fish samples reared with *moringa* treated water. Cadmium was below detection limit in the *moringa* treated water sample. Lead also increased from 0.18mg/g in the flesh of fish in wastewater and 0.27mg/g in the flesh of fish in *Moringa* treated wastewater. The maximum tolerance limits by W.H.O., 1993are 0.05mg/g in fish and 0.01mg/l in water. Lead metal ion was observed to be below maximum permissible limit set by W.H.O., in all fish samples. A lower concentration was found in the flesh of samples grown in wastewater, while lead was below detection limit in the treated flesh sample. High concentration of cadmium and lead observed in this study may therefore be from other anthropogenic sources apart from the wastewater. This result contradicts that of Aladesanmi and Awotoye, 2014 which reported higher concentration of lead, chromium and nickel in the muscles of fish samples but the cadmium level was below detection level in all samples. Abd El Majeed Ahmed, 2008 also reported that the concentration of the studied elements in water and flesh of *Clarias lazera* from sewage showed significantly higher level than the White Nile and was found to be higher than permissible level of the WHO (1993).

Tables 3 and 4 reported microorganism isolated from water and fish reared in water samples:

SAMPLE CODE	MICROORGANISM ISOLATED
Borehole water	*Staphylococcus aureus, Salmonella spp., Shigella spp., Vibrio spp., Streptococcus spp., E. coli, Aspergillus spp., Penicillium spp., Cladosporium spp., Fusarium spp., Aeromonas spp., Pseudomonas spp.,*
Wastewater	*Streptococcus spp., Bacillus spp., E. coli, Staphylococcus aureus, Salmonella spp., Aeromonas spp., Shigella spp., Pseudornonas spp., Latobacilllus spp., Proteus spp., Enterobacter spp., Vibrio spp., Serattia spp., Klebsiella spp., Fusarium spp., Aspergillus spp., Penicillium spp., Cladosporium spp.,*
Moringa treated wastewater	*E. coli, Staphylococcus aureus, Salmonella spp., Shigella spp., Pseudomonas spp., Preteus spp., Vibrio spp., Streptococcus spp., Klebsiella spp., Aspergillus spp., Penicillium spp., Fusarium spp., Cladosporum spp., Bacillus spp.,*

Table 3: Microorganism isolated from water

SAMPLE CODE	MICROORGANISM ISOLATED
Clarias in borehole water	Streptococcus spp., Pseudomonas spp., Salmonella spp., E. coli, Fusaroium spp., Aspergillus spp., Penicillium spp.,
Clarias in Wastewater	Bacillus spp., E. coli, Staphylococcus aureus, Salmonella spp., Clostridum lockleadii, Shigella spp., Vibrio spp., Pseudomonas spp., Fusaroium spp., Enterobacter spp., Serattia spp., Cladosporium spp.,
Clarias in Moringa treated wastewater	Streptococcus spp., Aeromonas spp., Clostridium lockleadii, Pseudomons spp., Fusarium spp., Klebsiella spp., Aspergillus spp., Penicillium spp., Proteus spp.,

Table 4: Microorganism isolated from fish reared in water samples

The following microorganism were isolated from all water samples used in this study *Staphylococcus aureus, Salmonella spp., Shigella spp., Vibrio spp., Streptococcus spp., E. coli, Aspergillus spp., Penicillium spp., Cladosporium spp., Fusarium spp., Aeromonas spp., Pseudomonas spp.*,while *Pseudomonas spp., Fusaroium spp.* were common to all fish samples.
This study collaborate the report of Buras *et al*. (1987) that generally fish usually contain high numbers of bacteria (including pathogenic micro-organisms; *Pseudomonas, Aeromonas, Salmonella* etc.) in the digestive tract, gills and flesh. The bacteria isolated in the gastrointestinal tract of catfish were *Pseudomonas fluorescens, Bacillus spp, Aeromonas hydrophila, Flavobacterium rigense*. But two new microorganisms were found in the fish samples which were *Aeromonas spp., Clostridium lockleadii* this is in agreement with the report of Abdulkader (2011) in Jimoh *et al.,* 2014 that motile *aeromonas* are frequently isolated from both healthy and diseased fish as well as from other aquatic animals. *Moringa* seed was able to remove the following microorganism from the wastewater *E. coli, Staphylococcus aureus, Salmonella spp., Shigella spp., Vibrio spp., Cladosporum spp., Bacillus spp.* While the following were removed from the fish samples *Bacillus spp., E. coli, Staphylococcus aureus, Salmonella spp., Clostridum lockleadii, Vibrio spp., Fusaroium spp., Enterobacter spp., Serattia spp., Cladosporium spp.*

Tables 5 and 6 reported total microbial count in water and fish samples

Sample Code	Total Bacteria Count (Cfu/ml)	Fungal Count (Cfu/ml)	Salmonella Count (Cfu/ml)	Vibrio Count (Cfu/ml)	Total Coliform Count (Cfu/ml)
Borehole water	5.8×10^5	1.8×10^5	1.9×10^5	2.7×10^4	3.9×10^1
Waste water	8.6×10^5	2.7×10^5	3.6×10^5	6.3×10^4	7.1×10^1
Moringa Treated Water	6.2×10^5	2.3×10^5	2.7×10^5	3.6×10^4	4.8×10^1

Table 5: Total Microbial analysis in water samples used for breeding (cfu/ml)

Sample Code	Total Bacteria Count (Cfu/ml)	Fungal Count (Cfu/ml)	Salmonella Count (Cfu/ml)	Vibrio Count (Cfu/ml)	Total Coliform Count (Cfu/ml)
Borehole water	3.6×10^7	1.1×10^5	2.1×10^5	1.8×10^4	1.7×10^1
Wastewater	5.7×10^7	1.9×10^5	2.5×10^5	3.5×10^4	5.6×10^1
Moringa treated Wastewater	4.1×10^7	1.4×10^5	1.6×10^5	2.1×10^4	2.3×10^1

Table 6: Total Microbial analysis in the fish samples (cfu/ml)

The treatment also was able to reduce total Bacteria Count, Fungal Count, *Salmonella* Count, *Vibrio* Count, and total *Coliform* Count in the wastewater and the fish reared in it. The microbial count was reduced in the fish samples reared in moringa treated water as compared to the count in wastewater: total Bacteria count by 1.6×10^7 Cfu/ml, Fungal count 0.5×10^5 Cfu/ml, Salmonella Count 0.9×10^5 Cfu/ml, Vibrio Count by 1.4×10^4 Cfu/ml and Total Coliform Count by 3.3×10^1 Cfu/ml.

4. Conclusions and Recommendation

It was observed that lead and cadmium concentration increased in both the flesh and gills of *Clarias lazera* the indication was that anthropogenic activities around the study area may be a cause of the increase. Also *moringa* seed may not be very effective in removing the lead and cadmium. *Moringa* seed was able to remove a number of microorganisms and the total microbial load was reduced appreciably with the treatment.

Based on the present work and the understanding that fish cannot be availed contamination free for all people at all times the following are recommended, strict monitoring of heavy metal pollutants should be carried out on fish ponds and other fishing facilities to identify and quantify heavy metals of possible concern present in fish samples to reduce it. Toxicological study should be carried out to determine the risk that the average consumer is exposed to, using differences sources of fish. Other bioremediation methods to remove heavy metals should be utilized.

5. References

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