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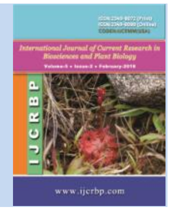

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Removal of Heavy Metals and Microbial Loads from Fish Pond using Biopolymer Crab Shell Chitosan and Egg Shell

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ABSTRACT

Water is the most important resource for aquaculture and can be a significant source for contamination. The conditions that fishes are cultured may be potentially stressful, causing existing infections to become more severe and precipitate disease outbreaks which may also compromise the fitness of such fish for human consumption. Heavy metals is one of challenge in reusing of wastewater for aquaculture production, this is because they are non-degradable and often accumulate through trophic level causing a deleterious biological effects.The concentrations of heavy metals in four different locations of fish pond in Ado Ekiti were investigated. The concentration of heavy metals in the fish pond (without a coagulant) ranges from Pb (0.12-0.01mg/l), Cr (0.12-0.47mg/l), Fe (0.14-0.34mg/l), Zn (1.81-1.37mg/l) Cd (0.07-0.02mg/l) and some heavy metals are also detected in the fish sample investigated despite the levels of the heavy metals detected in fish are not remarkable to cause acute health consequences, but chronic adverse health effects due to prolonged bioaccumulation and long term exposure through fish consumption can be inevitable. Treatment of the fish ponds with natural coagulants (chitosan and egg shell) has a greater efficiency on the removal of these pathogenic organisms and heavy metals. From the tested water samples, chitosan from crab shell remove the pathogenic organisms more than the egg shell. The effectiveness of the chitosan may be attributed to the flocculation and bactericidal activities, therefore chitosan and egg shell may serves as substitutes in treatment of fish ponds.

Introduction

Water is a life sustaining drink and is essential for the survival of all living organisms. However, there are toxic contaminants present in water that cause many life threatening health risks. So, water should be protected from contamination for public health cause and environmental reasons. Agriculture, as the single largest user of fresh water on a global basis and as a major cause of degradation of surface and groundwater resources through erosion and chemical runoff, has cause to be concerned about the global implications of water quality. The associated agro food-processing industry is also a significant source of organic pollution in most countries. Today, aquaculture is growing rapidly: according to the FAO/NACA WHO (1999), aquaculture provides 47% (51 million tons) of the global human fish consumption. In order to keep up with population growth and increasing *per capita* fish consumption, aquaculture output is set to increase by a further 60%–100% over the next 20–30 years (Aktar et al., 2010).

Heavy metals is one of challenge in reusing of wastewater for aquaculture production, this is because they are non-degradable and often accumulate through trophic level causing a deleterious biological effects (Aktar et al., 2010). Under certain environmental conditions heavy metals can accumulate up to toxic concentrations and cause ecological damage. In most cases fish is used as indicative factors in estimation of metal pollution in aquatic the aquatic system because they lie at the top of the aquatic food chain and may accumulate large amount of some metals from the water (Abdel-Baki et al., 2013). Contamination of heavy metal in fish ponds and its health hazard effects to the human gain more concern to food scientists. Almost all heavy metals are potentially harmful to most living organisms after exposure and adsorb certain level of it (Aktar et al., 2011).

The most reported toxic heavy metals to human health from contaminated aquatic organisms are cadmium (Cd), Mercury (Hg) and Lead (Pb) and some persistent organic pollutants (POPs) as a results of pollution from agriculture, industries, mining, household effluents and vector control (Polder et al., 2014). Among other effects, heavy metals may interfere with the major functions of the endocrine system of human and were termed endocrine disrupters. Presence of faecal coliform in fish intended for human consumption may constitute a potential danger of causing disease (Ampofo and Clerk, 2010). *Escherichia coli*, *Staphylococcus aureaus* and *Salmonella* are within group of pathogenic bacteria which cause food poisoning that lead to public health problem associated with fish and fishery products (Henson and Humphrey, 2009).

This study evaluated the use of chitosan and crab shell as an alternative coagulant in removal of heavy metals and microbial load associated with fish ponds.

Materials and methods

Experimental design

Fish were reared in five different water qualities ranging from wastewater to clean water. Both African catfish were stocked at a rate of five fish/m^2 of water for 90 days. Water analysis for heavy metals concentration (Cd, Pb, Cr, As, Zn, Mn and Fe) were analyzed from each study site. Also, at the end of the experimental period the fish were harvested and analyzed for heavy metal concentration, microbiological load (*Salmonella*, *E. coli*, and *Staphylococcus aureus*).

Water samples collection

Water samples were collected from five fishponds stocked with the African catfish (*Clarias gariepinus*) sited at different locations in Ado Ekiti. 100ml of water were collected in sterile glassware containers with stoppers. The water samples for microbial analysis were transported in ice-packs and processed between 2-3 hrs after collection.

Preparation of chitosan from crab shell

The raw materials i.e., crab shell was bought from Oja Oba Market Ado Ekiti. It was then dried, crushed and decalcified by treatment with 10% HCl, the acid being changed every day. The dorsal cover of the shell was peeled off on the third day. The resulting chitin protein complex of the dorsal cover was deproteinized by the treatment of 10% (w/v) NaOH AT 103 to 105°C in an autoclave. The septa region was deproteinized in 6h treatment under similar conditions. The resulting slurry was neutralized with 15% KOH solution in a large beaker and the precipitate was then filtered and thoroughly washed with hot water and deionized water. The resulting chitosan precipitates was dried at 105°C and ground to pass through a standard 40 mesh sieve.

Water sampling/sample preparation

The water samples were filled in sample bottles in triplicate from each experimental sites. There after the sample fixed at pH of 2 by nitric acid and samples were kept refrigerated at 4°C before being transferred to Afe Babalola University laboratory for heavy metals analysis.

Fish sampling/sample preparation

The experimental fish were harvested from cages and immediately chilled before kept frozen at -18°C before gathered to the laboratory for further analytical procedures. Before analysis, frozen fish were thawed in warm clean water then de-headed and filleted using sharp knife,and wasdried over 24 hours at 80°C in heraeus thermo scientific oven. The sample then grinded for20 minutes at 180 rpm using planetary mono mill (pulverisette 6) to get fine particles, the meal was used to make pellets after mixing with binder (starch). Pellets were inserted in X-Ray fluorescence (Spectro xepos – EDXRF) for analyzing the sample.

Microbial analysis

The microbial load of the water from the fish ponds wasdetermined by performing a ten-fold serial dilution of the sample in test tubes containing sterile distilled water. The total viable bacterial count was determined using the pour plate technique cultured in duplicates. The plates were incubated at 35°C for 48 hrs. The colonies were counted and expressed in colony forming unit per ml (cfu/ml) and values were estimated by means of duplicate determination. The isolated colonies were streaked on nutrient agar and pure cultures of the bacterial isolates were subjected to various morphological and biochemical characterization tests (Cheesbrough, 2006) to determine the identity of the bacteria isolates with reference to Bergey's Manual of Determinative Bacteriology (Buchanan and Gibbons, 1974).

Results and discussion

Table 1. Levels of heavy metals in water samples.

S/N	Pb (mg/l)	Cd (mg/l)	Mn (mg/l)	Cr (mg/l)	Fe (mg/l)	As (mg/l)	Zn (mg/l)
A1	0.12	0.07	0.01	0.37	0.26	<0.01	1.70
A2	0.08	0.04	<0.01	0.47	0.34	<0.01	1.81
A3	<0.01	0.05	0.03	0.36	0.28	<0.01	1.68
A4	<0.01	0.02	0.04	0.12	0.14	<0.01	1.37

$A_{1-}A_4$ = pond at different location in Ado Ekiti; PL = Permissible limit.

Table 2. Levels of Heavy metals in fish samples.

S/N	Pb (mg/l)	Cd (mg/l)	Mn (mg/l)	Cr (mg/l)	Fe (mg/l)	As (mg/l)	Zn (mg/l)
A1	0.00	0.00	0.00	0.38	0.38	0.00	27.14
A2	0.001	0.00	0.00	0.48	0.37	0.00	23.45
A3	0.00	0.00	0.001	0.63	0.674	0.00	22.32
A4	0.00	0.00	0.001	0.63	0.674	0.00	28.14

Table 3. Microbial count in water samples.

S/N	*Salmonella spp.*	*E. coli*	*Staphylococcus aureus*
A1	- ve	+ve	+ve
A2	-ve	+ve	+ve
A3	-ve	+ve	+ve
A4	-ve	-ve	+ve

Table 4. Effect of using coagulants (chitosan) on the metals and microbial count.

(a) Metals:

S/N	Pb (mg/l)	Cd (mg/l)	Mn (mg/l)	Cr (mg/l)	Fe (mg/l)	As (mg/l)	Zn (mg/l)
A1	Nil	0.01	Nil	0.08	0.01	Nil	1.12
A2	Nil	0.00	Nil	0.04	0.03	Nil	0.67
A3	Nil	0.00	0.01	0.05	0.27	0.00	0.147
A4	Nil	Nil	Nil	0.01	0.01	Nil	0.148

(b) Microbial count:

S/N	*Salmonella spp.*	*E. coli*	*Staphylococcus aureus*
A1	- ve	-ve	-ve
A2	-ve	-ve	-ve
A3	-ve	-ve	-ve
A4	-ve	-ve	-ve

Table 5. Effect of using natural coagulant (egg shell) on the metals and microbial count.

(a) Metals:

S/N	Pb (mg/l)	Cd (mg/l)	Cu (mg/l)	Cr (mg/l)	Fe (mg/l)	As (mg/l)	Zn (mg/l)
A1	0.07	Nil	0.01	0.28	0.21	Nil	1.12
A2	0.01	Nil	Nil	0.06	0.18	Nil	1.34
A3	<0.001	Nil	0.05	0.12	0.28	Nil	1.12
A4	Nil	Nil	0.01	0.08	0.24	Nil	1.18

(b) Microbial count:

S/N	*Salmonella spp.*	*E. coli*	*Staphylococcus aureus*
A1	- ve	+ve	-ve
A2	-ve	-ve	+ve
A3	-ve	+ve	+ve
A4	-ve	-ve	-ve

The concentrations of heavy metals in four different locations were investigated. The concentration of heavy metals in the fish pond (without a coagulant) ranges from Pb (0.12-0.01mg/l), Cr (0.12-0.47mg/l), Fe (0.14-0.34mg/l)Zn(1.81-1.37mg/l) Cd(0.07-0.02mg/l) and some heavy metals are also detected in the fish sample investigated with highest value of Zn. These concentrations differences of heavy metals between this study and others can be due to the ecological needs, surrounding environment, rate of metabolism, feeding behavior of the fish, exposure time and seasonal variation, despite the fact that these values observed are within the permissible limit of Cheesebrough (2006). they can reach dangerous trend if the source of these metals are not controlled.

To control these metals, two natural coagulants were used and tested on the water samples as shown in Table 4 and 5. The results shows that the natural coagulant chitosan have higher tendency and serve as effective ways in removing heavy metals from the pond compared to egg shell but the egg shell possess higher tendency of removing cadmium than chitosan and this may be due to abundant of calcium carbonate in the egg shell which have high infinity of binding to the cadmium in the samples.

The heavy metals like copper, nickel, mercury, lead, zinc, arsenic are extremely toxic and bio persistent in nature. The total removal of heavy metals by the chitosan may be due to tendency to bind/chelate with metal ions like cadmium, copper, lead, mercury, etc. The metal binding efficiency of chitosan depends on the availability of amine groups for interaction with metal ions.

The Table 3 revealed the presence of *Escherichia coli* and *Staphylococcus aureus* in all the four fish ponds investigated before the addition of natural coagulant. The presence of the above organism may pose a threat to the health of the fishes and the consumers and this is in agreement with findings reported by Molokw and Okpokwasilli (2002) and these could suggest a possible faecal contamination of the fish ponds.

Treatment of the fish ponds with natural coagulants (chitosan and egg shell) has a greater efficiency on the removal of these pathogenic organisms. From the tested water samples, chitosan from crab shell remove all the pathogenic organisms more than the egg shell. The effectiveness of the chitosan may be attributed to the flocculation and bactericidal activities which has been reported as a bridging mechanism for bacterial coagulation by and its molecules has a tendency to bind on the microbial cell surface and form an impervious layer around it (Varma et al., 2004). Thus, these may cause cell permeability and causes leaking of the intracellular constituents and death of microbial cells.

Conclusion

The results of this study revealed the quality of fish Ponds in terms of heavy metal and microbial contamination from four different location in Ado Ekiti and the use of natural coagulant chitosan and egg shell as a substitutes in removing the above effects. The levels of the heavy metals detected in fish are not remarkable to cause acute health consequences, but chronic adverse health effects due to prolonged bioaccumulation and long term exposure through fish consumption can be inevitable. The microbial load and heavy metals can be reduced by using Chitosan and egg shell as a natural coagulant in treatment of fish ponds. The public should be enlightened on the possibility of being susceptible to health problems when feeding a contaminated fish with heavy metals and harmful microorganisms.

Conflict of interest statement

Authors declare that they have no conflict of interest.

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