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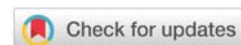
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## Review Article

# Biosorption of Cadmium from Freshwater Cultured with (Nile Tilapia) using Neem Leaf Aqueous Extract (NLAE). A Review

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## Abstract

In the quest to achieve economic prosperity and industrialization, sustainable water management, agriculture, biodiversity resources and food security have been marginalized. These have birthed serious intolerable environmental challenges such as heavy metal contamination and wastewater discharge into water bodies, particularly cadmium, lead and mercury. All these pose a serious threat to water bodies or the environment. These could adversely affect the biodegradation that demands bio-friendly remediation. A total of 180 healthy *Oreochromis niloticus* were used for this comparative research to evaluate neem leaf aqueous extract and pulverized neem leaf biosorption of cadmium in contaminated aquaria. This research focused on the review of neem leaf aqueous extract as a primary alternative for biosorption of heavy metals contamination in aquaculture or the environment. Following the trend of results obtained in recent research, the result depicted stressful acclimatization, death of *Oreochromis niloticus* and irreversible damage to muscle, liver, and kidney tissues or photomicrographs cultured with Cadmium. This is due to the chemical composition of the neem leaf aqueous extract being acidic and has a short shelf life. The reviewed findings showed that pulverized neem leaf is considered eco-friendly, a primary biosorbent for biosorption and is cost-effective in lead-contaminated aquariums cultured with cats and fish. The research results revealed normal acclimatization, insignificant bioaccumulation below normal range, drastic low mortality rate, chemical composition within range and physical and morphological characteristics of pulverized neem leaf are supportive factors for heavy metal binding. Photomicrographs of muscles, liver and kidney in contemporary research conducted with cadmium and lead showed that damage is reversible, unlike neem leaf aqueous extract that caused irreversible damage to muscles, liver and kidney tissues of fish.

## Introduction

Heavy metals have a substantial harmful impact on aquaculture and the environment [1]. Heavy metal ions get introduced into streams by industrial activities and their waste products during mining, refining ores, tanneries, batteries recycling, paper making and paint production among others and these pose serious threats to water bodies or the environment [2]. Toxic heavy metals have a devastating effect on the ecological balance of the recipient environment and the diversity of aquatic organisms [3]. According to the United States Agency for Toxic Substances and Disease Registry (ATSDR) [4], cadmium is second and seventh in toxicity and severity respectively.

[5] defined heavy metal as a dense metal that is usually toxic at low concentrations. Most heavy metals have a high atomic number, atomic weight and specific gravity greater than 5.0. Examples of heavy metals include lead, mercury, cadmium, chromium, iron, zinc and arsenic among others. Water may become contaminated by the accumulation of heavy metals and metalloids through emissions from the rapidly expanding industrial areas, mine tailings, disposal of high metal wastes, leaded gasoline, paints, land application of fertilizers, pesticides, wastewater, coal combustion residues and spillage of petrochemicals [6,7]. In water bodies, these heavy metals are absorbed by fish and other organisms in the food chain and amplified by animals that feed on fish (bioaccumulation and biomagnification). Heavy metals non-biodegradable in nature

make their threat multiplied by their accumulation in the environment through the food chain [8]. Lead and Cadmium affect fish organs like the liver, kidney muscular tissues, skin, blood and nervous system. The effects could be sub-lethal or lethal depending on the concentration of the accumulated metals in the fish or animals [9]. The conventional methods for the removal of heavy metal contamination from wastewater include chemical precipitation and oxidation, ion exchange, membrane separation, reverse osmosis and electrodialysis. These methods are costly, not very effective, and require high energy input. The search for alternative technologies for the removal of toxic metals or heavy metals from wastewater has focused attention on biosorption [9].

Biosorption is a physicochemical process that takes place on certain bio-aqueous and biomass, which allows contaminants removal on biosorbents' surface [10] while aqueous binding is through the soluble chemical compound; hydroxyl and carboxyl that attracts the heavy metals that are positively charged [11]. It is said to be effective and economical, because of its relatively low cost.

Neem trees are known to possess pharmacological properties which include; antibacterial, antifungal, antiulcer, repellent, pesticidal, and detoxifying agent [12–14]. One of the most promising natural compounds is Azadirachtin (AZA), an active compound extracted from the neem tree (*Azadirachta indica*), whose antiviral, antibacterial, and antifungal properties have been known for 2000 years [15,16]. Neem has been used successfully in aquaculture systems to control fish predators and treatment of large numbers of bacterial and parasitic fish diseases [17]. The agricultural sector is the mainstay of our economy in Nigeria which supply fish protein to the citizenry and also serves as a major source of revenue to the farmers and government alike. The present review aimed to evaluate the efficacy of aqueous neem extract on the histopathology of *Oreochromis niloticus* cultured in contaminated water with cadmium.

The most potent contaminants of the environment are heavy metals, they are discharged into the aquatic ecosystems through the effluents of several industries, resulting in severe aquatic pollution. This type of harmful heavy metal contamination in aquaculture systems has attracted great attention throughout the world. This research reported the bioaccumulation of different heavy metals into fishes, their toxic effects, and possible bioremediation techniques to protect the fishes from heavy metal contamination. Additionally, strategies to remove heavy metals from aquatic ecosystems are through effective bioremediation and biosorption of heavy metals [18].

Recent research investigated heavy metal levels in water with Nile Tilapia and observed conductivity, total dissolved solids, total suspended solids, and dissolved oxygen were within WHO and USEPA limits for potable water. Cu (0.028 – 0.112 mgL<sup>-1</sup>), Pb (0.002 – 0.009 mgL<sup>-1</sup>), and Zn (1.46 – 3.79 mgL<sup>-1</sup>) were below the maximum permissible limits by both WHO and USEPA, while cadmium, chromium, and nickel were

not detected in the water samples. Cu (2.56 – 3.54 mg/kg), Zn (22.93 – 32.13 mg/kg), and Ni (0.39 – 0.48 mg/kg), are within acceptable levels, while Cd and Cr were also not detected in the fish samples. However, Pb (1.71 – 2.30 mg/kg) was higher in the fish samples than the threshold limit (0.3 mg/kg) by FAO and WHO. In accordance, Pb with bioconcentration factor (BCF: 402) was more highly bio-accumulated in the fishes than Cu (BCF: 54.4) and Ni (BCF: 10.97). The results recommend the river water for utility purposes but raised concerns about the consumption of Nile tilapia fishes in the lake [19].

Pathological damage of waterborne lead toxicity in wild Nile tilapia collected from a lead-contaminated area (the Mariotteya Canal: Pb= 0.6 ± 0.21 mg L<sup>-1</sup>) and a farmed fish after 2 weeks of experimental exposure to lead acetate (5–10 mg L<sup>-1</sup>) in addition to evaluating the efficacy of Neem Leaf Powder (NLP) treatment in mitigating symptoms of lead toxicity. A total number of 150 fish (20 ± 2 g) were separated into five groups (30 fish/group with three replicates). G1 is a negative control without any treatments. Groups (2–5) were exposed to lead acetate for 2 weeks at a concentration of 5 mg L<sup>-1</sup> (G2 and G3) or 10 mg L<sup>-1</sup> (G4 and G5). During the lead exposure period, all groups were reared under the same conditions, while G3 and G5 were treated with 1 g L<sup>-1</sup> NLP. Lead toxicity induced DNA fragmentation and lipid peroxidation and decreased the level of glutathione and expression of heme synthesis enzyme delta aminolaevulinic acid dehydratase (ALA-D) in wild tilapia, G2, and G4. NLP alleviates the oxidative stress stimulated by lead in G3 and showed an insignificant effect in G5. The pathological findings, including epithelial hyperplasia in the gills, edema in the gills and muscles, degeneration and necrosis in the liver and muscle, and leukocytic infiltration in all organs, were directly correlated with lead concentration. Thus, the aqueous application of NLP at 1 g L<sup>-1</sup> reduced oxidative stress and lowered the pathological alterations induced by lead toxicity according to [20].

Nile tilapia weighted at this range (35.29 ± 1.21) was subjected to: control (without Cr or probiotics), hexavalent Cr (potassium dichromate) treatment (10 mg/L), probiotics treatment (1 ml/L), and combination of Cr and probiotics treatment (10 mg Cr/L and 1 ml probiotics/L) for six weeks (42 days). The results showed that growth performance (weight gain; WG, specific growth rate; SGR) and feed utility (feed conversion ratio; FCR) were inhibited significantly in the Cr-treated group compared to the control, while those parameters were found to be regained in a probiotics-treated group as to control. In the case of hemato-biochemical parameters (hemoglobin and glucose), Cr-exposed fish showed lower hemoglobin and higher glucose levels; however, the use of probiotics showed their level as control. The number of cellular (teardrop, twin, spindle, and echinocyte shaped) and nuclear (notched nuclei, nuclear buds, nuclear bridges, and karyopyknosis) abnormalities in erythrocytes were higher in the Cr-exposed group. However, probiotic addition leads to a notable decrease in these abnormalities. Similarly, higher deformities were observed in the gills and intestines of the fish exposed to Cr, while the administration of probiotics reduced these abnormalities. Overall, probiotics appear to have the potential to mitigate the negative physiological effects of Cr on Nile tilapia according to [21].

## Objectives

The specific objectives of the work are as follows:

1. To compare the efficacy of pulverized Neem and aqueous extract on the removal of cadmium in the contaminated aquarium with Nile Tilapia
2. To determine pathological damages in some key organs and tissues of Nile Tilapia
3. To ascertain whether PNL or NLAE could take in more of the contaminants in water

## Acclimatization

According to [1], *O. niloticus* exposed to Neem Leaf Water Extract (NLWE) showed respiratory distress, gasping, gulping the atmospheric air erratic swimming with some nervous manifestations in the form of fish moving in all directions of aquaria and some of them swam in circular directions were noticed during determination the LC<sub>50</sub> of NLWE, while fish exposed to Neem Leaf Powder (NLP) being normal without any abnormal sings, cadmium showed slimy body with dark skin colour, with signs of restlessness some fish suffered from asphyxia and jumped outside water, finally loss of appetite, escape reflex and settle down to the bottom, sluggish movement.

## Major chemical compounds in neem leaf aqueous extract

These chemical compounds include: Azadirachtin; a tetranortriterpenoid compound with a molecular formula of  $C_{35}H_{44}O_{16}$ . It is the primary compound responsible for the biosorption of Cd and Pb, Nimbin; a triterpenoid compound with a molecular formula of  $C_{30}H_{46}O_6$ . It contributes to the biosorption process by forming complexes with Cd and Pb, Quercetin; a flavonoid compound with a molecular formula of  $C_{15}H_{10}O_7$ . It has been found to chelate Cd and Pb ions, facilitating their removal and Glycosides; neem leaves contain various glycosides, such as nimbose and nimbinin, which contribute to the biosorption process [22].

## Neem leaf aqueous extract physicochemical biosorption

Bio-aqueous sorption is driven by the ion exchange of neem leaf compounds with heavy metals, allowing for their removal and chelation in neem leaf compounds through the formation of complexes with heavy metals, reducing their availability. Absorption is easier in water-soluble compounds in neem leaf extract and more easily absorbed by living organisms, allowing for better binding to heavy metals. Less toxic neem leaf water extract is generally considered less toxic compared to neem leaf powder, making it a safer option for environmental remediation [22].

## Chemical composition and physical characteristics of neem leaf powder

The chemical and chemical compounds found in neem leaf powder are; azadirachtin, nimbin, Quercetin, glycosides,

and hydroxyl and carboxyl functional group of compounds as defined in neem leaf extract [22].

## Physical characteristics and morphological characteristics

The physical characteristics of neem leaf powder are high surface area; pulverized neem leaves have a large surface area, which provides more binding sites for heavy metal ions, and porosity; the porous structure of neem leaves allows for efficient adsorption of heavy metal ions, and particle size; the small particle size of pulverized neem leaves (typically < 100  $\mu$ m) increases the surface area and enhances Pb removal. Surface charge; the surface of neem leaves carries a negative charge, which attracts positively charged heavy metal ions. Functional groups; the presence of functional groups such as hydroxyl (-OH), carboxyl (-COOH), and amino (-NH<sub>2</sub>) on the surface of neem leaves facilitates the binding of heavy metal ions [22]. Leaf Structure; neem leaves have a thin cuticle, which allows for easy penetration of heavy metal ions. Trichomes; are present (hair-like structures) on neem leaves and may enhance heavy metal removal by increasing the surface area and Vein Structure: the vein structure provides additional binding sites for heavy metal ions [22].

## Limitation of neem leaf water extract

**Shorter shelf life:** Neem leaf water extract has a shorter shelf life compared to neem leaf powder, requiring more frequent preparation and application. Lower stability: the water-soluble compounds in neem leaf extract may be less stable over time, affecting their binding efficiency. More difficult to standardize. Neem leaf water extract can be more challenging to standardize due to variations in extraction methods and solute concentrations. The optimal pH for biosorption is between 5 and 7 while neem leaf water extract range between 3 and 4. These significantly affect the efficacy and efficiency of its biosorption in environments contaminated with heavy metals [22].

## Efficacy and efficiency advantages of pulverized neem leaf over neem leaf water extract

The advantages are considered based on the chemical composition, physical characteristics and morphological characteristics of the pulverized neem leaf or neem leaf powder as earlier discussed. The pulverized neem leaf has chemical and functional group compound, high surface area and the structure of pulverized neem leaf. It has a longer life span, and higher stability and standardization are possible [22].

These factors also influence the biosorption in pulverized neem leaves: pH, temperature contact time and neem leaf dosage. pH; the optimal pH for biosorption is between 5 and 7. Temperature; the biosorption process is temperature-dependent, with optimal temperatures ranging from 25 °C to 35 °C. Contact time; the biosorption process is time-dependent, with optimal contact times ranging from 30 minutes to 2 hours. Neem leaf dosage; the biosorption efficiency increases with increasing neem leaf dosage [22].



## Materials and methods

### Preparation of Neem Leaves (PNL) and aqueous extract for analysis

A total number of 180 healthy Nile Tilapia were bought from the Department of Fisheries and Aquaculture, University of Nigeria Nsuka, Enugu State, Nigeria and were randomly divided into twenty-five [23] plastic aquaria (40 litre sized plastic bowls).

*Azadirachta indica* leaves were obtained from the Admiralty University of Nigeria Research Farm and identified in the Botany Herbarium. They were taken to Biology Laboratory where they were washed repeatedly with distilled water to remove dust and impurities. Suddenly after being washed, some of the leaves were pulverized to get aqueous extract and stored. The washed leaves were allowed to dry at a Laboratory temperature of  $28 \pm 3^\circ\text{C}$ . The leaves were oven-dried at  $70^\circ\text{C}$  for 30 hrs till the leaves became dry and crispy with Gallen Kamp oven model 605 England. The dried leaves were pulverized into fine powder by using a mortar and pestle. The powdered material was sieved with a sieve of 0.25–0.5 mm pore size (NLP) and preserved in Ambian Bottles for use in subsequent experiments, according to the methods of [24].

### Experimental water

Water used was collected from the Admiralty University of Nigeria Borehole. The following physicochemical properties were gauged before and after the experiment; pH, and temperature before the experiment. The same physicochemical properties were also gauged at the end of the experiment.

### Determination of the physicochemical properties of the aquarium water

Temperature, PH, and electricity Conductivity were measured using Hanna Multi- Parameters water tester Model HI 98129. This was done by selecting or pressing the Mode Key and selecting the corresponding reading or figure displayed on the LCD of the meter. While the DO was measured using VWR Metre Model L89023. At the end of the experiments, the above parameters were similarly measured at Federal Medical Asaba, Delta State, Nigeria.

### Experimental design and set up

Two experiments were set up for the heavy metal (lead) and Nile Tilapia were divided into three groups of ten [13] with each comprising two experimental groups and a control. The first group consisted of ten [13] fishes exposed to 10 mg/l and 15 mg/l lead and 60g of Pulverized Neem Leaf (PNL) placed in a plastic bowl while the Nile tilapia aqueous extract of this concentration was used  $\text{LC}_{50}$ . The bowls served as aquariums with dimensions of 100 by 50 cm and each of the plastic bowls contained 40 litres of water. The treatment in each of the experiments was replicated three times and arranged in a completely randomized design in a room at a temperature of  $25 \pm 3^\circ\text{C}$ . The setup was allowed to run for six [25] weeks. The

lead concentrations and dosage of PNL used in this experiment were based on [26] who established 0.03mg/L – 0.0129 mg/L Cd levels of contaminations in some Nigerian rivers.

### Preparation of samples for analysis of Cadmium (Cd) in fish

The approximate grams of samples harvested was 10g (muscle, liver and gills) and were homogenized. 1 gram of the homogenate was used to achieve digestion. The 1gram was placed in a 100ml flask and mineralized under reflux using the mixture of 6ml, 2ml and 4ml of  $\text{HNO}_3$ ,  $\text{HClO}_4$  and  $\text{H}_2\text{O}_2$  and  $\text{H}_2\text{SO}_4$ ,  $\text{HClO}_4$ ,  $\text{NaMoO}_4$  and  $\text{H}_2\text{O}_2$  for lead and mercury respectively. The procedure took 6 hours until a clear solution was obtained. The procedure was prepared in triplicate and carefully transferred to a flask. The digest was then analyzed for Cd using an atomic absorption spectrophotometer (Model: AAS Buck Scientific 205) with aqueous calibration standards prepared from the stock standard solutions of the respective elements as described by [25].

### Histology

The fish were sacrificed to collect gills and liver for histological examination. The removed parts were immediately fixed in 10% neutral buffered formalin for 24 hours. The formalin-fixed samples were then subjected to dehydration in concentrations of ascending alcohol (70% – 100%), cleared in xylene, embedded in paraffin, and sectioned at  $5\ \mu\text{m}$ . The collected sections were then stained with hematoxylin-eosin and viewed with a light microscope.

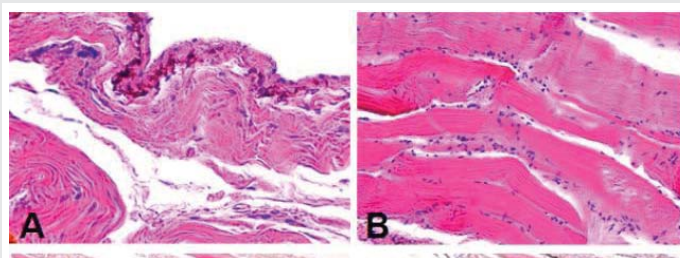
### Statistical method

All tissue data for lead collected were analyzed using the JMP version 13 [13]. It was analyzed ( $p < 0.05$ ) using a one-way analysis of variance (ANOVA). Significant differences in least square means were separated using a Tukey post hoc test. Physicochemical parameters data were subjected to one-way ANOVA.

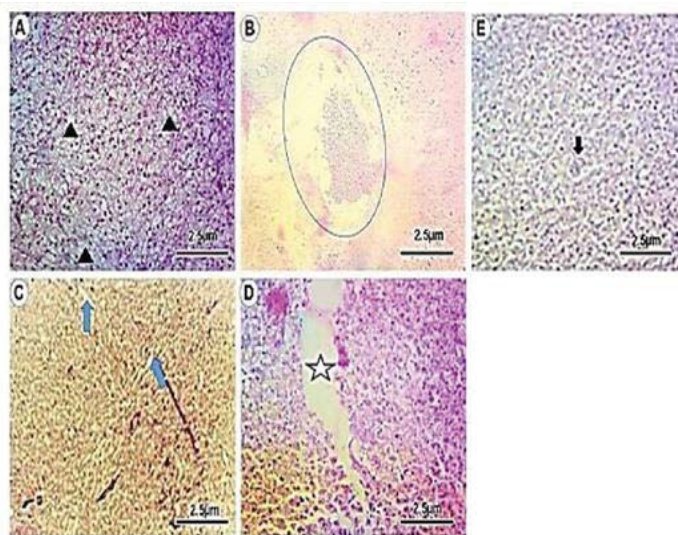
Histopathology of nile tilapia muscles exposed to Cadmium Figure 1.

Photomicrograph of *Oreochromis niloticus* Muscles Exposed to Cadmium with NLWE.

Histopathology of cats fish exposed to lead Figure 2.



**Figure 1:** Showing (A & B) skin and musculature of *O. niloticus* exposed to cadmium: there was diffused melanosis with inflammatory cells infiltration in the dermis. Source: [1].



**Figure 2:** Photomicrograph of the liver of juvenile *Clarias gariepinus* Fig. Photomicrograph of the livers of juvenile *Clarias gariepinus* exposed to a graded dose of lead (Pb), pulverized neem leaves (PNL). (A) Control: Shows the parenchyma composed of polyhedral hepatocytes typically with central nuclei. The nuclei of these cells are elongated and protrude into the sinusoidal lumen. (B) PNL and 15 mg/L of Pb: Shows the depletion of hepatic parenchyma and infiltration of lymphocytic cells into the parenchyma. (C) PNL and 10 mg/L Pb: Shows fatty degenerated parenchyma with focal necrosis (black arrow). (D) PNL and 10 mg/L Pb show fatty degenerated parenchyma with sinusoidal distortion (blue arrow). Biosorption was higher with higher PNL, the organs appeared reversibly and irreversibly damaged depending on the concentration of Pb. Histopathological examination revealed a congested gill filament vessel, partially.

Source: [11].

*gariepinus*) treated with neem (*Azadirachta indica*) and mango leaves (*Mangifera indica*) results showed Pb biosorption was observed in muscle, gills, and liver using pulverized neem leaves with no significant bioaccumulation was recorded [11]. The addition of Copper Nicotinate and Vitamin E in fish diets could protect the fish *O. niloticus* against Neem Seed Oil (NO) induced oxidative damage and histopathological changes showing recovery of fish organs. It was concluded that although botanical pesticides are considered less toxic/safe, they may provoke deleterious changes in the vital organs of the fish. Hence, precautions must be taken into account when botanicals are being used in fish production facilities [29].

## Recommendations

- I. Eco-friendly methods of digesting domestic and industrial waste should be encouraged by using pulverized neem.
- II. Ponds with heavy metal contaminations should try biosorption technology with pulverized neem leaves.
- III. Further research works should be done on closed contaminated aquatic habitats with pulverized neem and mango leaves to ascertain their efficacy in bioremediation of water pollution.

## Conclusion

Neem leaf aqueous extract (NLAE) and neem leaf powder all have binding capacities for heavy metals removal. In this research work, the limitation of neem leaf aqueous extract was discovered to have a high mortality rate, highly acidic, reduced surface area, and short life span in aquaculture while the pulverized neem leaf or neem leaf powder efficacy and efficiency were outstanding in biosorption of heavy metals in researches conducted with pulverized neem leaves. In conclusion, pulverized neem leaf has an edge over neem leaf aqueous extract and is considered an eco-friendly and primary biosorbent for use in water and aquaculture.

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## Discussion

This research work showed removal of cadmium in contaminated Nile Tilapia aquarium can be possible with the use of pulverized neem. However, significant irreversible damages were observed in muscles, gills, and liver samples of treatment treated with neem leaf aqueous extract. WHO [27] permissible level for cadmium is 0.03 mg/l. This means only safe water should be used for aquaculture.

The histological photomicrographs of *Clarias gariepinus* showed damages in the gills observed in PNL Pb appeared irreversible due to congestion and obliteration of the primary gill filament vessel, obliteration of secondary lamellae with insignificant bioaccumulation [11]. Similar findings were reported by [28] in the liver of heteroclaris juveniles exposed to lethal and sub-lethal concentrations of chlorpyrifos

The research results conducted on the removal of cadmium from freshwater-cultured Nile tilapia *Oreochromis niloticus* using Neem Leave Water Extract (NLWE) depicted significant bioaccumulation in the muscles, liver and kidney tissues. This can pose a threat to the environment, tilapia, carnivores, and humans that depend on fish as a source of food [1] while aqueous application of NLP at 1 g L<sup>-1</sup> reduced oxidative stress and lowered the pathological alterations induced by lead toxicity according to [20].

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