Toxicological Evaluation of Some Commercial Paints in African Catfish (ClariasGariepinus)

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Abstract. Commercial paints sold in Nigerian markets contain Lead which is hazardous to the health of humans and the environment. These toxic heavy metal, Lead in paints eventually find their way into the aquatic environment disrupting the ecosystem and causing harm to aquatic biota. The aim of this study was to determine and assess the biological effects of two Lead contaminated paints on the African catfish (Clariasgariepinus). Acute toxicity followed by chronic toxicity was carried out to investigate the effect of the Lead contaminated paints on lipid peroxidation, Alanine Transaminase (ALT), Aspartate Transaminase (AST), Alkaline Phosphate (ALP), protein and glucose in the exposed Clariasgariepinus. Lead level was also determined in the paints and tissue of the exposed fish. Results show that the coloured paints have higher Lead content than white paints, with FC orange paint having 97.88ppm level of Lead which was the highest. Acute toxicity experiment showed that the 96 hour LC50 value was 16.16mg/l for orange and 22.28mg/l for white, both of FC paint; yellow and white color of V paint has 17.16mg/l and 29.14mg/l respectively. FC paint (Orange) was most toxic. There was significant differences in the concentrations of ALP, AST, ALT, glucose, and protein between the exposed and control fishes. There was no significant difference in the levels of lipid peroxidation observed in the exposed and control fishes. Results show varying concentrations of Lead in the gills and skin of fishesexposed to Leadwhile control fish recorded no Lead level. Measures should therefore be taken to regulate the Lead content in commercial paints locally manufactured, the Lead content in the paint released into the environment is bio-accumulated in fishes as shown in this study which has health implications when consumed by humans.
1. Introduction
Fresh water pollution with various contaminants has been a serious environmental problem over the years [1]. The awareness of lead toxicity in paint dates back to decades and Painter’s colic or painter’s palsy was the term used to describe Lead poisoning. Goya and van Gogh are paint artists that were known to have been diagnosed with lead poisoning [2]. Although Leaded petrol was banned in many countries however Lead contaminated paint has been described as a major source of Lead exposure in humans and especially children, which is a major concern [3]. To give paint its colour, make it more durable and reduce the paint time of drying, lead is added to paint during manufacture. Lead like many other heavy metals is non-biodegradable and remain indoors, after leaded paint is applied on surfaces in homes, schools and many other areas. In old buildings where leaded paint has been applied over time, the lead particles may be discharged and this result in elevated levels of lead in the dust of such homes [4].

Following the challenges and high costs involved in removing leaded paint from residential and commercial buildings, the best way of protecting humans in homes is to prevent the use of leaded paints [4]. It is a major concern that many countries in Africa have not put in place measures to control the level of lead in paint [4]. In developed countries for example, there is effective regulation on use of leaded paint for as far back as in the 1970s and this regulation is not existing in many countries in Africa. Some researches have confirmed the presence of varying levels of the heavy metal lead in paints sold in African countries including Ghana, Nigeria and South Africa [5] [6]. A statement of caution on lead presence in paints is not visibly written on paint containers [4]. Accumulated toxic lead particles found inside and outside of homes are hazardous to human health. According to [7] and [8], high concentrations of lead were found in the blood of people living in painted houses when compared to levels in the blood of people who did not live in painted houses. [9] found lead levels as high as 515.9 g-1 in selected samples of commercial paints locally manufactured in Nigeria, this confirmed the presence of lead in these paints with a high risk to young children. It is therefore necessary to enforce and regulate the permissible level of lead in commercial paints sold in Nigeria to protect and ensure the safety and health of people as lead is known to be dangerous to humans. In an earlier study by [10], the researcher discovered that 96% of commercial paints sold in Nigeria had higher levels of lead which was above recommended limit with levels as high as 14,599 ppm. The researcher observed that the high levels of lead was determined by the color of the paint sold. The study recommended that presence of lead levels in homes need to be investigated and if results are positive, intervention programs should be put in place by the government to eliminate exposure risk and efforts made to create awareness and ensure regulations are enforced which will lead to removal of leaded paint used in homes [10].

Lead in paints eventually find their way into the aquatic environment disrupting the ecosystem and causing harm to aquatic biota. The heavy metal, lead can bio-accumulate in organisms especially Fish, and this happens through specific organs. According to [11] and[12] severe damage to the system can occur in fish and other aquatic organisms after exposure to lead. Fish is known to be sensitive to contaminants because of their ability to easily accumulate pollutants in their cells and organs during exposure [13]. Heavy metals have been known to bioaccumulate in the tissues of fish [14].The aim of this study was therefore to assess the chronic toxicity, bioaccumulation and biochemical responses of two leaded paints commercially sold in Lagos on the African catfish (Clariasgariepinus).

2. Materials and methods

2.1. Collection of Clariasgariepinus and test compounds
One hundred active juveniles (eight weeks old) of Clariasgariepinus were purchased, ad transported in a plastic container, from a fish farm, Premier Fisheries Limited, Ikorodu Local Government Area, in
Lagos. The container contained the pond water gotten from the farm. The fish was transported into an aquarium with a functioning aerator in the environmental biology laboratory in the Department of biological science. The test compounds which included two brands of commercial, commonly sold paints in the Lagos market mostly used by painters (FC paint and V paint). Different colors of the brands were purchased: FC paint: white and orange colors and V paint: yellow and white colors.

2.2. Acclimatization and feeding
The juvenile fish were transferred into plastic containers, containing water that has been previously de-chlorinated for 24 hours. The fish was acclimatized for fourteen days before using for the bioassay study. The fish were fed with fish feed known as coppens feed of 2mm size twice daily. The water was changed every 24 hours to reduce and remove accumulated fecal waste in the water. The fish were not fed a day prior to commencement of the bioassay study, this is to avoid accumulation of faecal matter in the bioassay which can affect the physico-chemical condition of the media.

2.3. Heavy metal analysis
Samples of the 2 different brands of paints in 4 colors were prepared according to standard methods and procedures, for the digestion and analysis of heavy metals [15]. One gram of each of the four samples were weighed using a weighing balance. Measurement of 50ml of nitric acid was taken and poured into a flask containing the paint samples, this was heated using hot plate in the fume cupboard for the digestion process until a clear solution was formed.

2.4. General Bioassay Techniques
Twenty four (20) bioassay containers were used for the bioassay study. Range finding test was conducted using various concentrations of the commercial paints purchased. The formulations of the paint were measured into 1000ml in a flask to achieve a stock solution of known strength, using distilled water. The stock solutions were diluted serially to get a solution of a lower concentration. In order to prepare the test media, a known concentration of the serially diluted stock solution was measured and poured into the container for the bioassay study. Two hundred live juveniles of Clariasgariepinus of similar age and size were distributed randomly into the experimental containers containing de-chlorinated water as described by [16].

2.5. Acute toxicity test
During the bioassay experiment, the fish was distributed into the containers with the paint sample solution and untreated solution. Assessment of mortality was done daily for 96 hours. All dead fishes were removed as soon as they were observed and showed no sign of movement in the containers. The pattern of active movement of the catfish and changes externally were observed in the fish. The Lethal Concentration 50 concentration of C. gariepinus for 96 hours was calculated using the probit analysis using SPSS 14.0

2.6. Chronic toxicity test
Following the acute toxicity test and deriving the 96 hours LC 50 value of all the paint brands, the sub-lethal concentrations were calculated using 1/10th and 1/100th of the 96 hours LC50 value. The fish were exposed to the treated concentrations of the sub-lethal values in replicates and control. The test media was changed every two days to a fresh media and this was done for a period of 30 days.

2.7. Tissue Preparation and Enzyme Assay
At specified periods, the required organs of the fish were removed for lipid peroxidation and other assays. The organs were removed and blood washed off in cold isolation medium (0.25 M sucrose, 5 mMtris HCL), weighed, homogenized and centrifuged as described by [17] and [18]. The supernatant was used for enzyme assays.
Lipid peroxidation analysis of the collected organs were determined according to method of [19]. The mixture was vortexed and the n-butanol layer was centrifuged at 3300 rpm and 25°C for ten minutes. The organic layer was obtained and the absorbance measured spectrophotometrically at 535 nm. Assay of Alanine Transaminase (ALT) activity was determined following the modified method described by [20]. Assay of Aspartate Transaminase (AST) activity was done using the same assay method described for ALT, the only exception was that ALT reagent was replaced with the AST reagent [20]. Determination of the assay for Alkaline Phosphate (ALP) was done according to the method described by [21] and [22]. Determination of Protein concentration was done using büriet method [23] and Bovine Serum Albumin (BSA) as standard.

2.8. Bioaccumulation of Lead in Fish tissues
The digested samples were analyzed for the level of lead using Atomic Absorption Spectroscopy (AAS). The tissue of the exposed and control fishes were also digested and analyzed for lead.

2.9. Statistical Analysis
The toxicity measurement indices were derived from the following:
\[
LC_{50} = \text{Median lethal concentration that causes 50\% response (mortality) of exposed organisms.}
\]
\[
T.F \quad \text{Toxicity factor} = \frac{96\text{h} \text{LC}_{50} \text{ value of other chemical}}{96\text{h} \text{LC}_{50} \text{ value of most toxic chemical}}
\]

3. Results

3.1. Heavy metal in paint samples
The results obtained from the digestion of the paint samples are presented on Table 1 below: The results of the analysis of the 2 commonly sold brands of paint sold in Nigeria showed that all of the paint samples contained lead. The lead content of V paint for each of the colors: white and yellow was Not Detected and 25.11 ppm respectively. The lead content of FC paint for each of the colors: orange and white, red and yellow were 97.78 ppm, and 6.22 ppm respectively. FC paint orange has the highest lead content while white coloured paints have the lowest lead content. It was obvious that lead was found present in all the paint samples except in V paint white color.

| Table 1. Mean concentrations of lead in paint samples |
|--------------------------------------------------|
| Paint brand | Mean concentration of lead (ppm) |
| V paint - white | ND |
| V paint - yellow | 25.11 |
| FC paint - white | 6.22 |
| FC paint - orange | 97.78 |

3.2. Acute and chronic toxicity of leaded paints against Clariasgariepinus.
The acute toxicity experiment showed that the 96 hr LC50 concentration was 16.16 mg/l for orange color and 22.28 mg/l for white color, both of FC paint (Table 2). Yellow and white color of V paint has LC50 value of 17.163 mg/l and 29.144 mg/l respectively. V paint yellow and FC orange were the toxic paints as reflected in the results. The toxicity factor was calculated as shown in Table 2. Considering the lethal concentrations, FC orange with value of 16.163 mg/l was the most toxic compound that was tested against Clariasgariepinus followed by V yellow, FC white and V white. Computed toxicity factor (96hLC50 ratios) showed that FC (Orange) was about 1.37x, 1.80x, 1.06x, more toxic than FC (White), V (White) and V (Yellow) respectively when tested against Clariasgariepinus.
Table 2. Acute toxicity and toxicity factor of two different paints exposed to *Clarias gariepinus*

| Toxicant       | 96 hour LC₅₀ (mg/l) | Toxicity factor |
|----------------|---------------------|-----------------|
| FC (Orange)    | 16.163              | 1.00            |
| FC (White)     | 22.288              | 1.37            |
| V (Yellow)     | 17.163              | 1.06            |
| V (White)      | 29.144              | 1.80            |

3.3. Levels of liver marker enzymes

Levels of liver marker enzymes of *Clarias gariepinus* exposed to different paints at 1/10th and 1/100th value of LC₅₀ are shown in Table 3. At 1/10th concentration of LC₅₀, levels of AST, and ALT in the liver of *Clarias gariepinus* exposed to V yellow paint, were significantly higher than control (Table 3). At 1/10th concentration of LC₅₀, levels of AST, ALP and ALT in the liver of *Clarias gariepinus* exposed to FC orange paint, were significantly different than control. The lowest value of ALT was observed in FC orange paint, lowest value of AST was observed in FC orange. At 1/100th concentration of LC₅₀, levels of AST, ALP and ALT in the liver of *Clarias gariepinus* exposed to V yellow paint, V paint white, FC orange, and FC white were significantly higher than control (Table 3). The lowest value of ALT was observed in V paint white, lowest value of AST was observed in FC orange.

Table 3. Levels of liver marker enzymes of *Clarias gariepinus* exposed to different paints at 1/10th and 1/100th value of LC₅₀

|                | AST            | ALT            | ALP            |
|----------------|----------------|----------------|----------------|
| 1/10th LC₅₀    |                |                |                |
| V paint yellow | 1299.80±14.14  | 47.40±0.57     | 32.30±0.42     |
| V paint white  | 534.90±5.66    | 20.20±1.41     | 24.70±0.99     |
| FC paint orange| 169.40±4.24    | 8.90±0.57      | 25.50±0.71     |
| FC paint white | 1163.50±4.24   | 41.30±1.41     | 48.70±0.99     |
| Control        | 520.30±14.14   | 16.90±0.85     | 34.70±0.28     |
| 1/100th LC₅₀   |                |                |                |
| V paint yellow | 1407.30±2.83   | 63.40±0.57     | 172.60±0.85    |
| V paint white  | 672.20±3.11    | 30.00±0.71     | 21.90±1.27     |
| FC paint orange| 661.30±1.41    | 31.30±0.42     | 21.60±0.85     |
| FC paint white | 920.20±14.14   | 42.50±0.71     | 76.70±0.99     |
| Control        | 520.30±14.14   | 16.90±0.85     | 34.70±0.28     |

a,b,c,d Mean (±Standard deviation) in the same column for 1/10th LC₅₀ and 1/100th LC₅₀ respectively having similar superscripts are not significantly different at p < 0.05.

3.4. Levels of glucose and protein in the liver

At 1/10th LC₅₀, the level of glucose in the liver was significantly higher in the *C. gariepinus* exposed to Value yellow paint (Table 4). This was lowest in the control. On the other hand, the level of protein in the liver of the *C. gariepinus* was significantly higher in the control and lowest in those exposed to all the paints. At 1/100th LC₅₀, level of glucose in the liver of the *C. gariepinus* was highest.
significantly in those exposed to V white paint. Liver glucose level at 1/100th value of LC50 was highest significantly for FC white than in the control. There was however no significant difference in the level of protein in the liver of \textit{C. gariepinus} exposed to the different paints and the control.

Table 4. Percentage (%) composition of glucose and protein in the liver of \textit{Clarias gariepinus} exposed to different paints at 1/10\textsuperscript{th} and 1/100\textsuperscript{th} value of LC50

|           | 1/10\textsuperscript{th} LC50 | 1/100\textsuperscript{th} LC50 |
|-----------|--------------------------------|---------------------------------|
| Glucose   | Protein                        |                                 |
| V paint yellow | 10.27±0.28\textsuperscript{a} | 0.15±0.03\textsuperscript{c}   |
| V paint white   | 6.34±0.06\textsuperscript{b}  | 0.56±0.04\textsuperscript{b}   |
| FC paint orange   | 8.46±0.57\textsuperscript{b}  | 0.56±0.06\textsuperscript{b}   |
| FC paint white   | 6.27±0.10\textsuperscript{b}  | 0.42±0.03\textsuperscript{b}   |
| Control     | 4.15±0.07\textsuperscript{d}  | 0.84±0.06\textsuperscript{a}   |
| V paint yellow   | 5.97±0.14\textsuperscript{b}  | 0.54±0.04\textsuperscript{a}   |
| V paint white   | 9.29±0.28\textsuperscript{a}  | 0.60±0.14\textsuperscript{a}   |
| FC paint orange   | 8.08±0.11\textsuperscript{a}  | 0.88±0.01\textsuperscript{a}   |
| FC paint white   | 6.27±0.10\textsuperscript{b}  | 1.11±0.14\textsuperscript{a}   |
| Control     | 4.15±0.07\textsuperscript{d}  | 0.84±0.06\textsuperscript{a}   |

\textsuperscript{a,b,c,d} Mean (±Standard deviation) in the same column for 1/10 LC50 and 1/100 LC50 respectively having similar superscripts are not significantly different at p < 0.05.

3.5. Levels of lipid peroxidation

There was no significant difference in the level of lipid peroxidation (malondialdehyde – MDA) recorded in the skin of the control \textit{C. gariepinus} and those exposed to the different paints at 1/10th LC50 (Table 5). On the other hand, at 1/10th value of LC50, level of MDA was significantly higher in the gills of \textit{C. gariepinus} exposed to FC white paint. Level of MDA was however not significantly different between the control and those exposed to V yellow paint, V white paint and FC orange paint. At 1/100th value of LC50, the level of lipid peroxidation recorded in the skin and gills of the \textit{C. gariepinus} was not significantly different between the control and those exposed to Value yellow paint, V white paint, FC orange paint and FC white paint.

Table 5. Level of Lipid peroxidation (Malondialdehyde – MDA [µmol/ml]) in the skin and gills of \textit{Clarias gariepinus} exposed to different paints at 1/10\textsuperscript{th} and 1/100\textsuperscript{th} value of LC50

|           | Skin   | Gill   |
|-----------|--------|--------|
| 1/10 LC50 |        |        |
| V paint yellow | 0.35±0.07\textsuperscript{a} | 0.29±0.01\textsuperscript{b} |
| V paint white   | 0.31±0.01\textsuperscript{a} | 0.33±0.04\textsuperscript{b} |
| FC paint orange   | 0.29±0.03\textsuperscript{a} | 0.31±0.01\textsuperscript{b} |
| FC paint white   | 0.17±0.03\textsuperscript{a} | 0.92±0.03\textsuperscript{a} |
| Control     | 0.21±0.01\textsuperscript{a} | 0.23±0.04\textsuperscript{a} |
| 1/100 LC50 |        |        |
| V paint yellow   | 0.56±0.04\textsuperscript{a} | 0.54±0.06\textsuperscript{a} |
| V paint white   | 0.33±0.04\textsuperscript{a} | 0.65±0.07\textsuperscript{a} |
| FC paint orange   | 0.75±0.07\textsuperscript{a} | 0.58±0.01\textsuperscript{a} |
| FC paint white   | 0.50±0.14\textsuperscript{a} | 0.46±0.04\textsuperscript{a} |
| Control     | 0.21±0.01\textsuperscript{a} | 0.23±0.04\textsuperscript{a} |
Mean (±Standard deviation) in the same column for 1/10 LC50 and 1/100 LC50 respectively having similar superscripts are not significantly different at p < 0.05

3.6. Bioaccumulation of Lead in gills and skin of C. gariepinus
The results of the accumulation of lead in the gills and skin of C. gariepinus exposed to different paints at 1/10th and 1/100th values of LC50 were used to calculate the Bioaccumulation Factor (Table 6 & 7). Lead was not detected in the gills and tissues of the control C. gariepinus. However, at 1/10th LC50, lead was significantly higher in the gills of C. gariepinus exposed to V white paint. The level of lead in the gills of C. gariepinus exposed to V yellow, FC white and FC orange were not significantly different. Similarly, at 1/10th LC50, there was no significant difference in the values of lead recorded in the C. gariepinus exposed to the different paints used. At 1/100th LC50, C. gariepinus exposed to FC white paint had significantly higher level of lead in the gills. Level of lead was not significantly different in the gills of C. gariepinus exposed to V yellow, V white and FC orange paints. On the other hand, lead was not detected in the tissue of C. gariepinus exposed to FC white paint. Tissue level of lead was however higher and not significantly different in the C. gariepinus exposed to V yellow and V white paints. This was lowest in the tissue of C. gariepinus exposed to FC orange paint.

Table 6. Bioaccumulation of lead (mg/100g) in the gills and skin of Clariasgariepinus exposed to different paints at 1/10th and 1/100th value of LC50

| Gill       | Skin       |
|------------|------------|
| 1/10 LC50  |            |
| V paint yellow | 0.03±0.00 \(^b\) | 0.11±0.01 \(^a\) |
| V paint white    | 0.29±0.00 \(^a\) | 0.21±0.01 \(^a\) |
| FC paint orange  | 0.08±0.00 \(^b\) | 0.22±0.00 \(^a\) |
| FC paint white   | 0.05±0.00 \(^b\) | 0.16±0.00 \(^a\) |
| Control         | ND         | ND          |
| 1/100 LC50     |            |
| V paint yellow  | 0.08±0.01 \(^b\) | 0.20±0.00 \(^a\) |
| V paint white   | 0.04±0.00 \(^b\) | 0.19±0.01 \(^a\) |
| FC paint orange | 0.08±0.00 \(^b\) | 0.04±0.01 \(^b\) |
| F paint white   | 0.59±0.71 \(^a\) | ND          |
| Control         | ND         | ND          |

4. Discussion
This results show that 3 out 4 of the paint samples under study do not exceed the standard limit of Pb in paint as stipulated in most developed and developing countries which are 90μg/g and 600μg/g. This is in contrast to findings made by [10]. However, this findings indicated that two brand of paints under study contained the element lead and have effects on catfish. A common trend was noticed in the death of the fish that increases as the concentration of the leaded paint that is exposed to C. gariepinus increases. Both FC paint (orange) and V paint (yellow) are more toxic to the fish than the other colors used in this experiment.

At 1/10th value of LC50, the level of glucose in the liver was significantly higher in the C. gariepinus exposed to V paint yellow than in the control which is in agreement with the finding of [24] who found increase in level of glucose in Clariasgariepinus after exposure to lead. On the other
hand, the level of protein in the liver of the *C. gariepinus* was significantly higher in the control than in those exposed to Value yellow paint. At 1/100th value of LC50, there was no significant difference in the level of glucose and protein in the liver of the *C. gariepinus* among the paints.

At 1/10th value of LC50, levels of AST and ALT were significantly higher in the liver of *Clariasgariepinus* exposed to Value yellow paint. These are higher than the one reported by [25]. In a study conducted by [26] they reported an increase in the concentrations of both AST and ALT measured in *C. gariepinus* after exposure to lead, their findings is similar to the findings reported in this study of AST and ALT increase.

The concentration of protein in the gills of exposed *C. gariepinus* had decreased. This decrease in protein content in the fish is in general agreement and the reported the conversion of protein into amino acids residues so as to increase amino acid pool [27]. Proteins are highly sensitive to heavy metals and hence indicators of heavy metals poisoning [28].

Significant difference was not observed in the level of lipid peroxidation (malondialdehyde – MDA) recorded in the skin of the control *C. gariepinus* and those exposed to the different paints at 1/10th value of LC50. The presence of Lipid peroxidation indicates that there is oxidative stress and this is used to measure the level of damage in cells of living organisms [29]. The increase in the MDA of skin and gills exposed to the different color of the two paints is in contrast to the work of [30] that stated that there was a decrease in lipid peroxidation in fish exposed lead. The result of bioaccumulation of lead in fish is in agreement with the findings of [31] who reported accumulation of lead in fish as high as 3.16 ug g⁻¹ collected from a polluted river. The author asserted that there are three areas in a fish body where heavy metals can accumulate, which are the gills, digestive tract and skin. Consuming such fish that have accumulated heavy metals from different polluted sources including leaded paint may exert toxic effects in humans. This will also have serious ecological impact on the reproductive sustainability of fish species in such contaminated waters, as these leaded particles eventually find their way into the aquatic environment.

5. Conclusion
This study has confirmed the presence of lead in paints sold in commercial markets in Lagos Nigeria. Indiscriminate disposal of these leaded paints in the environment can result in the accumulation of lead in the tissues of fish as shown in this present study. Consumption of these contaminated fish is a serious health concern. Effective measures should be put in place to regulate the level of lead in paints sold and manufactured in Nigeria.

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