

Depuration Technique of Xenobiotics with Reference to Accumulation and Elimination of *Paraquat Dichloride* in *Clarias Gariepinus*

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ABSTRACT

Background: *Paraquat dichloride* is a highly toxic herbicide which is still used in many developing countries. African cat fish (*Clarias gariepinus*) is a commercially important species in many countries and was selected assess accumulation and elimination of *paraquat dichloride* in its tissues.

Methods: Groups of ten fish with equal lengths and weights were exposed to varying concentrations of *Paraquat dichloride* for 28 days. After the exposure, the fish were transferred to uncontaminated water. Two fish were sampled for pesticide residue at the end of exposure period (28days) and 1, 7 and 14 days post exposure.

Results: In pesticide treated fish, the accumulation of *paraquat* increased with increases in the concentration of the toxicant and varied significantly between the treatments ($p < 0.05$). The herbicide depurated gradually with cessation of exposure and no pesticide was observed after 14 days.

Conclusion: Xenobiotics could be eliminated from aquatic organisms especially fishes and could be put into practice in areas at risk of pollutants. This novel approach can reduce the risks of biomagnification of poisons in sea food.

Keywords: *Clarias Gariepinus*, *Paraquat Dichloride*, Pesticides, Pollutants.

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INTRODUCTION

Paraquat dichloride is a highly toxic herbicide to fish and other aquatic organisms. In pure form, it exists as colorless crystals, but the technical product is composed of brownish crystals with a slight odour of sulphur dioxide. Technical grade paraquat is a mixture of two isomers - alpha-paraquat and beta-paraquat in a ratio of 7:3. This product contains 94% alpha-paraquat and beta-paraquat and other related compounds like paraquat alcohol, paraquat ether and paraquat sulphate

This herbicide does not dissolve easily in water but easily attaches to soil particles floating in the water or to soil at the bottom [1]. The small amounts of paraquat that dissolve in water breaks down over time and depending on the conditions in the water, it might take a day to several months. Some paraquat in surface water evaporates into air. Because it does not dissolve easily in water, only very small amount of paraquat is found in groundwater [1].

Evidences of threats to human health posed by pesticides especially paraquat dichloride are abundant and it has been banned or severely restricted in over 30 countries [2]. Safe application, however, cannot be guaranteed in developing countries where the chemical is still widely used. It is often applied to crops using sprayers and some may travel long distances in the air before it lands on crops, soil, or water. Paraquat on crops usually disintegrates within a few weeks. Paraquat found near hazardous waste sites is usually found in soil. Some of paraquat in soil evaporates and some breaks down in soil. However, it may stay in soil for several years before total decomposition.

Fish are relatively sensitive to changes in their environment and have a relatively long life span compared to other aquatic organisms. Therefore, they are good indicators of general health status of specific habitats or aquatic environments. The Africa cat fish, *C. gariepinus* was selected as the test organisms in this study, because this fresh

water fish is one of commercially important species for rapid aquaculture expansion in Nigeria and elsewhere in developing world [3]. *C.gariepinus* is popular as both aquarium and food fishes and is widely distributed all over the world. In West Africa, natural distribution covers the basins of the Niger, Benue, Volta, Gambia, Senegal, Nigeria and Chad. This fish inhabits rivers and their tributaries, lakes, estuaries and large ponds and can survive in salinity of up to 35%. The lower and upper lethal temperatures are between 12°C to 42°C. Spawning is induced at temperature of 22°C to 24°C. The male of this species grows 2 to 5 times faster than the female. It is omnivorous in diet, feeding on algae, diatom, insect larvae, fish eggs, fry of fish and detritus. This species is tolerant to extreme environmental conditions including water pH range of 6.5 – 8.0 and depth range 4-80cm [4]. Thus, we selected Africa cat fish to assess accumulation and elimination of paraquat dichloride in its tissues.

MATERIAL AND METHODS

The test was conducted under Organization for Economic Cooperation and Development (OECD) test guideline 407 [5]. One hundred and fifty juvenile *C. gariepinus* of mean weight (26.22 ± 0.1 gm) and length (15.20 ± 0.13 cm) were collected from the Department of Fisheries, Faculty of Agriculture, University of Benin, Benin City, Nigeria. They were acclimatized to laboratory conditions in glass tanks containing deionized water for two weeks before they were used for the experiments. The holding tanks were aerated with air pumps. They were cleaned and the water was renewed daily. Fish were fed 30% protein pellets, unconsumed feed and faecal wastes were removed and water was replenished regularly as recommended by [6].

The physicochemical parameters of the test media were obtained from the natural environment in the tropical rainforest. Deionized water was used for preparing test solutions. Stock solutions were prepared by dissolving 0.1ml Technical grade paraquat (94% alpha-paraquat and beta-paraquat) in 1 liter of deionized water. The concentrations of paraquat for the test were prepared from the stock solution via serial dilution. Stock and

test water concentrations were verified by Cecil HPLC system comprised of CE 1200 high performance variable wavelength monitor and CEII00 liquid chromatography pump and high-resolution gas chromatography (HRGC), using a Hewlett-Packard 5890 capillary gas chromatograph (Hewlett-Packard, Avondale, PA, USA) equipped with an electron capture detector (Hewlett-Packard).

The fish were exposed to the concentrations observed in the field. Groups of 10 fish with equal lengths and weights were exposed to different concentrations for 28 days at fixed times every day. During which freshly prepared test solutions were added on regular basis to maintain the concentration level after the waste have been siphoned out. The tanks were cleaned daily. The water was changed thrice weekly and aerated with air pump. Fish and water quality parameters (pH, temperature, dissolved oxygen) of the test solution were monitored throughout the experiment. Two fish were sampled for pesticide residue at the end of exposure period (28days) and at 1, 7 and 14 days post-exposure.

Chemical Analysis

Paraquat dichloride (97.5 % purity) and methanol (analytical grade) for high-performance liquid chromatography (HPLC) were obtained from Chemical Service (West Chester, PA, USA). High purity pesticide grade solvents (hexane, dichloromethane and the surrogate standard solution) were obtained from Merck (Darmstadt, Germany). Petroleum ether (analytical grade), Na₂SO₄ (99% purity), and NaOH (analytical grade) were supplied by Sigma-Aldrich (USA) and helium (purity 99.999 %) by Messer Techno gas (Czech Republic). Equipment included glassware, Cecil HPLC system comprised of CE 1200 high performance variable wavelength monitor and CEII00 liquid chromatography pump, UV detector with variable wavelength and stainless steel column (C18 Reverse phase) packed with Octasilica, vacuum pump, and ultrasonic check.

The samples were processed as described by Anastassiades M. et al. [7]

modified after Steinwandter [8]. In brief, samples were kept in iceboxes containing wet ice during transportation and later stored in a refrigerator at -40°C prior to solvent-solvent extraction method. Fifty (50) grams of the raw fish's fillet was weighed out, homogenized, and froze dried until ready for extraction. Twenty grams of the frozen dried tissue was transferred into a separatory funnel and its pH was measured. Fifty ml of 0.2 M sodium sulphate buffer was added to the sample, and pH was adjusted to 7 by adding drops of 0.1 N sodium hydroxide and HCL solutions. The neutralized sample was treated with 100 g sodium chloride to salt out the pesticides from the aqueous phase. Then 60ml triple distilled dichloromethane was added and shaken for two minutes while releasing pressure. The sample was allowed to settle for 30 minutes to enhance separation of the phases. The organic layer was collected in a 250 ml Erlenmeyer flask and stored at -40°C in a refrigerator. The extractions were repeated twice using 60 ml portions of dichloromethane and the extracts were combined and cleaned by passing through a Florisil column. The clean extracts were concentrated on a rotary evaporator to near dryness and reconstituted in HPLC hexane to 5 ml. The final samples were analyzed using Cecil HPLC system comprised of CE 1200 high performance variable wavelength monitor and CEII00 liquid chromatography pump and high-resolution gas chromatography (HRGC), using a Hewlett-Packard 5890 capillary gas chromatograph (Hewlett-Packard, Avondale, PA, USA) equipped with an electron capture detector (Hewlett-Packard). Quality control and quality assurance procedures included

replicate sampling, extraction and analysis for all samples

Statistical Analysis

Student's t-test and one-way analysis of variance (ANOVA) was employed to calculate the significance of the differences between control and experimental means and within various treatments using SPSS (14.0 version), SPSS Inc, Chicago, USA. P values of 0.05 or less were considered statistically significant [9]. Multiple line graphs were also used in this study for the pictorial representation of assessment endpoints.

RESULTS

Physicochemical Properties of the Test Media

The water quality parameters (pH, temperature, dissolved oxygen, turbidity and hardness) monitored during the exposure periods were not significantly different between various concentrations of the pesticide and the control. The parameters were almost uniform all through the study irrespective of the treatment (Table 1).

Bioaccumulation and Depuration

The results of bioaccumulation and depuration of the *paraquat dichloride* in *C. gariepinus* are shown in Figure 1 and 2 respectively. No residue was observed in the control group. The herbicide bioaccumulated in the treated fish irrespective of the concentrations and exposure durations. The accumulated residues varied significantly ($p < 0.05$) between various treatments and within the same concentrations at different time intervals.

Table1. Concentrations of physiochemical parameters of the test media of *C.gariepinus* exposed to different concentrations of *paraquat dichloride*.

Parameter	Ph	Temp.($^{\circ}\text{C}$)	DO(mg/l)	Turbidity (mg/L)	Alkalinity (mg/L)	Hardness(mg/L)
Con.($\mu\text{ g/l}$)	Mean \pm SD	Mean \pm SD	Mean \pm SD	Mean \pm SD	Mean \pm SD	Mean \pm SD
Control	7.20 \pm 0.00	26.67 \pm 1.15	8.12 \pm 0.10	0.23 \pm 0.04	17.40 \pm 0.72	31.33 \pm 1.15
2	7.27 \pm 0.25	27.00 \pm 1.00	8.16 \pm 0.09	0.23 \pm 0.01	17.33 \pm 0.42	31.33 \pm 1.15
4	7.07 \pm 0.12	26.33 \pm 0.58	8.34 \pm 0.11	0.23 \pm 0.02	17.30 \pm 0.36	31.00 \pm 1.00
6	7.17 \pm 0.29	27.00 \pm 1.00	8.73 \pm 0.12	0.25 \pm 0.02	17.30 \pm 0.81	30.67 \pm 0.58
8	7.16 \pm 0.25	26.63 \pm 0.58	8.13 \pm 0.12	0.26 \pm 0.03	17.73 \pm 0.12	30.67 \pm 1.15

The highest bioaccumulation of 9.1 $\mu\text{g/gdw}$ was recorded at the highest concentration of 8 $\mu\text{g/L}$, while the least concentration of 1.62 $\mu\text{g/gdw}$ was observed at the lowest concentration of 2 $\mu\text{g/L}$. Hence, accumulation increased with rising concentrations of the toxicant.

The depuration and elimination process was time dependent. The pesticide was eliminated from the fish tissues after 14 days in the pesticide free water and there was no significant difference in depuration between the different treatments and within the treatments at various time intervals.

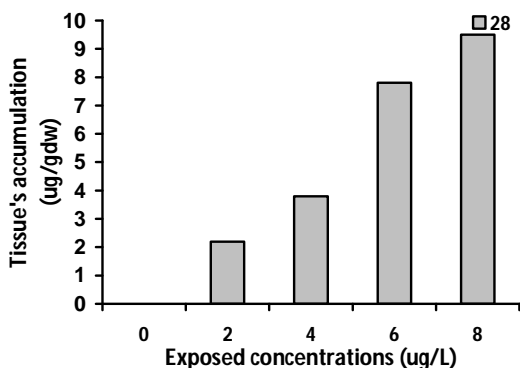


Figure 1. Bioaccumulation of *Paraquat dichloride* ($\mu\text{g/gdw}$) in *C. gariepinus* at 28 days exposure to different concentrations of the toxicant.

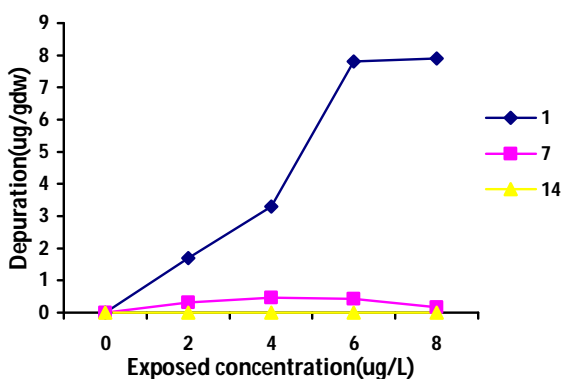


Figure 2. Depuration and elimination of *Paraquat dichloride* in *C. gariepinus* ($\mu\text{g/gdw}$) after 1, 7 and 14 days of cessation of exposure to different concentrations of the toxicant.

DISCUSSION

The nonsignificant changes in the water parameters of various experimental media reported in this study showed that sublethal concentrations of *paraquat* did not adversely lead to reduction in water quality where slight changes were observed. The physicochemical parameters were almost uniform in all the treatments. It is evident that physical and chemical properties of aquarium water were within the desirable range for fish culture.

The temperature range was 26.33 - 26.67°C, which is generally the temperature trend in water bodies in the tropical forest areas [10]. The recommended limit of dissolved oxygen (DO) for fish is 5mg/l [11], but conversely, Alabaster JS and Lloyd R [12] recommended a range of 2mg/l - 7mg/l. The range of DO was within the tolerable limits. The pH in this study 7.00 - 7.23 was within the acceptable pH range for drinking water which is between 6.5 - 8.5 [10], although WHO [13] has recommended 6.0 - 7.0. Total alkalinity showed a positive index of potential productivity for the water at 31.93 - 35.90 for fresh water species [10], which was within the range observed during this study.

The use of bioaccumulation and depuration to envisage in vivo response and recovery from contaminants has not been seriously considered in toxicology. Until now little is known about recovery time for complete depuration of xenobiotics in fishes and other aquatic organisms, especially the benthics under natural circumstances. It should be noted that the herbicide was completely eliminated after 14 days in herbicide free water. Also during this investigation, no mortality and no morphological changes were observed in the exposed fish.

Paraquat dichloride is known to bioaccumulate in the tissues of fish and other aquatic organisms and the estimates of tissue concentrations may be more valuable for the assessment of the situation of pesticides in natural environment [14, 15]. The European Union has banned import of fish from Tanzania, Uganda and Kenya due to high levels of *paraquat* residues [16]. Its Residues together with other chlorinated hydrocarbons

were also detected in animal samples from Benin, Nigeria, Cote d' Ivoire, Madagascar, South Africa, Kenya [17] and China [18].

The present toxicological experiment revealed that *Paraquat dichloride* has strong affinity to accumulate in the fish tissues. The higher the concentrations of the toxicant, the more the residual level in the fish's tissues, as observed after 28 days exposure. However when the fish were taken into pesticide free aquaria, residual level decreased which was proportional to the duration. The depuration of the pesticide was faster at higher concentrations and was eliminated after 14 days in pesticide free aquaria, irrespective of the treatments. This showed that the induction of the detoxifying enzymes is proportional to the concentrations of the toxicant in the fish. The higher the concentrations of xenobiotics, the higher the tendency to induce defense mechanisms. Enzymes play critical roles in the depuration and elimination of toxicant [19, 20]. The organs in the visceral region carry out the primary activities related to absorption, distribution and elimination. The enzymes involved mainly are cytochromes P-450s, glutathione-S-transferases, rhodanese, sulfotransferase, and other enzymes mainly belonging to mono-oxygenase system [21]. Our findings was in conformity with the observations of Canli M. and Mansouri B. et al. [22, 23]. Similar observations like high accumulation and depuration rates in fish were also noticed when fish, *Labeo rohita* and *Saccobrachus fossilis* were exposed to metasyntox; *Mugil cephalus* and *Mystus gulio* to *paraquat*, and *Clarias batrachus* in sub-lethal concentrations of dimethoate [24]. Also, Gundogdu A. et al. [25] observed that dimethoate bioaccumulated in liver and muscle tissues of *Clarias batrachus* and was eliminated following cessation of exposure.

CONCLUSION

Clarias gariepinus, appear to be very sensitive to waterborne *paraquat* exposure. Information regarding the effects of oral exposure to fish is very limited. Studies on farmed fish are needed in order to give appropriate safety recommendations for *paraquat* in fish feeds as well as the limit

needed to protect the fish in the wild. It is therefore imperative that efforts should be made to study the levels of pesticides in our natural bodies of water in order to provide baseline data, for formulating regulations and appropriate management options. This study has shown that *paraquat* is easily accumulated in fish and can be eliminated. It is hereby recommended that fish farmers in areas susceptible to environmental contamination, should expose the fish to water in a pond sited in a serene environment that is entirely free from contaminants before they are sold to the public for consumption.

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