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Concentrations and Bioconcentration Factors of Heavy Metals in Tissues of *Heteroclaris* juveniles exposed to Dye Effluents Mixed with Orange Peel

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ABSTRACT: The ability of metals in dye effluents to persist in the water bodies and in tissues could induce oxidative stress in fish. This paper therefore, investigate the concentrations and bio-concentration factors of heavy metals (Fe, Zn, Cu, Mn, Ni, Pb, Cr) in tissues of *Heteroclaris* juveniles exposed to dye effluents fed orange peel (0, 5 and 10%) using appropriate standard methods. Highest concentrations of the heavy metals were recorded in tissues of fish exposed to dye effluent fed 0% orange peel, while decrease in the concentration of the metals were shown in group of fish exposed to dye effluent fed on 5% orange peel. The most highly ($P < 0.05$) concentrated metal is Fe (16.986 ± 0.023) in the liver, while the least ($P < 0.05$) concentrated metal is Cr (0.128 ± 0.002) in the blood of dye-effluent-exposed fish fed 0%. However, all the concentrated metals in the tissues were all above the maximum permissible limits of World Health Organization standard, except in the blood and muscle with 0.128 of Cr and 0.272 of Pb. Dye-effluent-exposed fish showed highest bio concentration factors of 16.986, 12.102, 10.810, 9.798, 8.700 in liver, blood, gill, kidney and GIT respectively and 9.001 in muscle. This indicates that, metals in dye effluent bio concentrated in tissues of *Heteroclaris*, but 5% orange peel neutralized the metals. Dye effluent should therefore, be treated before discharged into water bodies.

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Increase in urbanization and industrialization has led to a tremendous increase in discharge of a wide diversity of pollutants into the water bodies and has caused undesirable effects on the different components of the aquatic environment and on fisheries (Gita *et al.*, 2017). Despite the economic benefits derived from this sector, uncontrolled discharge of untreated effluents from the industries, most especially the textile industries is alarming as a result of the large amount of water used during the processing of dyeing. The large volume of coloured water release into the aquatic environment caused

chemical pollution due to addition of different types of chemicals and dyes into the processing water (Gosavi and Sharma, 2014). The colours in the dye effluents make the water in the aquatic habitats turbid, thereby preventing sunlight energy from penetration, which affect the photosynthesis process, hence, reduction in the dissolved oxygen. Depletion of oxygen therefore, could lead to respiratory distress and hyperactivity and may eventually induce oxidative stress (Vafaei *et al.*, 2013). The low biocompatibility and un-biodegradability of most textile dyes such as Azo dyes make them persist in

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the aquatic environment. Increase in the use of dyes and their indiscriminate release of their effluents from the industries into the water bodies therefore prone major concern on the potential adverse on the aquatic lives and man through food chain. Toxicity of dye effluents is in relation to the colour, non-bio-degradability and presence of metals. When dye effluents are released into the water bodies, the harboring metals in the dye components are the primary source of toxicity, which are discharged and get bio-accumulated in the water bodies and eventually tissues of aquatic animals (Karthikeyan *et al.*, 2007 and Gosavi and Sharma, 2014). The ability of metals to persist and bioaccumulate in the tissues could cause damage to the tissues and eventually interrupt the functions of the affected tissues, most especially the gills, been the first ambient tissue e that has direct contact to any contaminants in the aquatic environment. This could alter the physiology and metabolic activities of the fish that may eventually lead to fish death and reduction in fish species diversity. The quality of the survived ones may be affected due to accumulation of these metals in the tissues and make fish unfit for human consumption. This could lead to loss of biodiversity that can have significant human health consequences since most of the world's population is dependent upon aquatic animals for food (Vitek *et al.*, 2007). Fish are used in ecotoxicological assessment because of their link in food chain, which is an important product in the fishery activities, that serve as the cheapest source of animal protein and is therefore important in the diet of the lowest income group (Alisson, 2001). It is highly nutritious, tasty and easily digested with a significant amount of all essential amino acids, which can be used to improve the overall protein quality of a mixed diet (FAO, 2005). "Heteroclaris" is an interspecific hybrid of *Clarias gariepinus* and *Heterobranchus bidorsalis* with a desired fast growth and are able to survive in poorly oxygenated water (Kori-Siakpere *et al.*, 2006). To protect the lives of these aquatic organisms, there is therefore the needs to investigate the adverse effects of the bioaccumulation and of the metals contained in the dye effluents and pave a way of managing and rescuing the fish from getting destroyed by the adverse effects of these pollutants. Several researches have been carried out and reported on the accumulation of different heavy metals in dye effluents such as cadmium, lead, copper, nickel, cobalt, chromium, arsenic, mercury, selenium, zinc, mercury, tin in various types of tissues (skin, gills, liver, spleen, and kidneys) of different freshwater fish (Vitek *et al.*, 2007 and Fabio *et al.*, 2015). However, there is sparse information of the management of the damages done to these tissues of fish by the

bioaccumulated metals from the dye effluents when released into the water bodies. Therefore the objective of this paper is to investigate the concentrations and bioconcentration factors of heavy metals (Fe, Zn, Cu, Mn, Ni, Pb, Cr) in tissues of *Heteroclaris* juveniles exposed to exposed to dye effluents mixed with orange peel.

MATERIALS AND METHODS

Experimental Setup: Juveniles of *Heteroclaris*, (mean weight of 19.00 ± 0.8 g and a standard length of 14.5 ± 3.0 cm) were obtained from a commercial fish farm in Ilorin Kwara State, Nigeria and transported in a well-aerated plastic-aquarium with the hatchery water in the early hours of the day to prevent stress that could lead to mortality, to the Laboratory in the Department of Zoology, Faculty of Life Sciences, University of Ilorin (Abdulkareem *et al.*, 2021). The fish were acclimatized in the Department of Zoology Laboratory for 14 days (USEPA, 1996) not fed prior to and after transportation to avoid indigestion. The pond water was partly replaced by an aerated chlorinefree borehole water under room temperature of 24°C. The water was completely renewed after 24 hours and unconsumed feed and fecal wastes were removed to prevent pollution, feeding commenced with commercial feeds (Skretting 2mm of 35% crude protein) at 4% of initial body weight twice daily (APHA, 2005). Water quality parameters such as pH, temperature, turbidity, total dissolve solids, biological oxygen demand, chemical oxygen demand, conductivity and dissolved oxygen were all monitored throughout the experimental period were measured following the standard method of water assessment by APHA (2005). The toxicant (Dye effluent) was obtained from a local textile industry in Ilorin, Kwara State, Nigeria. At the end of thirty day of exposure to dye effluent, the fish were sacrifice and organs were removed for metal bioaccumulation assay. Following the result of the acute toxicity, fish was exposed to sub-lethal concentration Dye effluents ($1/10$ of the calculated LC_{50}) and fed on diet supplemented with varying percentages of orange peel (0, 5 and 10%) to evaluate the ameliorative potentials of orange peel on Dye effluents-exposed fish. The orange peel (ameliorative agent) were thoroughly washed, air-dried, ground and incorporated in to three experimental diets at the following inclusion levels; 0 (control), 5, 10% respectively. The experiment was set into six (6) different groups (A, B, C, D, E F) in triplicates with eighteen plastic aquaria containing 10 litres of aerated dechlorinated borehole water with ten (10) fish each. Three groups (A, C and F) were not exposed to Dye effluent and fed on diet with varying

percentage (0, 5 and 10%) supplementation of orange peel respectively, while the remaining three groups (B, D and E) were exposed to one-tenth (1/10) of the calculated LC₅₀ value (76 ppm) of Dye effluent and fed on diet with varying percentage (0, 5 and 10%) inclusion of orange peel respectively. The renewal of the toxicant was done every 24 h to maintain the toxicity of the toxicant and the fish were fed twice daily.

Feed Formulation: The experimental diets were formulated into three different groups with the following ingredients: maize corn (30%), soya bean (20%), groundnut cake (18%), fish meal (30%), bone meal (0.4%), vitamin C (0.2%), methionine (0.4%), lysine (0.4%), salt (0.2%), premix (0.2%), orange peel (0%) which formed 100% balanced diet for the first group, while the major feedstuffs (Maize corn, soya bean, orange peel, groundnut cake and fish meal) were supplemented with 5% and 10% orange peel for second and third groups that formed the 100% balanced diet. The feedstuffs were finely ground, mixed, pelleted through a mincer of 2 mm die, air-dried and packed in airtight polythene bags prior use (Abdulkareem *et al.*, 2021). The oranges were purchased from a local market in Ilorin, Kwara State, Nigeria and peeled to remove the outer shell; sun-dried for 2 weeks and pounded with a pestle in a mortar to give the powdery form. The phytochemical tests of the peel was conducted using standard procedures to identify the constituent as de

Analysis of metal Concentration: Tissue samples such as blood, gill, kidney, liver, gastro intestinal tract (GIT), muscle, and were removed from the control and Dye effluent-exposed fish after 30 days. The tissues were put into Kjeldahl flask for digestion following the procedures adopted by Yi *et al.* (2011) and Ali *et al.* (2016). The digested tissues were diluted with de-ionized water and analyzed for metals concentrations using Perkin-Elmer (Model 2380) atomic absorption spectrophotometer subjected

Calculation of Bio-concentration factor (BCF): The bio concentration factor is a ratio and could be evaluated using the expression in equations 1 and 2.

$$CM_{mg/Kg} = \frac{C_{MW(mg/L)} * V_{Dig(L)}}{S_{wt}(Kg)} \quad (1)$$

Where $CM_{mg/Kg}$ = Concentration of metal in mg/Kg; C_{MW} = concentration of metal in water (mg/L); V_{Dig} = final volume of digested sample (L); S_{wt} = weight of sample in Kg

Hence, the BCF is presented in equation 2.

$$BCF = \frac{C_{MT(mg/Kg)}}{C_{MW(mg/Kg)}} \quad (2)$$

Where C_{MT} = concentration of metal in tissue (mg/Kg); C_{MW} = converted concentration of metal in water (mg/Kg)

Statistical Analysis: The data were analyzed with IBM SPSS version 20 Programme using analysis of variance (ANOVA) and Duncan's (1995) Multiple Range Test (DMRT) to test for the different treatment levels and to separate means. Means were presented as $\pm$ Standard error (SE).

RESULT AND DISCUSSION

Table 1 shows the content of metals in the blood of *Heteroclaris* exposed to dye effluents and ameliorated for the period of 30 days. There are little metal concentrations in the blood of control fish but the blood of *Heteroclaris* exposed to dye effluents has greater concentration of metals. Concentration of Fe (12.102) in the blood was the highest, followed by the Zn (11.490) among the metals, while the least concentrated metal was Cr (0.128). The content of these metals reduced in fish ameliorated with 5% and 10% orange peel. The order of accumulation of metals are $Fe > Zn > Cu > Mn > Ni > Pb > Cr$. Fe is the most accumulated metal and Cr is the least accumulated metal in the blood. Highest contents of metals were recorded in the tissues of *Heteroclaris* exposed to dye effluents only, with reduction in the contents of the metals in the groups ameliorated with orange peel after 30 days. The highest concentration of metals were recorded in group B that were exposed to dye effluents only compared to control groups A (0%), and ameliorated groups C and E fed on diet 5% and 10% orange peel only without any dye effluent. However, the concentration of metals in group D exposed to dye effluents but ameliorated with 5% orange peel recorded a decrease in the contents of metal in the gills. The trend of occurrence of metals in the gills of dye effluent-exposed-fish was $Fe > Cr > Mn > Zn > Cu > Pb > Ni$. Fe is the most accumulated metal while Ni is the least accumulated metal (Table 1). Accumulation of these metals in the gills of *Heteroclaris* exposed to dye effluents could be attributed to the direct adsorption of the dye effluents on the gill filaments or penetration of dyestuff across the gill membrane being the first tissue that has contact with the contaminant (Hao *et al.* 2013). A great increase in the levels of metals in the gills of Dye effluents-exposed fish conforms with the findings of Babatunde *et al.* (2012), Bawuro *et al.* (2018) and Ezeonyejiaku *et al.* (2014). The highest concentration of metals were recorded in group F that

were exposed to dye effluents and fed on 10% orange peel when compared to control groups A, C and E fed on diet 0%, 5% and 10% orange peel only without any dye effluent. However, the concentration of metals in group D exposed to dye effluents but ameliorated with 5% orange peel recorded a decrease

in the contents of metal in their kidney. The order of accumulation of metals in the kidney of dye exposed fish was Fe > Zn > Cu > Cr > Mn > Ni > Pb. Fe is the most accumulated metal while Pb is the least accumulated metal (Table 1).

Table 1: Concentration of metals in the tissues of *Heteroclaris* exposed to Dye effluent and ameliorated with orange peel for 30 days

Tissues	Group	Metals (mg/g)						
		Cu	Pb	Zn	Fe	Ni	Mn	Cr
Blood	A	1.398±0.002	0.105±0.001	1.916±0.002	1.599±0.002	0.522±0.003	0.009±0.002	0.312±0.003
	B	11.008±0.002	1.010±0.001	11.490±0.013	12.102±0.021	1.408±0.002	3.966±0.002	0.128±0.002
	C	1.979±0.001	0.241±0.001	1.578±0.002	1.981±0.002	0.328±0.001	0.192±0.002	0.321±0.004
	D	1.968±0.012	0.288±0.002	1.226±0.004	1.804±0.005	0.596±0.026	0.997±0.051	0.288±0.014
	E	12.001±0.002	0.933±0.003	11.983±0.008	11.908±0.002	1.978±0.007	4.198±0.002	0.401±0.003
	F	11.091±0.006	0.948±0.002	11.705±0.025	11.844±0.037	1.827±0.028	3.978±0.012	0.306±0.027
Gill	A	0.966±0.002	0.223±0.002	0.488±0.001	0.846±0.001	0.478±0.034	0.099±0.021	0.924±0.014
	B	1.930±0.001	1.788±0.003	5.902±0.002	10.81±0.003	0.982±0.015	6.471±0.013	7.631±0.018
	C	0.909±0.008	0.059±0.001	0.487±0.003	0.710±0.001	0.522±0.007	0.995±0.003	0.012±0.006
	D	0.642±0.021	0.218±0.020	4.998±0.002	0.989±0.100	0.299±0.002	0.211±0.006	0.096±0.002
	E	0.999±0.011	1.427±0.021	4.823±0.006	9.969±0.002	0.803±0.003	6.113±0.002	7.019±0.001
	F	1.926±0.002	1.536±0.001	0.194±0.006	10.03±0.002	0.899±0.004	6.405±0.007	7.097±0.009
Kidney	A	1.421±0.001	0.108±0.001	1.887±0.002	1.610±0.002	0.413±0.007	0.699±0.003	0.009±0.002
	B	5.225±0.004	0.563±0.001	8.423±0.001	9.798±0.006	2.669±0.002	2.918±0.001	3.982±0.001
	C	1.009±0.001	0.227±0.002	2.921±0.003	1.910±0.002	0.009±0.001	0.008±0.002	0.632±0.004
	D	2.098±0.002	0.279±0.012	3.114±0.004	0.096±0.001	2.977±0.002	0.996±0.001	0.889±0.004
	E	6.117±0.008	0.892±0.002	8.667±0.002	8.001±0.002	2.925±0.007	3.099±0.001	4.004±0.002
	F	5.297±0.001	0.808±0.012	8.009±0.007	8.045±0.015	0.009±0.026	3.114±0.020	3.981±0.026
WHO/FAO Standard (1996)mg/kg		5.000	0.300	30.000	2.000	0.600	1.000	1.000

Kidney is another target organ by metals because the kidney helps in the excretion of metals through bonding of metals to sequestering proteins such as metallothionein (Heath, 1987).

The liver of the control group-A recorded no metals content, while those exposed to dye effluents (Groups B, D & E) show higher contents of metals. However, the contents of metals in the fish of group D exposed to dye effluents and fed 5% orange peel shows great decrease in the contents of the metals with little reduction in group E fed on 10% orange peel. The concentration of metals decreased in the following sequence Fe > Cu > Zn > Ni > Mn > Cr > Pb. Fe is the most accumulated metal and Pb is the least accumulated metal in liver at the end of 30 days of exposure to Dye effluent (Table 2). In addition, high accumulation of the metals in the liver could be because of the roles of the liver in chemical assimilation, excretion and detoxification. The highest concentration of metals were recorded in group F that were exposed to dye effluents and 10% orange peel when compared to control groups A, C and E fed on diet 0%, 5% and 10% orange peel only without any dye effluent. However, the concentration of metals in group D exposed to dye effluents but ameliorated with 5% orange peel recorded a decrease in the contents of metal in their GIT. Fe is the most

concentrated metal in the GIT of the fish in group B, which recorded the highest metal concentration when compared to control groups while those of fish in group D exposed to Dye effluent and fed on 5% orange peel recorded a reduction in the concentration of metals. The order of accumulation of metals in the GIT of Dye effluent-exposed fish was Fe > Cu > Zn > Mn > Cr > Pb > Ni. Fe is the most accumulated metal while Ni is the least accumulated metal in the GIT (Table 2). Ability of the mucosa layer of the intestine to hold metals could also contribute to high metal accumulation in the GIT (Clearwater *et al.*, 2002), this result is similar report of Ishaq *et al.* (2011). There are little metal concentrations in the muscle of control fish but the muscle of *Heteroclaris* exposed to dye effluents accumulated greater amount of metals. Concentration of Cu (9.001) in the blood was the highest, followed by the Fe (8.2001) among the metals, while the least concentrated metal was Pb (0.272). The content of the metal reduced in fish ameliorated with 5% and 10% orange peel. The order of metals accumulation in the muscle is as Cu > Fe > Zn > Cr > Mn > Ni > Pb. Cu is the most accumulated metal and Pb is the least accumulated metal (Table 2). The concentration of Cu in the gills was below the FAO/WHO (1993) maximum permissible limit in fish tissues while the level of Cu in the liver, kidney, GIT, blood and

muscle of dye effluent-exposed fish was above the permissible limit in tissues of fish. The concentration of Pb in the gills, liver, kidney, blood, GIT and muscle was above the permissible limits in the tissues after 30 days of exposure to dye effluents. The level

of Zn in the gills, liver, kidney, blood, GIT and muscle of *Heteroclarias* exposed to dye effluent for 30 days was all below the maximum permissible limits of Zn in fish tissues.

Table 2: Concentration of metals in the tissues of *Heteroclarias* exposed to Dye effluent and ameliorated with orange peel for 30 days

Tissues	Group	Metals (mg/g)						
		Cu	Pb	Zn	Fe	Ni	Mn	Cr
Liver	A	0.9961±0.005	0.088±0.003	1.811±0.002	0.891±0.001	0.009±0.021	0.443±0.013	0.396±0.032
	B	8.068±0.003	0.812±0.002	5.0001±0.023	16.986±0.001	4.667±0.001	2.725±0.003	2.462±0.004
	C	0.998±0.002	0.088±0.002	0.300±0.001	0.790±0.001	0.399±0.004	0.441±0.001	0.296±0.006
	D	0.0406±0.003	0.096±0.001	1.913±0.024	0.047±0.021	0.118±0.001	0.169±0.001	0.209±0.003
	E	7.9091±0.002	0.789±0.001	4.811±0.003	16.045±0.002	4.098±0.007	2.661±0.002	3.171±0.001
	F	8.061±0.001	0.398±0.001	4.914±0.021	16.705±0.014	4.662±0.003	2.701±0.001	3.462±0.004
GIT	A	2.400±0.001	0.248±0.023	2.004±0.001	1.042±0.025	0.286±0.006	0.234±0.002	0.237±0.0001
	B	7.223±0.006	0.814±0.020	5.092±0.002	8.7001±0.015	0.692±0.041	3.882±0.002	1.116±0.0001
	C	1.482±0.003	0.136±0.002	1.366±0.012	0.013±0.025	0.266±0.023	0.888±0.001	0.117±0.0003
	D	1.008±0.005	0.211±0.001	1.009±0.002	0.8524±0.003	0.391±0.007	0.112±0.002	0.036±0.0023
	E	6.493±0.009	0.883±0.001	5.189±0.023	8.9401±0.002	0.701±0.003	4.001±0.009	0.996±0.0003
	F	6.934±0.002	0.891±0.004	4.991±0.002	9.012±0.004	0.535±0.019	3.971±0.024	1.120±0.0012
Muscle	A	2.466±0.002	0.292±0.017	1.994±0.004	1.001±0.023	0.269±0.002	0.923±0.005	0.766±0.0002
	B	9.001±0.001	0.272±0.001	6.088±0.001	8.2001±0.002	1.708±0.005	4.221±0.004	4.388±0.0003
	C	32.122±0.003	0.208±0.002	1.933±0.002	1.8796±0.002	1.322±0.015	0.371±0.017	0.136±0.0001
	D	1.116±0.001	0.109±0.001	1.994±0.004	1.0223±0.001	0.288±0.004	0.681±0.013	0.701±0.0016
	E	8.008±0.002	0.199±0.001	4.941±0.005	8.001±0.003	0.969±0.021	3.922±0.021	3.764±0.0018
	F	7.409±0.002	0.299±0.012	4.886±0.013	8.000±0.004	1.064±0.003	4.012±0.001	3.708±0.0006
WHO/FAO Standard (1996)mg/kg		5.000	0.300	30.000	2.000	0.600	1.000	1.000

The concentration of Fe in the gills, liver, kidney, blood, GIT and muscle of *Heteroclarias* exposed to dye effluents was above the acceptable limits of Fe in fish tissues set by WHO (1996), ITS standard (2000) and FEPA (2003). The observed levels of Ni in the gills, liver, kidney, blood, GIT and muscle of *Heteroclarias* exposed to dye effluents exceeded the WHO permissible limits for Ni in fish tissues. The content of Mn in the gills, liver, kidney, blood, GIT and muscle of *Heteroclarias* exposed to dye effluents was above the maximum recommended limits by FAO/WHO (1993). The concentration of Cr recorded in the gills, liver, kidney and muscle of *Heteroclarias* exposed to dye effluents was above the maximum permissible limits in fish tissues by ITS standard (2000) but concentrations in blood and GIT remain within permissible limits. The high content of metals in the tissues corroborate with the reports of Abdulkareem and Owolabi (2022) who reported bioaccumulation of Zn in the tissues of *Heterobranchus longifilis* exposed to ZnO-NPs. Adeyemi-Ale et al. (2021) also reported high contents of metals in the tissues of fish exposed to Automobile waste. Bioaccumulation of these metals in the vital organs of the fish could induced oxidative stress and cause physiological disorder (Owolabi and Abdulkareem, 2021). The use of these organs as biomarker to test the toxicity of Dye effluents could play a vital role in the management of aquatic ecosystem. Bio-Concentration Factor (BCF) of the

metals in the different tissues after 30 days of exposure to Dye effluents were revealed in Figs. 1-6.

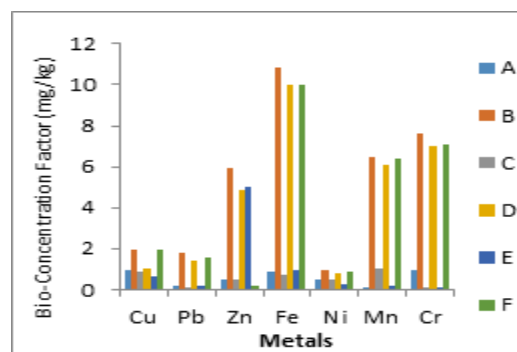


Fig. 1: Bio-Concentration factor of metals in the gills of *Heteroclarias* exposed to dye effluents for 30 days

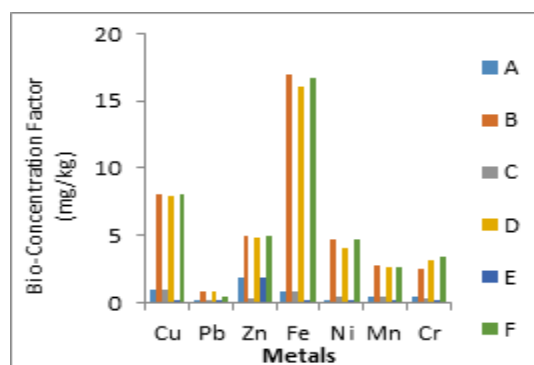


Fig. 2: Bio-Concentration factor of metals in the liver of *Heteroclarias* exposed to dye effluents for 30 days

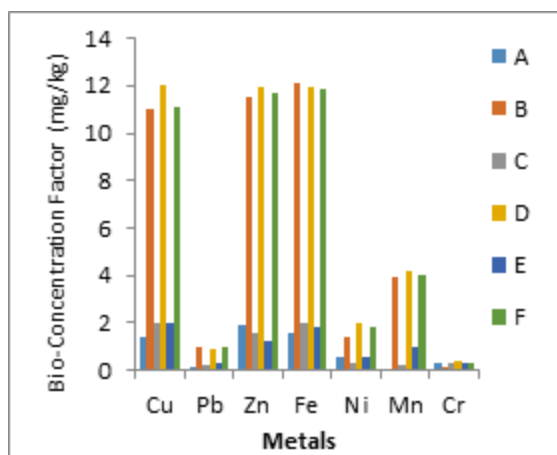


Fig. 3: Bio-Concentration factor of metals in the kidney of *Heteroclinus* exposed to dye effluents for 30 days

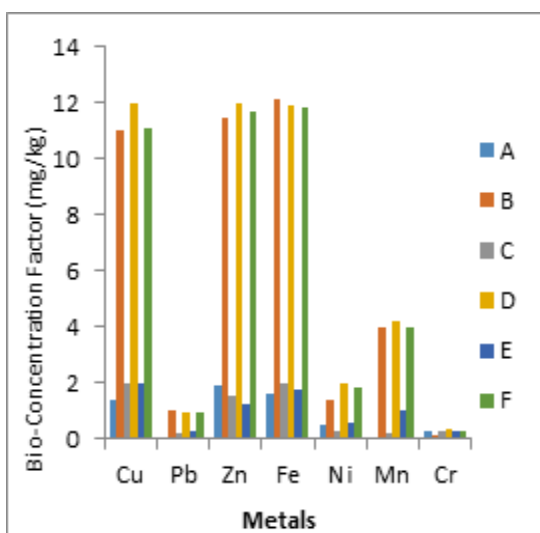


Fig. 4: Bio-Concentration factor of metals in the blood of *Heteroclinus* exposed to dye effluents for 30 days

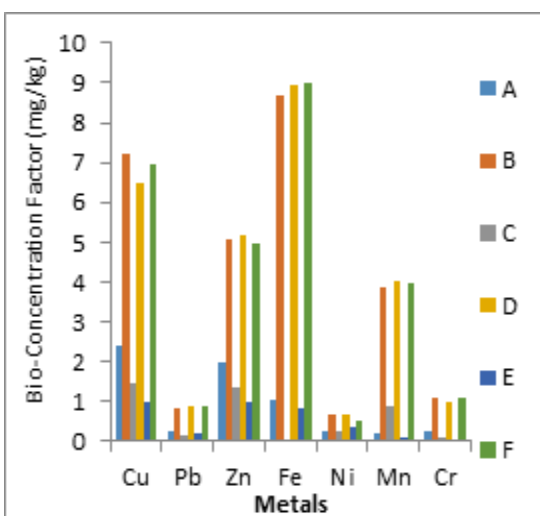


Fig. 5: Bio-concentration factor of metals in the GIT (Gastrointestinal tract) of *Heteroclinus* exposed to dye effluents for 30 days

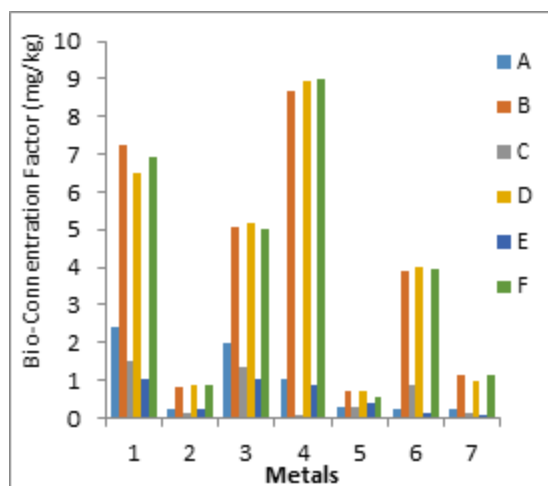


Fig. 6: Bio-Concentration factor of metals in the muscle of *Heteroclinus* exposed to dye effluents for 30 days

After the exposure period, the highest level of Fe was concentrated in blood, gill, kidney, liver, and GIT and Cu in the muscle (Figs. 1-6). While the least concentration of Cr was recorded in the blood, Ni in the gills and GIT with Pb in the kidney, liver and muscle in the tissues of fish in group B exposed to Dye effluents and only (Figs. 1-6). After the 30th day of amelioration, all the tissues in group D that was fed on 5% orange peel were able to release the accumulated metals greatly to a level that is within the recommended limits of metals in the tissues of fish (Figs. 1-6). The great reduction in the concentrations of metals in the organs of *Heteroclinus* fed on diet supplemented with 5% orange peel implies that 5% orange peel has the ability to neutralize the toxic effect of Dye effluents and reduce the content of accumulated metals in the organs, this corroborate with the reports of El Nemr *et al.*, (2009) who treated artificial Dye effluents with orange peel carbon.

Conclusion: The result of these findings indicates that metal contents in Dye effluents concentrated in fish tissues, but 5% orange peel is capable of neutralizing the toxic effects of Dye effluents and reduce the metals contents in the tissues of the fish.

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Data Availability Statement: Data are available upon request from the corresponding author.

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