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Uptake of Zinc by *Pteridium aquilinum* (Bracken fern) and Response of *Clarias gariepinus* Juveniles During Chronic and Sub-lethal Exposure

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Summary: Zinc is an essential trace element but can be toxic to fish at elevated concentrations. This study was carried out to assess the uptake of zinc by *Clarias gariepinus* juveniles and *Pteridium aquilinum* during chronic exposure to sub lethal concentrations. Two experiments (five treatments each: 0.0, 0.8, 1.60, 2.40 and 3.2mg/l zinc chloride) were undertaken simultaneously in static renewal bioassays for 28 days. One experiment contained 3-4 fronds of *P. aquilinum* per tank and the other without it. Each treatment had two replicates (Each having ten *C. gariepinus* juveniles mean weight 29 ± 2g, length 8 ± 3cm). Haematology, histology, zinc accumulation in tissues of *C.gariepinus* and *P. aquilinum* were recorded fortnightly. Packed Cell Volume, haemoglobin concentration, Red Blood Cells, White blood cells, Lymphocytes, Neutrophils, Mean Corpuscular Volume, Mean Corpuscular Haemoglobin Concentration and Mean Corpuscular Haemoglobin of *C. gariepinus* varied with increasing exposure period. RBC, WBC, LYM, and NEUT differed significantly ($p<0.05$) among treatments. Histology (Gills, Kidney and Liver) showed lesions of varying intensities depending on zinc concentration. Bioaccumulation of zinc in organs of *C. gariepinus* differed significantly ($p< 0.05$) with gills at 1.6mg/l Zn having the highest. Zinc in *P. aquilinum* differed significantly ($p<0.05$) with treatments, exposure period and concentration, with the highest concentration (22.91 ± 2.72 mg/g) at 1.6mg/l of Zn. *P. aquilinum* absorbed zinc from the water but did not show hyperaccumulator status in this study.

Keywords*:* Phytoremediation, Bioaccumulation, Bracken fern, *Clarias gariepinus*, Haematology, Histology

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INTRODUCTION

Contamination of the environment with pollutants is a major concern (Agbebi and Owoeye, 2012). Heavy metals are persistent, have toxic effects and can accumulate in organisms and aquatic ecosystems (Bhattacharya *et al*., 2011; Adamu *et al*., 2011; Babu *et al*., 2011); body tissues and organs (Babalola *et al.,* 2009) of organisms. Heavy metals are natural trace components of the environment, but their levels increase due to anthropogenic activities (Sprocati *et al.,* 2006). They affect the physicochemical characteristics, sediments, biological components of water, the quality and quantity of fish stocks (Singh *et al.,* 2006). As trace elements, heavy metals like copper, selenium and zinc are essential for fish but become toxic at higher concentrations. The effects associated with chronic exposure to heavy metals include mutagenicity, carcinogenicity, teratogenicity, immunosuppression, poor body condition and impaired reproduction (Govind and Madhuri, 2014).

Zinc (Zn), an abundant trace element, is found in almost every cell and is involved in nucleic acid synthesis, immune system, neurotransmission, cell signalling and several enzymes (Sfakianakis *et al.,* 2015; Hogstrand, 2011). It occurs in water as a free cation, soluble zinc complexes, or adsorbed on suspended matter (Authman *et al.,* 2015). Major sources of anthropogenic zinc discharges include electroplating, smelting, ore processing, drainage from mining operations, domestic and industrial sewage, road surface runoff, corrosion of zinc alloys or galvanized surfaces, and erosion of agricultural soils (Eisler, 1993).

Phytoremediation uses engineered or natural plants to remove, contain, or render harmless environmental contaminants like heavy metals, organic and radioactive compounds in soil or water (USEPA, 2000). Certain species of higher plants can accumulate very high concentrations of metals in their tissues without-showing toxicity (Bennett *et al.,* 2003). Ma *et al.,* (2001) reported the first arsenic hyperaccumulator – Chinese brake fern (*Pteris vittata*). Bracken fern (*Pteridium aquilinum*) has been used to accumulate copper (Olaifa and Omekam, 2014) and zinc (Olaifa and Ajagbe, 2017) from water in the presence of different manures.

Fish accumulate pollutants preferentially in fatty tissues or liver and the effects become apparent when concentrations in such tissues attain a threshold level (Authman *et al.,* 2015; Omar *et al.,* 2014). Accumulation depends on intake, storage and

elimination from the body (Abdallah and Morsy, 2013). Trace metals like copper, iron and zinc are readily concentrated in different fish tissues (Adewoye *et al.,* 2005). The main targets of water-borne zinc toxicity are the gills (Hogstrand, 2011), where the Ca^{2+} uptake is disrupted, leading to hypocalcemia and death (Niyogi and Wood, 2006). Also, fish's kidney is considered a target organ for Zn accumulation (Omar *et al.,* 2014). *Clarias gariepinus* is an important food fish and widely cultured in Nigeria, helping to enhance food security and provide income. This study was carried out to assess the uptake of zinc by *Clarias gariepinus* juveniles and *Pteridium aquilinum* during chronic exposure to sub lethal concentrations in water.

MATERIALS AND METHODS

Hydrated zinc chloride at five different concentrations 0.0, 0.8, 1.6, 2.40, and 3.2mg/l (Odiete, 1999) was used as toxicant in 20 litres of water for chronic toxicity test for 28 days. 300 juvenile *Clarias gariepinus* (mean weight 29 ±2g and length 8 ± 3cm) were acclimatized for two weeks, fed twice daily at 5% body weight and in good condition before the experiment. *Pteridium aquilinum* were obtained from the University of Ibadan campus and acclimatized (Olaifa and Omekam, 2014).

Two sets of experiments were carried out simultaneously using chronic, static renewal bioassay in completely randomized design (CRD). A total of 20 plastic tanks (0.39m x 0.27m x 0.26m, each equivalent to 27 L) was used. Each treatment had two replicates with 10 juvenile *C. gariepinus* per replicate. The first set -up contained 3-4 fronds on rhizome of *Pteridium aquilinum* (Olaifa and Omekam, 2014) while the second set up contained only the different concentrations of zinc. Zinc chloride was added to the water 24 hours before *P. aquilinum* and *C.gariepinus*. The fish were fed twice daily at 5 % of body weight during the experiments.

Initial and fortnightly physico-chemical parameters of water were determined using a water test kit (Pondlab 200 NT Laboratories Ltd, ME 18 5PP). The parameters monitored were temperature, hardness, alkalinity, pH, dissolved oxygen, nitrate, nitrite, and ammonia. Zinc was determined using Atomic Absorption Spectrophotometer (Perkin Elmer 3100).

Blood samples were drawn under pressure from the posterior caudal vein of the fish (Schmitt *et al.,* 1999) and 2ml transferred into heparinized bottles. Plasma was obtained from blood samples by centrifugation. The standard haemocytometer was used for both erythrocyte and leucocyte counts (Blaxhall and Daisley, 1973) using modified hyme's dilution fluid (Jain, 1986). The packed cell volume (PCV) was obtained using haematocrit reader. Haemoglobin concentration was evaluated using the cyanomethaemoglobin method (Schalm *et al.,* 1975). Haematometric indices including Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin Concentration (MCHC) and Mean Corpuscular Haemoglobin (MCH) were determined from the values obtained from red blood cell count, haemoglobin level and PCV values (Duncan *et al.,* 1994).

Fish samples from each treatment were fortnightly dissected and tissues (gills, kidney and liver) collected and fixed in Bouin's solution as a histological fixative for 24 h (Tao *et al.,* 1999). The specimens were dehydrated, cleared in xylene and embedded in paraffin wax before sectioning at 5 µm with a rotary microtome. The specimens were stained with hematoxylin and eosin Humason (1967). Sections were examined and photographically enlarged using light microscopy and photographed by a built-in camera.

The *P. aquilinum*, *C.gariepinus* and its tissues (gill, kidney and liver) were oven dried at 105°C in a Gallenkamp oven to a constant weight. The samples were ground into powder and further dried to a constant weight and 0.5g of each sample was obtained for digestion. The presence and concentration of Zn in the digest was determined with the aid of atomic absorption spectrophotometer (Perkin Elmer 3100).

Statistical analysis

All results were analysed with the computer statistical package (SPSS, version 17.0) using a One-way Analysis of Variance (ANOVA) at $p<0.05$ and Duncan's multiple Range Test.

RESULTS

The results obtained during the static, chronic exposure of *Clarias gariepinus* juveniles to sub lethal concentrations of zinc in water with and without *P. aquilinum* are presented in tables 1- 6 and figures 1-9.

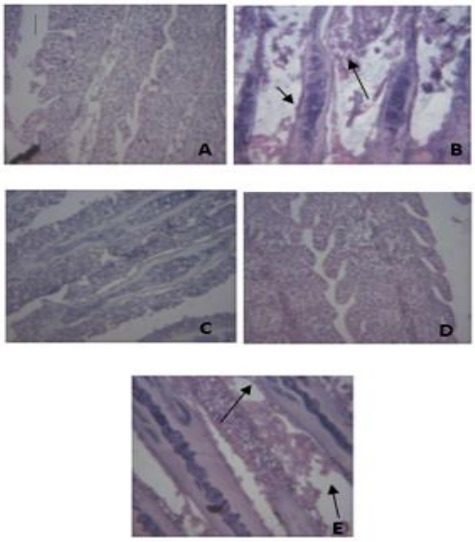

Figure 1: Histology of the Gills of *C. gariepinus* in experiment without *P. aquilinum* at week 4 (x400 magnification). A (Control, 0.0mg/l): No visible lesion seen; B= (0.8mg/l): Severe sub mucosal congestion and a moderate to severe mucosal erosion of the gill filaments, C (1.6mg/l): No visible

lesion seen, D (2.4mg/l): No visible lesion seen, E (3.2mg/l):
Moderate to severe mucosal erosion of the gill filaments.

Table 1: Water Quality parameters during a chronic exposure of *Clarias gariepinus* juveniles to sub lethal concentrations of zinc in water with and without *P. aquilinum*

		Without *P. aquilinum*			With *P. aquilinum*	
Parameters	**Conc. (mg/l)**	**Initial**	**2nd Week**	**4th Week**	**2nd Week**	**4th week**
Temperature (°C)	0.0	7.6	27.85 ± 0.15^{a}	27.65 ± 0.15^{a}	27.90 ± 0.10^{a}	27.70 ± 0.00^{a}
	0.8		27.90 ± 0.10^{a}	27.70 ± 0.05^{a}	27.85 ± 0.15^{a}	27.65 ± 0.05^{a}
	1.6		28.00 ± 0.00^{a}	27.75 ± 0.10^{a}	28.10 ± 0.10^{a}	27.60 ± 0.10^{a}
	2.4		28.00 ± 0.00^{a}	27.50 ± 0.10^{a}	27.80 ± 0.00^{a}	27.75 ± 0.05^{a}
	3.2		28.00 ± 0.00^{a}	27.80 ± 0.10^{a}	28.00 ± 0.00^{a}	27.85 ± 0.15^{a}
pH	0.0	7.6	7.50 ± 0.00^{b}	7.65 ± 0.15^{a}	7.00 ± 0.00^{a}	7.25 ± 0.25^{a}
	0.8		7.00 ± 0.00^{a}	7.55 ± 0.05^{a}	7.00 ± 0.00^{a}	7.55 ± 0.05^{ab}
	1.6		7.50 ± 0.00^{b}	7.90 ± 0.10^{a}	7.50 ± 0.00^{b}	7.90 ± 0.10^{b}
	2.4		7.90 ± 0.10^{c}	7.90 ± 0.10^{a}	7.25 ± 0.25^{ab}	7.90 ± 0.10^{b}
	3.2		7.65 ± 0.15^{bc}	7.70 ± 0.10^{a}	7.50 ± 0.00^{b}	7.70 ± 0.10^{ab}
DO (mg/l)	0.0	6.0	5.50 ± 0.50^{ab}	5.00 ± 0.00^{a}	5.50 ± 0.50^{ab}	5.00 ± 0.00^{a}
	0.8		6.00 ± 0.00^{ab}	6.00 ± 0.00^{b}	6.00 ± 0.00^{ab}	5.50 ± 0.50^{a}
	1.6		5.50 ± 0.50^{ab}	5.00 ± 0.00^{a}	5.50 ± 0.50^{ab}	5.00 ± 0.00^{a}
	2.4		5.00 ± 0.00^{a}	5.00 ± 0.00^{a}	5.00 ± 0.00^{a}	5.00 ± 0.00^{a}
	3.2		6.50 ± 0.50^{b}	5.50 ± 0.50^{ab}	6.50 ± 0.50^{b}	5.50 ± 0.50^{a}
Hardness (mg/l)	0.0	320.4	301.10 ± 19.30^{b}	231.40 ± 0.00^{a}	311.50 ± 8.90^{c}	231.40 ± 0.00^{a}
	0.8		284.80 ± 0.00^{b}	249.20 ± 0.00^{b}	240.30 ± 8.90^{a}	240.30 ± 8.90^{ab}
	1.6		267.00 ± 0.00^{ab}	240.20 ± 9.00^{ab}	267.00 ± 0.00^{ab}	249.20 ± 0.00^{b}
	2.4		293.70 ± 8.90^{b}	249.20 ± 0.00^{b}	293.70 ± 8.90^{bc}	249.20 ± 0.00^{b}
	3.2		240.30 ± 8.90^{a}	249.20 ± 0.00^{b}	240.30 ± 8.90^{a}	249.20 ± 0.00^{b}
Alkalinity (mg/l)	0.0	284.8	284.70 ± 0.10^{ab}	275.90 ± 8.90^{a}	275.90 ± 8.90^{a}	267.00 ± 0.00^{a}
	0.8		267.00 ± 0.00^{a}	275.90 ± 8.90^{a}	267.00 ± 0.00^{a}	267.00 ± 17.80^{a}
	1.6		328.80 ± 44.80^{abc}	267.00 ± 0.00^{a}	267.00 ± 0.00^{a}	267.00 ± 0.00^{a}
	2.4		356.00 ± 17.80^{bc}	258.10 ± 8.90^{a}	156.60 ± 128.20^{a}	258.10 ± 8.90^{a}
	3.2		373.60 ± 0.00^{c}	320.40 ± 17.80^{b}	147.70 ± 119.30^{a}	284.80 ± 17.80^{a}
Nitrite (mg/l)	0.0	0.0	0.10 ± 0.00^{a}	0.00 ± 0.00^{a}	0.25 ± 0.00^{a}	0.13 ± 0.13^{a}
	0.8		0.13 ± 0.13^{a}	0.00 ± 0.00^{a}	0.13 ± 0.13^{a}	0.00 ± 0.00^{a}
	1.6		0.25 ± 0.00^{a}	0.00 ± 0.00^{a}	0.20 ± 0.05^{a}	0.00 ± 0.00^{a}
	2.4		0.25 ± 0.00^{a}	0.13 ± 0.13^{ab}	0.23 ± 0.03^{a}	0.13 ± 0.13^{a}
	3.2		0.25 ± 0.00^{a}	0.25 ± 0.00^{b}	0.23 ± 0.03^{a}	0.25 ± 0.00^{a}
Nitrate (mg/l)	0.0	0.0	1.75 ± 0.25^{b}	0.25 ± 0.00^{a}	2.50 ± 0.00^{b}	1.75 ± 0.75^{a}
	0.8		1.00 ± 0.00^{a}	0.25 ± 0.00^{a}	1.00 ± 0.00^{b}	2.00 ± 0.00^{a}
	1.6		1.75 ± 0.25^{b}	0.25 ± 0.00^{a}	2.25 ± 0.25^{b}	1.00 ± 0.50^{a}
	2.4		2.25 ± 0.25^{bc}	0.38 ± 0.13^{ab}	2.25 ± 0.25^{b}	0.63 ± 0.38^{a}
	3.2		2.50 ± 0.00^{c}	0.50 ± 0.00^{b}	2.50 ± 0.00^{b}	2.00 ± 0.50^{a}
Ammonia (mg/l)	0.0	0.2	0.15 ± 0.05^{a}	1.10 ± 0.40^{a}	0.15 ± 0.05^{a}	0.85 ± 0.15^{a}
	0.8		0.15 ± 0.05^{a}	0.70 ± 0.00^{a}	0.15 ± 0.05^{a}	0.70 ± 0.00^{a}
	1.6		0.20 ± 0.00^{a}	0.70 ± 0.00^{a}	0.75 ± 0.25^{b}	0.60 ± 0.10^{a}
	2.4		0.50 ± 0.00^{b}	0.70 ± 0.00^{a}	0.85 ± 0.15^{ab}	0.60 ± 0.10^{a}
	3.2		0.35 ± 0.15^{ab}	0.70 ± 0.00^{a}	0.35 ± 0.15^{ab}	0.70 ± 0.00^{a}

Means and standard error on the same column with different superscripts are significantly different ($p<0.05$)

Table 2: Zinc Concentrations (mg/l) in water samples during experiments with and without *P. aquilinum*

			Without *P. aquilinum*		With *P. aquilinum*	
Parameters	**Conc. (mg/l)**	**Initial**	**2nd Week**	**4th Week**	**2nd Week**	**4th Week**
Zinc (mg/l)	Control (0.00)	0.02	0.01 ± 0.01a	33.9 ± 2.69ab	0.00 ± 0.00a	28.01 ± 1.90a
	0.8		0.3 ± 0.17abc	30.08 ± 1.02a	0.04 ± 0.01ab	31.17 ± 0.95ab
	1.6		0.18 ± 0.03ab	35.61 ± 0.51ab	0.07 ± 0.06ab	31.56 ± 0.48ab
	2.4		0.62 ± 0.16bc	36.64 ± 2.43ab	0.46 ± 0.18b	30.31 ± 0.40ab
	3.2		0.71 ± 0.19c	40.02 ± 2.71b	0.41 ± 0.19ab	32.41 ± 0.31b

Means and standard error on the same column with different superscript are significantly different ($p<0.05$)

Table 3: Mean Changes in Haematological Parameters of *C. gariepinus* Exposed to sub lethal Concentrations of zinc in water with and without *P. aquilinum* for 28 days

			Without *P. aquilinum*		With *P. aquilinum*	
Parameters	**Conc (mg/l)**	**Initial reading**	**2nd Week**	**4th Week**	**2nd Week**	**4th Week**
PCV (%)	Control (0.00)	25	24.00 ± 0.00^{bc}	19.50 ± 0.50^{c}	24.50 ± 0.50^{c}	21.50 ± 0.50^{a}
	0.8		20.50 ± 0.50^{a}	11.00 ± 2.00^{a}	24.50 ± 0.50^{c}	5.50 ± 5.50^{a}
	1.6		25.50 ± 0.50^{d}	14.50 ± 0.50^{c}	23.00 ± 0.00^{b}	21.50 ± 2.50^{a}
	2.4		23.50 ± 0.50^{b}	17.50 ± 0.50^{bc}	27.00 ± 0.00^{d}	16.00 ± 2.00^{a}
	3.2		25.00 ± 0.00^{cd}	25.50 ± 0.50^{d}	18.50 ± 0.50^{d}	16.50 ± 1.50^{a}
RBC ($x10^{12/l}$)	Control (0.00)	3.04	2.77 ± 0.09^{b}	3.28 ± 0.20^{b}	3.21 ± 0.05^{b}	4.42 ± 0.04^{c}
	0.8		2.56 ± 0.30^{b}	2.73 ± 0.13^{ab}	2.27 ± 0.01^{a}	3.38 ± 0.10^{b}
	1.6		1.94 ± 0.06^{a}	2.95 ± 0.09^{ab}	4.46 ± 0.40^{c}	4.55 ± 0.07^{c}
	2.4		2.81 ± 0.01^{b}	2.35 ± 0.13^{a}	2.31 ± 0.13^{a}	2.11 ± 0.07^{a}
	3.2		2.45 ± 0.17^{ab}	2.38 ± 0.26^{a}	2.95 ± 0.09^{ab}	2.19 ± 0.09^{a}
WBC ($x10^{9/l}$)	Control (0.00)	6.4	8.40 ± 0.40^{c}	6.60 ± 0.20^{a}	4.40 ± 0.40^{a}	7.10 ± 0.70^{b}
	0.8		4.40 ± 0.40^{a}	5.60 ± 0.80^{a}	10.20 ± 0.20^{d}	6.80 ± 0.40^{b}
	1.6		7.50 ± 0.30^{c}	6.00 ± 0.40^{a}	6.00 ± 0.40^{b}	9.30 ± 0.30^{c}
	2.4		6.20 ± 0.20^{b}	5.80 ± 0.20^{a}	7.80 ± 0.20^{c}	4.40 ± 0.40^{a}
	3.2		4.40 ± 0.40^{a}	9.10 ± 3.70^{a}	6.10 ± 0.30^{b}	8.80 ± 0.00^{c}
Hb (gm/dl)	Control (0.00)	8.2	7.90 ± 0.00^{b}	6.35 ± 0.15^{c}	8.05 ± 0.15^{c}	7.05 ± 0.15^{a}
	0.8		6.75 ± 0.15^{a}	3.55 ± 0.65^{a}	8.05 ± 0.15^{c}	5.05 ± 1.85^{a}
	1.6		8.40 ± 0.20^{c}	4.70 ± 0.20^{ab}	6.90 ± 0.00^{b}	7.05 ± 0.85^{a}
	2.4		7.75 ± 0.15^{b}	5.70 ± 0.20^{bc}	8.90 ± 0.00^{d}	5.20 ± 0.70^{a}
	3.2		8.20 ± 0.00^{bc}	8.35 ± 0.15^{d}	6.40 ± 0.20^{a}	5.40 ± 0.50^{a}
MCV (FL)	Control (0.00)	82	86.00 ± 3.00^{a}	59.50 ± 2.50^{bc}	76.00 ± 3.00^{b}	48.50 ± 1.50^{a}
	0.8		80.50 ± 7.50^{a}	40.00 ± 5.00^{a}	100.00 ± 5.00^{c}	46.50 ± 17.50^{a}
	1.6		112.00 ± 20.00^{a}	49.00 ± 0.00^{ab}	49.50 ± 4.50^{a}	48.50 ± 5.50^{a}
	2.4		83.00 ± 2.00^{a}	75.00 ± 2.00^{c}	116.50 ± 6.50^{c}	76.00 ± 12.00^{a}
	3.2		102.00 ± 7.00^{a}	108.00 ± 10.00^{c}	65.50 ± 3.50^{ab}	75.00 ± 4.00^{a}
MCH (pg)	Control (0.00)	26	28.00 ± 1.00^{ab}	19.50 ± 0.50^{ab}	24.50 ± 0.50^{b}	15.50 ± 0.50^{a}
	0.8		26.50 ± 2.50^{a}	13.00 ± 2.00^{a}	32.50 ± 1.50^{c}	15.00 ± 6.00^{a}
	1.6		43.00 ± 0.00^{c}	16.00 ± 0.00^{a}	15.00 ± 1.00^{a}	15.50 ± 2.50^{a}
	2.4		27.00 ± 1.00^{a}	24.50 ± 0.50^{b}	38.00 ± 2.00^{d}	25.00 ± 4.00^{a}
	3.2		33.00 ± 2.00^{b}	35.50 ± 3.50^{c}	21.50 ± 1.50^{b}	24.50 ± 1.50^{a}
LYM (%)	Control (0.00)	65	63.50 ± 0.50^{ab}	70.00 ± 2.00^{b}	68.00 ± 0.00^{c}	80.50 ± 5.50^{b}
	0.8		66.50 ± 4.50^{b}	66.50 ± 1.50^{ab}	60.50 ± 0.50^{b}	72.50 ± 2.50^{ab}
	1.6		58.00 ± 0.00^{a}	65.50 ± 0.50^{ab}	60.50 ± 0.50^{b}	68.50 ± 2.50^{a}
	2.4		65.00 ± 0.00^{ab}	65.00 ± 1.00^{a}	57.50 ± 0.50^{a}	67.50 ± 2.50^{a}
	3.2		60.00 ± 0.00^{ab}	65.50 ± 0.50^{ab}	66.50 ± 0.50^{c}	70.50 ± 0.50^{ab}
NEUT (%)	Control (0.00)	35	35.50 ± 0.50^{ab}	28.50 ± 2.50^{a}	30.50 ± 0.50^{a}	17.50 ± 5.50^{a}
	0.8		27.50 ± 0.50^{b}	32.50 ± 1.50^{ab}	38.50 ± 0.50^{c}	26.50 ± 2.50^{ab}
	1.6		41.00 ± 0.00^{a}	34.00 ± 0.00^{b}	38.50 ± 0.50^{c}	30.50 ± 2.50^{b}
	2.4		34.00 ± 0.00^{ab}	34.00 ± 1.00^{b}	41.50 ± 0.50^{d}	31.00 ± 3.00^{b}
	3.2		38.50 ± 0.50^{ab}	33.50 ± 0.50^{ab}	32.50 ± 0.50^{b}	28.50 ± 0.50^{ab}

Means and standard error on the same column with different superscript are significantly different ($p<0.05$). MCV = Mean Cell Volume, MCH = Mean Cell Haemoglobin, LYM = Lymphocytes, NEUT = Neutrophil

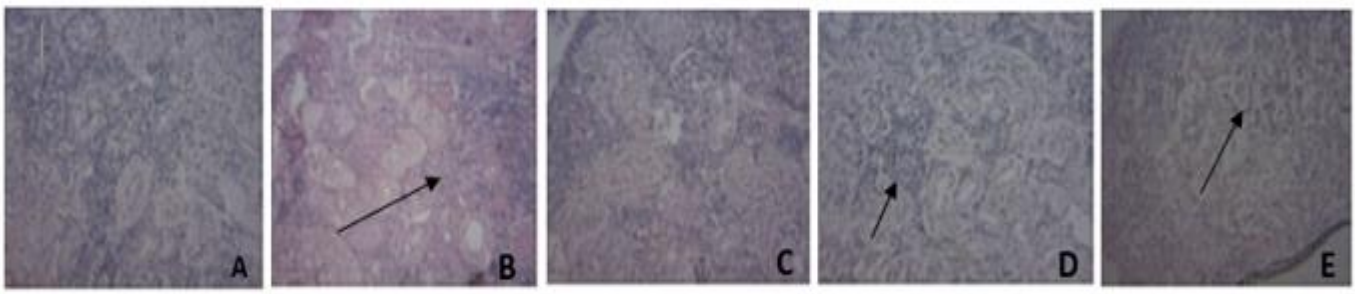

Figure 2: Histology of the Kidney of *C. gariepinus* in experiment without *P. aquilinum* at second week (x 400 magnification). A (Control, 0.0mg/l): No visible lesion seen. B (0.8mg/l): There was a severe sub mucosal congestion. C (1.6mg/l): No visible lesion seen. D (2.4g/l): Scanty hemopoietic tissue in the interstitium. E (3.2mg/l): There was severe diffuse tubular degeneration and necrosis, the hemopoietic tissue was not prominent.

Table 4: Concentration of Zinc in Organs of *C. gariepinus* with and without *P. aquilinum* (mg/g)

Tissues	Conc (mg/l)	Initial Reading	Without *P. aquilinum*		With *P. aquilinum*	
			2nd Week	4th Week	2nd Week	4th Week
Kidney	Control (0.0)	52.10	44.31± 5.06^{a}	27.69 ± 2.48^{a}	60.87 ± 0.50^{b}	42.19 ± 0.03^{bc}
	0.8		36.52 ± 0.83^{a}	35.91± 14.81^{a}	54.69 ± 5.43^{ab}	45.57 ± 5.65^{c}
	1.6		42.42± 2.87^{a}	23.45 ± 3.16^{a}	65.31± 0.20^{b}	30.16 ± 2.55^{ab}
	2.4		42.14 ± 7.02^{a}	29.70 ± 10.52^{a}	57.44± 2.18^{ab}	27.63 ± 2.02^{a}
	3.2		69.88± 5.57^{b}	47.68 ± 2.47^{b}	49.19 ± 9.82^{a}	28.02 ± 4.10^{a}
Gill	Control (0.0)	43.21	69.61± 1.69^{b}	27.93 ± 7.88^{a}	44.29 ± 4.97^{a}	33.96 ± 8.84^{a}
	0.8		60.36± 23.85^{b}	59.84 ± 36.33^{b}	29.78 ± 0.43^{a}	30.09 ± 9.52^{a}
	1.6		80.24± 1.87^{c}	41.69 ± 1.63^{b}	48.24 ± 10.98^{a}	26.10 ± 4.49^{a}
	2.4		61.51± 2.14^{b}	42.10 ± 0.09^{b}	42.29 ± 8.02^{a}	20.55 ± 1.37^{a}
	3.2		71.80± 0.59^{bc}	37.91 ± 1.73^{a}	59.34 ± 17.97^{b}	28.38 ± 7.77^{a}
Liver	Control (0.0)	69.21	61.65± 2.64^{b}	31.89 ± 0.18^{a}	74.26 ± 2.95^{c}	43.49 ± 6.68^{bc}
	0.8		68.35± 3.10^{b}	66.01 ± 23.82^{b}	71.28 ± 3.96^{c}	61.16 ±11.35^{b}
	1.6		65.47± 7.78^{b}	32.17 ± 0.02^{a}	72.68 ± 6.69^{c}	45.48 ± 3.77^{ab}
	2.4		64.97± 8.68^{b}	42.43 ± 6.82^{b}	72.47 ± 1.15^{c}	42.35 ± 5.84^{ab}
	3.2		68.65± 3.50^{b}	38.89 ± 1.01^{a}	64.37 ± 0.00^{b}	33.41 ± 2.20^{a}

Means and standard error on the same column with different superscript are significantly different (p<0.05)

Table 5: Zinc Concentrations in Whole C. *gariepinus* sample (mg/g) exposed to varying sub lethal concentrations of zinc

			Without *P. aquilinum*		With *P. aquilinum*	
Parameters	**Conc (mg/l)**	**Initial**	**2nd Week**	**4th Week**	**2nd Week**	**4th Week**
Zinc (mg/l)	Control (0.00)	89.99	70.67 ± 3.68a	19.85 ± 0.67ab	71.49 ± 1.76a	20.77 ± 0.96a
	0.8		80.48 ± 1.19a	17.67 ± 0.48a	71.57 ± 3.65a	21.84 ± 1.25a
	1.6		66.39 ± 1.60a	19.61 ± 0.40ab	76.28 ± 3.03a	20.35 ± 0.21a
	2.4		69.26 ± 2.01a	21.80 ± 0.73bc	21.80 ± 0.73bc	22.54 ± 0.58a
	3.2		70.50 ± 8.85a	24.62 ± 1.59c	69.54 ± 0.19a	21.08 ± 0.02a

Means and standard error on the same column with different superscript are significantly different (p<0.05)

Table 6: Zinc concentrations in *P. aquilinum* leaf-samples exposed to varying concentrations of zinc in water (mg/g)

			Exposure period (week)	
Parameter	**Conc (mg/l)**	**Initial**	**2nd Week**	**4th week**
Zinc (mg/l)	Control (0.0)	13.35	13.34 ± 0.43^{ab}	16.86 ± 0.18^{a}
	0.8		14.02 ± 0.70^{ab}	18.01 ± 0.37^{a}
	1.6		11.47 ± 1.26^{a}	22.91 ± 2.72^{b}
	2.4		13.01 ± 0.09^{ab}	19.54 ± 0.28^{ab}
	3.2		14.97 ± 0.24^{b}	20.66 ± 0.47^{ab}

Means and standard error on the same column with different superscripts are significantly different (p<0.05

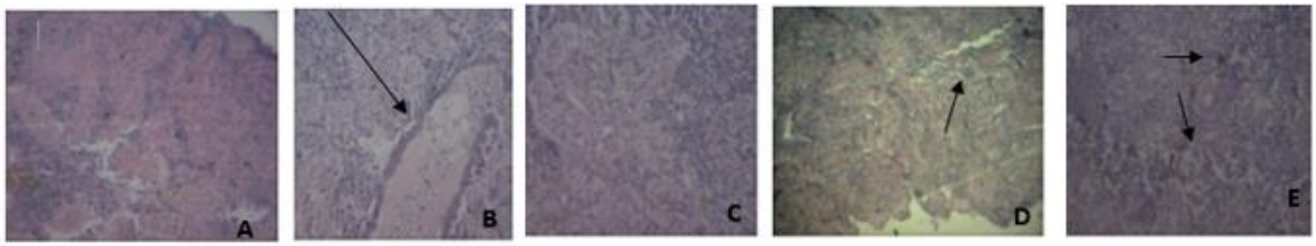

Figure 3: Histology of the Kidney of *C. gariepinus* in experiment without *P. aquilinum* at week 4 (X 400). A (Control (0.0mg/l)): No visible lesion seen. B (0.8mg/l): There was a focus of severe congestion. C (1.6mg/l): No visible lesion seen. D (2.4mg/l): There was mild interstitial congestion. E (3.2mg/l): There was severe diffuse tubular degeneration and necrosis, the hemopoietic tissue was not prominent.

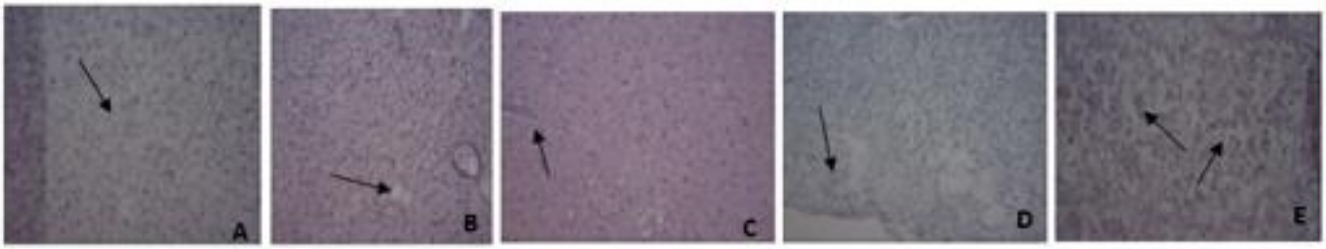

Figure 4: Histology of the Liver of *C. gariepinus* in experiment without *P. aquilinum* at week 2 (X 400). A (Control, 0.0mg/l): There was moderate to severe diffuse vacuolation of the hepatocytes. B (0.8mg/l): There was a very mild diffuse vacuolation of the hepatocytes. C (1.6mg/l): The portal channels were prominent and appeared enlarged. D (2.4mg/l): There was a focus on cellular infiltration in the parenchyma. E (3.2mg/l): There was severe vacuolar degeneration and necrosis of hepatocytes.

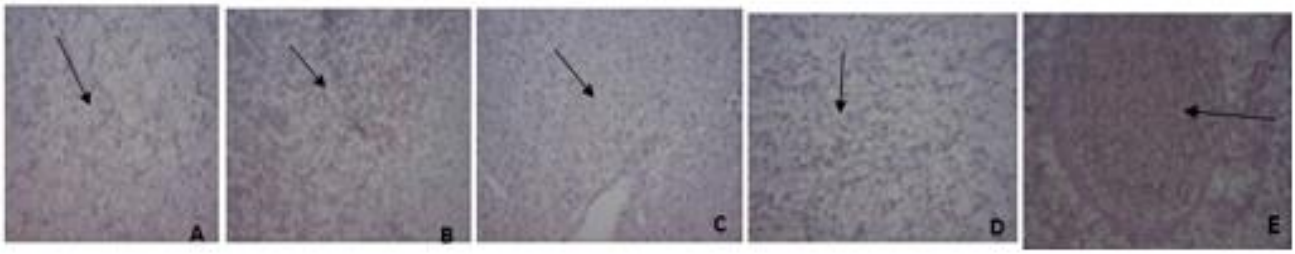

Figure 5: Histology of the Liver of *C. gariepinus* in experiment without *P. aquilinum* at week 4. A- Control (0.0mg/l): There was a pronounced diffuse vacuolation of hepatocytes B (0.8mg/l): There was mild, diffuse vacuolar degeneration and necrosis of hepatocytes. C (1.6mg/l): There was a mild diffuse hydropic change in the hepatocytes. D (2.4mg/l): There was diffuse vacuolation of hepatocytes. E (3.2mg/l): There was a severe portal congestion and severe diffuse vacuolation of hepatocytes.

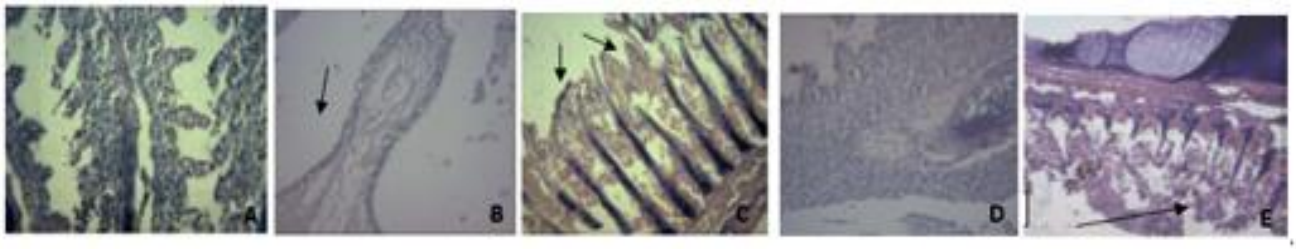

Figure 6: Histology of the Gill of *C. gariepinus* in experiment with *P. aquilinum* at week 4 (x 400). A (Control, 0.0mg/l): No visible lesion seen. B (0.8mg/l): the mucosal depth was moderately reduced. C (1.6mg/l): There was a moderate erosion of the mucosa. D (2.4mg/l): No visible lesion seen. E (3.2mg/l): There was severe mucosal erosion.

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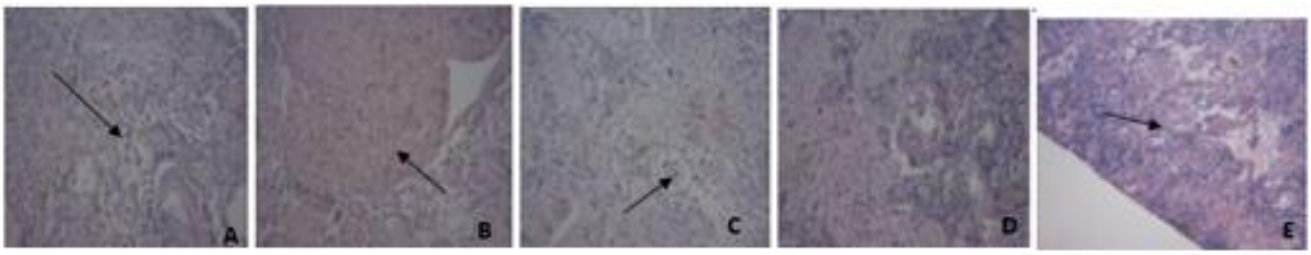

Figure 7: Histology of the Kidney of *C. gariepinus* in experiment with *P. aquilinum* at week 4 (X 400). A (Control, 0.0mg/l): There were few foci of tubular degeneration and necrosis. B (0.8mg/l): There was a very severe medullary congestion. C (1.6mg/l): Very mild multiple foci of interstitial congestion. D (2.4mg/l): There was a very mild interstitial congestion. E (3.2mg/l): No visible lesion seen.

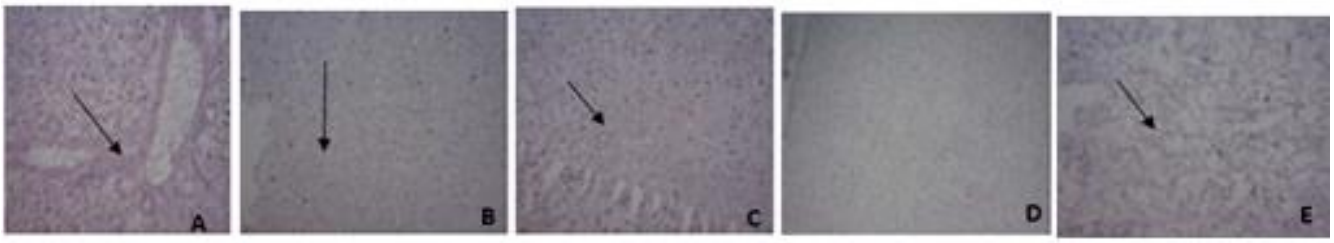

Figure 8: Histology of the Liver of *C. gariepinus* in experiment with *P. aquilinum* at week 2. A (Control, 0.0mg/l): There was moderate periportal vacuolar degeneration and necrosis of hepatocytes. B (0.8mg/l): No visible lesion seen. C (1.6mg/l): There were few foci of hepatic vacuolation. D (2.4mg/l): There was a severe diffuse vacuolation of the hepatocytes. E (3.2mg/l): There was a moderate, diffuse vacuolation of hepatocytes.

Figure 9: Histology of the Liver of *C. gariepinus* in experiment with *P. aquilinum* at week 4. A (Control, 0.0mg/l): There was a moderate diffuse vacuolation of hepatocytes. B (0.8mg/l): There was a moderate diffuse vacuolation of hepatocytes. C (1.6mg/l): There was a mild diffuse vacuolation of hepatocytes. D (2.4mg/l): There was a severe diffuse vacuolation of hepatocytes. E (3.2mg/l): There was a severe vacuolation of the hepatocytes, there is a very mild periportal fatty infiltration.

DISCUSSION

Aquatic bioassays are necessary to determine whether a potential toxicant is dangerous to aquatic life and if so, find the relationship between the toxicant concentration and its effect on aquatic animals (Olaifa *et al*., 2003). Zinc is an essential element required by plants and animals but at high concentrations, it exerts adverse effects by accruing structural damage, which affects the growth, development and survival of the fish.

Environmental factors such as water temperature, dissolved oxygen concentration, pH, hardness, salinity, alkalinity and dissolved organic carbon may affect and play significant roles in metal accumulation and toxicity to fish (Ebrahimi and Taherianfard, 2011; Linbo *et al*., 2009). Temperature, nitrite and ammonia showed no significant differences with and without *P. aquilinium*. All water quality parameters- temperature, pH, dissolved oxygen, hardness, alkalinity, nitrite, nitrate and ammonia were within normal range for the survival of *C. gariepinus*. The highest pH was reported between1.6 - 2.4mg/l zinc (Table 1). Though hardness and alkalinity appeared to be high, they were within the tolerable range of 50-400mg/l (Swann, 1997). High temperature and low dissolved oxygen tend to increase the toxicity of zinc (Bradley and Sprague, 2011) while hard waters can buffer the effects of heavy metals such as copper and zinc. The principal factors affecting toxicity of zinc are hardness and pH of water. Decreasing hardness and increasing pH increase the lethality of dissolved zinc (Ezeonyejiaku *et al*., 2012). There were significant differences ($p<0.05$) in zinc concentrations in water samples among treatments. Zinc concentration in water was highest (40 ± 2.71 mg/l) at 3.2mg/l zinc without *P. aquilinum* in week 4. Lower concentrations of zinc ($p<0.05$) were obtained in water at week 2 than 4 in both experiments with and without *P. aquilinum*.

The blood parameters (Table 3) of *C. gariepinus* exposed to varying concentrations of zinc chloride in terms of Pack Cell Volume (PCV), Haemoglobin (Hb), Red blood count (RBC), White blood count (WBC), Lymphocyte (LYM), Mean cell volume (MCV), Mean cell haemoglobin (MCH) showed significant differences ($p<0.05$) among treatments with and without *P. aquilinum*. PCV was significantly ($p<0.05$) higher (27.00 ± 0.00%) at 2.4mg/l of zinc and lowest at 0.8mg/l zinc (15.50 ± 5.50%) with *P. aquilinum*. Without *P. aquilinum*, the highest PCV (25.50 ± 0.50%) was recorded at 3.2mg/l and 1.6mg/l Zn in the fourth week and second weeks respectively, and lowest at 0.8mg/l Zn. PCV reduced significantly ($p< 0.05$) as exposure periods increased. RBC value was considerably higher (4.55 ± 0.07 trillion/l) with *P. aquilinum* at 1.6mg/l zinc. The highest WBC value (10.20 ± 0.20 billion/l) was recorded in the experiment with *P. aquilinum* at 0.8mg/l Zn.

Hb values decreased as the exposure time increased with higher values obtained in *C. gariepinus* at week 2 with and without *P. aquilinum*. Hb value was highest (8.40 ± 0.20 gm/dl) at 1.6mg/l of Zn and lowest (3.55 ± 0.23gm/dl) at 0.8mg/l Zn without *P. aquilinum*. The highest Hb value with *P. aquilinum* was recorded at 2.4mg/l of Zn (8.90 ± 0.00 gm/dl) and the lowest value (5.05 ± 1.85gm/dl). MCV reduced in all treatments as exposure period increased with the highest value of MCV (116.50 ± 6.50 FL) recorded in the experiment with *P. aquilinum* at 2.4mg/l Zn concentration, and the lowest (40.00 ± 5.00 FL) without *P. aquilinum* at 0.8mg/l concentration of Zn. Higher MCV values were observed in all treatments in week 2 than 4 except in 3.2 mg/l zinc concentration without *P. aquilinum*. In the fourth week, MCV values increased with increasing level of Zn in the two experiments. Mean Cell Haemoglobin (MCV) was highest at 1.6mg/l zinc without *P. aquilinum* ($p< 0.05$). LYM values ranged between 60.00 ± 0.00 % at 3.2mg/l of Zn and 70.00±2.00% in the control. The highest LYM value (80.50 ± 5.50 %) was recorded in the control while the lowest (57.50 ± 0.50 %) was recorded at 2.4mg/l of Zn with *P. aquilinum*. LYM values increased as exposure period increased with and without *P. aquilinum*. Neutrophil values reduced with increasing exposure period with and without *P. aquilinum* except for 2.4 mg/l zinc concentration without *P. aquilinum*.

Fish haematological parameters are often determined as an index of health status (Oshode *et al*., 2008). The reduction in hemoglobin and hematocrit values of the fish could be attributed to inadequate number of erythrocytes indicating anaemia (Maheswaran *et al*., 2008). Heavy metals might alter the properties of hemoglobin by decreasing their affinity towards oxygen- binding capacity, rendering the erythrocytes more fragile and permeable. This may result in cell swelling, deformation and damage (Witeska and Kosciuk, 2003). Increasing or decreasing numbers of white blood cells are normal reactions to metals such as zinc. Decreased number of white blood cells may be associated with an increased level of corticosteroid hormones (Kori-Siakpere *et al*., (2006). Changes in WBC count in stressed fish suppress the immune system and increase susceptibility to disease (Olurin *et al*., 2012). It has been reported that zinc decreased WBC count, neutrophiles and lymphocytes in carp (Witeska, 2005), WBC count in *O. mossambicus* (Buthelezi *et al*., 2000) and WBC count in *C. gariepinus* (Ololade and Ogini, 2009).

There were variations in the accumulation of zinc in the kidney, gills and liver of *C. gariepinus*. Zinc concentrations in the kidney of *C.gariepinus* were significantly ($p<0.05$) lower at week 4 than 2 with and without *P. aquilinum*. Similar trends were observed in

the gills and livers of *C. gariepinus*. The highest concentration of zinc in the kidney tissues was observed in 3.2mg/l zinc at week 2 while the highest concentrations in gills (80.24± 1.87) at 1.6mg/l zinc was at week 2, without *P. aquilinum*) and the lowest value (29.78±0.43 mg/l) was recorded in the treatment containing 0.8mg/l concentration of Zn with *P. aquilinum*.

Concentration of zinc in the liver of *C. gariepinus* was highest (74.26 ± 2.95mg/l zinc) at week 2 in the control. There were no significant differences ($p>0.05$) in zinc accumulation in the kidneys without *P. aquilinum* whereas significant differences ($p<0.05$) were observed in the fourth week with *P. aquilinum*. Among the experimental fish, the highest concentration of Zn (66.01 ± 23.82 mg/l) was recorded in the liver of *C .gariepinus* in 0.8mg/l zinc without *P. aquilinum* and lowest zinc concentration (20.55 ± 1.37mg/l) in the gill. There was a general decrease in zinc concentration in all the organs over the experimental period with the lowest concentrations at week 4. Zinc concentrations in the whole *C. gariepinus* (Table 5) sample showed no significant differences ($p>0.05$) across the treatments of the two experiments at week 2 but significant differences ($p<0.05$) were observed in week 4 without *P. aquilinum*.

Concentrations of Zn in the organs of *C. gariepinus* reduced with increase in exposure period with the highest concentrations in the gills at two weeks and lowest in the kidney. The gills of fish form the first point of uptake of metals including zinc and the liver appears to be one of the most important sites for Zn accumulation. These results are similar to Zn bioaccumulation in different tissues of several fish species exposed to Zn (Bawa-Allah and Saliu, 2015). The levels of Zn in liver at the end of both experiments in week 4 may be ascribed to the binding of Zn to metallothionein similar to earlier reports (Murugan *et al*., 2008). Ganesan and Karuppasamy (2015) suggested that Zn is taken up through the gill and possibly transported to the storage organs like liver and kidney. The differences in the level of accumulation in the different organs of the test fish can primarily be attributed to the differences in the physiological role of each organ (Ganesan and Karuppasamy, 2015).

Significant differences ($p<0.05$) were observed in the accumulation rates of zinc in *P. aquilinum* across the treatments (Table 6). Zn concentrations in the ferns increased with increasing exposure period. In the second week, the highest concentration (14.97 ± 0.24mg/l) was recorded at 3.2mg/l concentration of $ZnCl_2$. However, at the conclusion of the experiment, the highest value (22.91 ± 2.72mg/kg) was recorded in the treatment containing 1.6mg/l concentration of Zn. Compared to the control, *P. aquilinum* accumulated higher concentrations of zinc with respect to exposure periods and concentration. *P. aquilinum* accumulated substantial amount of zinc similar to other reports (Ma *et al*., 2001; Mkumbo *et al*., 2012). However, *P. aquilinum* failed to show its potential as a hyper accumulator of zinc. The changes observed in the *C.gariepinus* could be due to inadequate time interval (24 hours) between the addition of zinc, *P.aquilinum* to water and the introduction of *C. gariepinus*.

Histological structures (figures1-9) of *C. gariepinus* exposed to different concentrations of zinc showed lesions with varied intensities. Erosion of the gill filaments were observed in the treatments with high concentrations of zinc as exposure period increased. Severe mucosal congestion and a moderate to severe mucosal erosion of the gill filaments were observed in the gills of *C.gariepinus* among the treatments in the two experiments. Since gill is the first site of uptake of metals, its function gets reduced as a result of oxygen depletion which leads to necrosis of the filaments.

The kidneys showed alterations ranging from interstitial congestion to tubular degeneration and necrosis. Multiple foci of interstitial congestion, foci tubular degeneration and necrosis were observed in the kidney of *C. gariepinus* under chronic exposure to sub lethal zinc concentrations with and without *P. aquilinum*. These observations in the kidney of the *C.garipinus* in treatments with high level of Zn are similar to Javed (2003) who reported that in more severe cases, the degenerative process leads to tissue necrosis which promotes metabolic abnormalities in fish.

The most common lesions in the liver of the *C. gariepinus* under chronic exposure to sub lethal concentrations of zinc were vacuolar degeneration in the hepatocytes, focal area of necrosis, mild diffuse hydropic change in the hepatocytes and periportal fatty infiltration. These observations were similar to Ibrahem (2012). The livers of the fish in both experiments with and without *P. aquilinum* showed severe alterations, but damage was more severe in the treatments without *P. aquilinum*. The alterations in the livers could be due to impaired uptake of oxygen by the gills. This is in agreement with the observations of Kori-Siakpere *et al.,* (2006) who stated that oxygen deficiency as a result of gill degeneration is the cause of the cellular degeneration in the liver.

Conclusion

Sub lethal and chronic exposure of *C. gariepinus* to zinc in aqueous medium did not result in mortality but resulted in changes to organs in spite of uptake by *P. aquilinum.* Lower concentrations of zinc in water tended to produce higher concentrations in fish over time. This tended to agree with the notion that phytoremediation, though a green technology, requires ample time to function optimally.

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