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Patrick Ozovehe Samuel

Fisheries and Hydrobiology
Unit, Department of Animal
Biology, Federal University of
Technology, Minna, Niger State,
Nigeria

Francis O Arimoro

Fisheries and Hydrobiology
Unit, Department of Animal
Biology, Federal University of
Technology, Minna, Niger State,
Nigeria

Adesola V Ayanwale

Fisheries and Hydrobiology
Unit, Department of Animal
Biology, Federal University of
Technology, Minna, Niger State

Hadiza L Mohammad

Department of Biochemistry,
Federal University of
Technology, Minna, Niger State,
Nigeria

Corresponding Author:

Patrick Ozovehe Samuel

Fisheries and Hydrobiology
Unit, Department of Animal
Biology, Federal University of
Technology, Minna, Niger State,
Nigeria

Remediative roles of vitamins A, C and E on some growth parameters of *Clarias gariepinus* (Burchell, 1822) fingerlings exposed to lead nitrate in semi-static system

Patrick Ozovehe Samuel, Francis O Arimoro, Adesola V Ayanwale and Hadiza L Mohammad

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Abstract

Persistent environmental pollutants over the years have had deleterious effects on aquatic biota and by extension other living organisms on earth. The present study attempted to determine the roles of some vitamins in reducing the effects of Lead (Pb) in terms of some observed differences in the growth parameters of *Clarias gariepinus*. *C. gariepinus* fingerlings (whose initial weight ranged from 3 to 11g) were subjected to sub-lethal concentrations of Pb (00, 26mg/L, 44mg/L, 61mg/L and 79mg/L). Vitamins A, C and E at 26mg/L in each case were administered across all bud, respectively. The growth parameters measured every 7th day for a period of 12 weeks were standard lengths (SL), total lengths (TL) and weight. The following treatment groups were used: Pb (Pb only), PbVA (Pb+vitamin A), PbVC ((Pb+vitamin C) and PbVE (Pb+vitamin E) with four treatments (T₁-T₄) and replicates in each case. The specific growth rate (SGR), weight gain (WG) and percentage weight gain (% WG) were calculated from the weight. From the results: The highest SL in the Pb only, PbVA, PbVC and PbVE treatments were 14.90cm, 12.60cm, 12.30cm and 19.40cm, respectively. The highest TL in Pb only, PbVA, PbVC and PbVE, respectively were 17.60cm, 14.10cm, 13.90cm and 22.30cm. The highest weight value in the Pb only, PbVA, PbVC and PbVE were 36.09g, 21.64g, 23.48g and 83.26g, respectively. T₁ and T₃ had the highest %WG and SGR in comparison to the control in both Pb only and PbVE treatment groups. In the PbVE there was general improvement in weight values in all treatments. The outcome of this research suggests that vitamin E can attenuate the effects of Pb toxicant and out-perform the un-exposed samples; hence, optimized concentration of the vitamin can serve as remedy in heavy Pb intoxication.

Keywords: ameliorative roles, lead treatment groups, length-weight relationship, morphometric parameters, vitamins, weight derivatives

1. Introduction

The African cat fish, *Clarias gariepinus* is a tropical hardy species belonging to the Phylum Chordata, class Actinopterygii and family Clariidae. *Clarias* species is a widely distributed fish in Asia and Africa. In these areas, the fish is extremely popular on account of its tasty flesh, its unparalleled hardness, its rapid growth and its somewhat acceptable market price [1]. In Nigeria, *Clarias* species is an indigenous fish occurring in freshwater throughout the country. It is known that apart from tilapia, *Clarias* is the most abundant cultivated fish species in Nigeria [1]. The common species found are *Clarias gariepinus*, *Clarias anguillaris*, *Clarias buthupogon* and *Clarias lazera* [2].

Trace elements are either essential or non-essential. Heavy metals such as Fe, Cu, Zn, Ni, Co, Cr, and Mn are vital to human only at lower concentrations, but they become more toxic when they are taken up more than the bio-recommended limits [3]. It is also known that even essential metals may be toxic on the biological activities of organisms above certain concentrations [4].

Fish are particularly vulnerable and heavily exposed to pollutants due to feeding and living in aquatic ecosystems, because they cannot avoid pollutant harmful effects [5]. Heavy metals enter fish by direct absorption from water through their gills and skin, or by ingestion of contaminated food [6].

The dearth of information on the effects of Pb as well as the supplemented treatments with vitamins on the growth parameters of *Clarias gariepinus* as morphological manifestations of the physiological changes taking place in the organism due to the presence of the toxicant and vitamins necessitated this investigation. Previous study has shown that vitamin E can improve daily food intake, body weight gain and feed efficiency ratio [7]. It has also been shown that Cd and Pb can bioaccumulate in the head capsule and body muscle of *C. gariepinus* exposed to paint emulsion effluents which showed that fish can bioaccumulate these metals from polluted environment; culminating in the reduction or impairment of natural population size and could be sources of these metals to man [8] with deleterious effects over a long period of time since, among all the heavy metals, Cd, arsenic, mercury and lead pose highest degree of toxicity and that is of great concern to plants and human health [9]. This study therefore, determined the effects of the specific toxicant of interest (Pb) on some growth parameters of *C. gariepinus* and to what extent such effects can be abated or reduced in the presence of vitamin supplements at varying concentrations over a period of time.

2. Materials and Methods

2.1 Samples/materials collection and Acclimatization

A total number of six hundred and fifty (650) fingerlings of *Clarias gariepinus* were purchased from a commercial fish farmer and transported in 50L containers filled with water to the Old Farm Research Unit of the Department of Water, Aquaculture and Fisheries Technology, Bosso Campus, Federal University of Technology, Minna, Nigeria. The fishes were placed in fish ponds with water for acclimatization. The fishes were fed twice daily (morning_0800hr and evening_1600hr) with Blue Crown feed (3mm) for 14 days (2 weeks) period of the acclimatization. The holding water was changed every 2-3 days during the period. The vitamins A, C and E granules or pellets were purchased from commercial chemical stores. The toxicant, Pb(NO₃)₂ (500g) analar grade was purchased from commercial chemical stores and stored in a cool dry condition throughout the period of the experiment. This toxicant was administered according to the sub-lethal concentrations of the treatments during the chronic phase of the exposure [2].

2.2 Experimental Set-up

Five treatments including control with two replicates in each treatment were set-up for the Pb, Vitamin A, C and E; and the sub-lethal exposures (00 as Control, 26mg/L as T₁, 44mg/L as T₂, 61mg/L as T₃ and 79mg/L as T₄, respectively) were run for a period of twelve (12) weeks. The vitamin supplements (in each case) were taken as the lowest concentration of the toxicant and administered uniformly in every treatment. The first group of treatments was tagged Pb (Pb only with T₁-T₄ and replicates), second PbVA (Pb+vitamin A with T₁-T₄ and replicates), third PbVC ((Pb+vitamin C with T₁-T₄ and replicates) and fourth PbVE (Pb+vitamin E with T₁-T₄ and replicates).

2.3 Determination of Growth Parameters of *C. gariepinus* subjected to sub-lethal concentration of lead nitrate

2.3.1 Standard length

At every sampling day two randomly selected specimens were taken from each of the sub-lethal concentration exposure and replicate including the control for the determination of the

standard length in centimetres. The standard length was determined from the end of the mouth (face bone) to the posterior lobe (caudal peduncle) using a metre rule graduated in centimetres.

2.3.2 Total length

The total length of two randomly selected fish samples from each treatment and replicate including the control were taken at each sampling day of the chronic exposure. The total length was measured from the tip of snout to the end of the tail fin using metre rule graduated in centimetres and the average was taken in each case.

2.3.3 Weight

The weight of the fish was recorded using the electronic battery powered weighing balance (Digital Pocket Scale, 2*PCS, 3V AAA batteries). The fish was weighed in gram by placing them inside a basket whose weight has been taped to zero. The weight of the fish sample was determined from two randomly selected specimens from each treatment and replicate including the control. The specimen was weighed separately and the average taken to represent each treatment and replicate at each sampling day. From the measurements the following parameters were determined:

2.3.3.1 Weight gain

The weight gain (WG) was calculated as the difference between the final weight of fish and the initial weight in grams after the 12 weeks of exposure.

2.3.3.2 Percentage weight gain (%)

The Percentage weight gain was calculated thus:

$$\text{Weight gain (\%)} = \frac{\text{Final body weight} - \text{initial body weight}}{\text{Initial body weight}} \times 100$$

2.3.3.3 Specific growth rate

The specific growth rate (SGR) was calculated using the formula;

$$\text{SGR} = \frac{(\ln W_2 - \ln W_1)}{(T_2 - T_1)} \times 100$$

Where W₁ = initial weight, W₂ = Final weight, T₂-T₁ = Number of days of exposure.

2.4 Data Analysis

Length-Weight Relationship was determined using regression analysis and one way analysis of variation (graphically represented) of the length and weight measurements of the samples in all the treatments.

3. Results

3.1 Growth parameters of *C. gariepinus* exposed to sub-lethal concentrations of Pb toxicant and their respective supplemented treatments with Vitamins A, C and E

3.1.1 Standard Length of *C. gariepinus* exposed to sub-lethal concentrations of Pb toxicant and their respective supplemented treatments with Vitamins A, C and E

The standard length of the control samples ranged from 8.90cm to 13.40cm. The highest standard length in the Pb treatments was recorded in T₁ with 14.90cm at the 12th week of exposure. On the other hand, the lowest value was obtained

in T₂ with 7.50cm after the 1st week of exposure to the toxicant. There were slight gradual increases in length from week 1 to 12 in T₁. (Figure 1).

The highest standard length in PbVA (Pb treatments supplemented with vitamin A) was obtained in T₄ with 12.60cm while the lowest was recorded in T₂ at week 1 with 7.0cm. However, no particular trend was established as there were decreases or increases at one point or the other. (Figure 2).

The maximum standard length obtained in PbVC (Pb treatments supplemented with vitamin C) was 12.30cm in T₁ at the 10th week of exposure while the minimum was 7.50cm

recorded in T₄ at the 7th week of exposure. (Figure 3).

Unlike in other treatments above, there were marked increases in the standard length with improved growth throughout the PbVE (Pb treatments supplemented with vitamin E) when compared to the control samples. The marked improvements were recorded in T₁. The highest standard length was recorded in T₁ with 19.40cm while the lowest was recorded in T₃ with 7.20cm at the 12th week. There were general improvements in the standard length in all the treatments at the 9th week; while the same improvements were also recorded at week 12 in T₁-T₃. These improvements were significantly higher in T₁ than other treatments. (Figure 4).

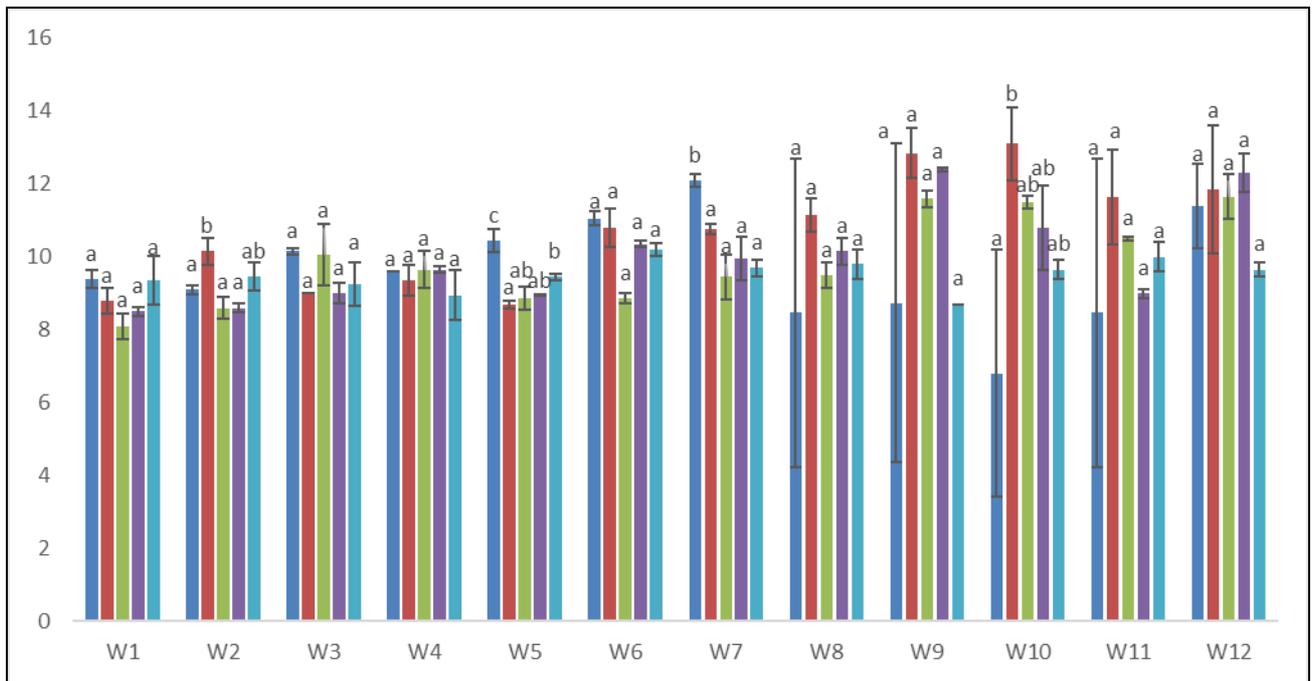


Fig 1: Mean values of standard lengths of *C. gariepinus* exposed to sub-lethal concentrations of Pb for a period of 12 weeks

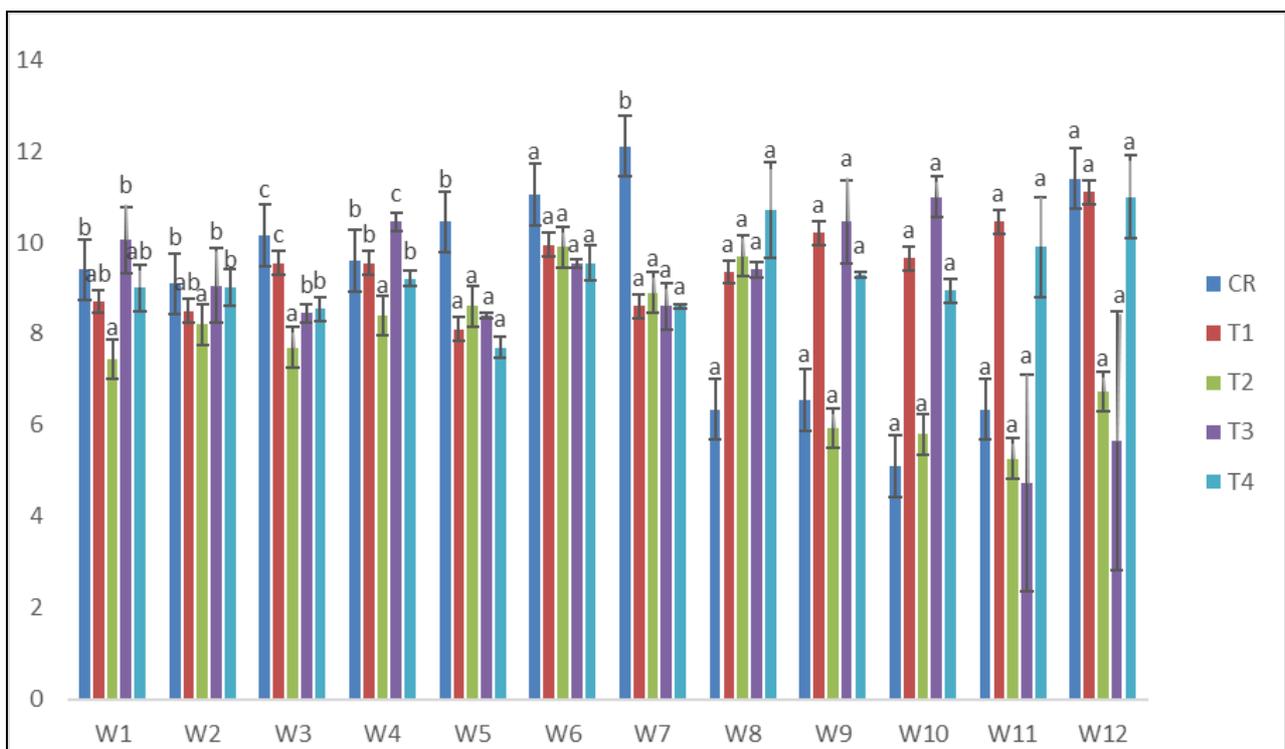


Fig 2: Mean values of standard lengths of *C. gariepinus* exposed to sub-lethal concentrations of Pb supplemented with vitamin A for a period of 12 weeks

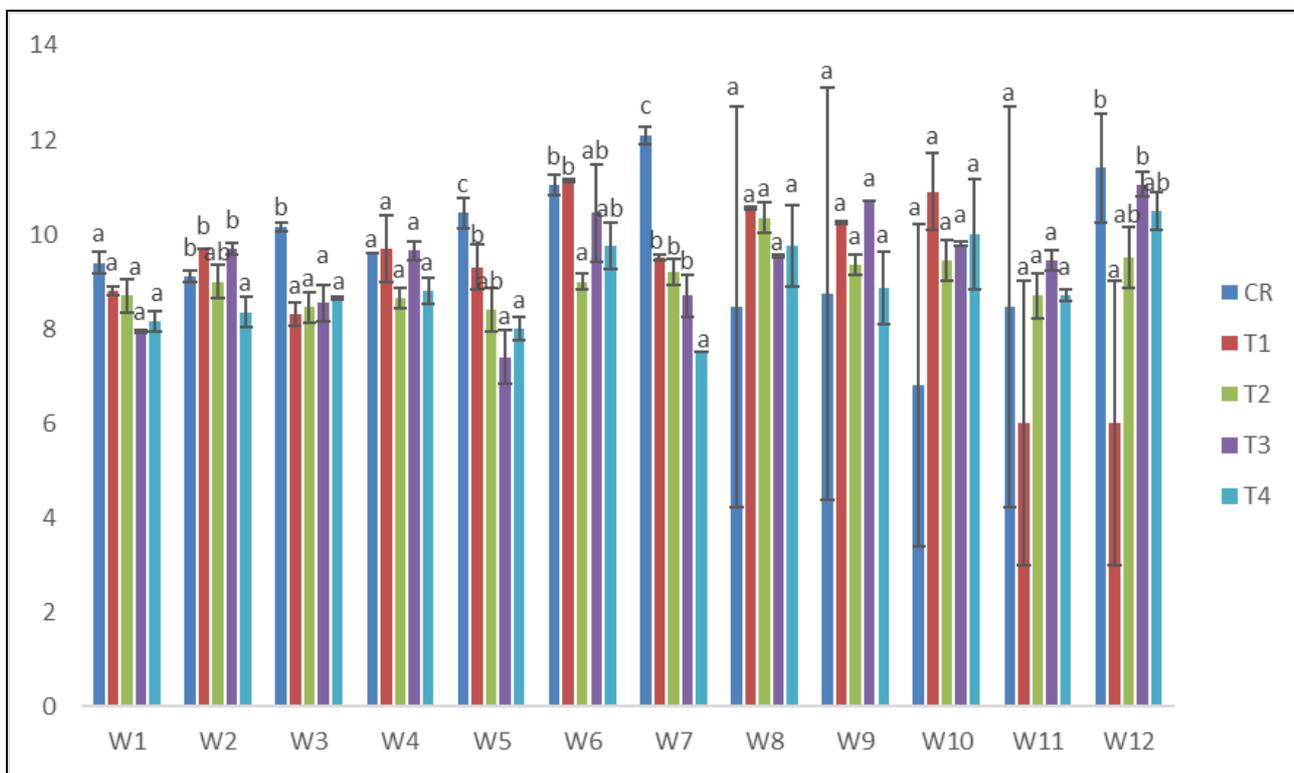


Fig 3: Mean values of standard lengths of *C. gariepinus* exposed to sub-lethal concentrations of Pb supplemented with vitamin C for a period of 12 weeks.

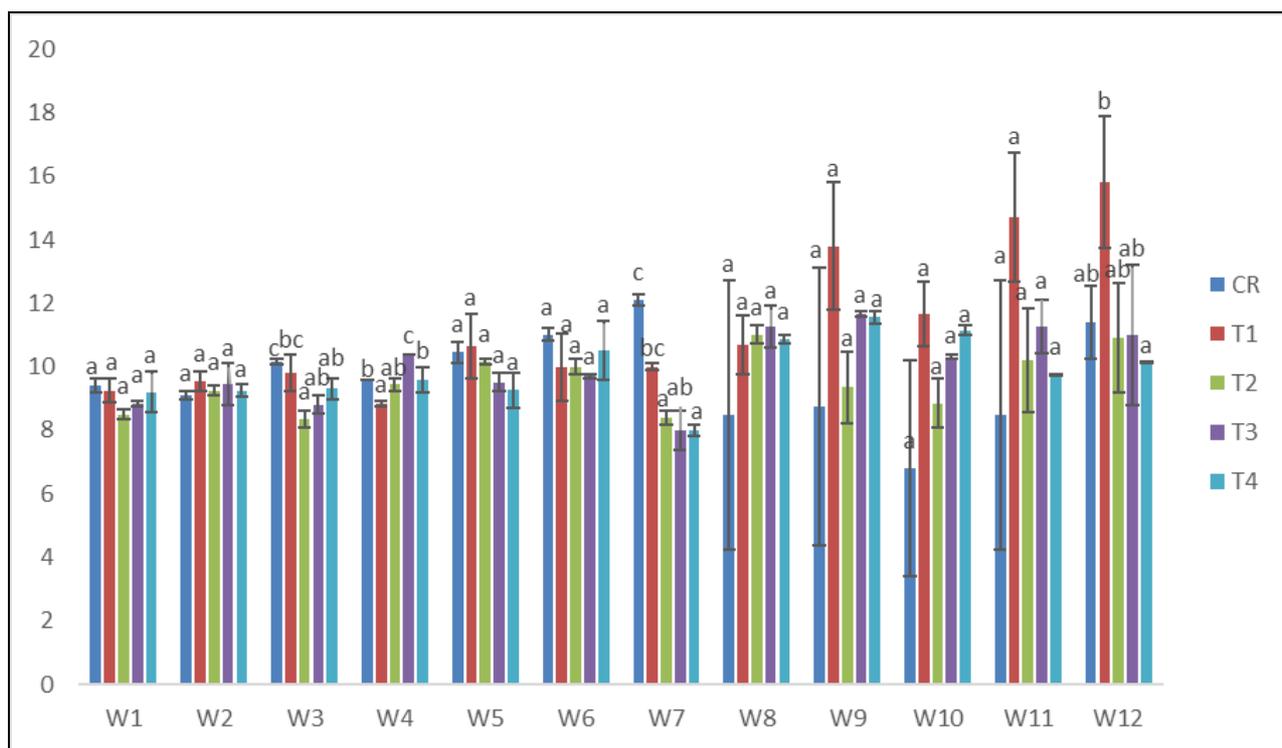


Fig 4: Mean values of standard lengths of *C. gariepinus* exposed to sub-lethal concentrations of Pb supplemented with vitamin E for a period of 12 weeks

3.2 Total Length of *C. gariepinus* exposed to sub-lethal concentrations of Pb toxicant and their respective supplemented treatments with Vitamins A, C and E

The control samples had 14.90cm as the highest total length at the 9th week while the lowest was recorded in the 1st week after exposure with 9.80cm. There were general increases in total length which were higher than what was observed in the control. The highest total length of 17.60cm was recorded in

T₁ at the 12th week; while the lowest was recorded in T₂ with 8.30cm at the 1st week after exposure. (Figure 5).

The highest total length obtained in PbVA was 14.10cm in T₄ at the 12th week of exposure; while the lowest was recorded in T₂ at the 1st week after exposure with 6.90cm. There was gradual increase in the total length from week 1 to week 12 in T₁. (Figure 6).

The lowest value recorded in PbVC treatments was 8.20cm in

T₃ at the 5th week; while the highest total length was 13.90cm in T₁ at the 10th week after exposure to the toxicant and the supplement. (Figure 7).

There were general improvements in all the treatments exposed to PbVE treatments. There was increase in the total

length from week 1 to 12 in treatments T₁-T₃. The highest total length was recorded in T₁ at the 12th week of exposure with 22.30cm which was significantly higher than other treatments; while the lowest was recorded in T₃ at the 12th week with 8.40cm. (Figure 8).

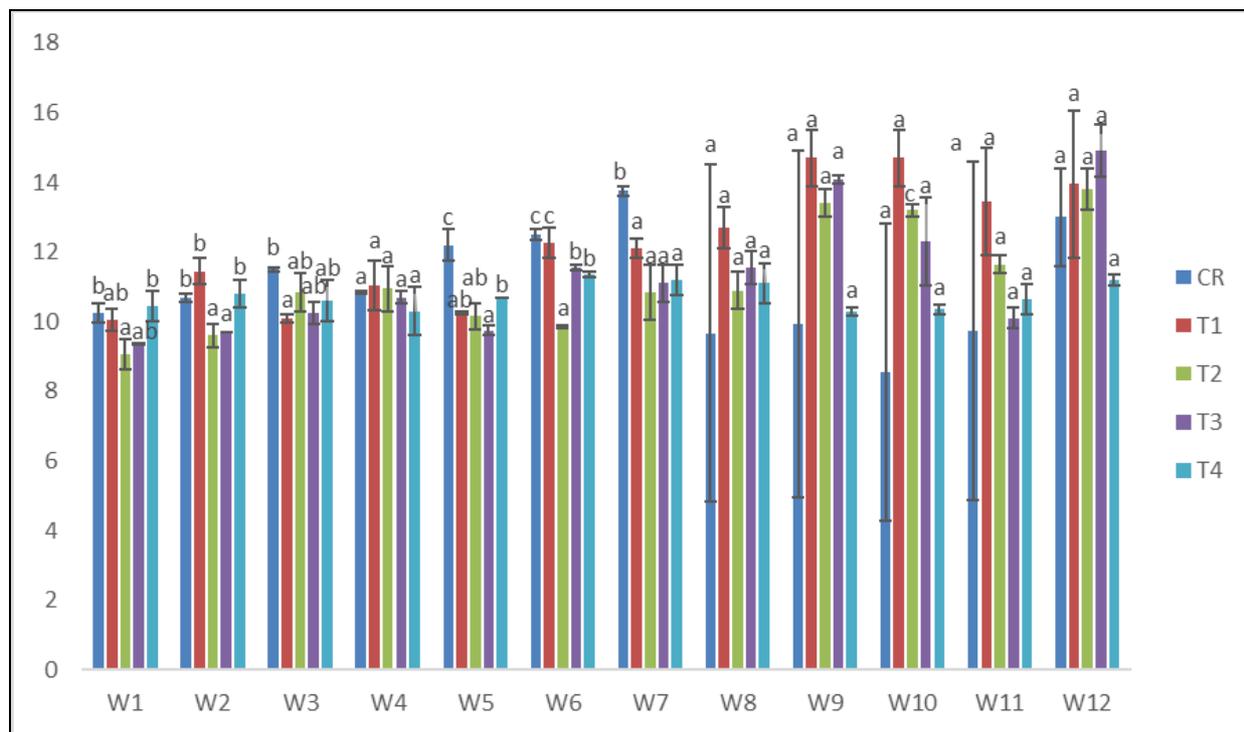


Fig 5: Mean values of total lengths of *C. gariepinus* exposed to sub-lethal concentrations of Pb for a period of 12 weeks

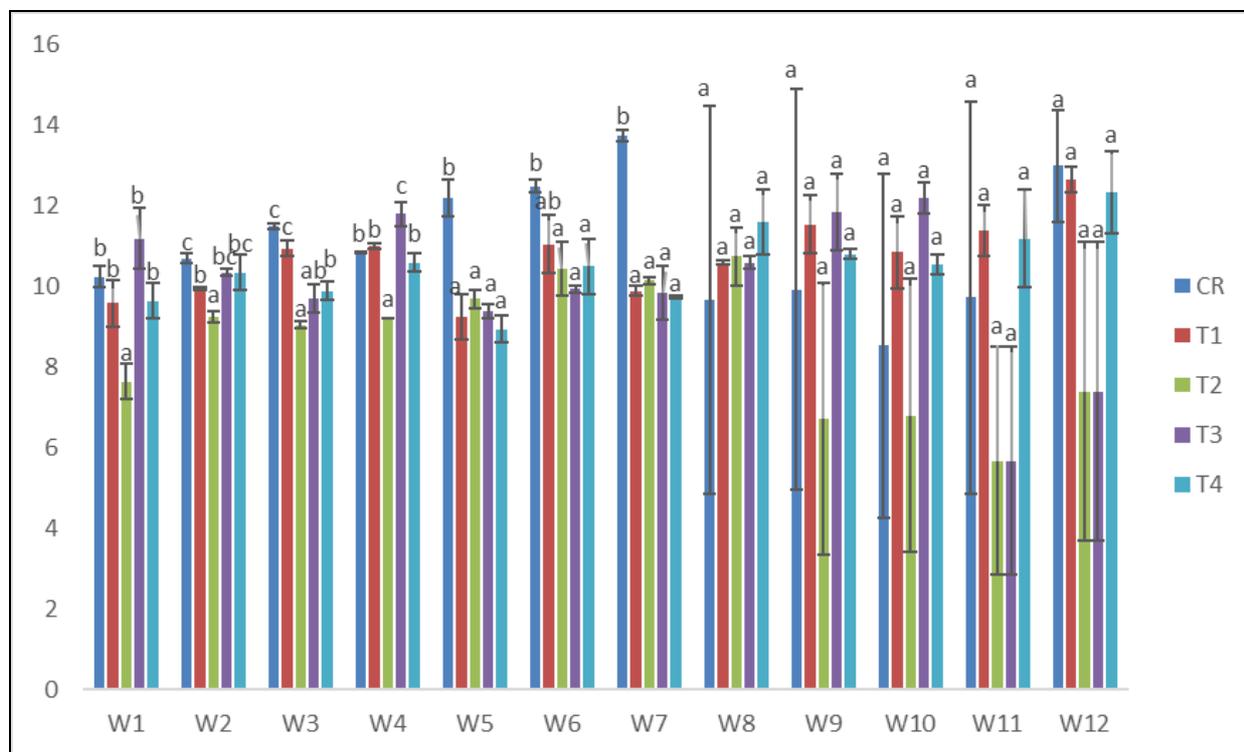


Fig 6: Mean values of total lengths of *C. gariepinus* exposed to sub-lethal concentrations of Pb supplemented with vitamin A for a period of 12 weeks

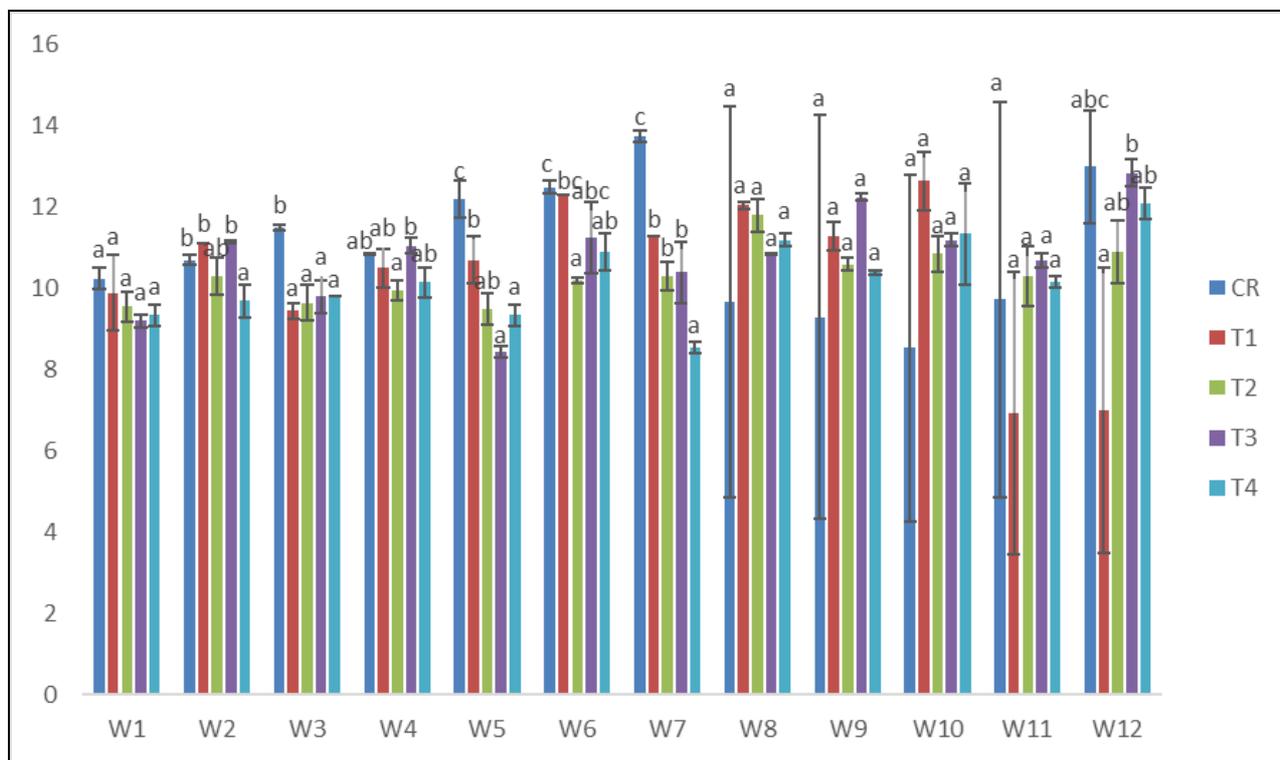


Fig 7: Mean values of total lengths of *C. gariepinus* exposed to sub-lethal concentrations of Pb supplemented with vitamin C for a period of 12 weeks

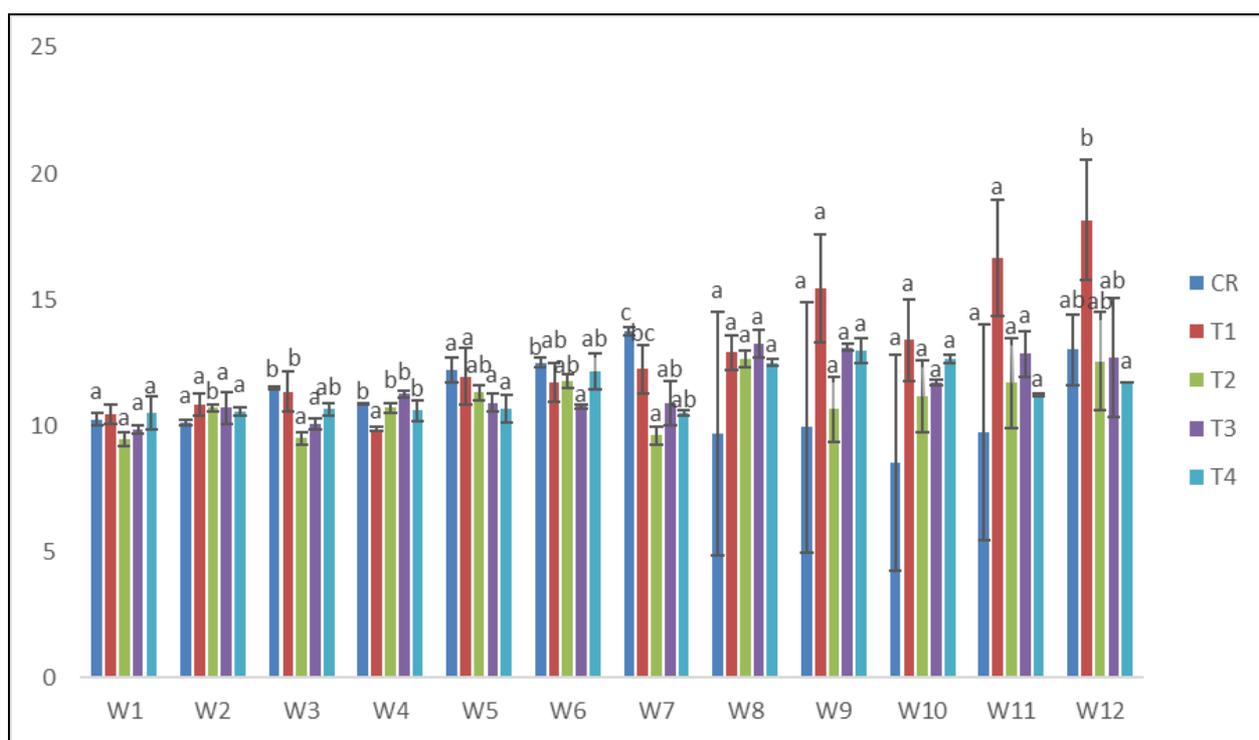


Fig 8: Mean values of total lengths of *C. gariepinus* exposed to sub-lethal concentrations of Pb supplemented with vitamin E for a period of 12 weeks

3.3 Weight parameters of *C. gariepinus* subjected to sub-lethal concentrations of Pb toxicant and their respective supplemented treatments with Vitamins A, C and E

The weight of the control samples ranged from 8.22g to 41.72g. There was general slight increase in weight values in T₁ of the samples exposed to Pb treatments from week 1 to 12. The highest weight value was 36.09g recorded at week 12 in T₁ while the lowest was 5.32g after the 1st week of exposure in T₂. T₁ and T₃ had the highest percentage weight

gain and specific growth rate when compared to the control. (Table 1 and Figure 9).

The highest weight value in samples exposed to PbVA treatments was measured from T₄ samples with 21.64g at the 8th week of exposure; while the lowest weight of 3.91g was recorded in T₂ after the 1st week of exposure. Contrary to what was obtained in Pb treatments, there was negative percentage increase in weight (%WG) and low specific growth rate (SGR) (Table 2 and Figure 10).

The maximum weight obtained in samples exposed to PbVC treatments was 23.48g in T₁ at the 10th week; while the lowest was recorded in T₂ after the 1st week of exposure with 3.84g. The highest%WG and SGR were recorded in T₃ treatments. (Table 3 and Figure 11).

In the samples exposed to PbVE there was general improvement in weight values in all treatments with marked

growth in T₁-T₃ with exceptional performance in T₁. The highest weight gain of the samples was recorded in T₁ (with 40.07g) at the 12th week with the value of 83.26g; while the lowest was recorded in T₂ at the 12th week also with 5.02g. There were also increased%WG and SGR in T₁ and T₃, respectively. (Table 4 and Figure 12).

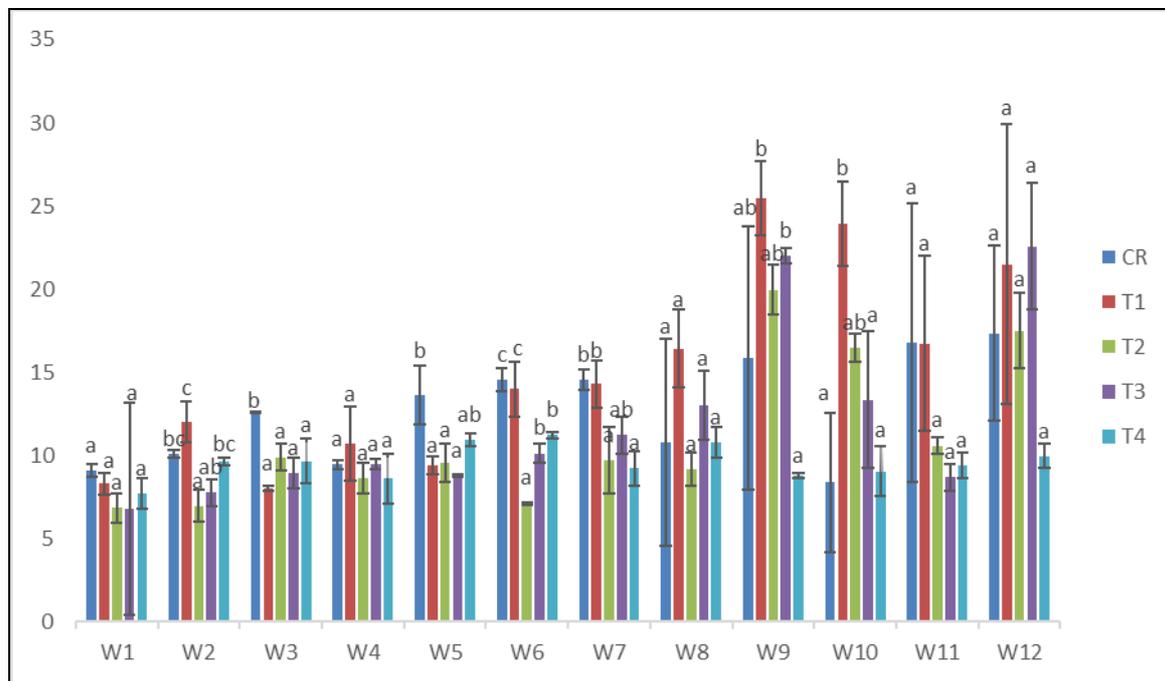


Fig 9: Mean values of weight of *C. gariepinus* exposed to sub-lethal concentrations of Pb for a period of 12 weeks

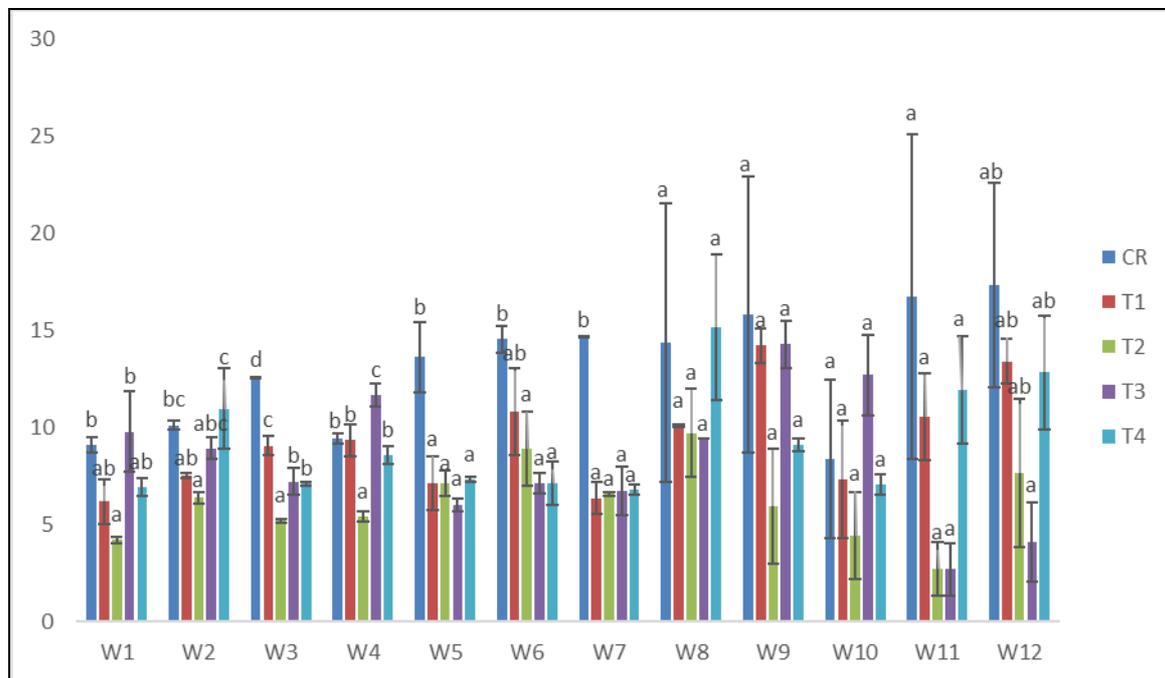


Fig 10: Mean values of weight of *C. gariepinus* exposed to sub-lethal concentrations of Pb supplemented with vitamin A for a period of 12 weeks

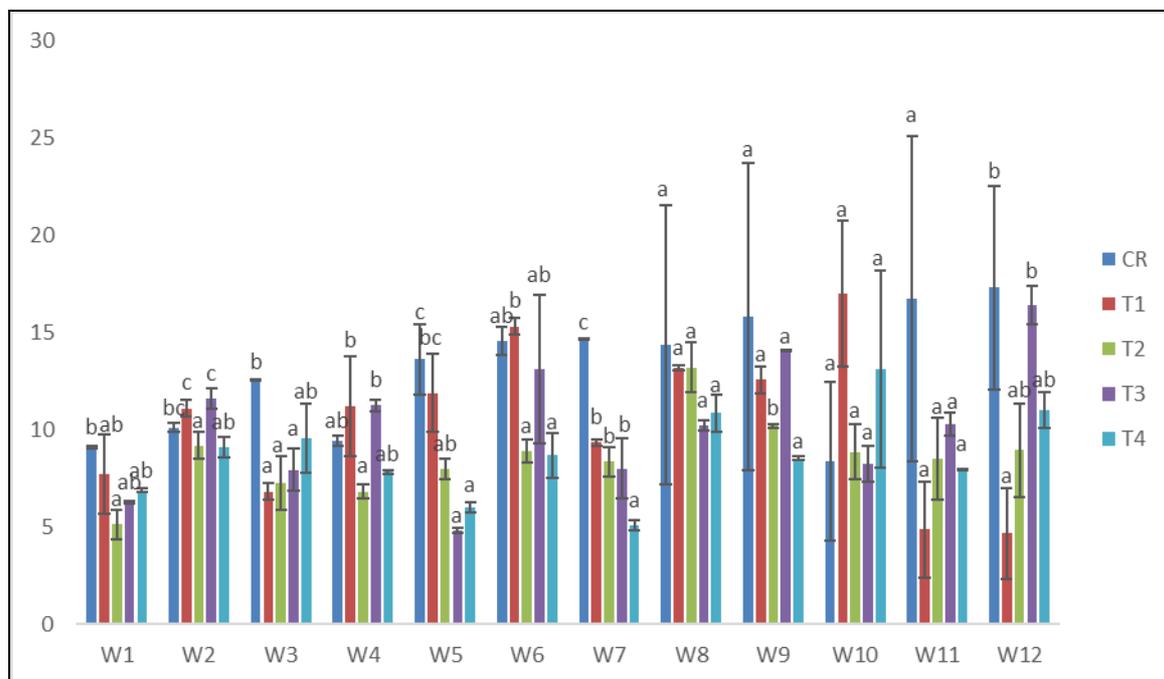


Fig 11: Mean values of weight of *C. gariepinus* exposed to sub-lethal concentrations of Pb supplemented with vitamin C for a period of 12 weeks

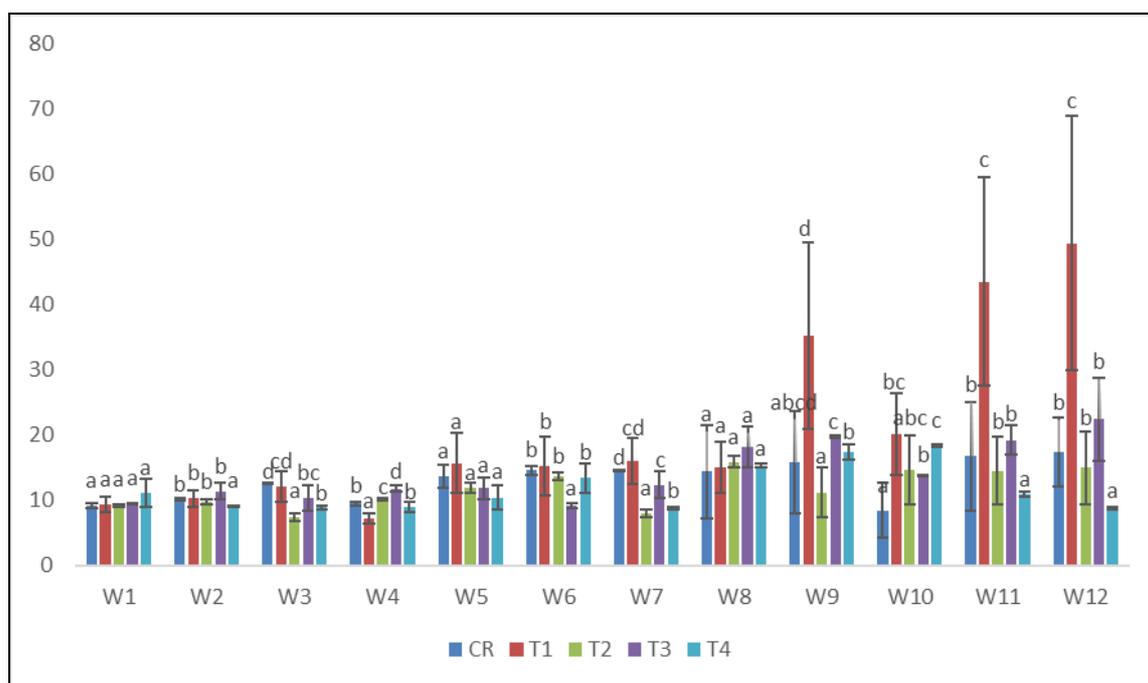


Fig 12: Mean values of weight of *C. gariepinus* exposed to sub-lethal concentrations of Pb supplemented with vitamin E for a period of 12 weeks

Table 1: Weight derivatives of samples of *C. gariepinus* exposed to sub-lethal concentrations of lead nitrate for a period of 12 weeks

Treatments	Final Weight	Initial Weight	Weight gain	%Weight gain	Specific Growth Rate (g/day)
C	17.33	9.10	8.23	90	3.23
T1	21.51	8.30	13.21	159	3.53
T2	17.49	6.84	10.65	156	3.29
T3	22.57	6.76	15.81	234	3.60
T4	9.95	7.68	2.27	30	2.46

Table 2: Weight derivatives of samples of *C. gariepinus* exposed to sub-lethal concentrations of lead nitrate supplemented with vitamin A for a period of 12 weeks

Treatments	Final Weight	Initial Weight	Weight gain	%Weight gain	Specific Growth Rate (g/day)
C	17.33	9.10	8.23	90	3.23
T1	13.43	6.18	7.25	85	2.92
T2	11.51	4.20	7.31	57	2.75
T3	6.13	9.79	-3.66	-37	1.60
T4	12.85	6.90	5.95	86	2.85

Table 3: Weight derivatives of samples of *C. gariepinus* subjected to sub-lethal concentrations of lead nitrate supplemented with vitamin C for a period of 12 weeks

Treatments	Final Weight	Initial Weight	Weight gain	%Weight gain	Specific Growth Rate (g/day)
C	17.33	9.10	8.23	90	3.23
T1	7.01	7.72	-0.71	-09	1.91
T2	8.96	5.51	3.81	74	2.37
T3	16.44	6.27	10.17	162	3.19
T4	11.01	6.88	4.13	60	2.63

Table 4: Weight derivatives of samples of *C. gariepinus* subjected to sub-lethal concentrations of lead nitrate supplemented with vitamin E for a period of 12 weeks

Treatments	Final Weight	Initial Weight	Weight gain	%Weight gain	Specific Growth Rate (g/day)
C	17.33	9.10	8.23	90	3.23
T1	49.41	9.34	40.07	429	4.59
T2	14.95	9.20	5.75	62	3.02
T3	22.46	9.39	13.07	139	3.58
T4	8.72	11.15	-2.44	-22	2.19

3.4 Length-Weight Relationships of *Clarias gariepinus* exposed to sub-lethal concentrations of Pb toxicant for a period of 12 weeks

The relationships between length and weight of *C. gariepinus*

were linear throughout the period of exposure in Pb treatments. T₂ samples in Pb treatments had the highest R² values (0.6796). (Figures 13-16).

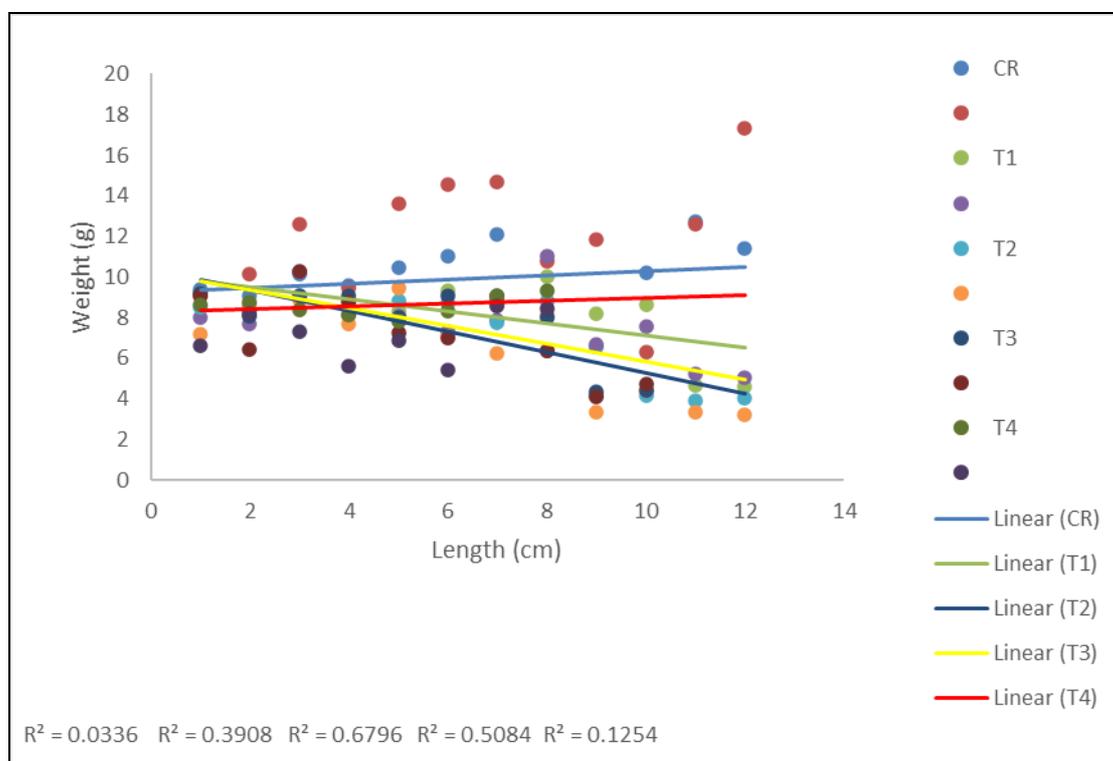


Fig 13: Length-Weight regression analysis of *C. gariepinus* exposed to sub-lethal concentration of lead (Pb treatments) for a period of 12 weeks

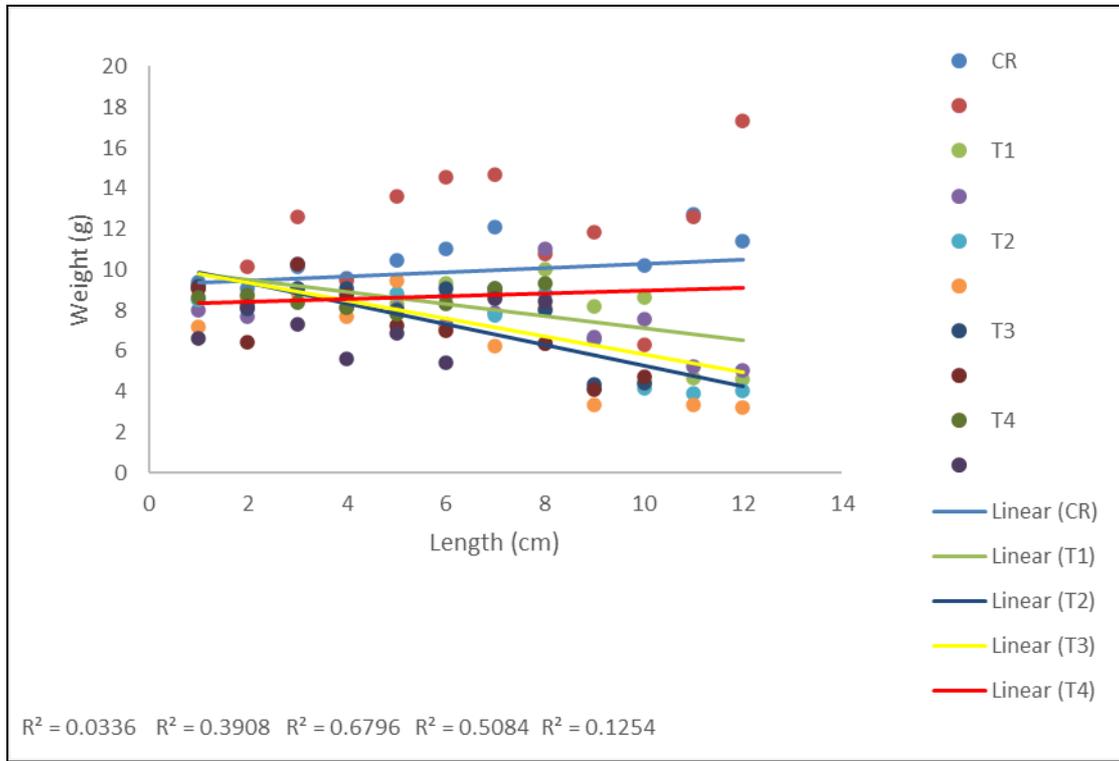


Fig 14: Length-Weight regression analysis of *C. gariepinus* exposed to sub-lethal concentration of lead supplemented with vitamin A (PbVA treatments) for a period of 12 weeks

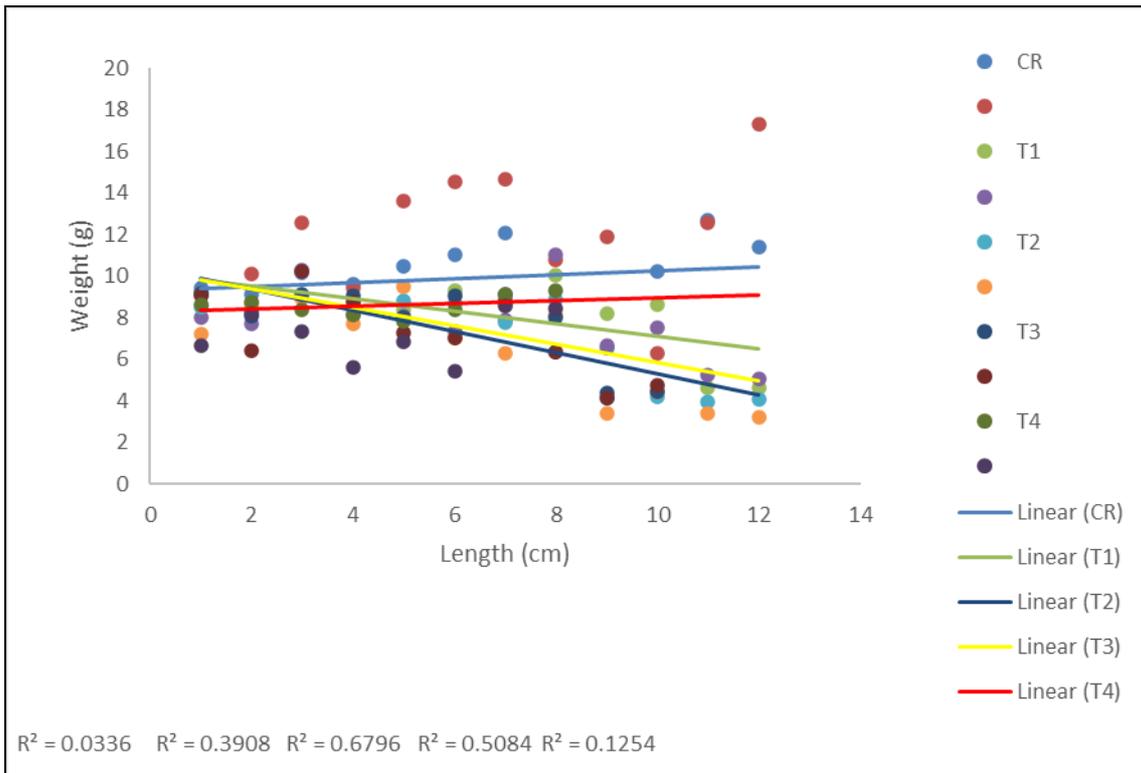


Fig 15: Length-Weight regression analysis of *C. gariepinus* exposed to sub-lethal concentration of lead supplemented with vitamin C (PbVC treatments) for a period of 12 weeks

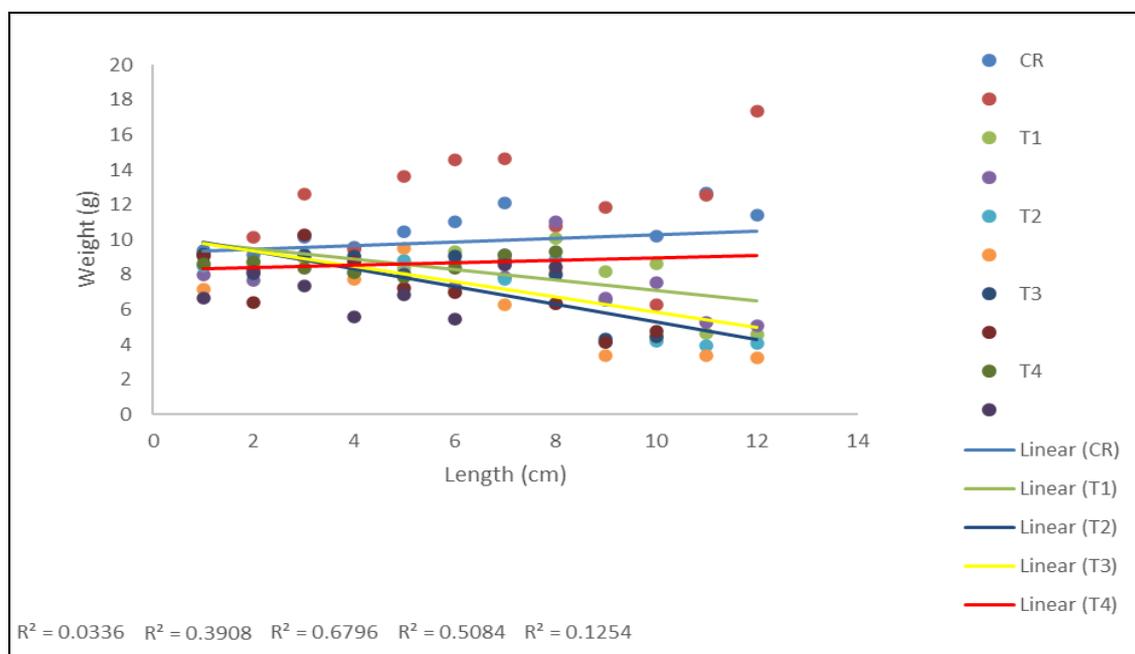


Fig 16: Length-Weight regression analysis of *C. gariepinus* exposed to sub-lethal concentration of lead supplemented with vitamin E (PbVE treatments) for a period of 12 weeks

3.5 Discussion

3.5.1 Growth parameters of *C. gariepinus* exposed to sub-lethal concentrations of Pb toxicant and their respective supplemented treatments with Vitamins A, C and E

The marked improvement in standard lengths of samples exposed to PbVE treatments in comparison to other treatments with the peak at T₁ (19.4 cm) probably buttresses the capacity of the vitamin to ameliorate the effects of the toxicant such that the fishes in this particular treatment performed better than even the samples of the control. In a related development, Osfor *et al.* [7] demonstrated that vitamin E could improve daily food intake, body weight gain and feed efficiency ratio. Similarly, Kadry *et al.* [10] showed that, fish fed diet supplemented with Vitamin E exhibited protective effects by minimizing the atrazine induced toxicity on female *Clarias gariepinus*, to the extent that the values obtained in other treatment were more or less similar to control group fish. Furthermore, the administration of vitamin E to lead-exposed fish has been reported to prevent the bioaccumulation of lead in tissues, and enhance the growth factor of fish [11].

In the samples exposed to PbVE there was general improvement in weight gain in all treatments with marked growth in T₁-T₃ with exceptional performance in T₁. The highest weight gain of the samples was recorded in T₁ at the 12th week with 83.26g. These marked increases in weight gain, %WG (429%) and SGR (4.59g/day) were in sharp contrast to what were obtained in the PbVA and PbVC treatments. This probably buttress the effectiveness of vitamin E in neutralizing or at best attenuating the effects of the toxicant, and consequently, culminating in improved growth. It is also likely that the vitamin serves as a nutrient booster for the survival of the fish as evident in T₁ with the lowest concentration of the toxicant. In a related development, Samuel *et al.* [2] reported how vitamin E ameliorated the effects of cadmium toxicant on *Clarias gariepinus* exposed to it but not as effective as the case of Pb toxicant probably because Cd was more deleterious. Trace elements (especially lead) in high concentration are naturally deleterious and are capable of eliciting myriads of physiological effects which are sometimes manifested physically. For instance, the effects of

Cd (100 µg/L) on the embryonic, larval or both stages of the ide (*Leuciscus idus*) showed that mortality rate, body size, various body morphometrics and deformities (vertebral curvatures and yolk sac deformities) were affected; and that the highest weight gain was found in control whereas the lowest weight gain was observed in the treatment with the highest concentration of the toxicant [12]. Ayegbusi *et al.* [13] also reported growth performance decline and obtained SGR value of 1.95±0.015mg/L when *Clarias gariepinus* samples were exposed to sub-lethal concentrations of PbCl₂ for a period of 21 days. In like manner, there was reduced fish growth when Nile Tilapia was subjected to various concentrations of Cd [14]. In addition to this, Oluwatosin *et al.* [15] reported that the sub-lethal exposure showed reduction in growth with an increase in PbCl₂ concentration and the specific growth rate in the control was higher than other treatments with PbCl₂. In another development, Han *et al.* [16] found that growth and hematological parameters determined decreased with increasing arsenic concentration, while the concentration of plasma components measured increased. Also, fish exposed to 10, 50 and 100µgHg/L showed a significant decrease in growth rates as from days 14 to 35 [17]. This was attributed to utilization of energy to overcome physiological stress induced by the toxicant, thus affecting fish growth. As the duration increases, there was lethargy and loss of appetite. This probably accounted in part the lack of weight gain in the later stages of the experiment as emaciation of the samples set in after utilizing the available energy to overcome stress. Consistent with this, Puvaneswari and Karuppasamy [18] posited that length of time of exposure affected the sensitivity of fish larvae and influenced the weight gain in *Heteropneustes fossilis* exposed to Cd. This could also be due to the fact that, growth reduction under metal contamination increased the energy costs due to increased metabolism [19]; and growth inhibition being a prominent effect of metal accumulation following chronic exposure [20].

Conclusions and Recommendations

The samples of *C. gariepinus* subjected to the sub-lethal

concentrations of lead nitrate elicited varying responses in the different treatment groups. The treatment groups that were supplemented with the vitamins displayed different levels of ameliorations of the deleterious effects of the toxicant especially in the lower concentrations at the end of the 12 weeks of exposure in terms of the standard and total lengths as well as the weight of the samples.

Amongst all the treatment groups, PbVE group recorded marked improvements especially in T₁ in terms of standard length (19.40cm), total length (17.60cm) and weight (83.26g). These improvements were significantly higher in T₁ than other treatments. There were also increased%WG and SGR in T₁ and T₃, respectively. The relationships between length and weight of *C. gariepinus* were linear throughout the period of exposure in Pb treatment groups. T₂ samples in Pb treatments had the highest R² values (0.6796) depicting how length and weight are interdependent.

The out-come of the research establishes the impacts of the vitamins in ameliorating the effects of the toxicant in the immediate environment of the fish. Therefore, the administration of the vitamins should be considered as a remedy in dealing with heavy metals intoxication given the fact that the responses are concentration and duration dependent both in terms of the toxicant and vitamins.

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