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Elemental and Proximate Analyses of Aquatic Animal Species Obtained in Ishiet River, Nigeria

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ABSTRACT

Concentrations of some metallic elements (Mg, Ca, K, Zn, Fe) and proximate compositions of two crayfish species (*Astacoides parastacoidea* and *Macrobrachium rosenbergii*) and two periwinkle species (*Pachymelania aurita* and *Tympanotonus fuscatus*), obtained in Ishiet River, Uruan, Nigeria, were analysed using standard procedures. Results from the proximate analyses revealed that the aquatic animals contain appreciable levels of the investigated parameters. In crayfish, the results varied from 0.25% ash in both species to 88.05% moisture in *A. parastacoidea.* In periwinkle, they varied from 0.10% ash in both species to 74.00% moisture in *P. aurita.* High positive correlation (r = 0.92) at p = 0.05 was observed between the proximate compositions in crayfish species. Correlation was perfect (r = 1) in periwinkle species. Results for the elemental analyses revealed that the essential elements (Mg, Ca, K) were higher in concentrations than the trace metals (Zn, Fe) in all species. The metals were perfectly correlated in crayfish species. Similar correlation was observed between the metals in periwinkles. The concentrations of Zn and Fe in each of the aquatic animals were below the levels that could cause toxicity in humans. In conclusion, the two aquatic animals species obtained in Ishiet are suitable for human consumption.

Keywords*:* Crayfish, periwinkles, proximate composition, metallic elements, Ishiet, Uruan.

INTRODUCTION

Aquatic animals are beloved delicacies, nutrient-rich and serve as good sources of protein, vitamins and minerals. They are rich in omega-3-fatty acids and useful in the reduction of common diseases (Andrew *et al*., 2016). Eating aquatic animals on a regular basis had been proven to ease the symptoms of arthritis, help preserve moisture in the skin, aid in reducing preterm deliveries and essential for central nervous system development (Mohapatra *et al*., 2009). Periwinkles and crayfish are among the popular aquatic animals consumed by the people living in the coastal region of Nigeria. Apart from being major delicacies, they equally serve as sources of economic empowerment for the people in the area. Although, periwinkles and crayfish are marine dwellers, they immigrate into the brackish water systems during their post-larval stages, grow fast and attain maturity in waters and lagoons. Their exposures to both marine and estuarine environments have effects on the body due to metal bioavailability from the natural as well as anthropogenic set up of the surroundings (Mohapatra *et al*., 2009). Fish is a valuable component of the human diet because it is easily digestible and contains high quality protein that provides a mixture of essential amino acids that our bodies cannot make themselves and must be supplied through the food. Globally, aquatic animal provides more protein than cattle, sheep or poultry (Oken, 2016). It has been established that periwinkles contain lots of nutritional values essential for people of all ages (Ayisi, 2014). Metallic elements are natural constituents of freshwater environment, generally found in very low

concentrations. Human activity has inevitably increased the levels of metallic ions in many of these natural water systems (Eisler, 1991). Mine drainage, oil and gas exploration, industrial (pesticides, paints, leather, textile, fertilizers and pharmaceuticals) and domestic effluents, agricultural runoff, acid rain have all contributed to the increased of metal loads in waters.

Some metallic elements are essential for our existence. They are known as major elements. Examples of such essential elements include K, Ca and Mg (Alan, 2016). Three quarters of all the elements that exist in the environment are metals. It is not surprising that many of these metallic elements are found within the living body, though mostly only in small amounts (Alan, 2016). Some of these are described as heavy metals. They are vital for some body functions. They do not exist in the body as elemental metals but as metallic salts. Examples are Fe and Zn which play a very important role in keeping the body working effectively (Ehigiator *et al*., 2012; Alan, 2016).

The focus of nutritionists and dieticians are in foods that are functionally active and which at the same time contain enough food nutrients. Crayfish and periwinkles belong to this class of functionally active food. Unfortunately, these groups of animals are widely polluted in their habitats due to all kinds of anthropogenic activities and natural factors such as acid rain and indeed oil spillage which can lead to pollution of the water bodies due to water runoff. This in turn affects the aquatic animals adversely which act as one of the vehicles for transferring trace elements into the food chain. Pollution due to metals is one of the global problems which represent a growing threat to the environment. Heavy metals are also considered as the major substances affecting the state of health in man and aquatic animals by reason of the increase in the use of chemicals in water bodies.

Proximate analyses are done to estimate the relative amount of protein, lipid, ash, carbohydrate, fibre and moisture in materials such as animals and plants based on the chemical properties of such materials (Uwah *et al*., 2015). According to Erebor (1998), food is any substance that gives the body energy and parameters for growth and repairs of worn out parts. A good food is known to contain carbohydrate, protein, fat and oil (lipid), water, vitamins and minerals in correct proportion.

Periwinkles and crayfish are among the popular aquatic animals consumed by the people living in the coastal region of Nigeria. Data and information on the concentrations of metallic elements and proximate composition of crayfish and periwinkles obtained in Ishiet River are scare, so there is every need for this study. The objectives of this study are to quantify the concentrations of some metallic elements (Mg, Ca, K, Zn, Fe) and proximate compositions of two crayfish species (*A. parastacoidea* and *M. rosenbergii*) and two periwinkles species (*P. aurita* and *T. fuscatus*) obtained in Ishiet River in order to ascertain the suitability of the aquatic animals for human consumption.

MATERIALS AND METHODS

Samples Collection: Freshly caught crayfish (*A. parastacoidea* and *M. rosenbergii*) and periwinkles (*P. aurita*, and *T. fuscatus*) samples were bought directly from the fisher men by the Ishiet River in Uruan, Akwa Ibom State, Nigeria. The crayfish and periwinkles species were identified and authenticated in the Department of Fisheries and Aquatic Environmental Management**,** University of Uyo.

Samples Preparation: The collected samples of crayfish and periwinkles were thoroughly washed with water to remove all kinds of dirt. The periwinkle samples were prepared by breaking the shells to extract the edible portions which were then oven dried at 40^{o}C for 45 hours. The crayfish samples were also oven dried at the same temperature. The dried samples were ground into powdered form, packed in opaque plastic bags (to prevent loss of nutrients and contamination), properly labeled and stored in air- tight container prior to the analyses.

Proximate Analyses: The standard methods described by AOAC (2010) were used for the determination of moisture, ash, crude protein, fibre, carbohydrate, crude lipids, nitrogen and dry matter contents of the crayfish and periwinkles samples. Thermal drying method was used for the determination of moisture contents of the samples. Dried weights (2.0 g) of each sample were taken in washed, dried and weighed crucibles and oven dried at 105°C for 3 hours, allowed to cool in a desiccator and reweighed. The percentage moisture content was calculated by computing the loss in weight on drying as a fraction of the initial weight of sample used and multiplied by 100 as indicated in Equation 1.

$$\text{Moisture (\% wet weight)} = \frac{b-c}{b-a} \times 100 \qquad \text{Equation 1}$$

Where: a = Weight of crucible, b = Weight of crucible + sample before oven drying,
c = Weight of crucible + sample after oven drying

Ash content of each sample was determined using the ignition method. Washed crucibles were pre-heated in a muffle furnace to about 600°C. Exactly 2.0 g of each of the oven-dried samples used in moisture determination were taken in the pre-heated, cooled and weighed crucibles, reweighed and heated at 600°C for two hours in the muffle furnace to burn off all the organic matters. They were allowed to cool in a desiccator and reweighed. The ash content (%) of each sample was calculated using Equation 2.

$$\text{Ash (\%)} = \frac{\text{Weight of ash}}{\text{Weight of sample}} \times 100$$

$$\text{OR, Ash (\%)} = \frac{c-a}{b-a} \times 100 \qquad \text{Equation 2}$$

Where: a = Weight of empty crucible, b = Weigh of crucible + sample before ashing,
c = Weight of crucible + ash

Determination of crude protein in the samples was done by first determining the total organic nitrogen (TON) using the macro-Kjeldhal method. This involved digestion, distillation and titration. Exactly 1.0 g of each sample was taken in digestion flask. Few granules of anti-bumps and 3.0 g of copper catalyst mixture (96% anhydrous sodium sulphate, 3.5% copper sulphate and 0.5% selenium dioxide) were added. Each sample was then digested by adding 20 mL concentrated sulphuric acid and heating on a heating mantle until a clear solution was obtained and allowed to cool. Each digested sample was then filtered and made up to 100 mL with distilled water. Exactly 20 mL of each of the diluted digests were distilled in a round-bottomed flask connected to a receiving flask containing 20 mL of 2% boric acid with methyl red indicator. Exactly 30 mL of 40% sodium hydroxide were injected into the flask and the ammonia formed was distilled by heating the flask. The distillation process was made to continue until the boric acid solution completely changed from purple to greenish – yellow. The boric acid mixture (containing the ammonium borate complex formed) was then titrated with 0.1N HCl to colourless end point and the sample titre noted. Blank determination was also carried out. The % TON was then calculated from Equation 3.

$$\%\text{TON} = \frac{(\text{Sample titre} - \text{Blank titre}) \times M_a \times TV_d}{\text{Weight of sample} \times V_d} \times 100 \qquad \text{Equation 3}$$

Where: M_a = Molarity of the acid; TV_d = Total volume to which digest was diluted,
V_d = Volume of digest distilled

The % Crude protein was obtained by multiplying the % TON content by a factor, 6.25 as shown in Equation 4.

$$\text{\% Crude protein} = \text{\% TON} \times 6.25 \qquad \text{Equation 4}$$

The value 6.25 is a general factor suitable for products in which the proportions of specific proteins are not well defined.

Determination of crude lipid content of each sample was done using Soxhlet type of the direct solvent extraction method. The solvent used was petroleum ether (boiling range 40 - 60°C). Exactly 2.0 g of the dried sample were taken in a soxhlet extraction thimble in a 20 mL capacity soxhlet extractor and extracted with 60 mL petroleum ether for 4 hours. The amount (%) of crude lipid was calculated from Equation 5.

$$\text{\% crude lipid} = \frac{\text{Weight gain in flask}}{\text{Weight of sample}} \times 100$$

$$\text{OR, \% crude lipid} = \frac{W_2 - W_1}{W_s} \times 100 \qquad \text{Equation 5}$$

Where: W_2 = Weight of beaker + sample, W_1 = Weight of empty beaker only,
W_s = Weight of sample

In the determination of crude fibre, 2.0 g of each sample were taken in petroleum ether for two hours and then boiled under reflux apparatus for 30 minutes with 200 mL of a solution containing 1.25% of H_2SO_4 per 100 mL solution. After series of filtering and washing with boiling water to remove the acid, the residue was transferred into a beaker and boiled for another 30 minutes with 200 mL of solution containing 1.25 g of NaOH per 100 mL. The final residue was filtered and washed with boiling water until neutral to litmus and then washed twice with ethanol and quantitatively transferred into a pre-weighed crucible, oven dried at 105ºC and then incinerated in a furnace at 550ºC for 2 hours. The crucible was removed, allowed to cool in a desiccator and reweighed. The crude fibre content was estimated according to Equation 6.

% Crude fibre = Weight loss on ignition (g) x 100

OR, $$\% \text{ Crude fibre} = \frac{1_a - 1_o}{\text{Original weight of sample taken}} \times 100$$ Equation 6

Where: 1_o = Weight of empty crucible
1_a = Weight of crucible and its contents after incineration

Total carbohydrate in each sample was estimated by the difference obtained after subtracting the sum of the percentages of all the other proximate components from 100. That is, Total Carbohydrate (%) = 100 – (% Moisture + % Crude protein + Fibre + % Crude lipid + % Ash). The calorific value (Kcal/100 g) of each sample was calculated by multiplying the value of the crude protein, lipid and carbohydrate by 4, 9 and 4, the Atwater factors for protein, fat and carbohydrate, respectively, as reported by Nostro *et al*. (2000).

Elemental Analyses: The concentrations of the metallic elements (Fe, Zn, Ca, Mg) in the samples were determined by standard procedures described by AOAC (2010). The procedures include ashing and digestion of samples, preparation of stock solution of each element and determination of the concentrations of the elements using their absorbance read from UNICAM solar 969, atomic absorption spectrophotometer (AAS). The concentration of each of the elements recorded in ppm was converted to milligrams (mg) by multiplying by the dilution factor and dividing by 1000. The dilution factor for all elements except Mg was 100. Dilution factor for Mg was 10000. For the determination of Mg, the original solution (the filtrate) was further diluted by taking 0.5 mL of it in a 100 mL volumetric flask and the volume made up to the mark with distilled water. For the determination of Ca, 1.0 mL of lithium oxide solution was added to the original solution to unmask Ca from Mg. K determination in the samples was done by flame photometry as described by AOAC (2010). The same wet digested sample solutions used in the AAS were used for the determination of K. Standard solutions of 20, 40, 60, 80 and 100 milli equivalent /L were used.

Data analyses: Statistical analyses were performed using the SPSS version 20.0. $P < 0.05$ was considered the level of significance.

RESULTS AND DISCUSSION

Proximate Compositions of the Crayfish and Periwinkles Species: The proximate compositions of the crayfish and periwinkles species analysed in this study are presented in Table 1. From the table, the proximate compositions (%) of the species of crayfish were: moisture 88.05, ash 0.25, crude fibre 1.10, crude protein 8.05, crude fat 1.25 and carbohydrate 1.30 in *A. parastacoidea*. In *M. rosenbergii*, moisture was 66.00, ash 0.25, crude fibre 1.45, crude protein 9.63, crude fat 1.40 and carbohydrate 25.28. The calorific value (kcal) was 48.65 and 152.20 for *A. parastacoidea* and *M. rosenbergii*, respectively. The moisture contents of 88.05 and 62.00% in the two crayfish species reported in this study were higher than 10.33 ± 0.29% reported by Ahmad *et al*. (2013). The ash contents of 0.25 and 0.25% in the crayfish

species were lower than the value of 17.30±0.77% reported by Ahmad *et al*. (2013) and in close range with the value of 1.52% reported by Nahid and Fayza (2009) in *Procambarus clarkii*. The crude lipid levels of 1.25 and 1.4% obtained in the crayfish species were lower than 3.83± 0.76 reported by Nahid and Fayza (2009). The crude lipid was however in close range with 1.76% reported by Adebayo-Tayo *et al*. (2006) in *P. clarkii*. Crude fat serves as an energy source as 1g of it supplies 9 Kcal of energy when oxidized (AOAC 2010). The crude fibre of 1.10 and 1.45% obtained in the two crayfish species in this study were in close range with 1.30 reported by Nahid and Fayza (2009). While the crude protein of 8.05 and 9.63% in the respective crayfish species were lower than 13.88% reported by Adebayo-Tayo *et al*. (2006) in *P. clarkii*.

Similarly, as presented in Table 1, the proximate compositions (%) of the periwinkles species were: moisture 74.00, ash 0.1, crude fibre 0.15, crude protein 8.05, crude fat 2.75 and carbohydrate 14.95 in *P. aurita*. In *T. fuscatus*, they were: moisture 73.00, ash 0.10, crude fibre 0.20, crude protein 7.70, crude fat 1.90 and carbohydrate 16.30. The calorific value (kcal) was 116.75 and 113.10 for *P. aurita* and *T. fuscatus*, respectively. The moisture content in each of the species of periwinkles was in close range with 78.00% in *T. fuscatus* and 74.00% in *P. aurita* reported by Adebayo-Tayo *et al*. (2006).

Figure 1 shows the correlation between the proximate compositions in the crayfish species. The proximate compositions of the two species of crayfish were found to be highly positive correlated with r = 0.92 at p = 0.05. Figure 2 shows the correlation between the proximate compositions in the periwinkles species. Here, the proximate compositions of the two species of periwinkles were perfectly and positively correlated (r = 1) at p = 0.05. These indicated that the parameters may have been influenced by the same anthropogenic factors as well as the same natural factors. Meaning that as one factor increases, the other also increases.

Table 1: Proximate Composition (%) of Crayfish and Periwinkle Species

Parameters	**Crayfish species**		**periwinkle species**	
	A. parastacoidea	***M. rosenbergii***	***P. aurita***	***T. fuscatus***
Moisture	88.05	62.00	74.00	73.00
Ash	0.25	0.25	0.10	0.10
Crude fibre	1.10	1.45	0.15	0.20
Crude protein	8.05	9.63	8.05	7.70
Crude fat	1.25	1.40	2.75	1.90
Carbohydrate	1.30	25.28	14.95	16.30
Calorific value (kcal)	48.65	152.20	116.75	113.10

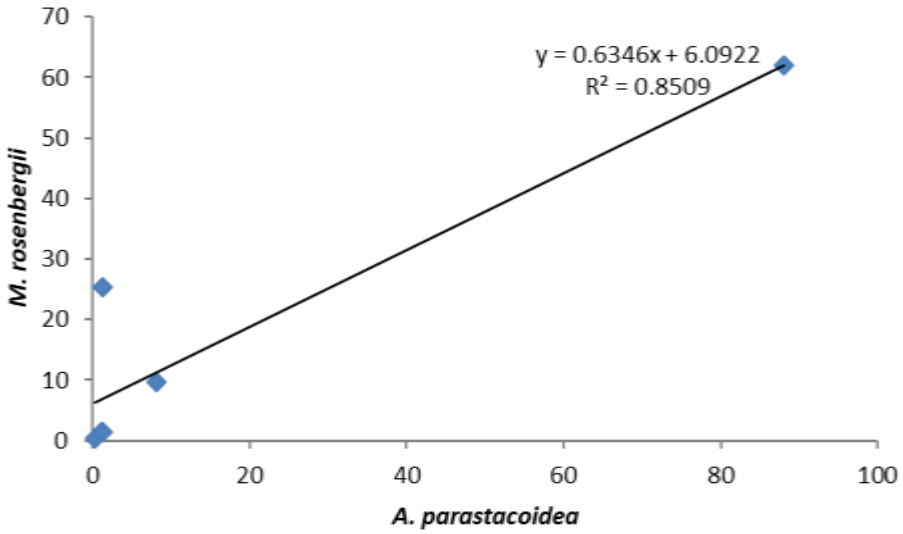

Figure 1: Correlation Between Proximate Compositions in Crayfish Species, (r) = 0.92.

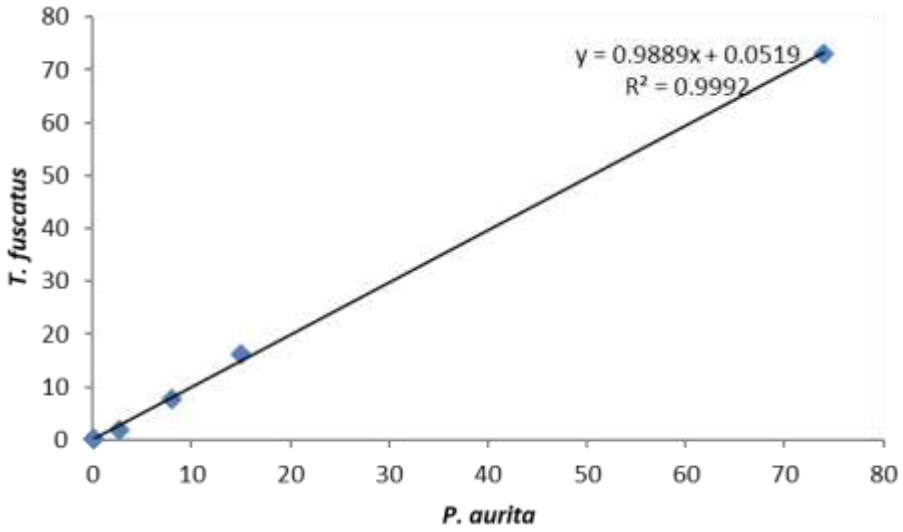

Figure 2: Correlation Between Proximate Composition in Periwinkle Species, (r) = 1

Concentrations of Metallic Elements in Crayfish and Periwinkles: The concentrations of the metallic elements in the two species of crayfish and periwinkles are presented in Table 2. For the crayfish species, Fe was 0.44 mg/100g in *A. parastacoidea* and 0.49 mg/100g in *M. rosenbergii*, Mg was 24.4 mg/100g in *A. parastacoidea* and 22.90 mg/100 g in *M. rosenbergii*. Ca was 28.20 mg/100g in *A. parastacoidea* and 27.95 mg/100g in *M. rosenbergii*. K varied from 21.09 mg/100g in *A. parastacoidea* to 21.00 mg/100g in *M. rosenbergii*, while Zn was 0.56 mg/100g in each of the two species. These values were low when compared with the Fe and Mg levels of 506.33 ug/g and 415.63 ug/g, respectively, in crayfish (*P. clarkii*), reported by Ahmad *et al*. (2013). For the periwinkles, Fe was 0.46 and 0.47 mg/100g in *P. aurita* and *T. fuscatus*, respectively, Mg was 174.90 and 174.30 mg/100g in *P. aurita* and *T. fuscatus*, respectively, Ca was 39.40 and 37.75 mg/100g in *P. aurita* and *T. fuscatus*, respectively, K was 36.80 and 38.30 mg/100g in *P. aurita* and *T. fuscatus*, respectively, while Zn was 0.46 and 0.47 mg/100g in *P. aurita* and *T. fuscatus*, respectively. Fe is a major component of haemoglobin in the blood. Fe deficiency is associated with poor diet, mal-absorptive disorders and blood loss. People with Fe deficiency usually have other

nutrients deficiencies. According to Ahmad *et al*. (2013), Mg plays a major role in the metabolic processes that take place in human systems and in the regulation of blood. It also functions as a co-factor to some enzymatic activities. Ca is a mineral that is present in relatively large amounts in the body. However, excessively high levels of Ca in the blood known as hypercalcemia can cause vascular and soft tissue calcification, hypercalcuiria (high levels of Ca in the urine) and kidney stones (Ahmad *et al*. 2013). Excessive exposure or intake of K may lead to a condition known as manganism, a neurodegenerative disorder that causes dopaminergic neuronal death and parkinsonian like symptoms. Zn is essential in animal and human systems. It has a protective effect against the toxicology of both Cd and Pb. Deficiency of Zn in the body is marked by retarded growth, loss of taste and hypogonadism, leading to a decrease in fertility (Ahmad *et al*. 2013).

Figure 3 shows the correlation between the concentrations of the metallic elements in the crayfish species. Positive perfect correlation with r = 1 at p = 0.05 was observed between the metallic elements in the crayfish species. Similarly, as presented in Figure 4, positive perfect correlation with r = 1 at p = 0.05 was observed between the metallic elements in the periwinkle species.

Table 2: Concentration (mg/100 g) of Metallic Elements of Crayfish and Periwinkles

Elements	Crayfish species		Periwinkle species	
	A. parastacoidea	***M. rosenbergii***	***P. aurita***	***T. fuscatus***
Zn	0.56	0.56	0.46	0.47
Mg	24.40	22.90	174.90	174.30
K	21.09	21.00	36.80	38.30
Fe	0.44	0.49	3.20	3.48
Ca	28.20	27.95	39.40	37.75

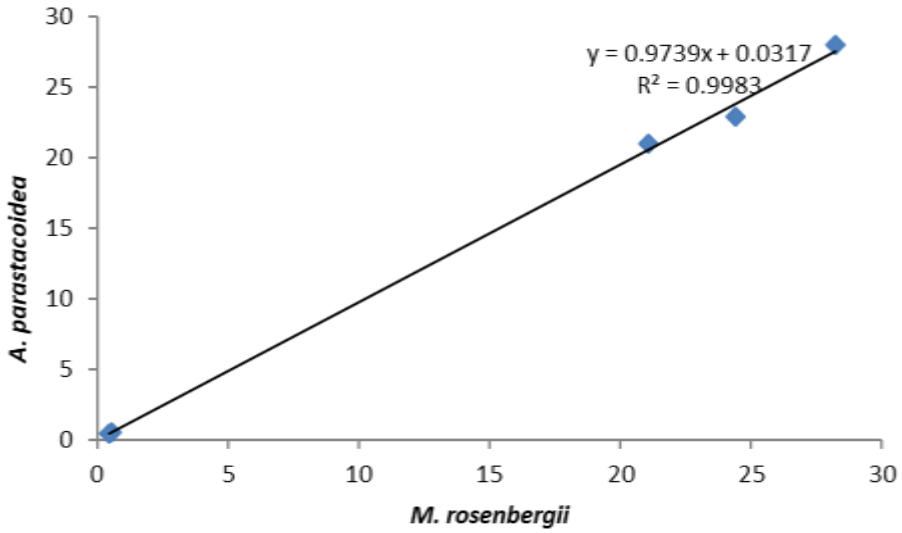

Figure 3: Correlation Between Metallic Elements in the Crayfish Species, (r) = 1.

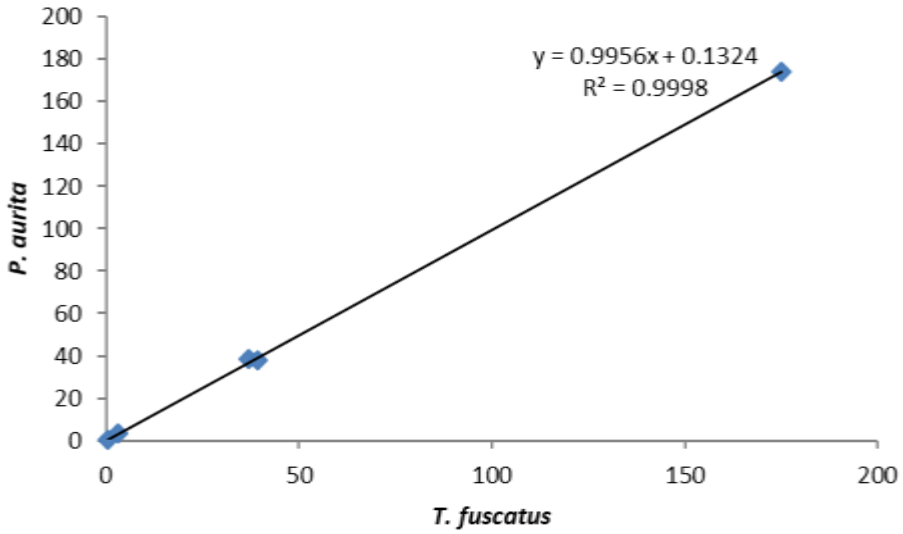

Figure 4: Correlation Between Metallic Elements in Periwinkle Species, (r) = 1.

CONCLUSIONS

Based on the analyses and results, it could be concluded that the crayfish and periwinkles species obtained in Ishiet River contain appreciable proximate compositions and variable concentration levels of the analysed metallic elements. The concentration levels of the metallic elements classified as essential metals (Mg, Ca and K) were higher when compared with those of the trace metals (Zn and Fe). There were high positive correlations at p = 0.05 between the proximate compositions in the crayfish species, while the proximate compositions in the periwinkles species were perfectly and positively correlated. Positive perfect correlations were observed between the essential elements in the two animal species. These indicated that all the parameters tested for in the aquatic animal species were being influenced by the same anthropogenic and natural factors. Meaning that as one factor increases, the other also increases. The Zn and Fe levels in the two animal species were below the levels that could cause toxicity in humans. Consequently, consumption of these two aquatic animal species may not pose health hazard to the consumers at the time of the study.

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