Survey Of Male And Female Frogs (*Pyxicephalus Edulis*) And Associated Toxic And Non Toxic Elements

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Research Article

Keywords: Male frog, female frog, element, *Pyxicephalus edulis*, sediment, PIXE

Posted Date: June 2nd, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-581624/v1>

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Abstract

The present research was performed to determine the concentration of heavy metals in the male and female specimens of the identified frog species: *pyxicephalus edulis* collected from the Igbekebo River in Igbekebo, Ese-odo local government. Adult frogs (male and female) were collected from the river bank, and sediment samples and water samples were also collected at five (5) separate locations in the river. The frogs were dried separately at 105°C for 6 hrs and then crushed into small particles (powder form). The sediment samples were air-dried for three days. Elemental components in frog samples and sediment samples were analyzed using Proton Induced X-Ray Emission (PIXE). Physiochemical parameters and heavy metals of the water samples were also analyzed. The findings showed that the concentrations of Si, P, Cl, Ni, Zn and Cd were higher in Male frog while Mg, Al, K, Ca, Ti, V, Cr, Mn, Fe, Co, Cu, Zr, Pb and Sn were higher in female frogs, the explanation for this variability is not known but may be due to variations in the genetic make-up of Male and Female frogs. The concentration of heavy metals in both male and female frogs was substantially higher relative to the available WHO limits. The mean concentration of elemental constituents in sediment was higher than the IAEA limit. The values of enrichment and the Igeo values were very high.

Introduction

The worldwide decline in amphibian species has shown over-exploitation as one explanation for a decrease in global amphibian populations (Stuart et al., 2008). It has been said by Niasse et al., (2004) that 281 amphibian species have the primary threat of utilization, 54% of which are already listed in their categories as vulnerable, endangered or critically endangered. A recent research by Warketin et al. (2009) sums up troubling data on Asian frog collected for use, with Mohnke (2011) reporting that a total of two million, 7800 and 610 frog species have been collected annually by 302 frog collectors in the southwest countries of Nigeria. A food scarcity and Africa behind other developed countries is one of the most apparent consequences of the world's population growth (Lameed, 2008). As a result, the production and supply of animal protein for feeding ever-expanding population decreased significantly (Akpan et al., (2009). Frogs have often been collected on a local scale as an important source of animal protein (Angulo, 2008; Mohnke et al. 2009) in a large number of African countries (such as Benin, Burkina Faso, Cameroon, the Ivory Coast (Ghana), Guinea, Namibia, Nigeria, South Africa and Togo). An increase in human population level continues to raise the current burden on wildlife. Frog species are generally extracted from wild animals in various areas of the world primarily for fruit, medicinal purposes and pet trade (Mohnke et al., 2011). Agriculture of these used frog species has traditionally been ignored in Africa and the world as a whole, although the rate of consumption continues to increase. Most frogs in advanced countries come from developing countries. Therefore, the rate of exports of frogs from developing countries (regardless of their conservation status) to developed countries has increased due to a high demand market. Onadeko et al. (2011) stated that frog legs were common in Europe and even eaten in countries where hunting frogs is prohibited legally. Frogs are called 'jumping chickens' in many regions in Asia (Indonesia) and Africa (Nigeria) as a taste similar to chicken (Altherr et al. 2011). Its availability, the taste for people and the need to meet the demand for protein are some of the key reasons for the annual consumption of billions of frogs. Leather and souvenirs, pet trades and culture, including traditional medicine are also collected for the purpose of collection (Kusrini and Alford, 2006; Gonwouo and Rodel, 2008). Overuse of these tools is, however, one of the main biodiversity challenges. Frogs became a trading commodity for more than 30 countries in the last decade, with trade in frog species worth about US\$ 48.7 million in 1998 (Teixeira et al., 2001). Pollution of heavy metals is harmful to our environment (Hazrat et al 2019). All of these elements are poisonous to human and animal microorganisms, they are necessary, not important. Excess heavy metal deposition in the body could result in vomiting, vision loss, thyroid, asthma, abdominal pain, sore throat, lung cancer, nose cancer, dizziness, and human heart problems (Tchounwou et al 2012). The aims of this study were to identify male and female frogs' species and to assess the elemental composition of male and female frogs as well as elemental composition and the physicochemical parameters of the sediment in the study area of Igbekebo. Relatively few studies in amphibian species in natural populations have been carried out concerning heavy metal accumulation (Sura et al. 2006). The *Rana ridibunda* Frog was used as a heavy metal contamination bioindicator (Efsthadiadou et al. 2007; Fenoglio et al. 2011; Loumbourdis et al. 2007; Simon et al. 2010). Amphibian species have been suggested as an important environmental indicator of pollution for the frog limb as being more sensitive than other aquatic vertebrate bioindicators, due to the fact that they have a permeable skin that is able ready to absorb contaminants from the atmosphere (Schuytema and Nebeker 1996; Simon et al. 2012)

Materials And Method

Study Area

In Ondo State, Nigeria, Igbekebo is headquartered in the Local Government Area. It is populated by the Western Apoi and Arogbo ethnic subgroups Ijaw (Izon). At the 2006 census, the population is 762 square kilometers and 154,978. Because of its fishing and lumbering activities, the Igbekebo river has a high financial influence. Due to the chemical and biological inputs in the river the river is highly contaminated and this can result in high concentrations of heavy metals. Igbekebo is an area of oil production that is very polluted by petroleum spillage due to bunkering in the field. It is an urban small rural village. Figure 1 indicates the various locations for sampling.

Sampling

Two adult frogs (male and female) were collected from the river bank and sediment samples were collected at five (5) different locations in the river. The samples of the frogs were labeled as samples A and B i.e. the male frog was labeled as sample A while the female frog was labeled as sample B, the sediment samples were labeled as location A, B, C, D, E.

Sample Preparation

The frogs were oven dried at 105°C separately for 6 hours then crushed and sent for analysis in small particles (powdery form). The sediment samples were air dried for three days and sent for analysis, and the water samples were also sent for analysis.

Metal analysis of some physio-chemical parameters of sediment Samples

Analysis was carried out with a 1.7 MeV SSDH Pelletron Accelerator for sample metal concentrations. The pellets of sample were then analyzed for the total content of the elements (expressed as mg/kg) by the Particle-Induced X-ray Emission (PIXE) technique (He et al., 1993; Johansson et al., 1995). The technical detection limit is from 0.1 to 10 mg/kg. Table pH and conductivity (Hanna 991300) meters have been used to measure the pH and electric conductivity of the ground sample. The nitrate and chloride were also determined using standard procedures (Ademoroti 1996). Detailed analyses had been reported by (Ediagbonya and Balogun, 2020a; Ediagbonya et al 2020).

Bioaccumulation factor

Bioaccumulation factor (BAF) explains the intake and distribution of the element in the organism after exposure in a given environmental matrix (Subotić et al., 2013); and has been used here to determine the level of frog with a higher risk to health (DeForest et al., 2007). The concentration of the sediment elements in the frog was measured as a ratio between the concentration of the frog element and the concentration in sediment (Liao and Ling, 2003; Javed and Usmani, 2013) and Bio-accumulation factor (BAF). $BAF < 1$ indicates no contamination of the frog; $1 > BAF \leq 10$, the frog is tolerant and $BAF > 10$, a hyperaccumulator (Ávila et al., 2017).

$$BAF = C(Frog)/C(sediment)$$

Health Risk Assessment

Risk was assessed using the target hazard quotient (THQ) and the hazard index (HI). The risk to human health derived from the ingestion of contaminated food as specified by FAO / WHO (2010) and the target hazard quotient (THQ) as set out in the USEPA Region III Risk-based Concentration Table (USEPA, 2015). The THQ was calculated using the formula given by (Singh et al., 2010) $THQ = Efr \times ED \times FIR \times C / RfDo \times Baverage \text{ wt} \times ATn \times 10^{-3}$

Efr is exposure frequency assumed to be 365 days year⁻¹, ED is exposure duration in 54 years an average lifetime for Nigeria, while the average body weight used was 65 kg for this study (Oguntona, 1998). FIR is average daily consumption taken as $1.95 \times 10^{-2} \text{ kg person}^{-1} \text{ day}^{-1}$. C is concentration of metal in frog sample in mg/kg, RfDo is the oral reference dose (Al, Ti, V, Cr, Mn, Co, Pb, Cd, Cu, Zn, Fe and Ni (USEPA, 2011, 2013; RAIS, 2017); and ATn is average exposure time for non-carcinogens and is taken as 365 days. The hazard index (HI) from the consumption of frog estimated as the sum of THQs of all the metals in the frog and was expressed as follows;

$$HI = THQ_{Al} + THQ_{Ti} + THQ_{V} + THQ_{Cr} + THQ_{Mn} + THQ_{Fe} + THQ_{Co} + THQ_{Ni} + THQ_{Cu} + THQ_{Zn} + THQ_{Cd} + THQ_{Pb}$$

Geo- Accumulation Index (Igeo).

The Geo Accumulation (Igeo) index is intended to measure the extent of sediment pollution as calculated by (Loska et al. 1997; Müller, 1979). It was used by $Igeo = \ln[C_n / 1.5 \times B_n]$ (Ediagbonya and Ayedun, 2018; Ediagbonya and Balogun, 2020). The calculated metal 'n' content in sediments is C_n and the background concentration of the same metal is B_n .

Enrichment Factor (EF)

To assess the extent of pollution in soil and sediments, the enrichment factor (EF) is used to determine excessive metal concentrations in sediments and soil. Al and Si were used as reference elements in this analysis. Few authors have used the reference feature of these components (Schiff and Weisberg, 199; Ediagbonya et al 2020b). The metal EF is classified as follows, according to Ergin et al., (1991):

$$EF = \frac{\left(\frac{X}{Al}\right)_{sediment}}{\left(\frac{X}{Al}\right)_{crust}}$$

Where X/Al is the heavy metal concentration ratio (X) to the concentration of Al.

Wedephol was taken from the reference crustal ratio of the shale value or lithology (1968)

Statistical data analysis

For the statistical analyses in this report, IBM Statistical Package for Social Sciences (SPSS) version 24.0 was used. Descriptive statistics at the various sampling sites, such as range, mean, standard deviation for the physicochemical parameters as well as heavy metals. One direction Variance Analysis was used to conduct the spatial variation of heavy metal means at the various sample sites where substantial difference was found to distinguish significant means was used by the Duncan Multiple Range Test (DMRT). The physicochemical parameters, using the Pearson correlation, were also associated with the toxic elements. The multivariate analysis conducted for the source detection of heavy metals was the principal component analysis. The significance standard was set at $p < 0.055$.

Results And Discussion

Table 1
Descriptive statistics of physiochemical parameters of soil samples in the sampled location

	A	B	C	D	E	p
Chloride	10.67 ± 0.78	9.68 ± 0.14	10.90 ± 0.17	9.68 ± 0.14	10.90 ± 0.17	0.043
pH	7.55 ± 0.21	7.70 ± 0.14	5.55 ± 0.21	7.70 ± 0.14	5.55 ± 0.21	0.000
µscm	888.50 ± 1.41	934.30 ± 2.12	1120.20 ± 0.71	934.30 ± 2.12	1120.20 ± 0.71	0.000
Milligram/litre	586.30 ± 2.83	613.20 ± 1.41	728.10 ± 2.12	613.20 ± 1.41	728.10 ± 2.12	0.000
Phosphate	8.71 ± 0.14	5.28 ± 0.14	5.12 ± 0.14	5.28 ± 0.14	5.12 ± 0.14	0.000
Nitrate	6.46 ± 0.14	6.52 ± 0.14	5.25 ± 0.21	6.52 ± 0.14	5.25 ± 0.21	0.001
Sulphate	19.13 ± 0.03	19.73 ± 0.03	16.94 ± 0.04	19.73 ± 0.03	16.94 ± 0.04	0.000

Table 1 shows the descriptive statistics on the physiochemical properties of soil samples at different locations. The highest mean chloride was recorded at locations C and E, i.e. 10.90 ± 0.17 (10.75–11.09) and the lower mean chloride was recorded at locations B and D 7.70 ± 0.14 (7.58–7.86) while the lower mean chloride was recorded at locations C and E 5.55 ± 0.21 (5.36–5.76). The highest mean electrical conductivity was recorded at locations C and E 1120.20 ± 0.71 (1119.50-1120.93 µscm) and the lowest mean µscm was recorded at location C and E 1120.20 ± 0.71 (1119.50-1120.93 µscm). Maximum mean was obtained at locations C and E 728.10 ± 2.12 (725.98-730.24 mg / l), while the smallest mean mg / l was shown at location A, 586.30 ± 2.83 (583.49-589.15). The maximum mean of phosphate was seen at location A, 8.71 ± 0.14 (8.59–8.37), while the smallest mean of phosphate was recorded at locations C and E, 5.12 ± 0.14 (5.00-5.28). The maximum mean of nitrate was reported at locations B and D, 6.52 ± 0.14 (6.40–6.68), while the smallest mean nitrate was recorded at locations C and E 5.25 ± 0.21 (5.06–5.46). The maximum of mean sulphate was recorded at locations B and S 19.73 ± 0.03 (19.90-19.78), while the smallest mean of sulphate was recorded at locations C and E, 16.94 ± 0.04 (16.90–17.00)

Table 2
Mean of the elemental concentrations in sediment in samples A-E (mg kg⁻¹)

	A	B	C	D	E	p
Na	4883.30 ± 7.07	4551.20 ± 7.07	5667.00 ± 0.71	6550.90 ± 7.78	4764.70 ± 2.12	0.000
Mg	23227.60 ± 7.07	22752.60 ± 9.90	24124.80 ± 2.83	22950.60 ± 3.54	25127.60 ± 7.07	0.000
Al	26427.20 ± 5.66	121552.80 ± 7.07	24291.00 ± 4.24	73994.50 ± 67264.66	24291.00 ± 4.24	0.076
Si	346361.80 ± 1.41	300682.70 ± 14.14	245744.90 ± 45.25	323527.75 ± 32293.64	245744.90 ± 45.25	0.003
P	460.40 ± 4.95	117.90 ± 7.07	339.40 ± 3.54	284.90 ± 243.24	339.40 ± 3.54	0.161
Cl	271.30 ± 3.54	88.00 ± 2.12	135.30 ± 3.54	180.15 ± 132.44	135.30 ± 3.54	0.150
K	1464.60 ± 18.38	5222.40 ± 7.78	1789.80 ± 4.24	3347.25 ± 2644.08	1789.80 ± 4.24	0.098
Ca	3159.20 ± 1.41	12337.20 ± 7.07	2568.20 ± 12.73	7745.20 ± 6487.00	2568.20 ± 12.73	0.067
Ti	70308.70 ± 2.12	4444.50 ± 12.02	35372.20 ± 2.83	70308.70 ± 2.12	19905.10 ± 21876.61	0.003
V	1277.60 ± 2.12	281.10 ± 2.12	660.90 ± 8.49	777.85 ± 704.63	660.90 ± 8.49	0.163
Cr	550.40 ± 20.51	528.00 ± 21.92	279.00 ± 0.71	538.70 ± 37.05	279.00 ± 0.71	0.000
Mn	3371.30 ± 2.83	451.40 ± 4.24	1728.90 ± 6.36	1908.85 ± 2065.39	1728.90 ± 6.36	0.169
Fe	121923.60 ± 31.11	84729.80 ± 4.95	100948.90 ± 8.49	103335.95 ± 26318.02	100948.90 ± 8.49	0.169
Co	260.70 ± 4.95	349.90 ± 7.78	383.00 ± 39.03	370.90 ± 4.24	416.60 ± 8.49	0.003
Cu	370.30 ± 2.12	239.70 ± 4.24	295.40 ± 2.12	239.70 ± 4.24	295.40 ± 2.12	0.000
Zn	220.20 ± 4.95	90.40 ± 3.96	136.60 ± 4.24	90.40 ± 3.96	136.60 ± 4.24	0.000
Sr	89.70 ± 10.61	329.50 ± 28.28	71.50 ± 2.83	329.50 ± 28.28	71.50 ± 2.83	0.000
Zr	2592.90 ± 6.36	223.80 ± 4.95	2261.30 ± 8.49	223.80 ± 4.95	2261.30 ± 8.49	0.000
Sn	127.60 ± 5.66	127.60 ± 5.66	70.50 ± 4.24	113.00 ± 3.54	113.00 ± 3.54	0.000
Pb	44.25 ± 1.91	44.10 ± 0.71	44.70 ± 1.41	45.95 ± 2.12	46.30 ± 1.41	0.574
Bi	27.90 ± 2.12	34.30 ± 2.12	34.30 ± 2.12	31.50 ± 1.41	30.30 ± 1.41	0.071
Sc	232.60 ± 2.83	218.35 ± 0.49	222.00 ± 1.70	219.75 ± 1.48	228.90 ± 8.06	0.057
Ni	87.35 ± 0.21	86.10 ± 0.71	84.85 ± 1.48	85.75 ± 1.34	87.90 ± 0.99	0.149
Cd	120.35 ± 0.21	91.35 ± 0.21	45.65 ± 0.31	98.35 ± 0.21	98.35 ± 0.21	0.001
Au	14.70 ± 0.14	14.70 ± 0.14	13.75 ± 0.21	13.75 ± 0.21	15.55 ± 0.35	0.002
S	489.25 ± 1.91	489.25 ± 1.91	472.80 ± 2.83	472.80 ± 2.83	506.80 ± 8.49	0.003
Y	41.50 ± 0.71	41.50 ± 0.71	38.50 ± 0.71	38.50 ± 0.71	41.00 ± 1.41	0.036

The above Table 2 showed the mean comparison of heavy metals in sediments. It showed that there is significant spatial variation in all the heavy metals except Al, P, Cl, K, Ca, V, Mn, Fe, Pb, Bi, Sc and Ni.

Table 3
Range and amount of elemental concentration in the sediment samples at Igbekebo
(mg/kg) compound with other studies.

Elements	Present study range (mean)	IAEA – 457	East Attica, Greece (Alexakis 2008)(mean)
Na	4551.20–6550.90(5287)	NA	8500
Mg	22752. 60–25127.60(23636.8)	NA	16083
Al	2429.00–121552.80(54111)	82660	36200
Si	245744.90–346361.80 (248121.4)	NA	NA
P	117.90–460.40(308.4)	NA	NA
Cl	88.00–271.30(162.01)	NA	NA
K	1464.60–5222.40(2722.77)	NA	7400
Ca	2568.20–12337.20(5675.60)	NA	122200
Ti	4444.50–70308.70(40067.84)	NA	2288
V	281.10–1277.60(731.67)	NA	NA
Cr	279.00–550.40(435.02)	144	285.8
Mn	451.40–3371.30(1837.87)	427	716
Fe	84729.80–121923.60(102364.51)	41,450	27,169
Zn	90.40–220.20(134.84)	425	169.98
Sr	71.50–329.50 (178.34)	137	166
Zr	223.80–2592.90(1512.62)	NA	34.62
Sn	70.50–127.60 (110.34)	NA	NA
Pb	44.10–46.30(45.06)	105	217.32
Bi	27.90–34.30(31.66)	NA	NA
Sc	218.35–232.60 (224.32)	NA	NA
Ni	84.85–87.90(86.39)	NA	NA
Cd	45.65–120(90.81)	NA	NA
Au	13.75–15.55(14.49)	NA	NA
S	472.80–506.80(486.18)	NA	NA
Y	38.50–41.50(40.20)	NA	NA
Co	260.70–416. 60(356.22)	NA	NA
Cu	239.70–370.30(288.10)	365	31.96

Table 3 showed that sodium, magnesium and titanium concentrations in Igbekebo River sediment ranged from 4551.20 to 6550.90, 22752.60 to 25127.60 and 4444.50 to 70308.90, respectively. With mean values of 2278785.45, 23940.10 and 37376.60, respectively. Mean concentrations of sodium, magnesium and titanium were higher in Eastern Attica, Greece (Alexakis 2008). However, the mean value of sodium, magnesium and titanium could not be compared with the value of the IAEA, as they were not available at the time of the research. Chromium, manganese, iron and strontium concentrations ranged from 279.00 to 550.40, 451.40 to 3371.30, 84729.80 to 121923.60, and 71.50 to 329.50, respectively, with mean values of 414.70, 1911.35, 103326.70 and 200.50, respectively, which were higher than those of both the IAEA-457 and East Attica, Greece (Alexakis 2008). Potassium and calcium concentrations ranged from 1464.60 to 5222.40. The mean potassium and calcium values were lower than those of East Attica, Greece (Alexakis 2008), but these mean potassium and calcium values could not be compared with the mean IAEA-457 because they are not available at the research point. Zirconium ranges from 223.80 to 2592.90 with a mean value of 1408.35, which is also high as that of East Attica, Greece (Alexakis 2008). Aluminum and copper concentrations ranged from 2429.00 to 121552.80 and 239.70 to 370.30, respectively, with mean values of 61990.90 and 305.00, which were lower than the average value of IAEA-457 and higher than the mean value of East Attica, Greece (Alexakis 2008). Zinc and lead concentrations range from 90.40 to 220.20 and 44.10 to 46.30, respectively, with mean values of 155.30 and 67.25 lower than the mean values of IAEA-457 and East Attica, Greece (Alexakis 2008). The order of the elemental concentration in the sediment sample is Au < Bi < Y < Pb < Ni < Sn < Cl < Sc < Cu < Cu < Cr < Cr < Cr < Cr < V < Cr < Mn < K < Na < Ca < Mg < Al < Ti < Fe < Si. High concentrations of these elements in sediment could affect animals, insects and micro-organisms living in water.

Table 4
Heavy metal concentration in both male and female frogs
species in Igbekebo mg/kg compared with WHO limits

Heavy Metals	Male frog	Female frog	WHO Limit
Mg	2282	2831.6	NA
Al	318.4	417.9	NA
Si	2348.5	1832.5	NA
P	85.9	74.8	NA
Cl	72.7	57.3	NA
K	1219.2	1303.1	NA
Ca	990.4	1006.8	NA
Ti	1934.5	2122.1	NA
V	89.3	107.2	NA
Cr	110.5	132.2	0.07
Mn	213.2	283.7	0.5
Fe	45752	65035.9	0.5
Co	88.9	101.1	NA
Ni	64.3	63.6	NA
Cu	61.1	102.4	NA
Zn	81	63	5
Zr	157.9	167.4	NA
Cd	20.9	28.1	NA
Pb	14.3	35.9	1
Sn	20.8	22.2	NA

During this study, male and a female frog were caught from oil producing community and both frogs were investigated for their elemental concentrations. The findings showed that in the samples high concentration elements in both frogs. Stolyar et al.2008 had also reported high concentration of elements in frogs living on the urban site in the Ukraine in comparisons between metal bioavailability in urban and rural frogs. Frogs are more sensitive to environmental toxins than other vertebrates because they do not allow foreign substances to be obstructed by egg membranes or skin (Duellman 1994; Snodgrass et al. 2003). frogs have been used as bioindicators for pollution monitorisation, they have the proclivity to accumulate heavy metals (Berzins and Bundy 2002; Haywood et al. 2004; Simon et al. 2012). The consumption of frog is a result of its availability, the taste for people and the need to meet the demand for protein are some of the key reasons for the annual consumption of billions of frogs. Frogs are also collected for leather production and souvenirs, pet trade and cultural purposes, including traditional medicine (Kusrini and Alford, 2006; Gonwouo and Rodel, 2008). From Table 4, Silicone (Si) had the concentration in both male frog and female while lead (Pb) showed the least concentration in both frogs, The female frog accumulated higher concentrations of elements than the male frog. This split difference could be as a result of their genetic makeup. The results of the study can be compared to other studies (Zocche et al 2013; Stolyar et al.,2008)

Table 5
Mean comparison of metals in male and female frogs

	Male	Female	P
Mg	2282.00 ± 10.00	2831.60 ± 500.00	0.130
Al	328.7 ± 344.18	417.90 ± 179.00	0.016
Si	2348.57 ± 348.50	1832.50 ± 500.00	0.216
P	85.93 ± 5.85	74.80 ± 10.00	0.171
Cl	72.73 ± 2.65	51.30 ± 10.00	0.023
K	1252.60 ± 270.65	1303.10 ± 300.00	0.839
Ca	990.53 ± 90.20	1006.80 ± 100.00	0.845
Ti	1934.50 ± 400.00	2122.07 ± 121.95	0.481
V	89.30 ± 9.00	107.20 ± 10.00	0.083
Mn	213.20 ± 13.20	283.70 ± 83.00	0.220
Fe	45752.00 ± 752.00	65035.90 ± 5000.00	0.003
Cu	61.07 ± 3.05	102.40 ± 10.00	0.002
Zn	81.00 ± 3.00	63.00 ± 10.00	0.040
Zr	157.90 ± 20.00	167.40 ± 10.00	0.503
Sn	20.80 ± 4.00	35.90 ± 10.00	0.072
Cr	110.50 ± 10.00	132.20 ± 10.00	0.057
Pb	14.30 ± 4.00	22.20 ± 5.00	0.099
Co	88.90 ± 8.90	101.10 ± 10.00	0.190
Ni	54.30 ± 4.00	63.60 ± 10.00	0.209
Cd	20.90 ± 10.00	28.10 ± 10.00	0.166

The mean comparison of metals in male and female frogs is shown in Table 5. There was a significant difference ($p < 0.05$) in the mean concentrations of Al, Cl, Fe, Cu and Zn, while the other elements did not show any significant differences ($p > 0.05$). In Al, Fe and Cu, females had higher concentrations than males, while in Cl and Zn, males had higher concentrations than females.

Table 6
Interrelationship between metals in Frog

	Mg	Al	Si	P	Cl	K	Ca	Ti	V	Mn	Fe	Cu	Zn	Zr	Sn	Cr
Mg	1	0.772	0.078	0.045	-0.260	0.618	0.616	0.459	.880*	.986**	.868*	.857*	-0.184	0.554	.963**	.865*
Al		1	-0.215	-0.285	-0.636	0.510	0.502	0.722	.944**	0.739	.931**	.945**	-0.571	0.718	.903*	.966**
Si			1	.996**	.885*	0.730	0.734	0.377	0.078	0.240	-0.357	-0.353	.918**	0.461	0.043	0.005
P				1	.919**	0.678	0.682	0.295	0.010	0.203	-0.405	-0.404	.947**	0.383	-0.011	-0.064
Cl					1	0.336	0.340	-0.077	-0.376	-0.122	-0.696	-0.702	.997**	0.001	-0.367	-0.445
K						1	.998**	.830*	0.731	0.737	0.343	0.355	0.410	.903*	0.675	0.681
Ca							1	.832*	0.730	0.736	0.340	0.352	0.414	.905*	0.674	0.680
Ti								1	0.797	0.543	0.463	0.493	-0.013	.988**	0.650	0.784
V									1	.889*	.881*	.891*	-0.298	.833*	.974**	.997**
Mn										1	0.797	0.789	-0.042	0.645	.955**	.863*
Fe											1	.999**	-0.636	0.487	.918**	.905*
Cu												1	-0.642	0.513	.919**	.916*
Zn													1	0.071	-0.289	-0.369
Zr														1	0.712	0.811
Sn															1	.968**
Cr																1
Pb																
Co																
Ni																
Cd																
*. Correlation is significant at the 0.05 level (2-tailed).																
**. Correlation is significant at the 0.01 level (2-tailed).																

Table 6 shows the interrelationship between metals in Frog. Most of the metals showed significantly high correlation with each other indicating similar sources.

Table 7
Enrichment value using Al and Si as reference elements and Igeo accumulation in location A to E

	A			B			C			D			E		
	Al	Si	Igeo	Al	Si	Igeo	Al	Si	Igeo	Al	Si	Igeo	Al	Si	Igeo
Al	1.00	0.01	-2.18	1.00	0.04	0.02	1.00	0.01	-2.30	1.00	0.02	-0.70	1.00	0.01	-2.30
Si	143.63	1.00	4.98	27.11	1.00	4.78	110.87	1.00	4.49	47.92	1.00	4.88	110.87	1.00	4.49
P	1.99	0.01	-1.19	0.11	0.00	-3.15	1.60	0.01	-1.63	0.44	0.01	-1.88	1.60	0.01	-1.63
Cl	4.56	0.03	0.01	0.32	0.01	-1.62	2.48	0.02	-1.00	1.08	0.02	-0.58	2.48	0.02	-1.00
K	0.17	0.00	-4.77	0.13	0.00	-2.93	0.22	0.00	-4.48	0.14	0.00	-3.58	0.22	0.00	-4.48
Ca	0.43	0.00	-3.39	0.37	0.01	-1.43	0.38	0.00	-3.69	0.38	0.01	-2.10	0.38	0.00	-3.69
Ti	46.27	0.32	3.35	0.64	0.02	-0.63	25.32	0.23	2.36	16.53	0.34	3.35	14.25	0.13	1.53
V	29.75	0.21	2.71	1.42	0.05	0.53	16.74	0.15	1.76	6.47	0.14	2.00	16.74	0.15	1.76
Cr	18.51	0.13	2.03	3.86	0.14	1.97	10.21	0.09	1.05	6.47	0.14	2.00	10.21	0.09	1.05
Mn	12.01	0.08	1.40	0.35	0.01	-1.50	6.70	0.06	0.44	2.43	0.05	0.58	6.70	0.06	0.44
Fe	7.82	0.05	0.78	1.18	0.04	0.26	7.04	0.06	0.51	2.37	0.05	0.55	7.04	0.06	0.51
Co	41.54	0.29	3.19	12.12	0.45	3.62	66.39	0.60	3.75	21.11	0.44	3.70	72.21	0.65	3.87
Cu	24.91	0.17	2.46	3.51	0.13	1.83	21.62	0.20	2.13	5.76	0.12	1.83	21.62	0.20	2.13
Zn	7.02	0.05	0.63	0.63	0.02	-0.66	4.74	0.04	-0.06	1.03	0.02	-0.66	4.74	0.04	-0.06
Sr	0.91	0.01	-2.33	0.72	0.03	-0.45	0.78	0.01	-2.65	1.19	0.02	-0.45	0.78	0.01	-2.65
Zr	49.06	0.34	3.43	0.92	0.03	-0.10	46.55	0.42	3.24	1.51	0.03	-0.10	46.55	0.42	3.24
Sn	29.71	0.21	2.71	6.46	0.24	2.71	17.86	0.16	1.85	9.40	0.20	2.53	28.63	0.26	2.53
Pb	6.70	0.05	0.56	1.45	0.05	0.56	7.36	0.07	0.58	2.48	0.05	0.62	7.62	0.07	0.63
Sc	5.03	0.04	0.15	1.03	0.04	0.06	5.22	0.05	0.08	1.70	0.04	0.07	5.38	0.05	0.12
Au	7.42	0.05	0.71	1.61	0.06	0.71	7.55	0.07	0.61	2.48	0.05	0.61	8.54	0.08	0.79
Y	4.83	0.03	0.09	1.05	0.04	0.09	4.88	0.04	-0.02	1.60	0.03	-0.02	5.19	0.05	0.07
Cd	1214.41	8.46	8.06	200.41	0.003	7.67	501.15	4.52	6.67	354.44	7.40	7.77	1079.69	9.74	7.77

From Table 7, location A: the geoaccumulation value as shown in Table 7; Al, P, K, Ca and Sr were unpolluted; Y, Au, Sc, Pb, Zn, Fe and Cl were slightly polluted; Mn was moderately polluted; V, Cr, Cu and Sn were moderately polluted; Zr, Co and Ti were severely polluted; and Si was extremely polluted. Location B; P, Cl, K, Ca, Ti, Mn, Zn, Sr and Zr were unpolluted; Au, Y, Sc, Pb, V, Fe and Al were slightly polluted; Cu and Cr were moderately polluted; Sn was moderately polluted; Co was severely polluted; Si was severely polluted, Cd was extremely polluted. Location C; Al, Y, Sr, Zn, Ca, P and Cl were unpolluted; Sc, Au, Mn, Fe, and Pb were slightly polluted; Sn, Cr and V were moderately polluted; Cu and Ti were moderately polluted; Co and Zr were severely polluted; Si was severely polluted. Location D; Zr, Y, Sr, Zn, Ca, K, P, Cl and Al were unpolluted; Mn, Fe, Pb, Au and Sc were slightly polluted; Cu, Cr and V were moderately polluted; Sn was moderately polluted; Co and Ti were severely polluted; Si was severely polluted. Location E; P, Al, Cl, K, Ca, Zn and Sr were unpolluted; Y, Au, Sc, Pb, Fe and Mn were slightly polluted; Cr, V and Ti were moderately polluted; Sn and Cu were moderately polluted; Zr and Co were severely polluted; Si was severely polluted. In this present study, the calculation of the enrichment factor was replaced by Al and Si. In the locations Cd was extremely polluted

The order of sediment enrichment in Location A when Al was used as a reference element was: Cd > Si > Zr > Ti > Co > V > Sn > Cu > Cr > Mn > Fe > Au > Zn > Pb > Sc > Y > Cl > P > Sr > Ca > K as shown in Table 8. When Si was used as a reference element, the sediment enrichment order was: Cd > Zr > Ti > Co > V = Sn > Cu > Cr > Mn > Fe = Au = Zn = Pb > Sc = Y = Cl > P = Al = Sr > Ca > = K. In Location B, the order of the enrichment value when Al was : Cd > Si > Co > Sn > Cr > Cu > Au > Pb > Fe > V > Y > Sc > Zr > Sr > Ti > Zn > Ca > Mn > Cl > K > P. When Si was used; Sn > Cu > Cr > Au > Pb = V > Co > Al = Fe = Sc = Y > Zr = Sr > Zn = Ti > Mn = Cl = = Ca > K = P > Cd. In Location C, the enrichment value when Al was : Cd > Si > Co > Zr > Ti > Cu > Sn > V > Cr > Au > Pb > Fe > Mn > Sc > Y > Zn > Cl > P > Sr > Ca > K. when Si was used: Cd > Co > Zr > Ti > Cu > Sn > V > Cr > Pb = Fe = Mn = Sc = Zn = Y > Cl = Al = Sr = P > K = Ca. In Location D, when Al was used the enrichment values were in order of: Cd > Si > Co > Ti > Sn > V > Cr > Cu > Pb > Au > Mn > Fe > Sc > Y > Zr > Sr > Cl > Zn > P > Ca > K. When Si was used: Cd > Co > Ti > V > Cu > Cr > Pb = Au = Mn = Fe > Sc > Zr = Y > Sn = Sr = Zn = Cl = Al > P = Ca > K. In Location E, when Al was used as reference element, the order of enrichment values were :Cd > Si > Co > Zr > Sn > Cu > V > Ti > Cr > Au > Pb > Fe > Mn > Sc > Y > Zn > Cl > P > Sr > Ca > K. When Si was used: Cd > Co > Zr > Sn > Cu > V > Ti > Cr > Au > Pb > Fe = Mn > Sc = Y > Zn > Cl > P = Sr = Al > K = Ca. Generally, It was observed that when Al gave higher values when used as reference element during computation of enrichment value when compared to that of Si.

Table 8
Bioaccumulation factor(BAF) target hazard quotient(THQ) and hazard index (HI) for both male frog and female frog

Element	BAF male frog	BAF female frog	THQ male frog	THQ female frog	RFD(USEPA 2013 2011)	RFD(RAIS2017)
Ti	0.48	0.053	0.00015	0.00016	4	
Cr	0.254	0.304	0.01105	0.01322	0.003	
Mn	0.116	0.154	0.0027	0.0036	0.024	
Fe	0.447	0.635	0.0196	0.0279	0.7	
Co	0.2496	0.284	0.00133	0.00152	0.02	
Ni	0.744	0.736	0.000965	0.000954	—	0.02
Cu	0.212	0.355	0.000494	0.000828	0.0371	
Zn	0.601	0.467	0.00008	0.0000603	0.3	
Al	0.059	0.0339	0.2388	0.3140	0.0004	
Cd	0.20	0.790	0.0258	0.02187	0.001	
Pb	0.32	0.797	0.00123	0.0007	0.0035	
V	0.122	0.147	0.00531	0.00638	—	0.00504
Zr	0.104	0.110	—	—	—	—
Mg	0.097	0.01198	—	—	—	—
Si	0.0095	0.0074	—	—	—	—
P	0.279	0.243	—	—	—	—
Cl	0.171	0.354	—	—	—	—
K	0.45	0.48	—	—	—	—
Ca	0.175	0.177	—	—	—	—
HI			0.307509	0.391195		

From Table 8 ,bioaccumulation factor in male frog was : Cd > Ni > Zn > Ti > K > Fe > Pb > P > Cr > Co > Cu > Ca > Cl > V > Mn > Zr > Mg > Al > Si while female frog : Pb > Cd > Ni > Fe > K > Zn > Cu > Cl > Cr > Co > P > Ca > Mn > V > Mg > Zr > Ti > Al > Si. Cd had the highest bioaccumulation and Silicon had the lowest in male frog while Pb was the highest in female frog In totality ,Pb was the highest bioaccumulation factor .The target hazard quotient as shown in Table 8 had decreasing order ; Al > Cd > Fe > Cr > V > Mn > Co > Pb > Ni > Cu > Ti > Zn while female frog :Al > Fe > Cd > Cr > V > Mn > Co > Ni > Cu > Pb > Ti > Zn. Aluminum had the highest quotient value and Zinc had the lowest value in both the male and the female frog. The hazard index of the female frog was higher than the hazard index of the male frog as shown in Table 8. The trend obtained in this study can be compared to the values reported by stolyar et al 2008.

Conclusion

This research has a significant effect on biodiversity and, in particular, human health, as human activities such as such as the deposition of waste in the river, fishing activities carried out with the use of toxic chemicals, it has been observed that Igbekebo is an oil-producing area, there is an oil spill on the river which contributes to the presence of a high concentration of heavy metals. As is well established in this work, concentration of elements in both male and female frogs was compared with the available WHO standard limits, which showed that the concentration of heavy metals in frog samples was significantly higher than available WHO limits, making frogs in the Igbekebo area harmful for consumption. It was also observed that Iron(Fe) was the highest concentration of 45752.0mg / kg and 65035.9mg / kg in male and female frogs, respectively, due to the natural occurrence of high concentrations of iron in water, the use of toxic chemicals for fishing and the high deposition of waste and metallic materials in the river. Furthermore, the results of heavy metals in sediment samples give convincing reasons for the high concentration of heavy metals in both male and female frogs.This study will support both the conservation authority and the consumption of these frog species, which are susceptible to pollution by heavy metals.

Declarations

Ethical Approval: The authors observed all ethics during the research and sought for the necessary approval

Consent to Participate: Not applicable

Consent to Publish:The authors give their consent to publish the manuscript

Authors Contributions: Ogunjobi Adedayo Johnson initiated the work and designed the topic, Edigbonya Thompson Faraday supervised the field work and the analytical work Adenikinji Charles Ademola, carried out the literature review, field work and the instrumental analyses, Edigbonya Thompson Faraday, designed the paper, reproduced the tables and did the editorial work

Funding: Partial funding by the institution

Availability of data and materials: Not applicable

Conflict of interest: The authors declare that they have no conflict of interest

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Figures

Figure 1

Map of Study Area