CONCENTRATIONS OF BTEX IN SHRIMPS OF OVWIAN-UDU RIVER, WARRI, DELTA STATE, NIGERIA AND HUMAN HEALTH IMPLICATIONS

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ABSTRACT

Concentrations of BTEX were determined in Shrimp (Macrobrachium macrobrachion) from Ovwian-Udu River in Warri, Delta State, Nigeria. Samples of shrimp were collected from March to May 2015. BTEX Concentration in whole shrimp was determined using Gas Chromatograph Agilent 6890 Series, with an FID detector. The mean concentration of benzene in the shrimp was 26.92±39.46 µg/kg. Toulene, Ethylbenzene, m-Xylene, p-Xylene and o-Xylene were not detected in shrimp. Target hazard quotients (THQs) for Benzene, Toluene, Ethyl benzene and Xylene in M. macrobrachion had values of 0.0020, 0.0000, 0.0000 and 0.0000. Although the total target hazard quotient was low, the values obtained for benzene were higher than the WHO standards for benzene in drinking water. With subsequent increase in benzene concentrations in shrimp, the human populations exposed are at risk. Regular assessment of water, sediment and fauna from Ovwian-Udu River, Warri for hydrocarbon contaminants, is recommended to safeguard human health and environmental intergrity.

Keywords: Health Risk, Fauna, Pollution, Target Hazard Quotient.

INTRODUCTION

Environmental pollution with petroleum and petrochemical products has been recognized as a significant and serious problem (Smith and Guentzel, 2010). Contamination of aquatic ecosystems by BTEX (benzene, toluene, ethylbenzene, and xylenes) has been observed in sediment, water and aquatic flora and fauna worldwide (Smith and Guentzel, 2010).

BTEX is the collective name for benzene, toluene, ethyl benzene, and xylenes, the volatile aromatic compounds often found in industrial discharges, petroleum oils and products (Anderson *et al.,* 2007). Benzene is used in the production of synthetic materials and consumer products, such as synthetic rubber, plastics, nylon, insecticides and paints. Toluene is used as a solvent for paints, coatings, gums, oils, and resins. Ethylbenzene is a gasoline and aviation fuel additive. They are also used extensively in

manufacturing processes (Baker *et al.,* 2001). Ethylbenzene may be present in consumer products such as paints, inks, plastics, and pesticides. Xylenes are used as a solvent in printing, rubber, and leather industries. The behavior of the four compounds is somewhat similar when released to the environment and thus they are usually considered as a group.

The main source of BTEX contamination is the leakage of gasoline from faulty and poorly maintained underground storage tanks. Other sources of BTEX contamination are releases from large bulk facilities, surface spills, and pipeline leaks. Exposure to BTEX can occur by ingestion, inhalation, and absorption through the skin.

Toxicity testing for BTEX compounds has also been conducted on aquatic organisms. Typically water concentrations in excess of 1 mg/L are required to produce acute toxic effects in organisms such as algae, daphnids and fish (Ameh *et al.,* 2013). Of the four BTEX compounds, benzene is the most toxic. Benzene increases the risk of cancer and other illnesses (Dougherty *et al.,* 2008). Toluene is readily absorbed from the gastrointestinal tract after ingestion, and is distributed preferentially in adipose tissue, then the kidneys, liver and brain. The main effect of toluene is on the brain and nervous system, with fatigue and drowsiness being the most obvious symptoms (Ameh *et al.,* 2011). The EPA considers that there is inadequate information to assess the carcinogenic potential of toluene (US EPA 2005). Toluene, Ethyl benzene and xylene have harmful effects on the nervous system (USGS 2006).

In recent years, world consumption of shrimp and other aquatic organisms has increased simultaneously with the growing concern of their nutritional and therapeutic benefits. The content of toxic BTEX in shrimp can counteract their beneficial effects. This may include serious threats like renal failure, liver damage, cardiovascular diseases and even death (Anderson *et al.,* 2004). In the last few decades, the concentrations of BTEX in shrimp and other aquatic organisms have been extensively studied in different parts of the world. In Nigeria, there exists a paucity of research on this.

The aim of this study is to assess the levels of BTEX in shrimps in Warri River around Ovwian-Udu Community and health risk to human who consume contaminated shrimps.

MATERIALS AND METHODS

Samplnig Site and Regime

Shrimp samples were collected from Ovwian-Udu River, located in Warri town Delta State, Nigeria (Latitudes $05^0$29'877"N and longitude $05^0$46'885"E from March to May 2015 (once a month) from three sampling points using locally fabricated shrimp traps. Samples were preserved in an ice chest.

Sample Preparation / Treatment

Samples were refrigerated at 4^0C for 24hrs and later oven dried at 35^0C for 48hrs, cooled and grinded. The dried samples were pulverized using a Thomas Willey Milling Machine and the powdered samples were weighed and stored in an air tight sample container for further use.

BTEX Extraction Procedure (EPA Method 5021)

Five grams of powdered shrimp tissue were added to 2g of anhydrous sodium sulfate ($NaSO_4$) and stirred with a stirring rod. Then 50ml of methanol was added to the sample and stirred with a magnetic stirrer for about 20 - 30mins. The extract was then ready to be cleaned up and fractionated using silica gel Solid Phase Extraction (SPE).

Silica gel clean ups

Glass wool was placed at the base of the syringe cartridge. Then 5g of silica gel was weighed into the syringe cartridge. The syringe cartridge was conditioned by rinsing with 5ml of the methanol and letting the methanol flow through the column until the head of the liquid in the column was just above the column frit. The collected methanol was discarded. The extract was loaded into the column, and elutant collected immediately in a 25ml volumetric flask. Prior to exposure of the column frit to air, column was eluted with an additional 5ml of methanol into the column again to remove completely the little amount of BTEX present in the sample. The final extract was transferred to labeled 2ml flask auto sampler vials with Teflon-Lined rubber caps.

BTEX was analysed using Gas Chromatograph Agilent 6890 Series, with an Agilent FID detector. Column dimensions was- capillary 30.0m x 320µm x 0.00 µm. Detector temperature was 300^{o}C, Inlet temperature was 200^{o}C, Pressure set point was 15.0 psi, The carrier gas was Helium. Oven temperature program used was 50^{o}C [hold 5min.] to 200^{o}C at 15^{o}C/min. to 210^{o}C at 2^{o}C/min. for 10min. The injection volume of the 2ml flask auto sampler vial with Teflon-Lined rubber cap was1.0 µl with a total cycle time run 19.67minutes for each sample injected.

External calibration was carried out using BTEXs standards. From the chromatogram, the retention times of the standards were used for the identification and quantization of the individual BTEXs. All other solvents used were of high purity analytical grade.

Target Hazard Quotient

The method for the determination of THQ was provided in the United States EPA Region III Risk based concentration table (US EPA 1989). The dose calculations were carried out using standard assumptions from an integrated United States EPA risk analysis.

Assumptions for the health risk calculations are;

1. Ingested dose is equal to the absorbed pollutant dose (USEPA 1989).
2. Cooking has no effect on the pollutants (Cooper *et al*., 1991).
3. The average body weight of a Nigerian is assumed to be 70 kg
4. Average lifetime of a Nigerian is 52 years.

THQ is determined by the following equation

$$THQ=\frac{EFrXEDtotXFIRXC}{RfDoXBWaXAXATn}x10^{-3}$$

Where EFr is exposure frequency (365 days/year); EDtot is the exposure duration 52 years, average lifetime); FIR is the food ingestion rate (g/day); C is the heavy metal concentration in crab/shrimp (µg/g);RfDo is the oral reference dose (mg/kg/day). BWa is the average adult body weight (70 kg) and ATn is the averaging exposure time for non carcinogens (365 days/year × number of exposure years assuming 52years).Since exposure to two or more pollutants may result in additive and/or interactive effects, total THQ in this study is treated as the arithmetic sum of the individual metal THQ values, derived by the method of Chien *et al.* (2002).

RESULTS

Table 1 shows the mean and standard deviation of the weight and length of shrimp. The mean weight of shrimp used for the experiment varied from 7.41-13.50g, while the mean length of shrimp used for the experiment varied from 6.00-10.54cm.

Table 2 shows the concentration of in BTEX in shrimp used for the study. The mean concentration of benzene in the shrimp was 26.92±39.46 µg/kg. Toulene, Ethylbenzene, m-Xylene, p-Xylene and o-Xylene were not detected in shrimp.

The oral reference dose for BTEX for shrimp is shown in Table 3 above. Target hazard quotients (THQs) for Benzene, Toluene, Ethyl benzene and Xylene in *M. macrobrachion* had values of 0.0020, 0.0000, 0.0000 and 0.0000. The total THQ (TTHQ) which measures the aggregated health risk due to uptake of BTEX via *M. macrobrachion* is 0.0020.

Regression statistics was adopted in testing the relationship between the weight and the total BTEX analysed in the whole tissue of shrimp. The weight of the fish and the total BTEX were taken as the independent and dependent factors respectively. No significant regression ($p>0.05$) was obtained in this test. The R^2 value which predicts the level of determination of dependent factor using the independent value was also low (0.002 or 0.2%)

DISCUSSION

The mean concentration of benzene in the shrimp was 26.92±39.46 µg/kg. Toulene, ethylbenzene, m-xylene, p-xylene and o-xylene were not detected. Regression statistics was adopted in testing the relationship between the weight and the total BTEX analysed in the whole tissue of shrimp. No significant regression ($p>0.05$), $R^2=0.002$ was obtained in this test.

Benzene has been reported to occur in fruits, fish, vegetables, nuts, dairy products, beverages, and eggs (EPA 1982). Benzene is toxic by all routes of administration. Hematotoxicity and immune toxicity have been consistently reported to be the most sensitive indicators of noncancer toxicity in both humans and experimental animals, and these effects have been the subject of several reviews (Aksoy, 1989; Goldstein, 1988, Snyder et al., 1993; Ross, 1996; U.S. EPA, 2002).

The most sensitive freshwater invertebrates include nymphs of the damselfly, *Ischnura elegans*, with a 48-hour LC_{50} of 10 mg/L (Sloof, 1983), and the water flea, with 48-hour LC_{50} of 31.2 mg/L for *Daphnia magna* (Bobra *et al*., 1983). The most sensitive fish species tested were salmonids, including rainbow trout, *Oncorhynchus mykiss*, with a 96-hour LC_{50} of 5.3 mg/L for juveniles (De Graeve *et al*., 1982), and coho salmon, *Oncorhynchus kisutch*, with a 96-hour LC_{50} of 9 mg/L for fry (Moles *et al*., 1979). Black *et al*. (1982) investigated the chronic toxicity of benzene to the early life stages of rainbow trout, leopard frog (*Rana pipiens*), and northeastern salamander (*Ambystoma gracile*). Eggs of each species were exposed continuously to benzene from within 30 minutes of fertilization (embryos) on through to 4 days post-hatch (larvae). This resulted in continuous exposures of 27 days for rainbow trout, 9 days for leopard frog, and 9.5 days for northeastern salamander. The LC_{50}s for continuous exposure were 8.3 mg/L for rainbow trout, 3.7 mg/L for leopard frog, and 5.2 mg/L for northeastern salamander.

Benzene is characterized as a class A human carcinogen following the 1996 EPA Guidelines for Carcinogen Risk Assessment (USEPA, 1996; USEPA, 2000). Benzene causes acute myeloid leukaemia (acute non-lymphocytic leukaemia), and there is limited evidence that benzene may also cause acute and chronic lymphocytic leukaemia, non-Hodgkin's lymphoma and multiple myeloma. Individuals who have experienced Benzene poisoning requiring treatment show a substantially increased risk of mortality from leukaemia (IARC 1987).

Chronic exposure to benzene can reduce the production of both red and white blood cells from bone marrow in humans, resulting in aplastic anaemia (PCS 1993). Both B-cell proliferation and T-cell proliferation are reduced by benzene. Decreased host resistance to infection has been reported in several laboratory animals exposed to benzene. However, other measures of immune toxicity have not been studied (PCS 1993).

Structural and numerical chromosomal aberrations have also been consistently reported in lymphocytes of workers exposed to benzene. Metabolites of benzene have been demonstrated to disrupt microtubule assembly *in vitro*, and also cause aneuploidy and chromosomal non-disjunction in human lymphocytes. This

may be significant in light of the fact that cytogenetic abnormalities involving the loss of all or part of chromosomes 5 and 7 have been associated with therapy-related myelodysplastic syndrome and leukemia (Irons *et al*., 1984; Lebeau *et al*., 1986).

The cancer slope factor (CSF) is an upper bound, approximating a 95% confidence limit, on the increased cancer risk from a lifetime oral exposure to an agent. The CSF for benzene ranges from 1.5×10^{-2} per mg/kg·day (0.015 per mg/kg·day) to 5.5×10^{-2} per mg/kg·day (USEPA, 1999; USEPA, 2000). TDI's or restriction levels have not been established by WHO or SCF because benzene is a human carcinogen. WHO (1993) derived concentrations in drinking water of 100, 10 and 1 µg/l, corresponding to excess lifetime cancer risk of 1 x 10^{-4}, 1 x 10^{-5}, and 1 x 10^{-6}, respectively and recommended a guideline value for drinking water of 10 µg/l.

Target hazard quotients (THQs) for Benzene, Toluene, Ethyl benzene and Xylene in *M. macrobrachion* had values of 0.0020, 0.0000, 0.0000 and 0.0000. The total THQ (TTHQ) which measures the aggregated health risk due to uptake of BTEX via *M. macrobrachion* is 0.0020. The TTHQ value was less than 1 suggesting that the consumption of *M. macrobrachion* is unlikely to cause adverse health effects to consumers.

However, the mean concentration of benzene reported in this study in the shrimp was high (26.92±39.46µg/kg). This could pose health risk to man through consumption of contaminated shrimp. Benzene has been reported to occur in food naturally, through migration from metallic covering layers of packaging material, or through contamination from the environment. It has been reported in several foods (eggs: 500–1900 µg/kg; rum: 120 µg/kg; irradiated beef: 19 µg/kg; heat-treated or canned beef: 2 µg/kg), and has also been detected in such foodstuffs as haddock, cheese, cayenne pepper, pineapple, and black currants (ATSDR 1999). Both epidemiological studies (Rinsky et al 1981, Rinsky 1989) and several case-studies showed that exposure to benzene was correlated with the occurrence of leukaemia (particularly acute myeloid leukaemia). Cytogenetic effects in peripheral lymphocytes were observed in human subjects with benzene haemopathy (WHO, 2003)

CONCLUSION

Concentrations of BTEX were determined in Shrimp (*M. macrobrachion)* from Ovwian-Udu River in Warri, Delta State, Nigeria. Although the target hazard quotient was low, the values obtained for benzene were higher than the WHO (2003) standards for benzene in drinking water. With subsequent increase in benzene concentrations in shrimp, the human populations exposed are at risk. Regular monitoring and assessment of water, sediment and fauna from Ovwian-Udu River, Warri for hydrocarbon contaminants, is recommended to safeguard human health and environmental intergrity.

REFERENCES

Agency for Toxic Substances and Disease Registry (1999). Toxicological profile for benzene. Atlanta, GA, US Department of Health and Human Services.

Aksoy, M. 1989. Hematotoxicity and carcinogenicity of benzene. *Environ. Health Perspect.* 82: 193-197.

Ameh, A.O., Mohammed-Dabo, I.A., Ibrahim, S., Ameh, J.B. (2013). Earthworm-assisted bioremediation of petroleum hydrocarbon contaminated soil from mechanic workshop. *African Journal of Environmental Science and Technology***7,** (6):531–539.

Ameh, A.O., Mohammed-Dabo, I.A., Ibrahim, S., Ameh, J.B., Azienge, C.D. and Tanimu, Y. (2011). Earthworm survival in used engine oil contaminated soil spiked with manure. *International Journal of Biology and Chemical Science,* 5(3):923–929.

Anderson, J. W., R. G. Riley, S. Kiesser, L. and Gurtisen, J. (2007). Toxicity of dispersed and undispersed Prudhoe Bay crude oil fractions to shrimp and fish. Proceedings 2007 Oil Spill Conference: (Prevention, Behavior, Control, Cleanup), Tenth Biennial, American Petroleum Institute, Washington, DC, pp. 235-240.

Baker, J. R., Jones, T. P., Jones, A. M. and Watson, H. C. (2001). Otter *Lutra lutra L.* mortality and marine oil pollution. *Biological Conservation,* 20:311-321.

Black, J.A., W.J. Birge, W.E. McDonnell, A.G. Westerman, B.A. Ramey, and D.M. Bruser, (1982). "The Aquatic Toxicity of Organic Compounds to Embryo-larval Stages of Fish and Amphibians", Research Report No. 133, University of Kentucky, Water Resources Institute, Lexington, Kentucky.

Bobra, A.M., W.Y. Shiu, and D. Mackay. (1983). "A Predictive Correlation for the Acute Toxicity of Hydrocarbons and Chlorinated Hydrocarbons to the Water Flea", *Chemosphere*, 12: 1121-1129.

Chien, L.C., Hung, T.C., Choang, K.Y., Yeh, C.Y., Meng, P.J., Shieh, M.J., Han, B.C (2002). Daily intake of TBT, Cu, Zn, Cd and As for fishermen in Taiwan. *Sci. Total Environ*, 285: 177-185.

Cooper , C.B., Doyle, M.E., Kipp, K. (1991). Risk of consumption of contaminated seafood, the Quincy Bay case study. *Environ. Hlth. Perspect*. 90: 133-140.

De Graeve, G.M., R.G. Elder, D.C. Woods, and H.L. Bergman. (1982)."Effects of Naphthalene and Benzene on Fathead Minnows and Rainbow Trout", *Arch. Environ. Contam. Toxicol*., 11: 487-490.

Dougherty, D., Garte, S., Barchowsky, A., Zmuda, J. and Taioli, E. (2008). NQO1, MPO, CYP2E1, GSTT1 and STM1 polymorphisms and biological effects of benzene exposure. *Toxicology Letters* **182** (3): 7–17.

Goldstein, B.D. 1988. Benzene toxicity. Occupational medicine. State of the Art Reviews. 3: 541-554.

IARC (1987). Summaries and Evaluations. Benzene. (Group 1). Lyon. International agency for research on cancer. P 120 (IARC. Monographs on the carcinogenicity of chemicals to humans. Supplement 7.http.www.inchem.org/documents/iarc/suppl7/bnzene.html.

Irons, R.D., R.W. Pfeifer, T.M. Aune, and C.W. Pierce. (1984). "Soluble Immune Response Suppresser (SIRS) Inhibits Microtubule Function *in vivo* and Microtubule Assembly *in vitro*", *J. Immunol*., 133 (4), 2032-2036.

Lebeau, M.M., K.S. Albain, R.A. Larson, J.W. Vardiman, E.M. Davis, R.R. Blough, H.M. Golomb, and J.D. Rowley. (1986)."Clinical and Cytogenetic Correlations in 63 Patients with Therapy-related Myelodysplastic Syndromes and Acute Nonlymphocytic Leukemia: Futther Evidence for Characteristic Abnormalities of Chromosomes no. 5 and 7", *J. Clin. Oncol*., 4:(3), 325-345.

Moles, A., S. Rice, and S. Korn. (1979)."Sensitivity of Alaskan Freshwater and Anadromous Fishes to Prudhoe Bay Crude Oil and Benzene", *Trans. Am. Fish. Soc*., 108: 408-414

PCS (1993). Benzene. Geneva world Organisation International Programme on Chemical Safety. Environmental Health Criteria 150. http://inchem.org/documents/ehc/ehc/ehc150.htm.

Rinsky R.A, Young R.J, Smith A.B. (1981). Leukemia in benzene workers. *American Journal of Industrial Medicine*, 2:217-245.

Rinsky RA (1989). Benzene and leukemia: an epidemiologic risk assessment. E*nvironmental Health Perspectives*, 82:189-192.

Ross, D. 1996. Metabolic basis of benzene toxicity. *Eur. J. Haematol*. 57: 111-118.

Slooff, W. 1983. Benthic macroinvertebrates and water quality assessment. Some toxicological considerations. *Aquat. Toxicol*, 4:73-82

Smith, K. L. and Guentzel, J. L. (2010). Mercury concentrations and omega-3 fatty acids in fish and shrimp: Preferential consumption for maximum health benefits. *Marine Pollution Bulletin,* 60 (9): 1615–1618.

Snyder, R., G. Witz, and B.D. Goldstein. 1993. The toxicology of benzene. *Environ. Health Perspect.* 100: 293-306.

US EPA (1982). Health effects assessment for benzene. EPA 540/1-86-037. Office of research and development. Office of health and environmental assessment. Environmental criteria and assessment office, Cincinnati OH 45268.

US EPA (1989).Risk assessment guidance for superfund. In: Human Health Evaluation Manual (Part A), Interim Final, vol 1. Office of Emergency and Remedial Response, US Environmental Protection Agency, Washington DC.

US EPA (1996). US Environmental Protection Agency. Proposed guidelines for carcinogen risk assessment. Federal Register 61: 17960-18011.

US EPA Method 5021(1996). Volatile organic Compounds in soil and other solids Matrices using Equilibrium headspace analysis, Revision 0, December 1996.

USEPA (1999). U.S. Environmental Protection Agency. Extrapolation of the benzene inhalation unit risk estimate to the oral route of exposure. National Center for Environmental Health, Office of Research and Development. Washington, DC. NCEA-W-0517. http://www.epa.gov/iris/supdocs/benzsup.pdf.

US EPA (2000). US Environmental Protection Agency.Benzene (CASRN 71-43-2). Integrated Risk Information System.http://www.epa.gov/iris/subst/0276.htm#quaoral.

U.S. EPA. (2002). Toxicological Review of Benzene (Noncancer Effects). Available online at: www.epa.gov/iris.

U.S. Environmental Protection Agency. (2005). *Integrated Risk Information System (IRIS) on Toluene*. National Center for Environmental Assessment, Office of Research and Development, Washington, DC.

USGS (2005). BTEX definition. United States Geological Survey. December 14, 2006.

WHO (1993). Guidelines for drinking water quality. 2 nd Edition, vol. 1. Recommendations. World Health Organization, Geneva.

WHO (2003). Benzene in drinking water. Background document for development of WHO Guidelines for drinking water quality. WHO/WSH/03.04/24.

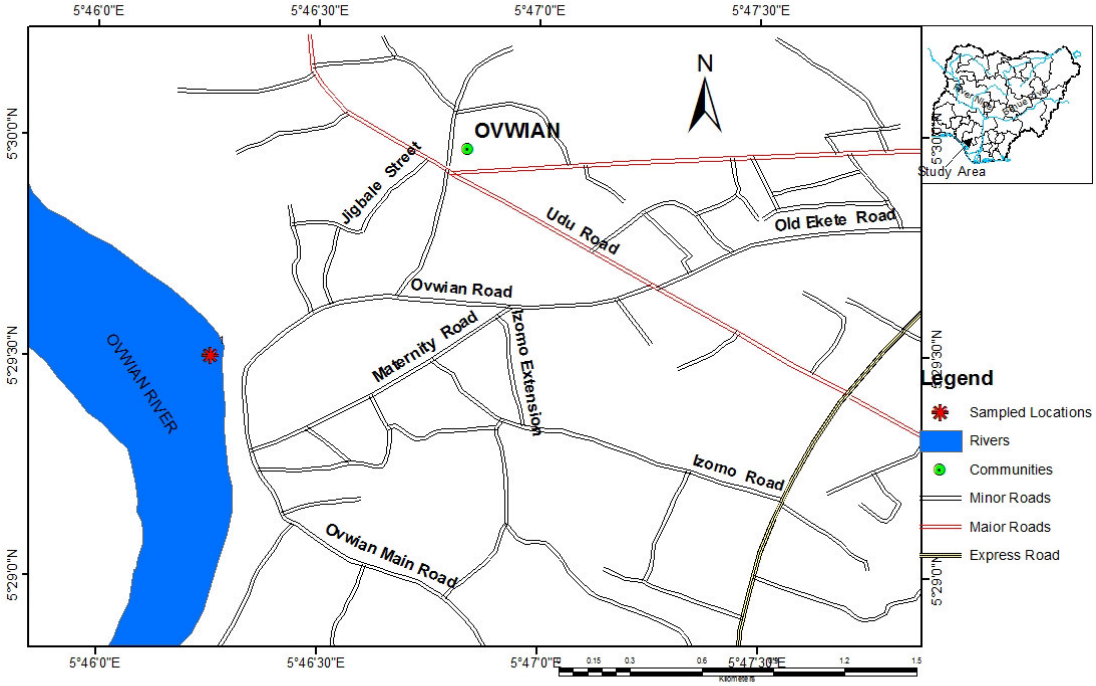

FIG. 1: MAP OF STUDY AREA SHOWING THE SAMPLES LOCATION

Fig 1: Map of study area

Table 1: Weight and length of the experimental shrimp

	Standard Length (cm)	**Weight (g)**
	$\bar{X}$±SD (Min-Max)	$\bar{X}$±SD (Min-Max)
Morphometrics	7.78±1.55 (6.00-10.54)	11.19±2.03 (7.41-13.50)

Table 2: Concentrations of BTEX in shrimp (µg/kg).

	Benzene	Toulene	Ethylbenzene	m-Xylene	p-Xylene	o-Xylene	Total
	$\bar{X}$±SD (Min-Max)	$\bar{X}$±SD (Min-Max)	$\bar{X}$±SD (Min-Max)	$\bar{X}$±SD (Min-Max)	$\bar{X}$±SD (Min-Max)	$\bar{X}$±SD (Min-Max)	$\bar{X}$±SD (Min-Max)
Concentrations	26.92±39.46 0.00-122.22	ND (0.00-0.00)	ND (0.00-0.00)	ND (0.00-0.00)	ND (0.00-0.00)	ND (0.00-0.00)	26.92±39.46 0.00-122.22

ND=Not Detected

Table 3: THQ of BTEX for Shrimp through consumption.

Contaminant	Concentration in Shrimp(mg/kg)	RfDo(mg/kg-d)	THQ
Benzene	0.026	0.004	0.0020
Toluene	0.00	008	0.0000
Ethyl Benzene	0.00	0.1	0.0000
Xylene	0.00	0.2	0.0000
TTHQ			0.0020

Table 3: Test of relationship between the total BTEX concentration in shrimps obtained from Ovwian-Udu River and their Weight.

	Regression Equation	**R^2 Value**	**p-Value**
Total BTEX	y = -865.0x + 36.60	0.002	p>0.05

p>0.05 – No Significant Regression
Independent factor = Weight of the shrimps
Dependent factor = Total BTEX

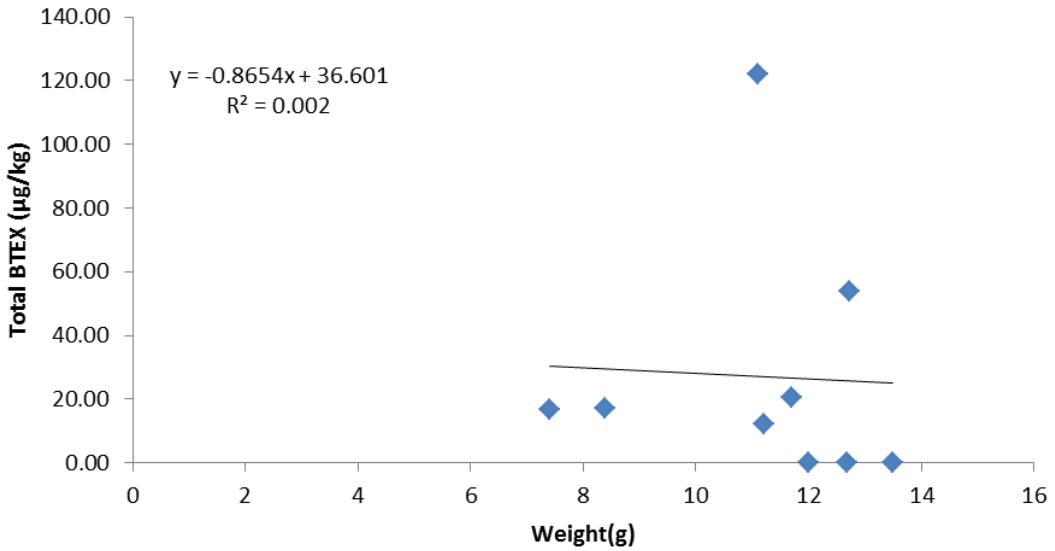

Figure 2: Relationship of BTEX concentration in shrimps obtained from Ovwianudu River and their Weight