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EVALUATION OF HEAVY METAL POLLUTION IN EDIBLE LARVAE OF AFRICAN PALM WEEVIL (*R. PHOENICIS*) IN BAYELSA STATE, NIGERIA

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Abstract

Bioaccumulation of some heavy metals and selected essential elements: Mercury (Hg), Lead (Pb), Cadmium (Cd), Copper (Cu), Zinc (Zn) and Manganese (Mn) was investigated in the edible larvae of African Palm Weevil (APW) (*Rhynchophorus phoenicis*) from polluted sites. Cadmium pollution was confirmed by the presence of 0.025mg/100g to 0.037mg/100g of cadmium in the larvae, above the limit of 0.02mg/100g was hazardous to health of consumers. Higher concentration of manganese (1.804mg/100g- 2.848mg/100g) found in the larvae was above the limit of 0.05mg/100ga risk to health. There was low concentration of zinc (2.79mg/100g-2.89mg/100g) and copper (0.07.40mg/100g-2.89 mg/100g) below the limit of 9.94mg/100g.No accumulation of lead and mercury in the larvae because it contained trace amount of 0.0001mg/100g of both heavy metals, which was below the limit of 0.0006mg/100g. Larvae of *R. phoenicis* are good biomarkers for monitoring heavy metal pollution in the environment. Effective control and management of toxicity of cadmium and manganese to save humans and biota in the affected communities and the environment is recommended urgently.

Keywords: Bioaccumulation, heavy metals, *R. phoenicis* larvae

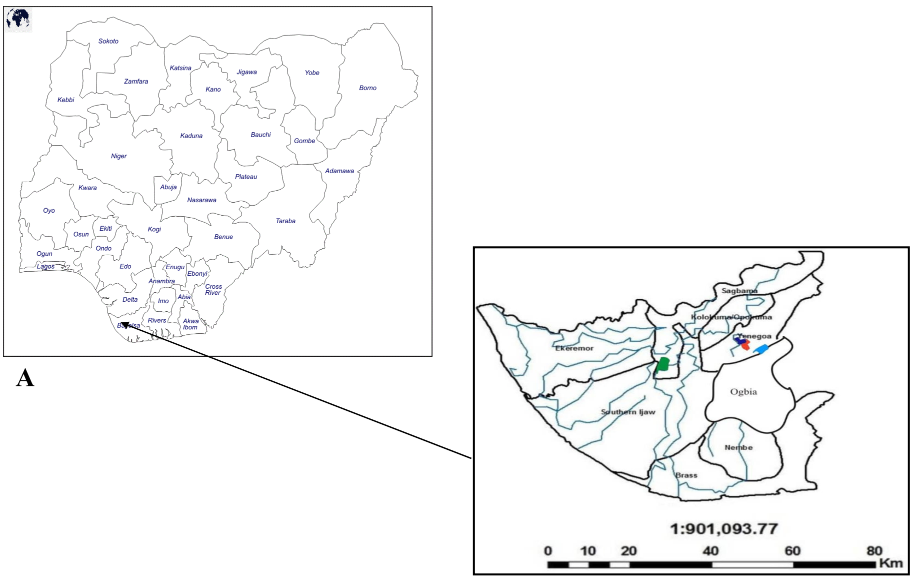
Introduction

The larvae of the African Palm weevil (*Rhynchophorus phoenicis*) are popularly known as "diet" in Warri and "Suya" in Bayelsa State. It is a highly cherished traditional delicacy in many parts of Nigeria and other countries in Africa (Thomas, 2003 and Ekpo, 2003). (Defoliart, 1992; Allotey and Mpuchane, 2003, Choon-Fah*et al,,* 2008). The Larvae of *R. phoenicis* are often hawked along major roads and markets across the Niger Delta States of Nigeria (Ekrakene and Igeleke, 2007). The mode of preparation of the larvae for eating differs from one ethnic group to another (Thomas *et a.l*, 2017). It is occasionally consumed in raw form roasted or fried but the fried form was more acceptable in the Niger Delta where it is usually eaten with tapioca or bread by the Urhobo in Delta State, whereas the Ijaw people eat it with soaked garri, farina and any staple food (Thomas 2003; Thomas, *et al*, 2005). The larvae are endowed with lot of nutrients, hence pregnant women eat it as a source of essential nutrients (Ekpo, 2003). The use of the larvae was beyond the nutritional value, as it was believed to possess some medicinal properties; hence the Itsekiri people in Delta State use the life larvae to cure some ailments in infants. It was also recommended for traditional healing of new born babies in some Ikwerre communities in Rivers State. A survey of its food uses showed that the larvae of *R. phoenicis* have different names in different ethnic groups, tribes and states in Nigeria, which confirms its widespread utilization as an important food in different parts of the oil rich palm belt, which include Bayelsa, Rivers, Cross River, AkwaIbom, Delta, Edo, Imo, Benue, Anambra and other states in southern Nigeria (Thomas 2003; Thomas and Briyai, 2019). Incidentally, the palm belt span across the oil-rich geological zone which equally produces commercial crude oil. Therefore, the larvae of the palm weevil had been associating with heavy metals released to the environment; hence, conscious effort is required to protect the masses from the intake of harmful pollutants, especially long-term exposure to the heavy metals. This study was carried out to ascertain the amount of some heavy metal in the edible larvae of African palm weevil in some impacted locations in Bayelsa State, where crude oil exploration had continued since 1956 till date

Materials and Method

Description of study area

The Gbaraing as gathering project location at Obunagha community in Gbarain Kingdom, Yenagoa, was set as site 1 sitting on a coordinate of Longitude 4.9514ºN, and Latitude 6.3492ºE. Site 2 was set at Etelebou flow station at Ogboloma Community in Gbarain Kingdom sitting on a Longitude of 5.0440ºN and Latitude of 6.3379ºE, while Site 3 wastaken at Tombia round about along Imiringi road within Yenagoa municipality sitting on a Longitude of 4.9514ºN and Latitude 6.3492ºE. The control site was taken at Toru-Ebeni forest in Sagbama Local Government Area sitting on a coordinate of Longitude 4.731ºN and Latitude of 6.1090ºE all in Bayelsa State of Nigeria (Fig 1).



A

B

Figure 1: A map (B) showing the study area and site locations depicted in 4 distinct colours as extracted from the map of Nigeria (A).

Site 1: Gbarain gas gathering project site, Obunagha in Gbarain Kingdom in Yenagoa Local Government Area.
Site 2: Etelebou flow station, Ogboloma in Gbarain Kingdom in Yenagoa Local Government Area.
Site 3: Tombia round about along Imiringi roadin Yenagoa municipality.
Site 4: Toru Ebeni forest in Sagbama Local Government Area.

Source of Materials

The larvae of *R. phoenicis* were obtained from infested raphia palms from the four locations (Fig. 1). In each site, three matured raphia palms (*Raphia hookeri*) that has been tapped for wine was cut down and left for over eight to twelve weeks on the ground at the various sites, after which it was harvested by cutting - open the infested portions of the trunk with an axe and matchete. The larvae were extracted manually from the tissues of the decaying palms and transferred in aerated plastic buckets with some quantity of the fibrous tissues and transported to the laboratory for analysis.

Heavy Metals Analyses Procedures

Samples were oven dried at 80-90°C and was grinded with a mortar pestle, 2g of the sample was weighed into a glass beaker (100m1); 5g of sample was transferred into 100ml glass beaker, while a mixture of 2m1 concentrated nitric acid ($HN0_3$), 10ml of concentrated hydrochloric acid (HCl) and about 20m1 distilled water was added. Samples were digested on a coming PC-351 model hot plate at medium to low heat until about 5m1 concentrated extract was left. The content of the beaker was left to cool for about 30 minutes. The sample solution was filtered and quantitatively transferred into 50m1 standard volumetric flask. Finally, the filtered solutions were marked up to the 50m1 graduation line using distilled water. The filtrate was used in the determination of the following elements (Zn, Pb, Cd, Ca, Mn and Hg) using the GBC 908PBMT model flame atomic absorption spectrophotometer (FAAS). The total metal concentration of each sample was reported in units of ppm (mg/kg) and converted to mg/100g. The results obtained are the means of triplicate samples with standard deviations.

Results

The results (Table 1) showed that the concentration of heavy metals was present in the larvae of *R.phoenicis* in the proportionate order: Zn>Mn>Cu>Pb>Fe ($P< 0.05$).The results showed that there was no accumulation of lead and mercury in the larvae obtained from all the studied sites because there was trace amount of 0.0001mg/100g of lead and mercury which was below the permissible limit of 0.0006mg/100g. There was low concentration of 0.07mg/100g of copper in the larvae collected from the control site, while the larvae of *R. phoenicis* collected from the polluted sites contained significantly higher concentration of copper: 1 (0.879mg/100g), 2(0.910mg/100g) and 3 (2.91mg/100g), respectively. These values revealed that there was gradual accumulation of copper in the edible larvae of *R. phoenicis* but not yet a risk to human life because it was below the permissible limit of 7.33mg/100g for copper. The results further showed that the larvae obtained from the polluted sites contained higher amounts of cadmium, ranged between 0.025mg/100g to 0.037mg/100g, while the larvae obtained from the control site contained 0.025mg/10g of cadmium. These values revealed that there was accumulation of cadmium in the larvae of *R. phoenicis* at all the studied sites, because it slightly exceeded the permissible limit of 0.02mg/100g of cadmium (Harvard, 2018).The larvae of *R. phoenicis* obtained from the polluted sites contained slightly higher concentration of zinc which ranged between 2.79mg/100g to 2.89mg/100g, while the larvae from the control site contained 2.790mg/100g of zinc. These values indicated that bio-accumulation of zinc had occurred in the study area but it was not yet a risk to the health of consumers and other animals as it was below the permissible limit of 9.94mg/100g (Harvard, 2018). Furthermore, the results showed that the larvae obtained from infested raffia palms from the control site contained lower amount of 1.804mg/100g of manganese, while the larvae obtained from infested raffia palms at the polluted sites contained significantly higher concentration of manganese: 1 (2.848mg/100g), 2(2.831mg/100g) and 3 (2.862mg/100g). These values showed that there was contamination of manganese in the edible larvae of *R. phoenicis* arising from accumulation of manganese in the soil of the studied locations***. This was of grave health concern to the life of the consumers of this edible insect because the amount of manganese exceeded the permissible limit of 0.05mg/100g of manganese.

Table 1: Heavy Metals Composition in *R. phoenicis* larvae from polluted and Control sites

Parameters (mg/100g)	**Polluted site 1 (OGGP)**	**Polluted site 2 (EFS)**	**Polluted site 3 (IMR)**	**Control site 4 (TE)**	**WHO limits (Harvard, 2018)**
Mercury	0.0001 ± 0.0^{a}	0.0001 ± 0.0^{a}	0.0001 ± 0.0^{a}	0.0001 ± 0.0^{a}	0.0006
Lead	0.0001 ± 0.02^{c}	0.0001 ± 0.02^{c}	0.0001 ± 0.02^{c}	0.0001 ± 0.02^{c}	0.0001-0.003
Copper	0.879 ± 1.7^{b}	0.910 ± 1.7^{c}	2.89 ± 3.1^{a}	$0.07.40\pm1.6^{a}$	9.94
Cadmium	0.037 ± 0.4^{a}	0.025 ± 0.3^{a}	0.037 ± 0.4^{a}	0.025 ± 0.9^{a}	0.02
Zinc	$2..80\pm3.1^{a}$	2.79 ± 3.0^{a}	2.89 ± 3.1^{a}	2.790 ± 3.0^{a}	9.94
Manganese	2.848 ± 3.1^{b}	2.831 ± 3.1^{b}	2.862 ± 3.1^{b}	1.804 ± 2.5^{a}	0.05

Different superscript (a, b, c, d) along row indicates a significant different among site compared to the control. (p< 0.05)
Key:
Site 1: OGGP – Obunagha – Gas gathering project site
Site 2: EFS – Etelebou Flow Station
Site 3: IMR – Imiringi Road, Yenagoa.
Site 4: TE – Toru – Ebeni bush

Discussion

The findings from this study revealed the presence of higher concentration of manganese which ranged between 1.804mg/100g to 2.862mg/100g in*R. Phoenicis* larvae was beneficial to consumers, because manganese is an essential element required for bone formation and strengthening of weakened bones in people who suffers from osteoporosis (WHO, 1977). In addition, manganese is a co-factor in the active sites of many enzymes involved in normal development, maintenance of nerves and immune cell functions, regulation of blood sugar and vitamins among others (Antonini *et al.,* 2006, Bailey *et al,* 2017 and Ben-shahar, 2018). However, when people are chronically exposed to manganese, it become a risk factor due to its toxic effect on many organ systems ("typical parkinsonian syndrome" or "manganism') arising from the accumulation of manganese in the brain. The above pathological condition was attributed to the presence of dopamine, GABA and glutamate neural signaling in insect brain which affects the foraging activities of pollinators (honey bees) (Ben-shahar, 2018). The presence of 2.79mg/100g – 2.80mg/100g of zinc in the larvae of *R. phoenicis* was of great nutritional importance because zinc is an essential element that is rarely present in diets of vegetarians. Therefore, vegans can be advised to consume the larvae of *R. phoenicis* as supplementary source of zinc to ameliorate chronic zinc deficiency. Zinc is required in the active-sites of about 300 enzymes involved in metabolizing glycosides and proteins, as well as insulin secretion by the pancreas, to enhance immunity and resolve sexual infertility, improve growth and cause faster rate of healing of wounds. Prolonged zinc deficiency was often associated with liver disease and pernicious anemia (ICMSF, 1986). Cadmium usually accumulates in the roots of terrestrial plants and bind to the cell wall, through which it is easily ingested by insects (grasshoppers) and aquatic invertebrates (especially molluscs) which feed on the plants for growth and reproduction. Similarly, in the case of the adult palm weevils, it lay its eggs inside the feeding holes which later hatch and develop through its life cycle in the tissues of an infested raffia or young oil palm (Kehinde*et al.,* 2017).

Atmospheric cadmium emitted to the air through gas flaring, deposits sparingly on the soil and absorbed through the roots of plants which are eaten by insects and other animals. Worst, in recent times, is the menace of illegal refining of crude oil obtained from vandalized pipelines by unemployed youths, as their means of survival, thereby contributing to the accumulation of cadmium and other heavy metals in the air and soil of Niger Delta (NCMCR, 2003). The frequent occurrence of crude oil spills, uncontrolled incineration of wastes at dumpsites, bush-burning by peasant farmers and occasional use of chemical fertilizers are additional sources of heavy metal emissions into the air (AMAP, 1998). Although, cadmium is not useful to plants and animal life, low concentrations are tolerated by binding it to special proteins to make it harmless. When it exceeds the permissible level of 0.02mg/100g, it affects the health of people by causing kidney damage and lung emphysema in humans (IPCS WHO, 1992a and 1992b).

The International Agency for Research in cancer confirmed that cadmium is one of the most carcinogenic elements in humans as it frequently causes lung cancer and occasionally causes prostate cancer in people who are exposed to it by their occupation (Waalkes, 2000 and IARC, 1993).

The results of this study have revealed that consistent accumulation of heavy metals is taking place in the soils of the impacted areasdue to systemic accumulation of pollutants emitted through gas flares from crude oil flow stations at Etelebou, plus pollutants from the Gbarain-Gas gathering plant that negatively affect the immediate host communities including: Obunagha, Okolobiri, Ogboloma, Koroama, Nedugo-Agbia, Polaku and all the neighbouring communities of Ekpetiama clan, especially Gbarantoru, Tombia and Akaibiri in Yenagoa local government area of Bayelsa State. The negative impact spreads over Imiringi road axis where massive oil exploration activities had continued at flow stations which flares gases in Ogbia local government area where the first commercial oil well was discovered at Oloibiri in 1957.

The results further revealed that Toru-Ebeni which was taken as a control site due to the non-existence of oil exploration activities, yet it suffered heavy metal pollution through anthropogenic sources (humans, animals, insects) and by natural geochemical actions of wind, water and other abiotic factors (Lee *et al.*, 2006). This implied that, the entire Bayelsa State is affected by chemical pollution by extension of the dispersion of the pollutants through air, surface and ground water resources (Ukpong *et al,* 2013*)*. It had been reported that heavy metal accumulation causes growth inhibition and reduces the fecundity and hatchability of eggs in insects. This means that bio-accumulation of cadmium and other heavy metals would affect the reproductivity of *R. phoenicis* which is a nutritious edible insect species that enhances the socio-economic wellbeing and livelihood of rural communities in the Niger Delta of Southern Nigeria, Cameron, Ghana, Kenya and other developing economies of Africa. Therefore, it calls for intervention by international agencies that are concerned with food security, social health and protection of the environment to respond by finding solution to the environmental menace of heavy metal pollution in Bayelsa state and indeed, the Niger Delta of Nigeria.

Conclusion

The findings of this study revealed that, the larvae of *R. phoenic is* contained significant amounts of cadmium (0.025-0.037mg/100g) which was above the permissible limit of 0.02mg/100g.Therefore, cadmium pollution was confirmed in the edible larvae of *R. phoenicis*, thereby exposing the rural communities to grave health hazards, arising from the carcinogenicity of cadmium which causes lung cancer and occasionally responsible for prostate cancer. So, all concerned authorities must respond to control the environmental threats to life suffered by the inhabitants of the communities in the affected areas. The edible larvae of *R. phoenicis* also contained high concentration of manganese which ranged between 1.84mg/100g to 2.83mg/100g which exceeded the permissible limit of 0.05mg/100g of manganese. Therefore, further studies on the impact of environmental manganese on the biology and physiology of *R.phoenicis,*as well as, its role on the ecological balance of the food web of the ecosystem should be conducted without delay. The edible larvae of *R. phoenicis* should be used as biomarkers of heavy metal contamination during environmental monitoring and impact assessment.

There is urgent need to adopt effective control measures for cadmium and manganese pollution to avert the grave consequences affecting the people of the immediate host communities of Gbarain, Ekpetima and Epie-Atissa clans in Yenagoa Local Government Area, and indeed the whole Bayelsa State from imminent danger of chemical pollution.

The concerned authorities of government should support studies on how to prevent further increase to hazardous levels in the accumulation of copper and other heavy metals, to save the environment and its inhabitants from disaster in Bayelsa State and the entire Niger Delta Area of Nigeria, where oil exploration and illegal refining activities are rampant in recent times.

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Appendix

Table 2: Susceptibility Profile of *Escherichia coli* as collected from all samples analyzed

Antibiotics Conc. (µg)	**Resistant n (%)**	**Intermediate n (%)**	**Susceptible n (%)**
Tetracycline (10) 0(0.00)	9(29.03)	22(70.97)	
Cotrimoxazole (25)	9(29.03)	8(25.81)	14(45.16)
Gentamycin (10)	0(0.00)	3(9.68)	28(90.32)
Cefuroxime (30)	25(80.65)	1(3.22)	5(16.13)
Chloramphenicol (10)	2(6.45)	0(0.00)	29(93.55)
Ceftriaxone (30)	7(22.58)	12(38.71)	12(38.71)
Cefotaxime (30)	20(64.52)	9(29.03)	2(6.45)
Ciprofloxacin (5)	10(32.26)	15(48.39)	6(19.35)
Amikacin (30)	8(25.81)	12(38.71)	11(35.48)
Vancomycin (30)	17(54.84)	1(3.22)	13(41.94)
Ceftazidime (30)	19(61.29)	5(16.13)	7(22.58)
Meropenem (10)	22(70.97)	2(6.45)	7(22.58)

Table 3: Susceptibility Pattern of Enterohemorrhagic Escherichia coli (EHEC) isolated from all sources in the study area

Antibiotics Conc. (µg)	**Resistant n (%)**	**Intermediate n (%)**	**Susceptible n (%)**
Tetracycline (10)	0(0.00)	4(36.36)	7(63.64)
Cotrimoxazole (25)	1(9.09)	3(27.27)	7(63.64)
Gentamycin (10)	0(0.00)	0(0.00)	11(100)
Cefuroxime (30)	10(90.91)	0(0.00)	1(9.09)
Chloramphenicol (10)	0(0.00)	0(0.00)	11(100)
Ceftriaxone (30)	3(27.27)	4(36.36)	4(36.36)
Cefotaxime (30)	8(72.73)	3(27.27)	0(0.00)
Ciprofloxacin (5)	4(36.36)	7(63.64)	0(0.00)
Amikacin (30)	0(0.00)	10(90.91)	1(9.09)
Vancomycin (30)	5(45.45)	0(0.00)	6(54.55)
Ceftazidime (30)	6(54.55)	3(27.27)	2(18.18)
Meropenem (10)	10(90.91)	0(0.00)	1(9.09)

Table 4: MAR Indices of *Escherichia coli* and Enterohemorrhagic *Escherichia coli* (EHEC) Isolated during the study

MAR Index	***Escherichia coli* N=31**	**Enterohemorrhagic *Escherichia coli* (EHEC) N=11**
0.0	1(3.23)	1(9.09)
0.1	0(0.00)	0(0.00)
0.2	3(9.68)	0(0.00)
0.3	12(38.71)	5(45.45)
0.4	7(22.58)	3(27.27)
0.5	3(9.68)	1(9.09)
0.6	2(6.45)	1(9.09)
0.7	2(6.45)	0(0.00)
0.8	1(3.23)	0(0.00)

KEY: Multiple Antibiotic Resistance (MAR)