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Full Length Research Paper

Toxicological effects of burrow pit effluent from a waste dump on periwinkle (*Tympanotonus fuscatus linne*)

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Burrow pit effluent collected from a waste dump site in the Niger Delta area of Nigeria was subjected to sublethal test using periwinkle (*Tympanotonus fuscatus linne*). This was to ascertain if the heavy metals and organic constituents in the burrow pit effluent bioaccumulated in the tissues of the organisms. The test was conducted using the Organization for Economic Cooperation and Development (OECD) protocol #218 in a sediment medium with varying concentrations of the test effluent. Low bioaccumulation potentials were observed for the metals in the tissues of the organisms. The determination of sixteen polycyclic aromatic hydrocarbons (PAHs) revealed only three components at relatively low concentrations in the tissues of the organisms at test termination of 28 days for test effluents concentrations (3.125, 12.5 and 50%). Concentrations for benzo [b] fluoranthene were (0.0017 – 0.0039 ppm), phenanthrene, (0.0021 – 0.0049 ppm) and pyrene, (0.0035 - 0.0081 ppm). However, the concentration of PAHs in the tissues of the organisms at test initiation was <0.0001 ppm. There was significant ($p < 0.05$) difference in the PAHs concentrations in the organisms exposed to the test effluent and the controls. This could lead to adverse ecological imbalance on a variety of aquatic species including bottom dwelling organisms inhabiting such environment if the release of untreated effluent is not controlled in the Niger Delta area of Nigeria.

Key words: Periwinkle (*Tympanotonus fuscatus linne*), sediment toxicity, effluent, sublethal toxicity (Bioaccumulation).

INTRODUCTION

The environment is perceived to be at risk from thousands of toxic substances and chemicals of both anthropogenic and natural origin. When hazardous substances are released into the environment, an evaluation is necessary to determine the possible impact of these substances on human health and other biota (Adams et al., 1992; Ogeleka et al., 2010). An important process through which chemicals substances can affect living organisms is bioaccumulation. Bioaccumulation is a process by which chemicals or substances are taken up by an organism either directly from exposure to a contaminated medium or by consumption of food containing the chemical or substance. Bioaccumulation means an increase in the concentration of a chemical or substance in a biological organism over time, compared to its concentration in the environment (Corl, 2001; Relyea and Diecks, 2008). Thus understanding the dynamic process of bioaccumulation is very important in protecting human beings and other organisms from the adverse effects of chemical exposure and has become a critical consideration in the regulation of chemicals (DPR, 2002; OECD, 2003).

The natural aquatic systems may extensively be contaminated with heavy metals released from industrial and other anthropogenic activities. Metals are non-biodegradable and are considered as major environmental pollutants causing cytotoxic, mutagenic and carcinogenic effects in animals (Ajayi and Osibanjo, 1981; Hayat et al., 2007; Hussain et al., 2011). Heavy metal contamination may have devastating effects on the ecological balance of the recipient environment and a diversity of aquatic

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organisms (Ashraj, 2005; Waqar, 2006; Farombi et al., 2007; Tawari-Fufeyin and Ekaye, 2007; Yilmaz et al., 2007).

Among animal species, fish and bottom dwelling organisms are the inhabitants that cannot easily escape from the detrimental effects of these pollutants (Vinodhini and Narayanan, 2008; Ezemonye et al., 2009). The mechanisms of heavy metal and organic excretion, deposition and detoxification in aquatic organisms are not capable of handling heavy metals in short time frames; thus these chemicals tend to accumulate specifically in metabolically active tissues and organs (Langston, 1989; Cicik and Engin, 2005; Ezemonye et al., 2007 a; Vinodhini and Narayanan, 2008). It is also known that physiological and biochemical parameters in aquatic organisms' blood and tissues could change when exposed to heavy metals and other toxic substances exerting an extra stress on the organisms (Sastry and Rao, 1984; Cicik and Engin, 2005; Davies et al., 2006).

Many aquatic pollutants such as polyaromatic hydrocarbons (PAHs) and their halogenated forms are chemically quiet stable; owing to their lipophilic nature, they can easily penetrate biological membrane and accumulate in organisms. Polyaromatic hydrocarbons (PAHs) consist of hydrogen and carbon arranged in the form of two or more fused benzene rings. They are important environmental pollutants because of their ubiquitous presence and carcinogenicity and are the most toxic of the hydrocarbon families (Tuvikene, 1995). The United State Environmental Protection Agency (USEPA) and the World Health Organisation (WHO) have identified 16 PAHs as priority pollutions while some of these e.g. benzo (a) anthracene, chrysene and benzo (a) pyrene are considered potential human carcinogens (EPA, 1980; Kanchanamayoon and Tatrahun, 2008).

Bioconcentration refers to the absorption or uptake of a chemical from a medium to concentrations in the organism's tissues that are higher than that in the surrounding environment. The degree to which a contaminant would concentrate in an organism is expressed as a bioconcentration factor (BCF). BCF refers to the concentration of a chemical in an organism's tissues divided by the exposure concentration (McGeer et al., 2003). It has been found that chemicals or substances displaying a half-life greater than 30 days, a bioconcentration factor (BCF) greater than 1000 or an octanol/water partition coefficient, log Kow value greater than 4.2 tend to be persistent and bioaccumulate (EPA, 2000; Ezemonye et al., 2007).

The aim of this study was to assess the effects of burrow pit effluent from a waste dump site in the Niger Delta area of Nigeria on bottom dwelling organism, periwinkle (*Tympanotonus fuscatus linne*). The toxicological end point of assessment was growth, bioaccumulation of heavy metals (copper, zinc, total iron, lead, chromium) and PAHs. The test species were chosen because they are abundant, sensitive and available all the year round in the Niger Delta ecological zone of Nigeria. They are of great economic importance since they play a role in the coastal food web serving as a source of nutrition for humans and other organisms (Beeby, 2001; Ciarelli et al., 1997).

MATERIALS AND METHODS

Area description

The waste dumpsite is in Ughelli area of the Niger Delta ecological zone of Nigeria. Ughelli is located in Ughelli North local Government Area of Delta State, Nigeria. The site boundary georeferences are latitude 05°28'55.6"N and longitude 005°48'34.0"E. The study site is a fresh water environment with temperature ranging between 21.5 and 36.9°C and relative humidity between 55 and 94%. An annual rainfall of 2900 mm is normal for the area. Freshwater swamp forest zone dominates but most of it has been cleared due to high human population density and rural to urban drift.

Collection and acclimation of test organisms

The effluent sample was collected from a waste dump site in Ughelli in the Niger Delta area of Nigeria and stored at 4°C before starting the experiment. Healthy test species of periwinkle were collected from a fresh water cultured farm in Ekrheranwhen in the Niger Delta. Ekrheranwhen is located in Ughelli North Local Government Area of Delta State, Nigeria (Latitude 05°32'43.6"N and longitude 005°55'04.6"E). The test organisms were acclimated under laboratory conditions for a period of seven days. The size of the organisms used for the sediment bioassay was 2.67 ± 0.20 g.

Experimental sublethal bioassay procedure

The 28 day experiment was carried out using the OECD #218 sediment toxicity bioassay protocol with spiked sediment (OECD, 2004). The test began with a range-finding test to determine the concentrations to be used in the definitive test. Approximately 24 hours before the test, the sediment samples were acclimated to 25°C and weighed. Triplicate treatment tanks for each concentration of the test effluent were prepared with 1 kg of the sediment. The prepared test solutions (3.125, 12.5, 50%) were added to the treatment tanks, homogenized and allowed to settle for 2 to 3 hours. The test organisms were placed in the dilution water to rinse off debris that may interfere with the test and ten (10) organisms were weighed to obtain the initial weight. The organisms were then gently transferred into each amber-coloured glass tank containing the sediment and test effluent. The controls were maintained in clean sediment and habitat water without the test effluent (Environment Canada, 1992). The treatment tanks were gently aerated using oil-free low whisper aerators. After 28 days the sediment was sieved and the periwinkles were rinsed in the dilution water and weighed to obtain the final weight.

Analysis of metals and polyaromatic hydrocarbons (PAHs)

For metal analysis, soft tissues were collected after the shells were removed. These were dried at 480°C for 24 h. The dried tissues were ground and known weights were digested in a mixture of nitric acid (5 ml), sulphuric acid (3 ml) and perchloric acid (3 ml). Five (5) trace metals namely lead, copper, total iron, chromium and zinc were determined. The heavy metal contents of the digest (habita

Table 1. Concentrations of heavy metals and organics in periwinkle after 28 days.

Parameter (ppm)	Concentration of effluent			
	Variable (%)			Control (%)
	3.125	12.5	50	0
Total iron	37.15 ± 0.11	43.09 ± 0.14	49.52 ± 0.16	34.01 ± 0.10
Zinc	0.34 ± 0.07	0.35 ± 0.04	0.44 ± 0.03	0.30 ± 0.04
Phenanthrene	0.0021 ± 0.002	0.0039 ± 0.007	0.0049 ± 0.010	<0.001
Pyrene	0.0035 ± 0.001	0.0065 ± 0.002	0.0081 ± 0.002	<0.001
Benzo[b] fluoranthene	0.0017± 0.001	0.0027 ± 0.001	0.0039 ± 0.001	<0.001

water, burrow pit effluent, soft tissues of test species and controls) were tested using atomic absorption spectrophotometer (AAS, Shimazu 6701 F model).

The samples collected for PAH analysis were crushed, dried with sodium sulphate and extracted with dichloromethane (DCM) for 4 h in a ratio of 1:10 sample: solvent. The subsequent extract was then concentrated to 1 ml by evaporation in a secured fume hood and passed through a fractionating column. The resulting extract was dried with sodium sulphate and placed in clean amber coloured vials rinsed with DCM. An appropriate volume (1 µl) was injected into a GC/MS (Agilent 5975C) for the analysis of the 16 different PAHs components. The GC/MS was calibrated using specific PAHs standards. Standard stock solutions (1 mg/ml) were prepared by dissolving 10 mg of the desired PAH in 10 ml DCM and stored at 20°C. All working solutions were freshly prepared by serial dilution with DCM.

Water chemistry

The determination of physico-chemical parameters of the dilution water was carried out to provide relevant information on possible changes that could result in potential hazards to the biological indicators. Physico-chemical constituents determined include; pH, temperature, dissolved oxygen (DO), salinity and conductivity.

Physiological effect

The physiological endpoint used in this assessment was growth. Mean weight of the total number of organisms used for the test was taken at initiation (day 0) and termination (day 28) of the test. It was computed using the formula:

$$\text{Mean weight} = \frac{\text{weight of organism at day 0} + \text{weight of organism at day 28}}{2}$$

RESULTS

The analysis of the concentration of contaminants in the test organisms is an important approach to assessing the bioavailability of substances and to evaluate their behaviour in the environment. The concentrations of physico-chemical parameters, heavy metals and PAHs in the burrow pit effluent, dilution water (control), and periwinkle soft tissues at days 0 and 28 are presented in Table 1, Figure 1 and 2.

The control and the burrow pit effluent recorded 5.60 ±0.28 and 7.21 ± 0.43 pH units respectively. Salinity was 0.04 ± 0.004 and 0.73 ± 0.03 ppt while conductivity was 290 ± 23 µS/cm and 2420 ± 82 µS/cm in the same order. The mean temperature during the experimental period in all the test was 27 ± 2°C with a 16:8 h light: darkness photoperiod. Dissolved oxygen concentrations recorded was 6.4 ± 0.4 mg/l. Concentrations in the control for copper, zinc and total iron were 0.02 ± 0, 0.05 ± 0.01 and 0.06 ± 0.02 mg/l respectively while in the burrow pit effluent <0.01, 0.06 ± 0.02 and 0.36 ± 0.02 mg/l respectively were recorded. Lead and chromium were not detected in the control and burrow pit effluent. Three components of PAHs (benzo [b] fluoranthene, phenanthrene, pyrene) out of the sixteen components determined, were found in the burrow pit effluent. There were no PAHs in the control sample; however, the PAHs concentrations for benzo [b] fluoranthene, phenanthrene and pyrene in the burrow pit effluent were 0.008 ± 0.001, 0.010 ± 0.001 and 0.059 ± 0.004 ppm respectively.

The pH of the native sediment (control) was 4.98 ± 0.5 pH units. It had an organic carbon content of 3.3 ± 0.13% while PAHs were < 0.001 ppm. Concentrations recorded for zinc, copper, iron, chromium and lead were 41.92 ± 1.2, 9.47 ± 0.89, 14213 ± 123, 6.32 ± 0.46 and < 0.01 ppm respectively.

Concentrations reported in the tissue of the control organism for copper, zinc and total iron were 0.07 ± 0.01, 0.30 ± 0.04 and 34.01 ± 0.10 ppm respectively while chromium and lead were not detected. Zinc and total iron concentrations in the test organisms for 3.125, 12.5 and 50% test effluent were 0.34 to 0.44 ppm and 37.15 to 49.52 ppm respectively (Table 1). Concentrations for PAHs were obtained mainly from three components namely benzo [b] fluoranthene, phenanthrene and pyrene. The concentration of PAHs in the tissues of the organisms at day 0 was <0.001 ppm while the concentrations of benzo [b] fluoranthene, phenanthrene and pyrene in the tissues of the organisms after 28-day exposure to the test effluent are given in Table 1. The lowest PAHs concentration was recorded in the organisms exposed to test effluent concentration of 3.125% (benzo [b] fluoranthene, 0.0017 ± 0.001 ppm) while the highest concentration was recorded in exposure concentration of 50% (pyrene, 0.0081 ± 0.002 ppm).

The mean weight of the periwinkle was higher in the

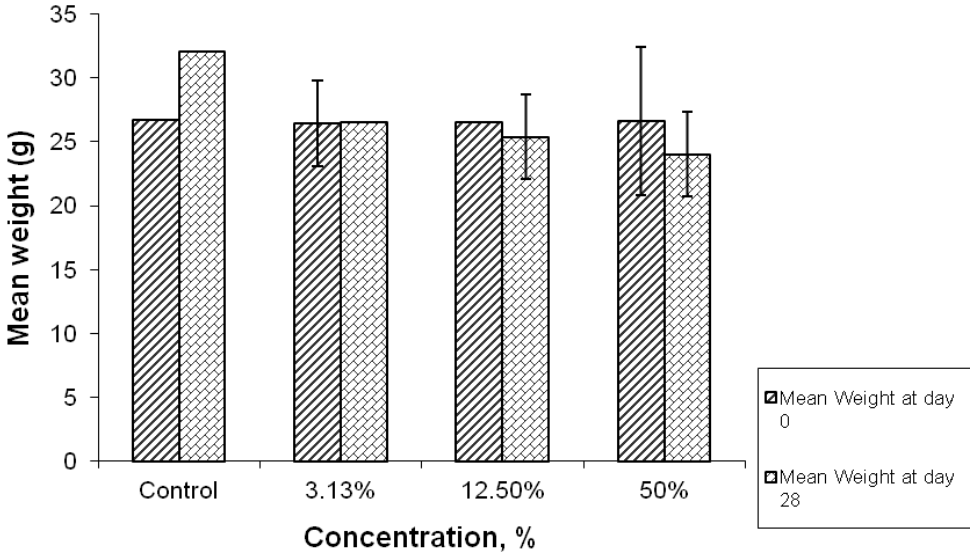

Figure 1. Mean weight ± SEM of periwinkle exposed to effluent at day 0 and 28.

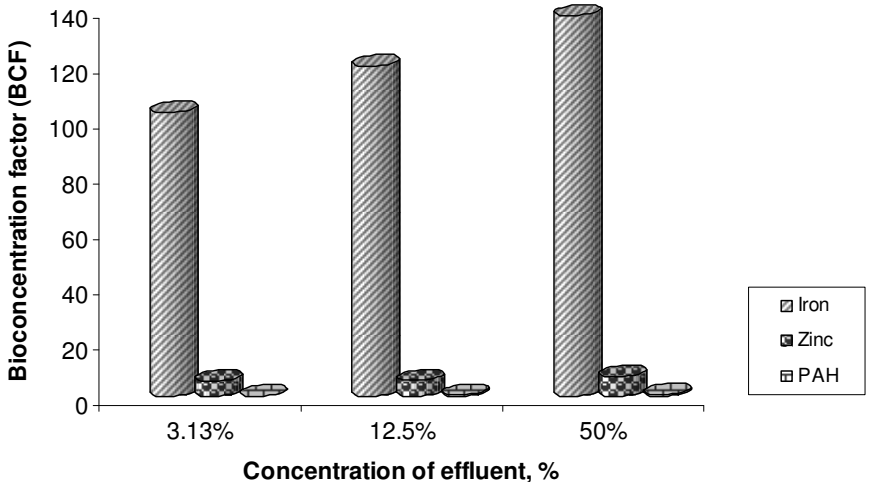

Figure 2. Mean Bioconcentration Factor (BCF) of burrow pit effluent at day 28.

control and the 3.125% test effluent than 12.5% and 50% test effluents concentrations (Figure 1). Bioconcentration factor (BCF) for total iron in the test organisms in the test effluent concentrations (3.125, 12.5 and 50%) were 103, 120 and 138 respectively. Zinc recorded a BCF ranging from 5.67 to 7.33. The test organisms showed levels of accumulation of PAHs, which varied from 0.22 in 3.125% to 0.63 in 50% test effluent (Figure 2).

DISCUSSION

Knowledge of heavy metal concentrations in aquatic organisms is important due to the nature of management and human consumption of these species. Bioaccumulation of metals in periwinkles can be considered an index of metal pollution in aquatic bodies (Davies et al., 2006). The baseline concentrations of metal components in the unexposed test organisms at test initiation indicate generally low levels than observed for the tissues of the organisms after uptake of the effluent. However, the observed accumulation of iron and zinc in the test organisms could be from the sediment since the values of these metals in the effluent were relatively very low in this study.

Certain factors may have influenced the differential uptake of metals in the test organisms. The chemical form of the metal, metal kinetics, route of exposure,

concentration and exposure duration may have affected the slight accumulation observed (Joel and Amajuoyi, 2009). Benthic organisms are the most directly impacted by contaminants, thus the removal of these aquatic components from the food web, due to pollution, may indirectly affect an ecosystem as a direct toxic effect since they serve as food for humans and other animals (Beeby, 2001). Invertebrates are generally more sensitive to pollutants than either fish or algae. Benthic species are particularly sensitive to water soluble contaminants, affecting the ability of the organisms to reproduce, avoid predators or feed (Bat et al., 1999; Chindah et al., 2001; Ezemonye et al., 2007 b). Consequently, when periwinkles are exposed to elevated metal levels in an aquatic environment, they can absorb the bioavailable metals directly from the environment via the tissue or through the ingestion of contaminated water, sediment and food (Burton, 1992; Trefry et al., 1996; Alexander, 2000). Uptake routes include: absorption primarily by passive diffusion with some active transport; the digestive system supports uptake of essential metals. Respiratory and body surfaces of aquatic organisms also support uptake of essential metals which are used for the maintenance of hyper (fresh) or hypo (marine) osmotic conditions. Rate of excretion / regulation determines toxicity / tolerance among populations. The results from this study compares favourably with works of Davies et al. (2006) and Chindah et al. (2009).

Very low concentrations of PAHs were found in the test organisms exposed to the contaminated effluent at test termination on the 28 day. The levels of PAHs in the organisms' tissues could have been induced by uptake from the burrow pit effluent since PAHs was not detected in the sediment at day 0. Polyaromatic hydrocarbons (PAHs) are readily absorbed by aquatic organisms during exposure to contaminated food, water and sediment, reaching levels higher than those in the ambient medium. However, PAHs do not accumulate in the same manner as some other lipophilic organic compounds, instead, they are converted to more water-soluble forms, which facilitate their subsequent slow excretion from the organism (Neff, 1985).

However, PAHs with a high molecular weight may cause sublethal effects; such as growth reduction, chronic diseases, reproductive impairment, at very low concentrations in biota: (5 to 100 ppb) in the tissue of the animal. Concentrations which are considered toxic (0.2 to 10 ppm) have been found in biota from heavily polluted areas (EPA, 2000; Kanchanamayoon and Tatrahun, 2008). Concentrations of PAHs in the aquatic environment are generally highest in sediment, intermediate in biota and lowest in the water column (CCME, 1992). In water, PAHs attach to sediment, impacting bottom-dwelling organisms like periwinkle, shrimp, oysters and plankton. As these organisms spend time in or near contaminated sediments, they accumulate PAHs in their tissues leading to harmful effects. A wide range of PAH-induced ecotoxicological effects in a diverse suite of biota, including microorganisms, aquatic biota, amphibians and terrestrial mammals have been reported (Delistraty, 1997; Ekundayo and Benka-Coker, 1994; Khan and Law, 2005). These studies assessed the effects of PAHs on survival, growth, metabolism and tumor formation, *i.e*. acute, developmental, reproductive toxicity, cytotoxicity, genotoxicity and carcinogenicity.

According to Canterford et al. (1978) it is useful to express results in terms of bioconcentration factor (BCF) when comparing the order of uptake of metals. In this study bioconcentration potentials were very low and varied with the test effluent concentrations. Although there was accumulation of zinc, total iron and PAHs in the tissue of the test organisms, BCF for these parameters were relatively very low i.e. less than 1000, thus are not persistent and could be eliminated from the test organisms with time if exposure is not continuous (EPA, 2000). However, the observed BCF indicates that periwinkles have potential to concentrate contaminants in their soft tissues (Ademoroti, 1996; Eja et al., 2003). The results from this study agreed with those obtained by Davies et al. (2006). The other parameters tested did not indicate any level of bioaccumulation in the tissues of the organisms.

In this study, mean weight of the periwinkle was higher in the control and the 3.125% test effluent than 12.5 and 50% test effluents concentrations. This indicates that the effect of the burrow pit effluent on the growth of periwinkle showed a slight reduction for organisms exposed to both 12.5 and 50% when compared to the control and 3.125%. Polycyclic aromatic hydrocarbons affect organisms through various toxic actions and they are being recognized with increasing frequency as major contributors to the hazard to aquatic life of contaminated sediments, particularly near areas of intense human activity (Neff and Burns, 1996; Neff et al., 2005; Pies et al., 2008).

Some authors suggested that PAHs detected in river sediments and water may contribute to endocrine disruption, which is supported by numerous studies on the physiological effects of PAH exposure on fish and other aquatic organisms (Lintelmann et al., 2003). Other studies have reported evidence of PAH exposure among fish populations (van den Heuvel et al., 1999), and cited PAHs as potential stressors causing increased mortality, growth and malformations in fish (Colavecchia et al., 2004; Heintz, 2007). In spite of the relatively low bioaccumulation levels recorded for metals and PAHs in the test organisms, it is important to caution that if the concentration of the components in the effluent increases, the amount inside the organism would also increase, as was observed in the organisms in the higher concentration, until it reaches a new equilibrium. Exposure to large amounts of the effluent for a long period of time, however, may overwhelm the equilibrium potentially causing harmful effects.

Conclusion

Information concerning effluent and chemical accumulation is fundamental in determining environmental quality guidelines by regulatory bodies. This will further assist in grouping substances that are potential hazards as well as qualifying the risk of chemicals and substances on the ecosystem and human health. Periodic surveillance is therefore essential to ensure ecosystem balance for these economically viable bottom dwelling organisms.

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