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CRITICAL REVIEW OF THE RELEVANCE OF BENTHIC ORGANISMS IN FORENSIC INVESTIGATION OF HYDROCARBON POLLUTION SOURCES

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ABSTRACT

Hydrocarbons have attracted so much attention due to their persistence in the environment, and bioaccumulation and carcinogenic properties. Their presence in the aquatic ecosystem does not only degrade aquatic habitat, water quality and aesthetics, but also generate additional costs for nations for containment and cleanup. Environmental forensics studies are triggered by environmental crimes and it involves the use of scientific investigative methods to identify responsible sources, and to ensure that justice occurs in order to compensate the damaged parties. Forensic evidence testing techniques are generally referred to fingerprinting, pollution fingerprints are used to identify the pollutant source or responsible party who will pay for the damages. In the aquatic environment, impacts of hydrocarbons on benthic organisms are well known. Benthic fauna and sediment are often examined for evidence of hydrocarbon exposure which may be used in litigation. In this review, the objective of is to examine the relevance of benthic organisms in forensic investigation of hydrocarbon pollution sources.

KEY WORDS : Benthos, Sediment, Hydrocarbon pollution, Forensic investigation.

INTRODUCTION

Hydrocarbons have attracted so much attention due to their bioaccumulation, carcinogenic properties, and persistence in the environment. Their presence in the aquatic ecosystem does not only degrade aquatic habitat, water quality and aesthetics, but also generate additional costs for federal, state and local agencies for containment and cleanup (GESAMP, 1990). Hydrocarbons are toxic to aquatic organisms due to their lipophilic character (low solubility), they tend to be adsorbed onto particles in aquatic ecosystem and ultimately accumulate in sediments (Bakhtiari *et al.*, 2009).

They are ubiquitous pollutants, which are rarely found as biosynthetic products and have a high toxicity for organisms due to their carcinogenic and mutagenic potentials (Bakhtiari *et al.*, 2010). Natural petroleum seeps and post-depositional transformations of biogenic precursors, as well as

through multiple human activities such as refueling operations, repair of boat engines, boat maintenance, leaching of creosote-treated pilings, and inputs from storm water runoff from parking lots and roads, and aerial deposition are their major access to aquatic systems (Yılmaz *et al.*, 2014). Other well known sources include; urban runoff, sewage disposal, industrial effluents, oil production and transportation operations (Boehm *et al.*, 2000).

In relation to environmental compartments of a watercourse, aquatic sediment and the associated organisms are major repository for most of the persistent chemicals introduced into surface waters (Bakhtiari *et al.*, 2009). Hence, sediments and benthos can be studied to determine the level and extent of pollutants such as hydrocarbons in aquatic ecosystems (Wenger and Isaksen, 2002). Many studies on the distribution and sources of both aliphatic and polyaromatic hydrocarbons have been conducted in different parts of the world (Meniconi

and Barbanti, 2007; Edokpayi *et al.*, 2016) and many of the studies revealed that chemical parameters such as indices, ratios and the occurrence of certain compounds can be used to establish levels of pollution, discriminate between biogenic or anthropogenic inputs and estimate the risk of harmful effects of hydrocarbons on aquatic organisms (Bakhtiari *et al.*, 2009; Ekpo and Wehner, 2009).

The world's aquatic systems have been under the influence of increasing degradation and exploitation of associated natural resources (Bender *et al.*, 1988; Ekpo *et al.*, 2012), and in the process waste and effluents from sundry sources end up in aquatic ecosystems, especially in the coastal areas, where together with urban and maritime activities are responsible for most environmental pollution events. Petroleum extraction, transportation and refining activities have contributed to the release of hydrocarbon in many aquatic systems. Owing to this, many studies have reported different levels of hydrocarbon contaminations in aquatic systems. Despite these enormous challenges posed by hydrocarbon contamination, not much has been achieved in the area of forensic studies to establish the origin of these contaminants. The aim of this work therefore, is to critically review literatures on environmental forensics with a view to establishing the relevance of benthic macroinvertebrates and sediment in environmental forensic investigation.

Determination of the primary sources of hydrocarbon in benthos

An array of physical, chemical and biological techniques has been developed to assist in the identification of sources of pollutants such as hydrocarbon in the aquatic environment (Meniconi and Barbanti, 2007; Bakhtiari *et al.*, 2009; Wang *et al.*, 2006). The primary goal of these techniques is to identify the origin of a contaminant of interest. For hydrocarbon in the aquatic ecosystem, biological attributes such as changes in the benthic community structure or molecular DNA, physiological processes are often employed. In some cases, a multi-evidential approach is used. Some of these analytical techniques are discussed below.

Gas Chromatography (GC)

Gas chromatography is probably the most basic and widely used analytical technique in environmental forensics today, and has been a standard analytical technique in many areas for the past years (Khalifa

et al., 2017). Good chromatography is the key to the success of environmental forensics. The chromatograms provide a fingerprint of the hydrocarbon present in benthic organisms. With various levels of fractionation, di-aromatic hydrocarbons can be separated from tri-aromatic hydrocarbons, or n-alkanes from branched and cyclic hydrocarbon or to isolate sulfur-containing compounds (Khalifa *et al.* 2017)

Gas chromatography-mass spectrometry (GCMS)

GCMS not only permits the separation of components present in hydrocarbon, but also aids the identification of the separated compounds. The technique has been effectively used in fingerprinting of hydrocarbon in aquatic biomonitoring using benthic organisms. It is not the goal of this paper to discuss all of the different types of mass spectrometers since they all have the same basic goal and that is, to identify components of hydrocarbon.

Biomarkers of hydrocarbon

The major classes of biomarkers used in petroleum exploration are equally applicable to environmental forensics. These include n-alkanes, isoprenoids, sesquiterpanes, tri-, tetra-, and pentacyclic terpanes, steranes, and a variety of other compounds, such as diamondoids and S-containing compounds, although not strictly biomarkers can be used in a similar manner. Not every class of biomarker will be present in every type of hydrocarbon product spilled into the environment, with the exception of crude oils which contain all the classes of biomarkers. For example, diesel, containing hydrocarbons in the C₁₀ to C₂₄ carbon number range will not contain steranes or pentacyclic terpanes in most cases, since these occur in the carbon number range beyond C₂₄. Gasoline contains few of the classical biomarkers since it is too volatile for most of these compounds to be present.

Stable isotopes

Carbon exists as two stable isotopes, and different hydrocarbon will have different carbon isotope distributions, depending on the extent of fractionation between the two isotopes during formation. The extent of fractionation, or the carbon isotope composition, is determined by completely combusting the material of interest to CO₂ and H₂O, removing the water, and then introducing the CO₂ into the isotope ratio mass spectrometer where the intensities of mass 45(13CO₂)/44(12CO₂) are

measured relative to an international standard. The resulting ratios are converted in a $\delta^{13}\text{C}$ value as defined by the following: $\delta^{13}\text{C} = (R - 1) \times 1000$; $R = 13\text{C}/12\text{C}$. A major leap occurred in the 1970s when a system was developed that would permit determination of the isotope composition of individual hydrocarbon species in complex mixtures (Beneteau *et al.*, 1999).

Petrogenic versus Pyrogenic Sources of hydrocarbon derivatives

The processes that originate hydrocarbon derivatives are both natural and anthropogenic, examples of natural sources are forest fires, petroleum seeps, post depositional changes of biogenic precursors and also biosynthesis origin of some plants and fungi (Zhu *et al.*, 2015). Anthropogenic sources include; combustion of fossil fuels, leaking of petroleum and derivatives, incineration of waste, production of coke, pitch, asphalt, cracking of petroleum and industrial effluents (Ekpo and Wehner, 2009).

A recent increase in concentrations of hydrocarbons in the environment may be associated with economic development and population increase, which leads to a greater number of sources of these compounds (Meniconi and Barbanti, 2007). The majority of the anthropogenic sources are related to exploration, transformation and combustion of fossil fuels (Zhu *et al.*, 2005). Another way to differentiate contamination sources of hydrocarbons in benthic organism is to employ diagnostic ratios (Bence and Burns, 1995). The employment of these ratios is based on the assumption that hydrocarbon isomers have similar physical and chemical properties and therefore will be transformed and degraded at the same rate, preserving the relation that is present in the emission (Wang *et al.*, 1999). These ratios have been widely adopted in several researches to identify possible hydrocarbon sources in animal and sediment samples (Wang and Stout, 2007).

Possible ways through which hydrocarbon gets into aquatic environment

Hydrocarbon can be introduced into aquatic ecosystems through the following ways;

Illegal Discharges

Some unscrupulous organizations or individuals may choose to dispose of their wastes containing hydrocarbon in an uncontrolled or illegal manner. It

may involve direct dumping of the waste in aquatic systems, leading to contamination of the environment. These activities are often done in secret to avoid detection by the authorities.

Fugitive Emissions or Discharge

In contrast to the above illegal discharge of waste containing hydrocarbon, emissions of hydrocarbon may be taking place without the knowledge of the owner of the source. Examples of this type of discharge include wind blown of on-site dusts and the wheels of Cars and Lorries picking up compounds on-site and then transferring them off-site. There is no malicious intent in this case, although these materials are making it off-site and the company is still liable under 'Strict Liability' (Philp, 2014).

Deliberate 'Fly-tipping'

Fly-tipping is the disposal of waste at an off-site location usually in one-off event and is often associated with small companies or individuals seeking to avoid the costs of disposing at correctly licensed sites, such wastes often include asbestos and may be laden with hydrocarbons.

Historical Discharges

Many countries have a significant industrial heritage and, since the industrial revolution began, there may have been several industries of different types occupying the same parcel of land. Previous practices may not have been as good as present ones, the atmosphere (dusts and smoke), waters and land may all have become contaminated with hydrocarbons that have appreciable environmental half-lives. That means they may still be available till date and redevelopment of such land may reveal different hydrocarbon derivatives from this industrial past.

Altered Environmental Processes

Historical contamination may be 'locked-up' in the sediments out of currently accessible aquatic systems. Naturally or anthropogenically induced changes in environmental processes, such as changes to the wave or current regime, may erode sediments laid down decades ago, and bring back into the current environmentally accessible region, hydrocarbons that have not been experienced in the recent past.

Identification of origin of hydrocarbon in environmental forensics

In environmental crime litigation, being able to identify correctly the parties responsible for hydrocarbon contamination is fundamental to success (Philp, 2014). Part of the remit of any practitioner of environmental forensics is to identify the origin of contaminants, which may be chemical or biological in nature, and demonstrate a pathway by which the materials may have reached that location. In addressing the problem of source identification, the following scenarios are considered;

A single source of hydrocarbon and a single process or route

In this case, the job of identifying the source of the contaminant is relatively easy. In such cases, simple presence or concentration information may be sufficient to demonstrate where the hydrocarbon has come from. Cases such as this are often restricted to man-made sources with very few possible sources and little or no material pre-existing in the environment.

Cases with multiple potential or actual sources of hydrocarbon

In this scenario, distinguishing one source of hydrocarbon from another adds to the complexity of the task. This is further complicated by the possibility of natural or historical occurrence of the hydrocarbon in the environment independent of any contamination event. If the sources were all of a similar nature, complex signature analysis relying on relatively subtle differences would be needed to differentiate unambiguously between potentially responsible parties (Murphy and Morrison, 2007; Mudge, 2009).

Cases of one source of hydrocarbon with different routes to the receptor sites

In this situation, the hydrocarbon may be of different path lengths or even from different media (e.g. air, water) and if it undergoes degradation in any way, the signature may change with time and path. It is also possible that there is a history of hydrocarbon contamination at a site with different sources responsible at different times. Therefore, it is important to have a handle that tells something about the age of the discharge. Differential degradation rates of components in a mixed

chemical source (e.g. BTEX components in petroleum-based fuels) may be able to provide some information on the time since discharge, but environmental processes may differ between locations and it may be that only relative values may be obtainable.

Cases where one source of hydrocarbon may contribute to several different receptors

Here, the concentration and signature of hydrocarbon at each site may be determined by the mechanism and time of the process that transports the compounds to the sinks. Analysis of the suite of compounds at the primary source may indicate a different chemical signature compared with other sites. This difference however, is an artefact or an indicator of the environmental transport process. A good example of this type of case is an oil spillage. Longer residence times in the environment will lead to the evaporation of the volatile components and simple Gas Chromatography-Flame Ionization detection (GC-FID) analysis may indicate two completely different aliphatic hydrocarbon profiles (Hegazi and Andersson, 2007). Careful choice of the key analytes is required in such situations.

Tools for origin apportionment

A major task in environmental forensics is the ability to link contaminants to a particular origin. Many techniques have been developed to assist in the identification of the origin of hydrocarbons (Murphy and Morrison, 2007; Mudge, 2009). Many of these are chemical in nature, while some rely on biological attributes such as changes in the biotic community or molecular DNA methods, where benthic organisms play major role, as mentioned earlier in source identification. Simple methods may work only in simple cases and more complicated cases often require a whole range of different analyses in order to provide evidence that passes the test of 'beyond all reasonable doubt'. Some of these techniques are outlined below;

Chemical Approaches

Most environmental investigations rely on chemical analyses of a range of different media to establish the origin of the hydrocarbons within the matrix. However, since majority of magistrates and judges do not have extensive environmental chemistry training, it is difficult to use chemical approaches in presenting hydrocarbon forensic evidence in court.

Presence or Absence

Some hydrocarbon derivatives are only produced synthetically and do not occur in nature. In this case, the simple presence of these compounds in any benthic specimen implies that it has been released from an anthropogenic source.

Ratios

The differential solubility and volatility of hydrocarbon components within a source mixture will lead to separation down the spill axis. Volatile components will be lost to the atmosphere, leaving larger compounds behind, water-soluble components will move with the water, leaving the less water soluble components bound to sediments, degradable components will be metabolized into other compounds or become part of the biomass of the benthic community, leaving the more refractile compounds in the system. Determining the ratios of these different components in benthos will provide an idea of the origin of the hydrocarbon.

Complex Signatures

Sometimes there are several potentially responsible parties to the hydrocarbon pollution, and there may be subtle differences between the complex signatures in each. These differences can be used to differentiate between each and post-analytical statistical methods such as Partial Least Squares (PLS) can apportion numerical proportions associated with them (Philp, 2014). The most frequently used multivariate method for such differentiation is the projection method of Principal Components Analysis (PCA), and this may demonstrate simply and clearly in a two-dimensional axis scheme the separation of potential sources of hydrocarbons.

Change of State

In some cases, clear gradients in hydrocarbon derivatives and their concentrations are not quite evident due to a combination of physical and chemical effects. In some cases, low water solubility of the contaminants may suggest that, these compounds were carried in suspension and deposited at sites where the current velocity was low (Mudge, 2009). In some environments, however, the natural changes that occur in hydrocarbons, directly influence the water solubility of the compounds. Some hydrocarbons fall into this category, as they pass through different

environments before getting into aquatic systems, the compounds may become immobilized on the sediments and eventually taken up by benthos (Hegazi and Andersson, 2007).

Stable Isotopes

In a few cases, it may not be possible to determine unambiguously the origin of the hydrocarbons in benthos based on their presence alone or even on their association in particular mixtures. In these cases, a more fundamental property of the compound is needed. Stable isotopes can provide that specificity in some instances. Stable isotope analyses are usually carried out for the carbon atom and reported as $\delta^{13}\text{C}$ values. This technique relies on a difference in the relative proportion of heavy and light isotopes in the hydrocarbon compounds from different origins such that they may be distinguished from each other (Wang *et al.*, 1999).

Biological and Ecological Approaches

In instances where chemical analyses are either not practicable or not possible for origin apportionment of hydrocarbon in benthic organisms, biological and ecological approaches can be adopted. For example, when the hydrocarbon discharge took place in the distant past and has either been metabolized or has simply been dispersed with water flow to concentrations below limits of detection. Because the spill was not reported at that time, samples were not taken for analysis. However, the benthic organisms living in the environment where the spill occurred would have responded to the spillage/discharge and may be altered in comparison with an assemblage unaffected by the spill.

Benthos and hydrocarbons relationship

Once hydrocarbons enter the aquatic environment and adsorbed into the sediment, they are readily taken up by benthic fauna through their gills or via ingestion (Baumard *et al.*, 1999; Liu *et al.*, 2015). The main mechanism underlying the impact of hydrocarbons is their direct binding to hydrophobic sites of macromolecules such as lipids, proteins, and nucleic acids, interfering with their normal functioning (Pampanin and Sydnes, 2013). Since benthos are in close association with aquatic sediment, which is major sink for pollutants they are of high priority for environmental pollution monitoring.

Establishing a link between evidence of exposure to hydrocarbons and their potential source requires

careful consideration of numerous factors such as; age, life stage, gender, reproductive status and the hydrocarbon exposure route (O'Connor, 1998). Hydrocarbons accumulated as whole-oil droplets by suspension feeders with little or no capacity to biologically transform these hydrocarbons retain considerable information regarding sources. It can also provide information regarding characteristic timescales of hydrocarbon accumulation, biological biomarker induction, depuration, and persistence of biomarker responses, which are also dependent on target species and exposure route (Boehm *et al.*, 1996).

Toxic action of hydrocarbons in benthos

Exposure to hydrocarbons, and especially to PAH, causes a variety of organism-level responses in benthic organism, depending on the mode of exposure, the species, the developmental status of the organism, and the time scale of exposure (Freedman, 1995; Carls *et al.*, 2002). The most widely known of these exposure indicators is cumulative mortality induced through narcosis. Oil pollution and PAH had once been regarded as minimally toxic based on the relatively high exposure concentrations required to kill test organisms within a few days. But within the last decade, the ecological consequences of reduced fitness have become more fully appreciated. Here, some of the toxic effects of hydrocarbon on benthos are listed.

- a. Reduction in the ability to acquire prey or to avoid predators in the wild.
- b. May lead to mortality prior to the first reproductive opportunity.
- c. The potential to harm a population or alter population structure through impaired development and growth inhibition.
- d. Immune suppression, or carcinogenesis.

As with the biological biomarkers, the spatial and temporal patterns of these responses may indicate particular hydrocarbon sources of exposure. Narcosis induced mortality arises when nonpolar contaminants such as hydrocarbons accumulate in the neural membrane lipids, inhibiting nerve transmission, and causing death through asphyxiation or heart failure. Benthic populations are also known to suffer lethal narcosis. Narcosis-induced mortality is usually associated with products with high BTEX contents such as gasoline and some diesel oils. Lauenstein and Daskalakis (1998), reported that chronic exposure to hydrocarbon contaminated sediments can elicit a

complex of;

- a. Cancerous tumors in benthic fish community.
- b. Liver neoplasms.
- c. Liver cancers.
- d. Alteration of population age structure of the affected benthic community.
- e. Carcinogenesis in benthic fish population.

Factors promoting the use of benthos in environmental forensic studies

Literature search on the possible factors promoting the use of benthos in environmental forensic investigation revealed the following (Lenihan *et al.*, 2003; Nanami *et al.*, 2005; Uwadiae *et al.* 2009; Wlodarska-Kowalczyk *et al.*, 2005; Uwadiae, 2017)

- Benthic macroinvertebrates play significant roles in the aquatic ecosystems. They are critical components of habitats and food chains supporting many aquatic biota.
- The ecological, biological, chemical, physiological and morphological attributes of benthos are determined by features of their immediate environment.
- They are secondary consumers and constitute an important link between primary producers and higher trophic levels for both autotrophic and detritus based food webs.
- They are composed of diverse taxa with a variety of reproductive modes and life history strategies.
- They are important food materials for planktonic and nektonic species.
- Benthic species are sessile unlike plankton and fish, and cannot physically avoid stressful environmental conditions. Thus they cannot evade, and must respond to, a variety of stressors, such as toxic contamination, eutrophication, sediment quality, habitat modification, and seasonal changes.
- The complexity of natural habitat stressors and ambient pollutant mixtures can be integrated by benthos, through physical contact with sediments, ingestion of sediment, bioaccumulation and biomagnification of contaminants in food chains and food webs, and expression of the synergetic effects of exposure to toxic chemicals like hydrocarbon.
- Distributions of benthic organisms can be predicted along environmental gradients and are usually defined by similar groups of species over broad latitudinal ranges.
- Benthic species composition, abundance, and

biomass are influenced by habitat conditions, including salinity, sediment type, and environmental stressors, both natural and anthropogenic.

- Information on changes in benthic population and community parameters due to habitat change can be useful for separating natural variation from changes associated with human activities.
- Benthic community studies have a long history of use in aquatic monitoring programs and have been proven to serve as an effective indicator for assessing the extent and magnitude of pollution impacts and habitat modification as well as for assessing the effectiveness of management measures.
- Response to contaminant and physical stressors among benthos have been widely documented.
- Most opportunistic species are known to be tolerant of chemical toxicants such as hydrocarbon, others are capable of thriving in physically disturbed habitats (e.g. high sedimentation, dredging operations, etc.) but not necessarily in contaminated areas.
- Experimental manipulation of habitats has shown that benthos with opportunistic life history strategies, respond positively to some stressors such as organic enrichment while other taxa respond negatively to both toxicants and excessive organic enrichment.
- The response of specific species to organic and toxic contamination is mediated by life history and feeding mode characteristics.

CONCLUSION

This review shows how direct or indirect assessments of hydrocarbons in benthic systems may provide evidence that is crucial to identifying hydrocarbon pollution sources. It also confirms the fact that benthos can be employed in monitoring studies, including long-term and large scale monitoring of hydrocarbon contaminant concentrations and toxicity assessments, bioeffects studies to determine the spatial extent and severity of hydrocarbon contamination and associated adverse biological effects which are critical in environmental forensic studies.

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