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Integrating Multiple Lines of Evidence in Ecological Risk Assessment: A Case Study of Crude Oil Pollution in the

Mangroves of Bodo Creek, Ogoniland, Nigeria

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Abstract

Crude oil pollution poses a significant threat to environmental integrity, particularly in coastal and marine ecosystems. This study employs a TRIAD approach, integrating multiple lines of evidence, to assess the ecological risks and human health hazards associated with crude oil pollution in Bodo Creek, Ogoniland, Nigeria. The investigation includes chemical analyses of contaminants, ecological evaluations, and risk assessments to determine the extent of environmental degradation following significant oil spills in 2008 and 2009. Chemical analyses revealed widespread contamination, with concentrations of heavy metals and Total Hydrocarbon Content (THC) exceeding regulatory standards at all sampling stations. Simple linear regression showed significantly that THC negatively correlated with species richness (p < 0.05, $R^2 = 0.76$). Polycyclic Aromatic Hydrocarbons (PAH), specifically benzo-a-pyrene (b-a-P) at all sampled stations exceeded both the Canadian Environmental Protection Agency (EPA) and background concentrations in rural Europe. The benthic fauna disturbance using AMBI software analysis of the biotic index indicated reduced species richness and diversity at all stations compared to pre-spill reference stations. The extent of mangrove destruction was severe, with complete loss at station 3 and significant reductions at other stations. The integration of findings in this study from Ecological Line of Evidence (EcoLoE) and Chemical Line of Evidence (ChemLoE) showed that Weight of Evidence (WoE) risk values exceeded 0.5 at all stations, signifying severe ecological damage and direct risks to human health due to strong food web interactions and local activities.

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Despite some natural degradation of THC since both spills, levels remain critically high, underscoring the ongoing environmental threat. This research underscores the broader challenges in managing oil pollution in coastal and marine environments and emphasizes the need for effective mitigation strategies to protect both ecosystems and the livelihoods of local communities reliant on these natural resources.

Keywords: Niger Delta; Crude oil pollution; Ecological risk assessment; Weight-of-evidence analysis; Mangrove swamp; Environmental contamination; Heavy metals; Polycyclic aromatic hydrocarbons (PAHs); Total hydrocarbon content (THC); Benthic community disturbance; Mangrove cover loss; Ecological impacts; Human health risks; Remediation efforts.

1. Introduction

Crude oil pollution resulting from spills during exploration and transportation activities represents a persistent challenge to environmental integrity according to [1, 2, 3]. Findings in [4, 5] reveal that the deleterious impact of such pollution on marine and coastal ecosystems constitutes a significant area of concern from both scientific and regulatory perspectives, both domestically and internationally. Holdway, [6] reports that oil spills can occur as chronic, low-level discharges over prolonged periods or as acute, high-volume releases, often associated with maritime accidents or catastrophic pipeline failures. Various factors, such as natural seepages, industrial accidents, equipment malfunctions, and human error contribute to oil pollution; as seen in [7, 8].

Although crude oil is biodegradable, its degradation can be protracted, particularly in certain environmental contexts such as vegetated swamps and coastal regions, according to [9, 10, 11]. The toxicological mechanisms of crude oil pollution encompass both physical and physiological impacts on affected organisms, leading to significant ecological disruption. The severity of these impacts is contingent upon the specific endpoints of interest for conservation and the unique characteristics of the affected ecosystems. Concerns that marine environments sustain substantial damage to benthic and planktonic communities, avian and marine mammal populations, as well as notable declines in vegetation and intertidal fauna of coastal habitats has been pointed out by [12,15]. There are direct health risks to human populations residing in proximity to polluted areas through exposure pathways such as dermal contact, ingestion, inhalation, and of course indirect risks via consumption of contaminated food and water sources according to [16]. Furthermore, [17, 18, 19] added that ecosystem degradation resulting from oil pollution can lead to the loss of critical ecosystem services, with implications for human well-being, particularly in vulnerable coastal communities.

1.1 Oil and the Ogoni People

Nigeria, as one of the leading crude oil producers in Africa and a major global exporter, exemplifies the intricate interplay between oil extraction and socio-environmental dynamics [20, 21]. The Niger Delta region, in particular, serves as the epicentre of Nigeria's oil industry, harbouring over 90 percent of the nation's oil reserves. With a population exceeding 20 million, the Niger Delta comprises a diverse array of freshwater and estuarine ecosystems, characterized by intricate networks of creeks and mangrove swamps amidst rainforest expanses, as seen in [22, 23, 24]

Among the indigenous communities inhabiting the Niger Delta, the Ogoni people have historically confronted the adverse impacts of oil exploitation, epitomized by the presence of a particular oil company since the mid-20th century [25]. Zabbey, [24] reported that the Ogoni populace are predominantly reliant on subsistence agriculture, fishing, and small-scale commerce. Nevertheless, grapples with poverty despite the abundance of natural resources in their homeland. The exploitation of oil resources in Ogoniland, coupled with inadequate environmental safeguards and recurrent pipeline vandalism, has precipitated environmental degradation and socioeconomic distress. Notably, the years 2008 and 2009 witnessed significant oil spills along the Trans Niger pipeline, traversing the Bodo Creek within Ogoniland, resulting in the release of Bonny Light crude—a high-grade Nigerian crude oil—into the surrounding ecosystem, causing extensive damage to brackish water habitats and mangrove swamps [18, 26].

Mangrove ecosystems play a pivotal role in supporting local livelihoods and ecological processes, serving as critical habitats for various species and offering invaluable ecosystem services [24]. However, they are particularly susceptible to the impacts of oil spills and other anthropogenic disturbances, which can compromise their ecological functions and resilience [19]. In response to mounting concerns over environmental degradation in Ogoniland, the United Nations Environment Programme (UNEP) initiated an assessment in 2008 to evaluate the extent of ecological damage and its ramifications for local communities [26, 27]. The findings underscored the urgent need for comprehensive restoration efforts, prompting the mobilization of diverse stakeholders, including governmental agencies, non-governmental organizations, and academic institutions, to develop remediation strategies and monitoring protocols. Despite significant financial commitments from the Nigerian government and other stakeholders involved, the implementation of restoration initiatives has been hindered by logistical and bureaucratic challenges, underscoring the complexities inherent in addressing the legacy of oil pollution in Ogoniland.

1.2 Ecological Risk Assessment (ERA)

Ecological Risk Assessment (ERA), initially conceived by the US Environmental Protection Agency (EPA) as a decision support tool, serves to estimate the probability of adverse ecological effects resulting from environmental stressors. It constitutes a blend of qualitative and quantitative analyses, comparing the presence of contaminants or stressors in the environment against established benchmarks of environmental quality. The overarching objective is to inform decision-making processes geared towards safeguarding both human health and environmental integrity [28, 29]. In 1992, the US EPA introduced a structured framework for Ecological Risk Assessment, providing a systematic approach for conducting assessments. While this framework is not prescriptive in nature, it offers a comprehensive guideline to aid risk assessors in conducting thorough evaluations. The following sections delineate the various stages of an ERA, drawing inspiration from the US EPA framework for ERA (1992) and insights from the textbook "Ecological Risk Assessment" by Suter [30].

1.2.1 Problem Formulation

The initial phase of an Environmental Risk Assessment (ERA) following the planning stage entails the compilation of extant information concerning the origin and nature of contamination, environmental

characteristics, as well as organisms and ecosystem functionalities at risk [31]. Subsequently, a conceptual framework is formulated to provide a speculative portrayal of contaminant dynamics within the environment, elucidating its origin, characteristics, mode of interaction, exposure pathways, and ultimate endpoints for protection and assessment. The determination of priority endpoints facilitates the identification of the most pertinent focus, with human health typically garnering primary consideration, prevailing over environmental welfare in most instances.

1.2.2 Exposure and Effect Assessment (Analyses)

This phase encompasses the quantification of exposure levels and the assessment of environmental stressor impacts [32]. Evaluation typically involves appraising contaminant fate and bioavailability alongside the potential repercussions of stressors, employing toxicity tests and ecological evaluations such as biodiversity indices and habitat degradation assessments. Findings are subsequently consolidated to facilitate risk characterization.

1.2.3 Risk Characterization

Risk characterization involves the synthesis of exposure levels with potential effects. Various methodologies exist for this purpose, with the toxic pressure calculation method being widely utilized, wherein environmental concentrations are juxtaposed against regulatory benchmarks to derive hazard quotients. Additionally, tools like toxicity bioassays and benthic quality indices contribute to risk characterization. Although no standard protocol dictates method selection, employing multiple lines of evidence enhances the assessment's robustness and aids in mitigating inherent uncertainties. The TRIAD method, for instance, integrates chemistry, toxicity, and ecology-based lines of evidence to conduct a weight-of-evidence risk analysis as explained by [33]. Uncertainties are typically quantified using standard deviations, with a threshold of <0.4 commonly accepted Reference [34].

1.2.4 Risk Management and Decision Making

The final phase of an ERA entails presenting assessment outcomes to decision-makers and risk managers. Deliberations involve a comprehensive examination of risk values and associated uncertainties, coupled with the consideration of management options and socio-economic factors. Stakeholders, including government officials and policymakers, engage in a nuanced evaluation of cost-benefit analyses to formulate effective management strategies.

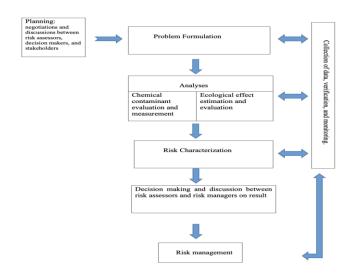


Figure 1: ERA Guideline Model. Adapted from the US EPA framework for ERA [35]

1.3 Weight of Evidence Analyses

This study employs a weight-of-evidence (WOE) analysis akin to a TRIAD approach, albeit with two distinct lines of evidence. The ecological risk stemming from oil pollution in Bodo Creek, Ogoniland, was evaluated utilizing WOE derived from two distinct avenues: 1) a Chemistry line, involving measured concentrations of oil and metals, followed by toxic pressure calculations, and 2) an Ecology line, entailing assessments of oil pollution impacts on the benthic invertebrate community and mangrove forest in Bodo Creek.

A recommended strategy when conducting WOE analyses is to adopt a tiered methodology, as outlined by Jensen & Mesman [36]. Employing a tiered approach enhances the efficiency of risk assessment, enabling a stepwise evaluation until uncertainty levels reach a threshold deemed acceptable for decision-making regarding remediation efforts. In the LIBERATION report by [36], three tiers are proposed as follows:

- Tier 1: This initial phase involves preliminary investigations encompassing all distinct lines of evidence. Tier 1 typically entails collation, assessment, and evaluation of existing data pertinent to the case study, potentially including on-site observations and physical assessments. Subsequently, an initial risk assessment is conducted based on available data, addressing questions regarding risk acceptability and associated uncertainty levels. If uncertainty is deemed high, indicating insufficient evidence for decision-making, proceeding to Tier 2 is warranted to gather additional evidence and mitigate uncertainty.
- Tier 2: This tier entails a more detailed assessment and screening, often necessitating increased sampling and more extensive chemical analyses (e.g., contaminant concentrations), as well as in situ and ex situ bioassays, toxicity testing, and ecological disturbance assessments. Typically, sufficient evidence is garnered at this stage to reduce uncertainty and provide environmental managers with a more confident risk assessment. However, if uncertainty persists, proceeding to Tier 3 is warranted.
- Tier 3: Advancing to this phase requires substantial resource and time investments. Uncertainty reduction entails further assessments, such as expanded sampling (increasing spatial and temporal

scales) and inclusion of additional measurable parameters linked to the stressor. In cases where evidence remains unacceptable or unreliable, iterative refinement of assessment details is necessary until an acceptable level of uncertainty is achieved. Subsequently, a more reliable risk assessment can be reported to environmental managers.



Figure 2: Illustration of the tiered approach flow chart utilized in a TRIAD assessment, adapted from Jensen & Mesman [36]

2. Material and Method

The seminal 2011 UNEP report on the environmental status of Ogoniland facilitated subsequent detailed investigations by disseminating critical information. Drawing upon data from the UNEP report, which encompassed a broader scope of Ogoniland, a more granular conceptual model focusing on the Bodo community was developed for this study (see Figure 3). This conceptual model elucidates the pathways of exposure and mechanisms of action of the stressor (crude oil), taking into account the environmental context and, importantly, the endpoints to safeguard—namely, human health and the ecologically vital mangrove swamps and brackish water creek supporting the livelihoods of the Bodo community members.

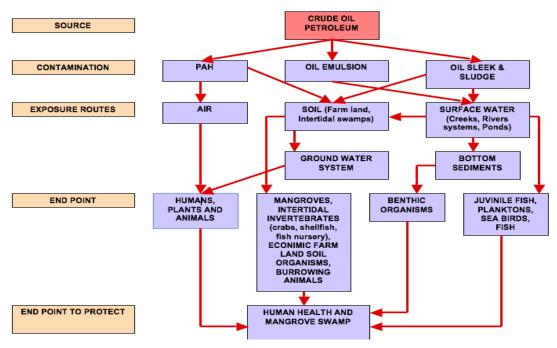


Figure 3: Conceptual model of actual pollution pathway in Bodo Creek

2.1 Site Description

A site-specific assessment was conducted in the Bodo Creek, targeting five selected sites within the mangrove swamps. Sediment samples were collected from each site and subjected to analysis for chemical parameters associated with crude oil, including Poly-cyclic Aromatic Hydrocarbons (PAHs), Total Hydrocarbon Content (THC), and potentially toxic heavy metals such as copper (Cu), lead (Pb), nickel (Ni), and cadmium (Cd). Additionally, ecological parameters were evaluated, encompassing (i) the structure of benthic invertebrate communities and (ii) the extent of mangrove cover depletion.

The study encompassed five distinct sites strategically chosen along the meanders of Bodo Creek, Nigeria. Among these, two sites, denoted as "stations 1 and 2," were situated within ostensibly pristine mangrove swamp regions of the creek, while the remaining trio of stations (3, 4, and 5) were positioned within areas heavily impacted by oil spillage and subsequent deforestation. Each station maintained an approximate separation of 1 kilometre, with the exception of stations 2 and 3, which exhibited a distance of approximately 6 kilometres along the creek's intricate path (refer to figures 2 and 3 for spatial representation). Additionally, to contextualize findings and ensure historical integrity, a reference station was established employing literature-derived pre-spill data from Bodo Creek [18, 37]. In instances where historical data from Bodo Creek was unavailable, data from analogous pristine environments within the Niger Delta or globally were employed (referenced accordingly in the methods and results sections), facilitating a comprehensive understanding predating the spillage.

Station 1: Designated "Penekiri" by the local community, this station is situated at Lon. N 04° 35" 37.1", Lat. E 007° 13" 54.9", approximately 7-8 kilometers downstream from Bodo community settlements. Characterized by lush red mangrove (*Rhyzophora racemosa*) stands adorning both banks, the area features a spectrum of mangrove maturity, interspersed with freshly cut stems, indicative of anthropogenic pressures. Notably, intertidal epifaunal organisms such as crabs and mudskippers inhabit the muddy soil, while subtle traces of oil were detected during quadrat sampling, underscoring the site's susceptibility to contamination despite its ostensibly pristine appearance.

Station 2: Located within the "Penekiri" area, this site, positioned at Lon. N 04° 36'' 03.7'', Lat. E 007° 13'' 36.9'', lies approximately 1 kilometer upstream from station 1. Comprising predominantly red mangroves (*Rhyzophora racemosa*) with scattered *Nypa fruticans* stands, the locale is inhabited by gastropods and crabs, with signs of ongoing mangrove wood harvesting. The soil, though muddy, exhibits greater organic content compared to station 1, reflecting subtle differences in ecological dynamics within the mangrove ecosystem.

Station 3: Referred to as "Sivibilagbara" locally, this station is positioned at Lon. N 04° 36'' 34.1'', Lat. E 0070 15'' 25.9''. Adjacent to a proposed bridge construction site and a community boat landing, the area bears the brunt of severe oil spillage-induced deforestation, with only sporadic mangrove patches remaining on elevated banks. The absence of intertidal organisms, coupled with the prevalence of heavily oiled mangrove stilt roots and sediment rich in organic matter and crude oil residue, paints a stark picture of ecological devastation.

Station 4: Identified as "Sivieva," this site, located at Lon. N 04° 36" 17.2", Lat. E 007o 16" 02.2", lies on the

opposite side of the bridge construction project. Nearly devoid of vegetation, save for mature *Avicennia* germinans trees on elevated terrain, the area bears witness to past abundance, evidenced by the presence of razor clam shells and fishing settlements. Despite the absence of intertidal organisms and the perceptible odour of volatile hydrocarbons, the sandy sediment suggests varying degrees of pollution impact compared to adjacent stations.

Station 5: Positioned at "Kozo" (Lon. N 04° 35''59.1'', Lat. E 007o 16'' 29.8''), approximately 800 meters downstream from station 4, this site epitomizes the transition from lush vegetation to barren mudflats, indicative of severe oil pollution-induced mangrove loss. Abundant shell remains underscore the area's previous ecosystem richness, now replaced by a desolate landscape marred by crude oil residues. Despite the sandy soil's higher organic content relative to station 4, the pervasive presence of oil lends a dark hue to the landscape, signalling significant ecological disruption.

In summary, the sampled sites within Bodo Creek offer a nuanced portrayal of mangrove ecosystems along a continuum of pristine to heavily impacted areas. The juxtaposition of pristine and degraded habitats provides invaluable insights into the multifaceted effects of oil pollution on mangrove ecosystems, underscoring the urgent need for comprehensive conservation and remediation efforts in the region.

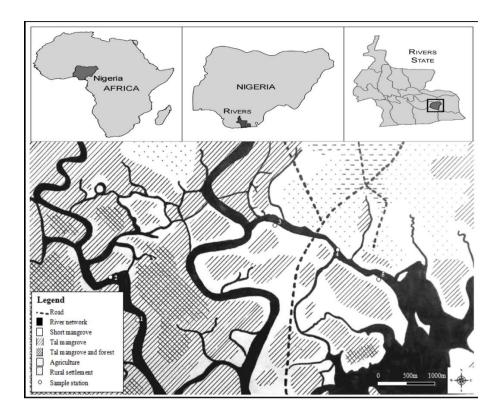


Figure 4: Map of Bodo Creek



Figure 5: Satellite map showing the five sampling stations extracted from Google Earth [38]

2.2 Sample Collection

Geographical coordinates of each station were meticulously recorded using a Garmin eTrex 10 GPS device to ensure accurate spatial documentation. Visual inspections and photographic documentation were conducted at each site to assess the extent of mangrove degradation.

At every station, a 20 x 20 cm quadrat was systematically positioned on exposed mudflats during low tide. Sediment samples were then meticulously collected to a depth of 20 cm using a spade. The sampling process was methodically replicated three times, with each sampling point spaced at least 20 meters apart. Subsequently, all sediment samples were consolidated into a single sack to ensure representative composited sampling.

A portion of the composited sediment was carefully transferred into air-tight 1-liter glass jars, sealed with aluminium foil lids, and stored in coolers with ice for subsequent sediment characterization and chemical analyses, focusing on Polycyclic Aromatic Hydrocarbons (PAHs) and Total Hydrocarbon content.

The remaining composited sediment samples underwent sieving through a 1 mm mesh sieve using creek water. The retained sieved material was then deposited into labelled 10-liter plastic buckets allocated per station. Water content within each bucket was measured, and a 10% formaldehyde solution, supplemented with eosin dye for organic matter staining, was added.

Sorting and taxonomical classification of benthic macrofauna (>1 mm) were conducted later, ex-situ. At each station, interstitial (pore) water, seeping into the dugout holes (20 cm depth) within each replicate quadrant, was carefully collected using an aluminium spoon. These samples were composited into 1-liter glass jars with airtight lids, covered with aluminium foil, and stored in thermos flasks with ice for subsequent ex-situ chemical analyses, focusing on heavy metals (Cu, Cd, Pb & Ni).

In-situ measurements of temperature (ambient and water), conductivity, salinity, and Total Dissolved Solids (TDS) in pore water were conducted using a handheld digital meter (Go n Do multi-meter CTS-406). The meter probe was submerged into 5 cm of interstitial (pore) water from each replicate, allowing for stabilization for a minimum of five minutes. Average readings across all replicates at each station were recorded. Similarly, pH measurements were conducted using a pH meter (WTW pH 330), and Dissolved Oxygen (DO) levels were assessed using a Milwaukee DO meter (MW 600).

2.3 Sample analyses

Organisms were meticulously identified down to the lowest taxonomic classification utilizing marine benthic invertebrate key manuals. Subsequently, all identified organisms underwent verification against the World Registry of Marine Species [39] database to rectify any outdated nomenclature and ensure the utilization of the most current species names available.

Species abundance was computed following the methodology outlined by [40], facilitating a rigorous and standardized approach to quantifying ecological dynamics within the study area.

$$A = \frac{(\Sigma n_i)}{(n_{sq})} 1/a_{sq} \tag{1}$$

Where A is abundance, n_i is the number of specimens found, n_{sq} is the number of squares (or replicates; which is 5 in this assessment), and a_{sq} is the area of the square (which is 20 cm x 20 cm in this assessment).

Chemical analyses of Cadmium, Lead, Copper, and Nickel were conducted utilizing the API RP45 heavy metals extraction technique [41], ensuring robust extraction and quantification methodologies.

For analysis of Polycyclic Aromatic Hydrocarbons (PAH) and Total Hydrocarbon (THC) content, sediment samples underwent extraction and analysis using Gas Chromatography-Mass Spectrometer (GC-MS), following standardized protocols PA 8270 and ASTM D 3921, respectively. These procedures were performed in accordance with ASTM (American Society for Testing and Materials) D. 3921–85 Standard Test Method for Oil, Grease, and Petroleum Hydrocarbons in Water, guaranteeing adherence to established industry standards.

Subsamples of both sediment and pore water were further analysed for additional quality control analyses and determination of total carbon, total organic carbon, and total nitrogen using an auto-analyser, thereby ensuring comprehensive characterization of the samples' chemical composition.

2.4. Calculating Risk Values from Chemical Line of Evidence (ChemLoE)

Step 1.1: Firstly, the Toxic Pressure of each of the heavy metals (Cu, Cd, Ni and, Pb), denoted as TP_{Metal} at all five sampled stations were calculated using the formula derived from [34].

$$TP_{Metal} = 1/[1 + Exp \left(1/(\log WQC - \log MEC)/SSD\right)] \tag{2}$$

Water Quality Criteria (WQC) for the various metals were calculated based on the intervention limit for underground water set by the Department of Petroleum Resources (DPR) Nigeria. To assess potential ecological risks, Species Sensitivity Distribution (SSD) for chemical pollutants was employed, utilizing the default value of 0.4 as recommended by [36].

Measured Environmental Concentrations (MEC) of metals at each station were determined, with MEC values for the reference station obtained from background concentrations reported in literature [42]. While direct measurements of pore-water concentrations for the heavy metals in Nigeria were unavailable, pore-water concentrations were predicted from MEC in sediment using similar formulae employed for ex-situ Solid Phase Microextraction (SPME) [43]. This approach facilitated a comprehensive evaluation of potential environmental impacts and provided valuable insights into the ecological dynamics of the study area.

At equilibrium;
$$C_{porewater} = C_{sediment} / Kd_{sediment-water}$$
 (3)

Where C is the concentration of the metals (pore water or sediment), and Kd is the partition coefficient of the various metals from sediment to water, which is obtainable from Alison & Alison [44].

Following the determination of Toxic Pressure for each metal at all sampling stations TP_{Metal} , these values were compared against acceptable background concentrations, derived from pre-spill data obtained from the reference station [45, 46]. This benchmark, denoted as TP_{Ref} serves as a reference point for assessing environmental impact. Risk values for each metal (R_{Metal}) according to the TRIAD method as seen in equation (4) below, provides insight into the risk levels of each metal at all stations, forming an integral component of the Chemical Line of Evidence (ChemLoE).

$$R_{Metal} = TP_{Metal} - TP_{Ref} / 1 - TP_{Ref}$$
 (4)

This methodology consistently yields a value of "0" for the reference station, representing minimal risk, while assigning increasing values from 0 to 1 for the remaining stations based on the degree of contamination for each metal. Here, "0" signifies the lowest risk level, whereas "1" indicates maximum risk, adhering to the principles of a TRIAD approach. This systematic evaluation facilitates the classification of environmental risk levels across different sampling stations.

Step 1.2. Subsequent to acquiring the risk values for each metal R_{Metal} at various stations, the toxic pressures for Total Hydrocarbon Content (THC) denoted as TP_{THC} , for all stations were calculated. The formula utilized in this step was derived from [34], ensuring rigor in the assessment of THC toxic pressure

$$TP_{THC} = MEC / EQC$$
 (5)

MEC = Measured Environmental Concentration EQC = Environmental Quality Criteria. In this calculation, the DPR (Department of Petroleum Resources) Nigeria's quality guideline was used. MEC values for the reference

station are background pre-spill concentration values from literature [45, 46]. The THC risk value R_{THC} for all stations were obtained using similar calculations as seen in the formula (4) above. This was then included as one of the chemical contaminant parameters in our ChemLoE.

Step 1.3. To obtain the toxic pressure and risk values for Poly Aromatic Hydrocarbons (PAH) denoted as TP_{PAH} and R_{PAH} respectively at all stations, step 1.2 above was followed.

Step 1.4. To obtain a final risk value for our ChemLoE, risk values of all chemical stressors were integrated using the integration method of [34].

$$1-10 \exp[(1-\log R_{Metal}) + (1-\log R_{PAH}) + (1-\log R_{THC})/Rn]$$
(6)

Where Rn represents the total number of chemical parameter stressors.

2.5 Calculating Risk Values from EcoLoE (Ecological Line of Evidence)

Step1. Evaluating Benthic Community Disturbance. The assessment of benthic community disturbance was conducted using the AMBI (AZTI-Marine Biotic Index) software, version 5, which classifies benthic organisms into five ecological groups based on their prevalence in stressed environments, ranging from 6 (Bad) to 0 (High), indicating severely impacted and normal environments, respectively. To enhance the assessment's robustness, the more advanced M-AMBI was employed, incorporating additional metrics such as the Shannon diversity index and species richness, aligning with the European Water Framework Directive standards [47]. Unlike the AMBI, the M-AMBI categorizes disturbance levels inversely from 0 (Bad) to 1 (High). Further methodological details on AMBI implementation can be found in [48].Reference data from the same geographical area pre-oil spill were utilized for comparative purposes, sourced from [49]; who collected samples at Bodo Creek during 2006 to 2008. Three sampled stations (referred to as stations 3, 4, and 5) in this study correspond to stations 1, 2, and 3 in [49]'s report. However, for consistency within our analysis, these stations were relabelled as Ref3, Ref4, and Ref5, respectively. To facilitate compatibility with the Weight of Evidence (WoE) calculation scale, M-AMBI results were converted to negative responses by subtracting each station's M-AMBI value from 1. Notably, only the reference station exhibiting the highest ecological status among the three (Ref.5) was considered in this computation. See (Figure 9).

$$BI = 1$$
- (M-AMBI value at station) (7)

Where BI = Biotic Index. A comparative analysis was then done by scaling the difference between the reference station and all five sampled stations. Similarly, to Step 1.1 of the ChemLoE above. The results of this step is denoted as $R_{Biotic\,Index}$ and as our first ecological stressor for the EcoLoE

$$R_{Biotic\ Index} = BI(station) - BI(Ref\ Station) / 1 - BI(Ref\ Station)$$
 (8)

Step 2. Determining Mangrove Cover Loss as an Ecological Stressor in EcoLoE. Following the initial assessment, the second ecological stressor in the EcoLoE framework involves estimating the extent of mangrove

cover loss. At each station, observations were made to identify the point where dead mangrove stems and roots transitioned from a perpendicular direction to the creek towards surviving mangroves, as illustrated in (Figure 6) below. Visual inspections supplemented by photographic documentation were employed to estimate the surface area of destroyed mangrove vegetation in square meters relative to the remaining vegetation within the delineated area.

The size of the mapped-out area of green vegetation at each station was determined using Google Earth Satellite Map [38]. Results from this assessment were subsequently converted into percentages to facilitate further calculations. To establish a background reference, a literature review and local ecological knowledge predating the spill in 2008 were consulted. A baseline of 95% mangrove vegetation cover, corresponding to a 5% impact, was adopted for the reference station instead of assuming 100% intact mangrove cover. This adjustment accounted for pre-existing factors that may have influenced mangrove health, such as agricultural encroachment, fuelwood harvesting, and construction activities by local communities.

After obtaining the percentage mangrove loss at all five stations, a comparative analysis was then done by scaling the difference between the reference which is a baseline value at 95 percent healthy mangrove and all five sampled stations. The results of this step is denoted as $R_{\rm \%Mangrove\ loss}$ and as our second ecological stressor for the EcoLoE

$$R_{\%Mangrove\ loss} = \% Mangrove\ Loss - 95\% / 1 - 95\%$$
 (10)

However, this methodological approach has inherent limitations. Notably, it does not consider the heterogeneity of vegetation species which may lead to variations and a mosaic in actual coverage. Additionally, the fragmented nature of mangrove forests, characterized by patches and separated by water puddles and small rivulets, further complicates accurate assessment. Therefore, caution must be exercised in extrapolating conclusions based solely on satellite mapping data, as it may not fully capture the complexities of mangrove ecosystems.

Despite these limitations, the estimation of mangrove cover loss serves as a critical component in elucidating the ecological impacts of the spill and guiding subsequent mitigation efforts.

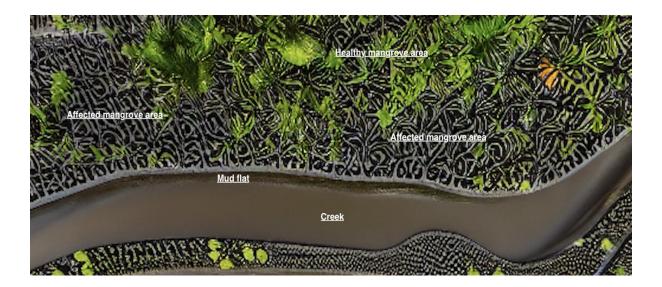


Figure 6: Simulation generated graphics of mangrove cover depletion at a sampled station

To obtain a final EcoLoE, the results from $R_{\text{%Mangrove loss}}$ and $R_{\text{Biotic Index}}$ were integrated, using the integration method of [34] below. This method compensated for the high dissimilarity in the values of % mangrove loss and biotic index at stations. Thus, giving the components of the EcoLoE ecological equal weight and representation.

$$1-10 \exp[(1-\log R_{\text{MMangrove loss}}) + (1-\log R_{\text{Biotic Index}})/Rn]$$
(11)

Where Rn represents the total number of stressors

2.6 Integrating ChemLoE and EcoLoE to Obtain the WoE (Weight-of-Evidence)

The culmination of the Weight-of-Evidence (WoE) analysis involves the integration of our two lines of evidence: ChemLoE and EcoLoE. Drawing inspiration from the methodology advocated by [34] in their report on integrating different lines of evidence within a TRIAD framework, we follow a similar approach tailored to our specific context. While traditional TRIAD analyses typically involve three lines of evidence, our WoE analysis, as previously outlined, focuses on combining two distinct lines of evidence to assess environmental impacts comprehensively.

Subsequently, the standard deviation (SD) between the ChemLoE and EcoLoE results was ascertained. A SD value less than 0.4 is considered acceptable, indicating a reasonable degree of agreement between the two lines of evidence. However, deviations exceeding this threshold may necessitate further investigation, as recommended by [34]. This critical evaluation ensures the robustness and reliability of our integrated analysis, providing valuable insights into the ecological consequences of the spill event.

3. Results

3.1 Physicochemical Parameters Analysis

Physico-chemical parameters across the five stations showed an average water temperature of 25.24 °C, with

standard deviation of ± 1.71 °C, average pH of 7.42 with a standard deviation of 0.64, average salinity of 13.08 ppt with a standard deviation of ± 2.43 ppt, average DO (Dissolved Oxygen) 2.34 mg/l with standard deviation of ± 0.63 mg/l, while the average measured TDS (Total Dissolved Solid) was 17.14 ppt with a standard deviation of ± 3.51 ppt. The sampling and collection of data was done in September 2016, which is characterized by frequent rainfall and high humidity in the Niger River Delta at this time of the year.

Table 1: Physico-chemical parameters *in-situ* at five sampled stations in Bodo Creek 24/09/2016

	Station1	Station 2	Station 3	Station 4	Station 5
Ambient Temperature ±1.0°C	26.3	26.3	24.8	24.6	24.6
Water Temperature $\pm~0.5^{\circ}\mathrm{C}$	23.5	27.1	23.4	25.7	26.5
PH ±0.1	7.5	6.5	7.3	8.3	7.5
EC μS/cm	30.7	28.7	24.0	26.0	17.9
Salinity ppt	15.3	13.9	14.3	12.9	9.0
DO ±0.1 mg/l	2.0	3.4	1.8	2.1	2.4
TDS ppt	21.1	19.2	16.2	17.4	11.8

3.2 Hydrocarbon parameters in sediment

The results below (tables 2 & 3), show the measured concentrations of PAH, THC and Heavy Metals at the field sites and background (reference) values before the oil spill obtained from literature reports used for the ChemLoE in the WoE (Weight-of Evidence) calculation in this assessment.

Table 2: Measured concentration of hydrocarbon parameters in sediment *ex-situ*, from five sampled stations at Bodo Creek 24/09/2016 and reference values from background concentration before spill

	Ref	Station1	Station 2	Station 3	Station 4	Station 5
TPH (mg/kg)	50.00	272.33	178.52	334.27	287.90	275.64
PAH (mg/kg)	0.72	0.30	2.79	0.86	0.61	0.30

The Department of Petroleum Resources (DPR) Nigeria has established a regulatory threshold for Total Hydrocarbons (THC) in sediment at 30 mg/kg, with background concentrations recorded at 50 mg/kg [45]. Additionally, the Canadian Environmental Protection Agency (EPA) has implemented a regulatory limit for Polycyclic Aromatic Hydrocarbons (PAH), specifically benzo-a-pyrene (b-a-P), in sediment at 0.6 mg/kg [50]. However, investigations in rural settlements within European countries such as Poland have revealed background concentrations of 0.3 mg/kg [46].

Table 3: Measured concentration of heavy metals in sediment pore water *ex-situ*, from five sampled stations at Bodo Creek 24/09/2016 and reference values from background concentration before spill

	Ref	Station1	Station 2	Station 3	Station 4	Station 5
Cu (mg/l)	0.01	0.087	0.068	0.031	0.050	0.031
Pb (mg/l)	0.04	0.811	0.679	0.541	0.541	0.541
Cd (mg/l)	0.01	0.088	0.096	0.052	0.045	0.031
Ni (mg/l)	0.02	0.384	0.417	0.287	0.352	0.254

The DPR Nigeria limit for underground water pollutants is 0.075mg/l for Pb, Cu, Ni, and 0.006mg/l for Cd. Using the SPME formula in equation 2 above; background (reference) pore water concentrations were obtained from average MEC values in sediment of a non-crude oil polluted site during the rainy season in Ikpoba River, Niger Delta Nigeria [51].

3.3 Integrated ChemLoE

The holistic assessment of chemical contaminant parameters (stressors) in our study, following the recommendation of [34] to assign equal weight to all parameters, reveals station 1 as posing the highest risk at 0.75 (see Table 4). Notably, stations 1 and 2 exhibit elevated metal concentrations in their interstitial waters (see Table 3). This phenomenon can be attributed to the composition of the soil, with stations 1 and 2 characterized by muddy soil types and high organic matter content compared to the sandy soil types observed at stations 4 and 5. This disparity suggests a potentially greater sequestration capacity for metals and organic pollutants in the former stations.

Table 4. Integrated risk values for ChemLoE

Stressor	Ref	Station1	Station 2	Station 3	Station 4	Station 5
Cu (mg/l)	0.00	0.90	0.87	0.71	0.83	0.71
Pb (mg/l)	0.00	0.90	0.89	0.85	0.85	0.85
Cd (mg/l)	0.00	0.86	0.87	0.76	0.72	0.61
Ni (mg/l)	0.00	0.82	0.83	0.77	0.81	0.74
PAH	0.00	0.32	0.01	0.73	0.38	0.26
THC	0.00	0.74	0.62	0.80	0.75	0.74
ChemLoE	0.00	0.75	0.71	0.73	0.69	0.63

Furthermore, station 3 exhibits the highest risk values for polycyclic aromatic hydrocarbons (PAH) and total hydrocarbons (THC), as well as maximum observed environmental concentration (MEC) values (see Table 2). This observation aligns with visual cues such as black discoloration, presence of crude oil, and the distinct odour of volatile hydrocarbons at the site. A pertinent scientific inquiry arises regarding the differential accumulation of metals vis-à-vis THC and PAH at stations 1 and 2, given their association with oil. Plausible explanations may include variations in the source and points of exposure, such as leakage from the Trans-Niger pipelines.

Additionally, the abundance of hydrocarbon-degrading bacteria at stations 1 and 2 may contribute to this phenomenon. While our study primarily focuses on the comprehensive risk assessment of the entire area rather than delineating pollution gradients, these observations warrant further investigation in future remediation studies. It is imperative to delve deeper into the mechanisms underlying these observations to inform targeted remediation strategies effectively.

3.4 Mangrove cover loss

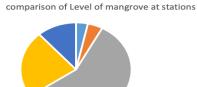
The following results detail the estimated mangrove cover loss at the five sampled stations in Bodo Creek, Ogoniland, as of September 2016. Additionally, the benthic community structure at these stations was assessed and compared to pre-spill background values from literature, utilizing the AZTI Marine Biotic Index (AMBI) software to derive M-AMBI results. Both the M-AMBI outcomes and the mangrove loss estimates were incorporated as ecological stressors in the EcoLoE of our Weight of Evidence (WoE) analysis, as presented in the result tables below.

Table 5: Result of estimated level of mangrove destruction, 24/09/2016

Stations	Mapped out	Vertical distance	Vertical distance of area of denuded	Impact
	area size	across forest patch,	mangrove starting, from point of observed	$\pm~0.5\%$
	$\pm 1 \text{ m}^2$	starting from creek	dead mangroves	
		± 1 m	± 1 m	
1	100	100	60	6
2	700	650	50	8
3	500	500	500	100
4	250	250	100	40
5	500	500	100	20

Station 3 experienced a complete (100%) loss of mangroves, a site previously identified as a protected mangrove area based on local ecological knowledge. The pie chart in Figure 7 highlights that Station 3 has the highest mangrove cover loss among all stations, with mangroves in this area entirely eradicated. This loss correlates with the maximum estimated concentration (MEC) of total hydrocarbons (THC) and polycyclic aromatic hydrocarbons (PAH) at this station, although the MEC of heavy metals remains low. The probable cause is the toxic effect of crude oil on mangroves, primarily through the physical smothering effect of hydrocarbons on mangrove stems. During the 2008 and 2009 spills, elevated levels of metals, PAH, and THC likely caused immediate damage to mangroves. Over time, metal concentrations may have decreased due to rainfall, reduced vegetation, and the hydrophilic nature of metals leading to leaching. In contrast, Stations 1 and 2 exhibit the highest mangrove cover and the lowest MEC of PAH and THC, supporting the hypothesis regarding the impact of hydrocarbons. However, it is essential to consider other anthropogenic pressures, such as deforestation, that might have influenced the mangrove loss pattern, particularly given the downstream location of Stations 1 and 2 relative to the other stations. These stations are farther from boat landing sites,

community settlements, and agricultural lands, which might have reduced human impact. The easier accessibility of Stations 3, 4, and 5 to these human activities suggests significant additional stressors on mangrove



1 = 2 = 3 • 4 = 5

Figure 7: Pie chart showing a comparison of level of impact of crude oil pollution on mangroves at stations

Table 7: Result of benthic quality index from (AMBI version 5)

stations	Phylum	class	Order	family	species Abu	ındance/20 <i>cm</i>
1	Arthropoda	Malacostraca	Decapoda	Grapidae	Sersama alberti	3
				Ocypodidae	Ulca tangeri	7
				Paguridae	Eupagurus spp.	1
2	Arthropoda	Malacostraca	Decapoda	Gecarcinidae	Goniopsis pelii	1
	Mollusca	Gastropoda	Mesogastropoda	Potamididae	Tympanotonus fusco	itus 27
	Annelida	Polychaeta	Aciculata	Neridae	Hediste diversicolo	r 1
			Capitellida	Capitellidae	Capitella capitata	1
					Notomastus laterice	eus 1
					Notomastus aberar	is 2
3	Arthropoda	Malacostraca	Decapoda	Grapidae	Sersama alberti	4
	Annelida	Polychaeta	Aciculata	Neridae	Hediste diversicolo	or 2
					Nereis pelagica	7
4	Mollusca	Bivalvia	Anguiformes	Lucinidae	Loripes rhizoecus	1
					Keletistes aberens	6
5	Annelida	Polychaeta	Aciculata	Neridae	Hediste diversicolo	or 74
					Nereis pelagica	3
					Nereis virens	3
			Capitellida	Capitellidae	Capitella capitata	a 26
					Notomastus abera	ns 7
					Notomastus lateric	eus 1
		Malacostraca	Isopoda	Idoteidae	Idotea spp	3
	Arthropoda		Decapoda	Panopeidae	Panopeus africanu	us 1

Table 7 presents the results from the AZTI-AMBI software analyses, including AMBI, Shannon diversity, richness, and M-AMBI indices. The AMBI index ranges from 0 (good) to 6 (bad), whereas the M-AMBI index ranges from 1 (high) to 0 (poor), in alignment with the European Water Framework Directive classifications of poor, moderate, good, and high status [47]. **Figures 8 and 9** provide graphical representations of species richness and diversity at various stations, compared to pre-spill samples from three stations. **Figure 10** displays a histogram of the M-AMBI results.

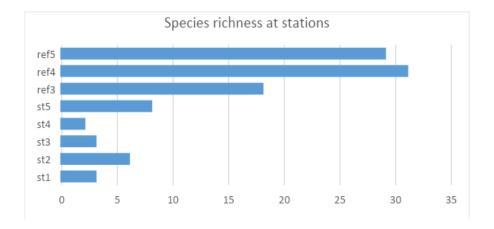


Figure 8: Bar chart of species richness/20cm2 at all stations obtained from AMBI calculator software

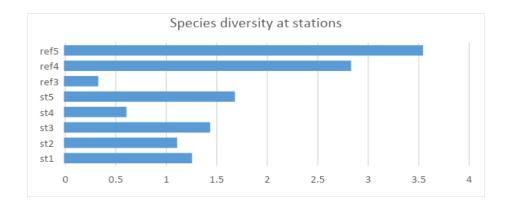


Figure 9: Bar chart of species diversity/ 20cm2at all stations obtained from AMBI calculator software

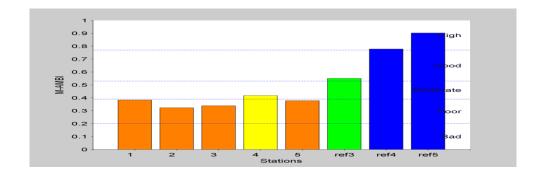


Figure 10: Histogram of M-AMBI results at stations, obtained from AMBI calculator software. Ref 3-5 are reference values, i.e. Historic monitoring data measured before the spills. (colours by default; orange-yellow-green-blue, in order of environmental status, bad to high)

3.5 Integrated EcoLoE

Table 6: Species composition and abundance of benthic infauna at stations, 24/09/2016

Stressor	Ref	Station1	Station 2	Station 3	Station 4	Station 5
Mangrove	0.00	0.01	0.03	1.00	0.37	0.71
Survival						
M-AMBI	0.00	0.56	0.64	0.62	0.54	0.85
EcoLoE	0.00	0.35	0.42	1.00	0.46	0.41

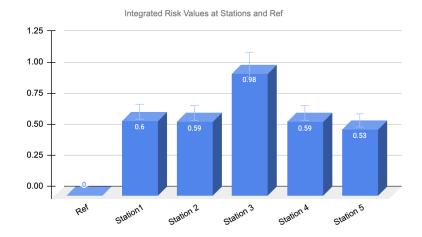


Figure 11: bar chart of final WoE risk values at stations

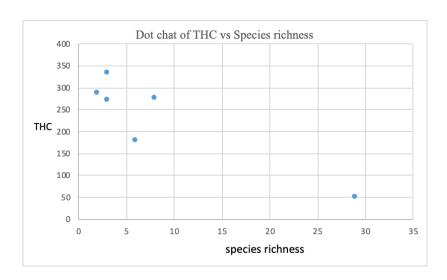


Figure 12: Regression analyses between THC and Species richness (p = 0.01, R2 = 0.76)

4. Discussion

The use of multiple lines of evidence in a TRIAD approach aims to enhance the confidence and acceptability of weight-of-evidence (WoE) assessments [52]. The variability in spatial and temporal scales across multiple

assessments underscores the importance of integrating diverse lines of evidence [53]. In this study, the integration of two distinct lines of evidence has provided a robust foundation for assessing the environmental status and risks associated with crude oil pollution in Bodo Creek. While it is not anticipated that different lines of evidence will yield identical results, their integration should bolster the assessment. The uncertainties in the final risk values from WoE analyses are quantified using the standard deviation of the different calculated lines of evidence. A standard deviation of less than 0.4 suggests low uncertainty, thereby indicating higher confidence in the derived risk values [34]. In this analysis, all stations exhibited standard deviations below 0.4, which strengthens the validity of the results.

The interpretation of the final results of an ecological risk assessment is subject to negotiation by regulatory authorities and stakeholders, often influenced by land use considerations [36]. Frameworks for interpreting results in multiple lines of evidence approaches in ecological risk analysis typically include questions such as whether there was significant biological degradation or if the contaminant levels exceed regulatory benchmarks Reference [54]. WoE risk values exceeding 0.2 generally indicate a polluted environment, while values above 0.5 suggest a severely impacted environment necessitating urgent remediation [34, 48]. In this study, all sampled stations in Bodo Creek exceeded WoE risk values of 0.5, indicating a critically high level of ecological risk. This poses a direct risk to human health due to strong food web interactions, including seafood consumption, fishing, intertidal shellfish harvesting, swimming, and other activities such as bathing and drinking locally sourced water.

Prior to sampling, stations 1 and 2 appeared pristine; however, chemical analyses and final WoE risk values revealed contamination, likely due to contaminant migration along the creek from major spill sites, particularly during tidal movements. The impact at station 3 was evident during sampling, with visible signs of petroleum hydrocarbon contamination, blackened crude oil, and dead mangrove stilt roots, which were corroborated by the final WoE risk values. AMBI calculations indicated reduced species richness and diversity at all stations compared to pre-spill reference stations (Ref3, Ref4, and Ref5). M-AMBI results suggested a moderate environmental status for station 4 compared to other stations (1, 2, 3, and 5); however, species richness and diversity data indicated otherwise (see **figure 9**), suggesting the presence of a sensitive species might have influenced the AMBI software's analysis. The final WoE analysis confirmed higher risk at station 4, comparable to stations 1 and 2. Unexpectedly, species diversity at station 3 was higher than before the spill, potentially due to recolonization by more crude oil-tolerant species, warranting further investigation.

The extent of mangrove destruction was severe, with complete loss at station 3 and significant reductions at other stations, despite some appearing healthy. This suggests additional anthropogenic pressures, such as local harvesting but also, invasive species contribute to mangrove loss in Bodo Creek.

Heavy metal toxic pressure calculations indicated high risk values before the final ChemLoE risk values, with concentrations of Cu, Pb, Cd, and Ni exceeding environmental quality standards at all stations, similar to PAH (Polycyclic Aromatic Hydrocarbons) levels. THC (Total Hydrocarbon Content) was the primary contaminant parameter in this WoE analysis due to its significant role in oil pollution [55]. Although all parameters were given equal weight, a separate statistical analysis using simple linear regression showed that THC negatively

impacts species richness (p=0.01, R²=0.76), corroborating the WoE results (see Figure 12).

Further, it is important to note that the MEC of THC in this 2016 assessment has significantly decreased through natural decomposition compared to the 2011 values reported by Zabbey & Uyi [45] at stations 1, 2, and 3, corresponding to stations 3, 4, and 5 in this assessment. Despite this reduction, the MEC values still exceed the regulatory standards set by the Department of Petroleum Resources (DPR) of Nigeria [45, 60].

Eight years after the most recent oil spills in Bodo Creek, it is evident that the environment has not returned to even half of its pre-spill condition. Tropical environments possess a natural resilience to crude oil pollution, primarily due to the activity of hydrocarbon-degrading bacteria and photolytic degradation [56, 57]. However, the specific environmental conditions of Bodo Creek hinder efficient photolysis in soil and sediment. The shading effect of the mangrove vegetation significantly reduces photolytic degradation, rendering this ecosystem particularly vulnerable to the impacts of oil spills [9, 58, 59].

5. Conclusion

Crude oil pollution from exploration and transportation activities presents a persistent challenge to environmental integrity [1 - 3]. This study underscores the critical importance of using multiple lines of evidence in a TRIAD approach to enhance the reliability of weight-of-evidence (WoE) assessments in evaluating the environmental impacts of crude oil spills in Bodo Creek. The integration of diverse lines of evidence provided a comprehensive assessment of the ecological risks and health hazards associated with crude oil pollution in this region.

Despite initial appearances of pristine conditions at some sampling stations, chemical analyses revealed widespread contamination, underscoring the insidious nature of pollutant migration and accumulation. The WoE risk values exceeded 0.5 at all sampled stations, indicating severe ecological damage and heightened risks to human health through direct and indirect exposure pathways. Heavy metal concentrations and Total Hydrocarbon Content (THC) levels were notably above regulatory standards, posing significant threats to both the ecosystem and local communities.

The observed reduction in species richness and diversity, along with the recolonization by more crude oil-tolerant species, highlights ongoing ecological shifts and the long-term impacts of oil pollution. The extensive mangrove destruction, particularly at station 3, further exemplifies the vulnerability of these critical habitats to oil spills and other anthropogenic disturbances.

This study also revealed that while natural decomposition processes have somewhat mitigated THC levels since 2011, they remain alarmingly high, failing to meet the regulatory standards set by the Department of Petroleum Resources (DPR) of Nigeria. The prolonged environmental degradation in Bodo Creek, exacerbated by inefficient photolytic degradation due to the shading effect of mangrove vegetation, underscores the ecosystem's vulnerability to prolonged oil pollution impacts.

The findings demonstrate the severe and persistent ecological risks in Bodo Creek, necessitating urgent and

sustained remediation efforts. The situation in Bodo Creek serves as a poignant example of the broader challenges faced in managing oil pollution in coastal and marine environments globally. Effective mitigation and restoration strategies are essential to safeguard the environmental health and well-being of local communities dependent on these ecosystems.

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