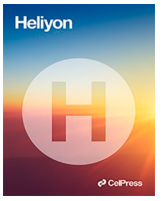
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Research article

Updating risk remediation-endpoints for petroleum-contaminated soils? A case study in the Ecuadorian Amazon region

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ABSTRACT

In Ecuador, the regulatory framework for the remediation of petroleum-contaminated soils is based on predefined concentration endpoints for a selected range of petroleum hydrocarbon compounds. However, such approach may lead to over or under-estimation of the environmental risk posed by contaminated soils. In this study, the end-point remediation criteria according to Ecuadorian Environmental legislation were evaluated using different approaches. The first one was based on Total Extractable Petroleum Hydrocarbons (TEPH) and the second one on Total Bioavailable Petroleum Hydrocarbons (TBPH). Both were compared with ecotoxicological determinations using EC_{50} -Microtox® bioassay at 5 and 15 min of exposure. The correlation (R^2) between EC_{50} values vs TEPH was of 0.2 and 0.25 for 5 and 15 min, respectively. Meanwhile, R^2 between EC_{50} and TBPH was of 0.9 and 0.65 for 5 and 15 min, respectively, demonstrating a stronger correlation. Our results suggest that a contaminated site where the concentration of the TEPH is higher than the relevant regulatory concentrations may be deemed to present an acceptable risk even though their concentrations exceed the target values in soils. The results also challenge the notion that hormesis is associated with TEPH, contrary to some literature. This study is the first in Ecuador to propose incorporating bioavailability into environmental regulations, highlighting the need for further research to establish realistic and achievable remediation goals based on toxicity studies involving various trophic levels.

1. Introduction

Soil is a non-renewable resource providing several vital functions such as habitat for living beings, gene pool, carbon sink, storage and filtration of many substances, as well as providing food, biomass and raw materials for human activities [1,2]. Soil contamination is a global threat to the food chain, public health, water, and air quality [3]. Petroleum is one of the dominant energy resources worldwide; however, this is also a source of soil contamination [4].

In Ecuador, petroleum represents more than 30 % of total exportations, contributing with 11 % of the Gross Domestic Product in 2022 [5]. Currently, the main production facilities are in the Amazon region (east of the country) and in minor proportion in the coastal one [6]. Since 1970, when petroleum extraction started in the Amazon region of the country, environmental damages such as

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the contamination of soil, rivers, and air have been attributable to oil production; consequently, social and economic impacts are a relevant problem [7].

Several remediation projects have taken place since 2005 by the Ecuadorian government. As stated in the Ecuadorian Environmental Legislation [8,9], the remediation endpoints for soils contaminated with waste oil production are based just on the concentration of Total Extractable Petroleum Hydrocarbons (TEPH), metals (Cadmium, Nickel, and Lead), and Polycyclic Aromatic Hydrocarbons. The fit for purpose principle defines the remediation endpoints according to the land use. In agreement with this principle, the land uses "Sensible Ecosystem" and "Agricultural" are more restrictive than "Industrial" (Table 1).

Since 2013 to the present, over 1300 oil-contaminated sites were cleaned, and approximately 1.6 million tons of soil were remediated in the Ecuadorian Amazon provinces of Orellana and Sucumbíos. Until 2019, 80% of the soil was remediated according to the Reglamento Ambiental para Operaciones Hidrocarburíferas Decreto Ejecutivo 1215 (RAOHE 1215) criteria while since 2020, the resting 20% the Acuerdo Ministerial 097 (AM 097) criteria was used. On the other hand, bioremediation techniques such as composting, windrows, and soil washing are applied. From them, windrows is preferred to treat larger volumes of soil; however, landfarming is more efficient in terms of the rate of petroleum hydrocarbon removal and for the time used in the process [10].

The soil remediation efficiency depends at least on four factors: recalcitrance of the molecule to be biodegraded, its bioavailability, the load of pollutant-degrading (micro) organisms, and environmental conditions for the growth of those (micro) organisms [11]. Recalcitrance refers to biochemical stability and does not depend on environmental conditions [12]; in this way, it is impossible to biodegrade a pollutant if it is recalcitrant, even if it is bioavailable [13]. Bioavailability refers to the portion of a substance to which a biological organism may have access under particular conditions and time [14]. Bioavailability depends on the transfer processes determined by the properties of the substance as well as soil and environmental characteristics [15].

Maximum permissible concentrations of potential pollutants are often used as soil quality and remediation end-points to ensure environmental and human health safety [16], as shown in Table 1. However, this approach does not consider partitioning pollutants such as petroleum hydrocarbons within the contaminated soils. Therefore, over the past decade, several studies pointed out the importance of considering the 'bioavailable' fraction of contaminants as it has significant implications for the risk assessment and remediation of contaminated media [17]. If it can be demonstrated that greater contamination levels can be left in soil without additional risk, lower remediation costs and smaller material volumes may be done diminishing the environmental footprint of remediation activities. It implies that a contaminated site where the bioavailable concentrations of the chemicals of concern are below the relevant regulatory concentrations may be deemed to present an acceptable risk even though their total concentrations exceed the target values in soils.

Determination of petroleum hydrocarbons concentration using chemical analysis is necessary to confirm that the remediation endpoints for contaminated soils are met. However, chemical analyses cannot quantify all the fractions of a pollutant, but only the ones which may react with the extracting-reagent [18]. They do not provide direct information about the toxicity of the residual hydrocarbon concentrations in soil [19]. In contrast, for at least 20 years, some countries were shifting their policies towards a risk-based approach to set up remediation goals focused on avoiding potential risks to human health and the environment, which may even evade inflated costs for soil and groundwater clean-up [20].

A better approach to assess the potential risks to human health and the environment is considering the effects of environmental samples on pre-selected organisms. It is known as the "Effect-Based Monitoring Strategy" (EBMS) [21]. Modern bioanalytic methods allow obtaining quantitative and qualitative information to determine the toxicity of environmental samples. Bioanalytics use organisms or their cells, to evaluate the potential effect of environmental samples [22]. The end-points to be studied determine the selection of one or various bioassays. For example, when whole-organisms are used, endpoints are related to mortality, growth or development disorders, using plants, algae, and animals like earthworms or other organisms [23].

One bioanalytic method uses the marine and naturally luminescent bacteria *Vibrio fischeri*. In short, different environmental sample concentrations confront the bacteria to measure its luminescence in terms of half maximal effective concentration (EC_{50}) in response to toxicity, allowing to obtain fast results and create databases for further comparisons [24]. Since the mid-1980s, the Solid-Phase Test (SPT) protocol has been developed using this bacterium in the so-called Microtox® SPT System to evaluate toxicity in soil and sediments. Results obtained through this method have shown consistency when compared with findings from invertebrate toxicity tests and macroinvertebrate field surveys. However, the suitability of its application must be evaluated on a case-by-case basis due to factors such as soil composition, the nature of potential toxicants, and sample handling conditions, all of which may influence the outcome [25].

In this study, we address a critical gap in current environmental regulations in Ecuador concerning the remediation of petroleum-contaminated soils. Unlike existing frameworks that rely solely on predetermined concentration endpoints for specific petroleum hydrocarbon compounds, our research introduces a novel perspective by evaluating the effectiveness of these criteria in accurately assessing environmental risks. Specifically, we investigate the applicability of two distinct approaches: one based on Total Extractable Petroleum Hydrocarbons (TEPH) and another on Total Bioavailable Petroleum Hydrocarbons (TBPH). By incorporating

Table 1
Remediation end-points for soils contaminated with petroleum according to two Ecuadorian Environmental Legislation: RAOHE 1215 and AM097.

Parameter	RAOHE 1215 (from 2001 to 2019)			AM 097 (from 2019 to the present)			
	Sensible ecosystem	Agricultural	Industrial	Residential	Commercial	Industrial	Agricultural
TEPH (mg/kg)	<1000	<2500	<4000	230	620	620	150

ecotoxicological assessments using the EC50-Microtox® bioassay at 5 and 15 min of exposure, we provide a comprehensive analysis that goes beyond conventional chemical analyses. Notably, this study marks the first attempt in Ecuador to integrate bioavailability considerations into environmental regulations, highlighting the necessity for further research to establish more realistic and achievable remediation goals based on toxicity studies involving various trophic levels.

2. Materials and methods

2.1. Soil preparation

A sample of 10 kg of uncontaminated soil from the surrounding EP PETROECUADOR Sacha Production Facilities (0° 20′ 7″ S 76° 52' 34" W) was collected and manually cleared of large particles such as stones, invertebrates, and plant debris. After, the soil was sieved through a 2 mm stainless mesh and dried at 105 °C for 12 h, obtaining a homogeneous material of 0.73 g cm^{-3}. After vigorous homogenization, six samples of 500 g each were collected, and the remaining soil was stored in a dark place at 30 °C and 45% relative humidity (RH) as a backup. Each soil sample was mixed with crude oil 23° American Petroleum Institute (API) gravity to get a target TEPH concentration according to the six remediation end-points detailed in Table 1: A: 150 mg/kg, B: 230 mg/kg, C: 620 mg/kg, D: 1000 mg/kg, E: 2500 mg/kg, F: 4000 mg/kg, and one negative control without TEPH. This process was followed according to the guidelines described in ILAC, 2005 [26] for the selection of stabilized material. TEPH analyses were done weekly for three weeks. Samples were used in the further assays if Analysis of Variance (ANOVA)-single factor (MS-EXCEL) determined no significant difference in TEPH concentration; new samples of standardized concentration were prepared until no variance in TEPH concentration was observed. Data obtained are showed in the Material Supplementary 1.

2.2. Hydrocarbons analysis

Briefly, 3 g of soil were homogenized by hand, dried at 105 °C for 12 h, and sieved through a 2 mm mesh; TEPH were then extracted using tetrachloroethylene 99.5 % (MERCK) and quantified according to the Environmental Protection Agency (EPA) method 8440 with a lab-based infra-red spectrophotometer (Thermo Scientific NICOLET IS 5).

2.3. Bioavailable analysis

TBPH were determined using 25 ml of a 1:1 mixture of 1-propanol (SIGMA-ALDRICH) and distilled water (CE < 10 µS cm^{-1}) as described by Dandie et al. (2010) [27].

2.4. pH, Electrical Conductivity (CE) and Dissolved Oxygen (DO)

These three measurements were determined using a lab-based multi-sensor device (METTLER TOLEDO SevenExcellence). pH was measured using the EPA 9045D method. During this work, at field conditions was not necessary to correct this parameter. CE indicates soil salinity [28] and was measured following the EPA 120.1 method, adapted for soil. DO was determined using the optical measuring principle with OptiOx sensor (METTLER TOLEDO).

2.5. Toxicity assays

Toxicity was evaluated using the Microtox® SPT procedure described by Cipullo et al. (2019) [29]. pH was adjusted to 7.0 ± 0.2

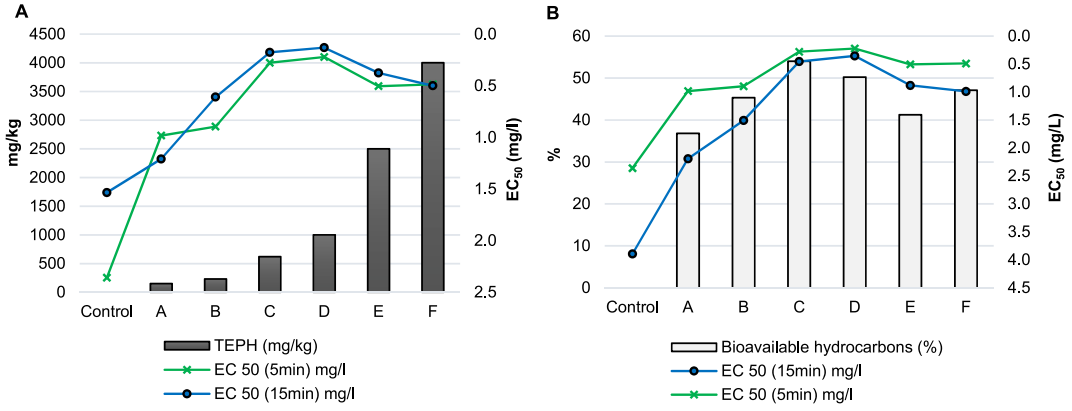


Fig. 1. Correlation between TEPH vs EC 50 (a) and TBPH vs EC 50 (b). Hydrocarbons concentration A: 150 mg/kg, B: 230 mg/kg, C: 620 mg/kg, D: 1000 mg/kg, E: 2500 mg/kg, F: 4000 mg/kg).

when needed with NaOH 0.1 N or HCl 0.1 N. Oxygen concentration was measured and regulated to be > 3 mg/l via agitation of the extract. EC_{50} was determined at 5 and 15 min in triplicates.

2.6. Statistical analysis

Results were analysed using descriptive statistics with MS-EXCEL tools [30].

3. Results and discussion

3.1. EC_{50} as function of TEPH concentration

The analysis of toxicity (EC_{50}) values across the various spiked soils, as per the six TEPH concentrations examined, indicates a decrease in soil toxicity with lower TEPH concentrations (see Fig. 1A). While there is a general trend of decreased soil toxicity with lower TEPH concentrations, intriguingly, two of the less contaminated samples (620 mg/kg and 1000 mg/kg) exhibit higher toxicity levels. Similar observations have been documented by Phillips et al. (2000), Xu & Lu (2010), Jiang et al. (2016), and Giovanella et al. (2021) [31–34] who also noted a lack of direct correlation between chemical composition and toxicity data. Supplementary Material 2 includes the raw data for reference.

In contrast, the bioavailable fraction of hydrocarbons showed a better correlation with toxicity (see Fig. 1B). As previously reported by Chen et al. (2019) [35], Microtox® response to petroleum-contaminated soils correlated strongly with water-soluble hydrocarbons, which are part of the bioavailable fraction. Notably, as the percentage of TBPH increases, so does toxicity, aligning with earlier reports [36,37].

Our findings also shed light on hormesis, a phenomenon elucidated by Schirrmacher (2021) [38], where low doses of stressors can stimulate organisms while high doses inhibit them. Interestingly, a hormetic response is observed concerning hydrocarbons when EC_{50} is associated with TBPH (Fig. 1B), contrasting with the absence of such relationship with TEPH (Fig. 1A). This suggests a stronger correlation between toxicity and TBPH, emphasizing the pivotal role of the bioavailable hydrocarbon fraction. Further research, including the development of appropriate dose-response models [39], is warranted to comprehensively grasp hydrocarbon-induced hormesis.

It is noteworthy that our findings diverge from attributing solely to the Microtox® SPT method, as hydrocarbon-induced hormesis had been previously documented. Agathokleous et al. (2020) [40] highlighted hormesis as a prevalent yet underexplored phenomenon in their review of 43 papers. Additionally, hormesis in plants growing on oil-polluted soils, such as *Medicago sativa* [39], *Salix viminalis*, and *Zea mays* [41], further corroborates its significance. Agathokleous et al. (2020) [42] further underpin this by examining 33 plant species and over 20 stress-inducing agents, affirming hormesis as a multifaceted response mechanism in plants, particularly driven by low-level stressors stimulating chlorophyll production to counteract higher stress scenarios.

3.2. Pertinence of proposing a paradigm shift on Ecuadorian environmental legislation

Surprisingly, soils with lower TEPH concentrations exhibited higher toxicity to Microtox® than those with higher TEPH concentrations. Bipolar plots between EC_{50} and TBPH versus EC_{50} and TEPH further showed a stronger correlation between toxicity and bioavailable fractions than with TEPH (see Fig. 2). These findings confirm the inadequacy of current criteria for setting end-points in the remediation of petroleum-contaminated soils, advocating for a shift towards bioavailability-based assessments. Such an approach not only enhances the efficacy of remediation projects but also mitigates negative environmental impacts associated with unnecessary remedial activities aimed at reducing concentrations that pose an acceptable risk.

Furthermore, our study highlights the importance of defining remediation endpoints based on the specific receptors requiring

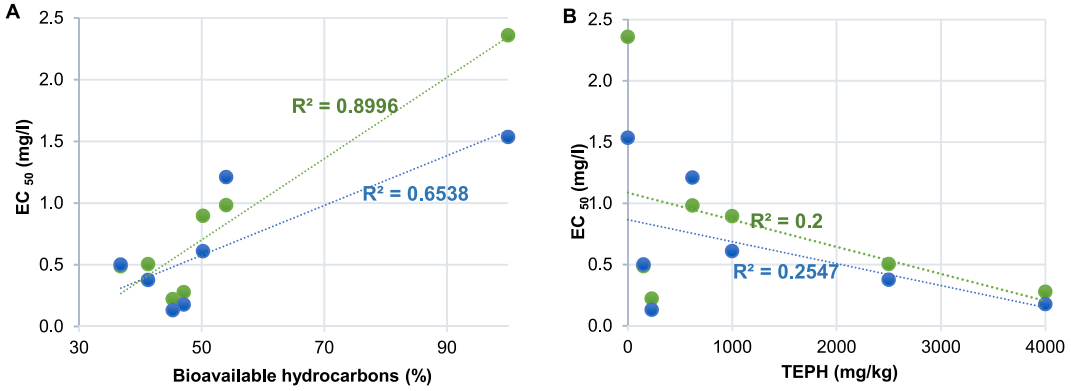


Fig. 2. Correlation between EC_{50} vs Bioavailable hydrocarbons (a) and EC_{50} vs TEPH (b). E EEC_{50} 5 min (mg/L). ———— Lineal regression EC_{50} 5 min. EC_{50} 15 min (mg/L). ————Lineal regression EC_{50} 15 min.

protection [43]. We observed a negative correlation between bioavailable hydrocarbons and EC_{50} ($R^2 > 0.65$), indicating that as bioavailability increases, toxicity decreases. However, the current legislative framework in Ecuador, which adopts a *"fit-for-purpose"* principle, fails to consider contributions from various sectors, such as wastewater treatment plants and the mining industry, resulting in the presence of Emerging Pollutants, metals, and agrochemicals in ecosystems [44,45]. Therefore, a more comprehensive approach is warranted, incorporating multi-sectoral responsibility for remediating polluted sites.

Considering the alternative of EBMS, tailored to Ecuador's diverse biogeographic regions, is therefore imperative [46]. Further analysis across these regions is essential to assess toxicity towards eukaryotic organisms at various trophic levels and different soils. Additionally, tools like broad-band and narrow-band vegetation indices such as Simple Ratio (SR), Normalised Difference Vegetation Index (NDVI), or Narrow-band indices like Red-edge Normalised Difference Index (NDVI705) can aid in detecting and mapping petroleum pollution, aiding in the targeted application of EBMS [47]. Integration of artificial intelligence techniques could further enhance the generation of robust information for setting appropriate remediation endpoints [29].

However, our study has limitations, primarily due to its reliance on laboratory-based experiments rather than field studies. While laboratory experiments offer controlled conditions and measurements, they may not fully capture the complex dynamics and variability present in real-world environments. Field studies could provide valuable insights into how the proposed paradigm shift in environmental legislation would operate in practical applications and how different environmental factors may influence the effectiveness of remediation efforts. Further to this, the study primarily focuses on the correlation between hydrocarbon concentrations and soil toxicity using a specific bioassay (Microtox® SPT), thus potentially overlooking other ecological impacts and indicators associated with petroleum contamination. Therefore, broader considerations and assessments are necessary to comprehensively address the environmental challenges posed by oil contamination in Ecuador.

Finally, to ensure the successful adoption of a paradigm shift in environmental legislation, it is essential to adopt principles of Open Science, making data and judgment criteria transparent and accessible to the public. This transparency is crucial in fostering public trust and acceptance, ultimately facilitating the implementation of effective remediation strategies [48].

4. Conclusions

Our study demonstrates the importance of re-evaluating the current regulatory framework for the remediation of petroleum-contaminated soils in Ecuador. We have demonstrated that total chemical concentration alone inadequately reflects soil toxicity levels, necessitating a paradigm shift towards bioavailability-based assessments. The observed discrepancies between total extractable fractions and bioavailable hydrocarbons highlight the inefficiency of existing criteria in accurately assessing environmental risks and guiding remediation efforts. By emphasizing the correlation between bioavailability and toxicity, our findings advocate for the incorporation of multi-sectoral responsibility and a more holistic approach to remediation endpoint definition. Furthermore, the potential adoption of EBMS tailored to Ecuador's diverse biogeographic regions offers promising avenues for targeted and effective remediation strategies. While our study underscores the limitations of laboratory-based experiments and the need for broader ecological considerations, it also highlights the significance of Open Science principles in fostering public trust and facilitating the implementation of evidence-based environmental legislation. Overall, our findings provide valuable insights for policymakers, stakeholders, and researchers, aiming to enhance the sustainability and efficacy of remediation efforts in mitigating the environmental impacts of petroleum contamination in Ecuador and beyond.

Data availability

Data are provided in Supplementary Material.

CRediT authorship contribution statement

Daniel Hidalgo-Lasso: Writing – review & editing, Writing – original draft, Project administration, Methodology, Investigation, Data curation, Conceptualization. **Karina García-Villacís:** Writing – review & editing, Writing – original draft, Funding acquisition, Data curation. **Jeaneth Urvina Ulloa:** Writing – review & editing, Investigation. **Darwin Marín Tapia:** Investigation. **Patricio Gómez Ortega:** Investigation. **Frederic Coulon:** Writing – review & editing, Methodology, Data curation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e30395.

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List of Abbreviations

AM097: Acuerdo Ministerial 097
ANOVA: Analysis of Variance
API: American Petroleum Institute
DO: Dissolved Oxygen
EBMS: Effect-Based Monitoring Strategy
CE: Electrical Conductivity
EPA: Environmental Protection Agency
NDVI: Normalised Difference Vegetation Index
NDVI705: Red-edge Normalised Difference Index
TEPH: Total Extractable Petroleum Hydrocarbons
TBPH: Total Bioavailable Petroleum Hydrocarbons
RAOHE 1215: Reglamento Ambiental de Operaciones Hidrocarburíferas Decreto Ejecutivo 1215
RH: Relative humidity
SPT: Solid-Phase Test
SR: Simple Ratio



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