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Multidisciplinary assessment of microbial fuel cells with river sediments historically contaminated by hydrocarbons amended with compost

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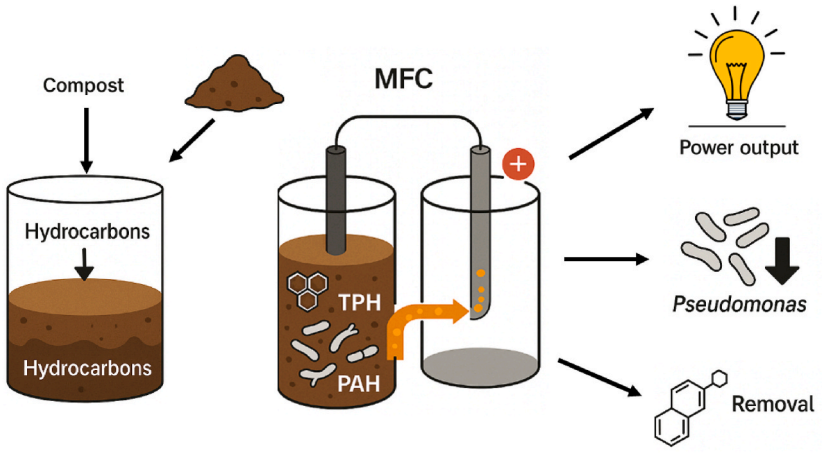
HIGHLIGHTS

- Compost addition enhanced power production and microbial activity in MFCs.
- MFCs achieved a peak density up to 173 mW/m^2.
- MFCs with compost promoted partial PAH degradation (up to 95 %).
- Some toxic transformation products may increase hydrocarbon bioavailability.
- Electrogenic and hydrocarbonoclastic bacteria (e.g., Pseudomonas) were enriched.

GRAPHICAL ABSTRACT



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ABSTRACT

A multi-disciplinary approach was adopted for the investigation of microbial fuel cells (MFCs), encompassing electrical, chemical, microbiological, biomolecular and spectroscopic analyses. This study involved a 4-month experiment utilising river sediment as the electrolyte, with a history of contamination by hydrocarbons (TPHs and PAHs). The MFCs were set up under various conditions, including the addition of compost (3 %) and in both open and closed circuit configurations. Batch conditions (no MFCs) were utilised as the control.

Electricity production in MFCs started after approximately 2 weeks in the presence of compost. These MFCs exhibited the most optimal electrical outputs over the experimental period. The outputs ranged from 173 to 8.2 mW/m^2 at the beginning and end of the testing period, respectively. Accordingly, the highest microbial activity was observed in MFCs with compost, particularly at the anode, where the highest percentage of electrogenic genera (e.g. *Pseudomonas*) were also detected. Interestingly, *Pseudomonas* encompasses species capable to degrade hydrocarbons, emphasising the metabolic versatility of these bacterial genera. As indicated by the Vis-NIR spectroscopy, a reduction in organic pollutants was observed at the end of the experiment. This finding was confirmed through chemical analyses, which detected varying removal percentages based on the condition and hydrocarbon type. The PAH removal rate was found to be up to 95 %, which suggests the presence of adapted microorganisms in the sediment. Ecotoxicological tests (with the bacterium *Aliivibrio fischeri* and the crustacean *Heterocypris incongruens*) found the absence of acute effects on both organisms. However, a significant inhibition growth was recorded for the crustacean at the end of the experiment, likely due to incomplete PAH degradation, leading to the formation of potentially toxic transformation products or their increased bioavailability.

Abbreviations

ASV	Amplicon sequence variance
BES	Bioelectrical system
CCV	Closed circuit voltage
DTD	Danube-Tisa-Danube
MFC	Microbial Fuel Cell
OCV	Open circuit voltage
PAHs	Polycyclic Aromatic Hydrocarbons
PCA	Principal Component Analysis
S	Sediment
SC	Sediment + Compost
TPH	Total Petroleum Hydrocarbons

1. Introduction

In recent years, there has been a growing interest in efficient, sustainable and circular methods for energy generation. In this context, Bio-Electrical Systems (BES) have gained popularity due to their efficiency, sustainability and cost-effectiveness. Among BES technologies, Microbial Fuel Cells (MFC) have gained considerable attention due to their capacity to generate energy for low-power devices, such as remote monitoring and biosensing applications [1]. MFCs are a type of bio-electrochemical system that exploit the extracellular electron transport ability of exoelectrogenic microorganisms. These microorganisms are capable of growting in anaerobic conditions and can catabolize organic compounds (including organic contaminants). The result of this process is the production and release of electrons. The latter are transported through a variety of mechanisms to the final acceptors, producing electricity [2–5]. The electrochemical reactions in a MFC are catalysed by microorganisms at both anode and cathode, converting organic compounds into electricity [6]. MFCs can be set-up in different configurations and applied to a wide range of applications and electrolytes [6,10,11]. However, their performance can be affected by both abiotic (e.g. temperature, pH, conductivity, contaminant presence) and biotic factors [4,7]. In this framework, terrestrial MFCs (using solid matrices such as soil or sediment as the electrolyte) are a particular challenge. In fact, only specific conditions could be suitable to produce electricity to support low power demanding electrical devices. Sufficient organic matter presence, high water content together with other abiotic factors can be critical for MFC activation and efficiency [8–10]. Moreover, contaminants in MFCs can hinder electrical output by being toxic to bacteria and disrupting electron transfer [11].

River dragged sediments have been found to be promising candidates for utilization as electrolytes in MFCs due to their organic carbon content, which can support microbial activity and power generation. Sediments typically contain between 0.4 % and 2.2 % organic carbon, which can ensure power generation [12–14].

These sediments, which are predominantly of terrestrial origin and result from processes such as erosion or the discharge of inadequately treated wastewater into rivers, necessitate dredging procedures. It is estimated that over 200 million m^3 are dredged annually in Europe [15]. However, more than half of this dredged sediment are potential reservoirs for toxic substances of anthropogenic origin, posing a significant financial burden on its management [16,17] and necessitating its disposal as a waste. For example, organic hydrocarbon pollutants (polycyclic aromatic hydrocarbons, PAHs; petroleum hydrocarbons, TPHs) [15], originating mainly from the petrochemical industry, can be present in dragged sediments. Due to their hydrophobicity nature, TPHs and PAHs rapidly settle in sediments turning them into long-term pollutant reservoirs [16]. It is acknowledged that a number of in-situ and ex-situ remediation techniques have been developed for these persistent sediment, however only a minority of these have been shown to be sustainable, simple to implement [17], or consistent with the principles of the green economy [18].

The power outputs from MFCs can be in the range of 10–100 mW/m^2 if the electrolyte is a pollutant-free sediment [19–22]. Therefore, the possible use of sediment contaminated with organic pollutants for producing energy using MFCs represents a big challenge.

Sediment bioremediation is based on the capability of some natural microbial populations to degrade contaminants, and they can also be present in MFCs [8,23].

Recent studies have tested MFCs with sediments artificially spiked with PAHs and TPHs. For example, a work with river sediment spiked with naphthalene, acenaphthene and phenanthrene with different configurations (aerobic and anaerobic conditions at cathode) [24] highlighted consistent power generations by the aerobic and anaerobic MFCs (60.2 ± 0.34 and 36.3 ± 0.37 $\mu W/cm^2$, respectively; external resistance used: 1500 Ω). A substantial PAH removal (36 % and 55.4 % in aerobic and anaerobic condition, respectively) was also achieved. About 94–99 % degradation of phenanthrene and pyrene spiked at high concentrations (10 and 5 mg/kg, respectively) was found after 8 months in MFCs with amorphous ferric hydroxide, with about 17 mV registered with an external resistance of 100 Ω, suggesting this technology can reach a high removal efficiency [25]. Most of the studies focused on PAHs or TPH

biodegradation using MFCs with soil or sediment spiked with these pollutants. To the best of authors' knowledge, a limited number of papers studied MFC efficiency using sediment that was aged, i.e. historically polluted with a wide range of chemicals.

Adding organic carbon sources can enhance MFC performance and promote degradation by supplying additional energy source to the microbial community. For example, the addition of nitrates or glucose in PAH-spiked (naphthalene, acenaphthene, and pyrene) sediments improved both power generation and contaminant removal efficiency [26,27].

Recently, vis-NIR spectroscopy has emerged as a valuable tool to predict the content of different contaminants [28–30] as well as other soil/sediment properties. There is no published paper yet on spectral soil/sediment investigations applied for MFCs. Recently, vis-NIR spectroscopy has emerged as valuable tool to predict the content of different contaminants [28–30] as well as other soil/sediment properties [30,31]. However, no paper have yet been published on spectral soil/sediment investigations applied for MFCs.

The aim of this work was to evaluate, through a multi-disciplinary approach combining electrical, chemical, microbiological, biomolecular, spectroscopic and ecotoxicological investigation techniques, the MFC efficiency in terms of electrical outputs, using a sediment historically contaminated by hydrocarbon pollutants, amended with a compost. Open (OC) or close circuit settings (CC) were tested. Moreover, batch conditions (no MFCs) were also performed as controls. The expe riment lasted about 4 months (117 days). Chemical analyses of the sediment revealed the presence of TPHs and PAHs (with 14 different types identified, from 2 up to 6 rings).

Vis-NIR spectroscopy was used to investigate spectral features of polluted sediments in all experimental conditions to highlight possible contaminant removal. Subsequent chemical analyses were used to confirm contaminant degradation. Additionally, the microbial community were evaluated at the start (in the sediment used to setup the experiment) and at the end of the experiment. The electrical outputs were registered over the experimental period. Finally, ecotoxicological analyses were performed with two different biotests (*Heterocypris incongruens* and *Allivibrio fischeri*) for assessing possible effects of the sediment before and after the MFC experiment.

2. Materials and methods

2.1. Multi-contaminated river sediment used as the electrolyte

The sediment (about 50 kg) used in this experiment was dredged from the navigable canal Bega, part of the Danube-Tisa-Danube (DTD) hydrosystem (Supplementary Materials, Fig. S1). The sediment characteristics are reported in a previous work [32] and in Supplementary materials, Table S1. The main characteristics are as it follows: total organic carbon, 2.27 ± 0.58 %; pH, 7.44 ± 0.21; total nitrogen, 2210 ± 124 mg/kg; total phosphorus, 1590 ± 164 mg/kg; conductivity, 441.5 ± 37.4 μS/cm. The sediment contained PAHs (920 ± 153 μg/kg) and TPHs (1799 ± 286 mg/kg) as the main organic pollutants. It was collected at the site, transported to laboratory, dried, sieved at 2 mm, and manually mixed and homogenized before its use for the MFC experiment.

A municipal solid waste compost (Progeva Spa, Laterza TA, Italy, certified based on ISO/IEC 17025) containing 26 % of organic carbon was mixed (3 %) with the half of the sediment. This type of compost was selected based on the findings from earlier research [9] which demonstrated its ability to enhance microbial activity in Microbial Fuel Cells, MFCs. This can be attributed to the provision of an additional organic carbon source for bacteria [9]. Altough a gradient study was not studied in this context, the 3% w/w compost amendment ratio was selected on the basis of previous research showing enhanced microbial activity and MFC performance at this particular concentration [9].

2.2. Set up of the microbial fuel cells

Each MFC consisted in a single chamber (Fig. 1). Graphite felt electrodes (8 cm of diameter, conductivity of 10 S/cm) were used for both anode and cathode. Electrodes were connected via stainless steel clips and sealed with epoxy resin to prevent corrosion. The external circuit included a data acquisition unit for daily measurements [33]. About 350 g of sediment was used to set up each MFC or used for each control batch condition (without putting the electrodes, NO MFCs). The sediment was completely saturated with distilled water (sediment moisture: about 30 %) for obtaining anaerobic conditions and MFC full capacity. Water content was maintained during the experimental period by daily water refilling.

MFCs were set-up in glass beakers (about 500 mL) with a little hole on the bottom to flow out the drained water (Fig. 1 and Supplementary Materials, Fig. S2). A layer (3 cm) of river pebbles (inert and not contaminated) was placed at the MFC bottom (Fig. 1, drainage layer); a layer (1 cm) of the historically contaminated sediment was then packed, and the anode was placed on the top of it. The MFCs were filled with the contaminated sediment (at least 5 cm, with or without compost) that was squished for removing air bubbles. Then, the cathode was positioned on the top, in contact with air (Fig. 1). Each anode and cathode were finally connected with an external circuit (Supplementary Materials, Fig. S2).

Some beakers (batch experiment), used as controls (NO MFCs), were set-up in the same way as the MFC but without electrodes. All MFCs and batch controls were put in the dark at 25 °C. The different experimental conditions and replicates are listed in Table 1.

Sediment samples at the start and end of the experiment (117 days, about 4 months) from each different condition were used for ecotoxicological, microbial and vis-NIR spectroscopic analyses and for chemical evaluations. The pH and Electrical Conductivity (EC, μS/cm) were also measured in all experimental conditions. Data are reported in Supplementary Materials, Table S2.

2.3. Electrical measurements

To monitor the performance of the MFCs, electrical measurements (voltage, power generation, external resistance) were made daily using a designed test station twice per day, as previously described [8]. The device was able to switch between open and closed-circuit conditions, varying the resistance and altering the charge and discharge periods. Two experimental periods were performed. The first period (PHASE I, from day 0 to day 79) was used to evaluate the polarization curves and measure the external resistance, which guarantees better external power outputs. Different external resistances (100, 300, 500, 1000, 2000, 3000, 5000 and 10,000 Ohm) were imposed during this period.was used to evaluate the polarization curves and measure the external resistance, which guarantees better external power outputs. Different external resistances (100, 300, 500, 1000, 2000, 3000, 5000 and 10,000 Ohm) were imposed during this period.

Subsequently, energy performance tests (durability tests, PHASE II) were conducted up to day 117, maintaining a constant external resistance (500 Ω for the sediment alone and 300 Ω for sediment with compost). The values of the resistance used in PHASE II were selected based on the optimal operating conditions obtained in PHASE I.

2.4. Vis-NIR spectroscopy

Spectral measurements were performed on air-dried sediment samples (MFCs and NO MFCs) at laboratory scale. Prior to measure, samples were dried for two days at about 24 °C to ensure the elimination of residues.

An ASD FieldSpec Pro spectroradiometer (Malvern Panalytical, UK), with a 350–2500 nm spectral range, was employed for spectral acquisitions. The collection of reflectance spectra was carried out according to

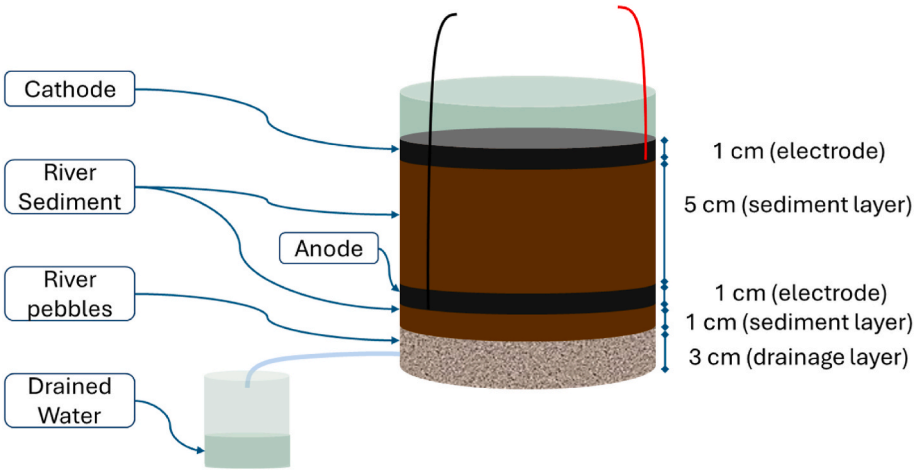

Fig. 1. Schematic representation of the MFCs setup in this experiment.

Table 1
Experimental conditions of the MFCs set up with only sediment (MFC S) and with compost (MFC SC) and control batch conditions (NO MFC SC and NO MFC S with or without compost, respectively). At least 3 replicates for each condition were set up.

Condition	Acronym	Name of the cells/ batch
MFC + Sediment	**MFC S** (3 replicates)	**MFC S1, MFC S2, MFC S3**
NO MFC + Sediment, control batch condition	**NO MFC S** (3 replicates)	**NO MFC S1, NO MFC S2, NO MFC S3**
MFC + Sediment + Compost (3 %w/w, dry sediment)	**MFC SC** (6 replicates)	**MFC SC1, MFC SC2, MFC SC3, ….. MFC SC6**
NO MFC + Sediment + Compost (3 % w/w, dry sediment), control batch condition	**NO MFC SC** (3 replicates)	**NO MFC SC1, NO MFC SC2, NO MFC SC3**

[46].

Spectral data acquired on MFC S and NO MFC S were expressed as mean absorbance values; then the visual analysis of the calculated second derivatives of absorbance spectra were performed.

2.5. Chemical analyses

Total petroleum hydrocarbons (TPHs) in sediment were analysed by a gas chromatograph with a flame ionization detector (GC-FID, Agilent 6890N), according to the ISO16703:2004.

PAHs were extracted from the sediment using the EPA3550b method, with the removal of elemental Sulphur (EPA3660b). The extracts were purified using the silica gel (EPA3630c). PAH analyses were conducted by a gas chromatography with a mass detector (Agilent 7890A/5975C), following the EPA8270C protocol. Further details on the specific chromatographic conditions are reported in a previous work [34]. Qualitative analysis (GC/MS scan analysis) were performed scanning the mass range from 35 to 550 atomic mass units (amu). The results were obtained using the Deconvolution Reporting software (DRS) with the NIST Mass Spectral Library and AMDIS System. Semi-quantitative analysis from the GC/MS scan analysis was performed using phenathrene-d10 as the internal standard. Degradation was calculated as the percentage of removed pollutant compared to the initial ("start") concentration.

2.6. Microbial community analyses

Microbial analyses were performed at the start (T = 0 day) and at the end of the experiment (117 days). The total microbial abundance (No. cell/g dry sediment) was analysed using the direct count method with DAPI (4',6-diamidino-2-phenylindole) as the DNA intercalant. Cell viability (%live/live + dead) was assessed using two fluorescent dyes (SYBR Green II and propidium iodide) to distinguish between viable (green) and dead (red) cells. Both microbial abundance and viability were analysed under a fluorescence microscope (DM 4000B Leica Microsystems GmbH, Wetzlar, Germany). Details are reported in previous works [35,36] and in the Supplementary Materials.

Microbial activity was evaluated as dehydrogenase (DHA) using a colorimetric determination of the 2,3,5-triphenylformazan (TPF) obtained by reduction of the colourless 2,3,5-triphenyltetrazolium chloride (TTC) by microorganisms. Details on this procedure are previously reported [37,38].

Additionally, the Prokaryotic community composition was evaluated by sequencing the 16S rDNA of the DNA extracted from sediments. A metabarcoding approach was used, which allows the taxonomic identification of complex environmental samples by 16S rRNA gene sequences analysis [39] as reported in a previous work [9]. Sediment DNA was extracted from aliquots of the initial sediment and sediment plus compost used for MFC set-up. DNA was also extracted at the end of the experiment from sediment collected from MFCs at different points: anode, cathode and bulk sediment. More details about the DNA extraction and sequencing analysis are reported in Supplementary Materials. Primers used for next generation sequencing (for Prokaryotes) are reported in Supplementary Materials, Table S3.

All analyses were performed in 3 replicates for each condition and sampling point.

2.7. Ecotoxicological tests

The sub-chronic test with the benthonic ostracod *Heterocypris incongruens* was performed for evaluating the possible ecotoxicity of the contaminated sediment in presence/absence of the compost at days 0 and at 117. For this purpose, the direct-contact test with the Ostracodtoxkit F™ (MicroBioTests Inc., Mariakerke, Belgium) was used in accordance with manufacturer's instructions and the standard protocol (ISO 14371:2012), using *H. incongruens* batches n. HI200519. The acute and sub-chronic effects (% mortality and % growth inhibition) were recorded after exposition (six days at 25 °C in the dark) to sediment samples. A sample was considered toxic if the mortality was >20 %, or/ and the growth inhibition was >30 %, as foreseen by ISO 14371:2012 [40]. The mortality was reported as Abbot mortality (with the Abbott's correction %) [41]. A detailed description of the procedure is reported in previous works [42] and in Supplementary Materials.

The acute ecotoxicity test with the aquatic luminescent bacterium

Aliivibrio fischeri was performed at days 0 and at 117. The bioassay was executed following the manufacturer's instructions and a standardised procedure (UNI EN ISO 11348–3:2019). The tests were performed on water extracts (1:4 sediment: distilled water, three replicates), as previously described [40]. The endpoint (bioluminescence inhibition %) was measured using the Microtox analyser (Model 500, Modern Water, UK) after 30 min of exposure and calculated with the Microtox Omni® software (V 4.2). More details are reported in Supplementary Materials.

2.8. Statistical analyses

PAH and TPH data are expressed as average values with their standard deviations. All electrical measurements and microbiological data are reported as average values and their standard errors.

Overall data were statistically analysed using the one-way ANOVA following Dunn's post-test ($p < 0.05$ was considered statistically significant) with the Statistica 14.1.0.8 software.

The effects of compost on the prokaryotic community in the initial conditions (Sediment and Sediment + Compost) and MFCs and the differences between Anode and Cathode were evaluated with a Principal-Coordinate Analysis of the ASVs, based on Bray-Curtis distances. A multivariate ANOVA with permutations (PERMANOVA) was applied to assess significance. Pairwise PERMANOVA was performed using the function pairwise.perm.manova (RVAideMemoire package) for evaluating the significance of ASV changes in the prokaryotic composition in all experimental conditions.

The Principal Component Analysis (PCA) was run to graphically synthesize the complexity of the dataset and visualise the contributions of degradation rates of PAHs and TPHs; microbiological data such as microbial abundance (DAPI), Microbial activity (DHA) and electrical data (OCV and power). PCA was performed with the R software, using the FactoMineR and factoextra packages. The $p < 0.05$ limit was used in all data analyses for their significance level.

3. Results and discussion

3.1. Electrical performance

The Maximum Power Density [mW/m^2] measured in the PHASE I (0–79 days, testing different external resistances) for each MFC is reported in Fig. 2A and Supplementary Materials, Fig. S3A. The values represent a performance peak during a 'short' period (15 s, power that can be used just to instantly supply a little sensor), when the circuit is closed (close circuit condition, CC, discharge); the circuit was charged in

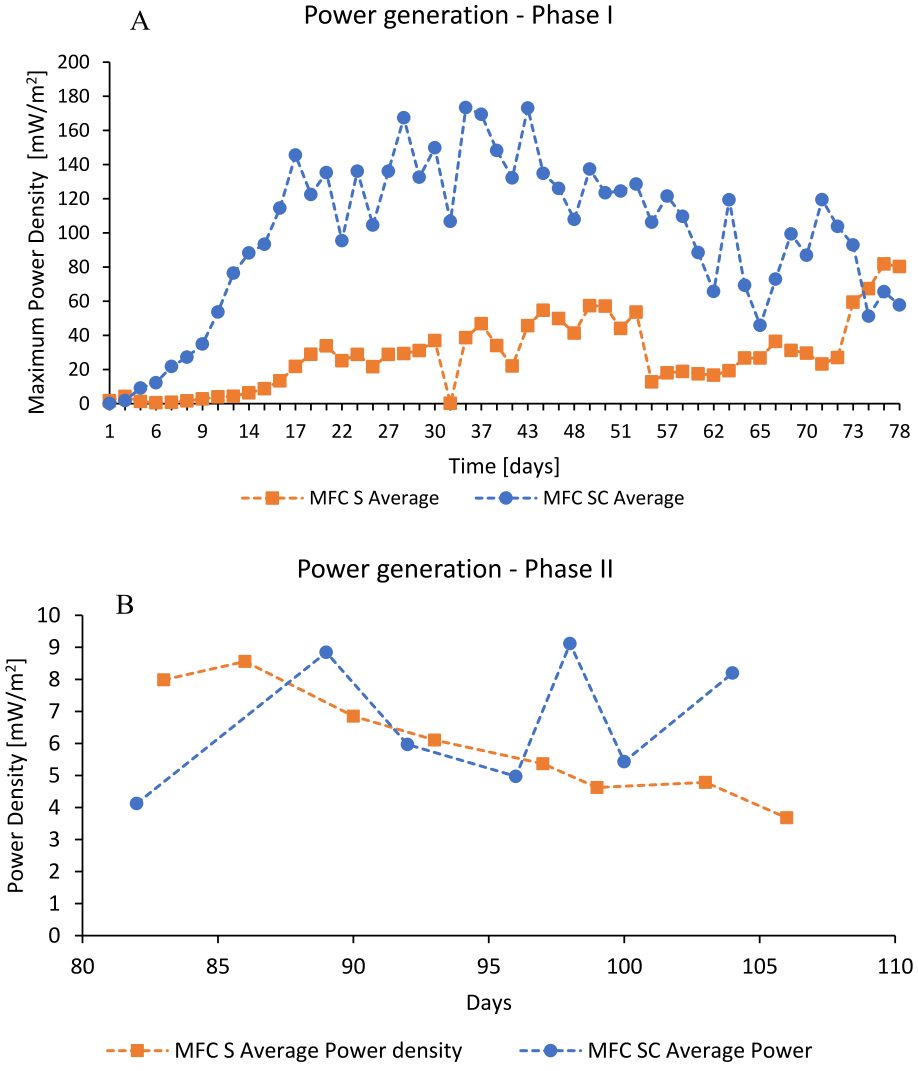

Fig. 2. (A) Power generation, expressed as Maximum power density (mW/m^2), reported as a mean of MFCs in presence or absence of compost, during the PHASE I of the experiment (from day 0 to day 79), where different external resistance were imposed. (B) Average power density generation (mW/m^2) in the MFC S and MFC SC conditions during the PHASE II of experiment (80–117 days).

OCV in the subsequent 900 s.

A relatively brief lag phase (compost presence: 2 weeks; compost absence: 3 weeks) was required for the MFC activation, i.e., reaching of stable operating conditions (Fig. 2A and Supplementary Materials, Fig. S3A), probably due to beneficial effects of compost for the microbial community developed at the anode, as already found by other authors [9]. This activation time was shorter than a previous work where the same compost was used in a PCB-spiked soil [11]. This can be ascribed to the microbial community of the present sediment already adapted to the pollutants.

Apart from two MFCs (SC1 and S1, one with compost and the other without) which exhibited a low and fluctuating trend in energy performance (probably due to inadequate adhesion of the cathode to sediment), the MFCs demonstrated consistent electrical performance over an extended period following activation (see the supplementary materials, Fig. S3). The highest average power outputs were recorded as 173.4 mW/m^2 and 81.8 mW/m^2 recorded from the MFCs with and without compost, respectively. The MFCs assembled with compost displayed an initial lower external resistance (300 Ω with compost) than the MFCs without it (1000 Ω). However, the external resistance to produce the maximum power density decreased in MFCs without compost over the experimental time to values (300–500 Ω) similar to those in MFCs with compost, leading to an increase in output power (Fig. 2A).

The MFC performance in terms of polarization curves in presence/absence of compost evaluated during the last week of tests (day 70 and day 79) are reported in Fig. 3.

A beneficial effect (in terms of electrical output) of compost on MFC performance was observed. In fact, an average Open Circuit Voltage (OCV) of 818.3 ± 15.8 mV was registered in compost presence versus 592.1 ± 172.5 mV in compost absence. Besides a higher OCV, the trend plotted for the six MFCs with compost was more uniform, reaching a mean current density and closed-circuit voltage output of 354.4 ± 218.1 mA/m^2 and 322.4 ± 25.4 mV, respectively, versus 641.6 ± 50.6 mA/m^2 and 178.1 ± 109.5 mV for MFCs without compost (Fig. 3). This result indicates that the activity of electroactive bacteria can increase in a similar way in all the MFCs tested due to the addition of an organic carbon source, as already found by other authors [10,43].

Subsequent tests (PHASE II, from 80 to 117 days) were carried out with a continuous operation to check MFCs durability, setting a constant value of the external resistances (300 Ohm in presence of compost and 500 Ohm in its absence). These resistances were selected from the polarization curve results performed daily in the PHASE I and were those that allowed the maximum power outputs. In the PHASE II, the electric circuit was permanently closed (to reproduce a continuous electrical supply), establishing an equilibrium between the electron transfers by bacteria through the sediment and the current generated at the anode.

Durability tests showed that the MFCs set-up with compost showed an increasing trend in electrical outputs (Fig. 2B; Supplementary Materials, Fig. S3B). This was probably due to the additional organic matter available for microorganisms which enhanced the electrochemical properties. At the start and at the end of the PHASE II, the average power outputs of the MFCs with compost increased from 4.1 to 8.2 mW/m^2. On the contrary, in compost absence, the average power density strongly decreased at the end of the experiment (from 8 to 3.7 W/m^2). These efficiency differences can be attributed to higher amounts of organic carbon available for electrogenic bacteria in compost presence reducing the rate of electron transfer due to the availability exo-electrogen microorganisms.

The power outputs registered here were higher than those reported in other works with MFCs set up with sediment spiked with PAHs. For example, in previous studies 3–6 mW/m^2 were measured under an external resistance of 1500 Ω and about 17 mV with an external resistance of 100 Ω. In those cases, contaminant addition had a negative effect on the activity of the non-adapted microbial community [24,25].

The historically PAH contaminated sediment tested in this study achieved a maximum power density of two-fold higher than that of another study [44] where the authors imposed a higher external resistance (470 Ω) than the one used in this experiment (300 Ω). This strongly affected the MFC power performance; in fact, a low internal resistance is reflected in higher molecular kinetics leading to increased performance. The different external resistance adopted could be attributed to the different organic matter amount: 12.3 % in this work (different organic pollutants) vs 5.3 % of Yu et al. (only anthracene, phenanthrene, pyrene) that enhanced microbial activity, providing a better proton transport. Physical-chemical parameters such as pH, conductivity, and the role of cathode have recently been identified as key factors in sediment MFC performance [9]. In our study, both the pH (7.44 ± 0.21) and electrical conductivity (EC, 441.5 ± 37.4 μS/cm) of the initial sediment (Supplementary Materials, Table S2) are into ranges favorable for proton transport and microbial activity. The pH did not substantially change from the start to the end of the experiment (Supplementary Materials, Table S3). However, the EC of the electrolyte, which is crucial for efficient power generation, was different in presence or absence of compost, both at the start and at the end of the experiment (Supplementary materials, Table S3). The EC, which allows for easier ion movement reducing internal resistance and enhancing power outputs [45] was higher in the presence of compost, and this probably also favoured the electrical outputs of the MFCs.

3.2. Spectral results

Vis-NIR spectroscopy analyses performed on initial sediment samples and on MFC and batch sediment (in presence/absence of compost) samples made it possible to highlight variations of second derivative

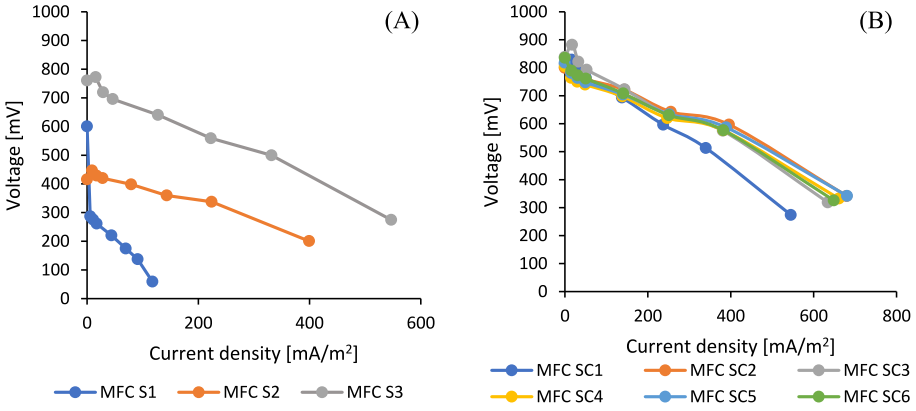

Fig. 3. Polarization curves (Voltage, mV plotted vs Current density, mA/m^2). (A) MFC S without compost; (B) MFC SC with compost. Results were recorded in the last week of the PHASE I (day 70–79), used for testing different external resistances.

spectra among the initial and final data and between the anode and cathode samples (Supplementary Materials, Figs. S4–6). A shift and a reduction in absorbance bands in the Vis range (400–780 nm) was observed in all MFCs (in presence and absence of compost) at the end of the experiment and were higher for anode samples. In accordance with previous woks, these absorption bands are attributable to electron excitations related to Fe oxides, organic matter and moisture content [46, 47]. Moreover, variations in depth and position of the absorbance bands in the NIR range (780–2500 nm) were observed; in this spectral range exist OH overtones and C-H + C-H, C-H + C-C, OH + overtones and combinations [47]. At 4 months of experiment, the reduction of the bands observed in all anode and cathode MFC samples, and the absence of the same bands in all batch samples (Supplementary Materials, Figs. S4–6), could be related to the decrease in water content of sediment and to the partial degradation of organic pollutants (PAHs and TPHs). To confirm these results, specific chemical analyses on TPH and PAH concentration at the start and at the end of the experiment were performed. Overall, vis-NIR spectroscopy allowed to provide a qualitative characterization of degraded environmental matrices such as the polluted river sediments used in this experiment by investigating their spectral behaviours.

3.3. TPH analyses

Fig. 4 reports total petroleum hydrocarbon (TPH, μg/kg) at the start and at the end of the experiment. A 15–30 % degradation was observed, without no significant difference among the conditions.

A lower removal percentage was found for TPH if compared to PAHs. This can be attributed to the relatively short experimental time (117 days) and the higher TPH persistence.

The results found in this experiment were in line with previous works showing that sediment aging and weathering processes lead to the preferential removal of lighter TPH fractions, while heavier molecular weight hydrocarbons accumulate in the sediment over time [48–50], presumably increasing their adsorption processes to sediment.

Therefore, these results indicate enrichment of high molecular weight TPH, which is generally more resistant to microbial degradation. The results in this study could also reflect the low bio-accessibility of these compounds, particularly those with molecular weights > C25, which are less likely to be metabolized by microorganisms in the absence of specific environmental conditions or long-term treatments.

These findings underline the complexity of biodegradation in sediment environments, where the balance between microbial activity and the chemical properties of pollutants plays a significant role in determining the rate of hydrocarbon removal. Further studies examining the long-term biodegradation dynamics of high molecular weight TPH fractions, along with more intensive treatment managements, may provide deeper insights into the potential for complete remediation of petroleum-contaminated sediments.

3.4. PAH analyses

Fig. 5 reports the amount (μg/kg) of PAHs at the start and at the end of experiments in the various conditions. At day 0, concentrations of low molecular weight (with 2–3 rings), were lower than those of high molecular weight PAHs (with 4–6 rings, Fig. 5A and B and C). Overall, PAHs with 2–3 ring represented less than 10 % of the total PAH and this can be ascribable to their higher biodegradability because they are more susceptible to microbial degradation due to their relatively low hydrophobicity and higher bioavailability [15]. The majority of the 2–3 ring PAHs were likely removed before sediment sampling, because they were readily metabolized by microorganisms. High molecular weight PAHs are known to have a high persistence and to be more resistant to microbial attack due to their larger size and higher environmental recalcitrance [49].

Overall, PAHs with 2–3 rings decreased at the end of the experiment up to 40 % compared to the initial analyses (Fig. 5A, T = 0), with no significant difference ($p>0.05$) among different conditions. More than 95 % of naphthalene and 35 % of phenanthrene were removed in all treatments. Significant removal of fluorene (60 %, $p<0.05$) was observed in anode compartment in MFCs with compost and in batch condition (NO MFC). No significant anthracene removal was detected in all conditions ($p>0.05$).

Overall, $\sum$4 ring PAHs degraded up to 80 % in all conditions. Interestingly, in the batch condition with compost a degradation rate of $\sum$4 ring PAHs was only of 50 %. Among PAH with 4 rings, fluoranthene and pyrene degraded at ≈90 % and 70 %, respectively, with no significant difference ($p>0.05$) between the different conditions. Interestingly, compared to batch condition, a significantly higher ($p<0.05$) benzo(a)anthracene and chrysene degradation was observed in MFCs with compost, with no significant differences between anode and cathode compartments ($p>0.05$). This result could be due to the presence of a more degradable carbon source, which had a priority in the microbial digestion pathway. A similar result was found for MFCs in compost absence, with a significant accumulation of benzo(a)anthracene and chrysene ($p<0.05$) in the cathode compartment. This could indicate a possible movement of these compounds towards cathode by electroosmosis (which can occur in water presence), with an electroosmotic flow which, in turn, facilitated the hydrocarbon and microorganism movement in the same direction of the fluid, from the anode to the cathode [51,52]. Compared to the fluoranthene and pyrene, benzo(a) anthracene and chrysene require a longer biodegradation time [53] and

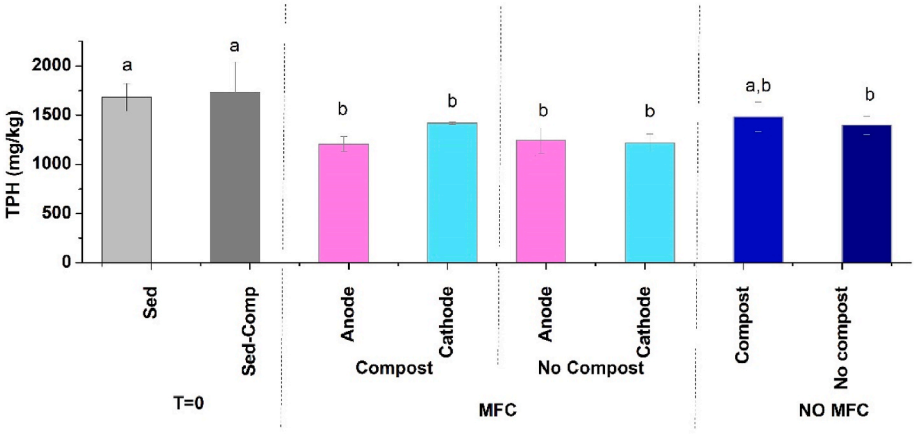

Fig. 4. TPH concentrations at the start and at the end of the experiment in all conditions and, in the case of MFCs, in the different compartments (anode and cathode). Small letters refer to statistical significance between the applied treatments.

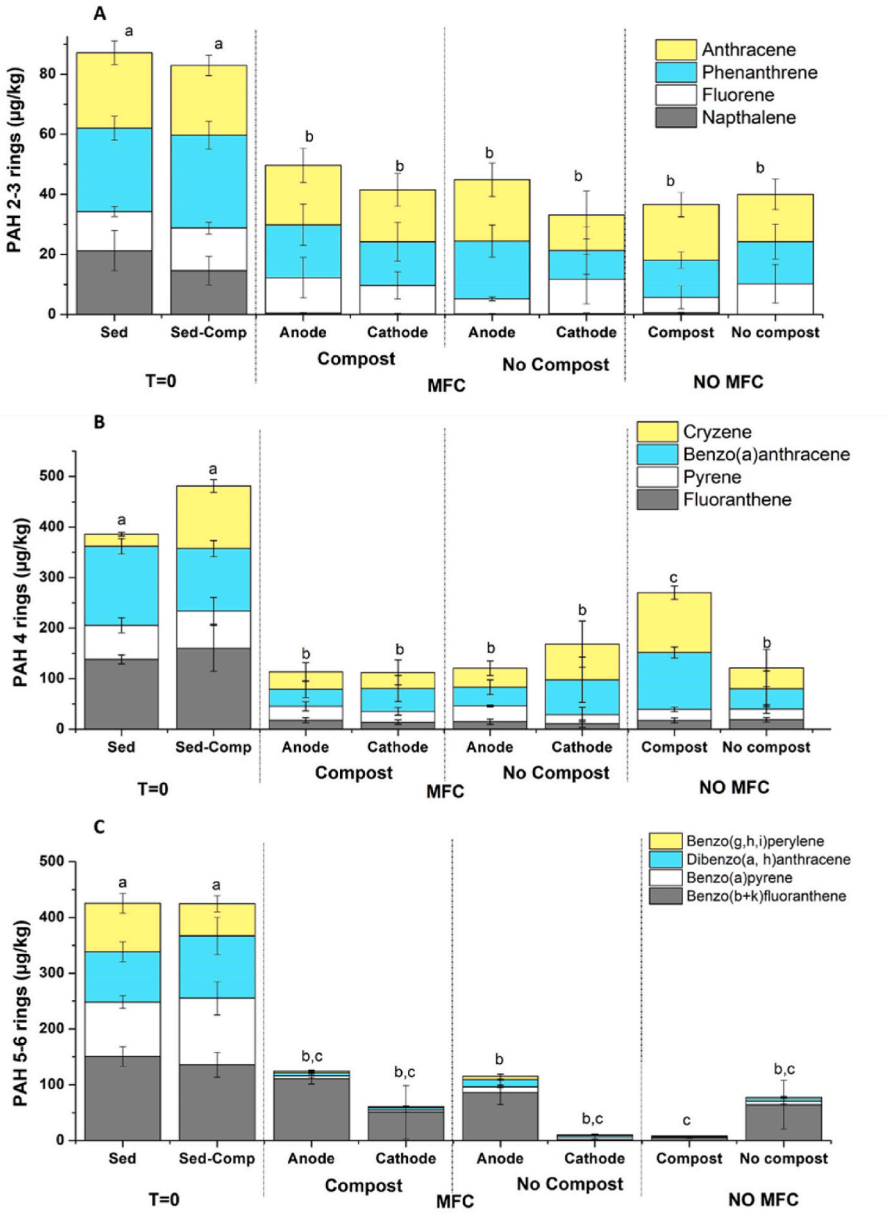

Fig. 5. PAH concentrations at the initial and final stage of the MFC experiment in all experimental conditions and in the different MFC compartments.
A: PAH with 2–3 rings, such as naphthalene, fluorene, anthracene, and phenanthrene.
B: Concentrations of PAH with 4 rings: fluoranthene, pyrene, chrysene, benzo(a)anthracene.
C: Concentrations of PAH with 5–6 rings: benzo(b+k)fluoranthene, benzo(a)pyrene, dibenzo(a,h)anthracene, benzo(g,h,i)perylene).
Small letters refer to statistical significance between the presented PAH groups, the same letter means that between observed values there is no significant difference.
Conditions: MFC S: MFC + Sediment. MFC SC: MFC+Sediment+Compost (3 %w/w, dry sediment). NO MFC SC and NO MFC S: NO MFC + Sediment (batch conditions) with or without compost (3 %w/w, dry sediment).

have a lower solubility [54]. In our experiment, the biodegradation of PAHs was not complete at the end of the 4-month experiment and the formation of some potentially toxic PAH metabolites is likely, as also found by other authors. During the chrysene biodegradation, the 1-hydroxy-2-naphthoic acid metabolite could be formed, and this can cause an increase in ecotoxicity. Similar results were observed for other PAHs [55,56]. Since benzo(a)anthracene, one of most toxic PAHs, degraded during the experiment, an increase in its available fraction probably occurred, which in turn can increase ecotoxicity of sediments.

Regarding 5–6 ring PAHs (Fig. 5C), a decrease of approximately ≈95 % was observed for benzo(a)pyrene, dibenzo(a,h)anthracene and benzo (g,h,i)perylene, with no significant differences ($p>0.05$) between the various conditions. Differently, benzo(b+k)fluoranthene showed a significant lower degradation in the MFC anode compartment in both compost presence or absence, and in the batch experiment without compost ($p<0.05$). Although there are few data comparing the biodegradation of higher molecular PAHs, benzo(a)pyrene was found to degrade more than benzo(b+k)fluoranthene [57]. The lower degradation in the anodic compartment could indicate that benzo(b+k)fluoranthene was more likely to degrade under aerobic conditions; moreover, due to its lower solubility and higher hydrophobicity, its movement in the fluid direction from anode to cathode was very limited. Further investigations are needed to confirm this hypothesis. In addition, to enhance the petroleum hydrocarbon removal and electricity generation in these soil MFCs, the addition of biochar can play an important role as stated by Li et al. [58].

3.5. Microbiological analyses

Microbial abundances, viability and activity are shown in Fig. 6.

The microbial number (1.3 10^8 cells/g) in the sediment at the start of the experiment (T0 day) was relatively high and this value remained substantially similar over the experimental time. However, the initial sediment cell viability was very low (3.6 %), showing that most bacteria were not active. At 117 days, owing to water and compost presence both viability and dehydrogenase activity increased (Fig. 6A and B and C). Microbial activity showed significantly higher ($p < 0.05$) values in compost amended sediment and at the anode (219 μg TPF/g sediment) of the microbial fuel cells (MFC SC) and in the batch condition (NO MFC

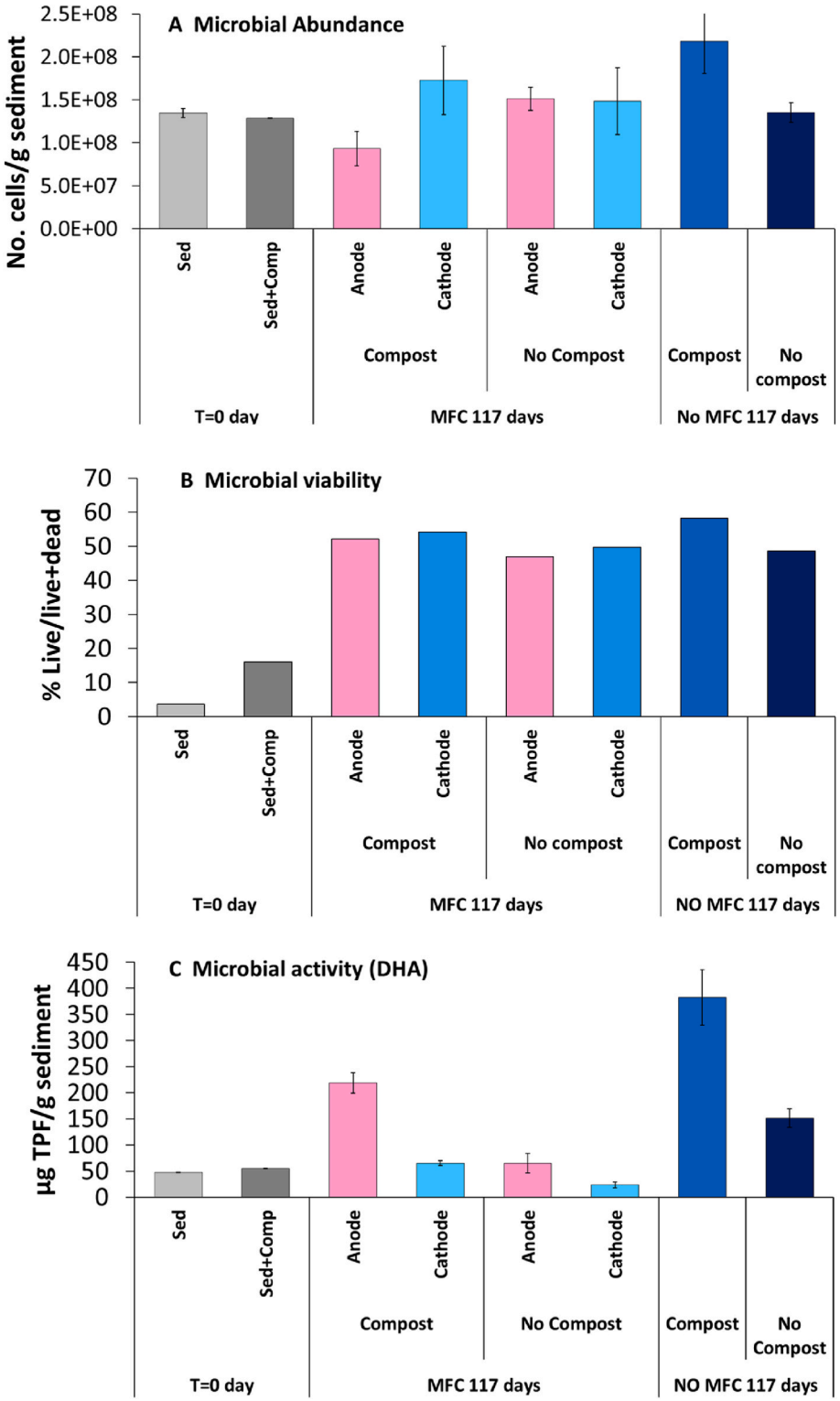

Fig. 6. A: Microbial abundance: No. of cells/g; B: Microbial viability: % of Live/live + dead cells; C: Microbial activity: dehydrogenase activity (μg TPF/g) in the sediment (Sed) and sediment plus compost (Sed + Comp) at the start (T = 0 day) and at the end (117 days) of the experiment in the MFCs and batches (NO MFC) conditions. In the case of MFCs, the analyses were performed at both anode and cathode area.

SC), in line with the higher electrical outputs. This was presumably due to the beneficial compost effect which stimulated overall microorganisms, especially exoelectrogenic bacteria, as recently demonstrated [12]. Compost presence, independently from the MFCs, stimulated bacterial activity and favoured more than other conditions some hydrocarbon removal (e.g. benzoa(a)pyrene and benzo(g,h,i)perylene). However, as their biodegradation occur, their bioavailability also increases and this can be a tricky phenomenon, because on one hand they are removed, but at the same time they can be more toxic [59].

The taxonomic identification by 16S rRNA gene sequences analysis, using amplicon sequence variant (ASV) of the microbial community at different microbial fuel cell compartments show that exoelectrogenic microorganisms developed on the anode (due to water addition and anaerobic conditions), significantly different from those found at the cathode (Supplementary materials, Fig. S7).

Significant differences were found among the various experimental conditions by the PCA based on Bray-Curtis distances of the ASVs of the prokaryotic community (Supplementary Materials, Fig. S8). A shift ($p < 0.05$) in ASV distribution was observed between the initial sediments (0 day), with and without compost, and the MFC conditions. The compost presence selected microbial populations involved in electrochemical activity and hydrocarbonoclastic bacteria and induced an ASV change in their distribution inside each MFC (Supplementary Material, Fig. S8). In fact, a higher presence of electrogenic bacteria, such as *Geobacter* (1.4 %), *Fulvivirga* (5.4 %) and *Pseudomonas* (10 %) was found on the anode in compost presence (MFC SC, Supplementary Materials, Fig. S7). These bacteria have a catalytic capacity to exchange electrons with solid substrates (i.e. electrodes) [59] and were already found in other works [61,62]. Interestingly, *Pseudomonas* was the most abundant genus at anode in compost presence, and this can be ascribed to the high versatility of this genus which is both an electrogenic and hydrocarbonoclastic bacteria [4,63]. *Pseudomonas* is a facultative anaerobic bacterium able to produce both energy [60] and degrade some PAHs, including pyrene [63] and naphthalene [62]. The latter compound in fact reduced more than 95 % at the anode in compost presence, showing the potential versatility of this genus which can be both an electrogenic and hydrocarbonoclastic bacterium. Interestingly, a Deltaproteobacteria bacterium (bacterium RIFCSPLOWO2_02_56_12) potentially able to degrade aromatic hydrocarbons [64] was also detected in all sediment samples at the start of the experiment (1.8 %) and in a relatively higher percentage (average value: 9 %) at 117 days (at anode and cathode, in batch condition, in presence and absence of compost). This was in line with the hydrocarbon degradation which occurred in all conditions.

Numerous studies have shown that several bacterial communities in contact with hydrocarbons rapidly shift to bacterial species able to degrade and utilize these compounds as sources of carbon and energy (hydrocarbonoclastic bacteria) [65]. Hydrocarbonoclastic bacteria have evolved (owing to their contact with hydrocarbons) adaptive mechanisms to tolerate the presence of hydrocarbons, such as the ability to emulsify and metabolize them, activation of DNA repair mechanisms, production of molecules involved in quorum sensing and biofilm mechanisms. However, hydrocarbon degradation is a complex phenomenon which depends on bacterial metabolism and hydrocarbon bioaccessibility, which is quite low in historically contaminated substrates. The presence of hydrocarbonoclastic bacteria was revealed in the studied sediment. These bacteria were adapted to hydrocarbon toxic effects. However, these bacteria were modulated by the experimental conditions, which favoured differently microbial activity, and the removal and bioaccessibility of contaminants.

Overall, in this experiment MFCs did not further promote total hydrocarbon degradation, differently on how found by other authors [24,25] using spiked sediments. However, the electrical performance increased significantly (especially in compost presence) because electrobacteria, adapted to the historical contamination were not negatively affected by it.

Biodegradation of hydrocarbon is potentially possible, however owing to their complexity it cannot rely on single microbial species, but requires versatile consortia which promote their bioaccessibility through production of biosurfactants; therefore hydrocarbon bioremoval can be improved using bioaugmentation strategies [63].

The Principal Component Analysis (PCA) performed considering PAH and TPH reduction between 0 and 117 days, microbial abundance, microbial activity, and electrical measurements under the various conditions is shown in Supplementary Materials, Fig. S9. The PCA explained 57.2 % of the total variance. The dimension 1 accounted for 33.8 % of the variance; the positive segment of the plot for this dimension is closely related to the reduction in several PAH (e.g. benzo(b+k)fluoranthene, 46 %; benzo(g,h,i)peryphene, 96 %; naphtalene, 99 %; benzo (a)pyrene, 95 %) suggesting a reduction in both MFCs and no-MFCs conditions. These results show that hydrocarbonoclastic bacteria were present in the historically contaminated sediment. The OCV, Power, TPHs and Benzoanthracene lay on negative part of segment 1. The Dimension2 accounts for 23.4 % of the variance and is related to pyrene reduction (66 %). However, the various conditions promoted differently hydrocarbon transformation particularly at the aerobic cathode. Moreover, some PAHs (e.g. crysene; benzo(a)anthracene) decreased more in compost presence.

3.6. Ecotoxicological analyses

The ecotoxicological test performed with a direct contact between *H. incongruens* and the historically contaminated sediment showed no effects in terms of mortality. However, at the end of the experiment a significant growth inhibition (higher than 30 %, Fig. 7), except at the cathode of MFC (in absence of compost), was recorded. This can be due to a possible contact/adsorption of some bioavailable or transformed PAHs on the test organisms, as found for other benthonic organisms [63].

The ecotoxicological tests performed at the start and at 117 days with the *Aliivibrio fischeri* bacterium using water extracts obtained from sediment samples, showed no inhibition effects (values always <20 %) in all conditions. This result was presumably due to low water solubility of hydrocarbons [63] which did not leach in the sediment elutriates. Although ecotoxicological tests showed significant growth inhibition of *H. incongruens* in compost-amended conditions, specific transformation products were not chemically identified. However, similar studies have focused on chrysene and other PAH degradation intermediates, such as

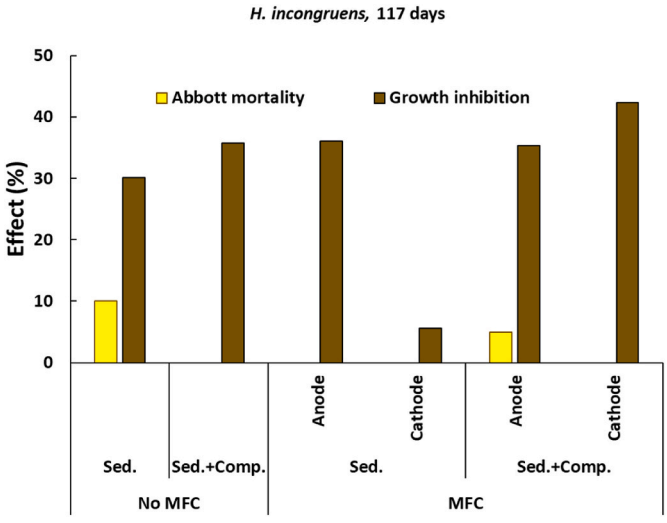

Fig. 7. Ecotoxicological effect on the crustacean *Heterocypris incongruens* at the end of the experiment. The results are expressed as Abbot mortality (yellow) and Growth inhibition (brown). Threshold value for mortality: 20 %; threshold value for the growth inhibition: 30 %.

1-hydroxy-2-naphthoic acid, with increased toxicity [54]. Future studies are necessary to investigate the nature of these by-products.

4. Conclusions and future perspectives

This study aimed to achieve the challenging goal of using a river sediment historically contaminated by hydrocarbons (TPHs and PAHs) as the electrolyte for the MFC setup.

MFCs with only the aged-contaminated sediment were able to produce energy rapidly without the electrogenic bacteria having to adapt to the toxic substances in the sediment. Therefore, the sediment acted as a suitable electrolyte for transferring hydrogen ions from the anode to the cathode. Adding 3 % of compost to sediment, MFC activation for electricity production started in a shorter time than in its absence. Moreover, compost promoted electrical performance, microbial activity, and degradation of some hydrocarbons. Although no gradient-based optimization for compost addition was performed, the power outputs of MFCs assembled with 3 % of compost showed positive trends for the electrical outputs due to additional organic matter used by microorganisms throughout oxidation processes. These results were in accordance with the highest voltage and power outputs measured. Future research on dose-dependent responses would be useful to determine optimal compost concentrations for balancing power generation and hydrocarbon degradation. The compost also influenced positively electrogenic bacteria development and activity, made it possible a long durability of the MFC performance. In fact, the average power density for the MFCs set up with compost showed an increase up to the end of the experiment, while electrical performance significantly dropped in its absence.

The performance of the MFCs with the historically contaminated sediment was not negatively affected by hydrocarbon presence because resident microorganisms were adapted to their presence. Differently from other works, where hydrocarbons were spiked, this technology did not improve overall hydrocarbon removal, presumably because aged contamination influence their bioaccessibility. Interestingly, MFCs and compost promoted differently bioavailability and biodegradation of some hydrocarbons comparing to others.

MFCs cannot be used alone as a bioremediation tool for historically contaminated sediments. However, if they are combined with compost and bioaugmented with effective hydrocarbonoclastic bacterial consortia or plant species (which can produce biosurfactants for hydrocarbon desorbing) it would be possible to obtain good electrical performance and increase hydrocarbon degradation. Moreover, the practical implementation of these MFCs poses specific engineering challenges, including the supply and transportation of compost, electrode fouling, and system scalability. Furthermore, the durability of the electrodes and the heterogeneity of the sediments have to be considered when evaluating the performance of the system. At present, MFCs cannot compete with conventional remediation technologies in large-scale applications. However, while further investigation is required, their integration into decentralised or low-cost monitoring and remediation configurations for contaminated sites can be considered very promising.

CRediT authorship contribution statement

Snežana Maletić: Writing – review & editing, Supervision, Methodology, Formal analysis. **Domenico Borello:** Writing – review & editing, Supervision, Project administration, Methodology, Funding acquisition, Conceptualization. **Gabriele Guglielmo Gagliardi:** Writing – original draft, Validation, Investigation, Formal analysis, Conceptualization. **Carlotta Cosentini:** Writing – original draft, Validation, Investigation, Data curation. **Valeria Ancona:** Methodology, Investigation, Formal analysis, Conceptualization. **Anna Barra Caracciolo:** Writing – review & editing, Supervision, Methodology. **Livia Mariani:** Investigation. **Gian Luigi Garbini:** Methodology, Investigation, Formal analysis. **Alessandra Narciso:** Investigation, Formal analysis. **Andrea Visca:** Investigation, Formal analysis. **Natalia Leone:** Investigation, Formal analysis. **Ludovica Rolando:** Investigation, Formal analysis. **Srđan Rončević:** Investigation, Formal analysis. **Marijana Kragulj Isakovski:** Investigation, Formal analysis. **Jelena Beljin:** Investigation, Formal analysis. **Paola Grenni:** Writing – review & editing, Supervision, Methodology, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Domenico Borello, other authors reports travel was provided by EU Cost Action PHOENIX CA 19123. Snezana Maletic reports financial support was provided by Republic of Serbia Ministry of Education Science and Technological Development. Domenico Borello, Gabriele Gagliardi, Carlotta Cosentini reports financial support was provided by Next Generation EU - NEST. Paola Grenni reports financial support was provided by NextGenerationEU - NBFC. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jpowsour.2025.238307.

Data availability

Data will be made available on request.

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