International Journal of Research Publication and Reviews

Journal homepage: www.ijrpr.com ISSN 2582-7421

Constructed Wetland Technology for Remediation of Heavy Metals and Crude Oil Polluted Soil Using *Paspalum Conjugatum* P.J. Bergius

***Ikeanumba, Michae Okwudiri*[1], *Orji, Justina Chibuogwu*[2], *Nweke, Christian Okechukwu*[3], *Adieze, Ifechukwu Enyinnaya*[4]**

[1.] *Biology Department, Alvan Ikoku Federal University of Education. Owerri, Nigeria*
[2.] *Microbiology Department, Federal University of Technology, Owerri, Nigeria*
[3.] *Microbiology Department, Federal University of Technology, Owerri, Nigeria*
[4.] *Microbiology Department, Federal University of Technology, Owerri, Nigeria*

ABSTRACT

The use of constructed wetland system for bioremediation of crude oil and heavy metals polluted soils especially in the tropics is a new area of study with a lot of potentials. The study was carried out over a period of five (5) months in a wetland system containing soil polluted with 10% crude oil and heavy metals (Zn = 39.4 mg/l, Ni = 10.20 mg/l, Cu = 29.4 mg/l and Pb = 11.2 mg/l) amended with different types of biostimulators, which are 1% native soap, 1% poultry manure and 1% combined native soap solution. All the setup had Paspalum conjugatum p.j. Bergius (plant) as the phytoremediator plant. Results obtained shows that amendment of the polluted soil with native soap gave the best result when the sediments of the microcosm wetland soil was assayed for the presence of residual heavy metals at the end of the study duration. This was followed by combined amendment of native soap solution and poultry manure, next was poultry manure, then plant with polluted soil only while the least result was from unamended soil. Molecular analysis of the microbial isolates identified Pseudomonas xiamenensis, Acinetobacter baumannii, Alcaligenes cloacae, Enterobacter cloacae, Pantoea dispersa, Lysinibacillus fusiformis, and Kocuria palustris as hydrocarbon-degrading bacterial species while biochemical ass identified the hyudrocarbon degrading fungi species are Penicillium spp. Aspergillus spp, and Fusarium. Results obtained from analysis of the soil sediment and residual water from constructed hybrid wetland shows that total petroleum hydrocarbon (TPH), THC and heavy metals were all significantly reduced from the soil and water while heavy metals significantly increased in the tissues of Paspalum conjugatum p.j. Bergius. This study shows that hybrid constructed wetlands are a promising technology for the remediation of environments contaminated with crude oil and heavy metals, offering a balance of effectiveness, sustainability, and cost-efficiency through enhanced contaminant removal

Keywords: Bioremediation, Phytoremediator, constructed hybrid wetland, biostimulator and hydrocarbon utilizing bacteria

1. INTRODUCTION

The necessity for sustainable wastewater and land treatment technologies that are environmentally friendly, easy to operate, less energy-intensive, and cost-effective is increasingly critical in light of growing environmental crises. These include pollution, water shortages, climate change (Hartemink, 2006), rapid population growth, and other pressing issues. Constructed wetlands (CWs) have emerged as a viable solution for achieving wastewater treatment objectives by harnessing natural components and processes. This approach minimizes reliance on energy-intensive mechanical systems and reduces technological complexity. Additionally, CWs leverage natural processes to effectively transform hazardous chemicals (Yergeau, Sanschagrin, Beaumier, & Greer, 2012).

Constructed wetlands (CWs) for wastewater treatment are engineered systems designed to harness natural processes for the removal of pollutants from wastewater. While CWs utilize many of the same mechanisms found in natural wetlands, they do so within a more controlled environment (Vymazal, 2022).

The 1980s and 1990s marked a significant period for the rapid expansion of engineered wetlands for wastewater treatment worldwide. Initially, the technology spread gradually, primarily through personal sharing of experiences until the early 1980s. However, in the late 1980s and early 1990s, a series of international conferences dedicated to this technology were organized in Europe, Asia, Australia, and both North and South America, facilitating its broader adoption and dissemination.

By the end of the 20th century, constructed wetland technology had proliferated across all continents, encompassing a variety of constructed wetland types. Research in the early 21st century focused on various design and operational features that could enhance pollutant removal efficiency (Vymazal, 2022; Wu, Kuschk, Brix, Vymazal, & Dong, 2014).

2. Materials and Methods

Collections of samples

Soil

The soil samples for this study are (i) uncontaminated soil (ii) contaminated soil through simulation.

Uncontaminated soil sample

The uncontaminated soil sample was collected from the back of Okemini Naval barrack, Rumuolumini, in Obio Akpor Local Government area of Rivers State (this site is not known to be polluted with petroleum hydrocarbon or by any other industrial pollutants), surface soils (0-20 cm) were randomly collected using a shovel. It was bulked to form a composite sample and then transported to laboratory; the soil sample was dried, and sieved through a 2 mm mesh to ensured uniformity. Sieved samples were stored in plastic bottles and covered with stoppers until analysis

Source of crude Oil

The crude oil type that was used for this work, was Bonny light crude oil obtained from the Port Harcourt Refinery Company Limited Eleme, in Rivers State. The crude oil was collected in twenty-five liters (25 L) plastic container and stored at room temperature in the laboratory until it when used.

Sample pollution

Soil pollution

Uncontaminated soil obtained as stated in Section 3.1 above was polluted using method adopted by Adieze, Orji, Nwabueze, & Onyeze, (2012). The crude oil was dissolved in acetone (3:1), and mixed with 10% of total soil. The crude oil laddered soil which served as the stock was added to the bulk of the soil and mixed to obtain the final concentrations of 3% (30 g/kg), 7% (70 g/kg) and 10% (100 g/kg) crude oil in the soil. The mixed crude oil enriched soil was stirred several times for 2 days to remove acetone (Adieze et al., 2012).

Water sample pollution with heavy metals

A liter (1 L) of deionized water was polluted with fixed concentrations of heavy metals (Zn, Cu, Ni and Pb) by dissolving their hydrated salts ZnSO4.7H2O, CuSO4.5H2O, Ni(NO3)2.6H2O and Pb(NO3)2 respectively to achieve concentrations above the maximum permissible limits for discharge waste water effluent into Nigerian environment as shown in Table 3.1 below, (Federal Ministry of Environment (FME), 1992). An equivalent of 10 L was obtained and stored in a 10 L clean and dried container. A 100 ml of this solution was added to a kg of the crude oil polluted soil, thoroughly mixed with a hand trowel to create a heavy metal and crude oil polluted soil.

Experimental design

Preliminary screening of plants species for tolerance to crude oil and heavy metals in wetland soil

The preliminary study was carried out in a greenhouse and involved the screening of eight (8) different species of plants for tolerance to different concentrations of crude oil and heavy metals pollutants in the CW soil. The two (2) plants that showed best tolerance to the pollutants (crude oil and heavy metals) were selected for further studies in 12% crude oil and heavy metals. The protocols for selection of heavy metals and crude oil tolerant plant species involved the determination of the following plant growth indices;

1. Plants’ shoot height,
2. Plants’ leaf width and,
3. Plants’ total biomass (wet weight)

Effect of different amendments on the remediation of the pollutants (10% crude oil and heavy metals)

A total of eight setups were constructed in duplicates, each representing a constructed wetland system. These setups utilized black ornamental bags measuring 29 cm × 59 cm, filled with 3 kg of soil that was contaminated with 10% crude oil and heavy metals. Each setup was treated with various amendments, as detailed in Table 2. The treatments included:

- 1% native soap solution
- 1% poultry manure
- Combination of both 1% poultry manure and 1% native soap solution

The purpose of these different treatment protocols is outlined in Table 2. This study was carried out for a duration of five (5) months and the soil sediment was assayed for residual heavy metals. The setup was aimed to identify which treatment yields the most effective results, potentially applicable to real constructed hybrid wetland macrocosms.

Constructed hybrid wetland (CHW) design and operation

A vertical surface flow hybrid constructed wetlands system (CWS) was designed for this study, comprising two cylindrical container troughs. Each trough represents a constructed wetland system, incorporating essential components like polluted soil, gravel, and waterlogged soil, simulating real-world CWS conditions. The first trough is positioned 128 cm above the second, allowing gravitational water flow when the connecting tap is opened. Each container has a capacity of 25 liters. The first CWS contains polluted soil amended with 1% natural soap solution, while the second contains unpolluted soil, but treated with combined 1% natural soap solution and 1% poultry manure, bioaugmented with hydrocarbon-utilizing microorganisms (bacteria and fungi) previously isolated, as described by Okpokwasili & Amanchukwu, (1988). Both CWS units were planted with Paspalum conjugatum, the plant that demonstrated the most desirable traits in preliminary tests as shown in Tables 2 and 3.

A 50-liter reservoir was constructed to manually control and distribute equal volumes of heavy metal-laden water into the constructed wetlands. Water flow was regulated through inlet and outlet valves, allowing for controlled water movement in and out of the vertical surface constructed wetlands via a 50 mm polyvinyl chloride (PVC) pipe. The retention time for the remediation process in each CWS was 30 days. After this period, the treated water was directed into the second CWS for further treatment by opening the second inlet valve. Following an additional 30 days of treatment, the outlet valve was opened to collect the remediated water, which had undergone microbial and phytoremediation, into a conical flask.

The collected water was transferred into a dry glass container with a screw cap and sent to the laboratory for analysis. Every 30 days, both the treated water and 5 g of soil sediment samples were taken from various parts of the first constructed wetland. These samples were thoroughly mixed to create a homogeneous sediment mixture, accurately representing the soil sediment in the CWS. The samples were then taken to the laboratory for analysis of residual crude oil and heavy metal concentrations.

The samples analysis for this study were carried out at Aigberua Ayotunde of Analytical Concept LTD, headquartered at Poultry Road, near the 2nd Railway, Odani Green City, Elelenwo, Port Harcourt, Rivers State. The diagram of the constructed wetland is displayed in figure 1.

Microbiological analysis

Microbial populations in the soil samples were assayed by standard plate count technique. The total aerobic heterotrophic culturable microbial populations present in the soil samples during the study were estimated by spread plate techniques of Pelczar & Chan, 1977.

Enumeration and isolation of hydrocarbon utilizing species was determined by the modified method of Okpokwasili & Amanchukwu, (1988). Characterization of the isolates followed the procedures in the Bergey’s manual of determinative bacteriology (Holt, 1994).

Molecular identification of isolated hydrocarbon utilizing strains

Bacterial genomic DNA extraction

Total DNA isolation and isolates identification were carried out at Nucleometrix Molecular Laboratory, Yengoa, Bayelsa State. The method as described by Saitou & Nei, (1987), was adopted for molecular identification of isolates

DNA quantification

The extracted genomic DNA as described above was quantified using the Nanodrop 1000 spectrophotometer.

16S rRNA Amplification

The 16s RRNA region of the rRNA genes of the isolates were amplified using the 27F: 5'-AGAGTTTGATCMTGGCTCAG-3’ and 1492R: 5'-CGGTTACCTTGTTACGACTT-3’ primers on an ABI 9700 Applied Biosystems thermal cycler at a final volume of 50 microlitres for 35 cycles.

Sequencing

Sequencing was done using the BigDye Terminator kit on a 3510 ABI sequencer by Inqaba Biotechnological, Pretoria South Africa.

Phylogenetic Analysis

Obtained sequences were edited using the bioinformatics algorithm Trace edit, similar sequences were downloaded from the National Center for Biotechnology Information (NCBI) data base using BLASTN.

Sample analysis for the constructed hybrid wetland

In-situ measurements were carried out for pH, temperature and turbidity using hand held instruments. A potable HACH conductivity meter was used for electrical conductivity and temperature and a HANNA instrument LP 2000 turbidity meter was used for turbidity determination. The samples that cannot be analyzed were refrigerated.

Determinations of metals in the soil and plant tissue

(i). Soil sample preparation and digestion procedure

Soil samples were prepared and digested according to USEPA Method 3050b, as described by Aktaruzzaman, Fakhruddin, Chowdhury, Fardous, & Alam (2013).

(ii) Plant sample preparation and digestion procedure

Plant samples were digested with nitric acid for heavy metal determination following the method of DB53/T 288-2009, as described by Allen, Grimshaw, & Rowland (1986).

Determination of heavy metal concentrations in plant samples

Atomic Absorption Spectroscopy (AAS) (Model: AA-6401F, Shimadzu, made in Australia), was used for the determination of heavy metals in plant tissues. To provide element specific wavelengths, a light beam from a lamp whose cathode is made of the element being determined was passed through the flame.

Extraction and determination of residual crude oil concentration in soil samples.

Soxhlet extraction, following EPA Method SW-846 3540 as described by Adeniji, Okoh, & Okoh (2017), was used for extracting and determining residual crude oil in soil samples.

Column cleanup and separation

The technique of column cleanup was employed to separate organic analytes from interfering substances of varying polarity that may have been co-extracted with the analytes as adopted by Zemo, O'Reilly, Mohler, Tiwary-Magaw, Synowiec, (2013).

GC analysis

The concentrated aromatic fraction obtained was transferred into a labeled glass vial with a Teflon rubber crimp cap for gas chromatography (GC) analysis, following the method described by Frysinger, Gaines, & Reddy (2002).

TPH in hybrid constructed wetland water

The U.S. Environmental Protection Agency (UEPA) Method 1664 was used to determine Total Petroleum Hydrocarbons (TPH) in water from a constructed hybrid wetland.

Statistical analysis

Statistical tools used for this study are SPSS version 22 and Microsoft Excel 2010.The mean and standard error (SE) values of two ($n = 2$) or ($n = 3$) replicates was calculated and the difference between treatments tested by a one-way ANOVA. If the difference was significant, the student's t-test comparisons was carried out to determine where the difference in sample means lie. The expression 'significant', as used in the text, refers to statistical significance at $p \leqslant 0.05$.

Table 1 - Respective concentrations of heavy metals in constituted polluted soil samples and their NFME permissible limits in soil

Metals	Exp Conc. (mg/l)	Limit for agric. land (mg/l)* (NFME, 1992)	Limit for nonagric. land (mg/l)* (NFME,1992)
Zn	39.4	5.0	0.03
Ni	10.2	0.2	0.2
Cu	29.4	1.0	4.0μ/l
Pb	11.2	0.05	1.8μ/l

Table 2 Experimental set up for the preliminary study for the selection of test plants in response to hydrocarbon and heavy metals pollution

Treatment	Purpose	Number of pots
Unpolluted soil +plants	Control	1
Crude oil polluted soil (3%) + plants+ heavy metals	Effect of 3% crude oil and heavy metals on the different species of plants	8
Crude oil polluted soil (7%) + plants+ heavy metals	Effect of 7% crude oil and heavy metals on the different species of plants	8
Crude oil polluted soil (10%) + plants+ heavy metals	Effect of 10% crude oil and heavy metals on the different species of plants	8
Crude oil polluted soil (12%) + plant + heavy metals	Effect of 12% crude oil + heavy metals on the selected plant from the above experimental set up	1

Table 3 Different amendment on 10% crude oil and heavy metals polluted soils and their purpose

Treatment	Purpose
UPS + SP Only	control
PS (CO + HM+S) Only	effect of microorganisms on remediation of the polluted soil
PS +SP	Effect of crude oil and heavy metals on the selected plant growth.
NS+ PS + SP	Effect of amendment with natural soap on indigenous hydrocarbon utilizing microorganisms and phytoremediation of crude oil and bioaccumulation of heavy metals by selected plant
PD + PS + SP	Effect of poultry droppings on indigenous hydrocarbon utilizing microorganisms and phytoremediation of hydrocarbons and bioaccumulation of heavy metals by selected plant
NS +PD +PS + SP	Combined effects of native soap and poultry droppings on indigenous hydrocarbon utilizing microorganisms and phytoremediation of hydrocarbons and bioaccumulation of heavy metals in selected plant
PS + NS	Effect of native soap on bioremediation of hydrocarbons and bioaccumulation of heavy metals by microorganisms.
PS + PD	Effect of poultry droppings on bioremediation of hydrocarbons and bioaccumulation of heavy metals by microorganism.

KEY:UPS → Unpolluted soil, PS → Polluted soil with crude oil and heavy metals, CO → Crude oil, HM → Heavy metal, SP → Selected plant (*Paspalum conjugatum* p.j. Bergius),

NS → Native soap. PD → Poultry droppings (manure).

Results

Table 4 Biochemical characterization and probable identity of hydrocarbon utilizing bacterial (HUB) isolates

S/N	organisms	GS	Shp	Pg	Es	Mt	Ct	Ox	VP	MR	Ur	O/F	Ci	In
M1	*Pseudomonas* spp.	-	rod	y	-	+	+	+	-	-	+	-	+	-
M2	*Acinetobacter* spp.	-	rod	w	-	-	+	-	-	-	-	-	+	+
M3	*Enterobacter* spp.	+	rod	w	-	+	+	+	-	+	+	+	+	-
M4	*Alcaligenes* spp.	-	rod	c	-	+	+	+	-	+	+	+	+	-
M5	*Erwinia* spp.	-	rod	y	-	+	-	-	+	-	-	+	+	-
M6	*Bacillus* spp.	-	rod	c	+	+	+	+	-	-	+	-	-	-
M7	*Micrococcus* spp.	+	cocci	y	-	-	+	-	-	+	-	+	-	-
M9	*Pseudomonas* spp.	-	rod	y	-	+	+	+	-	-	+	-	+	-
M10	*Erwinia* spp.	-	rod	y	-	+	-	-	+	-	-	+	+	-
M11	*Micrococcus* spp.	+	cocci	y	-	-	+	-	-	+	-	+	-	-
M12	*Pseudomonas* spp.	-	rod	y	-	+	+	+	-	-	+	-	+	-
M13	*Pseudomonas* spp.	-	rod	y	-	+	+	+	-	-	+	-	+	-
M14	*Acinetobacter* spp.	-	rod	w	-	-	+	-	-	-	-	-	+	+
M15	*Acinetobacter* spp.	-	rod	w	-	-	+	-	-	-	-	-	+	+

M16	*Alcaligenes* spp.	-	rod	c	-	+	+	+	-	+	+	+	+	-
M17	*Bacillus* spp.	-	rod	c	+	+	+	+	-	-	+	-	-	-
M18	*Pseudomonas* spp.	-	rod	y	-	+	+	+	-	-	+	-	+	-
M19	*Alcaligenes* spp.	-	rod	c	-	+	+	+	-	+	+	+	+	-
M20	*Pseudomonas* spp.	-	rod	y	-	+	+	+	-	-	+	-	+	-
M22	*Erwinia* spp.	-	rod	y	-	+	-	-	+	-	-	+	+	-

Key: Es. – Endospore; Gs. − Gram stain; Shp. − Shape; Pg. − Pigmentation; Mt. − Mortality; Ct. − Catalase test; Ox. − Oxidase; Vp. − Voges-proskauer; MR. − Methyl Red; Ur. − Urease; O/F. − Oxidation/Fermentation; Ci. − Citrate utilization; In. − Indole; C − Cream; W−White; Y − Yellow; plm − Pleomorphic shape, sph − Spherical shape

Table 5 Molecular equivalent of biochemical identified hydrocarbon utilizing bacteria (HUB) isolates

S/N	Probable identity of isolates from biochemical tests	Molecular identity of isolate
1	*Pseudomonas* spp.	*Pseudomonas xiamenensis*
2	*Acinetobacter* spp.	*Acinetobacter baumanii*
3	*Alcaligens* spp.	*Alcaligenes cloacae*
4	*Enterobacter* spp.	*Enterobacter cloacae*
5	*Erwinia* spp.	*Pantoa dispersa*
6	*Bacillus* spp.	*Lysinibacillus fusiformis*
7	*Micrococcus* spp.	*Kocuria palus*

Table 6 Morphological and biochemical characteristics and probable identity of hydrocarbon utilizing fungal isolates from pollutes soil sample

S/N	Isolate	Type of hyphae	Type of spore	Sucrose fermenter	Maltose fermenter	pigmentation
G1	*Penicillium* spp.	sepatate	Smooth conidiophore	−	−	green
G2	*Aspergillus* spp.	septate	Smooth chain conidiophore	−	−	black
G3	*Fusarium* spp.	septate	Oval	−	−	white

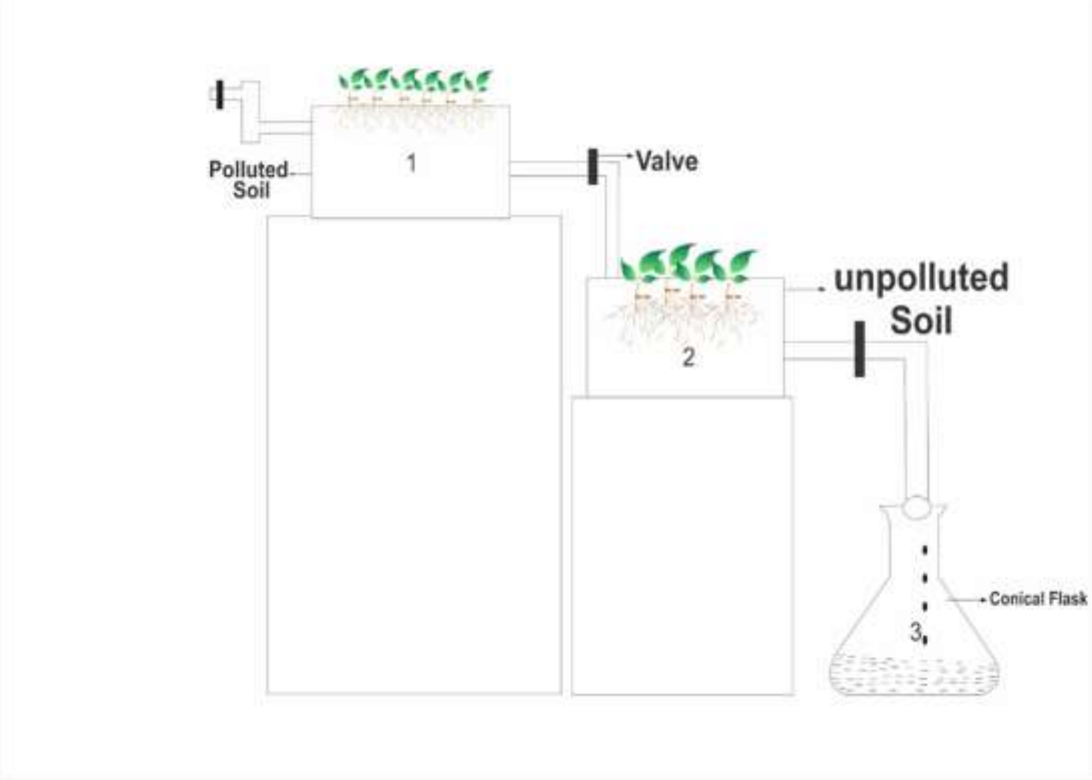


Fig 1 Constructed hybrid wetland system

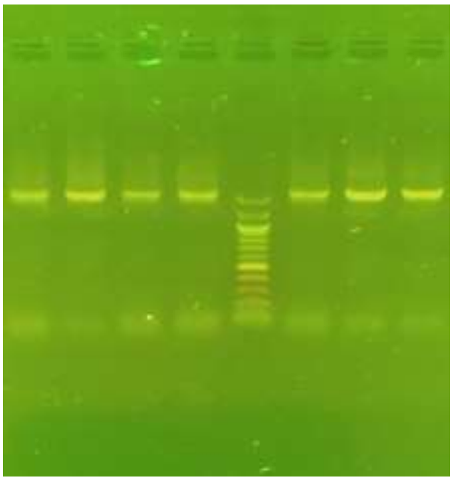


Figure 2: Agarose gel electrophoresis showing the plasmid bands. Lane 1-7 showing the 16SrRNA bands at 1500bp while lane L represents the 100bp molecular ladder

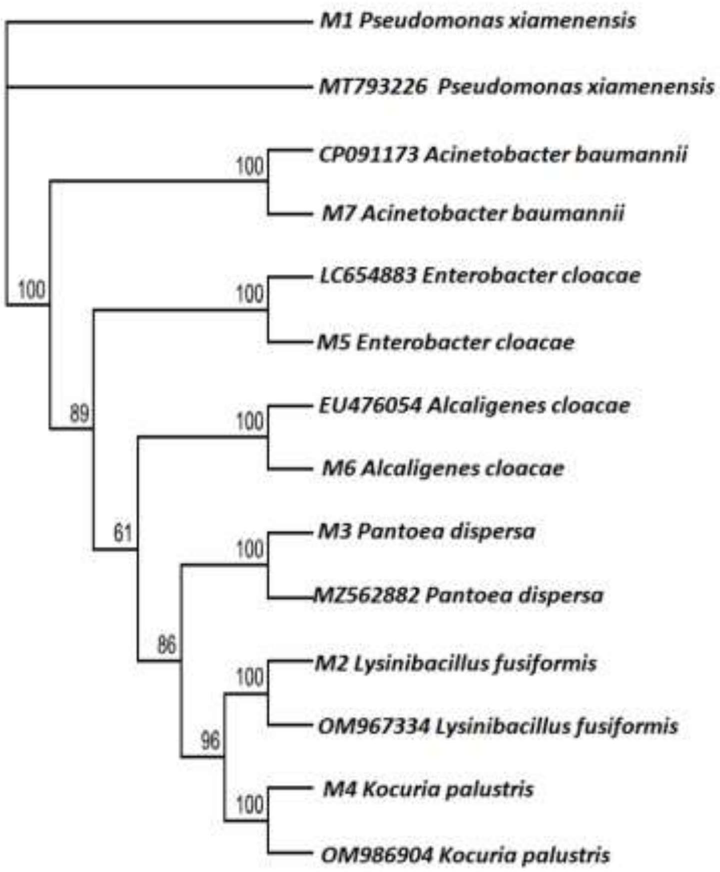
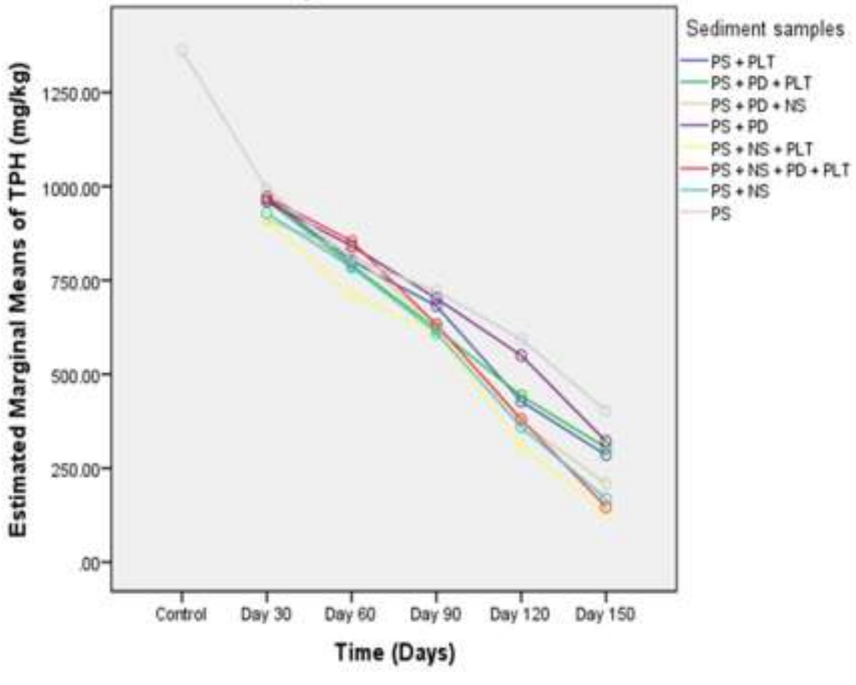


Fig 3 Phylogenetic tree of the microorganisms



Non-estimable means are not plotted

Figure 4: Residual concentration of total petroleum hydrocarbon (TPH) in the soil sediments from different microcosm constructed wetland with different soil amendments.

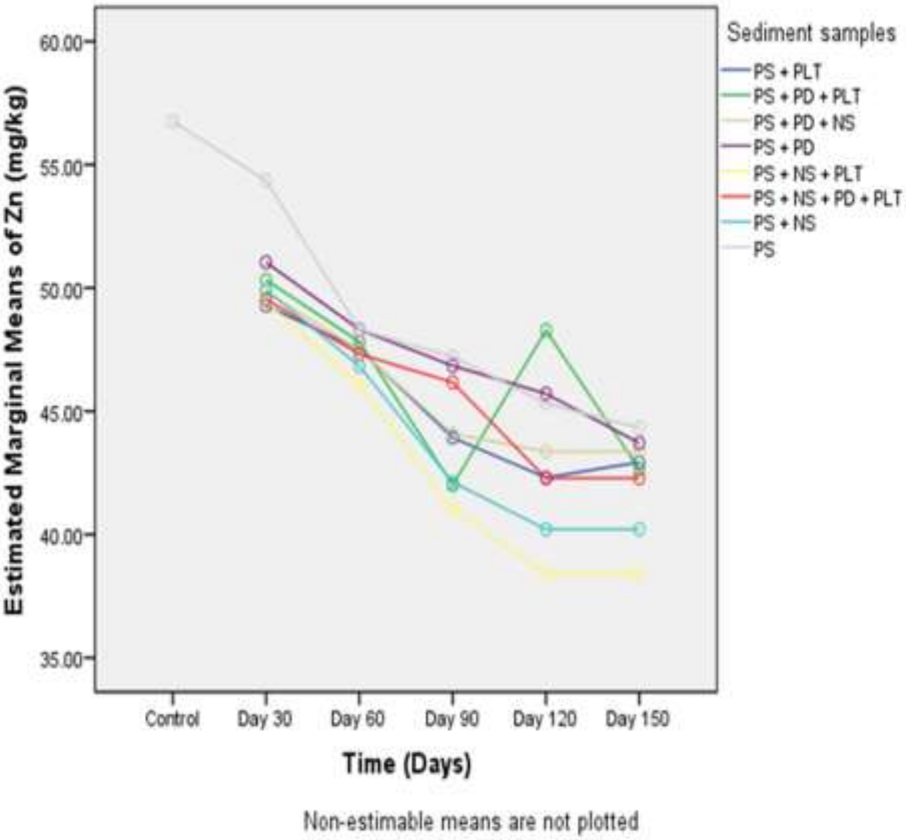


Figure 5: Residual concentration of Zinc (mg/kg) in the soil sediments from different microcosm hybrid constructed wetland with different soil amendments.

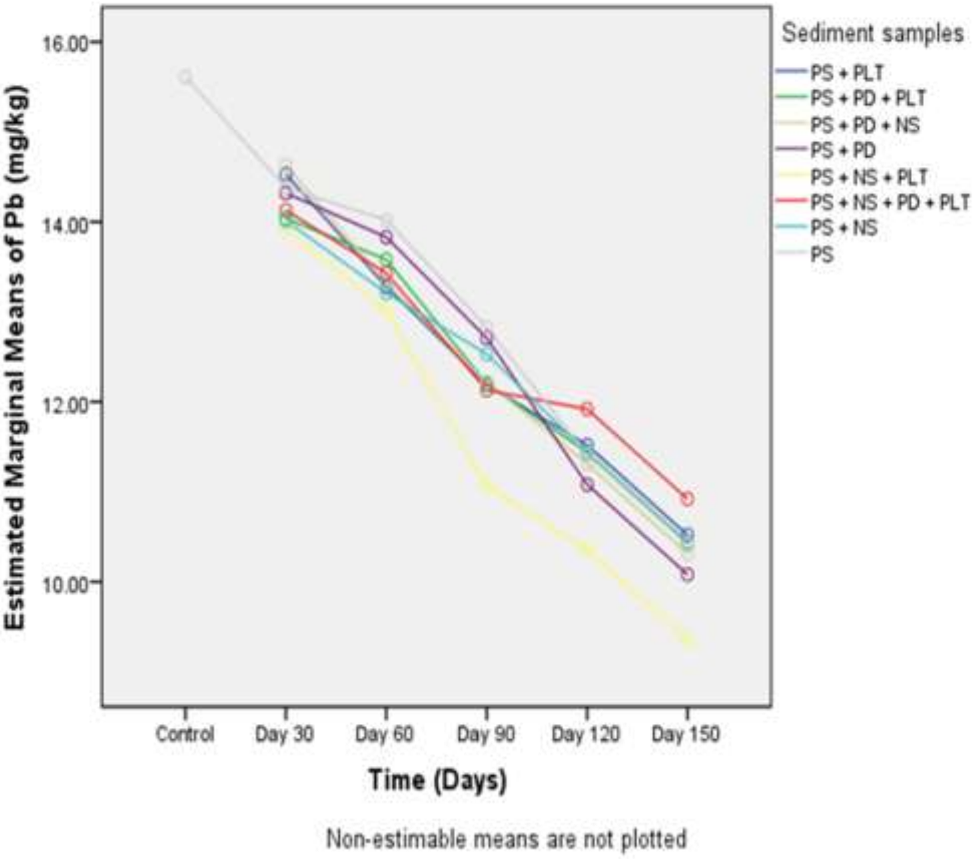


Figure 6: Residual concentration of Lead (mg/kg) in the soil sediments from different microcosm constructed wetland with various soil amendments

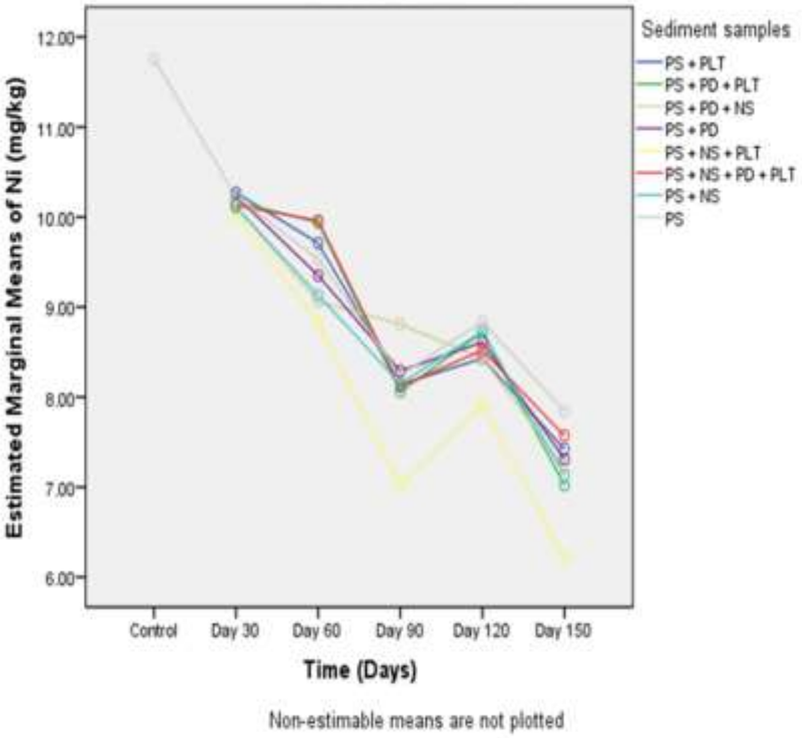


Figure 7: Residual concentration of Nickel (mg/kg) in the soil sediments from different microcosm constructed wetland with various soil amendments

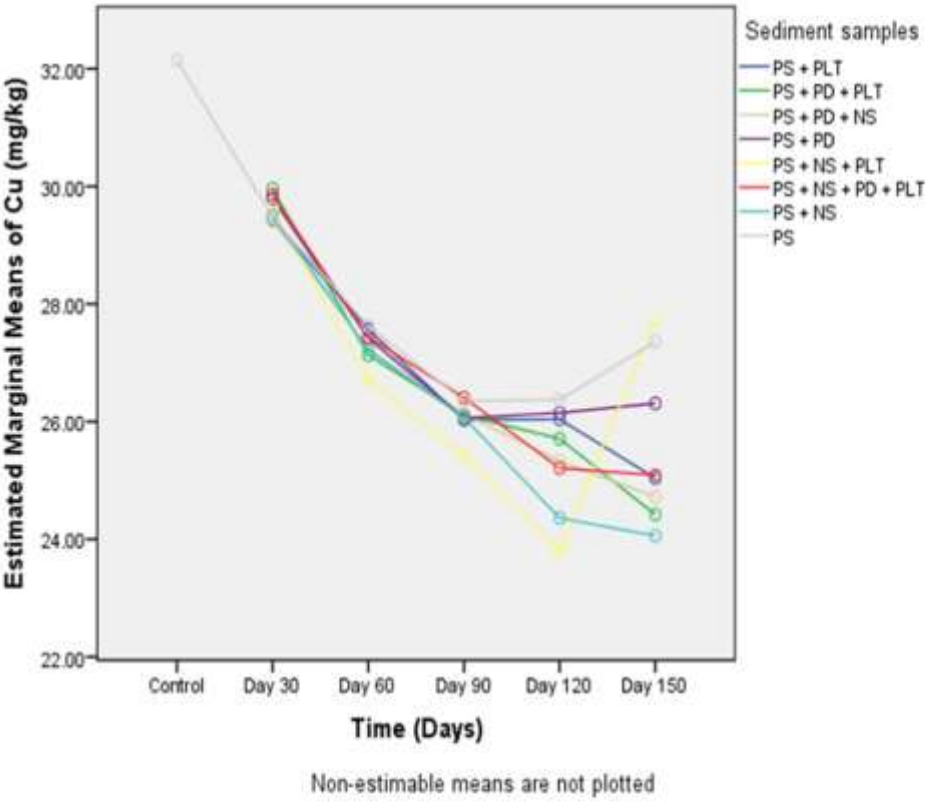


Figure 8: Residual concentration of copper (cu) in the soil sediments from different microcosm constructed wetland with various soil amendments

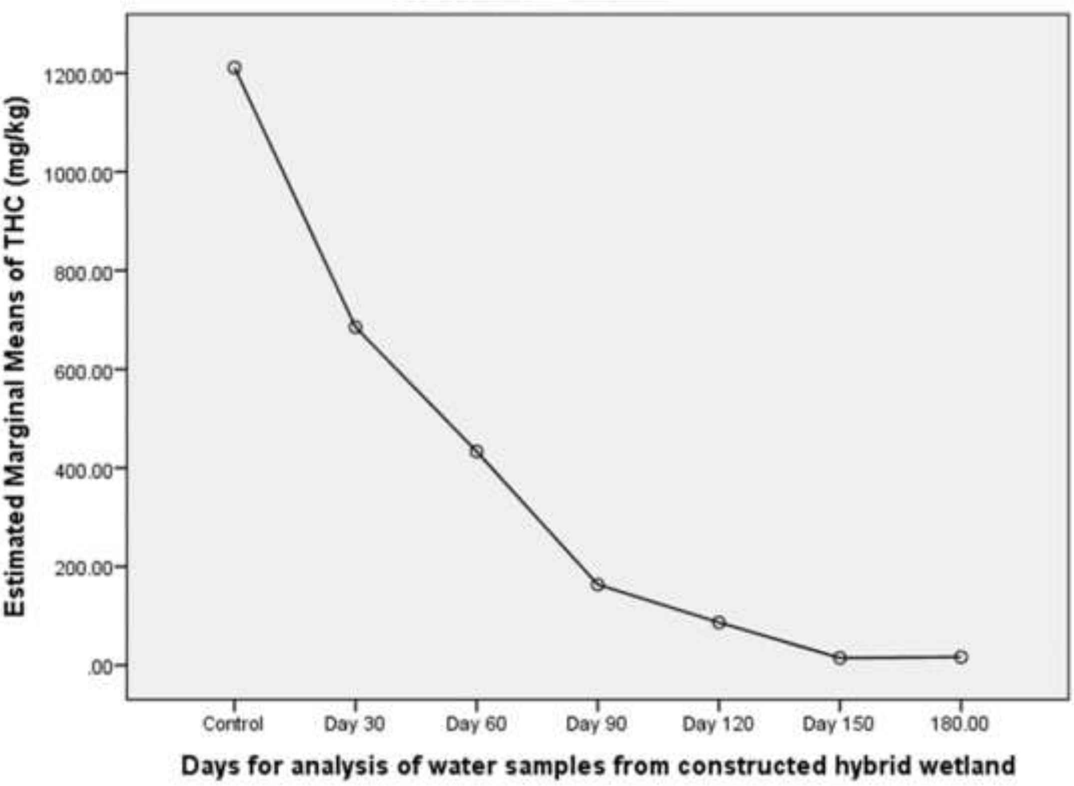


Figure 9: THC content in residual water sample from construction hybrid wetland

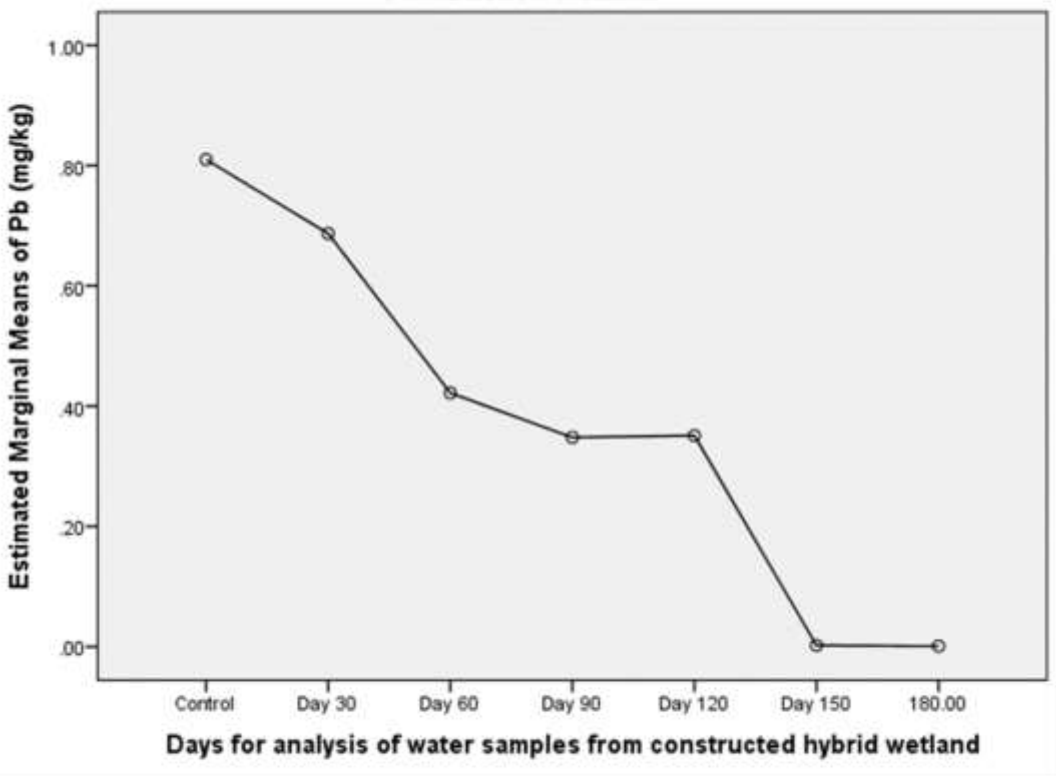


Figure 10: Concentration of Pb (mg/kg) content in residual water sample from construction hybrid wetland

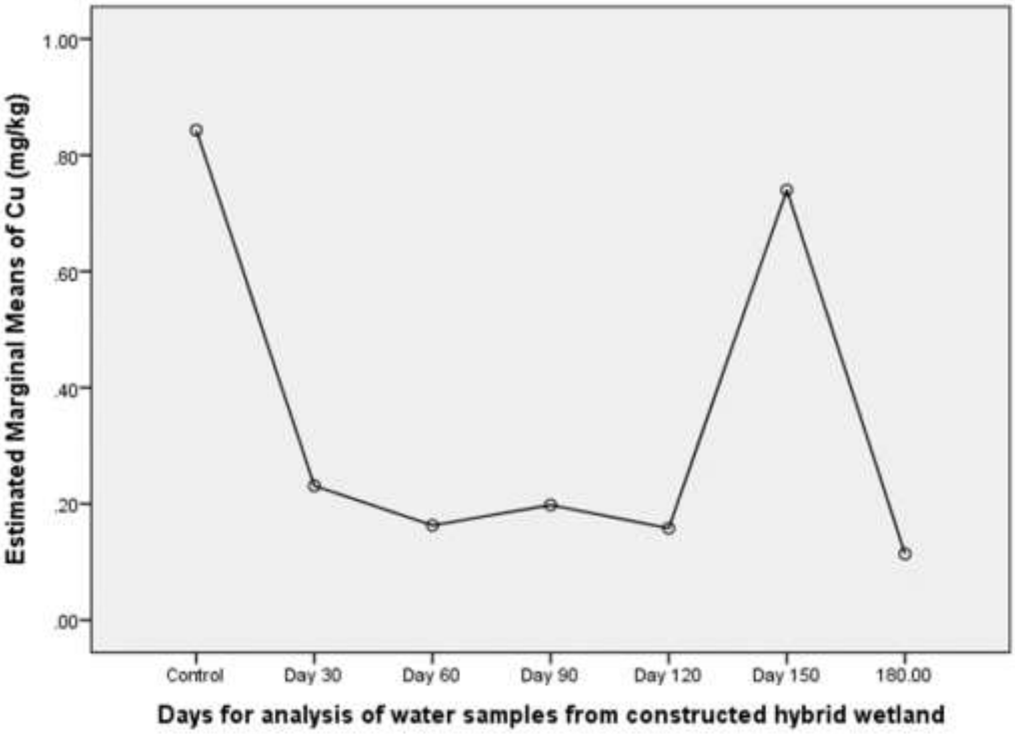


Figure 11: Concentration of Cu (mg/kg) content in residual water sample from construction hybrid wetland

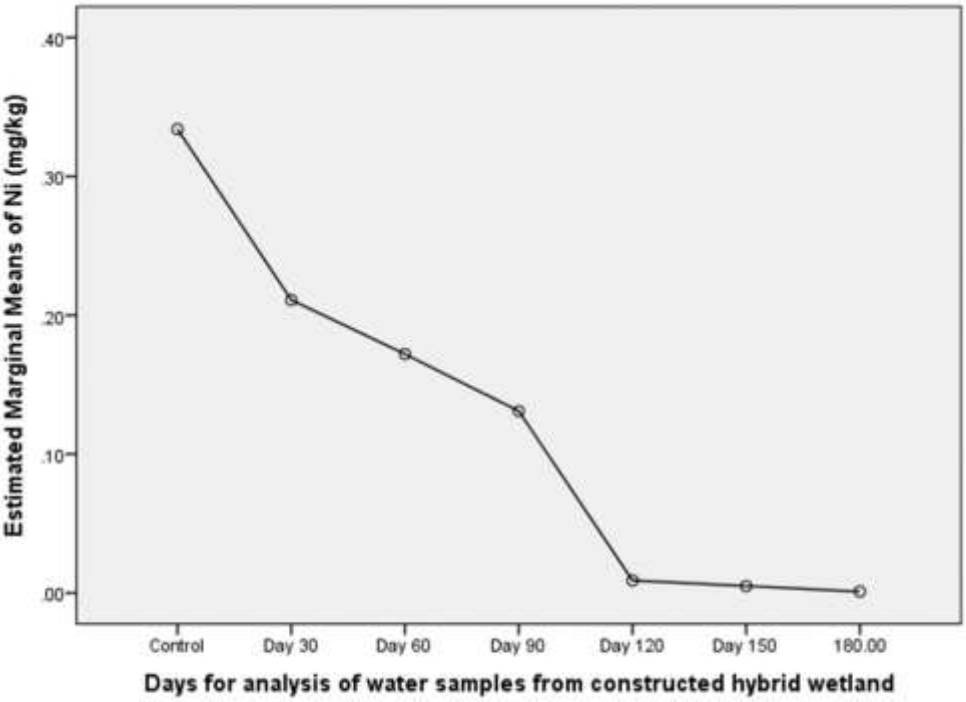


Figure 12: Concentration of Ni (mg/kg) content in residual water sample from construction hybrid wetland

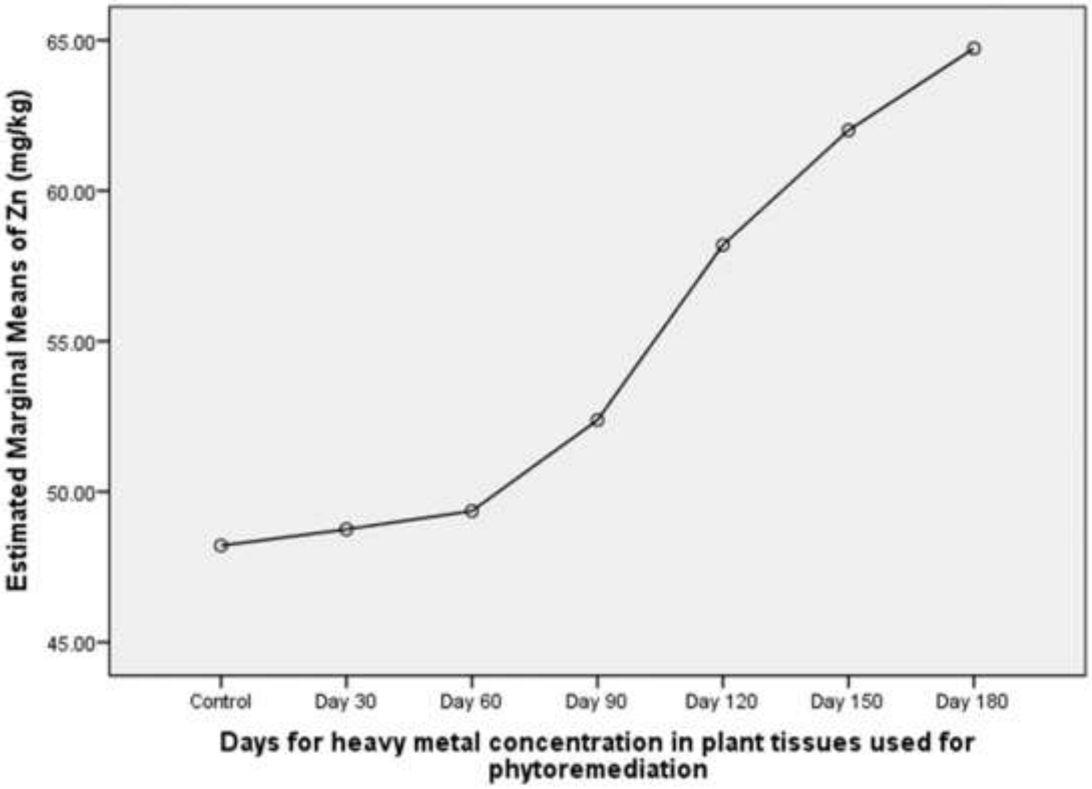


Figure 13: Zn (mg/kg) content in plant tissue used for phytoremediation studies in a constructed hybrid wetland

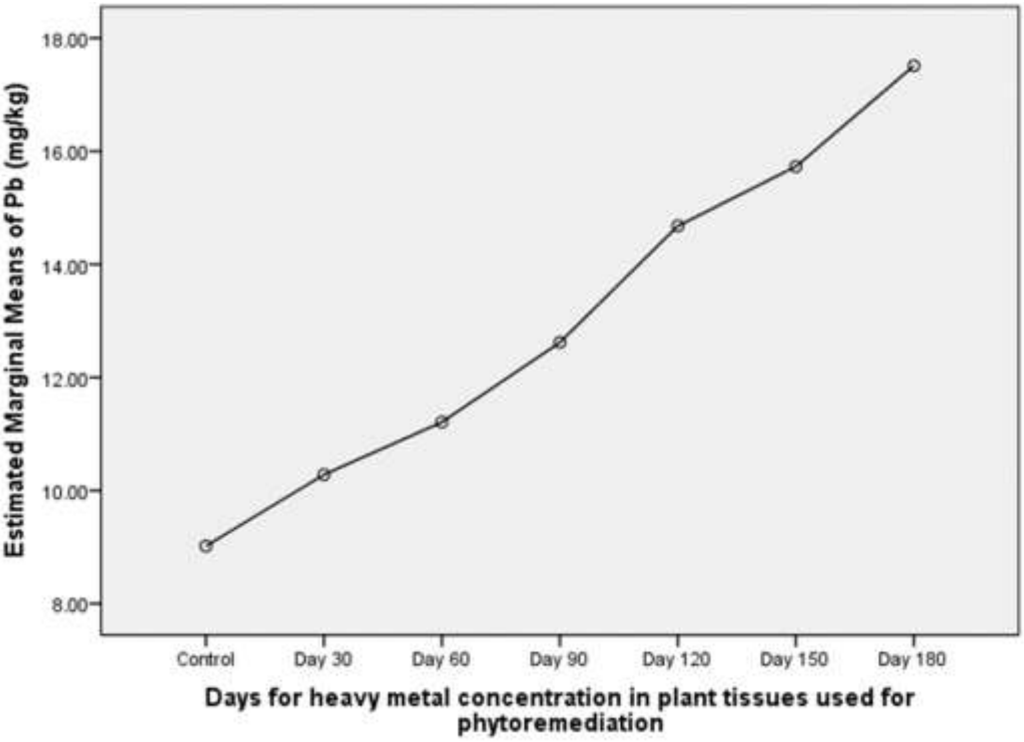


Figure 14: Pb (mg/kg) content in *P. conjugatum* P.J. Bergius tissue used for phytoremediation studies in constructed hybrid wetland

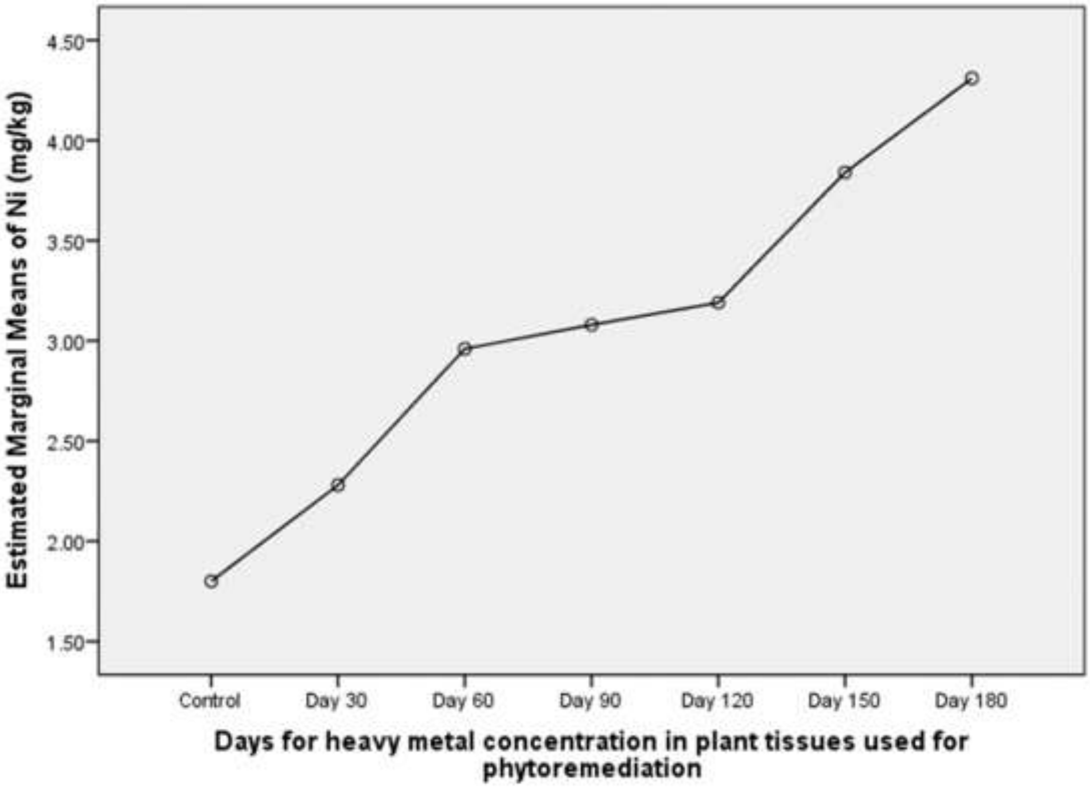


Figure 15: Ni (mg/kg) content in *P. conjugatum* P.J. Bergius tissue used for phytoremediation studies in constructed hybrid wetland

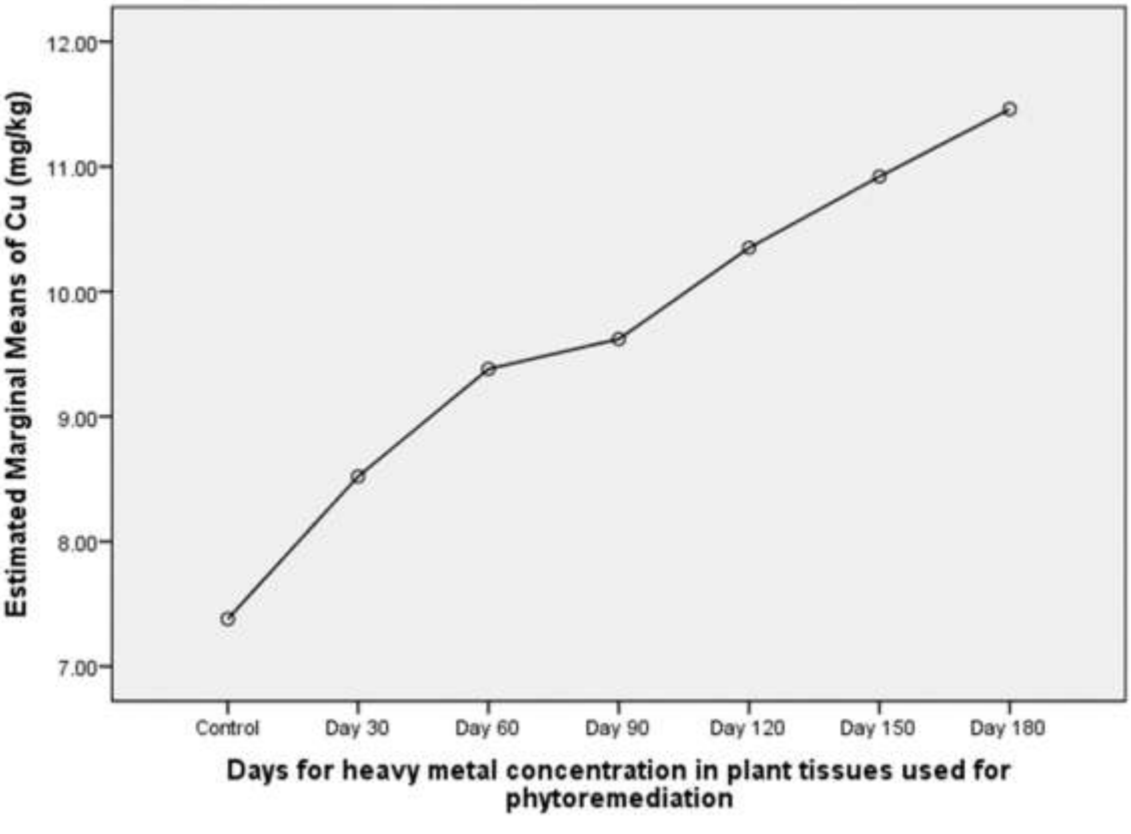


Figure 16: Cu (mg/kg) content in *P. conjugatum* P.J. Bergius tissue used for phytoremediation studies in constructed hybrid wetland

Discussion

The residual concentration of Total Petroleum Hydrocarbon (TPH) in soil sediments from microcosm constructed wetlands polluted with crude oil and heavy metals and phytoremediated over 150 days with various amendments: 1% poultry droppings (PD), 1% natural soap solution (NS), *P. conjugatum* P.J. Bergius (PLT), and combinations of % poultry droppings (PD), 1% natural soap solution shows that all treatments resulted in a decline in TPH concentrations over time with phytoremediation of the polluted soil sample with combined effect of natural soap and plant (*Paspalum conjugatum* P.J.Bergius) (PS + NS + PLT) shows the most significant reduction in TPH than all other treatments, suggesting a synergistic effect of multiple amendments. This finding is in line with the previous studies by Hoang, Lamb, Sarkar, Seshadri, Lam, Vinu, Bolan, (2022). This study assessed the effects of a synthetic surfactant (Triton X-100) and a triterpenoid saponin (from red ash leaves, Alphitonia excelsa) on plant growth and TPH biodegradation in the rhizosphere of two native wild species (a shrub, Hakea prostrata, and a grass, Chloris truncata). Results obtained shows that at high concentration of Triton X-100 dramatically inhibited the growth of the two plants under study (reducing biomass and photosynthesis) and the microbial activity in the rhizosphere. In contrast, saponin administration greatly boosted TPH elimination (up to 60% in C. truncata at 1000 mg/kg due to greater plant growth and related microbial activity in the rhizosphere). Their study demonstrated that natural soap reduced TPH concentrations more effectively than poultry manure in soil contaminated with high levels of crude oil; plants treated with natural soap showed improved physiological parameters, including increased chlorophyll content and better overall health and enhanced degradation of hydrocarbons was attributed to the improved solubilization of oil by natural soap, facilitating microbial degradation processes.

Total hydrocarbon (THC) content in water sample from hybrid construction wetland was determined and the results showed that THC levels drop significantly over time, with a sharp decrease between the control and day 30 (down to around 800 mg/kg), continuing to decrease steadily to near zero by day 150 and maintaining low levels up to day 180. The sharp decline in THC concentration suggests effective phytoremediation. The combination of plants and natural soap significantly enhanced the degradation and removal of hydrocarbons from the water. This is in agreement with the previous studies of Uloaku, Abbey, & Ifelebuegu, (2022). This systematic review discusses various remediation methods for oil-contaminated soils, emphasizing the effectiveness of combining plant-based treatments with natural surfactants like soaps. Hoang Son, , Lamb, Sarkar, Seshadri, Lam, Vinu. & Bolan, (2022) in their study titled "Plant-derived saponin enhances biodegradation of petroleum hydrocarbons in the rhizosphere of native wild plants," the authors found that the combination of plant roots and saponins significantly improved the biodegradation of petroleum hydrocarbons, highlighting the synergistic effect of these natural surfactants in remediation processes. The authors highlight that such combinations can significantly enhance the degradation of hydrocarbons in contaminated environments. This is consistent with the rapid decrease in THC observed in the figure.

The concentration of lead (Pb mg/kg) content in water sample from hybrid construction wetland was determined and results showed that there was a significant decline in Pb concentration, the steady decline in Pb concentration demonstrates the effectiveness of the phytoremediation by hybrid constructed wetland in removing lead from crude oil and heavy metals polluted water and soil. This agreed with the previous study of Kadlec & Wallace (2008); Sheoran & Sheoran, (2006); they demonstrated that the dynamics of heavy metal removal in wetlands often show rapid initial uptake, followed by a slower, more stable phase as the system reaches equilibrium

The concentration of copper (Cu) (mg/kg) in water samples from a hybrid constructed wetland was measured, revealing a significant decrease in Cu levels within the first 30 days, indicating rapid initial uptake and removal of Cu from the water. However, around Day 150, a noticeable spike occurred, with Cu concentration rising to approximately 0.70 mg/kg. This increase could be due to several factors, such as the release of Cu from sediment, disturbances within the wetland system, or seasonal effects that influence biological activity in the wetland. This observation aligns with the findings of Kadlec & Wallace (2008), who noted that heavy metals might be released from sediments under certain conditions, leading to temporary spikes in metal concentrations in the water column. After the spike, a sharp decline followed, with Cu concentration dropping to around 0.05 mg/kg by Day 180. This trend is consistent with the studies of Almuktar, Abed, & Scholz (2018) and Pilon-Smits (2005), who conducted long-term research on phytoremediation. They demonstrated that constructed wetlands can significantly reduce heavy metal concentrations over time, though regular monitoring and maintenance are required to manage fluctuations.

The concentration of nickel (Ni) (mg/kg) in water samples from a hybrid constructed wetland was monitored over a period of 180 days, showing a slow but steady decline, reaching near zero by the end of the study. This finding aligns with previous studies, including those by Vymazal, Švehla, Kröpfelová, Němcová, & Suchý, (2010); Ali, Khan, & Sajad, (2013); Kadlec and Wallace (2009), and Marchand, Mench, Jacob, & Otte, (2010). Marchand et al. (2010), which reported that hybrid constructed wetlands exhibit high removal efficiency for heavy metals like Cd, Ni, Pb, Cu, Cr, and Zn.

Zinc (Zn mg/kg) content in plant tissue used for phytoremediation microcosm studies in the hybrid constructed wetland shows that Zn concentration in plant tissues increases from approximately 48 mg/kg at the control stage to around 65 mg/kg by day 180. This trend indicates that the plants are progressively accumulating zinc over time, which is a characteristic outcome of effective phytoremediation. Phytoremediation is a well-documented method for the removal of heavy metals from contaminated environments. Plants used in this process absorb metals through their roots and accumulate them in their tissues which was documented through the previous work of Vymazal, (2010) which discussed the mechanisms by which plants absorb and accumulate heavy metals, supporting the observed increase in Zn concentration in plant tissues over time.

The lead (Pb mg/kg) content in *Paspalum conjugatum* P.J. Bergius tissue used for phytoremediation in hybrid constructed wetland polluted with crude oil and heavy metals was determined and the results shows that the concentration of Pb in the plant tissues increases significantly from the control stage to day 180 of the study. This agreed with the previous studies of Samecka-Cymerman et al, (2004) who agreed that the capacity of plants to accumulate lead is well-documented stating that certain plant species are particularly effective at uptake of lead from contaminated environments and storing it in their tissues.

Nickel (Ni) content in *Paspalum conjugatum* P.J. Bergius tissue used for phytoremediation microcosm studies in hybrid constructed wetland polluted with crude oil and heavy metals was determined and the results showed that Ni (mg/kg) content in plant tissue increases with time is consistent with the findings from several studies on the phytoremediation of heavy metals in constructed wetlands. The studies by Mihailović, Niketić, & Tomović, (2020) and other authors reported that certain hyperaccumulator plants show significant increases in nickel concentration in their tissues over time, particularly in the later stages of growth.

The copper (Cu) content in *Paspalum conjugatum* P.J. Bergius tissues was measured during phytoremediation microcosm studies in a hybrid constructed wetland polluted with crude oil and heavy metals. The results showed a progressive increase in Cu (mg/kg) concentration in plant tissues over time. This observation is consistent with the work of Ali et al. (2013), whose review on the mechanisms of heavy metal uptake by plants supports the idea that metal accumulation in plant tissues typically increases over time during phytoremediation.

Conclusion

This study shows that hybrid constructed wetlands are a promising technology for the remediation of environments contaminated with crude oil and heavy metals, offering a balance of effectiveness, sustainability, and cost-efficiency through enhanced contaminant removal, the combination of different types of wetland systems (e.g., vertical and horizontal flow) in hybrid constructed wetlands improves the efficiency of contaminant removal compared to single-system wetlands. Synergistic Effects; The integration of various physical, chemical, and biological processes in hybrid systems enhances the degradation and uptake of pollutants, leading to better overall treatment performance. Utilizing a variety of plant species in hybrid wetlands supports the breakdown and absorption of both organic and inorganic pollutants, improving the phytoremediation potential. Hybrid wetlands promote diverse microbial communities that contribute to the biodegradation of hydrocarbons and the transformation and immobilization of heavy metals. These systems are considered sustainable and cost-effective solutions for the treatment of polluted soils, leveraging natural processes to achieve high levels of pollutant removal.

Recommendations

The results obtained from this study recommends the use of hybrid constructed wetland for the treatment of soil and water bodies polluted with crude oil and heavy metals. The study also suggests the use of *P. conjugatum* P.J. Bergius as a phtoremediator plant due to its numerous advantages over some grasses which includes ability to tolerate high concentration (10%) crude oil and heavy metals pollution, high growth rate, easy to propagate and ability to tolerate dual environmental conditions because it can thrive well in both normal and waterlogged soils and requiring little or no fertilizer for its proliferation. This study recommends that every industry in Niger Delta and Nigeria as a whole involved in production of goods and services should adopt the methodologies applied in this study for the treatment of their waste and effluents before it's discharge into the environment be it soil or water bodies.

Acknowledgements

This work has been supported by Tertiary Education Trust Fund (TETFund). Special thanks to Professor Orji, Justina Chibuogu , Professor Nweke, Christain Okechukwu and Professor Adieze, Ifechukwu Enyinnaya for their invaluable guidance and supervision throughout the study. Their expertise and encouragement were pivotal to the successful completion of this work.

References

Adeniji, A. O., Okoh, O. O., & Okoh, A. I. (2017). Petroleum hydrocarbon fingerprints of water and sediment samples of Buffalo River Estuary in the Eastern Cape Province, South Africa. *J. of Analytical Methods in Chem.*, 26, 293-298.

Adieze, I. E., Orji, J. C., Nwabueze, R. N., & Onyeze, G. O. C. (2012). Hydrocarbon stress response of four tropical plants in weathered crude oil contaminated soil in microcosms. *Inter. J. of Environ. Studies*, *69*,490-500.

Aktaruzzaman, M., Fakhruddin, A.N.M., Chowdhury, M.A.Z., Fardous, Z. & Alam, M.K. (2013). Accumulation of Heavy Metals in Soil and their Transfer to Leafy Vegetables in the Region of Dhaka Aricha Highway, Savar, Bangladesh. *Pakistan Journal of Biological Sciences, 16,* 332-338

Ali, H., Khan, E., & Sajad, M. A. (2013). Phytoremediation of heavy metals—concepts and applications. *Chemosphere*, *91*, 869-881.

Almuktar, S. A., Abed, S. N., & Scholz, M. (2018). Wetlands for wastewater treatment and subsequent recycling of treated effluent: a review. *Environ. Sci. and Pollut. Res., 25*,23595-23623

Frysinger, G., Gaines, R. & Reddy, C. (2002). GC × GCA New Analytical Tool For Environmental Forensics. Environmental Forensics - ENVIRON FORENSICS. 3, 27-34.

Hartemink, A. E., Veldkamp, T., & Bai, Z. (2008). Land cover change and soil fertility decline in tropical regions. *Turkish Journal of Agriculture and Forestry*, *32*(3), 195-213.

Hoang Son, A.., Lamb, D., Sarkar, B., Seshadri, B., Lam S. S., Vinu. A. & Bolan, N. S. (2022). Plant-derived saponin enhances biodegradation of petroleum hydrocarbons in the rhizosphere of native wild plants. *Environ. Pollut., 292,*118309.

Hoang Son, A.., Lamb, D., Sarkar, B., Seshadri, B., Lam S. S., Vinu. A. & Bolan, N. S. (2022). Plant-derived saponin enhances biodegradation of petroleum hydrocarbons in the rhizosphere of native wild plants. *Environ. Pollut., 292,*118309.

Hoang Son, A.., Lamb, D., Sarkar, B., Seshadri, B., Lam S. S., Vinu. A. & Bolan, N. S. (2022). Plant-derived saponin enhances biodegradation of petroleum hydrocarbons in the rhizosphere of native wild plants. *Environ. Pollut., 292,*118309.

Holt, J.G. (1994) Bergey's manual of determinative bacteriology. 9th Edition, Lippincott Williams and Wilkins, Baltimore.

Kadlec, R.H., & Wallace, S. (2008). *Treatment Wetlands*. CRC Press.

Marchand, L., Mench, M., Jacob, D. L., & Otte, M. L. (2010). Metal and metalloid removal in constructed wetlands, with emphasis on the importance of plants and standardized measurements: *A rev. Environ.l pollut., 158,* 3447-3461.

Mihailović, N., Niketić, M., & Tomović, G. (2020). Comparative Study of Hyperaccumulation of Nickel by *Alyssum murale* and Other Species. *Polish J. of Environ. Studies*, 29,227-234.

Nigeria Federal Ministry of Environment (NFME) (1992) https://www.placng.org/lawsofnigeria/laws/F10.pdf

Okpokwasili, G. C. & Amanchukwu, S.C. (1988). Petroleum hydrocarbon degradation by *Candida* species. *Environ. Int*., *14*, 243-247.

Pelczar, M. J. & Chan, E.C. 1977. Laboratory Exercises in Microbiology, 4th edition, McGraw Hill, Inc.

Pilon-Smits, E. (2005) Phytoremediation. Annu. *Rev. Plant Biol., 56*, 15-39.

Saitou, N., & Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Molecular Biol. and evol., 4*,406–425. https://doi.org/10.1093/oxfordjournals.molbev.a040454

Samecka-Cymerman, A., Stepien, D., & Kempers, A. J. (2004). Efficiency in removing pollutants by constructed wetland purification systems in Poland. *J. of Toxicol. and Environ. Health, Part A, 67*,265-275.

Uloaku, M.-I., Abbey, S. J., & Ifelebuegu, A. O. (2022). A systematic review on the effectiveness of remediation methods for oil contaminated soils. *Environ. Sci., and Pollut. Res.,* 29,12345-12367.

Vymazal, J. (2010) Constructed wetlands for wastewater treatment. *Water, 2,530*–549

Vymazal, J. (2022). The Historical Development of Constructed Wetlands for Wastewater Treatment. *Land, 11*,2-29

Vymazal, J., Švehla, J., Kröpfelová, L., Němcová, J., & Suchý, V. (2010). Heavy metals in sediments from constructed wetlands treating municipal wastewater. *Biogeochemistry, 101,* 335-356.

Vymazal, J., Zhao, Y.& Mander, Ü. (2021). Recent research challenges in constructed wetlands for wastewater treatment: A review. *Ecol.Eng., 169,* 1-7.

Wu, S., Kuschk, P., Brix, H., Vymazal, J.& Dong, R (2014). Development of constructed wetlands in performance intensifications for wastewater treatment: A nitrogen and organic matter targeted review. *Water Res., 57,40–55.*

Yergeau, E., Sanschagrin, S., Beaumier, D.& Greer, C. W. (2012). Metagenomic analysis of the bioremediation of diesel-contaminated canadian high arctic soils. *PLoS ONE* ,7:1-10