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ENVIRONMENTAL MANAGEMENT & CONSERVATION | RESEARCH ARTICLE

Appropriate technology for soil remediation in tropical low-income countries - a pilot scale test of three different amendments for accelerated biodegradation of diesel fuel in Ultisol

Henrik Haller[1]*, Anders Jonsson[1], Joel Ljunggren[2] and Erik Hedenström[2]

Abstract: Polluted land in marginalized regions, such as tropical low-income countries and sparsely populated regions in industrialised countries, demand special remediation strategies that are energy-efficient, locally adapted, economically viable. Strategies for appropriate bioremediation technology under such circumstances can be based on locally available resources in combination with *in situ* bioremediation technologies to keep energy and material costs down. A pilot scale experiment was set up to test the application of three organic by-products from the local industry (whey, pyroligneous acid and compost tea) to enhance the natural biodegradation of diesel in ultisol. Biweekly applications of 6 mL whey kg^{-1} soil significantly increased the degradation rate but no positive effect on degradation was found with any of the other amendments. Tropical climate is favourable for biodegradation but many tropical soils are rich in clay which can inhibit the bioavailability of the pollutant which in turn may be decisive for biodegradation kinetics.

ABOUT THE AUTHOR

Dr. Henrik Haller and Professor Anders Jonsson belongs to the ecotechnology department and conduct research related to sustainable remediation of contaminated soils as well as broader applications of multifunctional land use, nature-based solution and ecosystem restoration. Henrik´s main research has mainly focused on the Global South and he has many years of practical experience of tropical agroforestry and ecosystem restoration. Erik Hedenström is professor in organic chemistry and leads a research group that works with applied green chemistry. Ph. L. Joel Ljunggren is a civil engineer in biotechnology and genomics but works multidisciplinarily within the fields of biology, chemistry and computer programming. The research reported in this paper belongs to a more extensive research cooperation project whose objective is to assess the development of multifunctional land use strategies that yields ecosystem products such as food, fresh water, wood, fibre, and simultaneously addresses the deteriorated health of ecosystems.

PUBLIC INTEREST STATEMENT

Soil pollution in low-income countries and remote regions in industrialised countries need innovative remediation strategies that are energy-efficient, locally adapted and cheap in order to come about. Locally available waste products from farms are cheap resources that can be used to stimulate the breakdown of soil pollutants. We tested the effect of three organic by-products (whey, pyroligneous acid and compost tea) in an experiment. The test showed that whey was efficient in stimulating the breakdown of diesel oil if applied in high enough quantities. Our results indicate that whey treatment can be suitable for treating petroleum-contaminated soils in low-income countries especially in tropical regions.

If low cost is a crucial factor, our results indicate that whey treatment has the potential to be an appropriate technology for treating petroleum-contaminated soils in tropical regions.

Subjects: Environment & Agriculture;; Environmental Studies & Management;; Food Science & Technology;;

Keywords: bioremediation; whey; pyroligneous acid; compost tea; tropical regions; low income countries; nature-based solutions

1. Introduction

Soil contamination by pollutants from the petroleum sector is a worldwide problem due to spillage, improper handling and transport of liquid fuel and oil (FAO, 2018; Kuranchie et al., 2019; Lawal, 2017; Mansour et al., 2017; Nkansah et al., 2017; Olive, 2018; Riser-Roberts, 1998). In marginalized regions, such as low-income countries in the Global South and sparsely populated regions in industrialised countries, economic incentives are small for soil remediation to take place (Haller et al., 2018; Jonsson & Haller, 2014; Lans-Ceballos et al., 2018). Such locations demand special strategies that are energy-efficient, locally adapted, economically viable and rely on the soil ecosystems capacity for self-organization. Strategies for appropriate bioremediation technology under such circumstances should be based primarily on solar energy and the embodied chemical energy of the organic pollutant itself to power the degradation process (Haller et al., 2018). Using locally available resources such as waste products in combination with *in situ* bioremediation technologies are also appropriate ways to keep environmental impacts as well as energy and material costs down (Haller, 2017; Joshi & Ahmed, 2016; Sharma et al., 2015). The innately high soil temperatures in tropical regions make implementation of *in situ* soil remediation particularly interesting since the activation energy of many biochemical transformations is in the order of 50 kJ mol^{-1} which implies that every 10°C increase in temperature gives an approximate doubling of the degradation rate. Soil remediation strategies that consider and capitalize on these ecosystem services have the potential to address several of the sustainable development goals (SDGs) if carefully designed in cooperation with local communities (Haller et al., 2018).

Biostimulation is a remediation technology that seeks to optimize soil conditions for pollutant-degrading microorganisms by aeration, addition of nutrients and adjustment of pH and temperature (Adams et al., 2015; Margesin et al., 2000). By-products from agriculture, livestock, fishing and forestry can be used as the main feedstock. Economies of low-income countries in the Global South are typically land use-based and this sector often accounts for 50% of employment (Ruane & Sonnino, 2011). Land use generates a number of by-products of little economic value that frequently create serious environmental problems when disposed of (Reddy & Yang, 2005). Animal feed has been proposed as an outlet for some of these by-products but due to low levels of protein, high levels of moisture and some anti-nutritional factors, i.e. presence of tannins and other polyphenols, this practice is limited (Aregheore, 2000; Ulloa et al., 2004). Being inexpensive and readily available sources of carbon, nutrients and bioactive compounds (Ayala-Zavala et al., 2011), many of these by-products can potentially be used to stimulate microorganisms to degrade toxic compounds under controlled conditions (Gadd, 2001). A considerable number of experiments with organic by-products as amendments for bioremediation have been conducted. The most common by-products include molasses (Boopathy et al., 1994; Nikolopoulou & Kalogerakis, 2008), bagasse (Dzul-Puc et al., 2005), corn cobs (Wu et al., 2008), manure (Kästner et al., 1995), blood meal (Fischer et al., 1998; Wang et al., 2017), fish bone meal (Walworth et al., 2003), straw (Cai et al., 2007; Laine & Jorgensen, 1996), and rice husks (Forss et al., 2013; Tarley & Arruda, 2004). The low mobility of solid amendments compared to liquids however can be an obstacle for efficient bioremediation in large scale *in situ* conditions since the amendments are not easily mixed with contaminated subsoil. Liquid amendments have a higher mobility and can thus be expected to more efficiently reach deeper soil layers (Pant et al., 2011; Scheuerell, 2004).

Three liquid by-products from farming operations are; whey, pyroligneous acid (PA) and compost tea (CT) (Haller et al., 2018). In our previous research, whey has shown positive results on biodegradation of diesel fuel hydrocarbons in soil (Östberg et al., 2006, Östberg, Jonsson et al. 2007, Östberg, Jonsson et al. 2007, Vilches, Bylund et al. 2010; Jonsson & Ostberg, 2011). An inventory of waste products to be used as amendments for *in situ* bioremediation in developing countries indicated that PA and CT would be appropriate amendments because of their documented stimulating effect on microorganisms and their liquid nature (Haller et al., 2012). PA and CT have, to the best of our knowledge, not been tested as amendments for soil bioremediation of organic pollutants. CT has been shown to significantly increase soil microbial respiration and dehydrogenase activity in similar tropical soils (Haller et al., 2017; Pant et al., 2011); and applications of PA have significantly increased basal respiration and microbial biomass in highly weathered tropical soils (Steiner et al., 2008). Previous results using whey as an amendment were achieved from laboratory experiments only and there is a need to scale up the experiments to be able to fully assess the potential of these amendments under more field-like conditions. The aim of this study was therefore to examine the ability of whey, PA and CT to enhance diesel degradation in a tropical soil (ultisol) in a pilot scale experimental station.

2. Material & methods

2.1. Experimental station

A research station was built on the experimental farm Casa Montesano, 349 m above sea level, in the municipality of Villa Sandino in the province of Chontales, Nicaragua. The region has a humid, tropical climate with an average precipitation of 2000 mm per year. The annual average temperature is between 25°C and 28°C. The soil on the experiment site is acidic with a pH range of 4.8–5.3 and classified as ultisol according to USDA's soil taxonomy system. Soil samples of the topsoil (0–30 cm depth) and the subsoil (30–60 cm depth) layer were sent to A&L Eastern Laboratories in Richmond, Virginia, U.S.A. for texture and chemical analysis. The results are presented in Table 1. The average soil bulk density of the top 60 cm used in the experiment was 1.32 g cm^{-3}.

2.2. The pilot scale experiment

The purpose of the pilot scale experimental station is to facilitate the simultaneous testing of rather large quantities of contaminated soil. The soil quantity was estimated to be large enough to compensate for the inhomogeneity of the soil giving results that are representative for *in situ* remediation conditions. Twenty-four compartments were built in concrete with a smooth cement plaster in two sets of 12 compartments each. The compartments were built to hold 150 L of soil each; 50 cm wide × 50 cm deep × 60 cm high (Figure 1). Agriculture soil (Table 1) from the experimental farm was placed inside each compartment causing as little damage to soil structure as possible. A lid structure of untreated *Tabebuia rosea* wood was bolted to the compartments to keep the soil from falling out. The compartments were placed under a roof to avoid rainfall and direct sunlight. The whole construction was also fenced to keep animals out. The soil moisture was kept between 0.3 and 0.5 g water g^{-1} soil which resembles standard conditions during the rainy season that last for approximately 9 months in this region.

2.2.1. Diesel contamination

The soil was spiked with commercial petroleum diesel fuel to a concentration of 5.00 g diesel kg^{-1} dw soil which is approximately twice the concentration found at a disused petrol station (Vilches, Bylund et al. 2010). The diesel was manually and uniformly dispersed on top of the soil and left to seep through the soil for 2 days before the first sampling. Prior to the addition of diesel, debris was removed and subsequently replaced to resemble field conditions.

2.2.2. Experimental setup

Each amendment was manually applied with a watering can, biweekly on day 3, 18, 32, 46, 60, 74, 88 after diesel contamination at levels described in Table 2. The compartments that received less liquid than the highest application levels (CTH; 5 L per compartment) were compensated for by adding spring water

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Table 1. Physical and chemical characterization of the soil at the experimental site

	Topsoil	Subsoil
pH (H_2O)	5.3	4.8
Organic Matter (%)	6.8	3.5
CEC (meq 100 g^{-1})	9.3	10.00
Soil texture		
Clay (%)	46.4	46.0
Silt (%)	29.6	23.6
Sand (%)	24.0	30.4
Chemical composition		
N (total %)	0.14	-
P (Mehlich III, ppm)	22	13
K (available, ppm)	187	65
Ca (ppm)	843	634
Mg (ppm)	194	227
Na (ppm)	26	30
S (total, ppm)	19	18
Fe (ppm)	82	37
Cu (ppm)	1.1	0.8
Zn (ppm)	2.9	1.5
Mn (ppm)	132	121
B (ppm)	0.6	0.3

in order to assure that the 5 L of liquid was added to all compartments (including 5 L of water for the control compartments). The amount of whey used was based on levels on which positive effects on diesel fuel hydrocarbon degradation had been observed in previous experiments (Jonsson & Ostberg, 2011; Östberg et al., 2006, Östberg, Jonsson, Lundström et al., 2007, 2007). The PA treatment level was based on amounts that had shown growth-stimulating effect of *Pleurotus ostreatus* (Yoshimura et al., 1995),

Figure 1. The interior of the experimental station with its 24 concrete compartments.

Table 2. Treatment levels of the three amendments

	Description	Amounts added (kg^{-1} soil dw)
1 WL	Whey low	0.6 mL
2 WH	Whey high	6 mL
3 PAL	Pyroligneous acid low + lime	0.1 mL
4 PAH	Pyroligneous acid high + lime	1 mL
5 CL	Compost tea low	2.5 mL
6 CTH	Compost tea high	25.3 mL
7 C	Control	-

and the CT treatment level was based on amounts that promoted soil microbial respiration and dehydrogenase activity (Pant et al., 2011).

2.2.3. The amendments

2.2.3.1. Compost tea. A cow manure-based vermicompost was used. The cow manure was collected from the adjacent farms and processed during a minimum of 2 weeks by red wrigglers (*Eisenia fetida*). The compost tea was made in a commercial vortex airlift bioreactor from *Keep It Simple Organics*. The below listed ingredients were brewed for 18 h and applied to the soil without previous filtering immediately after brewing in 40 L clear spring water: *2 L vermicompost, 0.2 L sugar cane molasses, 100 mL soluble seaweed (Ascophyllum Nodosum) extract powder, 100 mL granulated humic acid and 25 mL fish hydrolysate.*

2.2.3.2. Whey. The whey was made from local raw milk mixed with the commercial rennet *Super cuajo Luna M.*, composed of enzymes extracted from the fungi *Rhizomucor miehei*. The dry weight of the whey was 62.8 g L^{-1}.

2.2.3.3. Pyroligneous acid. The PA was made in an artisanal kiln from wood of *Erythrina sp.* (75%) and *Inga sp.* (25%) in a slow pyrolysis process that lasted 5 days. Due to the acidity of the PA (pH 3.8), the pH was adjusted to 6.0 ± 0.2 by addition of dolomite lime, 1.66 g L^{-1}.

2.2.4. Sampling

Soil samples were taken at day 2, 45 and 101. The first two sampling occasions aimed to catch an expected more rapid degradation during the initial phase of the experiment (based on previous results from laboratory experiments, e.g., Östberg, Jonsson, Bylund et al. (2007)) and the final point aimed to catch the degradation after an extended time (100 days). At each sampling occasion, samples were taken at 4 spots in each compartment with an iron-manganese steel gouge auger of 13 mm diameter from Eijkelkamp Agriresearch Equipment. A cylindrical soil sample of the entire depth was extracted from the auger with a spatula and placed on a clean paper. All instruments were cleaned with an acetone drenched cloth and subsequently washed with neutral phosphate-free detergent and rinsed with water between samplings. Each sample was subsequently divided into two 30 cm sections that were mixed with the corresponding section from the other four samples from each compartment and stored in 15 mL glass vials. The combined samples (one for each depth and compartment) were stored at −20 °C in the dark until analysis.

2.2.5. Chemical analysis

The soil samples were extracted by pressurized fluid extraction according to the United States Environmental Protection Agency (EPA) standard 3545A. Eight grams of soil (wet weight) was mixed with 4 g diatomaceous earth and placed in 22 mL stainless steel extraction cells. Internal standards, 0.5 mL of hexamethylbenzene (HMB) solution (0.023 mol L^{-1} dissolved in acetone/pentane 50/50) from Sigma Aldrich® and 0.5 mL 1-chlorooctadecane solution (0.028 mol L^{-1} in *n*-hexane) from Sigma Aldrich was added to each extraction cell with a Hamilton® syringe. Pesticide quality *n*-hexane and acetone were used as solvents. Three millilitres of the extracts was purified by column chromatography using 1 g silica gel and 2 g sodium sulphate in Pasteur pipettes stuffed with glass wool at the bottom.

Experimental settings for Gas Chromatography with Flame-Ionization Detection (GC-FID) were as stated in EPA 8015 c, with minor changes. Briefly, injection of 1 µl was done with an MPS2 autosampler (Gerstel). Oven and column were, 6890 GC (Agilent) fitted with an Agilent HP-5 column (30 m × 0.32 mm ID, 0.25 µm film thickness). The following GC program was used: Inlet 200°C, splitless, constant flow 2.2 mL min^{-1} (He), initial oven temperature 45°C, 3 min → 275°C (12°C min^{-1}), hold 12 min.

2.2.6. Data and statistical analysis

The chromatographic interval between the retention time of C_{10} (7 min) and C_{28} (23.5 min) was integrated with GC Chemstation (Rev. B.04.03 [16]), according to the EPA method with a fixed baseline starting from C_{10} until C_{28}. The sum of integrated peaks was divided into two equidistant groups between C_{10} and C_{28} (retention time 7–23 min). For brevity, the groups with the lower and higher carbon numbers were named low boilers (retention time: 7–15.25 min) and high boilers (retention time: 15.25–23 min), respectively. HMB was used as internal standard for low boilers and 1-chlorooctadecane for the high boilers. Correction with internal standard and dry weight extracted was as follows:

$$c = \frac{\sum_{n=1}^{n}(A_n) - As}{As * d}$$

Where: c = corrected sum of total area

A_n = peak area of peak n

As = Area of internal standard

d = Dry weight after extraction

The degree of degradation was calculated as percentage of the initial values (day 2) of diesel range organics (C_{10}-C_{28}). Student´s unpaired t-test with control and the three amendments was used to determine the statistical significance of the data.

3. Results & discussion

The results obtained from the experiment illustrate a relatively slow natural attenuation of the diesel range organics (DRO) during the 101 days of the experiment (Figure 2). Statistically significant ($p < 0.05$) acceleration of DRO degradation compared to the control was observed at both day 45 and 101 for the high-level treatment with whey (WH). This effect is consistent with previous results from laboratory experiments on phenanthrene degradation in diesel contaminated soil (Jonsson & Ostberg, 2011) and degradation of diesel fuel in soil from an abandoned petrol station in Sweden (Vilches, Bylund et al. 2010) and suggest that the results from the previous laboratory scale experiments on whey are applicable in field-like conditions. The treatment with the lower level of whey (WL) did not show any positive effect on degradation rates compared to the control, nor did the other two amendments (PA and CT) in either of the two treatment levels. At day 45, PAL seems to have an enhancing effect on the diesel degradation but at day 101 this effect is no longer sustained. This suggests that the optimum treatment level was exceeded by the repeated applications since PA has an inhibitory effect on microorganisms at high concentrations (Yoshimura et al., 1995). If PA is to be used to stimulate indigenous soil biota, its optimum treatment level must be thoroughly assessed under different conditions, considering that the contaminant itself may present an additional inhibitory effect on microorganisms. The absence of positive effect from CT may be attributed to poor transport of the microorganisms present in the CT through the soil. Ultisol like many tropical soils has a high clay content (Chagas-Spinelli et al., 2012) which may restrict access to oxygen (because of the

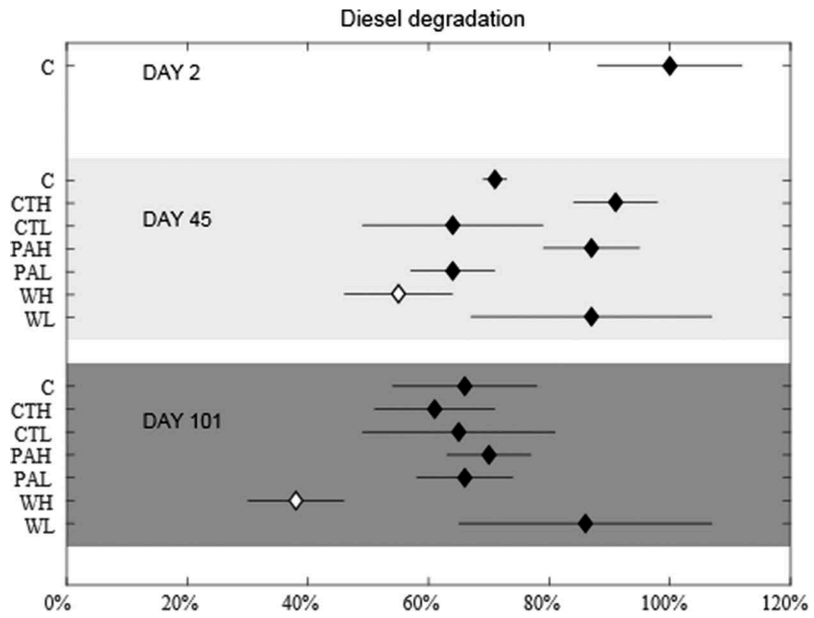

Figure 2. Effect of the different amendments on the degradation of the total diesel range hydrocarbons. The X-axis indicate the percentage of the diesel that remained in the soil. The white rhombi indicates statistical significance (based on the data before normalisation) and the errors bars indicate standard deviation of the normalized data. The abbreviations reflect the type of amendments and treatment level (high/low) according to the following; C = Control, CTH = Compost tea high, CTL = Compost tea low, PAH Pyroligneous acid high, PAL = Pyroligneous acid low, WH = Whey high, WL = Whey low.

dense pore structure of clay) and decrease bioavailability of the pollutant (Ferguson et al., 2003; Sako & Nimi, 2018) and thus obstruct the degradation. It may also hamper the vertical migrations of the organisms present in the ACT. A laboratory scale experiment conducted in the same soil as in this experiment showed that the vertical migration of many organisms present in CT is limited below a depth of 20 cm especially if the bulk density is high (Haller et al., 2017).

Overall degradation rate was relatively slow, even after whey addition. The constituents of diesel range organics were divided into low-boilers (7 < retention time ≤ 15.25) min and high-boilers (15.25 < retention time ≤ 23.5) min but no difference in degradation rate between the two fractions was detected. The slow degradation rates may be attributed to oxygen and nutrient deficiency (no additional nutrients were added to balance the C:N:P-ratio after the addition of the carbon-rich diesel) and the ultisol used in the experiment is innately nutrient-poor. When physico-chemical factors such as temperature, pH and bioavailability of the pollutant are apt for bacterial activity and degradation, the limiting factor of degradation of organic pollutants in soil is habitually deficient concentrations of nitrogen and phosphorus (Welander, 2005) but in this experiment oxygen may have been the most influential factor. Aerobic conditions are necessary for biodegradation of aliphatic, cyclic and aromatic hydrocarbons since the initial steps of catabolism by bacteria and fungi rely on the oxidation of the substrate by oxygenases, for which molecular oxygen is required (Alexander, 1999; Leahy & Colwell, 1990). Chagas-Spinelli et al. (2012) reported that aeration had a stronger effect on diesel PAH degradation in soil than addition of nutrients and microorganisms by the end of a 129-day experiment in a comparable tropical clay soil (oxisol). Deficient oxygen levels due to restricted diffusion of gases through soil pores in the dense clay soil may have been a contributing factor to the slow degradation rate together with nutrient deficiency. Other reasons for the relatively slow degradation rates may include reduced bioavailability of the diesel due to sorption of diesel fuel hydrocarbons to organic matter and especially clay particles in the soil matrix (Alexander, 1999; Chagas-Spinelli et al., 2012). Microcosm experiments have showed that degradation of *n*-hexadecane (a typical diesel fuel hydrocarbon) was considerably slower in loamy sand (silt and clay 18.6%) compared to sand (silt and clay 3.2%) after treatment of the diesel fuel contaminated soils with fermented whey (Östberg, Jonsson, Lundström et al., 2007).

A number of laboratory scale experiment corroborate that whey can promote the degradation of diesel but this experiment suggests the whey method may be appropriate even at field scale. In rural areas in low-income countries and other regions where economic incentives for bioremediation are few and money is scarce, even a moderate but significant effect of a remediation method may be of great importance if the method is cost-effective and environmentally and socially beneficial. The whey method may be one such cheap and simple way to enhance the degradation of diesel in places where the temporal factor is not decisive. Earlier work carried out by our research group has indicated that the development of soil bioremediation solutions for marginalized regions (Haller et al., 2018) requires innovative thinking and that appropriate solutions may offer co-benefits to the local society that opens up for a more sustainable development.

4. Conclusions

Treatment of diesel contaminated ultisol with 6 mL whey kg^{-1} dw in a pilot-scale experiment significantly increased the diesel degradation rate compared to the control ($p < 0.05$). No consistent effects on diesel degradation rates were observed after treatment with compost tea or pyroligneous acid. The absence of positive effect from the pyroligneous acid and compost tea may be attributed to an inhibitory effect on microorganisms at high concentrations of pyroligneous acid and poor vertical transport of compost tea microorganisms through the soil. Less than 40% of the diesel range organics was remaining in the soil after 101 days of whey treatment. Tropical climate is favourable for biodegradation but many tropical soils are rich in clay which can inhibit the bioavailability of the pollutant which in turn may be decisive for biodegradation kinetics. If low cost is a crucial factor, our results indicate that whey treatment has the potential to be an appropriate technology for treating petroleum-contaminated soils in tropical regions, especially in marginalized regions where economic incentives are small for soil remediation to be effectuated.

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