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SOIL TREATMENT TOWARDS STRESS-TOLERANT BIOREMEDIATION STRATEGY FOR PETROLEUM HYDROCARBON-CONTAMINATED SOILS IN COLD CLIMATES USING ZEOLITE AS A REMEDIATION AGENT: A PRELIMINARY STUDY

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Abstract: Petroleum hydrocarbon (PHC) contamination in soil environments in cold climates has been a significant concern in Canada. However, the ground in cold regions remains frozen or partially frozen for many months of the year, and seasonal temperature cycles significantly affect field monitoring, management and remediation practices. Current remedial strategies and on-site activities, regardless of the type of remediation technology, have been considered effective only during short summers (e.g., 2–4 months per year) due to the colder climate, slower contaminant degradation rates, and limited accessibility to remote areas during the freezing months. Nonetheless, we have consistently observed the meaningful extension of hydrocarbon biodegradation activity in freezing and frozen contaminated soils. This study suggests that our soil treatment specialized for stress-tolerant biostimulation in seasonally freezing and thawing PHC-contaminated soils is effective at sub-zero temperatures. We used analcime zeolite, a natural clay mineral, to retain unfrozen liquid water, immobilize nutrients and hydrocarbons (substrate), and provide unfrozen water-insulated pores, surface areas and microsites in partially frozen and frozen PHC-contaminated soils. Amending PHC-contaminated soils with zeolite retains unfrozen liquid water and changes the soil freezing characteristic curve (SFCC) of the soils. We also showed the excellent compatibility of the Canadian analcime zeolite with amendments of conventional inorganic nutrients and carbon-based soil amendments for enhancing indigenous microbial populations in field-aged, PHC-contaminated soils at low temperatures (10 °C).

1 INTRODUCTION

Petroleum hydrocarbons (PHC) are the most frequently occurring contaminants in Canada (Princz et al. 2012, Sanscartier et al. 2009), where over 22,000 contaminated and suspected sites have been identified (Federal contaminated sites inventory 2019). Approximately 60% of federal contaminated sites are directly or indirectly impacted by PHC contamination. This has been a significant environmental concern in northern soil environments, where cold-climate soils are fragile and susceptible to PHC contamination (Siciliano et al. 2008). Generally, PHC contamination occurs due to uncontrolled, accidental and chronic contamination associated with intensified industrial and anthropogenic activities (Aislabie et al. 2004). Unfortunately, the cost of remediation for remote cold sites, including sub-polar and polar regions, is often prohibitively high due to low temperatures, short summers, harsh environments, remoteness, and associate field logistics (Braddock et al. 1997, McCarthy et al. 2004).

Bioremediation has been frequently considered a viable tool to clean-up of PHC-contaminated soils in cold climates (Aislabie et al. 2004, Eriksson et al. 2001, Filler et al. 2008, Lovley 2003). These soils are often deficient in inorganic nutrients such as nitrogen (N) and phosphorus (P) (Aislabie et al. 2004) and it has been reported that stimulating *cold-adapted*, indigenous, hydrocarbon-degrading bacteria (psychrophilic and psychrotolerant) is feasible by adding N and P (i.e., biostimulation). Biostimulation strategies have produced many successful outcomes for PHC degradation in cold climates, from laboratory treatability studies to field implementations (Aislabie et al. 2004, Braddock et al. 1997).

Most biostimulation studies have been conducted in the range of 0–15 °C, or at a fixed sub-zero temperature (e.g., -5 °C) at the laboratory microcosm-scale (Børresen et al. 2007, Braddock et al. 1997, Eriksson et al. 2001, Walworth et al. 2001). Recently, our group has produced new biostimulation *field* data showing extended biodegradation activity into the winter season in a cold climate region where ambient temperatures reached -35 °C (Kim et al. 2018). The outdoor biopile experiment was conducted at a pilot scale (~ 3 tones) and over the winter near Saskatoon, Saskatchewan. Both treated and untreated (control) biopiles were subjected to *in situ* seasonal freezing, deep freezing and subsequent thawing (Kim et al. 2018). In the biologically treated biopile, microbial respiration activity was detected in both the partially frozen and deeply frozen soil phases. Significant biodegradation of petroleum hydrocarbons occurred in the biostimulated PHC-contaminated soils. It is speculated that unfrozen liquid water in freezing and frozen contaminated soils plays a key role in extending the sub-zero microbial activity to the long freezing months (Chang et al. 2011, Kim et al. 2018). Detectable unfrozen water was more abundant in biologically enhanced, PHC-contaminated soils, than in the untreated PHC-contaminated soils (control soils) (Chang et al. 2011, Kim et al. 2018). Therefore, in the present study, we focused on a freezing-tolerant biostimulation strategy based on increasing the retention of *unfrozen* water using Canadian analcime zeolites.

Zeolites are natural clay minerals that are abundant in nature in over 50 different species (Hedström 2001). They have a three-dimensional structure composed of silicate and aluminate (Mumpton 1999). The most common species of zeolite are clinoptilolite, chabazite, phillipsite, analcime, modernite, stilbite and laumontite (Wang and Peng 2010). Origin and formation determine the different species of zeolite (Hedström 2001) and different species have a slightly different chemical compositions and physical properties, which dictate their efficiency for a particular use (Mumpton 1999). Zeolite is of great interest to researchers because of its unique cage-like structures and properties, giving it a high cation exchange capacity (adsorption) and making it useful for its nutrient holding capacity and ability to act as a molecular sieve (Ming and Allen 2001). Such properties make zeolite a practical material in a range of applications, and they have already been used widely for various environmental uses: dye removal from waste effluents (Wang and Zhu 2006), wastewater treatments (Wang and Peng 2010), heavy metal soil pollution (Shi et al. 2009), nuclear waste site remediation (Cortés-Martínez et al. 2010), improving soil grading and fertilizer efficiency and preventing nutrient leaching (Ming and Allen 2001), the construction of permeable bio-reactive barriers to remove PHC from soil water (Freidman et al. 2017), the desalination of salinized groundwater (Gibb et al. 2017), and many more.

However, the compatibility and potential roles of zeolites in treating PHC-contaminated cold-climate soils, along with conventional biostimulation nutrients (N and P), have not yet been extensively understood. The specific objective of this study is to investigate the effects of the Canadian analcime zeolite on biostimulation and unfrozen water retention. Using PHC-contaminated cold-climate site soils, this study has produced positive results showing enhanced microbial activity and water retention under water-stressed conditions (both soil drying and freezing), due to the zeolite amendment combined with inorganic nutrients.

2 MATERIALS AND METHODS

2.1 Site soil characterization

The site soils are field aged, PHC-contaminated cold-climate soils. The site soils are poorly graded sandy soils with gravels based on the Unified Soil Classification System (USCS). The pH of the site soils was determined using $CaCl_2$ extract (Gregorich and Carter 2007). Viable heterotrophs were enumerated using

R2A agar plates and hydrocarbon-degrading bacteria were enumerated using Bushnell Hass agar spiked with 0.5% diesel.

2.2 Zeolite-assisted water retention during soil drying

The soil drying process is analogous to soil freezing in terms of changing water availability (Koopmans and Miller 1966, Spaans and Baker 1996). We conducted a soil drying experiment using the diesel-spiked sands that were inoculated with hydrocarbon-degrading bacteria (*Dietzia maris*) and amended with zeolite. Water retention was assessed by measuring time-variable water content during soil drying as well as the growth of viable hydrocarbon-degrading bacteria inoculated to the diesel-spiked Ottawa sands with *and* without zeolite (control). All of the microcosms were amended with Bushnell Hass (BH) media as nutrient sources except carbons. The spiked diesel provides a sole carbon source for the growth of bacteria. The Canadian analcime zeolite was obtained from ZMM Canada Minerals Corp. (BC, Canada). The dosage of the zeolite as the soil amendment was 2% by weight. The initial water content in the control soil microcosms without the zeolite amendment was successfully maintained. The control microcosm (0% zeolite) was served as the baseline for comparison with the zeolite-amended soils. The experiment was run for 11 days. Corresponding soil samples were aseptically collected on Day 11. Viable hydrocarbon-degraders were enumerated (CFU/g; colony forming unit per gram of soil). The viable microbial populations in the diesel-spiked sands amended with zeolite represent the survivors from soil drying (water scarcity) due to water retention.

2.3 Zeolite-assisted water retention during soil freezing

Soil freezing experiment was carried out to determine how the zeolite amendment in PHC-contaminated soils influences the retention of unfrozen liquid water. Field-aged, PHC-contaminated soils were amended with 2% (w/w) analcime zeolite. The particle size of the zeolite was ~44 µm (mesh size-325). The soil microcosms were subjected to freezing temperatures from 4 to -10 °C at a seasonal rate of -1 °C/day. Volumetric unfrozen water content and soil temperature were measured using 5TM-EM50 probes (Decagon Devices, Pullman, USA). The soil freezing characteristics curve (SFCC) of the soil microcosms were generated.

2.4 Zeolite-assisted biostimulation

We also examined the compatibility of the analcime zeolite with the conventional nutrient-amendment. A commercial 20:20:20 N-P-K fertilizer (20% total N: 20%P_2O_5:20% K_2O; Plant Prod®) was employed for the nutrient amendment. Zeolite along with a carbon amendment (ZMM Canada Mineral's T-Carbon), were used for the additional soil amendment. Field-aged, PHC-contaminated soils were treated with the 20:20:20 N-P-K fertilizer (200 mg N/Kg), 2% (w/w) analcime zeolite and 2% (w/w) carbon amendments at a microcosm-scale. The soil microcosms, including untreated control microcosms, were incubated at 22 and 10 °C for 14 days. The soil samples were aseptically collected for downstream analyses, including the viability assessment for estimating the growth of heterotrophs at 22 and 10 °C.

3 RESULTS AND DISCUSSIONS

3.1 Zeolite-assisted biostimulation potential

The site soils showed significant numbers of indigenous heterotrophs (4.3 x 10^7 CFU/g) and hydrocarbon-degrading bacteria in the diesel range (2.2 x 10^7 CFU/g). The presence of indigenous hydrocarbon-degrading populations in the site soils suggests the biostimulation potential of the site soils by supplying the inorganic nutrient solutions. It is speculated that zeolites positively influence in retaining water, immobilizing nutrients, and providing the additional surface areas for microbial habitats in the site soils.

3.2 Zeolite-assisted water retention for microbial survivors during soil drying

The diesel-spiked sand microcosms with zeolite additions of 2% (w/w) showed the significant survival of *D. maris* (2.9 x 10^6 CFU/g). The double asterisks in Figure 1 (**) refers to data that was statistically different from the control soils ($p < 0.0001$). The diesel-spiked sand microcosms *without* zeolite that were exposed to drying showed significant inhibition in the growth of the hydrocarbon degraders under water-stressed conditions.

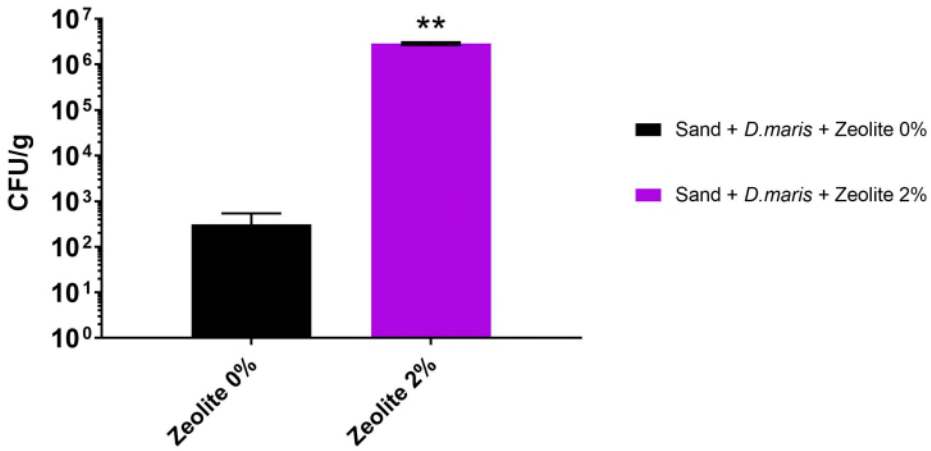


Figure 1: Improved bacteria viability due to zeolite in water stressed condition

The zeolite dosage in the second experiment setup (Figure 2) was 5% (w/w), and other factors (bacteria, nutrient concentration, diesel and soil matrix) were the same as those in the first setup (Figure 1). The initial water content was maintained at 15%. Water content during soil drying was estimated by weight loss (water evaporation). The Canadian zeolites hold more water, supporting the growth of hydrocarbon-degrading bacteria in diesel-contaminated sands. Soil drying does not inhibit the growth of hydrocarbon-degrading bacteria when the zeolites are applied to diesel-contaminated soils. This is likely due to the delayed rates of soil drying caused by the retention of water available for bacterial growth. Retaining water in the zeolite-soil matrix is important in extending the contact time between bacteria and the substrate (oil contaminant) and other nutrients, which may support the formation of biofilms under water-stressed conditions (e.g., during drying and freezing).

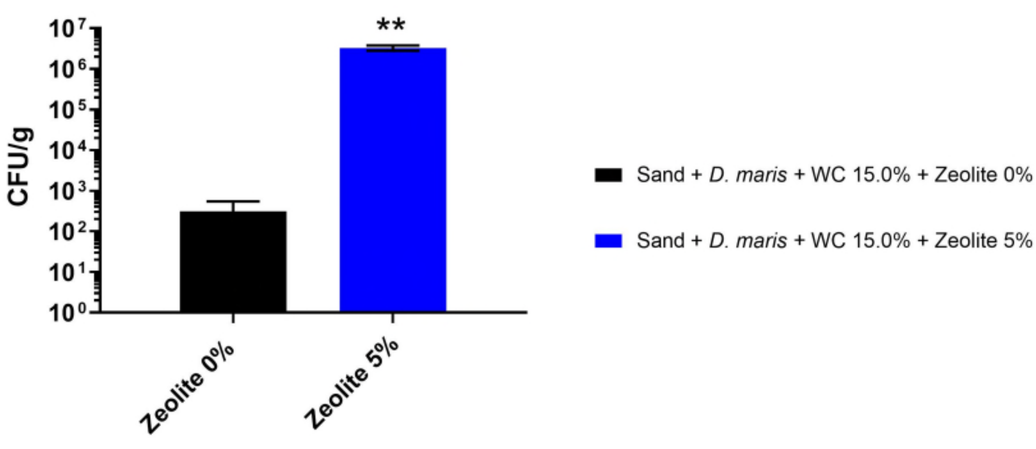


Figure 2: Improved water retention due to zeolite additions

3.3 Zeolite-assisted unfrozen water retention

Figure 3 shows the soil freezing characteristics curves (SFCC) for the zeolite-amended PHC-contaminated soils, along with the control set (without zeolites). Zeolites are beneficial in shifting the freezing characteristics of PHC-contaminated soils, significantly improving the retention of unfrozen liquid water in freezing and frozen contaminated soils. We will continue to analyze the effects of surface area and particle size distributions of the variety of the Canadian zeolites to accurately understand the effect of the different zeolites on soil freezing characteristics. Based on the current datasets and SFCC for the PHC-contaminated soils amended with the Canadian analcime zeolite, optimizing the dosage and type of zeolites is a promising approach for maximizing the effectiveness of the amendments in a stress-tolerant bioremediation strategy for PHC-contaminated soils in cold climates.

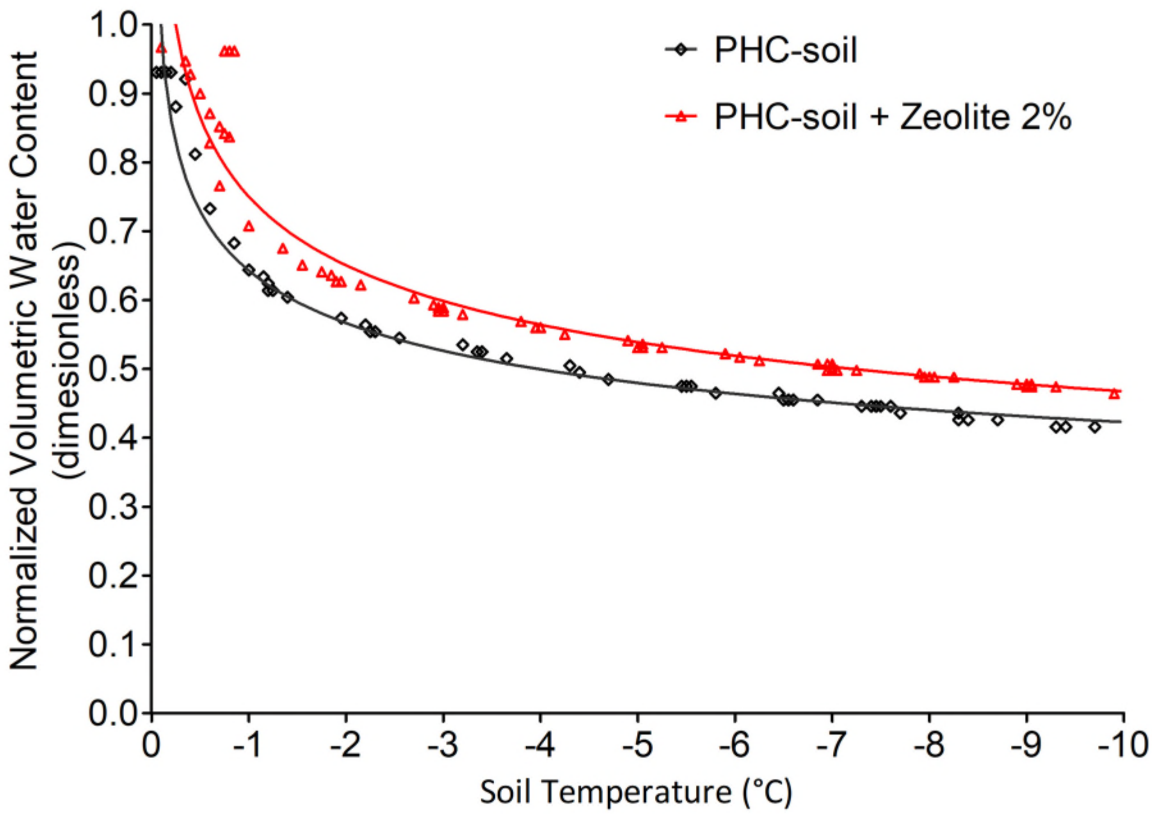
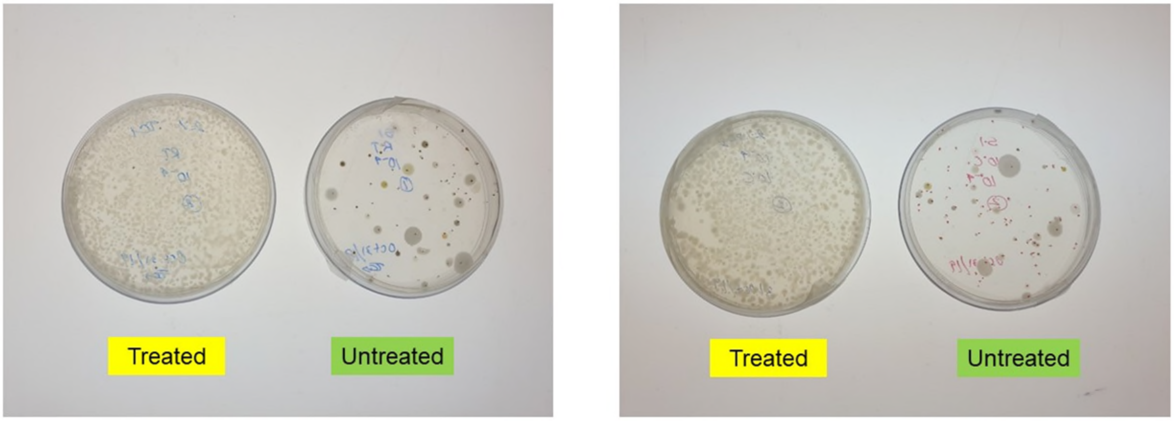


Figure 3: Non-linear regression analyses (curve-fitting) for the generation of SFCC for the PHC-contaminated soils amended with zeolite

3.4 Excellent compatibility of zeolite with inorganic nutrient supply for biostimulation

We examined the compatibility of the Canadian analcime zeolite with the water-based nutrient supply and additional carbon amendments for enhancing microbial activity. The zeolite amendment improved water retention and provides additional surface areas. But, zeolites might significantly adsorb some cations in the nutrient solution, potentially reducing the nutrient availability for microbial stimulation. As shown in Figure 4, the viability assessment, however, showed the excellent compatibility of analcime zeolite with the nutrient supply (20:20:20 N-P-K fertilizer) and carbon amendments at 22 and 10 °C.



22 ºC 10 ºC

Figure 4: Significant growth of bacteria in response to amendments

4 CONCLUSION

The site soil characterization indicated significant presence of indigenous hydrocarbon degraders suggesting the potential for using a bioremediation strategy assisted by nutrient addition. This study shows that the Canadian analcime zeolite shows excellent compatibility with indigenous hydrocarbon-degrading bacteria, and its addition likely introduces protective habitats for them under extreme water-stressed conditions (very low water content). Zeolite application may have multiple advantages for the bioremediation of PHC-contaminated soils, such as nutrient retention, unfrozen liquid water retention, and improved soil grading for the formation of protective microbial habitats. We will further investigate the effects of the analcime zeolite on enhancing bioremediation potential for PHC-contaminated soils in freezing and frozen soil phases under the sub-zero temperature regime. Overall, the current data suggest the significant potential of applying zeolites to enhance the bioremediation of oil-contaminated soils under water-stressed conditions.

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