

ISOLATION OF HYDROCARBON DEGRADING BACTERIA FROM GROUNDWATER CONTAMINATED WITH CRUDE OIL IN JAFFNA, SRI LANKA

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Crude oil is one of the most significant pollutants in the environment as it is capable of causing serious damages to human and the ecosystem. Prolonged exposure and high oil concentration especially in drinking water may damage biological organs such as liver, kidney, bone marrow and increase the risk of cancer. Thus, the WHO recommendation is 1 mg L^{-1} as maximum permissible level for oil in drinking water. In this study, crude oil degrading bacteria were isolated from crude oil contaminated ground water in Chunnakam area in Jaffna, where unauthorized crude oil discharge has been taken place. Due to the absence of perennial rivers or major water supply schemes in Jaffna peninsula, groundwater is the predominant water resource supplying water for domestic, industrial and agricultural needs. Triplicate samples were collected from each selected locations at Chunnakam area in Jaffna. The residual hydrocarbon present in the collected groundwater samples were extracted using n-hexane and analyzed using a spectrophotometer (2UVD10053, Labomed, inc. U.S.A) at the wave length of 400 nm. Quantification of crude oil was done using a calibration curve. Ten bacterial strains were isolated by enrichment culture method using standard pour plate. Crude oil degradation kinetics were studied by introducing 0.5 mL of overnight starved bacterial suspensions into sterile groundwater samples in triplicate at final concentration of 0.01 mg L^{-1} and 0.1 mg L^{-1} respectively. The samples were incubated at 28°C , shaking at 100 rpm and 0.5 mL sub sample aliquots were removed at 2 days interval for a period of 14 days. Analyses of residual hydrocarbon were performed by the spectrophotometer (400 nm). The results revealed that crude oil contamination levels range from 0 to 1.021 mg L^{-1} . Based on the morphological and biochemical tests, bacterial isolates were identified as *Bacillus* spp. (B1, B2, B3, B4, B5), *Pseudomonas* spp. (PS1, PS2, PS3) and *Micrococcus* spp. (M1, M2) respectively. The highest degradation percentage of crude oil was detected at 14 days incubation by the bacterium *Bacillus* sp. (B2) (0.01 ppm ; 89%, 0.1 ppm ; 86%) whilst degradation potential of remaining strains were recorded as: *Bacillus* sp. (0.01 mg L^{-1} ; 65%- 82%, 0.1 mg L^{-1} ; 55% - 77%), *Pseudomonas* sp. (0.01 mg L^{-1} ; 58% - 77%, 0.1 mg L^{-1} ; 53% - 72%) and *Micrococcus* sp. (0.01 mg L^{-1} ; 51%- 54%, 0.1 mg L^{-1} ; 39% - 47%). The contamination of the Jaffna ground water may be a severe problem as the aquifer is the main source of water for the Chunnakam area in Jaffna. Therefore, these bacterial strains could be used as a useful bioremediation tool in the removal of crude oil from the aquatic environment.

Keywords: Aquifers, *Bacillus* sp., crude oil, *Pseudomonas* sp., *Micrococcus* sp., microbial degradation

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