



ENHANCING THE ABILITY OF THREE DIFFERENT PLANT SEEDS TO GERMINATE UNDER ELEVATED CONCENTRATIONS OF OIL SLUDGE USING THREE DIFFERENT BACTERIAL ISOLATES

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Abstract

One of the major problems face the environment is the pollution caused by electricity power stations which result in a heavy contamination by oil sludge resulting in Oil Sludge Contaminated Soil (OSCS). This contamination affects negatively the eco system and the human health alike. The present investigation was carried out to study the effect of different concentrations of untreated oil sludge on the potential of seed germination and seedling growth of *Vigna unguiculata*, *Vicia faba* and *Brassica niger*. Seven concentrations of oil sludge (200, 500, 1000, 1500, 2000, 2500, 3000 or 3500 µl/ml) were used. Three bacterial isolates were isolated from contaminated site and identified as *Acinetobacter radioresistens* (S1), *Pseudomonas aeruginosa* (S2) and *Pseudomonas putida* (S3), and then used as inoculums added to Petri dishes where seeds germinated. Results indicated that seeds of the three plant species are dose-dependent, since a reduction in germination percentages occurred compared to controls, however, *V. unguiculata* exhibited higher germination percentage than *V. faba* and *B. niger* while the lowest was recorded in *B. niger*. Seeds of the studied plant species were also germinated in Petri dishes containing Whatmann filter paper soaked with distilled water resulted in 100, 80 and 76% germination percentages. After treatment with elevated concentrations of oil sludge, seed germination decreased gradually until totally inhibited at 3500, 2000 and 1500 µl respectively. Inoculation with isolated bacterial strains isolated from contaminated sites showed notably an increase in seeds germination percentages even at the above inhibitory concentrations. Seeds of *V. unguiculata* and *B. niger* seed germinated in the above concentrations after adding bacterial inoculums, while *V. faba* exhibited no seed germination. Thus, results suggest that *V. unguiculata* displays better tolerance to oil sludge than others. Inoculation with the bacterial strains to the sludge contaminated water enhanced the germination percentages, suggesting that these strains could be a candidate for biodegradation of hydrocarbons with a noticeable growth of *V. unguiculata*.

Keywords: Bioremediation, seed germination, oil contaminated soil, *Vigna unguiculata*, *Vicia faba*, *Brassica niger*, Bacterial strains.

Introduction

Oil sludge is one of the most significant hazardous solid wastes generated from oil industry in Iraq. It is mainly by-product generated from the electricity power stations. Various petroleum products are common soil contaminants and often contain potentially hazardous chemicals, particularly the polycyclic aromatic hydrocarbons (Huang *et al.*, 2004) amount of oil sludge consisted of many hazardous chemicals, such as petroleum hydrocarbons (PHCs) and heavy metals (HMs), which are of a great concern for the potential toxicity to human, have been spread into the local environment (Wang *et al.*, 2018). The spent lubricant, otherwise called waste engine oil, is usually obtained after servicing and subsequent draining from automobile and generator engines, is more widespread than crude oil pollution (Odjegba and Saiq, 2002). Petroleum waste is a complex mixture containing alkanes, aromatics, nitrogen, sulfur, oxygen, and asphaltene fractions. Therefore it is difficult for single-species of bacteria to biodegrade all components of oil, but only degradation of certain types of petroleum compounds. Microbial community allows a higher degradation rate for some oil fractions. Moreover, some substances can be decomposed only by metabolism (Milic *et al.*, 2009). The toxic components in oil sludge may cause nutrient deficiency or limit seed germination the growth of seedlings and plants (Mart *et al.*, 2009). There have been many remediation methods applied to polluted lands, especially those with petroleum-polluted lands. Several oil

companies in Indonesia, especially in the upstream sector, have used bioremediation methods to treat the oil-polluted land. Bioremediation methods also widely used in cases of coast pollution caused by oil tanker carrier accidents (Tuhuloula *et al.*, 2019). Microbial bioremediation of hydrocarbon and water contaminated soil has emerged as a promising technology in recent years. Several studies have shown that *Pseudomonas aeruginosa*, *Pseudomonas putida*, *Acinetobacter spp.*, *Flavobacterium spp.*, *Yokenella spp.*, *Alcaligenes spp.*, *Roseomonas spp.*, *Sphingobacterium spp.*, *Capnocytophaga spp.*, *Moraxella spp.*, *Corynebacterium spp.*, *Streptococcus spp.*, *Providencia spp.*, etc., are common hydrocarbon degraders (Mandri and Lin, 2007). The potential of using phytoremediation, a process whereby green vegetation is used to remediate petroleum-polluted soils, is examined, and has proven to be one of the most efficient, cost-effective and environmentally friendly technologies. Phytoremediation should be strongly encouraged. It is an innovative technology that uses plants to remove environmental contaminants such as heavy metals and organic compounds (Mouhamad *et al.*, 2012; Ansari *et al.*, 2015) Plants are utilized to absorb, accumulate and detoxify contaminants through physical, chemical or biological processes and have become a wide spread practice (Jilani and Kan 2006; White *et al.*, 2006). This technology has been applied to both organic and inorganic pollutants present in soil (solid substrate), water (liquid substrate) (Ghosh and Singh, 2005). The aim of the current experimental work is to examine seed germination of three

plant species under hydrocarbon pollution with the aid of hydrocarbon biodegrading bacterial strains isolated from polluted soil nearby power stations.

Materials and Methods

Sample collection

Soil samples were collected randomly from oil sludge contaminated soil nearby an electrical power station in Najaf province, Iraq.

Isolation and identification of microbes from soil samples

The microbial strains were isolated from the collected soil samples by serial dilutions. Selected colonies were identified by using morphological, cultural and biochemical characteristics (Aneja and Wilkes, 2002) Gram's staining (Bailey and Scott 1966) motility test, Biochemical confirmation test according to Bergey's manual of systemic bacteriology classification (Garrity 2012).

Oil sludge as a carbon source in liquid medium

Flasks containing 50 ml of selected bacterial strains were inoculated with sterile nutrient broth supplemented with 2% oil sludge while the control was inoculated with 50 ml of the medium plus 2 ml oil sludge. Cell turbidity was measured at 540 nm using colorimeter (Kumar *et al.*, 2006).

Seed germination

Seeds of three plant species including *Vigna unguiculata*, *Vicia faba* and *Brassica niger* were dipped in 0.05% potassium permanganate solution for 10 min, then rinsed with distilled water 3 times in order to remove the disinfectant solution. They were tested for their ability to germinate in distilled water contaminated with oil sludge. Plant seeds were collected from plants grown in the previous season. The oil sludge was obtained from a nearby contaminated soil of the electrical power station in province Najaf, Iraq. Aqueous medium (distilled water) was mixed with different concentrations of oil sludge and used for germinating all types of seeds to observe the effect of oil sludge on seed germination. The following concentrations of oil sludge were prepared 200, 500, 1000, 1500, 2000, 2500, 3000, or 3500 $\mu\text{l/ml}$. Aliquot of 10 ml was added to distilled water inside Petri dishes containing Whatmann type 1 filter paper. Each Petri dish was containing 7 seeds of *V. unguiculata*, 5 seeds *Vicia faba* or 40 seeds of *B. niger* individually. Each treatment was carried out with 3 replicates. After one week of seed sowing, germination percentages were recorded. The effect of oil sludge was expressed as a percentage of control (table 1). The same volume (100 $\mu\text{l/ml}$) of bacterial inoculums was added to Petri dishes containing the above elevated concentrations of oil sludge.

Results and Discussion

The effects of oil sludge on seed germination for the three plants under investigation are shown in figures 1, 2 and 3 respectively. Various responses of the plant species to the contaminant appear to be dose dependent.

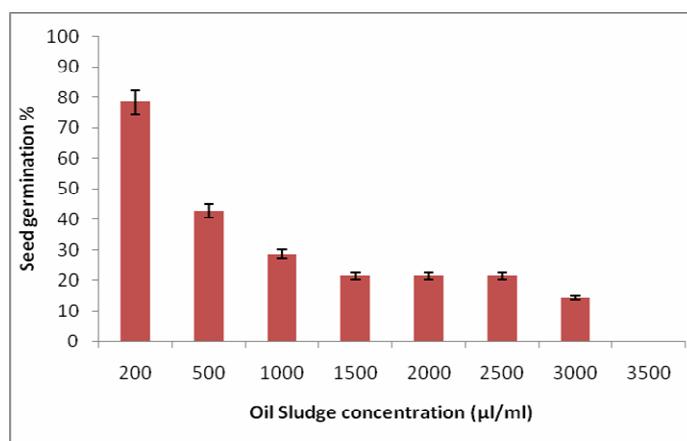


Fig. 1 : *Vigna unguiculata* seed germination expressed as percentages of control in distilled water supplemented with elevated concentrations of oil sludge after one week of seed sowing.

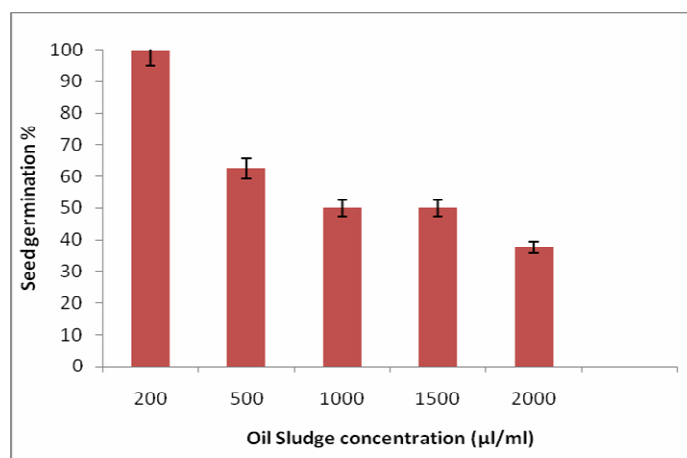


Fig. 2 : *Vicia faba* seed germination expressed as percentages of control in distilled water supplemented with elevated concentrations of oil sludge after one week of seed sowing.

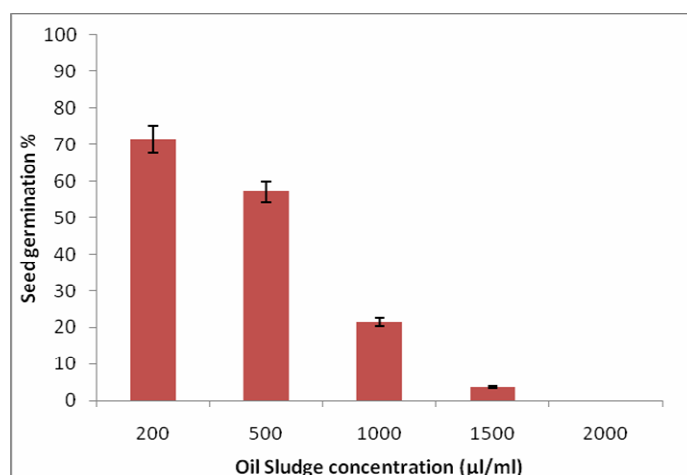


Fig. 3 : *Brassica niger* seed germination expressed as percentages of control in distilled water supplemented with elevated concentrations of oil sludge after one week of seed sowing.

A continuous decline in responses to the contaminant in terms of germination percentage in all species. They recorded exhibiting on dose dependent response to the contaminant, they recorded 14.7, 42.2 and 4.3 % for *V. unguiculata*, *Vicia faba* and *B. niger* respectively.

Reduction in seed germination in all species differed when compared to controls, however, *V. unguiculata* was the lowest and *B. niger* displayed the highest response for seed germination. Germination in *V. faba* and *B. niger* completely

inhibited at 2000 $\mu\text{l/ml}$ while recorded 3500 $\mu\text{l/ml}$ in *V. unguiculata*.

Table 1 : Effect of elevated concentrations of oil sludge on seed germination of *Vigna unguiculata*, *Vicia faba* and *Brassica niger* after one week of seed sowing in Aqueous medium (distilled water) mixed with different concentrations of oil sludge.

Concentration of oil sludge ($\mu\text{l / ml D.W}$)	Seed germination (% of control)		
	<i>Vigna unguiculata</i>	<i>Vicia faba</i>	<i>Brassica niger</i>
200	83.1	70	76.3
500	45.2	55.5	61.2
1000	30.5	55.5	22.5
1500	22.1	42.2	4.3
2000	22.1	0	0
2500	22.1	0	0
3000	14.7	0	0
3500	0	0	0

Seed germination is not only influenced by the external factors such as moisture, temperature and physical, chemical properties of medium, hydrocarbons with small molecules often have high toxicity, which can enter cell through permeable membranes, and then reduce the biological enzyme activity and impede nitrogen absorbing (Sun *et al.*, 2002). On the other hand, hydrocarbon with large molecules can obstruct the pore of seed coat, and further influence respiration (Cheng and Li, 2007). So the high concentrations oil sludge, the more seed germination inhibition, even stops germination completely.

There is no adequate information regarding the precise mechanisms affecting the reduction in germination rate, however, there are several possible mechanisms suggested by various authors. According to Henner *et al.*, 1999; some volatile fractions having less than 3 rings are found in spent oil. These compounds are known to have severe inhibitory impact on germination of several plant species. Another group of chemical compounds which are found abundantly in spent oil are polycyclic aromatic compounds (PAHs) which have indirect secondary effects including disruption on seed-water – air relationships (Renault *et al.*, 2000). Vwioko and Fashemi 2005, concluded that a reduction in germination rate could be resulted from coating of oil on seed coat, and hence a reduction in seed imbibitions. Various studies have shown that the dynamics of solubilization and ionic exchange in soils have been negatively affected under the influence of spent oil, and thereafter affects negatively on seed germination and plant growth.

Results shown in fig 4 indicated that addition of bacterial isolates to the medium enhanced the seed germination of *V. unguiculata* reached the upmost by adding *A. radioresistens* (S1) followed by *P. aeruginosa* (S2), while no effect with *P. putida* (S3) was noticed in addition to the mixture of the three bacterial isolates. Figure 5 indicated that addition of bacterial isolates to the medium negatively affected *Vicia faba* seed germination. While addition of bacterial isolates to the medium enhances the *B. niger* seed germination and the best results was by adding *P. putida* (S3) followed by *P. aeruginosa* (S2), while *A. radioresistens* (S1) and mixture of all the three bacterial showed negative effects (Fig. 6). Oil degrading bacteria secrete surface-active

substances allowing the adherence to oil droplets and formation of an emulsion in an aqueous environment (Colin *et al.*, 2014). The interaction between bacteria and oil sludge is a complex biochemical process and chemical reaction. These reactions depend on multiple variables within the interface of a multi-component system consisting of organic aqueous and inorganic components (Xue *et al.*, 2015).

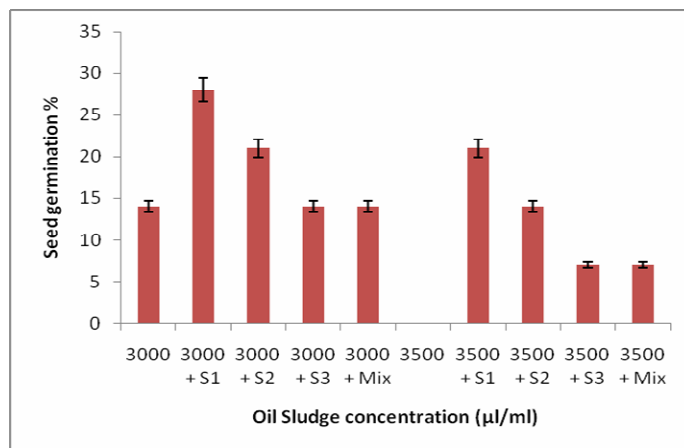


Fig. 4 : Effect of bacterial inoculums on *Vigna unguiculata* seed germination supplemented with high concentrations of oil sludge.

Acinetobacter radioresistens (S1), *Pseudomonas aeruginosa* (S2), *Pseudomonas putida* (S3) and (Mix) is a mixture of all three bacterial isolates.

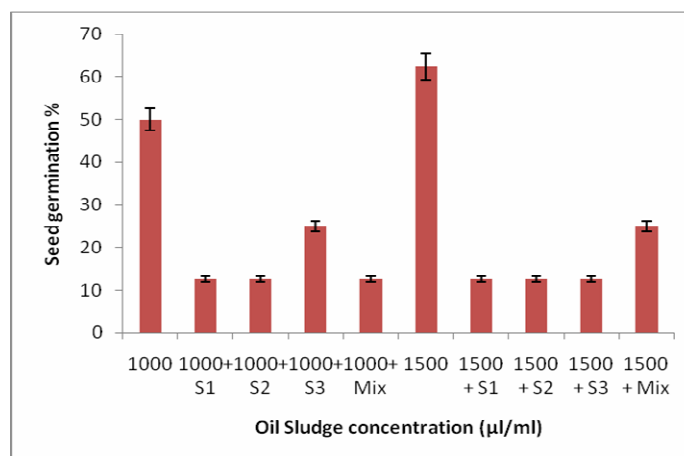


Fig. 5: Effect of bacterial inoculums on *Vicia faba* seed germination supplemented with high concentrations of oil sludge.

Acinetobacter radioresistens (S1), *Pseudomonas aeruginosa* (S2), *Pseudomonas putida* (S3) and (Mix) is a mixture of all three bacterial isolates.

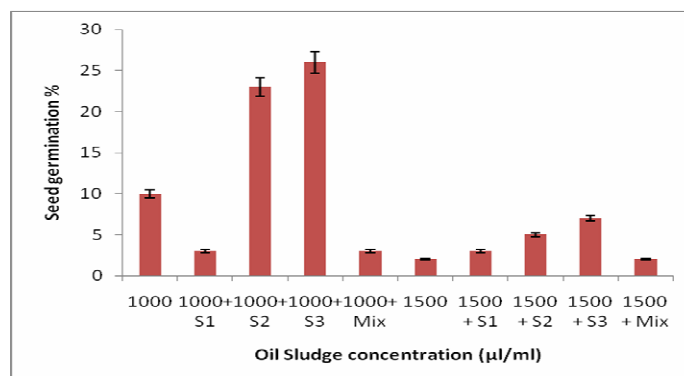


Fig. 6: Effect of bacterial inoculum on *Brassica niger* seeds germination supplemented with high concentrations of oil sludge.

Acinetobacter radioresistens (S1), *Pseudomonas aeruginosa* (S2), *Pseudomonas putida* (S3) and (Mix) is a mixture of all three bacterial isolates.

Conclusion

Oil sludge exhibited negative effects on seed germination of the studied plants. Germination decreased with increasing oil sludge and seedling emergence was inhibited. Bacterial isolates used hydrocarbons as energy source and hence responsible for the degradation of oil contaminates and improved seed germination.

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