Investigating the concomitant removal of hydrocarbons and heavy metals by highly adapted *Bacillus* and *Pseudomonas* strains

Zulfa Ali Al Disi, Mohammad Al-Ghouti, Nabil Zouari

Qatar University, Doha, Qatar

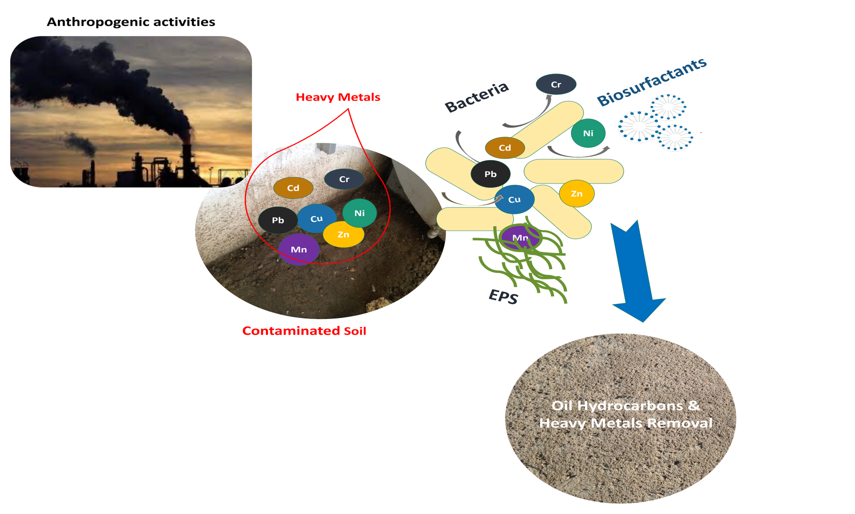
Faculty and Postdoc, Energy, Environment

Abstract

This study investigates the concomitant removal of hydrocarbons and heavy metals by highly adapted Bacillus and Pseudomonas strains. In regions characterized by harsh conditions such as Qatar, the weathering processes would affect the content, status, and distribution of these contaminants. It was shown in the weathered soil from Dukhan oil wastes dumpsite that 14 heavy metals exceeded the EPA limits. Moreover, it was demonstrated that soil organics did not affect the distribution of the metals in the soil. However, most of the heavy metals were strongly bonded to the residual and the iron-manganese oxide fractions. Eighteen bacterial strains isolated from highly weathered oily soils were able to grow with heavy metals concentrations up to 3 mM and above for some. Seven selected strains (4 Bacillus and 3 Pseudomonas) showed the ability to remove almost 60 to 70% of most of the heavy metals when used at 1 mM. Moreover, they removed up to 75% of the diesel range organics. These results are of interest for selecting bacterial strains, which can overcome the toxicity of hydrocarbons and heavy metals and remove them concomitantly.

Introduction

Anthropogenic activities in the petroleum sector have been releasing deteriorating pollutants into nature all over the globe (Arora, 2018). Such pollutants are mainly hydrocarbons and heavy metals, especially from fuels, which deposit into soil and water causing rapid effects on the environment and living organisms (Ukaogo et al., 2020). Thus, rising the need of providing treatment methods to tackle these issues with minimum damage to the ecosystem is emerging. In nature, there are various hydrocarbon-degrading bacteria, which are metal-tolerant microbes as well (Xingjian, et al., 2018). Weathering processes, strongly accentuated in arid areas such as in Qatar, were considered as an additional limitation of oily-soils bioremediation. Indeed, weathering contributes to the continuous changes of the soil pollutants (Jiang, et al., 2016). Hydrocarbons become more recalcitrant to biodegradation and heavy metals change their status and distribution in different forms, which make them more toxic and less bioavailable (Al-Kaabi, et al., 2017). In this study, different bacterial strains isolated from harsh Qatari soils were used to investigate their role in the removal of oil heavy metal when cultured on diesel hydrocarbons. Different parameters were studied to attain the most efficient strains and their optimal conditions for concomitant degradation of diesel hydrocarbons and removal of heavy metals.



Methods

1. Collection of soil samples
2. Sequential extraction and analysis of heavy metals in Dukhan soils
3. (ICP-OES Analysis) of the heavy metals
4. Growth kinetics of the selected hydrocarbon-degrading isolates at 1 mM heavy metals
5. Colony-forming unit (CFU) determination
6. Diesel range organics (DRO) extraction (GC-FID) analysis

Acknowledgments

This study was made possible by the grant PDRA5-0424-19007 from the Qatar National Research Fund (a member of the Qatar Foundation). The authors acknowledge all the staff of the Central Laboratories Unit (QUCLU) of the college of Arts and Sciences at Qatar University, Environment Science Center-Qatar University (QU-ESC) for providing support in performing gas chromatography and ICP-OES analyses.

Results

Table 1: Heavy metals in soil and in sequentially extracted fractions: exchangeable (EXC), carbonate (CA), iron-manganese oxide(FMO), residual (RES), and organic matter (OM) fractions.

Heavy metal	Total (ppm)	EPA limits (ppm)	Without washing with CH_2Cl_2					After washing with CH_2Cl_2				
			RES (ppm)	EXC (ppm)	CA (ppm)	FMO (ppm)	OM (ppm)	RES (ppm)	EXC (ppm)	CA (ppm)	FMO (ppm)	OM (ppm)
Cu	3.93	1.30	3.83	0.004	ND	0.002	ND	0.15	ND	ND	0.001	ND
Cr	24.76	0.10	22.2	0.06	0.2	0.9	0.09	2.7	ND	ND	0.9	0.08
Fe	642.8	-	284.4	0.06	2.1	372	1.5	254.8	1.5	ND	379	0.99
Mn	106.20	-	67.8	0.27	6.9	24.6	3.6	87.8	2.4	1.2	27	2.4
Ni	7.69	0.20	2.5	0.12	0.6	3.52	0.36	2.9	0.12	ND	3.77	0.3
Zn	39.92	0.50	16.5	1.32	2.4	20.3	0.052	17.1	2.6	0.9	21.1	1.2
Pb	9.96	15.00	7.2	2.15	ND	ND	ND	6.9	3.9	ND	ND	ND
Al	591	-	326.1	0.18	2.7	276	13.2	299	7.2	ND	275.5	8.7
Ba	601.8	0.002	477	3.1	4.2	119.3	3.3	468	2.9	ND	122.2	2.7
Mg	1188	-	953	2.6	2.1	128.7	1.9	998	1.9	ND	133.5	2.2
Ca	33.20	-	ND	ND	31.9	ND	ND	ND	ND	ND	34.1	ND
Cd	5	5	4.3	0.06	0.18	0.03	0.06	4.44	0.03	0.27	0.09	0.04
Co	ND	-	0.02	0.002	0.02	0.013	ND	0.015	0.001	0.02	0.014	ND
Ag	0.498	0.1	0.3	0.015	0.021	0.015	0.03	0.33	0.015	0.024	0.015	0.03
V	29.97	-	25.2	1.89	0.563	1.18	0.08	27.3	0.032	1.63	0.21	0.09

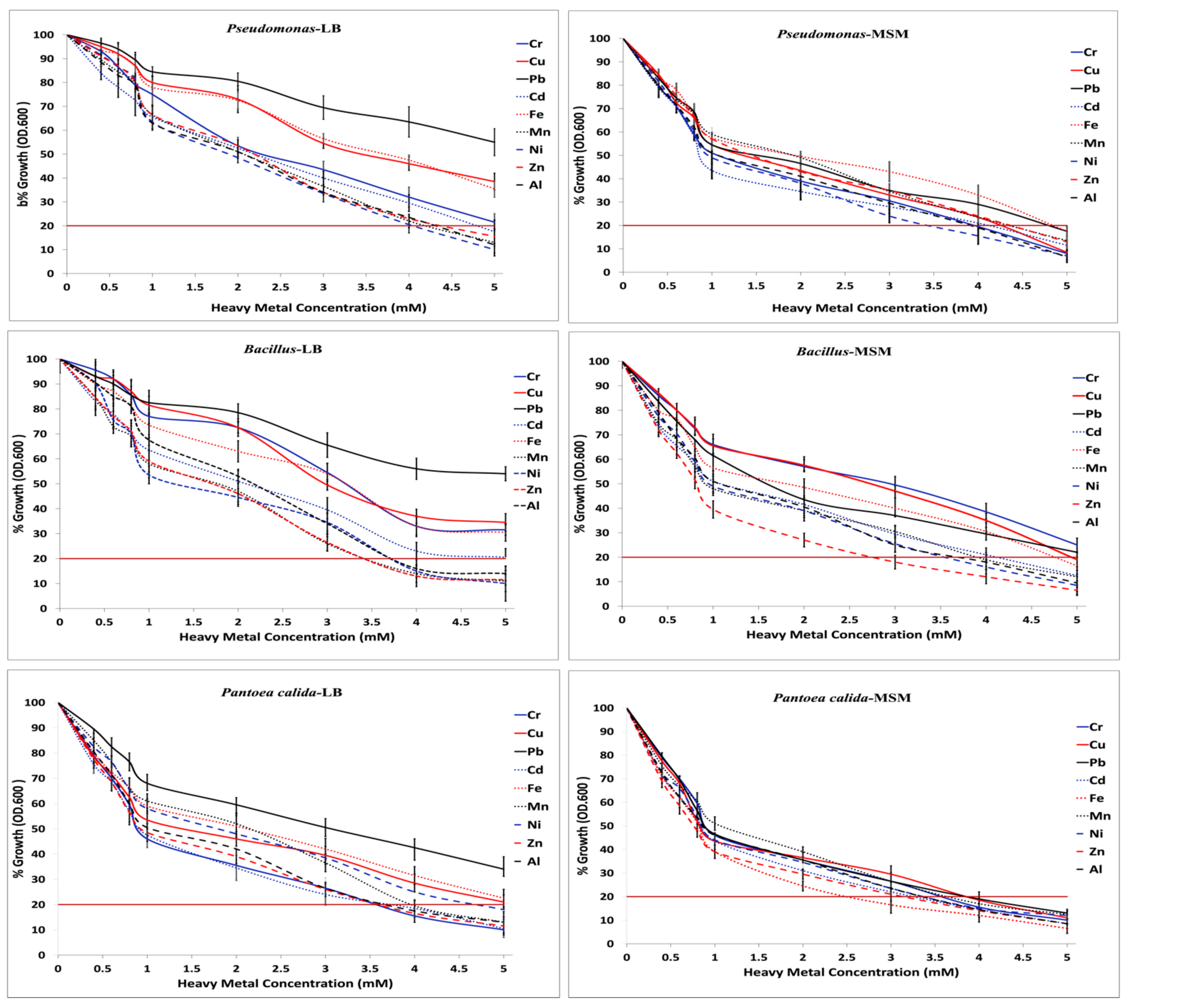


Figure 1: Growth of selective strains, each on LB medium and Diesel-MSM medium supplemented with different concentrations of heavy metals (0. 4, 0. 6, 0. 8, 1, 2, 3, 4, and 5 mM).

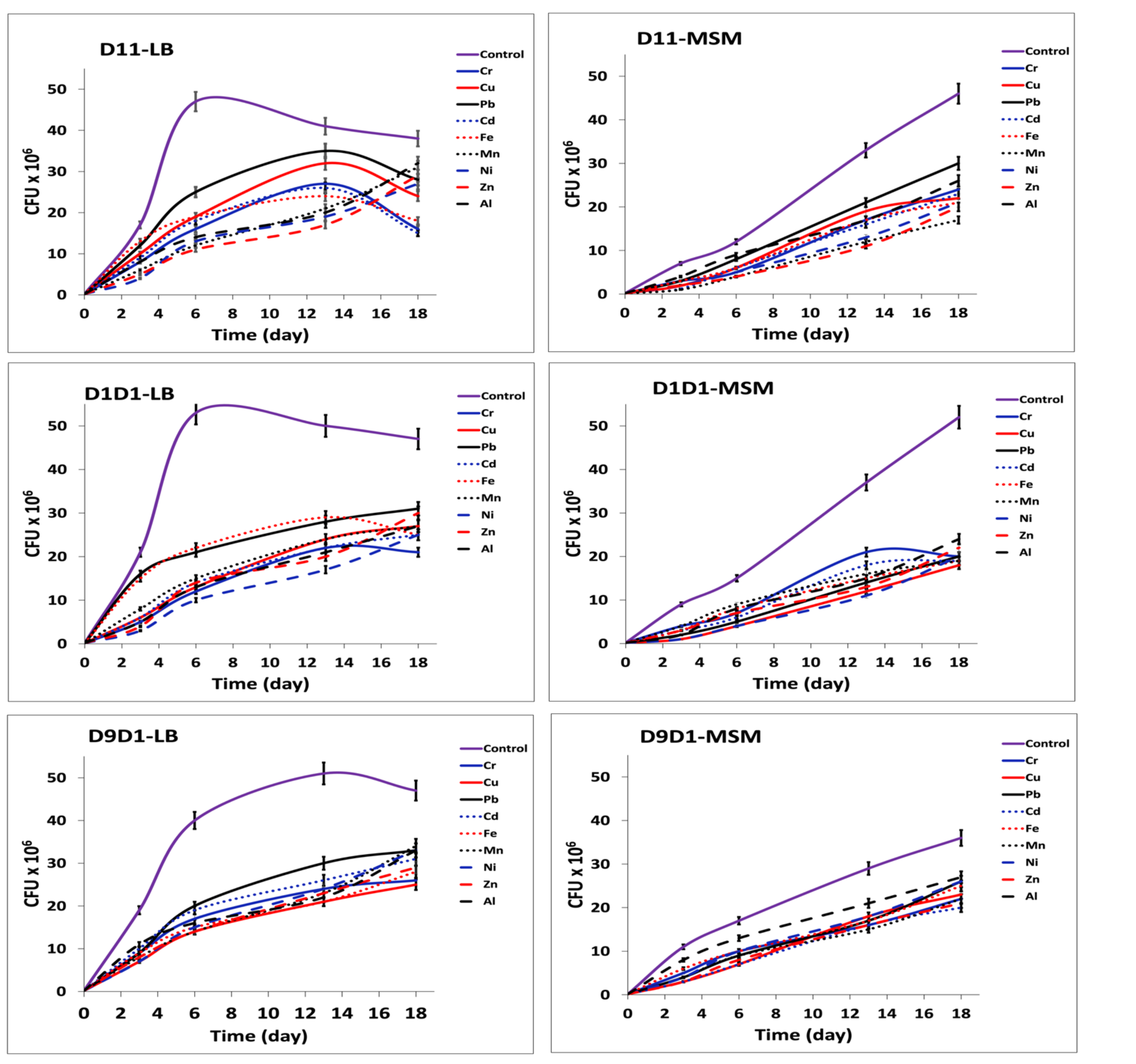


Figure 2: Effect of the heavy metals on the growth rate of selected bacterial species cultured in LB medium and diesel-MSM medium supplemented with 1 mM heavy metal.

Table 1: Bacterial strains identified by ribotyping or MALD TOF

Code	Strain identity	Accession number	MALDI TOF score
D9S2	Pseudomonas stutzeri	KX180912.1	2.11
D1D2	Bacillus licheniformis	KY962349.1	2.06
D5D1	Pseudomonas aeruginosa	KY040017.1	2.20
Z8D1	Morganella morganii	KU942503.1	2.10
Z3S1	Bacillus licheniformis	AF549498.1	2.04
D1S1	Bacillus sp.	KY911251.1	2.10
D2D2	Bacillus subtilis	MH071337.1	2.00
D4D3	Pantoea calida	KX036541.1	2.07
D4S2	Pseudomonas puteola	NR114215.1	2.03
Z7S1	Providencia rettgeri	CP027418.1	2.10
D1D1	Bacillus sp.	MG855692.1	2.02
Z4D1	Bacillus licheniformis	LN995452.1	2.05
D9D1	Pseudomonas stutzeri	KY849415.1	2.20
D7D1	Bacillus sp.	KT945027.1	1.80
Z6S1	Providencia rettgeri	CP027418.1	2.15
D11	Bacillus sonorensis	-	1.97
D12	Bacillus cereus	-	2.23
D13	Pseudomonas stutzeri	-	2.09

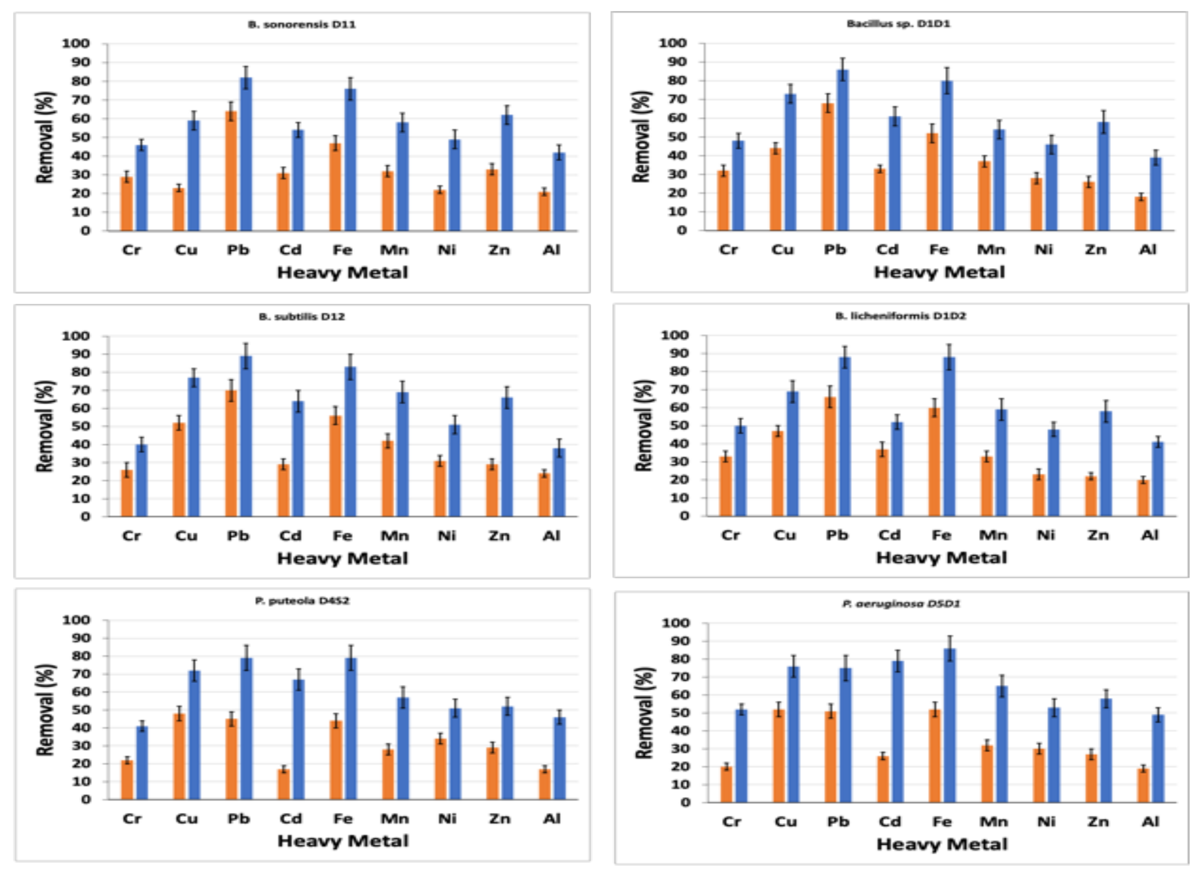


Figure 1: Heavy metals removal in each liquid culture of the strains after 10 days (orange) and 18 days (blue) of incubation.

Table 3: Diesel range hydrocarbon removal in the cultures performed with the selected strains in 5% Diesel-MSM containing 1 mM heavy metals.

HM	Incubation (d)	B. sonorensis D11	Bacillus sp. D1D1	B. subtilis D12	B. licheniformis D1D2	P. puteola D4S2	P. aeruginosa D5D1	P. stutzeri D9D1
Cr	10	38±3	41±3	37±2	43±4	37±4	34±3	37±3
	18	66±5	62±5	61±6	64±5	61±6	66±6	67±6
Cu	10	41±3	47±4	50±5	43±3	42±4	44±4	49±5
	18	58±5	63±6	67±6	71±6	69±6	70±7	71±6
Pb	10	41±3	49±4	47±4	41±3	40±3	41±3	49±4
	18	67±5	68±6	72±6	68±6	69±6	65±5	69±6
Cd	10	39±3	37±4	33±3	31±3	29±3	35±4	37±3
	18	60±5	63±5	65±6	59±5	60±5	59±6	64±6
Fe	10	46±5	49±4	50±5	52±5	49±5	51±5	49±4
	18	69±6	70±6	70±5	73±6	66±6	68±5	71±6
Mn	10	37±4	39±3	44±4	39±4	38±4	40±3	36±3
	18	59±4	64±5	68±6	58±5	59±6	62±5	56±4
Ni	10	29±3	31±3	30±3	33±4	32±3	30±4	33±3
	18	51±5	49±5	54±5	56±5	56±6	54±5	59±6
Zn	10	39±4	36±3	39±3	40±4	39±3	37±3	40±3
	18	63±5	58±5	65±5	62±5	62±5	68±5	62±5
Al	10	26±2	28±2	32±3	30±2	29±3	33±3	39±4
	18	43±3	44±4	43±3	47±5	46±5	48±5	49±5

Conclusion

- The organic fraction of the pollution did not strongly affect the distribution and the heavy metals in the soil.
- Bacterial strains isolated from highly weathered oily soils exhibited tolerance to heavy metal's toxicity up to concentrations above 5 mM.
- ***Bacillus*** **and Pseudomonas strains showed the ability to remove up to 70% of heavy metals and 75% of diesel range organics.**
- The results confirmed the high tolerance and adaptation to the high concentration of diesel (5%) and that of each heavy metals (1 mM).

References

Al Disi, Z., Jaoua, S., Al-Thani, D., Al-Meer, S., & Zouari, N. (2017). Considering the Specific Impact of Harsh Conditions and Oil Weathering on Diversity, Adaptation, and Activity of Hydrocarbon-Degrading Bacteria in Strategies of Bioremediation of Harsh Oily-Polluted Soils. *BioMed Research International, 2017*, 11.

Al-Kaabi, N. S., Kristensen, M., Zouari, N., Theis I. Solling, S. S., Al-Ghouti, M., & Christensen, J. H. (2017). Source identification of beached oil at Al Zubarah, Northwestern Qatar. *Journal of Petroleum Science and Engineering, 149*, 107-113.

Arora, N. K. (2018). Bioremediation: a green approach for restoration of polluted ecosystems. *Environmental Sustainability volume, 1*, 305–307.

Jiang, Y., Brassington, K. J., Prpich, G., Paton, G. I., Semple, K. T., Pollard, S. J., & Coulon, F. (2016). Insights into the biodegradation of weathered hydrocarbons in contaminated soils by bioaugmentation and nutrient stimulation. *Chemosphere, 161*, 300-307.

kaogo, P. O., Ewuzie, U., & Onwuka, C. V. (2020). Environmental pollution: causes, effects, and the remedies. *Microorganisms for Sustainable Environment and Health*, 419-429.

Xingjian, X., Wenming, L., Shuhua, T., Wei, W., Qi Qige, J. P., Xinmei, G., . . . Hongwen, Y. (2018). Petroleum Hydrocarbon-Degrading Bacteria for the Remediation of Oil Pollution Under Aerobic Conditions: A Perspective Analysis. *Frontiers in Microbiology, 9*, 2885.