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THE APPLICATION OF BIOREMEDIATION OF OIL SLUGE IN AL-DURA REFINERY IN SOME PLANT

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ABSTRACT

Our research was accomplished after fifth months of continuous working from the 10th of February to the 10th of July.16th samples were collected from different places at Al-Dura refinery which included tanks, soils, refinery operation stages, waste accumulation area. The results could be summarized as follows: 50% of the collected samples had solid and semisolid in nature included soil and oily sludge. While the other 50% of the collected samples had a liquid in nature. The pH of the collected samples ranged from 4 to 14. The percentage of heavy metals concentration in the sludge waste samples were zinc 0.0017, ferrous 0.04, magnesium 0.0038, tin 0.0004, chromium 0.0003, cupper 0.0009, nickel 0.0006 and lead 0.0001. While the heavy metals concentration in the sludge tanks were 0.002, 0.042, 0.0064, 0.0002, 0.0016, 0.0007 and 0.0001 respectively. All the collected samples showed heavy growth after culturing on different agar media with pH=7 incubated for 24 hours at 37 C°. Different microbes were isolated included bacteria (both Gram –ve and +ve), fungi yeast and algae which their isolation percentages were 100%, 20%, 5%, 1% respectively. Depending on the microscopic examination, growth on different agar media and results of the biochemical tests, many isolated species were identified according to Bergey's manual for bacterial isolates. The Gram-ve isolated bacterial species were *Pseudomonas aeruginosa*, *Pseudomonas fluorescence*, *E. coli*, *Providencia*, *Xanthomonas* and *Proteus* while Gram+ve bacterial species were *Micrococcus*, *Methylococcus*, *Bacillus* and *Peptococcus*. The isolated fungi were *Penicillium*, *Aspergillus*, *Mucor* and *Rhizopus*. The isolated yeast was *Saccharomyces*.The isolated alga was *Spirogera*. Rapid – One system was applied to the bacterial strains selected for bioremediation process.

Keywords: Bioremediation, heavy metals, oil sluge, plant, parameter.

INTRODUCTION

Refining process releases numerous different chemicals into the atmosphere. On the side from air pollution there are also wastewater alarms[1].Sludge is a common term for solids separated from suspension in a liquid. It can also refer to the dense suspension obtained from unoriginal drinking water treatment[2].Oil sludge can be a major funder to internal incineration machine problems. Sludge is usually accumulate with use. Ways to minimize sludge production and accumulation includes accomplishment recurrent oil changes, using synthetic oil, and following the manufacturer's instrument care predictable[3]. Bioremediation bring up to the use of microbes to reduce pollutants that fake environment and particularly human risks, it has become an accepted remedy for cleaning polluted soil and water [4].

In such cases, soil and groundwater from the impure place are elated to a place (like a bioreactor), where conditions encouraging for biological degradation can be measured and improved[5]. Interface between heavy metals and plants is founded together heavy metals extraction or elimination by plants. Both processes are called phytoextraction which due to heavy metals accumulation and stabilization in plant biomass, preventing their further spread to deeper soil layers and groundwater[6,7].

In this study we are trying to create a balanced system containing different biotic and abiotic agents in order to remove the toxic products from our environment in a better safe way.

EXPERIMENITAL

Materials

1. Sample collection in sterile containers. Aseptic Hygiene when dealing with samples (gloves, masks, and aseptic lab environmental techniques and instruments).

2. Electronic Rapid One System Biochemical analysis for Identification from Remel/USA and other biochemical tests were also applied like urease test, Citrate utilization test, Motility, IMVIC and Triple sugar fermentation test. Agars and broth media were previously prepared according to the manufacturing company, it included the types as listed below:

Table-1: Agars and broth media

No.	Agar media	Manufacturing Company
1	Mineral Salt Agar media	(Prepared According to [4])
2	Nutrient agar media	Himedia
3	Brain-Heart Infusion agar media	Himedia
4	Blood Agar media	Himedia
5	MacConkey agar media	Himedia
6	Sabrauod Agar media	Himedia
7	PCAH* Agar media	(Prepared According to [4])
8	Brain Heart Infusion broth	Himedia

3. One bag of planting soil containing minerals including: Nitrogen, Phosphorous, Carbon, Calcium, Potassium, Magnesium with pH of 7.2%.
4. Pots of No.115 (450mm×200mm×140mm) (Made in China).
5. Seeds for planting included: Onion [Spring-Ishikura], Tomato [Shirley F1] (Made in United Kingdom) (As in this picture).
6. Water with Ozone treatment

Fig.-1: Seeds for Onion, Tomato plant

Methods

a. After preparing isolation agar media Petri dishes a twice inoculums of 0.1 ml of each sample were placed on each type of the agar media then incubated overnight in the incubator at 37°C. The Isolated microbes were identified by direct examination of the colony special characteristics and with microscope after applying the suitable procedure for preparing each examined slide. The biochemical tests were applied after isolation.

b. Applying Electronic Rapid One system biochemical analysis for highly specific identification of the bacteria. Applying each isolated colony for PCAH decomposing on PCAH media for further qualification.

c. The two qualified strains that were previously activated were inoculated on Brain Heart Broth media which was mixed with (1-5) ml of the oily sludge consequently with pH=7 in shaker incubator with 50 cycle/min for 24 hours at 37C°.The total aromatic hydrocarbons rate was determined in the broth media before and after 24 hours of growth were done in ISSC Laboratory.

d. Chemical analysis of heavy metals for the decomposed sludge were done in ISSC Laboratory .

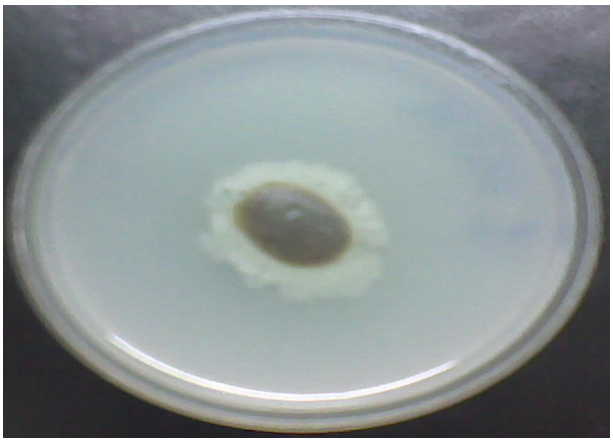
RESULTS AND DISCUSSION

A previous research was accomplished to treat the waste products produced from refining processes in Al-Daura refinery. The waste was treated successfully but the heavy metals accumulated and might be toxic for the living ecosystem in the soil. This problem is our major problem we decide to solve through this search Table-2.

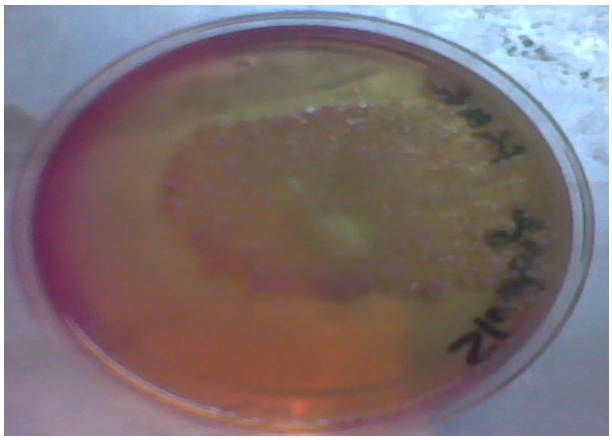
Table 2: The heavy metals was measured in the soil before process

Sample	Zn%	Fe%	Mg%	Sn%	Cr%	Cu%	Ni%	Pb%
Sludge waste	0.0017	0.04	0.0038	0.0004	0.0003	0.0009	0.0006	0.0001
Sludge tanks	0.002	0.042	0.0064	0.0002	0.0002	0.0016	0.0007	0.0001

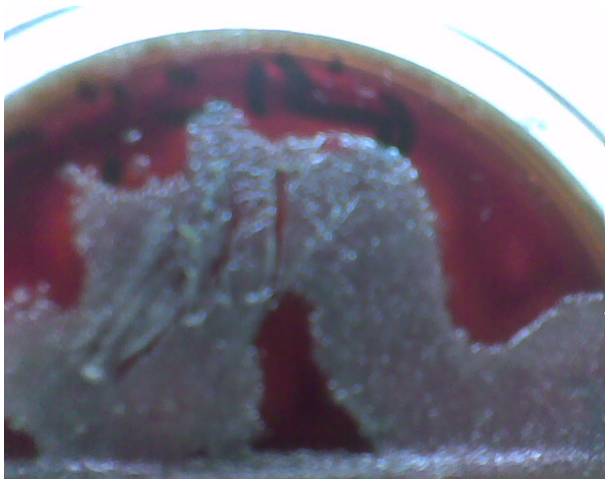
Providencia as a qualified bacterial strain grown on different agar media, which included Mineral salt agar, MacConkey agar with pale non lactose fermenting colonies, hemolysis on Blood agar, and PCAH agar, that showed the highly decomposing area by this bacterial strain (Figure-2).

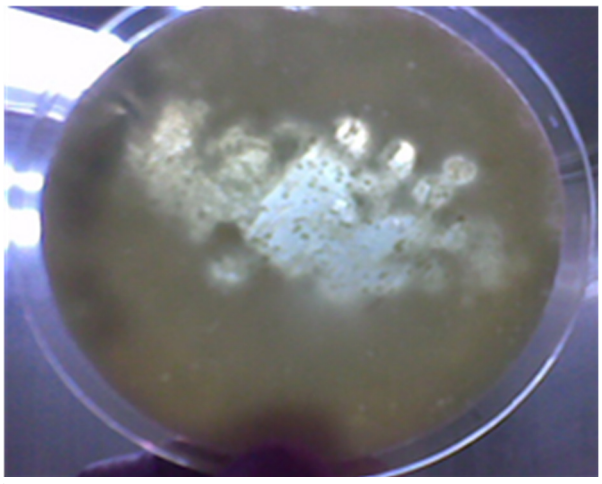


on Mineral Salt agar

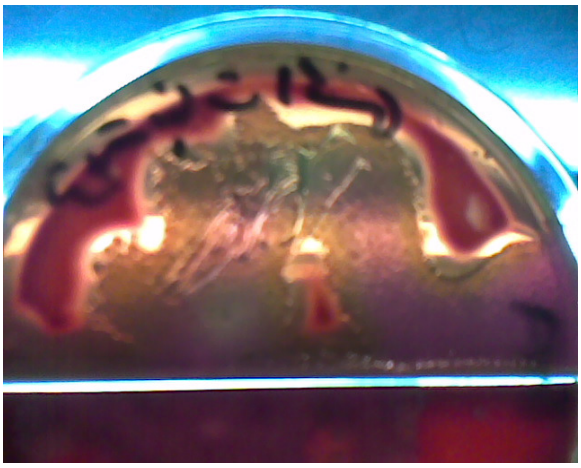


on MacConkey agar





on Blood agar



on PCAH agar

Fig.-2: Growth of the *Providencia* qualified strain on different agar media.

The complex consistency of the samples and its specific properties increased the ability of the growth of fastidious extreme tolerated microbes than other sensitive ones. Figure-3 showed the isolation rates in all samples, and how bacteria prevailed on other microbes according to the machineries they possess to live in such environment[8]. Similar results documented the dominance of bacteria than other microbes in highly concentrations of carbon[9,10].

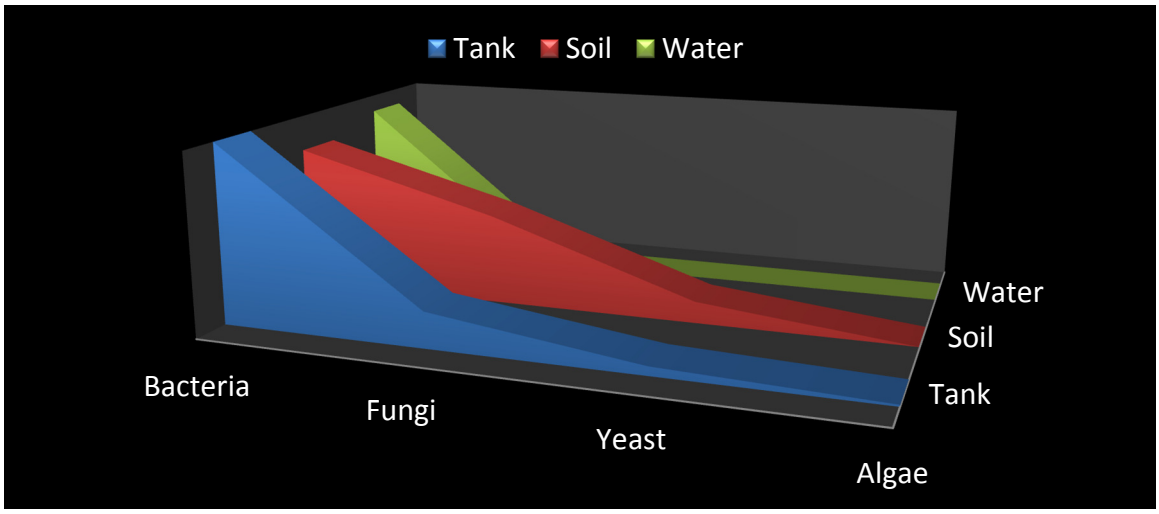


Fig.-3: Isolation percentages from the collected samples

All the isolated microorganisms had been identified according to the microscopic examination, biochemical test results and growth on different agar media which were listed in Table-3.
From the results we can put our hypothesis that the bacteria were the most isolated organism responsible for PCAH decomposition according to their pathway of degradation for complex molecules to get its energy, after changes in pH which would open the pathway for other degradable microbes as fungi, yeast and also algae to reach the Balanced environmental ecosystem in this low nutrient environment.Certain intelligences designated the structure and underlying forces of bacterial community elaborate in bioremediation of crude oil[11,12]; in this alteration a few group of bacteria were detected to rise the richness in response to oil corruption. During alternative revision off bacterial community on oil polluted soil[13] in Italy, informed that sequential stages of activation on bacterial population occur during bioremediation

treatment of oil in soil.Other studies documented similar results of the bacteria reducing the toxicity in soil contaminated with oil derivatives[14].

The heavy metals that were measured in the broth media before and after sludge consuming process refers that some of the heavy metals were reduced but not in high rates for *Providencia* and *Penicilium* as qualified strains (Table-4).

Table-3: The identified species isolated from the collected samples.

Type of the Microbe	Species	
Bacteria	Gram -ve	Gram +ve
	Pseudomonas aeruginosa	*Micrococcus*
	Pseudomonas fluorescence	*Methylococcus*
	E. coli	*Bacillus*
	Providencia	*Peptococcus*
	Xanthomonas	
	Proteus	
Fungi	*Penicillium*, *Aspergillus*, *Mucor* , *Rhizopus*	
Yeast	*Saccharomyces*	
Algae	*Spirogera*	

Table-4: The concentration of heavy metals in the broth media cultivated with *Providencia* and *Penicilium*.

5ml sludge inoculum		Sample No.	Zn%	Fe%	Mg%	Sn%	Cr%	Cu%	Ni%	Pb%
Before		1	0.0017	0.04	0.0038	0.0004	0.0003	0.0009	0.0006	0.0001
		2	0.002	0.042	0.0064	0.0002	0.0002	0.0016	0.0007	0.0001
After	*Providencia*	3	0.0025	0.045	0.024	0.0001	0.0002	0.0018	0.0003	0.0001
	Penicilium	4	0.000018	0.0001	0.0002	0.0002	0.0002	0.0001	0.0001	0.0001
1ml sludge inoculum		Sample No.	Zn%	Fe%	Mg%	Sn%	Cr%	Cu%	Ni%	Pb%
Before		1	0.0002	0.0007	0.0017	0.0003	0.0001	0.0002	0.0003	0.0002
		2	0.0001	0.0017	0.0012	0.0001	0.0001	0.0002	0.0001	0.0001
After	*Providencia*	3	0.0001	0.0004	0.0015	0.0001	0.0001	0.0008	0.0001	0.0001
	Penicilium	4	0.0001	0.0004	0.0037	0.0002	0.0001	0.0004	0.0001	0.0001

According to the documented studies, heavy metals in the soil had been reduced by mycorrhized fungus populations[15].Our results showed that *Penicilium* as the most qualified fungi in biodegradation process, when compound inoculums of bacterial and fungal content, the bioremediation would be faster and the rate of the heavy metals would be reduced. Also as a natural response, the ecosystem would be more balanced in the presence of two organisms opposing each other; as a result it would prevent the uncontrolled growth of each organism which were recorded by many studies suggesting to increase the microbial diversity of the inoculums in order to creat a multiple optional ecosystem for cleansing process[16]. In sterile conditions of pot experiment abed net of oily sludge was placed in the sterile soil followed by a filter bed of the inoculums was more successful for the plants than mixing the soil with the sludge directly. Using filter bed was more successful for the growing plants than inoculating the soil directly (Figure-3).Three types of seeds were planted including veggies (Onion [Spring-Ishikura], Tomato [Shirley F1]) and a herb garden (Coriander [Cilantro-for leaf]) they were all successful to develop a growing seedlings after 21 days which would refer to the poly aromatic hydrocarbon consumption in the soil by the microbial inoculums within the soil reaction that made the seedling grows a natural plants.

CONCLUSION

The existence of different microbial flora in soil sample collected with different chemical contents. Gram negative bacteria were more degradable than Gram positive bacteria. The development of the cultivated seeds depends on the way of mixing the soil with the sludge and the microbial inoculums. Two kinds of

veggie seeds and herb garden seeds showed a successful growing seedling. Heavy metals varied according to the active movement of those metals through the microbial flora cell wall. The rates of heavy metals inside the seedlings according to the plant resistant.

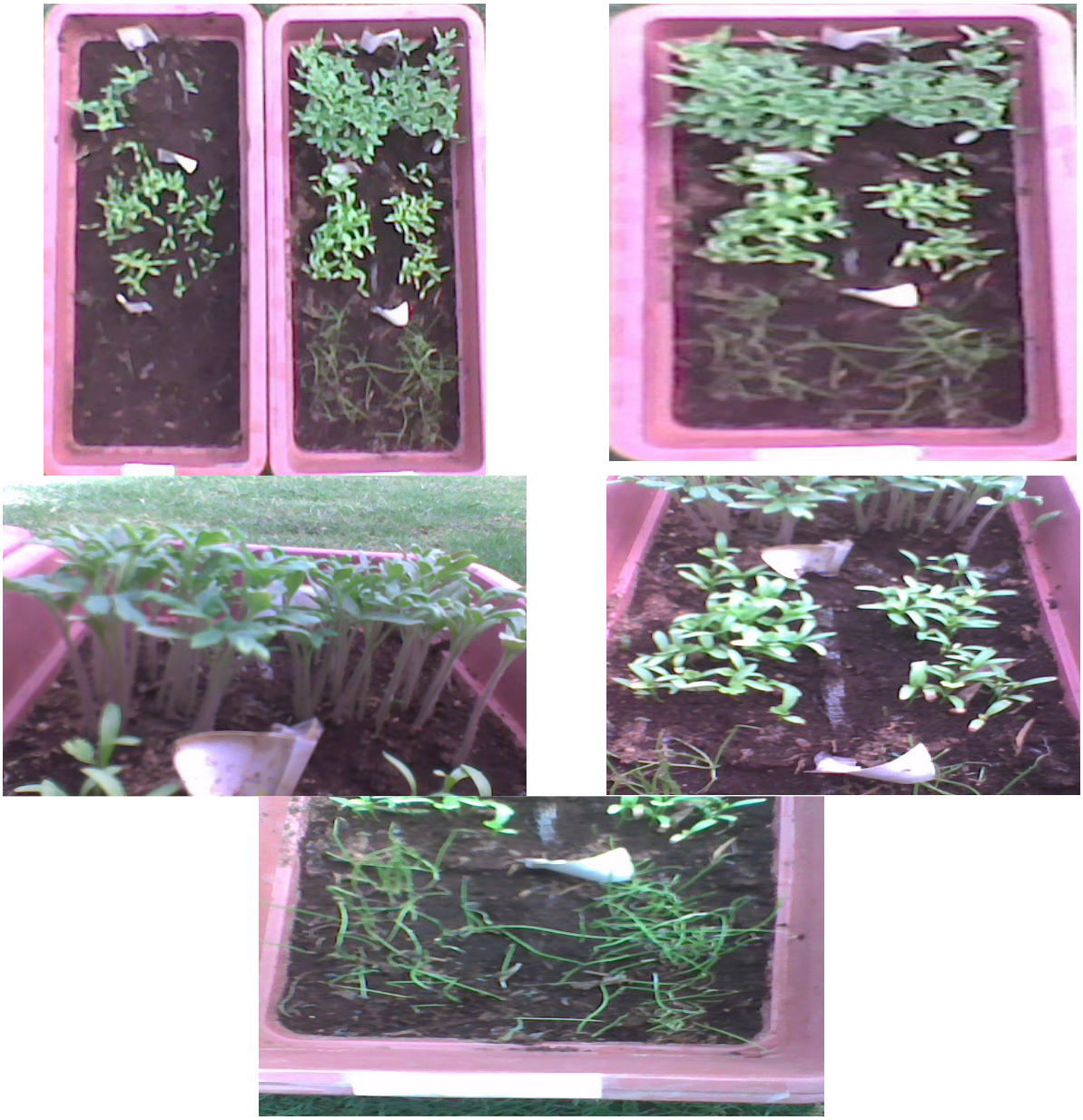


Fig.-4: development of the cultivated seeds

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