

Effect of Crude Oil Concentrations on Growth and Photosynthetic Pigments of *Helianthus Annus* Bio-Augmented with *Micrococcus luteus* and *Pseudomonas aeruginosa*

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Received: 02 July, 2020

Accepted: 03 September, 2020

Abstract: Bioremediation of soil contaminated with organic chemicals is a challenging problem in the environmental scenario. On the basis of identification of remediation capability of *Helianthus annus* (sunflower) assisted with *Pseudomonas aeruginosa* and *Micrococcus luteus* were used to investigate the soil contaminated with petroleum hydrocarbons (crude oil) at varying concentrations (25,50,75,100,125, &150ml/ kg of soil) in a pot experiment carried out in a green house. According to both qualitative and quantitative analysis of parameters including plant height, stem girth, number of leaves, leaf area and photosynthetic pigments including chlorophyll a, b and carotenoid indicated that *H.annus* showed peculiar tolerance to the higher concentration of crude oil concentration in the soil containing both micrococcus and pseudomonas (MP) (combined) consortium showing positive synergetic effect on *H.annus* in remediating the petroleum hydrocarbons at all concentrations more significantly at higher concentration of crude oil (150ml/kg) of soil. The results show significant increase in growth of (both *Micrococcus* and *Pseudomonas*) MP treated plant as compare to control plants; in control the leaf area 18.716 ± 1.256 , stem girth 3.40 ± 0.10 and plant height 90.72 ± 4.062 whereas in MP treated plant leaf area 19.65 ± 0.292 , stem girth 3.580 ± 0.048 , height 98.20 ± 3.852 respectively. On the whole *Helianthus annuus* is commercial oil yielding crop that can be effectively applied to phytoremediation of petroleum hydrocarbons assisted with *Micrococcus* and *Pseudomonas* is a significant treatment for remediating the soil contaminated with petroleum hydrocarbon.

Keywords: Sunflower, bioremediation, crude oil contaminants, phytoremediation.

Introduction

Petroleum refineries are most alluring for any national headway and upgraded individual fulfillment. In any case, the waste made by these causes unacceptable taint impacts (Ojumu et al., 2005). Most activities related with the age of oil-related products can introduce a certifiable natural issue like water, air, noise, warm, solid and land pollution (Abbriano et al., 2011; Beyer et al., 2016). The progression of oil adventures in new edges domains, the undeniable spillage that for the most part occurs in the midst of routine errands and records of extraordinary accidents in the midst of transportation has called for greater contamination of soil, rendering a critical issue especially in thickly populated countries, for instance, Pakistan.

Disposal of spent balm in essential openings, water channels, open plots, and properties is the more run of the mill practice especially by motor mechanics in oil siphons and workshops in the midst of changing of oil from the vehicles, control generators and diverse machines. In Pakistan, the present strategy for the unusual exchange of this waste oil fabricates defilement scenes in nature and it has been believed to be spread factor of crude oil defilement (Atuanya, 1987). Moreover rapid urbanization and industrialization have genuinely augmented the creating load of invention contaminants upon the soil. Genuine classes of common

contaminants of maritime and terrestrial organic networks are Poly-cyclic hydrocarbons (PAHs). These blends sway the extensive scale and scaled down scale vegetation and speak to a real hazard to human prosperity due to their genotoxic, mutagenic and malignant growth causing potential (Ashok and Musarrat, 2000) and persistence in the surroundings for a longer duration of time.

The presence of natural compounds in soil, such as hydrocarbons, poly-aromatic hydrocarbons, Polychlorinated Biphenyls, phenols, chloro-phenols, toluene, trinitrotoluene benzene, herbicides and pesticides inhibit growth and metabolic pathways of soil-associated microbes, even at very low concentrations (Oleszczuk, 2006; Porteous et al., 2006; Mackinnon and Duncan 2013; Sun et al., 2013). Furthermore, natural compounds can enter the food chain, due to the poisonous nature they can induce mutagenicity and carcinogenicity in animals and human beings (Mahanty et al., 2011; Guo et al., 2012; Havelcova et al., 2014). Therefore, the removal of these organic compounds from soil and water is one of the essential troubles in the discipline of environmental sciences and engineering (Cameselle et al., 2013; Chigbo et al., 2013; Wei et al., 2014; Zhu et al., 2014).

Crude oil is a fairly complicated mixture and a great source of variety of hydrocarbons (Cooney et al., 1980).

These crude oil compounds are divided into three generic groups such as saturated hydrocarbons, aromatic hydrocarbons and poly organic compounds (Huesemann and Moore, 1993). Crude oil is physical, chemical and biologically hazardous to the soil as it carries many toxic compounds (Franco et al., 2004). These toxic compounds penetrate the soil pores and thus restrict water and air transport that would be crucial for organic matter conversion (Caravaca et al., 2003). It inhibits the seed germination and plant growth in corn, wheat, and oats (Dorn and Salanitro, 2000), poisonous to the lettuce and oat root elongation (Henner, et

al.,1999). It produces toxic effects on the plant length, weight, and leaf chlorophyll content of the plant through lowering the leaf area (Hanson et al, 1997) generally, petroleum hydrocarbon compounds bind to soil components and is hard to dispose of or degrade.

In veneration of oil pollution, soil remediation techniques aim to prevent the further spread of pollutant and also its removal from the soil. The methods for removal of oil contamination include physio-chemical, thermal and organic techniques which includes bioventing, soil washing, rhizo-filtration, etc. are no

Table 1 Monthly Correlation between growth parameters of *H. annuus*.

1ST MONTH						2ND MONTH						3RD MONTH					
TREATMENT	PARAMETER	HEIGHT	L. AREA	LEAVES	GIRTH	TREATMENT	PARAMETER	HEIGHT	L. AREA	LEAVES	GIRTH	TREATMENT	PARAMETER	HEIGHT	L. AREA	LEAVES	GIRTH
25	HEIGHT	----	----	----	----	25	HEIGHT	----	----	----	----	25	HEIGHT	----	----	----	----
	L. AREA	-0.088	----	----	----		L. AREA	0.162	----	----	----		L. AREA	0.103	----	----	----
	LEAVES	-0.526*	0.091	----	----		LEAVES	0.757**	-0.062	----	----		LEAVES	0.543*	-0.087	----	----
	GIRTH	0.017	0.702	----	----		GIRTH	0.001	0.795	----	----		GIRTH	0.013	0.714	----	----
		0.620**	0.154	-0.32	----			0.628**	0.534*	0.283	----			0.575**	0.423	0.284	----
50	HEIGHT	0.004	0.517	0.172	----	50	HEIGHT	0.003	0.015	0.226	----	50	HEIGHT	0.008	0.063	0.225	----
	L. AREA	0.254	----	----	----		L. AREA	0.593**	----	----	----		L. AREA	0.334	----	----	----
	LEAVES	0.279	----	----	----		LEAVES	0.006	----	----	----		LEAVES	0.15	----	----	----
	GIRTH	0.388	-0.25	----	----		GIRTH	0.558*	.687**	----	----		GIRTH	-0.047	.535*	----	----
		0.091	0.295	----	----			0.011	0.001	----	----			0.845	0.015	----	----
75	HEIGHT	.775**	0.187	0.398	----	75	HEIGHT	.730**	0.700**	0.741*	----	75	HEIGHT	.581*	.700**	.668**	----
	L. AREA	0.001	0.481	0.082	----		L. AREA	0.001	0.001	0.001	----		L. AREA	0.016	0.001	0.001	----
	LEAVES	0.341	----	----	----		LEAVES	0.362	----	----	----		LEAVES	0.44	----	----	----
	GIRTH	0.141	----	----	----		GIRTH	0.117	----	----	----		GIRTH	0.052	----	----	----
		.758**	.468*	----	----			.664**	0.425	----	----			-0.093	0.425	----	----
100	HEIGHT	0.012	0.087	----	----	100	HEIGHT	0.001	0.062	----	----	100	HEIGHT	0.697	0.062	----	----
	L. AREA	.758**	.468*	1.000*	----		L. AREA	.664**	0.425	1.000*	----		L. AREA	-0.093	0.425	1.000**	----
	LEAVES	0.012	0.087	0.001	----		LEAVES	0.001	0.062	0.001	----		LEAVES	0.697	0.062	0.012	----
	GIRTH	0.012	0.087	0.001	----		GIRTH	0.001	0.062	0.001	----		GIRTH	0.697	0.062	0.012	----
		0.303	----	----	----			0.111	----	----	----			0.275	----	----	----
125	HEIGHT	0.194	----	----	----	125	HEIGHT	0.641	----	----	----	125	HEIGHT	0.241	----	----	----
	L. AREA	0.303	----	----	----		L. AREA	0.111	----	----	----		L. AREA	0.275	----	----	----
	LEAVES	0.194	----	----	----		LEAVES	0.641	----	----	----		LEAVES	0.241	----	----	----
	GIRTH	.465*	0.23	----	----		GIRTH	.354	-0.148	----	----		GIRTH	0.016	-0.08	----	----
		0.039	0.329	----	----			0.126	0.534	----	----			0.948	0.737	----	----
150	HEIGHT	-0.492*	0.087	-0.42	----	150	HEIGHT	0.633**	-0.143	.574**	----	150	HEIGHT	-0.054	-0.262	.450*	----
	L. AREA	0.028	0.715	0.064	----		L. AREA	0.003	0.546	0.008	----		L. AREA	0.823	0.264	0.046	----
	LEAVES	0.028	0.715	0.064	----		LEAVES	0.003	0.546	0.008	----		LEAVES	0.823	0.264	0.046	----
	GIRTH	0.028	0.715	0.064	----		GIRTH	0.003	0.546	0.008	----		GIRTH	0.823	0.264	0.046	----
		-0.032	----	----	----			0.418	----	----	----			-0.176	----	----	----
175	HEIGHT	0.893	----	----	----	175	HEIGHT	0.067	----	----	----	175	HEIGHT	0.457	----	----	----
	L. AREA	0.04	0.426	----	----		L. AREA	0.067	----	----	----		L. AREA	0.457	----	----	----
	LEAVES	0.868	0.061	----	----		LEAVES	0.067	----	----	----		LEAVES	0.457	----	----	----
	GIRTH	0.04	0.426	----	----		GIRTH	0.067	----	----	----		GIRTH	0.457	----	----	----
		0.268	-0.23	0.223	----			0.081	0.371	----	----			0.269	0.371	----	----
200	HEIGHT	0.253	0.338	0.345	----	200	HEIGHT	0.740**	0.361	.635**	----	200	HEIGHT	-0.015	0.02	0.587**	----
	L. AREA	0.253	0.338	0.345	----		L. AREA	0.001	0.118	0.003	----		L. AREA	0.951	0.933	0.006	----
	LEAVES	0.253	0.338	0.345	----		LEAVES	0.001	0.118	0.003	----		LEAVES	0.951	0.933	0.006	----
	GIRTH	0.253	0.338	0.345	----		GIRTH	0.001	0.118	0.003	----		GIRTH	0.951	0.933	0.006	----
		-0.004	----	----	----			0.289	----	----	----			-0.047	----	----	----
225	HEIGHT	0.987	----	----	----	225	HEIGHT	0.216	----	----	----	225	HEIGHT	0.845	----	----	----
	L. AREA	0.987	----	----	----		L. AREA	0.216	----	----	----		L. AREA	0.845	----	----	----
	LEAVES	-0.155	.529*	----	----		LEAVES	0.216	----	----	----		LEAVES	0.845	----	----	----
	GIRTH	0.513	0.017	----	----		GIRTH	0.216	----	----	----		GIRTH	0.845	----	----	----
		0.073	-0.05	-0.18	----			0.203	0.184	----	----			0.202	0.229	----	----
250	HEIGHT	0.758	0.652	0.447	----	250	HEIGHT	0.713**	0.028	0.022	----	250	HEIGHT	-0.219	-0.251	-0.195	----
	L. AREA	0.758	0.652	0.447	----		L. AREA	0.001	0.906	0.926	----		L. AREA	0.333	0.286	0.41	----
	LEAVES	0.758	0.652	0.447	----		LEAVES	0.001	0.906	0.926	----		LEAVES	0.333	0.286	0.41	----
	GIRTH	0.758	0.652	0.447	----		GIRTH	0.001	0.906	0.926	----		GIRTH	0.333	0.286	0.41	----
		0.113	----	----	----			-0.317	----	----	----			-0.097	----	----	----
DW	HEIGHT	0.635	----	----	----	DW	HEIGHT	0.173	----	----	----	DW	HEIGHT	0.683	----	----	----
	L. AREA	0.635	----	----	----		L. AREA	0.173	----	----	----		L. AREA	0.683	----	----	----
	LEAVES	-0.198	-0.35	----	----		LEAVES	0.173	----	----	----		LEAVES	0.683	----	----	----
	GIRTH	0.402	0.136	----	----		GIRTH	0.173	----	----	----		GIRTH	0.683	----	----	----
		-0.094	0.123	0.267	----			0.342	0.2	----	----			0.043	0.141	----	----
DW	HEIGHT	0.695	0.604	0.256	----	DW	HEIGHT	0.212	-0.526*	-0.01	----	DW	HEIGHT	-0.141	-0.517*	0.05	----
	L. AREA	0.695	0.604	0.256	----		L. AREA	0.212	-0.526*	-0.01	----		L. AREA	-0.141	-0.517*	0.05	----
	LEAVES	0.695	0.604	0.256	----		LEAVES	0.212	-0.526*	-0.01	----		LEAVES	-0.141	-0.517*	0.05	----
	GIRTH	0.695	0.604	0.256	----		GIRTH	0.212	-0.526*	-0.01	----		GIRTH	-0.141	-0.517*	0.05	----
		0.369	0.017	0.973	----			0.369	0.017	0.973	----			0.369	0.017	0.973	----

longer effective in elimination of natural pollutants (Knox et al., 1986). These techniques are laborious, expensive (Eckenfelder and Norris, 1993) and are not eco- friendly (Gautam, M., et al (2017).

In this dilemma Bio-remediation is the most attractive choice in which plant life and their associated microorganisms are used to degrade, include or render harmless contaminants in soil or groundwater (Duffy, T. M., & Cunningham, D. J. (1996). In essence, bioremediation employs human initiative to enhance the natural attenuation of contaminated sites. The techniques on natural, synergistic relationships among plants, microorganisms, and the environment, it does not require intensive engineering strategies or excavation

Phytoremediation has been used appropriately to remediate inorganic and organic contaminants in soil and groundwater. Various plants, inclusive of canola rapeseed plant (*Brassica napus* L.), oat (*Avena sativa*), and barley (*Hordeum vulgare*), sunflower (*Helianthus annuus*), lawn grass (*Cynodon dactylon*) (Banuelos et al.,

and microbes that leads to the rhizosphere effect. Some of the common microorganisms used as bio aid include *Pseudomonas*, *Arthrobacter*, *Alcaligenes*, *Corynebacterium*, *Flavobacterium*, *Achromobacter*, *Micrococcus*, *Mycobacterium*, and *Nocardia* are stated as the most active bacterial species in the degradation of hydrocarbons in soil (Bossert and Bartha, 1984). *Pseudomonas*, *Arthrobacter*, and *Achromobacter* regularly manifest in greater numbers inside rhizosphere soil than bulk soil (Walton, and Curry, (1994)). *Pseudomonas aeruginosa* was checked for its stress tolerance ability. Tewari and Arora (2014) reported the potency of plant growth promoting rhizobacteria for reclamation of stressed prone soil generated due to climatic variability and anthropogenic activities, to enhance crop production. *Micrococcus luteus* has been found to have plant growth-promoting properties under stress condition (Arun et al., 2012)

In this study sunflower and two different strains of bacteria are used to evaluate their synergetic effect on growth and photosynthetic pigment of plant grown

Table 2 Two-way anova (Duncan's Multiple Range Test) indicating effect of different crude oil concentration (C), treatments (T) and their interactions (C x T), from height, leaf area, girth, no. of leaves and photosynthetic pigments of *Helianthus annuus*

Factors	DF	<i>Helianthus annuus</i>							
		Height	Leaf area	Girth	No. of leaves	Chl A	Chl B	Chl A+B	Carotenoid
Concentration (C)	6	26.666***	32.115***	47.217***	36.812***	2.234*	68.760***	23.105***	6.457***
Treatment (T)	3	8.627***	15.828***	6.932***	2.380 ^{ns}	80.856***	246.229***	168.633***	29.402***
C x T	18	1.908*	1.367 ^{ns}	2.933***	1.717*	3.144***	12.395***	7.328***	1.546 ^{ns}
Error	112								

F- value***= significant at <0.001, ** = significant at < 0.01, *= significant at < 0.05, ns= non-significant

1997; Ebbs and Kochian, 1997; Brown et al., 1994). Alamo switchgrass (*Panicum virgatum*) (Entry and Watrud, 1998).

Plants and microorganisms are involved, each proximately and eventually, in the degradation of petroleum hydrocarbons into products (e.g., alcohols, acids, carbon dioxide, and water). These compounds are normally less toxic and less persistent in the surroundings than the parent compounds (Eweis et al., 1998). The microorganisms can degrade petroleum hydrocarbons independently. It is the interaction between plant and microorganisms (i.e., the rhizosphere effect) which serve as the principal mechanism responsible for petrochemical degradation. There is a significant form of data available related to the indirect roles performed through the plants in the degradation of petroleum hydrocarbons. These roles comprised of: (i) the supply of root exudates that cause the rhizosphere effect and embellish co-metabolic degradation, (ii) the release of root-associated enzymes successful of reworking organic pollutants, and (iii) the physical and chemical consequences of flowers and their root structures on soil stipulations (Gunther et al. 1996). These root exudates are the association between flowers

under hydrocarbon in soil.

Pakistan is an agricultural country and key fraction of the economic system is based on the manufacturing of a number of agricultural products inclusive of vegetables and fruits, cereals and vegetable oil. Due to diverse agronomic traits such as tolerance to varied climatic and soil conditions Sunflower is used in diverse situations for remediation of toxic compounds including hydrocarbons from soil (Paniego, et al., 2007).

The present study aims to investigate:

To evaluate the phytoremediation potential of *Helianthus annuus* assisted with *Pseudomonas aeruginosa* and *Micrococcus luteus* for remediation of total petroleum hydrocarbons contaminated soil by investigating the growth and chlorophyll content

Materials and Methods

Plant Material and Soil

The study was carried out in the green house of Biotechnology Lab of PCSIR Lab Complex Karachi.

The plant used in the study was *Helianthus annuus* (Agsum- 5264 Thailand) obtained from seed certification section of Plant Protection department, Malir Halt Karachi, Pakistan. Washed and autoclaved sandy loam soil packed in plastic bags was used for the experimental study. The crude oil used was obtained from Hydrocarbon Development Institute of Pakistan (HDIP), Korangi Karachi.

The chemical composition of the crude oil used in this research is given in Table 1:

Appearance/ color	Dark brownish liquid
Density at 15°C	0.944 g/cm ³
Specific gravity at 60°F	0.945
API gravity	18.2
Reid vapor pressure	7Kpa
Viscosity	75.9
Moisture	3175ppm
Pour point	-10°C
Flash point	95°C

The experimental soil (5kg/ pot) was packed into plastic pots (19cm in diameter and 22cm in height) each pot containing five seeds, after germination of the seeds thinning of the plants was performed leaving single plant per pot to avoid inter specific competition among the plants

The bacterial strains used in the study were *Micrococcus luteus* (ATCC 49732) culture obtained from Microbiology Labs of PCSIR Lab Complex Karachi and *Pseudomonas aeruginosa* (ATCC 27853) culture from Microbiology Labs of PCMD, ICCBS Karachi.

One ml of bacterial culture (*Micrococcus luteus*) was added to 50ml of Trypton Soya Agar media (Oxoid) in a conical flask and incubated for 24 hrs.in an orbital incubator at 37°C and 150 rpm. The optical density (OD) at an absorbance of 0.5 at 600nm was measured and then harvested as reported by (Wu *et al*, 2014)

One ml of bacterial culture (*Pseudomonas aeruginosa*) was added to 100 ml Luria Bertani (LB) media (Oxoid) supplemented with 1% (w/v) crude oil for acclimatization in a conical flask and is incubated for 48hrs. At 37°C in dark, centrifuged it twice for 20mins. at 10000 rpm by an orbital shaker as reported by (Fatima *et al*. 2015).

Experimental Design

The pot experiment was arranged in the set of 4 treatments as described below:

Control = uncontaminated soil + plant
T1= contaminated soil + plant (untreated soil)
T2= contaminated soil + plant + <i>Pseudomonas aeruginosa</i>
T3= contaminated soil + plant + <i>Micrococcus luteus</i>

T4= contaminated soil+ plant+ *Pseudomonas aeruginosa*+
Micrococcus luteus

In this experiment 6 specific concentrations of crude oil have been used that are 25ml, 50ml, 75ml, 100ml, 125ml, 150ml and 5 replicates for each treatment had been designed. Plants developing in sandy loam soil with NPK fertilizer (20:20:20) fertilizer was used as the nutrient source supplied weekly to the plants.

The pots were kept in the green house under the prevailing natural conditions (28°C- 30°C) 30-60% relative humidity) on ground in completely randomized design. Two hundred milliliters of distilled water were supplied to each pot on every alternate day (Basumatary *et al*, 2012).

The study was conducted for the period of 3 months plant girth, height and leaf area was recorded after 30, 60 and 90 days. The plants were harvested 90 days after planting and analyzed for the chlorophyll pigment

Growth parameters were monitored a month after the germination (Odejegba and Sadiq 2002). Plant height were measured with the help of measuring tape (cm) from the soil level to the terminal apex of the plant. The stem girth was measured by digital Vernier caliper. The number of leaves were counted, while the leaf area was determined with the leaf area meter (C1-202 Area meter CID, Inc. USA). All these measurements were carried out monthly until the termination of the experiment.

Chlorophyll a (Chl a), chlorophyll b (Chl b) and total chlorophyll (TC) concentrations were determined following the method of Shabala *et al*. (1998) and the total carotenoids (Cx+c) concentration was determined following the method of Lichtenthaler (1987).

Statistical analysis

All Statistical analysis of the collected data was performed using two-way analysis of variance (ANOVA). The Duncan multiple range test (1955), was used to determine the statistically significant difference between treatments at $p < 0.05$. All data is shown as means $\pm$ standard deviation of five independent replicates of every treatment using a completely randomized design (CRD).

Results and Discussion

The chlorophyll production in the fresh leaves of *H.annuus* in control plants and bio- augmented plants were estimated. Chlorophyll 'a' content of *H. annuus* leaves shows (Fig. 1) significant increase in P, M and MP treatments ($p < 0.05$) at different concentrations of crude oil as compare to control. Overall progressive increase in the chlorophyll a content (24.51 ± 0.019 , 28.16 ± 2.64 and 28.36 ± 2.76 mg g⁻¹ of FW) was observed in these treatments especially at higher concentrations (100,125 and 150ml) respectively. The M treatment shows marked increase in the chlorophyll a content at 100 ml (27.03 ± 3.69 mg g⁻¹ of FW) as compare to

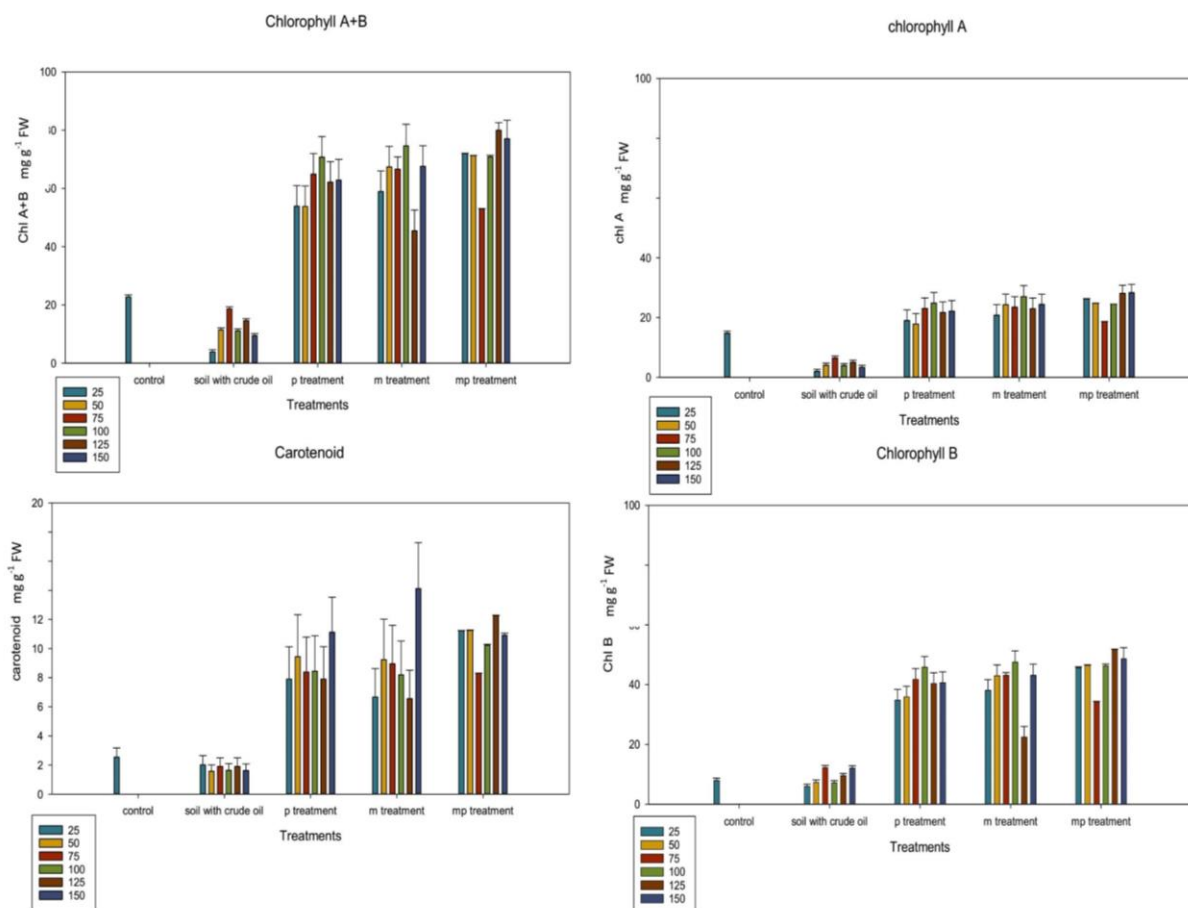


Fig: 1 Chlorophyll A, B total chlorophyll and carotenoid content of plant of MP, P, M and untreated soil treatments grown under different concentrations of crude oil

control, P and MP treatments, whereas MP treatment overall shows marked increase as compare to the other three treatments respectively. Similar trend was observed in carotenoid content (Fig. 1) where M treatment showed marked difference at 150ml (28.36 ± 2.76 mg g⁻¹ of FW) as compare to the rest of the three treatments (control, P and MP). The Chlorophyll 'b' content shows highest activity at higher concentration (100, 125 and 150 ml) in MP treatment (46.34 ± 0.54 , 51.81 ± 0.046 and 48.66 ± 3.69 mg g⁻¹ of FW) as compare to M, P and control treatments. Similar trend was observed in total chlorophyll content as well. Statistical analysis of the result stated significant variance ($p < 0.05$) in treatments, concentrations of crude oil and positive interaction of plant species to the stress. The chlorophyll content of *H. annuus* control at all concentrations of crude oil was lower as compare to the plants treated both *Pseudomonas* and *Micrococcus spp.* The result showed significant increase in the chlorophyll content of plant treated with MP at higher concentrations of crude oil (100, 125, 150ml), the mean with SD chlorophyll content was observed to be 77.60 ± 16.63 , 79.98 ± 5.89 and 77.03 ± 14.32 μ g/g as compare to the control plants having mean value with SD as 11.13 ± 1.58 , 14.62 ± 1.58 and 9.48 ± 1.57 at same concentrations. Similar results were reported by

Odjegba and Sadiq (2002) that also showed negative effects of crude oil on chlorophyll synthesis in leaves of *A.hybridus* plant grown in contaminated soil

As per correlation analysis (Table 1) in growth parameters height of the *H. annuus* in the first month showed marked difference in P treatment ($p < 0.05$) as compare to control, untreated, M and MP treatment whereas in 2nd and 3rd month the height of the plant showed substantial increase in MP treatment as compare to the other three treatments respectively. On the contrary, the other growth parameters including stem girth, number of leaves and leaf area in all three months showed substantial increase in MP treatment as compare to the remaining three treatments.

The results showed marked difference between control and other bio-augmented soil. The control plant showed reduced growth as compare to the plants assisted with microbial consortium. The height of the control plant confirmed decreased growth at higher concentration of oil. Similar results were investigated by (Anoliefo and Vwioko, 1995) in *Capsicum annum L.* and *Lycopersicum esculentum* and stated the definite retardation of mean height and leaf area of *C. annum* in soil treated with higher concentration of oil.

After Four weeks *H. annus* (Fig 3) shows reduction in the leaf area and the number of leaves in the plants grown untreated soil at higher concentration of crude oil. By the end of the 12th week (3rd month) the reduction in the leaf area and the number of leaves were observed to decrease persistently (Smith et al., 1989) mentioned that insufficient aeration resulting from oil contamination in soil caused stem rot, which consequently reduced (Mohammed and Muhammed 1990) had been of the opinion that reduction in leaf area could be due to general reaction of plant to pollutants at physiological and biochemical levels. Petroleum

week similar consequences were observed in terms of stem girth of the plant (Fig. 2).

The effect of bioremediation was examined with *Pseudomonas aeruginosa* and *Micrococcus luteus*. On phytotoxicity as measured by *H. annus* growth, significant differences were observed in treated and untreated soil. At the end of the 4th week (1st month) of the experiment, the height of the plant shows significant increase in the soil treated with *Pseudomonas spp.* As compare to the *Micrococcus* and MP treated soil. Later in the 2nd and 3rd month of the experiment the MP treated soil showed substantial increase in the height of the

Growth parameters

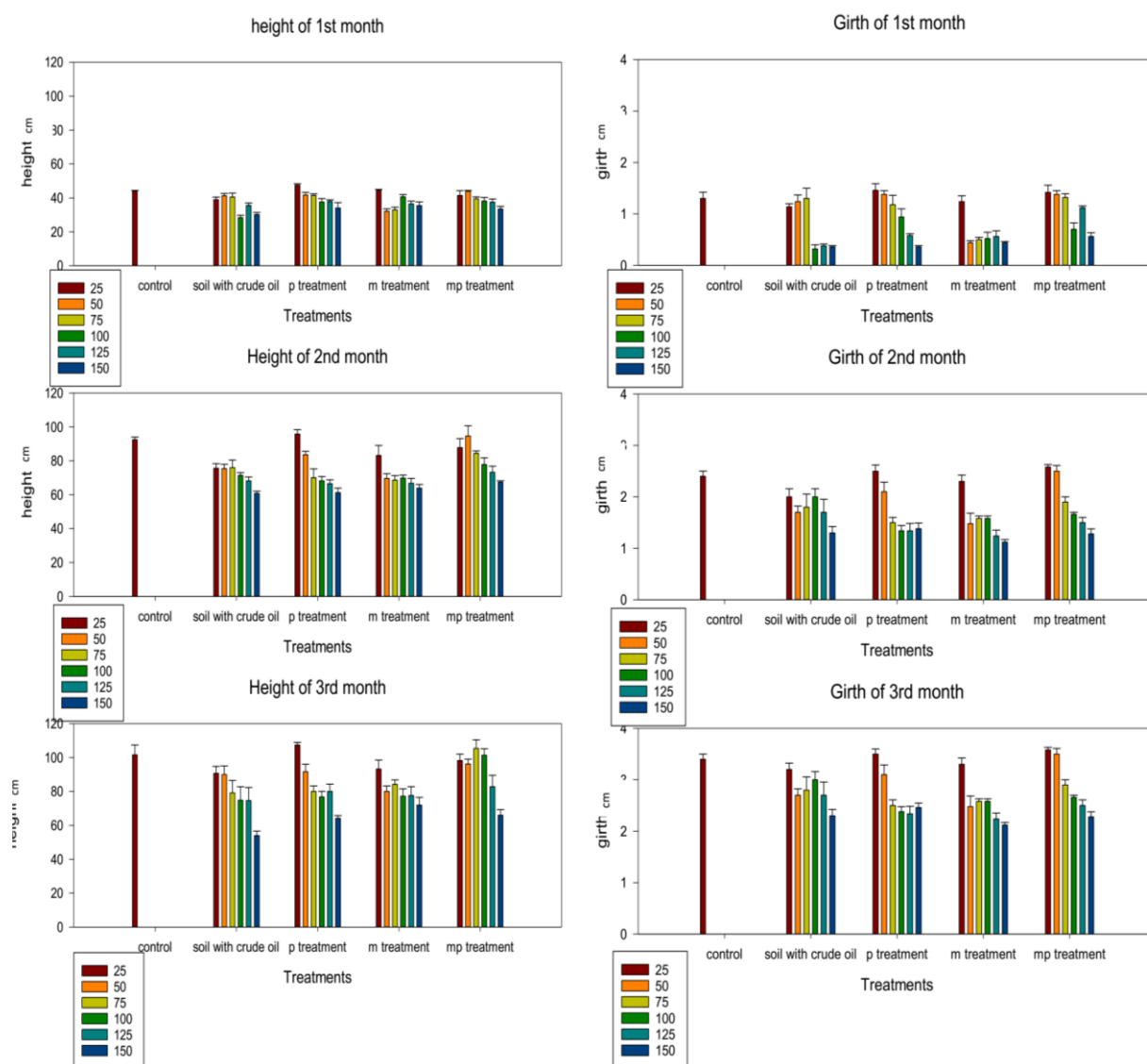


Fig. 2: Height and stem girth of plant of MP, P, M and untreated soil treatments for 1st, 2nd and 3rd months grown under different concentrations of crude oil

hydrocarbons produces negative effect on plants in terms of reduction of growth parameters such as root length and leaf area Rahbar et al., (2012). Plant growth in control plants in comparison to the plants treated with MP consortium produced significant proliferation in the number of leaves and leaf area of *H. annus* from four weeks after planting till the end of the experiment 12th

plant as compare to the other 3 treatments. It indicated that more hydrocarbons were degraded in presence of *Micrococcus* (Sandrin and Maier, 2003) and *Pseudomonas spp.* as compare to the other treatments.

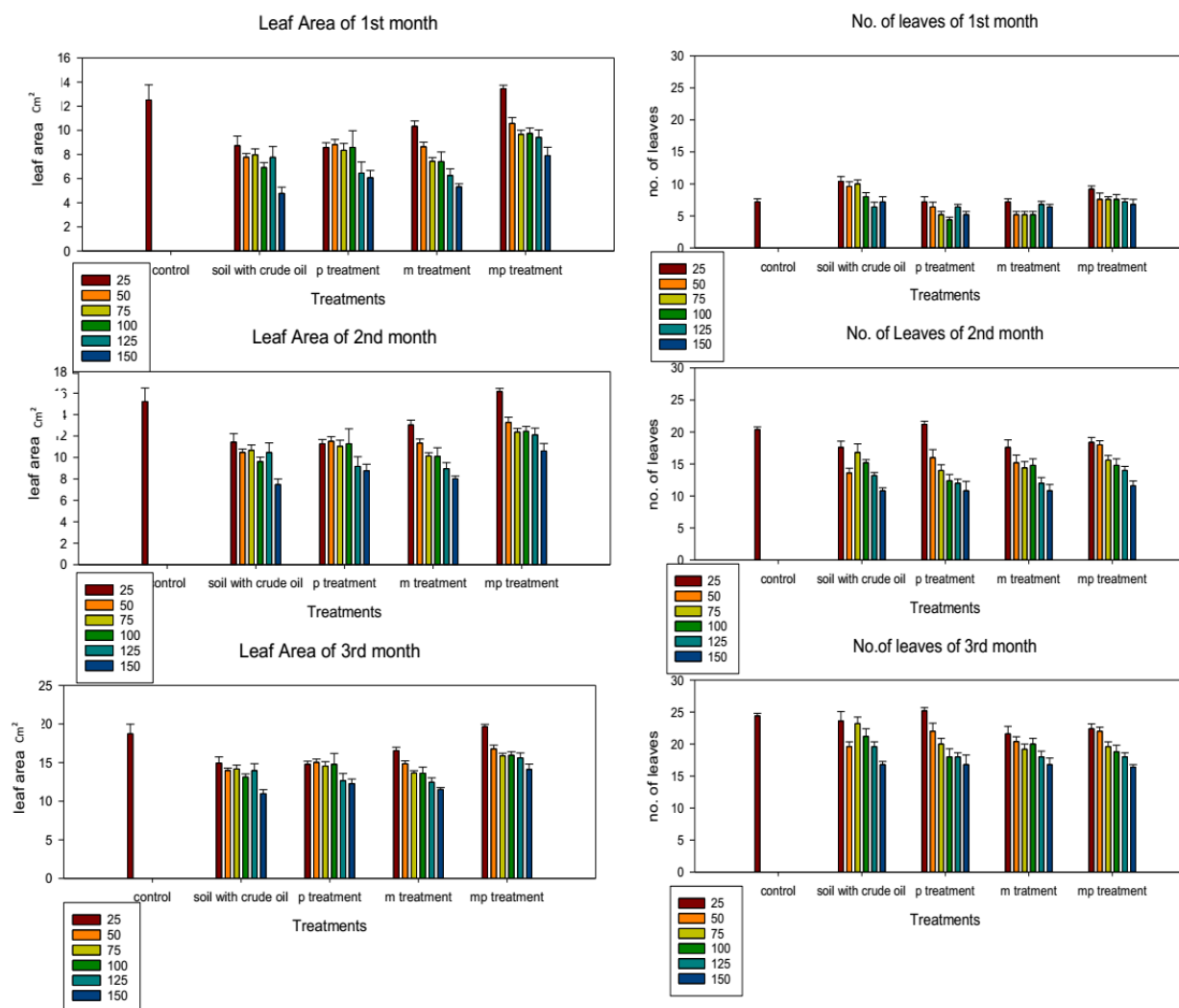


Fig:3 leaf area and No. of leaves of plant of MP, P, M and untreated soil treatments for 1st, 2nd and 3rd months grown under different concentrations of crude oil

Conclusion

The present study reveals novelty of the research regarding the synergistic effect of two bacteria *Pseudomonas aeruginosa* and *Micrococcus luteus* bio-augmented with *Helianthus annuus* grown in soil contaminated with different concentrations of crude oil exhibit distinct increase of growth and photosynthetic activity of the plant. However, in comparison with *Micrococcus* and *Pseudomonas*, the combined effect of both spp. MP (*Micrococcus* and *Pseudomonas* spp.) showed substantial increase in growth and photosynthetic activity of plant especially at higher concentration of crude oil.

Acknowledgement

Special thanks are owned to the Hydrocarbon Developing Institute of Pakistan (HDIP) Korangi Karachi and PCSIR Lab Complex Karachi, Pakistan for the skillful and technical assistance.

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