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Effect of bioremediated soil on growth of different plant species

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ABSTRACT

The bio-soil park at Ahmadi was established to assess the suitability of bio-remediated soil for greenery landscape planting in Kuwait. Two parallel experimental plots were made using bio-remediated and agricultural soil. The growth of four grass species (*Cynodon dactylon, Paspalum vaginatum, Pennesetum rubra,* and *Zoysia tenuifolia)* and five ground cover species (*Carissa grandiflora, Gazania uniflora, Ipomea palmata, Rosemarinus spp.* and *Wedelia trilobata)* in bio-remediated soil was compared with agricultural soil. The experiment was conducted using split-plot technique in triplicate randomized complete block design (RCBD). Periodic plant samples were collected and prepared for statistical analyses. The growth was determined on the basis of above ground biomass production of the plant species. It was concluded that the two soil types were similar in their effect on plant growth, as all the plant species showed non-significant growth differences. It is therefore concluded that bioremediated soil may successfully be utilized for greenery development programs.

Keywords: Agricultural, biomass, bioremediated, greenery, landscape.

INTRODUCTION

The Iraqi occupation of Kuwait in 1990 resulted in widespread oil pollution. Long term consequences of the Iraqi aggression include soil compaction, surface sediment disruption, soil corruption with oil, ground water pollution and deteriorated growth and visual impact of vegetation. Over 500 oil wells were set on fire by Iraqi soldiers during the occupation and withdrawal period (Al-Awadi *et al.* 1995). It was the massive release of oil that made the Gulf war the most notorious and created one of the worst man-made environmental disasters of all time. Blazing and gushing oil wells spewed approximately 60 million barrels of oil onto the land forming over 330 oil lakes that covered an area exceeding 49 km^2 (Al-Zalzaleh *et al.* 2001). The comparison of remote sensing Landsat Thematic mapper data taken in 1995, with that of 1987, indicated both heavily saturated and lightly contaminated soils (Kwarteng & Al-Ajmi 1996, Kwarteng 1998).

Negative impact of oil pollution on soil

In addition to its direct effects, oil pollution affects the soil properties indirectly, including oxygen depriviation due to greater microbial activities in contaminated soils, increased water repellence, disruption of soil texture and alteration of the soil-water relationship (Guo & McNabb, 1992, Sawatsky & Li 1997, Li *et al.* 1997). Under these conditions, an anaerobic environment is created in the contaminated soil which may lead to the generation of toxic compounds such as H_2S. Oil-degrading microorganisms also compete with plants for nutrients. These factors ultimately affect plant growth and development. Water repellency in hydrocarbon-contaminated soils is influenced by its water content and becomes problematic only in low-moisture regimes (McNabb *et al.* 1992). Dekker & Ritsema (1994) introduced the concept of soil moisture levels to describe the water content at which soils are no longer water repellent. Sawatsky and Li (1997) suggested critical water contents of 20% (contaminated soil) and 18% (bioremediated soils) above which the sorptivity of contaminated soil was near that of control soil. Infilteration of water into air-dried, contaminated soils was up to the order of magnitude slower than the control soil and remained constant over time. It is important to ascertain the effects of these changes on the growth and landscape qualities of ornamental plants.

Negative impact of oil pollution on plant growth

Petroleum contamination of agricultural soils generally has strong negative effects on plant growth. The mode in which petroleum molecules act on plants is a complex phenomenon involving both contact toxicity and indirect deleterious interactions with other molecules. The extent of toxicity depends on the type, polarity and molecular weight of the hydrocarbons (McGill *et al.* 1981). Low boiling petroleum components are readily removed from the biological surface layer in moist, well-drained soils through evaporation and leaching (Hunt *et al.* 1973). Oil pollution increases the anther sterility and meiotic irregularities and inhibition (Malallah *et al.* 1996a, 1996b, 1997, 1998).

Preliminary research conducted at Kuwait Institute for Scientific Research by Zaman and Al-Sidrawi (1993), Al-Houty *et al.* (1993) and Omar and Zaman (1995) highlighted the negative impact of oil pollution on the structure, species composition and productivity of native vegetation of Kuwait. High levels of heavy metals and hydrocarbons were detected in plants covered with oil mist and oil spills (Zaman & Al-Sidrawi 1993). The phenological procession of Kuwait' desert plants was altered due to black clouds, which affected the temperature and rainfall patteren (Zaman 1998). The severity of the negative impact varied in general with species, and the quantity and quality of oil pollutatnts. Most perennial plant species were severely damaged by oil pollution (Zaman 1998). Phytotoxicity tests with bioremediated soil showed differential responses to residual pollutant levels, particularly when the contamination was heavy. Broad beans and alfalfa were more sensitive to residual hydrocarbns than barley and bermuda grass (Al-Awadi *et al.* 1998b). The quality of grass is affected by soil texture and structure. Root biomass can affect the top growth. Plants grown in sandy soil exhibit a different root system than if they are grown in clayed soil. This difference may have a significant impact on the physiological activities of plants. The availability of nutrients from organic matter has pronounced results on the physiological and morphological traits of plants (Al-Zalzaleh 1999). Poor plant growth may result from the presence of high levels of toxic elements, and the damage from the presence of hydrocarbons in the soil. The effects of crude oil on plant growth are not well established.

MATERIALS AND METHODS

Site Selection

The experiment was laid out in a park-like setting for research as well as recreational purposes. The site was selected in the Ahmadi Governorate as suggested by the Kuwait Oil Company (KOC). The Bioremediated Soil Park is situated across the road from the Governorate building. This site was selected due to its adequacy according to the site selection criteria, dramatic topography, higher visual exposure and the benefit of a parking lot serving the adjacent kindergarten.

Field study

The experimental material consisted of bioremediated soil,agricultural soil and plant species.

Bioremediation of oil-contaminated soil

In 1994, Kuwait Institute for Scientific Research initiated a joint research program with Japan Petroleum Energy Center (PEC) to demonstrate biological technologies for the remediation and rehabilitation of oil lakes. Bioremediation is an innovative technology which can be very effective in treatment of petroleum hydrocarbon contamination. The technology is based on exploitation of the ability of soil microorganisms to degrade petroleum hydrocarbon constituents and use them as carbon source for growth and energy. (Atlas 1981, Balba *et al.* 1996). The field demonstration of soil bioremediation was carried out on oil-contaminated soil excavated from an oil lake bed in the greater

Burgan oil field. Four different bioremediation technologies, (a) Landfarming, (b) Windrow Composting Piles, (c) Static Bioventing Piles, and (d) Windrow Composting Using Pile-turner, were used for bioremediation.

Total extractable matter

Before extraction, anhydrous. Na_2SO_4 was added according to the moisture content and 1-10 gm of soil sample were extracted with 35 ml CH_2Cl_2 using the extraction unit. The evaporated CH_2C1_2 was collected, the oil extract was weighed and TEM calculated.

Extraction

Anhydrous Na_2SO_4 was added to 1-10 gm soil sample according to the moisture content, soil was then extracted with 70ml CH_2Cl_2 using the sonicator method (45 mins sonication). Deuterated Internal Standard containing 4 PAR's were spiked in the sample before extraction to check the recovery data. After extraction, the oil extract was filtered and the extract solvent was exchanged to cyclohexane. The exchange was performed by adding 4 ml of cyclohexane following reduction of the sample extract to 1-2 ml. The final extract volume was 2.0 ml.

Silica gel clean up (Method 3630 C)

The column was prepared with a slurry of 10g of activated silica gel in methylene chloride and placed into a 10 mm chromatographic column. Anhydrous sodium sulfate (1-2 gm) was added to the top of the silica gel.

Application of bioremediated soil

The top 60-cm layer in the experimental area was replaced with bioremediated soil and compacted uniformly to provide the required anchorage for plants. The total area for bioremediated soil treatment consisted of 5,600 m^2. The top 60-cm layer in the remaining area was replaced with agricultural soil which consisted of 7,600 m^2 including the separater.

Plant material

A broad range of plant material was selected to test the suitability of bioremediated soil. Plants were grouped into two categories: ground covers and turf grass. Each group contained the species included in the plant palette of the National Greenery Plan (NGP) of Kuwait. Four grass species (*Cynodon dactylon, Paspalum vaginatum, Pennesetum rubra,* and *Zoysia tenuifolia)* and five ground cover plant species (*Carissa grandiflora, Gazania uniflora, Ipomea* *palmata, Rosemarinus spp.* and *Wedelia trilobata)* were selected for the present study. These species were selected due to their vital importance in the Kuwait greenery and landscape projects.

Planting

Planting holes, appropriately 30 X 30 X 30 cm in size were prepared for ground covers and plants measuring 1-gal or 15-cm containers were planted.

Sods or stolons of turf grasses were planted directly in the ground. Container-grown plants or specimen plants were used for planting. After placing the plants in the planting holes, the vacant spaces were backfilled with a 1:1 (v/v) mixture of soil and sphagnum peat moss to enhance the water holding capacity and improve physical properties of the soils.The mixture was pressed lightly to provide adequate anchorage for the plants.

Fertilizer application & irrigation

The fertilizer 12-8-8 at 80g/m^2 was applied four times a year to ground covers whereas for turf grasses 15-15-15 at 168g/m^2 per month was applied. Brackish water mixed with fresh water (1:1) was used for irrigation. Irrigation water was applied through either drip irrigation i.e., for ground covers, or sprinklers i.e., turf grasses. The quantity of water applied to each group was varied according to the plants' requirements.

Experimental design and data recording

The experiment was laid out in a split-plot design with soil type (i.e., agricultural soil and bioremediated soil) as the main plots and species as subplots. Each plant group was tested separately. Plant species were evaluated on a plot area basis. Plant samples were taken after every three months and dried in the oven at 80^{0}C.

RESULTS AND DISCUSSION

Properties of bioremediated and agricultural soils

Landfarming proved to be the most effective bioremediation technology, followed by windrow composting and bioventing technologies (Al-Daher & Al-Awadhi 1998). These methods resulted in significant reduction in total petroleum hydrocarbons (TPH) and total alkanes. The physical and chemical characteristics of bioremediated and agricultural soil are given in Table 1. The physical and chemical characteristics of the bioremediated and agricultural soils showed that the bioremediated soil was darker (due to its petroleum contents) in color and had a relatively higher electrical conductivity (EC) and lower pH than the agricultural soil. The low pH is due to total petroleum hydrocarbon (TPH) and lower $CaCO_3$ contents. The TPH and total extractable matter (TEM) were higher in the bioremediated soil. Particle size distribution indicated a coarse sandy textural class for both soils. Primary soil particles were dominant in the sand fraction. In both soils, similar distributions of various fractions of sand were found. However, they differed slightly in their silt and clay fractions. The bioremedated soil consisted of relatively more coarser fractions (> 2 mm) composed mainly of white to brownish quartz grains.

Table 1: Physical and chemical properties of bioremediated and agricultural soil used in the biosoil park

Parameter	Unit of Measurement	Bioremediated Soil	Agricultural Soil
EC (1:2)	dSm^{-1}	3.34	0.30
pH (1:2)	-	7.41	8.35
$CaCO_3$	% eq	6.04	8.44
SAR	$(mmols/l)^{0.5}$	7.02	0.85
Munsell color	dry	2.5Y 4/1 (dark gray)	2.5Y7/2 (light gray)
Textural class	USDA (1993)	Sand	Sand
TPH (FTIR)	%	0.56	0.06
TEM (FTIR)	%	1.24	0.07
CM	%	3.77	0.79
Moisture	%	0.64	0.72

EC = Electrical Conductivity; SAR = Sodium Adsorption Ratio; TPH = Total Petroleum Hydrocarbons; TEM = Total Extractable Matter; FTIR = Fourier transform infrared; CM = Combustable Matter

Turf grasses

The data recorded on the growth performance, as measured by the extent of plant cover and biomass production are presented in Table 2. The standard analysis of variance technique (Steel & Torrie 1960) was applied for statistical analysis. The analysis of variance (ANOVA), as shown in Table 3, clearly indicates that the Fischer ratio calculated (FRc) for soil is less than the Fischer ratio tabulated (FRt) at the 5% alpha level, which confirms that the two soil types are similar in their effect on biomass production. However, the FRc for plant species is higher than the FRt at 5% alpha levels, which indicates a significant difference between the means for the plant species. The FRc for interaction (S X PS) is smaller than the FRt at a

5% alpha level, which confirms the absence of interaction between the soil and the plants. The least significant difference (LSD) test was applied to separate the means; the results are presented in Table 4.

Table 2: Performance of ground covers and turf grasses in bioremediated and agricultural soils

Species	Bioremediated Soil Plant Coverage Biomass (DW, g/m^2)			Agricultural Soil Plant Coverage Biomass (DW, g/m^2)		
Day	90	120	210	90	120	210
Ground covers						
Carissa grandiflora	75	385	119	70	265	72
Gazania uniflora	80	1249	134	80	1559	106
Ipomoea palmata	95	515	46	95	173	41
Rosemarinus sp.	50	176	66	70	90	76
Wedelia triloba	100	1225	110	90	1096	82
Turf grasses						
Cynadon dactylon	100	1058	99	100	559	166
Pennisetum rubra	100	2356	358	100	848	286
Pasphalim vaginatum	100	1700	110	100	706	119
Zoysia tenufolius	90	746	97	90	1285	113

DW: Dry Weight

Table 3: ANOVA table for biomass production of different turf grass species in bioremediated and agricultural soil.

SOV	DF	SS	MS	FRc	FRt 5%	FRt 1%
Blocks	2	19651.84	9825.92			
Soil	1	155.04	155.04	0.01ns	18.51	98.5
Main plot error (BS)	2	48395.67	24197.84			
Main plots	5	68202.55				
Plant Species (PS)	3	190255.76	63418.59	5.27*	3.49	5.95
S × PS	3	15164.44	5054.81	0.42ns	3.49	5.95
Sub plot error, S (B-1) (PS-1)	12	144443.48	12036.96			
Total	23	418066.23				

SOV = Source of Variation; DF = Degrees of Freedom; SS = Sum of Squares; MS = Mean Squares; FRc = Fischer Ratio Calculated; FRt = Fischer Ratio Tabulated ; ns = Not Significant; * = Significant.

Table 4: Least significant difference test for treatment means of turf grass species

Sr. No.	Treatment	Mean
1	*Pennesetum rubra*	1930.9 a
2	*Cynodon dactylon*	794.6 b
3	*Paspalum vaginatum*	687.9 bc
4	*Zoysia tenuifolia*	631.4 c

Treatment means sharing a common letter are not statistically significant at a 5% level of alpha.

Ground covers

The data recorded on the growth performance, as measured by the extent of plant cover and biomass production are presented in Table 2. The analysis of variance (ANOVA) as shown in Table 5 indicates that the Fischer ratio calculated (FRc) for soil is less than the Fischer ratio tabulated (FRt) at 5% levels of alpha, which confirms that the two soil types are similar in their effect on biomass production. However, the FRc for plant species is higher than the FRt at both levels of alpha (5% and 1%), which indicates highly significant differences among the means for plant species. The FRc for interaction (S X PS) is smaller than the FRt at the 5% level of alpha, which confirms the absence of an interaction between soil and plants. Duncan's New Multiple Range (DMR) Test was applied to separate the means; the results are presented in Table 6.

Table 5: ANOVA table for biomass production of different ground cover species in bioremediated and agricultural soil.

		Biomass Produced (g/m^2)				
SOV	DF	SS	MS	FRc	FRt	
					5%	1%
Blocks	2	1801.07	900.54			
Soil	1	2904.77	2904.77	1.76ns	18.51	98.5
Main plot error, BS	2	3300.39	1650.20			
Main plots	5	8006.23	1601.25			
Plant Species (PS)	4	20144.58	5036.15	6.37**	3.01	4.77
S × PS	4	2977.92	744.48	0.94ns	3.01	4.77
Sub plot error, S(B-1)(PS-1)	16	12655.08	790.94			
Total	29	43783.81	1509.96			

SOV = Source of Variation; DF = Degrees of Freedom; SS = Sum of Squares; MS = Mean Squares; FRc = Fischer Ratio Calculated; FRt = Fischer Ratio Tabulated; ns = not Significant; ** = Highly Significant; Standard Error = 30.23.

Table 6: Duncan's new multiple range test for treatment means

Sr. No.	Treatment	Mean
1	*Gazania uniflora*	119.8 a
2	*Wedelia trilobata*	95.98 a
3	*Carisa grandiflora*	95.32 ab
4	*Rosemarinus spp.*	70.72 bc
5	*Ipomea palmata*	43.58 c

Treatment means sharing a common letter are statistically not significant.

The nonsignificant results for both the soil types and absence of interaction between soil type and plant species indicates that in spite of the variable trends the growth of plant species was not affected by soil types. The plant species were however, highly different in their respose to the soil. The study also confirmed that it is possible to grow ornamental landscaping plants in bioremediated soil in arid regions. The information in this study is useful in long-term studies of plant growth in polluted soil.

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تأثير التربة المعالجة حيويا على نمو أصناف مختلفة من النباتات

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خلاصة

أنشئ منتزه الأحمدي للتربة المعالجة حيويا لتقييم استخدام التربة المعالجة لأنشطة الزراعة التجميلية في الكويت. وقد تم عمل مقارنة حقلية لنمو النباتات في التربة الزراعية والتربة المعالجة حيويا، والنباتات التي تمت تجرتهم هي أربع أصناف من النجيل (cynodon dactylon, Paspalum vaginatum; pennesetum rubra, and Zoysia tenuifolia) وأيضا تم زراعة زراعة خمسة أصناف من مغطيات نباتية للتربة وهي (Carissa grandiflora, Gazania uniflora, Ipomata, Rosemarinus spp, and Wedelia Trilobata).

وقد أوضحت الدراسة عدم ظهور الدراسة عدم ظهور اختلاف في نمو النباتات للتربة المعالجة والتربة الزراعية من خلال المعاملات الإحصائية للجزء الخضري للنبات لكل صنف. ومن خلال هذه الدراسة تبين أن بالإمكا استخدام التربة الملوثة بالنفط الخام بعد معالجتها حيويا في أنشطة الزراعة التجميلية.