

Effects of crude oil on the growth of horsetail tree (*Casuarina equisetifolia*)

تأثير النفط الخام في نمو الكازورينا *Casuarina equisetifolia*

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Abstract :

A field experiment was conducted between March, 2005 and December 2007 to study the effects of different levels of crude oil (0.0 , 0.5 , 1.0 , 2.0 , 3.0 liter/ seedling) on the growth of (*Casuarina equisetifolia*) under Baghdad district /Iraq environmental conditions. The results showed the adverse effects of crude oil on the plant growth. Seedlings of the experiment showed various symptoms of effects after exposing to crude oil pollution ranging from leaf paleness, yellowing, browning and drying to death depending on the level of crude oil used and period of exposure. These symptoms reflected interruption in the plant growth as a result of pollution effects on physiological processes carried out by plants. The plant growth was reduced significantly in low levels (0.5 and 1.0 liter/seedling) while the result with high levels was the death of 75% and 100% of seedlings polluted with 2.0 and 3.0 liter/seedling respectively. The present study approved that (*Casuarina equisetifolia*) could tolerate a specific levels of crude oil pollution, therefore it was suggested that this tree could be served as a plant bioremediation for soil polluted by crude oil.

الخلاصة :

أجريت تجربة حقلية بين آذار ٢٠٠٥ و كانون الثاني ٢٠٠٧ لدراسة تأثير مستويات مختلفة من النفط الخام (٠.٥ ، ١.٠ ، ٢.٠ ، ٣.٠ لتر/ شتلة) على نمو شتلات الكازورينا تحت الظروف البيئية لمنطقة بغداد/ العراق. أظهرت النتائج تأثيرا عكسيا للتلوث بالنفط الخام على نمو النباتات. ظهر على الشتلات أعراض مختلفة بعد تعرضها للتلوث تراوحت بين شحوب لون الأوراق، اصفرارها، تلونها باللون البني ومن ثم جفافها وموتها، كل ذلك بالاعتماد على مستوى التلوث الحاصل وطول الفترة التي تلت التلوث. عكست الأعراض تأثيرا في العمليات الفسيولوجية المؤداة من قبل النبات. انخفض معدل نمو النبات معنويا عند المستويات الدنيا (٠.٥ ، ١.٠ لتر /شتلة)، في حين كانت النتيجة موت ٧٥% ، ١٠٠% من الشتلات عند التلوث بالمستويين ٢.٠ ، ٣.٠ لتر/شتلة على التوالي. أشارت التجربة الحالية الى ان *Casuarina equisetifolia* له القدرة على تحمل مستويات معينة من التلوث بالنفط الخام لذا اقترحت الدراسة إمكانية استخدام هذا النوع كمصلح بايولوجي للترب المعرصة لهذا النوع.

Introduction :

Plants have different response to pollution depending on their inherent characteristics, stage of growth, chemical and physical structures and concentration of pollutant, in addition to the environmental conditions under which the plant is grown. Exposing the plant to chemical, physical, or biological pollution might have different reflections on plant growth companying with clear symptoms. The action of pollutants in the growth processes of higher plants has some time revealed by a study of the visible symptoms or anatomical effects or by realizing the influences of different levels of pollutants on the chemical composition of the affected plant. Crude oil is one of the most serious types of environmental pollution, especially in oil producing countries, where substantial amounts of oil spill to soils and water bodies occasionally. In Iraq,

soil crude oil pollution is the most frequent one because of explosions of oil pipelines and oil storages due to the current unstable security situation.

Crude oil pollution has adverse effects on soil fertility and plant production. It could reduce or stop plant growth leading to death as a result of forming a physical barrier and coating the roots. Such effects lead to prevent the exchange of gases (oxygen and carbon dioxide), absorbing water and ion exchange between soil and root surface, or it may directly toxic to the plant through the effect of one or more of their components. Sometimes death does not occur immediately, but plant might be affected by the changes in its physiological processes or behavior patterns that eventually lead to death. Plant poor growth as a result of crude oil pollution could be attributed to suffocation of plants caused by exclusion of air by oil or exhaustion of oxygen by increased microbial activity, interference with plant-soil-water relationships and toxicity from sulfides (Udo and Feyemi , 1975). Baek, et al, 2004 referred to phototoxic of crude oil to some plants. Dejong, 1980 reported that crude oil spillage on soil makes it unsatisfactory for plant growth. According to Agbogidi, et al, 2006^a soils polluted with crude oil significantly affected the performance of plant and the effects were proportional to the concentration of oil applied, other new study has shown that oil spills lower soil fertility and cause poor growth of plants (Pyagbara, 2007).

Casuarina equisetifolia (horsetail or beef wood tree) is a tree with long needle-like branches and cone-like fruit and it proved to have capability of growing on wide range of soils and even poor soils because it is a nitrogen fixer (Whistler and Elevitch, 2006). This tree is one of best suited plants in Iraq and has established wonderfully well since a long time ago (Chakravarty, 1976). The tree introduced to Iraq for plantations and roadside and might be exposed accidentally to crude oil spills. Systematic documentation of the effects of crude oil on plant growth in Iraq is lacking. The present study has been undertaken to evaluate the effects of crude oil applied at various levels on the growth of *Casuarina equisetifolia* under Iraqi environmental conditions.

Materials and Methods

Field experiment was conducted in the experimental area of the Natural History Research Centre & Museum, Baghdad University, Bab-Al-Muadham campus, Baghdad. Forty five one-year old seedlings of *Casuarina equisetifolia* were purchased from local nursery and planted in the field on 5th of March 2005 with a distances of one by one meter in clay soil. Planting process was done in the way that the roots of plants had an equal depth from ground surface to assure regularity of treatments. Seedlings were left to grow normally in the field until fourth of December 2005. Plants were irrigated when it was necessary by narrow channels 20 cm away from plant stumps. Then twenty equal length seedlings were selected and five levels of light Basra crude oil (0.0 (L0), 0.5 (L1), 1.0 (L2), 2.0 (L3) and 3.0 (L4)) liter / seedling were added on the soil surface around the base of plant stem. In order to assure regular distribution of crude oil around the treated plants a ring of plastic tube with a diameter of 30 cm was put on the soil surrounding each seedling then the crude oil was distributed equally and evenly inside the ring . The experiment was conducted by using complete randomized design (C.R.D), while the treatments were replicated four times. Recognizable observations and photographs were taken when were necessary during the experiment period. On December 2007 the effects of crude oil pollution on growth has evaluated by using height of plant and stem diameter as a parameters for growth and the results obtained were statistically analyzed by using LSD for testing mean differences through Statistica program (99 Edition).

Results and discussion

Seedlings of *Casuarina equisetifolia* showed various symptoms of effects after exposing to crude oil pollution (Table 1). Symptoms recognized during different periods after treatments correlated to the level of pollution. Seedlings grew in soil exposed to high levels (L3 and L4) showed the first symptoms of effects after two weeks when the top leaves of L3 turned from normal green color to paleness, and to paleness with resultant some yellow coloration for L4. Low levels (L1 and L2) did not show any abnormal symptoms comparing with control (L0). After three months (February, 2006) paleness of leaves appeared on all seedlings of L1 and L2, while color of seedlings turned to light brown with poor growth and to browning with terminated growth for L3 and L4 treatments respectively. After six months (May, 2006) all the seedlings of L4 and most seedlings of L3 were started drying and eventually died (Fig1). *Casuarina equisetifolia* was proved to be tolerant plant to the low levels of crude oil pollution, while unable to survive with high levels. Fig 2 shows that percent of survived plants after six months from pollution were 100, 100, 100, 25, 0.0 for L0, L1, L2, L3, and L4 respectively.

Observations collected after one year (December 2006) were demonstrated that all seedlings of L1 were able to survive and recover to the normal green color and become difficult to recognize the effect of pollution visually by color comparing to control plants. The seedlings of L2 and the only survived seedling of L3 seemed smaller, weaker compared with L1 and L0. The visual symptoms obtained from the current experiment were accompanied by the adverse effect of crude oil on the growth of *Casuarina equisetifolia* seedlings. These abnormal symptoms might be reflected interruptions in the plant growth as a result of pollution effect on physiological processes carried out by plants. These influences led to reduce the plant growth of seedlings grew in low levels of pollution (up to 1.0 liter/seedling) and led to death of all replications grew under high levels (3.0 liter/seedling) and death most of seedling grew under 2.0 liter/seedling of crude oil. Plant growth as represented by height of plant and stem diameter after two years (December 2007) reflected the same trend of effect of crude oil. Table 2 represents the statistical analysis of plant heights and stem diameters of L0, L1 and L2 only, while L3 and L4 were excluded due to the death of their plants, that to prevent any effects on accuracy and reliability of the analysis. Data indicated significant reduction of growth as measured by height and diameter caused by pollution. The length reduced from 379.0 cm for L0 treatment to 353.3 cm and 202.0 cm for L1 and L2 respectively, while the diameter reduced from 6.34 for L0 to 4.62 and 2.76 cm for the same above treatments, the only survived seedling of L3 showed lesser values than all treatments having lower levels of pollution.

Seedlings of *Casuarina equisetifolia* grew with pollution up to 1 liter / seedling were able to survive even after two years from pollution in spite of early symptoms of effects appeared. However; seedlings of L1 behave differently where the height of seedlings was not significantly affected by oil pollution while the diameter was significantly reduced (Table 2). Only 25% of seedlings of L3 were able to survive from death during the two years of exposing to pollution. This finding could be referred to different self capabilities of seedlings of this species to tolerate the current oil pollution level.

Although, we did not find any literature dealing with effects of crude oil pollution on growth of this tree species, many researchers reported adverse effects of crude oil pollution on different plants, (Udo and Feyemi, 1975; Agbogidi et al, 2006). Different hypothesis were given to interpret the causes of adverse effects of crude oil on plants, Udo and Fayemi (1975) related the effects to suffocation of plants caused by exclusion of air by increased microbial activity, interference with plant-soil-water relationships, and toxicity from sulfides and excess available Mn produced

during the decomposition of the hydrocarbons. Gill et al (1992) reported that the adverse effects noticed on the treated plants by oil might be due to unfavorable conditions created in the soil resulting in drought conditions as well as non-availability of nutrients. Agbogidi et al (2006) attributed the crude oil effects on plants to one or a combination of some factors; disruption of plant-water relations, direct impact on plant metabolic processes and toxicity to living cells. Also, reducing oxygen exchange between atmosphere and the soil, thus affecting root functioning, nutrients immobilization, heavy metal build-up and an alteration in physical, chemical and biological properties of soil.

According to the current study the death of seedlings grew at high levels over 2.0 liter/ seedling did not happen immediately after exposing to crude oil pollution but after nearly six months. This finding could be indicated to accumulation effects of oil pollution on plant and not to immediate effects of toxicity, which lead to direct death. The adverse effects of crude oil on the growth noted in the current experiment, might be attributed to one or more of following factors , disruption of plant-soil- water relationship, reduced soil oxygen since the oil covers the particle surfaces and fills the pore spaces of the soil. Direct harm effects on root systems as a result of direct contact with oil could be a reason, too. Other factor, which should not be excluded, is the toxic effects of some hydrocarbon compounds produced during crude oil decomposition by soil microorganisms with time, which might be absorbed by roots of plant. The reduction of the plant growth as a consequence of the crude oil pollution could be lasted more than two years (time of current experiment) because of the low rate of crude oil decomposition in soil. Finally the results of the current study also clearly pointed out to *Casuarina equisetifolia* as a tolerant plant for crude oil and can be used as bioremediation and could be planted in polluted areas of oil refineries in Iraq to reduce the pollution and to ameliorate the air from volatile hydrocarbons raised from such areas.

Table 1: Symptoms of effects of crude oil pollution on *Casuarina equisetifolia* seedlings at different time.

Time of observation after treatment	Levels of pollution (liter/ seedling)	Observable effects	Percent of dead seedlings
Two Weeks	L0 (0.0)	Null	None
	L1(0.5)	Null	None
	L2 (1.0)	Null	None
	L3(2.0)	paleness of the top leaves	None
	L4(3.0)	paleness of the top leaves with resultant some yellow coloration	None
Three Months	L0 (0.0)	Null	None
	L1 (0.5)	Leaf paleness	None
	L2 (1.5)	Leaf yellowing	None
	L3 (2.0)	Leaves turned to light brown with reduced growth	None
	L4 (3.0)	Leaf browning with terminated growth	None

Six Months	L0 (0.0)	Null	None
	L1 (0.5)	Leaf yellowing and reduced growth	None
	L2 (1.0)	Low density leaves with recognized yellow color and reduced growth	None
	L3 (2.0)	Leaves turned to reddish brown color and stunted	75%
	L4 (0.5)	Browning and drying all seedling parts	100%

Table 2: Height and Diameter of *Casuarina equisetifolia* seedlings as measured after two years of pollution by crude oil.

Treatment symbol	Level of pollution (Liter / Plant)	Mean of Plant Height (cm)	Standard Deviation	Mean of Stem Diameter (cm)	Standard Deviation
L0	0.0	379.0 a	49.86	6.34 a	1.43
L1	0.5	353.3 a	97.15	4.62 b	0.69
L2	1.0	202.0 b	56.86	2.76 c	0.81
L3	2.0	187.0 *	-	2.50 *	-
L4	3.0	0.0	-	0.0	-

Means with different letters are significantly different at $p < 0.01$.

* Refers to one replicate, and 0.0 refers to death of all replications.

Conclusion

The research has demonstrated that crude oil pollution has acute effect on *Casuarina equisetifolia* causing significant changes of plant appearance with subsequent reduction of growth. Visual symptoms on plants ranged from paleness, yellowing, browning, and drying of plant. The effects being proportional to level of pollution. Also, these effects could last for more than one growth season. The study suggests that the species could serve as a bioremediation after soil crude oil pollution. Further researches are required on other plant species.

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Fig 1: Effects of different levels of crude oil on growth of *Casuarina equisetifolia* seedlings as observed after six months.

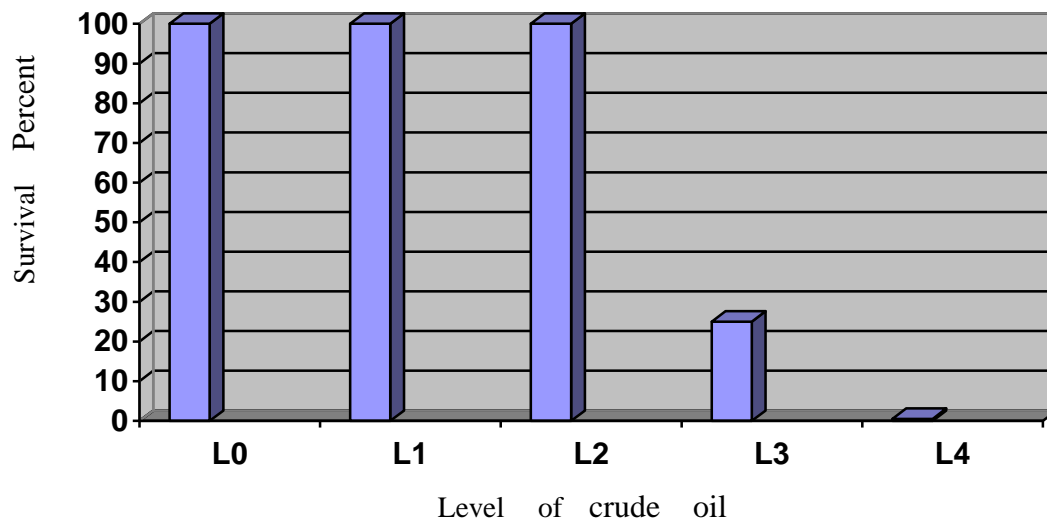


Fig 2 : Effect of different Level of crude oil t of survived trees after six months of pollution.

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