Seedling Emergence and Biomass Growth of Oleaginous and Other Tropical Species in Oil Contaminated Soil

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Abstract: Germination rate in contaminated soils cannot be used as the sole indicator to select suitable species for phytoremediation trials, since it does not predict the plant development after germination. As a result, most screening tests for selection of specie with phytoremediation potential to treat contaminated soils include germination rate and biomass production. These screenings take several months and considerable space in greenhouses. As an attempt to reduce the time required for screening, seedling emergence and root and aerial biomass growth in a very early stage of plant development were investigated in soil contaminated with crude oil. The experimental design was based on a factorial of 6x6x50 where seeds from six tropical species (*Ricinus communis* - castor bean; *Helianthum annus* - sunflower, *Glycine max* - soybean; *Acacia holosericea* - candelabra wattle; *Brachiaria brizantha* – braquiaria; *Tibouchina granulosa* - quaresmeira) were placed in sterilized sand contaminated with crude oil at 6 oil concentrations (0% - control, 0.05%, 0.5%, 2%, 4% and 6% weight/dry weight) in boxes with 50 seeds. Four replicates resulted in a total of 7200 seeds (1 200/specie) in 144 experimental units kept in a greenhouse under light and temperature controlled conditions. It was observed that depending on the oil concentration and specie, germination can be significantly ($\alpha = 0.05$) postponed or reduced and root as well as aerial dry biomass can be reduced. Root biomass increase was observed for oleaginous species soybean, sunflower and castor bean. Candelabra wattle was the only specie not significantly affected. The results support the hypothesis that more than one type of oil-soil-plant interaction might occur. The ranking in terms of tolerance to the crude oil, considering all variables analyzed was: *A. holosericea* > *G. max* = *B. brizantha* > *H. annus* > *R. communis* > *T. granulosa*. The low cost and short time required by the screening procedure proposed make it useful and effective for testing many species simultaneously, as the first step of a full-scale phytoremediation trial.

Keywords: Screening, phytoremediation, phytotoxicity, petroleum hydrocarbons, crude oil.

INTRODUCTION

Phytoremediation - the use of plant-microorganisms systems for soil and groundwater decontamination - is among the fastest growing areas of environmental remediation research, technology development and implementation [1]. Even though investigations regarding the potential of tropical plants for phytoremediation are still scarce, the potential of this technology for tropical and subtropical areas is expected to be high due to prevailing climatic conditions favoring plant growth and microbial activity [2]. Although it has not been proved yet that plants suffering toxic effects of the presence of contaminants in soil are not capable to remediate it, the plant's capacity to germinate and to produce biomass in the presence of the contaminant is necessary. However, tolerance to the soil contaminant is not always followed by phytoremediation capacity. Phytotoxicity can be defined as any change in the normal development of cultivated or native species, due to the presence of a chemical compound in the soil. The adverse effects of crude oil (petroleum) and in particular, some target groups of petroleum hydrocarbons on plant development result in a number of physical and/or chemical interactions with the plant system that go from reduction of phytotranspiration and carbon fixation processes to plant death [3].

These adverse effects have been the basis for the development of phytotoxicity tests where the purpose is to establish how harmful a chemical substance is to the environment and, specifically to plants by using recommended pre-tested species [4-6]. This is not the case of phytoremediation investigations, where the focus is to identify species capable to survive and even remove or degrade soil or groundwater contaminants.

Regarding chemical effects, heavy oils are usually less toxic than light oils, the second group having apparently easier penetration into the plant, preventing leaves to grow and sprouts regeneration [7]. Some studies report the reduction of seedling emergence and plant growth in the presence of polycyclic aromatic hydrocarbons-PAHs in newly contaminated soils or in solutions [8-11]. According to Henner *et al.* (1999) [10], the phytotoxicity of sites contaminated with crude oil or by-products de is mostly caused by volatile compounds. If so, aged contaminated soils are expected to be less toxic to plants than newly

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contaminated soils, due to low water solubility and strong adsorption of the large remained petroleum hydrocarbons to the soil matrix [12]. It has also been demonstrated that petroleum hydrocarbon toxicity to microorganisms, animals and aquatic plants is enhanced by the action of radiation [13, 14]. Reduced biomass in the presence of contaminants has been observed by Merkl *et al.* (2004) [2] that conducted an experiment for 30 weeks. Smith *et al.* (2006) [8] observed no significant effect on germination rate but growth reduction of seven oleaginous and grass species after 12 weeks in the presence of polycyclic aromatic hydrocarbons-PAHs in soils. Similar behavior – no effect on germination but biomass reduction - was described by Besalatpour *et al.* (2008) [15] with another group of species. These investigations suggest that germination alone is not sufficient to indicate phytotoxicity and to predict subsequent plant performance in contaminated soils. Therefore, germination rate cannot be used as the only indicator during screening of suitable crops for phytoremediation field trials. One constraint when searching for species with phytoremediation potential is that most studies assessing biomass production requires several months of plant development.

The objective of this investigation is to evaluate the effect of the crude oil on the seedling emergency (germination rate) and to check if a short screening period of five weeks would be enough to detect differential toxicity effects on root and aerial biomass growth. The purpose was to assess the potential for using the selected species in phytoremediation experiments. Six species were tested: a grass frequently found in degraded Brazilian soils; a specie native from Atlantic rainforest, a specie recommended for recovering degraded soils and three oleaginous in the Brazilian biodiesel production program.

MATERIALS & METHODOLOGY

Studied Species

The oleaginous species tested are included in the Brazilian biodiesel program: (a) *Helianthus annu*s (sunflower) variety Embrapa 2000, (b) *Glycine max* (soybean) variety M-Soy 5826 and (c) *Ricinus communis* (castor bean) variety Guarani, native to Asia, drought-resistant, found in tropical and sub-tropical regions, which is three times more productive per hectare than soybean. Castor bean also produces lubricating oils used in high-rotation motors. Once confirmed that biodiesel producing crops can grow in contaminated soils – which are inappropriate for food production – and in the best scenario, can remediate them, the competition between biodiesel crops and food species for agricultural land might be reduced. The other three species tested were: (d) *Brachiaria brizantha* (a grass known in Brazil as braquiaria) variety Marangatu, native to Africa, introduced into most tropical countries used for hay and silage, which has attributes such drought resistance, ability to spread and suppress weeds and to grow in shad, often found in contaminated areas; (e) *Acacia holosericea* (candelabra wattle, native from Australia) which belongs to the genus *Acacia* used for timber production and more recently suggested for recovery of Brazilian acid and degraded soils [16]; (f) *Tibouchina granulosa* (quaresmeira), evergreen shrub native to the Brazilian Atlantic Rainforest, that might be suitable for remediation of areas where protection restrictions prevent plantation with exotic species.

Experiment Setup

Crude oil from Alagoas basin, Brazil (Table **1**) was added to a fine sand soil (0.20-0.05 mm), previously sterilized at 120 ^{o}C (1 atm) during 1 h. The sand was mixed with crude oil at the concentrations: 0% (control), 0.05%; 0.5%; 2%; 4% and 6% (weight/dry weight). High density polyethylene-HDPE boxes (19 x 11 x 5.5 cm) previously disinfected where filled with 400 g of sand each. Six oil concentrations, six species and four replicates (with 50 seeds each) resulted in 200 seeds and 24 experimental units per specie. Seeds of *R. communis, B. brizantha* and *A. holosericea* were scarified with the purpose of promoting germination, according to the Brazilian Seeds Analysis Manual [17]. The HDPE boxes were kept in shelves in a cultivation room (24-25 ^{0}C, 98% of moisture and photoperiod of 8-12 h). During five weeks of monitoring, seeds with fungi were counted and discarded; emerged seedlings were counted, shoots were washed, roots were separated from the aerial part and both parts were dried at 60 ^{0}C for 24 h and weighted to measure dry biomass.

Germination Rate

The germination was counted as: early germination, late germination and total germination. Seeds that germinated within the time interval for specie studied was established in the Brazilian Seeds Analysis Manual [17] were counted as early germination. Seeds that germinated after that period were counted as late germination in each oil concentration, and compared to the control. The total germination rate was the total number of seedlings emerged during five weeks period among all seeds, excluding those with fungi, which were discarded. The germination index for each specie and oil concentration was the ratio between the average germination rates in that oil concentration divided by the average germination rate in the control, according to Eq. (1):

$$\textit{Germination Index} = \frac{\textit{Average germination rate at X}}{\textit{Average germination rate at the control}} \quad (1)$$

where X is the oil concentration in % (weight/dry weight)

Dry Biomass

The root and aerial dry biomass of early germinated seedlings were measured separately for all species but *T. granulosa* that had only the total (root and aerial) dry biomass considered.

Table 1. Characteristics of the Crude Oils from Alagoas Platform, Brazil

Relative Density (g ml^{-1})	^{o}API	Class	TPHs (mg g^{-1})	BEXT (mg g^{-1})	PAH (mg g^{-1})
0.824	40.2^{0}	light	727.366	31.467	2.688

^{o}API=[(141.5/specific gravity)-131.5]; TPH=total petroleum hydrocarbons; BTEXT=benzene, toluene, ethyl-benzene, xylene, trimethyl-benzene; PAH = polycyclic aromatic hydrocarbons.

Statistics

Many variables didn't follow normal distribution according to the normality test and data transformation (log transformation, etc.) did not result in normal distribution. Therefore, instead of using regression modeling, Kruskal-Wallis (a non-parametric alternative to the one-way analysis of variance) was chosen to test the equality of medians. For those variables where the hypothesis H0 was rejected (at $\alpha = 0.05$), Mann-Whitney was performed for each treatment (oil concentration) compared to the control.

RESULTS

Germination Period and Total Germination Rate

Fig. (**1**) shows the germination periods for all species, from the first to the last seedling emergence. Fig. (**2**) shows the total germination rates for different species at different concentrations of oil. *H. annus* and *R. communis* (6% of oil) and *T. granulosa* (2%, 4% and 6% of oil) showed a significant decrease in the total germination rate compared to the controls. The total germination rates of the other species (*A. holosericea, G. max* and *B. brizantha*) were not significantly affected in any oil concentration.

Early and Late Germination

When total germination was separated in early and late germination, effects of the oil on the germination speed were observed. *B. brizantha* had a significant decrease of early germination at 6%, which was compensated by late germination, the reason the total germination was not affected. Fig. (**3**) shows the germination index obtained for all studied species. The germination index for *A. holosericea, G. max, B. brizantha* and *H. annus* were close to 1, in oil concentrations up to 4% with a decrease at 6%.

Biomass

Fig. (**4**) shows descriptive statistics and Table **2** shows the results of the statistical test for comparison between medians for root and aerial biomass of early germinated plants at different oil concentrations and control.

Root Biomass

A significant increase in root biomass was observed in two oleaginous species: *H. annus* (from 0.05% to 4% in early germinated seeds and at 4% in late germinated seeds); and *G. max* (from 0.05% to 6% in early germinated seeds). The third oleaginous specie studied *R. communis* showed an apparent increase of dry root biomass at oil concentrations from 0.05% to 2% but not statistically significant.

Aerial Biomass

A significant decrease in aerial biomass in early-germinated *B. brizantha* (4% and 6% of oil) was observed.

DISCUSSION

Regarding the effect oil-contaminated soils have on germination rate, Dominguez-Rosado *et al.* (2004) [18] described an increasing germination rate of *Glycine max* when seeds were sown in soil contaminated with lubricating oil at low concentrations (0.1% and 1%). Merkl *et al.* (2004) [2] described the same behavior for *Mimosa orthocarpa* in the presence of 5% of crude oil. They attributed this positive effect on germination to changes in water infiltration due to the hydrophobic properties of oil, resulting in soil water retention and less moisture losses. Similar to what happened with *B. brizantha* seeds in the present experiment, Udo and Fayemi (1975) [19] observed that one of the plant responses to the presence of oil is the delay in germination. Another response to the presence of oil in soil is the reduction of

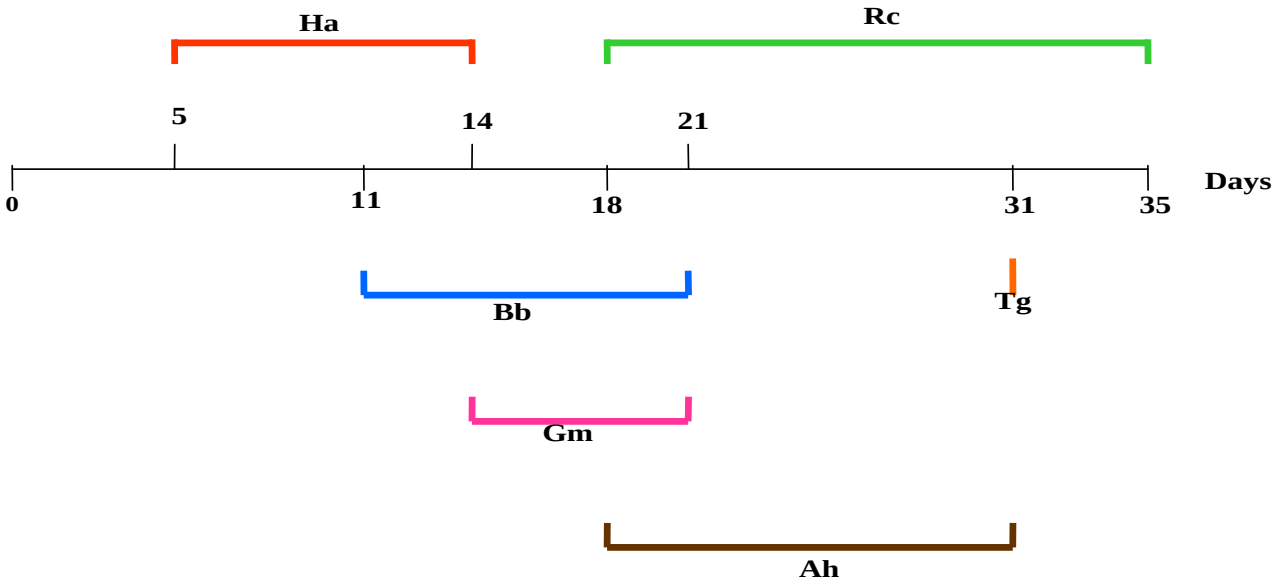

Fig. (1). Germination period registered for six species, including early and late germination. Ah = *Acacia holosericea;* Bb= *Brachiaria brizantha;* Gm = *Glycine max;* Ha = *Helianthum annus;* Rc = *Ricinus communis;* Tg = *Tibouchina granulosa.*

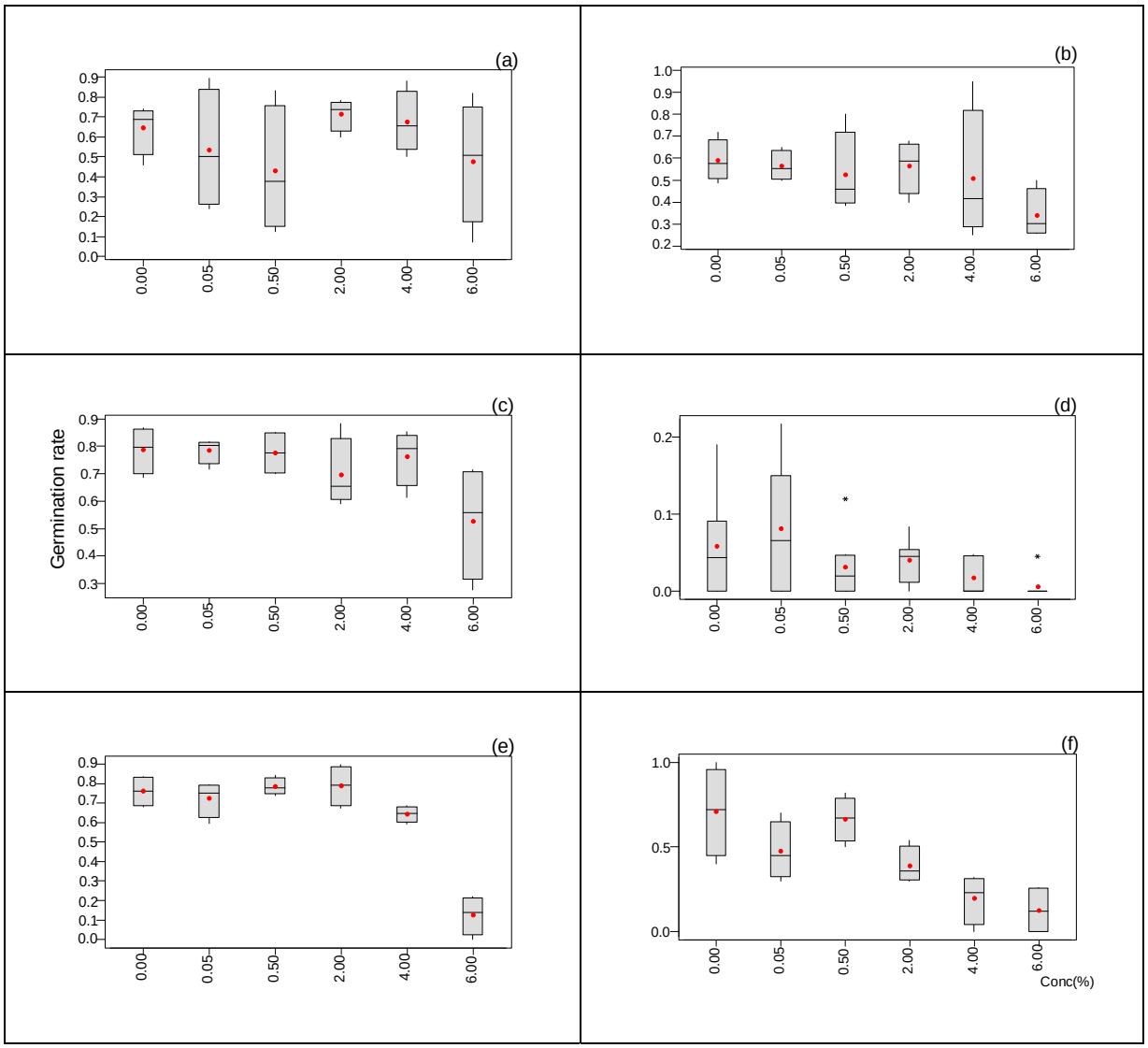

Fig. (2). Box plots for total germination rate under different oil concentrations. (**a**) *A. holosericea*; (**b**) *B. brizantha*; (**c**) *G. max*; (**d**) *R. communis*; (**e**) *H. annus*; (**f**) *T. granulosa.* Red dots and horizontal lines inside boxes are the average and the median respectively. Upper and lower limits of each box mean upper (third) quartile (*Q3*, $x_{.75}$) and lower (first) quartile (*Q1*, $x_{.25}$) respectively. Lower and higher perpendicular lines outside each box indicate the smallest non-outlier observation and the highest non-outlier observation respectively. Points outside the box plots in (**d**) are outliers.

germination. However, as long as no significant reduction of biomass occur, such as the case of *quaresmeira* in this experiment, germination might occur in clean soil and seedlings might be later transferred to the contaminated area and the specie can still be tested in phytoremediation trials.

The fact that only oleaginous species showed significant (sunflower, soybean) or apparent (castor bean) increase of root biomass in the presence of low concentrations of oil might have relevant implications and requires further investigations. To explain the phenomenon of root biomass increase observed in these oleaginous species, the following mechanisms previously described in the literature might be considered: (a) the oil was absorbed and accumulated in the vacuoles and cellular walls [20] and the procedure used for biomass drying (24 h at 60 ^{o}C) was not enough to eliminate

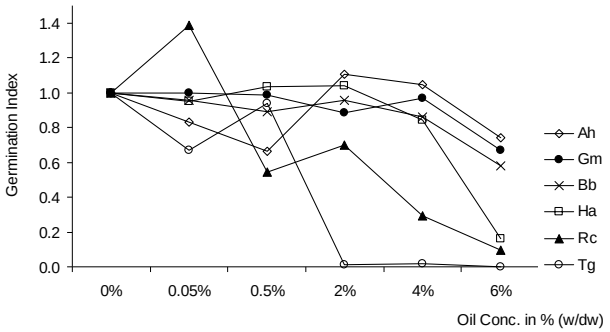

Fig. (3). Germination indexes for each specie and oil concentration. Ah = *A. holosericea;* Bb= *B. brizantha;* Gm = *G. max;* Ha = *H. annus;* Rc = *R. communis;* Tg = *T. granulosa.*

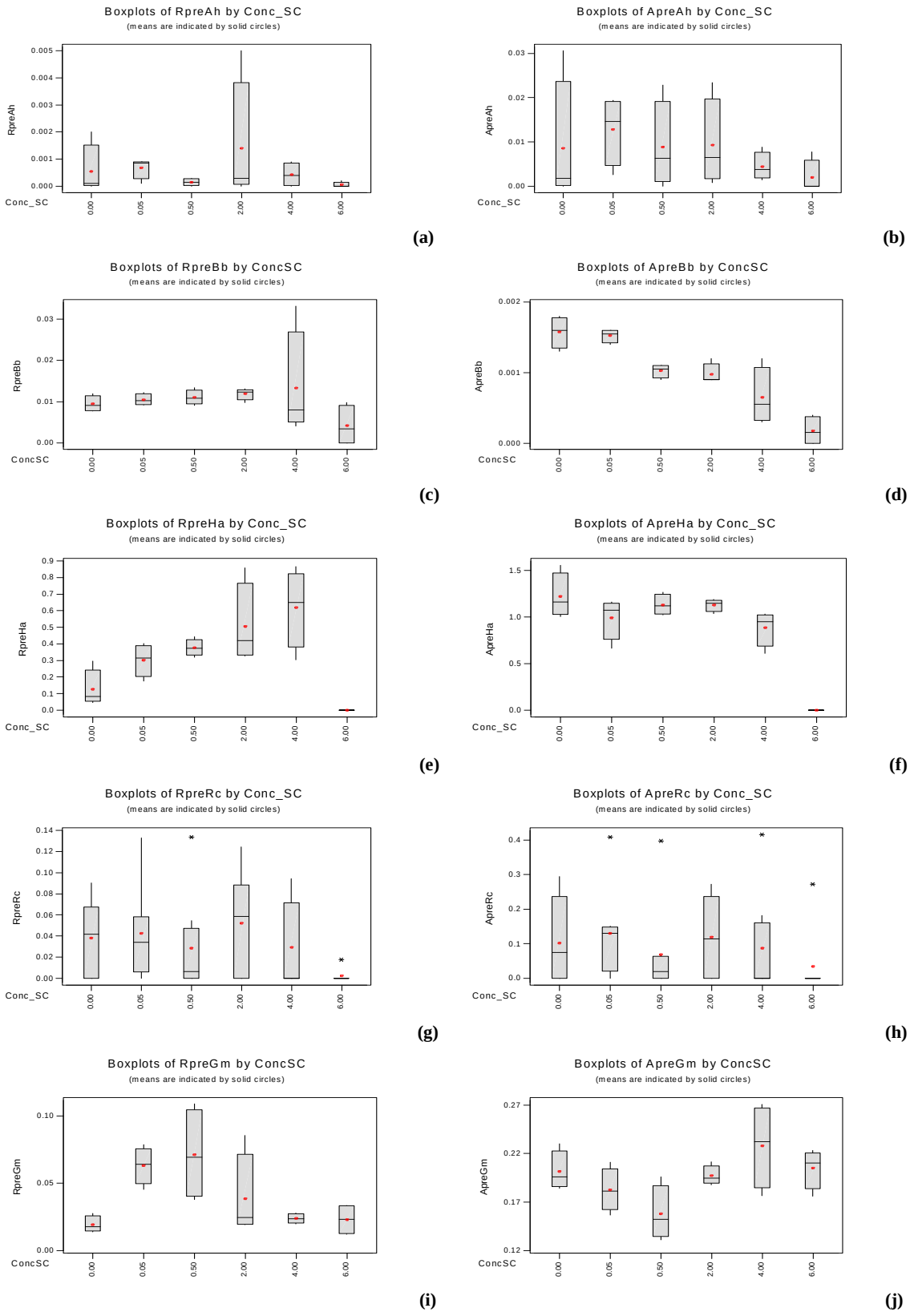

Fig. (4). Box plot: non-parametric statistics and mean values (red dot inside boxes) for dry root biomass (**a**, **c**, **e**, **g**, **i**) and dry aerial biomass (**b**, **d**, **f**, **h**, **j**) of early germinated plants in oil-contaminated soil (0, 0.05, 0.5, 2.0, 4.0, 6.0%). (**a**, **b**) *A. holocericea*; (**c**, **d**) *B. brizantha*; (**e**, **f**) *H. annus*; (**g**, **h**) *R. communis* and; (**i**, **j**) *G. max.*

it; this hypothesis could be tested in the future by comparing microscopy images of histological preparation of plant tissues or comparing the ashes produced at 550 ^{o}C for 16h [21]. Another possible explanation is that (b) higher root biomass in the presence of oil is related to the utilization of the petroleum hydrocarbon compounds as carbon source, stimulating the root growth [18]. Transversal histological cuttings obtained from fresh aerial and root portions of sunflower seedlings growing in the control soil (0%), and 0.05% and 4% oil-contaminated soil observed with optical microscopy (x 1.000, no coloration applied), showed dark-brown coloration in the parenchyma of those seedlings growing in contaminated soil. These images were confirmed in a second experiment with soybeans, after 30 days of growth in soil contaminated with 3% of crude oil. Gill and Nyawuame (1992) [22] observed the presence of crude oil at cellular level in seedlings of *Chromolaena odorata* and reported the absence of oil in vascular tissues, suggesting that the oil transport mechanism occurred *via* extracellular (diffusion through intercellular walls and voids).

Table 2. Kruskal-Wallis Non-Parametric Test for Equality of Medians Applied to Dry Biomass in Early Germination

Specie	Root Biomass	Aerial Biomass
Brachiaria brizantha	p=0.296	**p=0.022**
Helianthus annus	**p=0.019**	p=0.202
Glycine max	**p=0.014**	p=0.144
Acacia holocericea	p=0.93	p=0.814
Ricinus communis	p=0.085	p=0.234
	Total biomass	
*Tibouchina granulosa**	p=0.168	

Values of $p < 0.05$ (in bold) mean that the oil had significant effect on that particular variable, in at least one of the tested concentrations.
*Due to the small size of the shots, only total biomass was considered.

Regarding aerial biomass it has been previously reported [23] that at least for some species, aerial biomass growth is less affected than root biomass growth by the presence of oil in soil. Hernandez-Valencia and Mager (2003) [23], for instance, found that aerial biomass was more affected than root biomass in both *G. max* and *H. annus* plantlets growing in oil-contaminated medium.

CONCLUSIONS

Depending on the oil concentrations and plant species, different significant effects were observed when seeds of *G. max, H. annus, R. communis, A. holocericea, B. brizantha* and *T. granulosa* were sown in oil-contaminated sand soil under controlled environment: (i) no effect (*A. holocericea*); (ii) inhibition of germination (*H. annus* and *R. communis* at 6% of oil and *T. granulosa* at 2%, 4% and 6% of oil); (iii) delay in germination (*B. brizantha*); (v) decrease of aerial biomass (*B. brizantha*); (vi) increase in root biomass (*H. annus, G. max*). Taking into consideration four criteria in decreasing order of importance: (1º) absence of any effect on the variables studied; (2º) no significant decrease of germination; (3º) no significant decrease of biomass; the species were ranked as following, regarding their tolerance to the presence of the oil in the soil and, therefore, their potential to be used in phytoremediation schemes: (1st) *A. holocericea*; (2nd) *G. max* and *B. brizantha*; (3rd) *H. annus*; (4th) *R. communis*; (5th) *T. granulosa*. Having in mind that tolerance does not obligatorily mean phytoremediation capacity, additional investigation is needed to assess phytoremediation potential and specific properties such as rizodegradation, phytoextraction, phytodegradation, phytovolatilization. Since oleaginous species showed tolerance to germinate and to grow in the presence of oil, growing biofuel crops in contaminated areas could be considered as a strategy to reduce competition with food crops over arable and clean land. Therefore further investigation about the phytoremediation potential of these species should be carried out.

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REFERENCES

[1] E. K. Dzantor, and R. G. Beauchamp, "Phytoremediation, Part I: Fundamental basis for the use of plants in remediation of organic and metal contamination", *Environ. Pract*., vol. 4, pp. 77-87, Cambridge University Press, June 2002.

[2] N. Merkl, R. Schultze-Kraft, and C. Infante, "Phytoremediation in the tropics - The effect of crude oil on the growth of tropical plants", *Bioremediat. J*., vol. 8, no. 3-4, pp. 177-184, July 2004.

[3] M. F. M. Pedrozo, E. M. Barbosa, H. X. Corseuil, M. R. Schneider, and M. M. Linhares, "Ecotoxicologia e avaliação de risco do petróleo", *Centro de Recursos Ambientais*, vol. 12, pp. 246, 2002.

[4] OECD, "Terrestrial plants: Growth test", *OECD Guidelines for testing of chemicals*, Paris: No. 208. April 1984.

[5] U.S. EPA, "Ecological Effects Test Guidelines", OPPT 850.4200. *Seed Germination/ Root Elongation Toxicity Test*, EPA 512-C-92-154, April 1996.

[6] A. Baumgarten, and H. Spiegel, "Phytotoxicity (Plant tolerance)", Horizontal-8, *Agency for Health and Food Safety*. Vienna, Austria, p. 35, April 2004.

[7] S. R. Pezeshki, W. Hester, Q. Lin, and J. A. Nyman, "The effects of oil spill and clean-up on dominant US Gulf coast marsh macrophytes: A review", *Environ. Pollut.,* vol. 108, pp. 129-139, January 2000.

[8] M. J. Smith, T. H. Flowers, H. J. Duncab, and J. Alder, "Effects of polycyclic aromatic hydrocarbons on germination and subsequent growth of grasses and legumes in freshly contaminated soil with aged PAHs residues", *Environ. Pollut.*, vol. 141, no. 3, pp. 519-525, July 2006.

[9] B. Smreczak, and B. Maliszewska-Kordybach, "Seeds germination and root growth of selected plants in PAH contaminated soil", *Fresen. Environ. Bull*., vol. 12, pp. 946-949, September 2003.

[10] P. Henner, M. Schiavon, V. Druelle, and E. Lichtfouse, "Phytotoxicity of ancient gas work soils. Effect of polycyclic aromatic hydrocarbons (PAHs) on plant germination", *Org. Geochem.*, vol. 30, pp. 963-969, August 1999.

[11] C. H. Chaîneau, J. L. Morel, and J. Oudot, "Phytotoxicity and plant uptake of fuel oil hydrocarbons", *J. Environ. Qual.,* vol. 26, pp. 1478-1483, December 1997.

[12] M. Kriipsalu, M. Marques, D. R. Nammari, and W. Hogland, "Bio-treatment of oily sludge: The contribution of amendment material to the content of target contaminants and the biodegradation dynamics*", J. Hazard. Mater.*, vol. 148, pp. 616-622, September 2007.

[13] G. T. Ankley, S. A. Collyard, P. D. Monson, and P. A. Kosian, "Influence of ultraviolet light on the toxicity of sediment contaminated with polycyclic aromatic hydrocarbons", *Environ. Toxicol. Chem.,* vol. 13, pp. 1791-1796, November 1994.

[14] L. Ren, X. –D. Huang, B. J. McConkey, D. G. Dixon, and B. M. Greenberg, "Photo induced toxicity of three polycyclic aromatic hydrocarbons (fluoranthene, pyrene, and naphthalene) to the duckweed *Lemna gibba* L. G-3", *Ecotox. Environ. Safe*, vol. 28, pp. 160-171, July 1994.

[15] A. Besalatpour, A. H. Khoshgoftarmanesh, M. A. Hajabbasi, and M. Afyuni, "Germination and growth of selected plants in a petroleum contaminated calcareous soil", *Soil Sediment Contam.*, vol. 17, no. 6, pp. 665-676, November 2008.

[16] F. C. Balieiro, L. E. Dias, A. A. Franco, E. F. C. Campello, and S. M. Faria, "Acúmulo de nutrientes na parte aérea, na serapilheira acumulada sobre o solo e decomposição de filódios de *Acacia mangium* Willd", *Ciência Florestal*, *Santa Maria*, vol. 14, no. 1, pp. 59-65, January 2004.

[17] Brasil, Ministério da Agricultura e Reforma Agrária, "Regras para análise de sementes", MARA, Brasília, 1992.

[18] E. Dominguez-Rosado, J. Pichtel, and M. Coughlin, "Phytoremediation of soil contaminated with used motor oil: I. Enhanced microbial activities from laboratory and growth chamber studies", *Environ. Eng. Sci*., vol. 21, no. 2, pp. 157-168, March 2004.

[19] E. J. Udo, and A. Fayemi, "The effect of oil pollution of soil on germination, growth and nutrient uptake of corn", *J. Environ. Qual.*, vol. 4, pp. 537-540, June 1975.

[20] S. C. McCutcheon, and J. L. Schonnor, "Phytoremediation", John Wiley & Sons, New Jersey, pp. 355, 2003.

[21] S. Amir, M. Hafidi, G. Merlinda, H. Hamdi, and J. C. Revel, "Fate of polycyclic aromatic hydrocarbons during composting of lagooning sewage sludge", *Chemosphere*, vol. 58, pp. 449-458, January 2005.

[22] L. S. Gill, H. G. K. Nyawuame, and A. O. Ehikhametalor, "Effect of crude oil on the growth and anatomical features of *Chromolaena odorata* (L) K. & R. *Chromolaena odorata", Newsletter*, no. 6 pp. 1-6, 1992.

[23] I. Hernández-Valencia, and D. Mager, "Uso de *Panicum maximum* y *Brachiaria brizantha* para fitorremediar suelos contaminados con un crudo de petróleo liviano", *Bioagro*, vol. 15, pp. 149-155, May 2003.

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