BIORESEARCH BULLETIN
Sapience Research Labs

ORIGINAL PAPER OPEN ACCESS

Isolation and selection of filamentous Fungi from petroleum contaminated soil

Fraga ME, Zonta E and Balieiro FC.

Received: 20 September 2010 / **Accepted:** 02 December 2010 / **Published online:** 02 February 2011

Abstract

Background: The selection of plant species with phytoremediation potential is as essential as microorganisms are for the success of decontamination programs of areas affected by organic exploration and oil production spills. Based on a greenhouse study aimed at assessing the initial growth of three plant species [Mamona (*Ricinus communis*), Acácia (*Accia holocerisea*) and Mimosa (*Mimosa caesalpiniifolia*)] grown in the presence and absence of crude oil (petroleum), a study was carried out in order to isolate and select filamentous fungi which have potential to grow and degrade this contaminant.
Results: After a growth period of 47 days of the plants, soil samples were collected for treatment, and only comparisons between the contamination effects on the number of colony-forming units (CFU) of species and genera of fungi isolates were evaluated by the Student's t test. The presence of oil in the soil significantly decreased the number of CFU (44.39×10^3 vs. 8.83×10^2) and, consequently, the diversity of soil fungi. Two hundred and thirteen filamentous fungi were isolated from contaminated and uncontaminated soil samples. In uncontaminated soil, 12 genera and 29 species were identified, whereas in contaminated soil eight genera and 12 species were identified. Penicillium was the most frequent genus found in uncontaminated soil; however, it was not isolated from contaminated soil.
Conclusion: The existence of species that grow with or without the presence of oil allow inferences on their use as an contamination indicator or on how these hydrocarbons are degraded, having potential for the treatment of environments.

Keywords: Phytoremediation, Degradation, Hydrocarbons, Mycoremediation.

M.E. Fraga
Núcleo de Pesquisa Micológica e Micotoxicológica (NPMM), Departamento de Microbiologia e Imunologia Veterinária (DMIV), Universidade Federal Rural do Rio de Janeiro (UFRRJ), Seropédica, Rio de Janeiro, Brasil.
e-mail: fraga@ufrrj.br.
E. Zonta
Departamento de Solos, UFRRJ, Seropédica, Rio de Janeiro, Brasil.
F.C. Balieiro
Embrapa Solos, Rio de Janeiro, Rio de Janeiro, Brasil.

Web address:
http://bioresonline.org/archives/A132.pdf

Article citation:
Fraga ME, Zonta E and Balieiro FC. 2011. Isolation and Selection of Filamentous Fungi from Petroleum Contaminated Soil. Bioresearch Bulletin 4: 227-235.

INTRODUCTION

Many techniques can remediate soils contaminated by organic compounds, specially those that use plants and microorganisms, due to their ease growth and handling and cost, because they use photosynthesis as energy source and their metabolic (and co-metabolic) routes to degrade different compounds, including petroleum and their derivatives (Kuiper *et al*., 2004; Pasqualino et al., 2006).

Bacteria, yeasts and filamentous fungi are efficient agents to degrade of a large number of organic substances, commonly found in effluents generated by oil refineries (Atagana *et al.,* 2006; Santos *et al*., 2008), presenting as a powerful alternative to conventional treatment methods (Ururahy, 1998). Some studies have been developed, aiming at stimulating the activity of native microorganisms in the degradation of hydrocarbons (Hart, 1996; Evans *et al.*, 2004) or the introduction of potentially active microorganism strains that degrade hydrocarbons at the polluted site (Baddock *et al*., 1997; Kramer., 2005). According to Gadd (2004), fungi secrete a great range of efficient enzymes into the environment, which are used to help with their nutrition and which, consequently, are responsible for the degradation of several refined or processed natural substances. However, the degradation of hydrocarbons in soil may be limited by the inability of indigenous microorganisms to efficiently metabolize these substances, due to a lack of nutrients for the degrading microbiota or to a low bioavailability of hydrocarbons to degrader microorganisms, because of their sorption into the soil's mineral and organic solid phase (Johnsen *et al*., 2005). According to Chaillan *et al.* (2004), *Aspergillus* and *Penicillium* are the most commonly found fungi in tropical soil, which are able to degrade hydrocarbons. Conceição *et al.* (2005) cited that these genus form a group of microorganisms that definitely possess mechanisms to resist adverse environmental conditions, and some of them have the ability to degrade oil residues. Bento *et al*. (2001) has isolated *Aspergillus* species from diesel samples from refineries, storage tanks, as well as from fuel and injection pumps of vehicles. The *Trichoderma* genus is mentioned as being able to degrade hydrocarbons (Launem *et al*., 1995; Colombo *et al*., 1996). Silva *et al*. (2003) carried out a selection of fungi found in estuarine sediments which had the ability to degrade hydrocarbons, and they also observed the presence of *Trichoderma, Aspergillus* and *Mucor* genera.

In Brazil, several vegetal species have been studied in order to recover degraded soils (Franco and Faria, 1997; Rodrigues *et al*., 2009). Leguminous forest trees with potential to associate with diazotrophic bacteria and mycorrhizal fungi have earned attention due to their more efficient strategy to capture N (in particular) and P, resulting in an accelerated growth of these species, in addition to an intensification of the biogeochemical cycle of the system and improvement in the quality of the degraded substrate (Franco and Faria, 1997; Reis, 2006). Therefore, this study was developed in consideration to the strong soil microbiota interaction with the rhizosphere of plants and to the poor availability of information on microorganisms that are able to grow in environments contaminated by oil. We utilize an experiment with seedlings of three species (Mamona (*Ricinus communis*), Acácia (*Accia holocerisea*) and Mimosa (*Mimosa caesalpiniifolia*)) that was growing under contaminated and non contaminated soil and attempted to isolate and select fungi lineages that had potential to degrade oil under contaminated soils. The growth ability of isolates in a culture medium containing 1% oil was tested, aiming at their application in the interaction process between bioremediation and phytoremediation.

MATERIAL AND METHODS

Sampling procedure

The samples were obtained from a phytoremediation experiment, performed under greenhouse conditions in Seropédica Municipality, at Rio de Janeiro State, Brazil, between September and October 2006. The substrate used was obtained from a subsuperficial soil layer (10-20 cm), found in the UFRRJ area. This soils was classified as a Planosol Ecology Serie, according to the Brazilian soil classification (Ramos *et al*., 1973) or as Abruptic Arenic Ochraquult, according to Soil Survey Staff (2006). A randomized block design with 4 repetitions and 6 treatments was used. The treatments were composed of a combination of 3 species *(Acacia holocerisea*; *Mimosa caesalpiniifolia* and *Ricinus comunis)* and two levels of contamination (0 and 4% oil, volume base). The soil was contaminated by crude oil (Ref. BB01-Petrobras) with the aid of a concrete mixer. The mixture was incubated for 15 days, in order to allow adaptation of the biota specialized in the

degradation of xenobiotics. At that moment, samples of contaminated and uncontaminated soil were collected for chemical characterization and correction of the relation C:N:P into 100:10:1 values. The analyses were performed in Soil Fertlity Laboratory at the Universidade Federal Rural do Rio de Janeiro (UFRRJ). Once the results of the analyses were obtained, phosphate fertilization was made using monoammonium phosphate (MAP) in uncontaminated soil, and MAP and urea in contaminated soil, in order to adjust the C:N:P relations as previously mentioned and suggested by Deuel and Holliday (1997). Glass pots were used, which received 0.8 dm^3 of soil, and the humidity level was maintained near to the field capacity of the soil. The seedlings, germinated in sandboxes, were then transplanted (2 specimens per pot for leguminous species, and only 1 specimen for mamonas, due to seedling size.

At each final collection of the experiment, at the 47th day after transplanting, soil samples were collected from each pot, stored into paper bags and sent for analyses.

Sample Processing

Five grams of soil were resuspended in 45 mL peptone 0.1 % containing 0.1 % Tween 80 inside Erlenmeyer flasks, and then agitated for 30 min with Shake at 150 rpm (Gomes *et al*., 2001; Markovina *et al*., 2005).

Microbiota Quantification

Martin was the culture medium used (Domsch *et al*., 1993). Plating was made using 0.1 mL aliquots of the respective dilutions. Later, a drop -plating technique with 5 repetitions was used. Petri plates were incubated at 25º C. Their reading was based on the presence or absence of Colony-Forming Units (CFU) after seven days (Cattelan *et al*., 1997).

Isolation and Identification

The isolation and identification were based on the macroscopic and microscopy morphological study made in Potato Dextrose Agar medium (BDA) and, whenever necessary, the microorganisms were inoculated in media according to each group and/or their sporulation was stimulated in Saccharose Glucose Agar (SGA) and Oatmeal Agar (AA). Additionally, they were also based on alternating lighting cycles (12/12), incubation period and slide culture (Domsch *et al*., 1980; Barnett *et al*., 1999; Davet and Rouxel, 2000; Watanabe 2002). For identification of species, specific bibliographies were consulted, such as Raper and Fennell (1965), Klich and Pitt (1988), Pitt (2000), Samson *et al.* (2000) and Klich (2002). Fungal colonies selected for identification were picked and stored in tubes containing Malt Extract Agar (MEA) at 4^o C.

Selection of Oil-Tolerant Fungi in Solid Medium

The potential fungi degrader was tested according to the methodology used by Conceição *et al*. (2005), modified. The experiments were performed on Petri plates containing Mineral Medium (MM), added with 1 % oil after autoclaving. In the sequence, the plates were incubated in the dark for 7 days at 25^o C. At the end of this incubation period, colony growth was observed. The entire experiment was carried out in duplicate. Fungal growth was relatively quantified into no growth (-), small (+), medium (++) and large (+++) by visual qualification based on a 5 mm inoculum, with readings made on the 7th culture day (**Fig 1**).

Statistical Analyses

For statistical analyses, the sources of variation were the presence or absence of

Descriptive Scale of Relative Fungal Growth (CFR)

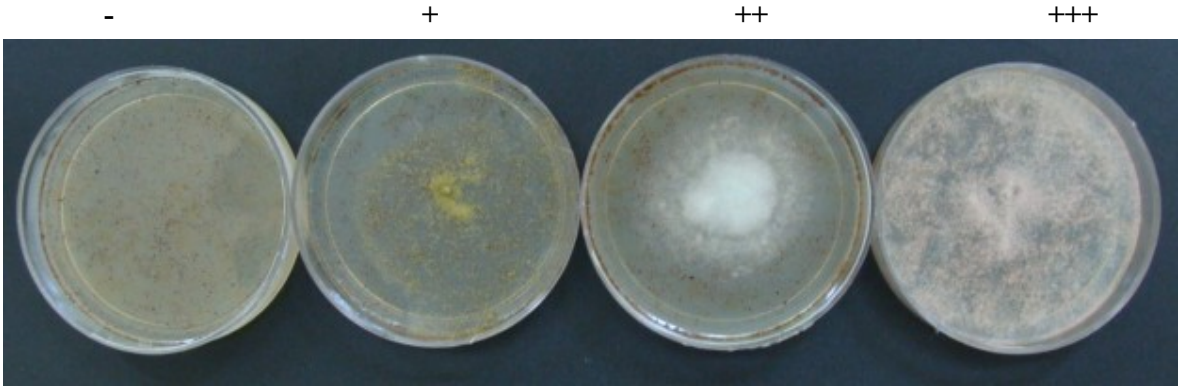

Fig 1: Tolerance scale of fungi in mineral medium (MM), added with 1% oil for 7 days at 25° C.

contaminants in the medium. For the purpose, the Student's t test at 5% was used to detect differences in the fungi diversity among the treatments. Based on this assumption, even if the samples were collected from pots containing distinct vegetal species, these collections seemed very similar in form and number and represented an opportunity of isolation of a higher number of species or genera, in comparison to collections from pots containing only one vegetal species.

RESULTS

The contamination of Planosol by oil modified significantly and negatively the number of CFUs; in average, the CFUs in uncontaminated and contaminated soil samples corresponded to 44.39 x 10^3 and 8.83 x 10^2, respectively (**Figure 2**).

Fig 2: Number of colony fungi units (CFU) (average ± standard error) in non-contaminated (NCS) and contaminated (CS) soil. * Significant difference between treatments with t-Student test at 5% of probability.

Two hundred and thirteen filamentous fungi were isolated from contaminated and uncontaminated soil samples. Twelve genera and 29 species were identified in uncontaminated soil, and the greatest fungi diversity was found in Class Ascomycetes. On the other hand, 8 genera and 12 species were identified in contaminated soil. *Penicillium* spp. was the genus more frequently found in uncontaminated soil; however, it was not isolated from contaminated soil.

The *Aspergillus flavus, A. niger, Cladosporium cladosporioides, Curvularia clavata,*

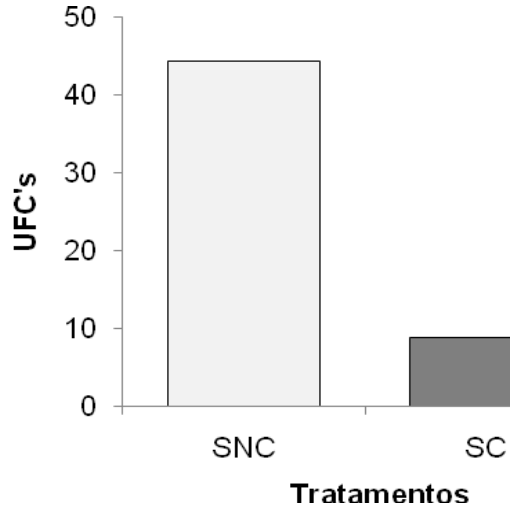

Fig 2: Number of CFUs in samples from contaminated (SC) and uncontaminated (SNC) Planosols.

Fusarium roseum, F. solani, Mucor mocrosporus and *Trichoderma harzianum* species developed in both contaminated and uncontaminated soil conditions. On the other hand, the *Trichoderma pseudokoningi, T. koning, Pythium ultimum, Rhizopus* sp. and *Mucor circinoides* species were found in contaminated soil only (**Table 1**).

For tolerance tests in MM, a medium that is poor in carbon source, this was an alternative to observe fungal growth under extreme conditions and for a possible utilization of oil as carbon source. *Aspergillus niger, Cladosporium cladosporioides* and *Curvularia clavata* either did not grow (-) in the medium, were considered sensitive to oil or were not able to use oil as carbon source. Their absence could be interpreted as a contamination indicator. A certain variation was observed regarding the non-growth and growth index of some *A. flavus, Fusarium roseum, F. solani, Trichoderma harzianum, T. koningi* and *T. pseudokoningii* isolates. The 21.4 *Aspergillus flavus* isolate, 7.5 *Fusarium roseum* isolate, 18.2 *Trichoderma harzianum* isolate *and* 13.4 *T. koningi* isolate showed the highest growth index (**Table 2**).

DISCUSSION

It is likely that the microorganisms evaluated may have co-metabolized the existing hydrocarbons under the conditions offered, even if they may have used a second carbon source to maintain their growth and reproduction while hydrocarbons were simultaneously degraded.

Studies on the isolation of filamentous fungi in environments containing oil or its subproducts found a very similar diversity of genera to that found in our study, such as: *Fusarium, Candida, Aspergillus* and *Penicillium* (Cerniglia, 1997; Bento *et al*., 2005; Chaillan *et al*., 2004). Araujo and Lemos (2005), in their study with soils contaminated by 5 % oil were able to isolate several species from the *Aspergillus, Penicillium, Paecilomyces* and *Fusarium* genera which, in their majority, were able to degrade petroleum hydrocarbons. Later, Reiche and Lemos (2006) isolated several filamentous fungi from soil, which were able to degrade crude oil. In this study, degradation was tested by means of mineral medium containing crude oil and mineral medium containing crude oil plus glucose. Ravelet *et al.* (2000) isolated many fungi species that were able to degrade polycyclic aromatic hydrocarbons. The species isolated were *Coniothyrium fuckelii,*

Table 1: List of fungi species isolated from uncontaminated soils and soils contaminated by 4% oil.

SNC	SC
Acremonium sp.	
Aspergillus flavus	*Aspergillus flavus*
A. fumugatus	
A. niger	*A. niger*
A. oryzae	
A. penicilioides	
A. terreus	
Cladosporium cladosporioides	*Cladosporium cladosporioides*
Curvularia clavata	*Curvularia clavata*
Dictyoarthrinium sacchari	
Eurotium chevalieri	
Fusarium oxysporum	
F. roseum	*Fusarium roseum*
F. semitectum	
F. solani	*F. solani*
Monocillium sp.	
	Mucor circinoides
Mucor hiemalis	
M. microsporus	*M. microsporus*
Penicillium chrysogenum	
P. citreonigrum	
P. decumbnes	
P. funiculosum	
P. islandicum	
P. janczeniskii	
P. restrictum	
P. rostratum	
P. simplicicimum	
P. viridicatum	
	Rhizopus sp.
	Pythium ultimum
Talaromyces sp.	
Trichoderma aureoviride	
T. harzianum	*Trichoderma harzianum*
Verticillium lecanii	
	T. koningii
	T. pseudokoningii

SNC: Uncontaminated Soil; **SC:** Contaminated Soil.

Gliocadium virens, *Mucor racemosus*, *M. racemosus var. sphaerosporus, Penicillium simplicissimum, P. janthinellion, Phialophora alba, Phialophora hoffmannii, Scopulariopsis brumptii* and *Trichoderma hazianum.*

Mollea *et al.* (2005) used fungi to optimize the degradation of PAHs and they demonstrated that *T. harzianum* was not able to biodegrade PAHs, whereas *Penicillium chrysosporium*, under the same testing conditions, biodegraded PAHs. These results differ from our findings, according to which *Trichoderma* species were able to grow in mineral medium containing crude oil and *Penicillium* species were not able to grow in soil contaminated by crude oil. Microorganisms may not degrade a certain contaminant at first, but this

Table 2: Tolerance of fungi isolated from contaminated soil, in MM containing 1% oil and incubated for 7 days at 25° C.

Fungus	Isolate	Growth Index
Aspergillus niger	21.3	-
	19.4	-
A. flavus	7.3	-
	13.3	+
	14.1	+
	17	+
	19.1	++
	20	++
	21.4	+++
Cladosporium cladosporioides	16.1	-
	22.1	-
Curvularia clavata	18.l	-
Fusarium roseum	4.1	+
	4.2	++
	4.4	+
	4.6	+
	4.7	++
	7.1	+
	7.5	+++
	7.6	+
F. solani	7.3	+
	12.3	++
	13.7	++
Mucor circinoides	2.3	CM
M. microsporus	6.7	CM
Pythium ultimum	2.1	CM
Rhizopus sp.	4.2	CM
Trichoderma harzianum	5.4	++
	5.16	-
	7.4	-
	12.4	++
	17.5	-
	18.2	+++
	19.5	++
	19.6	+
	21.5	-
T. koningii	13.4	+++
	14.3	+
	17.1	+
	19.3	+
	21.1	+
T. pseudokoningii	7.2	++

CM: Dead Culture.

can happen after a certain period. These characteristics are associated with the adaptation of growth enzymes of biodegrading populations and with genetic mutations (Brito *et al.*, 2004).

Colony growth rate assessments have been used to investigate the growth of filamentous fungi and to determine their effects on crude oil (Meysami and Baheri, 2003; Chaillan *et al*., 2004). Based on studies of the potential bioaugmentation effect on the removal of petroleum-based pollutants, Santos *et al.* (2008) obtained satisfactory results for fungal isolates used in the degradation of different pollutants through colony growth. In our study, we propose a methodology to be used as a tool to determine the biodegradation potential of fungal isolates, based on the development of colonies in relation to time.

Fungi are potential degraders under adverse conditions, such as in environments with low pH and poor in nutrients. They are present in any contaminated system and may use oil compounds and their recalcitrant derivatives as an energy source. Moreover, when compared with yeasts and bacteria, they have a higher ability to adapt to media with a low water activity.

CONCLUSIONS

Biological recovery processes of soils contaminated by hydrocarbons and/or their derivatives have been based on the stimulation of native microorganisms and, in some cases, on the increase of the microbial population, through incorporation of native/exogenous organisms. Success has been rarely achieved in the latter case, owing to the fact that many microorganisms available in the market are not native. These microorganisms do not seem to be competitive in comparison with the native microbial population, which is already adapted to the environment. One alternative would be the isolation of species from contaminated soil and their posterior growth and reintroduction into the same system. The problem is that conventional isolation methods are only able to extract a small part of viable microorganisms from the environment, thus limiting the achievement of species of interest. This fact will lead to a future development of better studies with these fungi, as well as with those that grow in both conditions, for specific purposes of use in biodegradation.

In our study the *Penicillium* was the most frequent genus found in uncontaminated soil; however, it was not isolated from contaminated soil. The existence of species that grow with or without the presence of oil allow inferences on their use as an contamination indicator or on how these hydrocarbons are degraded, having potential for the treatment of environments.

REFERENCE

Atagana HI, Haynes RJ, Wallis FM. 2006. Fungal Bioremediation of creosote contaminated soil: a laboratory scale bioremediation study using indigenous soil fungi. *Water, Air, and Soil Pollution* 172:201-219.

Araujo FSM, Lemos JLS. 2005. Isolamento e identificação de fungos degradadores de petróleo. X Jornada de Iniciação Cientifica do CTEM, Rio de Janeiro. 8p. http://www.cetem.gov.br/serie_anais_X_jic.htm

Barnett HL, Hunter BB. 1999. Illustrated genera of imperfect fungi. 4 th ed., Am. Phytop. Soc. Press, Saint Paul, MN. 218.

Bento FM, Camargo FA, Okeke BC, Frankenberger WT. 2005. Comparative bioremediation of soils contaminatedwith diesel oil by natural attenuation, biostimulation and bioaugmentation. *Bioresource Technology*, 96 (9):1049-1055.

Bento FM, Gaylarde CC. 2001. Biodeterioration of stored diesel oil: studies in Brazil. *International Biodeterioration & Biodegradation*, 47:107-112.

Brito NN, Zamora PP, Neto ALO, Battisti A, Paterniani JES, Pelegrini RT. 2004. Utilização de fungos na remediação de efluentes industriais**.** IV Fórum de Estudos Contábeis, Faculdades Integradas Claretianas, Rio Claro, SP. 1-55.

Braddock JF, Ruth ML, Cattedall PM, Walworth JL, McCarthy KA. 1997. Enhancement and Inhibition of Microbial Activity in Hidrocarbon Contaminated Arctic Soils: Implication for Nutrient Amended Biorremediation. *Environmental Science Technology* 31(7):2078-2084.

Cattelan AJ, Gaudêncio CA, Silva TA. 1997. Sistemas de rotação de culturas em plantio direto e os microrganismos do solo, na cultura da soja, em Londrina. *Rev Bras Cienc Solo* 21:293-301.

Cerniglia CE. 1997. Fungal metabolism of polycyclic aromatic hydrocarbons: past, present and future applications in bioremediation. J *In Microbiol Biotechnol* 19:324-333.

Chaillan F, Fleche AL, Bury E, Phantavong Y, Crimount P, Saliot A, Oudot J. 2004. Identification and biodegradation potential of tropical aerobic hydrocarbon-degradading microorganisms. *Res Microbiol*, 155:587-595.

Colombo JC, Cabello M, Arambarri AM. 1996. Biodegradation of aliphatic and aromatic hydrocarbons by natural soil and pure cultures of imperfect and lignolytic fungi. *Environmental Pollution* 94:355–362.

Conceição DM, de Angelis DA, Bidoia ED, de Angelis D. 2005. Fungos filamentosos isolados do Rio Atibaia, SP e refinaria de petróleo biodegradadores de compostos fenólicos. *Arq Inst Biol*, 72(1):99-106.

Davet P, Rouxel F. 2000. Detection and isolation of soil fungi. INRA Science Publishers, Plymouth, UK. 188.

Deuel LE, Holliday GH. **1997.** Soil remediation for the petroleum extraction industry. PennWell, 2nd. Ed., TULSA, USA., 242.

Domsch KH, Gams W, Anderson TH. 1993. Compedium of soil fungi. Academic Press, London. 860.

Evans FF, Seldin L, Sebastian GV, Kjelleberg S, Holmstron C, Rosado AS. **2004.** Influence of petroleum contamination and bioestimulation treatment on the diversity of *Pseudomonas* spp., in soil microcosms as evaluated by 16S rRna based PCR and DGGE. *Letters in Applied Microbiology*, 38 93-98.

Gadd G. **2004.** Mycotransformation of organic and inorganic sbstrates. *Mycologist* 18(2):60-70.

Gomes NCM, Heuer H, Schonfeld J, Costa R, Mendonça-hagler L, Smalla K. 2001. Bacterial diversity of the rhizosphere of maize (*Zea Mays*) grow in tropical soil studied by temperature gradient gel eletrophoresis. *Plant and Soil.*, 232:167 -180.

Hart S. 1996. In Situ Bioremediation: Defining the limites. *Environmental Science Technology* 30 (9):398A-401A.

Johnsen AR, Wick LY, Harms H. 2005. Principles of microbial PAH-degradation in soil. *Environ Poll*, 133(1):71-84.

Launen L, Pinto L, Wiebe C, Kiehlmann E, Moore M. 1995. The oxidation of pyrene and benzo[a]pyrene by nonbasidiomycete soil fungi. *Canadian Journal of Microbiology* 41:477–488.

Klich MA. 2002. Identification of common *Aspergillus* species. CBS, Utrecht, Netherlands. 116.

Klich MA, Pitt JI. 1988. A laboratory guide to common *Aspergillus* species and their teleomorphs. CSIRO, North Ryde, Australia. 116.

Kuiper I, Lagendijk EL, Bloemberg GV, Lugtenberg BJ. 2004. Rhizoremediation: a beneficial plant-microbe interaction. *Mol Plant Microbe Interact***,** 17(1):6-15.

Krämer, U. 2005. Phytoremediation: novel approaches to cleaning up polluted soils. *Current Opinion in Biotechnology*, 16:133-141.

Markovina A, Pitt JI, Hocking AD, Carter DA, Mcgee PA. 2005. Diversity of the Trichocomaceae in the Katandra Nature Reserve, Central Coast, NSW, Australia. *Mycol Res*, 109:964-973.

Meysami P, Baheri H. 2003. Pre-screening of fungi and bulking agents for contaminated soil bioremediation. *Adv Environ Res*, 7:881-887.

Mollea C, Bosco F, Ruggeri B. 2005. Fungal biodegradation of naphthalene: microcosms studies. *Chemosphere*, 60(5):636-43.

Pasqualino JC, Montane D, Salvado J. 2006. Synergic effects of biodiesel in the biodegradability of fossil-derived fuels. *Biomass and Bioenergy* 30:874879.

Pitt JI. 2000. A laboratory guide to common *Penicillium* species. Food Science Australia a Joint Venture of CSIRO and AFISC, Australia. 197.

Ramos DP, Castro AF De, Camargo MN. 1973. Levantamento detalhado de solos da área da Universidade Federal Rural do Rio de Janeiro. *Pesquisa Agropecuária Brasileria* 8:1-27. (Série Agronomia).

Raper KB, Fennell DI. 1965. The genus *Aspergillus*. Williams & Wilkins, Baltimore. pp. 686.

Ravelet C, Krivobok S, Sage L, Steiman R. 2000. Biodegradation of Pyrene by Sediment Fungi. *Chemosphere*, 40:557–563.

Reiche AP, Lemos JLS. 2006. Estudo do potencial de degradação de petróleo de linhagens de fungos isoladas de solo nordestino. In: XIV Jornada de Iniciação Científica, Centrode Tecnologia Mineral – CETEM/MCT. 55.

Reis LL. 2006. Monitoramento da recuperação ambiental de áreas de mineração de bauxita na Floresta Nacional de Saracá-Taquera, Porto Trombetas (PA). Universidade Federal Rural do Rio de Janeiro, Seropédica, RJ, 159. (PhD Thesis).

Rodrigues RR, Lima RAF, Gandolfi S, Nave AG. 2009. On the restoration of high diversity forests: 30 years of experience in the Brazilian Atlantic Forest. *Biological Conservation*. 142:1242-1251.

Soil Survey Staff. Keys to soil taxonomy. 10 ed. USDA-SCS, Washington. **2006**. 332.

Samson RA, Hoekstra ES, Frisvad JC, Filtenborg O. 2000. *Introduction to food and airborne fungi*. 6th ed., CBS, Baarn, The Netherlands. 389.

Santos EO, Rosa CFC, Passos CT, Sanzo AVL, Burkert JFM, Kalil SJ, Burkert CAV. **2008.** Pre-screening of filamentous fungi isolated from a contaminated site in Southern Brazil for bioaugmentation purposes. *African Journal of Biotechnology* 7(9):1314-1317.

Silva M, Cerniglia CE, Pothuluri JV, Canhos VP, Esposito E. 2003. Screening filamentous fungi isolated from estuarine sediments for the ability to oxidize polycyclic aromatic hydrocarbons. *World Journal of Microbiology & Biotechnology* 19:399-405.

Ururahy AFP, Pereira Jr N, Marins MDM. 1998. Desempenho de um Biorreator do Tipo CSTR no Processo de Degradação de Borra Oleosa. *Boletim Técnico PETROBRÁS*, 41(¾):125-132.

Watanabe T. 2002. Pictorial atlas of soil and seed fungi morphologies of cultured fungi and key to species. 2 ed.. CRC Press, Londom, UK. 486.