visible scientific programme. We have acquired expertise both in Antarctic science and logistics. Like many developed or developing countries, India is also an active partner in Antarctic explorations under the umbrella of various international governing bodies like ATCM, COMNAP, SCALOP, SCAR, etc. True to the spirit of the Antarctic Treaty System, the Indian Antarctic Programme is embarking upon mutually beneficial scientific collaborations with various Treaty nations.

Having gained sufficient expertise in organizing annual Antarctic expeditions for the last 25 years, it is most appropriate at this juncture to venture into the northern hemisphere, i.e. the Arctic region to undertake the aforesaid scientific studies. There is a pressing need for establishing bi-hemispheric approach in understanding the vital issues related to environment/climatic changes. The Svalbard Treaty may offer a unique opportunity to India to explore the possibilities of carrying out various scientific activities using the facilities at Svalbard Island as a research station. This correspondence does not necessarily speak on any policy with regard to the Arctic programme. It only points to the scientific wealth that the region can offer India.

1. The fourth Assessment Report (AR4), United Nations Intergovernmental Panel on Climate Change, 2007.
2. Rajan, S. and Khare, N., In Proceedings of the National Seminar on Four Decades of Marine Geosciences in India – A Retrospective, Geological Society of India, 2002, pp. 70–76.
3. MacDonald, G. M. *et al.*, *Quat. Res.*, 2000, **53**, 302–311.
4. Overpeck, J. *et al.*, *Science*, 1997, **278**, 1251–1256.
5. Manabe, S. and Stouffer, R. J., *Quat. Sci. Rev.*, 2000, **19**, 285–299.
6. Polyakov, I. V. and Johnson, M. A., *Geophys. Res. Lett.*, 2000, **27**, 4097–4100.
7. Polyakov, I. V., Johnson, M. A., Colony, R. L., Bhatt, U. and Alekseev, G. V., *Geophys. Res. Lett.*, 2002, **29**, 1878.
8. Stein, R. *et al.*, *Quat. Sci. Rev.*, 2004, **23**, 1485–1511.
9. Outridge, P. M., Stern, G. A., Hamilton, P. B., Percival, J. B., McNeely, R. and Lockhart, W. L., *Geochim. Cosmochim. Acta*, 2005, **65**, 4881–4894.
10. Knutti, R., Meehl, G. S., Allen, M. R. and Stainforth, D. A., *J. Climate*, 2006, **19**, 4224–4233.
11. Cortijo, E., Duplessy, J.-C., Labeyrie, L., Duprat, J. and Paillard, D., *C. R. Geosci.*, 2005, **337**, 897–907.
12. Leuschner, D. C. and Sirocko, F., *Quat. Sci. Rev.*, 2000, **19**, 243–254.
13. McDowell, P. F. and Edwards, M. E., *Quat. Sci. Rev.*, 2001, **20**, 63–76.
14. Funder, S., Hjort, C. and Kelly, M., *Quat. Int.*, 1991, **10–12**, 107–122.

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Isolating diesel-degrading bacteria from air

Air is rich in microorganisms, many of which are important to humans. In the present study, we have devised a method of isolating diesel-degrading bacteria from air, which is a simpler, faster and more efficient method of isolating these microorganisms compared to the conventional method of isolation from diesel contaminated soil[1] or water through enrichment culture techniques.

For isolation of diesel-degrading microorganisms from air, the experiment was set up in 250 ml flasks containing 100 ml Bushnell–Hass medium[2] ($MgSO_4$, 0.20 g/l, $CaCl_2$, 0.02 g/l; K_2HPO_4, 1 g/l; NH_4NO_3, 1 g/l; $FeCl_3$, 0.05 g/l; KH_2PO_4, 1 g/l; pH 7.0), supplemented with increasing concentrations of diesel (2–8%) and left open for isolating strains from air against a blank which was well covered. The flasks were left open for 10 days and manual shaking was done at regular intervals. These cultures were then plated on nutrient agar medium and incubated at 28°C overnight.

Flasks having 2 and 4% diesel concentration indicated observable growth after 4 days. After 6 days, other flasks containing higher percentage of diesel also showed growth and emulsification, which was lesser as compared to the ones with lower concentration (data not shown). The emulsification activity was determined using xylene emulsification method[3]. The temperature at which the experiment was carried out varied between 15°C and 33°C. Isolation from air at such variable temperatures indicated that our strain might have a wide range of thermal tolerance. Plating of the cultures from flasks having variable diesel concentrations on nutrient agar medium gave identical colonies, which indicated the presence of a single strain in all the flasks (Figure 1 *a*). The same isolate when plated on King's medium[4] (peptone, 20 g/l; glycerol, 10 g/l; K_2HPO_4, 1.5 g/l; $MgSO_4 \cdot 7H_2O$, 1.5 g/l; agar, 15 g/l; pH 7.2) showed fluorescence in the short-wavelength (254 nm) ultraviolet light (Figure 1 *b*). A common characteristic of the fluorescent pseudomonads is the production of pigments that fluoresce under short-wavelength ultraviolet light, particularly after growth under conditions of iron limitation.

The isolate was tentatively identified as *Pseudomonas* strain based on morphological and biochemical characteristics. In order to compare the bacterial characteristics of the air isolate with those isolated from diesel-contaminated soils, we selected 50-year-old diesel contaminated sites of the Himachal Pradesh Roadways Transport (HRTC), Shimla, Himachal Pradesh, India. Samples were collected using quadrant method in sterile containers and inoculated into enrichment cultures.

The isolate from air was compared with the 11 strains obtained from diesel-contaminated soil through morphological and biochemical studies. The air isolate along with other isolates obtained from the soil showed indications of belonging to the genus *Pseudomonas*. Further studies to identify these isolates are in progress.

The usefulness of the bacterial strain isolated from air for diesel spill site remediation was evaluated by studying its effect on mineralization of diesel in *in vitro* studies.

Percentage of diesel contamination in the soil and estimation of diesel utilization by microbial strains were studied using gravimetric and chromatographic analysis[5]. The diesel fractions were analysed by gas chromatography (Agilent Model 6820) with a flame ionization detector (GC-FID) using a 15 m long DB1 column (0.53 mm by 0.5 μm film thickness). The carrier gas was nitrogen at a flow rate of 45 ml/min. The injector and detector were maintained at 300°C and the oven was programmed to a temperature gradient from 80°C to 130°C at an increment of 30°C/min and held for 5 min, and finally ramped to 270°C for 20 min. Hydrocarbon degradation in the samples was calculated by integration of the peak areas under the resolved chromatogram over the entire run.

To test the diesel-degrading potential of the air isolate, an experiment was set up which continued for 9 days. Growth was monitored on a daily basis at an interval of 24 h, by performing microbiuret test[6].

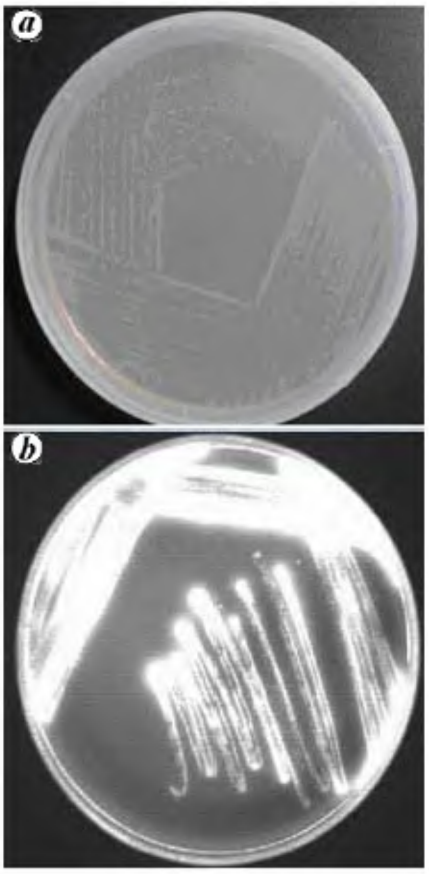

Figure 1. Plated bacterial colonies on nutrient agar medium (***a***) and showing fluorescence on King's medium (***b***).

Percentage of diesel degradation was calculated using the following expression:

$$100 * (Cc - Ct)/Cc,$$

where *Cc* and *Ct* are diesel content before and after treatment respectively.

In the case of flasks containing 2, 4 and 8% diesel content, the diesel content was reduced by 49, 66, 36 and 7% respectively (Table 1). From the data it can be seen that maximum diesel utilization by the air isolate had taken place in the flask containing 4% diesel concentration, followed by 2% concentration. A significant percentage of diesel has also been utilized in the flask containing 6% concentration, but the percentage degradation was lesser compared to 2 and 4% concentration. Minimum diesel utilization had taken place in the flask containing higher percentage of diesel, i.e. 8%. If we compare the data with the growth pattern (Figure 2), it can be seen that there is a correlation between diesel utilization and growth of the isolate at different diesel concentrations.

Data obtained by growth pattern of the isolate (Figure 2) suggested that our isolate exhibited best growth at 2–4% diesel concentrations, followed by 6 and 8%. Growth in 2 and 6% diesel concentrations peaked around 168 h and declined thereafter, whereas the flask containing 4% diesel was still in the growth phase, which indicated that 4% is the optimum concentration for the growth of this isolate. Even at 8% concentration, the strain was in the growth phase, but at a slow pace. This result suggested that 2 to 4% diesel concentration favour better growth of diesel-degrading bacterium compared to higher concentrations (above 6%).

No reliable conclusions could be drawn from emulsification activity test. But visibly 4% diesel concentration showed the best emulsification (data not shown).

The daily pH measurements (Figure 3) showed a shift of pH towards the alkaline side with the growth of bacteria at all diesel concentrations. Increase in pH may be due to release of by-products during hydrocarbon degrdation[7] or due to the denitrification process in *in vitro* cultures of *Pseudomonas aeruginosa*, as observed in another study[8]. Chemical analysis of the hydrocarbon by-products and bacterial secretions may be done in future.

Isolation of diesel-degrading bacteria from air has proved to be a unique and simple method compared to the conventional method of isolating such bacteria from diesel-contaminated soil and water. This is because the time taken to obtain the potential isolates is comparatively less and there is no risk of loss of certain strains due to serial dilutions as in the case of enrichment culture technique. The air isolate was able to grow at various concentrations of diesel, suggesting its possible exploitation in future bioremediation processes. We optimized the diesel concentrations on which our isolate showed the best growth. Qualitative analysis of the fractions being utilized by the bac-

Table 1. Percentage diesel utilization by air isolate while growing in increasing concentrations of diesel. Values are mean of three replicates ± SD

Treatment	Diesel utilized (%)
Bushnell–Hass medium + 2% diesel	49 ± 3.01
Bushnell–Hass medium + 4% diesel	66 ± 3.51
Bushnell–Hass medium + 6% diesel	36 ± 4.04
Bushnell–Hass medium + 8% diesel	7 ± 1.52

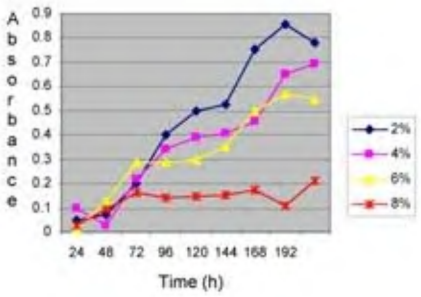

Figure 2. Growth of air isolate on increasing percentage of diesel in the medium.

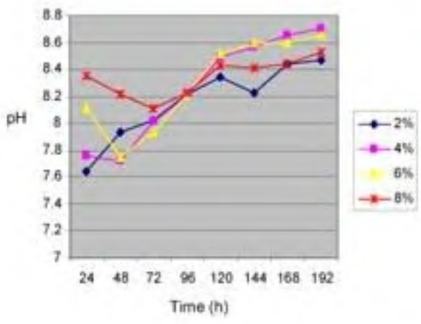

Figure 3. pH change during growth of air isolate on increasing percentage of diesel in the medium.

teria will be done using GC–MS. Further studies are under way to characterize this isolate.

1. Hong, Ji., Kim, J., Choi, O., Cho, K-Suk. and Ryu, H., *World J. Microbiol. Biotechnol.*, 2005, **21**, 381–384.
2. Bushnell, I. D. and Hass, H. E., *J. Bacteriol.*, 1941, **41**, 653–658.
3. Banat, I. M., Samarah, N., Murad, M., Horne, R. and Banerjee, S., *J. Microbiol. Biotechnol.*, 1991, **7**, 80–88.
4. King, E. O., Ward, M. K. and Raney, D. E., *J. Lab. Clin. Med.*, 1954, **2**, 301–307.
5. Chhatre, S., Purohit, H., Shanker, R. and Khanna, P., *Water Sci. Technol.*, 1996, **34**, 187–193.
6. Itzhaki, R. F. and Gill, D. M., *Anal. Biochem.*, 1964, **9**, 401–410.
7. Rahman, K. S. M., Rahman, T. J., Banat, I. M., Lord, R. and Street, G., In *Environmental Bioremediation Technologies* (eds Singh, N. S. and Tripathi, D. R.), Springer, Berlin, 2007, pp. 391–398.
8. Chawala, C. and Lu-Kwang, J., *Appl. Environ. Microbiol.*, 1999, **66**, 493–498.

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Ingress of lantana in dry tropical forest fragments: Edge and shade effects

Invasion of native communities by exotic species is considered the second largest threat to global biodiversity[1]. Since the tropics are highly populated, they experience greater pressure of invasive species, as the process of plant species invasion gets exacerbated with increased anthropogenic interventions. Seasonal tropical forests are especially susceptible to invasion[2]. In India, dry tropical forest accounts for 28.6% of the total forest cover[3]. These forests are under immense anthropogenic pressure due to rapid industrialization and related land-use changes in the past few decades[4], leading to forest fragmentation[5]. Lantana (*Lantana camara* L.) had been introduced in India in the early 19th century as an ornamental plant[6]; but now it is growing densely throughout India. Field observations in the dry tropical forests of India indicated that lantana is spreading fast. However, it is not uniformly distributed across a forest fragment[7]. The objective of the present study was to assess the spread of lantana across forest fragments and to establish a relationship between lantana cover and tree canopy opening due to dry tropical forest fragmentation.

The study area lies on the Vindhyan plateau, Sonebhadra District, Uttar Pradesh (24°13′–24°19′N; 83°59′–83°13′E) and is 315–485 m asl[8]. Climate is tropical monsoonal, with mean annual rainfall of 821 mm. Soils are Ultisols and extremely poor in nutrients[9], and support tropical dry deciduous vegetation[4].

Nine forest fragments (sites), varying between >1 and 10 ha in size were selected to represent a range of canopy cover and vegetation conditions. At each site, ten quadrats (10 × 10 m) were sampled along a belt transect from the edge to the interior to measure tree canopy and lantana cover. The quadrats along the belt transects were segregated into edge, middle and interior in terms of species composition by the PCA ordination technique (data not shown here). At each site, three quadrats were selected from each of the above regions for soil sampling and collected soil samples were further analysed for physical and chemical characteristics[10].

Triplicate seedlings of lantana were exposed to different shade treatments in the Botanical Garden, Banaras Hindu University (BHU), Varanasi (25°18′N lat., 80°1′E long.) for assessing growth at 60 and 90 days per shade treatment. A separate set of three seedlings was used for recording the initial growth parameters.

Natural shades were imposed on the seedlings by placing pots under tree shades providing low (70–100% sunlight), medium (30–60% of full sunlight) and high shade (10–20% of full sunlight) regimes[11]. Light intensity at the low-shade level was 1600–1720 µmol/sq. m/s PAR at 11.00 am on a cloud-free day, as measured using LCpro portable photosynthetic system (ADC, UK). All the pots were maintained at 50% soil moisture.

Seedlings harvested at 0, 60 and 90 days were brought to the laboratory, washed and oven-dried (at 80°C) for biomass and relative growth rate (RGR) estimation as follows[12]:

$$\text{RGR (mg/g/d)} = \frac{\ln W_2 - \ln W_1}{t_2 - t_1},$$

where W_1 is the total plant dry weight at time t_1 and W_2 the total plant dry weight at time t_2.

Relationships between distance from the edge and tree canopy cover, as well as lantana cover were examined through regression analysis. ANOVA and Tukey's range was used to examine the effect of shade regimes on the relative growth rate of seedlings.

In the present study, tree canopy and lantana cover varied from 23 to 65% and from 1 to 52% from the edge to the interior of the fragment (Figure 1). Significant positive and negative relationship of tree canopy cover ($r^2 = 0.87$, $P = <0.0001$) and lantana cover ($r^2 = 0.89$, $P = <0.0001$) was observed with the distance from edge to interior respectively. Thus tree canopy cover and lantana cover were negatively associated with each other ($r^2 = 0.85$, $P = 0.001$). Being an opportunistic species, lantana is favoured by disturbance-induced structural changes in the community, such as the amount of biomass destroyed[13] or changes in soil characteristics or both[14]. In the dry de-