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ENVIS CENTRE ON BIOGEOCHEMISTRY

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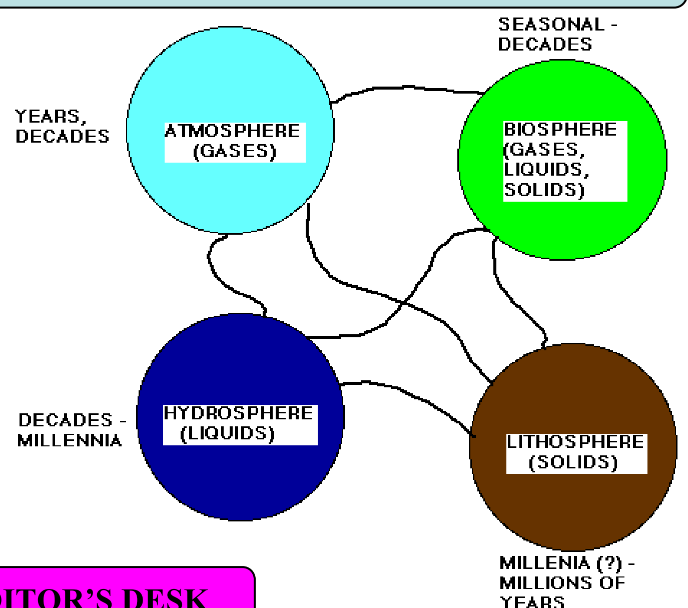
Content



FROM EDITOR'S DESK

This issue high lights both positive and negative roles, microorganisms play in the environment. In first article, the importance of microorganisms in nutrient cycling and the process of bio remediation has been emphasized. The second article describes the environmental impacts of antibiotic resistant strain MRSA and the preventive and curative measures to check its prevalence in India.

In addition, in present news letter covers some recent research publications on biogeochemistry.

Prof. V.K. Jain
Editor

THE PROCESS OF BIOREMEDIATION BY MICROORGANISMS: AN OVERVIEW

ABSTRACT : Microorganisms are widely distributed in geographical areas with diverse morphology ranging from single celled to multicellular. Some of these are capable to grow in extreme environmental conditions specially bacteria and fungi. These organisms play importnat role with respect to environmental degradation of natural and anthropogenic chemicals and wastes and are also involved in recycling of environmental resourses In this review we have discussed about the role of these microorganism in environment and also discuss their ecological characteristics which make them suitable to be used for bioremediation.

Introduction: Environment is the interrelationship between human beings, their natural surroundings and the environment they create. Due to anthropogenic activities a lot of hazardous waste which are dumped in our environment which disrupts the homeostatic environment. To cope with these nature has provided biotools (biocatalyst) as they have potential to grow in toxic environment (Acidophile **,** Alkaliphile**,** Anaerobe**,** Endolith**,** Halophile**,** etc) and they have capability to degrade or detoxify the pollutant. Such accumulation often occurs in inhospitable, toxic environments that lack the nutrients, water or appropriate electron acceptors that would be needed to support the growth of microorganisms capable of remediation. The ideal decontamination machinery would be able to cope with these conditions, and could explore the contaminated environment thoroughly and track chemicals even into pores and organic matter[1]. Chemicals precipitate, adsorb to surfaces or accumulate in organic matter and in tiny pores of solid matrices, leading to a decline in their bioavailability[2]. Fungi, alone or in collaboration with bacteria and plants, display many of these features and could be important components of biotechnologies designed to remediate polluted soil, water and air[1]. In this review we have focused on microorganisms specially suited for nutrient recycling and remediation process.

Bacterial bioremediation and recycling of nutrients:

Land utilization of biosolids and applications of fertilizers and pesticides have contributed to a continuous accumulation of heavy metals in many aquatic and near-surface systems[3]. Bioremediation, which involves the use of living microbes to remove heavy metals, has been considered to be a safe and economic alternative to physicochemical strategies due to their ability of self-replenishment, continuous metabolic uptake of metals after physical adsorption, and the potential for optimization through development of resistant species and cell surface modification[4]**.**The fate of toxic metallic cations in environment depends largely on their interactions with microorganisms. Metal ions can be immobilized by functional groups such as carboxyls, phosphomonoesters, phosphodiesters, amines and hydroxyls that are native to the proteins, lipids, and carbohydrates on the cell walls of organisms[5]. Cyanobacteria have been found to be of interest in metal adsorption processes. Cyanobacteria are photosynthetic prokaryotes commonly found in natural environment and are suggested to have some added advantages over other microorganisms for removing heavy metals because of their large surface area, greater mucilage volume with high binding affinity and simple nutrient requirements[6]. Cadmium known to be a Human Carcinogens receives increasing attention as one of the most toxic heavy metals[7]**.** When a plant dies, an animal dies, or an animal expels waste, the initial form of nitrogen is organic. Bacteria, or in some cases, fungi, convert the organic nitrogen within the remains back into ammonium (NH_4^+), a process called ammonification or mineralization. All the nitrogen assimilated, excreted are recycled in different form of nitrogen. This in biological term is called nitrogen cycle. Atmospheric nitrogen must be processed, or "fixed" to be used by plants. Some fixation occurs in lightning strikes, but most fixations is done by free-living or symbiotic bacteria. These bacteria have the nitrogenase enzyme that combines gaseous nitrogen with hydrogen to produce ammonia, which is then further converted by the bacteria to make their own organic compounds (**Fig. 1**). Most biological nitrogen fixation occurs by the activity of Mo-nitrogenase, found in a wide variety of bacteria and some Archaea. Mo-nitrogenase is a complex two component enzyme that contains multiple metal-containing prosthetic groups[8]. Some nitrogen fixing bacteria, such as *Rhizobium*, live in the root nodules of legumes (such as peas or beans). Here they form a mutualistic relationship with the plant, producing ammonia in exchange for carbohydrates. Nutrient-poor soils can be planted with legumes to enrich them with nitrogen. As a result of extensive cultivation of legumes (particularly soy, alfalfa, and clover), growing use of the Haber-Bosch process in the creation of chemical fertilizers, and pollution emitted by vehicles and industrial plants, human beings have more than doubled the annual transfer of nitrogen into biologically-available forms[9]. In addition, humans have significantly contributed to the transfer of nitrogen trace gases from Earth to the atmosphere, and from the land to aquatic systems. Human alterations to the global nitrogen cycle are most intense in developed countries and in Asia, where vehicle emissions and industrial agriculture are highest[10].

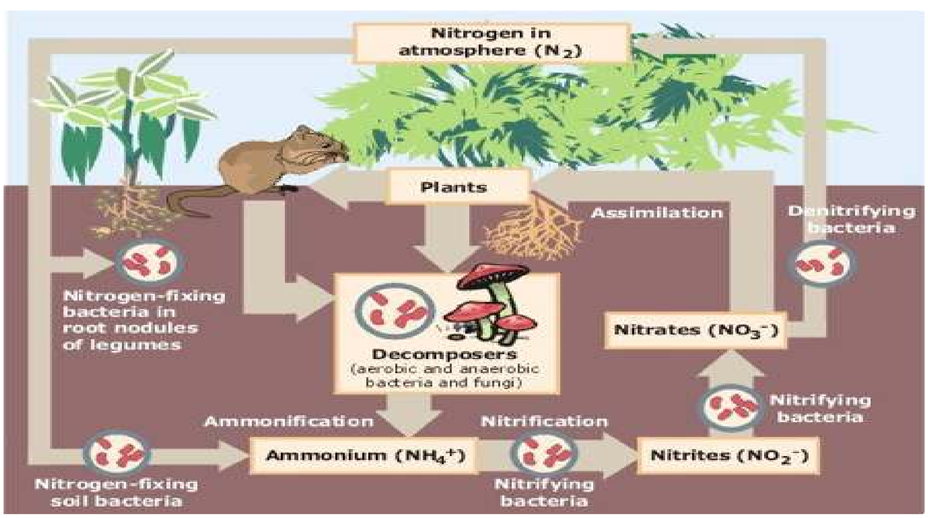


Fig. 1. Schematic representation of the flow of nitrogen through the environment. The importance of bacteria in the cycle is immediately recognized as being a key element in the cycle, providing different forms of nitrogen compounds assimilable by higher organisms.

Fungi as efficient biocatalyst for bioremediation

Fungi falls under the kingdom fungi which consist of hetrotrophic, absorptive eukaryotic organism. There body consist of tubular body called hyphae which are cylindrical, thread-like structures 2–10 µm in diameter and up to several centimeters in length. Some of them grow at extreme environmental conditions wherein other organisms are fatal. Their growing hyphae tips produce extracellular enzymes to digest the nutrients available to them. One of the primary roles of fungi in the ecosystem is decomposition, which is performed by the mycelium. The mycelium secretes extracellular enzymes and acids that break down ligninand cellulose, the two main building blocks of plant fiber. These are organic compounds composed of long chains of carbon and hydrogen, structurally similar to many organic pollutants. The key to mycoremediation is determining the right fungal species to target a specific pollutant. Certain strains have been reported to successfully degrade the nerve gases VX andsarin. The low specificity of many fungal enzymes enables the organisms producing them to co-metabolize structurally diverse compounds belonging to different pollutant classes, the most prominent example for this being *Phanerochaete chrysosporium* degrading benzene, toluene, ethylbenzene and xylenes (BTEX) compounds, nitroaromatic and *N*-heterocyclic explosives (TnT and RDX, respectively), organochlorines (chloroaliphatics, chlorolignols, chlorophenols, polychlorinated biphenyls and PCDDs), PAhs, pesticides, synthetic dyes and syntheticpolymers [11,12,13].

Box 1. Advantages of bioremediation (*Pure Appl. Chem.*, Vol. 73, No. 7, pp. 1163–1172, 2001. © 2001 IUPAC)

• Bioremediation is a natural process and is therefore perceived by the public as an acceptable waste treatment process for contaminated material such as soil. Microbes able to degrade the contaminant increase in numbers when the contaminant is present; when the contaminant is degraded, the biodegradative population declines. The residues for the treatment are usually harmless products and include carbon dioxide, water, and cell biomass.

• Theoretically, bioremediation is useful for the complete destruction of a wide variety of contaminants. Many compounds that are legally considered to be hazardous can be transformed to harmless products. This eliminates the chance of future liability associated with treatment and disposalof contaminated material.

• Instead of transferring contaminants from one environmental medium to another, for example,from land to water or air, the complete destruction of target pollutants is possible.

• Bioremediation can often be carried out on site, often without causing a major disruption of normalactivities. This also eliminates the need to transport quantities of waste off site and the potential threats to human health and the environment that can arise during transportation.

• Bioremediation can prove less expensive than other technologies that are used for clean-up of hazardous waste.

Conclusion and future direction

At present lots of hazardous waste is being dumped in our environment which is quiet recalcitrant to degradation. For example for PAH, halogenated compounds, endoceine disrupting agents etc); to become biologically degraded they must be exposed to microorganism (especially fungi and bacteria). In situ treatment is beneficial over ex situ (BOX 1) as its leaves the environment intact without leaving impact on surrounding. In-

Situ bioremediation treats contaminated soil or groundwater in the location where it was found. Usually microorganisms are pumped into the soil or ground water so the contaminated soil and water can be broken down. Ex-Situ bioremediation needs the contaminated water to be pumped out and the contaminated soil to be removed. The contaminated water and soil are treated with microorganisms and then after the materials are degraded the soil and water are placed in a bed with oxygen and rich nutrients for further degradation Although bioremediation holds great promise for dealing with intractable environmental problems, it is important to recognize that much of this promise has yet to be realized. Specifically, much needs to be learned about how microorganisms interact with different hydrologic environments. As this under-standing increases, the efficiency and applicability of bioremediation will grow rapidly.

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MRSA, Environmental impacts and Preventive measures

Background

Two news items which consistently appear in newspaper are the climate change and the occurrence of multi drug resistant bacterial strains in various environmental sites - be it hospital, poultry, food, or drinking water. Development of antibiotic resistance is not a very surprising news as it is an inevitable consequence of irrational use of any chemical reagent to kill bacteria. As we all evolve bacteria also evolve in response to antimicrobial agents and become resistant. Due to this rapid development of resistant bacterial strains, the arsenal to fight microbes is becoming weak day by day. The gene codes for resistance are very likely to spread among other microbes and this is a major worry for scientist and doctors. The last and only hope was the vancomycin a glycopeptides which was good enough to tackle multidrug resistant bacteria. The first vancomycin intermediate sensitive strain was already reported in 1996 in Japan and ever since vancomycin resistance strains are being reported from various part of the world including USA, France, UK, Italy. Recently in India, occurrence of bacteria with supergene New Delhi betalectamase gene-1 or NDM-1 has been reported and drawing lot of attention world wide. If we talk about the Indian scenario the problem of antibiotic resistance can be more serious and bigger because infectious disease accounts for 40% of all diseases in Asia and in India there is no surveillance system like other countries and hence it is an alarming issue.

What is the superbug MRSA and the threats due to this?

MRSA stands for Methicillin Resistant *Staphulococcus aureus*. It is a strain of *Staphylococcus aureus* gram positive bacteria discovered by surgeon Sir Alexander Ogston in 1880 from abscess. It grows in grape like cluster and is golden in colour. It is a facultative anaerobe, catalase positive and coagulase positive bacteria. It is usually found in the nasal cavity and on the skin of 20% of human population as commensal but it is dangerous if the barriers are broken. In case of wounds and cuts it may go inside the body and can cause serious damage like endocarditis, toxic shock syndrome and sepsis. Its treatment started with discovery of Penicillin in 1928 and was considered as wonder drug and following it various antibiotics related and non-related to penicillin were discovered. Penicillin kills bacteria by blocking its cell wall synthesis or more scientifically by blocking peptidoglycan cross linking by binding to enzyme called transpeptidase which is a key factor in cell wall synthesis. With course of time bacteria found the way to fight with penicillin and it genetically evolved with an enzyme called β-lactamase, which has capacity to degrade the β-lactam ring of penicillin. A little modified version of penicillin called methicillin came in to existence in 1959 having no affinity for β-lactamase so initially bacteria was quite susceptible to methicilin and was under process to adapt to the methicillin until it happened in 1961 when first MRSA was reported from UK. A gene named mecA conferred resistance to methicillin by producing modified transpeptidase (penicillin binding protein 2a or PBP-2a) enzyme having low affinity for methicillin and also other β-lactam drugs like cephalosporin and carbepenem.[1, 2]

MRSA infectins are treated with non-βlactam drugs, such as clindamycin and co-trimoxazole. Resistance to these antibiotics has led the use of Linezolid, however resistance has also been reported for this. The treatment of invasive MRSA is currently done by vancomycin a glycopeptides which has many side effects including neuro and nephrotoxicity and resistance to drug has also been reported. [3]

Therefore, MRSA is a serious issue as it endangered human life if remains untreated. The gene code for resistance, generally lies in the mobile regions of the plasmid of bacteria, and is more prone to spread to other bacterial strains of same genera and to others also. As reported the NDM-1 is considered as supergene code for resistance and occurrence of such gene in drinking water can transmit to the other water born pathogens and make them resistant to the available antibiotics. According to the news in USA in a poultry meat store occurrence of MRSA was found in one in four meat samples. Is it not a worrisome situation [4, 16]?

World wide research is going on to develop new tools to treat the MRSA and various approaches like use of phage therapy, nanoparticles and host defense pepitides are currently under exploration phase. However, main focus is the development of naturally occurring, evolutionary conserved host defense peptides also known as antimicrobial peptides (AMPs) as therapeutics. More than 800 AMPs have been discovered so far and they have been potentially active against microbes ever since their occurrences in the host body and it will be difficult for bacteria to evade them. Several of them are under clinical trials for various bacterial born infections. However, their exact mode of bacterial killing inside the host body is still a mystery which still remains unexplored and poses a hurdle in the way of drug development. For instance; defensin such as AMP found in human body in gut lining and in neutrophils is very active in vitro study in absence of salt while its activity is lost on addition of salt which raises the question that how it acts inside the body in presence of salt, and how it delivers at the site of infection.[5,6]

Factors responsible for MRSA development:

Irrational drug use. Apart from medicine tablets antibiotics are also available in creams, syrups and lotions and on applying, mass of microbes expose to such antibiotics unnecessarily and they start evolving slowly as the dose is not enough so as to kill them all and this is one way how bacteria becomes resistant to drugs.

Secondly, not using the prescribed amount of antibiotics also is one of the major causes of making bacterial strains resistant. Patients stop taking antibiotic as they get better after one or two dose of antibiotic and they don't complete the course, thus all the microbes are not eradicated and in presence of low drug concentration they prevails and become more robust to fight the antibiotics.

Thirdly, the animals are often given the antibiotics in low doses and the inhabiting microbes take benefit and develop resistantce to such antibiotics and these microbes transfer to humans on improper consumption of meat obtained from such animals. For example; the case of reports of resistant staphlycoccus in 1 in 4 of meat samples [16,17].

Lastly but most important cause of making directly or indirectly bacterial strain resistant is the availability of antibiotics over- the- counter without any physician's recommendation. Specially in India, people start taking antibiotic on their own and without knowing the actual use and dose of antibiotic.

Hospital environment and spread of resistance:

The proper cleaning of hospital and the frequent use of disinfectant is recommended to avoid the transfer of infection from patients to environment and to other patients. In absence of such kind of measures, the microbes persist in the environment and become resistant. In hospital environmental sites like dustbins where used needles and empty antibiotic bottles are thrown, the chances of bacterial exposure to various antibiotics are more and help bacteria to become more robust. The prevalence of MRSA is of high intensity in surgical wards and the intensive care units.[12]

Mishandling of resistant strains in laboratory:

There have been reports of environmental contamination of microbiology laboratories working with resistant strains. The surface and sinks are the main sites of strains deposition and improper discard of the cultures are also the source of spread of resistant strains to other sites. [13]

Prevalence of MRSA in India:

Based on the available literature it is evident that MRSA is not uncommon in various tertiary care hospitals in India. However, much research is required to explore this issue in detail and surveillance of all Indian hospitals is needed to be performed. A pilot study was done to survey the prevalence of MRSA simultaneously at bthree centers across India, Delhi Mumbai and Bangalore during 1996. And out of the total 739 cultures of *S. aureus*, 235 (32%) were found to be multiply resistant with the individual figures for resistance being 27% (Bombay), 42.5% (Delhi) and 47% (Bangalore) [7]. According to the reports the prevalence of MRSA in surgical wound infections at All India Institute of Medical Sciences (AIIMS) during 2001-02 was determined. The analysis of 2,080 pus samples was done. A high incidence of *S. aureus* was observed. The MRSA prevalence rate was 44% of all *S. aureus* isolates [9]. A recent study done to check the prevalence of MRSA and VRSA in the AIIMS from year 2004 to 2008 shows that the percentage of MRSA isolates with a vancomycin MIC of 1 $\mu g\ ml^{-1}$ gradually decreased from 51.5 % during 2004 to 37.7 % in 2008. And the percentage of MRSA isolates with a vancomycin MIC of 2 $\mu g\ ml^{-1}$ gradually increased from 40.6 % in 2004 to 61.3 % in 2008 showing a significant trend of increasing minimum inhibitory concentration of vancomycin [3]. Another study done in Punjab in January 2008-2009, reports that out of total 250 *S.aureus* isolates, 47% were MRSA and out of the MRSA the 74% were multi drug resistant [12]. Similar observation was obtained for pattern of MRSA in Southern region of India. During a period of one year (2007-2008) study was conducted in tertiary care hospitals in Kanchipuram and Mangalore. A total of 237 isolates of *S. aureus* were studied and 29.1% were found to be methicillin-resistant. Another important observation was MRSA isolates showed greater resistance to multiple drugs [10, 11]. A study conducted in a tertiary care hospital in Wardha shows the 51.8% MRSA out of 280 *S.aureus* isolates collected from different samples. All the above studies were conducted in various part of India and revealed the existence of MRSA in tertiary care hospital. And very recently in April 2011 Times of India reported a study done on drinking water of Delhi which revealed the occurrence of drug resistant microbes containes supergene NDM-1. However, a detailed survey is needed to be conducted to get a better idea about the antibiotic resistance pattern of microbes at each tertiary care hospital and the other environmental sites like air , water, soil, and different communities across the country.

Environmental impacts of MRSA:

Application effect of disinfectant and environmental consequences:

The MRSA has spread everywhere and the use of disinfectant and other cleaning reagents is going to be very high and an improper use of such reagents pose a potentially negative impacts on health and the environment. The over use of disinfectant will eventually leak to environment and could be dangerous for agriculture, for aquatic life and can come back to human life by various sources for example food, water.

Ecotoxicology due to Glutaraldehyde: Glutaraldehyde, a biocide frequently used in hospitals and industries, is highly toxic to fresh water fish, and release of such chemicals can put a stress on the aquatic biodiversity.

Pharmaceuticals and personal care products (PPCPs) and adverse effects on Health and ecology:

PPCPs refers, in general, to any product used by individuals for personal health or cosmetic reasons or used by agribusiness to enhance growth or health of livestock. PPCPs comprise a diverse collection of thousands of chemical substances, including prescription and over-the-counter therapeutic drugs, veterinary drugs, fragrances, and cosmetics, for example antibiotic used for orchard pathogen. Trace levels of PPCPs are presently found in Canadian surface water and groundwater, and drinking water [18]. Studies have linked exposure to disinfection by-products to a range of adverse health effects including certain forms of cancer and reproductive health outcomes. Emerging research shows that PPCPs, too, can react with the same disinfectants. For example, new chemical by-products, often of unknown properties and toxicity, are created when disinfectants mix with common PPCPs, such as the estrogenic steroids used in contraceptives, anti-inflammatory agents such as ibuprofen, the antibacterial agent triclosan, and ultraviolet (UV) filters. All pharmaceutically active compounds are developed to target specific biological activity and are not meant for dispersion into the environment.

Preventive measures and cures:

WHO collaborates with governments of various countries and gives supporting shoulder. Helps in developing preventing policies, and helps governments build proper labs and research facilities and with multi-sectoral coordination that is required to tackle this problem.

Standard Precaution Center for Disease Control (CDC) Guideline for Isolation Precautions: Preventing Transmission of Infectious Agents in Healthcare Settings 2007, Contact Precaution like proper hygien and cleaning should be maintained in hospital environment.

Patient and visitors Education

Patient education is a critical component of MRSA case management. Healthcare professionals should educate patients and visitors on methods to avoid MRSA transmission to close contacts. Visitors of Infected Patients should avoid touching catheters or wound sites and should wash their hands before leaving an infected person's room. In addition to above measures, workshop should we conducted in general public and the adverse effects of the overuse and misuse of antibiotics should be advised. Restrictions on the use of antibiotics and problematic ingredients in personal care products are also easier to justify than restrictions on the use of pharmaceuticals.

Green environmental cleaning:

Green cleaning requires effective cleaning. And purpose is to protect human health from the adverse effect of cleaning products. Additionally, it reduces the environmental issues associated with the entire cleaning process. Green cleaning includes traditional way of cleaning and various guidelines of green cleaning include the restricted use of disinfectant to high risk areas etc. Thus, it does not prohibit the use of disinfectants and in situations where MRSA is of concern, the appropriate U.S. Environmental Protection Agency (EPA) registered disinfectant and cleaning protocols should be followed [17].

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Some recent Publications in Journals Related to Environmental Biogeochemistry

Thuan-Quoc Thach, Chit-Ming Wong, King-Pan Chan, Yuen-Kwan Chau, G. Neil Thomas, Chun-Quan Ou, Lin Yang, Joseph S.M. Peiris, Tai-Hing Lam, Anthony J. Hedley 2010 Air pollutants and health outcomes: Assessment of confounding by influenza. Atmospheric Environment, Volume 44, Issue 11, Pages 1437-1442

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Shar Samy, Lynn R. Mazzoleni, Subhashree Mishra, Barbara Zielinska, Anna G. Hallar. 2010. Water-soluble organic compounds at a mountain-top site in Colorado, USA Atmospheric Environment , Volume 44, Issue 13, 1663-1671.

Yuri Bedjanian, Mai Lan Nguyen, Georges Le Bras 2010Kinetics of the reactions of soot surface-bound polycyclic aromatic hydrocarbons with the OH radicals, Pages 1754-1760 Atmospheric Environment , Volume 44, Issue 14,

S.A. Power, C.M. Collins. 2010. Use of Calluna vulgaris to detect signals of nitrogen deposition across an urban–rural gradient. Atmos. Environ., Volume 44, Issue14, 1772-1780

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Beatriz C.M. Guimarães, Jan B.A. Arends, David van der Ha, Tom Van de Wiele, Nico Boon, Willy Verstraete. 2010. Microbial services and their management: Recent progresses in soil bioremediation technology. Applied Soil Ecology Volume 46, Issue 2, 157-167.

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