

# Bioremediation, Biodiversity and Bioavailability

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**Scope and target readership:** *Bioremediation, Biodiversity and Bioavailability* accepts reviews and original papers that apply ecological concepts, theories, models and methods to the management of biological resources (primarily plant), through the use of applied ecological problems to test and develop basic ecological theory, and primary fields of applied ecology: conservation biology, global change, pollution biology, wildlife and habitat management, land use and management, aquatic resources, restoration ecology, nuisance species, and the effects of genetically modified organisms. *Bioremediation, Biodiversity and Bioavailability* also welcomes papers in chemical ecology that integrate ecology and chemistry in an attempt to increase our understanding of the biological significance of natural products, embracing the evolutionary biology of chemically-mediated biotic interactions (mechanistic approaches and environmental aspects), biotic controls on the chemistry of the environment, geochemical control of the structure and function of ecosystems. Cycles – in particular their controls – are considered, either of individual elements or of specific classes of natural or anthropogenic compounds in ecosystems. Trophic relationships, intra- and interspecific communication, competition, and other kinds of chemical communication in all types of interactions between organisms will be considered, but preference will be given to plant systems. Mechanistic approaches should deal with the identification, biosynthesis and metabolism of substances which carry information and with the elucidation of receptor- and transduction systems, biochemical, molecular and physiological techniques. All aspects of biological diversity, its description, analysis, conservation, management, sustainable development in a conservation framework, and controlled rational use are welcome.

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**Cover photos:** Top, left: Distribution of *Total Annual Precipitation* attribute in Castilla y Leon (Ruíz-Potosme *et al.*, pp 23-35); Bottom, left: SEM micrograph of CO<sub>2</sub> active carbon of *Tetracarpidium conophorum* (Nwosu *et al.*, pp 89-95); Right Effect of chromium (A) and copper (B) on *Pongamia* seed germination and seedling growth after 6 weeks (Kumar *et al.*, pp 43-48).

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**Number 1**

**Adônis Moreira, Larissa A.C. Moraes, Nand K. Fageria (Brazil)** Potential of Rubber Plantations for Environmental Conservation in Amazon Region (pp 1-5)

**ABSTRACT**

**Mini-Review:** The Amazon is the largest tropical rainforest in the world. Its conservation is important to avoid world climate changes, especially rising atmospheric temperature, release of greenhouse gases and control of the water cycle. The objective of this review is to discuss the potential of planting rubber trees as a source of income and C storage and to demonstrate the advantages of their introduction in a sustainable form to reduce the concentration of CO<sub>2</sub> in the atmosphere. In the humid tropical Amazon, in upland soil areas, rubber monoculture plantations present the lowest estimated evapotranspiration compared with a natural forest. The potential carbon sequestration in total dry weight of adult rubber plantations is estimated in 275.1 ton ha<sup>-1</sup>. In the latter case this is comparable with the average values found for primary forests (240 ton ha<sup>-1</sup>) and tropical agroforestry systems (95 ton ha<sup>-1</sup>), respectively. Another advantage is the carbon accumulation in soil cultivated with rubber trees in monoculture and polyculture plantations. The cultivation of rubber trees by small farmers will have less impact on fauna because these farmers will maintain more extensive forest plots. Furthermore, if smallholdings can have at least one perennial crop as a main source of income, communities in the Amazon will reduce the rate of deforestation in comparison with that necessary for short-cycle annual crops.

**K. Padmalatha, K. Jayaram, N. L. Raju, M. N. V. Prasad, Rajesh Arora (India)** Ethnopharmacological and Biotechnological Significance of *Vitex* (pp 6-14)

**ABSTRACT**

**Review:** *Vitex* (Verbenaceae) is a large genus that has a plethora of ethnopharmacological uses. The various species of *Vitex* have been used to treat a range of human ailments, particularly related to insects, fungi, bacteria, snakes and poisonous spiders and diseases associated with menstruation and gynaecological problems. Several secondary metabolites like flavonoids, iridoid glycosides, terpenoids and labdane diterpenes have been reported in different species of *Vitex*. *Vitex trifolia* and *Vitex negundo* can be propagated vegetatively for cultivation on desecrated lands to produce huge biomass for commercial applications. This review emphasizes the phytochemical and ethnobotanical knowledge on some species of *Vitex* to highlight their traditional and modern usage.

**Pranay Bantawa, Swapan Kumar Ghosh, Soumen Moitra, Partha Dev Ghosh, Tapan Kumar Mondal (India)** Status and Conservation Threats of *Picrorhiza scrophulariiflora* Pennell. (Scrophulariaceae): An Endangered High Valued Medicinal Plant of the Indo-China Himalayan Region (pp 15-22)

**ABSTRACT**

**Original Research Paper:** *Picrorhiza scrophulariiflora* Pennell. is an endangered medicinal herb of the Indo-China Himalayan region which urgently needs to be conserved *in situ* by preventing animal grazing as well as unsystematic harvesting of its rhizomes. Habitat preferences, population structures, existing status, conservation threats, correlation of soil characters with aerial and underground biomass as well as ethno-medicinal values of Sikkim Himalayas were studied. The present study revealed that analytical features such as density, frequency, abundance, relative density, relative frequency, abundance-frequency ratio, etc., were low in all populations, which indicates the requirement of immediate conservation planning. Open pastures, rocks, crevices, rocky terrain and moist areas were found to be the preferred habitat of this species. However, animal grazing, random collections of its rhizome as well as other associated rare medicinal species such as *Bergenia ciliata*, *Rhododendron anthopogan*, *Rheum australe* and *Saussurea nepalensis* by locals were the major threats to existing populations which has led to a drastic change in the original phytosociological structure of *P. scrophulariiflora*, resulting in rapid habitat loss. However, in order for conservation and sustainable utilization through cultivation, an attempt has been made to identify elite germplasm with higher underground biomass. Additionally a correlation between aerial and underground biomass growth and important soil characteristic such as available nitrogen, organic carbon, moisture content, soil pH and altitude gradients was also established.

**Norlan M. Ruíz-Potosme, Enrique Relea-Gangas, Salvador Hernández-Navarro, Jesús Martín-Gil, Adriana Corrêa-Guimarães, Pablo Martín-Ramos, Luís M. Navas-Gracia (Spain)** Selection of Environmental Parameters for *Pinus halepensis* in Castilla y León (Spain) through Geostatistical Techniques (pp 23-35)

#### ABSTRACT

**Original Research Paper:** Planning and management of biomass (including resources, biofuels and composting processes) require knowledge of the areas of tree species distribution. Geographic information systems (GIS) and geostatistical techniques have proved to be very useful tools to develop models of climate estimation, habitat determinations, biomass quality, changes in soil organic carbon storage, potential areas and connectivities. In this study, (i) several physiographic, climatic, edaphic and forestry parameters have been elaborated from the sampling data; (ii) a space-correlation analysis has been prepared by means of a digital elevation model (DEM); and (iii) a trend analysis has been carried out for each environmental variable under study. In Castilla y León (central Spain), semivariograms for climate and physiographic variables are unimodal, quasi-symmetrical, continuous and show a gradual evolution. In the plains of Castilla, with presence of forest masses of *carrasco* pine (*Pinus halepensis*), the distributions are more continuous and present totally homogenous distribution surfaces. In order to study on a parametrical autoecology model the ecological aptitude of our territory to establishing stable *carrasco* pine stands, we have developed a habitat classification system for *P. halepensis* in Castilla y León based on multiple regression analysis. The optimal areas are located in the provinces of Palencia and Valladolid, in the Duero Cenozoic Basin and the species that shows the best quality is located in one unit known as the “*Facies Cuestas*” which has a clear lacustrine character. A connectivity network for our species is presented with the aim of promoting a Regional Forest Rearrangement Plan.

**Mmbofheni Stanley Liphadzi (South Africa), Mary Beth Kirkham (USA)** Partitioning and Accumulation of Heavy Metals in Sunflower Grown at Biosolids Farm in EDTA-facilitated Phytoremediation (pp 36-42)

#### ABSTRACT

**Original Research Paper:** A field trial was conducted on a farm that annually received biosolids for 25 years in Manhattan, Kansas, in the USA. The aims of the trial were to investigate translocation and accumulation of heavy metals in organs of sunflower (*Helianthus annuus*) in EDTA-facilitated phytoremediation and to determine if plant density affects translocation and accumulation of heavy metals in sunflower plant parts. Two plant densities of 20,000 and 60,000 plants per hectare were grown on the biosolids farm. Four EDTA application rates, 0, 0.5, 1.0, and 2.0 g per kg soil as treatments were applied during the flowering stage and treatments were replicated four times. Plant organs were harvested separately at the end of the growing period, and the plant samples were analysed for the concentration of heavy metals. The concentration of toxic heavy metals (Cd, Ni, and Pb) in the roots decreased as a result of EDTA application but increased in aerial plant parts. High transpiration rate of the upper leaves in plants at 60,000 plants per ha, indicated by low stomatal resistance, enabled plants to retranslocate most of the toxic heavy metals from the roots to the upper leaves. However, plants grown at 20,000 plants per ha had reduced toxic metals in upper leaves because most of its metals could not be translocated to upper leaves due to high stomatal resistance in those leaves. EDTA had little or no effect on the concentration of essential metals (Cu, Fe, Mn, Zn) in plant roots. However, high plant density enhanced the accumulation of Zn in the top half of the stem.

**Sunil Kumar, Urmil J. Mehta, Sulekha Hazra (India)** *In Vitro* Studies on Chromium and Copper Accumulation Potential of *Pongamia pinnata* (L.) Pierre Seedlings (pp 43-48)

#### ABSTRACT

**Original Research Paper:** *Pongamia pinnata* (L.) Pierre is an oil-producing tree species. The potential of seed-derived pongamia oil as biodiesel has been identified but its potential for phytoremediation of contaminated sites and for phytoextraction of heavy metals remains unexplored. The objective of the present study was to determine the effect of chromium (Cr[VI]) and copper (Cu) on growth and metal uptake in different parts of *Pongamia pinnata* seedlings grown *in vitro* in medium containing Cr or Cu. *Pongamia* seeds were cultured in MS medium supplemented with various concentration of Cr (0-800  $\mu$ M) and Cu (0-400  $\mu$ M). After 6 weeks of incubation shoot height and root length of the seedlings were noted. The results demonstrated that growth of pongamia seedlings exposed to Cr(VI) concentrations ranging from 0 to 800  $\mu$ M were not affected whereas Cu (0-400  $\mu$ M) affected the root growth. Metal analysis carried out by atomic absorption spectroscopy demonstrated maximum accumulation of Cr in seed coat followed by root, leaves and cotyledons. In Cu the pattern was different. Cu content was optimum in seed coat followed by leaf, root and cotyledons. Least metal content was detected in stem in both treatments either in chromium or copper. High metal content in the seed coats demonstrates its ability to selectively absorb metal from the medium and retain it. This

property of the seed coat may be exploited for selective absorption of toxic metals from liquid waste.

**Dnyaneshwar R. Shinde, Tukaram D. Nikam, Vikram S. Ghole (India)** Utilization of Biomass of *Nostoc* Species for Production of Pigments and Adsorption of Heavy Metal Ions Cu(II), Cd(II) and Cr(VI) from Aqueous Solution (pp 49-54)

#### ABSTRACT

**Original Research Paper:** The dried biomass of species of cyanobacteria *Nostoc entophyllum*, *N. punctiforme* and *N. elliposporum* was assessed for phycobiliprotein and carotenoid content. The biomass left after extraction of pigments was utilized for the adsorption of heavy metal ions. The phycobiliprotein content of these species ranged from 32.1–51.1 mg g<sup>-1</sup> on a dry weight (dw) basis, while the total carotenoids content varied from 9.7 to 14.5 mg g<sup>-1</sup> dw. The pH-dependant adsorption of metal ion showed that optimum adsorption of Cu(II) and Cd(II) takes place at pH 4-6, and that of Cr(VI) at pH 1-2. Kinetics of adsorption of metal ions on biomass of these species showed that initial rate of adsorption of metal ions was fast and within 120 min in which 78-88% of Cu(II), 56-62% of Cd(II) and 52-64% of Cr(VI) was adsorbed by the *Nostoc* species biomass. For more than 90% adsorption, the time required was about 6 h for Cu(II), 36-48 h for Cd(II) and 48-60 h Cr(VI). Column experiments showed that the biomass of these species act as efficient adsorbents of Cu(II), Cd(II) and Cr(VI) with more than 30 mg g<sup>-1</sup> adsorption capacity towards Cu(II) by the biomass of all three species.

#### Number 2

**Ahmed Abdel-Megeed (Saudi Arabia), Rudolf Mueller (Germany)** Degradation of Long Chain Alkanes by a Newly Isolated *Pseudomonas frederiksbergensis* at Low Temperature (pp 55-60)

#### ABSTRACT

**Original Research Paper:** Biodegradation of polluted temperate and cold temperature environments may require the activity of psychrophilic and psychrotrophic bacteria, because their low temperature growth range parallels the ambient temperatures encountered in these environments. In this present study a mixed population of microorganisms from an ice sample from Spitzbergen was cultivated by enrichment culture technique which exhibited high efficiency to assimilate and mineralize C<sub>10</sub> to C<sub>22</sub> n-alkanes as the sole source of carbon and energy at both 4 and 20°C. *Pseudomonas frederiksbergensis* was isolated from this mixed culture, characterized and identified according to the cell wall fatty acids analysis and 16S rDNA sequence. The isolate was psychrophilic, with a growth temperature ranging from 4 to 20°C, an optimum growth temperature of 15°C and an optimal pH of 7.

**Lesedi Lebogang, Joanne E. Taylor, Tabo Mubyana-John (Botswana)** A Preliminary Study of the Fungi Associated with Salt pans in Botswana and their Anti-microbial Properties (pp 61-71)

#### ABSTRACT

**Original Research Paper:** The ability of fungal isolates from semi-arid salt pans in Botswana to produce secondary metabolites with antimicrobial properties was investigated. One hundred and eleven fungal isolates from 15 samples from 5 salt pans were isolated using the dilution plate method on PDA, amended with soil extracts and supplemented with streptomycin sulphate (0.3 g/l). Their antimicrobial activity was tested by confrontation and agar-well diffusion methods using attenuated *Escherichia coli*, *Staphylococcus aureus*, *Bacillus megaterium* and fungi, *Candida albicans* and *Aspergillus niger* as test organisms. *Aspergillus* spp. (26.1%), *Fusarium* spp. (18.0%), *Dendryphiopsis* sp. (13.5%), *Alternaria alternata* (7.2%) and *Phoma* spp. (5.4%), were the most common fungi isolated. In the confrontation test, almost 100% of the fungal isolates inhibited the growth of at least one test organism. Approximately 43% of the culture supernatants in malt-extract broth showed antimicrobial activity against at least one test organism in the well diffusion assays. *Candida albicans* was the least sensitive, being inhibited by only two (4.2%) fungal supernatants from the Tshane salt pan. *Bacillus megaterium* and *S. aureus* were the most sensitive and were inhibited by 81.3 and 70.8% of the fungal supernatants, respectively. *Escherichia coli* was inhibited by 12.5% and *A. niger* by 37.5% of the fungal supernatants. The most biologically active fungal isolate was Tsp22 (*Aspergillus terreus*), which inhibited the growth of all test organisms. Ten isolates showing broad activities were selected and further analysed for the minimum inhibitory concentration, which demonstrated that antimicrobial activity increased relative to increased volumes of the supernatants. Polymerase chain reaction was used to amplify the internal transcribed spacer region of the 10 selected isolates and two phylogenetic trees were created to confirm isolate identification.

**Madhumita Behera, Jagneshwar Dandapat, Chandni C. Rath (India)** Isolation, Characterization and Screening of Bacterial Isolates from Similipal Biosphere Reserve Forest Soil for their Metal Tolerance Capacity and Extracellular Enzymatic Activities (pp 72-78)

#### ABSTRACT

**Original Research Paper:** In total 50 bacteria were isolated from 15 soil samples collected from Similipal Biosphere Reserve through spread and pour plate technique. The CFU of bacteria per gram of soil ranged between  $10^6$  and  $10^9$ . The isolates were identified and assigned to the genera *Bacillus* (38, 76%), *Pseudomonas* (8, 16%), *Micrococcus* (2, 4%) and *Geomicrococcus* (2, 4%). Isolates were characterized for their growth in different media and pH. The isolates grew well at acidic pH, although growth was observed over a wide pH range. Surprisingly a high degree of multiple antibiotic resistance (0.142-0.428) was reported while studying the antibiogram pattern of the isolates. Isolates were screened for their metal tolerance capacity against heavy metals such as Hg, Cd, Cr, Ni, Cu, Zn, Co and Pb. When the isolates were screened for the production of industrially important enzymes by the plate assay method it was reported that 25 (51.02%), 24 (48.97%), 18 (36.73%), 18 (36.73%) and 28 (57.14%) of the isolates were positive for amylase, protease, lipase, phosphatase and DNase, respectively. Soil bacteria growing at acidic pH, having metal tolerance capacity and producing industrially important enzymes could be novel elements for different industrial processes.

**Saeed Minoui, Dariush Minai-Tehrani (Iran)** Effect of Triton X-100 on Bioremediation of PAHs of Medium Crude Oil in Soil (pp 79-83)

#### ABSTRACT

**Original Research Paper:** Polycyclic aromatic hydrocarbons (PAHs) are a group of chemicals that occur naturally in coal, crude oil, and gasoline. PAHs can also be released into the air during the burning of fossil fuels, garbage, or other organic substances. PAHs are found throughout the environment in the air, water, and soil, and can persist in the environment for months or years. Some PAHs are harmful to organisms. In this study, during a four-month period the effect of different concentrations of Triton X-100 (0-0.25%) on the bioremediation of medium crude oil obtained from Tehran refinery and its PAH components was studied in the soil obtained from a cultivation site near the Tehran refinery. Triton X-100 at low concentrations (0.01-0.025%) had a greater effect on the reduction of total PAHs than at a high concentration (0.05-0.25%). The highest crude oil and total PAH reduction was observed at 0.025% followed by 0.01% Triton X-100 samples, while the lowest was observed at 0.25% Triton X-100. The HPLC pattern of samples showed that naphthalene and acenaphthylene were reduced in all the samples as well as in the dry control. The highest reduction of three- and four-ringed PAHs such as phenanthrene, anthracene, fluoranthene, pyrene and chrysene was observed at 0.025% followed by 0.01% Triton X-100; least reduction occurred with 0.25% followed by 0.1% Triton X-100.

**Talaat El Sebaï, Dalia Barakat, Wafaa M. Abd El-Rahim, Hassan Moawad (Egypt)** Biodegradation of Atrazine in Fertile Nile Delta Clay Soil under an Intensive Multicrop Rotation Farming System (pp 84-88)

#### ABSTRACT

**Original Research Paper:** Biodegradation of atrazine in Nile Delta agricultural soils previously treated with atrazine under a maize cropping rotation system was investigated under controlled laboratory conditions. Two techniques were used to follow atrazine biodegradation kinetics: i) incubated soil samples and ii) soil suspension (slurry technique). An enrichment technique and atrazine agar indicator plates were used to isolate atrazine-degrading bacteria. Both tested soils showed high and similar ability to degrade atrazine, in which atrazine dissipated completely without detection of any metabolites in soil suspensions at 7 and 3 days after the first and second enrichment, respectively. Atrazine degradation rate constants ( $k$ ) were significantly increased by repeated enrichment: they increased from 0.4 and 0.5  $d^{-1}$  after the first enrichment to 1.3 and 1.2  $d^{-1}$  after the second enrichment for Soil I and II, respectively. Also, atrazine biodegradation kinetics was strongly affected by atrazine application number where atrazine degradation rate constants ( $k$ ) significantly increased by increasing pre-treatment number. However, the atrazine half-life ( $T_{1/2}$ ) was decreased (but not significantly) by previous treatment times where it was 2.45, 2.32, 2.11, and 1.17 days for T0, T1, T2 and T3 soil samples, respectively. These data suggest the presence of an adapted microbial population in these soils able to metabolise atrazine. This hypothesis was confirmed by isolation of seven bacterial strains characterized by their capacity to degrade a high atrazine concentration in the presence of a carbon source.

**Friday Onyekwere Nwosu, Bamidele Iromidayo Olu-Owolabi, Kayode Oyeboade Adebawale (Nigeria), Thomas Henle,**

**Uwe Schwarzenbolz (Germany)** Pore Structure and Surface Functional Groups on Six Tropical Fruit Nutshell Active Carbons (pp 89-95)

#### ABSTRACT

**Original Research Paper:** Six fruit nutshells, namely: *Thevetia nerriofolia*, *Hura crepitans*, *Hyphaene thebaica*, *Pentachlethra macrophylla*, *Tetracapidium conophorum* and *Cocos nucifera* were carbonised, oxidized with atmospheric air and activated in CO<sub>2</sub> at 840°C for 3 h. The iodine number range of active carbon (AC) was 822-1804 mg/g while BET surface area was within the 551-2738 m<sup>2</sup>/g range. The ranges of micropore and mesopore volumes were 0.100-0.210 and 0.017-0.053 cm<sup>3</sup>/g, respectively. The dependence of burn off, porosity and BET surface area of ACs on NaOH titratable surface functional groups indicated linearity. The phenol/lactone and carbonyl surface functional groups ranged from 6.002 to 8.294 and from 3.970 to 5.908 mmol H<sup>+</sup> equiv/g carbon, respectively. FT-IR spectra revealed the presence of OH, C=O, and C-O functional groups on surface of the prepared ACs. Their SEM micrographs show pores and crevices all over their surface. These carbons might have potential ability to remove metals and organic compounds from waste water.

**B. Leena Grace (India)** Fish Availability and Environmental Criteria of Kadinamkulam Backwater (pp 96-99)

#### ABSTRACT

**Short Communication:** The topographical alterations of shore lines due to the construction of new ports, recreational developments and pollution hazards lead to changes in environmental conditions. These changes favour less the selection of native fishes that result in a decline of biodiversity. Kadinamkulam backwater (latitude of 8° 35' – 8° 40' N and a longitude of 76° 45' – 76° 52' E) lies almost parallel to the adjoining Arabian sea in the Kerala coast of India. The present study focuses the relationship of physico-chemical features, dissolved gases, nutrients and sediment characteristics of the estuary with the availability of fin and shellfishes. Monthly collection of fish species, water samples and sediments were made for a period of one year. The results were correlated by Karl Pearson correlation analysis. The significance with each environmental parameter and availability of fishes are presented. The results suggest that this approach can facilitate biodiversity management by enabling the optimal utilization of limited management resources and raises awareness of the importance of estuarine biodiversity.

**H. T. Raghavendra Gowda, Vijaya Kumara (India)** Line Transact Method for Estimating Encounter Rate of Large Mammals in a Dry Deciduous Forest of Lakkavali Range of Bhadra Wildlife Sanctuary, Karnataka (pp 100-103)

#### ABSTRACT

**Research Note:** The encounter rate of large mammals were estimated using a line transect method in the Lakkavalli range of Bhadra Wildlife Sanctuary during April 2007 to March 2008 in the Lakkavali range for four species of large herbivorous mammals: chital (*Axis axis*), sambar (*Cervus unicolor*), barking deer (*Muntiacus muntjak*) and gaur (*Bos gaurus*). The range was divided into several zones based on location and habitat types. From 4 permanent vehicle transects of 6-10 km and from a total of 31 km in different habitat transects, the number of individuals was higher during pre- and post-monsoon seasons.