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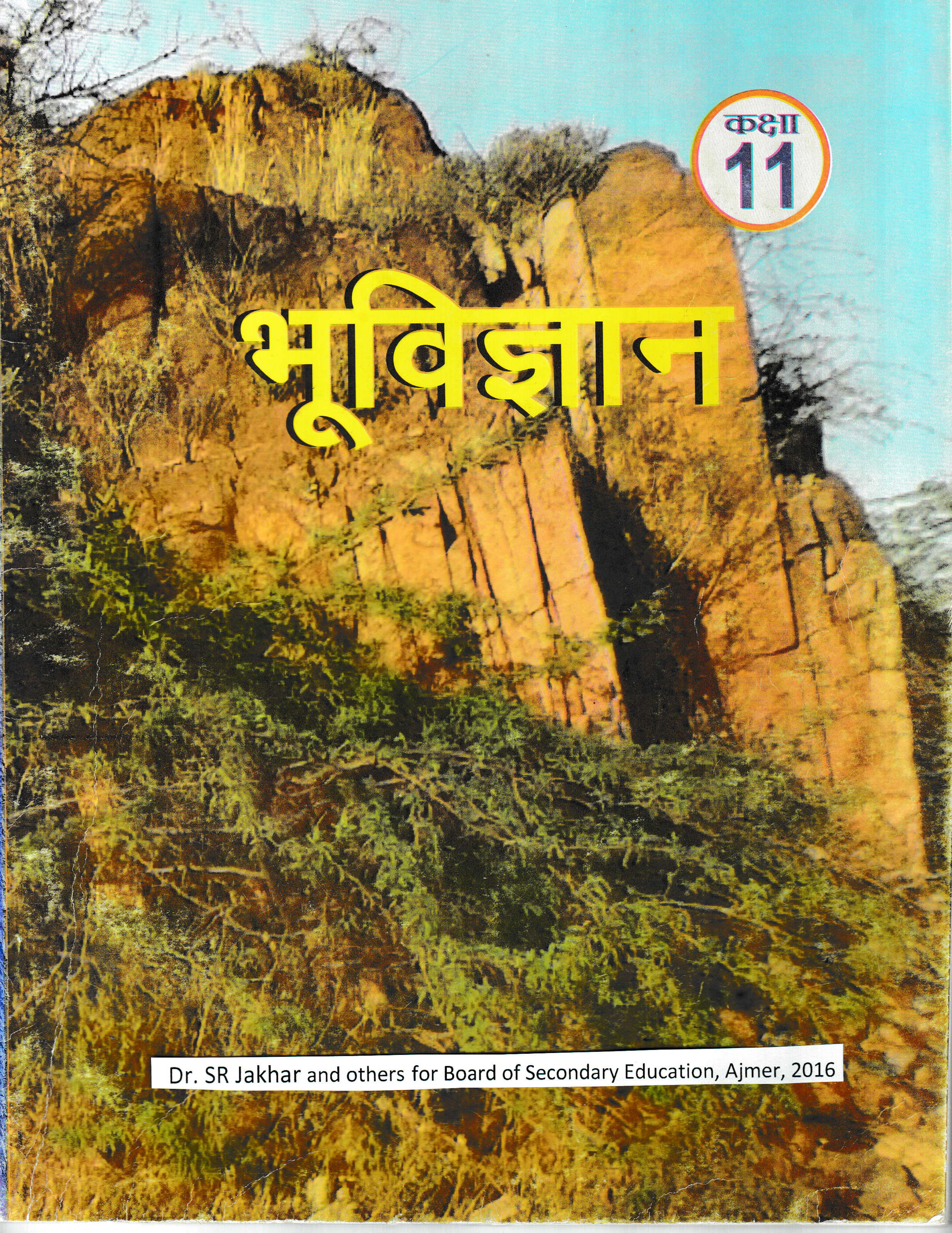

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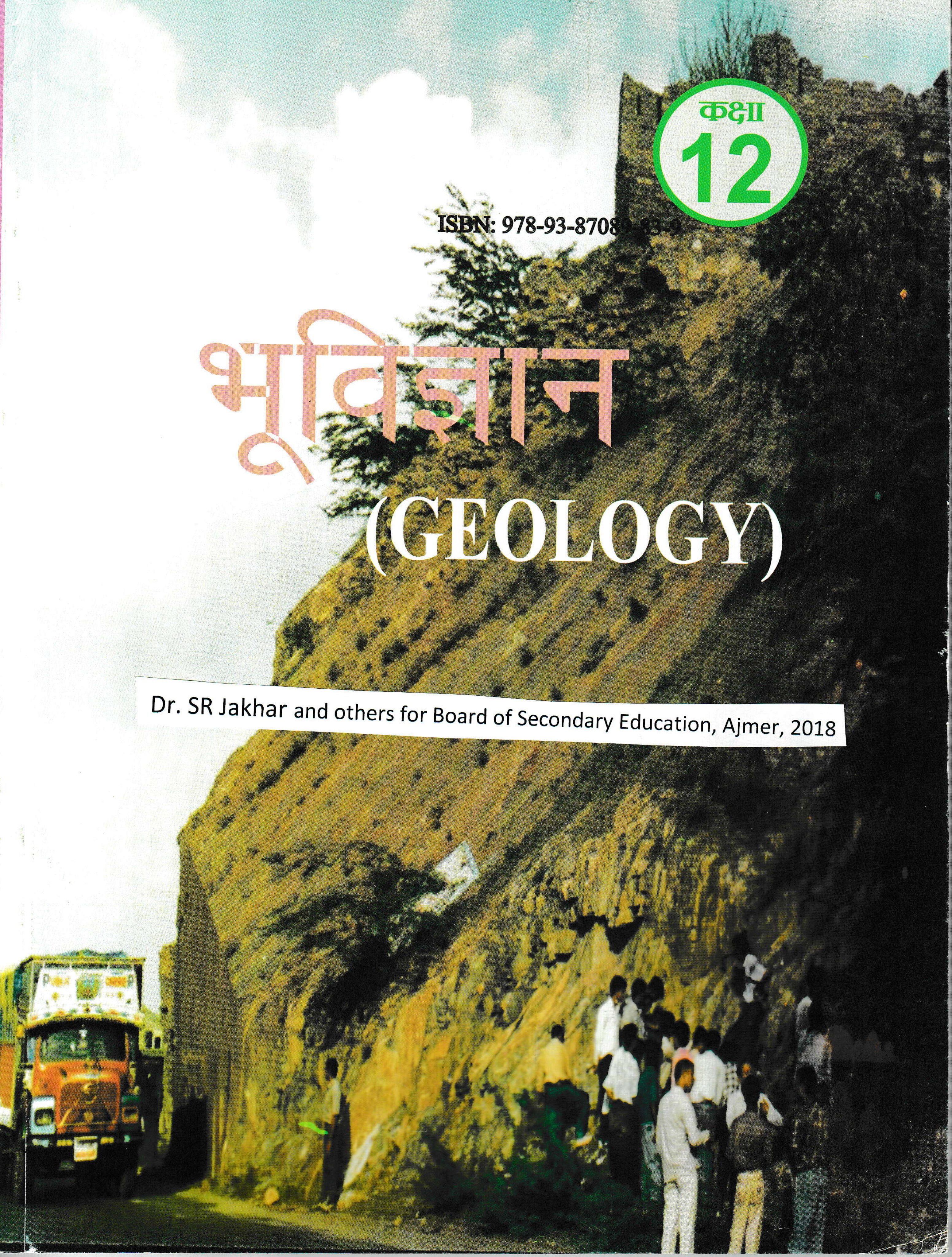

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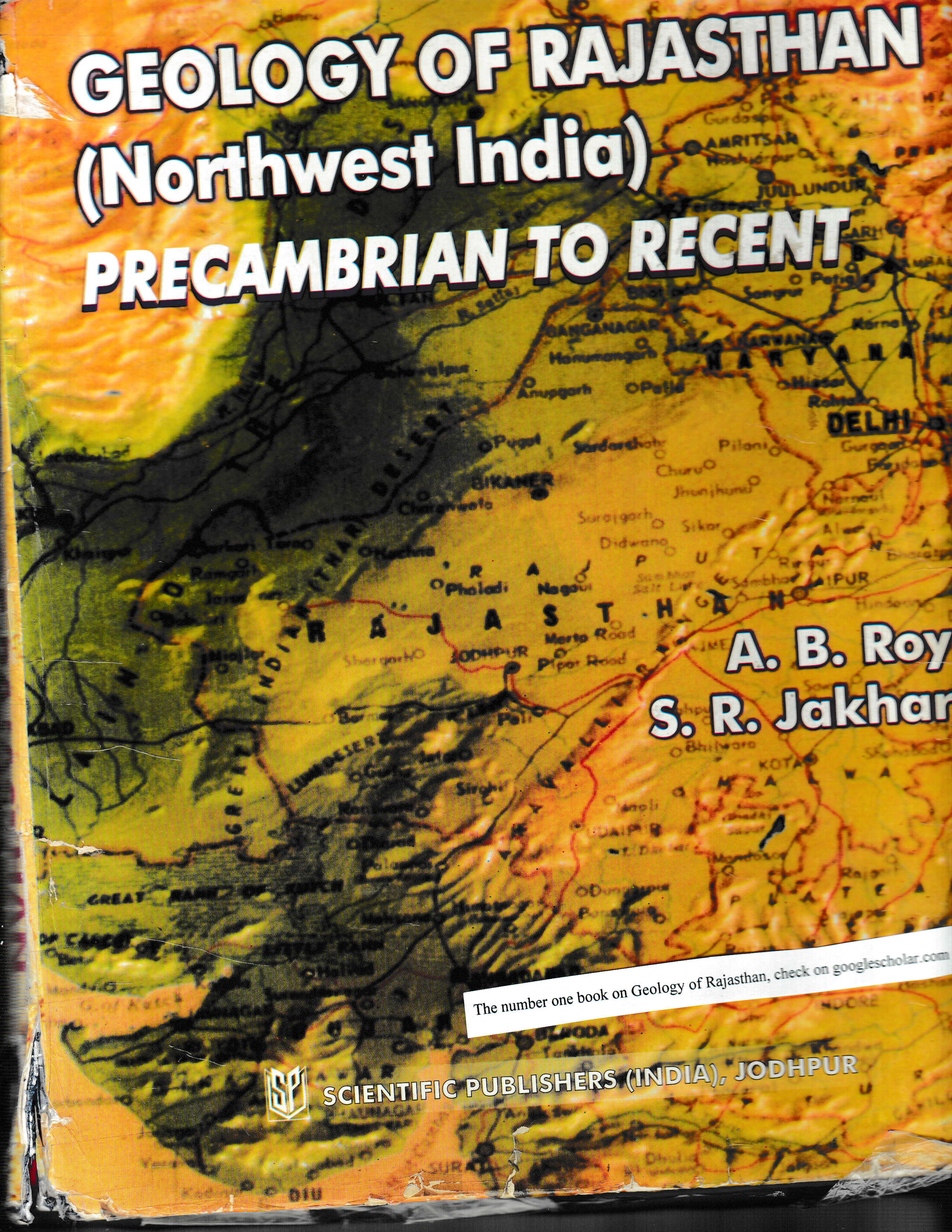

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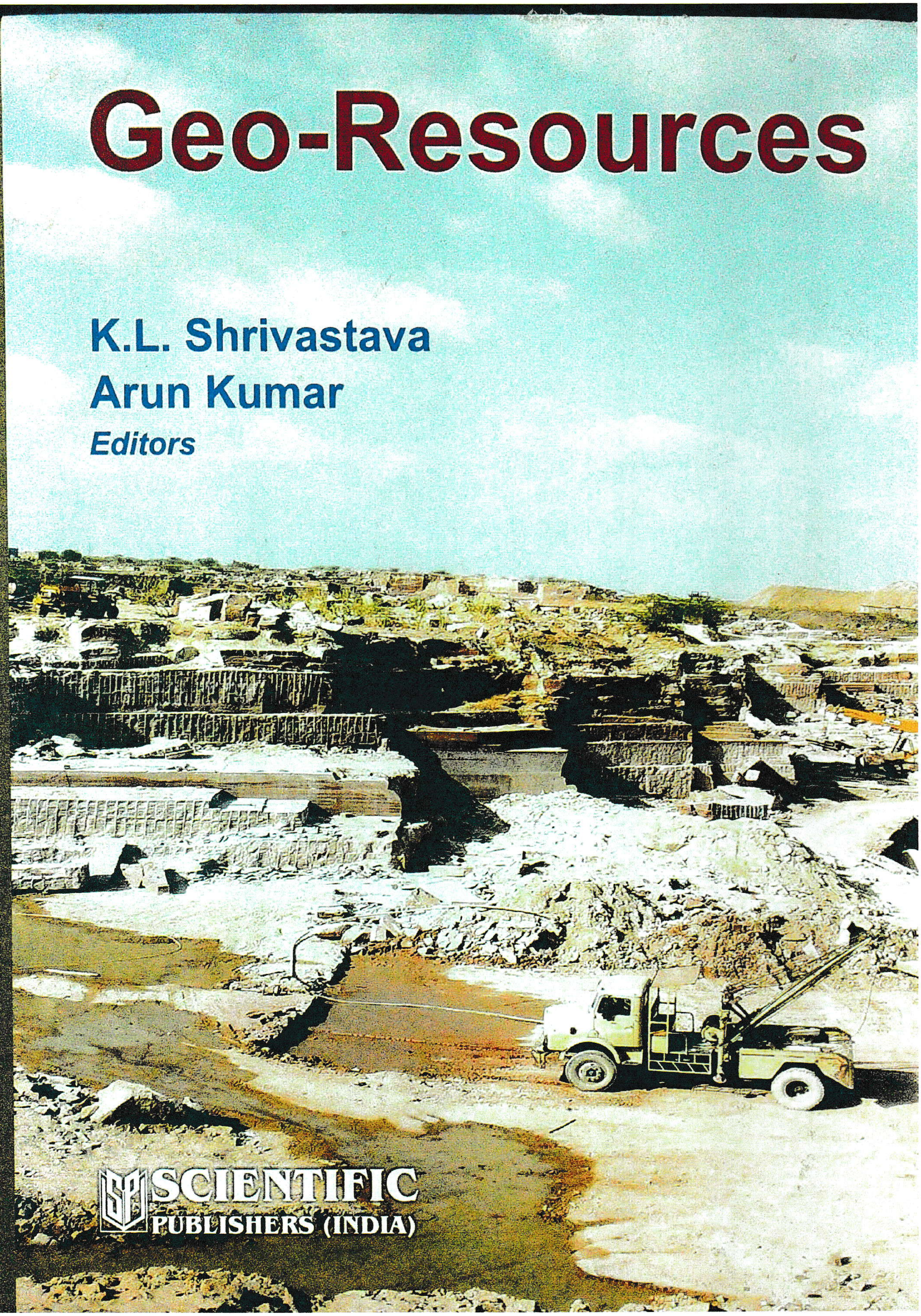

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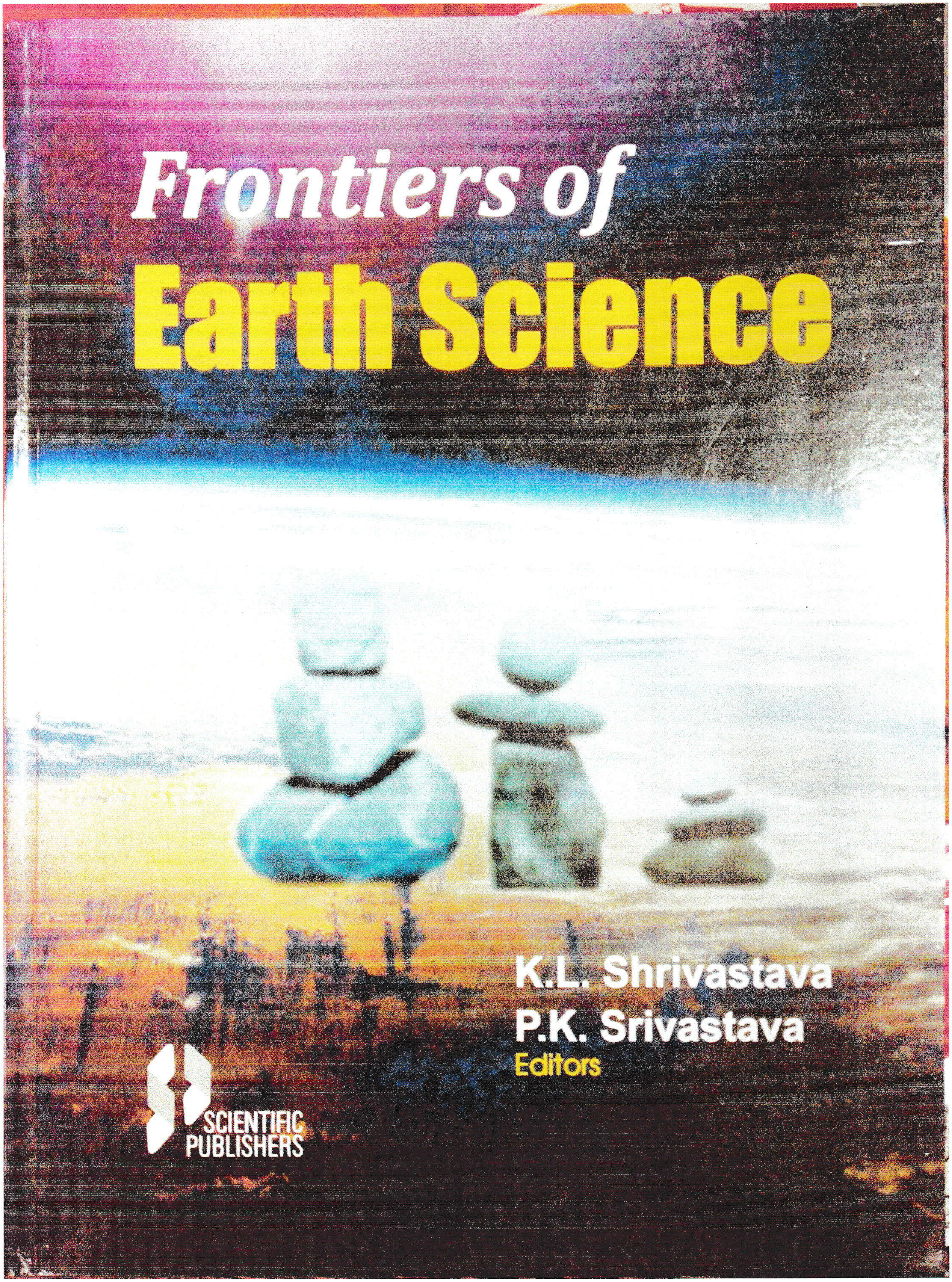

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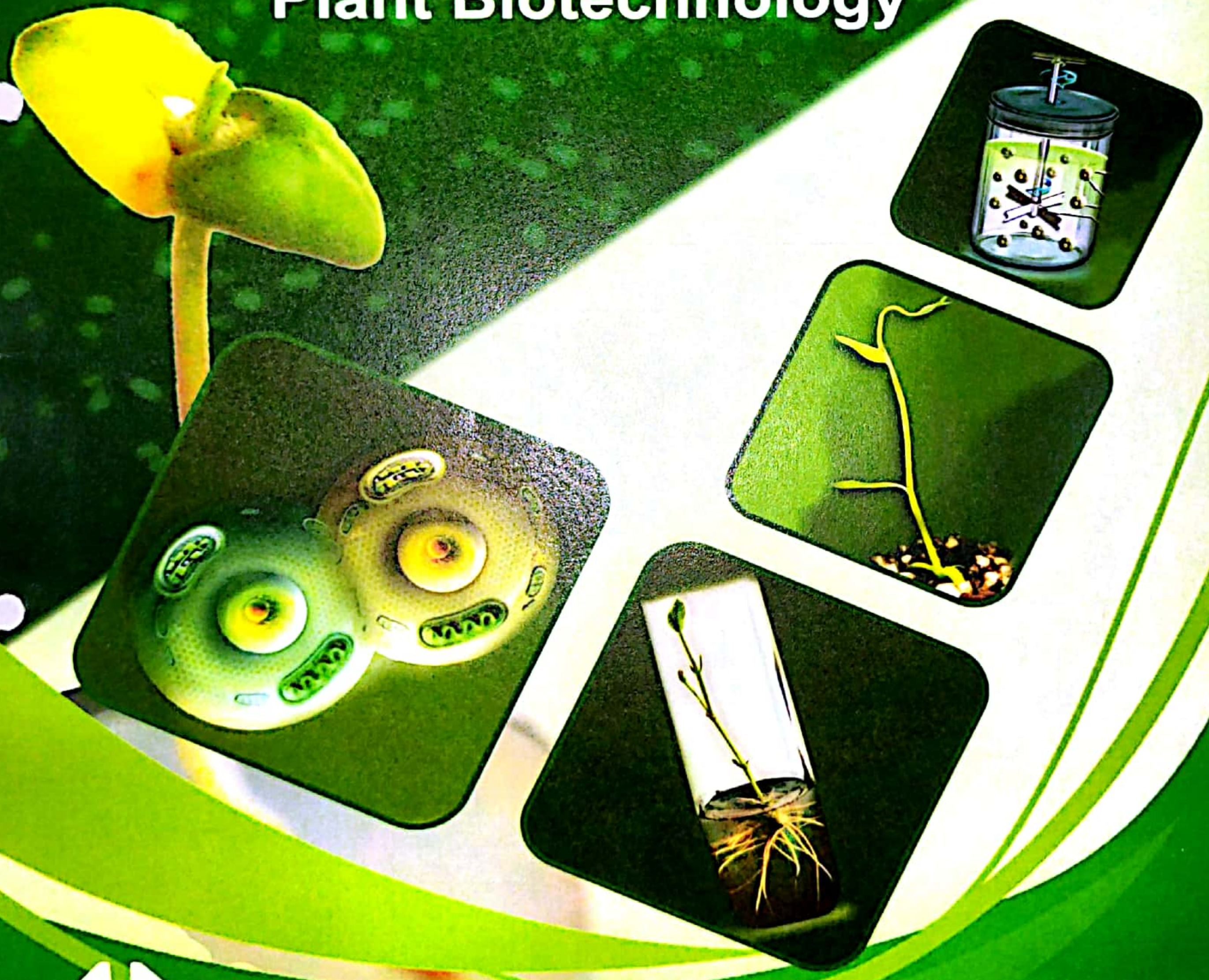

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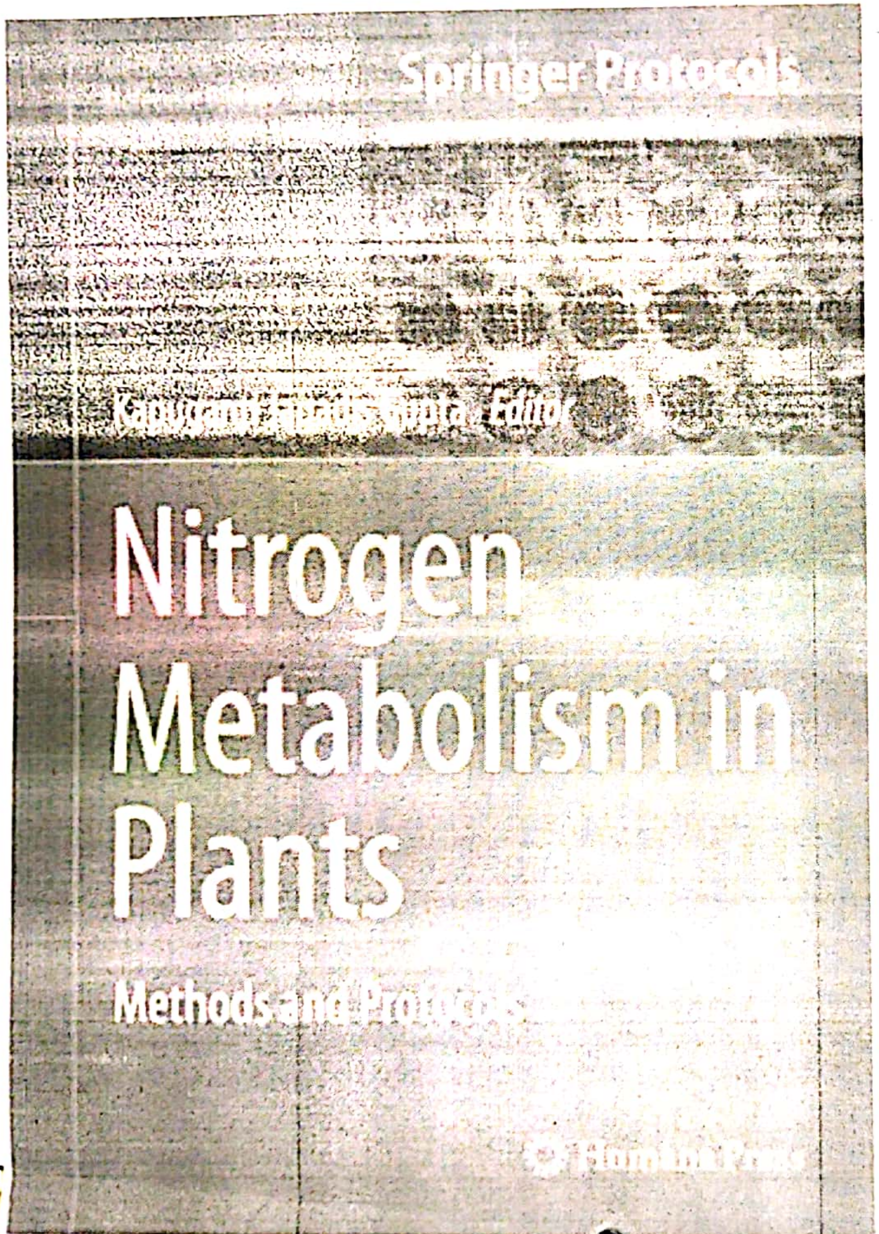
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Chapter 12

Methods for Isolation and Characterization of Nitrogen-Fixing Legume-Nodulating Bacteria

Nisha Tak, Garima Bissa, and Hukam S. Gehlot

Abstract

Symbiotic nitrogen fixation (SNF) is a characteristic feature of nodulating legumes. The wild legumes are comparatively less explored for their SNF ability; hence, it is essential to study nodulation and identify the microsymbiont diversity associated with them. This chapter aims to describe the methodology for nodule hunting; trapping, isolation, and characterization of root nodule bacteria (RNB) at phenotypic, genotypic, and symbiotic levels. The documentation of nodulating native legume species and the rhizobial diversity associated with them in various parts of world has gained attention as this symbiotic association provides fixed nitrogen, improves productivity of plants in an ecofriendly manner. Before field-based applications the symbiotic bacteria need to be assessed for their N fixing ability as well as characterized at molecular level. The phylogeny based on symbiosis-essential genes supplemented with the host-range studies helps in better understanding of the symbiotaxonomy of rhizobia. More efficient symbiotic couples need to be screened by cross-nodulation studies for their application in agricultural practices.

Key words Legume root-nodules, Rhizobia, DNA fingerprinting, Housekeeping and symbiotic genes, Phylogeny, Host-range

1 Introduction

The Leguminosae (Fabaceae) is the third largest family of flowering plants with about 750 genera and more than 19,500 species, traditionally divided into three subfamilies, of which Mimosoideae and Papilionoideae comprises of many nodulating genera in contrast to Caesalpinioideae with few nodulating genera [1, 2]. As per the recent classification of legumes the six subfamilies proposed are Caesalpinioideae, Cercidoideae, Detarioideae, Dialioideae, Duparquetioideae, and Papilionoideae [3]. The species within four (Cercidoideae, Detarioideae, Dialioideae, and Duparquetioideae) newly described sub-families are nonnodulating. The members of the old Mimosoideae have been nested in mimosoid clade within the redefined Caesalpinioideae which now comprises nodulating genera in tribes Ingeae, Mimoseae, Caesalpiniae, and Cassiae [4–6]. Legumes

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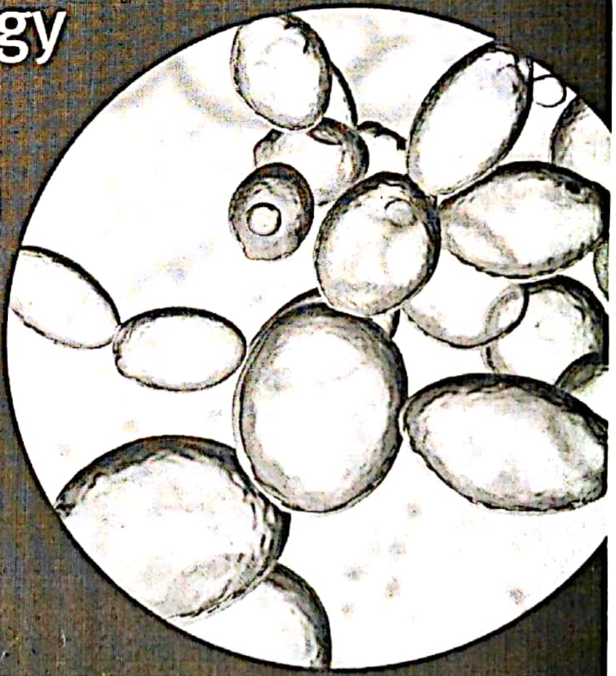

Recent Advances in Application of Fungi and Fungal Metabolites: Biotechnological Interventions and Futuristic Approaches

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Contents

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CHAPTER

4

Proteomic analysis of fungal species in response to various antifungal agents for novel drug development

Subhajit Dutta[a], *Shweta Jha*[b]

[a]Department of Biotechnology, National Institute of Technology, Durgapur, India, [b]Plant Functional Genomics Lab, Biotechnology Unit, Department of Botany, UGC—Centre of Advance Study, Jai Narain Vyas University, Jodhpur, India

4.1 Introduction

For the last few decades, fungal pathogens have been reported to cause different types of human diseases, especially among patients who are immunocompromised. Immunocompromised patients include recipients of organ transplants, cancer patients, AIDS patients (Nucci and Marr, 2005), etc., whose immune system is very weak, having a much reduced ability to fight off infectious diseases. Fungal infection ranges from acute to systemic, and the initiation stage of infection shows quite a similarity with the shifting status of the host immune system. Barelle et al. (2006) explained this with an example of the *Candida* species. These fungal species are normally present in the mouth and gastrointestinal tracts of healthy individuals, but behave as a potential opportunistic pathogen in immunocompromised patients, causing diseases such as candidiasis as well as candidemia. Ball et al. (2019) reported a mortality range of around 60%–90% in neutropenic patients infected with invasive aspergillosis (IA) caused by the opportunistic fungal pathogen *Aspergillus fumigatus*.

There are very few effective therapies available for treating fungal infections. This is due to the proximity of both the groups, i.e., fungi and human host, from an evolutionary point of view. Few commonly known antifungal agents, namely azoles, allylamines, echinocandins, polyenes, and miazines, are available on the market and these agents inhibit the fungal growth and development by hindering the DNA synthesis process and act through targeting the cell wall and plasma membrane of fungal cells (Odds et al., 2003). Most of the antifungal pharmaceutical drugs such as amphotericin B (AMB), voriconazole (VCA), and itraconazole (ICA/ITC) possess serious side effects, namely nephrotoxicity (Safdar et al., 2010), visual disturbances (Bayhan et al., 2015), and congestive heart disease (Vollenbroich et al., 2014), respectively, in the host. Moreover, some drugs, such as echinocandins, are sold at a higher price and therefore have limited clinical use (Neoh et al., 2014). Various findings have reported fungal pathogens becoming resistant toward antifungal drugs that are commonly available on the market. The fungal species *Candida auris* has become resistant to the antifungal drug fluconazole, thereby posing a challenge to the treatment regimens followed by doctors and biomedical researchers to treat patients infected with the fungi (Perfect, 2017; Geddes-Mcalister and Shapiro, 2019). Therefore, there is an urgent requirement to develop novel antifungal drugs that can be more reliable with minimal or no side effects. The focus should be on interpreting the mechanism of fungal pathogenicity and identifying novel therapeutic targets to develop new antifungal drugs to treat fungal infections. Two novel strategies that can exterminate the fungal infections are drug repositioning and usage of combinational therapy, where the concept of drug repositioning involves the exploration of existing drugs for new therapeutic targets and purposes.

Ball et al. (2019) reviewed the contributions of proteomics in the area of fungal pathogenesis, host-fungal interaction, and antifungal development. Technological developments such as mass spectrometry (MS) have resulted

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Bioremediation of Pollutants

From Genetic Engineering to Genome Engineering

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Progress, prospects, and challenges of genetic engineering in phytoremediation

4

*Shweta Jha**
Plant Functional Genomics Lab, Biotechnology Unit, Department of Botany (UGC-CAS), J.N.V. University, Jodhpur, India
*Corresponding author

4.1 Introduction

The rise in various industrial and agricultural anthropogenic activities resulted in a dramatic increase in the concentration of different pollutants in the soil, air, and water, causing environmental pollution. This represents a major global threat to the entire ecosystem, including deterioration of the quality of groundwater, soil properties, human and animal health. A wide range of remediation technologies has been employed to treat polluted sites, including physical, chemical, and biological methods. However, the conventional decontamination techniques like mechanical or chemical treatment and incineration are much more expensive and harmful for soil structure and its microbial fauna, as well as lac a sufficient environmental compatibility and public acceptability. Here, in situ biological remediation methods present a promising and more ecofriendly alternative approach, which consists of the use of microorganisms (bioremediation), and plants (phytoremediation) for efficient cleanup of soil, offering many benefits related to cost and environmental safety (Dhankher et al., 2011; Pandey and Souza-Alonso, 2019). Bioremediation using microbes has its own limitations like, the results of efficient microbial biodegradation ability under controlled lab conditions may not be replicated in actual field conditions. In addition, constant inoculation of microorganisms and application of nutrient media along with maintenance of optimal pH for proper microbial growth are essential over a long time, thereby increasing the maintenance cost of the site.

In this context, phytoremediation [*phyto* (Greek) = plant; *remedium* (Latin) = restoring balance], has been identified as an alternative method for in situ remediation of the contaminated site. It refers to the use of living plants to accumulate, detoxify, or modify harmful pollutants to nonhazardous compounds in the soil, air, or water. Certain hyperaccumulators naturally contain this ability to concentrate and metabolize toxic elements/compounds from the environment, like *Brassica* and *Allium* species, *Salix* spp. *Poplar* spp., *Pteris vitata*, *Helianthus annuus*, *Medicago sativa*, *Thlaspi caerulescens*, etc. (Koźmińska et al., 2018; Pandey and Bajpai, 2019). The common properties of a good hyperaccumulator are high biomass, fast

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JOGINDER SINGH

Professor, Department of Biotechnology, Lovely Professional University, Phagwara, Punjab, India

PRAVEEN GEHLOT

Professor, Department of Botany, Jai Narain Vyas University, Jodhpur, Rajasthan, India

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CHAPTER

20

Mechanistic evaluation of bioremediation properties of fungi

Manish Mathur[a] *and Praveen Gehlot*[b,*]

[a]ICAR-Central Arid Zone Research Institute, Jodhpur, Rajasthan, India, [b]Department of Botany, JNV University, Jodhpur, Rajasthan, India *Corresponding author.: drpg73@rediffmail.com

20.1 Introduction

There is a consensus that the indiscriminate utilization of agrochemicals, hydrocarbons, and pharmaceutical products has accelerated the problems related to land degradation (soil, vegetation, and microorganism communities), aquatic resources, and human and livestock health. Hazardous chemicals of particular concern pertain to petroleum hydrocarbons, incessant organic pollutants (halogenated organic compounds and polycyclic aromatic hydrocarbons), dyes, pesticides (herbicides and insecticides), and heavy metals. The characteristics of these chemicals are as follows.

20.1.1 Petroleum hydrocarbons (PHs)

They are a complex mixture of various compounds, namely saturated aromatics, resins, and asphaltenes. Among them, the saturated fraction have straight and branched chains of alkenes and cycloalkanes (naphthenes). The major aromatic portion includes volatile hydrocarbons such as toluene, benzene, and xylene. Fatty acids, phenols, ketones, esters, and porphyrins are the major constituents of asphaltene while pyridines, quinolines, carbazoles, sulfoxides, and amides are the major constituents of the resins. Depending on the level of PHs exposure, the human immune system as well as the circulatory, reproductive, respiratory, and endocrine systems are significantly affected. Similarly, in plants, studies have shown that glucose assimilation, stomatal opening and closing, and transpiration rate are significantly decreased in petroleum-contaminated soil (Han et al., 2016). The population and abundance of various soil microbes are also affected considerably with PHs contamination (Hazim and Al-Ani, 2019).

20.1.2 Polycyclic aromatic hydrocarbons (PAHs)

The term PAH denotes the compounds having carbon and hydrogen atoms only. Chemically, they comprise two or more benzene rings bonded in cluster, linear, or angular arrangements. The major source of PAHs in the environment is the incomplete combustion of hydrocarbons such as fossil fuels, the accidental discharge of petroleum or the use and dumping of petroleum products, and firewood gasification and liquefaction. Their toxicity is generally carried out through their interfaces with the cellular membrane function as well as enzymatic systems (Abdel-Shafy and Mansour, 2016).

20.1.3 Halogenated organic compounds

These are the substances containing hydrogen and carbon, but where hydrogen atoms have been replaced by halogens such as fluorine, iodine, chlorine, and bromine. Pentachlorophenol (PCP), trichloroethene (TCE), 2,4-dichlorophenoxyacetic acid (2,4-D), polychlorinated biphenyl (PCB), and dioxins are examples of xenobiotic halogenated organic compounds. The major portions of these compounds in the environment are generated through the use of chlorine within the pulp

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Professor, Department of Biotechnology, Lovely Professional University, Phagwara, Punjab, India

PRAVEEN GEHLOT

Professor, Department of Botany, Jai Narain Vyas University, Jodhpur, Rajasthan, India

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Professor, Department of Botany, Jai Narain Vyas University, Jodhpur, Rajasthan, India

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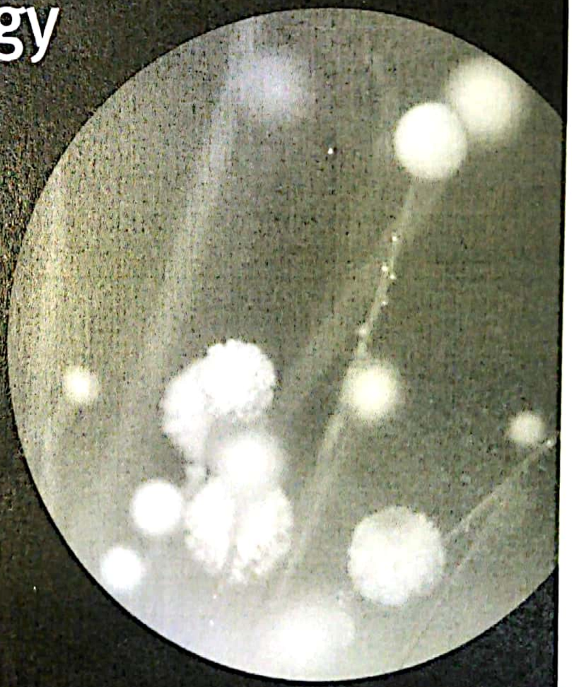

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Professor, Department of Biotechnology, Lovely Professional University, Phagwara, Punjab, India

PRAVEEN GEHLOT
Professor, Department of Botany, Jai Narain Vyas University, Jodhpur, Rajasthan, India

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CHAPTER

18

The role of antioxidants and ROS scavenging machinery in wild mushrooms

*Rachana Dinesh**

Department of Botany, Jai Narain Vyas University, Jodhpur, Rajasthan, India
***Corresponding author. E-mail: rachanadinesh.dinesh@gmail.com**

18.1 Introduction

The fruiting bodies of fungi belong to their higher group, basidiomycetes and ascomycetes (Chang and Miles, 1992) and are both edible and nonedible. The word mushroom was originated from the Latin word mucus (Baker, 1989). The saprotrophic, parasitic, or mycorrhizal nature provides mushrooms with special ecological importance. Edible mushrooms include both cultivated and wild mushrooms and are consumed worldwide. Mushrooms are rich in nutrition and are known for the preparation of delicious foods. In many Asian countries, they are used in curries, soups, and broths. In India, barbecued wild edible mushrooms are also consumed in the form of various delicious food preparations. Mushrooms are also used as medicines (Isildak et al., 2004) to treat various human ailments.

Water and molecular oxygen are the most important constituents for life on Earth. The balance of molecular oxygen is maintained by two main metabolic processes—photosynthesis and respiration—that occur in autotrophs. In addition to its constant liberation as a result of photosynthesis, molecular oxygen is also fixed into different compounds by enzymatic and nonenzymatic processes. In its dioxygen state, the oxygen molecule has two unpaired electrons with parallel spin. Upon physical or chemical activation of this dioxygen, the spin of one of the electrons changes and makes it highly reactive. The monovalent reduction of oxygen, that is, highly reactive superoxide (Elstner, 1982), is the first form of reactive oxygen species (ROS). The superoxide takes part in both oxidation and reduction reactions and can easily be dismuted into H_2O_2. The hydroxyl radical exhibits higher chemical reactivity and creates damage inside the cell. This chapter aims to highlight the nutritional importance of mushrooms and to discuss their roles as antioxidants.

18.2 Nutritional and medicinal value of wild mushrooms

Mushrooms are good source of vitamins, proteins, volatile oils, tocopherols, phenolic compounds, flavonoids, and organic acid. Consuming mushrooms is like getting a food therapy, which can be a solution to various ailments including high blood pressure and high cholesterol as well as the prevention of cancer. The nutritional and medical values of mushrooms are attributed to the presence of various useful chemical compounds (Manzi et al., 2001). Different species of wild mushrooms are consumed all over the world, and some are grown while some are foraged. *Agaricus bisporus, Lentinula edodes, Volvariella volvacea, Auricularia nigricans, Auricularia indica, Pleurotus tuber-regium, Pleurotus australis*, etc., are some of the popular cultivated mushrooms that are consumed by a good number of people. The wild mushrooms that are foraged and edible are expensive as compared to cultivated mushrooms. A lot of people choose to make them a part of their diet because of their earthy flavor. The consumption of foraged edible mushrooms is becoming quite popular due to their nutritional and medicinal properties (Manzi et al., 2001).

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CHAPTER

4

Fungal degradation of bioplastics: An overview

Indu Singh Sankhla[a,*], *Ghanshyam Sharma*[a], *Alkesh Tak*[b]

[a]Microbiology Lab, Department of Botany, University of Rajasthan, Jaipur, India, [b]BNF Lab, Department of Botany, Jai Narain Vyas University, Jodhpur, India

*Corresponding author. E-mail: induiitkgp@gmail.com

4.1 Introduction

Petroleum-based plastics are widely used in all fields of industry due to their exceptional properties and performance; they are highly resistant to temperature, pressure, UV light, and chemical solvents (Gawande et al., 2012). Plastics are ubiquitous in modern-day life. In 1950, worldwide plastic production amounted to 1.5 million tons. In 2015, production reached 388 million tons (Ryberg et al., 2018). If the rate of plastics production continues at the present rate, its production will be double within the next 20 years. The use of plastic in the United States, Europe, and India is 109, 65, and 9.7 kg/person/year, respectively (Jogdand, 1999). In the coming years, this figure is likely to rise. Some unique properties of plastics, like higher molecular mass, unusual bonds, and a high number of aromatic rings and halogen substitutions, make them resistant to microbial attack (Alexander, 1981). Therefore plastics are continuously accumulating in the environment, causing various types of pollution. Although recycling of plastics is an environmentally attractive solution, as per a United Nation's report from 2019, only a small fraction of all produced plastic waste is recycled (9%) and incinerated (12%), while the rest (79%) ends up in landfills or is left scattered across the ecosystem. Every piece of plastic so far produced is still existing somewhere in the environment. Unplanned dumping and extensive accumulation of plastic waste in the environment leads to high risk to plants, animals, and human health as well as severe environmental pollution.

To resolve these plastics-based problems, investigators have been looking for a novel alternative polymer that can be used as a suitable replacement for petrochemical-based plastic. One solution is the production of bioplastic, a form of plastic derived from bio-based polymers. It can be a good alternative to or complete replacement for conventional plastics due to its biodegradability, nontoxicity, and bio-based as well as eco-friendly nature. Attention on bio-based bioplastics has increased due to environmental alarms and the realization that global fossil fuels are limited in nature. The complete replacement of conventional plastic by bioplastic can reduce fossil energy use, pollution, and emission of greenhouse gases, which might be helpful in solving environmental and social problems caused by the plastics industry.

In recent years, there has been remarkable growth in the number of publications and citations related to bioplastic production and applications. Bioplastics are made up of a special type of biopolymer derived from renewable resources such as vegetable oil, corn starch, soybean proteins, sugars, potatoes, and others. They are also produced by a range of organisms including bacteria, fungi, and plants (Pei et al., 2011). However, the bacterial group is the dominant producer. At present, bioplastics represent about 1% of the total global plastic market (335 million tons), with an annual growth rate of 3%–4% (European Bioplastic, 2019). As the demand for bioplastic products and applications grows, so does the market.

As biopolymers are natural products that are synthesized and degraded by different organisms, bioplastics can also be metabolized by various groups of organisms. Generally, the decomposition of bioplastic is carried out by bacteria and fungi. Many reports and reviews on bioplastic degradation by bacteria have been published (Kasuya et al., 1998; Siracusa, 2019). However, research on fungal degradation of bioplastics is not well compiled. It is well known that fungi play a key role in the degradation of natural products such as cellulose, hemicellulose, and lignin, therefore the bioplastic degradation ability of fungal species should be acknowledged. This chapter sheds light on fungal degradation of various bioplastics and discusses recent research and progress related to this area.

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जयनारायण व्यास विश्वविद्यालय
जोधपुर

डॉ. अनिल कुमार श्रोत्रिय
विभागाध्यक्ष
भौतिक शास्त्र विभाग
श्री प्र.सिं.बा. राजकीय महाविद्यालय
शाहपुरा (भीलवाड़ा)

डॉ. उत्तम पालीवाल
असिस्टेंट प्रोफेसर
भौतिक शास्त्र विभाग
जयनारायण व्यास विश्वविद्यालय
जोधपुर

डॉ. एस.के. बरवड़
असिस्टेंट प्रोफेसर
भौतिक शास्त्र विभाग
जयनारायण व्यास विश्वविद्यालय
जोधपुर

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दोलन एवं तरंगे	शर्मा, शर्मा, शर्मा, सिंह, विजय
उष्मागतिकीय एवं सांख्यिकीय भौतिकी	शर्मा, मिश्रा, मल्होत्रा, विजय, शर्मा
गणितीय भौतिकी एवं आपेक्षिकता का विशिष्ट सिद्धान्त	नगावत, सिंह, सिंह, श्रोत्रिय
इलेक्ट्रॉनिकी एवं ठोस प्रावस्था युक्तियाँ	शुक्ला, सिंह, शर्मा, शर्मा
क्वाण्टम यान्त्रिकी एवं स्पैक्ट्रोस्कोपी	शर्मा, अग्रवाल, विजय, जाखड़
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चौड़ा रास्ता, जयपुर–302003
फोन : 2319198, 2319094
9829051646, 9314523113
E-mail: jaipur.jph@gmail.com, alokagarwal.jph@gmail.com, ajay.agarwal151269@gmail.com

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लेखक

डॉ. एस. एल. मीणा

असिस्टेंट प्रोफेसर, भौतिकी विभाग

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