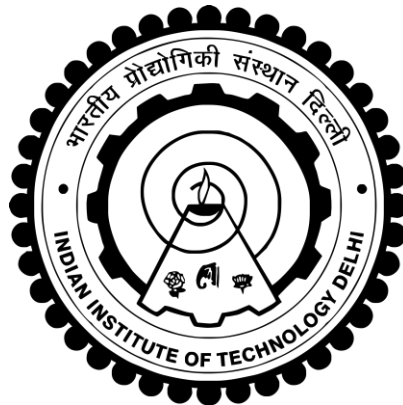


# **BIOREMEDIATION OF METAL CONTAMINANTS UNDER METAL PESTICIDE MATRIX**

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**CENTRE FOR RURAL DEVELOPMENT AND TECHNOLOGY**

**INDIAN INSTITUTE OF TECHNOLOGY DELHI**

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by

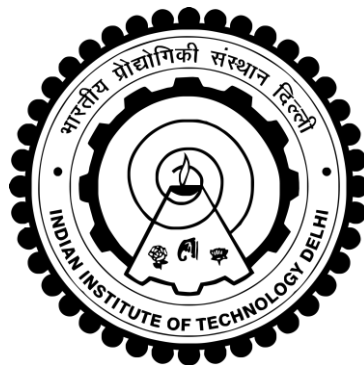
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Centre for Rural Development and Technology

Submitted

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to the



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## CERTIFICATE

This is to certify that the thesis entitled “**Bioremediation of metal contaminants under metal pesticide matrix**” being submitted by **Ms. Priyadarshini Dey** to the Indian Institute of Technology Delhi for the award of “**Doctor of Philosophy**” is a record of bonafide research work carried out by her. She has worked under our guidance and supervision and has fulfilled the requirements for the submission of this thesis. To the best of our knowledge the results contained in this thesis have not been submitted in part or full to any other university or institute for award of any degree or diploma.

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**Priyadarshini Dey**

## ABSTRACT

In the last few decades there has been a surge in the hazardous chemicals contamination in the water bodies in various parts of India. Consequently bioremediation of these hazardous chemicals by microorganisms has emerged as a lucrative option. However, remediation of a cocktail of metals and pesticide have been poorly understood. Advancement in the various physicochemical and robust molecular techniques such as 1D-SDS-PAGE followed by LC-MS/MS analysis as well as metabolite identification by <sup>1</sup>H-NMR can shed light on the various cellular strategies adopted by a microorganism. However, these advanced techniques have rarely been employed towards study of remediation of complex metal mixtures or metal pesticide mixture.

With this background, the present thesis work consists of four chapters, aiming to understand the complete process of metal/multimetal and pesticide mixture remediation by an isolated fungus. The first chapter comprises of the literature review on the occurrence of heavy metal/pesticide in the various water bodies in India. This section also comprises of various bioremediating microorganisms used by researchers till date and the latest proteomic and metabolomic tools available to study the mechanism of bioremediation.

The third chapter begins with a comprehensive tests for the analysis of the selected six heavy metals (Cd, total Cr, Cu, Ni, Pb and Zn) and the physicochemical parameters of the samples collected from the designated sites of the river Yamuna flowing across the peri urban and urban points of Delhi for a period of one year covering various seasons of the year. The ten designated sites had a consistent occurrence of Cd during the monitoring season of September, 2012.

Next, five fungal strains viz., *Aspergillus terreus* AML02, *Paecilomyces fumosoroseus* 4099, *Beauveria bassiana* 4580, *Aspergillus terreus* PD-17, *Aspergillus fumigatus* PD-18, were screened for simultaneous multimetal removal. Highest metal tolerance index for each individual metal viz., Cd, Cr, Cu, Ni, Pb and Zn (500 mg/L) was recorded for *A. fumigatus* for the metals (Cd, 0.72; Cu, 0.72; Pb, 1.02; Zn, 0.94). Thereafter, the strains were exposed to multiple metal mixture (Cd, Cr, Cu, Ni, Pb and Zn) of various concentrations (6, 12, 18, 30 mg/L). Compared to other strains, *A. fumigatus* had higher cube root growth (k) constants indicating its better adaptability to multimetal stress. After 72 h, multimetal accumulation potential of *A. fumigatus* ( $27.59 \pm 0.09$  mg/L) were higher than the other fungal strains at initial multimetal concentration of 30 mg/L. The metal removal varied from 72-89 % for other tested strains as compared to 91% for *A. fumigatus*. However, considering the post treatment concentrations of individual metals in multimetal mixture (at all the tested concentrations), *A.*

*fumigatus* demonstrated exceptional performance and could bring down the concentrations of Cd, Cu, Ni, Pb and Zn below the threshold level for irrigation prescribed by Food and Agriculture Organization (FAO). Further, this strain was studied for its ability to uptake metals in the simultaneous presence of 30 mg/L lindane pesticide. The performance of the strain with the introduction of pesticide decreased to 67% metal removal and as a result the metals Cd, Ni and Cu were not brought down below the permissible limits. An interesting observation was that Pb and Zn uptake by *A.fumigatus* were enhanced in the presence of lindane.

The mechanism for removal of 30 mg/L each of individual metals, 30 mg/L of multimetal and 30 mg/L of multimetal along with 30 mg/L of lindane were investigated in a three fold manner by various physiological, proteomic and metabolomic techniques. The different physiological techniques comprised of SEM, TEM-EDX, FTIR, XRD and XPS. The SEM studies revealed that the 30 mg/L individual metals exerted toxicity on the fungal strain when compared to the biotic control. The mycelia in the metal treated cases were highly twisted and shrunk. The TEM-EDX mapping along with the metal desorption study by cell disruption technique showed ~ 46 % Pb deposition on the cell wall and membrane and 51 % Cu, 51 % Ni and 54 % Zn in the cytoplasm in case of 30 mg/L individual metals. The FTIR studies confirmed the roles of carboxyl, hydroxyl and amide groups which are components of the fungal cell wall for metal binding. The XRD study implicated the precipitation of the metals Cd and Ni as phosphates in the fungal cell when exposed to the metals individually and at higher concentration than in the case of the metals present in multimetal or at lower concentrations.

The proteomic study gave support for the expression of the proteomes related to the enzymes responsible for the various metal uptake phenomena such as organic acid production for metal chelation extracellularly, transporter proteins, molecular chaperones like Hsp70 responsible for unfolding of folded proteins in case of metal stress. Enzymes responsible for production of antioxidants viz. superoxide dismutase and various intracellular metal chelating molecules of phytochelatin such as glutathione synthase were shown to be involved in the metal detoxification process.

The metabolomic study revealed the production of primary metabolites which aid in reducing the effect of contaminant stress in a fungus. The metabolite betaine which is an osmoregulant was produced in case of all the exposures. Overall, this study demonstrated that the same microorganism *A.fumigatus* has different strategies for removal of different metals and pesticide individually as well as in combination. This understanding leads to the scope for improvement and enhancement in a microbial remediating system for efficient contaminant removal.

Thus this research work is novel as there are limited studies on the mechanisms of multimetal as well as pesticide remediation by a fungus. Hence principally this work is a fundamental study to elucidate the comprehensive mechanism of metal/pesticide remediation with the aim to extrapolate the findings of this research work to various field applications such as design of immobilised enzyme systems for remediation of contaminants or production of useful metabolites under various contaminant exposures.

## सार

पिछले कुछ दशकों में भारत के विभिन्न हिस्सों में जल निकायों में खतरनाक रसायनों के प्रदूषण में वृद्धि हुई है। नतीजतन सूक्ष्मजीवों द्वारा इन खतरनाक रसायनों के बायोरेमेडीकरण एक आकर्षक विकल्प के रूप में उभरा है। हालांकि, धातुओं और कीटनाशकों के एक कॉकटेल के उपचार को काफी समझा गया है। 1 डी-एसडीएस-पेज जैसे विभिन्न भौतिक-रासायनिक और मजबूत आणविक तकनीकों में प्रगति, एलसी-एमएस / एमएस विश्लेषण के साथ-साथ 1 एच-एनएमआर द्वारा मेटाबोलाइट पहचान के साथ-साथ सूक्ष्मजीव द्वारा उठाए गए विभिन्न सेलुलर रणनीतियों पर प्रकाश डाला जा सकता है। हालांकि, इन उन्नत तकनीकों को शायद ही कभी जटिल धातु के मिश्रण या धातु कीटनाशक मिश्रण के उपचार के अध्ययन के लिए नियोजित किया गया है। इस पृष्ठभूमि के साथ, वर्तमान थीसिस के काम में चार अध्याय शामिल हैं, जिसका उद्देश्य एक पृथक कवक द्वारा धातु / बहुआयामी और कीटनाशक के मिश्रण की पूरी प्रक्रिया को समझना है। पहले अध्याय में भारी धातु / कीटनाशक की घटना पर साहित्य की समीक्षा शामिल है। भारत में विभिन्न जल निकायों इस खंड में अभी तक शोधकर्ताओं द्वारा उपयोग किए जाने वाले विभिन्न जैव-निदान सूक्ष्मजीवों और जैव-निदान के तंत्र का अध्ययन करने के लिए उपलब्ध नवीनतम प्रोटिओमिक और मेटाबोलामी उपकरण शामिल हैं।

तीसरा अध्याय चयनित छह भारी धातुओं (सीडी, कुल सीआर, कू, नी, पीबी और जेएन) के विश्लेषण के लिए एक व्यापक परीक्षण के साथ शुरू होता है और यमुना नदी के नामित स्थलों से एकत्र किए गए नमूनों के भौतिक-संबंधी पैरामीटर वर्ष के विभिन्न मौसमों को कवर करने के लिए एक वर्ष की अवधि के लिए दिल्ली के पेरी शहरी और शहरी अंक। सितंबर 2012 के मॉनिटरिंग सीज़न के दौरान दस नामित साइट्स सीडी की लगातार घटनाएं थीं।

अगला, पांच कवक के लक्षण, जैसे एस्परगिलस टेरियस एएमएम 2, 2, पेसिलोमायसस फ्यूमोसोरेस 4099, बीवेरिया बास्सियाना 4580, एस्परगिलस टेरियस पीडी -17, एस्परगिलस फ्यूमिगेटस पीडी -18, एक साथ मल्टीमेटल हटाने के लिए जांच की गई। धातु के लिए ए। फ्यूमिगेटस (सीडी, 0.72; कू, 0.72; पीबी, 1.02; प्रत्येक व्यक्ति के लिए अधिकतम धातु सहिष्णुता सूचकांक अर्थात् सीडी, सीआर, कू, नी, पीबी और जेएन (500 मिलीग्राम / एल) दर्ज किया गया था। Zn, 0.94)। उसके बाद, विभिन्न सांद्रणों (6, 12, 18, 30 मिलीग्राम / एल) के कई धातु मिश्रण (सीडी, सीआर, क्यू, नी, पीबी और जेएन) के संपर्क में थे। अन्य प्रकार के उपभेदों में ए फ्यूमिगेटस के उच्च घन जड़ विकास (कश्मीर) स्थिरांक बहुमेटल तनाव के लिए बेहतर अनुकूलन क्षमता को दर्शाता है। 72 घंटे के बाद, ए फ्यूमिगेटस (27.5 9 ± 0.0 9 मिलीग्राम / एल) की बहुआयामी संचय क्षमता 30 मिलीग्राम / एल की प्रारंभिक मल्टीमेटल एकाग्रता में अन्य फफूंद उपभेदों की तुलना में अधिक थी। मेटाइड हटाने के लिए 72-89% से अन्य परीक्षण किए गए उपभेदों में भिन्नता हुई, जबकि ए फ्यूमिगेटस के लिए 91% की तुलना में।

हालांकि, बहुमेटल मिश्रण (सभी परीक्षण सांद्रता में) में व्यक्तिगत धातुओं के बाद उपचार सांद्रता पर विचार करते हुए, एफ्यूमिगेटस ने असाधारण प्रदर्शन का प्रदर्शन किया और निर्धारित सिंचाई के लिए सीमा स्तर से नीचे सीडी, कू, नी, पंजाब और जेएन की सांद्रता को कम कर सकता है खाद्य और कृषि संगठन द्वारा (एफएओ)। इसके अलावा, इस तनाव का अध्ययन 30 मिलीग्राम / एल लिंडने कीटनाशक की एक साथ उपस्थिति में धातुओं को तेज करने की क्षमता के लिए किया गया। कीटनाशक की शुरूआत के साथ तनाव का प्रदर्शन 67% धातु हटाने की कमी हुई और परिणामस्वरूप धातु सीडी, नी और क्यू को अनुमत सीमा से कम नहीं लाया गया। एक दिलचस्प अवलोकन यह था कि एफ्यूमिगाटस द्वारा पीबी और जेन अपलिंक को लिंडने की उपस्थिति में बढ़ा दिया गया था।

30 मिलीग्राम / एल प्रत्येक व्यक्ति की धातुओं को हटाने, 30 मिलीग्राम / एल बहुमेटल और 30 मिलीग्राम / एल बहुमेटल को हटाने के लिए 30 मिलीग्राम / एल लिन्टेन के साथ तंत्र को विभिन्न शारीरिक, प्रोटिओमिक और मेटाबोलामिक तकनीक । एसईएम, मंदिर-एडीएक्स, एफटीआईआर, एक्सआरडी और एक्सपीएस के विभिन्न शारीरिक तकनीकों एसईएम के अध्ययन से पता चला है कि जैविक नियंत्रण की तुलना में 30 मिलीग्राम / एल व्यक्तिगत धातुओं ने कवक के तनाव पर विषाक्तता लगाई थी। मेटलिया के मामले में मेटलिया मामलों में बहुत ही मोड़ और सिकुड़ थे। सेल-डिटेक्शन तकनीक के द्वारा धातु-डिस्कॉप अध्ययन के साथ मंदिर-एडीएक्स मैपिंग ने सेल दीवार और झिल्ली पर 46% पीबी बयान और 51 मिलीग्राम घनत्व, 51% नी और 54% जेएन को 30 मिलीग्राम / एल व्यक्ति के मामले में साइटोप्लाज्म में दिखाया। धातुओं। एफटीआईआर के अध्ययन ने कार्बाक्सिल, हाइड्रॉक्सिल और एमाइड समूहों की भूमिका की पुष्टि की है जो धातु बंधन के लिए फंगल सेल दीवार के घटक हैं। एक्सआरडी के अध्ययन में धातुओं के सीडी और नी के फंगल कोशिकाओं में फॉस्फेट को फेंका गया जब धातुओं से अलग-अलग और बहुसंख्यक या कम सांद्रता में उपस्थित धातुओं के मामले में उच्च एकाग्रता पर सामने आये।

प्रोटिओमिक अध्ययन ने मेटल टैक्सेशन के लिए कार्बनिक अम्ल उत्पादन जैसे एक्स्ट्रायल्युलर, ट्रांसपोर्टर प्रोटीन, आणविक संरक्षक जैसे एचएसपी 70 जैसे धातुओं की तेज गति के लिए एंजाइमों से संबंधित प्रोटीओमों की अभिव्यक्ति के लिए समर्थन दिया है, जो धातु तनाव के मामले में जोड़ प्रोटीन के खुलासे के लिए जिम्मेदार हैं । एंटीऑक्सिडेंट के उत्पादन के लिए जिम्मेदार एंजाइम अर्थात् सुपरऑक्साइड डिस्मूतासेज और ग्लूटाथिऑन सिन्थेस जैसे फाइटोकेलैटिन के विभिन्न इंट्रासेल्युलर मेटल चैलेट अणुओं को धातु की सृजन प्रक्रिया में शामिल होना दिखाया गया था।

मेटाबोलाइक अध्ययन ने प्राथमिक चयापचयों के उत्पादन का खुलासा किया जो कवक में संदूषक तनाव के प्रभाव को कम करने में सहायता करता है। मेटाबोलाइट betaine जो सभी एक्सपोजर के मामले में ओसमोरगुलेट का उत्पादन होता है। कुल मिलाकर, इस अध्ययन दिखा दिया है कि एक ही सूक्ष्मजीव *A.fumigatus* विभिन्न धातुओं और कीटनाशक को हटाने के लिए विभिन्न रणनीतियों को व्यक्तिगत रूप से और साथ ही combination.This समझ कुशल संदूषक removal. Thus के लिए एक माइक्रोबियल remediating प्रणाली में प्रगति एवं संवृद्धि के लिए गुंजाइश की ओर जाता है के रूप में है यह शोध कार्य उपन्यास है क्योंकि मल्टीमैटल के तंत्र के साथ ही कवक द्वारा कीटनाशक के उपचार पर सीमित अध्ययन होते हैं। इसलिए मुख्यतः यह काम इस शोध कार्य के निष्कर्षों को विभिन्न क्षेत्रीय अनुप्रयोगों जैसे कि दूषित पदार्थों के उपचार के लिए अबाधित एंजाइम प्रणाली के डिजाइन या उपयोगी चयापचयों के उत्पादन के लिए एक्सट्रॉल करने के उद्देश्य से धातु / कीटनाशक उपचार के व्यापक तंत्र को स्पष्ट करने के लिए एक मूलभूत अध्ययन है।

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