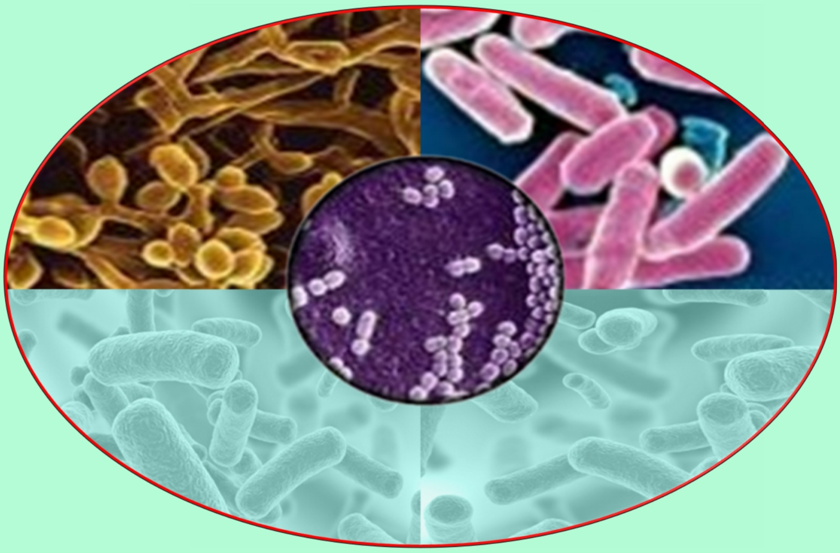
ENVIS RESOURCE PARTNER ON ENVIRONMENTAL BIOTECHNOLOGY

SUPPORTED BY:
MINISTRY OF ENVIRONMENT, FOREST& CLIMATE CHANGE
GOVERNMENT OF INDIA, NEW DELHI

ISSN : 0974 2476 Volume-40 Number-1, 2022

NEWSLETTER
ON
BIOREMEDIATION OF ENVIRONMENTAL TOXIC SUBSTANCES USING PROBIOTICS



DESKU ENVIS RP, UNIVERSITY OF KALYANI, NADIA, WEST BENGAL
Email: desku-envis.nic.in, Phone: +91-33-25828750
Website:http://www.deskuenvis.nic.in

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EDITORIAL

Now the environment has been severely polluted by heavy metals and is accumulating on the earth crust. Heavy metals such as mercury, arsenic, lead, silver, cadmium, chromium, etc., which have anthropogenic activities introduce large quantities in different environmental. Heavy metal pollution is increasing day by day due to industrialization, urbanization, mining, volcanic eruptions, weathering of rocks, etc. Accumulation of rich concentrations of heavy metals in environment can lead to affect the human, animal, and plant health.
So, bioremediation of heavy metals requires for protection of soil quality, air quality, water quality, human health and animal health.

Different microbial strains have developed to reduce metal toxicity and can uptake the heavy metal via different physiological and biological methods.

This newsletter highlights on the bioremediation of different heavy metals such as cadmium, lead, arsenic, and chromium by probiotics.

Prof. Kausik Mondal
Dr. Subhankar Kumar Sarkar

IN THIS ISSUE:

ENVIS RP on Environmental Biotechnology, University of Kalyani.

Bioremediation of toxic heavy metal using probiotics - An ecofriendly tool

Md. Golam Ambiya, Sumit Nath, Salma Haque, and Kausik Mondal
Aquaculture Laboratory, Department of Zoology, University of Kalyani, Kalyani, Nadia, West Bengal, India-741235

Abstract

The levels of heavy metals in the environment have increased day by day due to rapid industrialization, urbanization, intensive farming and anthropogenic activities, contaminating food and water and harming life in all parts of the world. Consumption of heavy metal-contaminated food and water poses a serious health risk. The indiscriminate discharge of heavy metal-laden industrial effluents into water bodies and soil is now posing life-threatening health risks to humans. Conventional heavy metal remediation techniques are not only costly, but they are also ineffective in low metal concentrations. Microbial assisted heavy metal remediation has emerged as a low-cost and simple alternative. Bioremediation is the use of microorganisms to degrade or reduce the concentration of hazardous wastes on a contaminated site. Biological treatment systems can be used to clean up contaminated sites like water, soil, sludge, and streams. Bioremediation is becoming a popular and successful management technique for treating and restoring the environment in an eco-friendly way. This paper reviews the role of naturally occurring probiotic bacteria in heavy metal remediation.

Key words: Heavy metals, Probiotics, Bioremediation

Introduction

An important environmental issue is the heavy metal poisoning of water bodies and soil, which is a result of the world's growing industrialisation and urbanisation. The natural process of metal transportation between soil and water consolidates metal contamination, affecting areas of the natural ecosystem (Runnells *et al*., 1992). Because of the severity of heavy metal contamination and its potential negative health impact on the public, tremendous efforts have been made to purify water containing toxic metal ions. Through the processes of bioconcentration, bioaccumulation, and biomagnification, heavy metals get into the food chain and reach to the top, having a negative impact on human health (Ahmed *et al*., 2017). Heavy metal pollutants cannot be broken down like other organic pollutants, especially those that exist as fundamental elements. For eliminating harmful heavy metals from contaminated sources, numerous traditional physicochemical techniques have been developed up to the present day. These methods include ion exchange, electrochemical treatment, evaporation, reverse osmosis, precipitation, adsorption on activated coal, and many others. However, the majority of approaches, particularly for metals at low concentrations or in large solution volumes, are ineffective and extremely expensive (Chaalal *et al*., 2005). Additionally, these compounds may lead to the production of a variety of hazardous by-products. Therefore, less expensive and eco-friendly biological treatments should be taken into consideration as alternatives to traditional heavy metal clean-up techniques (Congeevaram *et al*., 2007). Microorganisms are used in bioremediation technology to reduce, eliminate, contain, or transform contaminants found in soils, sediments, water, and air. Because of its advantages over traditional methods, bioremediation of heavy metals using microorganisms has received a lot of attention in recent years. In the current scenario of massive heavy metal pollution, microbial assisted remediation is a ray of hope. Among biological species, bacterial bioremediation is being considered because they can readily and quickly adapt to new environments and grow under either aerobic or anaerobic circumstances, and since more information on the structure of bacterial cells and biochemical processes is also accessible (Kargar and Shirazi., 2020).

Bioremediation is the most effective method for reducing or eliminating toxic pollutants. The application of microbes to ponds, known as 'bioremediation' is the current approach to improving water quality in aquaculture. Microbiologists have recently reported that probiotic microorganisms have the ability to detoxify heavy metals by both *in vitro* and *in vivo* (Table 1). The objective of this review is to investigate and summarise the heavy metal remediation by using probiotics.

Toxicity of cadmium and its bioremediation by probiotics

Cadmium is a non-essential element with no known biological function, and even at very low concentrations, it can be toxic to humans. Cadmium can accumulate in humans which has a 10–30year half-life in tissues, especially in the kidneys (Johri *et al*., 2010).Cadmium concentration increases 3,000 fold when it binds to the cystein-rich protein (metallothionein), forming the cystein-metallothionein complex. This cystein-metallothionein complex causes hepatotoxicity in the liver and circulates to the kidney, where it causes nephrotoxicity after accumulating in renal tissue. Ibrahim *et al.* (2006) investigated the ability of two common probiotics, *Lactobacillus rhamnosus* LC-705 and *Propionibacterium freudenreichii,* to bind and absorb lead and cadmium in solution. According to Cheng and Fan (2021), *Lactobacillus rhamnosus* and *Bifidobacterium longum* have a high biosorption capacity for Cd and Hg. The probiotic stains *L. rhamnosus, L. plantarum, L. acidophilus, and Bifidobacterium angulatum* effectively removed Cd from heavy metal contaminated water (Elsanhoty *et al*., 2016). Arivalagan *et al*. (2014) reported that *Bacillus cereus* KTSMBNL43 showed maximum absorption of Cd^{2+}at pH 6.0, temperature 35°C.Halttunen *et al*. (2007) found that *Bifidobacterium longum* 46, *Lactobacillus fermentum* ME3, and *Bifidobacterium lactis* Bb12 probiotics effectively removed cadmium at pH levels ranging from 2 to 6. *B. longum* 46 had the maximum removal of cadmium (54.7 mg of metal per g of dry biomass) for a contact time of 1 hour, followed by L. fermentum ME3 and B. lactis Bb12. *Bacillus* species such as *B. subtilis* (Gayathramma *et al*., 2013) and *B. safensis* (Priyalaxmi *et al*., 2014) have the ability to reduce cadmium levels via bioremediation. According to Jaafar (2019), the probiotic bacteria *Pediococcus pentosaceu* has a removal efficiency for Pb of 62.10-68.39% at concentrations of 25 and 50 ppm, respectively, and a removal efficiency for Cd of 52.71-11.25% at the same concentrations. Zhai *et al*. (2013) showed that *Lactobacillus plantarum* CCFM8610 has protective effects against acute cadmium toxicity in mice. Living *Lactobacillus plantarum* CCFM8610 can effectively reduce intestinal cadmium absorption, tissue cadmium accumulation, renal and hepatic oxidative stress, and hepatic histopathological changes. *Bacillus cereus* and *Bacillus thuringiensis* have been shown to increase Cd and Zn extraction from soil and soil polluted with metal industry effluent (Chibuike and Obiora, 2014).

Toxicity of Lead and its bioremediation by probiotics

Lead toxicity and exposure can also occur as a result of consuming contaminated food/water or ingesting lead particles. Lead is capable of bioaccumulating in both the blood and the bones (Somervaille *et al*., 1988). It has a half-life of about 30 days in the blood, but it can stay in the skeletal system for years, making lead toxicity a persistent issue (Heard and Chamberlain 1984; Manton *et al*., 2000).In the human body, Pb exposure causes neurologic and haematological dysfunctions, cardiovascular, hepatic, and renal damage, and reproductive disorders. It is especially hazardous to young children (Rossi, 2008). According to Belapurkar *et al*. (2016), *Bacillus coagulans* may play a role in the *in vitro* bioremediation of Cr (VI) and Pb (II). *L. bulgaricus* KLDS1.0207, which has a great Pb binding capability and Pb tolerance. The protective effects of *L.*

bulgaricus KLDS1.0207 against acute Pb toxicity in mice were evaluated by prevention and therapy groups. *In vivo* results showed that *L. bulgaricus* KLDS1.0207 treatment could reduce mortality rates, effectively increase Pb levels in the faeces, alleviate tissue Pb enrichment, improve the antioxidant index in the liver and kidney, and relieve renal pathological damage (Li *et al.*, 2017). These findings indicate that *L. bulgaricus* KLDS1.0207 may be useful as a probiotic against acute Pb toxicity. *Lactobacillus reuteri* P16 exerts a protective effect against lead toxicity in common carp by enhancing growth and hemological parameters, reducing oxidative stress, and by modulating gene expression (Giri*et al.*, 2018). Zhi*et al.* (2018) reported that *Lactobacillus plantarum* CCFM8661 alleviates Pb toxicity by decreasing blood and tissue Pb concentration through abrogation of oxidative stress in mice model. *B. licheniformis* NSPA5, *B. cereus* NSPA8, and *B. subtilis* NSPA13 reduced lead metal concentrations by 78%, 87%, and 86% (221.227, 130.565, and 145.231 ppm) from the original 1000 ppm concentration, respectively (Zhi *et al.*, 2018).

Toxicity of Arsenic and its bioremediation by probiotics

Arsenic (As) is a heavy metal and a member of group V of the periodic table of elements. In nature, arsenic exists in four oxidation states (+5, +3, 0, and –3), with pentavalent arsenate [+5, As(V)] and trivalent arsenite [+3, As(III)] being the most common inorganic forms of arsenic in the environment. Both of these forms are toxic to humans and the environment, but As (III) is more toxic than As (V) (Oremland and Stolz 2003). Microorganisms in soil can reduce arsenite under anoxic conditions to the volatile compounds arsine ($AsH3$) and methylarsines, which are the most toxic forms of arsenic (Mateos *et al.*, 2006). Arsenic is widely distributed in the environment as a result of both natural and anthropogenic activities, and it is frequently found in food, soil, and airborne particles (Obinaju, 2009). The primary sources of exposure are drinking water and food. High concentrations are found in the liver, kidney, lungs, and skin. Aside from these, small concentrations have been found in bone and muscles, with chronic exposure causing accumulation in hair and nails. The toxic effects of As are thought to be caused by mitochondrial damage, altered DNA repair, altered DNA methylation, oxidative stress, cell proliferation, co-carcinogenesis, and tumour promotion (Obinaju, 2009). Inorganic arsenic compounds may cause neurotoxicity in both the peripheral and central nervous systems. Neurotoxicity is typically characterised by sensory changes, muscle tenderness, and progressive weakness from the proximal to distal muscle groups (Klaasen and Watkins III, 2003).

Bhakta *et al.* (2010) observed that *Pediococcus dextrinicus* (As99-1, As100-2, and As101-3) and *Pediococcus acidilactici* (As102-4, As105-7, and As112-9) showed a broad spectrum of As resistance as well as good removal efficiency, implying that these lactic acid bacteria could be used as potential As removing probiotic agents within the animal system. Chi *et al.* (2017) demonstrated that As exposure may trigger horizontal gene transfer and increase the presence of antibiotic resistance genes in the gut microbiota of mice. Bacteria involved in As resistance or detoxification, such as *Lactobacillus johnsonii, Phyllobacterium, Parasporobacterium, Mucispirillum schaedleri*, and *Alistipes*, became more prevalent in mice exposed to As (Gokulan., 2018). Rahman *et al.* (2014) reported that *Lysinibacillus sphaericus* B1-CDA strain accumulates As amounted to 5.0 mg g^{-1} of the cells dry biomass and thus reduced the arsenic concentration in the contaminated liquid medium by as much as 50%. Singh and Sharma (2010) showed that *L. acidophilus* was able to bind and remove arsenic from water at concentrations of 50-1000 ppb.

Toxicity of Chromium and its bioremediation by probiotics

Chromium is found in rocks, animals, plants, and soil. The three most common chromium forms are Cr (II), Cr (III), and Cr (VI). The oxidation of chromium (II) compounds produces hexavalent chromium compounds (chromium VI) (ATSDR, 1999). Because of its oxidation state, hexavalent chromium is 100 times more toxic than trivalent chromium. It is also much more soluble in water, allowing it to easily seep into groundwater (Fu *et al.*, 2014). When chromium is inhaled, it is absorbed in the lung and transferred across cell membranes into the gastrointestinal tract (ATSDR, 1999). Studies suggest that the toxicity of Cr (VI) compounds is caused by the destruction of cellular components. The production of free radicals causes cell destruction (ATSDR, 1999). *Arthrobacter aurescens* can degrade agricultural pesticides in the soil and reduce hexavalent chromium, which can cause severe irritation in humans (Fu *et al.*, 2014). Ameen *et al.*, 2020 showed that *Lactobacillus plantarum* MF042018 removed 30.20.5% of the Cr^{2+} from the broth medium. Singh *et al.* (2013) reported that *Bacillus cereus* FA-3 strain reduces the Cr (VI) under a wide range of temperatures (25 to 40°C) and pH (6 to 10) with optimum at 37°C and initial pH 8.0.*Bacillus coagulans* could tolerate up to 512 ppm Cr (VI), with a 93% reduction in Cr (VI) in MRS broth after 72 hours of inoculation (Belapurkar *et al.*, 2016). According to Monachese *et al.* (2012), *Bacillus* species are useful because they have high chromium-binding activity and the ability to export the metal out of a cell, reducing damage to the body by decreasing cell concentration.

Toxicity of nickel and its bioremediation by using probiotics

Ni(II) is a more toxic and carcinogenic metal than Ni (IV). Nickel enters water, air, and soil through natural sources such as volcanic emissions, weathering of rocks and soil, and solubilization of nickel compounds from soil, as well as anthropogenic sources such as the release of nickel-containing effluents from industries such as the electroplating, battery, catalyst, and electronic equipment industries (Duda-Chodak and Blaszczyk, 2008). Nickel enters the human body through inhalation, ingestion, and skin absorption (Duda-Chodak and Blaszczyk, 2008). Nickel is transported in the blood by binding primarily to albumin but also to histidine and α2-macroglobulin (Glennon and Sarkar, 1982; Kasprzak *et al.*, 2003). *Lactobacillus plantarum* MF042018 was able to efficiently remove Ni^{2+} from the broth medium by 33.8±0.8% (Ameen *et al.*, 2020). Abdel-Monem *et al.* (2010) discovered that the highest biosorption efficiency of nickel by living biomass was achieved at 117.2mg Ni^{2+} ml-1 for *Bacillus subtilis* 117S, where 60.86% of nickel was removed, and at 234.4mg Ni^{2+} ml-1 for *Pseudomonas cepacia* 120S, where 54.84% of nickel was removed. In nickel bioremediation, immobilised *B. coagulans* also exhibited a high adsorption capacity of 68.4 mg/g of biomass (Lei *et al.*, 2014).

Table.1. Probiotics are used in the bioremediation of various heavy metals.

Sl No.	Probiotics	Heavy metals	References
1	*Bacillus cereus*	Cr, Cd, Pb & Zn	Ghaima*et al.*, 2013
2	*Lactobacillus plantarum* CCFM8610	Cd	Zhai*et al.*, 2013
3	*Bacillus cereus, B. amyloliquefaciens, B. icheniformis, B. subtilis,*	Cd, Zn, Cu, Pb	Issazadeh*et al.*, 2011
4	*Bacillus cereus* sys1	Cu, Cd	Sonawdekar and Gupte., 2020
5	*Bacillus clausii*	Cr, Pb, Cd & Ni	Goyal *et al.*, 2020
6	*Bacillus licheniformis* NSPA5, *B. cereus* NSPA8, & *B. subtilis* NSPA13	Pb, Cr, Cu	Syed and Chinthala, 2015
7	*Lactobacillus plantarum* MF042018	Cd, Pb	Ameen *et al.*, 2020
8	*Lactobacillus rhamnosus* LC-705, *Propionibacterium freudenreichii subsp. shermani* JS	Cd, Pb	Ibrahim *et al.*, 2006

9	*Lactobacillus reuteri* P16	Pb	Giri*et al.*, 2018
10	*Lactobacillus delbrueckii subsp. bulgaricus* KLDS1.0207	Pb	Li *et al.*, 2017
11	*Lactobacillus plantarum* CCFM8661	Pb	Tian *et al.*, 2012
12	*Pediococcus dextrinicus* & *P. acidilactici*	As	Bhakta *et al.*, 2010
13	*Bifidobacterium longum* 46, *B. lactis* Bb12 & *Lactobacillus fermentum* ME3	Cd, Pb	Halttunen*et al.*, 2006
14	*Pseudomonas cepacia* 120S & *Bacillus subtilis* 117S	Ni	Abdel-Monem*et al.*, 2010
15	*Bacillus cereus*	Cd	Arivalagan*et al.*, 2014
16	*Bacillus coagulans*	Cr, Pb	Belapurkar*et al.*, 2016
17	*Lactobacillus plantarum* & *L. fermentum*	Pb, Cd	Kirillova *et al.*, 2017
18	*Pediococcus pentosaceus*	Pb, Cd	Jaafar, 2019
19	*Lactobacillus rhamnosus* GG (LGG) & *Bifidobacterium longum* (BL)	Hg, Cd	Cheng and Fan, 2021
20	*Lactobacillus acidophilus, L. rhamnosus, L. plantrium Bifidobacterium angulatum,* & *Streptococcus thermophiles*	Cd, Pb, As	Elsanhoty *et al.*, 2016

Mechanism of heavy metal bioremediation by probiotics

Bioremediation of heavy metals often involves four general strategies: biosorption, bioaccumulation, sequestration and biotransformation (Fig.1).

Bacterial biosorption is a low-cost and effective method for removing pollutants from wastewater, including non-biodegradable elements such as heavy metals. Bacterial biomass can consist of both living and non-living cells. The efficiency of biosorption is determined by heavy metal ions and bacterial species. (Hassan *et al.*, 2010). The bacterial cell wall serves as the primary physical interface between metal ions and bacterial biomass. The overall negative charge imparted by anionic functional groups (such as amine, hydroxyl, carboxyl, sulphate, phosphate) present in Gram-positive and Gram-negative bacteria confers metal-binding capacity on or within the cell wall(Sherbet, 1978). Extracellular processes are used by dead biomass cells to remove heavy metals. These interactions are caused by functional groups, such as carboxyl, phosphonate, amine, and hydroxyl groups on the cell wall. (Doyle, 1980).

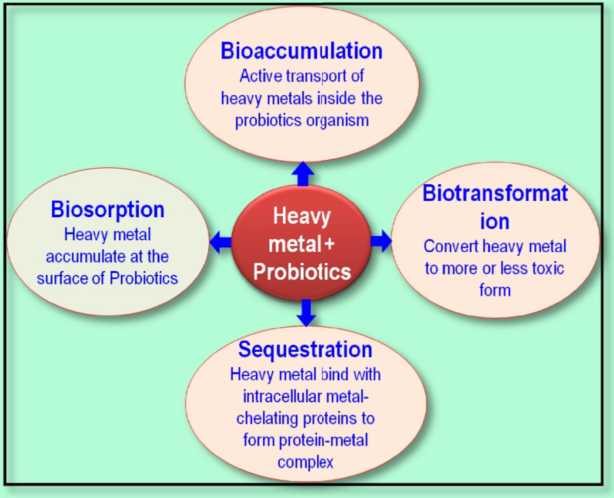

Fig. 1. Different strategies of heavy metal bioremediation followed by probiotics.

By complexation, the carboxyl groups can bind Cd on the surface (Yee and Fein, 2001). The amino groups have demonstrated effective Cr removal via chelation and electrostatic interactions (Kang *et al.*, 2007). Metal binding by anionic surface groups has been reported for *B. subtilis*. Exopolysaccharides (EPS) are also produced by *Lactobacillus rhamnosus* GG and a few *Bifidobacterium longum* strains (Landersjo *et al.*, 2002; Nagaoka *et al.*, 1995). These molecules contain a variety of charged groups, such as carboxyl, hydroxyl, and phosphate groups. The number of ligands that can bind cationic metals like cadmium and lead may increase if lactobacilli can produce EPS with a higher proportion of negatively charged groups. One more defence mechanism against HM stress used by probiotics is bioaccumulation. It is a metabolically active process that transports

HMs into intracellular space and then undergoes sequestration and biotransformation process (Chen *et al.*, 2022). Heavy metal sequestration is the process by which heavy metal bind with the intracellular metal-chelating proteins i.e., metallothioneins (MTs) and phytochelatins (PCs) to form protein–metal complex, whereas, by the process of biotransformation toxic heavy metals transformed into nontoxic forms by various detoxifying enzymes (e.g., Hg reductase and As methyl transferase). Some probiotic strains, including *Xanthomonadaceae*, *Comamonadaceae*, *Pirellula*, *Cloacibacterium*, and *Deltaproteobacteria* FAC87, convert methylated Hg to the less soluble form Hg^0, reducing absorption in the gastrointestinal tract (Bridges *et al.*, 2018; Rowland *et al.*, 1984). Diverse enzymatic transformations have been identified as critical resistance strategies for probiotics to combat heavy metal toxicity. *Faecalibacterium prausnitzii* is a commercialised probiotic with the ability to synthesise methyltransferase, an As-detoxifying enzyme (Qin *et al.*, 2009). Similarly, *Bacteroides* and *Faecalibacterium* secrete ArsC, a reductase that converts toxic As (V) to less toxic As (III) within the intestine.

Conclusion and future prospects

The role of microorganisms suggests an easy and affordable alternative for their remediation in the current scenario of heavy metal pollution causing hazardous effects on human life. Every microbe has different growth requirements (temperature, pH, and nutrients), so it is necessary to separate those types that can be easily cultured in a lab with few requirements and can be used to treat a variety of pollutants. This study provides an integrated understanding of the function and relationships of the microorganisms found in heavy metal-contaminated environments. Further research on the primary target of gene transfer within biofilms for heavy metal remediation is required.These would facilitate the development of improved techniques for heavy metal bioremediation within the ecosystem. Future research should concentrate on the ability of probiotics to bind a variety of heavy metals at physiologically relevant concentrations in humans, as well as the extent to which levels can be reduced over time.

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Obervations of World Environment Day-2022

World Environment Day celebrated every year on 5th of June. It is the biggest annual event in the world coined through United Nations Environment Programme (UNEP) to mark the environmental awareness among the people. This year the theme of World Environment Day 2022 is "**Only one Earth**". This year's campaign highlights the need to reset the balance with nature through transformative changes in how we eat, live, work and move around. For healthy living, environment plays an important role and it provides us air, water, food, etc. Environment is just like our neighbourhood; its surrounding conditions influence us and modify growth and development. It is one of the main prime actions to protect our environment.

Fig. 1. Celebration of World Environment Day in the University Premises

This year DESKU ENVIS Resource Partner on Environmental Biotechnology celebrated the World Environment Day, in collaboration with Department of Zoology and Department of Botany, University of Kalyani along with all university communities through plantation programme.

The plantetion programme was started at 11.10 am with the administration of Honb'le Vice Chancellor Prof. (Dr.) Manas Kumar Sanyal. The Hon'ble Vice chancellor inaugurated the world Environment Day by planting the tree saplings. Registrar, Deans, Head of the Departments, Officers, faculties, ENVIS staffs, students and research scholars were participated in the programme. More than 100 members of the University of Kalyani took part in the programme and planted the tree saplings.

Fig. 2. Inauguration of the programme by Honb'le Vice Chancellor Prof. (Dr.) Manas Kumar Sanyal through Plantation

A national seminar on Celebration of "World Environment Day" was organized on the occasion of World Environment Day at 6.00 p.m onwards. Though this year the theme is "**Only One Earth"** so the seminar highlights the need to reset the balance with nature through transformative changes in how we eat, live, work and move around. For healthy living, environment plays an important role and it provides us air, water, food, etc. Environment is just like our neighbourhood, its surrounding conditions influence us and modify growth and development. It is one of the main prime actions to protect our environment.

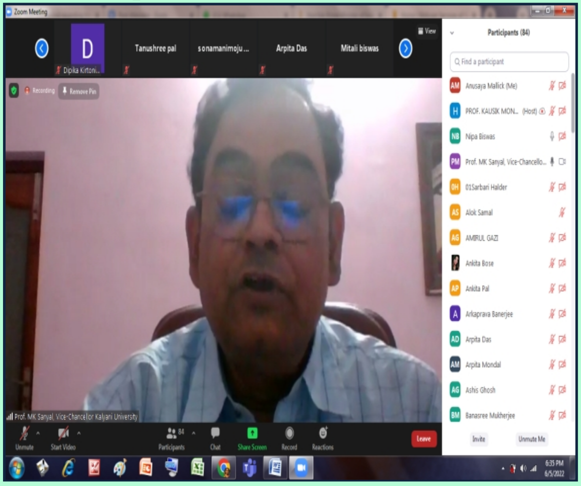

Fig. 3. Inauguration of the Webinar by Honb'le Vice Chancellor Prof. (Dr.) Manas Kumar Sanyal

FORTHCOMING EVENTS

Event	Date	Place & Correspondence
World Congress on Industrial Biotechnology (WCIB)	15-16th July, 2022	Blantyre, Malawi http://conferencefora.org/Conference/32631/WCIB/
10th World Congress and Expo on Green Energy	July 18-19, 2022	Netherlands , United Kingdom https://greenenergy.environmentalconferences.org/
International Conference on Agriculture, forestry, Biotechnology and Food Science (ICAFBFS)	22nd July, 2022	Sangli, Maharashtra, India http://scienceglobe.org/Conference/10727/international-conference-on-agriculture-forestry-biotechnology-and-food-science/
International Conference on Environment, Agriculture and Biotechnology (ICEABT)	6th August, 2022	Faridabad, Haryana, India http://academicsconference.com/Conference/24364/international-conference-on-environment-agriculture-and-biotechnology/
4th European Conference and Expo Future of Biofuels 2022	19-20 October, 2022	Copenhagen, Denmark https://fortesmedia.com/future-of-biofuels-2022,4,en,2,1,17.html

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