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It is certified that the work contained in the thesis titled "Removal of Cadmium, Lead and Hexavalent Chromium from Wastewater" has been carried out under my supervision and that work has not been submitted elsewhere for a degree.

It is further certified that the student has fulfilled all the requirements of Comprehensive Examination, Candidacy and SOTA for the award of Ph.D. Degree.

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DECLARATION BY THE CANDIDATE

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Symbols and Abbreviations

mM	milli mole
q_e	uptake Capacity
C_i	Initial Cr (VI) concentration
C_e	Equilibrium Cr (VI) concentration
mL	Millilitre
mg	Milligram
L	Littre
t	Time
C_a	Heavy metal ion concentration in the desorbed solution
V_a	Desorbed solution volume
PFO	Pseudo first order kinetics
PSO	Pseudo second order kinetics
ASTDR	Agency for toxic substances and disease registry
CLP	*Citrus limetta* peels
ChCLP	Chitosan coated *Citrus limetta* peels
SEM	Scanning electron microscopy
EDX	Energy dispersive X- ray analysis
XPS	X-ray photoelectron spectroscopic
AFM	Atomic force microscopy
FTIR	Fourier transformation infra-red
ICP-OES	Inductively coupled plasma optical emission spectrometry
Cr (VI)	Hexavalent chromium
Cr (III)	Trivalent chromium

Cd (II)	Cadmium
Pb (II)	Lead
V	Volume
W	Weight
r_0	Radius of adsorbent
∂	Thickness of film
D	Diffusion coefficient
R or r	Spherical radial coordinate
ϕ_b	Adsorption flux
ϕ_d	Desorption flux
S	Surface ligands
C_s	Subsurface ligands
C^*	Dimensionless concentration
C	Bulk concentration
C_0	Initial bulk concentration
ς	Dimensionless distance
r_s	Drop radius
S^*	Dimensionless surface concentration
S_∞	Maximum surface concentration
τ	Dimensionless time
k	Ratio of the surface concentration at equilibrium to bulk concentration
n	number of experiments
q_t	Biosorption or adsorption capacity at time *t*
k_s	Equilibrium rate constant

k'_2	Equilibrium constant
a	Initial biosorption rate
β	Extent of surface coverage
Q^0	Maximum uptake capacity
b	Langmuir isotherm constant
k_f and n	Freundlich isotherm and affinity constants
R	Universal gas constant
T	Temperature
A_T	Temkin isotherm constant related to heat adsorption
b_T	Temkin isotherm constant related to the maximum binding energy
K_H and n_H	Halsey isotherm constants
θ	Degree of surface coverage
n_{FH}	number of ions occupying adsorption sites
K_{FH}	F-H equilibrium constant
ΔG^0	Gibbs free energy
ΔH^0	Enthalpy change
ΔS^0	Entropy change
q_H	Maximum uptake capacity of Hill isotherm
$K_{D,}$ and n_H	Hill isotherm constants
RH	rice husk
FeRH	Ferrous ion coated rice husk
CMNPs	Chitosan coated MnO_2 nanoparticles
Al	Aluminium
As	Arsenic

Co	Cobalt
Cu	Copper
Mn	Manganese
Fe	Iron
Ni	Nickel
APHA	American Public Health Association
BOD	Biological oxygen demand
CWE	Coal washery effluent
EDTA	Ethylenediaminetetraacetic acid
A	Growth rate
K_r	Growth per day
B	Growth at time t
β_0	Growth at time $t=0$
Cm	Centimetre
µl	Microliter
M	Molar
Nm	Nanometer
HNO_3	Nitric acid
$HClO_4$	Perchloric acid
PBS	Phosphate saline buffer
PDA	Potato dextrose agar
KBr	Potassium bromide
ROS	Reactive oxygen species
GSH	Reduced glutathione

Rpm	Revolution per minute
SDS	Sodium dodecyl sulphate
SOD	Superoxide peroxidase
H_2SO_4	Sulphuric acid
USEPA	United States Environmental Protection Agency
WHO	World Health Organization

Preface

The heavy metal ions like hexavalent chromium, cadmium, lead, arsenic, nickel are well-known carcinogens that emalgate in the effluent of leather/ tanning industry, textile dyeing production, electroplating and rubber manufacturing plant, chrome plating and coal washery unit. The removal of heavy metal ions from contaminated water is necessary due to their toxicity. Various conventional methods such as membrane filtration, chemical precipitation, electro-dialysis, reverse osmosis and electrochemical precipitation have been used for the removal of toxic metal ions from domestic and industrial wastewater. These methods are not cost-effective at large scale, leads to the generation of secondary chemical sludge and are less effective when less concentration of heavy metal is present in the effluent. However, biosorption has been considered as an inexpensive and highly effective method for the removal of toxic heavy metal ions like Cr (VI), Cd (II) and Pb (II) from the liquid phase. A large number of biomaterials such as activated carbon, lignite, wheat grain husk, rice husk, banana peels, and nanoparticles have been utilized as biosorbents for the removal of heavy metal ions. Bacterial strains isolated from the contaminated site have also showed effective heavy metal removal property. White rot fungi like *Pleurotus florida* mushroom has emerging application in heavy metal removal due to its high tolerance property against toxic metal ions.

In the present work, iron ions were doped on the rice husk for adsorption-cum-reduction of lethal Cr (VI) to less toxic and partially soluble Cr (III). Energy dispersive X-ray analysis and X-ray photoelectric spectroscopy disclosed the effective doping of iron on rice husk and significant reduction of Cr (VI) into Cr (III). Physico-chemical analysis exhibited that the surface of iron doped rice husk was rough and in houses amino and hydroxyl moieties in majority together with high organic content. The maximum removal of Cr (VI) ions was observed in the acidic range as the value of point zero charge was 3.32. The study of dimensionless numbers(φ, N_k and λ) disclosed that the Cr (VI) adsorption on iron coated rice

husk was principally controlled by diffusion. Only 1.32% and 0.18% deviation in experimental results was observed for undoped and iron doped rice husk, respectively in correlation plot of artificial neural network. The values of film and pore diffusivity coefficients along with Bangham's model study revealed that the biosorption was film diffusion limited for iron ions coated rice husks. The maximal removal of Cr (VI) was found to be 81.56 % for iron doped which was much higher than uncoated (43.28 %) rice husk. The iron doped rice husk had high regeneration capacity up to several cycles of simultaneous adsorption and desorption. Isotherm, kinetic and thermodynamic modeling revealed that adsorption of Cr (VI) ions on the surface of iron doped rice husk was multilayer, chemisorptive, spontaneous, endothermic and favourable.

In the next objective, chitosan coated MnO_2 nanoparticles were synthesized through green route using curcumin and *Citrus limetta* peel extract. X-ray photoelectron spectroscopic analysis of chromium loaded nanoparticles confirmed the biosorption of Cr (VI), Cd (II) and Pb (II) onto biosorbent surface. X-ray photoelectron spectroscopic analysis also revealed that most of the Cr (VI) ions were reduced to Cr (III) on the biosorbent surface. Scanning electron and transmission electron microscopic images showed that these nanoparticles were spherical in shape, crystalline, ranged from 14 to 24 nm in size. The Fourier-transform infrared spectroscopy analysis indicated hydroxyl, carboxyl and amino groups on its surface. The dynamic light scattering showed a size distribution of nanoparticles as 299.50 ± 25.32 nm. High Brunauer- Emmett-Teller specific surface area (76.19 m^2/g) of these nanoparticles provided more active sites for binding of heavy metal ions. Energy dispersive X-ray and ultimate analysis indicated the presence of Mn, O, C, H, and N. These elements were the major components of these nanoparticles and confirmed the presence of chitosan on nanoparticle surface. Elemental mapping indicates to successful biosorption of Cr (VI), Cd (II) and Pb (II) on the surface of chitosan coated MnO2 nanoparticles. The lower value of pH_{zpc} (3.17) proved

the suitability of acidic pH values for conducting biosorption experiments for Cr (VI) and pH above 3.17 was suitable for biosorption of Cd (II) and Pb (II). The study of biosorption dynamics by using dimensionless numbers for Cr (VI), Cd (II) and Pb (II) ions showed that biosorption was mixed diffusion and transfer controlled for single metal ion system as well as ternary metal ion system. The artificial neural network modeling has been performed to compare experimental and predicted values. It has been found that both the experimental and predicted values for biosorbent seemed to be in agreement with each other showing a high R^2 value in the range of 0.95 - 0.99 for Cr (VI), Cd (II), Pb (II) ion in the single metal system and R^2 was 0.88 in ternary metal ion system. In both single and ternary metal ion system, better goodness of fit of Freundlich isotherm ($R^2 = 0.99$) for Cr (VI) biosorption revealed multilayer Cr (VI) biosorption on the heterogeneous surface. Langmuir isotherm ($R^2 = 0.99$) best fitted in the Cd (II) and Pb (II) biosorption which indicated monolayer coverage of heavy metal ions on the homogeneous surface. Pseudo-second order ($R^2 = 0.99$) (chemisorption) kinetics best fitted in Cr (VI), Cd (II) and Pb (II) biosorption in both single and ternary metal ion system. The thermodynamic study of Cr (VI), Cd (II) and Pb (II) biosorption of both single and ternary metal ion system showed that biosorption was endothermic and spontaneous and entropy driven. Chitosan coated MnO_2 nanoparticles showed high Cr (VI), Cd (II) and Pb (II) removal efficiency upto 92.19 %, 98.01 %, 94.40% at 100 mg/L initial metal ion solution and 1 g/L biosorbent dosage in a single metal ion system. In the ternary metal ion system, biosorbent was able to removal 53.90 % Cr (VI), 48.09 % Cd (II) and 57.96 % Pb (II) in the ternary metal ion system. Chitosan coated MnO_2 nanoparticles showed high regeneration capacity which could be used in several adsorption-desorption cycles.

In this objective, *Citrus limetta* was coated with chitosan polymer for removal of Cr (VI), Cd (II) and Pb (II). Energy dispersive X-ray and elemental mapping indicated the homogeneous distribution of Cr (VI), Pb (II) and Cd (II) together with other major elements

like carbon, nitrogen and oxygen which are considered as a major elemental constituent of chitosan and play a significant role in heavy metal biosorption as well as Cr (VI) biotransformation. The surface of the synthesized adsorbent was observed to be rough and porous. pH_{zpc} of chitosan coated *Citrus limetta* peel biomass was estimated as 3.69. Atomic forced microscopy showed that the roughness of chitosan coated *Citrus limetta* peel biomass increased up to 146.62 nm as compared to uncoated one (28.62 nm). X-ray photoelectron spectroscopic analysis confirmed the biosorption of Cr (VI), Cd (II) and Pb (II) on the chitosan coated *Citrus limetta* peel biomass. The Fourier-transform infrared spectroscopy analysis indicated that functional groups such as hydroxyl, carboxyl and amino groups actively participated in the biosorption of Pb (II), Cd (II) and Cr (VI) onto chitosan coated *Citrus limetta* peel biomass. The dynamics of heavy metal biosorption onto chitosan coated *Citrus limetta* peel biomass was described by dimensionless numbers for both single ternary metal ion system which indicated that biosorption was mixed diffusion and transfer controlled. It was observed that both the experimental and predicted values for chitosan coated *Citrus limetta* peel biomass were consistent with each other, with high R^2 values ranging from 0.92 to 0.99 for Cr (VI), Cd (II), and Pb (II) ion in single metal system and an R^2 value of 0.86 for the ternary metal system was obtained. Freundlich isotherm ($R^2 = 0.99$) isotherm model best fitted in biosorption data of Cr (VI) in both single and ternary metal ion system which indicates the multilayer biosorption of Cr (VI) onto heterogeneous chitosan coated *Citrus limetta* peels biomass. Langmuir isotherm was best fitted in the Cd (II) and Pb (II) biosorption in both single and ternary metal ion systems. Kinetic study indicated that pseudo second order kinetic model suitably to fitted in the biosorption data of Cr (VI), Cd (II) and Pb (II) of both single and ternary metal ion systems. Thermodynamics indicated that Cr (VI) adsorption was spontaneous, endothermic and entropy driven. Results showed that chitosan coated *Citrus limetta* peels biomass has Cr (VI), Cd (II), and Pb (II) removal efficiency up to 99.34 % Cr (VI), 99.92 %

Cd (II), 99.58 % Pb (II) in single metal ion system and 56.19 % Cr (VI), 62.29 % Pb (II), 68.29 % Cd (II) in the ternary metal ion system at 100 mg/L initial metal ion concentration and 10 g/L chitosan coated *Citrus limetta* peels biomass. A combination of two batch reactors in series was capable in removing 100 % heavy metal ions in single and ternary metal ion system. Chitosan coated *Citrus limetta* peels biomass also showed high regeneration capacity and can be used over several biosorption cycles for the removal of Cd (II), Cr (VI) and Pb (II).

Also, a heavy metal tolerance bacteria was isolated from the wastewater collected from Baliya Nala (drain) Singrauli, Madhya Pradesh, India wherein coal mining units discharge their effluents after treatment. The bacterial isolate showed maximum sequence similarity with the *Microbacterium paraoxydans* and it was submitted to the NCBI GenBank under the accession no. MN650647. The bacterial isolate was named *Microbacterium paraoxydans* strain VSVM IIT (BHU) accession no. MN650647. The scanning electron microscopic and energy dispersive X-ray analysis indicated that the cells of the bacterial isolate which were grown in Luria-Bertani broth containing Cr (VI), Cd (II) and Pb (II) got enlarged after the bio-accumulation of heavy metal ions, whereas Si, O and C ions were observed on the cell surface. The Fourier-transform infrared spectroscopy indicated that several functional groups on the biosorbent surface participated in the biosorption of heavy metal ions. During the bacterial mediated adsorption, some portion of heavy metal ions accumulated in the bacterial cells and few metal ions bound to the bacterial surface and the presence of heavy metal in bacterial cells was confirmed through X-ray photoelectron spectroscopic. The optimum growth was observed at 37ºC and pH 7. It was observed that the presence of both single and ternary metal ions in the solution enhanced the production of antioxidant enzymes such as glutathione S-transferase, catalase, superoxide dismutase and peroxidase within the cells. The concentration of these enzymes increased with the increase in heavy metal concentration in the growth medium. The study of heavy metal removal dynamics for bacteria using dimensionless numbers for Cr (VI),

Cd (II) and Pb (II) ions showed that biosorption was mixed diffusion and transfer controlled for single and ternary metal ion system based on the value of N_K (9.09×10^{-3}, 9.83×10^{-3} and 9.32×10^{-3} for Cr (VI), Cd (II) and Pb (II) ions in single metal ion system, 9.49×10^{-3}, 9.92×10^{-3} and 1.23×10^{-2} for Cr (VI), Cd (II) and Pb (II) ions in ternary metal ion system. It has been found that both the experimental and predicted values for novel bacterial isolate seemed to be in agreement with each other showing a high R^2 value in the range of 0.94 - 0.98 for Cr (VI), Cd (II), Pb (II) ions in single metal ion system and ternary metal ion system. The bacterial isolate showed a maximum heavy metal ion removal efficiency of 99.96 % Cr (VI), 94.96 % Pb (II), 84.96 % Cd (II) at 50 mg/L for single metal ion system and 91.62 % Cr (VI), 89.29 % Pb (II), 83.29 % Cd (II) at 50 mg/L of each metal ions in the ternary metal ion system.

Additionally, bioremediation of heavy metal ions from the coal washery effluent by *Pleurotus florida* was investigated. Metal ion concentration in the fruit body and substrate (paddy straw) was determined by inductively coupled plasma optical emission spectrometry. The fitness functions of exponential and linear extension growth models were evaluated mathematically (goodness of fit) at boundary conditions at t = 0 and at t = t. The surface characterization of *Pleurotus florida* showed that the surface of *Pleurotus florida* was rough, heterogeneous in nature together with negatively charged functional groups like carboxyl, hydroxyl, ketonic and esters. The elemental composition of *Pleurotus florida* revealed the abundance of carbon and oxygen in biomass compared to other elements such as nitrogen and phosphorus. Growth modeling of *Pleurotus florida* revealed the fact that experimental and theoretical values of linear extension growth rate k_r (mm d^{-1}) constant were not only close to each other but also they ranged from 1.36 to 2.21 mm d^{-1} and 1.41 to 2.03 mm d^{-1}. This showed the supremacy of the linear growth extension model over the exponential rate model. The metal toxicity stress markers like metallothionein (35.21 µg/g), superoxidase dismutase (9.4 U/mg), lipid peroxidase (2.5 nmol/mg), catalase (2.15 Pkat/mg) and reduced glutathione (28.09 µg/g)

had higher level expression in the fruit bodies of *Pleurotus florida* grown in coal washery effluent as compared to control. The simultaneous increase of metallothionein concentration from 2.50 to 35.21 µg/g with the increase in the concentration of metal ions in the term of CWE concentration (25 to 100%) in media showed the bioaccumulation of metal ions in the intracellular space. The maximum heavy metal uptake capacity of *Pleurotus florida* was found as 10.65, 1.12, 13.46, 3.0, 1.21, 19.11, 0.47 and 0.23µg/g for Pb, Cr, Cd, Zn, As, Mn, Ni and Ti, respectively. The maximum heavy metal removal from substrate (paddy straw) was found 99.53 % (Cd), 70.85 % (Cr), 77.77 % (Ni), 76.23 % (Zn), 42.63 % (Mn), 52.10 % (Pb), 49.07 % (Ti) and 51.66 % (As). The negatively charged rough surface, high amount of carbon and oxygen in biomass and the induced production of intracellular metal stress markers on exposure to heavy metals illustrated the immense bioaccumulation ability of *Pleurotus florida* in coal washery effluent.

On the basis of present work, we concluded that biosorbents developed were inexpensive, non-toxic and highly effective for the removal of hexavalent chromium, cadmium and lead. The LCA study for the removal of Cr (VI), Cd (II) and Pb (II) from contaminated wastewater helps to define the environmental impacts accompanying during handling, production, use, and recycling.