CHAPTER 2

Review of Literature

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2.0 Review of Literature

Environmental contaminants are the substances that are present in the natural environment at higher level compared to their permissible limits [Masindi and Muedi, 2018]. Industrialization and urbanization are the major causes of environmental pollution. The utilization of natural resources at careless rate, creates disturbance in the environment and causes several problems related to it [Gautam et al., 2016]. There are several types of pollutants such as organic, inorganic, metallic, gaseous and biological pollutants which cause pollution in air, water and soil [Martin and Johnson, 2012]. The heavy metal pollution in the fresh and wastewater is due to several natural and anthropogenic activities which has harmful effect on the animals and plants [Kinuthia et al., 2020].

2.1 Heavy metal pollution: Source and toxicity

Heavy metals are defined on the basis of higher atomic or higher density. The 'heavy metal' word has been used to describe metalloids or metallic elements which indicate toxic effects on human and other living organisms [Singh et al., 2011]. The heavy metals like As, Cr, lead Pb and Cd are toxic to the humans but few heavy metals are not toxic such as Au [Tchounwou et al., 2012]. The density of heavy metals is commonly greater than 5 g/cm^3 for example Ti, Cr, Fe, Co, Ni, Cu, Cd, Zn, Hg and Pb [Wu et al., 2016]. The exposure of heavy metal ions to the human body is generally through four ways; ingestion of metal contaminated food, drinking of contaminated water, through skin and inhalation in metal contaminated air [Jaishankar et al., 2014]. Metal compound tend to form covalent bonds which are responsible for extreme toxic nature of metalloid compounds. Heavy metals can get covalently attached with organic groups and form lipophilic compounds or ions [Jan et al., 2015]. Due to lipophilic nature of these metallic compound, they can easily cross cell membrane and enter into the

intracellular space. These metallic compounds cause toxic effect when they interact with cell organelles [Briffa et al., 2020].

Several industrial processes like leather tanning, chrome plating, batteries manufacturing, glass industries and pharmaceutical industrial process are considered as major heavy metal source which add toxic metal ions into the environments [Wuana and Okieimen, 2011]. According to international lead association, about 10.54 million tons Pb (II) have been produced of which 85.10 % has been used in the batteries, 5.5 % in pigments, 1.4 % in ammunition, 1.3 % in alloys, 0.9 % in cable sheathing, and rest of lead is used in the miscellaneous (2.1 %) [ILA, 2017]. Pb (II) is also used in the petrol as tetraethyl and tetramethyl agents in the form of antiknocking compounds [Quinn and Sherlock, 1990]. Cr (VI) is used for the production of steel, wood preservation, chrome plating, pigments and electroplating [Lunk 2015]. Cd (II) is generally used in the electroplating and battery industry [Panakkal and Kumar, 2014]. CPCB has demarcated the maximum discharge limit of Cr (VI), Cd (II) and Pb (II) in the industrial effluent (Table 2.1).

Table 2.1 Maximum limit of Cr (VI), Cd (II) and Pb (II) in the industrial effluent.

Heavy metals	**CPCB, 2000 (Dischargeable limit in industrial effluent)**
Cr	1.00-2.00 mg/L
Cd	0.20-2.00 mg/L
Pb	0.10 mg/L

Living organisms such as bacteria, fungi and microalgae can detoxify heavy metal ions by hiding the toxic metal site with intracellular proteins or by forming as insoluble granules. The accumulated heavy metals can be excreted out of the body through excretion system or remain in the storage form for a long period [Jan et al., 2015]. The heavy metal ions accumulated in the body tissue cause biological and physiological complications. Some metals

are necessary for metabolic functions and are called as essential substances. However, increased concentration of essential metals after certain limit show toxic effects on the body [Shahid et al., 2014; Jomova and Valko, 2011]. United States environmental protection agency (USEPA), USA has demarcated the maximum permissible limit of Cr (VI), Cd (II) and Pb (II) in the drinking water. The permissible limit of Cr (VI), Cd (II) and Pb (II) in the drinking water are shown in the Table 2.2.

Table 2.2 The maximum permissible limit of Cr (VI), Cd (II) and Pb (II)

Heavy metals (mg/ L)	**US EPA, 2018**	**WHO, 2011**
Cr (total)	0.10	0.05
Cd (II)	0.005	0.003
Pb (II)	0.015	0.01

Beyond these permissible limit, heavy metal causes several toxic effects. These heavy metals are considered as potential carcinogens [Yuan et al., 2016; Balali-Mood et al., 2021].

Cr (VI) is well known toxic agents and its toxicity highly depends on the oxidation states and ionic species [Bokare and Choi, 2011]. It is a powerful oxidizing agent and shows much more toxicity than Cr (III). Cr (VI) passes through cell membrane and enter into the intracellular space and subsequently reduced in to Cr (III) [Balali-Mood et al., 2021; Costa, 1997]. During the reduction process, ROS are generated which cause cell toxicity. The reduction of Cr (VI) is considered as detoxification process when it occurs at a distance from nucleus and other cell organelles or outside of the cell. If Cr (VI) reduction occurs within cell, it induces oxidative mediated toxicity and damages the cell organelles and mutation in the DNA take place [Wakeel et al., 2020; Dayan and Paine, 2000]. In case, Cr (VI) is converted into Cr (III) outside of the cell, the reduced Cr (III) and other intermediates components are unable to transport into the cell compartment and hence toxic effect is not observed [Cohen et al., 1993].

Several studies have reported that Cr (VI) causes carcinogenicity and multiple organ damage such as liver and cardiac failure and renal damage [Kim and Na, 1991]. Gumbleton and Nicholls, 1988 investigated that Cr (VI) induced kidneys damage in the rats after sub-cutaneous injection of Cr (VI). Bagchi et al., 1997 reported that Cr (VI) induced hepatic mitochondrial, microsomal lipid peroxidation and increased lipid metabolites in the urine when Cr (VI) was administrated orally. Other important toxic effects of Cr (VI) are respiratory cancer, chromosomal abnormalities and DNA strand breaks [Costa, 1997; Wise et al., 2004].

The main route of Cd (II) exposure in the human is inhalation, ingestion and drinking of Cd (II) contaminated food and water [Genchi et al., 2020]. The chronic inhalation of Cd (II) leads to change in the pulmonary functions, reduction in olfactory functions and emphysema [Rahimzadeh et al., 2017]. Ingested Cd (II) causes abdominal pain, loss of consciousness, vomiting, nausea, hepatic injury, renal failure, gastrointestinal erosion and burning of sensation [Baselt and Cravey, 1995; Baselt, 2000]. It is also responsible for pulmonary adenocarcinomas, single DNA strand damage, disruption in synthesis of proteins and nucleic acids [Waalkes and Berthan, 1995, Waalkes et al., 1996; Mitra, 1984].

The natural and anthropogenic activities such as mining, burning of fossil fuels, manufacturing of batteries and glasses are the major source of Pb (II) (https://fas.org/sgp/crs/misc/R46420.pdf). Toxic effects of Pb (II) in the children comes from dust and packed food products due to its coating on interior surface of packing materials [Lanphear et al., 1998]. The major affected organs in the body due to Pb (II) toxicity are kidney, liver and other soft tissues such as brain and heart [Flora et al., 2006]. Pb (II) toxicity massively impacts on nervous system. Poor attention, headache, dullness, irritability and memory loss are the early symptoms of Pb (II) poisoning in central nervous system [CDC, 2001; ATSDR, 2000].

2.2 Removal of heavy metal ions

Various technologies are accessible for minimization of heavy metal concentration from wastewater. The most widely used physiochemical technologies are ion exchange, coagulation, precipitation, adsorption, membrane separation and reverse osmosis [Khulbe and Matsuura, 2018]. Precipitation is considered to be a well-known technique for removal of Cr (VI), Pb (II) and Cd (II). The precipitation is done by varying pH with the help of various reagents [Qasem et al., 2021; Kurniawan and Chan, 2006]. These physiochemical techniques are expensive, generate secondary chemical sludge and these methods are only effective when heavy metal concentration high in the water (above 2mM) [Devi et al., 2012; Anirudhan and Sreekumari, 2011]. Considering these disadvantages of physiochemical methods, there is urgent need to develop cost effective and eco-friendly methods for successful removal of heavy metal from water [Wołowiec et al., 2019].

Biological removal of heavy metals are very attractive in comparison to other conventional methods as biological methods are inexpensive and highly efficient at low concentration of heavy metal ions in wastewater [Qasem et al., 2021]. Several biological agents such as plants biomass, agricultural waste, microbial biomass, green synthesized nanoparticles, fruit waste and biopolymers have been used for removal of heavy metal contaminations from liquid phase [Kumar et al., 2020; Abdelbasir et al., 2020]. The living organisms such as bacteria, algae and fungi have also emerging role in the removal of heavy metal [Tarekegn et al., 2020]. Fungi, bacteria and microalgae can uptake heavy metals from the surrounding medium into their intracellular environment [Vieira and Volesky, 2000]. It has been reported that various microbial species can transform toxic Cr (VI) into less toxic Cr (III). Microorganisms can easily uptake Cr (III) into intracellular space due to its less solubility in water and low toxicological properties [Mishra et al., 2012]. Several heavy metal bioremediation methods like biosorption (using dead biomass), phytoremediation (plant

mediated heavy metal removal), bioreduction (conversion of oxidation states of heavy metal ions), and bioaccumulation (uptake of heavy metal ions into intracellular space) have been attempted in past [Mishra et al., 2015].

2.2.1 Biosorption

The phenomenon of biosorption is considered to be metabolically independent and generally performed by dead biomass. In this process, toxic metal ions bind or accumulate on the surface of biosorbent [Volesky and Holan, 1995; Gadd, 2007]. The mechanism of biosorption is shown in Figure 2.1.

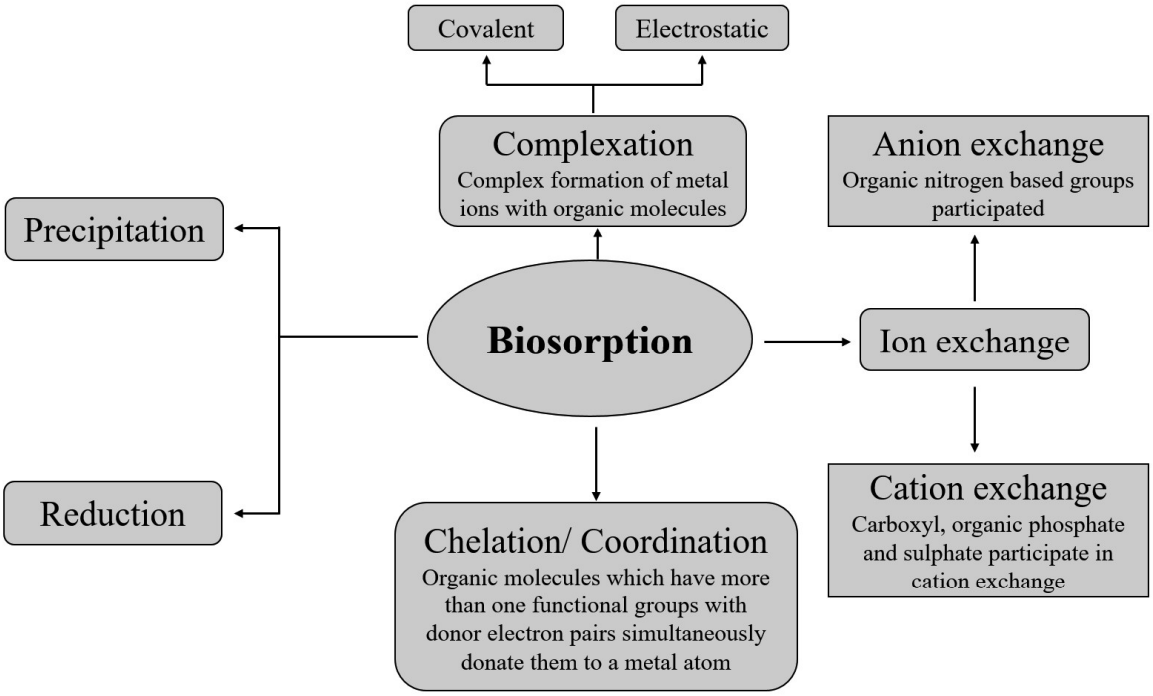

Figure 2.1 Mechanism of heavy metal biosorption

The ion-exchange occurs between the heavy metal ions and protons at the binding sites. Anion and cation exchange mediated mechanism occur in the ion-exchange [Aksu et al., 1992]. The coordination and complex formation involves the covalent binding and electrostatic forces. The complex formation occurs on the surface of biosorbent via interaction of heavy metals and active functional surface groups [Silva et al., 2003]. Precipitation take place when the pH of solution varies or the concentration of heavy metal ions increases in the solution up to their

saturation index. In some cases, biomass produce compounds which are involved in the precipitation of heavy metal ions [Chen et al., 2006]. Chelates are the complex formation of heavy metal ions and surface legends of biosorbents and the phenomenon of complex formation known as chelation [Davis et al., 2003]. The reduction of heavy metal ions is one of the important mechanism in biosorption. The more lethal metal species reduce into less toxic species such as Cr (VI) reduced into Cr (III) [Han et al., 2007; Li et al., 2020].

However, it is also performed by living biomass. Living cells can bind with heavy metal ions metabolically independent or by passive adsorption through surface complexation on the cell surface [Malik, 2004; Peng, 2018]. Biosorption can be described as biological ion exchange in which functional groups like amino, amide, imidazole, sulfonate and carboxyl groups bind with toxic metal ions [Wang and Chen, 2006]. The pKa value also affects the binding tendency of functional groups along with toxic metal ions [Volesky, 2007]. The raw adsorbent material should be inexpensive and easily available in nature [Verma and Sharma, 2017]. Diversity of functional groups present on the biosorbent surface also determines its quality. For an ideal biosorbent, the functional groups should be in high density [Ojuederie et al., 2017]. The surface morphology of biosorbent is also considered as important character which play an important role in the adsorption of heavy metal ions. Rough and porous surface provide more surface area for binding of heavy metal ions on biosorbent surface [Zhang et al., 2020b]. It is very important to characterize surface morphology and functional groups of biosorbent. Various techniques such as FT-IR, SEM, EDX, NMR and XRD are available for the characterization of biosorbent [Chojnacka, 2010; Tsezos et al., 1997].

Many other factors influence the biosorption such as types of biomass, occurrence of other metal ions (competing ions such as cations and anions), temperature and pH [Duwiejuah et al., 2020]. Generally, decrease in pH value causes competition between positively charged metal ions. However, elevation in pH causes surface deprotonation and exposes surface binding

groups [Duwiejuah et al., 2020; Dias et al., 2001]. The regeneration of biosorbent can be also achieved by using desorption. Recovery of metal ions was done by varying pH of medium [Naja and Volesky, 2010; Jobby et al., 2018]. In addition, biosorption mechanism of Cr (VI) is a complex process in which anionic hexavalent chromium ions bind with positively charged groups and also reduce into Cr (III) chromium ions through various pathways [Netzahuatl-Munoz et al., 2015]. Biosorption-cum-bioreduction of Cr (VI) ions generally occurs in three steps.

First step of biosorption is binding of negatively charged Cr (VI) ions with the surface functional groups having positive charge. The second step of biosorption is reduction, where Cr (VI) is reduced to Cr (III) with the help of an electron donor groups. The third step includes release of Cr (III) ions into solution due to an electronic repulsion between the Cr (III) with other positive charged groups or binding of the Cr (III) ions with other negative groups of biosorbent [Park et al., 2005; Park et al., 2006; Deng et al., 2009]. The preparation of biosorbent biological waste is shown in Figure 2.2.

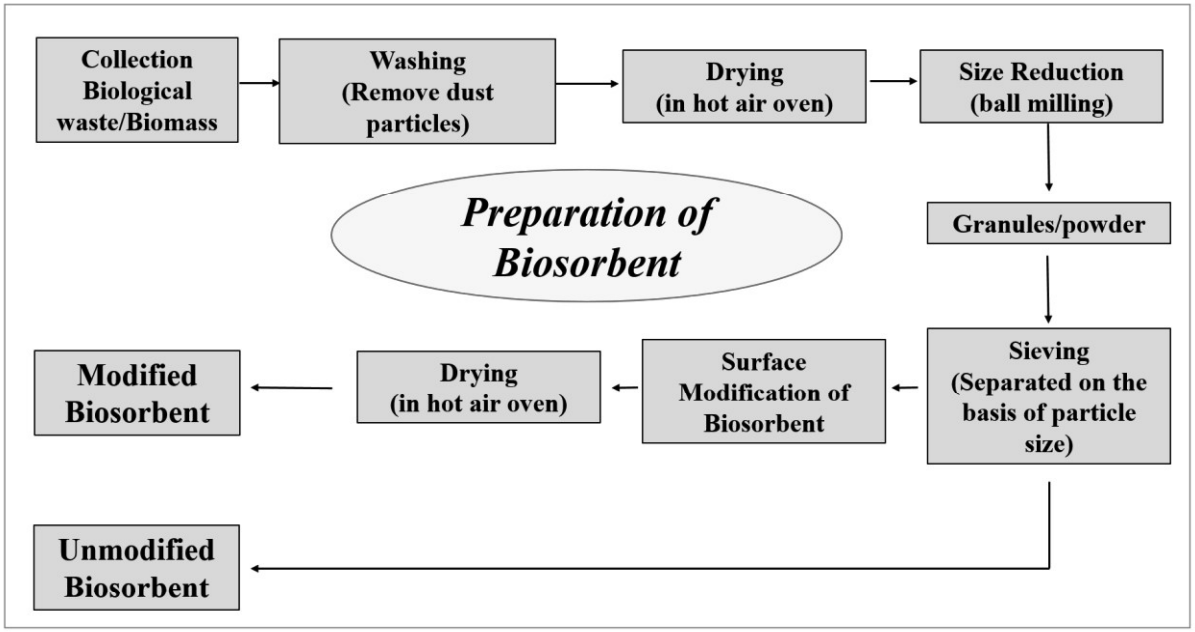

Figure 2.2 Schematic representation of biosorbent preparation

Biosorption method can be also performed in the suspension medium on immobilized biomass. Immobilization improves the rigidity, strength, age of biosorbent and overall enhances the heavy metal removal efficiency [Moghal et al., 2020]. Several types of matrices are available and are used in immobilization methods such as polysulfide, alginate, polyurethane and polyacrylamide [Khanpour-Alikelayeh et al., 2021; Pal and Maiti, 2020]. Various types of plants (lignocellulosic biomass) and microbial biomasses have been immobilized on these matrices, for example, *Chlorella homosphaera* (Biomass)-sodium alginate matrices [Bayat et al., 2015; Moghal et al., 2020]. Lignocellulosic biomass shows effective binding with heavy metal ions. The raw lignocellulosic materials used for biosorption is typically inexpensive, easily and widely available [Kumar et al., 2021]. The biosorption capacities of lignocellulosic materials, microbial and algal biomasses are shown in Table 2.3, 2.4 and 2.5.

Table 2.3 Cr (VI) biosorption capacity of different biosorbent derived from algal, fungal, lignocellulosic materials/plants biomass, bacterial biomass.

Biosorbent	**Biosorption capacity (mg/g)**	**pH**	**Temperature (°C)**	**Biosorbent dose (g/L)**	**Initial conc. (mg/L)**	**References**
*Bacillus salmalaya*139SI	20.35	3	25	1	120	[Dadrasnia et al., 2015]
Opuntia biomass	18.5	2	20	0.5	10	[Fernandez-Lopez et al., 2014]

Opuntia biomass	16.5	2	20	0.5	10	[Fernandez-Lopez et al., 2014]
Cotton waste	6.75	2	25	3	20	[Boosaeidi et al., 2017]
Barberry waste	7.89	2	25	5	40	[Boosaeidi et al., 2017]
Macadamia nutshell grinded powder	45.23	2	--	0.5	100	[Pakade et al., 2017]
Trewia nudiflora fruit peels powder	294.12	1-2	20	0.75	22-248	[Bhattachary a et al., 2013]
Ceramium virgatum dry biomass	26.5	1.5	20	10	10	[Sari et al., 2008]
Groundnut hull modified biomass	131	2	28	4	8.3	[Owalude et al., 2016]
Chlorella pyrenoidosa	142.86	5	30	0.1	50	[Rezaei et al., 2011]

Chlorella minutissima (immobilized)	57.33	2	30	200	100	[Singh et al., 2012]
Oedogonium hatei biomass	31	2	45	0.8	50	[Gupta et al., 2009]
Holly modified sawdust	18.86	7	--	6	60	[Siboni et al., 2011]
Acacia albida barks	2.98	2	37	20	7.5	[Gebrehawaria et al., 2015]
Pine needles powder	48	2-3	25	10	50	[Hadjmohammadi et al., 2010]
Dictyota dichotoma biomass	9.02	4	27	20	40	[Nandhagopal et al., 2018]
Cupressus lusitanica Bark	305.4	1.5	28	10	100	[Netzahuatl-Munoz et al., 2015]

Table 2.4 Cd (II) biosorption capacity of different biosorbent derived from algal, fungal, lignocellulosic (plants biomass), bacterial biomass.

Biosorbent	**Biosorption capacity (mg/g)**	**pH**	**Temperature (°C)**		**References**

Okara waste	14.80	6.2	70	[Hiew et al., 2021]
Foxtail millet shell	12.48	5	25	[Peng et al., 2018]
Heat-inactivated marine *Aspergillus flavus*	174.25	7	20	[Mahmoud et al., 2016]
Black gram husk	49.97	5	--	[Saeed et al., 2005]
Morus alba L. pomace	21.69	6	40	[Serencam et al., 2013]
Pomelo fruit peel	13.35	5.5	30	[Dinh et al., 2020]
Maize corncob	105.6	6	--	[Garg et al., 2008]
Jatropha oil cake	86.96	6	--	[Garg et al., 2008]
Sugarcane bagasse	69.06	6	--	[Garg et al., 2008]
Wheat straw biochar	69.80	5	25	[Liu and Fan, 2018]
Klebsiella sp. biomass	170.4	5	30	[Hou et al., 2015]

Extracellular Polymeric Substances (EPS) Synthesized by Microbactan	97	7	28	[Camacho-Chab et al., 2018]
Alga *Anabaena sphaerica* biomass	111.1	5.5	25	Abdel-Aty et al., 2013

Table 2.5 Pb (II) biosorption capacity of different biosorbents

Biosorbent	**Biosorption capacity (mg/g)**	**pH**	**Temperature (°C)**	**References**
Heat-inactivated marine *Aspergillus flavus*	207.2	6	20	[Mahmoud et al., 2017]
Black gram husk	39.99	5	--	[Saeed et al., 2005]
Pomelo fruit peel	47.18	5.5	30	[Dinh et al., 2020]
Citrus grandis peels	2.13	3	50	[Yu and He et al., 2018]
Pea (*Pisum sativum*) Peels	140.84	6	30	[Haq et al., 2017]
Gingelly Oil Cake (thermally activated)	105.26	--	--	[Nagashanmugam and Srinivasan, 2010]

Meranti sawdust	34.24	6	30	[Rafatullah et al., 2009]
Solanum melongena leaves	71.42	5	40	[Yuvaraja et al., 2014]
Araucaria heterophylla (green plant) biomass	9.64	5	30	[Sarada et al., 2013]
Azadirachta indica A. Juss seeds	17.96	5.5	--	[Costa et al., 2020]
Alga *Anabaena sphaerica* biomass	121.95	3	25	[Abdel-Aty et al., 2013]

In addition to biomasses, nanoparticles have gained lot of attention in the field of heavy metal removal. Nanoparticles also have applications in electron devices, health care, energy, agriculture and wastewater treatment [Yang et al., 2019; Ahmed et al., 2016]. In general, nanoparticles are small in size within nanoscale (1-100 nm) and small size of nanomaterials provide large surface area as compared to bulk materials [Khan et al., 2019]. Along with small particle size, nanoparticles have some other important properties such as quantum and macro quantum tunnel effect [Khan et al., 2019; Yang et al., 2019]. These specific properties of nanoparticles are responsible for their strange reactivity and adsorption property. These properties of nanomaterials are favourable for heavy metal removal from the contaminated water [Yang et al., 2019; Vidu et al., 2020]. There are several types of nanoparticles synthesized for the removal of heavy metal ions and few examples of these nanomaterial and their heavy metal adsorption capacity is shown in Table 2.6.

Table 2.6 Nanomaterials and their heavy metal adsorption capacity

Nanoparticles/ nanocomposites	**Heavy metal**	**Biosorption capacity (mg/g)**	**pH**	**Temperature (°C)**	**References**
Chitosan functionalised magnetic nanoparticles	Pb (II)	498.6	6	30	[Christopher et al., 2017]
Chitosan schiff's base@Fe_3O_4 (CSB@Fe_3O_4)	Pb (II)	83.33	5	50	[Weijiang et al., 2017]
CuO nanostructures	Pb (II)	115-125	6.5	--	[Farghali et al., 2013]
ZnO montmorillonite nanocomposite	Pb (II)	88.50	4-6	--	[Sani et al., 2017]
Cerium dioxide nanoparticles (CeO2 NPs)	Pb (II)	4.99	6.8	30	[Wang et al., 2016]
Iron oxide-tea waste nanocomposite	Pb (II)	18.83	--	25	[Khanna et al., 2020]

Nanoscale zerovalent iron (nZVI)	Pb (II)	1667	4.5	35	[Zhang et al., 2013]
Carboxymethyl cellulose bridged chlorapatite nanoparticles	Cd (II)	150.2	7	--	[Li et al., 2021]
Alumina nanoparticles	Cd (II)	24.20	8	27	[Koju et al., 2018]
CNSR coated magnetic nanoparticles	Cd (II)	54.6	10	30-50	[Devi et al., 2017]
Oxide-silica composite	Cd (II)	43.45	6	50	[Mahmoudi et al., 2020]
Folic acid-coated graphene oxide nanocomposite (FA-GO)	Cd (II)	103.1	5.5	25	[Eftekhari et al., 2020]
Mesoporous silica nanoparticles (MSNs)	Cr (VI)	42.2	2	25	[Jang et al., 2020]
Amorphous silica nanoparticles (ASNs)	Cr (VI)	34.0	2	25	[Jang et al., 2020]

Cu/Fe bimetallic nanoparticles	Cr (VI)	689.4	3.5	25	[Ye et al., 2021]
Magnetite/Zeolite-X Composite	Cr (VI)	3.69	2	50	[Adegoke et al., 2018]

2.2.2 Bioaccumulation: Removal of heavy metal using living cells

Bioaccumulation is defined as accumulation of toxic metal ions into intracellular space [Diep et al., 2018; Deng et al., 2007]. It is slower and more complex process than biosorption as various metabolic pathways are involved in it [Diep et al., 2018]. Bioaccumulation is a metabolic and energy-dependent process which is performed by living cells [Timkova et al., 2018]. Bioaccumulation minimizes steps of biosorbent preparation such as harvest of biomass, drying of biomass, preparation (washing and crushing) and storage of biomass [Ahluwalia, 2014]. However, bioaccumulation is hyper sensitive to experimental conditions [Ali et al., 2019]. The pollutants present in the medium can aggregate on the surface of organisms which effects the bioaccumulation [Yilmazer and Saracoglu, 2009]. Several fungi, bacteria, algae and plants species have been identified which have showed important role in the removal of heavy metals.

2.2.2.1 Bacterial species: Role in bioremediation of heavy metal ions

Among these living systems, the bacteria have prodigious heavy metal resistance and can bio-accumulate metal ions within their intracellular space through their cell surface receptors [Jacob et al. 2018]. Bacterial genera such as *Pseudomonas* [El-Naggar et al. 2020], *Klebsiella* [Tekerlekopoulou et al. 2013], *Microbacterium* [Humphries et al. 2005] and *Bacillus* [Li et al. 2020b], which are highly heavy metal resistant have been isolated. Additionally, bioremediation mediated by bacteria is considered as an eco-friendly and inexpensive method [Ibrahim et al., 2012]. Bacterial strains found in the heavy metal contaminated sites commonly

harbour specific heavy metal resistance [Liu et al. 2012]. Researchers have isolated *Microbacterium* species from heavy metals contaminated sites. Henson et al., 2015 reported that *Microbacterium* sp. (Cr-K29) is capable of removing Cr (VI) up to 88%. Pattanapipitpaisal et al., 2001 isolated Cr (VI) reducing *Microbacterium liquefaciens* from contaminated site and evaluated its bioremediation capability. The authors observed that *Microbacterium liquefaciens* removed 81% of Cr (VI) from contaminated water. Bacteria use several pathways for the heavy metal removal, like either they utilize heavy metals as an electron acceptor or detoxify it by producing soluble enzymes [Ahemad, 2015]. ROS are generated when microbial cells are exposed with toxic heavy metals. ROS damage the cell organelles or affect several metabolic functions which effects the normal cell functions [Kubrak et al. 2010; Kumar et al. 2013]. Thus, antioxidant activity of bacterial cells actively participate in the detoxification and bioremediation of Cr (VI) [Joutey et al. 2015]. The Cr (VI) removing microbial species are shown in the Table 2.7.

Table 2.7 Bacterial species and their Cr (VI) efficiency.

Microorganism	**Removal efficiency (%)**	**Optimum pH**	**Optimum temperature**	**Initial Cr (VI) concentration (mg/L)**	**References**
Bacillus subtilis	96	9	30	50	[Mangaiyarkarasi *et al.*, 2011]
Cellulosimicrobiumfunkei AR8	100	7	35	100-250	[Karthik et al., 2017]
Pseudomonas aeruginosa strain-1	86	4-7	37	0.028	[Pandian et al., 2014]

Pseudomonas fluorescens	99	7	37	1000	[Devi et al., 2012]
Bacillus cereus IST105	75	-	-	100	[Iyer et al., 2004]
Rhodococcus erythropolis	89.54	5	28	100	[Banerjee et al., 2017]
Staphylococcus capitis	89	7	37	--	[Zahoor and Rehman, 2009]
Bacillus sp. JDM-2-1	86	6	37	--	[Zahoor and Rehman, 2009]
Pseudomonas GT7	40.8	7	30	--	[Zhang et al., 2016]
Pseudomonas putida	95.68	6.5	37	--	[Balamurugan et al., 2014]
Bacillus subtilis (Bacteria)	95.19	7	37	--	[Balamurugan et al., 2014]
*Acinetobacter*sp.	75	7	75	--	[Srivastava and Thakur, 2007]

Table 2.8 and 2.9 shows the Pb (II) and Cd (II) removing efficiency of bacterial strains

Table 2.8 Pb (II) removal efficiency of bacterial strains.

Microorganism	Removal efficiency (%)	Optimum pH	Optimum temperature	Initial Pb(II) concentration (mg/L)	References
Bacillus sp. (phosphate solubilizing bacteria)	95	-	-	300	[Zhang et al., 2019]
Oceanobacillus profundus KBZ 3-2	97	6	30	50	[Mwandira et al., 2020]
Acinetobacter sp. strain THKPS16	71.2	5	35	35	[Ma et al., 2015]
Citrobacter sp. Strain MKH2	95.06	-	30	80	[Mohseni et al., 2014]
Bacillus sp. Strain Q3	93.8	5.8	38.8	115.4	[Heidari and Panico, 2020]
Bacillus sp. Strain Q3	76.4	6.2	34.3	127.4	[Heidari and Panico, 2020]
Bacillus sp. Strain AS2	99.5	4.5	30	500	[Cephidian et al., 2016]
Bacillus spp. PPS 03	85.64	7	35	2.04	[Singh and Chopra, 2014]

Bacillus spp. PPS 04	87.57	7	35	2.04	[Singh and Chopra, 2014]
Cedecea sp strain SC19	60.7	7	37	600	[Wang et al., 2020b]
Arthrobacter sp. GQ-9	56.60	5.5	28	100	[Wang et al., 2020b]

The Cd (II) removal microbial species are shown in the Table 2.9.

Table 2.9 Cd (II) removal efficiency of bacterial strains.

Microorganism	**Removal efficiency (%)**	**Optimum pH**	**Optimum temperature**	**Initial Cd (II) concentration (mg/L)**	**References**
Bacillus sp. Strain Q3	58	5	38.6	50.6	[Heidari and Panico, 2020]
Bacillus sp. Strain Q3	78	5	38.3	50	[Heidari and Panico, 2020]
Cedecea sp strain SC19	51	7	37	120	[Wang et al., 2020b]
Stenotrophomonas maltophilia ZZC-06	81.43	6	30	10	[Chen et al., 2016]

Pseudomonas azotoformans strain JAW1	44.67	6	30	25	[Choinska-Pulit et al., 2018]
Enterobacter sp. strain WS12 (RZ1)	87.75	7	35	0.1	[Abbas et al., 2014]
Enterobacter sp. strain WS12 (RZ2)	85.11	7	35	0.1	[Abbas et al., 2014]
Enterobacter sp. S2	95	--	32	1000	[Mitra et al., 2018]
Pseudomonas sp. M3	70	7	35	550	[Abbas et al., 2014]
Pantoea agglomerans strain UCP1320	100	6	35	10	[Acioly et al., 2018]

2.2.2.3 Microbial reduction of Cr (VI)

It is considered to be an important phenomenon for minimizing the Cr (VI) toxicity. Cr (VI) has a high level of toxicity due to more solubility, permeability and higher oxidation state as compared to Cr (III). In the reduction, Cr (VI) is reduced into less toxic and poorly soluble Cr (III) [Baldiris et al., 2018]. Microbial reduction of Cr (VI) is found to be most useful and practical technique. These methods are inexpensive and environmentally friendly as compared to other physiochemical methods [Raspor et al., 2000; Deng et al., 2010; Wang et al., 2012].

The enzymes and chemicals are secreted from several groups of anaerobic and aerobic bacteria which reduce Cr (VI) [Donati et al., 2003]. Anaerobic Cr (VI) reduction is a very slow process. This is mainly dependent on membrane-bound enzymatic system. The enzymes participating in the aerobic Cr (VI) reduction is mostly found in soluble form in the cytosol [Somasundaram et al., 2009; Puzon et al., 2002].

The Cr (VI) reduction efficiency is influenced by many factors like types of microorganism and the availability of Cr (VI) concentration in the microbial growth medium. The aerobically or anaerobically reduction of Cr (VI) also depends on temperature, media components and pH of medium [Alam and Malik, 2008; Xu et al., 2013]. There are three mechanism of Cr (VI) reduction [Ngwenya and Chirwa, 2011].

(1) Anaerobic reduction of Cr (VI): Under the anaerobic condition, cell protoplasm's components is involved in Cr (VI) reduction. The cell components such as vitamins, heme containing proteins, carbohydrates, amino acids as well as glutathione performs a main role in the Cr (VI) reduction. These cellular components act like an electron donor for Cr (VI) [Ahemad, 2014].

(2) Aerobic reduction of Cr (VI): Reduction of Cr (VI) ions depend on the extracellular soluble reductase enzymes. This reduction process is dependent on the NADPH. Chromate reducing enzymes are secreted from several groups of microbial species such as *Pseudomonas putida* PRS2000 and *Desulfovibrio vulgaris*. These chromate reductase enzymes utilize different electron donors located inside or outside of bacterial cell [Loryuenyong et al., 2014; Belchik et al., 2011]. It is an energy-dependent and highly regulated process. Extracellular chromate reduction does not require intake of chromium ion into the bacterial cell. In this process, bacterial cell protects from chromate mediated DNA damage. This is the main advantage of extracellular chromate reduction [Ahemad, 2014].

(3) Membrane-associated reductase-mediated reduction of Cr (VI) requires H_2 or glucose as electron donor components. In this process, Cr (VI) accepts terminal electrons from electron transport chain [Cheung et al., 2006; Baldiris et al., 2018]. Many researchers have reported glucose as external electron donor in this process [Qian et al., 2016; Rahman and Thomas, 2021]. Glucose enhances the enzymatic activity of chromate reductase about 3.5 folds [Ibrahim et al., 2012].

Researchers have reported few bacterial species and their chromate reductases gene [Baldiris et al., 2018]. The chromate reduction mechanism has been most widely studied in *P. Putida* (ChrR gene) and *E. coli* (YieF gene) [Ackerley et al., 2004]. ChrR and YieF were considered to be flavoproteins of bacterial cytoplasm which reduces Cr (VI) into Cr (III) completely. The Chromate reductase-mediated reduction of Cr (VI) depends on the various factors such as temperature, availability of electron donor group, for example, ChrR of *P. putida* reduces Cr (VI) at 70°C and YeiF of *E. coli* reduces Cr (VI) into Cr (III) at 35°C [Ackerley et al., 2004].

2.2.2.3 Mycoremediation: Fungal mediated removal of heavy metal ions

Fungus can adopt easily in their surrounding environment and are capable to decompose organic/ inorganic materials under natural condition [Archana et al., 2015]. They can be cultivated under highly stressed condition such as extreme pH, temperature and salt concentration [Hamba and Tamiru, 2016]. Macro-fungi such as white rot fungi have ability to uptake massive amount of toxic metal ions in their fruit bodies, this property of fungi (mushroom) make them appropriate for extraction of heavy metal ions from the contaminated sites [Ogbo and Okhuoya, 2011]. The heavy metal removal efficiency of major fungal species have been shown in Table 2.10.

Table 2.10 Fungi and their heavy metal removal capacities

Microorganism	**Heavy metal**	**Initial heavy metal concentration (mg/L)**	**Removal efficiency (%)**	**References**
Paecilomyces sp.	Cr (VI)	50	100	[Juan et al., 2010]
Penicillium sp. N3	Cr (VI)	60	93	[Fukuda et al., 2008]
Aspergillus sp. N2	Cr (VI	60	75	[Fukuda et al., 2008]
Rhizopus sp. CUC23	Cr (VI	80	72.3	[Bibi et al., 2018]
Aspergillus fumigates ML43	Cr (VI	80	72.9	[Bibi et al., 2018]
*Penicillium radicum*PL17	Cr (VI)	80	60.1	[Bibi et al., 2018]
*Fusarium proliferatum*FBL 1	Cr (VI)	80	53.0	[Bibi et al., 2018]
Absidia cylindrospora	Pb (II)	50 mg L^{-1}	59.00	[Albert et al., 2018]
Pleurotus Ostreatus	Pb (II)	45.4 mg L^{-1}	35.60	[Vaseem et al., 2017]
Pseudochlorococcum typicum	Pb (II)	20 mg L^{-1}	86.00	[Shanab et al., 2012]

Porphyra leucosticte	Pb (II)	10 mg L^{-1}	90.00	[Ye et al., 2015]
Drechslera hawaiiensis	Pb (II)	90 mg L^{-1}	99.26	[El-Gendy et al., 2017]
Absidia cylindrospora	Cd (II)	50 mg L^{-1}	68.00	[Albert et al., 2017]
Agaricus macrospores	Cd (II)	10 mg kg^{-1}	13.00	[Garcia et al., 2005]
Pseudochlorococcum typicum	Cd (II)	20 mg L^{-1}	70.00	[Ye et al., 2015]
Porphyra leucosticte	Cd (II)	10 mg L^{-1}	70.00	[Ye et al., 2015]
Drechslera hawaiiensis	Cd (II)	30 mg L^{-1}	99.26	[El-Gendy et al., 2017]

Pleurotus sp. (macro-fungi) is more advantageous in terms of heavy metal removal as compared to other mushroom species. *Pleurotus* sp. is considered superior for the treatment of contaminated water and soil [Zheng et al., 2007; Vaseem et al., 2017]. Additionally, *Pleurotus* sp. has a unique quality of effortless cultivation on various types of solid substrates (biomass) under extreme environmental conditions. They are good source of proteins, vitamins, minerals, nutrients and also have many therapeutic applications like immune-stimulatory, anti-inflammatory, anti-oxidant and anti-cancerous [Synytsya et al., 2009; Barros et al., 2007; Kim et al., 2007; Sarikurkcu et al., 2009].

Apart from this, the cell wall components of mushroom play an important role in the heavy metal removal due to presence of heavy metal binding sites and these active binding

sites are responsible for the accumulation of metal ions in intracellular space [Gadd, 2007]. The waste materials such as agricultural waste and plant residues containing cellulose, hemicelluloses and lignin are fragmented by extracellular enzymes of *P. florida* species like lignin peroxidase, cellulase and laccase [Kumla et al., 2020; Kumar and Chandra, 2020]. These enzymatic degraded materials are taken up by the fungal cell followed by intracellular digestion by enzymatic system. The aforementioned enzymatic system in spite of its structural complexity and heterogeneity have showed that this organization is non-specific in nature, which is also responsible for degradation of variety of toxic compounds including polycyclic aromatics, polychlorinated biphenyls and dioxins [Hestbjerg et al., 2009; Kapahi et al., 2017].

2.2.2.4 Heavy metal detoxification mechanism in living cells: Metal binding protein and antioxidant system

Heavy metal ions enter into the living cells and bound with the various metal binding proteins such as metallothionein and few ions precipitate into the cell [Jaishankar et al., 2014]. Intracellular toxic heavy ions into the cells bind with the sulfhydryl group of enzymes and leads to cellular toxicity [Malik, 2004; Peng et al., 2018]. The bioaccumulated harmful heavy metal ions promote the production of several cellular products such as metallothionein. These metal binding metallothionein proteins are rich in thiol groups and these thiol groups interact with the intracellular toxic metal ions [Permyakov, 2021]. Metallothionein proteins are less active and is not involved in cell metabolic process, thereby binding of toxic heavy metal ions with these proteins are the non-toxic or less toxic [Igiri et al., 2018; Tamas et al., 2014]. These proteins are frequently synthesized when toxic metal ions present in the surrounding environment of microorganism. This is the reason why many adopted microorganisms are more efficient than the non-adopted one. More bioaccumulation is achieved by microbial species isolated from heavy metal contaminated sites or polluted environments. Bioaccumulation is a

very efficient process and it can be enhanced by enhancing the expression of metal binding proteins through genetic engineering techniques [Chojnacka, 2010].

Antioxidants, superoxide peroxidase (SOD), lipid peroxidase, reduced glutathione (GSH) and catalase of play an important role in the detoxification of heavy metal toxicity and intracellular accumulation of metal ions [Zafar et al., 2007]. The antioxidants are responsible for producing ROS and for minimizing metal toxicity. The activity of these enzymes increases linearly with the heavy metal exposure. Therefore, these enzymes are needful in minimizing the heavy metal toxicity and helpful in enhancing the accumulation of heavy metals in the intracellular space [Das et al., 2007]. Toxic metallic components present in the mushroom substrate also interact with the extracellular enzymes and enter into the fungal cell by different ways such as diffusion, through outer cell transporter such as phosphate transporters, sulphate transporter etc. The uptake of metals from liquid environment is the simplest situation. *Pleurotus* species can bio-accumulate heavy metal ions from the substrate in their mycelia [Yetis et al., 1998].

2.2.3 Modelling approaches for heavy metal biosorption

2.2.3.1 Adsorption Kinetic Study

Adsorption is affected by the properties of adsorbent materials and reaction time. Adsorption kinetics play a vital role in the design of the treatment system. Generally, pseudo-first-order and second-order kinetic models are helpful in kinetic studies. Pseudo-first-order indicates towards physical adsorption and the pseudo-second-order kinetic model reveals to the chemisorption of heavy metal ions on the adsorbent's surface.

2.2.3.2 Adsorption Isotherm Study

Isotherms consider the relationship between adsorption capacity and residual concentration of heavy metal ions at a fixed temperature. The Freundlich, Langmuir, Temkin, Halsey, Harkin-

Jura (H-J), D-R, Redlich-Peterson and Jovanovic isotherm models have been widely used in adsorption.

The basis of Langmuir isotherm is monolayer adsorption on homogenous adsorbent by neglecting any surface interaction between two absorbed molecules. Freundlich adsorption isotherm model defines multilayer adsorption on the heterogeneous surface of adsorbent.

Temkin isotherm provides uniformly distributed binding energies over the population of surface binding adsorption. This predicts that the adsorption heat of all the molecules in a layer is decrements linearly with the coverage binding sites by the adsorbate. This model also indicates that adsorption is determined by the uniform distribution of the binding energies, up to threshold binding energy. D-R isotherm indicates that adsorption of heavy metal ions depends on the adsorbent structure. Halsey isotherm defines multilayer adsorption at a relatively larger distance from the adsorbent surface. Harkin-Jura (H-J) isotherm represents the possibility of multilayer adsorption on the adsorbent surface. Jovanovic isotherm assumes mechanical contact between adsorbate and adsorbent. Redlich-Peterson (R-P) isotherm is a combination of Langmuir and Freundlich isotherm that does not follow monolayer adsorption.

2.2.3.3 Thermodynamics Study

The parameters of thermodynamics such as change in enthalpy (ΔH°), Gibbs free energy (ΔG°), and entropy (ΔS °) can be analyzed at various temperatures. A positive value of ΔH° means adsorption is endothermic and is increased by raising the temperature. A negative result or value of ΔG° suggests that adsorption is a spontaneous and is increases with the rise in temperature.

2.2.3.4 Artificial Neural Network (ANN)

When given relevant input data, an ANN is capable of predicting an output pattern [Yildiz, 2018]. The LM algorithm has been frequently used as a training method in engineering fields.

In order to predict the output function, the feed-forward back-propagation network type is applied in conjunction with the L-M algorithm. The network was trained until it had the smallest number of epochs. Thereafter, the experimental data is combined with the network simulation. The experimental findings are compared with predicted output function. On the basis of data training, testing and validation, the mean square error (MSE) of the ANN model for the Cd (II), Cr (VI) and Pb (II) ions in the ternary metal-ion system get depicted.

ANN modelling was used by Ghosh and Sinha 2015 to optimize the reduction of copper by *Stenotrophomonas maltophilia* PD2 biomass and found R^2 of 0.958 from the trained network. Similarly, Talib et al., 2019 was used ANN to investigate the removal of Cr (VI) by *Acinetobacter radioresistens* strain NS-MIE and reported a R^2 of 0.99. Additionally, Ahmad et al. (2014) used ANN to predict the biosorption efficiency of immobilised *Bacillus subtilis* for removing Cd (II) ions and discovered an R^2 of 0.997 between the experimental data and model output. By experimenting with various functions, Khan et al., 2017 obtained R^2 in the range of 0.95 to 0.99 after executing ANN for modelling biosorption of Pb (II) ions.

2.2.3.5 Adsorption Dynamics and diffusivity coefficients

Metal ion adsorption at the solid-liquid interface is governed by bulk diffusion, surface (film diffusion), intra-particle diffusion and rearrangement [Imaga and Abia, 2015]. The rate determining step can be any of the preceding. Ion rearrangement is a reasonably quick process and is not recognized as a rate-control step. Additionally, dimensionless numbers can used to describe the kind of diffusion that occurred during the adsorption. On the basis of values of diffusion coefficient and dimensionless numbers it can be describe that heavy metal adsorption is mix diffusion or transfer controlled. Heavy metal biosorption on adsorbent surface can be controlled by rearrangement, film and bulk diffusion and intra-particle diffusion [Imaga and Abia, 2015].

Thesis Objective

The specific objectives for thesis are as follows.

I. Selection, preparation and characterization of biosorbent

II. Batch biosorption study: (a) Biosorption study at various parameters, (b) Kinetics, (c) Isotherm and (d)Thermodynamics

III. Dimensionless number and Artificial Neural Network (ANN) modeling

IV. Isolation and characterization of heavy metal resistant bacteria from wastewater

V. Heavy metal removal using bacteria isolate

VI. Bioremediation of heavy metal ions by *Pleurotus florida*