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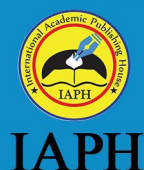
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Low-cost biochar-based heavy metal removal from water and its application towards minimizing bioaccumulation into the fish ecosystem: An optimization-based sustainability study

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Every day, we observe thousands of harmful chemicals and xenobiotics being released in an unregulated way by different human activities that are polluting our waterbodies. A large part of it are heavy metals.

Heavy metal contamination levels have been established to be in high concentrations in South Asian countries like India, Pakistan, Bangladesh, China etc.

This causes heavy metal poisoning, especially in the fish ecosystem. Fishes being a source of diet to humans becomes a cause for damaging human health.

Hence, here we have experimented the use of bamboo leaf biochar a very cost effective and environmental sustainable material abundantly available which shows great potential in removal of heavy metals through adsorption.

We designed an experiment of preparing a biochar optimised for maximum heavy metal adsorption using design of experiments and response surface methods. We then tested this biochar adsorption on high lead concentration to observe its effect on gangetic koi fish saplings health.

This book will provide an insight on the potential of biochar and its effectiveness of removing heavy metal concentration that too being a cheap and environmentally safe alternative.

**Shrubawati Sarkar**



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## ABSTRACT

Bamboo is a common plant native to Asia that has been used in a variety of industries. It also produces a large volume of leaves that go to waste and are not used for any useful purposes; it is often considered a bio-waste and is incinerated or dumped because its applications have not yet been fully explored. Ultimate analysis was performed to determine the Carbon, Oxygen, Hydrogen and Nitrogen content of bamboo leaf. The impact of bamboo leaves on the production of biochar has been investigated and described in this study. Performing proximate analysis Fixed carbon (FC) content, Ash (A) content and volatile matter (VM) content were determined to be  $(15.395 \pm 0.145) \%$ ,  $(2.745 \pm 0.131) \%$  and  $(81.861 \pm 0.258) \%$  respectively. Bulk density was reported to as  $(0.322333 \pm 0.001528) \text{ gm/cm}^3$ . X-Ray diffraction (XRD) results revealed the presence of various phases; Fourier transform infrared spectroscopy (FTIR) results showed various modes of vibrations viz. O-H stretching bending of other bonds; It appears that, the bamboo leaf biochar can have suitable properties for its use as an alternative source of bioremedic application for removing heavy metals from wastewater. A fish model was also developed by making the different concentration of lead contaminated water and using 'Gangetic koi' as a fish specimen. Its high porosity and carbon content suggest its application as activated carbon also; after physical or chemical treatments. The current study aims to push the boundaries of bamboo leaf utilization beyond being an unutilized bio-waste to become a value-added product with long-term applications.

**Keywords:** Toxicity; Heavy metals; Fish; Bioaccumulation; Biochar; Bioremediation; Biomass characterization; FTIR; XRD; Optimization.



# Chapter 1 | INTRODUCTION

---

## 1.1. Background:

Thousands of chemicals and xenobiotics have been emitted into the environment by mankind to suit the needs of the contemporary period, polluting the ecosystem. Pollution is a constant and frightening intrusion into the aquatic environment across the world from both spontaneously existing and manmade causes [1]. The constant discharge of a huge number of organic and inorganic pollutants such as dyes, heavy metals, surfactants, medicines, pesticides, and personal care items into water bodies from industry and municipalities is deteriorating the world's water resources [2]. The majority of these pollutants are extremely persistent and are converted to a recalcitrant state. The unregulated release of these toxins is an issue because of their potential negative effects on ecosystems [3]. Flocculation, adsorption, ultrafiltration, reverse osmosis, ion-exchange, electrochemical treatment, solvent extraction, and flotation are some of the traditional technologies used to remove inorganic contaminants from wastewater across the world. These technologies have a number of drawbacks, including inefficiency in removing pollutants at low concentrations and converting pollutants completely into biodegradable or less deleterious co-products, high energy and chemical consumption, process complexity, and high operational and maintenance costs and so on [4,5]. To be promoted and implemented on a broad scale, an effective and profitable treatment procedure must fulfil both environmental and economic requirements, incorporating low-cost and readily available materials into various treatment processes might reduce overall treatment costs while increasing process efficiency.

Water scarcity affects billions of people throughout the world. The issue is ubiquitous, but it is particularly acute in poorer nations. More than a million are at danger of excessive heavy metals exposure, which occurs mostly through drinking water but also through the airborne metalloid in places where coal is burned and industrial emissions are present. This is mostly owing to such communities' lack of sanitary services and lack of proper water treatment [6]. Heavy metal contamination in aquatic environment has become a significant concern in so many countries including Bangladesh, India, China, Mongolia, Nepal, Cambodia, Myanmar, Afghanistan, Korea and Pakistan [7]. This has also been documented to pollute marine, freshwater, and groundwater ecosystems [8]. Polluted water can cause the extinction of beneficial species, either directly by damaging aquatic organisms or indirectly by disrupting

biological food chains, including fish and their habitats and behavioral patterns [9]. Fish, the primary aquatic vertebrates, are considered bio-indicators of the aquatic medium. They play an important role in water pollution monitoring because the sudden death of fish indicates heavy pollution, and the consequences of exposure to sub chronic levels can be measured in terms of biochemical, physiological, and histological responses of the fishes [10,11]. Fish are the most abundant source of a largely healthy diet. Diet-borne contaminants passed down the food chain, on the other hand, put them in jeopardy.

Heavy metals are frequently employed in a variety of sectors and are commonly found in water. When the concentrations of heavy metals in medium exceed a specific threshold, the medium becomes poisonous to the species that dwell in it. Heavy metals such as lead (Pb), mercury (Hg), cadmium (Cd), chromium (Cr), and arsenic (As) are among the harmful contaminants acquired by fish. Heavy metals have a proclivity for accumulating in fish organs and muscle tissue. Heavy metals accumulate all through the food chain and may damage human health, in addition to their poisonous and destructive effects on aquatic creatures [12]. Because of their persistent nature and cumulative impact, heavy metals have been identified as potent biological toxins among pollutants. Heavy metals have been demonstrated to influence physiological functions and biochemical parameters in tissue and blood in a variety of fish studies [13]. Heavy metals are toxic to aquatic creatures, and they can bioaccumulate in the food chain, causing human ailments. They can be found in the environment as a result of natural processes as well as pollution caused by human activity. Heavy metal bioaccumulation and subsequent organ distribution in fish is very interspecific, according to the literature [14].

Though global biomass production is projected to be between 145 and 150 billion tons per year, only 10 - 15 percent (%) of such a vast biomass store is used for energy generation, and a large fraction of these stocks faces serious storage, management and disposal challenges. There is a need to convert biomass into a form that may be used for a variety of productive reasons, such as energy production and climate change mitigation, in addition to all of these connected features [15]. Biochar is a carbon-rich substance made from biomass through pyrolysis, which is the thermal breakdown of organic stuff with a little quantity of oxygen at temperatures below 700 °C. Although the biochar manufacturing method is like that of charcoal and comparable materials, its use and production sources set it apart from other similar products [16]. Biochar is gaining popularity across the world due to its vast range of environmental applications, including greenhouse gas reduction, resource conservation, power production, waste management, pollutant neutralization, soil fertilizing, and water filtering. Because of its complex and varied physical and chemical makeup, biochar is a rather adaptable substance. The chemical composition of biochar is determined by the pyrolysis circumstances as well as the biomass type. As a result, biochar lacks a well-defined chemical makeup. Carbon

is one of the most important components of biochar, though depending on the biomass type, biochar may also contain inorganic chemicals such as Ca, Mg, K, and inorganic carbonates [17]. Due to certain characteristics, including such large steady-state approximation (SSA), which represents the overall rate of the reaction of a series of complex reactions, nano-material content, porous structure, and abundant surface functional groups, biochar has been evaluated to be efficacious adsorbents for removing a variety of contaminants (SFG). Heavy metals, organic contaminants, nitrogen, and phosphorus have all been shown to be reliably eliminated from wastewater using biochar. Biochar's use in wastewater treatment is growing every year. Biochar may be used for a variety of remediation procedures, resulting in excellent pollutant elimination, thanks to its adaptability and different technical production possibilities [18].

Biochar, an environmentally friendly and low-cost substance made from organic wastes such as agricultural wastes, forestry residues, and municipal wastes, is gaining popularity, as indicated by its expanding usage in a variety of environmental applications. Pyrolysis, hydrothermal carbonization (HTC), gasification, and torrefaction are all methods for converting organic wastes into biochar [19]. Pyrolysis is the most common process for making biochar, but chars from gasification, torrefaction and HTC do not typically match the description of biochar defined in the European Biochar Certificate requirements (EBC). Biochar and its activated extracts have been reported as very efficient materials for removing various toxins, including potential pathogens, inorganics such as heavy metals, and organic contaminants such as dyes, due to their enhanced properties such as rich carbon content, increased surface area, high cation/anion transfer capacity, and stable structure [20]. The ability of biochar to eliminate contaminants from wastewater is directly related to its adsorption capacity, which is determined by physio-chemical properties such as elemental composition, surface area, pore size distribution, surface characteristics, and cation/anion exchange capacity. These physical and chemical properties vary depending on the nature of the feedstock as well as the preparation methods and conditions. Although biochar has a promising future in wastewater treatment, the possible negative effects of biochar use should be considered as well. Biochar may include different heavy metals and other pollutants that might be released during its application in aqueous solutions, depending on the nature of the feedstock and the conversion procedure used to make it [21].

## **1.2. Aim of the thesis:**

Accordingly, after an exhaustive elaborate literature search, the aim of this thesis was framed and mentioned below;

1. Screening and optimization of the process parameters for lignin removal followed by preparation of low-cost bio-adsorbent using waste biomass.
2. Application of the prepared biochar for heavy metal removal from contaminated water.



3. Determination of the efficacy of the prepared biochar towards bioremediation by adopting the fish model.

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## Chapter 2 | REVIEW OF LITERATURE

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A thorough analysis of the literature on the use of waste biomasses as an alternative bioremediation technology was undertaken, and the findings were grouped into the three categories below (most relevant works have been mentioned).

- i. Contamination of heavy metals and their transport in the aquatic ecosystem.
- ii. Bioaccumulation of heavy metals in aquatic habitats, notably fish and its negative impacts on human health.
- iii. Utilization of biomass in the form of biochar for removal of heavy metals from water.

### **2.1. Contamination of heavy metals and their transport in the aquatic ecosystem:**

#### **2.1.1. Heavy Metals**

Soils may become contaminated by the accumulation of heavy metals and metalloids through emissions from the rapidly expanding industrial areas, mine tailings, disposal of high metal wastes, leaded gasoline and paints, land application of fertilizers, animal manures, sewage sludge, pesticides, wastewater irrigation, coal combustion residues, spillage of petrochemicals, and atmospheric deposition [1, 2].

There are 35 metals related to community and occupational exposure among the elements of the periodic table. Heavy metals are represented by 23 of them. Heavy metals are also classified as trace elements due to their existence at trace amounts (ppb to less than 10ppm) in a variety of environmental matrices [3]. The indestructible nature of heavy metals as pollutants, as opposed to organic pollutants, and their tendency to accumulate in the environment, particularly in the bottom sediments of aquatic habitats in association with organic and inorganic matter, are the two most important factors that contribute to their deleterious effects as pollutants. They can be found in a number of chemical compounds as well as in their elemental form. Those that are volatile and attach themselves to small particles can be transferred in great quantities. The metabolic cycles and equilibrium of several heavy metals have been substantially altered as a result of human activity.

Copper, lead, and zinc output grew tenfold between 1850 and 1990 [3]. Metals including cobalt (Co), copper (Cu), chromium (Cr), iron (Fe), magnesium (Mg), manganese (Mn),

molybdenum (Mo), nickel (Ni), selenium (Se), and zinc (Zn) are vital nutrients that are needed for a variety of biochemical and physiological activities, according to research [4]. Other metals with no established biological functions include aluminum (Al), antimony (Sb), arsenic (As), barium (Ba), beryllium (Be), bismuth (Bi), cadmium (Cd), gallium (Ga), germanium (Ge), gold (Au), indium (In), lead (Pb), lithium (Li), mercury (Hg), nickel (Ni), platinum (Pt), silver (Ag), strontium (Sr), tellurium (Te), thallium (Tl), tin (Sn), titanium (Ti), vanadium (V) and uranium (U) [5].

Heavy metals have a wide range of chemical characteristics and are frequently employed in electronics, machinery, and daily artifacts, as well as in high-tech industries. As a result, they can infiltrate the marine systems and food chains of people and animals through a range of anthropogenic as well as natural causes.

#### **2.1.1.1. Lead**

Lead is a metal belonging to group IV and period 6 of the periodic table with atomic number 82, atomic mass 207.2, density 11.4 g cm<sup>-3</sup>, melting point 327.4°C, and boiling point 1725°C. It is a naturally occurring, bluish-gray metal usually found as a mineral combined with other elements, such as sulphur (i.e., PbS, PbSO<sub>4</sub>), or oxygen (PbCO<sub>3</sub>), and ranges from 10 to 30 mg kg<sup>-1</sup> in the earth's crust [6]. Typical mean Pb concentration for surface soils worldwide averages 32 mg kg<sup>-1</sup> and ranges from 10 to 67 mg kg<sup>-1</sup> [7]. Lead ranks fifth behind Fe, Cu, Al, and Zn in industrial production of metals. About half of the Pb used in the U.S. goes for the manufacture of Pb storage batteries. Other uses include solders, bearings, cable covers, ammunition, plumbing, pigments, and caulking. Metals commonly alloyed with Pb are antimony (in storage batteries), calcium (Ca) and tin (Sn) (in maintenance-free storage batteries), silver (Ag) (for solder and anodes), strontium (Sr) and Sn (as anodes in electrowinning processes), tellurium (Te) (pipe and sheet in chemical installations and nuclear shielding), Sn (solders), and antimony (Sb), and Sn (sleeve bearings, printing, and high-detail castings) [8].

Ionic lead, Pb (II), lead oxides and hydroxides, and lead-metal oxyanion complexes are the general forms of Pb that are released into the soil, groundwater, and surface waters. The most stable forms of lead are Pb (II) and lead-hydroxy complexes. Lead (II) is the most common and reactive form of Pb, forming mononuclear and polynuclear oxides and hydroxides [9]. The predominant insoluble Pb compounds are lead phosphates, lead carbonates (form when the pH is above 6), and lead hydroxides [10]. Lead sulfide (PbS) is the most stable solid form within the soil matrix and forms under reducing conditions, when increased concentrations of sulfide are present. Under anaerobic conditions a volatile organolead (tetramethyl lead) can be formed due to microbial alkylation [9].

### 2.1.1.2. Chromium

Chromium is a first-row d-block transition metal of group VIB in the periodic table with the following properties: atomic number 24, atomic mass 52, density 7.19 g cm<sup>-3</sup>, melting point 1875°C, and boiling point 2665°C. It is one of the less common elements and does not occur naturally in elemental form, but only in compounds. Chromium is mined as a primary ore product in the form of the mineral chromite, FeCr<sub>2</sub>O<sub>4</sub>. Major sources of Cr-contamination include releases from electroplating processes and the disposal of Cr containing wastes [11]. Chromium (VI) is the form of Cr commonly found at contaminated sites. Chromium can also occur in the +III oxidation state, depending on pH and redox conditions. Chromium (VI) is the dominant form of Cr in shallow aquifers where aerobic conditions exist. Chromium (VI) can be reduced to Cr (III) by soil organic matter, S<sup>2-</sup> and Fe<sup>2+</sup> ions under anaerobic conditions often encountered in deeper groundwater. Major Cr (VI) species include chromate (CrO<sub>4</sub><sup>2-</sup>) and dichromate (Cr<sub>2</sub>O<sub>7</sub><sup>2-</sup>) which precipitate readily in the presence of metal cations (especially Ba<sup>2+</sup>, Pb<sup>2+</sup>, and Ag<sup>+</sup>). Chromate and dichromate also adsorb on soil surfaces, especially iron and aluminum oxides. Chromium (III) is the dominant form of Cr at low pH [12].

### 2.1.1.3. Arsenic

Arsenic is a metalloid in group VA and period 4 of the periodic table that occurs in a wide variety of minerals, mainly as As<sub>2</sub>O<sub>3</sub>, and can be recovered from processing of ores containing mostly Cu, Pb, Zn, Ag and Au. It is also present in ashes from coal combustion. Arsenic has the following properties: atomic number 33, atomic mass 75, density 5.72 g cm<sup>-3</sup>, melting point 817°C, and boiling point 613°C, and exhibits complex chemistry and can be present in several oxidation states (-III, 0, III, V) [11]. In aerobic environments, As (V) is dominant, usually in the form of arsenate (AsO<sub>4</sub><sup>3-</sup>) in various protonation states: H<sub>3</sub>AsO<sub>4</sub>, H<sub>2</sub>AsO<sub>4</sub><sup>-</sup>, HAsO<sub>4</sub><sup>2-</sup>, and AsO<sub>4</sub><sup>3-</sup>. Arsenate and other anionic forms of arsenic behave as chelates and can precipitate when metal cations are present [13]. Metal arsenate complexes are stable only under certain conditions. Arsenic (V) can also coprecipitate with or adsorb onto iron oxyhydroxides under acidic and moderately reducing conditions. Coprecipitates are immobile under these conditions, but arsenic mobility increases as pH increases [14]. Under reducing conditions As (III) dominates, existing as arsenite (AsO<sub>3</sub><sup>3-</sup>), and its protonated forms H<sub>3</sub>AsO<sub>3</sub>, H<sub>2</sub>AsO<sub>3</sub><sup>-</sup>, and HAsO<sub>3</sub><sup>2-</sup>. Arsenite can adsorb or coprecipitate with metal sulfides and has a high affinity for other sulfur compounds. Elemental arsenic and arsine, AsH<sub>3</sub>, may be present under extreme reducing conditions. Biotransformation (via methylation) of arsenic creates methylated derivatives of arsine, such as dimethyl arsine HAs(CH<sub>3</sub>)<sub>2</sub> and trimethyl arsine As(CH<sub>3</sub>)<sub>3</sub> which are highly volatile. Since arsenic is often present in anionic form, it does not form complexes with simple anions such as Cl<sup>-</sup> and SO<sub>4</sub><sup>2-</sup>. Arsenic speciation also includes organometallic forms such as methyl arsenic acid (CH<sub>3</sub>)AsO<sub>2</sub>H<sub>2</sub> and dimethylarsinic acid (CH<sub>3</sub>)<sub>2</sub>AsO<sub>2</sub>H. Many As compounds adsorb strongly to soils and are therefore

transported only over short distances in groundwater and surface water. Arsenic is associated with skin damage, increased risk of cancer, and problems with circulatory system [15].

#### **2.1.1.4. Zinc**

Zinc is a transition metal with the following characteristics: period 4, group IIB, atomic number 30, atomic mass 65.4, density 7.14 g cm<sup>-3</sup>, melting point 419.5°C, and boiling point 906°C. Zinc occurs naturally in soil (about 70 mg kg<sup>-1</sup> in crustal rocks) [16], but Zn concentrations are rising unnaturally, due to anthropogenic additions. Most Zn is added during industrial activities, such as mining, coal, and waste combustion and steel processing. Many foodstuffs contain certain concentrations of Zn. Drinking water also contains certain amounts of Zn, which may be higher when it is stored in metal tanks. Industrial sources or toxic waste sites may cause the concentrations of Zn in drinking water to reach levels that can cause health problems. Zinc is a trace element that is essential for human health. Zinc shortages can cause birth defects. The world's Zn production is still on the rise which means that more and more Zn ends up in the environment. Water is polluted with Zn, due to the presence of large quantities present in the wastewater of industrial plants. A consequence is that Zn polluted sludge is continually being deposited by rivers on their banks. Zinc may also increase the acidity of waters. Some fish can accumulate Zn in their bodies when they live in Zn-contaminated waterways. When Zn enters the bodies of these fish, it can bio magnify up the food chain. Water-soluble zinc that is in soils can contaminate groundwater. Plants often have a Zn uptake that their systems cannot handle, due to the accumulation of Zn in soils. Finally, Zn can interrupt the activity in soils, as it negatively influences the activity of microorganisms and earthworms, thus retarding the breakdown of organic matter [17].

#### **2.1.1.5. Cadmium**

Cadmium is located at the end of the second row of transition elements with atomic number 48, atomic weight 112.4, density 8.65 g cm<sup>-3</sup>, melting point 320.9°C, and boiling point 765°C. Together with Hg and Pb, Cd is one of the big three heavy metal poisons and is not known for any essential biological function. In its compounds, Cd occurs as the divalent Cd (II) ion. Cadmium is directly below Zn in the periodic table and has a chemical similarity to that of Zn, an essential micronutrient for plants and animals. This may account in part for Cd's toxicity; because Zn being an essential trace element, its substitution by Cd may cause the malfunctioning of metabolic processes [18]. The most significant use of Cd is in Ni/Cd batteries, as rechargeable or secondary power sources exhibiting high output, long life, low maintenance, and high tolerance to physical and electrical stress. Cadmium coatings provide good corrosion resistance coating to vessels and other vehicles, particularly in high-stress environments such as marine and aerospace. Other uses of cadmium are as pigments, stabilizers for polyvinyl chloride (PVC), in alloys and electronic compounds. Cadmium is also present as

an impurity in several products, including phosphate fertilizers, detergents, and refined petroleum products. In addition, acid rain and the resulting acidification of soils and surface waters have increased the geochemical mobility of Cd, and as a result its surface-water concentrations tend to increase as lake water pH decreases [19]. Cadmium is produced as an inevitable byproduct of Zn and occasionally lead refining. The application of agricultural inputs such as fertilizers, pesticides, and biosolids (sewage sludge), the disposal of industrial wastes or the deposition of atmospheric contaminants increases the total concentration of Cd in soils, and the bioavailability of this Cd determines whether plant Cd uptake occurs to a significant degree [20].

#### **2.1.1.6. Copper**

Copper is a transition metal which belongs to period 4 and group IB of the periodic table with atomic number 29, atomic weight 63.5, density 8.96 g cm<sup>-3</sup>, melting point 1083°C and boiling point 2595°C. The metal's average density and concentrations in crustal rocks are 8.1 × 10<sup>3</sup> kg m<sup>-3</sup> and 55 mg kg<sup>-1</sup>, respectively [16]. Copper is the third most used metal in the world [21]. Copper is an essential micronutrient required in the growth of both plants and animals. In humans, it helps in the production of blood hemoglobin. In plants, Cu is especially important in seed production, disease resistance, and regulation of water. Copper is indeed essential, but in high doses it can cause anaemia, liver and kidney damage, and stomach and intestinal irritation. Copper normally occurs in drinking water from Cu pipes, as well as from additives designed to control algal growth. While Cu's interaction with the environment is complex, research shows that most Cu introduced into the environment is, or rapidly becomes, stable and results in a form which does not pose a risk to the environment [22]. In fact, unlike some man-made materials, Cu is not magnified in the body or bioaccumulated in the food chain. In the soil, Cu strongly complexes to the organic implying that only a small fraction of copper will be found in solution as ionic copper, Cu (II). The solubility of Cu is drastically increased at pH 5.5, which is rather close to the ideal farmland pH of 6.0–6.5 [23]. Copper and Zn are two important essential elements for plants, microorganisms, animals, and humans. The connection between soil and water contamination and metal uptake by plants is determined by many chemical and physical soil factors as well as the physiological properties of the crops. Soils contaminated with trace metals may pose both direct and indirect threats: direct, through negative effects of metals on crop growth and yield, and indirect, by entering the human food chain with a potentially negative impact on human health. Even a reduction of crop yield by a few percent could lead to a significant long-term loss in production and income. Some food importers are now specifying acceptable maximum contents of metals in food, which might limit the possibility for the farmers to export their contaminated crops [24].

### 2.1.1.7. Mercury

Mercury belongs to the same group of the periodic table with Zn and Cd. It is the only liquid metal at stp. It has atomic number 80, atomic weight 200.6, density 13.6 g cm<sup>-3</sup>, melting point -13.6°C, and boiling point 357°C and is usually recovered as a byproduct of ore processing [11]. Release of Hg from coal combustion is a major source of Hg contamination. Releases from manometers at pressure-measuring stations along gas/oil pipelines also contribute to Hg contamination. After release to the environment, Hg usually exists in mercuric (Hg<sup>2+</sup>), mercurous (Hg<sup>2+</sup>), elemental (Hg<sup>0</sup>), or alkylated form (methyl/ethyl mercury). The redox potential and pH of the system determine the stable forms of Hg that will be present. Mercurous and mercuric mercury are more stable under oxidizing conditions. When mildly reducing conditions exist, organic or inorganic Hg may be reduced to elemental Hg, which may then be converted to alkylated forms by biotic or abiotic processes. Mercury is most toxic in its alkylated forms which are soluble in water and volatile in air [25]. Mercury (II) forms strong complexes with a variety of both inorganic and organic ligands, making it very soluble in oxidized aquatic systems [13]. Sorption to soils, sediments, and humic materials is an important mechanism for the removal of Hg from solution. Sorption is pH dependent and increases as pH increases. Mercury may also be removed from solution by coprecipitation with sulphides. Under anaerobic conditions, both organic and inorganic forms of Hg may be converted to alkylated forms by microbial activity, such as by sulfur reducing bacteria. Elemental mercury may also be formed under anaerobic conditions by demethylation of methyl mercury, or by reduction of Hg (II). Acidic conditions (pH < 4) also favor the formation of methyl mercury, whereas higher pH values favor precipitation of HgS(s). Mercury is associated with kidney damage [26].

### 2.1.1.8. Nickel

Nickel is a transition element with the atomic number 28 and atomic weight 58.69. In low pH regions, the metal exists in the form of the nickelous ion, Ni (II). In neutral to slightly alkaline solutions, it precipitates as nickelous hydroxide, Ni (OH)<sub>2</sub>, which is a stable compound. This precipitate readily dissolves in acid solutions forming Ni (III) and in very alkaline conditions; it forms nickelite ion, HNiO<sub>2</sub>, that is soluble in water. In very oxidizing and alkaline conditions, nickel exists in form of the stable nickelo-nickelic oxide, Ni<sub>3</sub>O<sub>4</sub>, that is soluble in acid solutions. Other nickel oxides such as nickelic oxide, Ni<sub>2</sub>O<sub>3</sub>, and nickel peroxide, NiO<sub>2</sub>, are unstable in alkaline solutions and decompose by giving off oxygen. In acidic regions, however, these solids dissolve producing Ni<sup>2+</sup> [27]. Nickel is an element that occurs in the environment only at very low levels and is essential in small doses, but it can be dangerous when the maximum tolerable amounts are exceeded. This can cause various kinds of cancer on different sites within the bodies of animals, mainly of those that live near refineries. The most common application of Ni is an ingredient of steel and other metal products. The



major sources of nickel contamination in the soil are metal plating industries, combustion of fossil fuels, and nickel mining and electroplating [28].

### **2.1.2. Sources of heavy metals**

The main sources of heavy metal pollution are mining, milling, electroplating, and surface finishing. Industries that release various toxic metals into the environment, such as Cr, Cu, Cd, Ni, Co, Zn, and Pb. Over the past few decades, the concentration of heavy metals in river water and sediments has increased rapidly. This is likely due to human activity, as these metals can be released into the environment through manufacturing, transportation, and other activities. The increase in the concentration of toxic metals in grains and vegetables grown in contaminated soils is alarming [29].

#### ***Metal Pollution from Mining and Processing Ores***

Environmental harm can occur during the excavation of a mine, the removal of ore from it, and the extraction and processing of minerals. Mining activities have the potential to devastate wildlife, crops, and residences, as well as cause soil erosion and contaminate streams through toxic drainage. Toxic elements such as arsenic (As), lead (Pb), cadmium (Cd), and Sulphur oxides, among others, are emitted by smelters, causing substantial air pollution. Surface mining generates eight times the amount of trash as underground mining, but deep mining can result in far more serious issues, such as earthquakes [30]. Not only can subterranean mines kill workers, but they also induce surface subsidence, creating holes into which highways and buildings may fall. Miners must dig deeper to find minerals that are depleted at the surface [31].

When pyrite (FeS) and other sulphide minerals are exposed to air oxygen and moisture, they oxidized, resulting in acid-mine drainage water. The discharge of acid-mine drainage from operating and abandoned mines, especially coal mines, has long been linked to major water quality issues. It dissolves harmful components in tailings and soils and transports them to streams and even underground. Elements such as iron (Fe), manganese (Mn), zinc (Zn), copper (Cu), nickel (Ni), and cobalt (Co) have comparatively high amounts in water [32].

#### ***Domestic Wastewater Effluents***

Large amounts of toxic elements are present in domestic wastewater effluents due to metabolic waste products, corrosion of water pipes and many household products including detergents. Wastewater treatment typically eliminates less than half of the metal content of the influent, leaving a large metal burden in the effluent. Metals abound in the sludge produced by wastewater treatment. The principal manmade sources of cadmium (Cd), chromium (Cr), copper (Cu), iron (Fe), lead (Pb), and mercury (Hg) contamination are residential wastewater and the dumping of domestic and industrial wastes [33].

### ***Flow of Stormwater***

Metal contamination in receiving water streams is a substantial source of stormwater runoff from developed areas. Many variables influence the metal concentration of urban runoff water, including city planning, traffic, road building, land use, and the physical features and climatology of the watershed [34].

### ***Agricultural Runoff***

Animal and plant residues, fertilizers, specialized herbicides and fungicides, and the use of sewage and sludge as plant nutrients all contribute to the metal concentration of agricultural runoff in sediments and soils [35,36].

### ***Industrial Wastes and Discharges***

Heavy metal concentrations in industrial effluents are generally significantly higher than their allowed limits in aqueous solutions, necessitating immediate treatment of metal-containing effluents prior to release into aquatic bodies. Metals and their concentrations in commercial effluent discharged are highly dependent on the sector profile [37]. Several sources of industrial effluents are on their way to the aquatic environment, where they will suffocate heavy metals. Metals and steel, pulp & paper, oil refineries, jute industries, rubber and leather industries, paints, varnishes, agro-chemicals and fertilizers industries, medicines, tanneries, dyers, cosmetics, and desiccants all employ arsenic or arsenical-based refining products [38].

Sources of all the above discussed heavy metals are summarized in *Table 2.1*.

**Table 2.1. Summarized list of some toxic heavy metals along with their source.**

<b>Sl. No.</b>	<b>Heavy Metals</b>	<b>Sources</b>
1.	Lead (Pb)	<ul style="list-style-type: none"><li>• Automobile emissions,</li><li>• Mining and plumbing,</li><li>• Coal combustion,</li><li>• Lead smelters &amp; lead arsenate pesticides.</li></ul>
2.	Cadmium (Cd)	<ul style="list-style-type: none"><li>• Fertilizer industry &amp; pesticide manufacturers,</li><li>• Cadmium–nickel batteries,</li><li>• Nuclear fission plants.</li></ul>
3.	Arsenic (As)	<ul style="list-style-type: none"><li>• Atmospheric deposition,</li><li>• Byproducts of mining activities,</li><li>• Chemical wastes,</li><li>• Metal smelters.</li></ul>
4.	Chromium (Cr)	<ul style="list-style-type: none"><li>• Metallurgical &amp; chemical industries,</li><li>• Cement and asbestos units,</li><li>• Steel fabrication,</li><li>• Electroplating.</li></ul>

5.	Iron (Fe)	<ul style="list-style-type: none"> <li>• Cast Iron, Wrought Iron, steel, alloys,</li> <li>• High intake of iron supplements &amp; oral consumption,</li> <li>• Machine manufacturing.</li> </ul>
6.	Mercury (Hg)	<ul style="list-style-type: none"> <li>• Organic mercurial's used in pesticides,</li> <li>• Mining and refining of mercury,</li> <li>• Paint &amp; paper industry,</li> <li>• Volcanic eruption.</li> </ul>
7.	Cobalt (Co)	<ul style="list-style-type: none"> <li>• Sedimentary and igneous rocks,</li> <li>• Processing of cobalt-bearing ores,</li> <li>• Fertilizer.</li> </ul>
8.	Zinc (Zn)	<ul style="list-style-type: none"> <li>• Oil Refining,</li> <li>• Galvanizing processes,</li> <li>• Zinc refineries,</li> <li>• Brass manufacture,</li> <li>• Metal plating.</li> </ul>

### Fertilizers

Historically, agriculture was the first major human influence on the soil [39]. To grow and complete the lifecycle, plants must acquire not only macronutrients (N, P, K, S, Ca, and Mg), but also essential micronutrients. Some soils are deficient in the heavy metals (such as Co, Cu, Fe, Mn, Mo, Ni, and Zn) that are essential for healthy plant growth [40], and crops may be supplied with these as an addition to the soil or as a foliar spray. Cereal crops grown on Cu-deficient soils are occasionally treated with Cu as an addition to the soil, and Mn may similarly be supplied to cereal and root crops. Large quantities of fertilizers are regularly added to soils in intensive farming systems to provide adequate N, P, and K for crop growth. The compounds used to supply these elements contain trace amounts of heavy metals (e.g., Cd and Pb) as impurities, which, after continued fertilizer, application may significantly increase their content in the soil [41]. Metals, such as Cd and Pb, have no known physiological activity. Application of certain phosphatic fertilizers inadvertently adds Cd and other potentially toxic elements to the soil, including F, Hg, and Pb [42].

### Pesticides

Several common pesticides used extensively in agriculture and horticulture in the past contained substantial concentrations of metals. For instance, in the recent past, about 10% of the chemicals have approved for use as insecticides and fungicides in UK were based on compounds which contain Cu, Hg, Mn, Pb, or Zn. Examples of such pesticides are copper

containing fungicidal sprays such as Bordeaux mixture (copper sulphate) and copper oxychloride [41]. Lead arsenate was used in fruit orchards for many years to control some parasitic insects. Arsenic-containing compounds were also used extensively to control cattle ticks and to control pests in banana in New Zealand and Australia, timbers have been preserved with formulations of Cu, Cr, and As (CCA), and there are now many derelict sites where soil concentrations of these elements greatly exceed background concentrations. Such contamination has the potential to cause problems, particularly if sites are redeveloped for other agricultural or nonagricultural purposes. Compared with fertilizers, the use of such materials has been more localized, being restricted to sites or crops [43].

### **Biosolids and manures**

The application of numerous biosolids (e.g., livestock manures, composts, and municipal sewage sludge) to land inadvertently leads to the accumulation of heavy metals such as As, Cd, Cr, Cu, Pb, Hg, Ni, Se, Mo, Zn, Tl, Sb, and so forth, in the soil [44]. Certain animal wastes such as poultry, cattle, and pig manures produced in agriculture are commonly applied to crops and pastures either as solids or slurries [45]. Although most manures are seen as valuable fertilizers, in the pig and poultry industry, the Cu and Zn added to diets as growth promoters and As contained in poultry health products may also have the potential to cause metal contamination of the soil [46].

Biosolids (sewage sludge) are primarily organic solid products, produced by wastewater treatment processes that can be beneficially recycled [47]. Land application of biosolids materials is a common practice in many countries that allow the reuse of biosolids produced by urban populations [20]. The term sewage sludge is used in many references because of its wide recognition and its regulatory definition. However, the term biosolids is becoming more common as a replacement for sewage sludge because it is thought to reflect more accurately the beneficial characteristics inherent to sewage sludge [48]. It is estimated that in the United States, more than half of approximately 5.6 million dry tonnes of sewage sludge used or disposed of annually is land applied, and agricultural utilization of biosolids occurs in every region of the country. In the European community, over 30% of the sewage sludge is used as fertilizer in agriculture [49]. In Australia over 175 000 tonnes of dry biosolids are produced each year by the major metropolitan authorities, and currently most biosolids applied to agricultural land are used in arable cropping situations where they can be incorporated into the soil [43].

#### **2.1.3. Regulatory limit of some selected heavy metals:**

Heavy metals are found in extremely minute levels in living organisms, but greater concentrations pose a health risk. *Table 2.2.* shows the permitted levels of lead, cadmium, arsenic, and mercury in various environmental matrices recommended by several worldwide

reputable authorities such as the US EPA (Environmental Protection Agency), WHO (World Health Organization), World Bank and India CPCB [15].

**Table 2.2. Regulatory limits of some heavy metals in wastewater recommender by some reputed authorities**

Heavy Metals	WHO limit in wastewater (effluent) [16,17]	US EPA limit in wastewater (effluent) [18]	India CPCB limit in public sewers [19]	World Bank limit in wastewater (effluent) [20]
Hg	0.001	0.00003	0.01	0.01
Cd	0.003	0.01	1.0	0.1
Pb	0.01	0.006	1.0	0.1
Cr	0.05	0.05	2.0	0.5
Ni	0.02	0.2	3.0	0.2

**2.1.4. Transport of heavy metals in aquatic environment:**

After thinking about heavy metals, they express their destiny and actions in many compartments. As previously stated, heavy metals are obtained from geological weathering and are contaminated by industrial processing, fossil fuel combustion, and waste disposal leaching, which are the primary causes of heavy metal contamination in the environment compartments. Heavy metals often have a glossy look, are strong electrical conductors, and enter chemical processes as positive ions [21]. They have a propensity to adsorb onto particulate particles or bond with organic groups, creating lipophilic molecules and ions. As a result, their distributions are discovered in animals, plants, and sediments. The fates and behaviors of heavy metals entering compartments have been shifting and moving between environments. Because of their physicochemical features, the majority of dissolved heavy metals carried by natural water systems are swiftly adsorbed onto particulate matter. Heavy metals trapped in bottom sediments, on the other hand, may not always stay that way, and can be released into water or live organisms as a result of chemical changes. Sinks and remobilization processes, as well as addressing as entering compartments, regulate their destinies and behaviors [22]. Heavy metal enrichment in aquatic solid substances as sinks is achieved through a variety of techniques like adsorption and co-precipitation, precipitation and incorporation in biological activity.

Chemically, heavy metals (Me+) react as positive ions. The sorptive properties of negatively charged particles, such as silicon hydroxides (SiOH-) and aluminum hydroxides (AlOH-) groups in clay minerals, ferrous hydroxides (FeOH-) and manganese hydroxides (MnOH-) groups in hydrous iron (Fe) and manganese (Mn) oxides, and carboxyl and phenolic OH- groups in organic substances, are used to explain their adsorption. Negative charge balancing is a selective mechanism that compensates for the preferential adsorption of

particular positive charges and the release of corresponding charges from other species [23]. Furthermore, due to intermolecular interactions, any fine-grained materials with a significant surface area per volume are capable of collecting heavy metal ions at the solid-liquid interface.

Every metal has its own solubility product equilibrium constant which is used to characterize a saturated solution – related ionic compounds (soluble salts) with relatively low solubility – in which a dynamic equilibrium occurs between the solid material and its aqueous ions. If a chemical's solubility product is surpassed, the compound will precipitate until the product of the ionic concentrations is not more than the solubility product's equilibrium constant. As a result of exceeding the solubility product, which is calculated from the multiplied result of ion products compared to its equilibrium constant of solubility product, precipitation will occur [24].

Metals are non-biodegradable, which means they can't be broken down into less hazardous components. Organisms detoxify by storing active metal ions in insoluble form in intracellular granules for long-term storage or expulsion in the faeces, such as metallothionein (binding covalently to Sulphur). Calcium, phosphorus, potassium, salt, chlorine, Sulphur, and other essential metals are required for ionic equilibrium and as constituents of amino acids and nucleic acids, among other things [21]. Non-essential metals like mercury, lead, and cadmium can harm organisms by causing critical metal deficits by competing for active sites in physiologically vital compounds. Importantly, zinc is a vital component of at least 150 enzymes, and iron is a component of hemoglobin, the oxygen-carrying pigment in red blood cells, as well as unimportantly, mercury and lead are bioaccumulated in living organisms.

## **2.2. Bioaccumulation of heavy metals in aquatic habitats, notably fish and its negative impacts on human health.**

Heavy metals have a proclivity for accumulating in aquatic creatures, particularly fish, which may then enter human metabolism via eating, posing major health risks. Bioaccumulation is defined as a rise in a chemical's concentration in a biological organism over time in comparison to the chemical's concentration in the environment. When an organism absorbs a harmful material at a faster pace than it is lost, this is known as overabsorption. When an organism absorbs a harmful material at a faster pace than it is lost, this is known as overabsorption. Numerous xenobiotics enter a food chain through this technique, affecting many living creatures at various trophic levels [25].

### **2.2.1. Different heavy metal uptake and accumulation processes in fish:**

Heavy metal poisoning of water has clearly been a major source of worry among environmental experts in recent years. Industrialization, exploitation, and the fast growth of urban populations have all had a significantly negative influence on the hydrobiological quality

of lakes, ponds, and rivers across the world. In Indian rivers, heavy metals including cadmium (Cd), copper (Cu), chromium (Cr), and lead (Pb) are abundant. Detectable arsenic (As) and mercury (Hg) pollution has not been identified in any of India's rivers. The numerous ambient forms of heavy metals (As, Hg, Cd, Cu, Cr, and Pb), as well as the way of absorption, accumulation, and dynamics in the fish body, have been the focus of this review.

When arsenical chemicals enter the body through food, they build up in the digestive tract. It travels from the GI tract to the liver, where the majority of the biotransformation occurs. Then it accumulates in other organs or tissues of the organism, such as the brain, gonads, and muscles, either directly or via gill circulation. During depuration trials, a little quantity of metal is also eliminated by faeces. Arsenical chemicals dissolved in water can also enter through the gills and be deposited directly into the brain, kidney, gonads, and other tissue via speciation [26]. Dietary intake accumulates in the digestive system until the end of exposure, while the concentration diminishes progressively throughout depuration.

Mercury may be absorbed by fish through their gills, skin, or digestive tract. Metallic mercury (Hg<sup>0</sup>) or mercury vapor (Hg<sup>0</sup>) can be oxidized into water soluble inorganic mercury (Hg<sup>2+</sup>), which is mostly consumed by fish, or reduced back to metallic mercury (Hg<sup>0</sup>). Microorganisms may commonly convert metallic mercury into organic mercury (methyl mercury or phenyl mercury) [27]. Mercury toxicity is determined by the status of mercury, environmental media, circumstances, the age and life history of the specimen, and the organism's sensitivity. Because organic mercury may be biomagnified through the food chain, it is the most harmful to aquatic creatures.

The divalent lead ion can compete with divalent calcium ion for gill entrance. After passing through the metal, it passes through the basal membrane and into the bloodstream, where it quickly accumulates in the liver [28]. Although just a little quantity of lead is defecated out, dietary intake of the metal causes buildup in the liver. The metal then accumulates in the kidney, causing significant damage to the organ. Because the metal may pass across the blood-brain barrier, it causes significant neurological damage. Accumulation of this metal into bones and scales has also been documented by several studies [29].

Copper is a necessary element for living creatures since it is involved in many biological processes such as oxidative phosphorylation, gene control, and works as a cofactor for enzymes. However, when the metal reaches its tolerance threshold in the surrounding aquatic medium, it becomes poisonous. The major routes of entrance are through the gills and food absorption, with just a small quantity of the metal being absorbed through the skin. Copper binds to plasma proteins and is transported to the fish's many organs after entrance. Copper becomes poisonous when an excessive amount of copper enters the cell and binds to cellular proteins and nucleic acids, causing natural metabolic pathways and gene expression to be disrupted [30]. Copper accumulates in the gills at increasing concentrations with prolonged high-concentration exposure, where it may be harmful.

### 2.2.2. Heavy metal deposition in fish causing major histological changes in the gill, liver, and kidney:

Heavy metal accumulation causes toxicity at the cellular, tissue, and organ levels. Chronic exposure to various heavy metals has a variety of negative effects on fish organs. The degree of metal infestation is determined by a histopathological analysis, albeit the effects are dose/concentration and duration of exposure dependent, organ sensitive, and organism specific [31]. Histopathology of sensitive organs has been highly suggested as a biomarker of stress owing to metal exposure in ecotoxicological research. Because the gill, liver, and kidney are the organs most vulnerable to metal pollution, histopathology examinations of these organs have become an inescapable technique for assessing environmental metal stress in *Table 2.3*. Several studies in piscian models have revealed several forms of heavy metal-induced tissue deterioration. Long-term exposure to heavy metals, even in trace levels, causes cellular disease marker enzyme leakage in several fish tissues [32].

**Table 2.3. Summarized table of some toxic heavy metal deposition in various organs of fish causing histopathological changes.**

Heavy Metals	Chronic metal exposure to sub-lethal amounts causes major histopathological changes in several organs.			Source
	Gill	Liver	Kidney	
Lead (Pb)	Hyperplasia, hypertrophy and destruction or disintegration of lamellar architecture, lamellar clubbing and fusion of lamellae.	Disarrangements of hepatic cords, shrinkage of hepatocytes, dilation of sinusoids, exudation of blood, loss of cell adherence of hepatocytes.	Degeneration of renal epithelium, vacuolization, nuclear pyknosis. Renal tube atrophy, oedema, necrosis	[33,34]
Copper (Cu)	Lifting of Lamellar epithelium, RBC exudes, Necrosis, fusion of adjacent lamella, hyperplasia, oedema	Necrosis, vascular hemorrhage, dilated sinusoids and vacuolar degeneration	Damage and degeneration of renal tubules, glomerular oedema, Necrosis,	[35]



Mercury (Hg)	Mild congestion and oedema in primary lamellae, hyperplasia, desquamation in epithelial lining secondary lamellae, hyperactivity of chloride and mucous cells, Increase in RBC, macrophages	Vacuolization, Hypertrophy of hepatocytes, intravascular hemolysis, nuclear pyknosis, congestion in central vein, necrosis	Hydropic swelling of tubules, pycnotic nuclei, swelling of proximal convoluted tubule with necrotic nuclei.	[36]
Arsenic (As)	Epithelial hyperplasia, lifting and oedema, lamellar fusion, desquamation aneurism, and necrosis	Focal lymphatic and macrophage infiltration, congestion, sinusoid dilation a & Swelling, vacuolization and shrinkage of hepatocytes, necrosis	Pycnotic nuclei, vacuolization of tubular cells, glomerular shrinkage, lumen enlargement, necrosis.	[34,37]
Cadmium (Cd)	Hyperplasia, increase in chloride cells, reduced and shortened length of secondary gill lamellae	Dissociation of hepatocytes, Necrosis, blood congestion in liver sinusoids, vacuolization	Disorganization and degeneration of renal epithelial cells, reduction in glomerulus, hypertrophy, dilation of bowman's capsule, focal necrosis	[38]
Chromium (Cr)	Lamellar disorganization, Necrosis in epithelial cells, atrophied central axis.	Hyperplasia, Necrosis of hepatocytes, Reduced N-C ratio.	Highly fenestrated Bowman's capsule, Constricted lumen of renal tubes, glomerular disorganization,	[33]

Heavy metals in water, sediment, fish, and other aquatic species have been the subject of several research. *Table 2.4.* includes a few of them including lead (heavy metal) concentration in some of the common fishes [39-42].

**Table 2.4. A report on accumulation of a toxic heavy metal (lead) in the organs of five common fish.**

Fish Species	Fish tissues	Lead (Pb) Conc.
<i>Cirrhinus mrigala</i>	Muscles	2.37 ± 0.21
	Gills	2.29 ± 0.35
	Liver	2.54 ± 0.05
<i>Cirrhinus reba</i>	Muscles	3.89 ± 0.41
	Gills	4.77 ± 0.34
	Liver	1.54 ± 0.06
<i>Cirrhinus catla</i>	Muscles	2.03 ± 0.11
	Gills	2.93 ± 0.51
	Liver	3.15 ± 1.22
<i>Labeo rohita</i>	Muscles	1.12 ± 0.03
	Gills	1.83 ± 0.06
	Liver	2.27 ± 0.22
<i>Mystus tengara</i>	Muscles	1.45 ± 0.06
	Gills	1.74 ± 0.09
	Liver	2.32 ± 0.74

### 2.2.3. Toxic effects of Heavy Metals in the Human health:

Humans are omnivorous creatures. Toxic heavy metals can be ingested through a variety of foods, including seafood, cereals, and vegetables. Heavy metal pollution in freshwater bodies including rivers, lakes, and streams causes bioaccumulation of these elements in freshwater fish, whereas heavy metal contamination in agricultural areas causes bioaccumulation of these elements in agricultural crops. Toxic heavy metal contamination of human food chains is a concern to human health. Contamination is a severe human health concern, as seen in cases from the twentieth century. In Japan, intake of Hg-contaminated fish and Cd-contaminated rice produced Minamata Illness (MD) and itai-itai disease, respectively. The heavy metals Cd, Pb, Hg, and As decrease the primary antioxidants in cells, notably thiol group (SH) antioxidants, and enzymes. Reactive oxygen species (ROS) such as hydroxyl radicals (HO•), superoxide radicals (O<sub>2</sub>•), and hydrogen peroxide may be increased by such metals (H<sub>2</sub>O<sub>2</sub>). Increased ROS production may wreak havoc on cells' natural antioxidant defenses, resulting in a situation known as "oxidative stress" [43]. Heavy metals, such as Cd, Pb, and Hg, are hazardous to the kidneys, especially the renal cortex. In terms of toxicity, the chemical form of heavy metals is crucial. The toxicity of mercury is primarily determined by the Hg speciation. Patients with cancer and diabetes had greater quantities of hazardous heavy

metals, such as Cr, Cd, and Pb, and lower concentrations of the antioxidant element Se, compared to healthy people.

Cd is a very hazardous heavy metal that persists in the environment and accumulates in food systems. One of the most dangerous heavy metals is Cd. Cd has been dubbed an "enigmatic metal" since it is poisonous to almost all living things. Cd induces nephrotoxicity by causing damage to the renal tubules. Cd may also harm bones (bone demineralization) [44]; the damage to bones can be direct or indirect, depending on whether it is caused by renal disease or not. Kidney impairment as a result of Cd exposure can alter the metabolism of bone-building components like calcium, resulting in osteoporosis. Because of its capacity to activate the estrogen receptor, ER, Cd exposure at ecologically relevant quantities may raise the risk of breast cancer. Even at very low doses, Cd has endocrine-disrupting effects and inhibits the production of sexual hormones. Women have greater amounts of Cd in their blood (BCd) and urine (UCd) than males, which might be attributed to increased Cd absorption in women due to poor iron status [45].

Because of their development and metabolism, children are more susceptible to Pb poisoning. Pb poisoning has a negative impact on the mental development and conduct of children aged 2-4 years. The blood Pb (BPb) levels of children are inversely connected with their Intelligence Quotient (IQ). Pb exposure in the environment has been linked to anemia in children. In children aged 1 to 5, a link has been discovered between lead in the blood (BPb) levels and slight to anemia. Blood Fe and hemoglobin (Hb) levels have been discovered to have a negative connection with BPb levels. Children in China with blood Pb levels between 7.5 and 10 g dL<sup>-1</sup> had Hb levels that were considerably lower than those with blood Pb levels less than 5 g dL<sup>-1</sup> [45,46]. Even at blood lead (BPb) levels less than 5 g/dL, Pb is nephrotoxic, especially in sensitive groups such as hypertensive, diabetic, and renal patients. Recent research of a large adult population in China discovered a link between elevated blood Pb levels and an increased occurrence of cardiovascular illnesses (CVD) [47].

### ***Methods Utilized for the treatment of wastewater***

Because of the increasing population and rapid pollution of water resources, wastewater treatment and reuse are an important issue. The efficient use of existing water resources and treatment of polluted water resources with affordable and cheap technologies have been the focus of scientists. Wastewater treatments are needed for three reasons; these are water source reduction, wastewater treatment, and recycling. Recently, during purification step, while primary treatment includes preliminary physical and chemical purification processes, secondary treatment depends on biochemical decomposition of organic solids to inorganic or stable organic solids. Finally, after the third step called tertiary treatment processes, wastewater is converted into good-quality water, and it can be used for drinking or medicinal supplies. At the end of this step, almost all the pollutants (up to 99%) can be removed from water. To produce

good-quality and safe water, all these three processes should be combined. Otherwise, it will not be possible to obtain safe water from the wastewater. Many advanced methods and techniques have been used for the recycling of safe water from wastewater, but economic and effective water treatment is still a serious problem. Treatment of wastewater and recycling technologies have been classified in three stages. Which are:

- Primary treatment
- Secondary method
- Tertiary method

### **Primary method**

To remove organic matter and suspended solids from wastewater by means of physical operations, for example, sedimentation and gravity separation, they are done in primary treatment stage. Preliminary treatment, which is described as preparation for secondary treatment, is in fact intended to produce a liquid waste suitable for biological treatment.

### **Screening separation method**

Screening separation method is used to remove solid waste from wastewater. It is the process where suspended and floating materials including wood, paper, kitchen refuse, pieces of cloth, cork, hair, fibers, and fecal solids are removed from wastewater. In wastewater treatment, screening is generally used as the first operation step. For this purpose, various size screens are used, and their size is selected as per the requirement. Finer particles such as sand and small pebbles can be eliminated by using screening separation method.

### **Filtration method**

About 0.1–0.5 mm pore size is used in filtration separation method, water is passed through a medium having fine pores, and the filtration process is completed. Various membranes and filters, for example, cartridges, can frequently be used to remove suspended solids, greases, oils, and bacteria from the wastewater. The main purpose of filtration separation method is to separate the small solids and remove oil (they can be reduced up to 99%). Filtered water is used for many purposes such as ion exchange, adsorption, or membrane separation processes. In pharmaceutical and biotechnological industries, to the production of pure water, filtration separation method has become the focus as promising separation tool for WWT. The used membrane has a key role due to selectivity, low fouling, and performance stability for long-term operation in the filtration separation method. Because of these advantages, this method and its performance are becoming more and more important. In addition, it is one of the important enrichment techniques for trace heavy metal ions along with simplicity and rapidity of the procedure. For all these reasons, many scientists have focused on this subject to develop and use alternative and effective membranes [24, 25].

### **Centrifugal separation method**

This method is provided for separating components of a fluid or solid particles, but it is used especially for suspending solid from wastewater. Various types of centrifugal machines have been used to remove suspended noncolloidal solids in the centrifugal separation method. To separate solids from wastewater, centrifugal devices of various sizes are used. Density of suspended solids is the most important parameter when separating solid materials by centrifugation. In addition, oils and greases can be reduced and separated during application of centrifugal separation method.

### **Coagulation method**

Coagulation processes are a particularly effective cleaning method for containing oil-in-water emulsions such as sea, lakes, and rivers besides most industrial wastes contain especially oil or petroleum. After sedimentation and gravity separation method, if there are non-settleable solids in wastewater, this is called processing coagulation with the addition of certain chemicals to precipitate these non-settleable solids and non-precipitating deposits. There are some natural coagulants such as aluminum salts, iron materials, alum, starch, and activated silica and also some polymers that can be used as coagulants. In this process, the most important controlling factors are contact time, temperature, and pH. In addition, during biological treatment processes, to remove microbes and any organics in the water, some certain coagulants can be added. Coagulation processes play an important role in recycling and removing pollutants from wastewater.

### **Sedimentation and gravity separation method**

Sedimentation and gravity separation method are based on the removal of suspended solids, grits, and silts from aqueous media. Suspended solid materials settle down to the bottom of the tank under the influence of gravity; this event may vary depending on solid size and density. Some chemicals can sometimes be added to accelerate the sedimentation process. Although this method can reduce suspended solids by only up to 60%, purification of wastes is a very useful separation application. Water treatment in this technique can be used in many areas such as water for membrane filtration processes and ion exchange method. It is generally applied prior to conventional treatment.

### **Flotation method**

To remove suspended solids including oils, greases, biological solids, and other solids from wastewater, a flotation separation method is used. In these processes, suspended solids are removed by adhering them with either air or gas. Various chemicals like alum and activated silica are used to successfully apply the flotation process to wastewater because they help flotation separation method. For paper and refinery industries, flotation separation method is an

effective method for wastewater treatment because suspended solids that oil and grease is can easily be removed (up to 75–99%) by these processes. Recently, to separate mixed plastic is too difficult using gravity separation; therefore, for wastewater treatment and recycling purposes, plastic flotation method has been used effectively [26].

### **Secondary treatment methods**

Secondary treatment techniques have been used to remove soluble and insoluble pollutants from wastewater as biological. The main objective of this process is to convert the organic and inorganic solids into fluorinated residues that are finely divided and dissolved in the wastewater and to remove of soluble and colloidal organics and suspended solids besides reducing BOD and COD through biological process. When water has a high microbe concentration like bacterial and fungal strains, secondary treatment techniques should be selected for treatment because organic matter is converted into other products via these microbes; besides, they detoxify toxic inorganic matter. After this process is applied to wastewater, toxic organic and inorganic substances can be removed [27].

### **Aerobic separation method**

In biological treatment processes, organic matter can be biodegradable by aerobic and facultative bacteria. Aerobic processes depend on temperature, the oxygen amount and availability of oxygen, and the biological activities of the bacteria. If bacterial growth is accelerated by adding some chemicals to the medium, the organic pollutant oxidation rate as biological will also be increased. Aerobic treatment techniques are the most effective method for removing suspended, volatile, and dissolved organics, nitrates, and phosphates besides BOD and COD. Because of the production of a huge amount of biosolids, aerobic treatment techniques have a big disadvantage; however, the biodegradable organic amount can be reduced substantially (up to 90%) using this method.

### **Anaerobic separation method**

Anaerobic decomposition, called putrefaction, occurs when free dissolved oxygen is not present in wastewater, and this process is called as anaerobic treatment technique. In this treatment technique, organic matters convert into other organics including sulfur and carbon by anaerobic and facultative bacteria. There are two metabolic phases named acidogenic phase and methanogenic phase in the anaerobic separation technique. Some gases such as methane, hydrogen sulfide, ammonia, and nitrogen can be released. To reduce the biological load of wastewater, this method is very vital [1].

### **Tertiary treatment methods**

To produce safe water that people can consume, tertiary water treatment techniques are very important, and they should be applied to wastewater. In this last step, wastewater is

subjected to final treatment using some vital techniques, and they are briefly summarized below.

### **Distillation method**

The distillation method is based on the principle that the water is evaporated to the boiling point and the steam is distilled by cooling. After this process, purified water can be obtained free from impurities up to 99% in addition to wastewater is also freed from the volatile pollution. The obtained water by the distillation method is usable in levels of laboratory applications and medicinal preparations. In addition, to prepare potable water from the sea, distillation separation method is an effective tool.

### **Crystallization method**

The crystallization method, which is based on the increasing principle of the concentrations of pollutants up to the crystallization point, is an effective method for obtaining quality water. Crystallization technique is useful to remove high concentrations of total dissolved solids including soluble organics and inorganics from wastewater, and it can be created either by mixing some solvents or by evaporation.

### **Evaporation method**

When compared to other techniques, evaporation separation method is a natural process and suitable method but only for small wastewater volumes due to its high energy consumption. However, this technique has some problems such as pollution, calcification, and foaming that have occurred in the presence of suspended solids and carbonates in the wastewater. Thus, to increase the evaporation rate and to reduce energy consumption, vacuum evaporation step can be used. Under natural conditions, water surface molecules escape from the surface, and they generally collected pure water. Recently, to recycle water process, mechanical evaporators and sometimes vacuum evaporation have also been used. Using evaporation separation technique is effective for the removal of pollutants including organic and inorganic compounds, but some volatile organic compounds may recirculate into the water during the evaporation phase. Evaporation treatment techniques are applied to various industry wastewaters like pharmaceutical, petroleum, and fertilizer industries.

### **Solvent extraction method**

Solvent extraction separation method is an important tool to dissolve pollutants from wastewater using various organic solvents like phosphoric acid. Acetone, methanol, hexane, ethanol, and acetonitrile are the most used organic solvents. In this technique, some organic solvents are added to the wastewater to facilitate contaminant removal. The technique is very effective to remove oils, greases, and various organics.

### **Precipitation method**

The precipitation method based on the principle that the solubility of the contaminants is reduced and the precipitates which are converted into the solid form are easily separated from the water surface is an effective method for removing metal ions and various organic contaminants from wastewater. Chemical precipitation is a physicochemical process and a very flexible approach to various pollutant removals and can be applied at several stages during wastewater treatment. In industrial applications, precipitation has been the most common technology for metals [32]. In this process, to reduce solubility of the dissolved pollutants, it can be carried out either by lowering the temperature of the water or by adding some chemicals like sodium bicarbonates and ferric chloride, but chemical addition is not preferred because it increases the cost. Common applications of precipitation separation method are wastewater treatment from chromium and nickel-plating industries and water recycling besides water softening and removal phosphate from water.

### **Filtration method**

Recently, from the industrial sources, a large amount of oily wastewater has been generated. The most serious pollutants are oil-in-water emulsions because of treatment cost and ineffective of using treatment methods [34]. Using microfiltration, a suspended solid pollutant that is a particle size from 0.04 to 1 mm can be removed. Microfiltration separation technique has been widely used to remove macromolecules, emulsion droplets, suspended particles, and microorganisms from various industrial fields including food, pharmaceutical, biotechnological, and petrochemical. In the last decade, membrane separations have been developed using various organic/inorganic membranes like ceramic membranes. It is becoming a promising technology for industrial processes and is utilized currently for oil field produced wastewater treatment. When compared to traditional treatment methods, they have some advantages including high oil removal efficiency, low-energy cost, and compact design. Perhaps the most important advantage is that it does not require any chemicals. Some materials such as cellulose, fiberglass, and cotton can be used as filters in filtration method. Recently, several researchers focused on the new inorganic membrane development, for example, natural mineral-based ceramic membranes, carbon membrane, and zeolite membrane [35].

## **2.3. Utilization of biomass in the form of biochar for removal of heavy metals from water.**

Biochar is pyrogenic black carbon derived from thermal degradation (e.g., pyrolysis) of carbon-rich biomass in an oxygen-limited environment. In recent years, biochar has received increasing attention due to its multi-functionality including carbon sequestration and soil fertility enhancement (Laird, et al. 2010), bio-energy production (Field, et al. 2013), and



environmental remediation (Mohan, et al. 2014a). Several recent publications have provided evidence of biochar's excellent ability to immobilize organic (Lattao, et al. 2014, Inyang, et al. 2014, Rajapaksha, et al. 2014) and inorganic pollutants (Meng, et al. 2014, Mohan, et al. 2014b, Yao, et al. 2011a) in soil and water systems. While most organic pollutants are biodegradable, inorganic pollutants, mainly heavy metals, are non-biodegradable and may be passed along the food chain through bioaccumulation. Biochar is increasingly being considered as an alternative agent in water treatment technologies for metal removal.

Development of biochar technology provides opportunities to satisfy the need of low-cost adsorbents for aqueous heavy metals. Mohan, et al. (2007) noted that removal efficiencies of lead (Pb) and cadmium (Cd) by oak bark char are comparable to that of Calgon F-400, a commercial activated carbon. Chen, et al. (2011) reported that biochar produced from wood or corn straw can effectively adsorb copper (Cu) and zinc (Zn) in aqueous solutions. Similarly, Kong, et al. (2011) reported 75-87% removal of mercury from aqueous solution by soybean stalk biochar. Further, the application of different engineering methods in biochar production, such as pretreatment of feedstocks or modification of char surfaces, has resulted in many high efficiencies and cost-effective engineered biochar with adsorption capacities comparable to or even surpassing some commercial activated carbons. For instance, previous studies (Inyang, et al. 2012a, Inyang, et al. 2011a) showed that biochar produced from anaerobic digested biomass have a much higher Pb sorption capacity than commercial activated carbon. Physical treatment of the feedstock, by pulverization prior to pyrolysis, can also greatly enhance the sorption ability of the biochar to Cu in aqueous solution (Tong, et al. 2011).

### **Biochar production and heavy metal removal ability**

Biochar can be produced from a wide range of feedstock materials, including agricultural and forest residues, industrial by-products and wastes, municipal solid waste materials, and nonconventional materials, such as waste tires, papers, and even bones.

### **Agricultural and forest residues**

The growing use of agricultural and forest residues as biochar feedstock is due to their abundance and low cost. In addition, conversion of waste biomass to value-added biochar products can reduce operation cost associated with disposal of these abundant waste agricultural and forest materials. Global annual production of agricultural residues has been estimated to be 500 million tons (Duku, et al. 2011). Many agricultural and forest residues generated are waste or by-products from harvesting and processing of crops such as sugarcane, corn, peanut, sorghum, and oil palm for food and bio-energy production. Often, most of the residues generated in many countries, including the United States, are scarcely utilized, and thus can serve as feed stocks for biochar production. Furthermore, agricultural and forest

residues, such as wood, yard and forest waste are attractive feedstock to produce biochar due to their ease of collection from residential collection services and wood mills.

The strong sorption ability of biochar produced from agricultural and forest residues may be attributed to their surface properties originating from the feedstock materials. For example, the abundance of oxygen containing groups (C=O, C-O, -OH) in oak bark biochar originating from polyphenolic tannins, flavonoids and suberin contents in the partially aromatized bark materials provided negatively charged surface sites (COO and OH<sup>-</sup>) for the attraction of Pb<sup>2+</sup> (Mohan, et al. 2007). Kumar et al. Kumar, et al. (2011) reported that volatilization of large amounts of cellulose from switch grass during pyrolysis creates porous carbon materials with surface functional groups strongly affiliated to aqueous U (VI). Pyrolysis of other agricultural residues, such as sugar beet tailings, may also yield electron donor functional groups (C-OH, C-O, C-O-R) because the feedstocks have complex heteropolysaccharides containing galacturonic acid, arabinose, galactose, and several pectin substances (Aksu and Isoglu 2005). These oxygen functional groups can promote the sorption of aqueous chromium, Cr (VI) on biochar by reducing Cr (VI) to Cr (III) to facilitate surface adsorption (Dong, et al. 2011). It has also been reported that elevated levels of cationic nutrients, such as Na, K, and Mg, in oak and pine wood feedstock materials can also increase the cation exchange capacities of the corresponding biochar and enhance their sorption of Pb through ion exchange process under acidic pH conditions (Mohan, et al. 2007).

Domesticated animal wastes are another abundant source of potential biochar-feed stock. Biochar produced from animal wastes such as poultry litter (including bedding materials, spilled feed, and feathers) and dairy manure generally have high amounts of ash and inorganic components that can bind heavy metals (Duku, et al. 2011, Cao, et al. 2011, Uchimiya, et al. 2010). Multiple mechanisms, including precipitation and surface complexation, have been reported to be responsible for the strong affiliations between animal waste-derived biochar and aqueous heavy metals (Cao, et al. 2011, Uchimiya, et al. 2010).

### **Industrial by-products**

Production of biochar from industrial wastes and by-products has attracted a growing interest recently. The feasibility of using waste materials collected from bio-energy facilities (e.g., digested residues) and wastewater treatment plants (sewage sludge) for biochar production has been investigated (Yao, et al. 2011a, Phuengprasop, et al. 2011, Yao, et al. 2011b). Anaerobic digestion or sewage treatment involves biodegradation of waste residues by a variety of microorganisms. The degradation of carbonaceous substrates results in concentrating cationic or metallic elements within the residues which when converted into biochar, may have high ion exchange capacity to remove heavy metals from aqueous solutions (Gu and Wong 2004, Hanay, et al. 2008).

### 2.3.1. Variations of the biochar used for wastewater treatment:

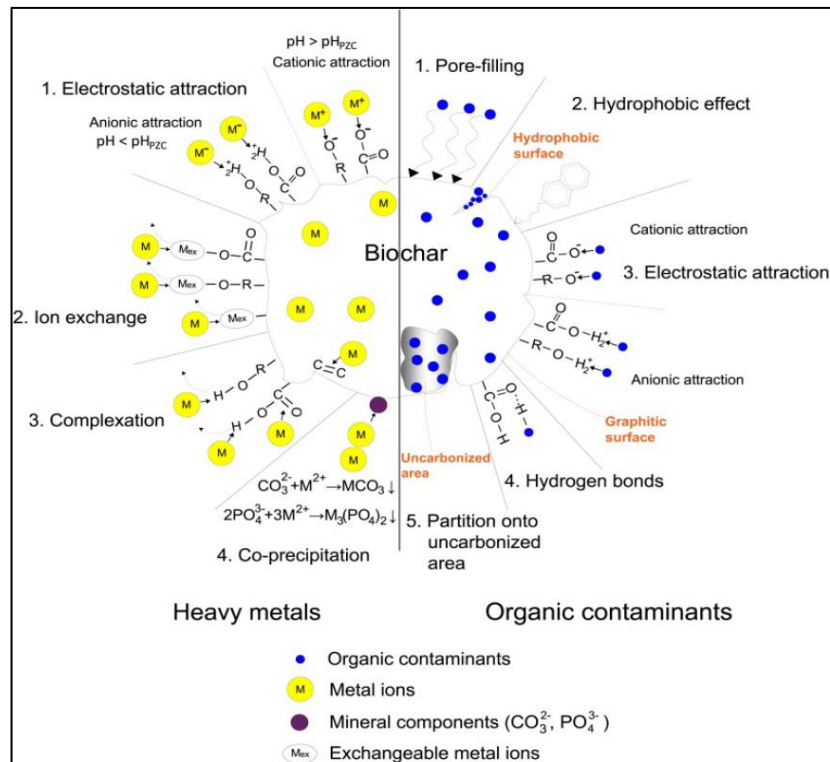
The features of biochar are directly influenced by variables such as temperature, maximum temperature and duration, feedstock, and air pressure within the chamber throughout the manufacturing process. Biochar, in addition to these manufacturing factors, can be chemically or physically changed to meet particular performance needs in certain cases [48]. Variations of biochar used for wastewater treatment are provided in *Table 2.5.* below, which differ from the generally used form of biochar (based on their name given in the data source).

**Table 2.5. Variation of biochar along with the treatment application process.**

Biochar type	Treatment application	References
Biochar based nanocomposites. Fe <sub>2</sub> O <sub>3</sub> -biochar nanocomposite.	<ul style="list-style-type: none"> <li>• Used for wastewater remediation,</li> <li>• Degradation of emerging organic pollutants</li> </ul>	[49]
Steam-activated biochar	<ul style="list-style-type: none"> <li>• Decolorization of cationic and anionic dye-laden wastewater</li> </ul>	[50]
Biochar & magnetic Fe <sub>3</sub> O <sub>4</sub> hybrids / “magnetic biochar	<ul style="list-style-type: none"> <li>• Pharmaceutical removal</li> </ul>	[51]
Photo-thermal biochar cakes (BCs)	<ul style="list-style-type: none"> <li>• Dye wastewater treatment</li> </ul>	[49,50]
Biochar based sorbents	<ul style="list-style-type: none"> <li>• Wastewater treatment.</li> <li>• Removal of heavy metals</li> </ul>	[49]
Biochar / layered double hydroxide (LDH) composite “Biochar/LDH composite”	<ul style="list-style-type: none"> <li>• Wastewater treatment; water purification</li> </ul>	[51]
Biochar filters	<ul style="list-style-type: none"> <li>• On-farm wastewater treatment</li> </ul>	[52]
Chitosan-modified biochar / Modified biochar/ Engineered biochar	<ul style="list-style-type: none"> <li>• Organic matter removal from biotreated coking wastewater.</li> <li>• Adsorption of emerging contaminants from wastewater.</li> <li>• Reclaiming phosphorus from secondary treated municipal wastewater</li> </ul>	[50]

### 2.3.2. Types of pollutants in the wastewater that can be removed by biochar:

Despite the fact that biochar is not a commonly used wastewater treatment technique, a large number of research on biochar's ability to remediate different contaminants is now underway. However, due to the variety of biochar changes and chemical and physical characteristics, as mentioned in the preceding section. It's hopeful that, in addition to removing typical pollutants like phosphate, ammonium, and heavy metals, biochar may also effectively remove antibiotics (mostly from cattle farm effluent) and organic industrial pollutants, which can be harmful and deadly in some situations. It should be emphasized that biochar is capable of eliminating a wide spectrum of organic and non-organic contaminants [53]. However, the effectiveness of biochar in removing contaminants differs depending on the pollutant. Because biochar is often negatively charged, removing negatively charged contaminants from wastewater, such as oxyanions, is more difficult for the basic form of biochar. As a result, specific biochar adjustments are required to ensure effective contamination removal. Electrostatic attraction, ion exchange, complexation, and co-precipitation are the most common sorption processes for heavy metals. Pore-filling, hydrophobic effect, electrostatic attraction, hydrogen bonding, and partition onto uncarbonized regions are the basic sorption processes for organic molecules.



**Figure 2. 1. Biochar sorption mechanisms of organic pollutants and heavy metals [53]**

In light of the previously researched biochar changes, it is clear that, in addition to these new modifications that permit increased pollutant removal efficiency, biochar contains a significant number of sorption properties. Based on the above facts, it can be deduced that the kind of contaminants, their chemical and physical characteristics, and the biochar's feedstock, production temperature, and other factors are all connected to the type of contaminants and their chemical and physical qualities [54].

### **2.3.3. Conventional Thermal Methods for the Conversion of Feedstock into biochar:**

Pyrolysis is one of the most common thermal methods for turning organic wastes into carbonaceous compounds that may be used to clean up wastewater. Pyrolysis breaks down lignin, cellulose, hemicellulose, and fat in the feedstock in an oxygen-free environment to increase the carbon content of the starting material by removing non-carbon molecules like oxygen and hydrogen. The primary elements governing the pyrolysis process are carbonization temperature, heating rate, nitrogen flow rate, and carbonization time. Increased hydrophobicity, surface area, and micropore volume come at higher pyrolysis temperatures (>500 C), making the biochar produced extremely suitable for the removal of organic contaminants. However, a lower pyrolysis temperature (500 C) resulted in biochar with smaller pore size, reduced surface area, and more oxygen-containing functional groups, making it better at removing inorganic contaminants [55].

Hydrothermal Carbonization (HTC) is one of the most appealing thermochemical conversion technologies for the creation of high energy density materials. This technology is particularly suited to wet feedstocks because it eliminates the need for an energy-intensive drying stage and allows for high conversion efficiency of biomass to a carbonaceous material with a high yield at low operating temperatures. HTC involves subjecting biomass in the presence of a liquid to temperatures ranging from 180 C to 300 C for many hours at a pressure ranging from 2 MPa to 10 MPa [56].

Gasification is a thermochemical process that converts biomass or other organic materials into a gas mixture known as "syngas" (85%) comprising H<sub>2</sub>, CO, CO<sub>2</sub>, and sometimes tiny hydrocarbons like CH<sub>4</sub>, a solid char (10%), and a liquid phase known as "tar" (5%). The unwanted byproducts of the gasification process include tar and char. The composition of the feedstock, reactor architecture, gasifying agent, and gasification temperature all influence the qualities of char produced by biomass gasification operations. In research, it's been also found that char generated by gasification of dealcoholized grape marc at 1200 C under airflow had a poor specific surface area (60 g/m<sup>2</sup>), which discourages its use as activated carbon without additional activation.

## *Utilization of response surface methodologies for the removal of heavy metals from wastewater*

Nowadays, because of rapid technological development especially in developing countries, environmental pollution is a serious problem for the ecosystem because wastewaters contaminated with toxic heavy metals are discharged directly or indirectly into the environment. Unlike most organic contaminants, heavy metals including As, Hg, and Cr are hazardous due to their nonbiodegradable nature [33, 45]. Thus, to protect the people and the environment, these hazardous ions should be removed from wastewater [46]. For example, while industrial wastewaters which contain Cr ions range from 0.5 to 270 mg L<sup>-1</sup>, inland surface water tolerance limits 0.1 mg L<sup>-1</sup>, and potable water Cr level should not exceed 0.05 mg L<sup>-1</sup> according to various health organization such as the WHO and EPA [47, 48]. To remove heavy metal ions from wastewater, many conventional techniques such as membrane filtration, reverse osmosis, ion exchange, chemical precipitation, electrodialysis, electrochemical treatment, and adsorption have been employed. While most of these methods suffer from operational costs for the treatment process and high capital, the adsorption method is better than the other methods due to its flexibility in design, simplicity of operation, and facile handling, and it is considered more efficient and economical [45, 49]. Since the dynamic characteristics of the adsorption process are complex, it is essential to have optimum working conditions to achieve optimum pollution removal efficiency. Process optimization is crucial to determine design parameters value, which is achieving the optimal obtained response level. The RSM is one of the most used methods because of its developing, improving, and optimizing of the processes especially in the presence of complex interactions. It is also used to determine the ideal points of independent variables that are effective under optimum conditions and to evaluate the interactions of these variables [50]. Its greatest advantage is the decreased experimental trial number required to interpret multiple parameters. Therefore, RSM optimization process contains three main steps: (a) appropriate experimental design selection, (b) model coefficient estimation using analysis of variance (ANOVA), and (c) model validation based on prediction and experimental runs of the process response validation of the final model [51]. This experimental design method for an adsorption process is more practical than other approaches because it allows for the opportunity to monitor and interpret interactions between variables and to describe the overall effect of the parameters on the process. The RSM has been successfully used; in addition, its greatest applications have been in industrial research [52].

There are numerous studies, and different results were obtained using various adsorbents reported such as by Anupama et al. [53]. They used a CCD with RSM for removing Cr(VI) from aqueous medium [53]. They investigated the effect of some parameters including pH and temperature on adsorption, and the optimum pH, time, and adsorbent dose were found to be 2.32, 25.76 min, and 1.79 g L<sup>-1</sup>. Also various adsorption kinetic models and isotherms were compared to find fit model. Jain et al. [54] studied Cr(VI) removal from aqueous solution using

BoxBehnken model with combined RSM approach by chemically treated *Helianthus annuus* flowers. They investigated three effective factors for Cr(VI) removal. It was reported that the optimum pH, adsorbent dose, and initial concentration of Cr(VI) were found to be 2.0, 5.0 g L<sup>-1</sup>, and 40 mg L<sup>-1</sup>, respectively [54]. In an another study [55], Box-Behnken design has been applied to evaluate operating variables interaction for Cr (VI), Ni (II), and Zn (II) ions adsorption on *Bacillus brevis*. They carried out a total of 17 experiments and used a quadratic model. Based on this model, it was reported that the regression equation coefficients were calculated, and the data fitted to a second-order polynomial equation for these metal ions removal with immobilized on *B. brevis*. According to another study, to evaluate and optimize Cr ions, adsorption on activated carbon experimental conditions using RSM as an efficient approach for predictive model building was performed by Sahu et al. [56]. A full factorial CCD was employed, and based on ANOVA, a high coefficient ( $R^2 = 0.928$ ) was obtained. In addition, satisfactory prediction of second-order regression model was derived. According to optimized process parameters, Cr(VI) removal percentage was obtained higher than 89% [56]. Kaplan Ince et al. [57] studied a batch experimental system for removal Pb(II) using clay, and optimized experimental approach was applied to some alcoholic beverages including beer and wine samples. Various effective parameters were investigated using a Box-Behnken experimental design methodology and RSM. They reported that the optimal conditions used for Pb(II) removal were pH of 5, contact time of 31 minutes, 75 mg for adsorbent dosage, and 100 rpm for agitation speed. Based on these results, maximum Pb(II) ion removal was calculated as 120 mg g<sup>-1</sup> from aqueous medium using an ETAAS [57]. Balan et al. (2009) examined the efficiency of Cd(II) removal from aqueous solutions using sphagnum moss peat as biosorbent. They carried out a CCD for experimental design to evaluate an analysis of results and to optimize process parameters including the pH of solution, biosorbent dosage, and Cd(II) initial concentration. The optimum values of experimental parameters were obtained as 4.72 for pH, 14.7 g L<sup>-1</sup> for biosorbent amount, and 13.64 mg Cd L<sup>-1</sup> for initial concentration of Cd (II) [58]. In another study, removal of Cr (VI) from simulated wastewater using RSM was examined by Bhatti et al. [59]. They investigated the performance of a laboratory scale electrocoagulation system for the removal of Cr (VI) using Al-Al electrodes. They obtained an interaction between voltage × time and amperage × time coefficient of determination as 0.8873 and 0.9270, respectively. For the optimization of process variables including pH, voltage, and treatment time, the RSM was used. Prediction model results were validated through laboratory scale batch experiments [59]. In another similar study, to remove arsenic from contaminated water by arsenite, an electrocoagulation method with stainless steel electrode was used. A response surface methodology approach was performed to optimize significant process variables such as treatment time and solution pH. They obtained pH as 5.2, treatment time ¼ 20 min for 10, and 55–100 mg L<sup>-1</sup> of initial arsenic concentration. It was stated that waste elimination with electrocoagulation is a sustainable treatment technology with quick start-up,

shorter treatment time, and minimum sludge generation [60]. An alginate-coated chitosan nanoparticle was carried out for heavy metal removal from industrial effluents by Esmaeili and Khoshnevisan [61]. To optimize the process of biomass for heavy metal removal from synthetic and industrial effluents containing nickel, an RSM approach was performed. Under optimum experimental conditions, which they obtained as a dose of 0.3 g biomass, pH of 3, 70 mg L<sup>-1</sup> of initial concentration nickel, and 30 min contact time, maximum removal efficiency of biomass was found as 94.48% [61]. The Cd removal from wastewater and simulated aqueous solution was examined by Iqbal et al. [62] using a polyurethane material as adsorbent. The effect of operating parameters including adsorbent dosage, pH of solution, and metal ion concentration was modeled by RSM combined with CCD. Experimental runs and independent variables optimum values for Cd adsorption were obtained as 305 mg L<sup>-1</sup> Cd ion initial concentration, pH 4.9, contact time 932 min, and adsorbent dose 1.3 g for polyurethane material. Based on the experimental results, to predict the response with good accuracy and reliability, it was mentioned that the RSM proved to be the best statistical model [62]. Ince and Kaplan Ince [63] examined the removal of Cr from industrial wastewater using RSM combined with CCD besides investigated as an efficient approach for examining predictive model building and optimization. To predictive regression models and optimize experimental variables, statistical design was modeled. The experimental parameters such as pH and agitation speed were selected for optimization. They obtained ideal Cr ion removal conditions as pH of 5.0, contact time 23.0 minutes, adsorbent dosage of 69.4 mg, and agitation speed of 135 rpm. The Cr removal efficiency was found at 23.16 mg g<sup>-1</sup>. Also, significant independent parameters and their interactions were verified by means of the ANOVA. The proposed adsorption process was applied to various industrial wastewaters. It was stated that a CCD method was identified to yield a maximum Cr ion removal of 99% [63].

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# Chapter 3 | METHODS AND METHODOLOGY

## 3.1. Biomass feedstock preparation:

Agricultural waste residue in the form of the bamboo leaf was chosen for the preparation of biochar. The various impurities like mud, soil, and sand were removed manually, cut into small pieces, dried under the sun, and stored in a hermetically sealed container at room temperature for further use. All the chemicals required in the study were obtained from Nice® Chemicals (Kerala, India). Double-distilled water was used for experimentation purposes which were prepared in-house (Borosil® distillation apparatus, Maharashtra, India).

## 3.2. Systematic classification of biomass:

Bamboo leaf has been selected as the preferable biomass and the systematic classification of the biomass is given below in a tabular form in table 3.1.

**Table 3.1. Systematic classification of biomass.**

<b>Kingdom</b>	:	<b>Plantae</b>
Clade	:	Tracheophytes
Clade	:	Angiosperms
Clade	:	Monocots
Clade	:	Commelinids
Order	:	Poales
Family	:	Poaceae
Genus	:	Bambusa
Species	:	<i>B. vulgaris</i>

## 3.3. Characterization of biomass:

The biomass specimen used in this study were characterized with reference to the structural, textural, and chemical characterization procedures. The literature indicates that bulk density is a cardinal physical property with reference to the logistic system linked to biomass transportation, handling, and management. It affects the impact storage requirement; The

various pretreated and untreated bamboo leaf specimens were characterized in terms of bulk density as per literature standard procedure [1].

The compositional constituents in terms of cellulose, hemicellulose, lignin, and extractive content were estimated as per standard literature reported protocol 2015 [2].

The proximate composition of the biomass under evaluation was determined by following standard test protocols in terms of ash content (A%), volatile matter (VM %), and fixed carbon (FC%) [3, 4].

Ultimate analysis determines the elemental mass percentage of carbon, hydrogen, oxygen, and nitrogen in biomass. The ultimate analyses of the biomass specimen were performed using It is carried out using a CHNS/O elemental analyzer as per the procedure given in ASTM D5373-08 and subsequently, the elemental ratios were computed [5].

The heating value (HV) of biomass indicates the energy content of biomass. The HV is usually measured in terms of the energy content per unit mass, or volume. The energy content of the biomass specimens was determined using an adiabatic bomb calorimeter (Petro Test Instrument, West Bengal, India) in terms of higher heating value (HHV) following the standard protocol [6].

Physicochemical properties of biomass and their thermal degradation profile were investigated by using a thermogravimetric analyzer (TGA). The biomass was subjected to pyrolysis up to 600 °C. A graph of weight % along with indicated the degradation behavior of selected biomass.

Further, to have an insight into the functional components of the biomass, FT-IR spectroscopic analysis was carried out as per standard literature reported protocol [7]. XRD analysis reveals the development of carbonized material increased with an increase in the terminal temperature of biochar.

### **3.4. Pretreatment of biomass and its optimization:**

Pretreatment of biomass with acid has been reported to be linked with high rates of reaction resulting in their hydrolysis. Acid pretreatment of biomass has been reported to hydrolyze the biomass components (up to 90%), which in turn helps in the disruption of their complex lignocellulosic structure. The alkaline process requires less severe conditions compared with the other pretreatment methods and lower amounts of inhibitors are formed in this pretreatment process. Sodium hydroxide (NaOH) was selected as the representative of alkali since the literature indicated that alkali pretreatment results in the structural modification of a spectrum of biomass. The time and MLR of the pretreatment were kept between 10 to 30 minutes and 0.05 to 1.0 respectively as suggested in the literature.

The Box Behnken Design (BBD) is a widely used experimental design in response surface approach to optimize the process parameters. BBD is a rotatable second-order factorial

design that has better prediction accuracy and requires fewer experiments as compared to the other experimental designs. Accordingly, a full quadratic BBD-based experimental array was considered in the study. Accordingly, a full quadratic model was defined from the experimental design array by using multiple linear regression analysis and analysis of variance (ANOVA). The three-dimensional response surface plots were obtained to identify the pretreatment parameter settings for the removal of lignin. The optimum pretreatment parameter level for maximizing the removal of lignin content was determined through numerical optimization by adopting the D-optimality procedure.

### 3.5. Validation of biochar using fish model:

The obtained biochar was activated by various physicochemical methods as mentioned above. For the adsorption study, three different concentrations of Pb (II) working solution of 10 ppm, 100 ppm, and 1000 ppm were prepared respectively. As per standards 10 ppm of lead in water is considered to be wastewater so we took that. Sometimes the water near industrial area also contains high lead concentration that varies from 100 ppm to 1000 ppm. Fish were kept in above mentioned three concentrations of lead-contaminated water. Two sets were prepared for each of these three concentrated water. One was kept normally and the another was treated with biochar.

### 3.6. Systematic position of selected fish:

*Anabas cobojius*, the ‘Gangetic koi’ was selected for the present investigation. The systematic position of the selected fish is described below.

**Table 3.2. Systematic classification of Gangetic Koi.**

<b>Kingdom</b>	:	<b>Animalia</b>
Phylum	:	Chordata
Class	:	Actinopterygii
Order	:	Anabantiformes
Family	:	Anabantidae
Genus	:	Anabas
Species	:	cobojius

### 3.7. Reason for selecting fish:

Selection of a suitable organism depends upon a number of factors. Some are described below.

1. It is a vitally important fish both economically and commercially.
2. Throughout the year, it is easily accessible in large quantities.



3. Throughout the year, the fish breed on their own.
4. It is easily adaptable to lab condition.
5. It does not require a lot of time and energy to maintain.
6. It has resistance to disease and other causes which weaken the fish (Transport and Wintering).
7. It is an ideal animal for toxicity studies in aquatic biology.

### **3.8. References:**

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# Chapter 4 | RESULT AND DISCUSSION

## 4.1. Biomass characterization:

The bulk density of the biomass has been reported to have a notable impact on biomass composition. A higher bulk density indicates a better lignin content, better logistics, and better heating value. The bulk density of the biomass under evaluation was determined by following standard literature reported protocol (as indicated in the material and methods section) and was found to be  $(0.322 \pm 0.002)$  gm/cm<sup>3</sup>. The obtained result gleaned that, the biomass under evaluation has a high bulk density. Accordingly, it may be corroborated that, biomass might have higher lignin content and accordingly, can be a very good feedstock for the preparation of biochar, which in turn may be used for the removal of heavy metals from wastewater.

### *Compositional analysis results:*

The compositional analysis of the biomass under evaluation was carried out in terms of lignin, cellulose, hemicellulose, and extractive content. The obtained result has been portrayed in Table 4.1. The cellulose content of the selected biomass (Bamboo leaf) is  $(18.299 \pm 0.642)$  %. The amount of hemicellulose from the compositional analysis is reported to be  $(15.329 \pm 0.409)$  %; whereas  $(23.668 \pm 0.888)$  % lignin is present. From Table 4.1 it may be conferred that the biomass has a significant amount of lignin present. The result obtained from the analysis of bulk density also indicated a similar drift. Accordingly, it may be conferred that the chosen biomass is a potential feedstock for biochar preparation which in turn may be used for heavy metal removal purposes from sewage.

**Table 4.1. Compositional analysis of biomass in terms of cellulose, hemicellulose, lignin, and extractive content.**

Biomass name	Cellulose content (%)	Hemicellulose Content (%)	Lignin Content (%)	Extractive Content (%)
Bamboo leaf	$18.299 \pm 0.642$	$15.329 \pm 0.409$	$23.668 \pm 0.888$	$8.659 \pm 0.309$

### *Proximate analysis and HHV results:*

The HHV of biomass (measured in terms of MJ/kg) is an insightful parameter for characterizing biomass. The HHV of the biomass under evaluation was found to be  $(16.737 \pm$

0.018) MJ/Kg. The obtained result was found to be in line with the results obtained from the bulk density analysis and compositional analysis.

The proximate analysis of the biomass was carried out in terms of FC, VM, and A in terms of % by following standard literature reported protocol as described in the earlier section. The results obtained from the proximate analysis have been presented in Table 4.2. The presence of higher volatile matter content may be corroborated with the presence of higher lignin content. Moreover, the presence of lower ash content also makes biomass suitable as the biochar feedstock. The presence of higher fixed carbon in the biomass may be corroborated by the presence of a higher heating value of the biomass under evaluation.

**Table 4.2. Proximate composition and calorific value of the biomass under consideration.**

Biomass	(FC) (%)	(VM) (%)	(A) (%)	HHV (MJ/Kg)
Bamboo leaf	15.395 ± 0.145	81.861 ± 0.258	2.745 ± 0.131	16.73748 ± 0.018811

**Ultimate analysis results:**

The ultimate analysis of the biomass under evaluation was performed by using standard literature reported protocol as mentioned in the earlier section. The obtained results have been presented in Table 4.3. The analysis was carried out in terms of carbon, hydrogen, nitrogen, and oxygen content. The presence of higher carbon and oxygen content in turn may be corroborated by the presence of higher volatile matter content and lignin content.

**Table 4.3. Ultimate analysis result of selected biomass (bamboo leaf) in terms of Carbon, Hydrogen, Oxygen, and Nitrogen content.**

Biomass sample	Carbon content (%)	Hydrogen content (%)	Oxygen content (%)	Nitrogen content (%)
Bamboo leaf	44.251 ± 0.056	5.651 ± 0.007	44.112 ± 0.079	1.790 ± 0.099

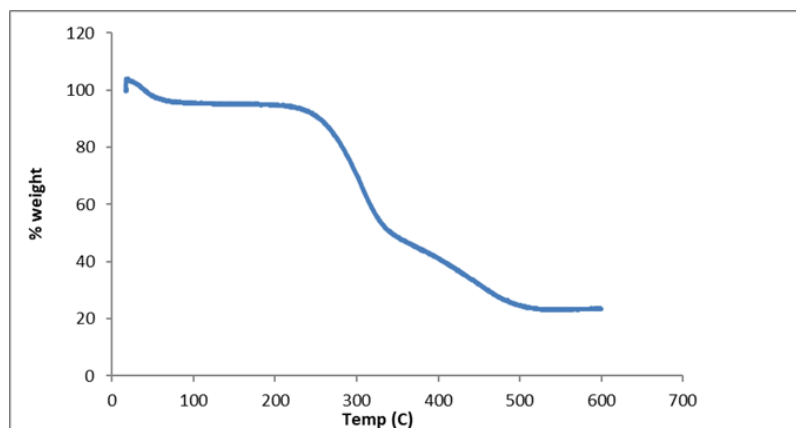
**TGA result analysis:**

The thermal degradation profile of the biomass evaluated in this study was carried out. The obtained result has been indicated in Figure 4.1. The biomass was subjected to controlled heating at the rate of 10 °C per minute under a nitrogen flow rate of 50 ml/ minute. The initial mass loss observed may be corroborated due to the loss of the moisture content. The second phase of mass loss may be accounted for due to the loss of the volatile components. The final stage of mass loss may be accounted for due to the loss of the ash content. The thermogram confirmed the presence of higher volatile matter content which was already decrypted from the proximate composition analysis.

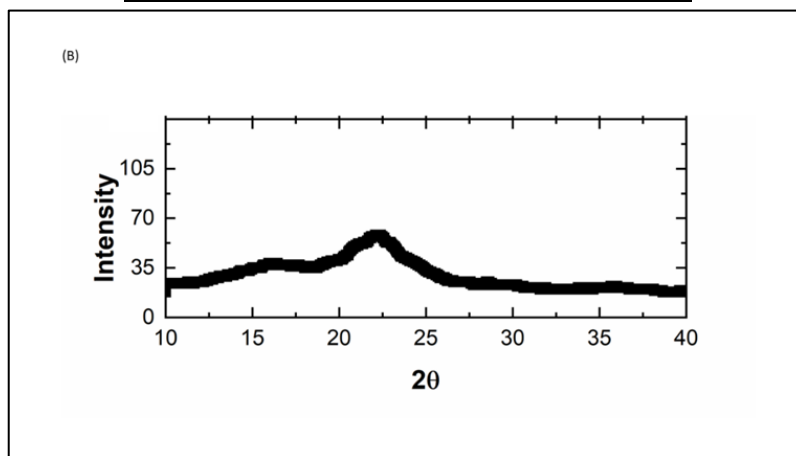
**XRD result analysis:**

The x-ray diffraction analysis was carried out to confirm the presence of holocellulose and lignin content in the biomass under evaluation within the range of 10-40 (2θ). The

presence of the peak at 16 ( $2\theta$ ) indicates the presence of lignin, and the presence of the peak at 23 ( $2\theta$ ) confirms the presence of the holocellulosic compartment.



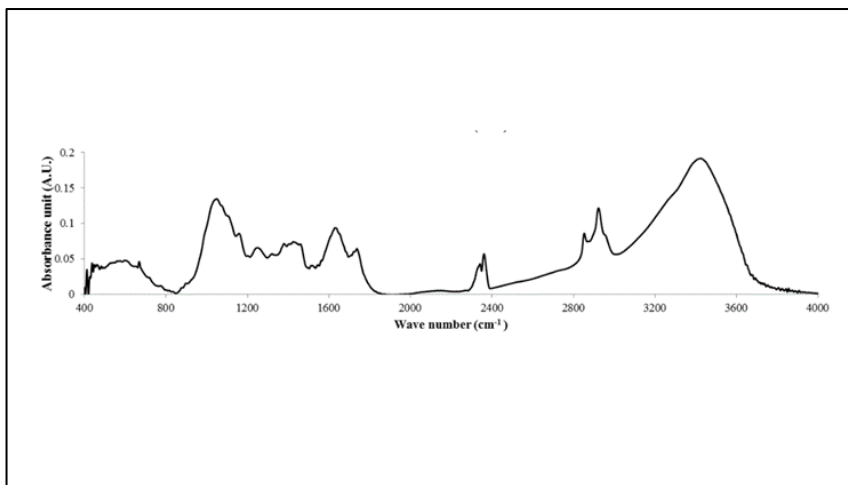
**Figure 4. 1. TGA analysis of bamboo leaf.**



**Figure 4. 2. X-Ray diffraction pattern of bamboo leaf.**

***FTIR result analysis:***

In order to have an insight into the presence of the chemical functionalities, FT-IR analysis was carried out with the biomass under evaluation. The FT-IR spectroscopy characterizes the functional group and fingerprint region of very small quantities of samples. The infrared spectra are obtained by using FT-IR spectrometer. The spectra may be taken in various wave-length regions as desired at the given room temperature. *Figure 4.3.* demonstrates a variety of wavenumber of  $400\text{ cm}^{-1}$  to  $4000\text{ cm}^{-1}$ . The absorbance unit (A.U.) also ranges between 0 to 0.2. Highest peak of absorbance unit is between  $3200 - 3600\text{ cm}^{-1}$ . The result of FTIR analysis suggested that there are a Broad and strong, O-H stretch from hydroxyl group of alcohols present.



**Figure 4. 3. FTIR spectra of bamboo leaf.**

## **4.2. Pretreatment and optimization results:**

The pretreatment is a prerequisite step that needs to be applied to any biomass prior to being used as the feedstock for any conversion process through either biochemical or thermochemical routes by partially degrading their recalcitrant nature. Biomass pretreatment also improves their characteristics in order to be applied to various purposes. In this study, the bamboo leaf was pretreated with the alkali reagent sodium hydroxide for the selective removal of lignin. Optimization of this pretreatment is described below.

Response Surface Approach/Methodology (RSM) was chosen instead of Machine Learning (ML) or Artificial Intelligence (AI), because, they require hard-core knowledge of coding languages like PYTHON, C++, etc. Programming languages may not be an easy task for people with different backgrounds other than computer science. Response Surface Methodology is easy to understand and is user-friendly. It provides us with an equation that proves to be fruitful. AI on the other hand gives no model equation. RSM may be of different types, like, Full-Factorial Design (FFD), Central-Composite Design (CCD), Box-Behnken Design (BBD), Taguchi model, etc.

Research has shown that among the many experimental design methodologies, a Full-Factorial design (FFD) is frequently criticized as unworkable owing to the necessity of a high number of experimental trials (88). Taguchi Model is found to produce a high Signal-to-Noise ratio (S/N ratio), and hence the results become unreliable (89). The Central-Composite design (CCD) and Box–Behnken design (BBD) are often used as experimental design approaches because they are not reliant on full or fractional factorial designs and have the desirable quality

of accurate prediction over the whole factor space addressed in research. However, the research indicates that, in terms of precision and accuracy, the BBD technique is far superior to the CCD for a complete quadratic response surface model with three or more components (90). BBD is a rotatable second-order factorial design that places points on the midpoints of the cubical design region's borders as well as points in the center. As a result, BBD was chosen for this investigation.

As a result, utilizing multiple linear regression analysis and analysis of variance, a comprehensive quadratic model was generated from the experimental design array (ANOVA). The statistically significant model terms were selected using the p-values associated with them (92). At a 95% confidence level, model terms with p-values greater than 0.05 were judged statistically insignificant. The statistical programmed Design-Expert (v11) was utilized for all of the statistical analyses in the study. The three-dimensional response surface plots were obtained to identify the parameter settings.

Three factor-three level BBD was explored for the optimization of the processing parameters utilizing the response surface technique in order to obtain maximum removal of lignin. Time, MLR and strength were evaluated to three levels and the array of distinct variables and their corresponding levels was used as the design points for the BBD matrix, as shown in *Table 4.4*.

**Table 4.4. BBD Matrix for the removal of lignin along with three factor level and their response.**

Factor level			Response
Time	MLR	Strength	
30	0.075	0.5	2.63
10	0.1	0.75	5.98
20	0.05	1	4.41
30	0.075	1	3.425
20	0.1	0.5	5.063
20	0.1	1	4.88
20	0.075	0.75	3.29
30	0.05	0.75	3.78
10	0.075	1	4.33
10	0.05	0.75	2.43
10	0.075	0.5	3.646
20	0.075	0.75	3.29
30	0.1	0.75	7.19
20	0.05	0.5	2.67

20	0.075	0.75	3.29
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After the designs were proposed and modeling was done, a Fit Summary was obtained as shown in *Table 4.5*.

**Table 4.5. Fit summary of lignin removal from selected biomass.**

Source	Sequential p-value	Lack of Fit p-value	Adjusted R <sup>2</sup>	Predicted R <sup>2</sup>	
Linear	0.0295		0.4192	0.0878	Suggested
2FI	0.8635		0.2683	-0.9528	
Quadratic	0.2294		0.4711	-2.0223	
Cubic			1.0000		Aliased

Design-Expert contains a variety of useful data tables to assist you in deciding which model to research further (the selection is actually made on the Model button screen). The software highlights and labels as "Suggested" the full-order model that meets the conditions given below. Design-Expert displays a warning regarding aliased models. In this circumstance, there aren't enough unique design points to estimate all of the model coefficients. Contour plots with misleading shapes may occur as a result of the least squares estimates not being unique.

The values obtained after optimization modelling tend to give a linear model as the best model. The ANOVA was performed with the experimental design array of three associated factors, indicated in *Table 4.6.*, *Table 4.7.* and *Table 4.8.* respectively.

**Table 4.6. Results of ANOVA for the linear model.**

Source	Sum of Squares	df	Mean Square	F-value	p-value	
<b>Model</b>	13.26	3	4.42	4.37	0.0295	significant
A-Time	0.0510	1	0.0510	0.0504	0.8264	
B-MLR	12.06	1	12.06	11.92	0.0054	
C-Strength	1.15	1	1.15	1.14	0.3089	
<b>Residual</b>	11.13	11	1.01			
Lack of Fit	11.13	9	1.24			
Pure Error	0.0000	2	0.0000			
<b>Cor Total</b>	24.40	14				

**Table 4.7. Fit statistics for ANOVA linear model.**

Std. Dev.	Mean	C.V. (%)	R <sup>2</sup>	Adjusted R <sup>2</sup>	Predicted R <sup>2</sup>	Adeq Precision
1.01	4.02	25.03	0.5437	0.4192	0.0878	6.1876

**Table 4.8. Result of ANOVA for the proposed modelling.**

Factor	Coefficient Estimate	df	Standard Error	95% CI Low	95% CI High	VIF
Intercept	4.02	1	0.2598	3.45	4.59	
A-Time	0.0799	1	0.3557	-0.7030	0.8628	1.0000
B-MLR	1.23	1	0.3557	0.4450	2.01	1.0000
C-Strength	0.3795	1	0.3557	-0.4034	1.16	1.0000

ANOVA was performed, and a FIT Statistics was obtained, which in turn will help in building up an equation (Table 4.9).

**Table 4.9. Final equation in terms of Actual factors obtained after performing ANOVA.**

Lignin removal	
-0.961608	
+0.007988	Time
+49.11500	MLR
+1.51800	Strength

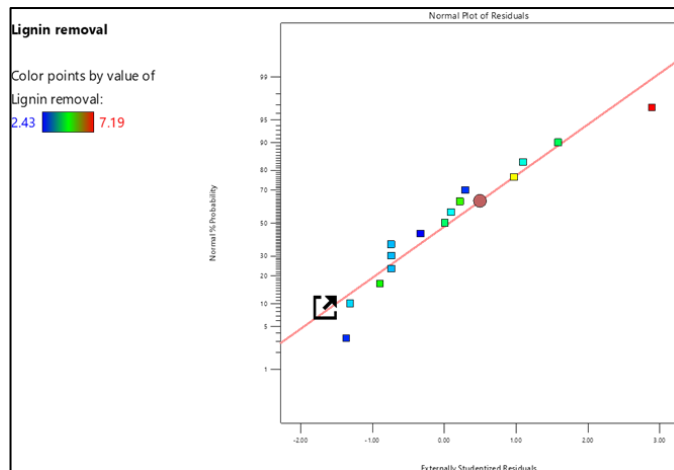
The accuracy of the model prediction was tested using the final response surface model shown in equation. The amount of lignin in the biomass sample following pretreatment at the same factor level settings was then evaluated experimentally. Following that, the acquired lignin removal was plotted against the experimental value. The achieved result is depicted in Figure 4.4.

Figure 4.4. also shows that the model proposed works for the better removal of lignin ranging from 2.43 to 7.19.

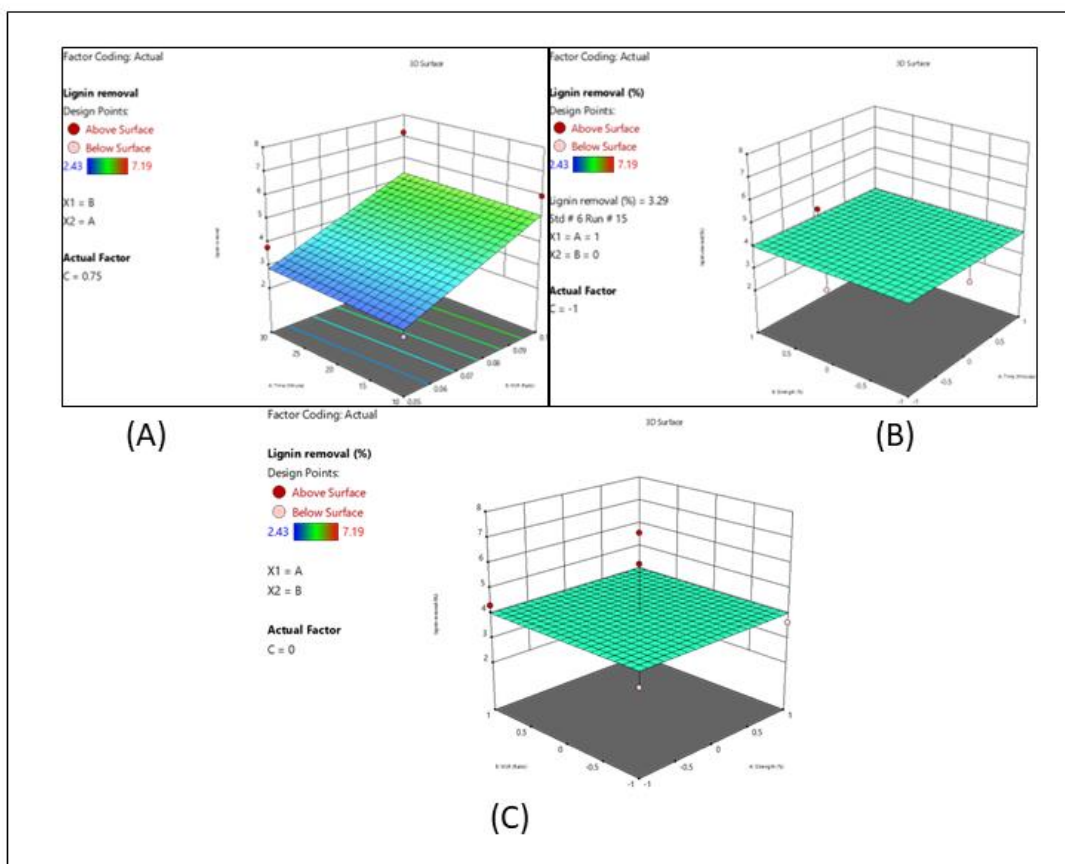
The final response surface model was used to determine the factor-level setting for the removal of lignin. Figure 4.5. depicts the three-dimensional response surface plots.

Finally, D-optimality was obtained with a removal efficiency 5.192 and the other values are mentioned in Table 4.10.





**Figure 4. 4. Plot showing predicted removal amount of lignin vs the experiment.**



**Figure 4. 5. Three-dimensional response surface plots of various parameters on the removal of lignin of pre-treated sample (two factors at a time). (A) Time and MLR conc. (B) Strength and time. (C) MLR and strength.**

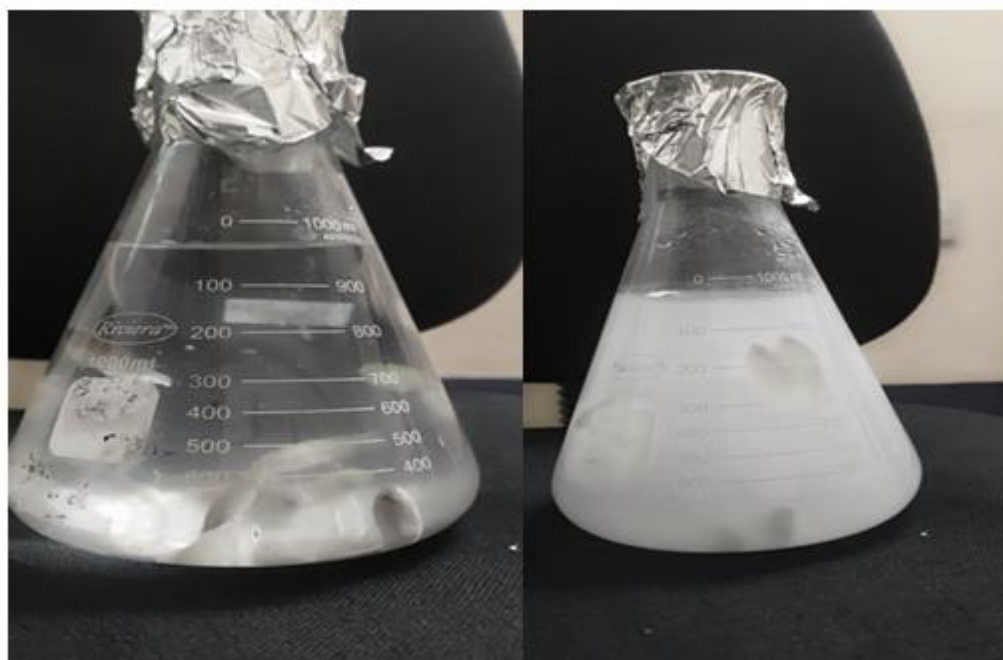
**Table 4.10. Conformation location: D optimality = 1**

Time	MLR	Strength	Removal efficiency
23.5	0.1	0.71	5.192

### 4.3. Validation of fish model:

As mentioned in the ‘methods and methodology’ three different concentrations of 10 ppm, 100 ppm, and 1000 ppm of Pb (II) solution was prepared and fishes were kept in them. Per 1 liter of stock solution, 8 fish were added. When the fish was kept in the set of 1000 ppm solution that was untreated with biochar, it was observed that the fish survived nearly 2-3 hours; for 100 ppm solution, the survival rate increased a little bit and finally, for the stock solution of 10 ppm the fishes were noticed not to be dead but according to various literature survey heavy metal lead (Pb) must be bioaccumulated in them causing several toxicities.

Per 1 liter of the stock solution 1gm of biochar was added in the remaining three sets of 10 ppm, 100 ppm, and 1000 ppm solution. A significant change in the survival time of fishes has been observed. No, fish in any of the sets were reported to be dead. Some images were taken while performing the experiment before and after treatment process mentioned in *Figure 4.6.*, *Figure 4.7.* and *Figure 4.8.*



**Figure 4. 6. Set of 1000 ppm before and after treatment.**



**Figure 4. 7. Set of 10 ppm before and after treatment.**

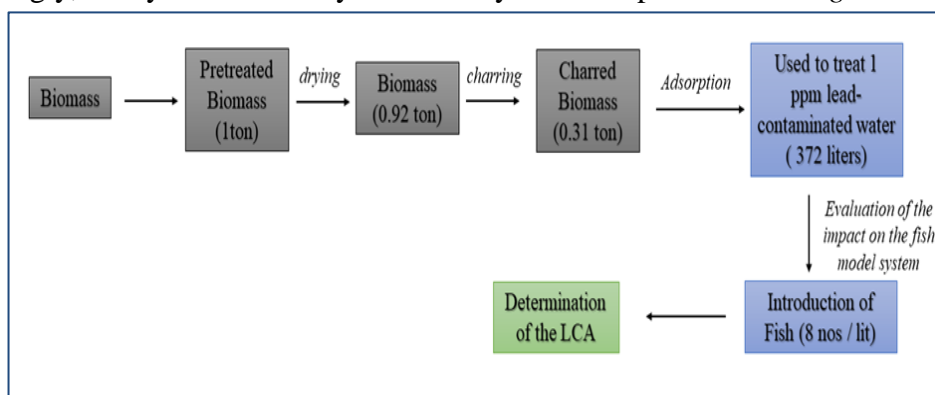


**Figure 4. 8. Set of 100 ppm before and after treatment.**

## Chapter 5 | LIFE CYCLE ANALYSIS (LCA)

The present study proposed a novel approach toward the utilization of a waste biomass category (chosen in the form of the bamboo leaf) for alleviating the heavy metal concentration in water which in turn prevented the accumulation of such heavy metals into the fishes and thereby prevent bioaccumulation. Accordingly, an ardent need was identified to investigate the overall sustainability of the proposed intervention. The comparison has been carried out with respect to various midpoint indicators namely, global warming potential (GWP), acidification potential (AP), eutrophication potential (EP), ozone depletion potential (ODP), human toxicity potential (HTP), and Terrestrial eco-toxicity potential (TETP) by Life Cycle Assessment (LCA) using GaBi® Software (educational version). LCA is a useful tool that helps in the quantitative investigation of the environmental impact contributed by a product or process throughout its life cycle [1]. It is also known as cradle to grave analysis. The LCA study was performed in accordance with the International Organization for Standardization (ISO) 14040 which includes four stages namely: 1) Defining the goals and scope of the study; 2) Inventory identification and quantification; 3) Evaluation of the potential environmental impacts, and 4) Interpretation of the results (ISO 14040).

Defining the life cycle inventory (LCI) is an important aspect of the life cycle assessment study. Accordingly, the system boundary of the study has been presented in *Figure 5.1*.



**Figure 5. 1. System Boundaries for the present study.**

The results obtained from the LCA simulation have been presented in *Table 5.1*. The result has been presented under the purview of the six midpoint indicators. The entire

simulation has been conducted by taking 1-ton biomass as the unit of operation. The indicators were chosen in order to ensure and cover a wider perspective regarding the detrimental impacts of the proposed intervention detailed in this study.

**Table 5.1. Stimulation of Life Cycle Analysis (LCA).**

Indicator	Unit	Value
Global warming potential (GWP)	kg CO <sub>2</sub> equivalent	0.98
Acidification potential (AP)	kg SO <sub>2</sub> equivalent	0.022
Eutrophication potential (EP)	kg Phosphate equivalent	0.00062
Ozone depletion potential (ODP)	Kg R 11 equivalent	0
Human toxicity potential (HTP)	Kg DCB Equivalent	0.0042
Terrestrial and ecotoxicity potential (TETP)	Kg DCB equivalent	2.01E-06

The data obtained from the sustainability analysis indicated that the proposed intervention has a minimum detrimental impact on the environment. The obtained values indicated that out of six midpoint indicators, the value of only GWP is relatively high. Such observation can be corroborated because heating of the biomass was a part of the biomass pretreatment process for selective lignin removal. The obtained values for the other five midpoint indicators evaluated in this study were significantly lower which in turn signifies that the overall process is environmentally sustainable and may be replicated for further scale-up, thereby promoting sustainable and green solutions.

### 5.1. References:

1. Khasreen, M. M., Banfill, P. F., & Menzies, G. F. (2009). Life-cycle assessment and the environmental impact of buildings: a review. *Sustainability*, 1(3), 674-701.

## Chapter 6 | CONCLUSION

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The present work was essentially motivated by the need to derive a cost-effective, economically less important biomass as a reliable resource or form of biochar in order to treat wastewater that contains very harmful heavy metals as successful implementation of any process for biomass conversion primarily depends upon the availability of low cost and good quality feedstock. Physicochemical properties of biomass and their thermal degradation profile were investigated by using a thermogravimetric analyzer (TGA). Bamboo leaf, the selected biomass for the investigation was characterized in various means like ultimate analysis, proximate analysis, bulk density, FTIR, XRD, etc. Several optimizations were performed and it showed the best result in order to remove the maximum amount of lignin. After the optimization was done the derived biochar from bamboo leaf (*Bambusa vulgaris*) was validated using a fish model. The fish model also showed a significant result. After performing the present investigation, I can conclude that biochar can be obtained from bamboo leaves, agricultural waste as well as lignocellulosic biomass and after pretreatment using alkali it has a very significant effect on the removal of heavy metal (lead) from wastewater or heavy metal contaminated water in an eco-friendly manner.

### ***Future scope:***

Several areas of interest with significant research potential have been identified based on the work contained in the thesis. It is recommended that future research should be focused on the following investigations:

- Extended studies on effects of other pyrolysis parameters like particle size, vapor residence time, sweep gas flow rate, etc. Further, the up-gradation of biochar can be studied by using catalytic modification.
- Extended studies may be carried out in the case of solid-state kinetic analysis of raw biomass.