PLANT-BASED BIOCHAR FOR ENVIRONMENTAL REMEDIATION: SYNTHESIS, CHARACTERISATION, AND APPLICATION

A PROJECT REPORT

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CERTIFICATE

We hereby certify that the project dissertation titled “Plant-based Biochar for Environmental Remediation: Synthesis, characterization and Applications” which is submitted by **Vijay Pratap Singh, 2K20/IBT/12**, Department of Biotechnology, Delhi Technological University, Delhi in partial fulfilment of the requirement for the award of the degree of Master of Technology, is a record of the project work carried out by the student under our supervision. To the best of our knowledge this work has not been submitted in part or full for any Degree or Diploma to this University or elsewhere.

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CANDIDATE’S DECLARATION

I, **Vijay Pratap Singh, 2K20/IBT/12** student of M. Tech (Industrial Biotechnology), hereby declare that the project Dissertation titled **“Plant-based Biochar for Environmental Remediation: Synthesis, characterization and Applications”** which is submitted by me to the Department of Biotechnology, Delhi Technological University, Delhi in partial fulfilment of the requirement for the award of the degree of Master of Technology is original and not copied from any source without proper citation. This work has not previously formed the basis for the award of any Degree, Diploma Associateship, Fellowship or other similar title or recognition.

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ABSTRACT

Biochar is a solidified, obstinate natural C-compound produced when plant *Nephrolepis auriculata* biochar is heated to temperatures ranging from 150 to 900 °C. Biomass feedstock is produced from a variety of biomass matter, like, farming deposits, wood cock, and civil strong contaminants, using a variety of warm medicines, the most widely used of which slow pyrolysis an account of its adequate is active circumstances and improvement of biochar flex. Regardless of the new presentation of the term 'biochar' for this material, roasted materials had a few uses the earlier because of their novel character (for example huge unambiguous exterior region, nanoporous, and surface assimilation capacities). This untimely utilization has firstly concentrated on the utilisation of biochar as a soil correction in farming, but another utilization in ecological emend design perhaps uniformly significant (for example for loam and aquifer analysis and stormwater channel media). Biochar is the best way to treat heavy metals and dye, eco-friendly and economical for the countries. Developing countries working on it and developed countries are already follow-up that natural remediation techniques. The purpose of this survey is to supply a point-by-point assessment of the design character and capacity applications of biochar as a designed matter for natural emend. Biochar, because of its profound factor and adjustable exterior science, has extraordinary capacity in a diversity of design functions, some of which are still unknown.

Keywords: Biochar, remediation, characterisation, synthesis.

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LIST OF SYMBOLS AND ABBREVIATIONS

DGT- Diffusive the Slope

CEC- Cation Exchange Capacity

CIA- Chemical Index of Alteration

IPU- Irrigation Potential Utilised

OD- Optical density

µm- Micro-meter

nm- Nano-eter

ºC- Degree Celsius

AC- Activated carbon

BC- Biochar

FT-IR- Fourier transform infrared

SEM- scanning electron microscope

CHNS- Carbon, Hydrogen, Nitrogen, Sulphur Analyser

RhB- Rhodamine-B

MB- Methylene Blue

Hg- Mercury

CHAPTER 1: INTRODUCTION

1.1 Background

Biochar is a multifaceted cause of environmentally friendly zest with the capacity to produce intensity, and power. The employ of biochar for vitality and as a farming change is not a novel concept: the conversion of bio-mass matter into dark carbon has been registered since the dawn of present-time science, and we have archaeological verification of much more seasoned utilization [1]. As genuine attentiveness to environmental replacement reduction and sustainable vitality beginning has fattened, further awareness has been attentive to the development of strong items from biomass (biochar) for carbon secludedness motive [2], [3]. Biochar is a dominantly stable, obstinate natural carbon (C) compound produced when biomass (feedstock) is heated to inversions typically between 150 and 1000 °C, under low (ideally zero) oxygen fixations [4], [5]. Biochar is required for the development of matter known as jet black carbons, which are undeniably delivered beside both synthetic and cosy swaps of the initial biomass matter and others have depicted the range of pyrolysis items remembered for this arrangement in words of enlarging of carbon gratified. On this continuum, biochar is addressed by burning buildups with O: C molar proportions less than 0.5 [6]–[8]. Recent audits have focused on biochar's agronomic applications. Considerably less thought has been given to the use of biochar in design utilizations, which may be a further actual utilization of the matter given the financial estimates of dark carbon creation for quickest horticultural use have been difficult for quite some time [9]–[11]. As a result, the utilisation of little value of biochar in specific applications is uplifted too to hold up a wide range depending on markets for biochar and work on the financial aspects of biochar manufacturing [12]. Regardless of whether the biochar is scorched for energy age or not, the general reason for carbon forfeiture is quite protected, regardless of actual biochar even though may not be straightforwardly integrated into the loam. The substantial and enzymatic character of biochar can differ remarkably based on the feedstock and thermodynamic change (creation) reaction [12], [13]. Appropriately, the appearance of bio-char in lots of area utilization is inextricably linked with twain the creation technique utilised with the synthesis of the fount matter. To greater acknowledge the current state of obtainable bio-chars and the intimation of their utility as a designed matter, the bond in the middle of biochar character, manufacturing, shape, and content arrangement should be accepted. To that finish, an outline of their present understanding concerning the impact of origin matter and creation advances on bio-char qualities is conferred. Due to its remarkable character, bio-char can be used in a broad extent of natural rehabilitation utilization. The anticipated applications, difficulties, and valuable open doors as bio-char utilize in natural rehabilitation are discussed.

1.2 Biochar feedstock

Various types of corn cob, sugar beet, corn tail, rice straw, waste wood, and wheat deposits have been utilized for biochar synthesis [14]. The synthesis and extent of various constituents varies depending on the type of biomass this includes lignin, trace amount of extractive along with cellulose and hemicellulose [15], [16]. Because of the differences in the amount of lignin and available carbon content present among original biochar sources, the amount of biochar which has been produced from biomass differ greatly, most of which is provided significantly returns consistently coming from lignocellulosic (wood-determined) biomass [18], [19]. In this regard, insufficient production efficiencies or potentially intermittent accessibility of specific feedstocks (for example, low-lignin biomass) could restrict their ubiquitous use [20], [21]. For example, the intermittent accessibility and geological spread of horticultural deposits make it difficult to operate biomass-specific power plants [22]. As a result, it may be preferable to use a combination of rural deposits, excrements, metropolitan natural food squanders, and woodcock to compensate for declined accessibility of the items during the cold weather months.

1.3 Conversion/Production Technologies

Sorption transformation for converting biomass into environmentally friendly potential articles along with biochar is classified as pyrolysis, gasification, and aqueous carbonization. This research depicts the working conditions (temperature and home time), common item yields, and carbon items in biochar created by various change procedures. Because of the pyrolysis conditions, the yields of various items vary greatly. Chen et al. (2012) studied the development of pyrolysis which employed the use of corn straw and discovered that with the increase in temperature from 250 to 650 °C inside a similar pyrolysis unit, the charcoal yield decreased dramatically, from 66.5 to 26.7 per cent. With the further rise in temperature from 650 to 950 °C no significant changes in charcoal yield were not observed [23]. There is a general decline in the yield of the strong residuals with rising creation temperature, with a normal range of 15 to 60 per cent of the dry-weight biomass in the strong residuals [24], [25]. Pyrolysis advancements can also be identified by the pyrolysis material's response season (ex: - easy and quick pyrolysis action). The following pyrolysis conditions favour high biochar yields: (I) high lignin, debris, and N content in the biomass, (ii) low pyrolysis temperature (400 °C), (iii) somewhat high cycle pressure, (iv) long fume home time, (v) broadened fume/strong contact, (vi) low warming rate, and (vii) massive biomass, (viii) advanced heat mix and molecule size [22]. Slow pyrolysis produces more biochar than quick pyrolysis, but quick pyrolysis produces more fluid. Even though slow pyrolysis frequently produces exceptional burn returns (up to 40% wt), the produced gas is a generally low-energy fuel with only minor warming worth (Brown et al. 2011). As a result, while slow pyrolysis is the most effective innovation for biochar production, it may not be the best innovation for generating associative energy from recovered pyrolysis gas [2]. One intriguing record is that many of the warm transformation innovations employ a moderately giant inversion (200 °C), necessitating a massive inceptive power process. However, substance aided pyrolysis, like corrosive treatment, can be carried out at temperatures of 100 °C and could be used to improvise the sustainability and reusability of biochar production (Barra et al., 2022), for example, evolved a practical method for preparing biochar that includes hydrolysis of waste including rice husk involving sulfuric acid under low temperature and environmental stress. At 95 °C for 6 hours, the created circle-like carbon materials with molecule sizes of 500 nm were obtained [26]. Biochar provided in acidic conditions could also be enacted for improvised sorption properties [27], especially provided that conventions for corrosive initiation of scorched material have previously been laid out utilising actuated charcoal [28].

1.4 Objective

This project aims to

- Synthesize biochar from *Nephrolepis auriculata*
- Characterize the synthesized biochar
- Check remediation efficiency of synthesized biochar against Mercury, Rhodamine-B and Methylene Blue

CHAPTER 2: LITERATURE REVIEW

2.1 Biochar Characterization

Biochars delivered through various change innovations have diverse properties because of the variety of working conditions and post-handling medications used. The tremendous changeability of biochars, as well as site-specific collaborations with soil and vegetation, has made it extremely difficult to clearly establish links between the physiochemical properties and relevance as a soil correction [29]. On account of this inconsistency, determining the best feedstock and manufacturing strategy for biochar for a specific utilization had remained largely dependent on the situation. To obtain proficient, sustainable, large-scale and economical biochar production with ideal sorptive properties, for example, research that should expand our now limited understanding of the overall impact of transformation innovation and gives feedstock on biochar properties [30].

2.1.1 Carbon Composition

Although biomass is incineration, labile natural material (for example, biomass) is successfully obliterated, as calcified carbon leftover in salvageable form.As higher temperatures are expected to consume more stable types of Carbon from different sources (for example, cellulose present in wood strands), the pyrolysis temperature attained freely direct how much labile Carbon is lost (estimated by the unpredictable matter substance) and thus at last fixed natural Carbon substance of the resultant scorch (generally equivalent to the debris content) [31]–[33]. Generally, debris feedstock rises with rising scorching inversion and term, whereas feedstock declined with increasing inversion and span. The C substance increases as the pyrolytic temperature rises, while the Oxygen and Hydrogen contents decrease, indicating that the burns are becoming more carbonised [34], [35]. Relative natural items among biochars produced at various inversions exhibit a fast decline of Oxygen and Hydrogen in the middle of 300 and 500°C. Mistakes in H and O content are presumed due to the cleavage and breaking of fragile bonds present within the biochar structure at high pyrolysis temperatures [36], [37]. One exception to this pattern was observed in corn-tail determined biochar: the estimated carbon content decreased with pyrolysis temperature, falling from 56.8 per cent at 300°C to 48.4 per cent at 500°C [38]

2.1.2 Non-carbonized Composition

N in biochar decreases with increasing pyrolysis temperatures [38]. In any case, directly was never a critical pattern in the middle of N feedstock and inversion in some tests. The N contents of wood singes were somewhat consistent, but grass scorches had the highest (1.24 per cent) at 400 °C, demonstrating the improvement of N-containing compounds [39], [40]. Indeed, in some tests, N content increased with roasting temperature and span, ranging from 0.97 to 1.73 [41]. The higher pyrolysis temperature and longer length of time were also accompanied by an increase in total P and total K [38]–[40]. Some pre-treatment methods also have an impact on the basic structure of bio-chars. It is remarkable that, except for K, the vast majority of the inorganic components in the build-up materials expanded after anaerobic absorption [42]. Typically, feedstock synthesis also takes a part in a significant character in deciding the essential component of bio-char. Compost-derived biochar, for example, is commonly rich in soil supplements such as N, P, Ca, Mg, and K. (Lee et al., 2020; Santra et al., 2020; Yang et al., 2021). Higher concentrations of N and P in wastewater muck biochar, as well as other small and large-scale supplements, have also been cited as the primary reason for the use of wastewater ooze for biochar production and agricultural use (Lee et al., 2020). In comparison to lignocelluloses' biomass, biochar from green growth was similarly low in C (20.5322.1%), high in N (1.94.0%), with H contents ranging from 2.5 to 4.1 per cent, and also high in a few inorganic components such as Na and K. (Yang et al., 2021).

2.1.3 Zeta-Potential and pH

A negative charge resides on the outer charcoal layer as it primarily comprises of BC and AC. This makes adsorption of phosphate and other negatively charged entities rather difficult (Yao et al. 2011). pH advantages increased with rise in temperatures of pyrolysis of biochar is within the range of 9.1 to 10.5 (Lee et al., 2020; Santra et al., 2020; Yang et al., 2021). Studies have stated that every time an unbiased biochar is synthesized and are acidic in their carbonized aqueous form (Chahal et al., 2019).

2.1.4 Viscosity and Surface Region

The rasping transmutation state and rise transmutation inversions outcome in a fatten amount of biochar morsel with more modest molecule size dispersions (*Kim et al.* 2012). *Kim et al.* identified non-identical dimensions area of the orifice in bio-char fragment using Scanning Electron Microscope (SEM) images, along all take part in some important part of the sorption characteristics of permeable materials. Casein biochar was described with mostly mesopores (3.7 to 41.9 nm) and full-scale pores (> 87.94 - 6809.93 nm) (*Purevsuren* et al. 2003). In prevalent, rising temperatures caused a spectacular increase in viscosity and exterior region (Bird et al. 2011; *Keiluweit et al*. 2010). Temperature is a significant variable in burn development because the exterior region is an important gauge of the roast take-up capacity.

2.1.5 Cation inter-change abilities

Abundantly bio-chars have approximately giant cation trade limit (CEC), owing to their pessimistic exterior charge and consequent preference for loam cations which majorly comprises of thrash metals (for example, Cr^{3+}, Pb^{2+}). As a result, a few examined have been regulated to investigate the sorption of cations into bio char [35], which has been the first tool by to employ biochar for heavy-metal tainting rehabilitation. Significant variation lying between 4.5 – 40 $molkg^{-1}$ in CEC is observed among various feedstock and biomass generated by earth [30]. In a comparison of lots of earthbound bio-mass bio-chars, the Cation interchange abilities of green growth biochar are moderately high. The CEC of biochar-modified loam is highly relative on the age, exterior useful character [46]

2.2 Biochar Utilization for Environmental Rehabilitation

The huge exterior region and cation trade limit are not entirely determined by starting materials, pyrolysis inversion, and any post-production handling [47]. A large exterior region is significant for the adsorption of both natural and man-made pollutants on biochar [47], which is an important system for reducing toxin versatility in contaminated soils. Several lab studies have been conducted to investigate numerous kind of bio-char and their ability to sorb man-made and natural foreign substances in lots of mediums and arrangements. They deliberated that have revealed that biochar has beneficial effects on toxin sorption. Regardless, variations in soil natural matter substance can cause special cases: Discovered that in loam with huge levels of broken down natural matter, the capacity to adsorb a specific animal variety is sometimes smaller in biochar revised loam than in unaltered reign loam [48]. Furthermore, some field examine [49], on enlarge escalate and lengthy time of biochar consolidation demonstrated that the biochar is absorb the toxic elements from the soil that is declined the rate of loam contaminant in crops and also in another conditions too, for example lakes, wet-lands, and defiled loam and silt). Additional advantages of bio-char when as opposed to enacted char ash as a therapeutic methodology, it is less expensive, has a negligible site unsettling influence as a latent, in situ treatment, and may save money if decontaminated residue can be cleaned without extensive digging or exhuming and removal.

2.3 Rehabilitation of man-made Contaminants

Biochar has a variety of good immobilisation properties that work with compound changes of heavy metals, and dye. For example, its mesoporous, nanostructured, composition, gesture surface utilitarian collecting, with a high CEC and a high pH. SEM, FT-IR, CHNS and XRD characterization disclosed that biochar has significant areas of strength for a preference for heavy metals (*Sun et al., 2018; Wen et al., 2021)*. Because of the expansion that biochar shows, the loam zeta-potential and CEC typically declines, with a loam pH rise. Studies have revealed the possibility of heavy metal immobilisation in biochar-corrected soils (for example *Bielská et al.*, 2018). Biochar has been shown to significantly reduce $HgCl_2$, RhB, and Methylene Blue concentrations in polluted soil frameworks [53]. Biochar has been shown to significantly decrease $HgCl_2$, RhB, and Methylene Blue concentrations in polluted soil frameworks. When compared to decreases seen in leachate from lab segment deliberate. Biochar has been used to recover smelling salts from wastewater streams [54]. Starting with the results of a pioneering study conducted by [51], in the lake near Southern part of India to numerate methyl mercury and mercury presence with time via diffusive method. Blends of slime and barnyard fowl compost-based biochar may be sufficient for low-temperature evacuation. The slope in thin film (DGT) tests has been uplifting: lipophilic Hg range looks to decline in residue hole H_2O in the existence of bio-char, implying ore or deposit immobilisation. More research is expected to evaluate the effect on mercury divisions, together in polluted environments along various heavy metals exist. Aside from being used as an adsorbent to fish out soil impurities, it can also be employed to deal with disintegrated groundwater pollutants [55]–[57]. The physicochemical properties of biochar suggest that it could act as a proficient adsorbent for leaching out uranium contaminating groundwater and as a receptive hindrance against remediation (*Kumar et al*. 2011). Nonetheless, repeated biochar utilization may reduce its efficacy. The arrangement of total biochar particles at higher concentrations limits the possible surface adsorption Cu [5]. High pH, CEC, and surface regions are common in biochar delivered at higher temperatures. In reality, low-temperature biochar has more dynamic destinations together with stable C-O entities [30]. C-O moieties are precious surface gathering point for multiple sorption/substances response possibilities [3], [5]. Biochar that is enacted after creation, on average, has a larger surface area [58]. KOH steam-enacted walnut shell biochar may be very effective at sorbing abundance Cu from water frameworks. However, this enactment step raises the overall material expense [58], [59], so it may only be appropriate for serious, limited scope applications in severely polluted areas. The systems for metal expulsion by biochar involve absorption, depletion, decomposition, and, immobilisation of thrash metals. The process of making biochar may also affect how metals behave. Large scale, nanoporous and miniature structures are found throughout biochar' grid could generate a less mobile reduced-state for metals [60]. Because of greater proclivity against pollutants and property to carry electrons towards adsorbed reactants, the minute graphene lattice which exists in biochar wields the ability to reduce as well as adsorb pollutants [61]. Overall, the redox centres of heavy metals increase, particularly after treatment with biochar, but the immobilisation impacts are diminished at higher beginning centralizations of heavy metals [57]. As would be adsorbed less quickly to soils and would be more portable in conditions with negative redox potential provided by biochar [62]. Furthermore, being soluble under decreasing conditions, the fixation for oxides of Mn and Fe(*Moreno-Jimenez et al*. 2012), will assumedly elevate in pore water with unfavourable redox potential. Supposedly, oxidation of biochar follows inside-out pattern [63]. This could result in different component maintenance between surface and interior destinations. This difference in endurance in the middle of the pores and the exterior could define why biochar has different expulsion effectiveness on different metal particles. According to the FT-IR examination, useful categories include in the elimination of $HgCl2$, RhB, and Methylene Blue include carboxylate and hydroxyl groups. Because the utilitarian gatherings for a few metals' adsorptions were synthetically comparable, rivalry for limiting destinations between various metals occurred, influencing immobilisation proficiency for separate ore in blended metal polluted soils. The immobilisation outcome of bio-char on thrash metals and other man-made contaminants in genuine contaminated water and loam should be evaluated going forward.

2.4 Rehabilitation of biological/biotic Contaminants.

Bio-char is a successful sorbent of natural mixtures, along with mixed within loam, it can consume natural impurities into the loam, changing their resistance to bio-deterioration. Biochar, for example, unequivocally sorption phenanthrene [64], hexachlorobenzene [65], and sulfamethoxazole [66] and significantly reduces the accessibility of PCDD/Fs estimated by polyoxymethylene (POM) aloof take-up and worm take- (*Chai et al.* 2012). The inclusion of biochar to swill ooze resulted in eminent reductions in broken up PAH concentrations (*Oleszczuk et al.* 2012). It has also been found, with applying biochar on loam pretentious, diligence; viability of pesticides such as isoproturon (IPU) (*Sopena et al.* 2012); benzo-nitrile [65], as well as herbicides such as (4-chloro-diminished especially with rising biochar part) of the dirt. Soil biochar fixations influenced the amount and degrees of phenyl-urea herbicide isoproturon (IPU) debasement. As a result, the excellent quantity of bio-char used in comparison to loam can be adapted to the site-responsive focuses. The character of biochar had an important influence on the sorption of natural impurities. Bio-chars with smaller compelling molecule sizes have a huge exterior part and carry-out superior in the world of natural composite surface assimilation and evacuation. The latest examination has revealed significant sorption of PCDD/Fs into biochar, particularly powdered. In comparison to biochar with enlarge molecule proportions, biochar with teeny molecule sizes (such as 0.074 mm) required less duration to achieve adsorption balance for simazine and atrazine both [66]. Biochar's high trichloroethylene adsorption limit of 700°C was assign for its increased fragrant and small extremity (*Ahmad et al.* 2012). Essentially, when biochar collate is delivered at 450°C, manufactured biochar at 850°C specifically declined plant accessibility to the two pesticides an account of its high proclivity to adsorb pesticide build-ups [64]. Surface enactment, as assumed, rises the exterior assimilation of natural pollutants because of the huge exterior extent of the actuated singe in comparison to untreated biochar of comparable arrangement and creation techniques. The fact that the worth for the non-identical absorbent was huge for the financially accessible enactment of carbon and the bio-chars synthesised in the research facility proposes that the exterior region of the carbon affected natural toxin sorption. The fact that the "n" expression was mostly under for unproductive bio-chars than for productive carbons suggested that solid absorption locales were more constrained in the unproductive bio-chars and that they were being immersed at huge toxin clusters.

CHAPTER 3: MATERIAL AND METHOD

3.1 Harvesting of plant leaves of *Nephrolepis auriculata*

Biological sample: Plant laves of *Nephrolepis auriculata*

Chemical: Concentrated H_2SO_4 (Conc. Sulphuric acid), Double distilled water.

Glassware: Conical Flask, Measuring cylinder, Beaker.

Instruments: Weighing balance, Heating Plate Centrifuge, Mortar pestle, Hot air oven, Pipette.

Miscellaneous: Centrifuge tube, aluminium foil, spatula, marker.

Procedure

- Taking 100 gm plant leaves of *Nephrolepis auriculata* from the Horticulture department at Delhi Technological University.
- Wash these leaves with double distilled water to remove dust and dirt.
- Washed plant leaves, leave to dry in a hot air oven for 2hrs at 60 degrees Celsius.
- Dried leaves of the *Nephrolepis auriculata* plant are crushed in a mortar pestle to make a fine powder of it.
- Fine powder of leaves mixed with concentrated H_2SO_4 in the gradient of 1:8 in a Glass Beaker of 50 ml.
- Glass beaker keep in the heating plate at 40 degrees Celsius at the initial point.
- Rise temperature slightly after every 30 min up to 180 degrees Celsius.
- Glass beaker heated at the heating plate for up to 8 hrs to remove complete H_2SO_4.
- After the removal of H_2SO_4 from plant powder make it complete samples.
- A sample is turned black and becomes scabrous Activated carbon after removal of H_2SO_4.

3.2 Washing of scabrous Activated Carbon

Biological sample: Activated carbon (Biochar)

Chemical: Double distilled water.

Glassware: Conical Flask, Measuring cylinder, Beaker.

Instruments: Weighing balance, Hot air oven, Filter, Pipette, pH meter.

Miscellaneous: Centrifuge tube, aluminium foil, spatula, marker.

Procedure

- Activated carbon is left for cooling for a few hours approximately 1-1.5 hrs.
- After the cooling of activated carbon was washed with the double distilled H_2O.
- Measure the initial pH of the activated carbon samples.
- After every washing cycle, frequent centrifugation at 400 rpm (Eppendorf centrifuge).
- At every centrifugation process, measure the pH level of activated carbon until it reached the pH level of 7 to 7.5.
- Once the pH level reached the range of 7 to 7.5 of activated carbon, then the removal of H_2SO_4 is 100%.
- Activated carbon sample put inside the hot air oven once again at 110 degrees Celsius.
- Samples at 110 degrees Celsius remove the complete moisture continent in 1 to 2 hrs.
- Dried samples are filtered out through a 90 µm sieve to achieve only fine Activated Carbon particles.

3.3 Analysis of heavy metals and dye

Biological sample: Activated carbon (Biochar)

Chemical: Double distilled water, Rh B, $HgCl_2$, Methylene blue.

Glassware: Conical Flask, Measuring cylinder, Beaker.

Instruments: Tip, UV spectrophotometer, Pipette, Shaker.

Miscellaneous: Centrifuge tube, aluminium foil, spatula, marker.

Procedure

- Take heavy metals ($HgCl_2$) and dyes (Rhodamine B and methylene blue) at different concentrations.
- Making a heavy metal at different levels of ppm (100-10, 5, 4, 3, 2, 1 ppm).
- Take dye Rhodamine B and methylene blue and make two different 10, 40 ppm concentrations.
- Heavy metal and dye dilute with water and make different ppm concentrations.
- Take a 2ml centrifuge tube to mix the dye and heavy metals.
- Take a sample for UV spectrophotometer and note down the initial reading at their concentrations.
- $HgCl_2$, Rhodamine B and methylene blue are put on a mechanical shaker for complete mixing, then take another reading.
- Take water as a reference for reading and compare heavy metal and dye reading.

3.4 Analysis of Biochar potential with heavy metals and dye

Biological sample: Activated carbon (*Nephrolepis auriculata* plant Biochar)

Chemical: Double distilled water, Rh B, $HgCl_2$, Methylene blue concentration.

Glassware: Conical Flask, Measuring cylinder, Beaker.

Instruments: Tip, UV spectrophotometer, Pipette, Shaker.

Miscellaneous: Centrifuge tube, aluminium foil, spatula, marker.

Procedure

- Take *Nephrolepis auriculata* plant Biochar mixed with dye and heavy metal at different ppm.
- Put the sample beaker into the mechanical shaker for proper mixing.

- After a few hours take a sample into a 2 ml centrifuge tube.
- Start centrifugation of sample at 400 rpm for 5 min.
- Take a sample of dye and heavy metals supernatant, and put it into a cuvette of UV spectrophotometer.
- Set a UV spectrophotometer according to sample wavelength and take a reading.
- Compare the reading of sample after mixing of biochar with before biochar and note down the remediation value.

3.5 Characterization of Activated carbon biochar

3.5.1 Using UV- vis spectrophotometer

Biological sample: Activated carbon (*Nephrolepis auriculata* plant Biochar)

Chemical: Double distilled water.

Glassware: Cuvettes.

Instruments: UV spectrophotometer, Pipette.

Miscellaneous: Tissue paper, Wash bottle, Pipette tips

Procedure

- Take distilled water bottle to clean the cuvette, set baseline in UV-vis spectrophotometer.
- Take water as a reference baseline, 2 ml of sample was taken in the cuvette and put in the sample holder to take a reading.
- The sample was scanned for the range of 200- 400 nm.
- The spectrum was seen for the peak between 200- 350 nm.
- That process repeated for frequent days until the biochar was stable.

3.5.2 Using Zeta-potential analyser

Biological sample: Activated carbon (*Nephrolepis auriculata* plant Biochar)

Procedure

- Take a 25mg biochar added to a 50 ml water centrifuge tube.
- Sample sent for the zeta potential analysis were treated in central instrumentation facility, department of chemistry, Delhi Technological University.
- Results are then examined as stated.

3.5.3 Using FT-IR analyser

Biological sample: Activated carbon (*Nephrolepis auriculata* plant Biochar)

Procedure

- Take sample in a 2ml centrifuge tube and dry properly.
- Sample sent for the FT-IR examination were treated in the University science instrumentation science, department of chemistry, Delhi University.
- Results are then examined as stated.

3.5.4 Using CHNS elemental analyser

Biological sample: Activated carbon (*Nephrolepis auriculata* plant Biochar)

Procedure

- Take sample in a 2ml centrifuge tube and dry properly.
- Sample sent for the CHNS elemental examination were treated in the University science instrumentation science, department of chemistry, Delhi University.
- Results are then examined as stated.

3.5.5 Using SEM analyser

Biological sample: Activated carbon (*Nephrolepis auriculata* plant Biochar)

Procedure

- Take sample in a 2ml centrifuge tube and dry properly.

- Sample sent to the SEM elemental examination were treated in the University science instrumentation science, department of chemistry, Delhi University.
- Results are then examined as stated.

3.6 Application

3.6.1 Degradation of $HgCl_2$

Biological sample: Activated carbon (*Nephrolepis auriculata* plant Biochar)

Chemical: $HgCl_2$ (100-10 and 5, 4, 3, 2, 1 ppm concentration), distilled water.

Glassware: Conical flask, Measuring cylinder.

Instruments: Weighing balance, Pipette, Shaker, UV- vis spectrophotometer.

Miscellaneous: Pipette tips, Cuvette, Tissue paper.

Procedure

- Take 25mg of biochar added into 100ppm concentration 50ml of $HgCl_2$.
- Sample put into mechanical shaker for 2 hrs for dilution.
- Take water for a baseline at a wavelength of 546 nm.
- 2ml sample of $HgCl_2$ load into cuvette at a wavelength of 546 nm.
- Examine the degradation of $HgCl_2$ and note down reading.
- Sample reading process was checked frequently after every 2 hrs.
- Different ppm concentrations of $HgCl_2$ sample are checked degradation same as previous.
- Note down all ppm concentration reading of sample at every 2 hrs.
- Repeat the same process until the sample was degraded at last.

3.6.2 Degradation of Rhodamine B

Biological sample: Activated carbon (*Nephrolepis auriculata* plant Biochar)

Chemical: RhB (10 and 40 ppm), distilled water.

Glassware: Conical flask, Measuring cylinder.

Instruments: Weighing balance, Pipette, Shaker, UV- vis spectrophotometer.

Miscellaneous: Pipette tips, Cuvette, Tissue paper.

Procedure

- Take 0.25g of biochar added into a 10ppm and 40ppm concentration of 50ml of RhB.
- Sample put into mechanical shaker for 2 hrs for dilution.
- Take water for a baseline at a wavelength of 553 nm.
- 2ml sample of RhB load into cuvette at a wavelength of 553 nm.
- Examine the degradation of RhB and note down reading.
- Concentrations of 10ppm and 40ppm of RhB sample degradation of the sample observed.
- Sample reading process was checked frequently after every 2 hrs.
- Repeat the same process until the sample was degraded at last.

3.6.3 Degradation of Methylene Blue

Biological sample: Activated carbon (*Nephrolepis auriculata* plant Biochar)

Chemical: Methylene Blue (10 and 40 ppm), distilled water.

Glassware: Conical flask, Measuring cylinder.

Instruments: Weighing balance, Pipette, Shaker, UV- vis spectrophotometer.

Miscellaneous: Pipette tips, Cuvette, Tissue paper.

Procedure

- Take 0.25g of biochar added into a 10ppm and 40ppm concentration of 50ml of Methylene Blue.
- Sample put into mechanical shaker for 2 hrs for dilution.
- Take water for a baseline at a wavelength of 664 nm.
- 2ml sample of Methylene Blue load into cuvette at a wavelength of 664 nm.

- Examine the degradation of Methylene Blue and note down the reading every 2 hours.
- 10ppm and 40ppm concentrations of Methylene Blue sample degradation of the sample observed.
- Sample reading process was checked frequently after every 2 hrs.
- Repeat the same process until the sample was degraded at last.

3.7 To check the Degradation of dye and heavy metal

Biological sample: Activated carbon (*Nephrolepis auriculata* plant Biochar)

Chemical: Methylene Blue, Rhodamine B (10 and 40 ppm), $HgCl_2$, distilled water.

Glassware: Conical flask, Measuring cylinder.

Instruments: Weighing balance, Pipette, Shaker, UV- vis spectrophotometer.

Miscellaneous: Pipette tips, Cuvette, Tissue paper.

Procedure

- Take heavy metals and dye multiple readings at the initial stage of observation.
- Sample observation has been taken according to the repeated time.
- Initial reading and the final reading of all samples were taken.
- Compare the reading of all samples to each other.
- Now examine the reading of each sample, then calculate.
- Using the formula to find the degradation percentage of heavy metal and dye.

CHAPTER 4: RESULTS

4.1 Synthesis of biochar from *Nephrolepis auriculata*

After evaporating H_2SO_4 and washing repeatedly with water and drying fine activated carbon was obtained in the form of biochar. A total of 6.66 grams of biochar was obtained from 100 grams of *Nephrolepis auriculata*. The total yield of biochar was calculated to be 6.66 %.

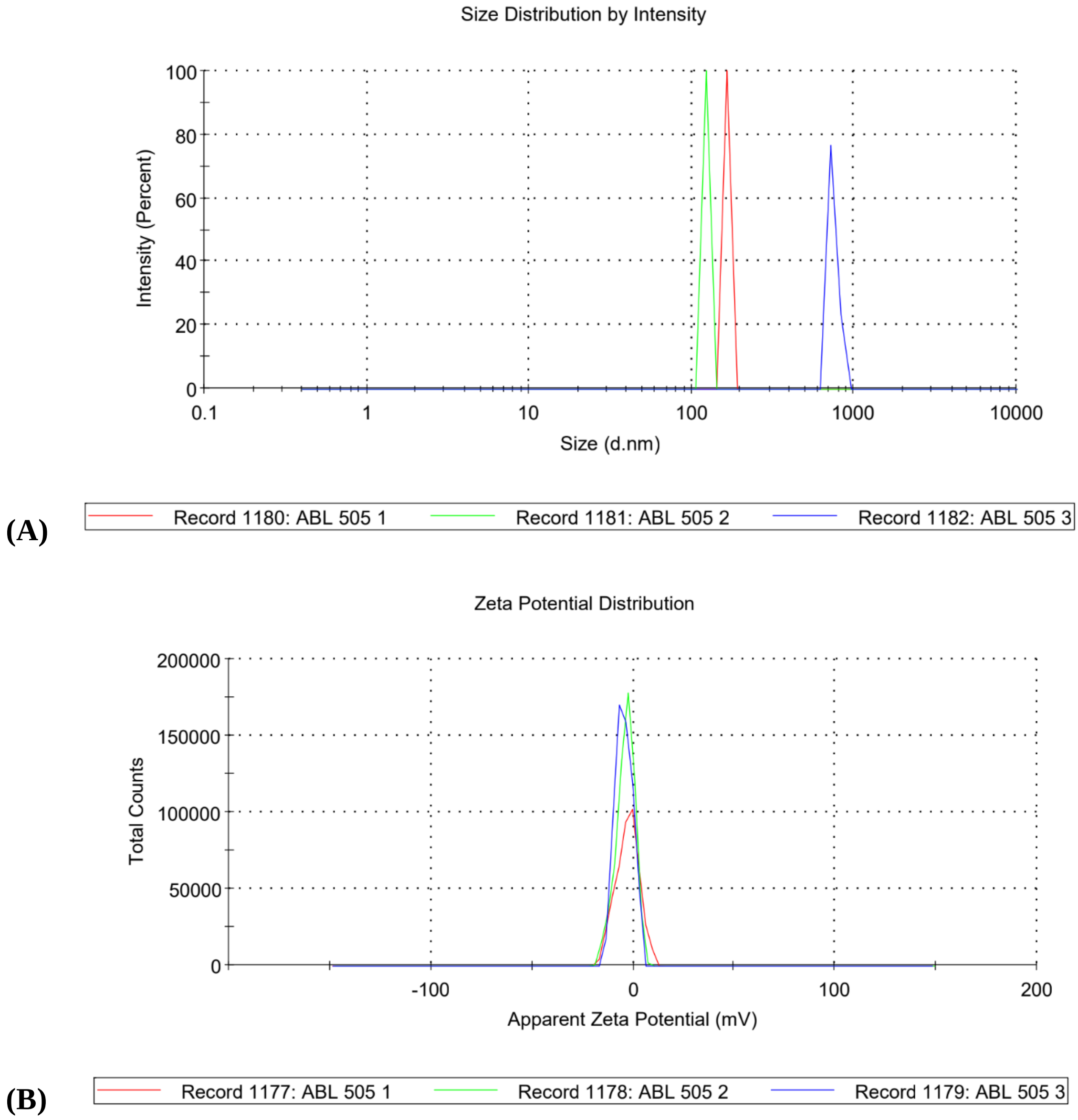
(A) **(B)**

Figure 1: Biochar synthesized from *Nephrolepis auriculata*. A) After vaporising H_2SO_4; B) After washing and drying.

4.2 Characterization of biochar

4.2.1 Zeta Potential Analysis

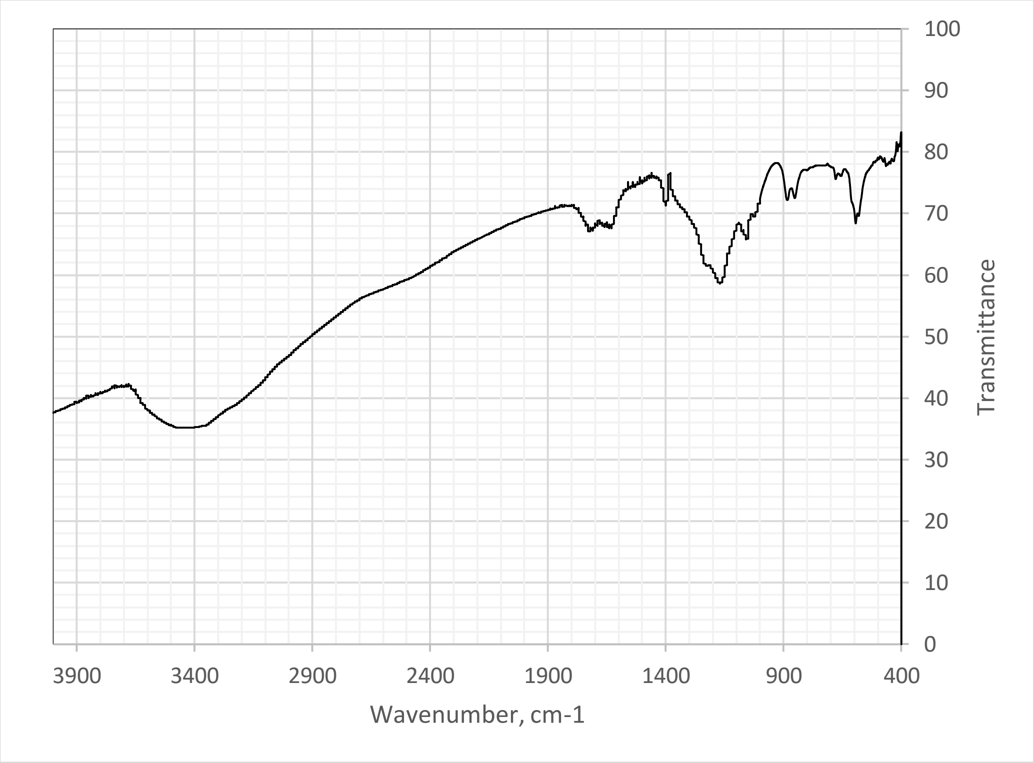
The diameter of finely grinded biochar powder was 3239 d.nm with a Pdl value of 0.147, which means that the majority of the biochar particles are of similar size. A zeta-potential value of -4.56 shows that the prepared biochar powder is unstable in water, as a result it readily flocculates in water.



Graph 1: Zeta-potential analysis result for *Nephrolepis auriculata* biocharA) Size distribution graph; B) Zeta potential distribution graph of biochar synthesized from *Nephrolepis auriculata.*

4.2.2 FTIR Analysis

Upon FTIR analysis, peaks corresponding to different functional groups were observed. Small peaks within the range of 2960 to 2850 cm^{-1} is corresponds to the presence of aliphatic formation of C - H stretches hinting the presence of membrane material like cellulose, lignin, and hemicellulose. Small peaks in the range of 2830-2670 cm^{-1} represents aliphatic C-H bending. Peaks appearing between 1610-1590 cm^{-1} occurred due to hemicellulose C=C stretches. Deformation of membrane material like cellulose and hemicellulose C-H bonds corresponds to the peaks between 1480-1410 cm^{-1}. C-O stretching in membrane material is represented by the peaks observed between 900-1120 cm^{-1}. Aromatic rings are represented by the peaks present in the between the range of 880-720cm^{-1}.



Graph 2: FTIR transmittance spectra of *Nephrolepis auriculata* biochar.

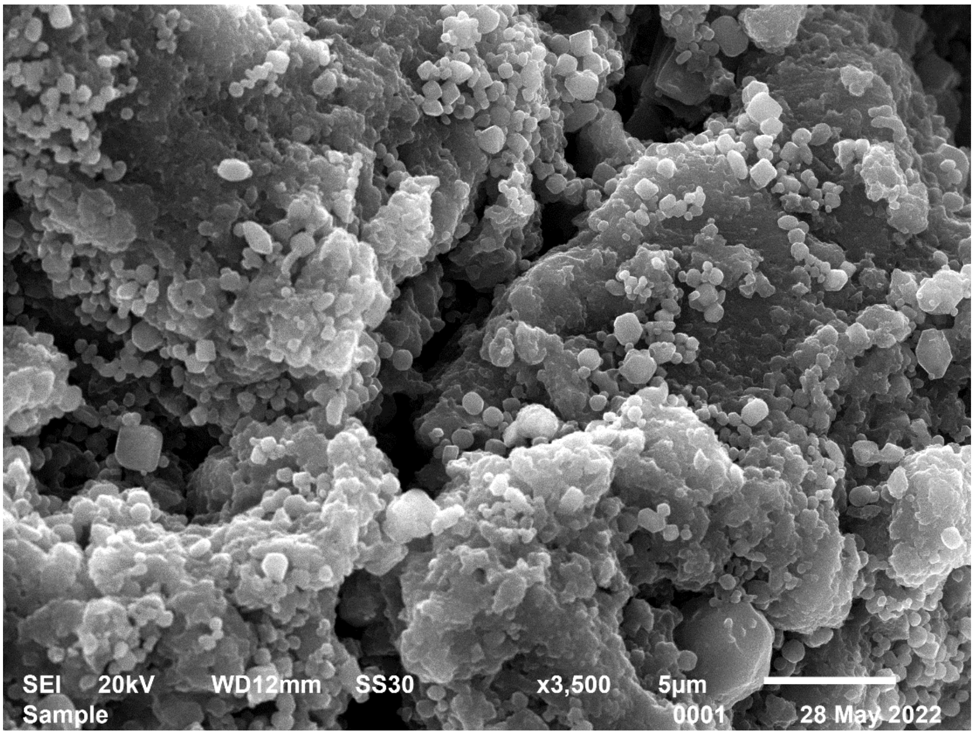
4.2.3 CHNS Elemental Analysis

Table 1: CHNS composition of biochar synthesized from *Nephrolepis auriculata*

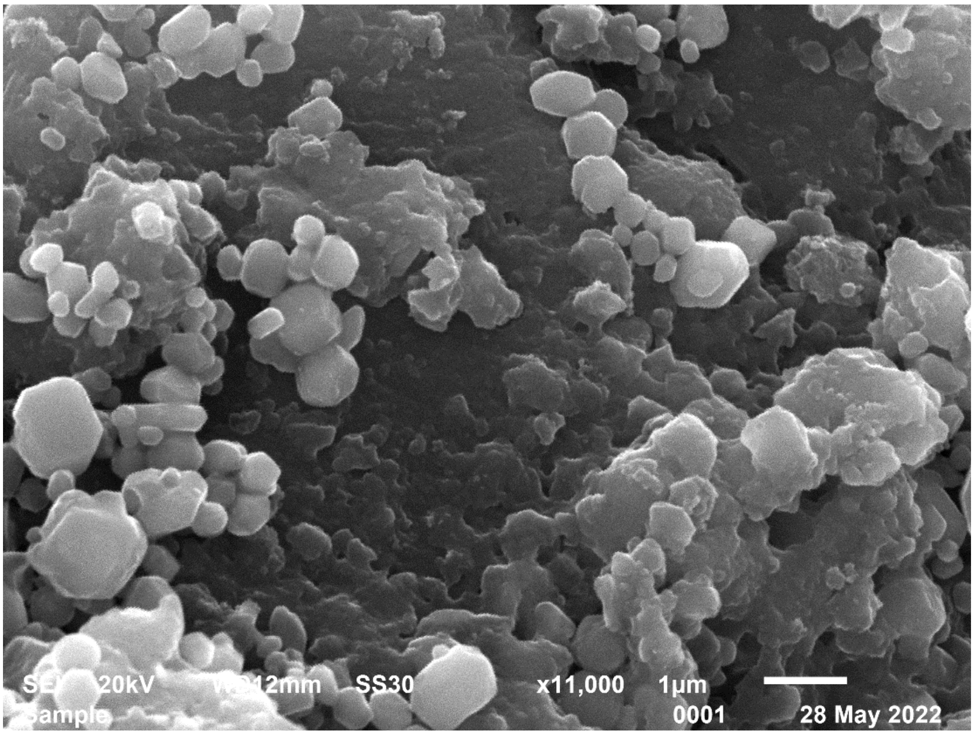
CHNS Composition	
Carbon %	19.08
Nitrogen %	2.77
Hydrogen %	3.31
Sulphur %	15.17
C/N Ratio	6.88
C/H Ratio	5.75

The sample consists of comparatively higher amount of carbon as compared to other 3 elements followed by sulphur which were 19.08 % and 15.17 % respectively.

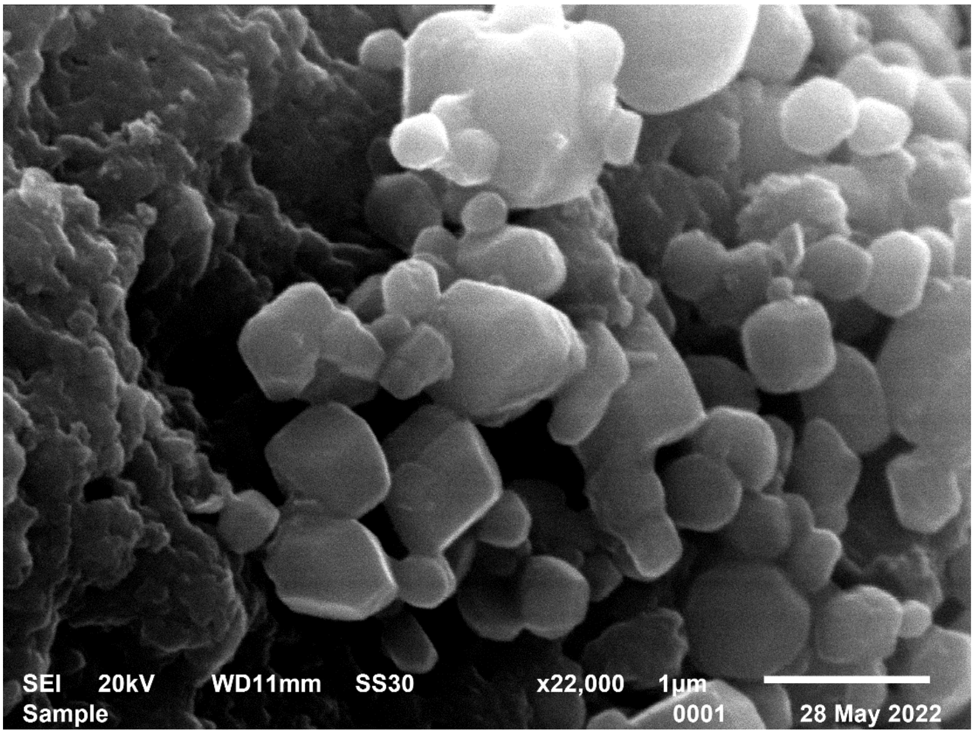
4.2.4 SEM



(A)



(B)



(C)

Figure 2: SEM result of biochar powder at A) 3,500x; B) 11,000x and C) 22,000x magnification.

Images from SEM revealed that the particles of biochar possess crystalline polygonal shape. Cracks and holes caused due to acid hydrolysis were also observed in SEM images.

4.3 Application

4.3.1 Degradation of Hg using Nephrolepis auriculata biochar

Table 2: Bio-remediation of Hg at different concentrations using *Nephrolepis auriculata* biochar

Hgcl2 Concertation (PPM)	Initial reading at 546 nm	Final reading at 546 nm	Remediation value	Remediation Percentage
100	0.0939	0.0503	0.0436	46.43237487
90	0.0879	0.0432	0.0447	50.85324232

80	**0.0698**	**0.0325**	**0.0373**	**53.43839542**
70	**0.0621**	**0.023**	**0.0391**	**62.96296296**
60	**0.0549**	**0.0202**	**0.0347**	**63.20582878**
50	**0.0489**	**0.0185**	**0.0304**	**62.16768916**
40	**0.0432**	**0.0157**	**0.0275**	**63.65740741**
30	**0.0373**	**0.0139**	**0.0234**	**62.73458445**
20	**0.0255**	**0.0128**	**0.0127**	**49.80392157**
10	**0.036**	**0.003**	**0.033**	**91.6666667**
5	**0.0116**	**0.0105**	**0.011**	**94.82758621**

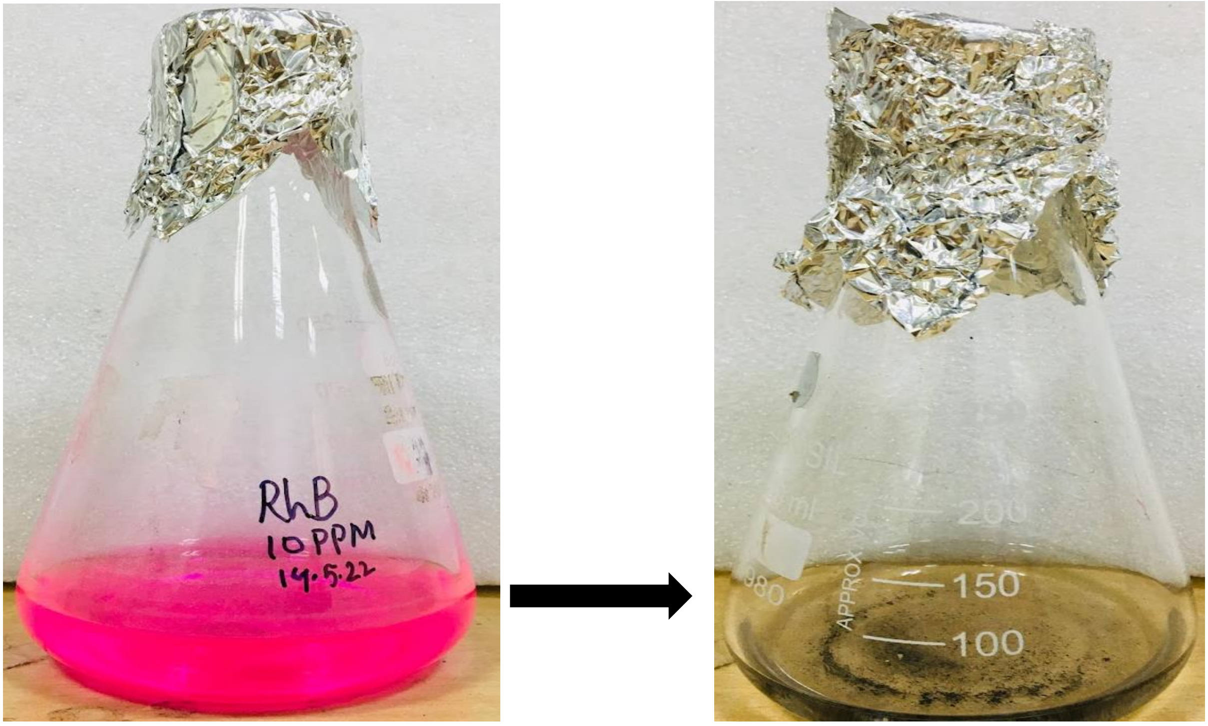
High remediation efficiency of 91.66 % and 94.82 % was observed in 10 ppm and 5 ppm $HgCl_2$ solutions respectively using *Nephrolepis auriculata* biochar. Around 50-60 % degradation of $HgCl_2$ was observed in rest of the concentrations. Lowest degradation was observed in 100 ppm $HgCl_2$ solution.

4.3.2 Degradation of RhB using Nephrolepis auriculata biochar

Table 3: Bio-remediation of Rhodamine-B using *Nephrolepis auriculata* biochar.

Absorbance at 553 nm	**RhB Concentration**		**Remediation Efficiency**	
	40-PPM	**10-PPM**	**40-PPM**	**10-PPM**
0 hour	0.1669	0.2655		
4 hours	0.0501	0.0625	69.98202516	76.45951036
8 hours	0.0477	0.0346	71.42001198	86.96798493
12 hours	0.0407	0.0326	75.6141402	87.7212806
24 hours	0.0269	0.0239	83.88256441	90.99811676
48 hours	0.0137	0.013	91.79149191	95.10357815
72 hours	0.0105	0.0062	93.70880767	97.66478343
96 hours	0.0064	0.0036	96.16536848	98.6440678
120 hours	0.0047	0.0002	97.18394248	99.92467043

After 5 days, 99.92 % and 97.18 % degradation of 10 ppm and 40 ppm Rhodamine-B was observed using *Nephrolepis auriculata* respectively. After 24 hours 90 % of the 10 ppm concentration of RhB had undergone degradation while for 90 % degradation 40 ppm RhB solution it took 48 hours.



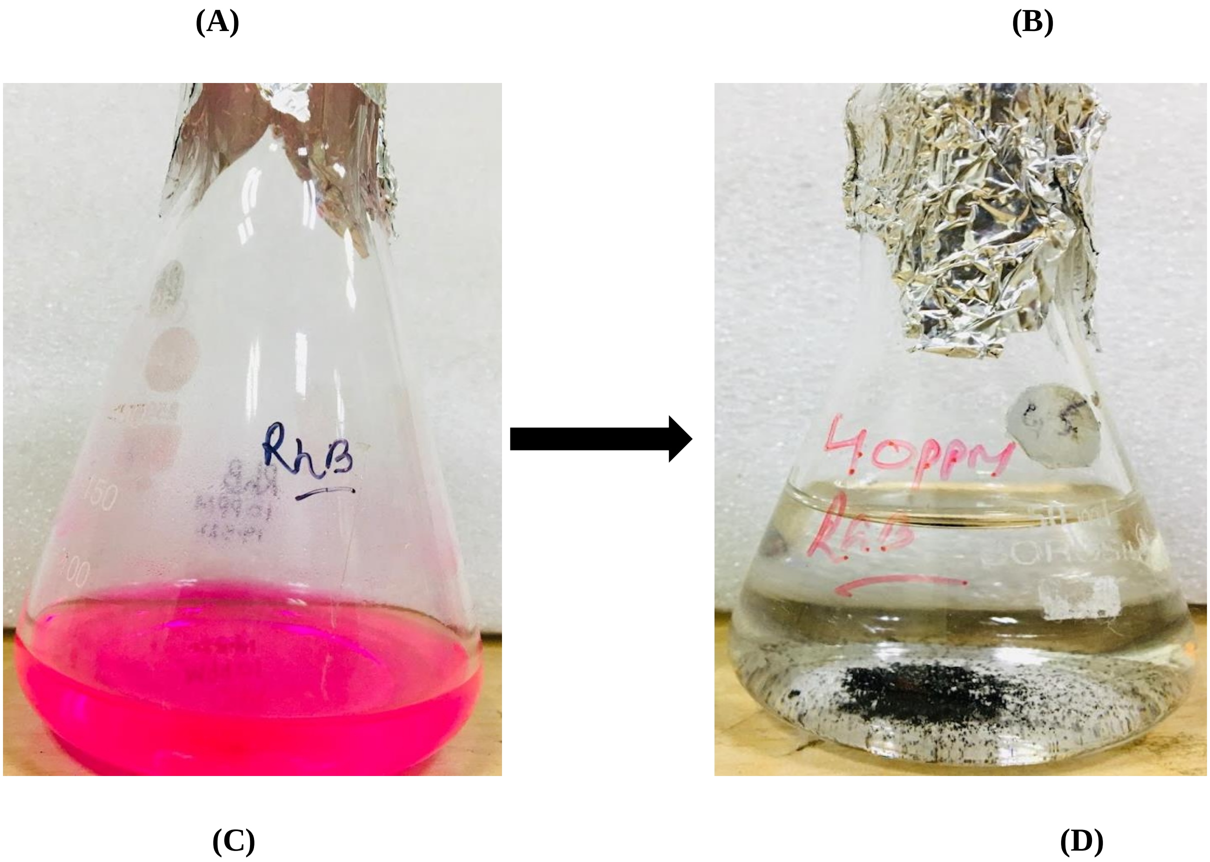


Figure 3: Remediation of Rhodamine-B using *Nephrolepis auriculata* biochar. A) and C) 10 ppm and 40 ppm Rhodamine-B solution at 0 hours respectively; B) and D) Remediated 10 ppm and 40 ppm Rhodamine-B solution after 120 hours.

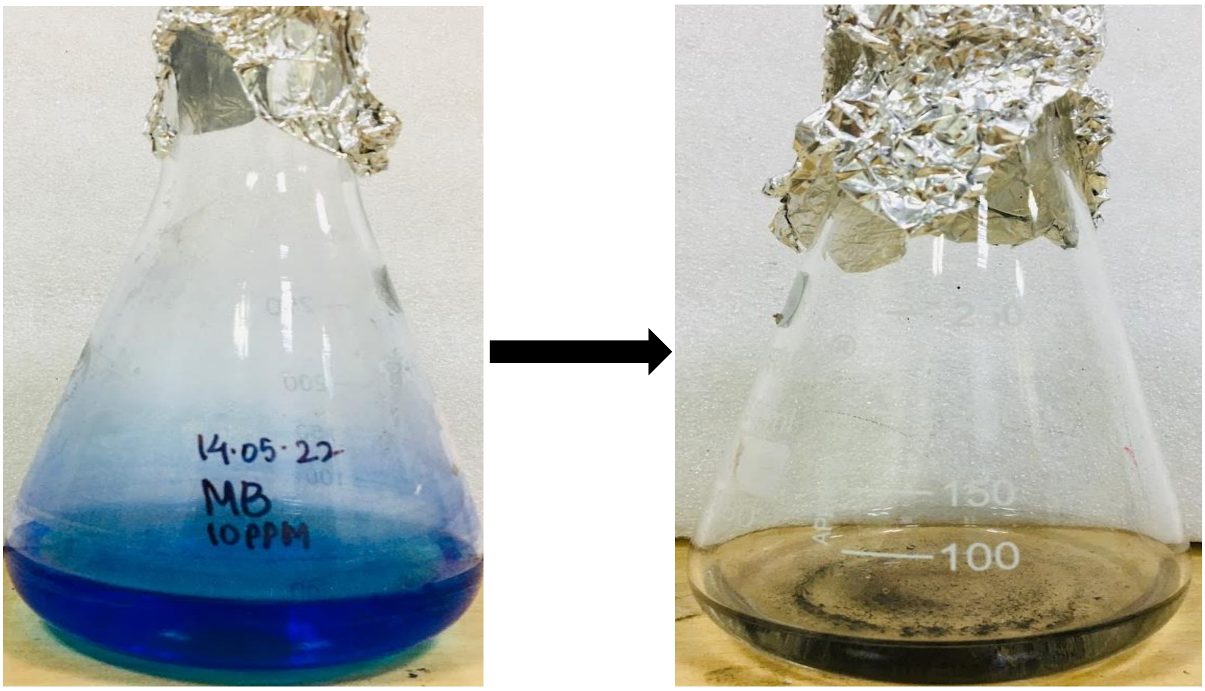
4.3.3 Degradation of MB using Nephrolepis auriculata biochar

Table 4: Bio-remediation of Methylene blue using *Nephrolepis auriculata* biochar.

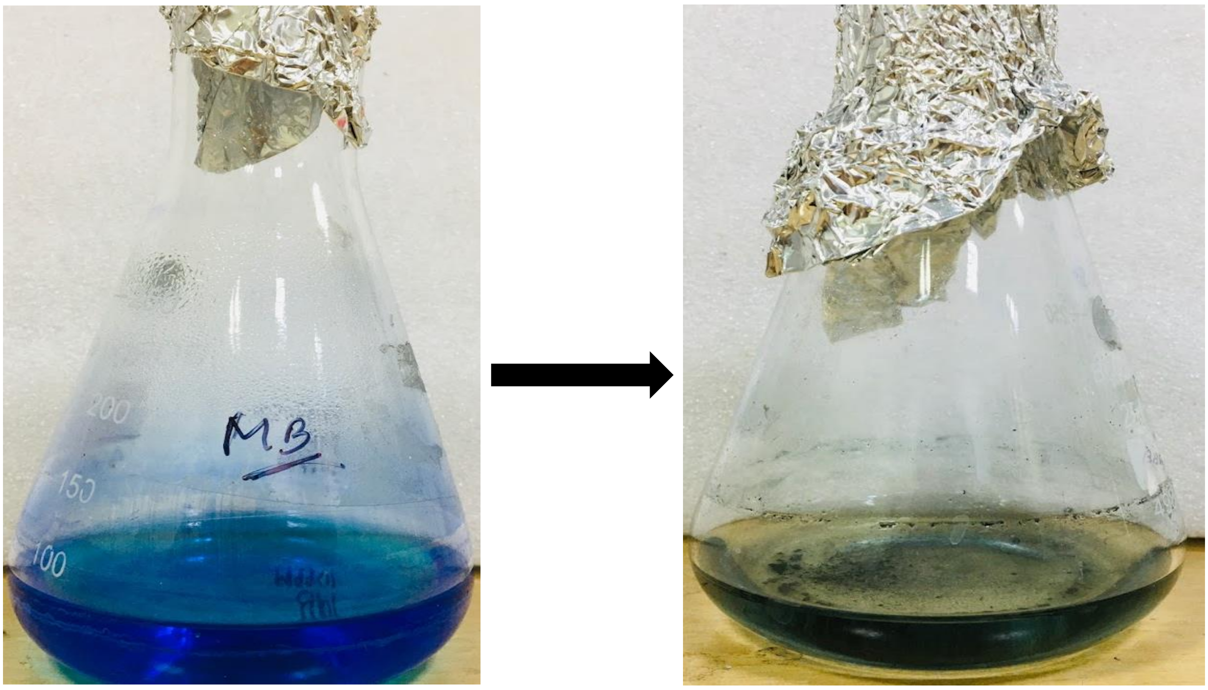
Absorbance at 663 nm	**MB Concentration**		**Remediation efficiency**	
	40-PPM	**10-PPM**	**40-PPM**	**10-PPM**
0 hour	0.259	0.3629		
4 hours	0.2316	0.3239	10.57915058	10.74676219
8 hours	0.2206	0.2879	14.82625483	20.66685037
12 hours	0.1669	0.1462	35.55984556	59.71341967
24 hours	0.1448	0.1205	44.09266409	66.7952604
48 hours	0.1309	0.0888	49.45945946	75.53044916
72 hours	0.1278	0.0652	50.65637066	82.03361808

96 hours	0.1101	0.0219	57.49034749	93.96527969
120 hours	0.101	0.002	61.003861	99.44888399

A higher degradation was observed in 10 ppm methylene blue solution which had a remediation efficiency of 99.44 % after 5 days. While only 61 % degradation was observed in 40 ppm sample during the same period. With time an increase in degradation of methylene blue was also observed for both the concentrations.



(A) **(B)**



(C) (D)

Figure 4: Remediation of Methylene Blue using *Nephrolepis auriculata* biochar. A) and C) 10 ppm and 40 ppm Methylene Blue solution at 0 hours respectively; B) and D) Remediated 10 ppm and 40 ppm Methylene Blue solution after 120 hours.

CHAPTER 5: DISCUSSION

After using acid hydrolysis method, 6.66 grams of biochar was synthesized from 100 grams of *Nephrolepis auriculata*. This process showed a total biochar yield of 6.66 %. Zeta potential analysis confirmed that the size of the biochar particles was 3239 d.nm. It also revealed that the particles are unstable in water and they readily flocculate. FTIR result showed bending and stretches of different C- bonds present and deformed ones formed in membrane materials. Crystalline structure along with cracks and holes were observed in SEM. Similar characterization of SEM and FT-IR results were shown by Reza et al. 2020, for biochar synthesized from *Pennisetum purpureum*. Biochar synthesized from *Nephrolepis auriculata* showed promising results in bioremediation of Hg, RhB and MB. Around 50-60 % remediation of $HgCl_2$ was observed for concentration 100 ppm – 20 ppm. More than 90 % degradation of $HgCl_2$ was observed for 10 ppm and lower concentrations. 10 ppm $HgCl_2$ showed 91.66 % degradation and 5 ppm showed 94.82 % degradation which is higher than the mercury remediation reported for biochar synthesized from different plants. For Rhodamine-B and Methylene blue remediation value increased with increase in time. After 120 hours, 97 % of 40 ppm concentration of RhB was degraded while almost 100 % of the 10 ppm concentration underwent degradation after treatment with *Nephrolepis auriculata* biochar. Similarly, almost complete degradation was observed in 10 ppm MB solution, but 40 ppm MB solution showed only 61 % degradation. The results showed that biochar can be considered as potential solution to tackle pollution caused due to heavy metal like mercury and dyes like Rhodamine-B and Methylene Blue. Since biochar is synthesized from waste generated biomass, it can also serve as a method to utilize the waste generated after the plant is processed. With further research it can also be used for bioremediating other polluting heavy metals and dyes.

CHAPTER 6: CONCLUSION

Biochar can serve as a productive way to utilize plant waste produced after farming, bioactive compound extraction and other such processes which generates plant waste. Not only does it reduce waste but biochar itself have many benefits. It can be used as bio-fertilizer, to prevent leaching of nutrients and cleaning of water. Since biochar possess large surface area it shows high adsorption. This high adsorption can be used to bio-remediate dyes and heavy metals present in the environment. This project highlighted the remediation potential of biochar synthesized from *Nephrolepis auriculata* against Mercury, Rhodamine-B and Methylene Blue. For 10 ppm concentration almost 100 % remediation was observed in both the dyes. Extend of dye degradation through biochar increased with time was also observed.90 % - 95 % degradation of $HgCl_2$ was observed in 10 ppm solution. The presence of C-bonds of membrane materials like cellulose and hemicellulose showed in FT-IR spectra conclude that biochar is majorly composed of membrane materials. This shows that biochar can be serve as a method to utilize plant waste. High remediation efficiency and utilization of plant waste makes biochar as a potential candidate for tackling pollution caused due to heavy metals and dyes and also preventing air pollution caused due to burning of plant waste.

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