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Research Article

Bamboo Plantations for Phytoremediation of Cadmium in Tannery Effluent: Plant Response and Nutrient Uptake

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Abstract: The rapid development of the industrial sector led to a greater level of heavy metal Cadmium in recent years. Food and breathing are the primary ways that Cadmium enters the body. Humans are exposed to long-term health hazards from Cadmium. Cadmium accumulates in immune cells, triggers inflammatory responses, and leads to a number of health problems. Compared to conventional physical and chemical techniques, phytoremediation is a useful, environmentally advantageous method of remediating contaminated soil. Among the different approaches available for phytoremediation, Phytoextraction is the most effective and successful method since it gets rid of heavy metals. Some bamboo species have been recognized as the most promising species for sequestering carbon and potentially impacting the phytoremediation of soils contaminated with heavy metals. This study aims to assess the growth patterns of the native Indian bamboo species (*Bambusa bambos, Bambusa vulgaris, and Bambusa balcooa*) when exposed to metal-contaminated water, such as tannery effluent, in the Tiruchirappalli area. The ability of bamboo sp. to phytoextract Cadmium from contaminated soil has been demonstrated through experimentation, and its antimicrobial and anti-cancer potency is tested after being subjected to phytoextraction since the heavy metals in the tannery effluent are predominantly carcinogenic. The laboratory conducted a preliminary study on bamboo plant growth to gauge its growth. To measure the tolerance of bamboo when being rinsed with a solution containing 100 mg Cd/l, the morphological characteristics, heavy metal uptake, and translocation potential of the three bamboo species were assessed. The distribution of Cadmium in different fragments of the bamboo plant has also been studied, along with the factors that affect the uptake mechanism. Among the native species, *Bambusa bambos is* found to have a greater phytoextraction capacity of heavy metals, especially Cadmium. The plant is also evaluated for its anticancer and antimicrobial activities following phytoextraction so that the stem and other parts of *Bambusa bambos* have been used as indigenous medicine by the tribal people, and this could be due to the presence of flavonoids and other associated compounds present in it.

Keywords: Bamboo Growth; Phytoremediation; Heavy Metal Tolerance; Wastewater Treatment; Heavy Metal Translocation; Anti-Microbial and Anti-Cancer Activity

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1. INTRODUCTION

One of the most hazardous industrial pollutants is tannery effluent. For every kilogram of raw material processed in leather manufacturing, 35 liters of water are used. The approximate quantity of tannery effluent produced is 75,000 $m^{3/}$day in Tamil Nadu. 4,00,000 tons of chemicals are required to process 7,00,000 tons of hides and skins annually. The leather used in the tanning process primarily absorbs around 20% of the chemicals, and the rest dissolves in the wastewater. [1] The most polluted wastewater comes from tanneries and contains significant levels of suspended particles, proteins, chlorides, trivalent chromium, nitrogen, sulfate, and sulfides and higher BOD and COD levels [1,2]. The quantity of wastewater discharged and its properties differ from one procedure to the next, from one tannery to another. Tasks are completed in batches in a tannery, and wastewater is intermittently discharged. Wastewater from beam house activities like soaking, liming, deliming, bathing, etc., is alkaline and contains hair, lime, TSS, TDS, sulfides, and BOD, which is mainly due to excess usage of calcium hydroxide without sufficient monitoring. Historically, untreated discharges from tanneries have caused significant water pollution and odor problems for the common people. The increased use of synthetic chemicals, such as insecticides, solvents, dyes, finishing agents, and novel processing chemicals, which entail toxicity and persistence issues, has given rise to additional problems more recently. Heavy metal contamination affects the quality of food, drinking water, and the natural environment[2]. Using effluent sewage for crop irrigation results in the build-up of several heavy metals in soil in most developing nations [3]. Heavy soil metals offer a huge ecological risk[4]. These heavy metals, including Cr, Ni, and Cd, are absorbed by plants, raising the likelihood that they will build up in human cells[5,6]. Since they harm living things, heavy metals at higher concentrations are defined as environmental pollutants[7]. One of the main hazardous substances created by leather tanneries is Cadmium[8]. It is a new environmental problem that contributes to several diseases[9]. Cadmium is carcinogenic and particularly harms the kidneys, liver, and lungs. DNA damage is also a result of this one. When compared to other methods of removing heavy metals from the soil, phytoremediation is a new process that is far less expensive and calls for less technical expertise. The ability of plants to collect heavy metals, their tolerance to higher concentrations of heavy metals, their ease of harvest, and composition growth all have a role in the selection of plants for phytoremediation. These plants are well adapted to ingest hazardous trace metals in contaminated soils and grow rapidly [10]. In addition to other nutrients, some plants have the capacity to take up and absorb heavy metals from the soil. The term "hyperaccumulators" refers to this. A phenomenon known as Phytoextraction also referred to as phytoaccumulation, photoabsorption, or phytosequestration, occurs when plant roots absorb pollutants and move to shoots or other above-ground biomass such as stems, leaves, and fruits[11,12]. Phytoremediation is a sustainable method used to purify wastewater and remove contaminants from the soil. Since it is affordable, environmentally friendly, and enhances the biological value of the region, this technology is becoming increasingly popular. Additionally, both organic and inorganic pollutants can be eliminated from the soil, air, and water with this technology[13]. This method is the most widely accepted in society. There is a great challenge to developing chemical compounds that can provide antibacterial activity in industrial processing, as they are toxic and harmful to the human body and health [14] in contrast to natural compounds that provide antibacterial properties and are considered more human-friendly. As *Bambusa bambos* (*Poaceae*) is available in abundance in India and is also an eco-friendly and multifunctional plant, the leaves and stems of the plant have been used in agriculture and medicine for thousands of years. Chemical constituents include Silica, potash, lime, alumina, choline, betaine, hydrate of silicic acid, nuclease, urease, a proteolytic enzyme, cyanogenetic glucoside, and an alkaloid[15]. Cancer is one of the leading causes of mortality worldwide, and the inadequacy of conventional chemotherapy indicates that new approaches are critically needed. This study aimed to develop an antimicrobial substance from bamboo in addition to its phytoextraction capacity and finally analyzed the anti-cancer potency of bamboo post phytoextraction.

2. MATERIALS AND METHODS

2.1. Plant Authentication and Voucher Reference

The native Indian bamboo species (*Bambusa bambos, Bambusa vulgaris, and Bambusa balcooa*) were collected from Mannampandal (latitude 11.1021361 and longitude 79.6941161), a village located in the Nagapattinam District of TamilNadu State in India. Mrs.Philomina, Department of Botany, A.V.C College, Mannampandal, authenticated the selected plant species. A voucher specimen of the plants has been deposited in the Rapinat Herbarium of the Department of Botany, St. Joseph's College, (Autonomous), Tiruchirappalli. The entire experimental portion of this work was completed in a controlled setting. Three pots with three different bamboo species, *Bambusa bambos, Bambusa vulgaris,* and *Bambusa balcooa* are designated for study to examine their adaptability, contamination level, and efficiency in removing Cadmium from soils and roots, rhizomes, stems, and leaves. Each pot measures D = 30 cm in diameter and h = 24 cm in height. For a single volume of 10 L, each pot has a horizontal surface area of 490 cm^2. The soil's density (D) was equivalent to 0.25 kg/l, and it had a mixture of blond, brown peat, natural vegetable conditioner, and humified organic matter, with a pH of 6.9 and a $C_{(org)}$ content of about 20% dry weight (DW) and 1% DW. Each container contained a total of 4 kilograms of soil.

2.2. Soil Preparation

At 75 °C, the soil is air-dried to a constant weight before being used to plant various bamboo species. Before chemical digestion, the samples were homogeneously mixed, and to eliminate coarse particles, they were sieved through a 2-mm sieve. The same amount of prepared soil (2 kg) was put into each pot. Each bamboo plant is then thoroughly rinsed with deionized water after being properly cleaned with water to eliminate any remaining soil and debris. After that, the soil in the pot was filled with plant materials. Then, the tannery effluent was applied to pot soils to develop cadmium contamination levels. To prevent overflow from the pots, the effluent was applied gradually. Finally, the pots were coated with effluent, left undisturbed for a few days, and then uniform amounts of water were poured into the pots to contaminate the soil with cadmium[16] evenly.

2.3. Measurement of Bamboo Growth

To assess growth performance every week, the heights of the three different bamboo species planted in pots were

measured[17]. After each measurement, a computer-aided drawing that showed the development of bamboo stems emerging from a shared subsurface rhizome system was updated. Additionally, any indication of abnormal plant growth was documented.

2.4. Chemical Digestion of Plant Materials and Soil Samples

In a fluorocarbon polymer (PFA/TFM) closed system oven, a sample of 0.5 g of plant materials was digested using 7 mL of concentrated nitric acid (HNO_3): 1 mL hydrochloric acid (HCl) in a ratio of 7:1. An extraction fume system was installed on the vessel liner. The oven unit had a quartz power system installed (1800 W). First, the clear liquid was diluted to 50 mL in vials that had been acid-washed after the vessel had cooled. Next, 1.5 g of ground-up dried soil samples were put into 100-mL digesting tubes. Next, Aqua regia was added, composed of 20 mL of concentrated HNO_3 and 70 percent HCl in a 1:4 ratios. A funnel was provided as a cover for the tubes, and a digestion block was used to conduct digestion at 160 °C in a fume chamber. It was heated until there was only 4 mL left in the tube. Still, another 20 mL of aqua regia was added to the process and evaporated to around 5 mL. Then, membrane filters (10µm) were used to filter the solution. Before total Cd analysis, the filtrate was then diluted with de-ionized and distilled water to a volume of 25 mL. The amounts of total Cadmium were then determined using spectroscopic analysis on all the digested samples, including the laboratory blanks. The temperature, light, and air moisture were controlled and kept at 20 °C throughout the experiments.

2.5. Determination of Physicochemical parameters of tannery effluent

The tannery effluent gathered from a tannery factory in Tiruchirappalli was characterized for fundamental parameters in the current investigation. The effluent was gray with an unpleasant odor, acidic in pH, and high in electrical conductivity (EC), total dissolved solids (TDS), hardness, chlorides, and sulfates due to high organic and inorganic load levels. The physicochemical characteristics were established by the requirements set forth by the Bureau of Indian Standards (BIS).

2.5.1. Sample Collection

The tannery creates semi-finished leathers that have been tanned. The Two-liter polythene containers were used to carefully collect and transport the effluent samples from the raw composite stream to the lab. The sample used in the present study is an effluent taken from the Sealathaar tanning industry in Tiruchirappalli and was aimed to analyze the characteristics of the effluent as per the standards of BIS and to study the phytoextraction potential of bamboo sp. to treat the pollutants present in the effluent.

2.5.2. Color & Odor

By comparing the sample visually, color is measured. Half of a wide-mouthed stoppered bottle is filled with sample, sealed, then shaken erratically for 5 seconds before the smell is detected. It was determined whether the sample's odor at the bottle's mouth was agreeable or repulsive.

2.5.3. pH

After calibration, the instrument was verified using a buffer solution whose pH was close to that of the sample[18].

2.5.4. Electrical Conductivity

Reagents: Standard potassium chloride solution

0.7456 g of anhydrous potassium chloride (0.01N) is dissolved in distilled water and made up to 1000 ml. At 25 °C, this solution has a specific conductance of 1,408 µs/cm[19].

Procedure

The electrical conductivity of around 50 ml of standard potassium chloride solution was measured at room temperature for calibration. After calibration, the electrical conductivity of 50 ml of the effluent sample was determined and expressed as µs/cm.

2.5.5. Total Dissolved Solids

The evaporating dish was prepared by heating it for one hour at 180 °C in an oven, cooling it in desiccators, weighing it, and storing it in desiccators. A pre-weighed Whatman filter was used to filter a 100 ml sample. The weight of the filterable residue was calculated using 40 filter papers. In a hot air oven, 100 ml of the sample is pipetted onto an evaporating dish previously weighed [20]. The evaporating dish is removed after the water in the residue has completely evaporated, and it is weighed after chilling in a desiccator.

2.5.6. Total Hardness

Principle

When calcium and magnesium salts are introduced to water, the monochrome black -T dye turns wine red at pH 10.0. It becomes complex when the EDTA titration is completed. The solution changes from wine red to blue at the complex formation's finish, marking the endpoint.

Reagents

i) Patton and Reeder indicator solution: 1g of Patton and Reeder reagent was dissolved in 100 ml of distilled water EBT indicator: 0.5 g of EBT indicator and 45 g of hydroxylamine hydrochloride were dissolved in 100 ml of spirit.
ii) Standard calcium solution: 0.1 g of $CaCO_3$ was dissolved in 100 ml of diluted HCl (1 ml = 0.4008 g of Ca).
iii) Buffer solution: 70 g of Ammonium chloride was added to 570 ml of 30 % ammonium solution in water and made to 1 liter using distilled water.
iv) 1N Sodium hydroxide: 40 g of NaOH was dissolved in 1 liter of distilled water.
v) 0.001M EDTA solution: 3.75 g of Disodium ethylene diamine Tetra-acetate dihydrate was dissolved in distilled water and made up to 1 liter.

Procedure

To 25 ml of sample in 250ml conical flask, add 25 ml of distilled water, 10 ml of buffer solution, and 5 drops of ferrochrome black T. The mixture was titrated against EDTA

solution till it turned from wine red to blue. Then, Ca equivalent to 1 ml of EDTA solution was calculated.

2.5.7. Biological Oxygen Demand

Principle

The sample is put into an airtight bottle and incubated for three days at the right temperature. The initial and final dissolved oxygen concentrations are measured, and the BOD is calculated from the difference between the two. All oxygen uptakes after the initial DO measurement, which occurs shortly after dilution, are considered when calculating BOD[21].

Reagents

i) Manganous sulfate solution: 480g of $MnSO_4.4H_2O$ was dissolved in distilled water, filtered, and made up to 1000 ml.
ii) Alkaline Iodide Solution: 500g of Sodium hydroxide and 135gm of sodium iodide were dissolved in distilled water and made up to 100 ml.
iii) Starch Indicator: 2g of Starch and 0.2g of salicylic acid were dissolved in 100 ml of hot water.
iv) Stock sodium thiosulphate solution: 25g of Sodium thiosulphate was dissolved in distilled water, and up to 1000ml 1.0g of sodium hydroxide was added as a preservative.
v)Standard sodium thiosulphate solution: 250ml of the Stock solution was dissolved in distilled water and made up to 1000ml.

Procedure

Two ml of manganese sulfate solution and 2 ml of alkaline iodide solution were added to the sample in a 300 ml BOD bottle. The material was completely blended before being given time to precipitate and settle. The stopper was removed when the mixture had settled, and 2 ml of concentrated sulfuric acid was introduced through the sides. The stopper was reinstalled to dissolve the released iodine, and the bottle was vigorously shaken. After adding 3–4 drops of starch indicator, 200ml of the solution was titrated against sodium thiosulphate solution. Dark blue to colorless is the endpoint.

2.5.8. Chemical Oxygen Demand

Principle

Most organic material is oxidized by a chromic and sulfuric acid solution that is heated. The effluent sample is refluxed in a known excess of potassium dichromate and a strongly acidic solution ($K_2Cr_2O_7$). Following digestion, the amount of $K_2Cr_2O_7$ consumed is estimated using the oxidizable matter's oxygen equivalent by titrating the remaining unreduced $K_2Cr_2O_7$ with ferrous ammonium sulfate.

Reagents

i) 0.25 N Standard potassium dichromate solution: 12.259 g of $K_2Cr_2O_7$ was dissolved in distilled water and diluted to 1000ml.
ii) Sulphuric acid reagent: 0.55 g of Silver sulfate was dissolved in 100 ml of conc. H_2SO_4.
iii) Ferroin indicator solution: 1.485 g of the 1,10-Phenanthroline monohydrate and 695 mg of $FeSO_4$. $7H_2O$ were dissolved in distilled water and diluted to 100 ml.
iv) 0.25 N Standard Ferrous Ammonium Sulphate (FAS): 98 g $Fe(NH_4)_2$ $(SO_4)_2.6$ H_2O was dissolved in distilled water. 20 ml Conc. H_2SO_4 was added, cooled, and diluted to 1000ml. This solution was standardized against standard $K_2Cr_2O_7$.
v) Mercuric sulfate ($HgSO_4$) and
vi) Potassium hydrogen phthalate (KHP)

Procedure

A 250 ml BOD container is filled with 50 ml of the sample. Then, 5 ml of the sulphuric acid and 1 g of $HgSO_4$ is added gradually. Then 0.25 N $K_2Cr_2O_7$ solution was added and combined with 55 ml solution in the BOD bottle. Then, a condenser was connected to the flask, which activated the cooling system. The remaining 70 ml of sulfuric acid reagent is added to the condenser's open end with continuous swirling and blending. The reflux condenser is then disconnected, and distilled water is added to the liquid to dilute it to about twice its volume**.** After cooling to room temperature, the ferroin indicator titrates excess $K_2Cr_2O_7$ with FAS. The same volume of ferroin indicator is used for all titrations even though the amount is unimportant. The endpoint is seen as a distinct color change from blue-green to reddish-brown. Finally, a blank containing the reagents and an equivalent volume of distilled water is refluxed and titrated against the sample in the same way [21].

2.6. Plant species and experimental design

A phytoremediation study was conducted on native bamboo species such as *Bambusa bambos, Bambusa vulgaris, and Bambusa balcooa* in earthen pots. This species was chosen due to its ease of availability and fast pace of growth. The experiment had four treatments, and three replications were created for each. A pot study is used to assess the ability of *Bambusa bambos, Bambusa vulgaris, and Bambusa* balcooa to remediate cadmium levels in tannery effluent, even at lower concentrations.

2.7. Seedlings Transplantation and Experiment Duration

Each container received a transplant of healthy seedlings of uniform height. Each pot was irrigated with a tiny amount of water after transplantation. For 84 days, the three types of bamboo *(Bambusa bambos, Bambusa vulgaris, and Bambusa balcooa)* were cultivated on contaminated soils. After the experiment, all the plants were removed from the pots, and their cadmium uptake was examined.

2.8. Determination of heavy metals in plants tissues

Air-dried soil and plant samples were ground, sieved, and exposed to acid digestion for heavy metal analysis. Nitric acid and perchloric acid (9:4) are used for soil decomposition, and nitric acid is used for plant parts (US Environmental Protection Agency 1996, 1998). Following digestion, filtered samples were subjected to inductive coupled plasma-optical emission spectroscopy (ICP-OES 6000, iCap 6300 DUO, Thermo Fisher, England). The concentration of heavy Metals (Cadmium) in the samples was reported using the following equation:

(V/M) mg kg^{-1}

V = final volume of sample in a volumetric flask (100 ml) and M = dry mass of digested sample.

2.9. Experimental Variables

The uptake and elimination of Cadmium from soil were determined by monitoring the many specified factors. For each cadmium treatment, the height of the plants was measured to examine the physical growth factors. After properly washing the plant, first with tap water and then with distilled water to remove soil and other unwanted particles, the fresh weight of the plant was determined. The moisture from these plants was removed by air drying them first, followed by an overnight oven drying at 120°C. The dry weight was then determined using an electric balance. Finally, an Atomic Absorption Spectrometer determined the Cadmium in soil and plant samples.

2.10. Analysis of Total Cadmium Levels

Utilizing inductively coupled plasma optical emission spectrometry, levels of total Cd were determined in the plant materials, effluent, and blank samples (ICP-OES). Therefore, total Cd levels were assessed in the effluent sample before bamboo species were transplanted, as well as in the corresponding roots, rhizomes, stems, and leaves after a growth period of three months. For each sample, the concentrations were given in milligrams per kilogram of dry weight (mg/kg DW).

2.10.1. Determination of bioconcentration

BCF and TF were calculated to identify the plants' metal phytoremediation potential. BAF is the ratio of metal in plant parts with the metal in the effluent, i.e., BCF = metal concentration in plant part/metal concentration in the effluent. It is the efficiency of plants to accumulate metals in their tissues from tannery effluent. Plants exhibiting high BCF and TF values >1 are phytoextractors, while those with BCF and TF values <1 are phytostabilizers[22].

2.10.2. Translocation Factor

The primary criterion for assessing and choosing plants for phytoremediation is the translocation factor (TF)[23]. TF measures how much heavy metals have moved from the roots to the plant's vegetative portion, which includes the leaves, shoots, and flowers. The following equation was used to determine TF for cadmium concentrations in the bamboo species that were selected for analysis.

$$TF = \frac{\text{Concentration of heavy metal in the root (mg/Kg)}}{\text{The concentration of heavy metal in the shoot (mg/Kg)}}$$

2.10.3. Biological Analysis

Using conventional serial dilution techniques, the tannery sample is examined for various microorganisms, including fungi, bacteria, actinomycetes, and nitrogen-fixing bacteria[24,25]. To examine fungi, actinomycetes, azotobacter, and rhizobium species, nutritional agar media, rose bengal chloramphenicol agar, Kenknight and Munaier's medium, Jensen's medium, and yeast extract mannitol agar medium were used, in that order[26].The colony-forming units can be determined by multiplying the number of colonies and the dilution factor, and their ratio to the total weight of the sample gives $CFUg^{-1}$.

2.10.4. Determination of carbon sequestration rate

The method by which plants permanently store atmospheric CO_2 is known as carbon sequestration. Different sections of plants, such as above-ground and below-ground biomass, contain different levels of carbon that have been sequestered. By multiplying the total biomass (AGB + BGB) by a factor of 0.5, the total carbon stock for the selected plant species can be calculated (Intergovernmental Panel on Climate Change[27]. The results will be given in kilograms of carbon per acre per year.

2.11. Determination of Anti-microbial activity of Bambusa vulgaris

The bamboo fiber used in this study was produced from Bambusa *vulgaris* with the following sreps:

bamboo splitting → alkali degumming → acid rinsing → water rinsing → dewatering → shaking → drying → combing.

In addition, some of the cotton fiber was treated with the antibacterial agent Eugenol, while another part of the cotton and all other fibers were untreated. The bamboo bundle used in this study came from the first step of the process, and bamboo powder was obtained by grinding the natural bamboo fiber into 40~60 mesh powder. In this study, three repeated specimens for each series of tests were prepared.

2.12. Microorganisms and media

Gram-negative bacteria, Escherichia coli (E. coli, 8099), Gram-positive bacteria, Staphylococcus aureus (S. aureus, ATCC 6538), and fungi Candida albicans (C. albicans, ATCC10231) were used as test organisms. Nutrient Broth and Nutrition Agar culture medium were prepared for the bacteria growth, and Sabouraud's Agar culture medium was used for the fungi culture. The buffer solution used for diluting was phosphate buffer (PBS, 0.03mol/L, pH=7.2~7.4).

2.13. Antibacterial test

In the test for investigating the natural antibacterial property of natural bamboo fiber and the effect of bamboo shape on it, the untreated cotton was used as the negative control sample, and the eugenol-treated antibacterial cotton was used as the positive control sample. The antibacterial properties of test samples were evaluated by calculating the bacteriostatic rate as per the following equation.

$$Y = \frac{Wt-Qt}{Wy} * 100$$

Where Y is the bacteriostatic rate, %, Wt is the average CFU per milliliter for the flask containing the negative control sample after 18h contact, and Qt is the average CFU per milliliter for the flask containing the test sample after 18h contact.

2.14. Determination of anti-cancer activity

2.14.1. Preparation of leaf extracts

The leaves were dried under the shade, then powdered with the mechanical grinder, and stored in an airtight container to prepare chloroform and hydro-alcohol extracts. The powdered leaves were extracted with Chloroform by the Soxhlet extraction method. Hydro-alcohol extract of 80%v/v was prepared by taking 140 grams of powdered leaves of *Bambusa bambos* in a 2.5-liter capacity glass container and adding 300ml ethanol and 700ml water. The mixture was shaken occasionally for 3-4 days. It was then filtered, solvents evaporated, and the extract was stored in a desiccator until needed.

2.14.2. Test for Cytotoxicity

The extracts at toxic concentration damage the cells and makes pores on the membrane through which trypan blue enters. The damaged cells are stained with a trypan blue stain and can be distinguished from viable cells. Since live cells are excluded from staining, this method is also known as the dye exclusion method[28].

2.14.3. Ehrlich's Ascites Carcinoma (EAC)

Varying concentrations (10 - 200µg/ml) of chloroform and hydro-alcohol extracts of leaves of *Bambusa bambos* were prepared. The cancer cells were aspirated from the cancer-bearing mice's peritoneal cavity and washed thrice with normal saline. The cell suspension (1 x 10^6 EAC cells in 0.1 ml) was added to tubes containing various concentrations of test extracts (10, 20, 50,100, and 200 µg/ml), and the volume was made up to 1ml using phosphate buffer saline (PBS). The control tube contained only EAC cell suspension. The assay mixtures were incubated for 3 hours at 37^0C and added with two drops of Trypan blue dye. Further % of dead cells were evaluated by the Trypan Blue Exclusion method.

$$\% \, Cytotoxicity = \frac{No.of \ live \ cells}{(No.of \ live \ cells + No.of \ dead \ cells)} \times 100$$

2.15. Quality Assurance and Control

To assess the correctness and reliability of the results, samples underwent analysis utilizing sufficient quality assurances and controls (QA/QC). To prevent sample contamination from the outside, precautions were taken. High-purity analytical grade reagents were utilized throughout the entire analytical process. Before usage, glassware was given a 0.5% (v/v) HNO_3 soak and numerous rinses in distilled and de-ionized water.

2.16. Statistical Analysis

The samples were examined thrice, and the results were presented as mean and standard deviation. In addition, the F test for statistical significance was performed to compare variables between and within the groups using one-way and two-way analysis of variance (ANOVA) at P 0.05.

3. RESULTS

3.1. Growth Pattern of Bamboo

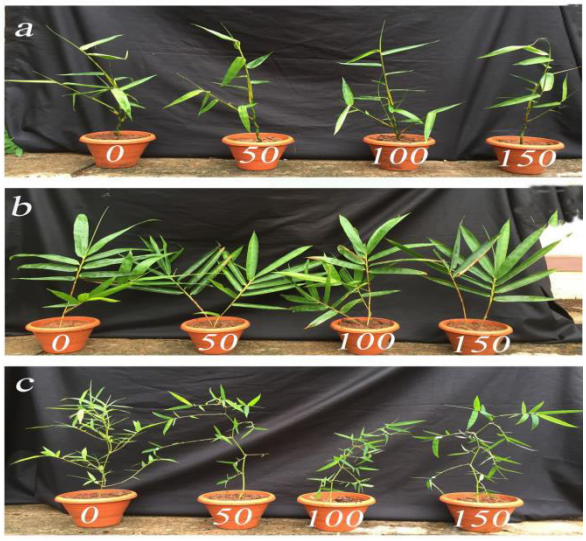

Fig 1: Growth of a.Bambusa bambos, b.Bambusa vulgaris, and c.Bambusa balcooa under different concentrations of Cadmium

Fig 1 illustrates the growth pattern of the selected bamboo species viz, a.*Bambusa bambos*, b.*Bambusa vulgaris*, and c.*Bambusa balcooa* after treatment with different concentrations of Cadmium. Control plants were grown by treating them with drinking water. There were three replicates for each treatment.

3.2. Growth Rate Test on Bamboo Plantations

Before starting the growth test, the bamboo species chosen for examination were examined for their environmental adaption in the effluent treated soil. The 84-day growth test was conducted in a lab-controlled setting, where the following variables were continuously tracked[29].

– Soil pH
– Exposure to light
– Homogeneity of the quantity of water to be supplied in all pots
– Temperature

It was feasible to determine the growth rate (gr) based on the growth of morphological elements like branches and total length. Bamboo plants demonstrated good flexibility in all conditions. The average growth rate for pots 3 and 4 was nearly equivalent to 9.56 mm/week, whereas it was similar for pots 1-2 and equal to 5.67 mm/week. The height of bamboo plants during the growth rate test is shown in Figure 2.

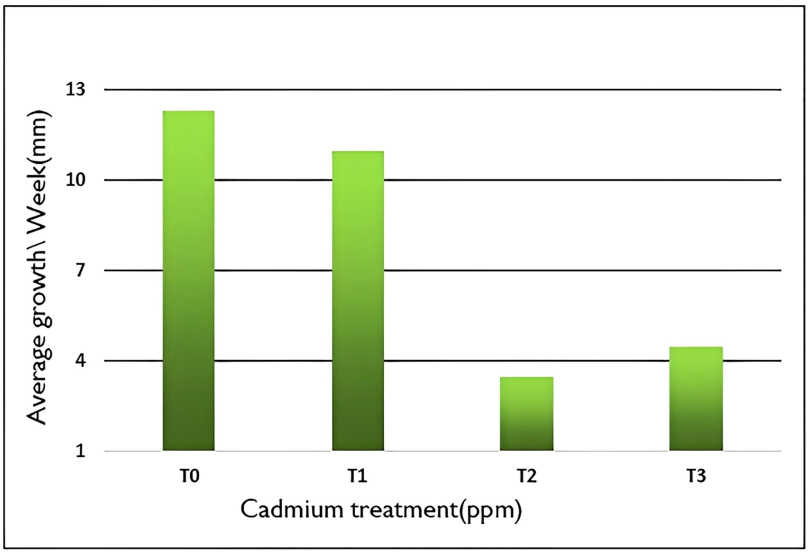

Fig 2: Average Height (cm) of Cadmium-treated *Bambusa bamboo*

Fig.2. illustrates the average height of Cadmium-treated Bam*busa bambos* (84 days). A faster growth rate was observed initially in the T1 (treated with 25 ml of effluent). However, with increasing concentration (T2-50 ml), the growth ceases in the subsequent weeks (3.89 mm/week). *Bambusa bambod* show a better tolerance against Cadmium (T3-100 ml), and the growth accelerates after four weeks (5.67 mm/week).

3.3. Contamination and Tolerance Test

Cd $(NO_3)_2.4H_2O$ was used to create an aqueous solution with 100 mg Cd/L to contaminate the soil of four pots. Although the growth rate in pots 1 and 2 was significantly reduced at a concentration of 100 mg Cd/L of effluent, the bamboo plants' vegetative functions of the bamboo varieties were still maintained. Necrosis of the upper part of the stem and the higher leaves was manifested only after 4 weeks in pot 2. The Bambusa bamboos in pot 2, polluted with an aqueous solution containing 100 mg Cd/L, exhibited no overt signs of contamination stress[30]. A faster growth rate has also been observed, and pot 2 had a stronger tolerance for bamboo than pots 3 and 4. After the fifth and sixth weeks, cadmium application led to substantial changes in tissue morphological characteristics, especially in pot 2, where some tissue necrosis was discovered due to greater cadmium consumption. Regarding volume decrease and the number of new shoots, these impacts were more pronounced on roots and rhizomes than on stems and leaves.

3.4. Cadmium Phytoextraction from the Soil

To counteract Cadmium's harmful effects, plants employ various strategies, accumulating the metal in their tissues through root absorption and subsequent transfer [31]. Following the trial, the residual amounts of Cd in the soils varied from 60 to 62 mg/kg DW for pot 3 and between 167 and 173 mg/kg DW for pot 2. Results of the soil study revealed that after growing plants on contaminated soils, the levels of Cadmium dramatically decreased, with clearance percentages of around 50%. Starting from pot 2, the percentage of Cadmium removed from the soil ranged from 45.8 to 47.4%.

3.5. Cadmium Distribution in Tissues

After the contamination test, samples of the leaves and roots of the plants in pots 1, 2, 3, and 4 were collected to do Inductively Coupled Plasma Optical Emission Spectroscopy to determine the distribution of Cadmium. The aerial portion of the plants in each pot had very low cadmium content. It shows that 84 days of Cd poisoning cannot push the roots

and rhizomes past their breaking point, allowing the metal to move to the aerial section. Cadmium was mostly discovered in the roots and rhizomes; thus, it is now known that *Bambusa bambos* concentrate it in the rhizome-root system by limiting transit in the aerial sections. Cadmium distribution in bamboo tissues in pots 1-4 is as follows: Rhizomes in pot 2 contain 153 mg of Cd, roots have 47 mg, stems have 25 mg, and leaves have 23 mg. These amounts translate to 72% for rhizomes, 20% for roots, 9% for stems, and 8% for leaves. For pots 3–4, there was a reduction in the translocation rate and cadmium in the leaves was around 5%[32]. When comparing the amount of cadmium mass maintained to the mass of plant tissues, the amount of cadmium per gram of root or rhizome is equivalent to 4.5 mg/g DW on average. The amount of cadmium per gram of stem or leaves is equivalent to 3.7 mg/g DW.

3.7. Height of Plants in Contaminated Soils

In three levels of cadmium (50, 100, and 150 ppm), the height of *Bambusa vulgaris*, *Bambusa bambos*, and *Bambusa balcooa* was measured. In the control plants, the plant height was 12.3 cm. *Bambusa vulgari*s planted in pot 3 reached the highest height, or 11 cm, followed by *Bambusa bambos* and *Bambusa balcooa*(Fig 2). The plants that had been exposed to greater concentrations of cadmium, i.e., 100 and 150 ppm, exhibited stunted development and lower height[33].

3.8. Fresh and Dry Weight of Bamboo Plants grown in Contaminated Soils

Bambusa bambos, Bambusa vulgaris, and *Bambusa balcooa* had a fresh weight of around 18.34 g in cadmium-contaminated soil at pot 1 (control plants), which declined as the cadmium content increased in pots 2 (50 ppm), pot 3 (100 ppm), and pot 4. (150 ppm). The fresh weight of *Bambusa bambos*, *Bambusa vulgaris*, and *Bambusa balcooa* has significantly decreased in values exceeding 50 ppm. The results reveal that the roots were more vulnerable to cadmium, as the plants exposed to the metal (50 ppm) started to wilt and eventually die. In addition, Cadmium was found to be more lethal at higher doses than at lower levels.

3.9. Concentration of cadmium in Bambusa bambos, Bambusa vulgaris, Bambusa balcooa

Each of the plant's parts had its level of cadmium uptake. Hyperaccumulators are plant species with exceptionally high metal concentrations in their above-ground body sections. As the amount of cadmium in the soil increased, the concentration of cadmium found in *Bambusa bambos, Bambusa vulgari*s, and *Bambusa balcooa* also increased remarkably.

3.10. Translocation Factor (TF)

The translocation factor evaluates the rate at which heavy metals are moved from roots to leaves and shoots. Compared to *Bambusa vulgaris* and *Bambusa balcooa, Bambusa bambos* had a higher rate of cadmium translocation. The main criteria for assessing and choosing plants for phytoremediation are BCF and TF[34]. With low cadmium concentrations, the translocation rate in pot 2 is approximately 1.3. It remained steady at the next concentration, 100 ppm in pot 3, but it significantly increased at 150 ppm. Furthermore, it remained above 1.3 and steady for 150, 200, and higher concentrations. If the translocation factor exceeds 1, the metal has moved from the root to the above-ground portion [35]. Only plant species with BCF and TF values larger than 1 have the potential to be employed for phytoextraction. [36]

Table 1:BCF and TF of Bamboo species grown on effluent-treated Soil

Species	BCF and TF of heavy metal(Cd)	
	BCF	TF
Bambusa bambos	3.83±0.12	2.06±0.04
Bambusa vulgaris	3.45±0.108	1.9±0.057
Bambusa balcooa	3.22±0.116	2.00±0.054

Table 1. illustrates the BCF and TF for the Bamboo sp. *Bambusa bambos,Bambusa vulgaris,Bambusa balcooa grown in Cadmium treated soil.* The data represented as mean ± SD of three independent experiments.

3.11. Physicochemical Characterization of Leather Effluent

The physicochemical characteristics of untreated tannery effluent have shown that it is acidic, has high BOD and COD levels, organic particle matter, and has an offensive odor and color. Dark ash color was seen in the raw effluent, which may have come from tanning procedures. In addition, microbial development or the breakdown of organic materials can provide an unpleasant stench. The tannery effluent's pH ranged from 4.8 to 5.5, which is extremely acidic and does not comply with the general guidelines suggested by CPCB (1995) for the discharge of effluents into land or irrigation purposes.

3.12. Antibacterial property

The results of the antibacterial test are shown in Table 2. As the negative control sample, the untreated cotton was ineffective against bacteria. In contrast, the antibacterial cotton was very effective against all test bacteria, with a bacteriostatic rate of over 99 % against E. coli and 100 % against S. aureus and C. albicans, indicating the dependability of this test. In addition, the results showed that natural bamboo fiber was ineffective against E. coli, S. aureus, and C.albicans since the bacteriostatic rate against all of them was 0. By comparison, the bacteriostatic rate of phytoextracted bamboo fiber was 75.8% against *Staphylococcus aureus* than the natural bamboo fiber.

Table 2: Bacteriostatic rate of Bamboo fibers

Sample type	Bacteriostatic rate (%)		
	Escherichia coli	*Staphylococcus aureus*	*Candida albicans*
Untreated cotton	**0**	**0**	**0**
NBF	0 (-68.9)	0 (-13.2)	0 (-41.3)
PBF	41.4	75.8	0 (-12.8)
Antibacterial cotton	>99%	100%	100%

Note: NBF=Natural bamboo fiber, PBF=Phytoextracted bamboo fiber

3.13. Cytotoxic activity

The results obtained from the cytotoxicity study revealed that chloroform extract and hydro-alcohol extract showed remarkable (dose-dependent cytotoxicity) anti-cancer activity against the test EAC cell lines. Chloroform extract showed 70% cytotoxicity compared to hydro-alcohol extract, which showed 50% cytotoxicity at the highest 200 µg/ml concentration.

Table 3: Cytotoxic activity of Chloroform and hydroalcoholic extracts of Bambusa bambos post phytoextraction of Cadmium from tannery effluent

Concentration of extract(µg/ml)	Cytotoxicity (%)	
	Chloroform extract	Hydro-alcohol extract
10	18	10
20	39	11
50	61	15
100	63	41
200	70	50

4. DISCUSSION

The process of transforming unfinished hides and skins into polished leather encompasses tanning. Depending on the type of raw material used and the final product, tanning is a difficult process involving over 130 different chemicals[37]. High total hardness, TDS, BOD, and COD levels in the tested tannery effluent will impact water quality and may result in bad taste and odor [38]. Discharging untreated effluents with such low pH values into ponds, rivers, or on land directly affects zooplankton and fisheries as low pH levels may affect the physiology of fishes[39]. The ability of a water sample to conduct an electric current is expressed numerically as electrical conductivity. The presence of inorganic materials and salts with good conductivity may cause the high conductivity of tannery effluent reported in this investigation. The processing of finished leather may have contributed to the high total suspended particles found in the tannery effluent[40]. Additionally, the presence of total suspended solids causes turbidity, which impairs light penetration in the aquatic system and affects the activity of photosynthesis. Furthermore, settling suspended particles on soil and soil fauna may cause a number of negative effects, including changes in the soil's porosity, texture, and capacity to hold water, as well as obstruction of gills in fishes. One of the crucial factors included in water pollution studies to assess the effect of wastewater on receiving waterways is biochemical oxygen demand[41]. The current investigation has demonstrated that tannery effluents have high biological oxygen demand values. The most accurate approach for calculating the total oxygen demand by the organic material in the effluent is chemical oxygen demand. In the current examination, a high quantity of COD (2529 mg/L) was found, exceeding the acceptable limit of 250 mg/L set by CPCB (1995) for effluent discharge into inland surface water[42]. It demonstrates that aquatic species cannot survive in wastewater. The hardness of the water is caused by ions, mainly calcium, sulfate, magnesium, and sodium. The findings showed that the ion concentrations were above the CPCB-recommended limit of 1000 mg/L. The water hardness measurement in the current study ranged from about 2870 mg/L, indicating that the water was exceptionally hard[43]. The lengthy soaking process used in the tannery could cause high chloride levels in the effluent[44]. Additionally, the tannery effluent's high ion and hardness content may result from the tannery effluents from various processing sources within the tannery being mixed with the aqueous system[45]. The tannery effluent was found to have an acidic pH, elevated total hardness, and high levels of TDS, BOD, and COD, according to the present study's findings. The present study also revealed that the tannery effluent was extremely contaminated and did not comply with the CPCB's prescribed standards (1995). Untreated tannery effluent discharge into the sewage system results in calcium carbonate buildup and clogs. Therefore, destroying the pollutants, or at the very least converting them to harmless substances, is superior to conventional methods. Further research using a greener bio method is currently underway. Bioremediation is an option that provides the potential to destroy or make harmless different contaminants of tannery effluent using natural biological activity. In this study, the plants grown in pots with soils with different levels of Cd treatments indicated that plant height and fresh and dry weight increased at low concentrations but decreased with high concentrations of Cadmium. The translocation factor[46] was lower than 1 for low levels of contamination but higher than 1 for higher levels of contamination. The concentration of Cd in plants increased gradually and became almost constant for higher contamination levels (T3) in all the three native species of Bamboo selected for the study. Phytoextraction by *Moso bamboo (Bambusa bambos)* has demonstrated good flexibility and great performance when compared to the other two species, *Bambusa vulgaris* and *Bambusa balcooa,* in removing cadmium from the soil, according to the observations. Also, the bamboo sp. involved in phytoremediation has potent anti-bacterial activity against a wide range of bacteria such as *E.coli, Staphylococcus aureus, and Candida albicans. Compared to the natural bamboo fiber,* the

bacteriostatic rate of phytoextracted bamboo fiber was 75.8% against *Staphylococcus aureus* and had an anti-cancer activity ranging from 60-80% for different cell lines. The anti-cancer activity of *Moso bambos* may be attributed to its antioxidant molecules and would have played a significant role in its cytotoxic nature. Additionally, Moso bambos should possess some free radical scavengers inside the tissues, which favor its anti-cancer activity[47].

5. CONCLUSION

Industrial waste has been disposed of in a dangerous way across the country due to a lack of technological, financial, and regulatory restrictions for managing hazardous wastes, risking the lives of people, animals, and plants. The current study aimed to determine *Bambusa bamboo*'s efficiency in removing Cadmium from dry and semi-arid soil that has been heavily contaminated by tannery effluent. The wide spectrum of pharmacological properties of natural chemicals obtained from bamboo, such as flavonoids, terpenes, alkaloids, polyphenols, etc., have generated a lot of interest recently[48]. As its molecular constituents, *Bambusa bambos* is reported to contain flavonoids, alkaloids, and phenolic acids[49]. The data presented indicates that *Bambusa bambos* also has potent anticancer properties. When administered as an anticancer drug, Bambusa bambos may be helpful in the treatment of numerous diseases caused due to reactive oxygen species. The presence of phenolic acids, well-known for their anti-oxidative effects, is likely responsible for the powerful anticancer action of Bambusa bambos. Along with the traditional applications of *Bambusa bambos*, more in vitro and in vivo study on isolated phytoconstituents is necessary to understand their mechanism of action.

6. AUTHORS CONTRIBUTION STATEMENT

Ms. Vidhya experimented the study. Dr. R. Kalaivani and Ms. Vidhya wrote the manuscript. Dr. R. Kalaivani supervised the work and conceived the original idea. Dr. R. Kalaivani and Ms. Vidhya developed the theoretical formalism, performed the analytic calculations, and performed the numerical simulations. Both authors, Dr.R. Kalaivani and Ms. Vidhya, contributed to the final version of the manuscript.

7. CONFLICT OF INTEREST

Conflict of interest declared none.

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