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# Employing Fabricated Loofah Sponge Supported Indiginous Chromium Sequestering Bacteria from Tannery Effluent-A Strategy for Heavy Metals Removal and Recovery

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***Abstract:-*** Anthropogenically introduced heavy metal from leather and tanning industrial effluents pose a major threat to all aquatic bodies, flora, fauna, and mainly human beings as they enter the food chain due to high bioaccumulation normality. The study deals with heavy metal mitigation by modified loofah sponge supported indigenous microorganisms. The tannery effluent and sludge were collected from Chrompet and Madhavaram, Chennai, Tamil Nadu. The chromium tolerant bacteria were screened on a selective-agar screening test, and the minimum inhibitory concentration was noted. Antibiotic susceptibility test results showed that indigenous micro-organisms were susceptible to 23 antibiotics, so it was considered for further analysis. The 16s rRNA sequencing with Basic Local Alignment Search Tool results showed *Klebsiella pneumoniae KPN0422* [accession number PQ066210]. The *Klebsiella pneumoniae KPN0422* strain showed up to 82.14 and 85.35% of chromium adsorption at 150 and 200 ppm, respectively. The lab scale approach showed modified loofah sponges immobilized Klebsiella pneumoniae *KPN0422* adsorbed 88.44% chromium, biosorption increased further up to 7.30% than free cells. So, in the future, the modified loofah sponge supported indigenous organisms, a green approach, can be used for chromium detoxification in tanneries and also in other industrial effluents and sites.

**Keywords:** chromium, Klebsiella, tannery, industry, heavy metal, loofah sponges.

## 1. Introduction

Electroplating, leather tannery, electroplating, steel manufacturing, dye, and pigment production, inorganic and organic chemicals manufacturing industry and textiles have widespread chromium (Cr)-based applications in many processes that resulted in soil and groundwater contaminations (Thacher et al., 2015). The partially treated or untreated chromium containing tannery effluents gets discharged legally or illegally to the water stream, and enter food chains, and causing many health hazards and even death at high dosages, so it leads to increasing public concern in all countries. Government agencies including the Environmental Protection Agency (EPA), the World Health Organization, and the Indian Standard Institution addressed chromium contamination, and the formulated limit for chromium in drinking water and limit was 0.1 mg/L (USEPA, 2003), 0.05 mg/L (Shah et al., 2009) and 0.05 mg/L (Indian Standards, 1993), respectively. According to ISI: The Bureau of Indian Standard (BIS) permissible discharge level of Cr (VI) from industrial effluent into inland water is 0.1 mg/L (Congeevaram et al., 2007). Potable water greatly decreases due to industrialization and urbanization, but the industrial development also propels country development by contributing to Gross domestic product (GDP), so without arbitrating industrial growth, heavy metal mitigation must be propelled, to safeguard the environment which aids in the good health of people around the world. For several decades, microorganisms were used for mitigating toxic pollutants (Chandra & Banik, 2021) by methylation, reduction, oxidation, etc., has reaped good results and attention among scientists in a few decades. The chromium is the tenth most abundant element,

located in group D with atomic number 24 and atomic mass 51.9961u, and it has no odour or taste, with a 27.7-year half-life for $^{51}Cr$, and their oxidation states differ from -2 to +6, but stability is observed in $Cr^{6+}$ and $Cr^{3+}$ states, where later is more toxic and persistent in the environment (Cheung & Gu, 2007a). The Cr (III) is one of the micronutrients for organisms' growth and metabolic activities, poorly soluble and less mobile (Xu et al., 2015), but Cr (IV) has high solubility and mobility and is reported to be carcinogenic and mutagenic in higher amounts (He et al. 2016; Fendorf, 1995; Cotton et al. 1999; Thacher et al. 2015; He *et al.* 2016). The chromium was reduced by bacteria to $Cr^{3+}$ in the presence of oxygen using the enzymes Cr6+ reductase (ChrR), chromate reductase (YieF) and TKW3 under aerobic and anaerobic conditions (Cheung & Gu, 2007b; Phillips & Taylor, 1976), for e.g., Enterobacter, Aspergillus, Shewanella, Pseudomonas, Desulfomacculum, Pantoea, Aeromonas, Sporophyticus, Bacillus, Phanerochaete, etc. have been reported as efficient chromium reducers. The highly persistent nature of heavy metal causes cell toxicity by hindering various enzymatic and metabolic reactions, by producing reactive oxygen species (ROS), by displacing biomolecules essential metal ions, or by blocking biomolecule functional groups and entering the food chain, so an economic, eco-friendly, and efficient alternative must be developed in developing countries for heavy metal mitigation (Tálos et al., 2012). Locally available cheap biocarriers with high efficiency not only act as biofilm forming matrices for structural integrity but also get shielded from stresses such as biocides or toxic contaminants presence, protozoa ingestion, desiccation, and exhibit intercell communication, promote microcolony formation and preservation, immunity against sensitive microorganisms, which is essential for biofilm performance (Lago et al., 2004). This is two-phase research; at first, pallavaram and madhavaram tannery effluent physicochemical assessments were presented. Secondly, microbial strains effectiveness is assessed through bioremediation experiments by involving indigenous microcosms to remediate heavy metal-contaminated effluents. Loofah sponge contains mainly cellulose with alcohol-hydroxyl groups (82.4%), lignin with phenolic hydroxyl groups (11.2%), and a small amount of hemicellulose and other substances (Luo et al., 2019), which can be easily modified with chemicals and has more energy adsorption capacity per unit mass with a unique framework that can be beneficial for solid-liquid separation and also can be reutilized for effluent treatment. The present work shows that sodium hydroxide, acetic acid, potassium permanganate and sodium hematophosphate modified loofah sponges, can serve as good matrices for indigenous microcosms to accelerate the mitigation of heavy metal such as chromium from tannery effluent. The study results can be useful for policy-makers, governments, scientists, and researchers as they present the idea of using heavy metal degrading smidgen microcosms as an efficient treatment rather than abundant microbes.

## 2. Objectives

The objectives of the study are to analyse the biocarrier property of novel modified loofah sponges immobilized indigenous organisms for chromium mitigation study in tannery effluents

## 3. Materials and methods

### 3.1 MEDIA AND CHEMICALS

Heavy metal salts of chromium were chosen, and about 2.82 g of potassium bichromate ($K_2Cr_2O_7$, HIMEDIA analytical grade) were dissolved in 1000 ml of distilled water to achieve a 1000 ppm dosage, and the stock solutions were sterilized and stored for further experiments.

### 3.2 SAMPLE COLLECTION

The chromium contaminated tannery effluent sample and tannery sludge samples were collected from Chrompet (latitude 12°57′51.8″N and longitude 80°07′58.1″E) and Madhavaram (latitude 13.1345N and longitude 80.2401E), Chennai, Tamil Nadu, in March 2024. The samples were transferred into a sterilized plastic container, and the cooling temperature was maintained till reaching the laboratory and stored at 4℃ in the laboratory for further use.

### 3.3 ISOLATION, SCREENING AND BIOCHEMICAL CHARACTERIZATION OF MICROORGANISMS

The indigenous microbes were isolated from tannery effluent and sludge using standard agar plate technique using sterilized nutrient agar plates and streaked for several times to get pure colonies. The plates were maintained at 37℃ for 2 days, and colony forming units were determined. The microbial colonies were screened for chromium tolerance by the agar dilution method (Cervantes & Ohtake, 1988) by supplementing media with 50 ppm of chromium. Plates were inoculated with overnight grown cultures incubated at 37ºC for a day, and growth was determined, and plates were streaked to get pure colonies. The biochemical tests such as the indole test, methyl red test, Voges Proskauer test, Simmons citrate, triple sugar iron test and lactose test, were carried out for isolated microbes.

### 3.4 PHYSICOCHEMICAL PARAMETERS OF TANNERY EFFLUENT

Physicochemical parameters of tannery effluent such as pH, colour, odour, dissolved oxygen, total organic carbon (TOC), salinity, conductivity, suspended solids, total dissolved solids (TDS), and heavy metal such as chromium, copper and nickel were determined by The Bureau of Indian Standards (BIS).

### 3.5 GROWTH CURVE OF INDIGENOUS ORGANISMS

The growth of bacteria was noted by calculating CFU in agar plates and 50 ppm chromium supplemented agar plates that was cultured with bacteria at 28℃ for 3-5 days. The growth of organisms in broth with and without 50 ppm chromium was determined by following the viable count method and measuring at OD 600 nm using a Shimadzu UV- Vis spectrophotometer (model 1780) in 1 and 2 days of incubation.

### 3.6 MINIMUM INHIBITORY CONCENTRATION

The plate dilution method was used to determine the minimum inhibitory concentration (MIC) of heavy metal against indigenous bacterial native growth. The stock solution of the chromium was prepared with potassium dichromate ($K_2Cr_2O_7$) by liquifying 2.88 g/L in distilled water. The MIC of bacteria noted at varying dosages ranging from 100, 200, 400, 800, 1000, 3000, and 5000 ppm against the isolated bacteria were noted (Kalaimurugan et al., 2020).

### .7 ANTIBIOTIC SUSCEPTIBILITY

The susceptibility of an isolated organism to different antibiotics was evaluated by Kirby Bauer's test. Antibiotic- impregnated discs (6 mm, dia. HIMEDIA) were placed with a minimum of 30 mm apart in dried Mueller-Hinton agar cultured bacteria isolate and incubated at 28 ºC for 24 h. The inhibition zones appeared were measured with HIMEDIA calibrated zone scale PW096, and organisms were classified as susceptible, resistant or intermediate to antibiotics as per Clinical and Laboratory Standard Institute guidelines (Survey, 2022). Discs containing the following antibiotics were used: Gentamycin (gen-10µg/disc), Tetracycline (TE-30µg/disc), Norfloxacin (NX-10µg/disc), Vancomycin (VA-30µg/disc), Chloramphenicol (C-30µg/disc), Ampicillin (AMP-10µg/disc), Colistin (CL-10µg/disc), Cefepime (CPM-30µg/disc), Ticarcillin/clavulanate (TCC-75/10µg/disc), Nystatin (NS-50µg/disc), Piperacillin/tazobactam (PIT100/10µg/disc), Amoxillin/clavulanic acid (AMC-30µg/disc), Cefotaxime (CTX-30 µg/disc), Cefuroxime (CXM-30µg/disc), AmphotericinB (AP-50µg/disc), Cefotaxime/clavulanic acid (CEC30/10- µg/disc), Ceftazidime (CAZ-30µg/disc), Imipenem (IPM-10µg/disc), Cefazolin (CZ-30µg/disc), Doripenem (DOR-10 µg/disc), Cefdinir (CDN-5µg/disc), Aztroneum (A0-30µg/disc), Levofloxacin (LE-5µg/disc), Cephoxotin (CN-30 µg/disc), Ceftizoxime (CX-30µg/disc), Netillin (NET-30µg/disc), Cephoxitin (CX-30µg/disc), Ciproflaxacin (CIP-5 µg/disc), Ceftriaxone (CI-30µg/disc), Fosfomycin (F-200µg/disc), Astronem (AT-30µg/disc), Ciproflaxacin (CF-30 µg/disc), Cefoperazone (CS-75 µg/disc), Gatifloxacin (GF-30 µg/disc). The isolate was determined for its multiple antibiotic resistance index (Sandhu et al., 2016).

**MULTIPLE ANTIBIOTIC RESISTANCE INDEX = NUMBER OF RESISTANCE ANTIBIOTICS ÷ TOTAL NUMBER OF ANTIBIOTICS**

### 3.8 MICROBIAL CHARACTERIZATIONS

The indigenous microbes isolated in nutrient agar plates were streaked for pure colony isolation, and genomic characterization of microbes was done by 16S rRNA sequencing, and the sequenced data were exposed to BLAST and classified using the NCBI database. The MEGA 11 software was used for phylogenetic analysis.

### 3.9 LOOFAH SPONGES FABRICATION PROTOCOL

The loofah sponges were fabricated as per previous study (Santhiya Jayakumar, Sharmila, 2024). In short, the loofah sponges were treated with 4% sodium hydroxide, 2% acetic acid, 0.6% potassium permanganate, 2% sodium hematophosphate and 30% glycerol to obtain modified loofah sponges. The This sponge has been proven to adsorb heavy metal, which are used as biocarriers and heavy metal sequestrants in this study.

### 3.10 BATCH STUDY

The bacterial optimization study was done in batch mode conditions at different pH, contact time, and temperature using a shaking incubator (Scigenics Biotech, India) at 180 RPM. The 100 ml of Luria Bertani broth augmented with 2 grams sucrose was sterilized, and after cooling, about 15 ml and 20 ml of 1000 ppm tock solutions were added to get 150 and 200 ppm dosages. The pure bacterial culture was inoculated at log phase with 0.1 OD at 600 nm in a shaker for chromium degradation. The sterilized loofah sponges, about 1 gram, was placed inside the media along with bacteria for chromium degradation. The analysis was carried out in triplicates (Srivastava & Thakur, 2007).

### 3.11 SEM and SEM-EDX

The surface morphology of fabricated loofah and immobilization of microbes was noted with scanning electron microscopy (SEM, AMETEK, US). The microbe immobilized loofah was precipitated with carrier-aluminium and under vacuum, using 15KV voltage, the sample was gold sputtered and analyzed with 2 µM and 10 µM imaging modes with a 20 mm distance. The samples were also analyzed for Energy Dispersive X-ray Analysis (SEM-EDX, AMETEK, United States) for the weight and atomic percentage of heavy metal in the sample.

### 3.12 ICP-OES

The filtrate was extracted after centrifuging the contents at 8000 rpm for 15 min (Eppendorf Centrifuge 5804 R, A444 swinging-bucket rotor), and supernatant was collected for analyzing the heavy metal. The presence of the chromium in the filtrate was analyzed using an analyte at wavelength 267.716 nm using Inductively coupled plasma optical emission spectrometry (ICP-OES, Agilent Technologies model 5800) (Kotelnikova et al., 2024) and analyzed with data software ICP-EXPERT. The chromium biodegradation was calculated (Srivastava & Thakur, 2007).

$$\boldsymbol{Chromium\ degradation}\ \% = \left\{\frac{\boldsymbol{initial\ chromium\ concentration\ (ppm) - final\ chromium\ concentration\ (ppm)}}{\boldsymbol{totalchromium\ present\ in\ media}}\right\} \times \mathbf{100}$$

## 4. Results

The tannery effluents and sludge were collected in the Chrompet and Madhavaram area in Chennai, Tamil Nadu, in March 2024. The samples were serially diluted and inoculated in enriched nutrient agar plates (10% effluent) supplemented with 50 ppm of chromium, using serial-dilution and plating techniques, at various dilutions from $10^{-1}$ to $10^{-10}$. Among 50 isolates, strain R6 has good growth, so the R6 colony was selected for further analysis. The colony forming unit was found to be $8 \times 10^{-1}$ CFU/ml in the 10-1 dilution plate, which is similar to the study by Ashraf et al (Ashraf et al., 2018). Microbes were characterized biochemically and morphologically (table 2) (Fig. 1).

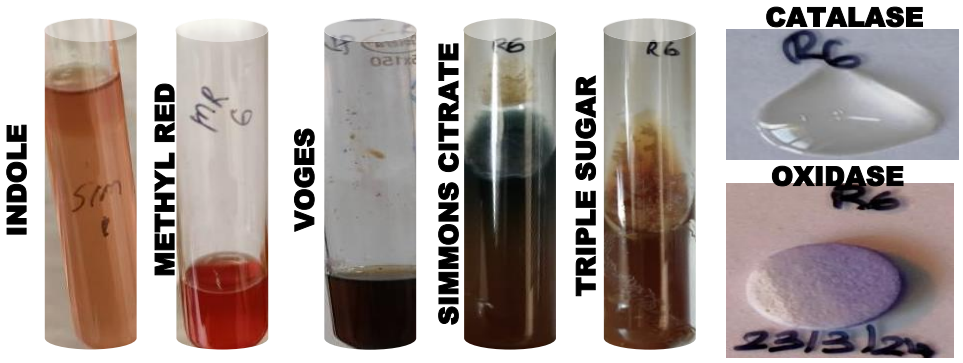


**Fig. 1: BIOCHEMICAL CHARACTERIZATION.**

The physicochemical parameters of raw tannery effluents collected were determined according to Bureau of Indian Standards (BIS) and are shown in Table 3. Though the heavy metal were less in raw data, many literature reviews show a higher percentage of chromium concentration in metals, so the study was continued for chromium detoxification.

**TABLE 1: HEAVY METALS MAXIMUM DISCHARGE LIMITS AND ITS HEALTH HAZARDS**

| S.NO | HM | MAXIMUM LIMIT OF DISCHARGE (mg/L) | | | HEALTH HAZARD |
|---|---|---|---|---|---|
| | | INDIA BIS | WHO | USEPA | |
| 1 | Cr | 0.05 (Cr (III, VI) | 0.01-0.09 | 0.1 | ALLERGIC DERMATITIS, DERMAL DISEASE, KIDNEY DISFUNCTIONS, LUNG TUMOR |

Many regulations have been implemented against HM discharge, such as the United States Environmental Protection Agency (USEPA) established Maximum Contaminant Level standards for some HM and the maximum limit of discharge framed by India BIS and the World Health Organization (WHO), as they are a major threat to the environment and human health. The maximum tolerance limit of tannery effluent containing HM established by India's BIS, WHO (Cotruvo, 2017), and USEPA (Gaur et al., 2021) and the associated health hazards are depicted in Table 1. The growth curve of the microbes decreased with an increase in chromium dosage, which is depicted in Fig. 2.

**TABLE 2: BIOCHEMICAL CHARACTERIZATION OF K. PNEUMONIAE STRAIN *KPN0422***

| BIOCHEMICAL TEST | K. pneumoniae strain *KPN0422* [accession number PQ066210]. |
|---|---|
| INDOLE TEST | -ve |
| VOGES PROSKAUER | + ve |
| METHYL RED | -ve |
| SIMMONS CITRATE | + ve |
| TRIPLE SUGAR IRON | No hydrogen sulphate formation, gas presence, or acidic in the top and base. |
| OXIDASE | -ve |

| | |
|---|---|
| **CATALASE** | **+ ve** |
| **LACTOSE, STARCH, UREA** | **+ ve, pink colony** |
| **COLONY MORPHOLOGY** | **Gram negative, rod shape, white, raised, circular, 1 mm size, transparent.** |

**TABLE 3: THE TANNERY EFFLUENTS PHYSICOCHEMICAL PARAMETERS**

| PARAMETERS | METHOD | RESULTS (mg/l) |
|---|---|---|
| **Total dissolved solids** | IS 3025 (Part 16) 2023 | 6668 |
| **Total suspended solids** | IS3025 (p17): 1984 (Realff-2017) | 2668 |
| **Total alkalinity** | IS3025 (part 23)-1986 (R-2019) | 893.45 |
| **Biological oxygen demand** | IS3025 (P-44):1993 (Realff.2019) | 1177.5 |
| **Chemical oxygen demand** | IS3025(P-46)-1993 (Realff-2019) | 3672 |
| **Total organic carbon** | TNTH/SOP/WATER/068 | 0.08 |
| **Sulphate** | IS 3025 (PART 24 SEC-1)-2022 (RA 2014) | 2471.5 |
| **Nitrate** | IS 3025 (PART 34) 1988 (RA 2019) | 88 |
| **Phosphate** | IS 3025 (PART 31)-1988 (RA 2019) | 1.04 |
| **chromium** | IS 3025 (PART 52)-2003 (RA 2014) | BQL (0.1) |

The bacterial growth curve in presence and absence of chromium was measured at OD600 nm using UV-Vis spectroscopy, in which growth retardation observed with increasing chromium percentage than native cells without the presence of the chromium. The log phase gets started at 6 hours for native cells, while cells with chromium showed a delayed log phase, which shows that organisms take time to adapt to the surrounding heavy metal habitat, and the death phase also started early for cells with chromium, while native cells showed a slow death phase, which is depicted in Fig 2.

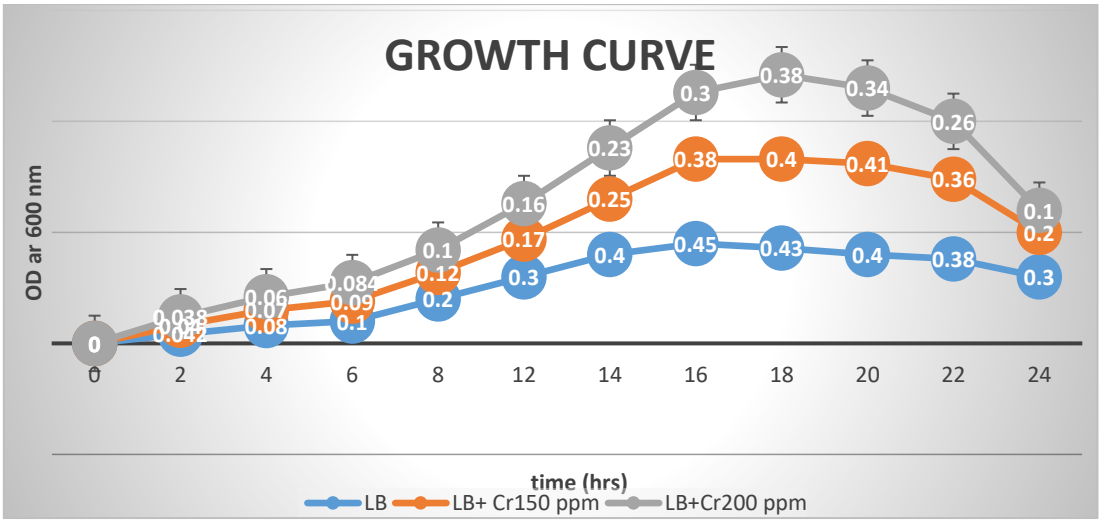


**Fig. 2: GROWTH CURVE OF K. PNEUMONIAE STRAIN *KPN0422* IN PRESENCE AND ABSENCE OF CHROMIUM**

## 2.6 MINIMUM INHIBITORY CONCENTRATION

The minimum inhibitory dosages of the Klebsiella pneumoniae strain *KPN0422* against various dosages of chromium metals prepared from stock solutions proved that the isolated bacteria were tolerant up to 5000 ppm, depicted in Fig. 3 and Table 4. Results noted in 2-3 days of incubation, as the growth is slow in the presence of the metals. Higher the percentage of metals causes more toxicity, and growth was retarded gradually when high ppm dosages of the metals were used.


**Fig 3: MINIMUM INHIBITORY DOSAGES- CHROMIUM**

**TABLE 4: THE MINIMUM INHIBITORY CONCENTRATION OF BACTERIA AT VARIOUS CHROMIUM SALT DOSAGES.**

| STRAIN/Cr (ppm) | NC | PC | Cr 200 | Cr 400 | Cr 800 | Cr 1000 | Cr 3000 | Cr 5000 |
|---|---|---|---|---|---|---|---|---|
| **Klebsiella pneumoniae KPN0422** | - | +++ | +++ | +++ | +++ | +++ | ++ | + |

The susceptibility of isolated organisms noted in Mueller Hinton cultured plates s as per Bauer-Kirby method for further usage of organisms in real time environments. The antibiotic impregnated discs were used against the growth of organisms which showed resistance to AP-50, NS-50, AMC-30, CL10, CX30, CN30, GF30, CAZ-30, AC30, COX200, AO30, CDN5, and susceptibility and intermediate to 23 antibiotics such as NX10, CZ30, DOR10, IPM10, CAZ30, CEC30/10, PIT100/10, TCC75/10, CL10, CTX30, CPM30, CXM30, CIP5, AK30, CS75, C30, TB10, NET30, LE5, FO200, CK10, AT30, K10. Among tested antibiotics, the organisms showed good susceptibility to Doripenem and colistin, so the organism considered was further depicted in table 5. The multiple antibiotic resistance index was found to be 0.4; the organisms have a high index which shows that the organism has a high risk of contamination. This study is shown to prove heavy metal detoxification by indigenous organisms and not encourage the specific organisms in real time usage as it is one of the high-risk organisms (Wang et al., 2021).

**TABLE 5: ANTIBIOTICS AND THEIR INHIBITION ZONE.**

| ANTIBIOTICS | SUSCEPTIBILITY-REFERENCE DIAMETER (CLSI) IN MM | INHIBITION ZONE (mm) | SUSCEPTIBLE/ INTERMEDIATE/ RESISTANT |
|---|---|---|---|
| NX-10 | 17 | 10 | INTERMEDIATE |
| CZ-30 | 23 | 12 | INTERMEDIATE |
| DOR-10 | 23 | 33 | **SUSCEPTIBLE** |
| IPM-10 | 23 | 18 | INTERMEDIATE |

| | | | |
|---|---|---|---|
| CAZ-30 | 21 | 15 | INTERMEDIATE |
| CEC30/10 | >23 | 21 | INTERMEDIATE |
| PIT100/10 | 25 | 12 | INTERMEDIATE |
| TCC 75/30 | 20 | 14 | INTERMEDIATE |
| CTX30 | 26 | 13 | INTERMEDIATE |
| CPM-30 | 25 | 25 | INTERMEDIATE |
| CXM30 | 20 | 15 | INTERMEDIATE |
| CIP5 | >21 | 10 | INTERMEDIATE |
| AK30 | >17 | 10 | INTERMEDIATE |
| CS75 | >17 | 14 | INTERMEDIATE |
| CL30 | >18 | 14 | **SUSCEPTIBLE** |
| TB10 | >15 | 13 | INTERMEDIATE |
| NET30 | 15 | 10 | INTERMEDIATE |
| LE5 | 21 | 21 | INTERMEDIATE |
| FO200 | 16 | 10 | INTERMEDIATE |
| CK10 | 20 | 19 | INTERMEDIATE |
| AT30 | 21 | 32 | INTERMEDIATE |
| K10 | 18 | 14 | INTERMEDIATE |
| CIP5 | >30 | 18 | INTERMEDIATE |

The isolated indigenous organisms 16 rRNA was sequenced and submitted to the NCBI-GENBANK database. The BLAST analysis of the sequence was done, and the organism was found to be *Klebsiella pneumoniae KPN0422* [accession number PQ066210]. The organism's evolutionary history and optimal phylogenetic tree were conducted by MEGA11 and inferred by the Neighbor-Joining method (Fig:4) and the Maximum Composite Likelihood method was used to compute the evolutionary distances (Tamura et al., 2004, 2021). Ten nucleotide sequences were analyzed. Pair wise deletion was used to remove all ambiguous positions, and the

final dataset has a total of 1531 positions.

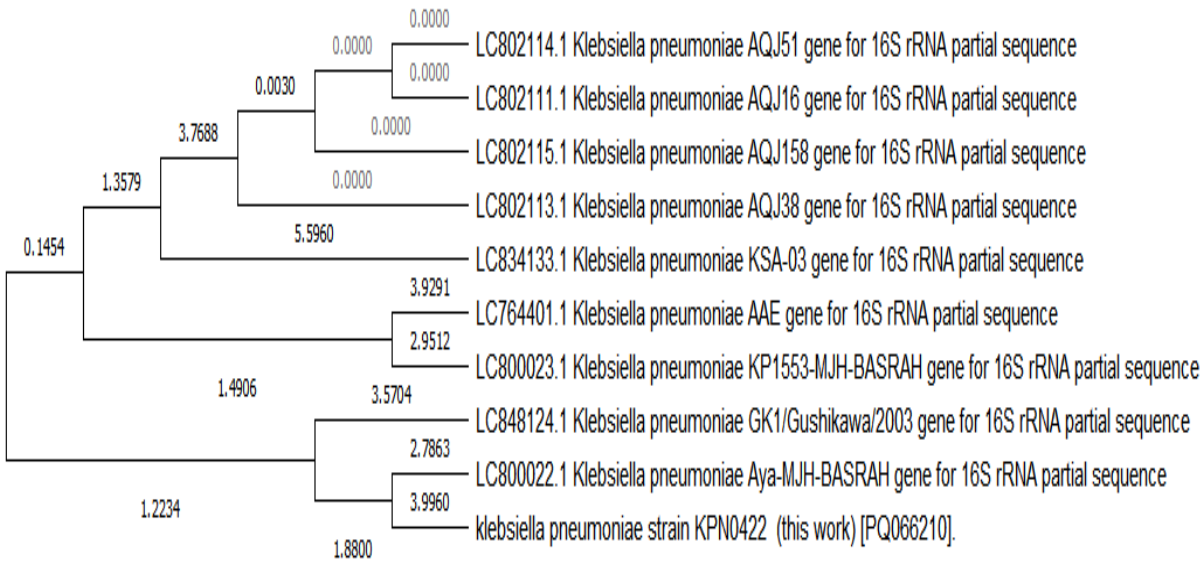


**Fig 4: KLEBSIELLA PNEUMONIAE KPN0422 PHYLOGENETIC TREE**

The fabrication of loofah sponges was done as per study (Santhiya Jayakumar, Sharmila, 2024), which proved that loofah sponges were natural copper sequestrants in modified form, so in this study, the real time approach of using modified loofah sponges as the indigenous organisms biocarriers and sequestrant at the same time. The optimization study was done in batch mode by inoculating inoculum at 0.1 OD at 600 nm. Media supplemented with 150 or 200 ppm of chromium was kept in a shaker with 1 ml of log phase inoculum for 6 days, and the optimum pH, temperature, contact time for chromium adsorption were noted.

**TABLE 6: CHROMIUM ADSORPTION BY K. PNEUMONIAE.**

| **ICP-OES ANALYSIS-CHROMIUM ADSORPTION (mg/L)** | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | **pH** | **pH6** | | | **pH7** | | | **pH8** | | |
| **DAYS** | **TEMPERATURE** | 28℃ | 37℃ | 42℃ | 28℃ | 37℃ | 42℃ | 28℃ | 37℃ | 42℃ |
| **1** | **150 PPM** | 78.62 | 101.3 | 143.4 | 73.92 | 98.4 | 131.3 | 39.05 | 121.34 | 148.72 |
| **3** | | 40.58 | 54.2 | 138.32 | 37.52 | 87.6 | 128.32 | 35.02 | 100.8 | 145.2 |
| **6** | | 28.27 | 45.58 | 123.2 | 26.79 | 73.30 | 126.23 | 33.04 | 85.98 | 139.6 |
| **1** | **200 PPM** | 161.26 | 156.93 | 175.27 | 125.60 | 148.63 | 198.70 | 145.23 | 165.24 | 198.26 |
| **3** | | 39.5 | 117.72 | 165.92 | 71.06 | 98.36 | 196.07 | 114.32 | 141.90 | 197.4 |
| **6** | | 38.2 | 92.43 | 161.50 | 29.3 | 83.9 | 195.20 | 97.20 | 94.28 | 196.2 |

The chromium adsorption by Klebsiella pneumoniae KPN0422 was dependent on initial metal concentration, pH, temperature, and contact time (Srivastava & Thakur, 2007). The optimized adsorption of chromium was

noted at pH 7 rather than pH 6 and pH 8. The adsorption of chromium was noted in order pH7>pH6>pH8. Further increase in pH decreased the chromium adsorption (Imai & Gloyna, 1990). The adsorption of chromium was dependent on initial chromium dosage, the increase in initial chromium concentration increased the adsorption up to 82.14% for 150 ppm, which increased further to 85.35% for 200 ppm, about a 3.2% increase in adsorption was noted. The adsorption was temperature dependent, the results showed that an increase in temperature decreased the chromium adsorption, the optimal temperature for chromium adsorption was 28 ℃. The chromium adsorption was increased with an increase in contact time, the maximum adsorption of heavy metal was adsorbed in 6 days. This study shows that chromium adsorption is favoured at optimal pH 7, at 28 ℃ and increased adsorption is noted at 200 ppm initial chromium dosage at 6 days of contact time. The loofah sponge immobilized Klebsiella pneumoniae KPN0422 was prepared by adding 1 ml of log phase culture and incubated in pH 7 Luria Bertani media at 28 ℃ in the shaker incubator for 6 hours, and after 0.1 OD achieved at 600 nm, 20 ml of 1000 ppm chromium was added to achieve a 200-ppm concentration. The chromium adsorption percentage at various pH, temperature, contact time and the initial metal concentration was shown in table 6 and degradation percentage was depicted in Fig 5.

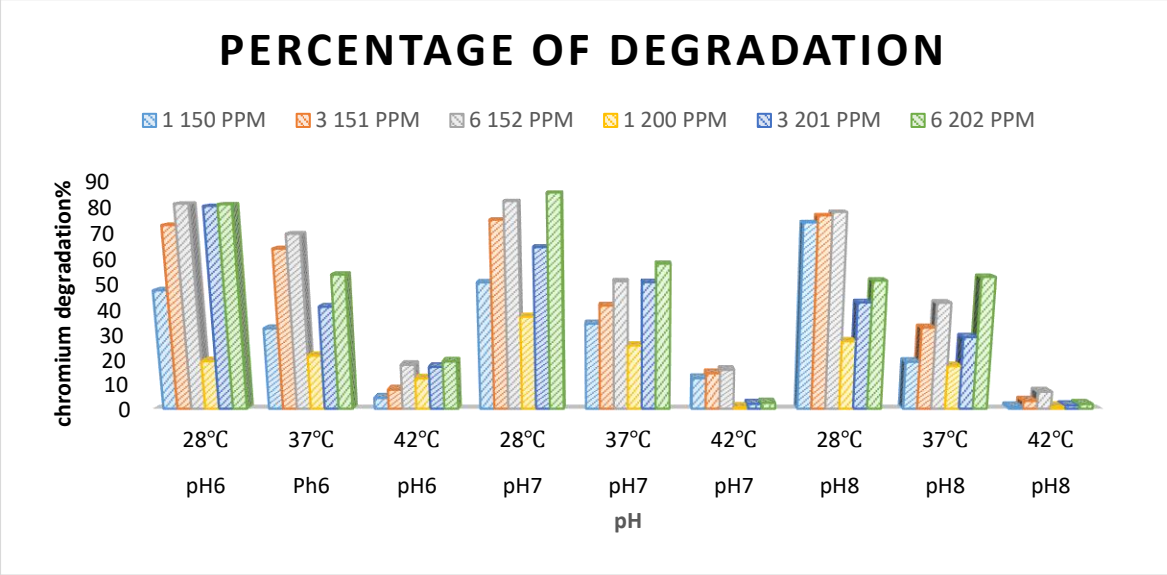


**Fig 5: PERCENTAGE OF CHROMIUM DEGRADATION OF FREE CELLS.**

The surface morphology of modified loofah sponge was devoid of the waxes and impurities and Klebsiella pneumoniae KPN0422 was nicely attached to the surface of the modified loofah sponges, and thick biofilm formation was observed. The presence of chromium was confirmed using SEM-EDX in modified loofah sponge, which proves that the modified loofah sponge used in this study was a good chromium adsorbent and acts as an excellent biocarriers for indigenous microbes.

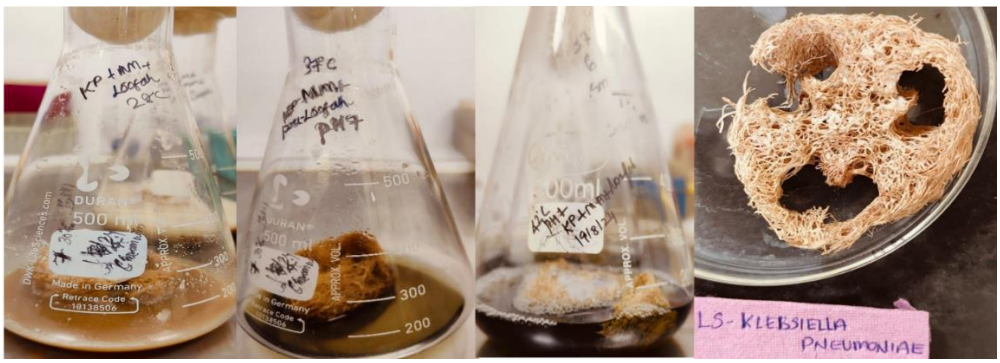


**Fig 6: (A) LOOHAH SPONGES IMMOBILIZED KLEBSIELLA PNEUMONIAE (B) CROSS SECTIONAL VIEW OF IMMOBILIZED LOOFAH SPONGES**

The loofah sponges immobilized with Klebsiella pneumoniae KPN0422 were analyzed for surface biofilm formation in SEM, and the presence of chromium was confirmed using the SEM-EDX analysis. The SEM and SEM-EDX show dense biofilm formation on loofah sponges' surfaces and the presence of chromium was observed on surfaces of microbes and the loofah sponges which proves the characteristic heavy metal adsorption property and showed successfully indulged as biofilm aiding biocarriers property of modified loofah sponges. The SEM-EDX and SEM photographs of loofah sponges, immobilized Klebsiella pneumoniae with chromium crystal lattice were noted in 4 µM and 2 µM imaging modes.

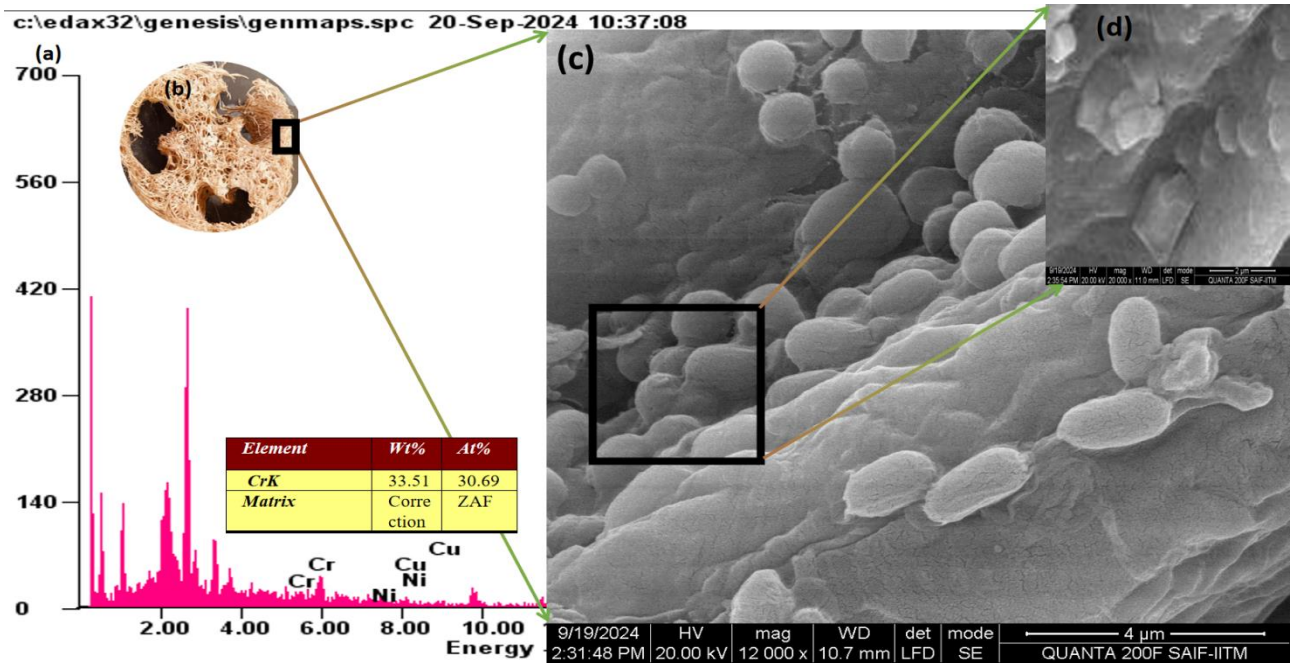


**FIG 7: (A) SEM EDX OF CHROMIUM (B) CROSS SECTIONAL LOOFAH SPONGE WITH K. PNEUMONIAE (C) 4 µM SEM PHOTOGRAPH (D) 2 µM SEM PHOTOGRAPH**

The ICP-OES analysis was done to establish the chromium concentration in the filtrate. The loofah sponge immobilized Klebsiella pneumoniae KPN0422 was tested at optimized conditions and adsorbed 88.44% of chromium, which showed up to 7.30% of increased adsorption than free cells. This study shows free cells at optimum pH adsorbed only 82% of chromium, but modified loofah sponges immobilized bacteria showed increased adsorption than free cells for 37℃, 42℃. Thus, immobilised cells shows better efficiency than free cells.

**5. Discussion**

The immobilized biofilm forming microbes show better potency to adsorb heavy metals such as chromium than free cells (Pallee Shree et al., 2023; Srivastava & Thakur, 2007), as the biofilm production hides the drug interacting sites and the organisms could withstand higher stress. This study considers modification technique as per previous study (Santhiya Jayakumar, Sharmila, 2024), as the modification involves chemicals such as the sodium hydroxide, sodium hematophosphate, acetic acid, and glycerol. Pristine loofah sponges composed of 55–90 % of cellulose, 8-22 % of hemi-cellulose, 10-23 % lignin, extractives >3.2 %, ash, and others 0.4% (Chen et al., 2019), as the impurity blocks the water adsorption, alkali treatment with sodium hydroxide was done (Begum et al., 2021). The sodium hematophosphate acts as a surfactant and glycerol acts as the wetting agent to remove all impurities and improves the tensile strength of the loofah sponges. The SEM and SEM-EDX analysis proved the efficient biocarrier property of modified loofah sponges along with heavy metals sequestrant property. The ICP-OES analysis proved the higher percentage of reduction in modified loofah sponges immobilised microbes than free cells at optimised conditions, this shows efficiency of biofilm forming organism. This study proves the indigenous bacteria efficiency in indigenous contaminants such as heavy

metals. Further research must be done to analyse all other contaminants reduction by novel modified loofah sponge immobilized indigenous organism.

## 6. Conclusion

The indigenous bacteria in this study were found to be K. Pneumonia KPN0422 strain which has an efficient heavy metal degradation property compared to other bacterium, as bacteria from other sources will not tolerate high heavy metal loads, while indigenous bacteria thrive in that habitat and have developed the property to thrive in the heavy metal present environment, which also safeguards the metabolic property unlike other cells. The SEM images show a good oval characteristic structure of bacteria, which shows the tolerability of organism without any distortions for heavy metal chromium. The SEM-EDX proved the presence of the chromium in the loofah sponges as well as microbial surface, which shows surface adsorption property of the microbes. The ICP-OES analysis proved that about 173.21 mg/g of chromium adsorption at 150 ppm while at 200 ppm adsorbed 175.7 mg/g, which further increased by providing biocarriers modified loofah sponges 177 mg/g. Thus, this study proves novel modified loofah sponges as biocarriers is good for acting as immobilizing stages and acting as natural fibers and uptakes a great amount of chromium. This study doesn't initiate using contaminants in real time waste-water treatment, as it leads to infection outbreaks. This study shows the ability of indigenous source bacteria as a best source of contamination detoxification or removal, and efficiently modified loofah sponges can be good sources of biocarriers for heavy metal contaminated environments as they propel the adsorption efficiency of organisms.

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### Author contribution

The authors confirm their contribution to the paper as follows: study conception and design, data collection, analysis and interpretation of data, draft manuscript preparation: Santhiya Jayakumar, supervision: Dr K J Sharmila. All authors reviewed the results and approved the final version of the manuscript.

### Competing interest

The authors, Mrs. Santhiya jayakumar, Dr K J Sharmila declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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