



Journal of Integrated SCIENCE& TECHNOLOGY

Chemically modified Ginger and Spirulina for bioremediation of Hexavalent Chromium from polluted water

Bhanu Revathi Kurella^{1*}, Aishwaraya Srinivasa Ramanujan¹, Bhagyashree Nagaraj¹, Rajeswari Narayanappa¹, Shinomol George Kunnel²

¹Department of Biotechnology, Dayanada Sagar College of Engineering, Affiliated to Visveswaraya Technological University, Belagavi, Bangalore, India. ²Department of life Sciences, Kristu Jayanthi College (Autonomous), Bangalore, India.

Received on: 05-Oct-2023, Accepted and Published on: 20-Dec-2023

ABSTRACT

Heavy metals are toxic pollutants that are persistent in air, water and soil. These metals are non-biodegradable and cause variety of disorders due to their bioaccumulation in living organisms. Chromium is one such hazardous heavy metal found in effluents of tanneries, electroplating industries, etc. The current study presents natural biosorbents and their chemical modification and



immobilization as a low-cost alternative for uptake of hexavalent Chromium. Ginger (*Zingiber officinale*) and *Spirulina maxima* are used as biosorbents in the current study. The physicochemical parameters required for biosorption were optimized by batch adsorption experiments to attain maximum adsorption of upto 70% in lake waters of Bangalore city. The maximum adsorption obtained by *Z. officinale* - 38%, 78%, 88% and *S. maxima*, 22%, 48%, 39% was obtained using natural, chemically modified and immobilized biosorbent respectively in lake water. Treatment in lake water not only reduced the metal concentration but also mitigated the oxidative stress in the liver homogenates of zebrafish, depicting the effectiveness of bioremediation methodology and biosorbent used in the current study.

Keywords: Atomic Absorption spectroscopy, bioremediation, biosorbents, chromium, Spirulina maxima, Zingiber officinale,

INTRODUCTION

Chromium is a major heavy metal pollutant prevalent in air, soil and water. In aqueous solutions, Chromium exists in two forms, the trivalent and hexavalent (Cr (VI))form. Hexavalent chromium is a highly reactive heavy metal and is found to cause neurological, reproductive, respiratory and metabolic disorders subsequent to the release of oxygen free radicals and acts as a carcinogen in the lungs and liver.¹ Cr (VI) is among one of the the 17 chemicals identified

*Corresponding Author: Dr. Shinomol George K. Faculty, Department of Life Sciences, Kristu Jayanthi College (Autonomous), Kothnur Post, Bangalore, Karnataka, India- 560077 Tel:8123343560 Email: shinojesu@gmail.com

Cite as: J. Integr. Sci. Technol., 2024, 12(4), 782. URN:NBN:sciencein.jist.2024.v12.782

©Authors CC4-NC-ND, ScienceIN http://pubs.thesciencein.org/jist

by the United States Environmental Protection Agency as biggest threats to human systems. It is also found to be one of the top 20 contaminants that are considered for bioremediation and removal from the natural environment.² In India, the permissible limit of Cr^{6+} in effluent waters is 2 mg/l according to the norms of the Central Pollution Control Board.¹

Industrial effluent waters that contain both chromium and other metal cations have harmful effects on the microbial communities that mediate wastewater treatment systems³. It is estimated that numerous workers are exposed every year to elemental Chromium and its compounds in their work environment. In humans and most animals, Cr^{3+} is a micronutrient that is involved in glucose, lipid and protein metabolism by facilitating the working of insulin. However, occupational exposure has posed to be a vast concern owing to elevated risk of diseases caused due to exposure. Chromium concentrations range between 1 -3000 ppm in soil, 5 - 800 ppb in sea water, and 26 - 52 ppm in small aquatic bodies such

as rivers and lakes. Unexpected or intentional intake of enormously high levels of Chromium (VI) has resulted in severe respiratory, cardiovascular, gastrointestinal, hematological, nephrological, hepatic and neurological effects in humans. Although the incidence of carcinogenicity in terrestrial mammals and humans seems to have stronger correlation to exposure, the mechanism of carcinogenecity may be due to oxidative damage which manifests as genotoxic damage resulting from chromosomal abnormalities, and DNA strand breaks.⁴

Adsorptive removal of Cr(VI) by biosorbents can drastically reduce the human health risks associated with polluted drinking water.5. Conventional methods for removal of heavy metals from industrial effluents include chemical precipitation, filtration, chemical redox conversions, ion exchange, electrochemical treatment, reverse osmosis, use of membrane technologies and recovery by evaporation⁶. These techniques may be ineffective or highly expensive mainly with the heavy metals concentration in solution ranging from 1-100 ppm7. Hence, it is crucial to formulate a novel, cost effective, and environment friendly process for mitigation of heavy metals in drinking water. Bioremediation of Chromium from polluted drinking water initially was proposed by subjugation of Cr(VI) to a lesser toxic form of Cr(III) which possesses better chances of elimination from human body8. However the chances of reoxidation of Cr to hexvalent form may not be completely overlooked.

Some of the biosorbents which were assessed for metal ion biosorption from aqueous media are bacteria, yeasts, Spirulina biomass, sea vegetable (Bladderwrack), by-products and biowastes from the agro, forestry and fishery industries.⁹ However, the uptake of heavy-metals by dead or inactive biological materials may also lead to secondary sludge that could cause additional harm and difficulties in disposal. Biosorbents used in this project are readily available, have medicinal and nutritional value and are economic.

Spirulina maxima belongs to the Phylum Cyanobacteria and constitutes carbohydrates (15%-25%), proteins (55%-70%), essential fatty acids (18%) minerals, and vitamins. It also contains photosynthetic pigments such as carotenoids, chlorophyll a and phycobiliproteins such as the phycocyanin. Spirulina is proposed to be an excellent food and dietary supplement, and also having anticancer, antiviral, and antioxidant properties.¹⁰ Its therapeutic protein, phycocyanobilin is a chromophore bound to phycocyanin, which is a potent inhibitor of NADPH oxidase, the key enzyme oxidative stress in many involved in detrimental neurodegenerative, fibrotic, or inflammatory disorders.¹¹ It was also found to mimic the activity of bilirubin in organisms.¹² Many marine and fresh water algae such as Chlorella, Dunaliella and Spirulina were experimented for heavy metal uptake from contaminated water.

Various aquatic and terrestrial plants are available for phytoextraction of Cr and among them are the water lettuce, duckweed, Indian mustard, drumstick, Mango leaves and Neem that are shown to have metal chelation property. *Zingiber officinale* (Ginger) is known to help relieve gastrointestinal irritation and suppress gastric contractions. It also has anti-inflammatory and broad spectrum anti-microbial properties. Ginger is found to possess more than 20 phenolic phytochemicals including gingerols and diarylheptanoids. They act on the pro-oxidant property of metals and bind to them. These adsorbents may be assessed to reduce the metal concnetration from water thereby reducing the oxidative stress caused by Cr^{6+} ions in aquatic life. Biosorption is however sensitive to physicochemical characteristics and may be needed to optimize and then tested in batch adsorptive experiments with fixed volume of water. In the current study, water samples from contaminated lakes in and around Bangalore were subjected to batch adsorption studies to adapt ex- situ remediation strategy for mitigation of pollutants using the biosorbents- *Zingiber officinale* and *Spirulina maxima*.

METHODOLOGY

PREPARATION OF THE BIOSORBENT

Natural biosorbent:

The biosorbents used for the biosorption of Chromium are *Spirulina maxima* and *Zingiber officinale* (Ginger). The washed ginger biosorbent was cut and sundried for 20 days alongside *Spirulina* powder. The sundried biosorbents were taken and ground individually, to obtain amorphous powder as shown in figure 1 below.



Figure 1(a). Sun dried biosorbent: Z. officinale 1 (b). Sun dried biosorbent: S. maxima

Chemical modification of *Z. officinale* and *S. maxima* biosorbent:

The chemical modification was performed for *Z. officinale* and *S. maxima* separately. The sieved ginger and *Spirulina* powder was treated with 0.1N NaOH and the slurry was mixed at 300 rpm using a magnetic stirrer for 1 hour at room tempertaure to eliminate additional alkali. Subsequently, the powders were sieved on a 40mm mesh, rinsed twice with water and treated with citric acid and dried at 50°C. The biomass was then filtered onto Whatman filter paper No. 41 and washed with distilled water to get rid of surplus citric acid. This biomass was further dried overnight at 50°C and was stored in airtight containers for further tests. This procedure was done to introduce anionic surface groups such as – OH- and –COO- for enhanced uptake of cationic Cr^{6+} ion.

Immobilization of biomass powder:

The dried biomass was suspended in distilled water and stirred with 3% sodium alginate. 100 ml aliquote of sodium alginatebiomass suspension was added dropwise to 0.2M CaCl₂ using syringe pump. The Calcium alginate beads formed were allowed to cure for hardening for 30 minutes. The beads were soaked in CaCl₂ solution for 4 hours to complete the gelling and was then washed with saline to remove excess Calcium ions.

Characterization of the dried biosorbent

The dried biosorbents *Z. officinale* and *S. maxima* (natural and chemically modified) biosorbents were characterized by subjecting them to FTIR (Fourier Transform Infrared) spectroscopy to identify the surface functional groups present on the cell wall of the adsorbent and thus the possibility of adsorption between the metal ion in the solution and the surface functional groups on the biosorbent¹³. The metal ions being positively charged tend to adsor b to the negatively charged moities present on the exposed part of the biosorbent. The FTIR spectra of the biosorbents were analyzed using IRpal 2.0 software for the identification of the functional groups.

PREPARATION OF CHROMIUM STOCK SOLUTION AND ESTIMATION OF CHROMIUM USING DIPHENYL CARBAZIDE METHOD

Chromium solution was prepared by dissolution of predetermined, weighed quantity of Potassium Dichromate $(K_2Cr_2O_7)$ in deionized water. 1000 ppm of Chromium solution was formulated by dissolving salt in one liter of double distilled water and serially diluted with double distilled water to obtain a concentration of 10 to 300ppm of Chromium solution. It was mixed with 0.25ml of phosphoric acid and pH was adjusted to 1.0 ± 0.3 and diluted to 100 ml using double distilled water. Further Diphenylcarbazide reagent was added and mixed well, incubated and absorbence was measured at 540 nm.

KINETIC BATCH STUDIES FOR ASSESSMENT OF BIOSORPTION

The batch adsorption experiment was conducted by contacting the biosorbents (*S. maxima* and *Z.officinale*) individually with the metal solution at room temperature $(27 \pm 0.1 \text{ °C})$ to optimize the physicochemical parameters viz. pH, biosorbent dosage, temperature and contact time for maximum biosorptive uptake of Chromium ions¹⁴. The percentage of biosorption was calculated using equation (1) as:

% biosorption = $(q_0-q_e)/q_o *100$

Where q_0 is the initial concentration of the Cr^{6+} ion an q_e is the equilibrium concentration of the ions in the solution after biosorption.

(1)

Effect of pH

The batch trials were conducted by contacting 1g biosorbent with 50 mg/l of Cr(VI) solution, at varied pH, range from 1 to 8. It was found from previous experiments, that at pH less than 2, the elevated proton level diminishes the metal sorption and above 6, the precipitation of metal ions is facilitated due to neutralization of surface negative charges. The pH was adjusted by using phosphate-citrate buffer. The mixture was agitated for an hour at room temperature, adsorbent was filtered, and the final pH for each sample was estimated again¹⁵. The equilibrium concentration of Cr ion in the filtrate solution was assessed colorimetrically by diphenyl semicarbazide method.

Effect of adsorbent dosage

The biosorbent levels were varied in order to obtain the optimum dosage of the biosorbent. For both the biosorbents used in this study (*S. maxima* and *Z. officinale*), the biosorbent dosage ranged from 0.01g to 2 g for a fixed volume of 50 mg/l of solution at the optimum pH previously determined. Natural, immobilized beads and chemically modified biosorbents were added separately to the Chromium solution at optimum pH, which was obtained from the previous results. The samples were incubated in the shaker incubator for one hour at room temperature, filtered, and the equilibrium concentrations of Cr^{6+} in the filtrate of all the samples were estimated spectrometrically, by testing the metal concentration in the samples, and thereafter the percentage biosorption was calculated using equation (1).¹⁶

Effect of Contact time

To obtain the optimum time for biosorption, experiments in batch mode, with an initial metal ion concentration of 50 mg/l at optimal pH and biosorbent dosage, which were obtained from the first two optimization tests were conducted. The prepared samples were incubated in the incubator, at different intervals of time (10, 20, 30, 50, 60,70,80 and 90 minutes). After removing the samples from the shaker incubator for every time interval, the samples were filtered and the filtrate subsequently tested for concentration of Cr(VI) colorimetrically by diphenyl semicarbazide method.¹⁷

Effcet of Temperature

The optimum temperature was estimated by performing batch experiments with the same concentration of 50 mg/l at optimum pH, optimum biosorbent dosage, and optimum contact time, for which maximum biosorption occurred, which were obtained from the previous optimized parameters. The prepared samples were incubated at different temperatures ($20^{\circ}C$ - $90^{\circ}C$) following which, the filtrate was tested for equilibrium concentration colorimetrically and percentage biosorption was calculated using the equation (1)¹⁸.

TREATMENT OF LAKE WATER SAMPLES

Lake water was collected from Bellandur and Madiwala lakes of Bengaluru, India according to American Public Health Association (APHA) standards of examination of water. The water was stored in sterile containers after acidification by nitric acid to prevent the deposition of metal ions due to precipitation and also to prevent any further microbial growth in the collected water. The optimum dosage of biosorbents prepared were weighed and added to 100ml of deionized water and was stirred to activate the extract. The solution was then poured into 1 litre of the lake water sample kept in conical flask and stirred for 5 minutes. The pH of the mixture was adjusted to the optimized value. The set up was kept undisturbed for the optimum contact time. After incubation time, the solution was filtered through a Whatman 41 filter paper. The procedure was reiterated for all the water samples treated with 6 different biosorbents (Immobilized, natural and chemically modified forms of ginger as well as Spirulina). The treated water samples were then stored at 4°C and used to estimate the concentration of Cr6+ in the untreated and treated water samples using Atomic Absorption Spectrometer (AAS) from Thermo Fischer Scientific using an air-acetylene flame.

ASSESSMENT OF HEPATOTOXICITY INDUCED BY CR (VI) IN ZEBRA FISH

Determination of LD50 value:

Chromium solution of 0.8,1.6,2.4, upto 12 mg/l was prepared and poured into 15 different tanks. A group of ten male wild type Zebra fish (*Danio rerio*)were introduced into each tank and a control group of fish without metal ion exposure was maintained. Observations were made every 24 hours. Viability of the fish was monitored and the LD50 range was determined.

The zebra fish were exposed to the lake waters individually and also separately in lake waters treated with biosorbents. The liver homogenates of the 24 hour exposed fish were examined to assess the protein damage by protein carbonyl assay as the biomarker of oxidative stress. A control group of fish were only exposed to the metal ion without addition of biosorbent to assess the mitigation of oxidative stress. *Z. officinale* was the only chosen biosorbent as *S. maxima* had less than half of its potency to chelate Cr(VI) ions. **Protein carbonyl assay:**

Protein carbonyls were assessed by the protocol described by Levine et al $(1990)^{19}$.100 mM of phosphate buffer was added to zebra fish liver homogenate and pH was adjusted to 7.4. The above solution was centrifuged and Trichloro Acetic acid (TCA) was mixed with the supernatant and centrifuged at 13700 rpm for 20 min (-4°C). The pellet obtained from above process was suspended in DNPH and incubated in dark for 1 hour. To the protein precipitate obtained, 20% TCA was added and centrifuged at 5000 rpm for 10 min. Consequently, the supernatant was discarded and precipitate was washed with acetone. The pellet was dissolved in guanidine hydrochloride solution to form a suspension and then protein carbonyls were estimated by UV visible spectrophotometry at 366 nm.

RESULTS AND DISCUSSION

CHARACTERIZATION OF DRIED BIOSORBENTS

The results illustrated in Figure 2 shows the FTIR spectra of the biosorbent. This result depicts the presence of the functional groups on the surface of the biosorbent particles.²⁰



Figure 2. FTIR spectrum of *Z.officinale* in its natural dry amorphous powder form.

From the FTIR spectrum, it may be concluded that the strong stretching at 3431 cm⁻¹ is due to the vibration of C=O, N-H and phenol groups. Also, the bands at 2928 cm⁻¹ and 1022 cm⁻¹ can be attributed to the vibration of $-CH_3$ and C-O groups respectively.

The FTIR result obtained after chemical modification of *Z*. *officinale* indicated modification in functional groups as depicted in peaks in the figure 3. The major peak at 3362.37 cm⁻¹ indicates a carboxylic acid - OH - broad dimer along with CO stretch at 1647.88 cm⁻¹ and Thiocarbonyl, ethers R-O-R CO stretch at 1080.62 cm⁻¹. These peaks indicate successful introduction of surface groups due to chemical modification. These results are consistent with that of previous studies using acid and base treated ginger root for uptake of divalent metal ions. which show similar peaks after chemical modification to prepare the biosorbent for metal uptake²¹. It is evident from earlier studies that presence of negatively charged metal ions.^{13,17} The presence of gingerols could be responsible for the hydroxyl groups on the biosorbent surface.²¹



Figure 3. FTIR spectrum of *Z. officinale* in its chemically modified form.

From the FTIR spectrum of natural and chemically modified *S. maxima*, it clear that –COOH and –OH groups are abundant and act as H^+ ion donors as biopolymers. The FTIR spectrum of *S. maxima* adsorbent powder is seen in figure 4. In the biosorption with Cr (VI) metal ions, therefore, deprotonated forms of the previously mentioned groups may be involved. In Figure 5, FTIR spectra of chemically modified *S. maxima* powder showed slight to marginal shifts in the peaks respectively from 3429, 2924, 2864,1433 and 1400cm⁻¹ to 3400, 2926, 1730, 1616 and 1411cm⁻¹. These shifts may occur due to changes related to the counter ions with the carboxylate and hydroxylate ions.²¹



Figure 4. FTIR spectrum of *Spirulina maxima* (natural powder) as potential biosorbent



Figure 5. FTIR spectrum of *Spirulina maxima* (chemically modified) as potential biosorbent

OPTIMIZATION OF BIOSORPTION PROCESS PARAMETERS

Effect of pH

It was observed from previous studies that pH of the solution affects the solubility of the Cr (VI) ions and thus the concentration of the counter ions adsorbed on to the functional groups on the biosorbent surface. Consequently, pH is found to be one of the most important parameter that mediates the biosorption and uptake of heavy metals from solutions.¹⁸ The effect of pH on the adsorption of Chromium ions onto Z. officinale was assessed and the results are indicated in Figure 6. It is evident that the maximum potency of biosorption for Cr⁶⁺ ions achieved were 54%, 74% and 39% at pH 2 when treated with Z. officinale in its natural, chemically modified and immobilized form respectively. It was found that most of the cations absorb with greater efficiency at acidic pH as seen with cone biomass of Pinus sylvestris, which showed 100% efficiency of biosorption at pH 1.23 Therefore, all the experiments for biosorption of Cr⁶⁺ were carried out at pH 2 using Z. officinale as the biosorbent. Similiar results were obtained in case of Cr6+ adsorption using chemically modified Walnut shell waste²². This may be attributed to displacement of H⁺ ions with more stronger Cr6+ ions onto the surface of biosorbent. The ionic strength and radius of Cr⁶⁺ are much higher than that of H⁺ which may lead to stronger binding onto the surface of biosorbent.^{7,8}



Figure 6. Effect of pH on the biosorption of Chromium by Z.officinale.



Figure 7. Effect of pH on the biosorption of Chromium by S. maxima.

The effect of pH value on the biosorptive removal of Chromium ions onto S. maxima was assessed and the results represented in figure 7. It is evident that the highest biosorption for Cr^{6+} reached 54% at pH 7 and 75% at pH 2 when treated with S. maxima in chemically modified form and immobilized form respectively compared to natural form of the algae. The above results indicate that the biosorption of Cr increases with rising pH value. At lower pH, the concentration of positive charge (protons) was enhanced on the surface of the biomass, that constraints the access of heavy metal ions to the surface of biomass due to repulsion of like charges. As the pH increases, the hydrogen ion concentration declines and the surface of the biomass is more negative²³.Reddy et al have shown that increased adsorption by S. maxima at higher pH could mean that surface charges have higher affinity towards Cr⁶⁺ at neutral pH only when most of the surface groups may be open to biosorption.15,17

Effect of dosage of biosorbents

Various dosages of bisorbent ranging from 0.01 to 2 g/100 ml were utilized to understand the effect of biosorbent dose on the uptake of Cr^{6+} ions as shown in figure 8 and 9. The results revealed that the adsorption efficiency of Cr^{6+} on *Z. officinale* and *S. maxima* were significantly affected by the dose of the biosorbent in the solution. The biosorption of Cr(VI) ions was enhanced with consequent increase in the biosorbent's dose and almost became constant at higher dosage when using *Z. officinale* but declined with subsequent increase in the dosage of *S. maxima*. This response in case of *S. maxima* could be interpreted by the formation of biomass aggregates at higher doses, which reduces the accessible surface area for biosorption. The effect of biosorption on the binding sites follows saturation kinetics with an increase in biosorbent dosage, which may be consequent to crowding effect.²³

It is seen that the highest adsorption for Cr(VI) for Z. officinale at 1g/100ml was with the chemically modified form(78%) compared to immobilized (40%) and natural (36%) form. Further it is also observed that the maximum biosorption for Cr+ with S. maxima was with immobilized form (70%) followed by chemically modified (48%) and natural (36%) forms at 1 g/100ml dosage. Therefore, the dose of 1g/100ml of solution was selected as the optimal dose for both the biosorbents for the remaining part of the study. Adsorption increases with increasing exposure to active sites. At higher concentrations, repulsive forces interfere with adsorption possibly due to more ligsnocellulosic compounds hindering adsorption.^{22, 24}



Figure 8. Effect of dose of Z. officinale on the biosorption of Chromium



Figure 9. Effect of dose of S. maxima on the biosorption of Chromium.

Effect of contact time

The time of contact between the biosorbent and metal ions is very crucial in the biosorptive removal of ions. The outcomes indicate that adsorption of Chromium using *Z. officinale* was rapid and then gradually increased till it almost attained equilibrium at 70 minutes with the biosorption rate of 50% and 45% for the natural and immobilized forms and started decreasing thereafter as seen in figure 10. For the chemically modified form 70% biosorption was observed at 70 minutes depicting its superior nature compared to the other two forms. The rate of biosorption became almost constant at 90 min for all the forms of ginger used. These results can be compared to the adsorption of Cr(VI) ions by *Kappaphycus*



Figure 10. Effect of contact time on the biosorption of Cr^{6+} by *Z.officinale.*

sps where the rate of biosorption decreased beyond 90 minutes. The equilibrium was attained at about 90 minutes beyond which saturation was observed²⁶.Similar results were observed in case of biosorption using walnut biomass for Cr^{6+} adsorption by Garg et al.²²

The outcome of contact time on biosorption of Cr(VI) ions by *S. maxima* is shown in figure 11. The results for the biosorption of Chromium using *S. maxima* was rapid and then gradually increased till it almost attained equilibrium at 80 min with the biosorption rate of 30%, 64% and 71% for the natural, chemically modified and immobilized forms respectively and started decreasing thereafter. With increase in contact time biosorption is enhanced due to slow kinetics of adsorption as Cr^{6+} molecules were observed to displace other ions and bind to the surface ions at a slower rate owing to gradual increase in rate of biosorption.



Figure 11. Effect of contact time on the biosorption of Cr^{6+} by *S. maxima.*

Effect of temperature

The effect of temperature on the removal of Cr in aqueous solution by Z. officinale and S. maxima was observed by changing the temperature between 10° C and 90°C. The data portrayed in the figure 12 below depicts a decrease in biosorption of metal ions by the biomass with an increase in temperature. Close observation of the graph shows that majority of the metal content has been adsorbed around 40°C for the biosorption of Cr⁶⁺ using Z. officinale with the highest rate of biosorption being 75% for chemically modified form followed by 54% for natural and 38% for immobilized form respectively. In the results obtained for biosorption using S. maxima in figure 13, it can be observed that the best temperature for biosorption is 20°C where the highest rate of biosorption was observed for immobilized algal biomass (74%). Natural and chemically modified forms showed 34% and 48% biosorption respectively. The magnitude continues to decline as temperatures are increased further. This is due to weakening of the attractive forces between surface of the biomass and heavy metal ions at increasing temperature, and consequently, the sorption decreases. At elevated temperature, the thickness of the boundary layer declines, owing to the enhanced tendency of the metal ion to escape from the surface of the biomass to the solution, which leads to a decrease in biosorption as temperature elevates.²⁵



Figure 12. Effect of temperature on the biosorption of Cr^{6+} by *Z. officinale*



Figure 13. Effect of temperature on the biosorption of Cr^{6+} by S. maxima

Lake water Treatment

The water samples that were collected from the polluted lakes were treated with the biosorbents and assessed for Cr⁶⁺ level before and after treatment using AAS. According to Environment Protection Agency EPA, permissible limit of hexavalent chromium in water is 0.05 mg/l. The Cr⁶⁺ level in untreated Bellandur lake water was found to be 0.161 mg/l and in untreated Madiwala lake water was found to be 0.133 mg/l which seems to be mildly alarming in terms of toxicity to the lake ecosystem. However, treatment with chemically modified Spirulina biomass of the Bellandur and Madiwala lake water samples reduced the metal concentration by 50% and 42.8% respectively. Further, uisng chemically modified ginger, we achieved a phenomenal removal of Cr6+ upto 96.3% and 88% for Bellandur and Madiwala lake respectively. It is to be noted that, highest removal was achieved in both the lakes in case of treatment with chemically modified biosorbents. In a similar study conducted by our group on these lake waters of Bangalore it was found that the leaf and bark samples of Moringa oleifera were effective in removal of Cr⁶⁺ upto 53% and 23 % respectively.²⁸ Garg et al reported 93.11% removal of Cr⁶⁺ at elevated temperature using citric acid modified walnut shell biosorbent.²² In real time studies using lake waters of Banglore, ours is the first study reporting Cr⁶⁺ removal to this extent. It is also to be noted that chemically modified ginger biosorbent shows greater than 90% removal of Chromium from these waters.

 Table 1: The concentration of chromium(vi) after the treatment with s.maxima

Lake	Concentration of Cr^{6+} (mg/l) after treatment with the biosorbent samples of <i>S. maxima</i>			
	Natural	Chemically modified	Immobilized	
Bellandur	0.122	0.080	0.097	
Madiwala	0.103	0.076	0.081	

Table 2: The concentration of Chromium after the treatment with z. officinale

Lake	Concentration of Cr ⁶⁺ (mg/l) after treatment with biosorbent samples of <i>Z. officinale</i>			
	Natural	Chemically modified	Immobilized	
Bellandur	0.102	0.006	0.007	
Madiwala	0.003	0.016	0.008	

TOXICOLOGY STUDIES WITH ZEBRAFISH

Lethal Dosage

Zebrafish are known to be more sensitive to toxins in aqueous environments compared to other organisms. Zebrafish were separately exposed in polycarbonate culture vessels to varying concentrations of Cr (VI). The Cr(VI) exposure was carried out on adult male zebrafish (greater than 6 weeks) for 24-72 hours. Lethality was determined by comparison with positive control without Cr(VI) and estimated the LD_{50} value. The percentage of deceased fish vs. concentration levels were tabulated as toxicity data as shown in table 3.

Table 03: Lethal dosage levels of zebra fish on exposure to cr(vi) at various time intervals

Time (hrs)		24	48	72
	Control	+++	+++	+++
Cr conc. (mg/l)	0.8	+++	+++	+++
	1.6	+++	+++	+++
	2.4	+++	+++	+++
	3.2	+++	+++	++
	4.0	+++	++	++
	4.8	+++	++	+
	5.6	+++	++	+
	6.4	+++	++	+
	7.2	+++	++	-
	8.0	++	+	-
	8.8	++	+	-
	9.6	++	+	-
	10.4	+	+	-
	11.2	+	-	-
	12			

+++: high activity, -: 50% mortality,++: moderate activity, --: 100% mortality, +: less activity.

The LD₅₀ range was determined to be 7.2 to 11.2 mg/l for a 72 hour period and 8.0 to 9.6 mg/l for 24 hour period. These results were aligned with the studies performed by Domingues et al.²⁹ It is vital to estimate the lethal dose for exposure of fish as chronic toxicity has to be determined. Sublethal concentration of toxic compounds also leads to its bioaccumulation in various tissues, thus displacing essential metal cofactors required for enzyme activity. These heavy metals also act as noncompetitive inhibitors for certain digestive enzymes such as amylases and proteases. The presence of metal ions also interferes with oxidative phosphorylation in mitochondria leading to formation of reactive oxygen species (ROS) and reactive nitrogen species (RNS) thereby leading to oxidative damage of proteins, lipids and DNA.5,12 Liver being the primary organ for metabolism, chronic exposure to Cr⁶⁺ leads to cellular damage and loss of vital storage, transport and catalytic proteins.4,30

Protein Carbonyl Assay

Oxidative stress is the result of increased formation of reactive oxygen species (ROS), due to failure in scavenging of ROS or when the mending of oxidatively modified molecules decreases. ROS such as hydroxyl radical, peroxyl radical, superoxide anion, and hydrogen peroxide are immensely reactive toxic oxygen moieties. The test of carbonyl groups in proteins was found to be a convenient method for detection and quantification of proteins modified by oxidative stress. As the chemically modified ginger biosorbent depicted increased Cr(VI) biosorption compared to S. maxima, ginger biosorbent was used for zebrafish studies. As evident from figure 14 and 15 given below, the control group of fish that was exposed to 4 mg/l of Cr(VI) showed the highest level of protein carbonyl formation indicating increased oxidative stress whereas treatment with biosorbent reduced the Cr(VI) content and thereby the protein carbonyls after 24 hour exposure. Similar decrease in oxidative stress biomarkers were observed in case of exposure of zebra fish to Lycopodim selago extract due to the presence of antioxidant compounds in the extract. The presence of bioactive secondary metabolites that possess antioxidant and metal chelatory activity as active ingredients in biosorbents is likely to decrease oxidative stress in exposed organisms.³¹



Figure 14. Protein Carbonyl assay of liver homogenates of Zebrafish exposed to Bellandur lake sample



Figure 15. Protein Carbonyl assay of liver homogenates of Zebrafish exposed to Madiwala lake sample

The results from the Protein Carbonyl assay illustrates that the biosorption of Cr⁶⁺ led to significant decrease in the protein carbonyl content, resulting in a decrease in heavy metal induced oxidative stress possibly due to up-regulation of antioxidant enzymes and thereby decrement in the oxidative stress markers.^{32,33} The decrease in protein carbonyls was found to be greater than 75% in the chemically modified ginger samples as observed from both the lake water samples compared to natural and immobilized ginger biosorbents. When the biosorbent was exposed to water for removing Cr(VI), bioactive compounds from the biosorbent would have leached into the water which could have entered the fish's body resulting in an in vivo chelation effect of Cr (VI) further in the liver samples.³⁴⁻³⁶ Hence, from the study, we interpret that the removal of Cr(VI) from the water by the biosorbentas well as the entry of leached biosorbent into the fish's body would have resulted in protection against oxidative stress. Similar results were observed by Valu et al using Lycopodim selago extract ³¹. Similiarly, Mezzomo et al (2019) found that taurine protects zebrafish against stress induced protein carbonyls by reducing the extent of protein carbonyls in zebrafish.³⁶

CONCLUSION

Biosorbents are effective in removal of Cr⁶⁺ from water and can be immobilized and reused for several cycles of biosorption. The functional groups on the biosorbent can be enhanced by chemical modification. The concentration of Cr⁶⁺ was reduced significantly using the biosorbents in the study-Zingiber officinale (ginger) and Spirulina maxima(Spirulina) with the former depicting better biosorption. The levels of Cr6+ can be reduced upto 96% upon optimization of physicochemical paramters such as pH, temperature, contact time, agitation rate and biosorbent dosage. On comparitive analysis, it was found that the effect of biosorption was in the following order: chemically modified > immobilized >natural form of biosorbent. Thee excellent results in biosorption by chemically modified biosorbent could be due to the availability of chelatory sites due to additional functional groups upon chemical modification. Hepatotoxicity studies on zebrafish indicate that they were on par with the previous studies conducted in case of LD₅₀ values. The reduction in protein carbonyls in Chromium exposed zebrafish indicates the potential of biosorbent in mitigating oxidative stress by adsorptive removal of Cr⁶⁺. These biosorbents

can thus be used for *in vitro* chelation and reduction of Cr⁶⁺ induced hepatotoxicity from polluted water sample.

Further, the use of immobilized biosorbents can be enhanced by performing recycle studies of batch biosorption and further analysis into adsorption- desorption cycles with the metal ion from its biosorbent and its effective recycle to various industries. The scope of this study can be further extended to other aspects of Cr mediated toxicity such as embryotoxicity, neurotoxicity, reproductive toxicity, nephrotoxicity etc.

ACKNOWLEDGMENT

The authors acknowledges the support from management and staff of the department of Biotechnology, Dayananda Sagar College of Engineering, and Department of Civil Engineering, B.M.S College of Engineering for Atomic Absorption Spectroscopy.

CONFLICT OF INTEREST STATEMENT

Authors declare that they do not have any conflict of interest for publication of this work.

References

- 1. Central Water Commission: Status of Trace and Toxic Metals in Indian Rivers -A Report by Ministry of Water Resources. **2014**.
- C.N. Mulligan, R.N. Yong, B.F. Gibbs. Remediation technologies for metal-contaminated soils and groundwater: An evaluation. *Eng. Geol.* 2001, 60 (1–4), 193–207.
- A. S.Stasinakis, N. S.Thomaidis, E.Cp. D.Mamais, T.D. A.Tsakon. Lekkas.Effects of chromium(VI) addition on the activated sludge process. *Water* 2140–2148.
- A.J. Paine. Mechanisms of chromium toxicity, carcinogenicity and allergenicity: Review of the literature from 1985 to 2000. *Hum. Exp. Toxicol.* 2001, 20 (9), 439–451.
- S.P.B. Kamaludeen, K.R. Arunkumar, S. Avudainayagam, K. Ramasamy. Bioremediation of chromium contaminated environments. *Indian Journal* of Experimental Biology. 2003, pp 972–985.
- S.S. Ahluwalia, D. Goyal. Microbial and plant derived biomass for removal of heavy metals from wastewater. *Bioresour. Technol.* 2007, 98 (12), 2243– 2257.
- A.U. Rajapaksha, R. Selvasembian, A. Ashiq, et al. A systematic review on adsorptive removal of hexavalent chromium from aqueous solutions: Recent advances. *Sci. Total Environ.* 2022, 809, 152055.
- M.R. Shaibur. Heavy metals in chrome-tanned shaving of the tannery industry are a potential hazard to the environment of Bangladesh. *Case Stud. Chem. Environ. Eng.* 2023, 7, 100281.
- B. Volesky. Biosorbents for metal recovery. *Trends Biotechnol.* 1987, 5 (4), 96–101.
- R.A. Soni, K. Sudhakar, R.S. Rana. Spirulina From growth to nutritional product: A review. *Trends Food Sci. Technol.* 2017, 69, 157–171.
- M.F. McCarty. ' "Iatrogenic Gilbert syndrome"- A strategy for reducing vascular and cancer risk by increasing plasma unconjugated bilirubin. In *Medical Hypotheses*; 2007; Vol. 69, pp 974–994.
- F. Jiang, S.J. Roberts, S.R. Datla, G.J. Dusting. NO modulates NADPH oxidase function via heme oxygenase-1 in human endothelial cells. *Hypertension*. 2006, pp 950–957.
- S. Dahiya, R.M. Tripathi, A.G. Hegde. Biosorption of heavy metals and radionuclide from aqueous solutions by pre-treated area shell biomass. J. Hazard. Mater. 2008, 150 (2), 376–386.
- A. Selatnia, A. Boukazoula, N. Kechid, M.Z. Bakhti, A. Chergui. Biosorption of Fe3+ from aqueous solution by a bacterial dead Streptomyces rimosus biomass. *Process Biochem.* 2004, 39 (11), 1643– 1651.

- D.H.K. Reddy, D.K.V. Ramana, K. Seshaiah, A.V.R. Reddy. Biosorption of Ni(II) from aqueous phase by Moringa oleifera bark, a low cost biosorbent. *Desalination*. 2011, pp 150–157.
- A.R. Türker. Separation, preconcentration and speciation of metal ions by solid phase extraction. *Sep. Purif. Rev.* 2012, 41 (3), 169–206.
- D.H.K. Reddy, Y. Harinath, K. Seshaiah, A.V.R. Reddy. Biosorption of Pb(II) from aqueous solutions using chemically modified Moringa oleifera tree leaves. *Chemical Engineering Journal*. 2010, pp 626–634.
- Y. Göksungur, S. Üren, U. Güvenç. Biosorption of cadmium and lead ions by ethanol treated waste baker's yeast biomass. *Bioresource Technology*. 2005, pp 103–109.
- R.L. Levine, D. Garland, C.N. Oliver, et al. Determination of Carbonyl Content in Oxidatively Modified Proteins. In *Methods in Enzymology*; Academic Press, **1990**; Vol. 186, pp 464–478.
- Q. Yu, J.T. Matheickal, P. Yin, P. Kaewsarn. Heavy metal uptake capacities of common marine macro algal biomass. *Water Res.* 1999, 33 (6), 1534– 1537.
- N.D. Shooto, E.B. Naidoo, M. Maubane. Sorption studies of toxic cations on ginger root adsorbent. J. Ind. Eng. Chem. 2019, 76, 133–140.
- R. Garg, R. Garg, M. Sillanpää, et al. Rapid adsorptive removal of chromium from wastewater using walnut-derived biosorbents. *Sci. Rep.* 2023, 13 (1), 6859.
- M. Aoyama. Comment on "Biosorption of chromium(VI) from aqueous solution by cone biomass of Pinus sylvestris." *Bioresour. Technol.* 2003, 89 (3), 317–318.
- L. Deng, Y. Zhang, J. Qin, X. Wang, X. Zhu. Biosorption of Cr(VI) from aqueous solutions by nonliving green algae Cladophora albida. *Miner. Eng.* 2009, 22 (4), 372–377.
- D.H.K. Reddy, K. Seshaiah, A.V.R. Reddy, S.M. Lee. Optimization of Cd(II), Cu(II) and Ni(II) biosorption by chemically modified Moringa oleifera leaves powder. *Carbohydrate Polymers*; **2012**, 88, 1077–1086.
- M.S. Rahman, K. V. Sathasivam. Heavy metal adsorption onto kappaphycus sp. from aqueous solutions: The use of error functions for validation of isotherm and kinetics models. *Biomed Res. Int.* 2015, 2015.
- Z. Aksu, Ü. Açikel, T. Kutsal. Investigation of simultaneous biosorption of copper(II) and chromium(VI) on dried Chlorella vulgaris from binary metal mixtures: Application of multicomponent adsorption isotherms. *Sep. Sci. Technol.* **1999**, 34 (3), 501–524.
- K.S. George, K.B. Revathi, N. Deepa, et al. A Study on the Potential of Moringa Leaf and Bark Extract in Bioremediation of Heavy Metals from Water Collected from Various Lakes in Bangalore. *Procedia Environmental Sciences*. 2016, pp 869–880.
- I. Domingues, R. Oliveira, J. Lourenço, et al. Biomarkers as a tool to assess effects of chromium (VI): Comparison of responses in zebrafish early life stages and adults. *Comp. Biochem. Physiol. - C Toxicol. Pharmacol.* 2010, 152 (3), 338–345.
- B. Pushkar, P. Sevak, S. Parab, N. Nilkanth. Chromium pollution and its bioremediation mechanisms in bacteria: A review. *J. Environ. Manage*. 2021, 287, 112279.
- M.V. Valu, C. Ducu, S. Moga, et al. Effects of the hydroethanolic extract of lycopodium selago l. On scopolamine-induced memory deficits in zebrafish. *Pharmaceuticals* 2021, 14 (6), 568.
- K. Kaur, S. Sharma, J.A. Malik. Chromium Pollution and Its Bioremediation: An Overview. *Microb. Biotechnol. Interv. Bioremediation Phytoremediation* 2022, 337–374.
- 33. A. Sharma, A. Tomer, J. Singh, B.S. Chhikara. Biosorption of metal toxicants and other water pollutants by Corn (Maize) plant: A comprehensive review. J. Integr. Sci. Technol. 2019, 7 (2), 19–28.
- J. Singh. Determination of DTPA extractable heavy metals from sewage irrigated fields and plants. J. Integr. Sci. Technol. 2013, 1 (1), 36–40.
- M.M. Authman. Use of Fish as Bio-indicator of the Effects of Heavy Metals Pollution. J. Aquac. Res. Dev. 2015, 06 (04), 1–13.
- N.J. Mezzomo, B.D. Fontana, T.E. Müller, et al. Taurine modulates the stress response in zebrafish. *Horm. Behav.* 2019, 109, 44–52.