Extraction and Characterization of Chitosan from Squid Species Using Chromium Heavy Metal Implications for Environmental Remediation

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**Abstract:**This study explores the potential of chitosan derived from squid bones as a highly efficient adsorbent for chromium ions in aqueous solutions. Initially, chitin extracted from squid bones through the deacetylation process end derivate chitosan, characterized using The SEM image of chitosan rough, porous structure with irregular flake-like features. Fourier Transform Infrared spectroscopy to assess its chemical composition and functional groups 3252.67 cm⁻¹ to 735.34cm⁻¹. The adsorption capacity of squid bone chitosan was evaluated through rigorous batch adsorption experiments, revealing substantial removal efficiencies. Notably, the adsorbent achieved impressive absorption rates, removing 95.2% of chromium ions at 750 ppm and 98.6% at 1000 ppm concentrations after 24 hours of contact time. The maximum biosorption of metal in squid waste chitosan adsorption of chromium metal at the PH of 8 and 10 (78.3%), (45.4 %) was observed. These results show the potential of squid bone-derived chitosan in addressing heavy metal contamination, highlighting its application in environmental remediation efforts. Future research includes refining extraction techniques to optimize chitosan yield and purity from squid bones, as well as exploring its broader applicability in wastewater treatment and environmental cleanup initiatives. By utilizing squid bone waste to produce effective adsorbents, this study contributes to sustainable solutions for managing chromium and other heavy metal pollutants in aquatic environments.

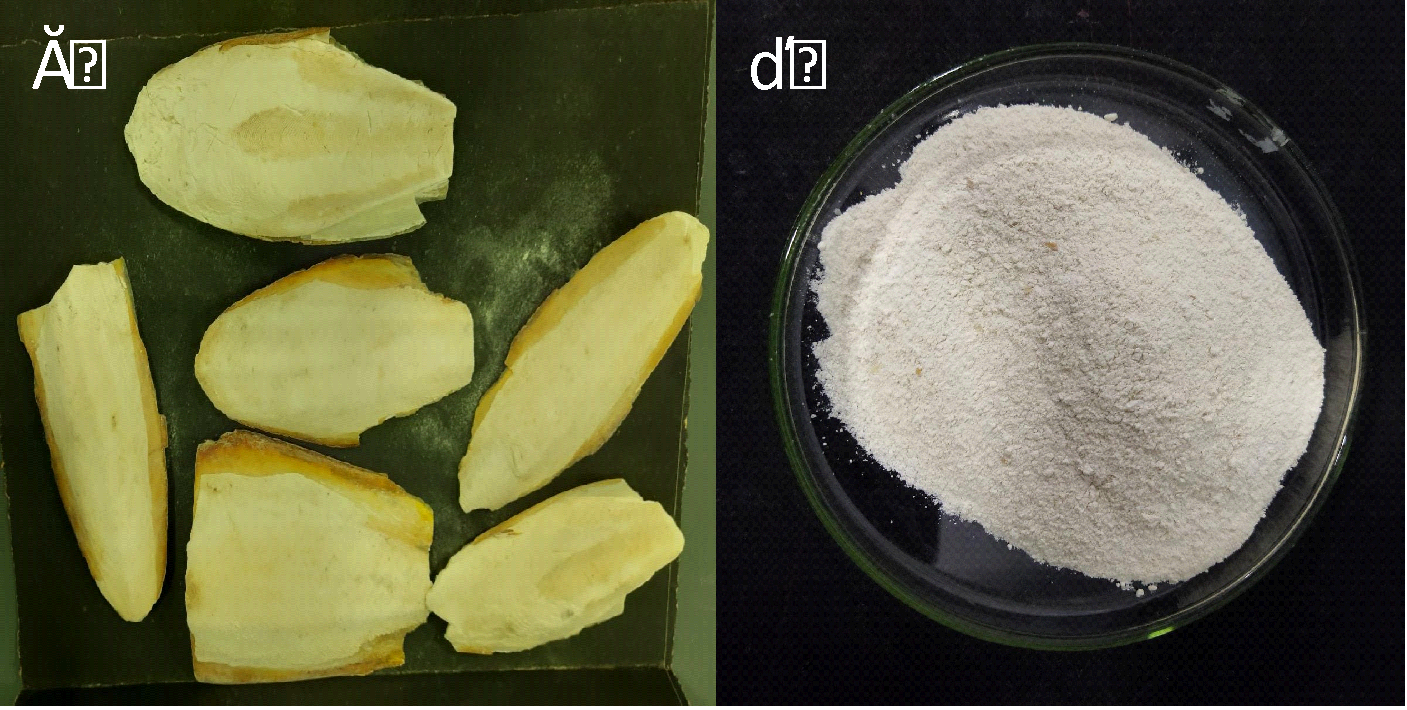
**Keywords: Squid bone, chitosan, pH range, Absorption, heavy metal, chromium, Bioremediation.**

# Introduction

Heavy metals are of significant concern in ecotoxicology due to their ability to persist over long periods, accumulate in organisms, and magnify through the food chain [(V. Kumar et al., 2019)](https://paperpile.com/c/X0UEmn/Kn4F). The occurrence of heavy metals in industrial wastewater has become a notable environmental issue [(Su et al., 2023)](https://paperpile.com/c/X0UEmn/K0orM). Industrial wastewater must undergo comprehensive treatment before being released into aquatic environments, given the toxicity of alloys such as Hg, Cr, and Zn to living organisms [(Pellis et al., 2022)](https://paperpile.com/c/X0UEmn/ZAEoK). Pollutants from natural and human sources, such as heavy metals, dyes, insecticides, oils, and pathogens, can contaminate oceans, and groundwater water [(Yu et al., 2022)](https://paperpile.com/c/X0UEmn/gPEhs) . Industrial activities like electroplating, battery manufacturing, nuclear power plants, pesticide production, mining, and textile dye manufacturing are significant sources of heavy metal ion discharge into the environment [(Karthik et al., 2023; Velusamy et al., 2021)](https://paperpile.com/c/X0UEmn/bibMR+SeiAF). Plants and seafood are significant contributors of metals within the food chain, playing a crucial role in human exposure to these contaminants [(Ahmed et al., 2022)](https://paperpile.com/c/X0UEmn/txeyR). Soluble heavy metals are absorbed by vegetation and tend to accumulate in their roots through uptake mechanisms before being translocated to edible parts such as fruits [(Li et al., 2023)](https://paperpile.com/c/X0UEmn/BtaRh). The marine fishery is vital to many countries' economies worldwide, with over 60% of processed fish categorized as by-products, posing disposal challenges for components such as organs, bones, muscle wastes [(Nag et al., 2022)](https://paperpile.com/c/X0UEmn/9eQXc)[(Ajay et al., 2023; Chokkattu et al., 2023; Padarthi et al., 2023)](https://paperpile.com/c/X0UEmn/Ilmal+f27JW+8ga0w). Researchers are currently developing methods to extract chitosan and other byproducts from fish processing for use in nutraceuticals and bioactive compounds [(Azelee et al., 2023)](https://paperpile.com/c/X0UEmn/JrJjW). The latter technique is highly regarded for its efficient adsorption of various hazardous contaminants due to its cost-effectiveness, and high efficiency [(Eltaweil et al., 2020)](https://paperpile.com/c/X0UEmn/oQXBJ). The byproducts of the seafood industry, particularly crab and shrimp exoskeletons, serve as essential biological resources for the extraction of chitin and the biopolymer chitosan [(Sathya et al., 2024)](https://paperpile.com/c/X0UEmn/ubGDG). Chitosan is produced through the deacetylation of chitin, a key polysaccharide characterized hydrophilic nature and the presence of amine groups[(Omer et al., 2022)](https://paperpile.com/c/X0UEmn/m8QR0)[(Dharman et al., 2023; S. Sindhu et al., 2023; Sreenivasagan et al., 2023)](https://paperpile.com/c/X0UEmn/ZnwsI+BLu7M+C10bD). Marine crustaceans, including mollusks, shrimp, crabs, and lobsters, exhibit unique physiological and biological traits [(Allapitchai et al., 2024)](https://paperpile.com/c/X0UEmn/cQO5U). Chitosan is highly effective in chelating ions, surpassing other natural polymers in its various industrial and environmental applications [(Benettayeb et al., 2022)](https://paperpile.com/c/X0UEmn/r65mT). Chitosan-based biosorbents are essential for efficiently removing a range of pollutants, such as metal [(Ahmed et al., 2022)](https://paperpile.com/c/X0UEmn/txeyR) ([(Duraisamy & Senior Lecturer, Department of Prosthodontics and Implantology, 2021)](https://paperpile.com/c/X0UEmn/dq2cU) . Chitosan is used as a drug delivery system and is efficacious in improving drug bioavailability and stability and reducing toxicity [(Mustafa et al., 2024)](https://paperpile.com/c/X0UEmn/k3vVh). This study comprehensively examines techniques for converting chitin to chitosan, emphasizing its application as a bio-adsorbent for heavy metal chromium, specifically utilizing chitosan derived from squid bone waste.

# Materials and methods

Squid bones were procured from a local fish market and washed thoroughly to eliminate any residual salt and surface contaminants. The bones were fragmented into smaller pieces and dried at 60°C for 48 hours. Once dried, the bones were pulverized using a mechanical pulverizer and passed through a 30-mesh sieve. The pulverized material was further dried in an oven to remove any remaining moisture and then stored in an airtight container for subsequent analysis and experimentation.Fifty grams of squid bone powder were mixed with 100 mL of 10% HCl (2M) and maintained at 60°C for 150 minutes. Afterward, the mixture was filtered through filter paper, and the residue was rinsed with distilled water until it reached a neutral pH.The squid bone powder was treated with 100 mL of NaOH (3M) at 80°C for 120 minutes to remove proteins. After filtration to separate the proteins, the filtrate was washed with distilled water until it reached a neutral pH. Squid bone was decolorized using a solvent mixture in a 1:2:3 ratio of chloroform, methanol, and distilled water. The decolorized sample was then kept in a hot air oven at 60°C for 24 hours. The resultant chitin was extracted and stored in a refrigerator for future use. Deacetylation process by treating chitin with 65% NaOH for 3 days at 30°C convert to chitosan. The mixture was centrifuged at 4000 rpm for 15 minutes to remove excess alkali. The supernatant was discarded, and the remaining pellets were washed with dis. water until achieving a neutral pH. The chitosan was then dried overnight and stored at ambient temperature for subsequent uses (Fig.1a, b).

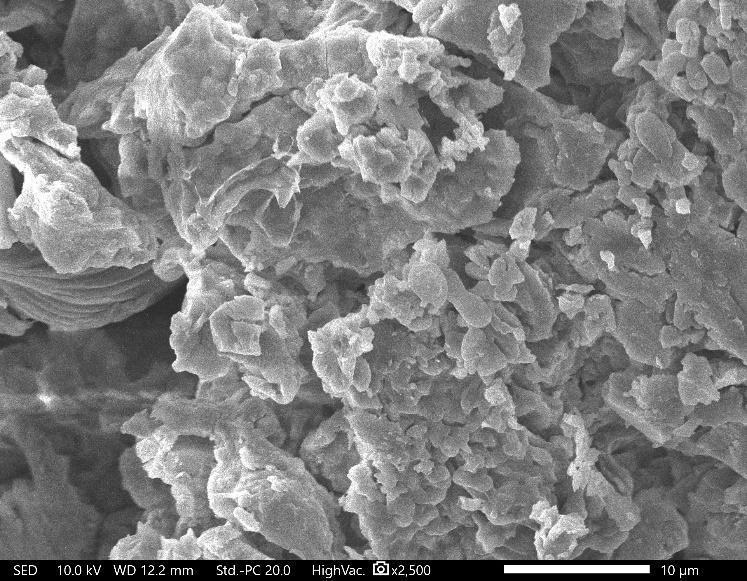


**Figure 1.** a) Squid bone, b) Chitosan

The surface morphology of chitin and chitosan, extracted from squid bones, was analysed using scanning electron microscopy, employing dried specimens of both materials [(Liu et al., 2012)](https://paperpile.com/c/X0UEmn/tvoB9).[(Jridi et al., 2019)](https://paperpile.com/c/X0UEmn/gKaFO) Cuttlefish bone chitosan underwent Fourier Transform Infrared spectroscopy analysis using the transmission technique. Each sample, weighing 2 mg, was ground with 100 mg of dried potassium bromide and compacted into 3 mm diameter disks. Infrared spectrophotometer, measuring absorbance across the range of 400 to 4000 cm⁻¹.A metal solution of chromium ions was prepared by dissolving 0.1 g of chromium in 500 ml of dis. water in a conical flask. The pH of the solution was subsequently adjusted from acidic to alkaline.Adsorption studies of chromium were carried out by adding chitosan derived from squid in three different concentrations (250 mg, 500mg, 750 mg, and 1000 mg) to 50 ml of chromium metal solution in three separate conical flasks. Similarly, 1 g of commercial chitosan was added to 50 ml of chromium metal solution, and all flasks were placed in an orbital shaker at 200 rpm for 6 to 24 hours. After 24 hours, the solutions were filtered using Whatman filter paper to separate the supernatant and the pellet. The supernatants from the different chitosan concentrations were stored, and the pellets were dried and transferred to storage containers for future use.The experiments were carried out across a pH range of 3 to 8, covering both acidic and basic conditions. Higher concentrations were not used to prevent precipitation. The initial pH of the solution was adjusted to acidic by adding HCl and to basic by adding NaOH, with pH values of 3 and 7 being set. Adsorption experiments involved mixing chitosan adsorbent derived from squid bone at four different concentrations (250 mg, 500mg,750 mg, and 1000 mg) with 50 mL of chromium metal solution in a conical flask. The mixture was then agitated in an orbital shaker at 450 rpm for 2 hours at room temperature. Afterward, the solution was filtered, and the filtrate was collected to measure the pH and ion concentration.

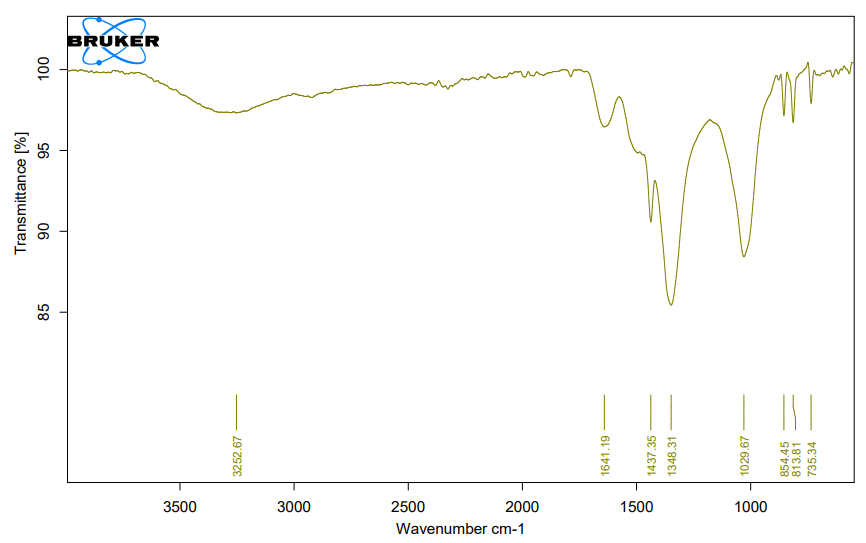
# Result and discussion

The SEM image of chitosan from squid waste shells at 2,500x magnification shows a rough, porous structure with irregular flake-like features. These micrometer-sized particles are slightly clumped together in processing conditions. Partial crystallinity is also visible, which might be linked to how the chitosan was processed (Fig. 2).



**Figure 2**. SEM Morphology Image of Squid-Derived Chitosan

The FTIR spectrum analysis of squid chitosan reveals its complex chemical composition through distinct absorption bands(Nikalje et al., 2024) (Chehelgerdi et al., 2023). A medium intensity band at 3252.67 cm⁻¹ corresponds to N-H stretching, indicating secondary amine groups s (Fig. 3). A strong band between 1641.19 cm⁻¹ is attributed to C=N stretching, suggesting the presence of imine or oxime groups. The medium band at 1437.35 cm⁻¹ is associated with O-H bending, indicative of carboxylic acid groups. Additionally, a strong absorption band at 1348.31 cm⁻¹ points to S=O stretching, revealing sulfate groups. The medium intensity band between 1029.67 cm⁻¹ corresponds to C-N stretching, characteristic of amine groups. Strong bands at 854.45 cm⁻¹, 813.81, and 735.34cm⁻¹ indicate C-H bending in 1,2,4-trisubstituted and 1,2,3-trisubstituted aromatic compounds, respectively.



**Figure 3**. FTIR Spectrum Image of Squid-Derived Chitosan

The study investigated the effectiveness of squid bone chitosan in removing chromium ions from aqueous solutions at various concentrations: 250 ppm, 500 ppm, 750 ppm, and 1000 ppm. The concentration of chitosan and the percentage of chromium ion removal. Specifically, the removal efficiency was 26.2% at 250 ppm, 48.4% at 750 ppm, and reached 82.5% at 1000 ppm. These results suggest that higher concentrations of chitosan offer more active sites for adsorption, leading to greater removal efficiency (Figs. 4 and 5). 

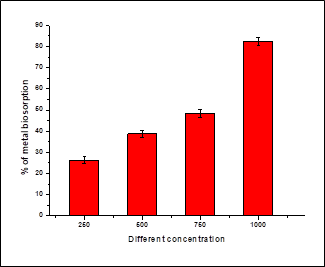
**Figure 4**. Initial stage of Chromium metal degradation

The study evaluated squid bone chitosan's ability to remove metal ions from water over time intervals of 6, 12, 18, and 24 hours. Results showed increasing removal efficiencies of 20.9% at 6 hours, 37.2% at 12 hours, 47.2% at 18 hours, and 89.4% at 24 hours (Fig. 5).



**Figure 5.** Final stage of Chromium metal degradation

The chromium biosorption was based on different pH ranges observed. The different pH ranges were recorded from acidic condition (2) to basic condition (10). The maximum biosorption of metal in squid waste chitosan adsorption of chromium metal at the PH of 8 and 10 (78.3%), (45.4 %) was observed (Fig. 6).



**Figure 6.** Different concentrations of chitosan absorption of metal chromium

# Discussion

These functional groups secondary amines, imine/oxime groups, carboxylic acids, sulfates, amines, and specific aromatic substitutions collectively highlight the molecular complexity and potential functional properties of squid chitosan[(Ramakrishnan et al., 2023; Shenoy & Maiti, 2023; J. S. Sindhu et al., 2023)](https://paperpile.com/c/X0UEmn/5UNrb+nviLh+x5ohH). The FTIR spectrum of chitosan from cuttlefish bone shows an absorption band at 1411 cm−1, as noted[(Hazeena et al., 2022)](https://paperpile.com/c/X0UEmn/O4krc). Compared to our studies [(Olafadehan et al., 2021)](https://paperpile.com/c/X0UEmn/uq8x8)found that the absorption bands at 1384,1428 cm−1 in shrimp and crab shell wastes are attributed to C-N stretching vibrations. According to [(Hardani et al., 2021)](https://paperpile.com/c/X0UEmn/SuVrs) the FTIR spectrum of the chitosan sample showed absorbance peaks at 3439 cm⁻¹ (OH group), 2922 cm⁻¹ (C-H group), 1641 cm⁻¹ (C=O amide group), and 1082 cm⁻¹ (C-O-C group). The Fourier-transform infrared spectrum of the chitosan-based hydrogel shows absorption bands at 3200 to 3400 cm⁻¹ [(Vilela et al., 2019)](https://paperpile.com/c/X0UEmn/l3k8e).Therefore, squid bone chitosan is a highly effective adsorbent for chromium ion removal, with the highest efficiency observed at 1000 ppm (Fig. 4). Similarly, previous study reveals Different doses of chitosan adsorbent were added, and the adsorbed Cr by raw chitosan, compared to untreated bananas during the dose tests were 6.588, 0.966 ppm [(Begum et al., 2020)](https://paperpile.com/c/X0UEmn/P5BdZ). An optimal adsorbent mass of 20 mg of chitin for the elimination of chromium from an H2O2 solution of 10 ppm [(Baharin et al., 2021)](https://paperpile.com/c/X0UEmn/IPwZt). [(Ablouh et al., 2019)](https://paperpile.com/c/X0UEmn/YI4sJ) Chitosan-coated C6H7NaO6 hybrid beads adsorb heavy metal Chromium from aqueous solutions, achieving adsorption capacities of 16 mg/g in chromium[(Kasabwala et al., 2021; Rajeshkumar & Lakshmi, 2021; Varghese et al., 2023)](https://paperpile.com/c/X0UEmn/WKHxR+bQxM9+HuVrv).The material effectiveness in adsorbing metal ions, with efficiency improving significantly with longer exposure periods[(Keerthana & Ramesh, 2021; Murugesan, 2021; Tiwari & Jain, 2021)](https://paperpile.com/c/X0UEmn/FVxHP+2rCUd+p5I37)[(Keerthana & Ramesh, 2021; Murugesan, 2021; Subramanian et al., 2021; Tiwari & Jain, 2021)](https://paperpile.com/c/X0UEmn/FVxHP+2rCUd+p5I37+B6G5y). These adsorption capabilities of squid bone chitosan highlight the critical role of time duration in optimizing its performance as an effective metal ion adsorbent. Similar results in [(Baharin et al., 2021)](https://paperpile.com/c/X0UEmn/IPwZt) determined that 12 minutes was the optimal contact time for removing Chromium ions with different time intervals. In[(Begum et al., 2020)](https://paperpile.com/c/X0UEmn/P5BdZ) study, the optimal duration for chitosan adsorption was found to be 180 minutes, with raw chitosan adsorbing Chromium at concentrations of 2.163 ppm, 0.859 ppm for banana, and 0.896 ppm for areca during the time tests.[(Xu et al., 2022)](https://paperpile.com/c/X0UEmn/E9KwJ) demonstrated that papermaking sludge-chitosan material efficiently adsorbs Chromium from wastewater, achieving saturation absorbs of 114.6 mg/g and 110.3 mg/g, indicating promising potential for regeneration and effective adsorption kinetics[(Evaluation Composite Restoration Posterior Teeth Proanthocyanidin Pretreatment Liner Using Fédération Dentaire Internationale Criteria: Split-Mouth Randomized Controlled Trial, n.d.; Pranati et al., 2021; Sakthi et al, 2021)](https://paperpile.com/c/X0UEmn/q28oR+Z0rs1+U99rV).Chitosan exhibits its maximum adsorption capacity for Cd²⁺ ions at pH 2, with a progressive decline in efficiency as the pH of the solution rises, particularly decreasing at neutral pH (7) and reaching the lowest adsorption at pH 8 [(Marzuki et al., 2019)](https://paperpile.com/c/X0UEmn/vTX2B)[(G. & Ganapathy, 2022; I. L. Kumar & Ramesh, 2021)](https://paperpile.com/c/X0UEmn/sJl7Z+8nDes)). Previous study the pH of the solution changed from 2.0 to 4.0, and the adsorption efficiency for Cr, Pb, and Cd increased notably, resulting in a stronger affinity for the metal ions[(Rahaman et al., 2021)](https://paperpile.com/c/X0UEmn/DbSoT). The effect of pH on Cu²⁺ adsorption by cellulose-chitosan composite was examined within the pH range of 1 to 6 [(Yang et al., 2021)](https://paperpile.com/c/X0UEmn/NdBsr). [(Chai et al., 2018)](https://paperpile.com/c/X0UEmn/XMQow) observed that the adsorption capacity of hydrogels for Chromium significantly increased at lower pH values, where the concentration of hydrogen chromate ions was higher.

# Conclusion

This study investigates the potential of squid bone-derived chitosan as an effective adsorbent for chromium ions in aqueous solutions. The research involves the extraction of chitin from squid bones, followed by deacetylation to produce chitosan. SEM and FTIR analysis is employed to characterize the structural properties of squid chitosan, highlighting key functional groups essential for adsorption mechanisms. Adsorption experiments reveal significant chromium ion removal capacities, particularly notable at higher concentrations and prolonged contact durations. The utilization of squid bone chitosan as a sustainable and efficient material for addressing heavy metal pollution provides valuable insights into its environmental remediation potential. Future studies may focus on optimizing production techniques and expanding the scope of applications for squid bone-derived chitosan in the environment.

# References

1. [Ablouh, E.-H., Hanani, Z., Eladlani, N., Rhazi, M., & Taourirte, M. (2019). Chitosan microspheres/sodium alginate hybrid beads: an efficient green adsorbent for heavy metals removal from aqueous solutions. *Sustainable Environment Research*, *29*(1). https://doi.org/](http://paperpile.com/b/X0UEmn/YI4sJ)[10.1186/s42834-019-0004-9](http://dx.doi.org/10.1186/s42834-019-0004-9)
2. [Ahmed, S., Fatema-Tuj-Zohra, Mahdi, M. M., Nurnabi, M., Alam, M. Z., & Choudhury, T. R. (2022). Health risk assessment for heavy metal accumulation in leafy vegetables grown on tannery effluent contaminated soil. *Toxicology Reports*, *9*, 346–355.](http://paperpile.com/b/X0UEmn/txeyR)
3. [Ajay, R., JafarAbdulla, M. U., Sivakumar, J. S., Baburajan, K., Rakshagan, V., & Eyeswarya, J. (2023). Dental alloy adhesive primers and bond strength at alloy-resin interface: A systematic review and meta-analyses. *The Journal of Contemporary Dental Practice*, *24*(8), 521–544.](http://paperpile.com/b/X0UEmn/f27JW)
4. [Allapitchai, J. F., Pitchai, A., & Ramasamy, P. (2024). Isolation and free radical scavenging ability of linear polysaccharides from cuttlebone of Sepia prashadi. *Cureus*, *16*(5), e60163.](http://paperpile.com/b/X0UEmn/cQO5U)
5. [Azelee, N. I. W., Digvijay, D., Ayothiraman, S., Noor, N. M., Abd Rasid, Z. I., Ramli, A. N. M., Ravindran, B., Iwuchukwu, F. U., & Selvasembian, R. (2023). Sustainable valorization approaches on crustacean wastes for the extraction of chitin, bioactive compounds and their applications-A review. *International Journal of Biological Macromolecules*.](http://paperpile.com/b/X0UEmn/JrJjW)
6. [Baharin, S. N. A., Istamam, H., Suhaimi, N. F., Jamion, N. A., Raaov, M., & Sambasevam, K. P. (2021). Synthesis and characterization of polyaniline/chitin (squid pens) for the removal of chromium (VI) from aqueous solution. *Malays J Chem*, *23*, 26–32.](http://paperpile.com/b/X0UEmn/IPwZt)
7. [Begum, H. A., Haque, A. K. M. M., Islam, M. D., Hasan, M. M., Ahmed, S., Razzak, M., & Khan, R. A. (2020). Analysis of the adsorption of toxic chromium (VI) by untreated and chitosan treated banana and Areca fiber. *Journal of Textile Science and Technology*, *06*(02), 81–106.](http://paperpile.com/b/X0UEmn/P5BdZ)
8. [Benettayeb, A., Ghosh, S., Usman, M., Seihoub, F. Z., Sohoo, I., Chia, C. H., & Sillanpää, M. (2022). Some well-known alginate and chitosan modifications used in adsorption: A review. *Water*, *14*(9), 1353.](http://paperpile.com/b/X0UEmn/r65mT)
9. [Chai, Q., Lu, L., Lin, Y., Ji, X., Yang, C., He, S., & Zhang, D. (2018). Effects and mechanisms of anionic and nonionic surfactants on biochar removal of chromium. *Environmental Science and Pollution Research International*, *25*(19), 18443–18450.](http://paperpile.com/b/X0UEmn/XMQow)
10. Chehelgerdi M., Chehelgerdi, M., Allela, O. Q. B., Pecho, R. D. C., Jayasankar, N., Rao, D. P. & Akhavan-Sigari, R. (2023). Progressing nanotechnology to improve targeted cancer treatment: overcoming hurdles in its clinical implementation. Molecular cancer, 22(1), 169.
11. [Chokkattu, J. J., Mary, D. J., Shanmugam, R., & Neeharika, S. (2023). Evaluation clove ginger-mediated titanium oxide nanoparticles-based dental varnish against Streptococcus mutans Lactobacillus Species: vitro study. *World J Dent*, *14*(3), 233–237.](http://paperpile.com/b/X0UEmn/8ga0w)
12. [Dharman, S., Maragathavalli, G., Shanmugam, R., & Shanmugasundaram, K. (2023). Curcumin mediated gold nanoparticles analysis its antioxidant, anti-inflammatory, antimicrobial activity against oral pathogens. *Pesquisa Brasileira Em Odontopediatria E Clínica Integrada*, *23*.](http://paperpile.com/b/X0UEmn/BLu7M)
13. [Duraisamy, R., & Senior Lecturer, Department of Prosthodontics and Implantology, (2021). Applications of chitosan in dental implantology - A literature review. *International Journal of Dentistry and Oral Science*, 4140–4146.](http://paperpile.com/b/X0UEmn/dq2cU)
14. [Eltaweil, A. S., El-Monaem, A., Omer, E. M., Khalifa, A. M., El-Latif, A., & El-Subruiti, M. M. (2020). Efficient removal of toxic methylene blue (MB) dye from aqueous solution using a metal-organic framework (MOF) MIL-101 (Fe): Isotherms, kinetics, and thermodynamic studies. *Desalination Water Treatment*, *189*, 395–407.](http://paperpile.com/b/X0UEmn/oQXBJ)
15. [*Evaluation Composite Restoration Posterior Teeth Proanthocyanidin Pretreatment Liner Using Fédération Dentaire Internationale Criteria: Split-mouth Randomized Controlled Trial*. (n.d.).](http://paperpile.com/b/X0UEmn/U99rV)
16. [G., K. E. V., & Ganapathy, D. (2022). Operator errors in failed composite restoration-A review. *Int J Dent Oral Sci*, *8*(7), 2941–2944.](http://paperpile.com/b/X0UEmn/8nDes)
17. [Hardani, P. T., Sugijanto, N. E. N., & Kartosentono, S. (2021). Heavy metals bioremediation by shells dust and chitosan derived from Belamya javanica Snail, an Eco-friendly biosorbent. *Research Journal of Pharmacy and Technology*, *14*, 1555–1560.](http://paperpile.com/b/X0UEmn/SuVrs)
18. [Hazeena, S. H., Hou, C.-Y., Zeng, J.-H., Li, B.-H., Lin, T.-C., Liu, C.-S., Chang, C.-I., Hsieh, S.-L., & Shih, M.-K. (2022). Extraction Optimization and Structural Characteristics of Chitosan from Cuttlefish (S. pharaonis sp.) Bone. *Materials*, *15*(22), 7969.](http://paperpile.com/b/X0UEmn/O4krc)
19. [Jridi, M., Nasri, R., Marzougui, Z., Abdelhedi, O., Hamdi, M., & Nasri, M. (2019). Characterization and assessment of antioxidant and antibacterial activities of sulfated polysaccharides extracted from cuttlefish skin and muscle. *International Journal of Biological Macromolecules*, *123*, 1221–1228.](http://paperpile.com/b/X0UEmn/gKaFO)
20. [Karthik, P., Ravichandran, S., Sasikala, V., Mukkannan, A., & Rajesh, J. (2023). Evaluation of MnO2 incorporated cellulose acetate membranes and their potential photocatalytic studies using Rhodamine-B dye. *Process Safety and Environmental Protection : Transactions of the Institution of Chemical Engineers, Part B*, *179*, 691–699.](http://paperpile.com/b/X0UEmn/SeiAF)
21. [Kasabwala, H., Nallaswamy, D., Subhashree, R., & Ahmed, N. (2021). Evaluation Of Overall Marginal Accuracy Of DMLS Copings Fabricated Using 3 Different DMLS Printing Machines. *Int J Dentistry Oral Sci*, *8*(7), 3335–3340.](http://paperpile.com/b/X0UEmn/bQxM9)
22. [Keerthana, T., & Ramesh, S. (2021). Knowledge, attitude and practice survey on awareness of the association between diet and dental erosion. *International Journal of Dentistry and Oral Science*, *8*(2), 1533–1540.](http://paperpile.com/b/X0UEmn/2rCUd)
23. [Kumar, I. L., & Ramesh, S. (2021). Knowledge, Attitude and Practices (KAP) survey of shade selection for indirect veneers. *Int J Dent Oral Sci*, *26*, 2856–2864.](http://paperpile.com/b/X0UEmn/sJl7Z)
24. [Kumar, V., Parihar, R. D., Sharma, A., Bakshi, P., Singh Sidhu, G. P., Bali, A. S., Karaouzas, I., Bhardwaj, R., Thukral, A. K., Gyasi-Agyei, Y., & Rodrigo-Comino, J. (2019). Global evaluation of heavy metal content in surface water bodies: A meta-analysis using heavy metal pollution indices and multivariate statistical analyses. *Chemosphere*, *236*(124364), 124364.](http://paperpile.com/b/X0UEmn/Kn4F)
25. [Liu, S., Sun, J., Yu, L., Zhang, C., Bi, J., Zhu, F., Qu, M., Jiang, C., & Yang, Q. (2012). Extraction and characterization of chitin from the beetle Holotrichia parallela Motschulsky. *Molecules (Basel, Switzerland)*, *17*(4), 4604–4611.](http://paperpile.com/b/X0UEmn/tvoB9)
26. [Li, Y., Rahman, S. U., Qiu, Z., Shahzad, S. M., Nawaz, M. F., Huang, J., Naveed, S., Li, L., Wang, X., & Cheng, H. (2023). Toxic effects of cadmium on the physiological and biochemical attributes of plants, and phytoremediation strategies: A review. *Environmental Pollution (Barking, Essex: 1987)*, *325*(121433), 121433.](http://paperpile.com/b/X0UEmn/BtaRh)
27. [Marzuki, I., Bachtiar, E., Sinardi, Alwi, R. S., Mudyawati, & Iryani, A. S. (2019). *Chitosan performance of shrimp shells in the biosorption ion metal of cadmium, Lead and Nickel based on variations pH interaction*. https://doi.org/](http://paperpile.com/b/X0UEmn/vTX2B)[10.31219/osf.io/kmr5h](http://dx.doi.org/10.31219/osf.io/kmr5h)
28. [Murugesan, A. (2021). Saravana Dinesh SP evaluation of shear bond strength of ceramic brackets with two different base designs: An in-vitro study. *Int J Dentistry Oral Sci*.](http://paperpile.com/b/X0UEmn/p5I37) <https://www.academia.edu/download/72981941/IJDOS_2377_8075_08_304.pdf>
29. [Mustafa, A., Indiran, M. A., Ramalingam, K., Perumal, E., Shanmugham, R., & Karobari, M. I. (2024). Anticancer potential of thiocolchicoside and lauric acid loaded chitosan nanogel against oral cancer cell lines: a comprehensive study. *Scientific Reports*, *14*(1), 9270.](http://paperpile.com/b/X0UEmn/k3vVh)
30. Nikalje, A. V., Tajane, S. T., Kocharekar, A., Vekariya, D., & Patil, H. (2024, April). Detecting Cancer through Analysis of Histopathological Images. In 2024 International Conference on Expert Clouds and Applications (ICOECA) (pp. 579-585). IEEE.
31. [Nag, M., Lahiri, D., Dey, A., Sarkar, T., Pati, S., Joshi, S., Bunawan, H., Mohammed, A., Edinur, H. A., Ghosh, S., & Ray, R. R. (2022). Seafood discards: A potent source of enzymes and biomacromolecules with nutritional and nutraceutical significance. *Frontiers in Nutrition*, *9*, 879929.](http://paperpile.com/b/X0UEmn/9eQXc)
32. [Olafadehan, O. A., Amoo, K. O., Ajayi, T. O., & Bello, V. E. (2021). Extraction and characterization of chitin and chitosan from Callinectes amnicola and Penaeus notialis shell wastes. *J Chem Eng Mater Sci*, *12*, 1–30.](http://paperpile.com/b/X0UEmn/uq8x8)
33. [Omer, A. M., Dey, R., Eltaweil, A. S., Abd El-Monaem, E. M., & Ziora, Z. M. (2022). Insights into recent advances of chitosan-based adsorbents for sustainable removal of heavy metals and anions. *Arabian Journal of Chemistry*, *15*(2), 103543.](http://paperpile.com/b/X0UEmn/m8QR0)
34. [Padarthi, L. C., Anumula, L., Chinni, S. K., Sannapureddy, S., & Govula, K. (2023). Evaluation Composite Restoration Posterior Teeth Proanthocyanidin Pretreatment Liner Using Fédération Dentaire Internationale Criteria: Split-mouth Randomized Controlled Trial. *International Journal Prosthodontics Restorative Dentistry*, *13*(4), 191–200.](http://paperpile.com/b/X0UEmn/Ilmal)
35. [Pellis, A., Guebitz, G. M., & Nyanhongo, G. S. (2022). Chitosan: Sources, processing and modification techniques. *Gels (Basel, Switzerland)*, *8*(7), 393.](http://paperpile.com/b/X0UEmn/ZAEoK)
36. [Pranati, T., Ranjan, M., & Sandeep, A. H. (2021). Marginal adaptability custom made cast post made different techniques-a literature review. *Int J Dentistry Oral Sci*, *8*(8), 3954–3959.](http://paperpile.com/b/X0UEmn/q28oR)
37. [Rahaman, M. H., Islam, M. A., Islam, M. M., Rahman, M. A., & Alam, S. M. N. (2021). Biodegradable composite adsorbent of modified cellulose and chitosan to remove heavy metal ions from aqueous solution. *Current Research in Green and Sustainable Chemistry*, *4*(100119), 100119.](http://paperpile.com/b/X0UEmn/DbSoT)
38. [Rajeshkumar, S., & Lakshmi, T. (2021). Biomedical potential of zinc oxide nanoparticles synthesized using plant extracts. *Int J Dent Oral Sci*, *8*, 4160–4163.](http://paperpile.com/b/X0UEmn/HuVrv)
39. [Ramakrishnan, M., Shanmugam, R., Neeharika, S., Chokkattu, J. J., Thangavelu, L., & Khanna, N. (2023). Anti-inflammatory activity and cytotoxic effect of ginger and Rosemary-mediated titanium oxide nanoparticles-based dental varnish. *World Journal of Dentistry*, *14*(9), 761–765.](http://paperpile.com/b/X0UEmn/nviLh)
40. [Sakthi, S., et al. (2021). Thymus vulgaris mediated selenium nanoparticles, characterization and its antimicrobial activity - an in vitro study. *International Journal of Dentistry and Oral Science*, 3516–3521.](http://paperpile.com/b/X0UEmn/Z0rs1)
41. [Sathya, I., Pitchai, A., Subhapradha, N., & Ramasamy, P. (2024). Chitosan from gladius of Doryteuthis sibogae and their capability to inhibit the blood clotting and its antibacterial effect against human pathogens. *Process Biochemistry (Barking, London, England)*, *146*, 109–114.](http://paperpile.com/b/X0UEmn/ubGDG)
42. [Shenoy, N. D., & Maiti, S. (2023). Evaluation marginal fit CAD/CAM crowns using CBCT digital scanners. *Annals Dental Specialty*, *11*(3-2023), 37–44.](http://paperpile.com/b/X0UEmn/5UNrb)
43. [Sindhu, J. S., Maiti, S., & Nallaswamy, D. (2023). Comparative analysis on efficiency and accuracy of parallel confocal microscopy and three-dimensional in motion video with triangulation technology-based intraoral scanner under influence of moisture and mouth opening - A crossover clinical trial. *Journal of Indian Prosthodontic Society*, *23*(3), 234–243.](http://paperpile.com/b/X0UEmn/x5ohH)
44. [Sindhu, S., Maiti, S., & Nallaswamy, D. (2023). Factors affecting accuracy intraoral scanners-a systematic review. *Annals Dental Specialty*, *11*(1-2023), 40–52.](http://paperpile.com/b/X0UEmn/ZnwsI)
45. [Sreenivasagan, S., Subramanian, A. K., Mohanraj, K. G., & Kumar, R. S. (2023). Assessment of toxicity of Green Synthesized Silver Nanoparticle-coated Titanium Mini-implants with Uncoated Mini-implants: Comparison in an Animal Model Study. *The Journal of Contemporary Dental Practice*, *24*(12), 944–950.](http://paperpile.com/b/X0UEmn/C10bD)
46. [Subramanian, E., Ravindran, V., & Jeevanandan, G. (2021). Comparison of amount of tooth reduction in primary first molar for stainless steel, zirconia and fibre-glass crowns–in-vitro study. *International Journal of Dentistry and Oral Science*, *8*(7), 3427–3430.](http://paperpile.com/b/X0UEmn/B6G5y)
47. [Su, C., Wang, J., Chen, Z., Meng, J., Yin, G., Zhou, Y., & Wang, T. (2023). Sources and health risks of heavy metals in soils and vegetables from intensive human intervention areas in South China. *The Science of the Total Environment*, *857*(Pt 1), 159389.](http://paperpile.com/b/X0UEmn/K0orM)
48. [Tiwari, A., & Jain, R. K. (2021). The effect of motivational and reminder therapy on the compliance of patients wearing fixed appliances. *Int J Dent Oral Sci*, *8*(7), 3303–3305.](http://paperpile.com/b/X0UEmn/FVxHP)
49. [Varghese, R., Maliael, M., & Subramanian, A. (2023). Antibacterial activity of nanoparticle-coated orthodontic archwires: A systematic review. *Journal of International Oral Health: JIOH*, *15*(1), 1.](http://paperpile.com/b/X0UEmn/WKHxR)
50. [Velusamy, S., Roy, A., Sundaram, S., & Kumar Mallick, T. (2021). A review on heavy metal ions and containing dyes removal through graphene oxide-based adsorption strategies for textile wastewater treatment. *Chemical Record (New York, N.Y.)*, *21*(7), 1570–1610.](http://paperpile.com/b/X0UEmn/bibMR)
51. [Vilela, P. B., Dalalibera, A., Duminelli, E. C., Becegato, V. A., & Paulino, A. T. (2019). Adsorption and removal of chromium (VI) contained in aqueous solutions using a chitosan-based hydrogel. *Environmental Science and Pollution Research International*, *26*(28), 28481–28489.](http://paperpile.com/b/X0UEmn/l3k8e)
52. [Xu, K., Li, L., Huang, Z., Tian, Z., & Li, H. (2022). Efficient adsorption of heavy metals from wastewater on nanocomposite beads prepared by chitosan and paper sludge. *The Science of the Total Environment*, *846*(157399), 157399.](http://paperpile.com/b/X0UEmn/E9KwJ)
53. [Yang, S.-C., Liao, Y., Karthikeyan, K. G., & Pan, X. J. (2021). Mesoporous cellulose-chitosan composite hydrogel fabricated via the co-dissolution-regeneration process as biosorbent of heavy metals. *Environmental Pollution (Barking, Essex: 1987)*, *286*(117324), 117324.](http://paperpile.com/b/X0UEmn/NdBsr)
54. [Yu, H., Wu, M., Duan, G., & Gong, X. (2022). One-step fabrication of eco-friendly superhydrophobic fabrics for high-efficiency oil/water separation and oil spill cleanup. *Nanoscale*, *14*(4), 1296–1309.](http://paperpile.com/b/X0UEmn/gPEhs)