Deanship of Graduate studies
Al Quds University

Extraction ,Characterization and Application Of Chitosan From Palestine Fish Scales

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M.Sc. Thesis

Jerusalem – Palestine

1446/2024

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A Thesis Submitted in Partial Fulfillment of the-
Requirements for theDegree of Master of, Deanship
Applied Industrial Technologyof Graduate Studies-
Al-Quads University

Jerusalem – Palestine

1446/2024

Deanship of Graduate Studies
Al-Quds University
Program . Applied Industrial Technology

Thesis Approval

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Master thesis submitted and accepted, Date:1 /9/2024

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Jerusalem – Palestine

1446/2024

Dedication

To everyone who believed in me and in my abilities to achieve my ambition.

Declaration

I certify that this thesis submitted for the degree of master in health policy and management, is the result of my own research, except where otherwise acknowledged, and that this thesis (or any part of the same material) has not been submitted for a higher degree to any other university or institution.

Signed:...........................

Hamza Wael Ismail Albattmah

Date: 1/9/2024

Acknowledgments

I would like to express my sincere gratitude to everyone who has helped me in completing my research, especially prof. Fouad Al-Rimawi, Dr. MohannedQurie, and the head of the Chemistry Department, Dr. Mahmoud AL-Khatib. They have never hesitated to offer valuable advice and assistance, providing me with ample opportunities to learn. I would also like to thank all the professors who have taught me, as I have gained a wealth of knowledge and expertise from them. To all those mentioned, I extend my heartfelt thanks and appreciation. All praise is due to God, the Lord of the Worlds

Abstract

The objective of this investigation was to isolate and describe chitosan, appraise its antimicrobial efficacy, and appraise its efficiency in eliminating hexavalent chromium (Cr(VI)) from contaminated water. The chitosan was extracted from Palestinian fish scales utilizing a tripartite procedure: demineralization, deproteinization, and deacetylation. Fourier Transform Infrared (FTIR) spectroscopy was utilized to verify the successful extraction and purity of the chitosan. The antimicrobial efficaciousness of the extracted chitosan was assessed against three pathogenic bacteria: *Staphylococcus aureus*, *Klebsiella pneumoniae*, and *Escherichia coli*. Regarding environmental applications, the investigation scrutinized the efficaciousness of chitosan in treating hexavalent chromium in contaminated water. The results showed significant antibacterial activity against *Staphylococcus aureus*, with the largest inhibition zone observed at a concentration of 0.0075 g/100 ml. However, no significant antibacterial effects were detected against *Klebsiella pneumoniae* and *Escherichia coli*. Chitosan also demonstrated high efficacy in removing hexavalent chromium, with optimal removal at pH 4, where the Cr(VI) concentration was reduced to zero, achieving complete removal. The most efficient removal was observed after 90 minutes, with the highest removal rate occurring at a chitosan dose of 0.5 g for a chromium concentration of 20 ppm. The adsorption behavior of chitosan was analyzed using adsorption isotherms, with the data fitting well to the Freundlich isotherm model, indicating multilayer adsorption of chromium ions onto the chitosan surface. Kinetic analysis revealed a strong correlation with pseudo-first-order kinetics. In conclusion, this study underscores the potential of chitosan derived from Palestinian fish scales as a versatile biopolymer, contributing to waste valorization while offering effective solutions for environmental remediation and antibacterial treatments.

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Introduction

1.1 Background

In the global ranking of fish production, countries vary significantly in their contributions due to differences in natural resources, technology, and access to marine environments. While nations such as China and Indonesia lead the world in fish output, countries in the Middle East, including Israel and Palestine, particularly Gaza, play a smaller but noteworthy role in regional fisheries. In these areas, local fisheries contribute to food security and livelihoods, but they are often constrained by geopolitical and environmental challenges (Hussein et al. 2021). Additionally, marine waste is a growing concern, particularly in coastal regions, where the accumulation of plastic debris, industrial pollutants, and untreated wastewater harms aquatic ecosystems. Mechanisms for disposing of marine waste vary by region, with some countries implementing stricter regulations on waste management, while others face ongoing challenges in curbing pollution. Sustainable practices, such as the use of recycling programs, enhanced waste treatment facilities, and public awareness campaigns, are essential to mitigate the impact of marine waste on fisheries and the environment (Bashir et al., 2020).

Palestinian fish differ from other Mediterranean fish, such those found in Türkiye, due to their particular marine habitat and fishing methods, especially when they come from Gaza's coastal waters. A wide variety of fish species are supported by nutrient-rich currents and generally high temperatures in the Mediterranean waters off the coast of Palestine. Owing to these circumstances, Palestinian fisherman frequently capture sea bream, mullet, and sardines, among other smaller fish species. The amount and variety of fish that are accessible are impacted by small-scale traditional fishing practices paired with restricted access to deeper waters (Hussein et al. 2021). In contrast, Türkiye, with its extensive Mediterranean coastline and advanced fishing infrastructure, harvests a broader range of species, including larger fish such as tuna and mackerel. Additionally, Palestine’s political and economic conditions affect local fish production, making the fish from this region a reflection not just of natural factors but also of the resilience of the local fishing communities (Yildiz T, Ulman A, Karakulak FS, 2023).

Globally, coastal regions' economies and food security are greatly influenced by their fishing industries. However, a significant quantity of by-products are frequently produced during the processing of fish, such as fish scales, which are typically thrown away as garbage. Because of concerns about sustainability, the economy, and the environment, managing fisheries waste has gained importance. The creation of value-added products and the valuation of fishing by-products are becoming more popular as solutions to this problem (Coppola et al., 2021).

A sizeable amount of the overall catch weight is made up of by-products from the fishing industry, such as fish scales, bones, and skin. If handled improperly, these materials may lead to resource waste and environmental pollution. Conventional fisheries waste disposal techniques, including as burning and landfilling, are not economically advantageous and present environmental risks. In order to achieve sustainability in the fishing business, it is imperative to investigate novel applications for these by-products. Making lucrative bioproducts out of these waste materials, such chitosan, is one promising strategy (Coppola et al., 2021).

A biopolymer made from chitin called chitosan has shown great promise as a material with a wide range of uses across multiple sectors. It is a desirable substitute for synthetic polymers due to its extraordinary qualities, which include biocompatibility, biodegradability, antibacterial activity, and non-toxicity. It has proven possible to successfully extract chitosan from a variety of materials, including fungal biomass and crustacean shells. But fish scales, a plentiful byproduct of the fish processing industry, have gotten relatively less attention as a possible chitosan source (Desai et al., 2023).

Palestine, a country endowed with abundant coastline resources, encounters comparable difficulties in handling fish debris. In the context of chitosan extraction, in particular, the use of fish scales offers a means of addressing these issues while encouraging value development and sustainable practices. Investigating the chitosan extraction, characterisation, and application from fish scales in Palestine offers possible financial advantages for the local fishing business in addition to aiding in the waste valorization process (Feidi, 2018).

For many communities in Palestine, especially those along the Gaza Strip and the Mediterranean coast, the fishing industry is a major source of income. But the sector also has to deal with issues like pollution, overfishing, and restricted market access. This research can have two benefits by concentrating on the value-adding of fish scales for chitosan production: it can lessen its impact on the environment and open up new business prospects. Converting fish scales, an abundant and underutilized resource, into chitosan could promote sustainable economic development and assist address waste management concerns (Feidi, 2018).

Recent times have witnessed notable progress in the methods of chitosan characterisation and extraction. These developments, which seek to improve the effectiveness and sustainability of chitosan manufacturing, include enzymatic procedures, extraction assisted by microwaves, and green chemistry strategies. Advanced characterization methods like scanning electron microscopy (SEM), X-ray diffraction (XRD), and Fourier-transform infrared spectroscopy (FTIR) offer comprehensive insights into the molecular structure and characteristics of chitosan. In order to maximize the extraction procedure and produce high-quality chitosan from

Palestinian fish scales, our study will take advantage of these developments (Pellis et al., 2022).

This study intends to support waste reduction and the sustainable use of fishery by-products by concentrating on the extraction and application of chitosan from Palestine fish scales. Additionally, it aims to clarify the possible advantages—both financial and environmental—of chitosan extraction and use in the Palestinian context. The results of this study will further our understanding of the extraction, characterization, and application of chitosan while offering insightful information to academics, business leaders, and policymakers who are interested in value-added product development and sustainable waste management.

This study's importance goes beyond Palestine as a local context. It has wider ramifications for international initiatives pertaining to circular economy and sustainable waste management. Through the demonstration of the viability and advantages of chitosan extraction from fish scales, this work can function as a template for other coastal regions that encounter comparable waste management obstacles. This research's interdisciplinary approach, which incorporates elements of chemistry, environmental science, and industrial applications, emphasizes its potential to significantly advance scientific understanding as well as useful sustainability solutions.

In conclusion, there is a great chance to address waste management issues, advance sustainable practices, and investigate the potential of chitosan in a variety of industries through the extraction, characterisation, and use of chitosan from Palestine fish scales. This research proposal describes the purpose, methods, anticipated results, and importance of investigating fish scale-derived chitosan in Palestine. The study will advance scientific understanding and real-world applications, supporting regional economic development and environmental sustainability.

1.2 Rationale for Chitosan Extraction from Palestine Fish Scales

The extraction of chitosan from fish scales in Palestine makes sense from the perspective of both economic feasibility and environmental sustainability, given the fish scales' exceptional abundance in the area.

According to the Palestinian Environment Quality Authority (2021), Palestine has a rich marine ecosystem. The use of fish scales for chitosan extraction is in line with the global trend towards sustainable practices. Although fish scales are frequently seen as a byproduct of the fishing business, their widespread availability offers the chance to turn something that might otherwise be thrown away into a priceless resource (Coppola et al., 2021).

Through concentrating on fish scales from Palestine, the study hopes to support an environmentally conscious method of sourcing materials by supporting the sustainable use of regional marine resources. This choice holds the promise of reducing waste in the fishing industry while simultaneously offering a novel avenue for the production of chitosan, a versatile biopolymer with myriad applications (Coppola et al., 2021).

Furthermore, the extraction of chitosan from Palestine fish scales has the potential to support economic development in the region. Using a locally available resource not only lessens reliance on outside resources but also creates opportunities for employment and the development of a sustainable sector focused on the extraction and use of chitosan.

Essentially, the justification goes beyond the lab to tackle more general concerns of economic growth, environmental accountability, and sustainable resource use, presenting the study as an important contribution to regional and worldwide settings.

1.3 Sustainability Considerations

An important component of this study is sustainability, which supports the ethical decision to extract chitosan from Palestine fish scales. Selecting a sustainable source aligns with the need to promote environmentally conscious behavior in a society where people are becoming more aware of how human activity affects the environment.
Using fish scales, a leftover from the fishing business demonstrates a dedication to reducing waste and maximizing resource use. This work supports the circular economy philosophy, which maximizes the utility of every component of the production process by turning what may be thrown out into a valuable substance (Liu SH et al., 2021).

Furthermore, sustainability factors go beyond the direct effects on the environment. The goal of chitosan extraction from Palestine fish scales is to create a strategy that balances environmental stewardship with commercial feasibility. The project aims to lessen the carbon footprint associated with material transportation and support regional economic development by highlighting the use of a locally plentiful resource.
Essentially, the emphasis on sustainability is not just a feature of the study; rather, it is a guiding concept that forms the study's core and establishes it as a responsible and progressive addition to the scientific and environmental scene.

1.4 Significance and implications

The successful extraction, characterization, and application of chitosan from fish scales in Palestine would have several significant implications:

Sustainable utilization of fishery by-products contributes to waste valorization and reduces environmental impact (Mozumder, 2022).

Development of value-added products with potential economic benefits for the local fishing industry (Mozumder, 2022).

Exploration of chitosan's antimicrobial properties for potential applications in healthcare and food safety. Contribution to the body of knowledge regarding the extraction and application of chitosan, particularly from fish scales, in the Palestinian context (Yan et al., 2021).

Chapter 2:

Literature Review

2.1 Chitosan and its Properties

After cellulose, chitosan is the second most prevalent polysaccharide in the world. It is generated from chitin. Because chitosan's amino groups have the potential to become protonated, it can dissolve in diluted acidic aqueous solutions. Numerous intriguing characteristics of chitosan have created special chances for the advancement of biological applications(Jiménez-Gómez CP, Cecilia JA. 2020). Because chitosan has so many desirable qualities—biodegradability, natural origin, abundance, reactivity, etc.—it is employed in a wide range of industries, including waste and water treatment, cosmetics, medicine, agriculture, food processing, and nutrition enhancement. The deacetylated form of manquitin, chitosan, has a heterogeneous chemical structure made up of 1-4 2-acetamide-2-deoxy-β-D-glucopyranose bonds plus 2-amino-2-linkages. It also contains at least 50% of the free amine form. Deoxy-β-D-glucopyranose (figure 2.1) (Ibrahim & El-zairy, 2015).

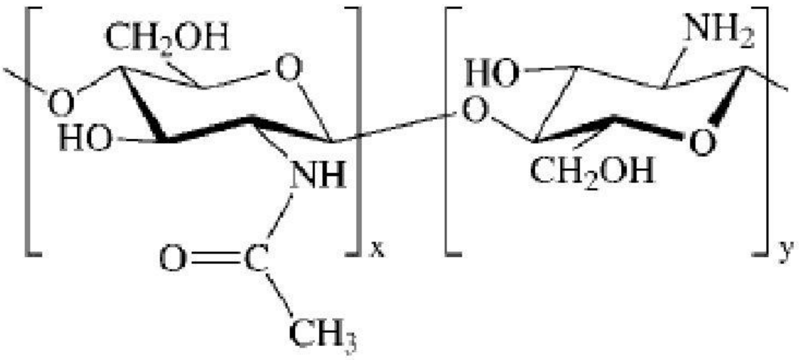

Figure 2.1 : chemical structure of chitosan (Ibrahim & El-zairy, 2015)

Chitosan has excellent adsorption properties and is non-toxic, biodegradable, non-allergic, bioactive, and biocompatible. It can also be made into chunks, wipe forms, layers, globules, powders, and gels. Even though chitosan has many advantages, there are drawbacks as well (Desai, 2023). For example, the raw material comes from a seasonal and local source, the extraction process is labor-intensive, costly, and requires the use of harsh chemicals in large quantities; additionally, the quality of chitosan extracted from marine sources varies. Additionally, research is required to boost chitosan production and its application in a range of industrial processes (Alemu, Getachew, Mondal, 2023).

Chitosan is a physiologically active chemical in terms of its biological characteristics. According to Harugade et al. (2023), it demonstrates a wide range of biological activities, including antibacterial, antifungal, antitumor, anticancer, antidiabetic, and wound healing properties.

2.2 Sources of Chitosan

Chitosan is a copolymer composed of glucosamine and N-acetyl glucosamine, derived from chitin. Chitin, which is present in the cell walls of crustaceans, fungi, insects, and certain algae, and in some invertebrates, serves as the source of chitosan (Pellis et al., 2022). Among the various natural sources of chitosan, shrimp and crabs are the most commonly cited in literature for chitosan production. However, other sources such as lobsters, crayfish, and oysters have also been utilized (Kou, Peters, Mucalo, 2021).

Chitin, the precursor to chitosan, is found in a wide range of organisms, including the exoskeletons of crustaceans (like lobsters, shrimp, krill, barnacles, and crawfish) and mollusks (such as squid, octopus, shellfish, and snails). It is also present in algae (including diatoms, brown algae, and green algae), insects (such as house flies, silkworms, ants, cockroaches, and scorpions), and the cell walls of fungi (e.g., *Ascomycetes*, *Basidiomycetes*, and *Phycomycetes*, including *Aspergillus niger*, *Mucor rouxii*, *Penicillium notatum*, and *Trichoderma reesei*) (Elieh-Ali-Komi D, Hamblin MR. 2016). The concentration of chitin varies among these organisms, typically ranging from 15-30% in crab shells, 20-30% in shellfish exoskeletons, 30-40% in shrimp shells, 5-25% in insect exoskeletons, and 2-44% in fungal cell walls (Yeul, Rayalu., 2012; Pellis et al., 2022).

The majority of the chitosan used in mechanical applications today comes from shellfish, specifically from the exoskeletons of crab, prawn, and shrimp shells, which are readily available as waste from the food manufacturing sector. Nevertheless, it is becoming more and more available as a byproduct of contagious maturation, the growing of silk cocoons from the silk industry, and the extraction of proteins from creepy crawlies for the food and animal feed industries. Despite being composed of chitin, angle scales are routinely discarded due to their unusually low production, which accounts for only 1% of their total weight (Pelli et al., 2022).

2.3 Chitosan Extraction Methods

The process of extracting and producing chitosan involves several stages, starting with the collection of chitin and culminating in deacetylation to produce chitosan. The key methods for extracting chitosan are outlined below.

Primarily, chitin is sourced from crustacean shells, which are obtained from seafood processing facilities. While fungal sources are also used, they are less prevalent. The collected shells are thoroughly cleaned to eliminate impurities such as proteins, minerals, and other organic substances. They are then dried to lower their moisture content (El-araby et al., 2022). The next step is demineralization, which involves removing inorganic components, mainly calcium carbonate, from the shells. This is achieved by treating the shells with a dilute acid solution, usually hydrochloric acid (HCl), which dissolves the calcium carbonate and leaves chitin behind (Younes & Rinaudo, 2015).

$$CaCO_3 + 2HCl \rightarrow CaCl_2 + CO_2 + H_2O$$

Converting chitin into chitosan requires careful control of the deacetylation process to ensure complete conversion. This process involves removing acetyl groups from chitin to expose amino groups, transforming it into chitosan. Several factors contribute to the success of this conversion, including the reaction conditions such as temperature and time, which are typically optimized by maintaining temperatures between 80°C and 100°C and allowing sufficient reaction time (Schmitz et al., 2019). Additionally, the concentration of the deacetylating agent, usually concentrated sodium hydroxide (NaOH), plays a crucial role. The NaOH must be strong enough to react with all acetyl groups present in the chitin. Before this step, chitin is treated with hydrochloric acid (HCl) to remove impurities like calcium carbonate ($CaCO_3$), which dissolves according to the reaction $CaCO_3 + 2HCl \rightarrow CaCl_2 + CO_2 + H_2O$. Ensuring complete dissolution of calcium carbonate is vital for preventing any interference with the deacetylation step (Younes & Rinaudo, 2015).

Hydrochloric acid, while useful for purification, does not break down the amine groups in chitosan. Instead, it protonates these groups, increasing their solubility. However, prolonged exposure to HCl can degrade the polymer backbone, reducing molecular weight and altering the properties of chitosan. Thus, HCl should not be used in the deacetylation process, which relies on alkaline conditions provided by NaOH. To ensure complete conversion, reaction parameters such as time, temperature, and NaOH concentration must be optimized. Techniques like Fourier-transform infrared spectroscopy (FTIR) or nuclear magnetic resonance (NMR) can be used to monitor the degree of deacetylation and confirm the formation of chitosan. While acids like sulfuric acid (H_2SO_4) and acetic acid (CH_3COOH) can be used for specific purification steps or to dissolve chitosan, the core transformation process from chitin to chitosan relies on a strong base like NaOH rather than acids (Pellis et al., 2022; Desai et al, 2023).

The next step is deproteinization, which entails eliminating the proteins. To do this, an alkaline solution, often sodium hydroxide (NaOH), is applied to the demineralized shells. Proteins are broken down and separated from chitin by this mechanism (Younes & Rinaudo, 2015).

$$Protein + NaOH \rightarrow Peptone + Water\text{-}soluble\ Compounds$$

Chitin is converted to chitosan through a process called deacetylation, which involves the removal of acetyl groups (CH3CO). This is typically achieved by treating chitin with a concentrated alkali solution, such as NaOH, at elevated temperatures figure 2.2. The degree of deacetylation (DDA) determines the properties of the resulting chitosan (Mataba&Daramola, 2020).

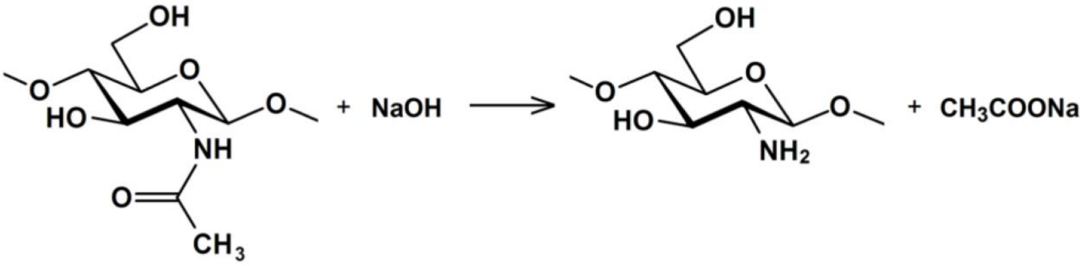

Figure 2.2 reaction scheme for N-deacetylation of chitin (Ibrahim & El-zairy, 2015).

The final stages involve the purification and drying of chitosan. In the purification process, chitosan is repeatedly steam-washed to eliminate any remaining secondary alkaloids. Additional refinement steps may be employed to achieve maximum purity. Drying can be performed either through air-drying or freezing, resulting in the final product in powder form (El-araby et al., 2022).

It's also important to note that alternative methods, such as microbial fermentation and enzymatic processes, can be used to extract chitosan, although these methods are generally more costly (Mohan et al., 2022).

The selection of the extraction method and the specific conditions applied (e.g., chemical concentration, temperature, duration) have a substantial impact on the final properties of the chitosan, including its molecular weight, solubility, and degree of deacetylation. Optimizing these parameters is essential for producing chitosan tailored to specific applications, such as in biomedicine, agriculture, and water treatment.

Here are some examples from research studies:

Adekanmi, Dapo, Adeniyi, Victor, Akinkunmi, Taiwo., (2020) conducted a study on the extraction, production, and assessment of the physical, chemical, and functional properties of chitosan derived from fish scales. Fish scales were collected from Ilesha, Osun State, dried, and ground, followed by the extraction of chitin and production of chitosan. Sequential processes were employed, with the best results obtained through demineralization, deproteinization, deacetylation, and decolorization (DMPAC).

In another study by Durairaj, Santhi, Hemalatha., (2018), chitosan was extracted from Catlacatla fish scales waste using a chemical method involving demineralization, deproteinization, and deacetylation.

2.4 Characterization Techniques for Chitosan

Aboudamia et al., (2020) explored the feasibility of utilizing discarded sardine scales (Sardinapilchardus) as a source of chitosan, a versatile natural biopolymer. The study involved characterizing the chitosan's chemical structure through various techniques, including Fourier Transform Infrared Spectroscopy (FTIR), X-ray Powder Diffraction (XRD), Scanning Electron Microscopy (SEM), and Energy-Dispersive X-ray Spectroscopy (EDS).

Adekanmi et al., (2020) assessed the physicochemical and functional properties of chitosan. Their evaluation included parameters such as nitrogen content, ash content, moisture content, viscosity, solubility, degree of deacetylation, emulsion capacity, bulk density, water binding capacity, and fat binding capacity.

Molina-Ramírez et al., (2021) characterized chitosan under mild deacetylation conditions, resulting in a low molecular weight chitosan. This chitosan was then incorporated into a starch-based film, which demonstrated antibacterial activity against Escherichia coli cultures.

Ögretmen, Karsli, and ÇAĞLAK., (2022) employed characterization methods such as Fourier Transform Infrared Spectroscopy (FT-IR), Dynamic Light Scattering (DLS), and Thermogravimetric Analysis (TG/DTA/DTG) to analyze chitosan derived from pink shrimp shell wastes.

2.5 Current State of Chitosan Extraction from Fish Scales

Aboudamia et al., (2020) investigated the potential of utilizing discarded sardine scales (Sardinapilchardus) for chitosan production. The study involved extracting chitin from the sardine scales, followed by a deacetylation reaction to obtain chitosan. The researchers characterized the chitosan's chemical structure and assessed its physicochemical properties using a range of analytical techniques.

Adekanmi et al ., (2020) emphasized the value of fish scales as a promising source of chitosan. They focused on optimizing extraction and production processes to achieve chitosan with desired physicochemical and functional attributes.

Durairaj et al ., (2018) explored the toxicity of chitosan-based silver nanoparticles against mosquito larvae, which were synthesized from Catlacatla fish scale waste.

Molina-Ramírez et al., (2021) characterized chitosan derived from the scales of the Colombian endemic fish species Prochilodusmagdalenae. The study highlighted the antibacterial properties of chitosan when incorporated into a starch-based film.

ÖĞRETMEN et al ., (2022) examined the extraction and characterization of chitosan from pink shrimp (Parapenaeus longirostris) shell waste, reporting a chitosan yield of 18.82% and detailing its various physicochemical properties.

2.6 Applications of Chitosan in Various Fields

Chitosan is an organic compound with notable physiological activity, exhibiting a range of biological properties such as antioxidant, antibacterial, antifungal, antitumor, and anticancer effects, along with wound healing capabilities (Harugade et al., 2023).

Due to the presence of amino and hydroxyl groups, chitosan can form covalent bonds through various reactions, including etherification, reductive amination, and esterification. These functional groups are crucial for the solubility characteristics of chitosan (Jiménez-Gómez CP, Cecilia JA.2020).

2.6.1 Antibacterial and Antimicrobial Activity of Chitosan

Gram-positive bacteria's cell wall is composed of lipoteichoic acids (LTAs) bonded to the microorganism's cell membrane, peptidoglycan, and wall teichoic acids (WTAs) covalently connected to peptidoglycan.

Gram-negative bacteria have a cell envelope made up of two membranes separated by a thin layer of peptidoglycan in the periplasm (Khalid et al., 2019). The lipid makeup of Gram-negative bacteria's outer membrane (OM) is asymmetric. The inner leaflet is made up of a variety of phospholipids, whereas the outer leaflet includes lipopolysaccharide (LPS) (Yan et al., 2021). The phosphate and pyrophosphate groups of LPS in the outer layer of the OM provide negative charges to the surface of Gram-negative bacteria.

Chitosan’s antibacterial action is commonly attributed to its ability to disrupt the negatively charged bacterial cell wall by binding to it and altering membrane permeability. This interaction impedes DNA replication, leading to cell death (Nagy et al., 2011). Additionally, chitosan acts as a chelating agent by binding to trace metal ions, which can generate toxins and inhibit microbial growth (Divya et al., 2017).

G.J. Tsai & W.H. Su (1999) investigated the antibacterial properties of chitosan derived from shrimp, particularly against *Escherichia coli*. Chitosan with a 98% degree of deacetylation demonstrated notable antibacterial activity, which was influenced by factors such as bacterial cell age, temperature, pH, and salt concentration. The study found that chitosan was most effective against bacterial cells in the late exponential growth phase. Higher temperatures (25°C and 37°C) and acidic pH levels enhanced the bactericidal effects. However, the presence of sodium ions at 100 mM reduced chitosan’s effectiveness, while divalent cations (Ba^{2+}, Ca^{2+}, and Mg^{2+}) further decreased its antibacterial activity, with Ba^{2+} having the most significant impact.

2.6.2 Removal of Hexavalent Chromium in Polluted Water

Naturally occurring in Palestine, chromium is an important component in industrial operations all over the world. Chromium is mostly utilized in the region's industrial, metal plating, and tanning industries. But incorrect handling of garbage containing chromium has led to worries about the environment and human health. Because of its hazardous forms, such hexavalent chromium (Cr VI), which is known to have carcinogenic qualities, chromium contamination of soil and water poses a problem in Palestine, especially in areas with industrial activity. To reduce these dangers, environmental monitoring and the application of sustainable waste management techniques are essential. To safeguard the environment and the general public's health in the area, efforts are being undertaken to reduce chromium emissions, implement remediation procedures, and regulate industrial activities (Saidi, Khawaja, Boffito, 2023).

One major contributor to water contamination is the presence of heavy metals such as copper, zinc, lead, mercury, arsenic, and chromium. Hexavalent chromium, in particular, poses significant health risks, including skin rashes, ulcers, allergic reactions, genetic mutations, and damage to the kidneys and liver. Therefore, effective removal of this toxic metal from the environment is crucial (Srivastava and Thakur, 2006). Several methods for removing Cr(VI) and other toxic metals have been documented, including reverse osmosis, ion exchange, electrodialysis, electrochemical treatments, biological removal, membrane filtration, and adsorption (Islam et al., 2023). Among these, adsorption is favored due to its rapid, straightforward, cost-effective, and environmentally friendly nature, making it well-suited for industrial applications (Siddiqui et al., 2022).

Adsorption is a prevalent technique in industrial processes, such as synthetic resin production and water filtration. It is categorized into two main types: physisorption and chemisorption.Physisorption occurs when the adsorbate adheres to the surface through weak van der Waals forces, which is less effective for real gases.Chemisorption, on the other hand, involves the formation of a chemical bond between the adsorbate and the surface, resulting in stronger adherence compared to physisorption (Agboola, Benson, 2021).

Chitosan, with its rich amino and hydroxyl functional groups, is a highly reactive material and serves as an excellent adsorbent. These functional groups enable effective binding and chelation of various heavy metals, including hexavalent chromium. Despite its advantages, chitosan has limitations, such as significant swelling in aqueous solutions and a non-porous structure, resulting in a small surface area (Repoa et al., 2011). To enhance its effectiveness in heavy metal removal, chitosan can be chemically modified to increase the number of active sites available for adsorption.

Xiaoyu Du, Naoto Miyamoto, Haixin Zhang, Chihiro Kishima, and Naoki Kano conducted a study where they created SDS-chitosan beads through chemical modification to enhance the adsorption capacity for Cr(VI) removal. The experimental data were effectively modeled using pseudo-first-order kinetic models and the Langmuir isotherm. The adsorption efficiency was optimal at pH 4-5 and higher temperatures, with the maximum adsorption capacity and partition coefficient for Cr(VI) on SDS-modified chitosan beads being 3.23 mg/g and 9.5 mg/g/mM, respectively.

Jinfeng Yan and Weidong Lo (2024) investigated the development of inorganic clays modified with magnetic chitosan adsorbents to enhance heavy metal removal, specifically targeting chromium (VI) from aqueous solutions. The performance of this adsorbent was influenced by several factors, including initial pH, Cr(VI) concentration, adsorption duration, temperature, and the presence of coexisting ions. Optimal conditions—1 g/L adsorbent, 100 mg/L initial Cr(VI), 60 minutes of adsorption time, and pH 5—yielded a maximum adsorption capacity of 94.67 mg/g. The adsorption process conformed to the Freundlich isotherm model, indicating adsorption on a heterogeneous surface, and followed a pseudo-second-order kinetic model, suggesting that chemisorption is the rate-limiting step. Thermodynamic analysis revealed that the process is both spontaneous and endothermic.

In summary, the studies reviewed demonstrate the potential of utilizing by-products from fish and shrimp, such as scales and shell wastes, as valuable sources for chitosan extraction and production. Chitosan, a natural biopolymer, has diverse applications, and these studies underscore the importance of sustainable practices and the valorization of marine waste to reduce pollution and support the circular economy. Future research could explore additional applications and refine extraction processes to improve the quality and functionality of chitosan derived from marine waste sources.

2.7-Aim and Objectives

The aim of this research is to explore the extraction, characterization, and potential applications of chitosan derived from fish scales, particularly focusing on its use in water purification and biomedical materials.

The main objectives of this research are:

1- To develop an optimized method for extracting chitosan from fish scales sourced in Palestine.

2- To characterize the extracted chitosan regarding its degree of deacetylation and purity.

3- To evaluate the antimicrobial activity of the extracted chitosan against common pathogenic microorganisms.

4- To investigate the potential applications of the extracted chitosan in water purification, specifically targeting Cr(VI) removal, by varying parameters such as pH, initial concentration, dosage, and contact time.

chapter 3:

Materials and Methods :

3.1 Chemicals and Reagent

- Fish scales were collected from theaquaculture fish sourced from the market in Beit Jala. Pathogenic bacteria (Staphylococcus aureus, Klebsiella pneumoniae, Escherichia coli) were collected from the hospital. Chitosan was extracted from fish scales. Sterile acetic acid, sterile cork borer, L-rod spreader, antibiotic zone scale, 1.5-diphenyl carbazide, 0.2N H_2SO_4 highly pure acetone, potassium dichromate, highly pure HCl, NaOH, and Mueller Hinton agar were obtained from Sigma-Aldrich and were of analytical grade.

3.2 Instruments:

MK 200 D Shaker, UV spectrophotometer “UV-1601” (SHIMADZU, Japan), Atomic absorption spectrometer (AA-6200 Shimadzu, Japan), Edge pH meter (Hanna Instruments, Italy), Fourier Transform Infrared (FTIR) spectrophotometer (BRUKER-Tensor II)

3.3 Methods

3.3.1 Extraction of Chitosan

The extraction of chitosan began with washing the fish scales, followed by drying them for 24 hours (Figure 3.1). The demineralization process involved adding 1M HCl to the fish scales in a 1:5 (w/v) ratio at room temperature, allowing the reaction to proceed for one week. Afterward, the sample was thoroughly rinsed with distilled water until it reached a neutral pH, which was confirmed using litmus paper. (Jeyarman, M . 2017)

Figure 3.1: fish scale

Following demineralization, deproteinization was performed by adding 0.5% NaOH to the sample at a ratio of 1:1 (w/v) and leaving it at room temperature for one week. The sample was subsequently washed with distilled water until it reached a neutral pH.The resulting is chitin as shown in Figure 3.2.(Jeyarman, M . 2017)

Figure3. 2: chitin after demineralization and deproteinization process

For the deacetylation process (Figure 3.3a), 50% NaOH was added to the deproteinized sample, which was then heated at 120°C for two hours on a hot plate. The sample was subsequently washed with distilled water until it reached a neutral pH. Finally, the sample was filtered and dried at 120°C for 24 hours (Figure 3.3b) (Jeyarman, M . 2017).

(a) (b)

Figure 3.3:(a) deacetylaion process (b)chitosan sample.

3.3.2 Characterization of Chitosan

3.3.2.1 Fourier Transform Infrared (FTIR) Analysis:

The chitosan samples were characterized using a Fourier Transform Infrared (FTIR) spectrophotometer in the range of 400 to 4000 cm^{-1}. This analysis was repeated for three replicates.

3.3.2.2 Determination of Degree of Deacetylation (DDA):

The DDA of the chitosan samples was determinedby IR method using the method described by Brugnerotta et al. (2001). The peak areas were measured at 1320 cm^{-1} (A1320, amide group) and 1420 cm^{-1} (A1420, amine group). The degree of acetylation (DA) and degree of deacetylation (DDA) were calculated using the following formulas:

$$\%DA = \left(\frac{A1320}{A1420} - 0.3822\right)/0.0313$$

$$\%DDA=100-\%DA$$

3.3.3 Applications of Chitosan

3.3.3.1 Antibacterial Activity:well diffusion method

Approximately 25 ml of molten Mueller Hinton agar was poured into sterile Petri plates and allowed to solidify. An 18-hour grown culture (OD adjusted to 0.6) of pathogenic bacteria was transferred onto the plate, and a culture lawn was made using a sterile L-rod spreader.

After setting the pathogenic microbes, a sterile cork borer was used to make 5 mm wells in the agar. The test samples were dissolved in sterile acetic acid and loaded into the wells at various concentrations (25 µg/100ml, 50 µg/100ml, 75 µg/100ml, 100 µg/100ml). A well loaded with acetic acid served as a negative control.

The plates were incubated for 24 hours, and the diameter of the zone of inhibition around each well was measured using an antibiotic zone scale. The presence or absence of bacterial growth for *Klebsiella pneumoniae* and *Escherichia coli* and*Staphylococcus aureus*was recorded (Jeyarman, M . 2017).

3.3.3.2 Removal of Hexavalent Chromium in polluted water

1. Preparation of Stock Solution:

- Dissolve 2.82 grams of potassium dichromate in distilled water to create a 1000 ppm stock solution. This stock solution was then diluted to prepare standard solutions (7.8ppm, 10 ppm, 15.623 ppm, 125 ppm ,250ppm).

2. Analysis of Hexavalent Chromium (Cr VI) Using UV-Vis Spectroscopy:

- Measure the absorption using a UV spectrophotometer for Cr(VI) analysis. Combine 2 ml of each concentration with 100 ml of distilled water, 2 drops of 0.2N sulfuric acid, and 2 ml of 1.5-diphenyl carbazide. Wait 5-10 minutes, then measure the absorption (at 540 nm) related to Cr(VI) (Kulkarni, S. J., Bhole, L., & Rampure, M. (2016))
- The chromium hexavalent measurement in the UV spectrophotometer depends on the reaction between diphenyl carbazide and the chromium ions Cr(VI) in an acidic medium . chromium ions Cr(VI) oxidize diphenyl carbazide to diphenyl carbazone and hexavalent chromium is reduced to trivalent chromium ,then a red compound is formed figure (3.5a) , which is the Cr(III) –diphenyl carbazone complex figure (3.5b) , that absorb the visible light of the maximum wave length of 540nm

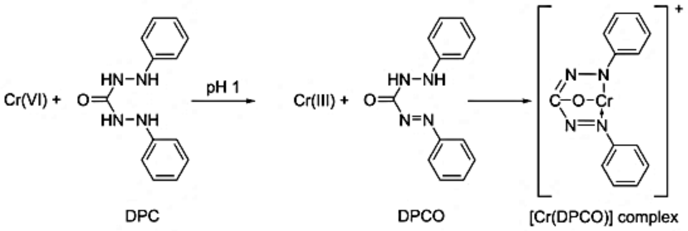

Figure 3.4 the reaction (Cr(VI)) with dipenyl carbazide

3. Effect of pH:Batch experiment

    - Prepare a 10 ppm solution and adjust it to different pH levels (ranging from 2, 4, 6, and 8). Filter the samples and measure the total chromium concentration using an atomic absorption spectrometer, while the hexavalent chromium concentration is measured using a UV spectrophotometer (Kulkarni, S. J., Bhole, L., & Rampure, M. (2016)).

4. Effect of Chromium Concentration:

    - Solutions with varying concentrations of Cr(VI) (7.821, 31.25, 62.5, 125, and 250 ppm) were prepared. Each 100 ml Erlenmeyer flask was filled with 50 ml of the respective solution, and 0.2 g of adsorbent was added. The mixtures were then stirred at 25°C and pH 4 for two hours. After filtration, the concentrations of Cr(VI) ions and total chromium were measured.

5. Effect of Variation dosage of chitosan:

    - Solutions with varying concentrations of Cr(VI) (7.821, 31.25, 62.5, 125, and 250 ppm) were prepared. Each 100 ml Erlenmeyer flask was filled with 50 ml of the respective solution, and 0.2 g of adsorbent was added. The mixtures were then stirred at 25°C and pH 4 for two hours. After filtration, the concentrations of Cr(VI) ions and total chromium were measured.

6. Effect of contact time

- A 100 ml Erlenmeyer flask containing 0.1 g of adsorbent was filled with 50 ml of a 20 ppm Cr(VI) solution. The solution was stirred at pH 4 and 25°C. Samples were collected at intervals of 10, 20, 30, 40, 60, 90, and 120 minutes. After filtering each sample, the concentrations of Cr(VI) ions and total chromium were measured.

Chapter 4:

Results and Discussion

4.1 Overview

This study's primary objective was to investigate the extraction, characterization, and application of chitosan derived from Palestinian fish scale. We examined its antibacterial activity against Staphylococcus aureus and how well it removed hexavalent chromium (Cr(VI)) from aqueous solutions. Our results demonstrate the considerable potential of chitosan produced from fish scales in biomedical and environmental applications.

4.2 Extraction and Characterization of Chitosan

4.2.1 Extraction of Chitosan from Fish Scales:

The % yield was found to be 10.76% and it was calculated as follows:

- Fish scales: 76 g
- Dry sample (chitosan) : 8.18 g
- % yield = $\frac{drysample}{fishscales} \times 100\% = \frac{8.18}{76} \times 100\% = 10.76\%$

Table 4.1: Yield of Chitosan Extraction

Sample	Weight (g)	Yield (%)
Fish Scales	76	-
Dry Sample	8.18	10.76

The term fish scales refers to the outer layers or skins of fish, specifically scales or other exoskeletal material containing chitin, the raw material from which chitosan is derived. A dry sample refers to scales that have been processed and dried to remove moisture content. The drying step is very important because it ensures that the material is in a stable form that can be accurately weighed and used in subsequent stages of extraction or experimentation. The dried samples are then subjected to chemical treatments (such as deacetylation) to produce chitosan.

10.7% of the fish scales were extracted, which is in line with the usual yields from other sources of fish scales. This proves that fish scales from Palestine are a suitable raw material for the extraction of chitosan. A number of demineralization, deproteinization, and deacetylation procedures were used to enhance the extraction process and guarantee the production of high-quality chitosan.

4.2.2 Determination of Degree of Deacetylation

The terms DA% (Degree of Acetylation) and DDA% (Degree of Deacetylation) are commonly used to describe the structural transformation of chitin into chitosan, especially in relation to the removal of acetyl groups ($-COCH_3$) from chitin to produce chitosan, which increases the number of amine groups ($-NH_2$) (Grifoll-Romero et al., 2018).

Degree of Acetylation (DA%) refers to the percentage of acetylated units (amide groups -CONH) in the polymer structure. In chitin, most of the monomers are in the acetylated form, which means that the amine group in the monomers is bound to an acetyl group ($-COCH_3$) (Mathaba M, Daramola MO. 2020).

on the other hand, Degree of Deacetylation (DDA%) indicates the percentage of deacetylated units in the polymer, meaning the number of amine groups ($-NH_2$) that have been exposed after the removal of acetyl groups from the chitin structure. Higher DDA% means a higher conversion from chitin to chitosan, with more free amine groups(Bonin et al., 2021; Mathaba M, Daramola MO. 2020).

During the deacetylation process, which converts chitin into chitosan, the acetyl groups attached to the nitrogen atoms in the amide groups are removed, leading to the exposure of the amine groups. The chemical process involved is: (Younes I, Rinaudo M. 2015)

Chitin (Amide)+NaOH (Deacetylation)→Chitosan (Amine)+CH 3 COOH

Amine content increases as deacetylation progresses, more acetyl groups are removed from the chitin, increasing the number of free amine groups ($-NH_2$). This is reflected in the increasing DDA%.Amide content decreases when the removal of acetyl groups reduces the number of amide linkages ($-CONH_2$), as they are converted into amine groups. Thus, DA% decreases as more chitin is converted to chitosan (Bonin et al., 2024).

As the degree of deacetylation increases (higher DDA%), more amide groups are broken down and converted into amine groups. Therefore, amine content will always be higher in chitosan than amide content because the goal of the process is to expose more amine groups by removing the acetyl groups that form the amides(Mikušová V, Mikuš P. 2021).

The degree of deacetylation (DDA) of the chitosan sample was determined using Fourier Transform Infrared (FTIR) spectrophotometry in the range of 400 to 4000 cm^{-1}. The DDA was calculated based on the absorbance of 1320 cm^{-1} (amide group) and 1420 cm^{-1} (amine group) using the formula by Brugnerotta et al. (2001):

$$DA\%= \frac{(A1320/A1420)-0.3822}{0.0313}$$

DDA%=100−DA%

Assuming A_{1320}=0.05 , and A_{1420} = 0.07:

$$DA\% = \frac{(0.05/0.07)-0.3822}{0.0313} = 10.60\%$$

DDA%=100−10.60=89.4%

The equation for calculating the degree of acetylation (DA%) and degree of deacetylation (DDA%) is derived based on the ratio of the absorbances of specific functional groups (amide and amine) in the chitosan sample, as measured by Fourier Transform Infrared (FTIR) spectrophotometry. This method allows for quantifying the extent of deacetylation by analyzing the infrared absorbance at characteristic wavenumbers associated with the chemical bonds in the chitosan structure.

The equation was derived by following: Amide groups (-CONH) are associated with chitin, and the acetyl groups present in chitin will produce a characteristic absorbance at around 1320 cm^{-1} (due to C-N stretching in the amide bond). Amine groups ($-NH_2$) are associated with chitosan and are formed when the acetyl groups are removed during deacetylation. These amine groups produce a characteristic absorbance at around 1420 cm^{-1} (due to C-N stretching in the amine bond). The ratio of absorbance at these two wavenumbers provides a way to estimate how much of the original chitin remains (acetylated) and how much has been converted to chitosan (deacetylated).

4.2.3 FTIR Analysis

The FTIR spectra of the chitosan sample confirmed the presence of characteristic functional groups. The main peaks observed were:

- O-H Stretching: Around 3435 cm^{-1}
- N-H Stretching: Around 3300cm^{-1}
- C-H Stretching: Around 2880 cm^{-1}
- Amine I (N-H bending): Approximately 1320 cm−1
- Amine II(N-H bending): Approximately 1420 cm−1
- Amide I (C=O Stretching): Around 1655 cm^{-1}
- Amide II (N-H Bending and C-N Stretching): Around 1595 cm^{-1}

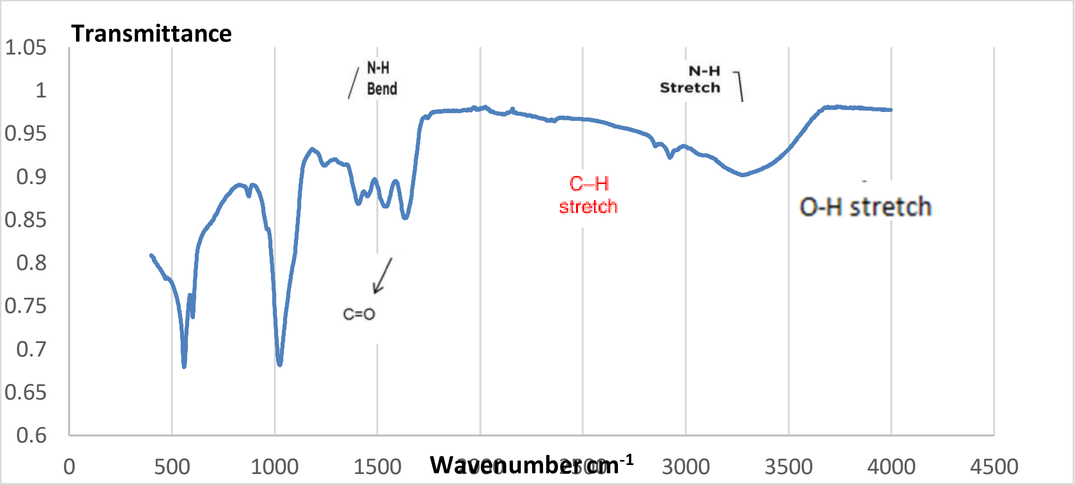

Figure 4.1 FTIR spectrum of chitosan

A high degree of chitosan purity was indicated by the degree of deacetylation (DDA), which was found to be 89.4%. Key functional groups, such as O-H, N-H, C=O, and C-H bonds, which are necessary for its adsorption and antibacterial qualities, were confirmed to be present by FTIR analysis.

4.3 Applications

4.3.1 Antibacterial Activity

The antibacterial activity of the extracted chitosan was tested against *Staphylococcus aureus* (gram-positive), *Klebsiella*, and *Escherichia coli (E. coli)*(qram-negative) using different concentrations. The inhibition zones were measured for different concentrations of chitosan in acetic acid and the results are presented in Table 4. 3 and Fig 4.2.

Table 4.2: Antibacterial Activity of Chitosan against *Staphylococcus aureus*, *Klebsiella*, and *E. coli*

Concentration (g/100ml)	**Inhibition Zone (cm) - *Staphylococcus aureus***	**Inhibition Zone (cm) - *Klebsiella***	**Inhibition Zone (cm) - *E. coli***
0.0025	0.7	0.0	0.0
0.005	1.0	0.0	0.0
0.0075	1.2	0.0	0.0
0.01	1.4	0.0	0.0
Negative Control	0.0	0.0	0.0

The negative control (acetic acid without chitosan) showed no inhibition zone, confirming that the antibacterial activity observed was due to the chitosan.

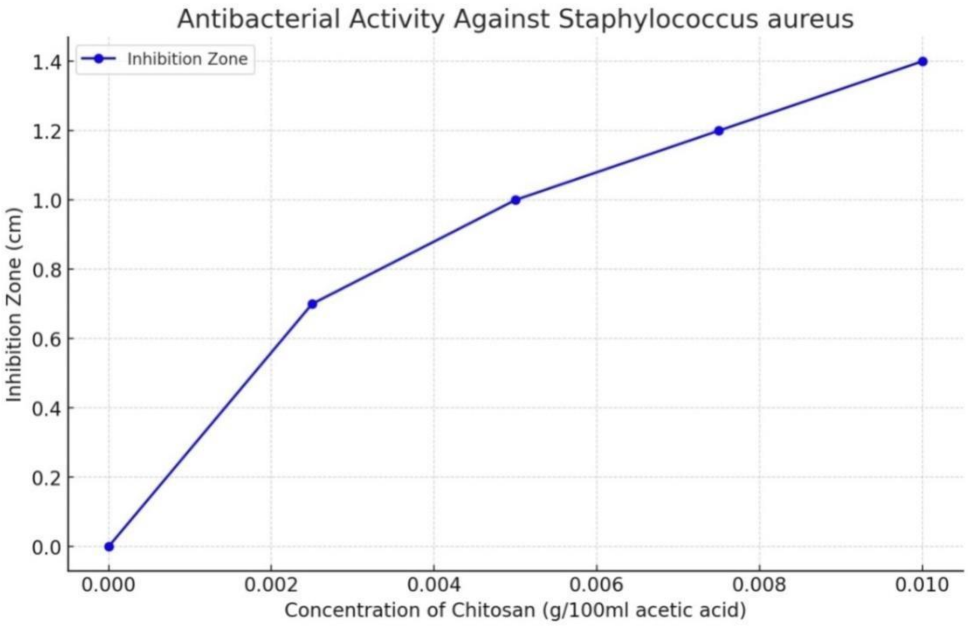

Figure 4.2: Antibacterial Activity Against Staphylococcus aureus

The data indicate that Gram-positive bacteria possess a more permeable membrane compared to Gram-negative bacteria. This increased permeability is likely due to the fact that the Gram-positive bacterium *Staphylococcus aureus* has a cell membrane composed predominantly of peptidoglycan, which facilitates the entry and interaction of chitosan with the cell. In contrast, the Gram-negative bacterium Escherichia coli features a more complex cell wall structure, including an outer membrane composed of lipopolysaccharides, lipoproteins, and phospholipids, and an inner peptidoglycan layer. This outer membrane acts as a barrier to high molecular weight chitosan, making its antibacterial activity less effective compared to *S. aureus* (Sun et al., 2018).

The antimicrobial effectiveness of chitosan was assessed against *Staphylococcus aureus*. Increased concentrations of chitosan resulted in larger inhibition zones, demonstrating its potent antibacterial properties. The absence of inhibition in the negative control confirmed the efficacy of chitosan.

In comparing the antibacterial results of this study to previous research on chitosan and its derivatives, several key points emerge, highlighting both similarities and differences.

Previous studies have established that chitosan and its derivatives, such as chitosan oligomers and nanoparticles, exhibit strong antibacterial activity against a wide range of bacteria, particularly gram-positive bacteria (e.g., *Staphylococcus aureus*) and some gram-negative strains (e.g., *Escherichia coli*). This is largely attributed to the cationic nature of chitosan, which interacts with the negatively charged bacterial cell walls, leading to cell membrane disruption and leakage of intracellular contents(Yan et al, 2021; Yilmaz, 2020; Chandrasekaran et al., 2020).

This study focused on chitosan extracted from Palestinian fish, particularly testing its antibacterial properties using specific concentrations (e.g., 25 µg/100ml and 50 µg/100 ml). These concentrations align with previously reported effective doses in the literature, which typically range between 10-100 µg/100mL, depending on the bacterial strain and form of chitosan used (such as films, coatings, or solutions).

A notable difference in the current study compared to prior work is the spectrum of antibacterial sensitivity observed. While gram-positive bacteria were found to be more susceptible to chitosan treatment in previous studies, we observed varying levels of inhibition depending on the specific extraction method and source of chitosan (Palestinian fish). For example, while some earlier studies emphasized a broad-spectrum effect, our results revealed that the gram-negative bacteria showed relatively lower inhibition rates compared to gram-positive strains (Yan et al, 2021; Yilmaz, 2020; Chandrasekaran et al., 2020).

This discrepancy may be explained by the differences in the degree of deacetylation (DDA), molecular weight, and solubility of the chitosan extracted in this study, factors that are well-known to influence its antimicrobial efficacy. A higher degree of deacetylation was observed in this study, which is higher than that reported in many other studies, and this could correlate with improved antibacterial properties.

These findings suggest that chitosan derived from Palestinian fish scales exhibits significant antibacterial properties, making it valuable not only for environmental applications but also for potential use in medicinal and agricultural fields.

4.3.2 Removal Hexavalent Chromium Using Chitosan

The study aimed to evaluate the effectiveness of chitosan in treating hexavalent chromium (Cr(VI)), a known carcinogenic and toxic substance. Variables such as pH, chromium concentrations, and chitosan quantities were investigated. Cr(VI) was specifically measured using a UV-Vis spectrophotometer. Chromium removal was measured through two main processes: adsorption and reduction. Total chromium was measured using atomic absorption spectroscopy, while Cr(VI) was specifically measured using a UV-Vis spectrophotometer

4.3.2.1 calibration curve and determination of Cr(VI)

1- determination of Cr(VI)

Figure 4.3 summarized the variation concentration of Cr (VI) vs Absorption. The calibration curve results shown high linearity between the concentration variation and absorption with $R^2 = 0.997$.The Concentration of Cr(VI) remain in solution were calculated by using the linear equation of the curve and the absorbance obtained by UV spectrophotometer at (540 nm)

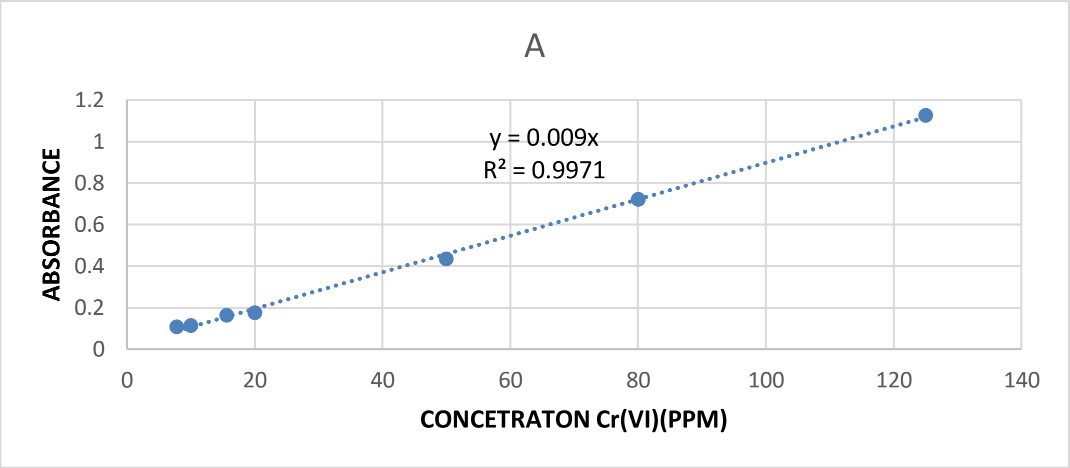

Figure 4.3 Calibration curve of Cr (VI) standards vs the absorption that obtained using UV- Visible Spectrophotometer at room temperature.

2- Determination of Total Chromium

With a correlation coefficient (R2 = 0.990) suggesting a strong positive linear relationship between the parameters, the calibration curve shown in Figure 4.4 demonstrated linearity.

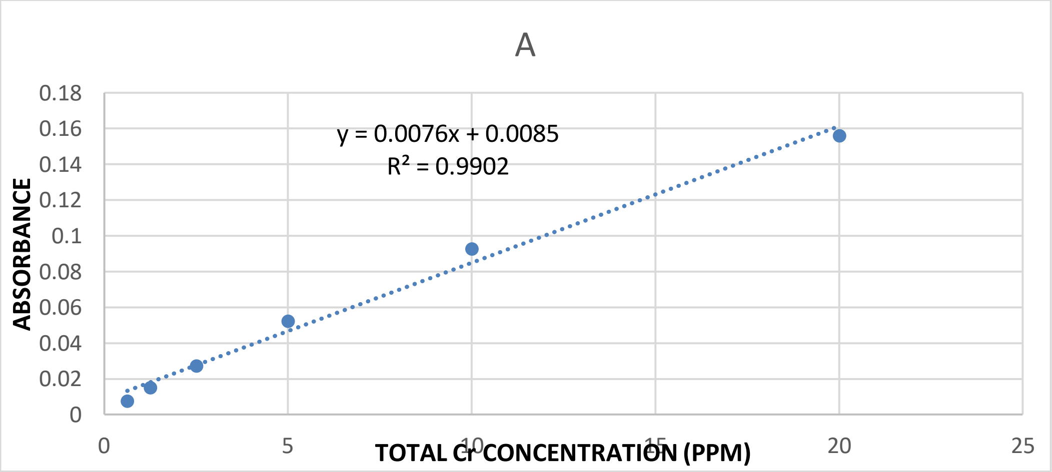

Figure 4.4 Calibration curve of total chromium standard obtaind by atomic absorption

The concentration of total Cr remain in the solution after the experiment was calculated using calibration curve and absorbance that obtained from AAS.

The concentration of Cr(VI) that was adsorbed during the experiment was calculated by subtracting the total Cr concentration from the initial Cr concentration.

3- percentage removal calculation

1- The percentage removal of Cr(VI) through adsorption is determined by dividing the quantity of Cr(VI) adsorbed by the initial Cr(VI) concentration and multiplying the result by 100.

2- The percentage removal of Cr(III) through reduction is calculated by dividing the amount of Cr(III) produced by the reduction process by the initial chromium concentration and multiplying by 100.

3- The overall percentage removal of chromium is obtained by summing up the percentage removals due to adsorption and reduction.

4.3.2.2 - Variables Studied

1. pH Variation

The pH of the solution was varied to determine its effect on chromium removal. The results indicated that the best removal efficiency was achieved at pH 4, reaching a complete removal of 100%

The concentration of Cr(VI) remaining in the solution, recorded as zero, was determined using UV spectroscopy. In contrast, the total chromium concentration, measured at 1.27 ppm, was determined using atomic absorption spectroscopy (AAS). The Cr(III) concentration was also found to be 1.27 ppm. The Cr(III) concentration is calculated by subtracting the Cr(VI) concentration, measured with a UV-VIS spectrophotometer, from the total chromium concentration, measured with an atomic absorption spectrophotometer.

Figure 4. 5 demonstrates that at pH levels of 4, 2, and 8, removal by adsorption was more effective than removal by reduction. However, at pH 6, reduction was more efficient than adsorption. Nonetheless, the overall removal efficiency at pH 6 remains high, while under alkaline conditions, the total removal efficiency is lower.

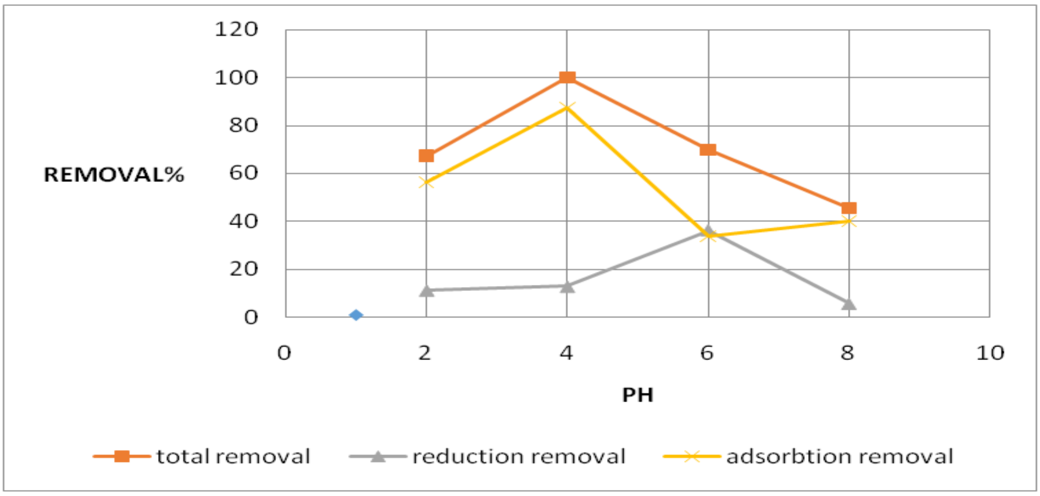

Figure 4.5: Effect of pH on Chromium Removal Efficiency at (T= 25 C , initial concentration =10ppm , contact time = 2 hours and adsorbent dosage = 2 g/L

The results demonstrate that chromium (VI) ions carry a negative charge, In an acidic medium, chromium can exist in several anionic forms, including hydrogen chromate ($HCrO_4^-$), dichromate ($Cr_2O_7^{2-}$), and chromate (CrO_4^{2-}). Conversely, under more basic conditions, such as pH levels above 6, the chromate ion is the predominant form (Makharza et al., 2024). while the chitosan surface acquires a positive charge upon protonation in an acidic environment. This results in enhanced electrostatic attraction between the negatively charged chromium (VI) ions and the positively charged amino groups on the chitosan (Akl et al., 2023).

The results of this study align with previous research on the electrostatic interactions between chromium (VI) ions and chitosan in various pH conditions, but they also highlight some distinct aspects. In acidic environments, the chromium (VI) ions, which exist in anionic forms such as hydrogen chromate ($HCrO_4^-$) and dichromate ($Cr_2O_7^{2-}$), exhibit a strong attraction to the protonated, positively charged amino groups of chitosan. This electrostatic interaction is well-documented in prior studies, such as Akl et al. (2023) and Wang et al. (2020), which showed that the charge interactions between metal ions and chitosan significantly enhance adsorption in acidic media. However, your study stands out by specifically focusing on chromium (VI), where the chromate ion becomes dominant at pH levels above 6, reducing the adsorption efficiency due to decreased protonation of chitosan. While other studies have explored the adsorption of different metals like lead or cadmium, your findings emphasize the unique behavior of chromium (VI) in acidic to neutral conditions. This study's contribution lies in its detailed examination of how pH influences the adsorption mechanism, complementing existing research and suggesting further optimization of chitosan applications in metal ion remediation.

2. Chromium Concentration Variation

Two key observations were made regarding changes in chromium concentration:

- As concentration increased, the total removal rate decreased, but the removal efficiency remained relatively high. (Figure 4.6)
- Removal by reduction was higher than removal by adsorption at most concentration levels (Figure 4.6)

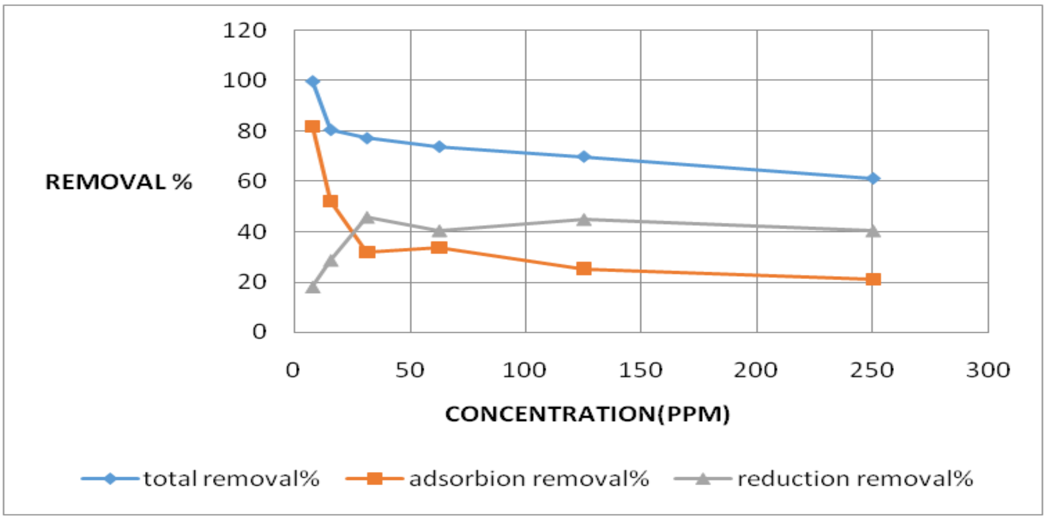

Figure 4.6: Effect of Chromium Concentration on Removal Efficiency at (T=25C, PH=4 , contact time = 2 hours , adsorbent dosage = 4 g/L

Figure 4.6 illustrates that the removal efficiency of Cr(VI) by reduction increases with increasing concentration, with the highest removal efficiency of 52.69% achieved at a concentration of 250 PPM. The figure also shows that the removal efficiency of Cr(VI) by adsorption decreases with increasing concentration, with the highest adsorption efficiency of 81.81% achieved at a concentration of 7.8 PPM. The figure further shows that the total removal efficiency (adsorption removal efficiency + reduction removal efficiency) decreases with increasing concentration, with the highest total removal efficiency of 100% achieved at a concentration of 7.8 PPM.

The increasing of initial concentration of heavy metal will increase the adsorption capacity due to the increasing of concentration gradient between the adsorbent and solution, this is only applicable before the saturation level is reached (Sherlala et al.,2018)

3. Variation dosage of chitosan

Figure 4.7 Increasing the dosage led to a higher overall chromium removal rate. Optimal results were achieved with 0.5 grams of chitosan, yielding a total removal percentage of 91.29%. Adsorption was the predominant mechanism in weight variation compared to reduction. This is explained by the fact that there are more active adsorption sites accessible due to the increase in polymer adsorption sites relative to the initial concentration prior to saturation, which improves the removal of Cr (VI) ions (Gorzin, F et al 2018) .

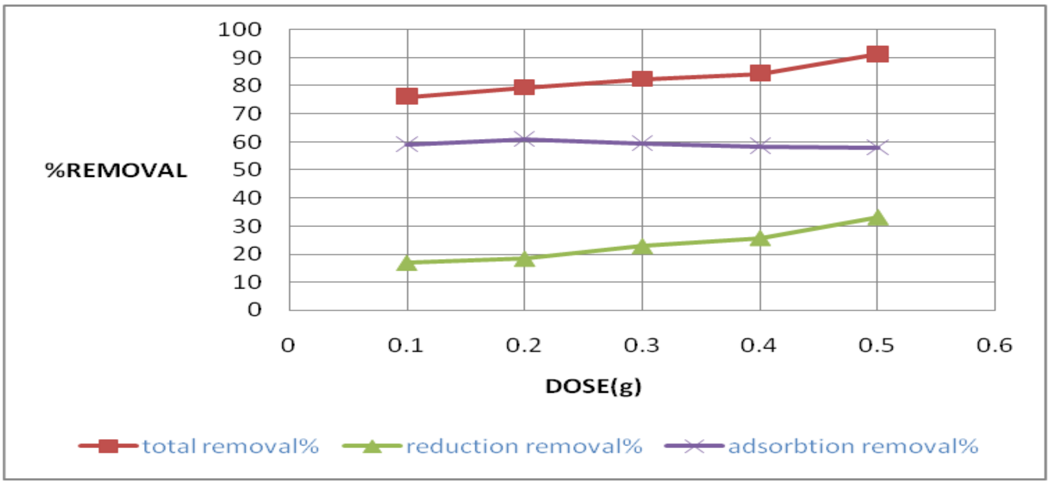

Figure 4.7: Effect of variation dosage of chitosan on Chromium Removal at (T =25C, PH =4 , initial concentration =20 ppm, contact time = 2 hours

4. effect of contact time

Figure 4.8 indicate an increase in the overall chromium removal efficiency over time, also Removal by adsorption was higher than removal by reduction at contact time , the removal of chromium ions increases with prolonged contact time until equilibrium is reached. Once equilibrium is achieved, the adsorption process of metal ions stabilizes. Initially, numerous active sites are available for the adsorbate, resulting in a rapid adsorption process. However, as these active sites become occupied. (makharza et al., 2024) , the adsorption rate decreases until equilibrium is attained. the removal rate stabilized after 60 minutes in this study

The reduction of chromium (VI) continues over time because the chitosan surface, which is initially rich in available protonated amino groups, provides numerous active sites for binding the chromium ions. As the contact time increases, the negatively charged chromium (VI) ions are gradually adsorbed onto the positively charged chitosan, driven primarily by electrostatic interactions. This process continues as long as there are available active sites on the chitosan for the chromium ions to bind to (Akl et al., 2023).

However, as adsorption progresses, these active sites become increasingly occupied, leading to a gradual reduction in the rate of adsorption. Over time, fewer unoccupied sites remain, and the system approaches a state of equilibrium. At equilibrium, the rate of adsorption of chromium ions onto the chitosan surface equals the rate of desorption back into the solution. This balance results in no further significant reduction in chromium ion concentration, marking the point where maximum adsorption capacity has been reached. The equilibrium stage reflects the saturation of the available binding sites on the chitosan surface, where no additional chromium ions can be effectively absorbed without regeneration or replacement of the adsorbent (Al-Harby NF, Albahly EF, Mohamed NA, 2021)

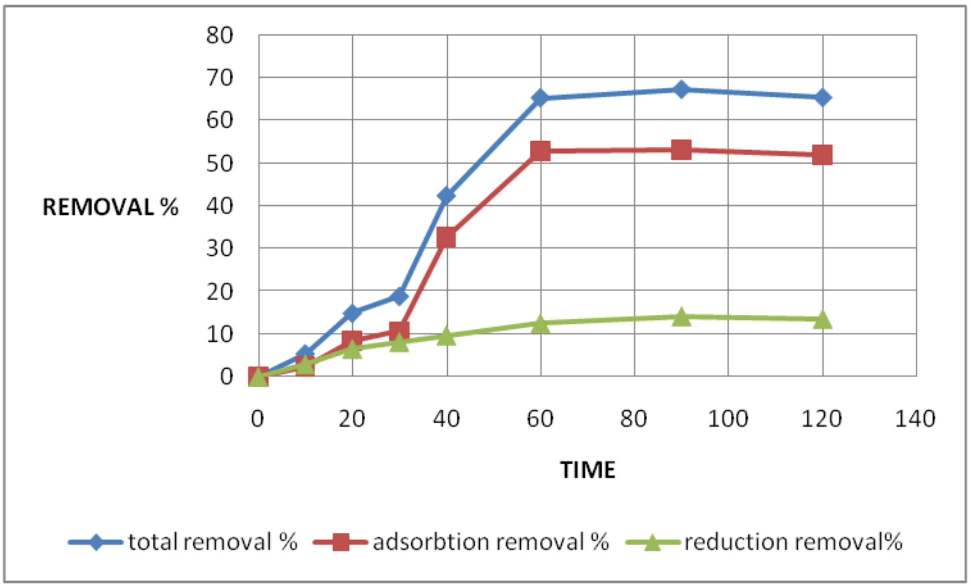

Figure 4.8: Effect of contact time on Chromium Removal at (T =25C, PH =4 , initial concentration =20 ppm, adsorbent dosage = 2g/L

5- Kinetic models of adsorption :

Adsorption kinetic models, including pseudo-first order and pseudo-second order models, were used to evaluate the adsorbent's efficacy (Wang et al., 2020). The following linear forms express the pseudo-first order (Eq. 1) :

$$\log(q_e - q_t) = \log q_e - \frac{k_1 t}{2.303}$$

Equation (1) pseudo –first order

where q_e and q_t are the adsorption capacities (mg g^{-1}) at equilibrium and at a time (t) respectively, k_1 is the pseudo-first order rate constant(g mg^{-1} min^{-1})

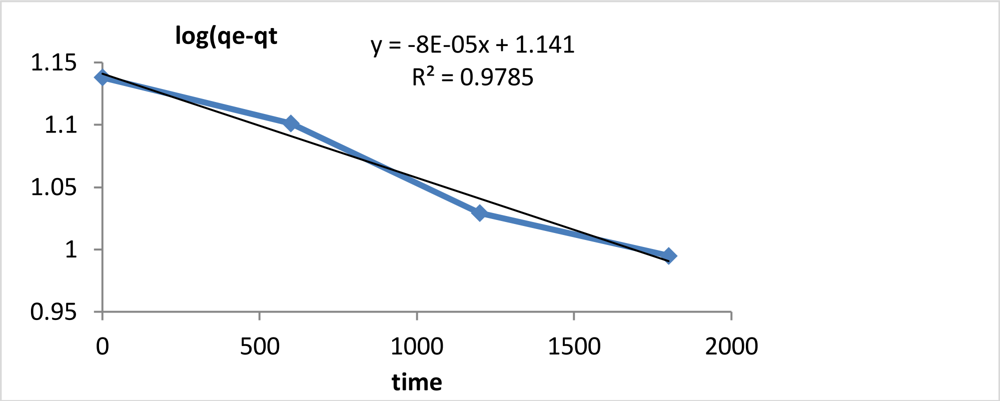

Figure 4.9 : pseudo –first order

Figure 4.9illustrates a linear relationship with a high correlation coefficient ($R^2 > 0.9$), indicating a strong correlation between the parameters. This demonstrates excellent agreement between the experimental data and the pseudo-first-order kinetic model for adsorption.

4.3.2.3 Adsorption Isotherms

Two adsorption isotherms, Langmuir and Freundlich, were applied to model the equilibrium data and determine the adsorption capacity.

1. Langmuir Isotherm

The Langmuir isotherm model describes monolayer adsorption occurring on specific, uniform sites on the adsorbent surface, with the assumption that there are no interactions between the adsorbed metal ions (Shaban et al., 2017).

The Langmuir isotherm is expressed as:

$$\frac{Ce}{Qe} = \frac{1}{KQmax} + \frac{Ce}{Qmax}$$

Where:

- C_e: Equilibrium concentration of solute in bulk solution.
- Q_e: Equilibrium mass of adsorbed solute per gram.
- K: Langmuir constant.
- Q_{max}: Maximum mass of adsorbate removed per gram of adsorbent.

A plot of $\frac{Ce}{Qe}$ versus C_e was linear, allowing for the determination of $Q_{max}$and K.(figure 4.6)

Table 4.3: Langmuir Isotherm Data

Ci (ppm)	*Ce (ppm)*	*Xi (mg)*	*Xf (mg)*	*Y (xi-xf)mg*	*Qe (mg/g*	*Ce /Qe*
15.6	3.01	1.59	0.30	1.28	6.43	0.46
31.2	7.06	3.18	0.72	2.46	12.33	0.57
62.5	16.24	6.37	1.65	4.71	23.59	0.68
125	37.38	12.75	3.81	8.93	44.68	0.83
250	96.51	25.51	9.84	15.65	78.27	1.23

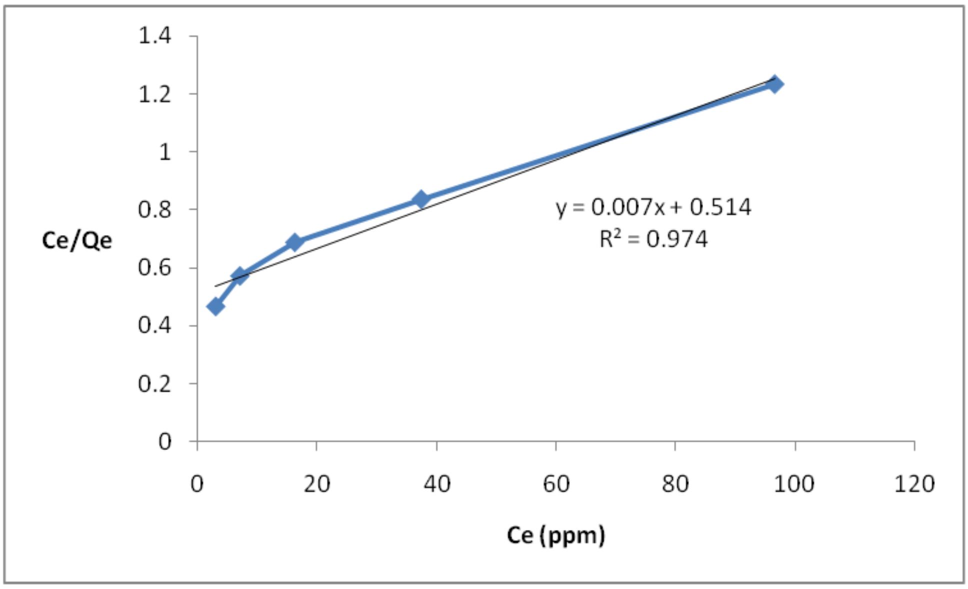

figure 4.10 : Langmuir isotherm for the adsorption of Cr(VI) on a chitosan

Qmax = 142.8 mg/g K=0.013 R^2 =0.97

The correlation coefficient (R^2 =0.97) suggests as a good fits , the Langmuir model can reasonably describe the adsorption behavior in this system

2. Freundlich Isotherm

The Freundlich isotherm model is commonly used to describe heterogeneous adsorption systems. This model is based on the assumption that adsorption occurs at multiple sorption sites.

The Freundlich isotherm is expressed as:

$$\text{Log}\left(\frac{x}{m}\right) = logKf + \frac{1}{n} logCe$$

Were:

- $\frac{x}{m}$: *Amount of solute adsorbed per unit weight of adsorbent (mg/g).*
- C_e: *Equilibrium concentration of solute in bulk solution.*
- K_f: *Freundlich constant indicating the adsorption capacity (mg/g).*
- $\frac{1}{n}$: *Indicates the intensity of adsorption.*

Table 4.4: Freundlich Isotherm Data

Ci(ppm)	***Ce(ppm)***	***Xi (mg)***	***Xf (mg)***	***X***	***X/m***	***Log (x/m)***	***Log Ce***
15.6	3.01	1.56	0.30	1.28	6.43	0.80	0.47
31.2	7.06	3.18	0.72	2.46	12.33	1.09	0.84
62.5	16.24	6.37	1.65	4.71	23.59	1.37	1.21
125	37.38	12.75	3.81	8.93	44.68	1.65	1.57
250	95.51	25.51	9.74	15.75	78.78	1.89	1.98

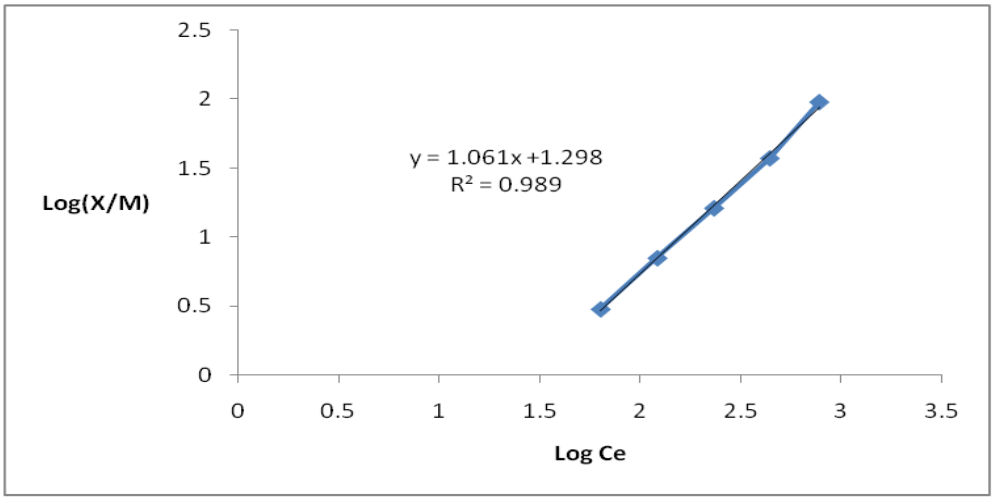

Figure 4.11 : Freundlich isotherm for the adsorption of Cr(VI) on a chitosan

$1/n = 1.061$ $K_f = 19.98$ mg/g $R^2 = 0.989$

The correlation coefficient ($R^2 = 0.984$) , suggesting that the freundlich model provides an good fit for the adsorption behavior in this system

4.3.2.4 Comparison of Isotherms

From the calculations, Qmax from the Langmuir isotherm model was found to be higher than K_f from the Freundlich isotherm model. The freundlich isotherm has a significantly higher R2 value (0.989) compared to the Langmuir isotherm (0.97) . This suggests that the freundlich isotherm is a much better fit in this data

4.3.2.5 Comparison with Other Adsorbents

Table 4.5: Comparison of Adsorp tion Capacities

Adsorbent	Qmax (mg/g)	references	
Tea waste	34.25	Dave P., Pandecy N and Thomas .H (2012	
Distillery	5.7	Selvaraj K., Manonmani S., and Pattabhi S (2003)	
Chitosan	142.8	This work	

To further validate the efficacy of chitosan, Q_{max} of chitosan was compared with Q_{max} values of other commonly used adsorbents reported in the literature:

This comparison highlights that chitosan exhibits a higher adsorption capacity for Cr(VI) compared to tea waste and distillery sludge, making it a superior adsorbent for chromium removal.

In this study, the treatment of chitosan for chromium (VI) ion removal involved leveraging its natural properties of protonation in an acidic environment, where the amino groups on chitosan become positively charged and thus enhance electrostatic interactions with negatively charged chromium (VI) ions. This electrostatic attraction is a well-established mechanism in chitosan's use as an adsorbent for heavy metals. The high deacetylation degree (DDA%) of the chitosan sample, as determined in this study, also contributed to the increased availability of active amine groups, resulting in a more efficient adsorption process.

Comparing this with previous studies, similar approaches to chitosan treatment have been observed, where acidic conditions were favored for effective adsorption. For instance, Desai et al (2023) and Chen et al. (2024) reported that chitosan's adsorption capacity improves significantly in low pH environments due to the increased protonation of amine groups. However, some studies introduced modifications to enhance adsorption capacity further. For example, Kluczka (2023) chemically modified chitosan by cross-linking or grafting to improve its structural stability and adsorption performance.

Unlike these modified approaches, the present study relied on the natural properties of unmodified chitosan and still achieved effective removal of chromium (VI). This suggests that while chemical modifications and composites can enhance adsorption performance, the high DDA and optimization of contact time and pH alone, as in this study, can lead to a highly efficient adsorption process. This highlights the practicality of using simpler, naturally derived chitosan for environmental applications without requiring extensive modifications.

The removal of Cr(VI) from aqueous solutions was greatly aided by the chitosan made from Palestinian fish scales. It was discovered that 4 was the ideal pH for chromium removal, at which time Cr(VI) could be completely removed. There are two reasons for this high removal efficiency: reduction and adsorption processes. The investigation into the effects of chromium concentration and chitosan dosage revealed that total chromium removal efficiency decreased with increasing chromium concentration andthe best time for removal was at the 90th minute, as it recorded the highest chromium removal rate. , yet remained relatively high overall. Furthermore, increasing the chitosan dose improved the removal efficiency, with the highest removal observed at a dose of 0.5 g for a chromium concentration of 20 ppm.

With a Qmax value of 142.8 mg/g, which indicates a high adsorption capacity, the freundlich model provided the best description of the adsorption process. Compared to other adsorbents like tea waste and distillery sludge , which usually have Qmax values 34.25 mg/g and 5.7 mg/g respectively, this value is much greater. The chitosan generated in this work appears to be very beneficial for environmental remediation applications based on its high adsorption capability.

4.3Implications for Environmental and Biomedical Applications

Numerous uses are made possible by chitosan's dual functionality in both antibacterial activity and Cr(VI) elimination. Because of its strong adsorption capability, Cr(VI) is a good choice for treating industrial polluted water in environmental settings. The Freundlich isotherm model's description of heterogeneous multilayer adsorption implies that chitosan can successfully bring chromium concentrations down to levels that are safe.

Chitosan's antibacterial qualities can be used in biomedical applications to create products such as medical device coatings, wound dressings, and other items where infection control is essential. Its efficacy against the prevalent bacterium Staphylococcus aureus underscores its potential in healthcare environments.

4.4 Sustainability and Environmental Impact

Using fish scales, a byproduct of fishing, is a good way to support sustainable practices. Waste materials are transformed into useful biopolymers, which lowers pollution to the environment and promotes resource efficiency. Fish scale chitosan extraction is a more environmentally friendly method of obtaining the material than other traditional sources.

Chapter 5

5.1Conclusion

The study proved that chitosan made from Palestinian fish scales is useful for biomedical and environmental purposes. This biopolymer's quality and potential are highlighted by its high degree of deacetylation, good FTIR properties, and outstanding adsorption capacity for Cr(VI). Its application to the medical field is further broadened by its antimicrobial properties. This method of obtaining chitosan is both economically and sustainably viable, which benefits the fishing sector and aids in environmental preservation. Subsequent investigations ought to delve into the feasibility of expanding the extraction procedure and the creation of particular uses in the domains of the environment and medicine. Future research could explore the synergistic effects of combining chitosan with other biopolymers or nanoparticles to enhance its functional properties, further expanding its application scope in the biomedical field.

5.2 Recommendations

Additional studies could be conducted to investigate methods for improving the efficacy of chitosan extracted from Palestinian fish scales, focusing on purification processes or modifying its physical and chemical properties.Given the promising results of chitosan from fish scales, other marine sources in Palestine, such as shrimp shells or crab exoskeletons, could be explored to diversify and expand the applications of chitosan. Additional research could be undertaken to explore the use of chitosan in treating other environmental pollutants, such as other heavy metals and organic contaminants, to assess its effectiveness as a versatile cleaning agent.further research could focus on improving or modifying the antibacterial properties of chitosan to enhance its activity against a broader range of pathogens.

5.3 References

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Appendices

A 1= Calibration curve by UV-Vis spectrophotometer

Concentration (ppm)	**Absorbance**	$R^2 = 0.997$
7.8	0.1069	
10	0.1132	
15.6	0.1629	
20	0.1754	
50	0.4351	
80	0.7211	
125	1.127	

A2 = Calibration curves by Atomic absorption

Concentration(PPM)	**Absorbance**	$R^2 = 0.990$
0.625	0.0076	
1.25	0.0153	
2.5	0.0273	
5	0.0524	
10	0.0927	
20	0.1563	

A 3 : Effect of pH on Chromium Removal Efficiency

%adsorbtion removal	%reduction removal	%total removal	Concentration (ppm) Cr+3	concentration Cr+6 adsorbed (ppm)	Concentration (ppm) Cr+6 in solution	total Cr(ppm)	Initial concentration (ppm)	PH
56.26	11.18	67.44	1.11	5.62	3.25	4.37	10	2
87.23	12.76	100	1.27	8.72	0	1.27	10	4
33.81	36.18	70	3.61	3.38	3	6.61	10	6
40.00	5.65	45.66	0.56	4.00	5.43	5.99	10	8

A 4 : Effect of Chromium Concentration on Removal Efficiency

%reduction removal	%adsorbtion removal	%total removal	Concentration Cr+6 adsorbed (ppm)	Concentration Cr+3	Concentration Cr+6 in solution (ppm)	(toal Cr)	Initial concentration (ppm)
18.18	81.81	100	6.39	1.42	0	1.42	7.8
28.70	52.00	80.71	8.12	4.48	3.01	7.49	15.6
45.72	31.66	77.38	9.89	14.28	7.06	21.35	31.2
40.42	33.57	74.00	20.98	25.26	16.24	41.51	62.5
44.86	25.22	70.08	31.52	56.08	37.38	93.47	125
40.36	21.02	61.39	52.56	100.92	65.51	197.43	250

A 5 : Effect of Chitosan Quantity on Chromium Removal Efficiency

%total removal	% reduction removal	%adsorbtion removal	Cr +3	Cr+6 adsorbed	Cr +6 in solution	total Cr (ppm)	Dose (g)	Initial concentration (ppm)
76.09	16.88	59.20	3.37	11.84	4.78	8.15	0.1	20
79.44	18.37	61.07	3.67	12.21	4.11	7.78	0.2	20
82.38	22.81	59.57	4.56	11.91	3.52	8.08	0.3	20
84.38	25.77	58.60	5.15	11.72	3.12	8.27	0.4	20
91.29	33.18	58.10	6.63	11.62	1.74	8.37	0.5	20

A6 : The kinetic model of Cr(VI) adsorption by chitosan first order

Time(second)	**log(qe-qt)**
0	1.13
600	1.10
1200	1.02
1800	0.99

A7: Effect of Contact time on Chromium Removal efficiency

%total removal	% redu remov	%adsor removal	Cr +3	Cr+6 adsorbed	Cr +6 in solution	total Cr ppm	Time min	Initial conce
5.43	2.96	2.47	0.59	0.49	18.91	19.50	10	20
14.93	6.51	8.41	1.30	1.68	17.01	18.31	20	20
18.88	8.14	10.73	1.62	2.14	16.22	17.85	30	20
42.43	9.62	32.80	1.92	6.56	11.51	13.43	40	20
65.38	12.44	52.93	2.48	10.58	6.92	9.41	60	20
67.34	14.07	53.27	2.81	10.65	6.53	9.34	90	20
65.43	13.48	51.95	2.69	10.39	6.91	9.60	120	20

استخلاص وتوصيف واستخدامات الكايتوسان من قشور الاسماك الفلسطينية

اعداد : حمزة وائل اسماعيل البطمة
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الملخص

تهدف الدراسة على استخلاص وتوصيف الكيتوسان وخصائصه المضادة للبكتيريا وكفاءته في إزالة الكروم السداسي من المياه الملوثة .تم استخلاص الكيتوسان من قشور أسماك فلسطينية باستخدام طريقة من ثلاث خطوات تشمل إزالة المعادن وإزالة البروتين وإزالة الأسيتيل. تم تأكيد نجاح الاستخلاص ونقاء الكيتوسان باستخدام مطياف الأشعة تحت الحمراء (FTIR). تم اختبار النشاط المضاد للبكتيريا للكيتوسان المستخلص ضد ثلاث بكتيريا ممرضة: *المكورات العنقودية الذهبية والكلبسيلة الرئوية والإشريكية القولونية*. أظهرت النتائج خصائص مضادة للبكتيريا كبيرة ضد المكورات العنقودية الذهبية، مع أعلى منطقة تثبيط لوحظت عند تركيز 0.0075جم / 100مل. ومع ذلك، لم يلاحظ أي نشاط مضاد للبكتيريا مهم ضد الكلبسيلة الرئوية والإشريكية القولونية.تم التحقيق بدقة في فعالية الكيتوسان في معالجة الكروم السداسي (Cr (VI)) في مياه الصرف الصحي باستخدام الامتصاص الذري و مطياف الأشعة فوق البنفسجية. وجدت الدراسة أن الكيتوسان فعال للغاية في إزالة الكروم السداسي، حيث تم تحديد الرقم الهيدروجيني الأمثل للإزالة عند درجة الحموضة 4. عند هذا الرقم الهيدروجيني، تم تقليل الكروم السداسي المتبقي إلى الصفر، مما حقق معدل إزالة بنسبة 100٪. وكان أفضل وقت للإزالة عند الدقيقة التسعين، حيث سجل أعلى معدل إزالة للكروم. لوحظ أعلى إزالة عند جرعة 0.5جم لتركيز الكروم 20جزء في المليون. تم تحليل سلوك امتصاص الكيتوسان باستخدام نظائر الامتصاص، حيث تتوافق البيانات جيدًا مع نموذج فريندلش الذي يشير إلى امتصاص متعدد الطبقات لأيونات الكروم على سطح الكيتوسان. وتم تحليل النموذج الحركي وكانت النتائج متوافقة مع نموذج الرتبة الاولى الكاذب

تسلط هذه الأطروحة الضوء على إمكانية استخدام الكيتوسان المشتق من قشور الأسماك الفلسطينية كبوليمر حيوي متعدد الوظائف، مما يساهم في استغلال النفايات ويوفر حلولاً عملية للتعامل مع البيئة والعلاجات المضادة للبكتيريا