

BIO-SORPTION STUDIES OF CHROMIUM (VI) IONS WITH MODIFIED CHICKEN FEATHERS

By

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fulfillment of the requirements for the degree of
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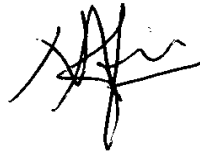
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Declaration

It is hereby declared that

1. The thesis submitted is my own original work while completing degree at BRAC University.
2. The thesis does not contain material previously published or written by a third party, except where this is appropriately cited through full and accurate referencing.
3. The thesis does not contain material which has been accepted, or submitted, for any other degree or diploma at a university or other institution.
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Abstract

Adsorption is one of the most important methods for cleaning industrial waste effluents. Adsorbents such as activated carbon have been used for a long time to remove heavy metal ions from wastewater. However, such adsorbents' high capital and regeneration costs have motivated researchers to develop new low-cost adsorbents. Biosorption is an emerging technology for the removal of heavy metals from industrial wastewater. The feasibility of using alkali-treated (0.5M NaOH) chicken feathers and raw chicken feathers (untreated), a waste from poultry as an adsorbent for chromium contaminated wastewater was studied. The batch adsorption tests were shown to be influenced by the concentration of the chromium solution, reaction temperature, solution pH, adsorbent dosage limit, contact time, and pre-treatment with sodium hydroxide. The parameters such as contact time, adsorbent dose, pH, and temperature were optimized and were found to be 3-5 hrs, 2-2.5 gms, pH \pm 2.0, and temperature 25°C, respectively. T-tests were conducted to determine the significant differences between the batch parameters to establish optimized conditions for adsorbent efficiency.

Keywords: Adsorbance; Chicken Feathers; Dosage; Concentration; pH; Temperature

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List of Acronyms

AAS	Atomic Absorption Spectrophotometer
CFs	Chicken Feathers
DO	Dissolved Oxygen
DOC	Dissolved Organic Carbon
EPA	Environmental Protection Agency
FDA	Food and Drug Administration
HCl	Hydrochloric Acid
HNO ₃	Nitric Acid
IARC	International Agency for Research on Cancer
MWCO	Molecular Weight Cut-Off
NRC	Nuclear Regulatory Commission
PPM	Parts Per Million
SCW	Skin-Cut-Waste
UV-VIS	Ultraviolet Visible

Chapter 1

Introduction

1.1 Background

Industries like leather, textile, etc. play a vital role in the manufacturing of basic needs. While this spells economic development; however, our prime concern is the wastewater generated by these tannery industries. Wastewater from tanneries, textile industries electroplating, etc. contains chromium and assorted heavy metals (Alemu et al., 2018; Ali et al., 2016; Anah & Astrini, 2017; Ansar & Durham, 2019).

Heavy metals are defined as metallic elements present in the human body (Rakhunde, Deshpande & Juneja, 2012). Heavy metals are vital for the proper functioning of biological systems and constitute essential nutrients for humans, and their deficiency or excess could lead to several disorders (Unceta et al., 2010). For instance, iron is a vital component of hemoglobin and cytochrome c; zinc is crucial for certain DNA-binding proteins; and selenium, manganese, copper, and zinc in trace amounts are essential for the proper performance of enzymes. Additionally, zinc and selenium ensure the boosting of the immune system and increase its functionality (Ansar & Durham, 2019). Arsenic is essential to promote growth and acts as a co-cidiostat (Ansar & Durham, 2019). Yet, excess amounts of these essential metals can have a deteriorating effect on human wellbeing as well as on the environment. A study conducted in India revealed that the permissible limits for cadmium, chromium, and copper were exceeded due to effluent discharge from textile and tannery factories (Ansar & Durham, 2019). These effluents released onto the land and discharged into the environmental water ultimately leach into groundwater. Strong evidence suggested contamination due to the accumulation of these undegradable toxic metallic components results in a series of hazards to the ecosystems (Ansar & Durham, 2019; Banu et al., 2013; Cheng, 2014).

A fairly high amount of chromium is released into the biosphere due to electroplating and leather industries. It can be said that the electroplating and leather industries are major dispensers of chromium. This huge quantity of chromium salts discharged as tannery waste has raised several ecological concerns (Ali et al., 2016; Dong et al., 2017; Zahid et al., 2006; Zahir & Ahmeduzzaman, 2012). Chromium occurs in the environment in various forms under various physio-chemical and morphological conditions. Based on these settings, Cr can display oxidation states from 0 to 6 in the environment. Among those oxidation states, only Cr(III) and Cr(VI) are stable (Asfaw, Tadesse & Ewnetie, 2017). The total mean concentration of chromium ranges from 7 to 150 PPM (Asfaw, Tadesse & Ewnetie, 2017). However, its content and circulation within the soil are contingent on the type of the soil's mother rock. The presence of additional amounts of Cr in the soil is produced by human activity. Chromium can exist as Cr(III) cation only in a strongly acidic and reducing medium, while Cr(VI) exists in a strongly basic and oxidizing medium as CrO_4^{2-} anion (Asfaw, Tadesse & Ewnetie, 2017; Soares et al., 2009).

There are numerous physical and compound techniques available for the removal of substantial metals including chromium from water. Adsorption utilizing activated carbon can expel chromium from wastewater. There have been extensive studies detailing the adsorption method of activated carbon in effluent treatment processes (Mohan, Singh & Singh, 2006; Paul, Nwoken & Anumonye, 2018; Song et al., 2016). Furthermore, other conventional approaches involve chemical precipitation and filtration, chemical oxidation, electrochemical treatment, reverse osmosis, evaporation, ion exchange, and sorption (Ince & Ince, 2019). However, most available methods may show economical and technical disadvantages such as high capital and operational costs, high sensitivity to operational conditions, significant energy consumption, or sludge generation (Ince & Ince, 2019). Based on these factors, it is necessary to develop effective, low-cost, and environment-friendly approaches for water treatment. The present

study is aimed at the selection of a low-cost bio-sorbent, which can adsorb chromium from the wastewater. The present investigation has carried out detailed batch studies with the selected adsorbent, chicken feathers. The effect of pH, contact time, adsorbent concentration, temperature, and absorbance rate were also investigated.

1.2 Importance of this Research

Currently, various methods including coagulation and precipitation, membrane filtration, advanced oxidation, adsorption, biological process, and photocatalytic degradation have been extensively applied for the treatment of dye-bearing wastewater. The treatment processes are usually chosen based on composition, characteristics, and concentration of material present in the effluents. Among these processes, adsorption ranks as one of the most effective methods. Its application follows a simple design, ease of operation and guarantees relatively high efficiency with the use of, for instance, activated carbon as an adsorbent. However, it is quite expensive and hence, its use is limited. For a long time now, researchers have been focusing on using relatively cheaper adsorbents to replace activated carbon. This is where the scientific usage of feathers as a renewable material offers both economic and environmental benefits. Development and utilization of low-cost and effective adsorbents such as the use of chicken feathers for the adsorption processes is a research focus in recent years. (Ince & Ince, 2019; Mohan, Singh & Singh, 2006; Paul, Nwoken & Anumonye, 2018)

1.3 Scope and Limitation of the Study

1.3.1 Scope of the study:

The study has been carried out at the BRAC MNS Department Labs, Dhaka. The study was carried out between March to October 2019. Post-lockdown, additional calibration readings were taken on 17th November 2020.

Some of the following limitations incurred during and after the completion of the study:

- 1 The study could have been better validated with the use of an Atomic Absorption Spectrophotometer (AAS). Only GENESYS 10S UV-Visible Spectrophotometer was available to detect the levels of heavy metal contamination.
- 2 Activated Carbon was not used for the comparative batch experiments due to it being expensive for the experiment.
- 3 The experiment and its subsequent thesis writing experienced delays due to the ongoing pandemic.
- 4 Due to the ongoing pandemic, the batch experiments were not duplicated using actual chromium-contaminated wastewater.
- 5 Due to the pandemic and restrictions in movement, further batch experiments using native chicken feathers could not be conducted.
- 6 Due to the pandemic and restrictions in movement, extensive batch experiments using extracted keratin protein solutions could not be conducted.

Chapter 2

Literature Review

2.1 Chromium Speciation

Chromium (Cr) is extensively used in the chemical industry for different applications such as dyeing, metal plating, or leather tanning and in chemical productions such as chemical synthesis and as catalysts (Nur-E-Alam et al., 2020). As a result, different species of chromium can be released into the environment (soil, surface, and groundwaters) and is then available to humans. Chromium can exist in various chemical forms between 0 and VI speciation state. Although, only trivalent and hexavalent chromium are stable enough to exist in the environment (Soares et al., 2009). Cr(III) is considered a necessary micronutrient in the human diet and is largely used as a nutritional supplement for humans and animals (Unceta et al., 2010). Nevertheless, it has been demonstrated that Cr(III) is capable of eliciting eczema at low concentrations (Nagaraj et al., 2008; Nur-E-Alam et al., 2020) and of causing DNA damage in cell-culture systems (Onchoke et al., 2016).

Comparatively, Cr(VI) is much more hazardous than Cr(III) in terms of both acute and chronic exposure. Its compounds are regulated through the Dangerous Substances Directive (67/548/EEC) (Unceta et al., 2010). It is suspected of being extremely hazardous after inhalation and oral exposure with deleterious effects on the respiratory tract, liver, kidney, gastrointestinal and immune systems. Furthermore, blood and dermal exposure may cause contact dermatitis, sensitivity, and ulceration of the skin (Soares et al., 2009; Unceta et al., 2010). Cr(VI) has been recognized as a highly toxic species and categorized as a human carcinogen by the US EPA (Environmental Protection Agency) and as a class I human carcinogen by the IARC (International Agency for Research on Cancer) (Unceta et al., 2010). The toxicological difference between Cr(III) and Cr(VI) is closely related to the chemical

properties of each species, which also determines the stability, mobility, and bioavailability of these species in the environment. The high redox potential of the Cr (VI)/Cr(III) couple supports Cr(III) stabilization in an acidic medium. On the other hand, under alkaline conditions the redox potential decreases, which suggests the stabilization of Cr(VI) under such conditions. (Gao et al., 2014; Kumar, Kakan & Rajesh, 2013)

Thermodynamically, Cr(VI) could only exist at relatively high pH. Also, Cr(III) can exist in numerous forms as a function of its pH. At pH between 0 and 4, trivalent chromium tends to develop hexacoordinate complexes with other complexing agents such as water, ammonia, sulfate, urea, and organic acids (Gao et al., 2014). Within pH 4–6, Cr(III) tends to form hydrolysis products simply abbreviated as $\text{Cr}(\text{OH})^{2+}$, $\text{Cr}(\text{OH})_2^+$, and $\text{Cr}(\text{OH})_3^\circ$. At pH higher than 6, Cr(III) precipitates to form $\text{Cr}(\text{OH})_3(\text{s})$ which is a stable species and one of the major forms of Cr(III) in the environment. Above pH 9 this precipitate is converted into the soluble $\text{Cr}(\text{OH})_4^-$ complex. Because of the high E° value of the Cr(VI)/Cr(III) redox couple, the oxidation of Cr(III) to Cr (VI) by natural oxidants is not typical, only manganese oxide appears to be an effective oxidant in the environment (Osasona et al., 2015; Soares et al., 2009; Unceta et al., 2010). Contingent on factors, specifically pH and their concentrations, chemical types of Cr(VI) range from chromate (CrO_4^{2-}) (pH 6.5–14) through hydrogen chromate (HCrO_4^-) and dichromate ($\text{Cr}_2\text{O}_7^{2-}$) (pH 0.7–6.5) to chromic acid (H_2CrO_4) (pH<0.7) (Unceta et al., 2010). HCrO_4^- and CrO_4^{2-} can be easily reduced to Cr(III) by different reducing agents, for example, Fe(III), phosphate, sulfide, and organic matter, e.g. humic acid (Soares et al., 2009; Unceta et al., 2010).

2.2 Characterization of the Adsorbent

Adsorption is one of the most significant approaches for cleaning industrial waste effluents. Adsorbents such as activated carbon have been used for a long time to remove heavy metal

ions from wastewater (Paul, Nwoken & Anumonye, 2018). However, such adsorbents' high capital and regeneration costs have motivated researchers to develop new low-cost adsorbents. Biosorption is an emerging technology for the removal of heavy metals from industrial wastewater (Cheng, 2014; Garcia-Sabido, Lopez-Mesas & Cavillo-Navarrete, 2014; Ince & Ince, 2019). Some biosorption materials show excellent sorption capacities of Cr from water (Sarin & Pant, 2006). Most Cr(VI)-ion sorbents operate at low pH values because the groups on the surface of the sorbents hydrolyze water molecules and form positively charged groups to attract Cr(VI) anions through electrostatic attraction. Amino groups are able to hydrolyze easily and consequently form positively charged groups at relatively higher pH values. Therefore, materials with more amino groups might be excellent in the adsorption of Cr(VI) under mild conditions (Genawi et al., 2020; Sharma et al., 2017; Song et al., 2016).

Keratin is a natural protein that has its source in animal hair, feathers, nails, hooves, beaks, claws, and horns. The keratin protein is a stable type of protein that cannot be dissolved in any organic or inorganic solvents. Also, it cannot be cleaved by protease enzymes such as trypsin or pepsin (Saucedo-Rivalcoba et al., 2010). The stable structural morphology of the keratin protein makes it an extremely versatile protein (Genawi et al., 2020). The keratin protein can be engineered into any bioproduct including bio-films, scaffolds, sponges, nanoparticles, and hydrogels. They can self-construct themselves to form a stable structure or morphology. (Schrooyen et al., 2001)

Presently, the keratin fiber extracted from chicken feathers is documented as an almost infinite source of high-performance materials (David & Leonor, 1934; David & Leonor, 1935; Schrooyen et al., 2001; Song et al., 2016). The chicken feathers possess 91% of keratin protein (David & Leonor, 1934; David & Leonor, 1935). The chicken feather discards are generated into the environment in tonnes. These feathers are dumped in the lands cause environmental issues such as the production of methane or greenhouse gases and pollution of the groundwater

sources (Gupta et al., 2012; Kamarudin et al., 2017; Schrooyen et al., 2001). It needs some effective management system. Studies show that keratin proteins, found copiously in bird feathers, sheep's wool, and human hair, are a fascinating and potentially advantageous renewable biopolymer. It consists of a variety of functional groups on the backbone and side chains of the proteins. It is considered an ideal component to fabricate a range of novel adsorbent systems for the separation of toxic pollutants via physisorption or chemisorption (Na Ayutthaya & Woothikanokkhan, 2013).

CFs constitute a fibrous proteinaceous material with a complicated structure showing a large surface area (Gupta et al., 2012; Na Ayutthaya & Woothikanokkhan, 2013; Nur-E-Alam et al., 2019). CFs are a potentially excellent adsorbent. In previous investigations, CFs were used to adsorb dyes, (Gupta et al., 2012; Na Ayutthaya & Woothikanokkhan, 2013) and the results suggested that chicken feathers are excellent bio-sorbents for the removal of dyes. CFs have also been used for the removal of heavy metals such as As(III)²³ and Zn(II), Cu(II), and Ni(II) from wastewater (Garcia-Sabido, Lopez-Mesas & Cavillo-Navarrete, 2014; Nur-E-Alam et al., 2019). The results show that chemically treated chicken feathers have larger sorption capacities than do untreated CFs (raw chicken feathers) (Beak, Ijagbemi & Kim, 2009; Garcia-Sabido, Lopez-Mesas & Cavillo-Navarrete, 2014; Gupta et al., 2012). However, in the previous studies, the changes in the structure and properties of the CFs were not clarified, and only bases were used to treat the CFs. Thus, the explanation of the changes in structure and property of CFs treated with NaOH is necessary, and treatment with chemicals having different groups might create additional functions for the raw CFs, such as hydrophilic and chelation properties. As previously mentioned, amino groups can form positively charged groups at relatively higher pH values; therefore, adding amino groups to the surface of sorbents can increase their sorption capacities for eliminating Cr(VI) ion from water (Beak, Ijagbemi & Kim, 2009; Gupta et al., 2012; Na Ayutthaya & Woothikanokkhan, 2013).

2.3 Extent of Chromium contamination in Bangladesh

Feeds and fertilizer production obtained from tanned skin-cut wastes is the most direct occurrence of chromium eco-toxicity leading to food chain contamination in Bangladesh (Nur-E-Alam et al., 2020; Khan et al., 2020). Tanning industries of Hazaribagh at Dhaka process some 220 tons of hide per day with the release of 600-1000 Kg tanned skin-cut waste (SCW) per ton processed hide (Hashem et al., 2015; Nur-E-Alam et al., 2020; Khan et al., 2020). The SCW is protein-rich and unscientifically utilized to produce poultry and fish feeds, and organic fertilizer (Khan et al., 2020; Sayed, Saleh & Hasan, 2005). Accumulation of chromium can happen within poultry products, fish, and vegetables, and further bio-magnify into the food chain. The target population is large. It is reported that feed ingredients produced from SCW contained chromium at 2.49%, which is quite high (Khan et al., 2020). As Cr(VI) is the most hazardous species of chromium; therefore, reduction of Cr(VI) to Cr(III) before being utilized as feed ingredients and fertilizers can safeguard the input of chromium from a toxicological point of view (Khan et al., 2020; Sayed, Saleh & Hasan, 2005).

In leather processing, tanning agents are used for the stabilization of the skins, and in most cases, basic chromium sulfate ($\text{Cr}_2(\text{SO}_4)_3$) is applied. The skins and the tanning agents are reacted in an aqueous solution and this process results in high chromium (Cr) concentrations in the wastewater. In the Hazaribagh area of Dhaka city, the capital of Bangladesh, tanneries started to operate in the 1960s (Anawar, Safiullah & Yoshioka, 2000) and during peak time up to 150 tanneries were operated (Khan et al., 2020; Zahid et al., 2006). Most of the tanneries used outdated and inefficient processing methods and lacked effluent control and wastewater treatment (Zahid et al., 2006; Zahir & Ahmeduzzaman; 2012). According to a study by Khan et al. (2020), the tanneries discharged an estimated daily average of 75 tons of solid waste and 21,600 m³ of environmentally hazardous liquid waste. It should be noted though the discharge amount varied widely between peak and off-peak production periods (Khan et al., 2020). This

discharge went directly to surrounding low-lying areas, the municipal drainage system, a natural canal, and an adjacent lagoon, which originally was a ditch and subsequently filled up with tannery waste during the last decades, and finally into the nearby Buriganga river (Saha & Ali, 2001; Sharmin, Miah & Zakir, 2020).

Several studies have investigated the environmental impact of the Cr waste discharged by the Hazaribagh tanneries (Anawar, Safiullah & Yoshioka, 2000). In the environment, Cr mainly occurs in two oxidation states, the trivalent Cr(III) and the hexavalent Cr(VI), with Cr(III) being the less toxic form (Unceta et al., 2010). Khan et al. (2020) noted that due to the specific hydrochemical conditions in the study area, Cr(III) seems to be the dominant species (Khan et al., 2020), and typically total chromium (total Cr) concentrations are reported in related studies (Hashem et al., 2015; Zahid et al., 2006). Maximum total Cr concentrations in the topsoil of the contaminated sites reached up to 28,500 mg/kg, and even at a depth of 10 m, total Cr concentrations of about 2,000 mg/kg were found in the filled-up lagoon soil (Karim et al., 2013). Total Cr concentrations in the Buriganga river sediments ranged from 100 mg/kg to more than 4,000 mg/kg. Total Cr concentrations in the Buriganga river water were reported to range from 0.001 to 2.66 mg/l, depending on the season, sampling location, and sampling time. (Karim et al., 2013)

Another similar study conducted by Anawar, Safiullah and Yoshioka (2000) examined the surface and subterranean waters of Hazaribagh industrial area, the western part of Dhaka city of Bangladesh (Karim et al., 2013). They found the area to be heavily contaminated with chromium, inorganic and organic matters. In this study, exposure assessment of chromium, pH value, alkalinity, calcium, and dissolved organic carbon in tannery effluents, surface water, and groundwater has been done (Anawar, Safiullah & Yoshioka, 2000; Karim et al., 2013). They collected samples from ten different sites in two seasons (spring and winter) according to a well-planned monitoring scheme that represented the entire area and an instant analysis was

done. The pH values of wastewater flowing through main outflows and groundwater varied from 8.90 to 11.00 which is higher than the acceptable limit proposed by the Bangladesh drinking water standard (Karim et al., 2013; Saha & Ali, 2001), very harmful for aquatic animals and unfavorable for microbiological growth for waste treatment. The calcium content of the samples (560.0-1600.0 PPM) was also very high to disrupt the sewerage system by deposition of calcium carbonate inside it (Anawar, Safiullah & Yoshioka, 2000). The high content of dissolved organic carbon (DOC) from 338.98 to 361.13 PPM was depleting the dissolved oxygen (DO) in the river Buriganga making the water ecosystem uninhabitable for fish (Anawar, Safiullah & Yoshioka, 2000). The chromium content of the chrome tan liquor and wastewater ranged from 5.19 to 72.80 PPM (Karim et al., 2013). This higher concentration can interfere with biological oxidation by tying up the enzymes essentially required for microbial growth and exceeding all of the national and international standards for safe disposal and drinking. The chromium concentration in surface water (5.19 PPM) on the low-lying lands discharged from main outflows showed identical value with that of groundwater (5.19 PPM) contributing to dietary intake (Karim et al., 2013). The daily intake of chromium, 377.45 µg/kg/d for the residents of that area, that was higher than maximum limits proposed by the USA, NRC (0.71-2.90 µg/kg/d) and FDA (120.00 µg /d), posed health concerns among the population and they were at risk of health hazards. (Ali, et al., 2016; Banu et al., 2013; Karim et al., 2013; Khan et al., 2020; Saha & Ali, 2001)

Chapter 3

Materials and Methodology

3.1 Materials needed: Activation of Chicken Feathers

1. Samples: Chicken feathers and wastewater from a dyeing industry
2. Sodium hydroxide solution
3. Detergent

3.2 Materials needed: Preparing a keratin solution

1. Sodium sulfide
2. Ammonium sulfate
3. Copper sulfate (for biuret test)
4. Potassium hydroxide (for biuret test)

3.3 Materials needed: Preparation of Standard Chromium Solution

1. Pure Potassium dichromate crystals
2. Sodium hydroxide
3. Hydrochloric acid

3.4 Equipment needed

1. Oven
2. Glass beakers, funnel, No 1 Whatman filter, biuret, pipette
3. UV-Visible Spectrophotometer; Thermo Fisher Scientific GENESYS 10S
4. Water bath
5. Incubator
6. Blender

7. Centrifuge
8. Hotplate with a magnetic stirrer

3.5 Methodology

3.5.1 Activation of Chicken feathers (CFs)

The procedure has been adapted from Gupta et al., (2012).

Pre-Treatment of the Chicken feathers (CFs): By thoroughly washing CFs with water and detergent to remove stains, oils, and grease before processing them. Later, drying under the sun or in the oven.

To determine the best possible way of cleaning synthetic wastewater, CFs will be used in two ways. One way would be to simply activate the CFs with NaOH and observe their efficiency against regular CFs (non-activated). The other way would be to dissolve the CFs and prepare a protein precipitate. This protein precipitate would be added to the synthetic wastewater.

Activation of the CFs: 10 gms of CFs are measured and kept aside. 4 gms of NaOH are measured and dissolved in 1 liter of water. The solution is then mixed with 10 gms of CFs, and stirred with a magnetic stirrer for 20 mins. After 20 mins, the solution is filtered. The activated CFs are dried in the oven. For the preparation of the adsorbent, again 1.5 gms of CFs are measured. The CFs are mixed with the wastewater sample. The mixture is stirred for 30 mins. Wait for 30 mins to see the results.

3.5.2 Preparation of Keratin Hydrolysate

The procedure has been adapted from Gupta et al., (2012).

Dissolving of CFs: 2L of 0.5M sodium sulfide solution is prepared in a 2L conical flask. 50g of the blended CFs are weighed and added to the sodium sulfide solution. The solution is heated to the temperature of 30 o C, pH is maintained between (10-13) pH and the solution is

continuously stirred for 6 hours. The solution is then filtered and centrifuged at 10,000 rpm for 5 minutes. The supernatant liquid is carefully collected and then filtered using filter paper to make it particle-free.

Preparation of ammonium sulfate solution: 700g of ammonium sulfate is dissolved in 1L deionized water. The solution is stirred until all the ammonium sulfate particles are dissolved. The solution is then filtered to make it particle-free.

Protein precipitation: The feather filtrate solution collected earlier would be placed in a beaker and stirred. Ammonium sulfate solution is added slowly dropwise. The ratio of feather filtrate solution and ammonium sulfate solution added is 1:1. The solution is then centrifuged at 10,000 rpm for 5 minutes and the solids particles are carefully collected. The supernatant liquids are collected separately and step 2 and 3 are repeated with it.

Protein purification: The solid particles collected are added into 100ml deionized water and stirred (washing). The solution is then centrifuged at 10,000 rpm for 5 minutes and the solids are gathered carefully. The collected solid particles are then dissolved in 100ml of 2M sodium hydroxide solution. The solution is then centrifuged again at 10,000 rpm for 5 minutes and all the liquids are collected carefully and stored while the solids are discarded. The precipitating, washing, and dissolving steps are repeated 3 times. The supernatant is preferably purified, using the dialysis method. A preferred method uses dialysis against stirred water using a dialysis membrane (Snakeskin® Dialysis Tubing, 10K MWCO, 16 mm) having a cutoff of about 10,000MW. The resulting solution is preferably concentrated to a concentration of about 0.1 gm/mL.

Biuret test to check the concentration of protein: 1% copper sulfate solution and 1% potassium hydroxide solution are prepared. The 5ml of the solution collected is mixed with potassium hydroxide solution with a 1:1 ratio. Three drops of copper sulfate solution are added

to the mixture solution. Changes in the solution is observed and recorded. The solution is analyzed under UV-VIS to obtain its absorbance.

3.5.3 Preparation of a standard chromium solution

Author's Calculations

A 1000 PPM standard chromium solution was prepared using chromium salt (Potassium dichromate; $K_2Cr_2O_7$).

Molecular wt. of chromium = 51.996

Molecular wt. of potassium dichromate = 294.185

The molecular formula of potassium dichromate is $K_2Cr_2O_7$

So, 103.992 Cr is present in 294.185 salt

1 Cr is present in $294.185/103.992 = 2.828$

For 1000 PPM it came out to be 2828.91 mg/L i.e., 2.828 g/L

Finally, 2.828 g was dissolved in 1 L water to get a 1000 PPM solution. After preparing a standard solution, 10 samples were taken from the standard solution.

As we know,

$C_1V_1 = C_2V_2$, and V_2 is fixed 50 ml flask volume.

For 10 PPM sample we got - $1000 \times V_1 = 10 \times 50$

$V_1 = 0.5$ ml

For 20 PPM sample we got - $1000 \times V_1 = 20 \times 50$

$V_1 = 1$ ml

For 30 PPM sample we got - $1000 \times V_1 = 10 \times 50$

$V_1 = 1.5 \text{ ml}$

For 40 PPM sample we got - $1000 \times V_1 = 10 \times 50$

$V_1 = 2 \text{ ml}$

For 50 PPM sample we got - $1000 \times V_1 = 10 \times 50$

$V_1 = 2.5 \text{ ml}$

For 60 PPM sample we got - $1000 \times V_1 = 10 \times 50$

$V_1 = 3 \text{ ml}$

For 70 PPM sample we got - $1000 \times V_1 = 10 \times 50$

$V_1 = 3.5 \text{ ml}$

For 80 PPM sample we got - $1000 \times V_1 = 10 \times 50$

$V_1 = 4 \text{ ml}$

For 90 PPM sample we got - $1000 \times V_1 = 10 \times 50$

$V_1 = 4.5 \text{ ml}$

For 100 PPM sample we got - $1000 \times V_1 = 10 \times 50$

$V_1 = 5 \text{ ml}$

After preparing the 10 samples, a UV-Visible spectrophotometer was used to take the readings. The readings were taken in triplicates. The absorbance acceptance criteria for standard chromium solution were collected from Test No. 101: UV-VIS Absorption Spectra, OECD Guidelines for the Testing of Chemicals, Section 1. The accepted wavelength is between (235-350 nm).

3.5.4 Preparation of stock solution of chromium ions for pH tests

The procedure has been adapted from Dong et al., (2011).

K₂Cr₂O₇ with H₂O and NaOH: The stock solution of (K₂Cr₂O₇) is prepared by dissolving (2.27 gm) of potassium dichromate in (150 ml) distilled water to get (0.05 M). The stock solution of (NaOH) has been prepared by dissolving (1 gm) (NaOH) in (50 ml) distilled water to get (0.5 M). The samples are prepared from up stock solutions, the first sample contains (20 ml) (K₂Cr₂O₇) with the value of pH (4.9). The second sample is prepared by adding (1 ml) (NaOH) to (20 ml) (K₂Cr₂O₇) to reach the value of pH (6.27). The third sample is prepared by adding (2 ml) (NaOH) to (20 ml) (K₂Cr₂O₇) to reach the value of pH (6.67). The fourth sample is prepared by adding (3ml) (NaOH)to (20 ml) (K₂Cr₂O₇) to reach the value of pH (7.13). And, so on and so forth was done to achieve a pH value of 10. All (pH) measurements is recorded by using (pH meter) Pocket Pro™+ Tester at (25 °C).

K₂Cr₂O₇with H₂O and HCl: The stock solution of (K₂Cr₂O₇) is prepared by dissolving (2.27 gm) of potassium dichromate in (150 ml) distilled water to get (0.05 M). The stock dilute solution of Hydrochloric acid is prepared by adding (0.5 ml) of (11.45 M) HCl to (150 ml) distilled water to get (0.038 M). The samples are prepared from up stock solutions, the first sample contains (20 ml) (K₂Cr₂O₇) value of pH (4.9). The second sample is prepared by adding (1 ml) (HCl) to (20 ml) (K₂Cr₂O₇) to reach the value of pH (3.4). The third sample is prepared by adding (2 ml) (HCl) to (20 ml) (K₂Cr₂O₇) to reach the value of pH (2.7). The fourth sample is prepared by adding (3 ml) (HCl) to (20 ml) K₂Cr₂O₇) to reach the value of pH (2.5). And, so on and so forth was done to achieve a pH value of 10. All (pH) measurements is recorded by using (pH meter) Pocket Pro™+ Tester at (25 °C).

3.5.5 Batch experiments for chicken feathers

Batch experiments were carried out in 100-mL beakers containing 70 PPM and 90 PPM of chromium solutions respectively. 2 g of chicken feather (dry biomass) and 70 PPM of 50 mL chromium solution were taken in each 100-mL conical flask. The desired pH of the respective solutions was maintained by adding 0.038 M HCl and/or 0.5 M NaOH. The contact time for each solution was maintained for 30 min. The temperature for all experiments was fixed at 25–26 °C. Adsorption experiments were conducted in different batches where the pH, adsorbent dose, contact time, initial chromium concentration, and temperature were changed. In these experiments, parametric ranges were done by changing pH from pH1 to pH10, adsorbent dose: 0.5 g to 4 g, contact time: 1–24 hours, initial chromium concentration, 50–100 PPM, and finally changes of temperature ranges from 25 to 55 °C. Influence of various operating parameters was studied by varying one parameter at a time and keeping the others constant. This is a serial adsorption process where the best removal of chromium for a parameter can be screened out and simultaneously fixed the value of that parameter following the next experiment. Then the chromium was analyzed by a UV-Visible spectrophotometer (GENESYS 10S).

3.5.6 Metal Analysis

The equation was adapted from Mondal, Basu & Das, (2019).

At the end of every step of adsorption, the remaining metal concentration was measured through the spectrophotometric method (Thermo Fisher Scientific GENESYS 10S). To estimate the percentage removal of chromium (VI) from an aqueous solution, the following equation was used.

$$\text{Percentage removal of Cr (VI)} = \frac{(C_{\text{initial}} - C_{\text{final}})}{C_{\text{initial}}} \times 100$$

where $C_{initial}$ and C_{final} are the concentrations of Cr(VI) at the beginning and at the end of the adsorption process.

3.5.7 Determination of pH_{ZPC}

The experiment was adapted from Mondal, Basu & Das, (2019).

The point of zero charge of the adsorbent (pH_{ZPC}) was determined by the solid addition method (Mondal, Basu & Das, 2019). A 50 mL of 0.1 M KNO_3 solution was transferred into a series of 100-mL conical flasks. The initial pH values of the solution were adjusted from 1.0 to 10.0 using HNO_3 (0.05, 0.1, and 0.5 N) or 0.1 N KOH. Then the fixed amount of chicken feather (2 g) was added to each 100 mL beaker and properly sealed. The flasks were then placed into a constant temperature water bath for 24 hrs. The pH values of the supernatant were noted after 24 hrs.

Chapter 4

Results and Discussions

This experiment yielded results based on multiple permutations performed using different concentrations of chromium (VI) ions, different amounts of chicken feathers, and setting up various artificial environmental factors. As discussed previously in the experimental plan section of the paper, a pure 1000 PPM standard chromium solution using potassium dichromate as salt was prepared. After preparing the standard solution, 10 samples from the standard solution were taken. A fixed volume of 50 mL was prepared for each concentration of chromium (VI) ions. Based on some initial experiments to determine which concentrations exhibited a clear difference in color change and absorbance rates; concentrations 70 PPM and 90 PPM were selected for the following experiments. The reason behind selecting the test sample concentrations of 70 PPM and 90 PPM was that any color change in the solution was noticeably visible. It might be argued that test samples of 50 PPM and 100 PPM would have been ideal concentrations to experiment upon. However, due to the former being a lower concentration and hence, any potential color change wasn't visible during batch experiments. Furthermore, batch experiments conducted on a 50 PPM chromium solution were prone to instrumentation errors more often than not. Also, batch experiments conducted using a chromium solution of 100 PPM gave similar results to batch experiments conducted using a 90 PPM chromium ion solution.

The following results displayed below offer knowledge about the various factors that played their significant role in decreasing chromium ions in water.

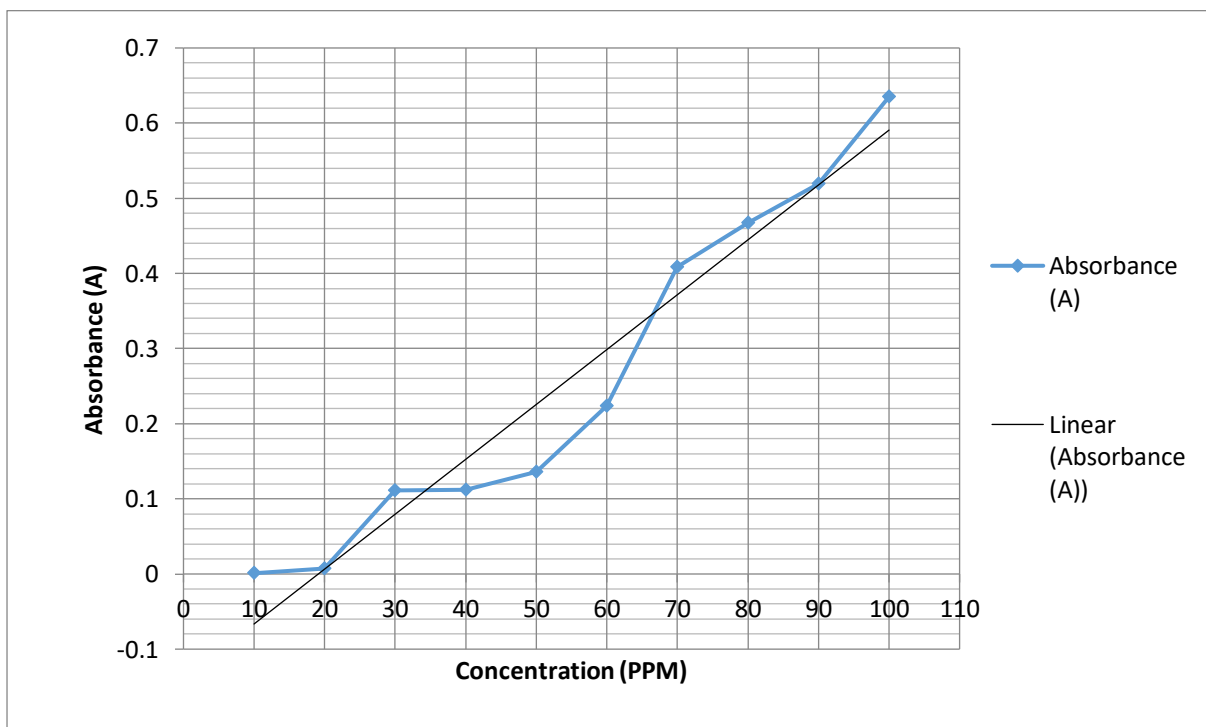
4.1 Standard curve or Calibration plot

A standard curve was plotted using a 1000 PPM chromium solution. The absorbance rates and the concentration of each 10 samples were measured in UV-Visible spectroscopy. The absorbance of each sample is presented in the following table.

Table 1: Standard curve plotted for Chromium (VI) ions using absorbance rates vs. concentration rates

Concentration (PPM)	Absorbance Rates (A)
10	0.001
20	0.007
30	0.111
40	0.112
50	0.136
60	0.224
70	0.409
80	0.467
90	0.519
100	0.635

Figure 1: Calibration plot based on Table 1



For experimental purposes, only 70 PPM and 90 PPM chromium test samples were selected. Due to the close absorbance values between (60-100) PPM, only concentrations 70 PPM and 90 PPM were selected for these experiments. This was done to identify any noticeable changes in the concentration of chromium in water when attempting to see the effect of adsorbent dosage, time, contact surface, pH, temperature, and movement. The values presented in the tables are average values calculated from triplicate readings.

4.2 Adsorbents Characterization

4.2.1. Analysis of pH_{ZPC}

The zero-point charge of chicken feathers showed 7.67. The charges on the surface of adsorbents depend on these pH_{ZPC} value below and above the pH_{ZPC} , the surface of the adsorbent changes to positive and negative charges, respectively. However, at the pH_{ZPC} point, the adsorbent surface has no charge at all (Mondal, Basu & Das, 2019). As per the literature, chromium species are present in different oxy-anionic forms such as CrO_4^{2-} , $HCrO_4^-$ or $Cr_2O_7^{2-}$ at acidic pH and the present experiment suggests that all the three adsorbents showed maximum Cr(VI) removal at acidic pH 1 and it is below the pH_{ZPC} value. Therefore, it can be suggested that Cr(VI) adsorption is favorable when the surface of the adsorbent is positive. An almost similar observation was reported by earlier researchers (Mondal, Basu & Das, 2019).

4.2.2. Spectrophotometric Study

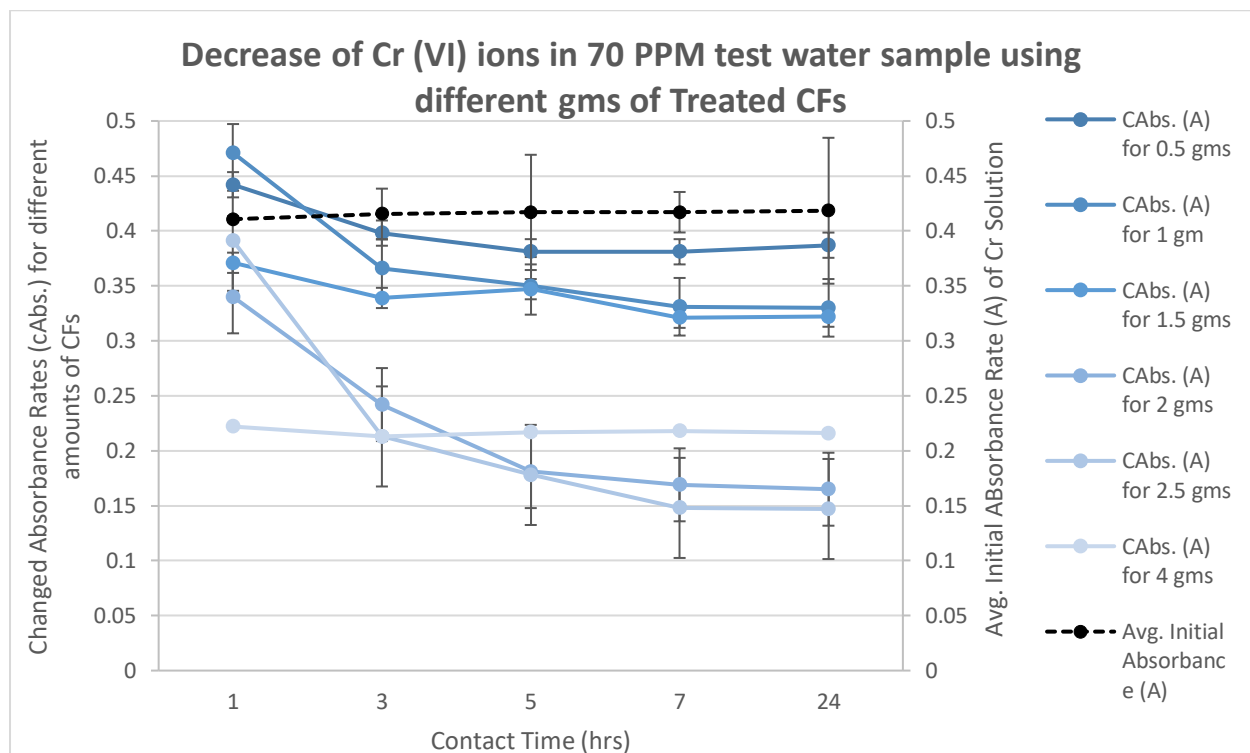
The following section covers the effects of using treated vs. untreated adsorbents, dosage, contact time, pH, and temperature on removing chromium ions from water. Before the adsorption of heavy metal using chicken feathers, the initial recorded values for 70 PM chromium solution were— ± 0.42 A (absorbance rate); 2.951 (pH). It should be noted that the batch experiments were conducted to determine the optimal conditions for the chicken feathers

to remove chromium from water. After a series of experimental permutations, it was determined that the removal worked best between temperatures, (25-45)°C. The subsequent analysis follows the results produced when the temperature was set at 25°C.

4.2.3. Effect of Adsorbent Dosage and Contact Time

- i. **Batch experiments using 70 PPM chromium solution:** In the first set of batch experiments, the effect of different amounts of chicken feathers (dosage limit) on a 70 PPM concentration chromium solution was observed (Figure 2). The following graph displays the relationship between the effect of adsorbent on the concentration of chromium ions in water.

Figure 2. Effect of Dose/Adsorbent (Treated Chicken feathers) on 70 PPM chromium solution.



Note:

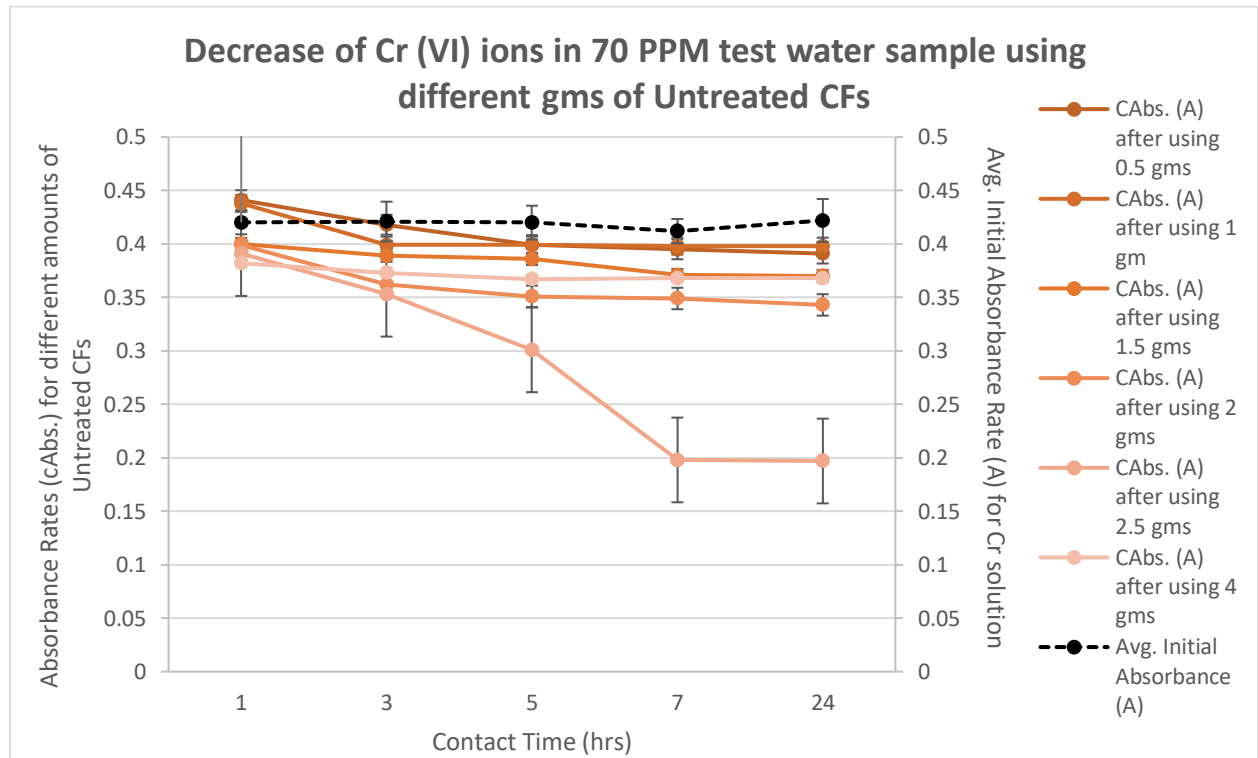
1. The average initial rate of absorption for a 70 PPM chromium solution is measured at ± 0.42 A.
2. The secondary axis plots the initial rate of absorption for a 70 PPM chromium solution.

The above graph displays how different combinations of the adsorbent (Treated chicken feathers) can affect the removal of chromium ions in water (Figure 2). Here, a concentration of

70 PPM chromium solution was taken. It is observed that as the quantity of the adsorbent is increased, the concentration of the solution also decreases. It is understood that the light absorbed by the solution decreases as the concentration within the solution decrease. Essentially, the rate of absorption decreases. It is noticed that after 3 hours the rate of absorption decreases considerably compared to the initial absorption rate. This occurs in each case when the quantity of the adsorbent is increased consecutively by 0.5 grams. However, it reaches a constant between 7 hours to 24 hours in each case when the quantity of the adsorbent is increased consecutively by 0.5 grams. This could mean that the effect of the adsorbent diminishes between 7 hours to 24 hours.

Furthermore, it is noticed that an exception to the rule occurs when 4 grams of adsorbent is added to the 70 PPM chromium solution. While the changed rate of absorption is still considerably lower than the initial rate of absorption; however, the change is quite constant even after the contact time is increased from 1 hour to 24 hours. This could mean that the amount of adsorbent used is quite higher for the volume of chromium solution used in this particular batch experiment. In theory, due to a higher quantity of adsorbent used, the concentration of chromium ions should have decreased drastically and rendered its concentration closer to that of water. However, in practice, it is seen that when using 4 grams of the adsorbent, the concentration of chromium ions decreases, and with that, the rate of absorption also decreases. It works quite well within the first hour of contact, after which the effect of the adsorbent diminishes and the effect of dosage becomes almost constant.

Figure 3. Effect of Dose/Adsorbent (Untreated Chicken feathers) on 70 PPM chromium solution.



Note:

1. The average initial rate of absorption for a 70 PPM chromium solution is measured at ± 0.42 A.
2. The secondary axis plots the initial rate of absorption for a 70 PPM chromium solution.

The above graph depicts the effect of different combinations of adsorbent (Untreated chicken feathers) have on the 70 PPM chromium ion solution (Figure 3). It is observed that as the quantity of the adsorbent is increased the concentration of the solution also decreases. This graph is quite similar to the previous graph. In this case, too, the rate of absorption decreases as the quantity of adsorbent is increased. Again, it is noticed that after 3 hours the rate of absorption decreases considerably compared to the initial absorption rate. This occurs in each case when the quantity of the adsorbent is increased consecutively by 0.5 grams. Again, it reaches a constant between 7 hours to 24 hours in each case when the quantity of the adsorbent is increased consecutively by 0.5 grams. However, compared to the decreasing changed rate of absorption for the Treated feathers adsorbent, the decrease in the rate of absorption for the untreated feathers is not as extreme. For example, when the contact time is 3 hours and 2 grams of untreated feathers are used, the rate of absorption is seen to be 0.362 A. Under similar

conditions, when Treated feathers are used as adsorbents, the rate of absorption is seen to be 0.242 A. This phenomenon is mimicked in each case as the contact time and the quantity of adsorbent is increased. This shows that treated chicken feathers work even better than untreated chicken feathers. Again, it is noticed that an exception to the rule occurs when 4 grams of adsorbent is added to the 70 PPM chromium solution. While the changed rate of absorption is still considerably lower than the initial rate of absorption; however, the change is quite constant even after the contact time is increased from 1 hour to 24 hours.

In figures 2 and 3, it is noticed that the adsorbents (Treated and Untreated) seem to work at optimum at 3 hours, with some action also seen between 5 hours to 7 hours after which the rate of absorption becomes constant. Also, the optimum quantity of adsorbents (both Treated and Untreated) seems to be between 2 grams to 2.5 grams. The effect of the dosage limit (2-2.5 grams) seems to contribute to a gradual decrease in the concentration of chromium ions as the contact time is increased from 1 hour to 7 hours.

T-tests were conducted to illustrate the relationship between the dosage limit and the changed rate of absorption for a 70 PPM chromium solution.

From the previous test experiments, it was evident that when the adsorbent dosages were set between (1.5-4.0) grams, the rates of absorption went down when compared to the initial absorption rate. However, the best dosage effect was seen when the dosages were set at (2.0-2.5) grams. Similarly, the optimum contact time for the adsorbent to bind with the chromium ions was determined at 3 hours. The following t-test results depict the relationship between the dosage effect of adsorbent on the concentration of chromium ions in water.

For the following t-tests, the concentration of the chromium solution was set at 70 PPM and the contact time was selected at 3 hours (Table 2).

- a. Hypothesis: Determining the relationship between the dosage limit of treated chicken feathers and the consequent changed rate of absorption.

H_0 : As the quantity of the adsorbent (treated chicken feather) is consecutively increased by 0.5 grams, the changed rate of absorption remains equal to the initial rate of absorption.

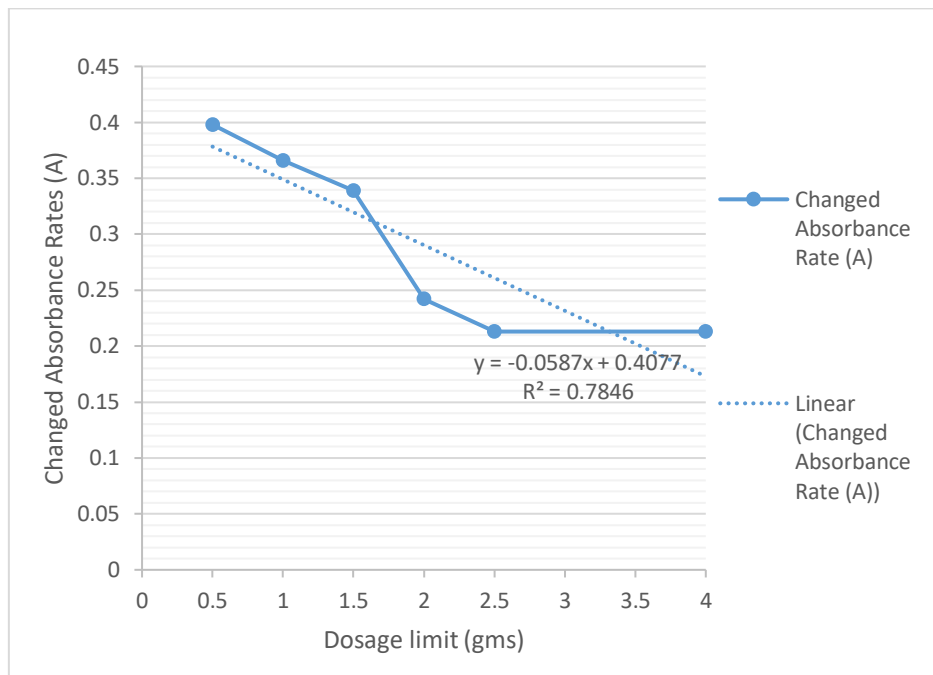
H_A : As the quantity of adsorbent (treated chicken feather) is consecutively increased by 0.5 grams, the changed rate of absorption does not remain equal to the initial rate of absorption.

Table 2. Relationship between the Dosage limit and the Changed rate of absorption.

t-Test: Two-Sample Assuming Unequal Variances		
	<i>Dosage (grams)</i>	<i>Changed Absorbance Rate (A)</i>
Mean	1.916666667	0.295166667
Variance	1.541666667	0.006768567
Observations	6	6
Hypothesized Mean Difference	0	
df	5	
t Stat	3.191876327	
P(T<=t) one-tail	0.012107051	
t Critical one-tail	2.015048373	
P(T<=t) two-tail	0.024214102	
t Critical two-tail	2.570581836	

Note: Treatment was conducted on a 70 PPM chromium solution. Here, the effect of dosages on the rate of absorption was seen when the contact time was set at 3 hours.

Figure 4. Linear plots showing the effect of dosage on chromium absorption (contact time=3 hours; concentration=70 PPM)



As the mass of the adsorbent increased, the rate of absorption decreased. The decreasing of the rate of absorption signaled the increase in the removal of chromium ions from the water sample. Looking back the Table 2, it is seen that the p-value is 0.024214102. Based on this it can be said that the p-value is less than 0.05, and therefore we may reject H_0 at $\alpha=5\%$ significance level. This means that as the quantity of the adsorbent is increased by 0.5 grams, the initial rate of absorption does not remain equal to the changed rate of absorption. Furthermore, the R^2 value is 0.7846 meaning that 78% of the data fit the regression model. The graph (Figure 4) supports the alternative hypothesis and illustrates that as the adsorbent dosage is consecutively increased by half-fold, the rate of absorption decreases concurrently (removal of chromium increases).

T-tests were conducted to determine the best type of adsorbent used in treating a 70 PPM chromium solution.

From the previous test experiments, it was evident that treated chicken feathers were the better adsorbents compared to untreated chicken feathers. The effect of untreated chicken feathers as

an adsorbent on the removal of chromium ions from water yielded mostly inferior results. To better judge the significance of these results, a paired t-test was conducted.

For the following t-test, again the concentration of the chromium solution was set at 70 PPM and the contact time was selected at 3 hours (Table 3).

- i. Hypothesis: Determining the effectiveness of % chromium removal between Treated and Untreated adsorbents.

H_0 : % removal of chromium ions from water samples is much higher with untreated adsorbent rather than treated adsorbent.

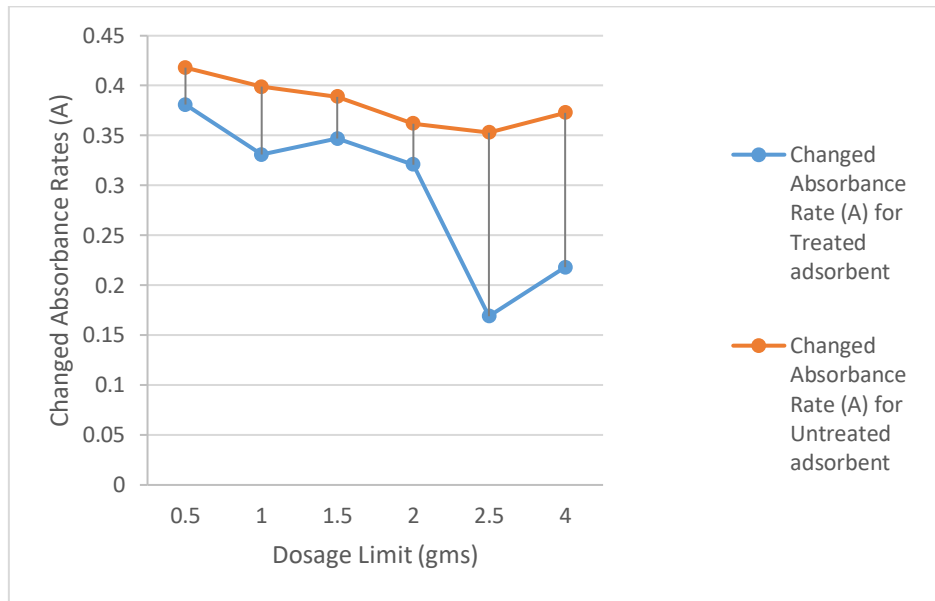
H_A : % removal of chromium ions from water samples is much lower with untreated adsorbent rather than treated adsorbent.

Table 3. Inferring the effectiveness between Treated and Untreated adsorbents.

t-Test: Paired Two Sample for Means		
	Changed Absorbance Rate (A) for Treated adsorbent	Changed Absorbance Rate (A) for Untreated adsorbent
Mean	0.2945	0.382333333
Variance	0.0067751	0.000591067
Observations	6	6
Pearson Correlation	0.789552473	
Hypothesized Mean Difference	0	
df	5	
t Stat	-3.317353489	
P(T<=t) one-tail	0.010534713	
t Critical one-tail	2.015048373	
P(T<=t) two-tail	0.021069426	
t Critical two-tail	2.570581836	

Note: Treatment was conducted on a 70 PPM chromium solution. Here, the effectiveness of the two different types of adsorbents was judged when the contact time was set at 3 hours.

Figure 5. Showing effectiveness of the two different types of adsorbents on chromium absorption (contact time=3 hours; concentration=70 PPM)

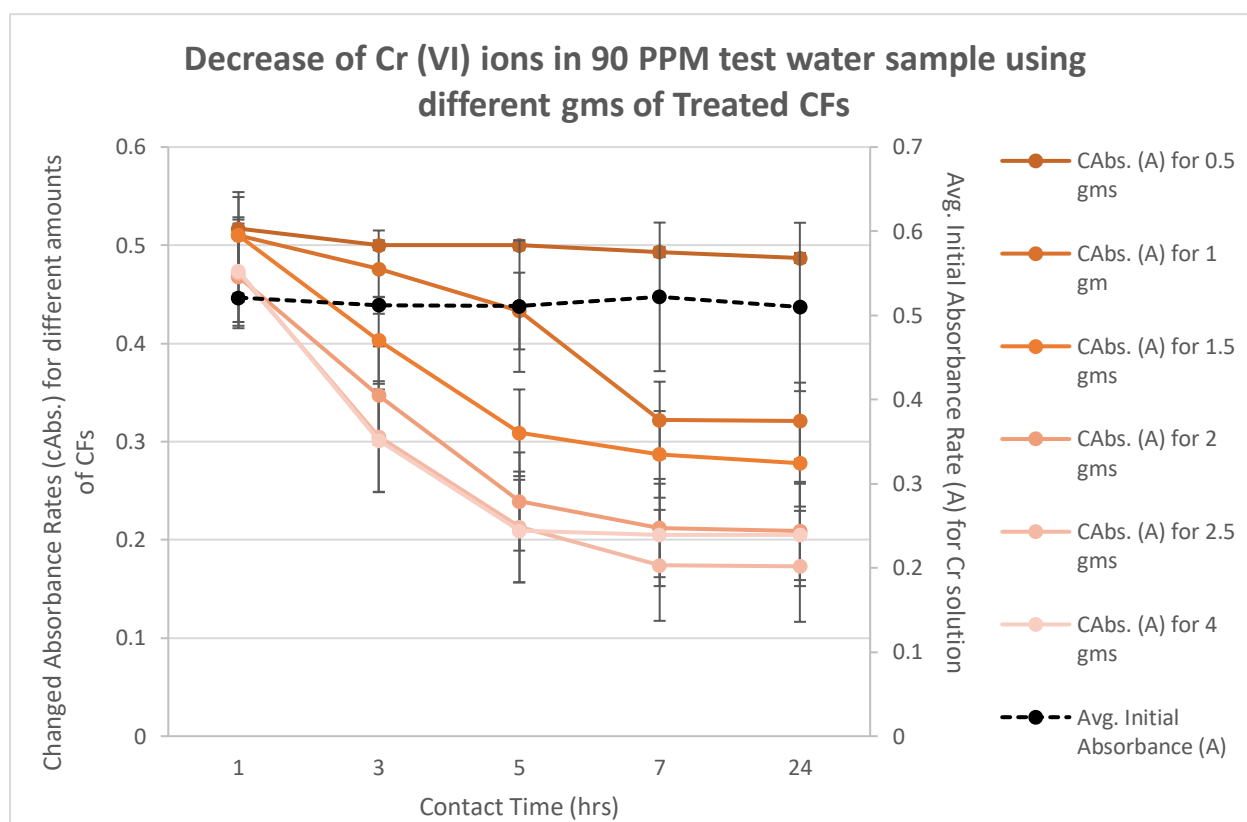


Based on the graph (Figure 5), it is seen that the changed rate of absorption for the Treated adsorbent is much lower compared to that of the Untreated adsorbent. Drawing from previous graphs, it was observed that the rate of absorption for a 70 PPM chromium solution is measured at ± 0.42 A. Looking at Figure 5, it is seen that the rate of absorption after adding Treated adsorbent decreases from ± 0.4 A until ± 0.2 A. On the other hand, the rate of absorption is quite high for test samples treated with Untreated adsorbent. This would imply that the rate of effectiveness between the two types of adsorbents is quite contrasting.

Looking back the Table 3, it is seen that the p-value is 0.021069426. Based on this it can be said that the p-value is far less than 0.05, and therefore we may reject H_0 at $\alpha=5\%$ significance level. This means that the effectiveness of the % removal of chromium ions from the test water samples is quite higher for the Treated adsorbent than that of the Untreated adsorbent. The graph (Figure 5) further supports the t-test results and accepts the alternative hypothesis. It illustrates that % removal of chromium ions from water samples is much lower with untreated adsorbent rather than treated adsorbent due to the changed rates of absorptions being quite higher than those of Treated adsorbent.

ii. **Experiments using 90 PPM chromium solution:** In the second set of batch experiments, the effect of different amounts of chicken feathers (dosage limit) on a 90 PPM concentration chromium solution was observed (Figure 6). The following graph displays the relationship between the effect of adsorbent on the concentration of chromium ions in water.

Figure 6. Effect of Dose/Adsorbent (Treated Chicken feathers) on 90 PPM chromium solution.



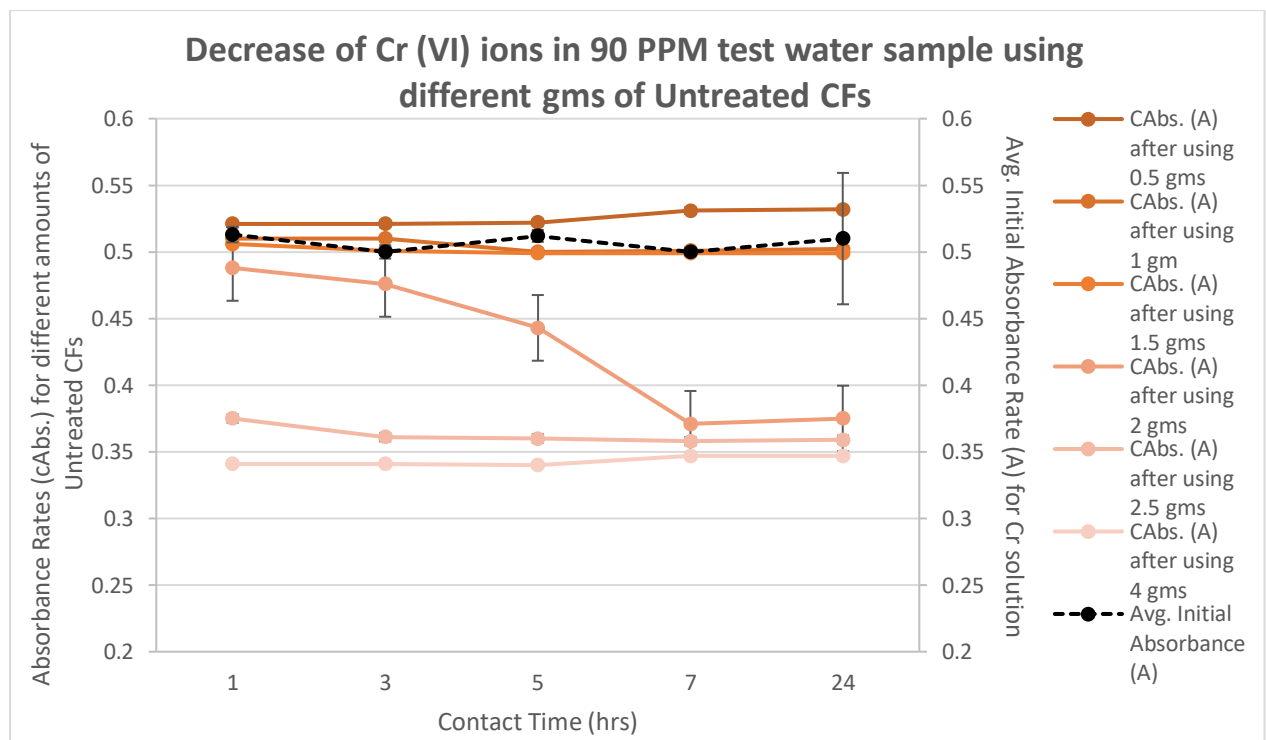
Note:

1. The average initial rate of absorption for a 90 PPM chromium solution is measured at ± 0.51 A.
2. The secondary axis plots the initial rate of absorption for a 90 PPM chromium solution.

The above graph displays how different quantities of the adsorbent (Treated chicken feathers) can affect the removal of chromium ions in water (Figure 6). Here, a concentration of 90 PPM chromium solution was taken. It is observed that as the quantity of the adsorbent is increased, the concentration of the solution also decreases. Essentially, the rate of absorption decreases. It is noticed that after 3 hours the rate of absorption decreases considerably compared to the initial absorption rate. This occurs in each case when the quantity of the adsorbent is increased

consecutively by 0.5 grams. However, it reaches a constant between 7 hours to 24 hours in each case when the quantity of the adsorbent is increased consecutively by 0.5 grams. This could mean that the effect of the adsorbent diminishes between 7 hours to 24 hours. Again. The optimum dosage limit is shown to be between 2.0-2.5 grams. Furthermore, it is seen that when using 4 grams of the adsorbent, the concentration of chromium ions decreases, and with that the rate of absorption also decreases. It works quite well until the 5-hour mark, after which the effect of the adsorbent diminishes and the effect of dosage becomes constant.

Figure 7. Effect of Dose/Adsorbent (Untreated Chicken feathers) on 90 PPM chromium solution.



Note:

1. The average initial rate of absorption for a 90 PPM chromium solution is measured at ± 0.51 A.
2. The secondary axis plots the initial rate of absorption for a 90 PPM chromium solution.

The above graph depicts the effect of different quantities of adsorbent (Untreated chicken feathers) has on the 90 PPM chromium ion solution (Figure 7). It is observed that as the quantity of the adsorbent is increased the concentration of the solution also decreases. This graph is quite similar to the previous graphs (Figures 2, 3, 6). In this case too, the rate of absorption decreases as the quantity of adsorbent is increased. It is noticed that any effectiveness of the

adsorbent is observed between 3 hours to 7 hours. Again, the rate of absorption decreases considerably compared to the initial absorption rate. This occurs in each case when the quantity of the adsorbent is increased consecutively by 0.5 grams. However, in the case of the effect of 0.5-gram dosage, it is seen that after the 7-hour mark the rate of absorption increases. This could be due to the inability of the keratin molecules to effectively bind to the chromium ions and that after some time the affinity between said molecules reduces. It could also be an instrumentation error.

When, compared to the decreasing changed rate of absorption for the Treated feathers adsorbent, the decrease in the rate of absorption for the Untreated feathers is not as extreme. For example, when the contact time is 3 hours and 2 grams of Untreated feathers are used, the rate of absorption is seen to be 0.476 A (Figure 7). Under similar conditions, when Treated feathers are used as adsorbents, the rate of absorption is seen to be 0.347 A (Figure 6). This phenomenon is mimicked in each case as the contact time and the quantity of adsorbent is increased. This shows that Treated chicken feathers work even better than Untreated chicken feathers.

In figures 6 and 7, it is noticed that the adsorbents (Treated and Untreated) seem to work at optimum at 3 hours, with some action also seen between 5 hours to 7 hours after which the rate of absorption becomes constant. Also, the optimum quantity of adsorbents (both Treated and Untreated) seems to be between 2 grams to 2.5 grams. The effect of the dosage limit (2-2.5 grams) seems to contribute to a gradual decrease in the concentration of chromium ions as the contact time is increased from 1 hour to 7 hours.

T-tests were conducted to illustrate the relationship between the dosage limit and the changed rate of absorption for a 90 PPM chromium solution.

From the previous test experiments, it was evident that when the adsorbent dosages were set between (1.5-4.0) grams, the rates of absorption went down when compared to the initial absorption rate. However, the best dosage effect was seen when the dosages were set at (2.0-2.5) grams. Similarly, the optimum contact time for the adsorbent to bind with the chromium ions was determined at 3 hours. The following t-test results depict the relationship between the dosage effect of adsorbent on the concentration of chromium ions in water.

For the following t-tests, the concentration of the chromium solution was set at 90 PPM and the contact time was selected at 3 hours (Table 4). The same hypothesis is assumed for the treatment of a 90 PPM chromium solution as it was for the treatment of a 70 PPM chromium solution.

- a. Hypothesis: Determining the relationship between the dosage limit of Treated chicken feathers and the consequent changed rate of absorption.

H₀: As the quantity of the adsorbent (Treated chicken feather) is consecutively increased by 0.5 grams, the changed rate of absorption remains equal to the initial rate of absorption.

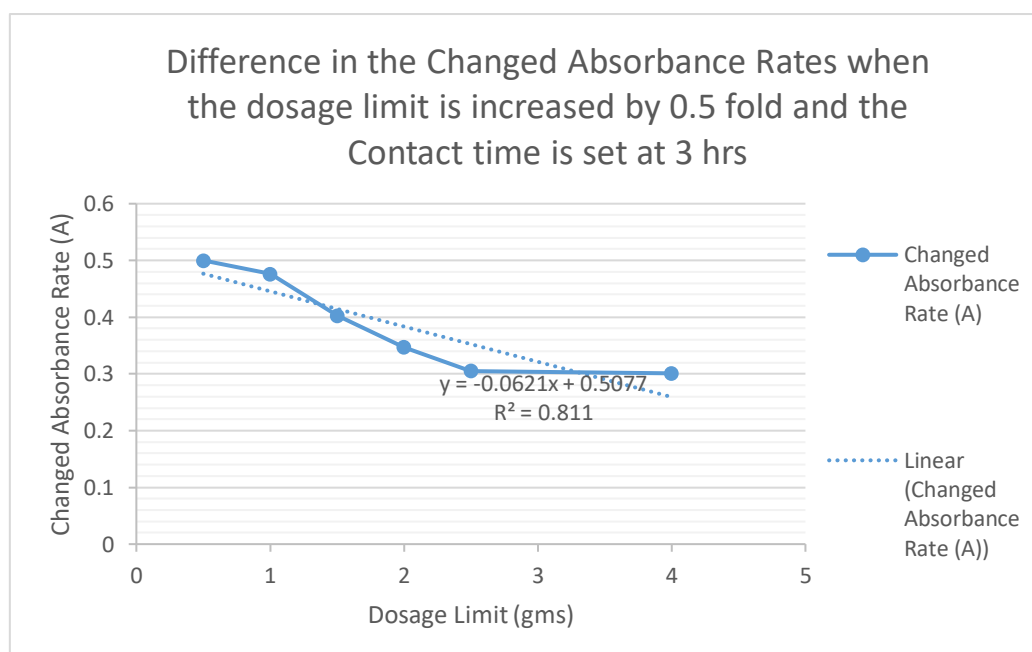
H_A: As the quantity of adsorbent (Treated chicken feather) is consecutively increased by 0.5 grams, the changed rate of absorption does not remain equal to the initial rate of absorption.

Table 4. Relationship between the Dosage limit and the Changed rate of absorption.

t-Test: Two-Sample Assuming Unequal Variances			
	Dosage (gms)	Changed Rate (A)	Absorbance
Mean	1.916666667		0.388666667
Variance	1.541666667		0.007329867
Observations	6		6
Hypothesized Mean Difference	0		
df	5		
t Stat	3.007279242		
P(T<=t) one-tail	0.014924334		
t Critical one-tail	2.015048373		
P(T<=t) two-tail	0.029848668		
t Critical two-tail	2.570581836		

Note: Treatment was conducted on a 90 PPM chromium solution. Here, the effectiveness of the two different types of adsorbents was judged when the contact time was set at 3 hours.

Figure 8. Linear plots showing the effect of dosage on chromium absorption (contact time=3 hours; concentration=90 PPM)



As the mass of the adsorbent increased, the rate of absorption decreased. The decreasing of the rate of absorption signaled the increase in the removal of chromium ions from the water sample. Looking back the Table 4, it is seen that the p-value is 0.029848668. Based on this it can be said that the p-value is less than 0.05, and therefore we may reject H_0 at $\alpha=5\%$ significance level. This means that as the quantity of the adsorbent is increased by 0.5 grams, the initial rate

of absorption does not remain equal to the changed rate of absorption. Furthermore, the R^2 value is 0.811 meaning that 81% of the data fit the regression model. The graph (Figure 8) supports the alternative hypothesis and illustrates that as the adsorbent dosage is consecutively increased by half-fold, the rate of absorption decreases concurrently (removal of chromium increases).

T-tests were conducted to determine the best type of adsorbent used in treating a 90 PPM chromium solution.

As it has been already established, Treated chicken feathers are the better adsorbents compared to Untreated chicken feathers and ash. The effect of ash as an adsorbent on the removal of chromium ions from water (test sample=90 PPM Cr(VI) ions) yielded the most inferior results. To better judge the significance of these results, a paired t-test was conducted.

For this purpose, the adsorbents have been categorized into Treated and Untreated adsorbents.

For the following t-tests, again the concentration of the chromium solution was set at 90 PPM and the contact time was selected at 3 hours (Table 5). The same hypothesis is assumed for the treatment of a 90 PPM chromium solution as it was for the treatment of a 70 PPM chromium solution.

- b. Hypothesis: Determining the effectiveness of % chromium removal between Treated and Untreated adsorbents.

H_0 : % removal of chromium ions from water samples is much higher with Untreated adsorbent rather than Treated adsorbent.

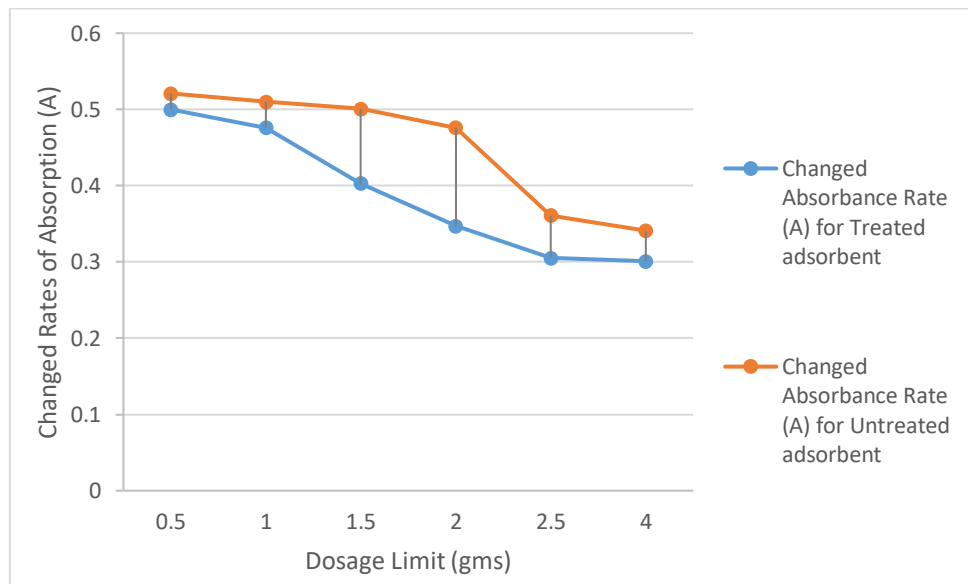
H_A : % removal of chromium ions from water samples is much lower with Untreated adsorbent rather than Treated adsorbent.

Table 5. Inferring the effectiveness between Treated and Untreated adsorbents.

t-Test: Paired Two Sample for Means		
	Changed Absorbance Rate (A) for Treated adsorbent	Changed Absorbance Rate (A) for Untreated adsorbent
Mean	0.388666667	0.451666667
Variance	0.007329867	0.006340667
Observations	6	6
Pearson Correlation	0.874073934	
Hypothesized Mean Difference	0	
df	5	
t Stat	-3.685954691	
P(T<=t) one-tail	0.007102642	
t Critical one-tail	2.015048373	
P(T<=t) two-tail	0.014205284	
t Critical two-tail	2.570581836	

Note: Treatment was conducted on a 90 PPM chromium solution. Here, the effectiveness of the two different types of adsorbents was judged when the contact time was set at 3 hours.

Figure 9. Showing effectiveness of the two different types of adsorbents on chromium absorption (contact time=3 hours; concentration=90 PPM)



Based on the graph (Figure 9), it is seen that the changed rate of absorption for Treated adsorbent is much lower compared to that of the Untreated adsorbent. Drawing from previous graphs, it was observed that the rate of absorption for a 90 PPM chromium solution is measured at ± 0.51 A. Looking at Figure 9, it is seen that the rate of absorption after adding Type A

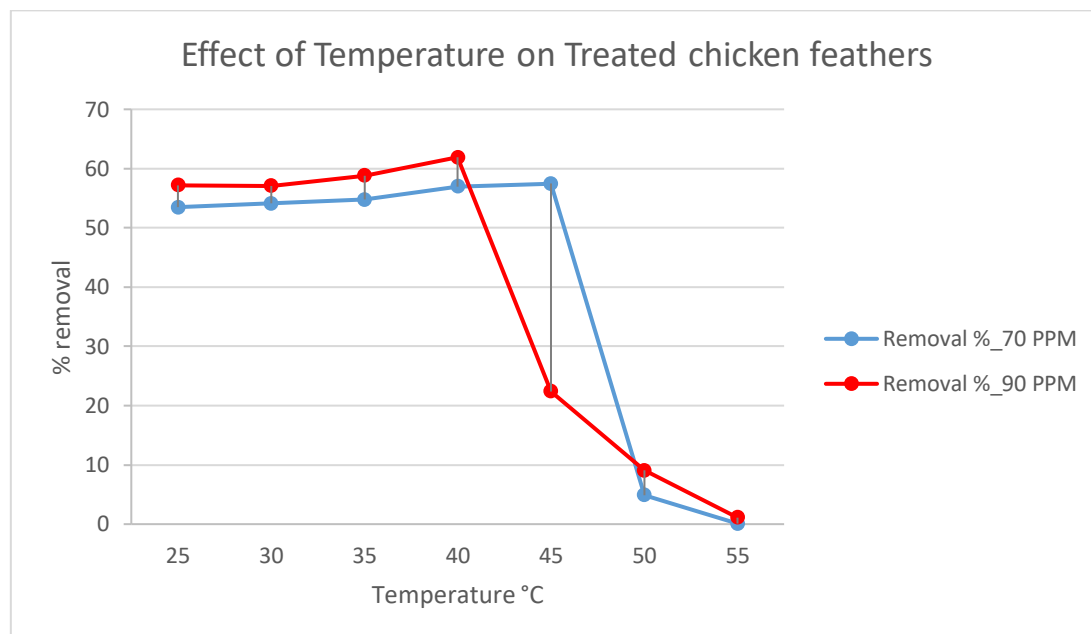
adsorbent decreases from ± 0.5 A until ± 0.3 A. On the other hand, the rate of absorption is quite high for test samples treated with Untreated adsorbent. This would imply that the rate of effectiveness between the two types of adsorbents is quite contrasting.

Looking back the Table 5, it is seen that the p-value is 0.014205284. Based on this it can be said that the p-value is far less than 0.05, and therefore we may reject H_0 at $\alpha=5\%$ significance level. This means that the effectiveness of the % removal of chromium ions from the test water samples is quite higher for the Treated adsorbent than that of the Untreated adsorbent. The graph (Figure 9) further supports the t-test results and accepts the alternative hypothesis. It illustrates that % removal of chromium ions from water samples is much lower with Untreated adsorbent rather than Treated adsorbent due to the changed rates of absorptions being quite higher than those of Treated adsorbent.

4.2.4. Effect of Temperature

Cr(VI) is removed from aqueous solution by varying the temperature from 25 to 55 °C under constant initial concentration (70 PPM/90 PPM), pH (± 2), contact time (3 hrs), and adsorbent dose (2.5 g) (Figure 10). Figure 10 reveals that the percentage of Cr(VI) removal increased from 53.47 to 57.44% when the temperature increased from 25 to 45 °C (for 70 PPM Cr(VI) solution). Also, the percentage of Cr(VI) removal increased from 57.21 to 61.91% when the temperature increased from 25 to 40 °C (for 90 PPM Cr(VI) solution). This enhancement of adsorption with increasing temperature is probably due to the diffusion of chromium (VI) through the external boundary layer and internal pores of chicken feathers (Gao et al., 2014). However, no effect on the removal efficiency was observed upon increasing the temperature further than 40-45°C. This could be because the protein had denatured under extreme temperatures, rendering no binding of chromium ions with the peptide bonds.

Figure 10. Effect of temperature on the adsorption of chromium (VI) ions. (Contact time=3 hrs; dosage=2.5 gms; pH=±2)



Note: For this experiment only treated chicken feathers were used as adsorbents. Previous batch experiments had already established that the optimal conditions for contact time, dosage, and pH were, 3 hrs, 2.5 gms, and ±2 respectively.

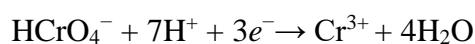
4.2.5. Effect of pH

The effect of PH on Cr(VI) uptake by the Treated chicken feather is presented in Fig. 11. As the pH of the solution was increased from 1 to 6, the adsorption of Cr (VI) decreased. Figure 11 demonstrates that the Cr(VI) removal increases with decreasing the pH of the medium. This is probably due to the modification of the surface in acid. At low pH values, the dominant species of Cr (VI) in solution is HCrO_4^- . This is converted to CrO_4^{2-} and $\text{Cr}_2\text{O}_7^{2-}$ ions as the pH increases. The adsorption free energy change for HCrO_4^- is lower than those of CrO_4^{2-} and $\text{Cr}_2\text{O}_7^{2-}$ ions. Therefore, HCrO_4^- is more easily adsorbed than CrO_4^{2-} and $\text{Cr}_2\text{O}_7^{2-}$ at lower pH values (Soares et al., 2009).

The several functional groups present in keratin protein, especially peptide backbone, such as disulfide($-\text{S}-\text{S}$), amino($-\text{NH}_2$), and carboxylic acid($-\text{COOH}$) transform positive charges after protonation (David & Leonor, 1934). Therefore, the surface positive charge can easily attract negatively charged chromium species (CrO_4^{2-} and HCrO_4^-) through electrostatic attraction.

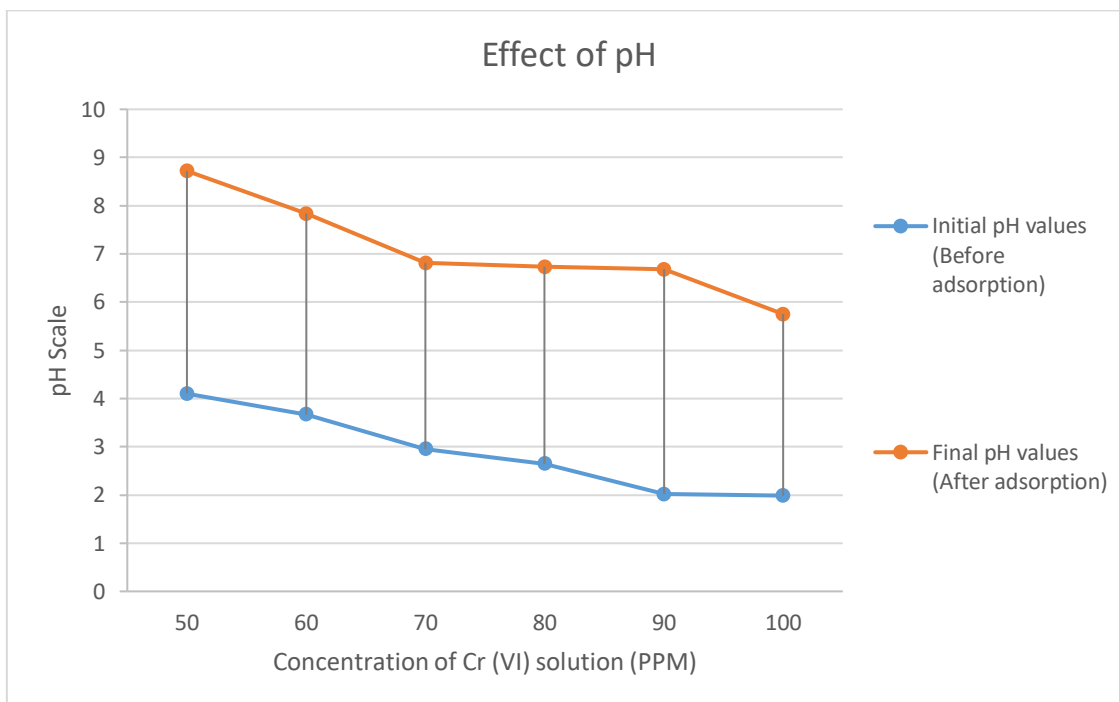
Moreover, the groups on the surface of keratin bio-sorbents can hydrolyze water molecules of higher concentration of hydrogen ions and subsequently transform them to a positive charge (Gao et al., 2014).

On the other hand, at higher pH, the absorbent surface transforms to negatively charged and it does not support attracting the negatively charged species of chromium. Therefore, higher pH does not support higher removal of Cr(VI) (Gao et al., 2014). Moreover, it is well established in several pieces of literature that biosorption of cation is favored at $\text{pH} > \text{pH}_{\text{ZPC}}$, while the biosorption of anions is favored at $\text{pH} < \text{pH}_{\text{ZPC}}$ (Mondal, Basu & Das, 2019). Soares et al., (2009) also reported that the Cr(VI) can be reduced to Cr(III) and the possible reduction can be explained by the following reaction:



Therefore, this phenomenon reduced the percentage of Cr(VI) removal in an aqueous medium also.

Figure 11. Effect of pH on the adsorption of chromium (VI) ions. (Contact time=3 hrs; dosage=2.5 gms; temperature=25°C)



Note: For this experiment only treated chicken feathers were used as adsorbents. Previous batch experiments had already established that the optimal conditions for contact time, dosage, and temperature were, 3 hrs, 2.5 gms, and 25°C respectively.

4.3 Protein Extraction Study

4.3.1. Observation

Standard biuret testing was conducted to test the presence of protein. The solution turned purple after the reagent is added and this is only possible if peptide bonds are present in it. The more peptide bonds in it, the higher the intensity of the purple color as seen in Figures 12 and 13. Also, the difference between the purple colors of the different solutions is visible seen in Figures 12 and 13. This is in accordance with the absorbance of the biuret solution and the amount of protein obtained at the end of the research. The higher the amount of chicken feather dissolved the higher the protein obtained.

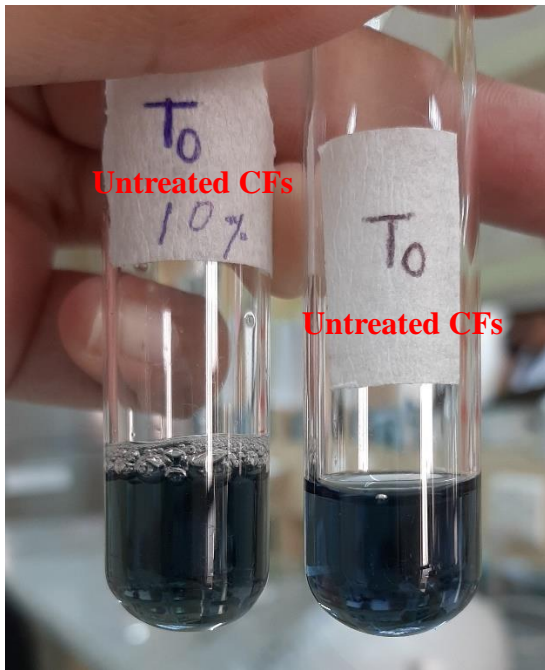


Figure 12: Standard Biuret Test for Treated and Untreated chicken feathers.

The color has changed to a deep violet-blue, indicating the presence of peptide bonds. The rich dark color is indicative of presence of higher protein content.

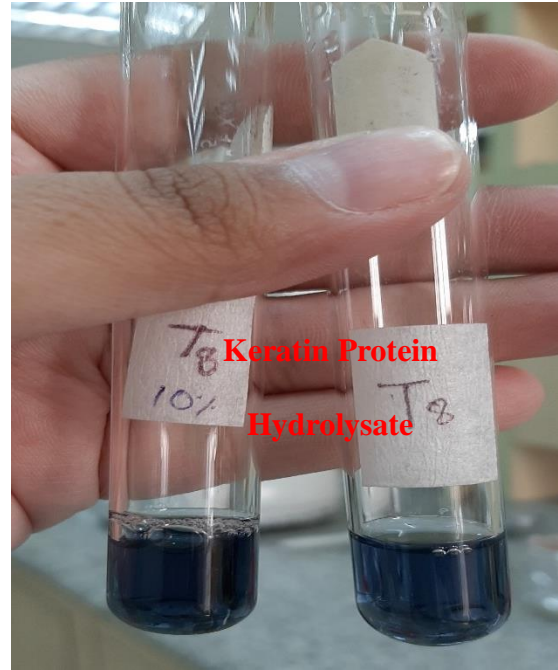


Figure 13: Standard Biuret Test for Keratin Protein Hydrolysate.

The color has changed to a deep violet-blue, indicating the presence of peptide bonds. The left test tube contains 2.5 gms of dissolved CFs, and the right test tube contains 2 gms of dissolved CFs. The left test tube displays a darker solution indicating the presence of higher protein content.

4.4 Determining Efficiency

To estimate the percentage removal of chromium (VI) from an aqueous solution, the following equation was used.

$$\text{Percentage removal of Cr (VI)} = \frac{(C_{\text{initial}} - C_{\text{final}})}{C_{\text{initial}}} \times 100$$

where C_{initial} and C_{final} are the concentrations of Cr(VI) at the beginning and at the end of the adsorption process.

Table 6. Percentage of Chromium Removal (Efficiency)

Adsorbent Type	Contact Time (hr)	Initial Concentration of the Cr solution (PPM)	Changed Concentration of the Cr solution (PPM)	Initial Absorbance Rate of the Cr solution (A)	Changed Absorbance Rate of the Cr solution (A)	% of Chromium Removal
Treated chicken feathers	5-7 hrs	70 PPM/ 90 PPM	18.67 PPM/ 37.92 PPM	0.41 A/ 0.52 A	0.148 A/ 0.173 A	73.33%/ 57.87%
Untreated chicken feathers	5-7 hrs	70 PPM/ 90 PPM	19.98 PPM/ 52.01 PPM	0.42 A/ 0.52 A	0.198 A / 0.375 A	71.46%/ 54.27%
Keratin Protein Solution	3-5 hrs	70 PPM/ 90 PPM	33.68 PPM/ 42.02 PPM	0.41 A/ 0.52 A	0.171 A/ 0.37 A	53.32%/ 51.89%

Note:

1. The dosage for all the adsorbent type is 2.5 gms. 2.5 mL was measured out for the keratin hydrolysate. Lowest concentration readings were recorded for each adsorbent type at this dosage limit.

Absorbance is proportional to the concentration of a solution. So, increasing the absorbance also shows higher protein concentration. As a result, a high protein concentration resulted in the binding of more chromium ions. From the above Table 6, it is evident that the extracted keratin solution (hydrolysate) works faster at removing the chromium ions from the solution compared to the adsorption time of treated/untreated chicken feathers.

Chapter 5

Conclusions and Way Forward

The present study has highlighted the use of chemically treated chicken feathers as an adsorbent to remove Cr(VI) ions by using a chemically treated chicken feather. For this purpose, the effect of five operating variables such as initial concentration, adsorption dose, contact time, and pH were evaluated. The results obtained for the removal of Cr(VI) were affected by all the mentioned operating variables. An optimum condition for Cr(VI) uptake of 70 PPM and 90 PPM at 25°C was achieved at adsorption dose 2-2.5 gms, contact time 3-7 hrs, and pH \pm 2. The removal efficiency for 70 PPM Cr(VI) solution using chemically treated chicken feathers was calculated at 73.33%, whereas the efficiency for untreated feathers stood at 71.46%. The removal efficiency for 90 PPM Cr(VI) solution using chemically treated chicken feathers was calculated at 57.87%, whereas the efficiency for untreated feathers stood at 54.27%. On the other hand, optimum conditions for Cr(VI) uptake of keratin hydrolysate at 25°C was achieved at adsorption dose 1.5-2.5 gms, contact time 3-5 hrs, and pH \pm 2. The removal efficiency for 70 PPM Cr(VI) solution using chemically treated chicken feathers was calculated at 53.32%, %, whereas the efficiency for adsorbing 90 PPM Cr(VI) solution stood at 51.89%. It was also observed that initially, the rate of adsorption is higher; however, as time passes the adsorption decreases with a decrease in chromium removal. A higher removal efficiency was expected for the keratin hydrolysate adsorbent. However, storage of said hydrolysate at 19°C for preservation may have affected the protein activity of the hydrolysate. As previously discussed in the paper, the dominant form of Cr(VI) in the pH range of 1–6 is HCrO_4^- . The highest adsorption capacity occurs in the pH range 2–3. Since in this pH range the protein is below its isoelectric point, a great number of cationic sites are present in the amino acid side chains and are able to bind negative ions. The optimal temperatures for the chromium ions to bind with the keratin protein were observed at 25 to 45 °C. This study showed that modified chicken

feathers could be used as a potential bio-sorbent for the removal of Cr (VI) ions from an aqueous solution under optimized conditions.

The pretreatment and modification of the natural keratin biomaterials aiming to improve their biosorption capacity were introduced and evaluated in this study. It should be noted that keratin-rich nanofiber and keratin colloidal solution also have good bio-sorption abilities (Shavandi et al., 2017). Although chemically modified keratin biomaterials or some keratin-rich materials having specific structure and properties can enhance the adsorption of heavy metals, the cost of chemicals used and methods for the modification also have to be taken into consideration to actually produce “low-cost” bio-sorbents in the real applications in Bangladesh. In conclusion, the widespread keratin biomaterials in industries for wastewaters treatment applications are strongly recommended due to their local availability, technical feasibility, engineering applicability, and cost-effectiveness. Despite this, the process of biosorption requires further investigation in the direction of modeling, regeneration of bio-sorbent, recovery of metal ions, and modification of the keratin biomaterials for the practical application in the Bangladeshi context.

Experiments about biosorption have shown significant contributions to the removal of contaminants from aqueous effluents. Keratin biomaterials (wool, feather, and hair) have received increasing attention for heavy metal removal due to their good performance, low cost, and large available quantities. The functional groups present in the surface of the keratin biomaterials such as carboxyl, hydroxyl, amino and sulfur-containing groups, etc. have some affinity in general for heavy metals. Based on the literature, we understand that different keratin biomaterials as bio-sorbents are used for the removal of heavy metals. Information reviewed was mainly focused on the biosorption performance of such bio-sorbents, their possible pretreatment and modification, their regeneration/reuse, about the modeling of the heavy metal

biosorption process (even kinetics and isotherm models), together with an evaluation of their potential application and their future in the market (Shavandi et al., 2017).

Most of the reported studies are performed in the batch process, which gives a platform for the further designing of continuous flow systems with possible industrial applications at the commercial level (Ince & Ince, 2019). Further research should be carried out to make the process economically viable at an industrial scale with a focus on metal recovery and regeneration of keratin biomaterials, especially in the case that they are modified with some specific pretreatment. Therefore, in the future, one trend is to use hybrid technology for the pollutants (Kumari & Shobha, 2015; Osasona et al., 2015; Shavandi et al., 2017). Another trend would be to develop the commercial bio-sorbents and to improve the biosorption process including the proper immobilization of biomaterials, improvement of their regeneration and reuse, an improvement on their possible pretreatment and modification, optimization of biosorption process under different conditions, and for different case studies (feed samples), etc. (Cheng, 2014; Ince & Ince, 2019).

The study could have been improved further in terms of comparing the heavy metal removal efficiency of the modified chicken feathers with that of the removal efficiency of activated carbon. The application of activated carbon to treat heavy-metal contaminated water usually follows a simple design, ease of operation, and guarantees relatively high removal efficiency. However, due to the onset of the covid-19 pandemic, as well as budgetary and time constraints batch experiments with an equivalent carbon source (Ash) for comparison studies could not be conducted. Results from such a study would help determine the economic viability of chicken feathers as a replacement to the expensive activated carbon in effluent treatment systems.

References

- 1 Alemu, A., Lemma, B., Gabbiye, N., Alula, M. T., & Desta, M. T. (2018). Removal of chromium (VI) from aqueous solution using vesicular basalt: A potential low-cost wastewater treatment system. *Heliyon*, 4(7), e00682. <https://doi.org/10.1016/j.heliyon.2018.e00682>
- 2 Ali, M. M., Ali, M. L., Islam, M. S., & Rahman, M. Z. (2016). Preliminary assessment of heavy metals in water and sediment of Karnaphuli River, Bangladesh. *Environmental Nanotechnology, Monitoring & Management*, 5, 27–35. <https://doi.org/10.1016/j.enmm.2016.01.002>
- 3 Anah, L., & Astrini, N. (2017). Influence of pH on Cr(VI) ions removal from aqueous solutions using carboxymethyl cellulose-based hydrogel as adsorbent. *IOP Conference Series: Earth and Environmental Science*, 60, 1–6. <https://doi.org/10.1088/1755-1315/60/1/012010>
- 4 Anawar, H. M., Safiullah, S., & Yoshioka, T. (2000). Environmental Exposure Assessment of Chromium and Other Tannery Pollutants at Hazaribagh Area, Dhaka, Bangladesh, and Health Risk. *Journal of Environmental Chemistry*, 10(3), 549–556. <https://doi.org/10.5985/jec.10.549>
- 5 Ansar, A., & Durham, J. (2019). Heavy Metals in Poultry Products in Bangladesh: A Possible Death Threat to Future Generations. *Journal of Social and Political Sciences*, 2(1). <https://doi.org/10.31014/aior.1991.02.01.53>
- 6 Asfaw, T. B., Tadesse, T. M., & Ewnetie, A. M. (2017). Determination of Total Chromium and Chromium Species in Kombolcha Tannery Wastewater, Surrounding Soil, and Lettuce Plant Samples, South Wollo, Ethiopia. *Advances in Chemistry*, 2017, 1–7. <https://doi.org/10.1155/2017/6191050>

- 7 Banu, Z., Chowdhury, M. S. A., Hossain, M. D., & Nakagami, K. (2013). Contamination and Ecological Risk Assessment of Heavy Metal in the Sediment of Turag River, Bangladesh: An Index Analysis Approach. *Journal of Water Resource and Protection*, 05(02), 239–248. <https://doi.org/10.4236/jwarp.2013.52024>
- 8 Beak, M. H., Ijagbemi, C. O., & Kim, D. S. (2009). Treatment of malachite green-containing wastewater using poultry feathers as adsorbent. *Journal of Environmental Science and Health, Part A*, 44(5), 536–542. <https://doi.org/10.1080/10934520902720132>
- 9 Chakraborty, R., Asthana, A., Singh, A. K., Verma, R., Sankarasubramanian, S., Yadav, S., Carabineiro, S. A. C., & Susan, M. A. B. H. (2020). Chicken feathers derived materials for the removal of chromium from aqueous solutions: kinetics, isotherms, thermodynamics, and regeneration studies. *Journal of Dispersion Science and Technology*, 1–15. <https://doi.org/10.1080/01932691.2020.1842760>
- 10 Cheng, J. (2014). Bioremediation of Contaminated Water-Based on Various Technologies. *OALib*, 01(01), 1–13. <https://doi.org/10.4236/oalib.preprints.1200056>
- 11 David, R. G., & Leonor, M. (1934). A Study of Keratin. *Journal of Biological Chemistry*, 106, 605-614
- 12 David, R. G., & Leonor, M. (1935). Derivatives of Keratin. *Journal of Biological Chemistry*, 112, 361-371
- 13 Dong, G., Zhang Y., Shan H., Xu J., & Xin, S. (2011). Spectrophotometric Study on Kinetics and Thermodynamics of Adsorption and Catalytic Transformation of K₂Cr₂O₇ to K₂CrO₄ by Natural Hermit Crab Shell Powder. *Chemical Sciences Journal*, 2. <https://doi.org/10.4172/2150-3494.1000030>
- 14 Dong, H., Deng, J., Xie, Y., Zhang, C., Jiang, Z., Cheng, Y., Hou, K., & Zeng, G. (2017). Stabilization of nanoscale zero-valent iron (nZVI) with modified biochar for

- Cr(VI) removal from aqueous solution. *Journal of Hazardous Materials*, 332, 79–86.
<https://doi.org/10.1016/j.jhazmat.2017.03.002>
- 15 Gao, P., Liu, Z., Wu, X., Cao, Z., Zhuang, Y., Sun, W., Xue, G., & Zhou, M. (2014). Biosorption of Chromium(VI) Ions by Deposits Produced from Chicken Feathers after Soluble Keratin Extraction. *CLEAN - Soil, Air, Water*, 42(11), 1558–1566.
<https://doi.org/10.1002/clen.201300669>
- 16 García-Sabido, D., López-Mesas, M., & Carrillo-Navarrete, F. (2014). Chicken feather fibres waste as a low-cost biosorbent of acid Blue 80 dye. *Desalination and Water Treatment*, 57(8), 3732–3740. <https://doi.org/10.1080/19443994.2014.986531>
- 17 Genawi, N. M., Ibrahim, M. H., El-Naas, M. H., & Alshaik, A. E. (2020). Chromium Removal from Tannery Wastewater by Electrocoagulation: Optimization and Sludge Characterization. *Water*, 12(5), 1374. <https://doi.org/10.3390/w12051374>
- 18 Gupta, A., Kamarudin, N. & Yeo, C., Chua, G. K., Bin, R., & Yunus, R. (2012). Extraction of Keratin Protein from Chicken Feather, 6, 732-737.
- 19 Hashem, M. A., Islam, A., Mohsin, S., & Nur-A-Tomal, M. S. (2015). Green environment suffers by discharging of high-chromium-containing wastewater from the tanneries at Hazaribagh, Bangladesh. *Sustainable Water Resources Management*, 1(4), 343–347. <https://doi.org/10.1007/s40899-015-0033-4>
- 20 Ince, M., & Ince, O. K. (2019). *Heavy Metal Removal Techniques Using Response Surface Methodology: Water/Wastewater Treatment, Biochemical Toxicology - Heavy Metals and Nanomaterials*. IntechOpen. <https://doi.org/10.5772/intechopen.88915>
- 21 Kamarudin, N. B., Sharma, S., Gupta, A., Kee, C. G., Chik, S. M. S. B. T., & Gupta, R. (2017). Statistical investigation of extraction parameters of keratin from chicken feather using Design-Expert. *3 Biotech*, 7(2). <https://doi.org/10.1007/s13205-017-0767-9>

- 22 Karim, M. R., Manshoven, S., Islam, M. R., Gascon, J. A., Ibarra, M., Diels, L., & Rahman, M. M. (2013). Assessment of an Urban Contaminated Site from Tannery Industries in Dhaka City, Bangladesh. *Journal of Hazardous, Toxic, and Radioactive Waste*, 17(1), 52–61. [https://doi.org/10.1061/\(asce\)hz.2153-5515.0000139](https://doi.org/10.1061/(asce)hz.2153-5515.0000139)
- 23 Khan, A., Michelsen, N., Marandi, A., Hossain, R., Hossain, M. A., Roehl, K. E., Zahid, A., Hassan, M. Q., & Schüth, C. (2020). Processes controlling the extent of groundwater pollution with chromium from tanneries in the Hazaribagh area, Dhaka, Bangladesh. *Science of The Total Environment*, 710, 136213. <https://doi.org/10.1016/j.scitotenv.2019.136213>
- 24 Kumar, A. S. K., Kakan, S. S., & Rajesh, N. (2013). A novel amine impregnated graphene oxide adsorbent for the removal of hexavalent chromium. *Chemical Engineering Journal*, 230, 328–337. <https://doi.org/10.1016/j.cej.2013.06.089>
- 25 Kumari A. R., & Sobha K. (2015). Cost effective and ecofriendly method for copper removal adsorption with Emu feather (*Dromaius novaehollandiae*) and Chitosan (*Agaricus bioporus*) composite. *Int J Chem Tech Res*, 8(4), 1769–1782
- 26 Mohan, D., Singh, K., & Singh, V. (2006). Trivalent chromium removal from wastewater using low cost activated carbon derived from agricultural waste material and activated carbon fabric cloth. *Journal of Hazardous Materials*, 135(1–3), 280–295. <https://doi.org/10.1016/j.jhazmat.2005.11.075>
- 27 Mondal, N. K., Basu, S., & Das, B. (2019). Decontamination and optimization study of hexavalent chromium on modified chicken feather using response surface methodology. *Applied Water Science*, 9(3), 1–15. <https://doi.org/10.1007/s13201-019-0930-z>

- 28 Na Ayutthaya, S. I., & Woothikanokkhan, J. (2013). Extraction of Keratin from Chicken Feather and Electrospinning of the Keratin/PLA Blends. *Advanced Materials Research*, 747, 711–714. <https://doi.org/10.4028/www.scientific.net/amr.747.711>
- 29 Nagaraj, P., Aradhana, N., Shivakumar, A., Shrestha, A. K., & K Gowda, A. (2008). Spectrophotometric method for the determination of chromium (VI) in water samples. *Environmental Monitoring and Assessment*, 157(1–4), 575–582. <https://doi.org/10.1007/s10661-008-0557-2>
- 30 Nur-E-Alam, M., Akter, N., Chakma, S., Fatema, K., Azad, A., Jaman Chowdhury, M., Abu Sayid Mia, M. (2019). Alkali Enzymatic Extraction of Keratin Protein from Chicken Feather Waste in Bangladesh. *Iranian (Iranica) Journal of Energy & Environment*, 10(4), 235-241. doi: 10.5829/ijee.2019.10.04.02
- 31 Nur-E-Alam, M., Mia, M. A. S., Ahmad, F., & Rahman, M. M. (2020). An overview of chromium removal techniques from tannery effluent. *Applied Water Science*, 10(9). <https://doi.org/10.1007/s13201-020-01286-0>
- 32 Onchoke, K. K., & Sasu, S. A. (2016). Determination of Hexavalent Chromium (Cr(VI)) Concentrations via Ion Chromatography and UV-Vis Spectrophotometry in Samples Collected from Nacogdoches Wastewater Treatment Plant, East Texas (USA). *Advances in Environmental Chemistry*, 2016, 1–10. <https://doi.org/10.1155/2016/3468635>
- 33 Osasona, I., Adebayo, A. O., Okoronkwo, A. E., & Ajayi, O. O. (2015). Acid and Alkali Modified Cow Hoof Powder as Adsorbents for Chromium (VI) Removal from Aqueous aqueous phase. *Iranica Journal of Energy & Environment*. Published. <https://doi.org/10.5829/idosi.ijee.2015.06.04.07>
- 34 Paul, E.D., Nwoken, N.C., & Anumonye, F.U. (2018). Biosorption of Heavy Metals (Cd²⁺, Cr³⁺, Cu²⁺, Ni²⁺, Pb²⁺ And Zn²⁺) From Aqueous Solution onto Activated

- Carbon Prepared From Chicken Feather. *ATBU Journal of Science, Technology and Education*, 6, 194-205.
- 35 Rakhunde, R., Deshpande, L., & Juneja, H. D. (2012). Chemical Speciation of Chromium in Water: A Review. *Critical Reviews in Environmental Science and Technology*, 42(7), 776–810. <https://doi.org/10.1080/10643389.2010.534029>
- 36 Saha, G. C., & Ali, M. A. (2001). Groundwater contamination in Dhaka city from tannery waste. *Journal of Civil Engineering, the Institution of Engineers, Bangladesh*, 29(2), 151-166
- 37 Sarin, V., & Pant, K. (2006). Removal of chromium from industrial waste by using eucalyptus bark. *Bioresource Technology*, 97(1), 15–20. <https://doi.org/10.1016/j.biortech.2005.02.010>
- 38 Saucedo-Rivalcoba, V., Martínez-Hernández, A. L., Martínez-Barrera, G., Velasco-Santos, C., & Castaño, V. M. (2010). (Chicken feathers keratin)/polyurethane membranes. *Applied Physics A*, 104(1), 219–228. <https://doi.org/10.1007/s00339-010-6111-4>
- 39 Sayed, S., Saleh, S., & Hasan, E. (2005). Removal of some polluting metals from industrial water using chicken feathers. *Desalination*, 181(1–3), 243–255. <https://doi.org/10.1016/j.desal.2005.02.022>
- 40 Schrooyen, P. M., Dijkstra, P. J., Oberthür, R. C., Bantjes, A., & Feijen, J. (2001). Stabilization of Solutions of Feather Keratins by Sodium Dodecyl Sulfate. *Journal of Colloid and Interface Science*, 240(1), 30–39. <https://doi.org/10.1006/jcis.2001.7673>
- 41 Sharma, S., Gupta, A., bin Tuan Chik, S. M. S., Gek Kee, C. Y., & Poddar, P. K. (2017). Dissolution and characterization of biofunctional keratin particles extracted from chicken feathers. *IOP Conference Series: Materials Science and Engineering*, 191, 012013. <https://doi.org/10.1088/1757-899x/191/1/012013>

- 42 Sharmin, S., Mia, J., Miah, M. S., & Zakir, H. (2020). Hydrogeochemistry and heavy metal contamination in groundwaters of Dhaka metropolitan city, Bangladesh: Assessment of human health impact. *HydroResearch*, 3, 106–117. <https://doi.org/10.1016/j.hydres.2020.10.003>
- 43 Shavandi, A., Silva, T. H., Bekhit, A. A., & Bekhit, A. E. D. A. (2017). Keratin: dissolution, extraction and biomedical application. *Biomaterials Science*, 5(9), 1699–1735. <https://doi.org/10.1039/c7bm00411g>
- 44 Soares, R., Castro Carneiroa, M., Inês Couto Monteiro, M., de Souza Henrique Junior, S., Veronesi Marinho Pontes, F., Irene Dias Da Silva, L., Alcover Neto, A., & Erthal Santelli, R. (2009). Simultaneous speciation of chromium by spectrophotometry and multicomponent analysis. *Chemical Speciation & Bioavailability*, 21(3), 153–160. <https://doi.org/10.3184/095422909x466095>
- 45 Song, D., Pan, K., Tariq, A., Azizullah, A., Sun, F., Li, Z., & Xiong, Q. (2016). Adsorptive Removal of Toxic Chromium from Waste-Water Using Wheat Straw and Eupatorium adenophorum. *PLOS ONE*, 11(12), e0167037. <https://doi.org/10.1371/journal.pone.0167037>
- 46 Sun, P., Liu, Z. T., & Liu, Z. W. (2009). Chemically Modified Chicken Feather as Sorbent for Removing Toxic Chromium(VI) Ions. *Industrial & Engineering Chemistry Research*, 48(14), 6882–6889. <https://doi.org/10.1021/ie900106h>
- 47 Unceta, N., Séby, F., Malherbe, J., & Donard, O. F. X. (2010). Chromium speciation in solid matrices and regulation: a review. *Analytical and Bioanalytical Chemistry*, 397(3), 1097–1111. <https://doi.org/10.1007/s00216-009-3417-1>
- 48 Zahid, A., Balke, K. D., Hassan, M. Q., & Flegr, M. (2006). Evaluation of aquifer environment under Hazaribagh leather processing zone of Dhaka City. *Environmental Geology*, 50(4), 495-504, <http://dx.doi.org/10.1007/s00254-006-0225-1>

49 Zahir, H., & Ahmeduzzaman, M. (2012). A case study on the impact of the leather industries on the ground water aquifer in Bangladesh. *International Journal of Civil & Environmental Engineering*, 12 (5), 52-56.