

DUCKWEED AND ITS VARIOUS BIOTECHNOLOGICAL APPLICATIONS

Submitted in partial fulfillment of the requirements for the award of Bachelor of
Technology degree in Biotechnology

By

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This is to certify that this Project Report is the bonafide work of Ms. SATHIYASREE M (Reg. No. 39230041) and Ms. Rajalakshmi D (Reg. No. 39230034) who carried out the project entitled "DUCKWEED AND ITS VARIOUS BIOTECHNOLOGICAL APPLICATIONS" under our supervision, from August 2022 to April 2023.


Internal Guide

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Submitted the Viva Voce Examination held on 11.05.2023


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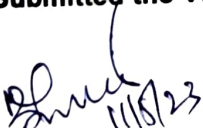

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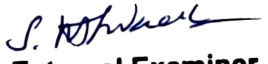
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DECLARATION

I, **SATHIYASREE M (Reg. No. 39230041)** hereby declare that the Project Report entitled **"DUCKWEED AND ITS VARIOUS BIOTECHNOLOGICAL APPLICATIONS"** done by me under the guidance of **Dr.Valli Nachiyar** (Internal guide) & **Dr.P.Prakash** (External guide) at **Sathyabama Institute of Science and Technology, Chennai** is submitted in partial fulfillment of the requirements for the award of Bachelor of Technology in Biotechnology.

DATE: 26.04.2023

PLACE: Chennai



SIGNATURE OF THE CANDIDATE

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ABSTRACT

The *Lemnaceae* family of duckweeds, which includes the genus *Lemna* and related species, contains the tiniest flowering plants which is a single, flat, oval leaf, technically a modified stem that floats on the surface of still-moving ponds, lakes, and sloughs. In this study, growth parameter for duckweed had been optimized. Since, duckweed has been known to have higher protein content it was extracted and used for the preparation of fish feed, where protein isolated from duckweed was used as feed for *Danio rerio*. And then cellulose was extracted and characterized from the duckweed, further coated with Superparamagnetic Iron Oxide Nanoparticles (SPIONs) and used for the removal of dye, antibiotics and heavy metals. Oil was also extracted and characterized using FTIR analysis.

Keywords: Duckweed; feed; protein; oil, cellulose

TABLE OF CONTENTS

CHAPTER NUMBER	TITLE	PAGE NUMBER
	ABSTRACT	i
	TABLE OF CONTENTS	ii
	LIST OF ABBREVIATIONS	v
	LIST OF FIGURES	vi
	LIST OF TABLES	vii
1.	INTRODUCTION	1
	1.1 Characteristics of Duckweed	1
	1.2 Applications	2
	1.2.1 Bioremediation	2
	1.2.2 Fish Feed	3
2.	LITERATURE SURVEY	6
	2.1 Duckweed and its nutritional components	6
	2.2 Duckweed in formulation of animal feed/ human foods	9
	2.3 Bioremediation application	11
	2.4 Duckweed in the production of biodiesel	14
3.	AIM AND OBJECTIVE	16
4.	MATERIALS AND METHODS	17
	4.1 Collection of duckweed	17
	4.2 Optimization of duckweed	17
	4.3 Extraction of protein cake from duckweed	17
	4.4 Characterization of the protein cake	17
	4.5 Preparation of fish feed	17
	4.6 <i>In-Vivo</i> study to determine the growth of fishes	18
	4.6.1. Collection and Maintenance of zebrafish	18
	4.6.2. <i>In-vivo</i> study using zebrafish	18
	4.6.3. Histopathological studies	18
	4.7 Extraction of cellulose from duckweed	19
	4.7.1 Coating of SPIONs over cellulose	19

4.7.2 Characterization of cellulose and SPIONs coated cellulose	19
4.8 Batch adsorption isotherm	20
4.8.1. Isotherm studies	20
4.9 Extraction of oil	21
4.10 Characterization of oil	21
5. RESULTS AND DISCUSSION	22
5.1 Collection and optimization of duckweed	22
5.2 Extraction and Characterization of Protein	22
5.3 <i>In-vivo</i> study using zebrafish	22
5.4 Histology studies	23
5.5 Characterization of cellulose and SPIONs coated cellulose	24
5.6 Batch Adsorption Isotherm	25
5.6.1 Optimization of adsorbate concentration: Cellulose as adsorbent	25
5.6.2 Optimization of adsorbate concentration: SPION- coated Cellulose as adsorbent	25
5.6.3 Optimization of adsorbent concentration: Cellulose as adsorbent	25
5.6.4 Optimization of adsorbent concentration: SPION- coated Cellulose as adsorbent	26
5.6.5 Optimization of pH - Cellulose as adsorbent	26
5.6.6 Optimization of pH – SPION coated Cellulose as adsorbent	26
5.6.7 Optimization of contact time: Cellulose as adsorbent	27
5.6.8 Optimization of contact time: SPION- coated Cellulose as adsorbent	27
5.7 Isotherm studies	27

	6. Extraction and characterization of oil	28
6.	SUMMARY	29
	CONCLUSION	29
	REFERENCES	30
	FIGURES AND TABLES	39

LIST OF ABBREVIATIONS

BCF	-	Bio Concentration Factor
BMI	-	Body Mass Index
BOD	-	Biochemical Oxygen Demand
Cd	-	Cadmium
COD	-	Chemical Oxygen Demand
EDX	-	Energy Dispersion X Ray Spectroscopy
FAME	-	Fatty Acid Methyl Esters
FTIR	-	Fourier Transform Infrared Spectroscopy
H ₂ SO ₄	-	Sulphuric Acid
LC-PUFA	-	Long-Chain Polyunsaturated Fatty Acid
Pb	-	Lead
PO ₄ ³⁻	-	Phosphate
RuBisCO	-	Ribulose-1,5-Bisphosphate Carboxylase
SEM	-	Scanning Electron Microscopy
TAG	-	Triacylglycerols
TDS	-	Total Dissolved Solids
TPC	-	Total Plate Count
UV-Vis	-	Ultraviolet Visible Spectroscopy
VFA	-	Volatile Fatty Acids
VFA	-	Volatile Fatty Acids
XRD	-	X Ray Diffraction Spectroscopy

LIST OF FIGURES

FIGURE NUMBER	TITLE	PAGE NUMBER
5.1	Optimization study	38
5.2	Observation of duckweed growth for 30 days	39
5.3	Extraction and Characterization of protein	41
5.4	Different types of fish feed	42
5.5	Graphical representation of a. Length, b. Weight, c. BMI of the zebrafishes.	43
5.6	H & E staining: Histology images of intestine of zebrafish exposed to a. Test 1 (Duckweed feed), b. Test 2 (duckweed orange feed)	44
5.7	H & E staining: Histology images of liver of zebrafish exposed to a. test 1 (Duckweed feed), b. test 2 (duckweed orange feed)	44
5.8	Sudan B Black staining: Histology images of stomach of zebrafish exposed to a. test 1 (Duckweed feed), b. test 2 (duckweed orange feed)	45
5.9	Sudan B Black staining: Histology images of liver of zebrafish exposed to a. test 1 (Duckweed feed), b. test 2 (duckweed orange feed)	45
5.10	Characterization of cellulose	46
5.11	Characterization of SPIONs coated cellulose	47
5.12	Optimization of cellulose	48
5.13	Optimization of SPIONs coated cellulose	49
5.14	Isotherm studies for cellulose	50
5.15	Isotherm studies for SPIONs coated cellulose	51
5.16	a. Two phases of oil (Aqueous phase and organic phase), b. Fourier-Transform Infrared Spectroscopy (FTIR) of oil	52

LIST OF TABLES

TABLE NUMBER	TITLE	PAGE NUMBER
1	Removal of various adsorbates using cellulose as adsorbent	52
2	Removal of various adsorbates using SPION- coated cellulose as adsorbent	52

CHAPTER – 1

INTRODUCTION

The monocotyledonous water plant family *Lemnaceae*, also referred to as water lens or duckweed, has the quickest growth rates of any flowering plants which is divided into 36 different species over five genera, including *Spirodela*, *Landoltia*, *Lemna*, *Wolffiella*, and *Wolffia* (Landolt, 2000; Landolt and Kandeler, 1987; Ziegler *et al.*, 2015; Sree *et al.*, 2016; Sree *et al.*, 2015b, Edelman *et al.*, 2020). Due to the high rate of biomass production, duckweeds can be used for a variety of practical purposes in biotechnology and the sustainability of food and feed (Landolt and Kandeler, 1987; Kaur *et al.*, 2018; Sonta *et al.*, 2019; Chen *et al.*, 2018; Zhao *et al.*, 2015; Appenroth *et al.*, 2017, 2018; Ren *et al.*, 2018). The taxonomy of these plants must be understood in order to optimize duckweed biotechnologically and for commercial use because of the reduction in size. *Lemnaceae dumort* is the family that includes duckweeds. Although it has been demonstrated convincingly that the *Lemnaceae* family and the *Araceae* family are closely related (Cusimano *et al.*, 2011; Nauheimer *et al.*, 2012; Choi *et al.*, 2017), it is discussed that it is not important to include the *Lemnaceae* family under the subfamily *Lemnoideae* in the *Araceae* family and that this group can be treated according to taxonomy. The family has been divided into the *Wolffioideae* (members without roots) and *Lemnoideae* subfamilies based on the morphological characteristics (members with varying numbers of roots). Normally, since taxa are paraphyletic, they would not be considered to be so (Bog *et al.*, 2019).

1.1 CHARACTERISTICS OF DUCKWEED

Duckweeds are well known as efficient resources for removing contaminants from wastewater because they can withstand harsh environments. They effectively accumulate pollutants by adsorption or absorption to filter sewage. Duckweeds are also found to remove impurities. Elimination of ammonium is one of the essentials for wastewater purification as it worsens eutrophication in ponds and creates nitrates in groundwater. In order to store ammonium ions as a helpful supply of nitrogen, *Landoltia punctata* takes ammonium from water. The ecology suffers when boron, a by-product of industrial manufacturing, is concentrated too much. Boron removal from water has also been credited to *L. gibba*. In duckweeds, boron is adsorbed to apiogalacturonans in the cell wall (Baek *et al.*, 2021).

Owing to its quick growth and strong nutrient removal capacity, *Lemna minor*, a member of the genus *Lemna*, is the species that is most prevalent and the subject of substantial research in wastewater treatment. *Lemna minor* can quadruple its biomass in two days if the climate is right and the growth medium's nutrients are balanced. *Lemna minor* takes up a lot of phosphorus, which is then stored in its body mass. However, compared to nitrogen, duckweed grows best with very little phosphorus. The duckweed prefers to absorb phosphorus in the form of phosphate (PO_4^{3-}) instead. A typical duckweed's dry mass contains 0.03 to 2.8% phosphorus while containing 0.8 to 7.8% nitrogen (Sharma and Kaur, 2017).

Duckweed can store large amounts of phosphorus in its biomass, which allows the plant to continue growing in phosphorous-deficient conditions. When duckweed decays, the phosphorus that was held in the plant biomass is easily accessible in the water. Literature demonstrates that *Lemna minor* has a variable ability for phosphorus absorption under various environmental circumstances. At phosphorus concentrations of 4 and 22 mgL^{-1} of growth media, duckweed grows most effectively.

In order to bioremediate domestic and industrial wastewater, duckweeds can accumulate heavy metals such as arsenic, chromium, lead, cadmium, cobalt, copper, selenium, nickel and zinc. To manage the oxidative stress brought on by heavy metals and lessen damage, they feature an enzymatic antioxidant system. By triggering anti oxidative systems, duckweeds reduce the stress that heavy metals inflict on the body and interfere with nutritional absorption. Duckweeds' chelating properties can also reduce the stress brought on by heavy metals like chromium (IV) (Zhou *et al.*, 2019).

1.2 APPLICATIONS

1.2.1 BIOREMEDIATION

A developing agricultural plant known as duckweed is utilized in the manufacture of biofuels, bio plastics, and even human food. It is also used to remediate wastewater and in phytoremediation. Duckweed has received a great deal of attention because of its distinctive traits, which include small size, quick life cycles, and simplicity of aseptic production. Various cellulose rich products have been used as adsorbents for the remediation. Cellulose was extracted and then it was coated with nanoparticles to observe the bioremediation using adsorptions and isotherm studies. The hulls of sunflower seeds were also used for the effective removal of ammonium from aqueous solutions. The Langmuir model also showed that 58% of

ammonium ions in 50 mg/L concentrations and 3 g/L sunflower seed hulls at pH 7 were removed within 15 min (Ismail and Hameed, 2016). Sayah *et al.* (2018) reported that the metal-removal capacity of cystamine-modified cotton increased by ~200% and of polyamines-modified cotton by ~400% for Cd(II), and Pb (II) metal ions.

It was one of the earliest plant models known to academia. Due to the great effectiveness of nitrogen and phosphorous absorption as well as the inexpensive investment and operating costs, duckweed-based wastewater treatment has been widely used. Duckweed biomass, which can produce up to 100 tonnes of dry matter per hectare per year, has lately been suggested as a sustainable and renewable feedstock (Cao *et al.*, 2018).

According to the study's findings in Iwano *et al.* (2020), a sewage treatment system that maximises biomass production without compromising treatment goals can be suggested. It would include primary sedimentation, a duckweed pond, and a tertiary treatment unit. To maximise duckweed development, the size and operational characteristics of the duckweed pond should be chosen primarily based on the nutrients present in the influent water.

Duckweed that floats freely, such as *Spirodela polyrhiza* and *Lemna minor*. Both were noted to be extremely effective duckweeds for phytoremediation due to their affordability, lengthy storage capabilities, low chemical and volume of biological sludge. Additional benefits include its portability, adaptability to various climatic situations, and quick reproduction rates. For *Lemna minor* and *Spirodela polyrhiza*, respectively, the percentage reduction in COD (82.2% and 68.8%), BOD (84.3% and 63.8%), phosphate (63.8% and 59.02%), ammonia nitrogen (75.4% and 55.9%), and nitrate (79.1% and 55.4%) was interesting. During the study period, duckweeds grew at their fastest rates at the pH range of 7-8 (Patel and Kanungo, 2010).

1.2.2 FISH FEED

In various Asian nations, including Laos, Thailand and Cambodia, duckweeds have historically been used as a source of human food. The starch, protein, fat, mineral, vitamin, and phytosterol contents, as well as the amino acid and fatty acid spectra, of representatives from all five known genera, with a focus on *Wolffia*, including all 11 species of the genus.

In diets based on cereal grains, mature fowl can use duckweed as a vegetable protein substitute, however very young birds had a slight weight loss as a result.

Duckweed is a little less effective protein and energy source for pigs than soybean meal. Duckweed meals as supplements to forages given to ruminants have not received much research, although there seems to be a lot of potential for their use as a source of minerals (especially P) and nitrogen. For the purpose of giving protein directly to the animal, duckweed protein must be treated to prevent microbial oxidation in the rumen. A diet for ruminants that includes both fresh duckweed and agricultural leftovers appears to provide a nutritional balance that can optimize the rumen (Leng *et al.*, 1995).

Various researchers such as Barbarino and Lourenço, (2005); Najafabadi *et al.* (2015); Nieuwland *et al.* (2021) have demonstrated methods for the extraction and purification of lipids from the microalgae by using a biphasic system was formed by adding water and hexane to the extracted crude oil and also extraction of protein from algae by The various extraction techniques (water exposure, grinding, etc.), protein precipitation using various concentrations of 25% trichloroacetic acid, and protein measurement using two separate techniques and two distinct protein standards were assessed.

For the fish and poultry industries, the plant might be a potential replacement in order to supply the rapidly expanding need for feed. The production of biomass, lipid, protein, carbohydrate, and mineral (Ca, Mg, Fe, Mn, and Zn) contents of *L. minor* are all examined in various study in relation to different nutrient concentrations (ppm). The altering N and P concentrations had a significant impact on the aforementioned metrics. In comparison to the control tank, the 30 ppm nitrogen and phosphorous tank produced the highest biomass yield of 172 g/m² per day. Higher amounts of protein, lipids, and carbohydrates were found in samples with nitrogen and phosphorous concentrations of 30 ppm, 20 ppm, and 10 ppm, respectively. Mineral concentrations like Ca, Mg, Fe, Mn, and Zn rose in environments with 20 ppm P and all N. While nitrogen and phosphorous was less effective at raising the mineral contents, it was more effective at increasing the content of proteins, carbohydrates, and lipids. Increases in N and P concentrations were positively correlated with the nutritional makeup of *L. minor* (Ullah *et al.*, 2022).

Continuous production is necessary for the utilization of *Lemna* species as prospective constituents in aqua-feed. Understanding this plant's dietary and environmental needs is essential for its sustainable cultivation. The culture medium has an impact on a plant's nutritional value as well. In various natural and laboratory

circumstances, duckweed clones grew at varying rates. Numerous investigations have demonstrated the presence of *Lemna* sp. in effluent water, septage-fed ponds, and domestic wastewater. For the production of commercial aqua-feed, *Lemna* sp. must be grown in clean water with a regular manuring schedule (Chakrabarti *et al.*, 2018).

CHAPTER – 2

LITERATURE SURVEY

2.1 DUCKWEED AND ITS NUTRITIONAL COMPONENTS

Duckweed is a surface-floating macrophyte that grows profusely in still, nutrient-rich tiny ponds, ditches, and marshes as well as in slowly moving water bodies and are common in tropical and subtropical regions. The low fiber content of the plant has a favourable effect on digestibility when used in animal feed since the entire plant body is made up of metabolically active non-structural tissue. Duckweed concentrates the minerals and produces protein while growing in water that has relatively high levels of N, P, and K. Many essential and non-essential amino acids (such as histidine, leucine, valine, isoleucine, methionine, phenylalanine, lysine, arginine, threonine, and tyrosine) as well as polyunsaturated fatty acids, beta-carotene, and xanthophylls have been documented to be present playing as a possible constituent as aquafeed (Chakrabarti *et al.*, 2018).

Certain varieties of duckweed have high protein contents nearly 45%, which supply all the required amino acids as per FAO recommendations and can aid in the growth and development of the human body. Additionally, duckweed contains a variety of functional and nutrient-rich carbohydrates like cellulose, starch and trace amounts of hemicellulose, and pectin. Additionally, duckweed contains a lot of vitamins, minerals, and phytochemicals, including lutein and beta-carotene, which are favourably linked to a lower risk of developing a number of chronic diseases. Duckweed can be utilized to extract proteins and phytochemicals for use as functional ingredients, or it can be used to partially replace wheat flour in traditional cereal-based diets (Xu *et al.*, 2022).

Primary metabolites like carbohydrates, proteins, and lipids are highly concentrated in duckweed. Protein content for *L. punctata* and *W. arrhiza* was 0.140.03 and 0.170.01 g/g dry biomass, respectively, and total lipid content was 0.060.01 and 0.090.01 g/g dry biomass. Particularly, the bulk of the FAs, or 58.38% of the total Fatty Acid Methyl Esters (FAME) for *L. punctata* and 44.46% of the total FAME for *W. arrhiza*, were polyunsaturated. They made up the bulk of FAME in *L. punctata* and *Wolffia arrhiza*, respectively, accounting for 44.93 and 22.17% of the total FAME in each species. Long-chain FAs were obtained in the majority of size 13-22 C (Sembada and Faizal, 2022).

Demann *et al.*, (2022) examined the nutritional content of three different batches of duckweed. *Lemna minuta* and *Lemna minor* having 17.5% crude protein (batch A), *Spirodela polyrrhiza* with 24.6% crude protein (batch B), and *Lemna obscura* containing 37.0% crude protein (batch C) were all mixed together in them. Treatment diets included 25, 50, and 75% of batch C along with 50% each of batches A and B. Broiler chickens (Ross 308) were fed all of the diets when they were 21 to 27 days old. When compared to the control, feed consumption was lower in the diets that contained 50% and 75% of batch C (109.3 and 74.9 g/day, respectively). Across duckweed batches, there were considerable differences in the standardized ideal digestibility of crude protein and amino acids, with values for methionine ranging from 49.9 to 90.4%. Batch C consistently had the maximum digestibility for all amino acids, while batch A consistently had the lowest. For batches A, B, and C, the varying tannin amounts were 2943, 2890, and 303 mg/kg, respectively. There was a considerable variation in the apparent ideal digestibility of phosphorus between all batches (50.8-78.9%). For broiler chicks, duckweed can be fed as a source of protein.

Duckweeds make excellent options for wholesome and secure meat protein alternatives for people. The European food market is likewise predicted to contain duckweeds. It is anticipated that switching to plant protein from animal protein will reduce energy use and greenhouse gas emissions. Iron homeostasis and folic acid concentration are maintained while the danger of iron deficiency is decreased when *W. globosa* is used as a meat substitute. Clinical nutrition investigations have shown that duckweeds are comparable to peas and cheese in terms of essential amino acid and vitamin B12 levels. Similar to the sodium/potassium ratio and fiber content, duckweeds have enough iron and zinc to meet the required intake. The World Health Organization (WHO) guidelines are met by the amino acid composition of *Wolffia sp.*, *Wolffiella sp.*, *L. minor*, *L. gibba*, *S. polyrrhiza*, *W. microscopica*, *Landoltia punctat*, and *Wolffiella hyaline* have higher ratio of ω -3 to ω -6 fatty acids making it ideal for food and feed.. However, it should be mentioned that consuming oxalic acid-rich duckweed species may result in kidney stones (Baek *et al.*, 2021).

According to reports, duckweed provides 20% to 30% more protein than cereal. Ribulose-1,5-Bisphosphate Carboxylase (RuBisCO), the primary protein in duckweed, provides a rich supply of all nine necessary amino acids. Also, because of its nutritional content, in vitro digestibility, and lack of allergens, RuBisCO protein is a strong choice as a functional meal. RuBisCO protein also possesses strong foaming ability, good

gelling and emulsification capabilities, and high solubility. Carotenoids, polyphenols such flavonoids and anthocyanins, 4% to 7% fat, 4% to 10% starch, and 4% to 10% fat are also present in duckweed. Duckweed contains between 60% and 63% of its total fatty acid content in polyunsaturated fatty acids, and between 41% and 47% of α -linolenic acid. Duckweed's starch contains 26.5% amylopectin and 35.7% amylose (Yahaya *et al.*, 2022; Chakrabarti *et al.* 2018; Lee *et al.*, 2016).

Up to 40% of the dry weight of another species called *Wolffia globosa* is made up of protein. After getting dried duckweed Protein Extract (PE) using Ultrasound-Assisted Extraction, Protein Concentration (PC) and protein solution (pH 3.5) were created by isoelectric precipitation (UAE). Alcalase enzyme was used to hydrolyze the PC and produce Protein Concentrate Hydrolysate (PCH). By lowering populations of *Candida albicans* and *Vibrio parahaemolyticus* by 0.43 and 1.31 log reductions (66.21 and 99.98%, respectively), PCH demonstrated antibacterial activities among all fractions. Moreover, at pH 8, the PE and PS had high solubilities of 90.49% and 86.84%, respectively. The PE, PC, PCH, and PS all had major molecular weights (Mw) of 25, 45, 63, and 100 kDa, with the PCH's Mw being somewhat lower (5 kDa). The two main amino acids found in the PCH, which contained the highest total amino acid content, were aspartic acid and glutamic acid. The findings demonstrated duckweed protein and hydrolysate's antibacterial and functional capabilities for the first time and demonstrated their potential for further development as functional food ingredients (Duangjarus *et al.*, 2022).

Also, when cultivated in strong light, duckweed has very high amounts of zeaxanthin and is rich in α -tocopherol and carotenoids. Moreover, duckweed exhibits a rare combination of rapid growth and high zeaxanthin levels. Moreover, duckweed has high phenolic contents. Duckweeds are a good source of protein since they contain all the necessary amino acids that people need. On comparison with soybean plants, which exclusively store protein in their seeds, Duckweed plants produce more protein overall per growing area (i.e., up to 20 times more) for a single layer of duckweed due to the fact that protein is stored throughout the entire plant. Duckweeds feature a nutrient-rich ratio of polyunsaturated ω -3 to ω -6 fatty acids, which have opposing effects on the initiation and termination of immunological responses (Polutchko *et al.*, 2022).

Certain nations in South Eastern part of Asia, notably Laos, Thailand, and Myanmar, regularly consume duckweed as a vegetable known as "Khai-Nam" The

predominant species utilised for human consumption are *Wolffia globosa* and *Wolffia arrhiza*. In spite of its enormous potential as a source of plant protein for human nutrition, duckweed is not commonly consumed in Western nations (Appenroth *et al.*, 2017; De Beukelaar *et al.*, 2019).

2.2 DUCKWEED IN FORMULATION OF ANIMAL FEED/ HUMAN FOODS

Ice creams were formulated with *L. minor* and its nutritional value and microbiological makeup were studied. The results of the nutritional analysis showed that the ice cream with 2% of dried *L. minor* had a significantly higher protein, fiber, and ash content than the control. Also, a Total Plate Count (TPC) was carried out for the microbiological examination of the ice cream made from duckweed. The findings indicated that 2% dried *L. minor* in prepared ice cream included a trace level of bacteria with colony of 3.82 CFU/g. Overall, the nutritional, microbiological, and metabolites profiles of the meal made from the *L. minor* plant show that duckweed is an excellent contender for future food (Yahaya *et al.*, 2022).

A study was conducted where five grades of duckweed included in the meals of the fish: 0% (LM0, control), 5% (LM5), 10% (LM10), 15% (LM15), and 20% (LM20) (LM20) used. The LM15 and LM20 diets for fish had significantly higher final weights and specific growth rates than other diets. In fish fed diet LM20, the feed conversion ratio was at its lowest. The LM0 therapy resulted in significantly increased amylase activity. The amount of duckweed in the diet had a linear relationship with the total protease, trypsin, and chymotrypsin activities. Carp fed diets LM15 and LM20 contained much more protein and essential amino acids than other diets. Fish feed diets made of duckweed had considerably higher lipid content than control fish. Duckweed incorporation in the diet and carp n-3 Long-Chain Polyunsaturated Fatty Acid (LC-PUFA) concentration were found to be directly correlated. By increasing dietary duckweed, the amount of desaturated and elongated linolenic acid (18:3n-3) products, such as 20:4n-3, 20:5n-3, 22:5n-3, and 22:6n-3, gradually rose. In fish feed duckweed, the amounts of monounsaturated fatty acids and n-6 PUFA dramatically decreased. Carp given diets LM10, LM15, and LM20 expressed more fads2d6, elovl2, elovl5, and elovl2 than control fish did. The addition of *L. minor* to the diet increased the protein, lipid, amino acid, and n-3 PUFA levels, improving the nutritional value of carp (Goswami *et al.*, 2022).

Fiordelmondo *et al.* (2022) made experiment using three experimental meals (L1, L2, L3) for rainbow trout were made with duckweed (*Lemna minor*) meal as a 10%, 20%, and 28% protein supply, respectively. Increased amounts of duckweed were added to the remaining protein sources to create the isonitrogenous (41%) and isolipidic (20%) diets, which served as the control diet. In 12 tanks, 540 fish with the mean body weight 124.5 +/- 0.7 g were randomly distributed among the four different diets. After 90 days of fishing, the fish were weighed to determine the most notable productive performances, fillet quality, and fatty acid profile. The final body weight of L3 fish was substantially lower ($p < 0.05$) than that of L1 trout (340.53 g), L2 trout (339.42 g), and LC trout (348.80 g) (302.16 g). The ultimate mean length, weight gain, specific growth rate, and food conversion rate all showed similar tendencies. Duckweed incorporation impacted somatic indices. Diets had no discernible changes on the fatty acids in L1, L2, or L3 of the fillet compared to LC. Based on this research, duckweed meal made from *Lemna minor* can be added to rainbow trout feed at a 20% protein substitution without having a negative impact on the fish's ability to grow.

Male CF-1 mice were fed semi-purified meals containing casein or diets in which 10% or 25% of the casein was replaced with duckweed protein in Roman *et al* (2021). The mice's food intake, growth, and final organ and adipose tissue mass were all measured. According to proximate analysis, the employed duckweed protein preparation included 39.9% protein (w/w), all of the required amino acids, with MET acting as the limiting amino acid. Average growth rates for the treatment groups were 0.21 g/day for the control diet, 0.24 g/day for the 10% duckweed protein diet, and 0.25 g/day for the 25% duckweed protein diet, respectively. Although both duckweed protein diets had daily food intakes that were 6.5% to 8.0% more than the control diet, there was no difference in feeding efficiency between the diets. There were no appreciable differences in the relative weights of the colon, liver, spleen, kidneys, heart, and epididymal fat between the therapy groups. The findings of this study demonstrate that laboratory mice's development rate and ultimate organ and adipose tissue weights are unaffected by substituting up to 25% of their diet's casein with duckweed protein.

8 Isonitrogenous (about 35% crude protein) and isocaloric (roughly 4.2 kcal g⁻¹) diets were created with raw and fermented duckweed (*Lemna polyrrhiza*) leaf meal included at levels of 10%, 20%, 30%, and 40%. A specific bacterial strain (*Bacillus* sp.) that was isolated from the intestine of a carp (*Cyprinus carpio*) that has

extracellular amylolytic, cellulolytic, proteolytic, and lipolytic activities was used to ferment leaf meal for 15 days at 37 °C. After fermentation, tannin and phytic acid levels in leaf meal decreased from 1.0% to 0.02% and 1.23% to 0.09%, respectively, and the fiber content dropped from 11.0% to 7.5%. In the fermented leaf meal, sugars, protein, and fatty acids are more easily obtainable. The response of rohu, *Labeo rohita*, fingerlings fed the experimental diets for 80 days was evaluated in contrast to fish fed a reference diet based on fish meal. When 30% fermented *Lemna* leaf meal was added to the diet, rohu fingerlings performed at their best in terms of growth response, food conversion ratio, and protein efficiency ratio. The development and feed utilisation rates of fish fed diets containing fermented leaf meal were typically higher than those of fish fed diets containing raw leaf meal (Bairagi *et al.*, 2002).

2.3 BIOREMEDIATION APPLICATION

Duckweed effectively reduces water eutrophication by utilising nitrogen, phosphorus, and inorganic nutrition from wastewater. Microorganisms are crucial to these processes because they help duckweed thrive and increase its resistance to stressors. Second, duckweed is a viable bioenergy feedstock because it can quickly accumulate significant amounts of starch, meeting the rising demand for the manufacturing of bioethanol. To increase the efficiency of turning starch into bioethanol, pretreatment conditions before fermentation can be improved. Duckweed is a suitable source of protein for food or animal feed because it has an amino acid profile similar to whey protein, which is readily absorbed and digested by people and other animals. Duckweed has also demonstrated significant promise as a natural plant factory for the manufacture of medications and dietary supplements (Liu *et al.*, 2021).

The pollutants removal from home wastewater using *Lemna gibba*, a promising duckweed. Sedimentation and duckweed tanks make up the wastewater treatment system for this study's duckweed. Both sedimentation and duckweed tanks' wastewater media samples had their temperature, pH, BOO, COD, TSS, TDS, EC, NH₃-N, NO₂-N, NO₃-N, faecal coliform, and total bacteria counts assessed. For the sedimentation tank, the average removal efficiency for TSS, BOO, COD, NH₃-N, NO₂-N, NO₃-N, TN, TP, TDS, EC, fecal coliform bacteria, and Total bacteria count were correspondingly 55%, 27%, 24%, 26%, 30%, 27%, 19%, 32%, 6%, 4%, 50%, and 39%. On the 66th day of operation, the average removal efficiencies for BOO, COD, TSS, TDS, EC, NH₃-N, NO₂-N, NO₃-N, TN, TP, faecal coliform, and total bacteria count

for the duckweed tank were 60%, 54%, 63%, 9%, 5%, 63%, 64%, 63%, 63%, 65%, 96%, and 98%. The tissue of the plants was examined. Duckweed growing in the system absorbed nitrogen and phosphorus at rates of 0.173 g/m² d and 0.032 g/m² d, respectively. About 1.2 g/m² d was the average growth rate of duckweed that was seen. The amount of wastewater present and the local climate directly affected the development of the duckweed and the removal of nutrients from the tank (Hashimi and Joda, 2010).

The potential of duckweed (*Lemna gibba*) in water containing heavy metals (Pb and Cd) at various pH conditions and metal loads. Three different Pb and Cd strengths of 2, 5 and 10 mg/L respectively were used in the system, each with a different pH value, to measure changes in metal concentration and metal uptake yield (5, 7 and 9). Pb and Cd had clearance rates of 60.1% (2 mg/L at pH 9) and 98.1% (10 mg/L at pH 7), respectively. For lead, the clearance rates were 41.6% (10 mg/L at pH 9) and 84.8% (2 mg/L at pH 7). The best metal removal was demonstrated by the duckweed setup at pH 7. In all cases, the rate of metal removal exhibited an inverse relationship with pH ($r^2 > 0.60$). The equivalent metal absorption yield for BCF Pb and BCF Cd was 403-738 and 445-616, respectively, per unit of dry biomass (q_m). The doses (mg/L) 5 and 10 at pH 5 are recommended by the q_m as the optimal combinations for the best removal. In light of these findings, *L. gibba* may be a viable option for the removal of heavy metals from polluted water bodies (Verma and Suthar, 2015).

Sekomo *et al.* (2012) has evaluated the use of duckweed and algae ponds as a post-treatment for textile wastewater under the presumption that variable pond characteristics, such as pH, redox potential, and dissolved oxygen, would lead to different heavy metal removal efficiencies. Two lab-scale systems, each consisting of three ponds in succession and seeded with either algae or duckweed (*Lemna minor*), were run for seven days at two different metal loading rates and light cycles (16/8 h light/darkness and 24 h light, respectively). Regardless of the metal loading rate and light regime, the Cr removal rates for the algal ponds were 98% and 94%, respectively. For Zn removal, pond-type effect could be shown. Zn removal performed well (70%) under the 16/8 light regime at low metal loading rates, but fell below 40% at higher metal loading rates. With the greater metal loading rate, but under a 24-h light regime, the removal efficiency increased once more to 80%. With removal efficiencies of 36% and 33% for lead (Pb), 33% and 21% for Cadmium (Cd), and 27% and 29% for Copper (Cu) in the duckweed and algal ponds, respectively, Pb, Cd, and Cu all displayed

somewhat similar patterns. This suggests that neither of the treatment methods is particularly effective as a polishing step for getting rid of these heavy metals.

Al-Khafaji *et al.* (2018) conducted a study on the removal of heavy metals from industrial effluent using *Lemna minor* system under constructed wetland system. For the clarifier and duckweed tanks, this plant was run with constant hydraulic retention times of 2 h and 10 days, respectively. The ability of duckweed to absorb Cd, Cr, Ni, and Pb from mixed industrial wastewater has been researched. According to the experiment findings, Cd, Cr, Ni, and Pb had average removal efficiencies of 44.93%, 32.26%, 74.48%, and 79.1, respectively.

During the course of two weeks, (Hodaifa *et al.* 2019) worked on the phytoextraction of copper, iron, zinc, lead and nickel from landfill leachate by duckweed (*L. minor*). Furthermore calculated were the bioconcentration factor and clearance efficiency. The study's findings demonstrated that *L. minor* greatly lowered the level of heavy metals in the landfill leachate. *L. minor* had a removal effectiveness of more than 70% for all the metals in landfill leachate, with copper having the highest value at 91%. Both the chemical and biological oxygen demands (COD and BOD) were reduced by 39% and 47%, respectively. The pH, total suspended solids (TSS), and Total Dissolved Solids (TDS), on the other hand, were all lowered by 13%, 33%, and 41%, respectively. Based on the Bio Concentration Factor (BCF) value, which was less than 1, the plant is a modest accumulator of these heavy metals, with the highest values for copper (0.84) and lead (0.81). Duckweed (*L. minor*) was named as a competitive alternative for the treatment of contaminants present in landfill leachate waste water. Fallahizadeh *et al.* (2019) showed that the combined solutions had a better removal effectiveness of heavy metals by duckweed than the individual solutions. While the removal rate remained constant in the combined solutions, the increased starting concentration also decreased the removal efficiency in the separate solutions. Moreover, the mixed solutions eliminated the heavy metals more quickly. Lead, cadmium, nickel, and chromium were removed in order from different solutions at the maximum removal time. Such a removal rate sequence was not seen in the combined heavy metal solutions.

Lemna minor L. was researched for its potential to be used in phytoremediation to recycle lead-contaminated water. The prepared stationary and recirculated set-ups at 3, 6, and 9 d used duckweed as a bioaccumulator of lead (Pb). The pH, BOD, DO, turbidity, temperature, and other physicochemical traits of the influent and effluent

were contrasted. In the recirculated setup, the greatest bioaccumulation of 62.8% was attained at 3 days. When improved effluent is taken into account, the analysis's findings showed that duckweed has the potential for phytoremediation. The Pb content in the effluent of the recirculated setup with duckweed was 0.93 mg/L, which was significantly lower than the original influent value of 2.5 mg/L. The study's findings suggested that duckweed may be a useful plant for removing lead (Pb) from water, which has important ramifications for treating water contaminated with heavy metals from a variety of sources (Ubuza *et al.*, 2020).

Kara *et al.* (2005) demonstrated the usage of *Lemna minor* (duckweed) to remove Cd from solution. In these systems, duckweed seems to be very beneficial. In just 4 days, the plant was able to remove 75–85% of the Cd from 100 mL of both types of waste streams, which had 3.0-7.0 mgL⁻¹ of the metal at an ideal pH of 6.5. Due to its widespread distribution, quick rate of development, simplicity of harvest, wide range of temperature tolerance, and prolonged growing and harvesting periods, it will be great candidate for wastewater treatment.

Spirodela polyrhiza and *Lemna minor* were noted to be extremely effective duckweeds for phytoremediation due to their affordability, lengthy storage capabilities, low chemical and volume of biological sludge. Additional benefits include its portability, adaptability to various climatic situations, and quick reproduction rates. For *Lemna minor* and *Spirodela polyrhiza*, respectively, the percentage reduction in COD (82.2% and 68.8%), BOD (84.3% and 63.8%), phosphate (63.8% and 59.02%), ammonia nitrogen (75.4% and 55.9%), and nitrate (79.1% and 55.4%) was interesting. During the study period, duckweeds grew at their fastest rates at the pH range of 7-8 (Patel and Kanungo, 2010).

2.4 DUCKWEED IN THE PRODUCTION OF BIODIESEL

Duckweed may be used to create the potential alternative fuels ethanol, butanol, and biogas, which can help reduce reliance on scarce crude oil and natural gas. High rates of nutrient (nitrogen and phosphorus) uptake, a high biomass output, and tremendous potential as a substitute feedstock for the generation of fuel ethanol, butanol, and biogas are all benefits of this aquatic plant. The production of starch through photosynthesis and the consumption of starch due to metabolism are the two main mechanisms that influence the accumulation of starch in duckweed biomass. Based on available technology, increasing photosynthesis comes at a relatively high

cost. To prevent starch degradation, significant research has been done. Selection of duckweeds, optimization of duckweed biomass production, improvement of starch accumulation in duckweeds, and utilization are some future research needs in production of biodiesel (Cui and Cheng, 2015).

Mu *et al.* (2020) have argued that 1% H₂SO₄ acid hydrolysis was preferable for pretreatment of duckweed biomass. 169.30 mL g⁻¹ dry weight of maximum hydrogen generation was measured at a temperature of 35 °C and a starting pH of 7.0. Volatile Fatty Acids (VFAs), such as acetate and butyrate, were found in the waste after the dark fermentation, with concentrations of 1.04 g L⁻¹ and 1.52 g L⁻¹, respectively. When *Chlorella saccharophila* FACHB-4 was grown mixotrophically using garbage as fuel, the maximal microalgal biomass and lipid production, respectively, were about 2.8 and 33 times larger than during autotrophic development. A green way for producing biofuel was the simultaneous creation of biohydrogen and waste use.

Souto *et al.* (2019) have discussed that In the meantime, the release of CO_x, SO_x, hydrocarbons, and particulate matter from fossil fuels has resulted in significant environmental pollution issues. Utilizing bioenergy instead of fossil fuels might significantly lower emissions of exhaust gases and particulate matter. Therefore, it is crucial to develop and use renewable energy sources, particularly bioenergy. It has been suggested that duckweed be used as a feedstock for the production of bioenergy products such biogas, ethanol, butanol, and hydrogen. As a promising bioenergy plant, it offers a number of distinguishing qualities, including as better output and no rivalry with agricultural crops.

CHAPTER – 3

AIM AND OBJECTIVE

3.1 AIM

To obtain biotechnological products such as protein, cellulose and oil from duckweed and to find its various application such as animal feed (Fish feed), cellulose, SPIONs over cellulose for environmental remediation and oil for fuels.

3.2 OBJECTIVE

- ✓ To collect and the optimize the growth of duckweed.
- ✓ To extract and characterize the following products from duckweed: protein, cellulose and oil.
- ✓ To prepare animal feed using protein lysate and to study their impact on fish growth.
- ✓ To coat the cellulose with SPIONs and to characterize.
- ✓ To study pollutant removal using Batch Adsorption studies and Isotherm studies for Cellulose and SPIONs coated cellulose.

CHAPTER – 4

MATERIALS AND METHODS

4.1 COLLECTION OF DUCKWEED

Initially, duckweed were purchased from Tarun fish farm, Chennai and it was washed thoroughly with distilled water, used for various studies.

4.2 OPTIMIZATION OF DUCKWEED GROWTH

The growth of the duckweed was determined with a control and test. Two trays were used for the study, one with water and other with the medium. The trays were covered with a cover punched with several holes on it. Initially, the count of the duckweed were noted and calculated for 10th, 20th and 30th day.

4.3 EXTRACTION OF PROTEIN CAKE FROM DUCKWEED

To eliminate the dirt and dust that were on the duckweed, it was thoroughly cleaned with distilled water. 0.1N sodium hydroxide and 170 mL of distilled water were combined with washed duckweed. To completely pulp the leaves, the mixture was blended for 1 minute at high speed after 30 seconds at low speed. A pH of 3.5 was achieved by filtering the solution and adding 1N of HCl. The protein curd in this solution was cooked until it seemed solid and the entire mass floated above the alcohol. After filtering, protein cake was taken out of the funnel. This was cleaned and dried. (Ghaly and Alkoaik, 2010).

4.4 CHARACTERIZATION OF THE PROTEIN CAKE

The protein was characterized using Fourier-transform infrared spectroscopy (Shimadzu, IRTACER 100, Kyoto, Japan).

4.5 PREPARATION OF FISH FEED

Initially, the obtained duckweed cake was taken and made into small pellets. Then it was dried until the moisture has removed. Secondly, the obtained duckweed cake was mixed with orange peel powder in the ratio of 1:1. It was made into small pellets and dried until the moisture has removed.

4.6 IN-VIVO STUDY TO DETERMINE THE GROWTH OF FISHES

4.6.1. COLLECTION AND MAINTENANCE OF ZEBRAFISH

Male *Danio rerio* were obtained from Tarun Fish Farm in Chennai and housed in a glass tank with continuous and enough aeration of the water. After three to four days of acclimation, the fish were introduced to the test environment. The standard is to feed dry food twice a day and change the water once a week.

4.6.2. IN-VIVO STUDY USING ZEBRAFISH

Danio rerio was used in an *in-vivo* investigation to make the determination. For the experiments, 30 one-month-old zebrafish were chosen at random. The fish were kept in a cycle of 12 h of light and 12 h of darkness. The water was kept at a constant 30 °C temperature. Every day, the pH and turbidity of the water were measured and maintained in accordance with the standards. Three tanks were used for the study where control was commercial feed, Test 1 was Duckweed feed and Test 2 was duckweed orange feed. The fishes received their appropriate meal twice daily (5mg). Every day at 10 a.m. and 10 p.m., the feed was administered, strictly adhering to the scheduling regimen. Fish length and weight are measured prior to the experiment. Fish were weighed and measured from head to tail. The digital weighing apparatus determined the weight of the fish. By dividing the fish's body weight by the square of its measured body length, the BMI of the fish was computed. These measurements were done on the 0th day, 10th day, 20th day and 30th day. The observations for the study were performed in triplicates (Ramalingam *et al.*, 2021).

4.6.3. HISTOLOGICAL STUDIES

Histology investigations were used to examine the changes that occurred in the fish's body after exposure to the various forms of diet. On the 30th day, fish from each test were removed and placed in 10% formaldehyde solution for examination. The fish's internal organs, including its liver, and intestine, were then stained with Hematoxylin and Eosin (H&E) and stained with Sudan B black. Using a confocal microscope, it was investigated (Leica DMI6000 B, Leica Microsystems, Stockach, Germany).

4.7 EXTRACTION OF CELLULOSE FROM DUCKWEED

Duckweed was washed thoroughly with distilled water to remove the dust and soil particles present on it. Washed duckweed was sundried for 2-3 days and ground into fine powder. 30g of fine duckweed powder was dissolved in 450mL of toluene and ethanol in the ratio of 2:1. It was then kept in the shaker at 150rpm for 20h at 25°C. it was filtered and the solvent was removed. Samples were washed with ethanol and filtered. It was followed by drying for 1 h at 100°C. The dried powder was mixed with 1.4% sodium chlorite and pH of the solution was adjusted to 4 by adding 5% of acetic acid. This solution was heated to 70°C and stirred continuously for five h at a speed of 500 rpm. To achieve a pH of zero, distilled water was used to filter and wash the sample solution. Afterwards it was dried for 16 h at 100 °C. The dry powder was dissolved in 600 mL of 5% potassium hydroxide, agitated continuously for 24 h at 500 rpm, and then heated for 2 h at 90 °C. It was dried to get cellulose after being rinsed with distilled water until the pH was neutral (Romruen *et al.*, 2022).

4.7.1 COATING OF SPIONS OVER CELLULOSE

In order to create a homogenous solution, 150 mL of ethanol was mixed with 15 mL of 3-Aminopropyl Triethoxysilane, a silane coupling agent, in a ratio of 1:10. Before adding 6 g of cellulose, 6 g of SPIONs were added to the mixture and sonicated for 30 min. It was sonicated for 30 min, followed by three rinses in water that had been nitrogen-purged and dried. (Prasad *et al.*, 2021).

4.7.2 CHARACTERIZATION OF CELLULOSE AND SPIONS COATED CELLULOSE

The cellulose and SPIONs coated cellulose was characterized using various analysis namely Kyoto, Japan), Fourier-transform infrared spectroscopy (Shimadzu, IRTACER 100, Kyoto, Japan), scanning electron microscopy (Zeiss Ultra Plus, Oberkochen, Germany), X-ray diffraction spectroscopy (PANalytical Corporation, X'Pert, Netherlands), Vibrating Sample Magnetometer (Lake Shore 7400).

4.8 BATCH ADSORPTION ISOTHERM

Batch adsorption studies were performed by taking dye, antibiotic and heavy metal such as crystal violet, rifampicin and chromium respectively as adsorbents, and cellulose and SPIONs coated cellulose as adsorbents. The absorbance of crystal violet, rifampicin and chromium were observed at 590nm, 460nm and 540nm respectively. A standard graph was plotted for all the adsorbates with varying concentrations from 1ppm to 10ppm in order to remove the adsorbate from the adsorbent. The concentration of the adsorbate was initially varied between 1ppm to 10 ppm, and then it was incubated for 1 h. After interaction, the solution was centrifuged at 500 rpm for 15 minutes and the absorbance of the supernatant was measured. The optimal adsorbate concentration is found to be the ppm with the maximum clearance percentage. The efficient removal was investigated using the optimum adsorbate concentration and an adsorbent concentration range of 0.01 g to 0.1 g. Under optimal adsorbate and adsorbent concentration, pH was optimized by shifting between 5 and 9. Similar to this, between the time range of 30 minutes and 150 minutes, the interaction duration between the adsorbent and adsorbate was maximized for the efficient removal. Further, the adsorption study of chromium was done using DPC method. After each optimization, 1 mL of supernatant was treated with 30 μ L of 2 N H_2SO_4 to cause Cr^{6+} to dissociate. This is followed by the addition of 0.5% of Di Phenyl Carbazide, which binds to Cr^{6+} to produce a reddish-violet colour and observed spectroscopically at 540 nm (Samrot *et al.*, 2019; Stover, 1928; Shigematsu *et al.*, 1978; Samrot *et al.*, 2022).

The adsorption removal of adsorbent was determined by using the formula

$$\% \text{ Removal} = \frac{\text{Initial concentration} - \text{final concentration}}{\text{Initial concentration}} \times 100$$

4.8.1. ISOTHERM STUDIES

Three different types of isotherms were used to evaluate the adsorption pattern and the mechanism of adsorbate removal by adsorbent. They are i) Langmuir, ii) Freundlich and iii) Tempkin isotherm. The parameter used for the determination are C_e , $C_i - C_e$ where C_i and C_e , are the initial concentration and final concentration of adsorbate, volume (V) in litres, Mass (M) in grams, $q_e = (C_i - C_e) V/m$, $1/q_e$, $1/C_e$, $\ln q_e$, $\ln C_e$, $(RT \ln (1 + (1/C_e)))$. These were calculated and the graph was plotted for each

isotherm. Freundlich and Tempkin isotherms were explored by plotting q_e vs. $\ln C_e$, and a graph between $1/q_e$ and $1/C_e$ was created for the Langmuir isotherm.

4.9. EXTRACTION OF OIL

Oil was extracted from duckweed according to the Najafabadia et al. (2015) with some modifications. Initially, the duckweed was washed thoroughly with running tap water and it was kept under the sun until it dries completely. 1g of dried duckweed was mixed with the 10mL of hexane/ ethanol in the ratio of 1:1 in a beaker. It was well sonicated for 20 minutes in an ice bath. The lipids from the duckweed were extracted by agitating the mixture with magnetic stirrer at 180 rpm for about 60 minutes at 25°C. It was then centrifuged for 10 minutes and supernatant was extracted. Pellet were removed. It was centrifuged for several times until no residual was obtained. The obtained supernatant were collected and specific amount of hexane and water was added to the mixture. It was stirred vigorously and left rest for 24 h at 25 °C. Finally the organic layer had been obtained for the further analysis.

4.10. CHARACTERIZATION OF OIL

The oil was characterized using Fourier-transform infrared spectroscopy (Shimadzu, IRTACER 100, Kyoto, Japan).

CHAPTER -5

RESULTS AND DISCUSSION

5.1 COLLECTION AND OPTIMIZATION OF DUCKWEED

Duckweed was collected from Tarun fish farm, Chennai. It was optimized and cultivation for the period of 30 days to observe the growth in two different trays; one tray with water as control and the other with the MS medium as test. Initially 5g of duckweed were taken and placed on the tray with different solutions (Figure 5.1). On the 10th day, the growth of duckweed were similar in count (Figure 5.2) and the water in the control were slightly grown with algae. On the 20th day of observation, a few leaves were started turning white colour and its growth was slightly higher when compared to the 10th day whereas many leaves in the test tray turned white in colour and it showed an increased growth count when compared to the previous observation day. On the 30th day, the growth of the control decreased and leaves started to die. But the duckweed in the test showed an increased growth count with healthy leaves (Figure 5.2). Hence, these duckweed plants were used for the further extraction and applications.

5.2 EXTRACTION AND CHARACTERIZATION OF PROTEIN

Protein were extracted from the duckweed and it was lyophilized for further studies. Protein of duckweed was determined using FTIR analysis to identify the functional groups present in the samples. The peak at the range of 2923.87cm⁻¹ corresponds to –CH₂ groups. The absorbance was determined at the peak of 1637.56 cm⁻¹ which indicates the characteristic band of primary amide group of proteins (-CO-NH₂) (Figure 5.3) (Gbassi *et al.*, 2012).

5.3 IN-VIVO STUDY USING ZEBRAFISH

Thirty fishes were taken for experimentation with three different feeds (Figure 5.4), observations of length, weight and BMI were shown in the (figure -5.5). Three different samples (Commercial feed, Duckweed feed and duckweed orange feed) were used for this study. Initially, the length, Weight and BMI of the fishes were calculated (pre feeding measurements). The length, weight and BMI of the control fishes were observed as 2.3± 0.057cm, 0.14± 0.025g and 0.027± 0.005g/cm²

respectively. The length, weight and BMI of the test 1 fishes were observed as 2.26 ± 0.23 cm, 0.24 ± 0.04 g and 0.048 ± 0.005 g/cm² respectively. The length, weight and BMI of the test 2 fishes were observed as 2.56 ± 0.05 cm, 0.29 ± 0.05 g and 0.044 ± 0.006 g/cm² respectively. There was no significant difference in the length, weight and BMI attained in the pre-feeding stage between the fishes. On the 10th day, the length, weight and BMI of the control fishes were observed as 2.4 ± 0.15 cm, 0.14 ± 0.015 g and 0.024 ± 0.004 g/cm² respectively. The length, weight and BMI of the test 1 fishes were observed as 2.36 ± 0.15 cm, 0.27 ± 0.02 g and 0.049 ± 0.009 g/cm² respectively. The length, weight and BMI of the test 2 fishes were observed as 2.6 ± 0.1 cm, 0.30 ± 0.02 g and 0.045 ± 0.004 g/cm² respectively. There is slight increase in test 2 when compared to the control and test 1. On the 20th day, the fishes in the control, test 1 and test 2 measurements were observed. The length, weight and BMI of the control fishes were 2.53 ± 0.05 cm, 0.15 ± 0.005 g and 0.023 ± 0.001 g/cm² respectively. 2.53 ± 0.11 cm, 0.30 ± 0.011 g and 0.047 ± 0.002 g/cm² are the length, weight and BMI of the test 1 fishes. 2.53 ± 0.11 cm, 0.28 ± 0.005 g, 0.044 ± 0.004 g/cm² are the length, weight and BMI of the test 2 fishes respectively. On the observation of 20th day, there was an increase in test 1 in all the parameters. The final day for the observation was on the 30th day, where the length, weight and BMI of the control fishes were 2.53 ± 0.05 cm, 0.20 ± 0.01 g and 0.032 ± 0.001 g/cm² respectively. The length, weight and BMI of the test 1 were 2.53 ± 0.05 cm, 0.31 ± 0.005 g and 0.048 ± 0.002 g/cm² respectively. The length, weight and BMI of the test- 2 fishes are the 2.53 ± 0.05 cm, 0.31 ± 0.005 g and 0.048 ± 0.001 g/cm² respectively. Ramalingam *et al.*, 2021 reported the normal BMI of the 5 – 8 months old fish was 0.015 ± 0.004 g/cm². Dai *et al.*, 2015 reported the length and weight of the zebrafish increases when it is provided with high-fat diet with cholesterol from the 7th day onward.

5.4 HISTOLOGY STUDIES

Histology analyses were performed for the fishes which had different types of feeds in order to determine the possible damages or accumulation of lipids. In Hematoxylin and Eosin (H&E) Staining, There were no changes and abnormalities observed in both intestine and liver (Figure 5.6 and 5.7) whereas in Sudan B Black staining, Intestinal abnormalities were not observed among the different dietary groups. There was minimal accumulation of lipid vacuoles in enterocytes and also in liver, regardless of the diet consumed (Figure 5.8 and 5.9). Fountoulaki *et al.*, 2017

reported that there was an increased lipid deposition in hepatocytes trend were observed in the diets with 20% and 15% lipid.

5.5 CHARACTERIZATION OF CELLULOSE AND SPIONS COATED CELLULOSE

The cellulose was extracted (Figure 5.10 a) and coated over SPIONs. It was analyzed using various characterizations. The UV analysis of cellulose (Figure 5.10 b) peak ranges between 200 to 400nm indicate the presence of polysaccharide. The peak at the range of 250nm indicates the presence of cellulose. A similar range of spectra was reported by Samrot et al. 2022, Cazón et al. 2022. On the another hand, the synthesized SPIONs were coated over the Cellulose. UV analysis were performed and identified the absorbance of SPIONs coated cellulose were between 260nm to 390nm (Figure 5.11b). Figure 5.10 c and Figure 5.11c illustated the Fourier Transform Infrared spectrum of cellulose and SPIONs coated cellulose. The peak at the range of 3357 cm^{-1} and 2912.51 cm^{-1} indicates the presence of hydroxy groups of cellulose and vibration of cellulose in the Cellulose sample (Bhuvaneshwaran *et al.*, 2019). Also the peak at the range of 2912.51 cm^{-1} indicates that there is weak peak of stretching vibrations of C-H bond. The main . the peaks of SPIONs coated cellulose at the range of 1311.59 and 1411.89 indicates the the bending of CH_3 and CH_2 (Samrot *et al.*, 2021c; Samrot *et al.*, 2019). The prominent peaks at the range of 2920.23 and 1411.89 indicates the presence of $-\text{CHO}$ group of aldehydes and $\text{CH}_3 - \text{CH}_2$ groups present in polysaccharides. The absorbance showed peaks near 500 to 550 which corresponds to the stretching vibrations of $\text{Fe}-\text{O}-\text{Fe}$ bond (Samrot *et al.*, 2018b). Scanning Electron Microscopy (SEM) is a technique to determine the surface structure of the samples. The images revealed that the cellulose were smooth with layers (Figure 5.10 d) EDAX analysis were determined for cellulose which revealed the presence of elements like carbon, hydrogen, calcium, potassium (Figure 5.10 e). The cellulose were slightly crystalline in nature and showed peaks from 20 to 30° which indicates the presence of cellulose (Figure 5.10 f) whereas SPIONs coated cellulose were also crystalline in nature with peaks at $2\theta = 32^\circ$ and 35° (Figure 5.7 d). Ma *et al.*, 2015 reported nanocellulose from *Xanthoceras Sorbifolia* Husks had sharp peaks with high crystallinity index. Figure 5.7e is the VSM analysis which there is no hysteresis loop formation and magnetization pass through the origin indicating the Superparamagnetic property in the SPIONs coated cellulose.

5.6 BATCH ADSORPTION ISOTHERM:

5.6.1 OPTIMIZATION OF ADSORBATE CONCENTRATION: CELLULOSE AS ADSORBENT

Optimization of adsorbate with highest removal of Dye like crystal violet, Antibiotic like rifampicin and heavy metal chromium was observed in the (Figure 5.12 a). The highest removal of crystal violet by cellulose was observed at 7ppm with the removal efficiency of 72.63% whereas the rifampicin was observed in 3ppm with the removal efficiency of 77.74%. The maximum removal of chromium was analysed to have highest removal percentage of 75.43% in 7ppm. A covalent interaction between the adsorbate and the adsorbent also might induce the removal of adsorbate by the adsorbent. Similarly, Samrot et al. (2022) reported that 72.85% was the maximum removal in the crystal violet for the muskmelon fiber with the adsorbent concentration of 0.01g. A cadmium ion (Cd^{2+}) or lead ion (Pb^{2+}) solution was used as wastewater for adsorption efficiency studies. A significant difference was observed between Cell-EDTA and Cell-CM in the pH range 1–7 compared to unmodified cellulose (Daochalermwong *et al.*, 2020).

5.6.2 OPTIMIZATION OF ADSORBATE CONCENTRATION: SPIONs- COATED CELLULOSE AS ADSORBENT

The adsorbate concentration for removing SPION-coated cellulose was optimized for dye, antibiotics and heavy metal (Figure 5.13 a). 0.01g of adsorbent removed 5ppm of crystal violet with the removal efficiently 80.46% whereas rifampicin removed 7ppm of rifampicin with the maximum removal of 66.10%. The maximum removal was observed in 4ppm of chromium with removal percentage of 76.37%. Similar results was observed in SPIONs coated muskmelon fiber with the removal percentage of 82.97% removed 7ppm of crystal violet (Samrot *et al.*, 2022). Samrot *et al.* (2022) reported that 0.01g of SPIONs coated fibers against 1ppm CV dye removed 42.71%.

5.6.3 OPTIMIZATION OF ADSORBENT CONCENTRATION: CELLULOSE AS ADSORBENT

Adsorbent concentration was optimized by identifying the best adsorbate concentration. A quantity of 7ppm of crystal violet was removed by 0.05g of cellulose

with the removal efficiency of 76.03% whereas 3ppm of rifampicin removed 0.06g of cellulose with the removal efficiency of 80.44%. Also, 0.03g of cellulose removed 7ppm of chromium with the removal percentage of 75.89% (Figure 5.12 b). . Martins et al. 2022 reported derivative of cellulose efficiently removed both anionic and cationic dyes with parameters of pH, contact time and solute concentration and identified the maximum adsorption of 201 mg g⁻¹ for orange II and 2403 mg g⁻¹ for crystal violet.

5.6.4 OPTIMIZATION OF ADSORBENT CONCENTRATION: SPIONs- COATED CELLULOSE AS ADSORBENT

Similarly, after optimizing the adsorbate concentration of SPION- coated Cellulose. Concentration of adsorbent was evaluated by varying from 0.01g to 1g. 0.03g of SPION- coated Cellulose removed 5ppm of crystal violet with efficiency of 81.83%. 7ppm of rifampicin was removed by 0.05g with the highest removal percentage of 66.97%. The highest removal of chromium of 81.96% was achieved with 0.07g of SPION- coated Cellulose (Figure 5.13 b). Samrot *et al.* (2018a) reported that chitosan-coated SPIONs have proven to be more efficient in the removal of chromium ions from the solution and showed greater efficiency than control.

5.6.5 OPTIMIZATION OF PH - CELLULOSE AS ADSORBENT

The optimized pH for the maximum removal of dye, antibiotic and heavy metal was evaluated (Figure 5.12 c). pH was varied from 5 to 9 and maximum removal after interaction of 1 h was found to be 77.33% at pH 8 in 7ppm of crystal violet. The maximum removal was seen in the alkaline pH of 8 where it removed 0.06g of 3ppm of rifampicin with the removal efficiency of 81.6% whereas 0.03g of cellulose removed 7ppm of chromium with the removal efficiency of 76.98%.

5.6.6 OPTIMIZATION OF pH – SPIONs COATED CELLULOSE AS ADSORBENT

An optimal pH was determined for the removal of dye, antibiotic and heavy metal (Figure 5.13 c). SPIONs- coated Cellulose removed 5ppm of crystal violet removed 81.83% with the pH of 7 whereas 0.06g of SPION- coated Cellulose removed 7ppm of rifampicin at 7ppm with the removal efficiency of 66.97%. At the pH of 8, the maximum removal was 82.28% which was observed at the 4ppm. Samrot *et al.*, 2021a reported that when exposed to the magnetic field, crystal violet dye can be removed completely from the aqueous medium along with SPIONs-a.

5.6.7 OPTIMIZATION OF CONTACT TIME: CELLULOSE AS ADSORBENT

Keeping the optimized concentration of adsorbate and adsorbent, pH as constant, contact time were varied and evaluated (Figure 5.12 d). This final optimization was done to check how much interaction time was required to achieve maximum removal efficiency. 78.28% was removed at 90 minutes of interaction time between the adsorbate and adsorbent at the pH of 8 whereas the maximum removal was 81.60% in 60 minutes with the 3ppm of rifampicin at the pH of 8. Similarly, 7ppm of chromium was removed at 60 minutes with a maximum removal efficiency of 76.98%. Pei *et al.*, 2021 reported that cellulose-based composite membrane had a maximum adsorption capacity can reach up to 194 mg/g which resulted in more efficient adsorption at lower heavy metal concentrations and higher pH value of feed solution for heavy metal ions removal.

5.6.8 OPTIMIZATION OF CONTACT TIME: SPIONs- COATED CELLULOSE AS ADSORBENT

0.03g of SPIONs- coated Cellulose removed 5ppm of crystal violet in 60minutes with the removal efficiency of 81.83% whereas 0.06g of SPIONs- coated Cellulose efficiently removed 7ppm of rifampicin in 90 minutes with the removal efficiency of 67.58%. Also, 4ppm of chromium removed 0.07g of SPIONs- coated Cellulose in 90 minutes with removal efficiency of 85.3% (Figure 5.13 d). It was reported that 98.92% of methylene blue removed 0.08g of latex coated SPIONs at 3 h of interaction time (Samrot *et al.*, 2021b)

5.7 ISOTHERM STUDIES:

The adsorption pattern of the adsorbate over adsorbate was determined through the three isotherm models such as Langmuir, Freundlich and Tempkin isotherm for the three different adsorbates like crystal violet, rifampicin and chromium (Figure 5.14 and Figure 5.15). This was done to identify the best model which suits the adsorption isotherm models (Table 1 and 2). In cellulose, the removal of crystal violet followed the Freundlich isotherm with the R^2 value 0.8261 indicating multilayer adsorption. The removal pattern of rifampicin and chromium by cellulose followed tempkin isotherm with varying R^2 values of 0.6353 and 0.4946 respectively. SPIONs-coated Cellulose removed crystal violet by the following tempkin isotherm with the R^2

value of 0.8829. SPIONs- coated Cellulose showed the highest removal in the rifampicin and chromium by following the Langmuir isotherm with the R^2 values of 0.9357 and 0.5854 respectively. Samrot *et al.*, (2021c) reported 0.1 g of cellulose fibers extracted from *Citrus sinensis* removed 1 ppm crystal violet dye at pH 8 after interacting for 5 h by following the Langmuir isotherm with R^2 value of 0.914.

6. EXTRACTION AND CHARACTERIZATION OF OIL

The oil was extracted from the duckweed and it was characterized using FTIR analysis to identify the functional groups present in the oil (Figure 5.16). The peaks at the range of 3340.71cm^{-1} indicated the presence of O-H which has the class compounds of phenols and alcohol. The peak at the range of 1637.56cm^{-1} indicated the stretching of C-H bonds where alkanes are present (Wauton and Ogbeide, 2021).

CHAPTER – 6

SUMMARY AND CONCLUSION

In this study, a subfamily of blooming aquatic plants called *Lemnoideae* is also referred to as duckweed has been cultivated and the growth was optimized, where the test with the MS medium showed an increased growth count with healthy leaves. This growth optimization was done for 30 days. Since, duckweed has been known to have higher protein content it was extracted and characterized using FTIR analysis. It was then used for the preparation of fish feed, where protein isolated from duckweed was used as feed for *Danio rerio* and orange peel was added to the duckweed protein as another feed for the fishes. Three different types of fish feeds (commercial feed, Duckweed feed and duckweed orange feed) were used for the observation of length, weight and BMI of the fishes which was observed for 30 days. Fishes consumed duckweed as feed (Test 1) showed enhanced growth compared to those two. Finally, cellulose was extracted and further coated with Superparamagnetic Iron Oxide Nanoparticles (SPIONs). It was characterized using UV, FTIR, SEM, EDAX, XRD and additionally VSM was carried out for SPIONs coated cellulose. It was used for the removal of dye, antibiotics and heavy metals. Isotherm studies were determined for the adsorbates crystal violet, rifampicin and chromium. Oil was also extracted and characterized using FTIR analysis.

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FIGURES AND TABLES



Figure 5.1 Optimization study a. Control (only water); b. Test (MS Medium)

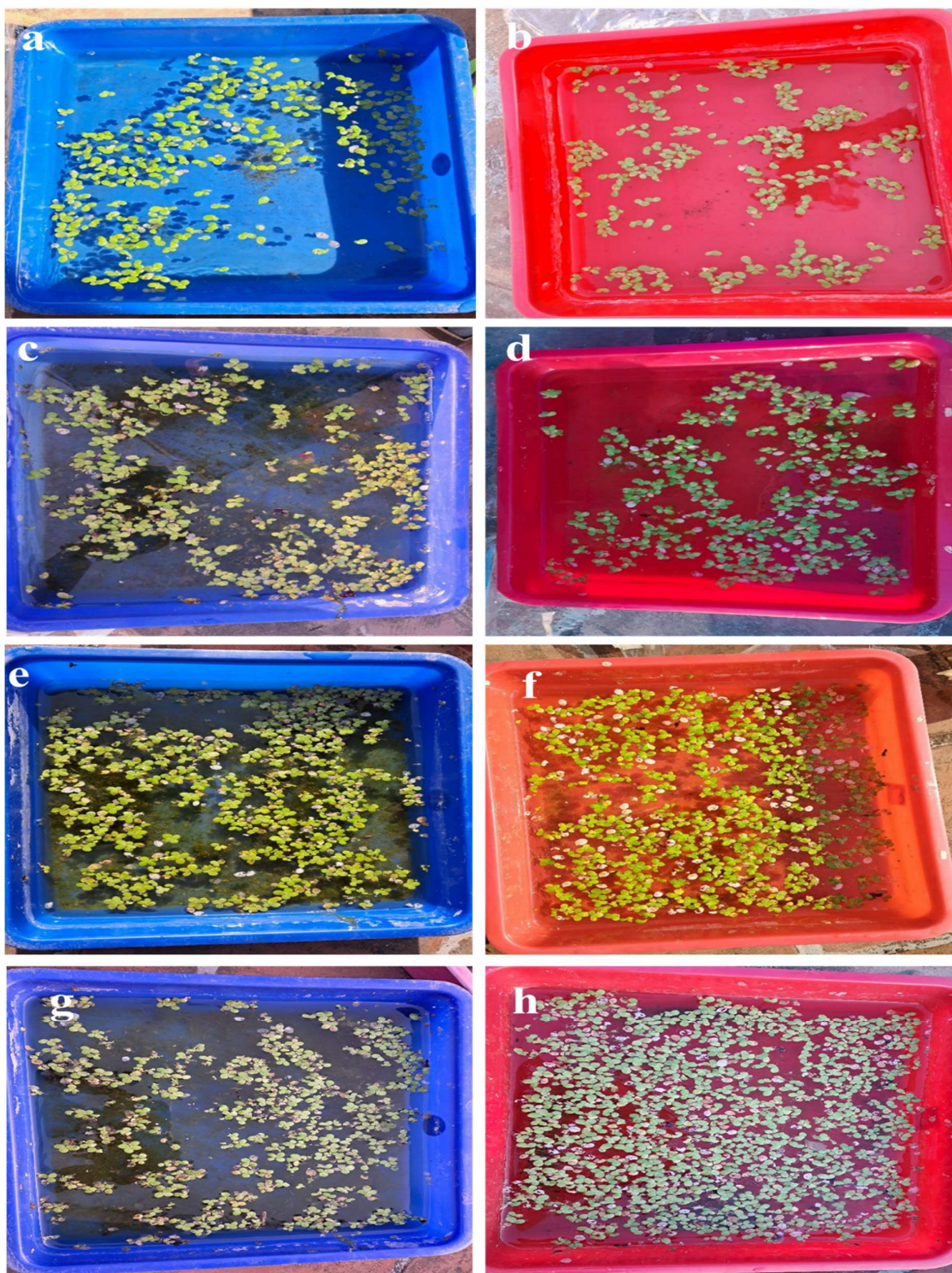


Figure 5.2 Observation of duckweed growth a. 0th day – Control, b. 0th day – Test, c. 10th day – Control, d. 10th day – Test, e. 20th day – Control, f. 20th day – Test, g. 30th day – Control, h. 30th day – Test.

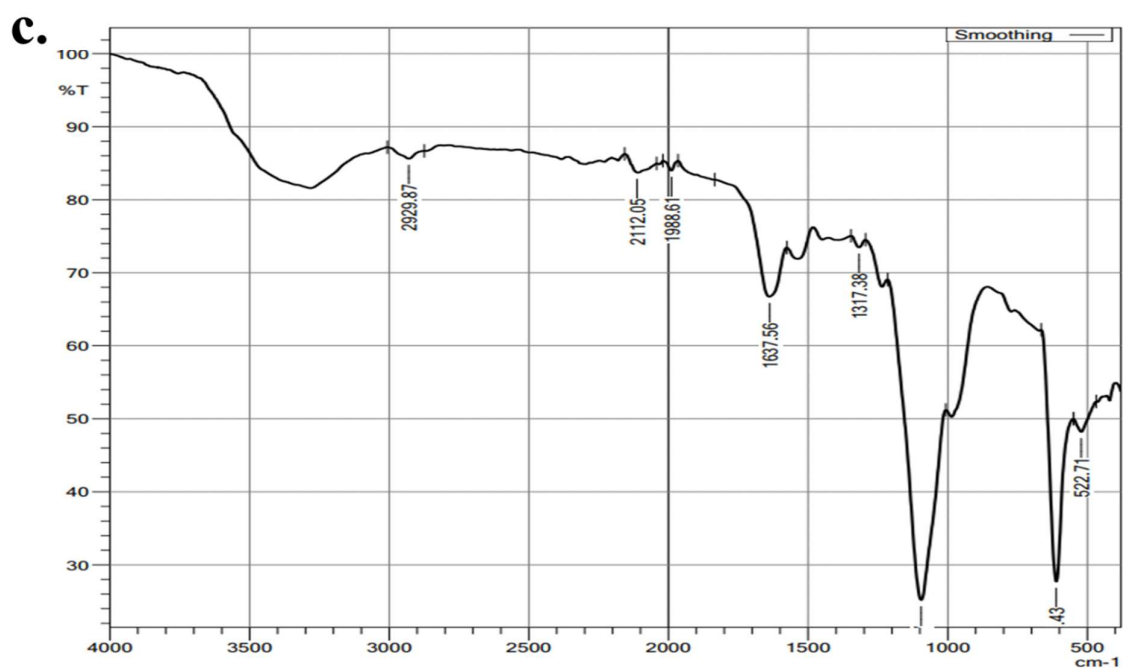
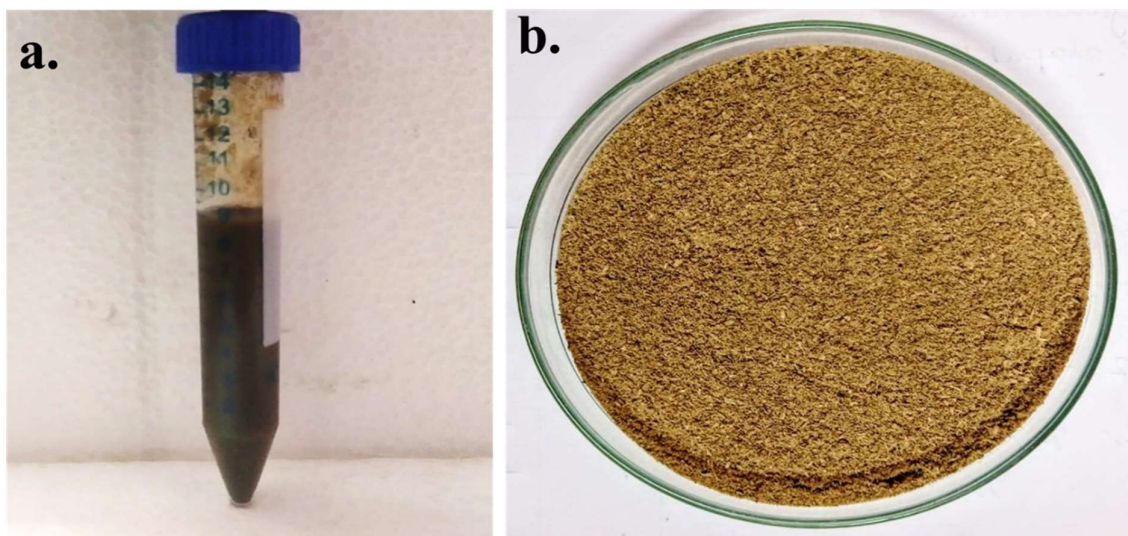


Figure 5.3 Extraction and Characterization of protein (Fourier Transform Infrared spectroscopy)

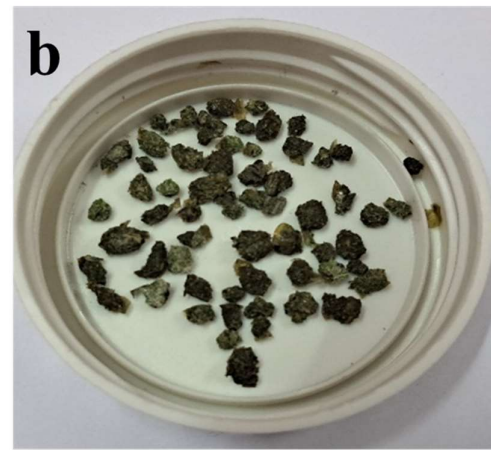


Figure 5.4 Different types of fish feed, a. Commercial Feed; b. Fish feed from duckweed; c. Feed from Duckweed and orange peel powder

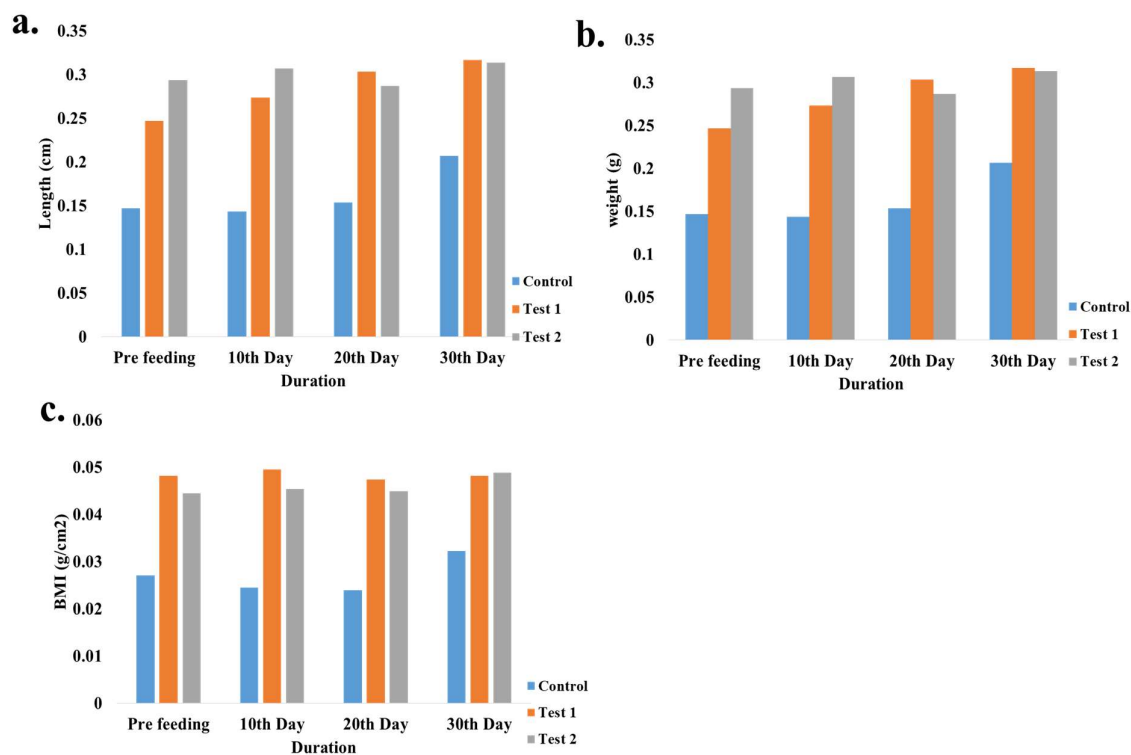


Figure 5.5 Graphical representation of a. Length, b. Weight, C. BMI of the Zebrafishes.

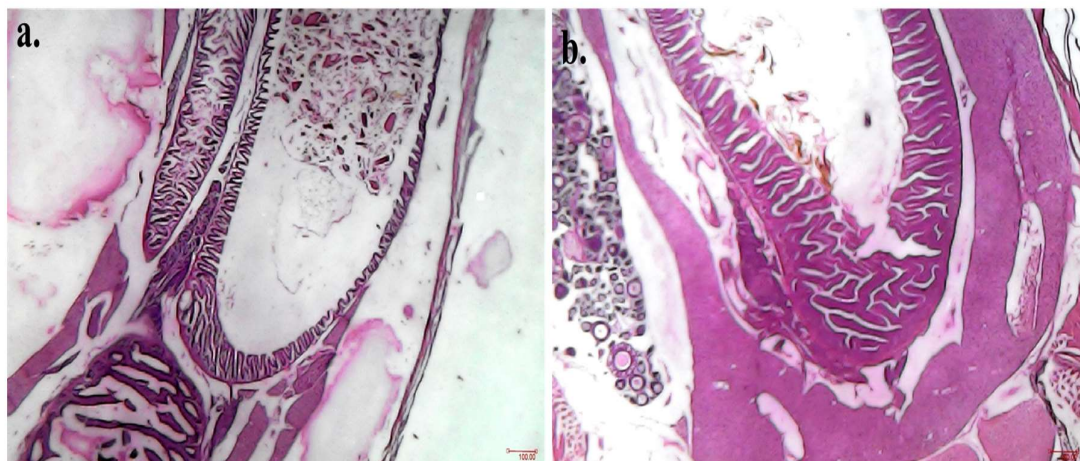


Figure 5.6 H & E staining: Histology images of intestine of zebrafish exposed to a. Test 1 (Duckweed feed), b. Test 2 (duckweed orange feed)

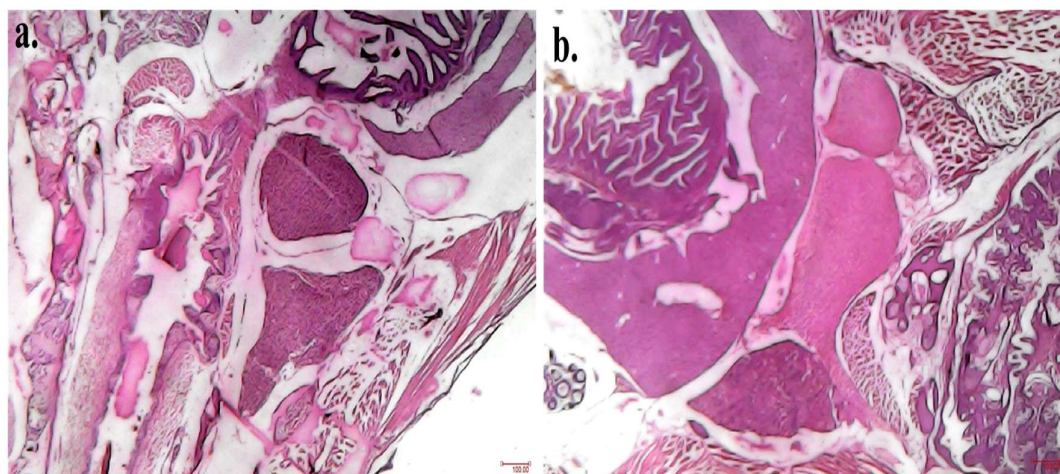


Figure 5.7 H & E staining: Histology images of liver of zebrafish exposed to a. test 1 (Duckweed feed), b. test 2 (duckweed orange feed)

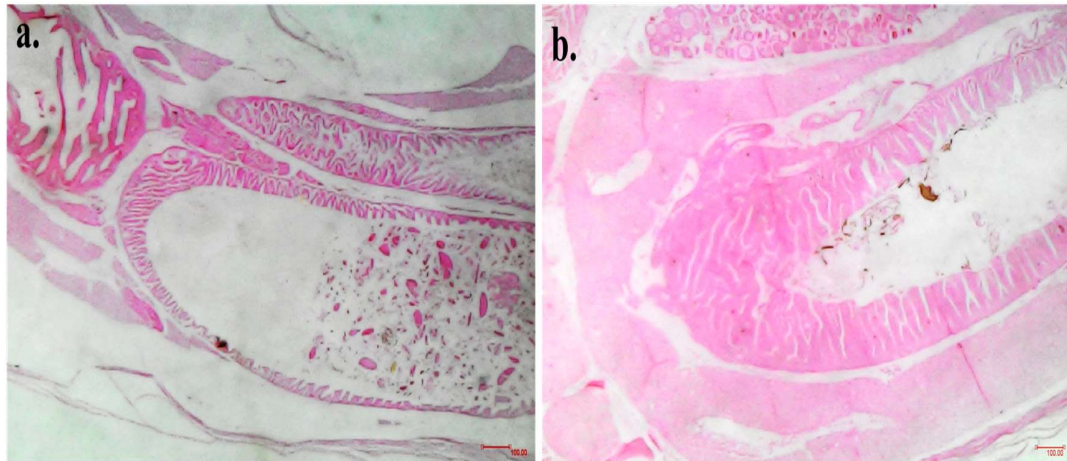


Figure 5.8 Sudan B Black staining: Histology images of stomach of zebrafish exposed to a. test 1 (Duckweed feed), b. test 2 (duckweed orange feed)

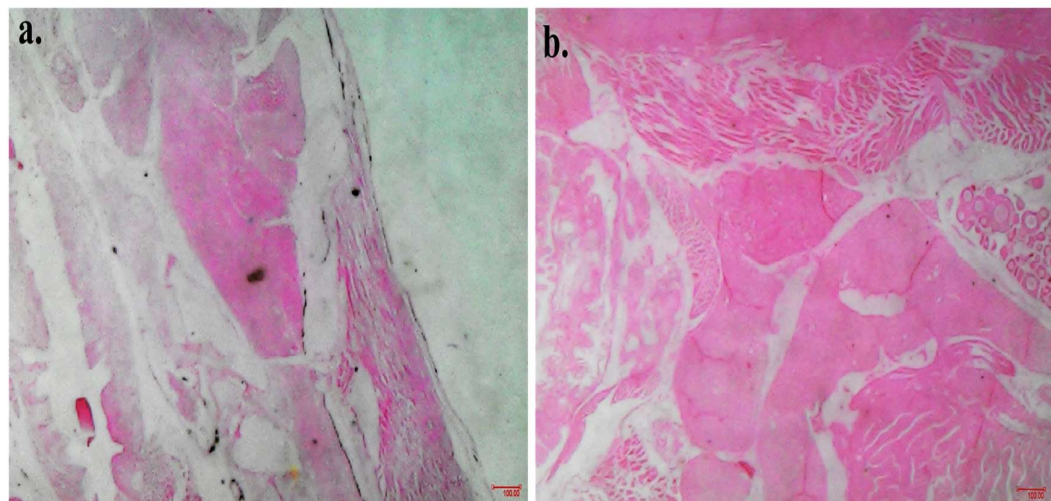


Figure 5.9 Sudan B Black staining: Histology images of liver of zebrafish exposed to a. test 1 (Duckweed feed), b. test 2 (duckweed orange feed)

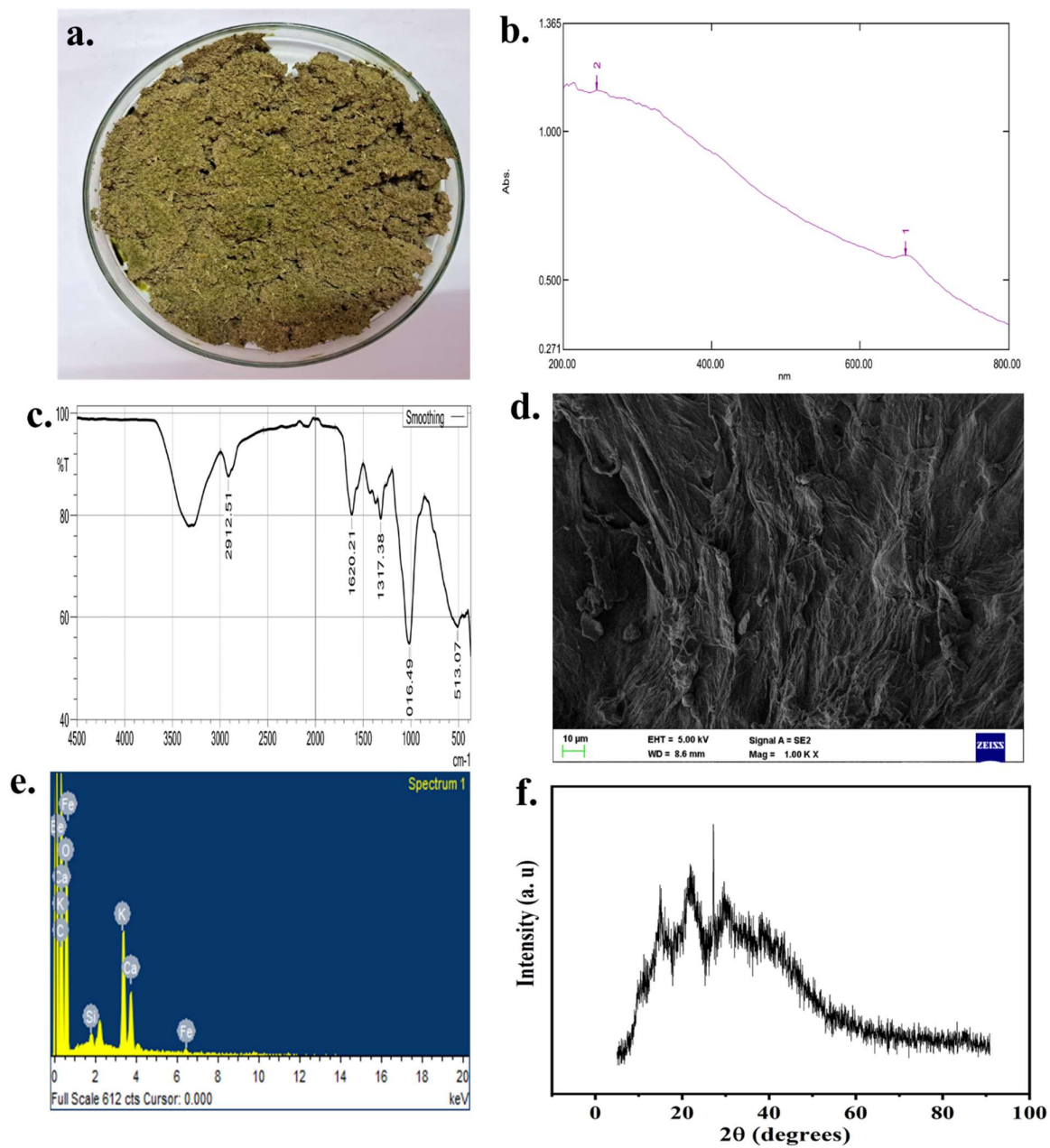


Figure 5.10 Characterization of Cellulose a. Extracted cellulose, b. UV analysis c. FTIR analysis, d. SEM analysis, e. EDAX analysis, f. XRD analysis

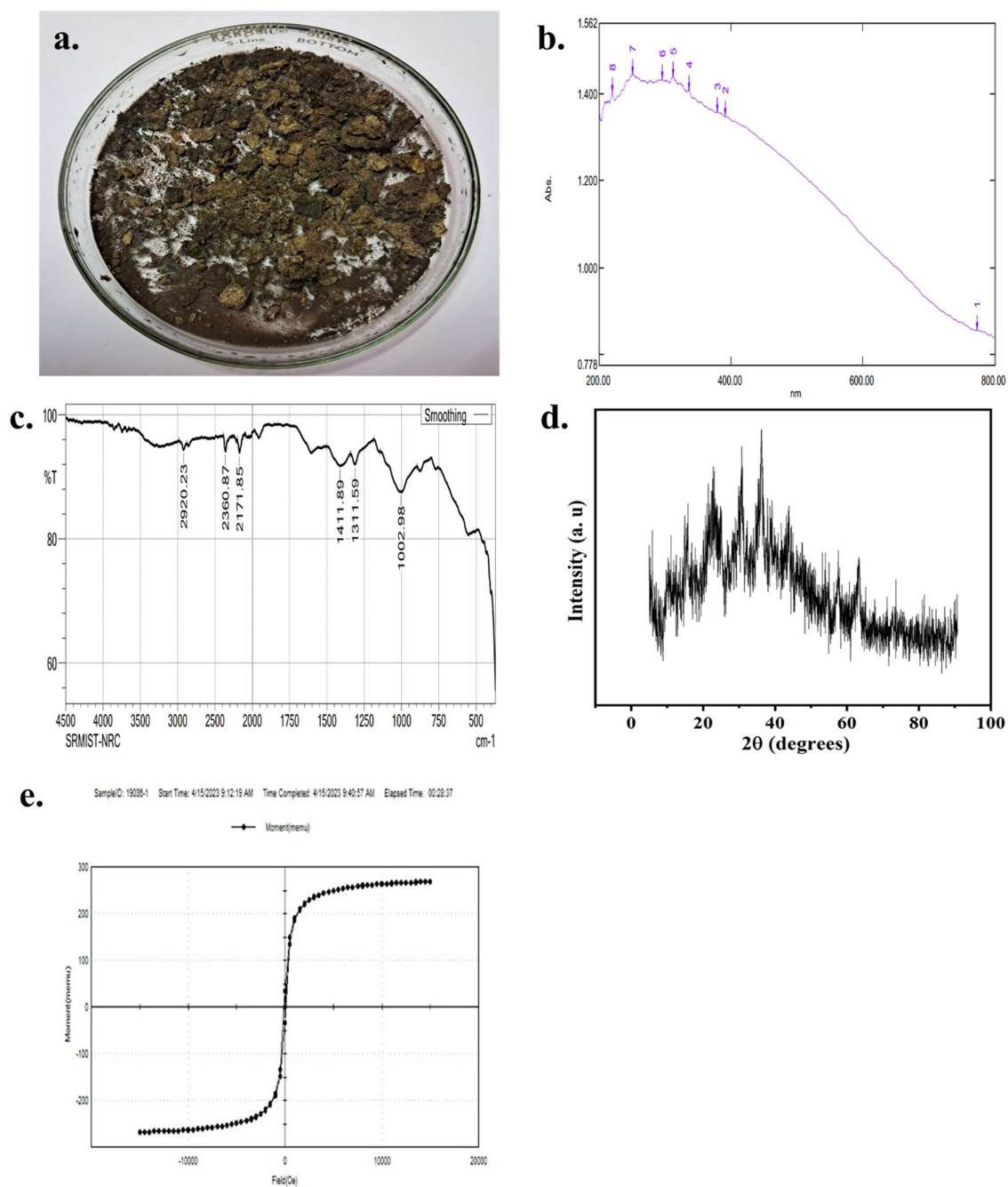


Figure 5.11 Characterization of SPIONs coated Cellulose a. SPIONs coated cellulose, b. UV analysis c. FTIR analysis, d. XRD analysis, e. VSM analysis

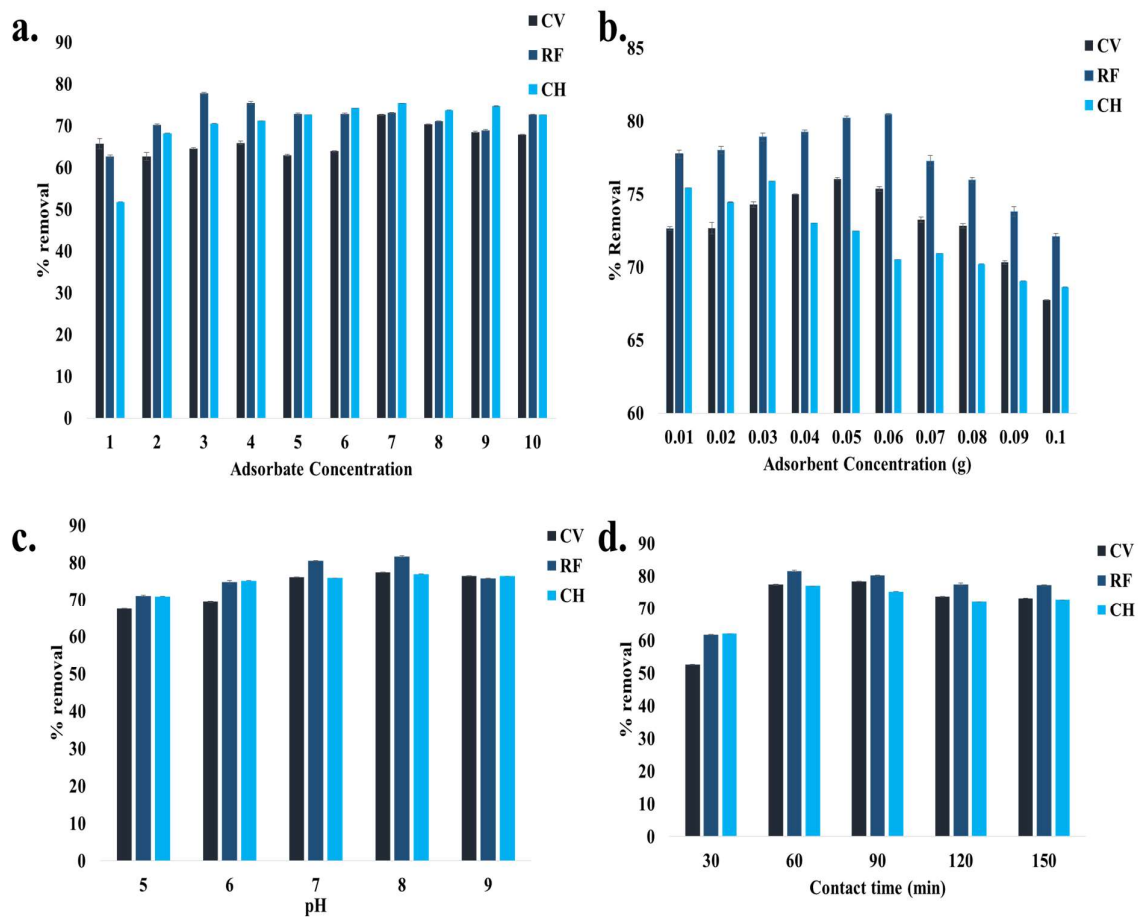


Figure 5.12 Optimization of cellulose a. adsorbate concentration, b. Adsorbent concentration, c. pH, d. Contact time

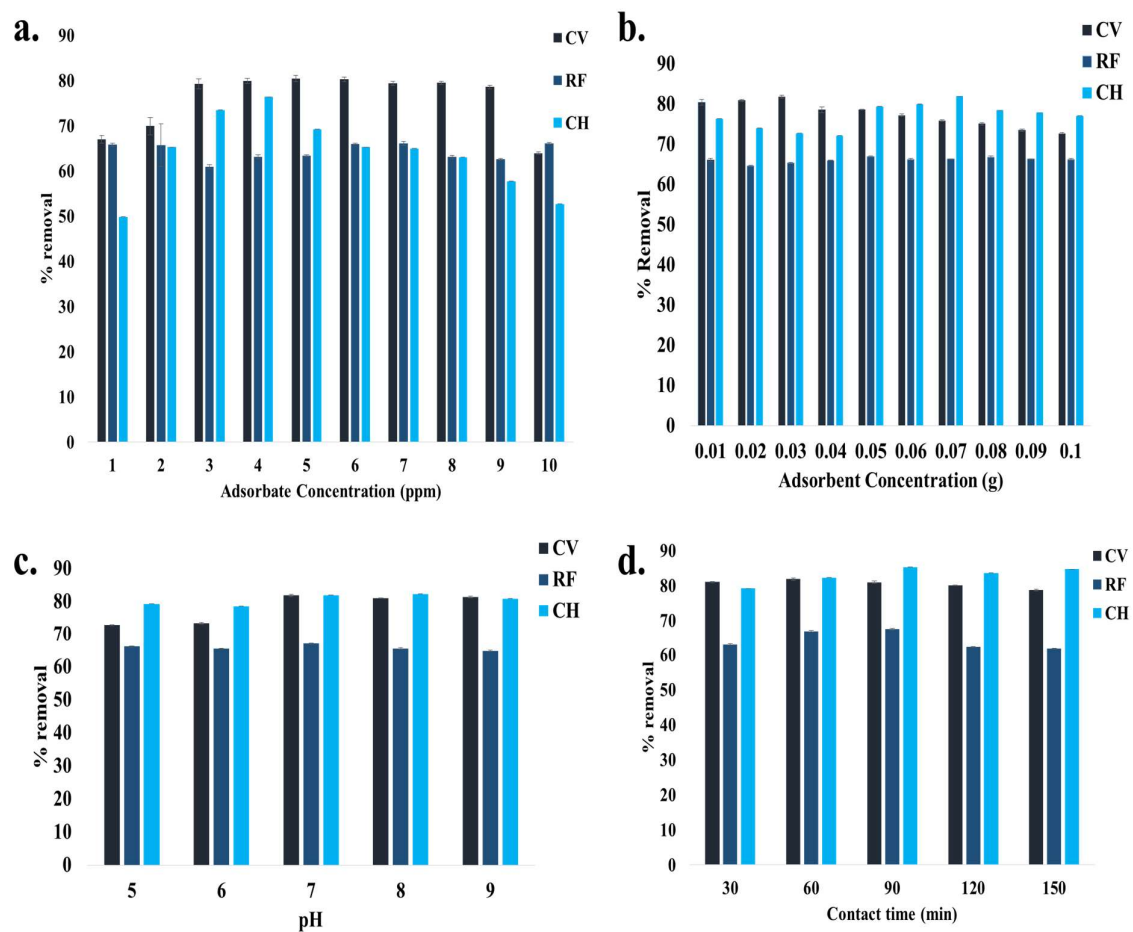


Figure 5.13 Optimization of SPIONs coated cellulose a. adsorbate concentration, b. Adsorbent concentration, c. pH, d. Contact time

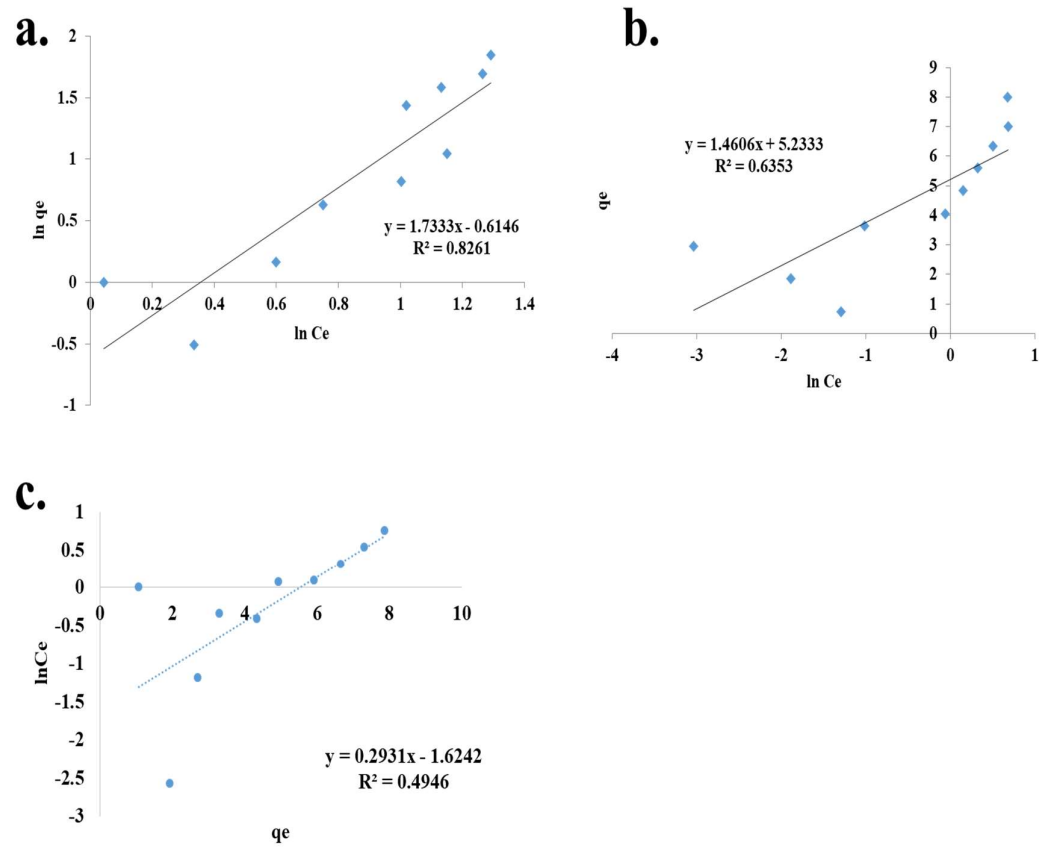


Figure 5.14 Isotherm studies for cellulose a. Crystal violet, b. Rifampicin, c. Chromium

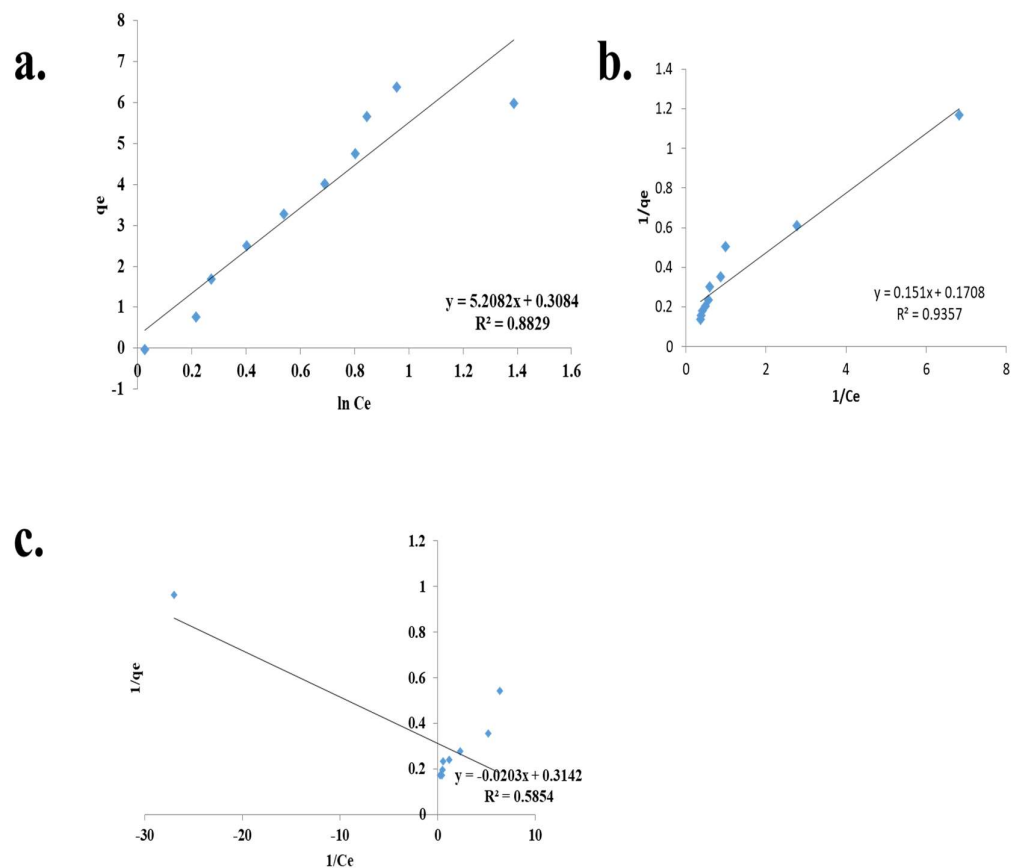


Figure 5.15 Isotherm studies for SPIONs coated cellulose a. Crystal violet, b. Rifampicin, c. Chromium

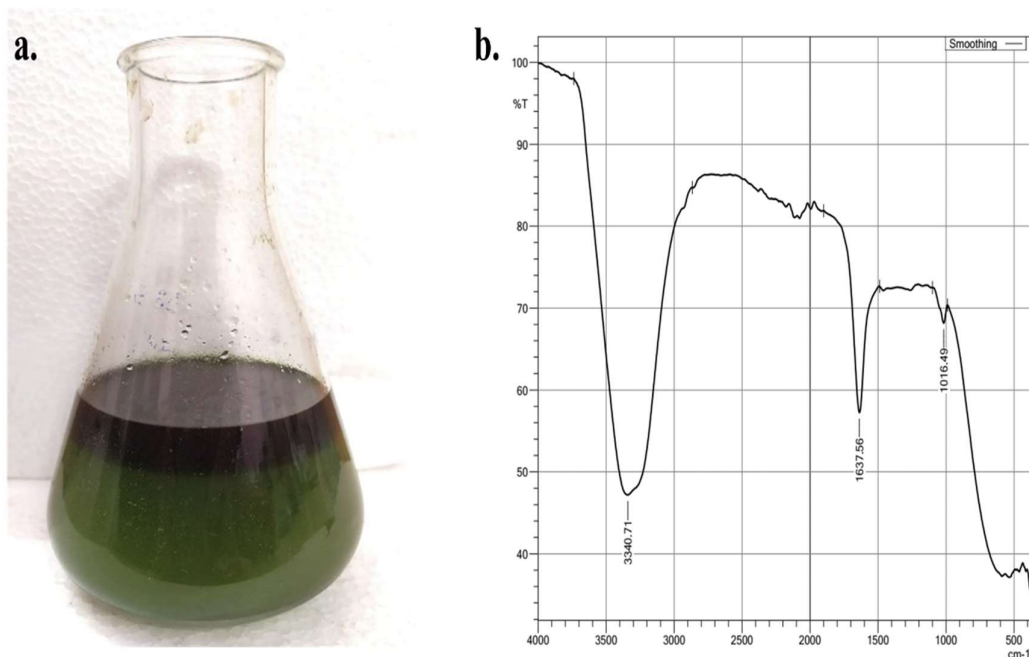


Figure 5.16 a. Two phases of oil (Aqueous phase and organic phase), b. Fourier-transform infrared Spectroscopy (FTIR) of oil

Table. 1 Removal of various adsorbates using cellulose as adsorbent

Adsorbate	R ² Value	Isotherm model
Crystal Violet	0.8261	FREUNDLICH
Rifampicin	0.6353	TEMPKIN
Chromium	0.4946	TEMPKIN

Table. 2 Removal of various adsorbates using SPIONs- coated cellulose as adsorbent

Adsorbate	R ² Value	Isotherm model
Crystal Violet	0.8829	TEMPKIN
Rifampicin	0.9357	LANGMUIR
Chromium	0.5854	LANGMUIR