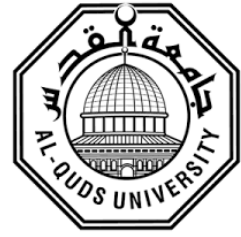


Deanship of Graduate Studies

Al-Quds University



**Production of *Spirulina platensis* using Cheap Local
Resources in Palestine**

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M.Sc. Thesis

Jerusalem - Palestine

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Production of *Spirulina platensis* using Cheap Local
Resources in Palestine

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A thesis submitted in partial fulfillment of requirements for
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Department of Earth and Environmental Sciences, Faculty of
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Al-Quds University
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Thesis Approval

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Jerusalem – Palestine

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Dedication

To my great parents – Amina & Walid
who care about me and support my work,

To my wonderful brothers – Omar, Mohammad, & Raad
who believed in me and in my work,

To all my friends, especially
Eng. Samer Jebreen for his valuable help

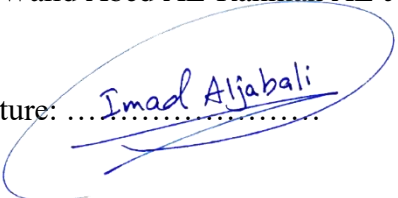
To all my teachers especially
Prof. Ibrahim Abbasi

Thank You All
Imad AL-Jabali

Declaration

I Certify that this thesis submitted for the degree of Master of Science in environmental studies is the result of my own research, except where otherwise acknowledged, and that this thesis (or any part of the same) has not been submitted for higher degree to any other university or institution.

Imad Walid Abed AL-Rahman AL-Jabali

Signature: 

Date: 7/5/2022

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Abstract

In this study five main experiments were used with different treatments to evaluate the possibility of the cheap aquaculture water such as Tilapia effluents to be used for Spirulina production, and to test if solutions made by plant ash be used for Spirulina production, also if brackish water and mining water have a good impact on Spirulina production, and to create a medium composed of cheap chemicals and fertilizers to be used for Spirulina cultivation, and to test if a mix made from local components be useful to produce Spirulina or not.

All experiments were monitored through growth and dry weight measurements including chemical and physical characteristics for the samples with a comparison with Zarrouk medium as a reference used for each experiment alone while all experiments were done for a period of 21 days to find the best media type that stay longer for commercial purposes. In all experiments, pH values were between 8 and 11, and EC was between 9.8-30 mS/cm, while temperature was at 30 °C and 35 °C, and light was at 1500 and 5000 Lux for 16h light and 8h dark.

The first experiment was done to evaluate the possibility of using wastewater from Tilapia-fish ponds in ADS to cultivate Spirulina. In this experiment, both treatments began with low concentrations, and the initial absorbance around 0.5 at 880nm. ZM tripled after 8 days while FW doubled in the same period and both treatments stayed at the same level till the end of the experiment. This experiment showed that Spirulina can grow in FW in a similar way like ZM with a price of 0.0015 ILS/L for FW compared to 8.9625 ILS/L for ZM.

The second experiment was done through running treatments of fish wastewater, diluted brackish water, plant ash solution, and a mix of them. In this experiment, all treatments began with low concentrations as in experiment one with absorbance around 0.6 at 880nm and most of it tripled after 14 days, and ZM was the lowest one among all; so, from this experiment FW, diluted BW, PAS 3% or a mix of it could be used to cultivate Spirulina with a very cheap price compared to ZM.

The third experiment was conducted to evaluate the same components as in experiment two with some modification and addition of new treatments such as mining water and creating new chemical mediums as IMJ and IMJ-1. In this experiment, all treatments began with high concentration with absorbance range between 1.6-1.9 at 880nm for all types. ZM and IMJ were the best types among all other treatments. FW stayed at the same level for the first week

and began to grow with fluctuation curve till the end of the experiment. IMJ with a price of 3.66 ILS/L is lower than ZM and could be a good alternative source.

The fourth experiment was run at the same time of experiment five. It was a comparison between ZM and IMJ-2. In this experiment, both treatments began with a moderate concentration with absorbance reading of 1.25 at 880nm. ZM doubled nearly after 14 days while IMJ-2 raised by 1.76 times at the same period. IMJ-2 was better than ZM till day 10 and a clear difference shown after that. IMJ-2 with a price of 1.38 ILS/L is lower than ZM and could be a very good cheap alternative source.

The fifth experiment was a comparison between ZM, FW, and the following types FW:BW (9:1), FW:MW (9:1), FW:PAS-3% (9:1), FW:IMJ-1 (9:1), and a mix composed of FW:(BW+MW+PAS-3%+IMJ-1) (9:1). In this experiment, all treatments began with moderate concentrations with absorbance around 1.25 at 880nm. ZM and Mix were the best types, while ZM, Mix, and FW:IMJ-1 took a similar behavior until day 10.

According to the results of the dry weight we found that it was not representative for the growth of Spirulina, and the reason is still unclear. All treatments that used in this study had lower cost than Zarrouk medium and with some optimization its' quality could be better. The main result that was obtained from all the experiments in this study is the ability of Spirulina to grow within a wide range of chemical parameters with a cheaper price. Therefore, this study recommends the use of water from fish farming in the production of spirulina after its examination.

إنتاج طحالب سبيروलिينا بـلاتنسييس بتكلفة منخفضة باستخدام مصادر محلية في فلسطين

إعداد: عماد وليد عبد الرحمن الجبالي

إشراف: أ. د. معتز علي القطب

الملخص

في هذه الدراسة، تم استخدام خمسة تجارب رئيسية مع معاملات مختلفة لتقييم إمكانية استخدام المياه رخيصة الثمن والنتيجة عن تربية الأسماك مثل أسماك البلطي في إنتاج السبيروليينا، واختبار ما إذا كانت المحاليل المصنوعة من رماد النبات تستخدم لإنتاج السبيروليينا، وأيضًا دراسة تأثير المياه متوسطة الملوحة والمياه الناتجة عن قص الحجر في المحاجر على إنتاج السبيروليينا، بالإضافة إلى إنشاء وسط مكون من مواد كيميائية وأسمدة رخيصة لاستخدامها في زراعة السبيروليينا، واختبار ما إذا كان المزيج المصنوع من مكونات محلية مفيدًا لإنتاج السبيروليينا أم لا.

تمت مراقبة جميع التجارب من خلال قياسات النمو والوزن الجاف بما في ذلك الخصائص الكيميائية والفيزيائية للعينات مع المقارنة مع وسط Zarrouk كمرجع مستخدم لكل تجربة على حدى، بينما أجريت جميع التجارب لمدة 21 يومًا للعثور على أفضل نوع من الوسائط والتي قد تبقى لفترة أطول وذلك للأغراض التجارية. في جميع التجارب، كانت قيم الأس الهيدروجيني بين 8 و 11 ، وكانت الموصلية للكهرباء بين 9.8-30 ملليسيمنز / سم ، بينما تم استخدام درجة الحرارة 30 درجة مئوية و 35 درجة مئوية، وتم استخدام مصدر اضاءة بشدة 1500 و 5000 لوكس لمدة 16 ساعة نهار و 8 ساعات ليل.

أجريت التجربة الأولى لتقييم إمكانية استخدام المياه الناتجة من أحواض أسماك البلطي (FW) في المشروع الإنشائي العربي لزراعة السبيروليينا. في هذه التجربة، بدأت كلا المعاملتان بتراكيز منخفضة، وكانت نسبة الامتصاص الأولى حوالي 0.5 عند 880 نانومتر. تضاعفت ZM ثلاث مرات بعد 8 أيام بينما تضاعف FW في نفس الفترة وبقيت كلا المعاملتان على نفس المستوى حتى نهاية التجربة. أظهرت هذه التجربة أن سبيروليينا يمكن أن تنمو في FW بطريقة مماثلة مثل ZM بسعر 0.0015 شيكل / لتر لـ FW مقارنة بـ 8.9625 شيكل / لتر لـ ZM.

أجريت التجربة الثانية من خلال معاملات FW والمياه متوسطة الملوحة المخففة (BW) ومحلول رماد النبات (PAS) ومزيج منهم (Mix). في هذه التجربة ، بدأت جميع المعاملات بتراكيز منخفضة كما في التجربة الأولى بنسبة إمتصاص حوالي 0.6 عند 880 نانومتر وتضاعف معظمها ثلاث مرات بعد 14 يومًا، وكان ZM هو الأقل بين الجميع؛ لذلك، نستنتج من هذه التجربة أنه يمكن استخدام FW ، BW المخفف، 3% PAS أو مزيج منها لزراعة سبيروليينا بسعر رخيص جدًا مقارنة بـ ZM.

أجريت التجربة الثالثة لتقييم نفس المكونات الموجودة في التجربة الثانية مع بعض التعديلات وإضافة معاملات جديدة مثل المياه الناتجة عن قص الحجر (MW) وإنشاء وسائط كيميائية جديدة مثل IMJ و IMJ-1 . في هذه التجربة، بدأت جميع

المعاملات بتركيز عالٍ مع نسبة إمتصاص بين 1.6-1.9 عند 880 نانومتر لجميع الأنواع. كان ZM و IMJ أفضل الأنواع من بين جميع المعاملات الأخرى. ظل FW عند نفس المستوى للأسبوع الأول وبدأ في النمو مع منحنى متذبذب حتى نهاية التجربة. يندر سعر تركيبة IMJ حوالي 3.66 شيكل / لتر وهي أقل من ZM ويمكن أن تكون مصدرًا بديلاً.

تم إجراء التجربة الرابعة في نفس وقت التجربة الخامسة. كانت التجربة عبارة عن مقارنة بين ZM و IMJ-2 في هذه التجربة ، بدأ كلا المعاملتان بتركيز معتدل مع نسبة إمتصاص 1.25 تقريباً عند 880 نانومتر. تضاعف ZM تقريباً بعد 14 يوماً بينما ارتفع IMJ-2 بمقدار 1.76 مرة في نفس الفترة. كان IMJ-2 أفضل من ZM حتى اليوم العاشر وظهر فرق واضح بعد ذلك IMJ-2. بسعر 1.38 شيكل / لتر أقل من ZM ويمكن أن يكون مصدرًا بديلاً رخيصاً

كانت التجربة الخامسة عبارة عن مقارنة بين ZM و FW والأنواع التالية (9: 1) FW: BW ، (9: 1) FW: MW ، (9: 1) FW: (BW + MW + PAS-3% + IMJ) ، ومزيج مكون من IMJ-1 (9: 1) ، FW: PAS-3% (9: 1) ، FW: (9: 1) (1) في هذه التجربة ، بدأت جميع المعاملات بتركيز معتدلة مع نسبة إمتصاص حوالي 1.25 عند 880 نانومتر. كان ZM و Mix من أفضل الأنواع ، بينما اتخذت ZM و Mix و IMJ-1: FW سلوكًا مشابهًا حتى اليوم العاشر.

وفقًا لنتائج الوزن الجاف وجدنا أنه لا يمثل نمو السبيرولينا، ولا يزال السبب مجهولاً. جميع المعاملات المستخدمة في هذه الدراسة كانت أقل تكلفة من وسط Zarrouk ومع بعض التحسين يمكن أن تكون جودتها أفضل. النتيجة الرئيسية التي تم الحصول عليها من جميع التجارب في هذه الدراسة هي قدرة سبيرولينا على النمو ضمن مجموعة واسعة من المتغيرات الكيميائية بسعر أرخص. لذلك توصي هذه الدراسة باستخدام المياه الناتجة عن تربية الأسماك في إنتاج السبيرولينا بعد فحصها.

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List of Abbreviations

ADS	Arab Development Society
BW	Brackish Water
CaCl ₂	Calcium Chloride
COVID-19	Corona Virus Disease 2019
EC	Electrical Conductivity
FeSO ₄ .7H ₂ O	Ferrous (II) sulfate heptahydrate
FW	Fish Wastewater
FAO	Food and Agriculture Organization
IMJ	Imad Aljabali Medium
IMJ-1	Imad Aljabali Medium Number 1
IMJ-2	Imad Aljabali Medium Number 2
ILS	Israeli Shekel
K ₂ HPO ₄	Dipotassium phosphate
KNO ₃	Potassium nitrate
L	Liter
m ³	Cubic Meter
MgSO ₄ .7H ₂ O	Magnesium sulfate heptahydrate
m	Meter
mS/cm	Micro Siemens per centimetre
mL	Milliliter
MW	Mining Water
MIX	Mix Solution
Na-EDTA	Disodium Salt (anhydrous) Ethylenediaminetetraacetic acid
NaHCO ₃	Sodium bicarbonate
NaCl-Salt	Sodium Chloride
NASA	National Aeronautics and Space Administration
ppm	Part per million
PAS	Plant Ash Solution
Pepsi Plant	Pepsi factory in Palestine that belong to the international Pepsi brand
pH	Quantitative measure of the acidity or basicity of aqueous solution Power of Hydrogen
S. platensis	<i>Spirulina platensis</i>
TDS	Total Dissolved Solids
UN	United Nations
WHO	World Health Organization
ZM	Zarrouk Medium
%	Percentage
\$	United States Dollar

Chapter One

Introduction & Background

- **1.1 Background**
- **1.2 Problem Statement**
- **1.3 Study Justification**
- **1.4 Study Goals**
- **1.5 Study Period**
- **1.6 Study Location**
- **1.7 Study Questions**

Chapter One: Introduction & Background

1.1 Background

Arthrospira (Spirulina) platensis is a filamentous cyanobacterium that has a high nutritional and economical value. (El-Kassas, 2015) (See Figure 1.1). It is now produced by several companies including pharmaceutical industries and sold all over the world as a high nutrition source in health food stores (Abdel-Daim, 2014; Alvarenga *et al.*, 2011), (Borowitzka *et al.*, 1988; Mingtao *et al.*, 1992). It is a rich source of protein, vitamins, and minerals. (Gumbo *et al.*, 2017) *Spirulina platensis* Production in China was first recorded at 19,080 tons in 2003 and increased sharply to 41,570 tons in 2004, with around 7.6\$ million and 16.6\$ million, respectively. (Ahsan, 2008). The global market size of Spirulina generated around \$348 million in 2018, and is estimated to reach around \$779 million by 2026. (Kunsel *et al.*, 2019)

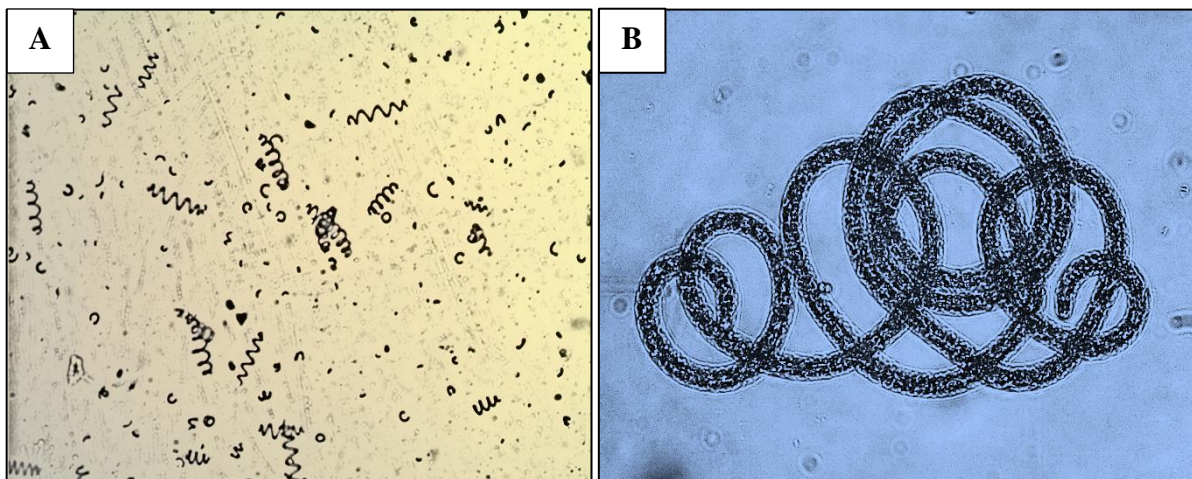


Figure (1.1): *Spirulina platensis* helical shape under microscope (A:4x, B:40x) in Aquatic Environmental Research Lab at AL-Quds University, Palestine.

1.2 Problem Statement

Introducing a new economical agricultural activity such as the production of *Spirulina platensis* to Palestine is vital to overcome the malnutrition in Palestine and introduce a new economical industry that could be sold worldwide for the benefit of Palestinian economy and local farmers. The main problem for Spirulina cultivation in Palestine is the high cost of the growth medium. Zarrouk medium (See table 1.1) (Zarrouk, 1966), the ideal medium, can cost around 8.9625 ILS/L if it prepared manually in the lab and if it used to produce *Spirulina platensis* for commercial scale as one dunum for example with 5 raceway ponds, 60 m³ each, it will cost according to our calculations for the growth medium only around 2,688,750 ILS for the initial farm preparation and need to add around 6 m³ monthly with a cost of 53,775

ILS and 645,300 ILS/year; so it is mandatory to find alternative and cheap source for the cultivation of *Spirulina platensis*.

Table (1.1): Zarrouk medium components (g/L) and their price ILS/L.

Zarrouk Medium Components		g/L	Mass (g)	Price (ILS) Sigma Aldrich Source	Price ILS/L	
NaNO ₃		2.5	1,000	426	1.065	
K ₂ HPO ₄		0.5	1,000	632	0.316	
MgSO ₄ .7H ₂ O		0.2	1,000	670	0.134	
CaCl ₂		0.04	1,000	1,124	0.045	
FeSO ₄ .7H ₂ O		0.01	1,000	886	0.008	
NaEDTA		0.08	1,000	1,298	0.103	
NaHCO ₃		16.8	1,000	385	6.468	
NaCl		1	1,000	374	0.374	
K ₂ SO ₄		1	1,000	449	0.449	
Micro Elements Solution	H ₃ BO ₃	0.62	1 mL	1,000	748	0.0005
	MnCl ₂ .4H ₂ O	0.012		1,000	1,032	
	ZnSO ₄ .4H ₂ O	0.044		1,000	591	
	Na ₂ MoO ₄	0.012		1,000	3,028	
	CuSO ₄ .5H ₂ O	0.02		1,000	856	
Sum					8.9625	

1.3 Study Justification

Introducing a new economic activity through the production of *Spirulina platensis* in Palestine using cheap local resources. The new activity will help to overcome the malnutrition in Palestine and introduce a new economical industry for the benefit of local labour.

1.4 Study Goals

The main goal of this Study is to conduct the basic operation and experiments on the cultivation and Production of large amounts of *Spirulina platensis* using Cheap, local, and environmentally friendly resources in a sustainable procedure.

The Specific Objectives are:

1. To gather the data and knowledge needed to perform the technical, economic and sustainability evaluations of *Spirulina platensis* production.
2. Adjusting of environmental factors (lighting, pH, mixing, etc.) to ensure the robust growth of *Spirulina platensis*.
3. Study the growth rate of *Spirulina platensis* in different water resources as: Fish water bonds, Brackish Water from the Jordan valley.
4. Study the growth rate of *Spirulina platensis* using low-cost nutrient resources such as local algae, cheap fertilizes and organic plant resources.

5. Study a mixture of optimum conditions of water Chemistry, Temperature and local resources that could introduce this valuable species to the Palestinian markets in low cost.

1.5 Study Period

All study experiments were done during COVID-19 pandemic in 2020 and 2021 as follow:

- Experiment One was done in June 2020.
- Experiment Two was done in October 2020.
- Experiment Three was done during March and April 2021.
- Experiments Four & Five were done during June and July 2021.

1.6 Study Location

Data collection and training were done in Aquatic Environmental Research Lab at Al-Quds University, while main experiments were done at my home in Jericho city due to COVID-19 and all measurements were taken at Agriculture Research Center Lab at Al-Quds Open University in the same city.

1.7 Study Questions

1. Is it possible to use aquaculture water as an alternative cheap medium for *Spirulina platensis* production?
2. Can plant ash be used as alternative cheap medium for *Spirulina platensis* production?
3. Can brackish water be used as alternative cheap medium for *Spirulina platensis* production?
4. Can mining water be used as alternative cheap medium for *Spirulina platensis* production?
5. Can fertilizers formula be used as alternative cheap medium for *Spirulina platensis* production?
6. Is it possible to formulate a new formula by mixing the above resources in different ratios for the optimum growth of *Spirulina platensis*?

Chapter Two

Literature Review

- **2.1 Literature Review**
 - 2.1.1 *Spirulina platensis* Discovery
 - 2.1.2 Life Cycle of *Spirulina platensis*
 - 2.1.3 Physical & Chemical Characteristics of *Spirulina platensis*
 - 2.1.4 *Spirulina platensis* Measurement Tools
 - 2.1.5 *Spirulina platensis* Components
 - 2.1.6 *Spirulina platensis* farming systems around the world
 - 2.1.7 *Spirulina platensis* in Palestine: Where do we stand?
 - 2.1.8 Fish Farms in Palestine
 - 2.1.9 Brackish Water in Jordan Valley
 - 2.1.10 Mining Stones in Palestine
- **2.2 Previous Studies**

Chapter Two: Literature Review & Previous Studies

2.1 Literature Review

2.1.1 *Spirulina platensis* Discovery

Spirulina platensis is an old microorganism that rediscovered in last few decades; P. J. Turpin isolated *Spirulina* from a freshwater sample in 1827 AD (Saranraj *et al.*, 2014). *Spirulina* was first isolated in the 16th century from Lake Texcoco in Mexico. (Al-Harbi, 2008; Habib, 2008) In 1990s; National Aeronautics and Space Administration (NASA) studied the cultivation of *Spirulina platensis* as a food source (Tadros, 1988) also World Health Organization (WHO) reported that *Spirulina platensis* has no risk and is a good food supplement for health. (Seyidoglu, 2017) *Spirulina platensis* has 50-70% protein, 5-10% lipids and 10-20% carbohydrates, all essential amino acid in complete balance, 10 vitamins especially vitamin B12, pro-vitamin A (β -carotene), and minerals such as iron. (Li *et al.*, 2012; Seshadri, 1993)

2.1.2 Life Cycle of *Spirulina platensis*

The life cycle of *Spirulina platensis* in laboratory culture is rather simple (Fig. 2.1) (Balloni, 1980); the spirulina whole cell is called Trichome; a mature trichome is broken to several pieces to form Necridia that will form hormogonia after several processes; while hormogonia rise to give a new trichome. *Spirulina* trichomes increase in numbers and lead to increase chlorophyll as well and that give it the unique green color.

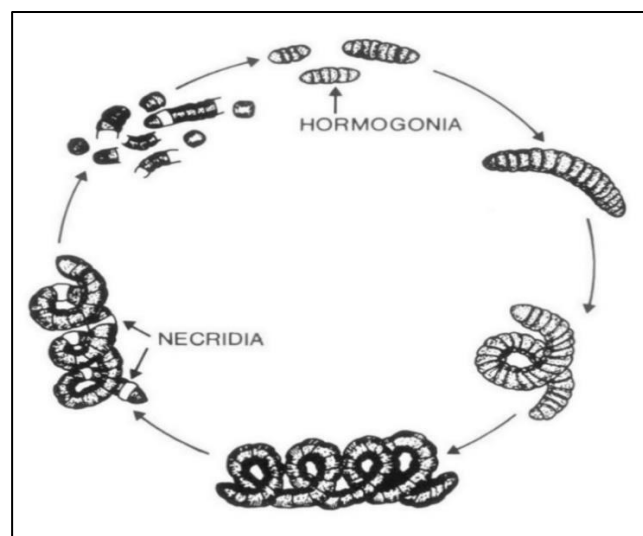


Figure (2.1): *Spirulina platensis* Life Cycle (Balloni, 1980; Ciferri, 1983)

2.1.3 Physical & Chemical Characteristics of *Spirulina platensis*

Spirulina platensis is naturally found in tropical regions inhabiting alkaline lakes (pH 11) with high concentration of NaCl and bicarbonates. (Cozza *et al.*, 2000; Volkmann, 2008) *Spirulina platensis* can live with pH (8.5-11) (Ahsan, 2008), and electrical conductivity of 20,000 $\mu\text{S}/\text{cm}$, while the best growth light of *Spirulina platensis* is on 5 Klux. It can also survive down to 1.5 Klux (E. Danesi *et al.*, 2004). *Spirulina* like other organisms need good and optimum temperature to reach their best growth, the best temperature for *Spirulina* growth is 35 °C (Torzillo *et al.*, 1991) Mixing of *Spirulina platensis* is a suitable way to support it with Carbon Dioxide and Oxygen, without mixing, the algae will go up toward the surface and in case of any unsuitable conditions it may sink and die.

2.1.4 *Spirulina platensis* Measurement Tools

Spirulina platensis growth can be measured and monitored through varied methods; such as cell concentration, Chlorophyll content, Turbidity (Secchi Disk), and using standard curve obtained from *Spirulina* dry weight and absorbance, also it can be measured by monitoring the change of color through spectrophotometer on wavelengths such as 560 and 880nm. (Delrue *et al.*, 2017)

2.1.5 *Spirulina platensis* Components

Spirulina platensis or Superfood as it called has the highest level of protein content among other protein sources, it has total protein content of 50-70g per 100g of *Spirulina platensis* powder while whole dried egg had 47% and soyabean has around 36%, also peanut, chicken, fish, and beef meat have a range between 17-26%, and that make *Spirulina platensis* on the top. (Henrikson, 1994)

Table (2.1): *Spirulina* protein content vs. different food protein content (Henrikson, 1994)

Food Protein Origin	Protein (%)
Spirulina powder	60-70
Whole dried egg	47
Beer yeast	45
Skimmed powdered milk	36
Whole soybean flour	36
Parmesan cheese	36
Wheat germ	27
Peanuts	26
Chicken	19-24
Fish	19.2-20.6
Beef meat	17.4

2.1.6 *Spirulina platensis* farming systems around the world

Spirulina platensis farming could be achieved in different methods. The most famous method is through raceway ponds. Raceway ponds came with different dimensions according to the volume of *Spirulina platensis*, it can be grown in very small scale (100 L) to large scales (35,000L) for each pond (Figure 2.2) (Company, 2021) / The size could be larger or smaller according to the purpose of use. Optimum growth could be only achieved under suitable and optimum growth conditions of temperature, light, mixing, pH, and the proper nutrients.

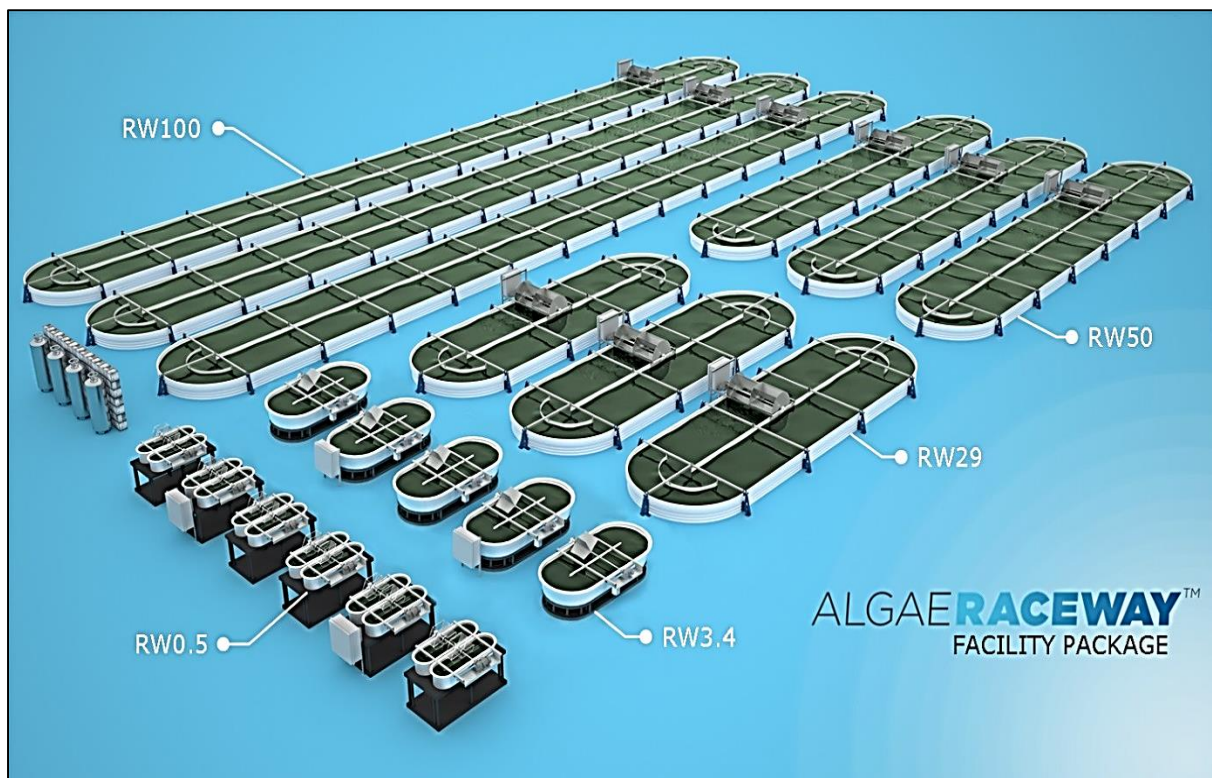


Figure (2.2): Raceway ponds different sizes; RW0.5 = 100L, RW3.4 = 950L, RW100 = 35m³ (Company, 2021)

Raceway ponds could be created from different materials, concrete, metal, polyvinyl chloride (PVC), or any other suitable material. *Spirulina platensis* could be cultivated in bioreactor systems that may composed of plastic bags, plastic columns, or glass.

Spirulina platensis used as food supplement, fish food, and in animal feed, but most of its' production went to food supplements. In 1976, a group of U.S. pioneers, founded the Earthrise company in California to develop spirulina as a world food resource. (Earthrise, 2021b) Earthrise produce different products based on *Spirulina* types ranges from 10-100\$, Earthrise operates the world's largest *Spirulina* farm (37 pond, 5000m² each) on around 437 dunum site (figure 2.3) to supply over 20 countries with *Spirulina* products. (Earthrise, 2021a)



Figure (2.3): Example of Earthrise farms in California, USA. (Earthrise, 2021a)

Another example of Spirulina farm is Nutrex Hawaii company (figure 2.4) that operates in Hawaii in the United States of America, Nutrex Hawaii was initiated in 1990 by Dr. Gerald Cysewski for production of Spirulina products, the company has many products and operates on an area of around 360 dunum. (Hawaii, 2021b) and beside USA Spirulina producing companies, France had created the Federation of Spiruliniers in 2009 that gather all Spirulina farmers in France.

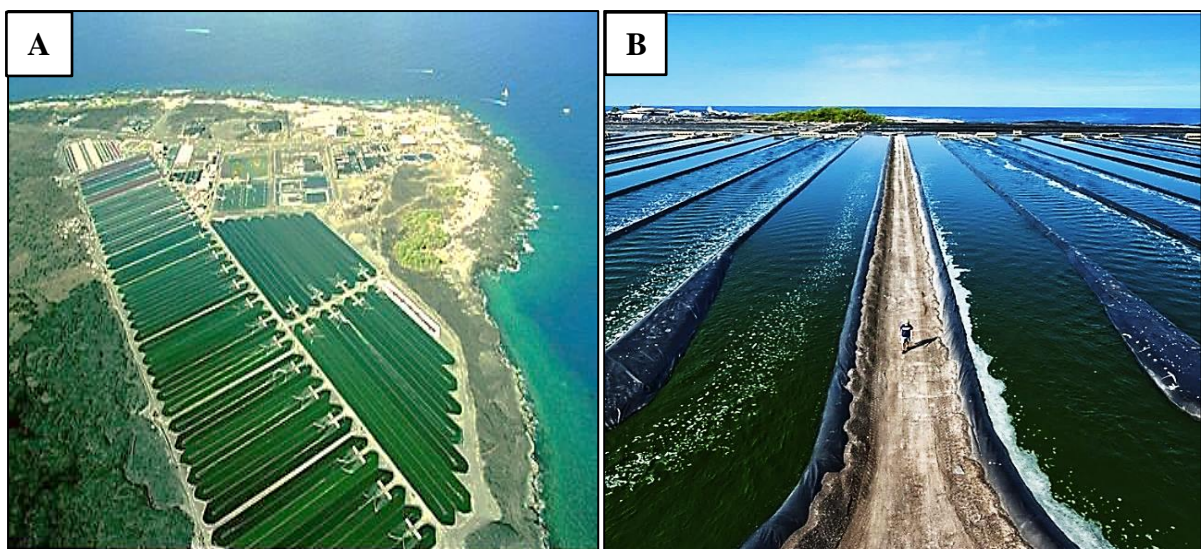


Figure (2.4): Nutrex Hawaii Farms in Hawaii, USA. (Hawaii, 2021a)

2.1.7 *Spirulina platensis* in Palestine: Where do we stand?

Spirulina platensis natural habitat is the alkaline-brackish lakes in the tropical regions and this kind of conditions is not present in Palestine and the case is same for it. The entrance of *Spirulina platensis* to Palestine was very difficult due to Israeli occupation, but in the last few years the Palestinian scientists in the universities and research centers were able to introduce it in the lab and few of them were able to cultivate it successfully, and this study is one of the first projects that worked to cultivate *Spirulina platensis* with cheap resources in Palestine, while during my work as a supervisor of *Spirulina platensis* project entitled “Joint Center of Excellence for Strategic Arid Land Food and Bioproduction – SAFAB” at Agricultural Research Center in Jericho as a cooperation between Al-Quds Open University and the higher council for innovation and excellence since 2018, *Spirulina platensis* was successfully cultivated in the lab, and upgraded *Spirulina platensis* from lab scale to pilot scale as shown in figure 2.5.

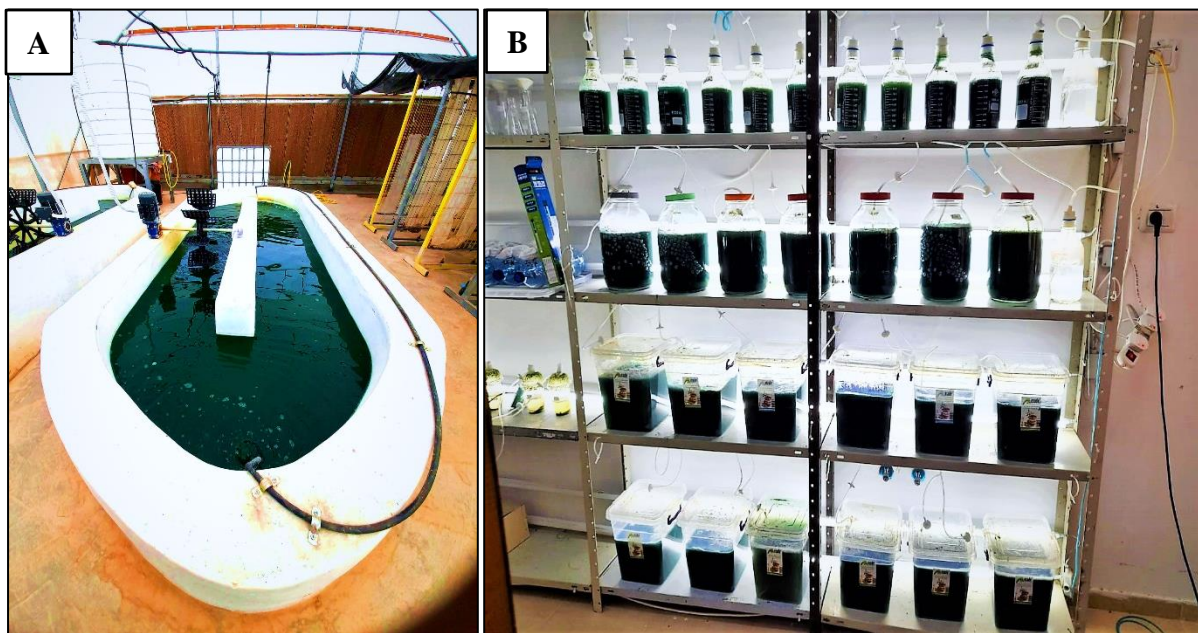


Figure (2.5): *Spirulina platensis* in Agricultural Research Center at Al-Quds Open University. (A) Raceway ponds as Pilot Scale, and (B) *Spirulina platensis* samples. (Aljabali, 2021)

2.1.8 Fish Farms in Palestine

Fish farming were introduced in Palestine since around 11 years with the established of Tilapia fish farms in Arab Development Society (ADS). ADS in the eastern part of Jericho city in Palestine. According to ADS website, it has total area of 8,084 dunum (Bsharat, 2021) and most of the land is rented to local Palestinian companies.

Tilapia farm in ADS has 23 ponds with estimated capacity of around 3000 m³ water and different pond sizes from 6 m³ to 300 m³ while around 400 m³ of water changed daily from all ponds. Fish farm is supplied with water from local well contains brackish water in Arab Development Society and fresh water from Jericho municipality. The farm itself is composed of around 6 large ponds and 4 Brazilian-type ponds (Figure 2.6) and around 13 ponds (Figure 2.7) for Tilapia cultivation and propagation. (Najjar, 2021) Tilapia rearing-water could be a proper and suitable alternative growth medium for *Spirulina platensis* because it is rich in nutrients with respect of other microorganisms that present in it and with the proper filtration and sterilization.

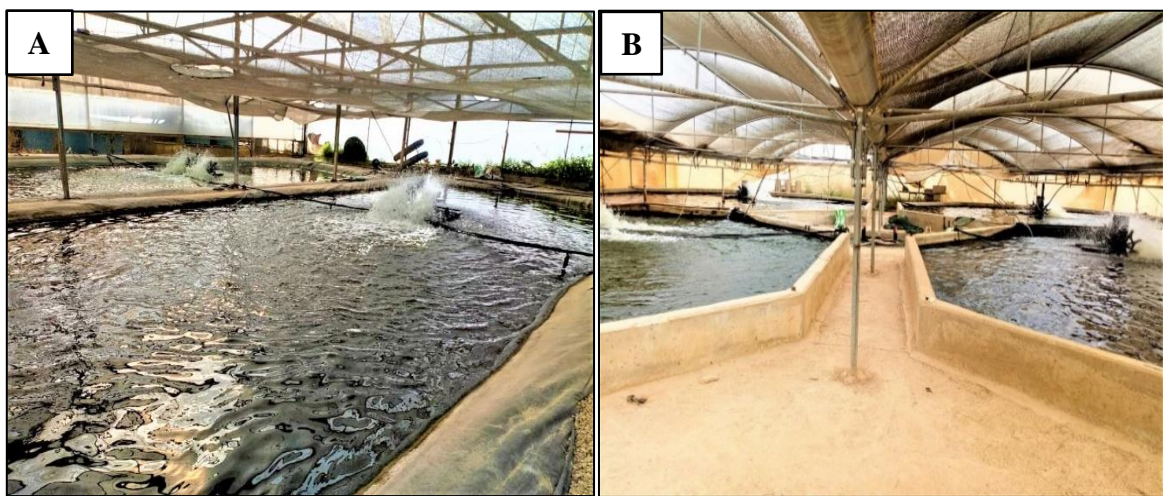


Figure (2.6): (A) Tilapia ponds, and (B) Brazilian Type of Tilapia ponds in ADS.



Figure (2.7): Tilapia Propagation Ponds in ADS.

2.1.9 Brackish Water in Jordan Valley

West bank and Gaza suffer from water shortage and water scarcity and because Jericho area is the representative area of the Jordan Valley, (Da'as *et al.*, 2013) it could be the best choice for studying the use of brackish water from local wells to cultivate algae instead of using it in agriculture because some plants in Jericho such as banana cannot tolerate brackish water.

In 2018, Pepsi plant or factory (belong to trademark Pepsi) was established besides of the agro-industrial park in Jericho city, and it uses brackish water from a local well. The water enter a treatment process in water treatment room, it enters chlorination process, then chlore removed using carbon filters, then water filtered using bag filters and treated by ultraviolet unit, then it filtered using reverse osmosis (RO) unit; the water enters the RO unit with total dissolved solids (TDS) of 5000ppm and exit the unit with TDS around 50ppm, while the unaccepted water (but can be treated) transport to mini-RO unit and enters the treatment cycle again and the unaccepted water (TDS is 10,000ppm) ejected from the mini-RO unit and transported to irrigate Palm date farms.



Figure (2.8): Reverse Osmosis main unit in Pepsi Plant in Jericho City.

2.1.10 Mining Stones in Palestine

The Stone (limestone) and Marble (trade name) Industry (SMI) is one of the most important and active industries in Palestine. (Salem, 2021) The stone or as it called the white gold industry has a great impact on the economy and it is profitable for the people in Palestine, but also it has a negative impact on the environment, the water that used in cutting and treatment of the stone is discarded on the surface of the ground. The water prevents the growth of many plants and that leads to endanger other organisms including humans; the mining itself destroys the land and the natural resources including the biodiversity. So, according to our measurements the water pH value was over than 8. The high pH value could be a good indicator to solve the negative effect of Mining stone toward the environment if the water used in Spirulina cultivation.

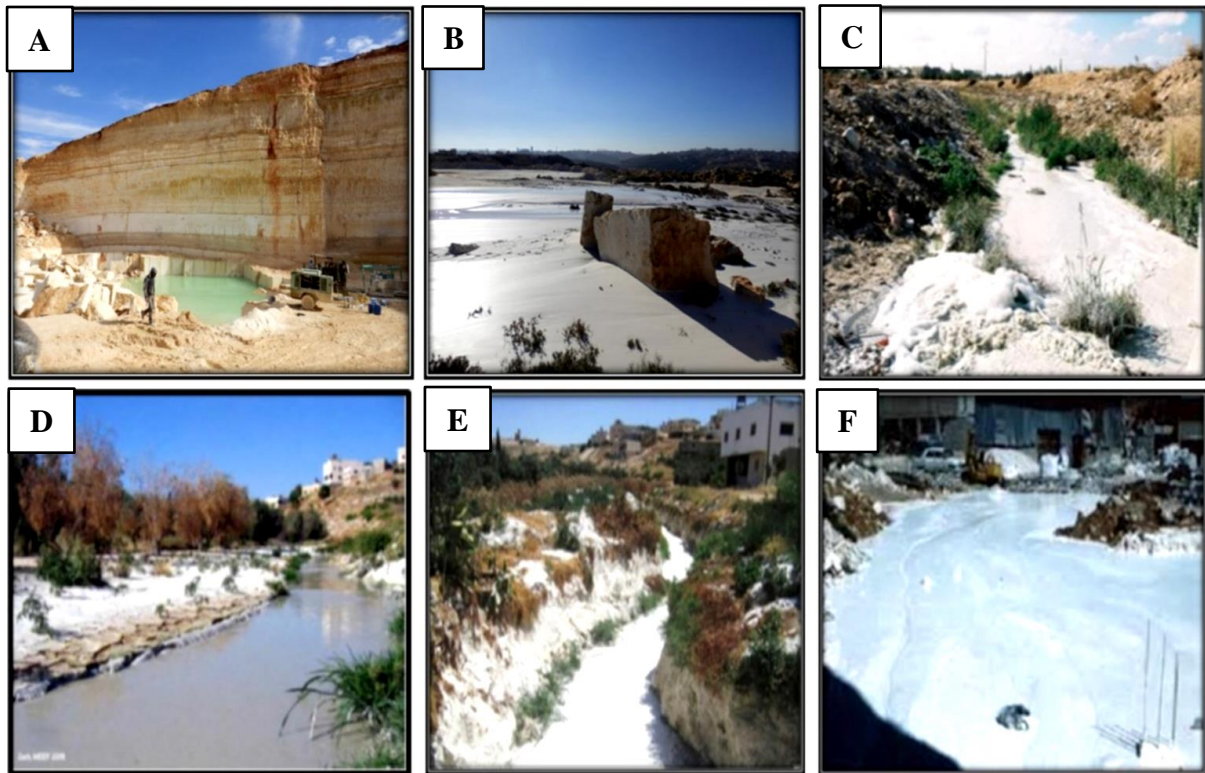


Figure (2.9): Examples of the wet and dry limestone slurry waste (LSW), generated from quarrying and stone-cutting sites, flowing to agricultural and residential areas in different localities of the West Bank. (Al-Joulani, 2008; Salem, 2021)

2.2 Previous Studies

Soni *et al.* (2019) work presented a novel experimental approach to maximize biomass yield, minimize evaporation rate and respiration losses in a laboratory scale closed reactor and open pond system. Lab scale, open pond, and closed reactor system were designed for spirulina cultivation under dry climatic conditions at Bhopal, India. Zarrouk media was used as standard and modified organic media was prepared by changing the nitrogen source. Temperature and other input parameters were maintained. Aeration was done manually in an open pond, and the air pump was used in the case of a closed reactor system. Biomass yield obtained from an open pond system was 11.34 g/l, and 12.28 g/l in the closed reactor system. Doubling time was also less in the closed reactor in comparison with the open pond system. Urea seems to be a promising alternative source of low-cost nitrogen for Spirulina cultures. From the experimental results, it is concluded that modified organic media and closed reactor system could be used for better biomass yield.

Sukumaran *et al.* (2018) studied the prospect of *Arthrospira platensis* cultivation in newly designed medium with commercial or industrial grade fertilizers under real environment. Consequently, growth and yield of *A. platensis* was investigated under outdoor condition using modified Kosaric medium (MKM) which was designed by substituting the major laboratory chemicals in standard Kosaric medium (SKM) with commercial grade baking soda, sea salt, urea, phosphoric acid, potassium hydroxide and Epsom salt. Urea as an alternative nitrogen resource to sodium nitrate was pulse-fed throughout the cultivation period. The algal growth was measured through optical density, biomass dry weight and chlorophyll A content. The algal yield was determined by calculating its productivity and specific growth rate. The growth and yield of *A. platensis* was significantly higher ($p < 0.05$) in MKM in terms of optical density with 2.541 ABS, biomass dry weight with 1.30 g/L, chlorophyll a content with 12.96 mg/L, productivity with 0.141 g/L. d⁻¹ and specific growth rate with 0.253 μ/d compared to SKM in eight days of cultivation period. The present finding showed the potential of MKM in lowering the medium cost up to 97% compared to SKM without compromising the algal yield under natural condition with proper cultivation techniques such as preadaptation and fed batch addition of urea in the late evening.

Delrue *et al.* (2017) investigated Spirulina biomass productivity, a modified Zarrouk medium was selected as it gave higher final dry weights and longer sustained growth than Hiri's and Jourdan's media. Then, to reduce Spirulina production cost, modified Zarrouk medium was

rationalized by testing different dilutions. It was found that modified Zarrouk medium could be diluted up to five times without impacting the growth rates in a 28-days batch cultivation. Higher dry weights were even observed after 21 days of batch cultivation (1.21 g/L for 20%-modified Zarrouk medium in comparison to 0.84 g/L for modified Zarrouk medium). Iron uptake was then investigated as one of the major contributors to *Spirulina* nutritional quality. An increase in iron content was obtained by replacing iron sulfate by iron EDTA at a concentration of 10 mgFe/L (2.11 ± 0.13 mgFe/g biomass for EDTA-FeNa.3H₂O at 10 mgFe/L compared to 0.18 ± 0.13 for FeSO₄.6H₂O at 2 mgFe/L). Impact of light intensity on *Spirulina* biomass productivity was also investigated in a 2 L Photobioreactor (PBR). Specific growth rates were calculated for Photosynthetically Photon Flux Densities (PPFD) from 85 to 430 $\mu\text{mol}/\text{m}^2\cdot\text{s}^{-1}$. At 430 $\mu\text{mol}/\text{m}^2\cdot\text{s}^{-1}$, photoinhibition was not observed and the specific growth rate was maximum (0.12/day). Finally, a 40-day cultivation experiment was conducted in a 1000 L PBR giving a maximum daily areal productivity of 58.4 $\text{g}/\text{m}^2\cdot\text{day}^{-1}$. A techno-economic analysis gave production cost two to 20 times higher for PBR (from 18.71 to 74.29 /kg) than for open ponds (from 3.86 to 9.59 /kg) depending on *Spirulina* productivity.

Rajasekaran *et al.* (2016) studied and evaluated the effect of modified Zarrouk medium on the growth response of 6 different *Spirulina* strains. Specific growth rate, doubling time, mean daily division rate, biomass, and chlorophyll-A contents were analyzed. Growth patterns of the strains were monitored continuously for 40 days. The results revealed significant differences in the growth parameters for different strains. *Spirulina platensis* (SP-6) and *Spirulina platensis* (CCMB) showed the maximum specific growth rates ($\mu = 6.1$, $\mu = 5.8$), doubling times ($T_d = 6.93$, $T_d = 6.87$), mean division rates ($k = 0.27$, $k = 0.23$) biomasses (5.1, 5.0 g/l) and chlorophyll A contents (78, 65 $\mu\text{g}/\text{ml}$) respectively, when compared with the other strains used in the study. Therefore, *Spirulina platensis* (SP-6) and *Spirulina platensis* (CCMB) strains can be suggested for large scale commercial cultivation with modified Zarrouk medium.

Danesi *et al.* (2011) studied the effects of light intensity and temperature in *S. platensis* cultivation with potassium nitrate or urea as nitrogen source, as well as the biomass chlorophyll contents of this *Spirulina platensis*, through the Response Surface Methodology. Experiments were performed at temperatures from 25-34.5 °C and light intensities from 15 to 69 $\mu\text{mol photons m}^{-2}\cdot\text{s}^{-1}$, in mineral medium. In cultivations with both sources of nitrogen, KNO₃ and urea, statistic evaluation through multiple regression, no interactions of such

independent variables were detected in the results of the dependent variables maximum cell concentration, chlorophyll biomass contents, cell, and chlorophyll productivities, as well as in the nitrogen-cell conversion factor. In cultivation performed with both sources of nitrogen, it was possible to obtain satisfactory adjustments to relate the dependent variables to the independent variables. The best results were achieved at temperature of 30 °C, at light intensity of 60 $\mu\text{mol photons m}^{-2}\cdot\text{s}^{-1}$, for cell growth, with cell productivity of approximately 95 $\text{mg}\cdot\text{L}^{-1}\cdot\text{d}^{-1}$ in cultivations with urea. For the chlorophyll biomass content, the most adequate light intensity was 24 $\mu\text{mol photons m}^{-2}\cdot\text{s}^{-1}$.

Chapter Three

Materials & Methods

- **3.1 Materials**
- **3.2 Methodology**
 - 3.2.1 Data Collection
 - 3.2.2 *Spirulina platensis* Cultivation
 - 3.2.3 Growth Medium Types
 - 3.2.4 Physical and Chemical Parameters
 - 3.2.5 Growth Medium & Experiment Preparation

Chapter Three: Materials & Methods

3.1 Materials

In this study general lab tools were used in all experiments. The devices include single beam UV/Vis spectrophotometer (Jenway 7305) and cuvettes, vertical autoclave, pH and EC meter, analytical balance (up to 120g, sartorius) and Whatman filter paper (90mm diameter).

While the tools include LED light (Cooled White, 1500 Lux, 5000 Lux), fish aerator, airline tubes (originally used for aquariums), cotton, distiller, aquarium airline separator, plastic Tupperware, plastic pipette (3mL), micropipette 1mL with tips, tubes (50mL), graduated cylinder (50mL, 100mL, 250mL, 500mL, 1000mL), flasks (100mL, 250mL, 500mL, 1000mL), and beakers (500mL, 1000mL, 2000mL)

The following chemicals were used in this study:

- **Sigma Aldrich:** NaNO₃, K₂HPO₄, MgSO₄·7H₂O, CaCl₂, FeSO₄·7H₂O, Na-EDTA, NaHCO₃, NaCl, K₂SO₄, HBO₃, MnCl₂·4H₂O, ZnSO₄·4H₂O, Na₂MoO₄, CuSO₄·5H₂O.
- **Local market:** Sodium bicarbonate, Salt table, Urea, Potassium Sulphate fertilizer (50%).

3.2 Methodology

3.2.1 Data Collection

Data and information about *Spirulina platensis* were gathered from scientific papers and books in 2019 and 2020 through searching via google scholar and reading various topics about Spirulina cultivation and production. While the training on *Spirulina platensis* cultivation was done in Aquatic Environmental Research Center at AL-Quds University after looking for videos on YouTube that shown Spirulina production methods around the world.

The main book used to learn about Spirulina entitled “Spirulina platensis (Arthrospira): Physiology, Cell-biology, and Biotechnology, edited by Avigad Vonshak”. While the main document used to understand Spirulina situation entitled “Spirulina A Livelihood and A Business Venture by Antonio Piccolo” that published by FAO.

3.2.2 *Spirulina platensis* Cultivation in AL-Quds University

Spirulina platensis was cultivated using Zarrouk medium (tables 3.1 and 3.2) in fish aquariums (figure 3.1) in Aquatic Environmental Research Center (AERC) at AL-Quds University at a temperature of 32 °C, pH of 10.5, sunlight of ~25K-Lux, and aeration using an

air pump. While during COVID-19 pandemic *Spirulina* samples were transferred to Jericho city (figure 3.2) and cultivated with similar growth conditions to establish the experiments.



Figure (3.1): *Spirulina platensis* in different aquariums in AERC at AL-Quds university.



Figure (3.2): *Spirulina platensis* samples in Jericho city.

3.2.3 Growth Medium Types

Spirulina platensis can be cultivated in different conditions, for that reason different growth medium types were created. Zarrouk medium was used the most to support the growth of *Spirulina platensis*. In this study Zarrouk medium was used as a reference medium. To achieve the purpose of this study, the following local materials were used to prepare a cheap local media:

1. Fish wastewater (not analyzed for its' composition) was used and collected from Arab Development Society Tilapia Farm in Jericho.

2. Brackish water (not analyzed for its' composition) gathered from Jordan Valley, especially from the rejected water from the Reverse Osmosis Unit in Pepsi Plant (Note: the water was diluted for the irrigation of Palm date trees in Al-Wadi Farms in Jericho).
3. Limestone water (not analyzed for its' composition) gathered from Hebron mining stone.
4. Plant ash (not analyzed for its' composition) created after burning of weed and grass leaves and fibers that collected from different parts in Jericho.
5. Different chemical formulas (IMJ, IMJ-1, IMJ-2) composed of fertilizers & chemicals.

3.2.4 Physical and Chemical Parameters

Physical and chemical parameters such as pH, EC, TDS, Salinity, turbidity, and color were tested for each growth medium type, also temperature and light intensity were measured too.

3.2.5 Growth Medium & Experiment Preparation

To understand the ecology and behavior of *Spirulina platensis* in different cheap local resources, the following experiments were investigated using Zarrouk medium as a reference.

3.2.5.1 Preparation of Zarrouk medium

Zarrouk medium (ZM) (Zarrouk, 1966) was prepared from the components shown in Table 3.1 and 1mL of the components in table 3.2 was added to the solution.

Table (3.1): Zarrouk medium main components

Component	Mass (g/L)	Source
NaNO ₃	2.5	Sigma Aldrich
K ₂ HPO ₄	0.5	Sigma Aldrich
MgSO ₄ .7H ₂ O	0.2	Sigma Aldrich
CaCl ₂ ,	0.04	Sigma Aldrich
FeSO ₄ .7H ₂ O	0.01	Sigma Aldrich
Na-EDTA	0.08	Sigma Aldrich
NaHCO ₃	16.8	Sigma Aldrich
NaCl	1.0	Sigma Aldrich
K ₂ SO ₄	1.0	Sigma Aldrich

Table (3.2): Zarrouk medium micro components

Component	Mass (g/L)	Source
HBO ₃	0.62	Sigma Aldrich
MnCl ₂ .4H ₂ O	0.012	Sigma Aldrich
of ZnSO ₄ .4H ₂ O	0.044	Sigma Aldrich
Na ₂ MoO ₄	0.012	Sigma Aldrich
CuSO ₄ .5H ₂ O	0.02	Sigma Aldrich

All components except NaHCO₃ were dissolved together in 500mL of distilled water while NaHCO₃ was dissolved separately in 500mL of distilled water (to prevent salt precipitation).

The two solutions were autoclaved at 121 °C and 105 KPa for 15 min. After it was cooled to room temperature, it was mixed to form 1000mL of the reference Zarrouk medium.

3.2.5.2 First Experiment Preparation

Fish Wastewater (FW) was taken from Tilapia fish farm in Arab Development Society, it was filtered 3 times to remove all large particles and autoclaved on 121 °C and 105 KPa for 15 min. FW was cooled at room temperature. 5 replicates, composed of 50mL of *Spirulina platensis* with 50mL of fish wastewater per each replicate, were prepared. Also, 5 replicates were prepared for ZM treatment (50mL of Zarrouk medium (ZM) with 50mL of *Spirulina platensis* for each replicate).



Figure (3.3): Preparation of *Spirulina platensis* samples.

All fish wastewater and the reference Zarrouk medium treatments (table 3.3) were incubated using white LED light (1500 lux) as a light source for a period of 16h light and 8h dark in an aerated flasks by using an air pump at a temperature of 30 °C. 10mL of distilled water were added daily to replace the evaporation loss. The experiment was run for 21 days, and the measurements were taken twice per week.

Table (3.3): First Experiment Treatments (mL)

First Experiment Treatments (mL)											
ZM Type	R 1	R 2	R 3	R 4	R 5	FW Type	R 1	R 2	R 3	R 4	R 5
Zarrouk Medium	50	50	50	50	50	Fish Wastewater	50	50	50	50	50
<i>Spirulina platensis</i>	50	50	50	50	50	<i>Spirulina platensis</i>	50	50	50	50	50
Total Volume (mL)	100	100	100	100	100	Total Volume (mL)	100	100	100	100	100

*R: Replicate

Measurements for all replicates were analyzed using single beam UV/Vis spectrophotometer (Jenway 7305) at 880nm. Dry weight for all samples was measured by taking 10mL from each sample and filtered it on Whatman paper. Samples analysis and drying was repeated every week.

3.2.5.3 Second Experiment Preparation

Zarrouk medium was prepared as in section 3.2.5.1, while Wastewater from fishponds was treated as in section 3.2.5.2, ZM and FW treatments were prepared as in section 3.2.5.2 with lower replicates number (table 3.4).



Figure (3.4): Second Experiment treatments.

Plant Ash Solution (PAS) was prepared with a concentration of 3%, and the solution was filtered 4 times and autoclaved at 121 °C and 105 KPa for 15 min, then and after the solution was cooled at room temperature it was mixed with *Spirulina platensis* for making 3 replicates as follow: 50mL of *Spirulina platensis* were mixed with 50mL of PAS 3% for each sample.

Brackish water (BW) from rejected RO-unit in Pepsi Plant was diluted with distilled water as 1:2 dilution factor and autoclaved at 121 °C and 105 KPa for 15 min, then and after the solution was cooled at room temperature it was mixed with *Spirulina platensis* for make 3 replicates as follow: 50mL of *Spirulina platensis* were mixed with 50mL of brackish water (1:2) for each sample.

Mix solution was prepared from FW, PAS 3%, and BW (1:2) with a ratio of 1:1:1, and the solution autoclaved on 121 °C and 105 KPa for 15 min and after the solution cooled it was mixed with *Spirulina platensis* to make 3 replicates as follow: 50mL of *Spirulina platensis* were mixed with 50mL of mix solution for each sample.

Table (3.4): Second Experiment Treatments (mL)

Second Experiment Treatments (mL)							
ZM Type	R 1	R 2	R 3	FW Type	R 1	R 2	R 3
Zarrouk Medium	50	50	50	Fish Wastewater	50	50	50
<i>Spirulina platensis</i>	50	50	50	<i>Spirulina platensis</i>	50	50	50
Total Volume (mL)	100	100	100	Total Volume (mL)	100	100	100
PAS Type	R 1	R 2	R 3	BW Type	R 1	R 2	R 3
Plant Ash Solution 3%	50	50	50	Brackish water 1:2	50	50	50
<i>Spirulina platensis</i>	50	50	50	<i>Spirulina platensis</i>	50	50	50
Total Volume (mL)	100	100	100	Total Volume (mL)	100	100	100
MIX Type	R 1	R 2	R 3				
MIX Solution	50	50	50				
<i>Spirulina platensis</i>	50	50	50				
Total Volume (mL)	100	100	100				

*R: Replicate

All samples were incubated under white LED light (1500 lux) as a light source for a period of 16h light and 8h dark in an aerated flasks by using an air pump at a temperature of 30 °C. 10mL of distilled water were added daily to replace the evaporation loss. Measurements for all replicates were analyzed using single beam UV/Vis spectrophotometer (Jenway 7305) at 880nm. Dry weight for all samples was taken every week by taking 10mL from each sample and filtered it on Whatman paper. Samples analysis and drying was repeated every week.

3.2.5.4 Third Experiment Preparation

Some of the third experiment treatments (Zarrouk medium, fish wastewater, brackish water) was like the previous section (3.2.5.3) with exception to the addition of new treatments as described below. Plant Ash Solution (PAS) was prepared with a concentration of 1.5%, and the solution was filtered 4 times and autoclaved on 121 °C and 105 KPa for 15 min, then and after the solution was cooled at room temperature it was mixed with *Spirulina platensis* to create 4 replicates as follow: 125mL of *Spirulina platensis* were mixed with 125mL of PAS 3% for each sample.

Mining water (MW), was taken from Mining stone from Hebron city, was autoclaved on 121 °C and 105 KPa for 15 min, then the solution was mixed with *Spirulina platensis* to create 4 replicates as follow: 125mL of *Spirulina platensis* were mixed with 125mL of mining water for each sample.

Imad Aljabali medium (IMJ) was prepared as described below and was dissolved in 1000mL of distilled water with addition of 1mL from micro-nutrients solution (See table 3.6), then autoclaved at 121 °C and 105 KPa for 15min. The solution was mixed with *Spirulina*

platensis to form 4 replicates as follow: 125mL of *Spirulina platensis* were mixed with 125mL of IMJ for each sample.

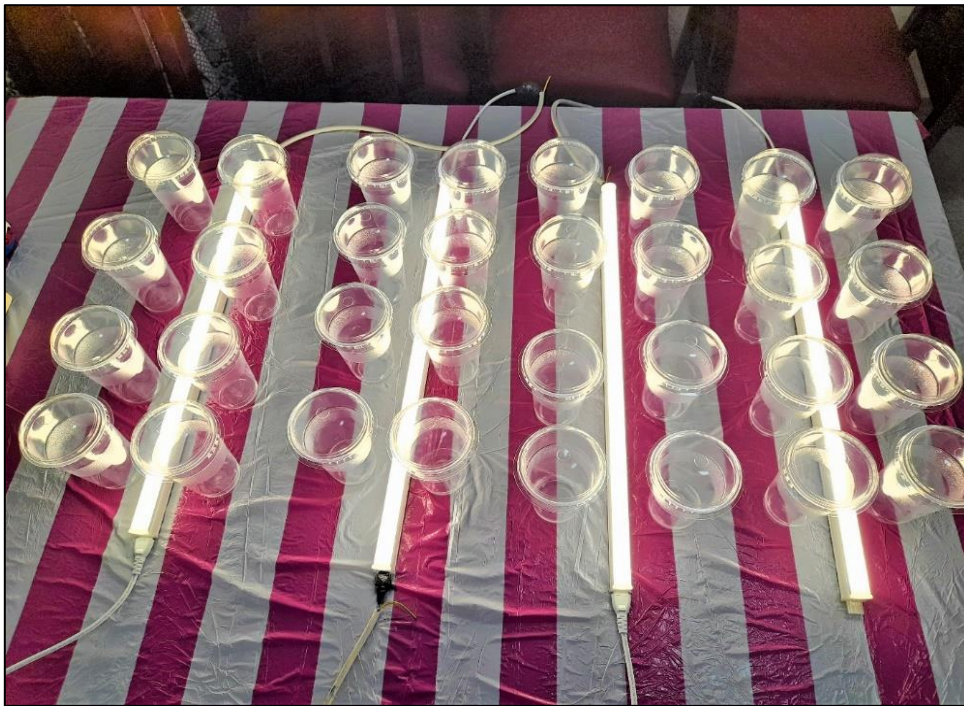


Figure (3.5): Third Experiment Preparation: Step 1.



Figure (3.6): Third Experiment Preparation: Step 2.

Table (3.5): IMJ medium main components

Component	Mass (g/L)	Source
KNO ₃	3.0	Sigma Aldrich
K ₂ HPO ₄	0.2	Sigma Aldrich
MgSO ₄ .7H ₂ O	0.15	Sigma Aldrich
CaCl ₂	0.04	Sigma Aldrich
FeSO ₄ .7H ₂ O	0.06	Sigma Aldrich
Na-EDTA	0.1	Sigma Aldrich
NaHCO ₃	5.2	Local Market
NaCl	2.5	Salt Table
K ₂ SO ₄	2.0	Sigma Aldrich

Table (3.6): IMJ medium micro components

Component	Mass (g/L)	Source
HBO ₃	0.62	Sigma Aldrich
MnCl ₂ .4H ₂ O	0.012	
of ZnSO ₄ .4H ₂ O	0.044	
Na ₂ MoO ₄	0.012	
CuSO ₄ .5H ₂ O	0.02	



Figure (3.7): Third Experiment Preparation: Step 3.

Imad Aljabali medium – 1 (IMJ-1) was prepared as present table (3.7) and was dissolved in 1000mL of distilled water with addition of 1mL from micro-nutrients solution (table 3.8) and autoclaved at 121 °C and 105 KPa for 15min, then the solution was mixed with *Spirulina platensis* to form 4 replicates as follow: 125mL of *Spirulina platensis* were mixed with 125mL of IMJ-1 for each sample.

Table (3.7): IMJ-1 medium main components

Component	Mass (g/L)	Source
NaHCO ₃	16.8	Local Market
NaCl	1.0	Salt Table
Urea	0.5	Fertilizer – Local Market
CaCl ₂ .2H ₂ O	0.04	Sigma Aldrich
FeSO ₄ .7H ₂ O	0.01	Sigma Aldrich
Na-EDTA	0.08	Sigma Aldrich
K ₂ SO ₄ (50%)	3.0	Fertilizer – Local Market
MgSO ₄ .7H ₂ O	0.2	Sigma Aldrich
K ₂ HPO ₄	0.5	Sigma Aldrich

Table (3.8): IMJ-1 medium micro components

Component	Mass (g/L)	Source
HBO ₃	0.62	Sigma Aldrich
MnCl ₂ .4H ₂ O	0.012	
of ZnSO ₄ .4H ₂ O	0.044	
Na ₂ MoO ₄	0.012	
CuSO ₄ .5H ₂ O	0.02	

Mix solution was prepared from PAS 1.5% and mining water with a ratio of 1:1, and the solution autoclaved on 121 °C and 105 KPa for 15 min and it was mixed with *Spirulina platensis* for 4 replicates as follow: 125mL of *Spirulina platensis* were mixed with 125mL of mix solution for each sample.

Table (3.9): Third Experiment Treatments (mL)

Third Experiment Treatments (mL)									
ZM Type	R 1	R 2	R 3	R4	FW Type	R 1	R 2	R 3	R4
Zarrouk Medium	125	125	125	125	Fish Wastewater	125	125	125	125
<i>Spirulina platensis</i>	125	125	125	125	<i>Spirulina platensis</i>	125	125	125	125
Total Volume (mL)	250	250	250	250	Total Volume (mL)	250	250	250	250
PAS Type	R 1	R 2	R 3	R4	BW Type	R 1	R 2	R 3	R4
Plant Ash Solution 1.5%	125	125	125	125	Brackish water 1:2	125	125	125	125
<i>Spirulina platensis</i>	125	125	125	125	<i>Spirulina platensis</i>	125	125	125	125
Total Volume (mL)	250	250	250	250	Total Volume (mL)	250	250	250	250
MW Type	R 1	R 2	R 3	R4	IMJ Type	R 1	R 2	R 3	R4
Mining Water	125	125	125	125	IMJ Medium	125	125	125	125
<i>Spirulina platensis</i>	125	125	125	125	<i>Spirulina platensis</i>	125	125	125	125
Total Volume (mL)	250	250	250	250	Total Volume (mL)	250	250	250	250
IMJ-1 Type	R 1	R 2	R 3	R4	MIX Type	R 1	R 2	R 3	R4
IMJ-1 Medium	125	125	125	125	Mix Solution	125	125	125	125
<i>Spirulina platensis</i>	125	125	125	125	<i>Spirulina platensis</i>	125	125	125	125
Total Volume (mL)	250	250	250	250	Total Volume (mL)	250	250	250	250

*R: Replicate

Measurements for all treatments were taken using single beam UV/Vis spectrophotometer (Jenway 7305) at 880nm. White LED light (5,000 lux) was used as a light source for a period of 16h day/8h night at 30 °C, and 20mL of distilled water were added daily to replace the

evaporated water. Dry weight for all samples was taken every week. This was done by taking 10mL from each sample and filtered it using Whatman paper.



Figure (3.8): Filtration Step for Dry Weight Calculation.

3.2.5.5 Fourth Experiment Preparation

Zarrouk medium was prepared as described in section (3.2.5.1) with 250mL as total volume for each replicate (125mL of *Spirulina platensis* + 125mL of Zarrouk medium). Imad Aljabali medium – 2 (IMJ-2) was prepared as follow:

Table (3.10): IMJ-2 medium main components

Component	Mass (g/L)	Source
NaHCO ₃	10.0	Local Market
NaCl	2.0	Salt Table – Local Market
Urea	0.35	Fertilizer – Local Market
CaCl ₂ .2H ₂ O	0.16	Sigma Aldrich
FeSO ₄ .7H ₂ O	0.07	Sigma Aldrich
Na-EDTA	0.08	Sigma Aldrich
K ₂ SO ₄ (50%)	3.0	Fertilizer – Local Market
MgSO ₄ .7H ₂ O	0.4	Sigma Aldrich
K ₂ HPO ₄	1.0	Sigma Aldrich

Table (3.11): IMJ-2 medium micro components

Component	Mass (g/L)	Source
H ₃ BO ₃	0.62	Sigma Aldrich
MnCl ₂ .4H ₂ O	0.012	
of ZnSO ₄ .4H ₂ O	0.044	
Na ₂ MoO ₄	0.012	
CuSO ₄ .5H ₂ O	0.02	

And with the addition of 1mL from micronutrients solution (See table 3.11), all components were dissolved in 1000mL of distilled water and autoclaved at 121 °C and 105 KPa for 15

min. After the solution cooled it was mixed with *Spirulina platensis* to form 4 replicates as follow: 125mL of IMJ-2 were mixed with 125mL of *Spirulina platensis* for each replicate.

Table (3.12): Fourth Experiment Treatments (mL)

Fourth Experiment Treatments (mL)									
ZM Type	R 1	R 2	R 3	R 4	IMJ-2 Type	R 1	R 2	R 3	R 4
Zarrouk Medium	125	125	125	125	IMJ-2	125	125	125	125
<i>Spirulina platensis</i>	125	125	125	125	<i>Spirulina platensis</i>	125	125	125	125
Total Volume (mL)	250	250	250	250	Total Volume (mL)	250	250	250	250

*R: Replicate

Measurements for all replicates were taken using single beam UV/Vis spectrophotometer (Jenway 7305) at 880nm, while white LED light (5000 lux) was used as a light source in a period of 16h day/8h night at 35 °C, and 20mL of distilled water were added daily to replace the evaporated water, and dry weight for all samples was taken every week, and it was done by taking 10mL from each sample and filtered it using Whatman paper.

3.2.5.6 Fifth Experiment Preparation

Zarrouk medium and fish wastewater was prepared and treated as mentioned in sections (3.2.5.1) and (3.2.5.2) respectively, while replicates prepared as described in table (3.13). All solutions were autoclaved at 121 °C and 105 KPa for 15 min

The rest of the treatments were prepared as follow:

- **FW-BW:** FW (90%) mixed with brackish water (10%).
- **FW-MW:** FW (90%) mixed with mining water (10%).
- **FW-PAS:** FW (90%) mixed with PAS 3% (10%).
- **FW-IMJ-1:** FW (90%) mixed with IMJ-1 (10%).
- **FW-MIX:** FW (90%) and brackish water (2.5%), mining water (2.5%), PAS 3% (2.5%), IMJ-1 (2.5%).

Measurements for all replicates were taken using single beam UV/Vis spectrophotometer (Jenway 7305) at 880nm, while white LED light (5000 lux) was used as a light source in a period of 16h day/8h night at 35 °C, and 20mL of distilled water were added daily to replace the evaporated water, and dry weight for all samples was taken every week, and it was done by taking 10mL from each sample and filtered it using Whatman paper.

Table (3.13): Fifth Experiment Treatments (mL)

Fifth Experiment Treatments (mL)									
ZM Type	R 1	R 2	R 3	R4	FW Type	R 1	R 2	R 3	R4
Zarrouk Medium	125	125	125	125	Fish Wastewater	125	125	125	125
<i>Spirulina platensis</i>	125	125	125	125	<i>Spirulina platensis</i>	125	125	125	125
Total Volume (mL)	250	250	250	250	Total Volume (mL)	250	250	250	250
FW-BW Type	R 1	R 2	R 3	R4	FW-MW Type	R 1	R 2	R 3	R4
FW-BW Medium	125	125	125	125	FW-MW Medium	125	125	125	125
<i>Spirulina platensis</i>	125	125	125	125	<i>Spirulina platensis</i>	125	125	125	125
Total Volume (mL)	250	250	250	250	Total Volume (mL)	250	250	250	250
FW-PAS Type	R 1	R 2	R 3	R4	FW-IMJ-1 Type	R 1	R 2	R 3	R4
FW-PAS 3% Medium	125	125	125	125	FW-IMJ-1 Medium	125	125	125	125
<i>Spirulina platensis</i>	125	125	125	125	<i>Spirulina platensis</i>	125	125	125	125
Total Volume (mL)	250	250	250	250	Total Volume (mL)	250	250	250	250
FW-MIX Type	R 1	R 2	R 3	R4					
FW-MIX Medium	125	125	125	125					
<i>Spirulina platensis</i>	125	125	125	125					
Total Volume (mL)	250	250	250	250					

*R: Replicate

Chapter Four

Results & Discussion

- **4.1 Results**
 - 4.1.1 First Experiment
 - 4.1.2 Second Experiment
 - 4.1.3 Third Experiment
 - 4.1.4 Fourth Experiment
 - 4.1.5 Fifth Experiment
- **Discussion**

Chapter Four: Results & Discussion

4.1 Results

4.1.1 First Experiment

The first experiment was done to evaluate the possibility of using wastewater from Tilapia-fish ponds in ADS to cultivate *Spirulina*. In this experiment, pH values of ZM and FW were over 10 and less than 11, and electrical conductivity was between 20-30 mS/cm, while temperature of the experiment was at 30 °C, and light was 1500 Lux for 16h light and 8h dark. Both treatments began with low concentrations, and the initial absorbance around 0.5 at 880nm. ZM tripled after 8 days while FW doubled in the same period and both treatments stayed at the same level till the end of the experiment. This experiment showed that *Spirulina* can grow in FW in a similar way like ZM with a price of 0.0015 ILS/L for FW compared to 8.9625 ILS/L for ZM.

4.1.1.1 Physical and Chemical Parameters

Chemical and physical parameters were measured for all treatments before mixing it with *Spirulina platensis* using pH & EC meter. The result is present in table (4.1). Zarrouk medium treatment had a whitish color and a little turbid appearance while fish wastewater treatment had a brownish color before filtration, The fish water trial was very turbid, but after filtration the effluent was clear and had a clear – transparent appearance.

Table (4.1): Chemical Parameters for First Experiment before mixing with *Spirulina platensis*

Chemical Parameters	Growth Medium Types	
	Zarrouk Medium	Fish Wastewater
pH	8.91	8.76
EC (μS/cm)	18,840	6,600
TDS (ppm)	12,435	4,356
Salinity (ppt)	10.76	3.30

After they mixed with *Spirulina platensis*, chemical parameters of the mixture measured and reported in table (4.2). Both treatments had a green color, and their appearance was turbid.

Table (4.2): Chemical Parameters for First Experiment after mixing with *Spirulina platensis*

Chemical Parameters	Growth Medium Types	
	Zarrouk Medium	Fish Wastewater
pH	10.12	10.65
EC (μS/cm)	29,700	24,900
TDS (ppm)	19,602	16,434
Salinity (ppt)	17.40	14.60

4.1.1.2 Measurements

All measurements were taken on 880 nm the maximum wavelength florescence for chlorophyll wavelength. Figure (4.1) describes the results of this experiment. Both wastewater fishpond and the Zarrouk medium treatments correlated very well with each other. *Spirulina platensis* had grown very well in fish wastewater, and during the first 4 days growth doubled, and it was increased to reach 1.309 for ZM treatment and 1.104 for FW treatment, and by day 8 the reading increased again to reach 1.917 for ZM treatment and 1.540 for FW treatment, and it grown in a similar way to reach 2.104, 1.980, 2.054, and 2.139 respectively for ZM treatment and 1.759, 1.651, 1.806, and 1.972 respectively for FW treatment for days 11th, 15th, 18th, and 21st. Data is shown in section (A), table (A.1) in the Appendix.

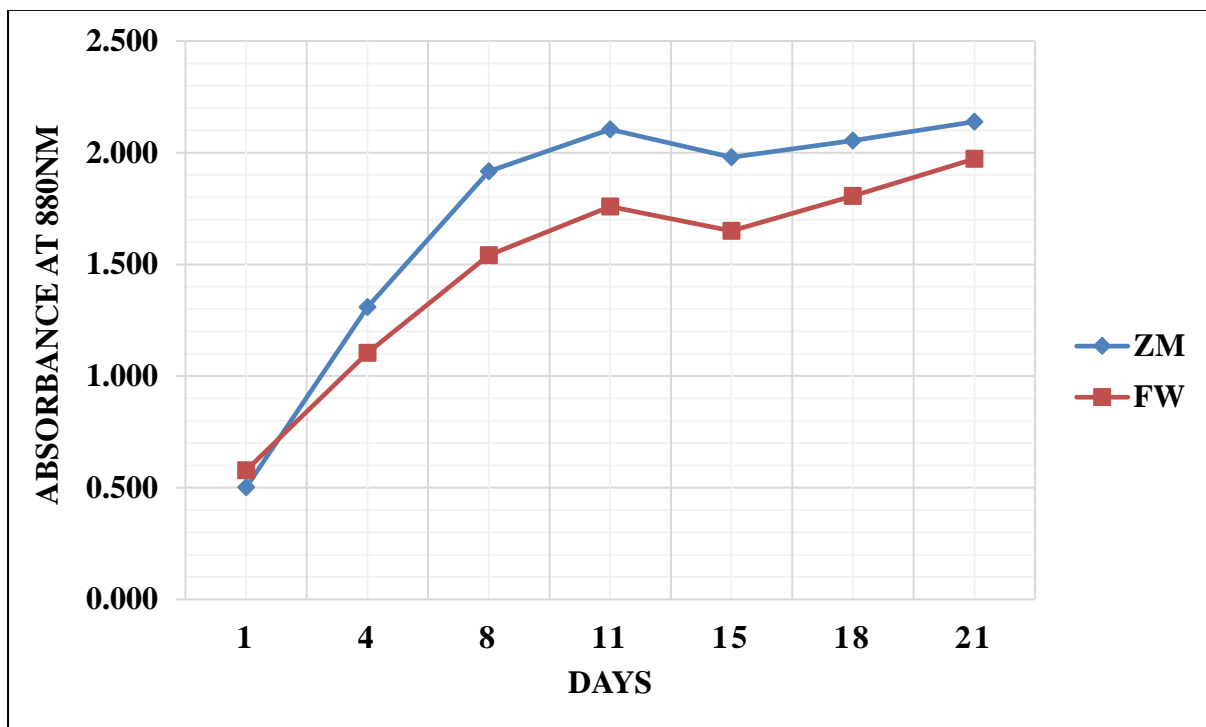


Figure (4.1): First experiment growth measurements at 880nm

4.1.1.3 Growth Rate (Percentage)

Growth rate percentage in this experiment was depended on the change in absorbance in figure 4.1 and it was calculated based on the following equation:

$$\text{Growth rate Percentage} = ((A_2 - A_1) / A_1) * 100$$

Spirulina platensis absorption of ZM treatment increased from 0.502 at 880nm in the beginning of the experiment with a growth rate of 161% in the first 4 days (See figure 4.2) to

reach 2.139 at 880nm in the end of the experiment with a growth rate of 4% after 21 days. The sum of growth rate with all conditions (increase and decrease) was 219% from day one with a huge increase in the first week. While *Spirulina platensis* cultivated in FW treatment increased from 0.578 at 880nm in the beginning of the experiment with a growth rate of 91% in the first 4 days to reach 1.972 at 880nm in the end of the experiment with a growth rate of 9% after 21 days. The sum of growth rate with all conditions (increase and decrease) was 157% from day one with a huge increase in the first week. For more explanation you can see data in the Appendix in section (A), table (A.2).

The decrease of the growth rate percentage for both treatments in day 11 was based on the decrease in day 15 while it raised again because the absorption reading increased in the next days. Growth rate curve in figure 4.2 begins with very high growth values (161% for ZM, 91% for FW) and it decreased gradually in an opposite way to the absorbance curve in figure 4.1 due to the increase in *Spirulina* cells number that reduced light to reach all *Spirulina* trichomes and it is logic that the raise in cells means increase of wastes and decrease in nutrients as food source.

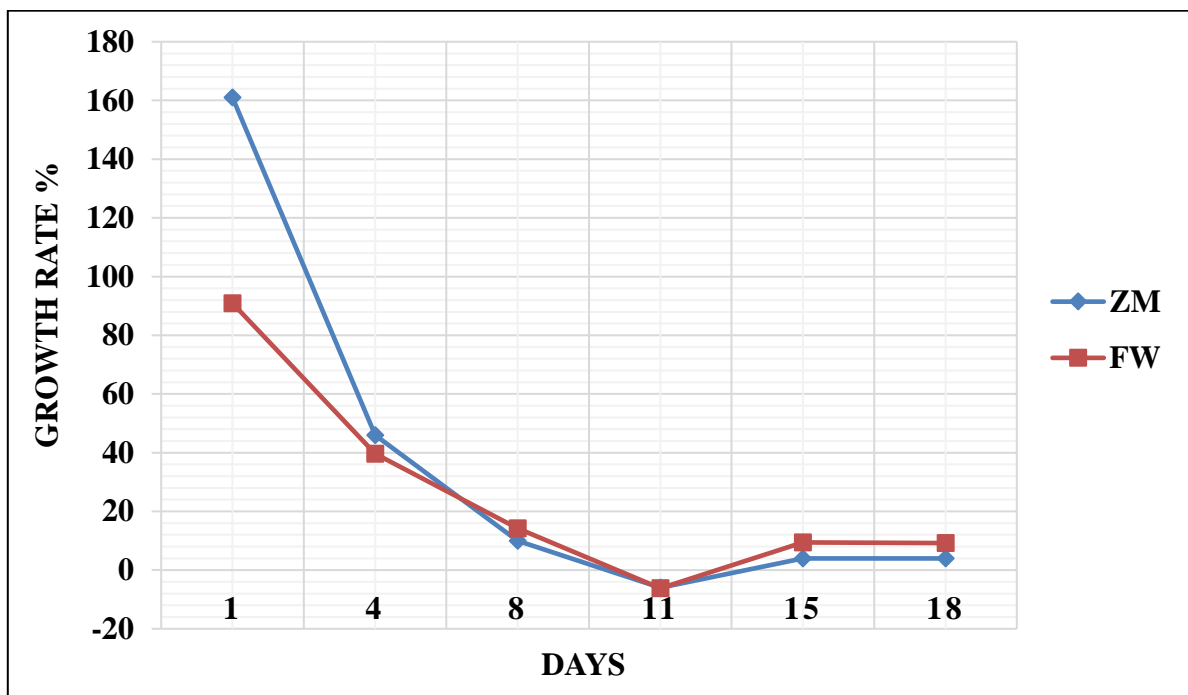


Figure (4.2): First Experiment Growth Rate (%) of Zarrouk medium (ZM) vs. Fish Wastewater (FW)

4.1.1.4 Dry Weight (g/L)

Spirulina platensis dry weight was very close and does not increase a lot in the first two weeks for unknown reason despite of it has a large increase in absorption reading at 880nm as seen in figure 4.1, while it increased sharply from 5.05g/L to 13.68g/L in the third week for

ZM treatment and from 5.34g/L to 8.34g/L for FW treatment, and in the fourth week dry weight decreased to 9.34g/L and 5.10g/L for ZM and FW treatments respectively although the absorption values were more than in the third week. According to the results of this experiment there is no relation between measurement of absorption reading and dry weight and the reason is not clear.

The data described in section (A), tables A.1 and A.2 in the appendix approved that Spirulina concentration of Spirulina cells increased 3.8 times from its' initial concentration after 8 days for ZM and 2.6 times for FW; so the logical dry weight should be 19.41g/L for ZM and 13g/L for FW after one week of the beginning of the experiment, and it should be the same in the third week because the absorption value is nearly similar between day 8 and day 15 for both treatments, while dry weight in the end of the experiment should be 21.54g/L for ZM and 16.64g/L for FW.

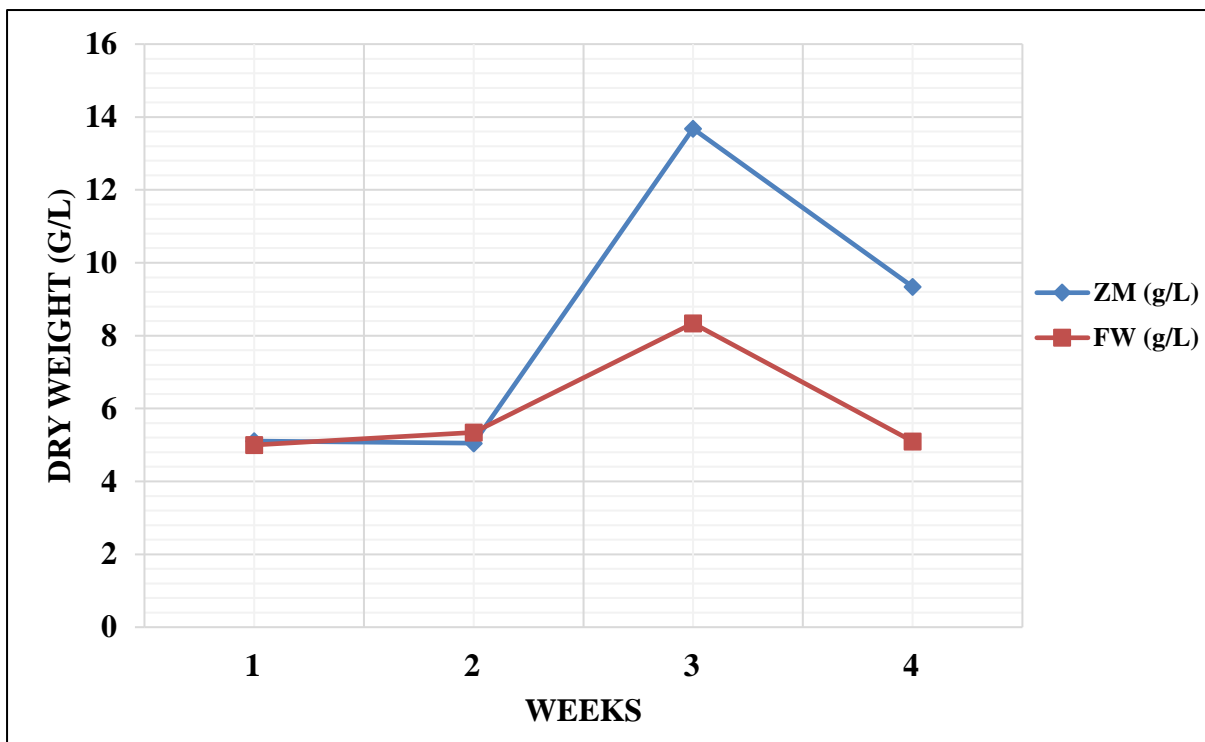


Figure (4.3): First Experiment Dry Weight (g/L) of Zarrouk medium vs. Fish Wastewater.

4.1.2 Second Experiment

The second experiment was done through running treatments of fish wastewater, diluted brackish water, plant ash solution, and a mix of them. In this experiment, pH values were over 9 and below 10, while EC values were between 14-19 mS/cm. While temperature was at 30 °C, and light was 1500 Lux for 16h light and 8h dark. All treatments began with low concentrations as in experiment one with absorbance around 0.6 at 880nm and most of it

tripled after 14 days, and ZM was the lowest one among all; so, from this experiment FW, diluted BW, PAS 3% or a mix of it could be used to cultivate *Spirulina* with a very cheap price compared to ZM.

4.1.2.1 Physical and Chemical Parameters

Second Experiment was also run for 21 days, but the measurements were taken every 3-4 days. Chemical parameters were taken for each medium type in this experiment as reported in table (4.3), while Zarrouk medium (ZM) had whitish color and its' appearance was slightly turbid and fish wastewater (FW) before filtration was very turbid and had a brownish color. After filtration, it had a clear appearance, also brackish water (BW) was diluted with distilled water with a dilution factor of two, it had a pure color with slightly turbid appearance. The 30g plant ash (PAS) was mixed with 1000mL of distilled water to form 3% solution, the solution had a turbid-black color while after filtration it had a clear slightly turbid appearance. The mix solution was created from mixing of fish wastewater, plant ash solution (3%), and brackish water (1:2), it had a clear appearance.

Table (4.3): Chemical parameters for Second Experiment before mixing with *Spirulina platensis*

Chemical Parameters	Growth Medium Types					
	ZM	FW	BW	BW 1:2	PAS 3%	MIX
pH	8.35	8.72	8.1	7.56	10.72	9.55
EC ($\mu\text{S}/\text{cm}$)	18,960	6620	21,500	11,140	12,580	10,250
TDS (ppm)	9,480	3320	10,800	5,570	6,300	5,140
Salinity (ppt)	10.82	3.50	12.3	5.95	6.78	5.45

Zarrouk medium after mixing with *Spirulina platensis* had greenish color and turbid appearance with chemical parameters (table 4.4) suitable for *Spirulina* growth. FW after mixing with *Spirulina platensis* had turbid appearance and greenish color. diluted BW after mixing with *Spirulina platensis* had a greenish color with a turbid appearance and parts of *Spirulina platensis* cells clumped together due to salinity shock. They were removed by filtration. PAS 3% after mixing with *Spirulina platensis* had a greenish color with a turbid appearance and the same was for mix solution.

Table (4.4): Chemical parameters for Second Experiment after mixing with *Spirulina platensis*

Chemical Parameters	Growth Medium Types				
	ZM	FW	BW 1:2	PAS 3%	MIX
pH	9.35	9.65	9.55	9.75	9.79
EC ($\mu\text{S}/\text{cm}$)	19,850	14,100	15,710	17,050	15,890
TDS (ppm)	9,940	7,050	7,850	8,530	7,950
Salinity (ppt)	11.35	7.72	8.69	9.60	8.79

4.1.2.2 Measurements

The result of experiment two is shown in figure (4.4). The figure illustrates a comparison between all treatments; Zarrouk medium (ZM), Tilapia wastewater (FW), diluted brackish water (BW 1:2), plant ash solution (PAS) (3%), and a mix solution for the cultivation of *Spirulina platensis*.

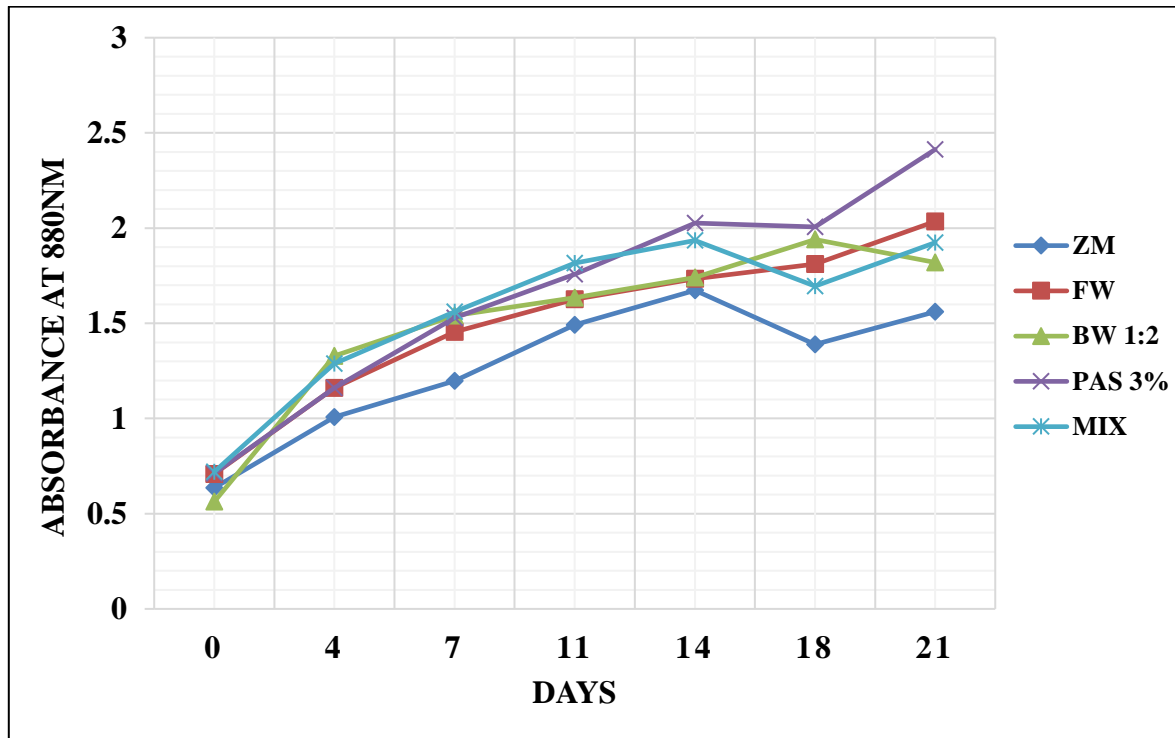


Figure (4.4): Second Experiment absorbance reading at 880nm for all treatments.

The data is also presented in the appendix (Section B, table (B.1)). *Spirulina platensis* starts with close concentration for all types, while ZM began with absorbance of 0.636 at 880nm and increased to reach 1.561 after 21 days, FW started with an absorbance of 0.709 and increased to reach 2.035 at the end of the experiment, while BW 1:2, PAS 3% and MIX began with 0.563, 0.712, 0.720, respectively and after 21 days it reached 1.740, 1.757, and 1.817 respectively.

4.1.2.3 Growth Rate (Percentage)

According to the absorption reading of this experiment, PAS (3%) was the best medium to cultivate *Spirulina platensis* on the long term while according to growth rate, diluted brackish water was the best medium to cultivate *Spirulina platensis* rapidly because it increased 167% in the first 4 days with an average of 41.75% for each day. It was decreased gradually to reach -6% in the end of the experiment and increased by 201% from its' initial concentration.

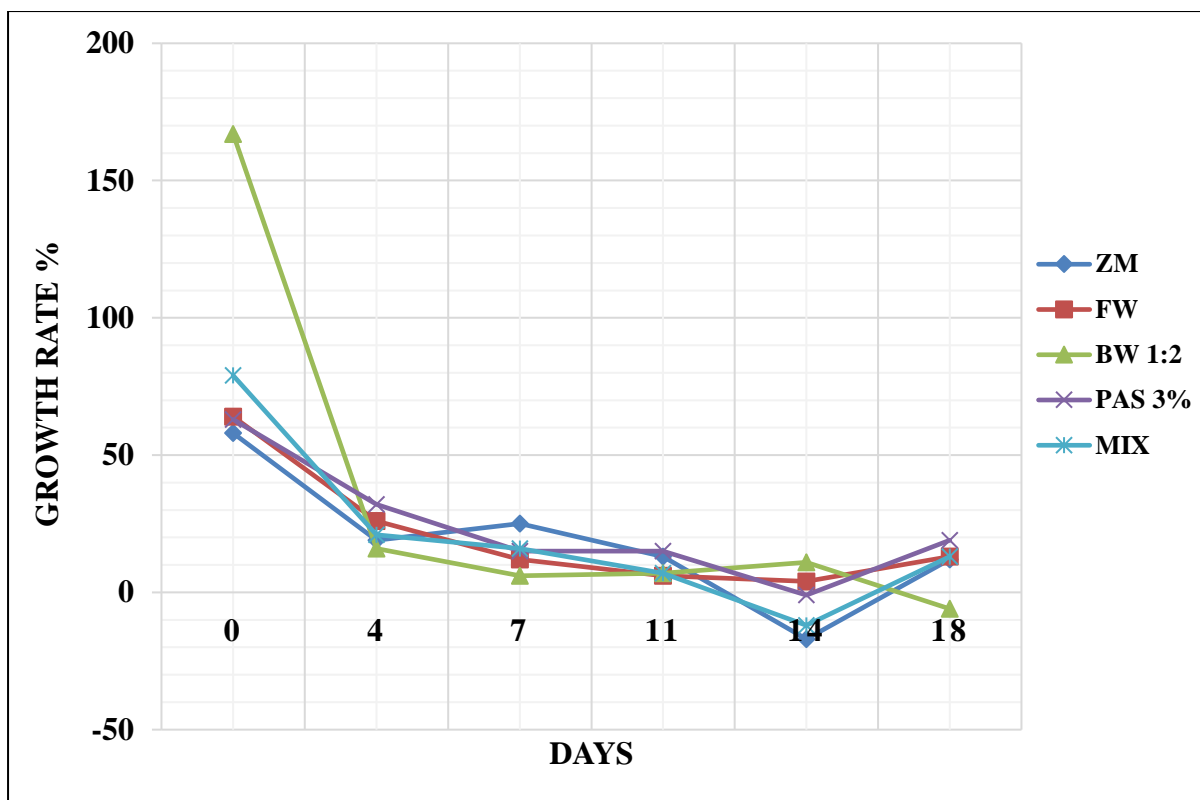


Figure (4.5): Second Experiment growth rate (%).

ZM treatment increased by 58% for the first 4 days and it was increased by 110% from its' initial concentration. FW increased by 64% for the first 4 days, it was increased by 125% from day one, while PAS 3% increased by 63% after 4 days and increased by 143% after cultivation from the first day. Mix solution was increased by 79% in the first 4 days with an average of 19.75% for each day and by the end of the experiment it was increased by 124% from the first day. (See Appendix: Section B, table B.2)

4.1.2.4 Dry Weight (g/L)

Spirulina platensis cultivated in 3% PAS and mix solution had the highest dry weight per liter. PAS (3%) begin with dry weight of 3.3 g/L and after 21 days it increased to 18.2 g/L with a positive difference of 14.9 g/L while mix solution begin with dry weight of 3.8 g/L and reached 18.8 g/L with a positive difference of 15 g/L after 21 days. ZM, BW 1:2, and FW started with 2.9 g/L, 4.4 g/L, and 2.8g/L respectively and increased to 10.0 g/L, 8.9 g/L, and 8.0 g/L respectively, while the lowest difference in dry weight was 3.6 g/L for DW 1:2 and the highest one was 15.0 g/L for mix solution. (See Appendix: Section B, table B.3)

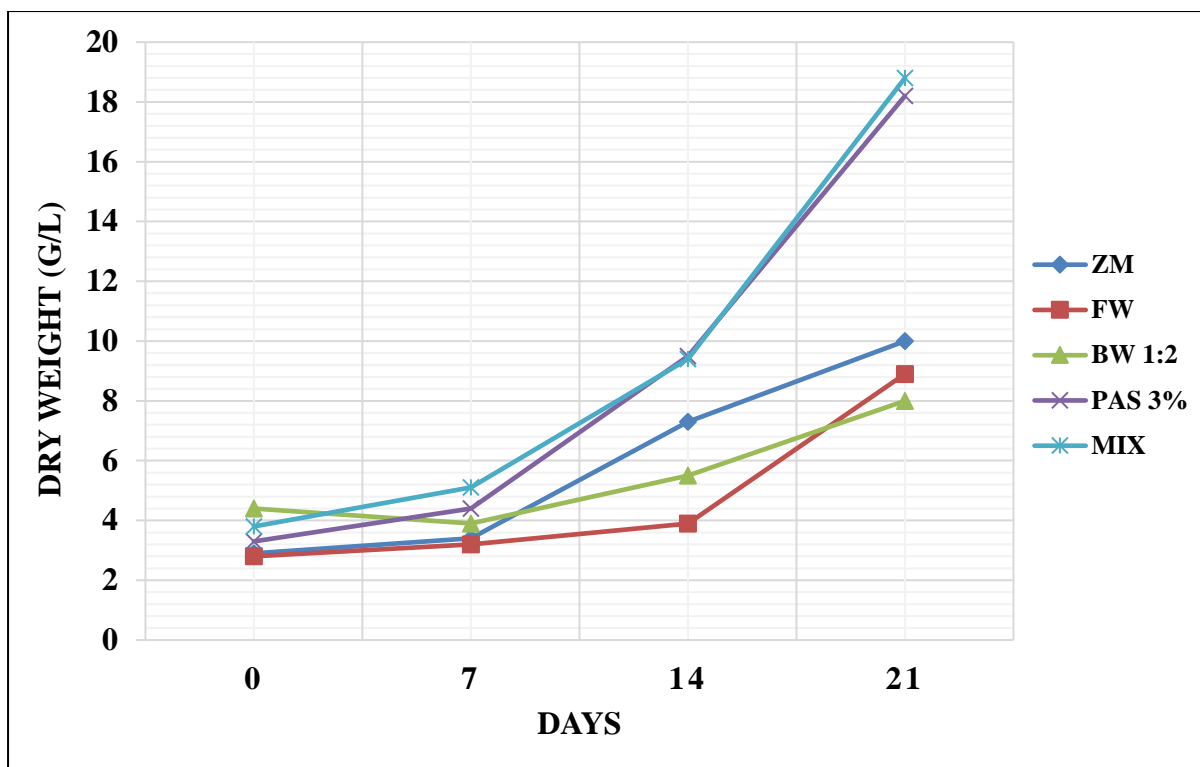


Figure (4.6): Second Experiment dry weight (g/L)

4.1.3 Third Experiment

The third experiment was conducted to evaluate the same components as in experiment two with some modification and addition of new treatments such as mining water and creating new chemical mediums as IMJ and IMJ-1. In this experiment, pH values were over 9 and below 10, while EC values were between 12-21 mS/cm. While temperature was at 30 °C, and light was 5000 Lux for 16h day and 8h night. All treatments began with high concentration with absorbance range between 1.6-1.9 at 880nm for all types. ZM and IMJ were the best types among all other treatments. FW stayed at the same level for the first week and began to grow with fluctuation curve till the end of the experiment. IMJ with a price of 3.66 ILS/L is lower than ZM and could be a good alternative source.

4.1.3.1 Physical and Chemical Parameters

Third Experiment was done for 21 days with ZM as a reference. The experiment included FW, BW (diluted 1:2), mining water (MW), PAS (1.5%), IMJ, IMJ-1, and mix solution. The chemical parameters were tested for each type before mixing with *Spirulina platensis* and it were reported in Table 4.5.

Table (4.5): Chemical Parameters for the third experiment treatments before mixing with *Spirulina platensis*

Chemical Parameters	Growth Medium Types							
	ZM	FW	BW 1:2	MW	PAS 1.5%	IMJ	IMJ-1	MIX
pH	9.03	8.72	8.41	8.78	9.63	9.08	9.26	9.25
EC ($\mu\text{S}/\text{cm}$)	20,300	6,610	10,830	2,760	1,905	15,530	15,500	2,330
TDS (ppm)	10,200	3,310	5,430	1,380	953	7,770	7,760	1,170
Salinity (ppt)	11.6	3.47	5.70	1.38	0.93	8.56	8.55	1.17

Table (4.5) above describes the chemical parameters of different growth medium types and as a comparison between its. It could be noticed that PAS (1.5%) has the lowest electrical conductivity and total dissolved solids from the natural types, but it has the highest pH value. This makes it the best solution to raise pH level, while FW, BW, and MW have lower pH value and higher electrical conductivity and total dissolved solids. The pH of the undiluted brackish water was 8.3, EC was 21,300 $\mu\text{S}/\text{cm}$, TDS was 10,700 ppm, and salinity was 12.2 ppt. At the same time ZM and IMJ treatments were very close in pH value and differ in EC, TDS, and salinity. Mix solution which was a combination between MW and PAS (1.5%). All medium types were whitish and had slightly turbid while FW and PAS were turbid before filtration, they were slightly turbid after filtration. After mixing growth medium types with *Spirulina platensis*, chemical parameters were tested and described in Table (4.6).

Table (4.6): Chemical Parameters for the third experiment treatments after mixing with *Spirulina platensis*

Chemical Parameters	Growth Medium Types							
	ZM	FW	BW 1:2	MW	PAS 1.5%	IMJ	IMJ-1	MIX
pH	9.05	9.64	9.68	9.86	9.86	9.67	9.70	9.89
EC ($\mu\text{S}/\text{cm}$)	21,000	13,230	15,010	12,490	12,230	18,150	18,100	12,140
TDS (ppm)	10,500	6,630	7,500	6,250	6,200	9,140	9,050	6,070
Salinity (ppt)	12	7.19	8.29	6.71	6.62	10.31	10.28	6.52

Table (4.6) describes the chemical parameters for different growth medium after mixing with *Spirulina platensis*. All pH values for all types were above 9 and lower than 10, and all electrical conductivity values were over than 10,000 $\mu\text{S}/\text{cm}$ and TDS values were over than 6,000 ppm while the lowest one was mix solution and the highest one was ZM in EC and TDS values and Mix, MW, PAS 1.5%, IMJ-1, BW, and IMJ in pH values respectively.

4.1.3.2 Measurements

Figure (4.7) describes the results of the experiment. ZM and IMJ were the best to cultivate *Spirulina platensis* with the preference to ZM while FW and IMJ-1 were the second and the rest were failed to grow *Spirulina platensis*. The growth rate had dropped down gradually

after the experiment begin. The behavior of some types was different from medium to another.

All types began with a close concentration, the absorbance of all samples was between 1.6 and 1.9. *Spirulina platensis* that grown in ZM increased from 1.927 in day 0 and increased gradually to reach its' maximum in day 15 and began to decrease gradually, while *Spirulina* grown in FW decreased in the first 3 days and then increased in day 5 and continue going to day 15, then it decreased again, and that grown in BW, MW, and mix solution failed to grow and that in BW failed down quicker than the one grown in lime mining water, in the same time *Spirulina* grown in IMJ and IMJ-1 treatments grown well and IMJ compete with ZM, but fertilizers failed after day 8, while the one grown in PAS (1.5%) started to grow in day 11 but failed down again. (See Appendix: Section C, table C.1)

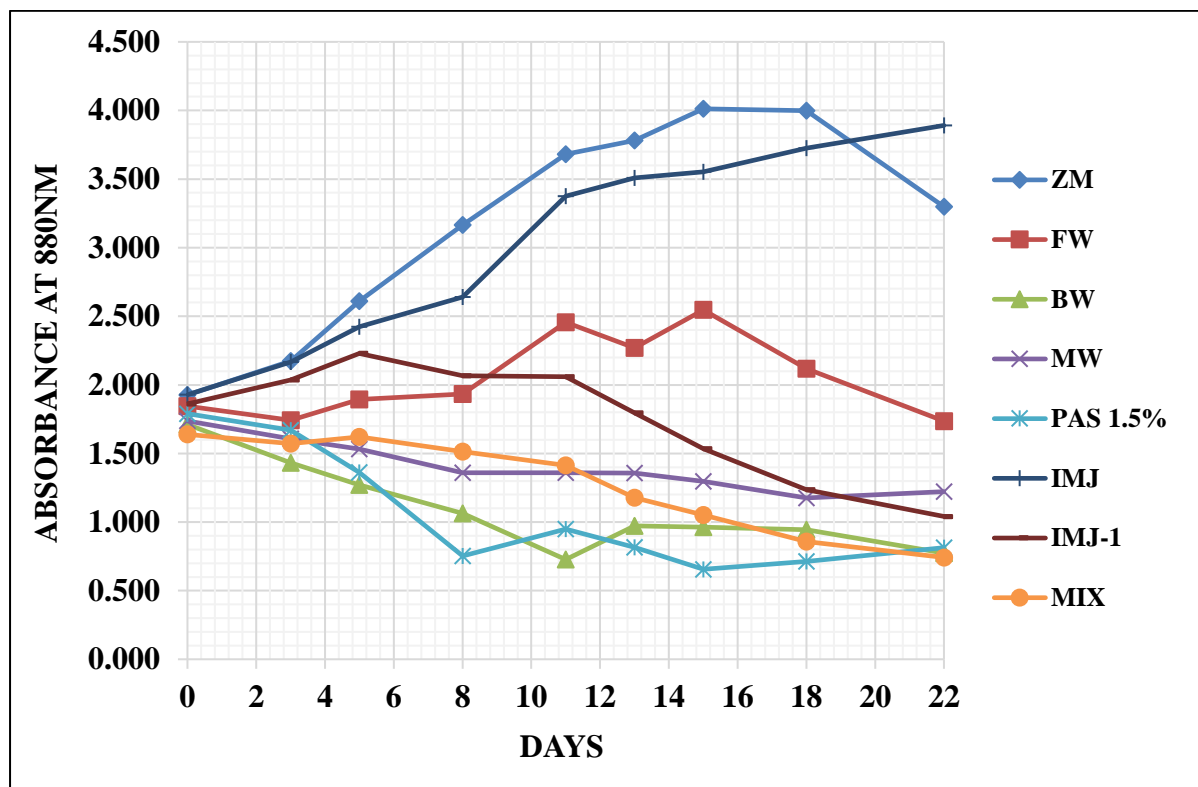


Figure (4.7): Third Experiment measurements at 880nm.

4.1.3.3 Growth Rate (Percentage)

Growth rate result is different from growth medium type to another; ZM and IMJ were the best types with a growth rate of 13% and 12% for ZM and IMJ respectively. In the first 3 days both medium had increased and decreased in the percentage of the growth, while the other types unfortunately grown negatively except some types such as FW and PAS (1.5%). (See Appendix: Section C, table C.2)

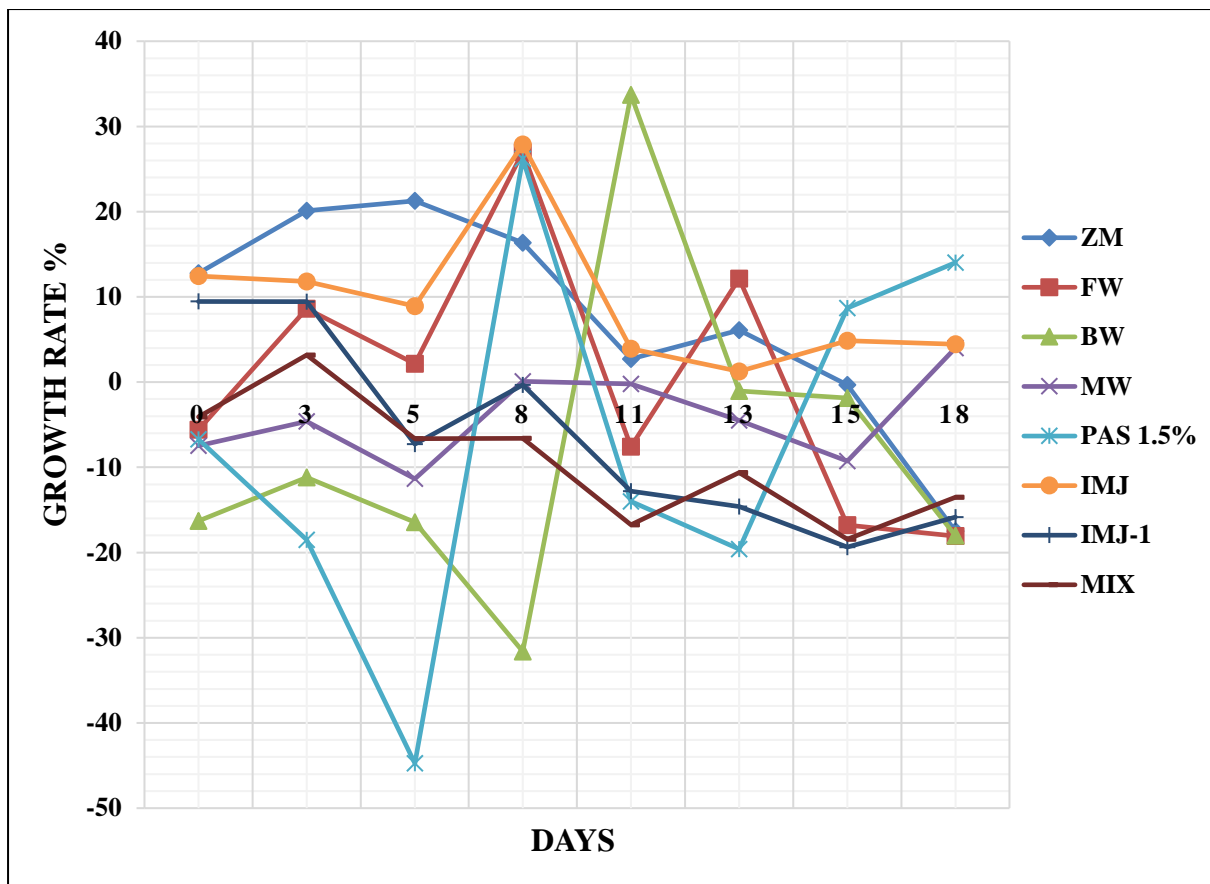


Figure (4.8): Growth rate (%) for Third Experiment.

4.1.3.4 Dry Weight (g/L)

Although some growth medium types were not satisfied for the growth of *Spirulina platensis* but they gave a good indicator for the dry weight, ZM as usual gave a higher dry weight than others and FW was competing with it and even surpassed it in the third week as present in figure (4.9), while the other types shown a good increase in dry matter that reached a minimum of 13g/L in the mix solution and a maximum of 35g/L in FW from the first and initial week to the third week. (See Appendix: Section C, table C.3)

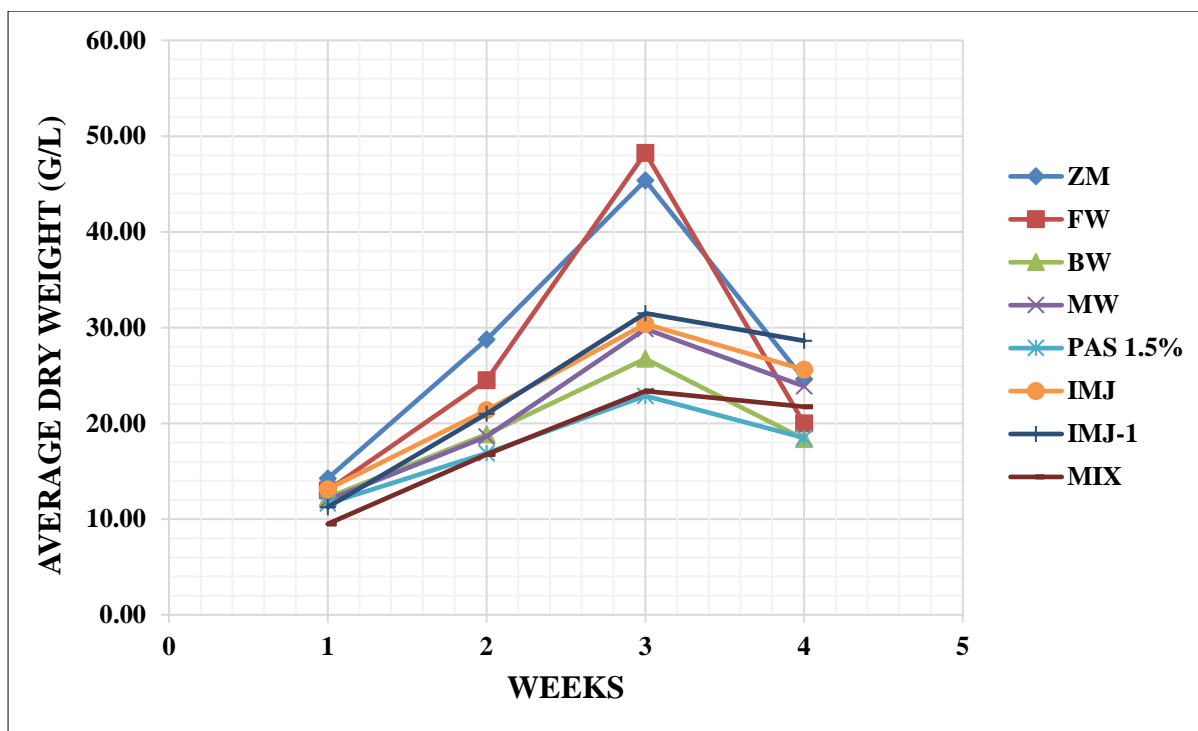


Figure (4.9): Dry weight (g/L) for Third Experiment.

4.1.4 Fourth Experiment

The fourth experiment was run at the same time of experiment five. It was a comparison between ZM and IMJ-2. In this experiment, pH values were between 9 and 10, while EC values were around 21 mS/cm. While temperature was at 35 °C, and light was 5000 Lux for 16h day and 8h night. Both treatments began with a moderate concentration with absorbance reading of 1.25 at 880nm. ZM doubled nearly after 14 days while IMJ-2 raised by 1.76 times at the same period. IMJ-2 was better than ZM till day 10 and a clear difference shown after that. IMJ-2 with a price of 1.38 ILS/L is lower than ZM and could be a very good cheap alternative source.

4.1.4.1 Physical and Chemical Parameters

Experiment four was also run for 21 days, it was a comparison between ZM as a reference medium with IMJ-2 medium. It was done simultaneously with the fifth Experiment. Table (4.7 and 4.8) describe the chemical parameters of the solutions, IMJ-2 chemical parameters were lower than ZM.

Table (4.7): Chemical Parameters for the fourth experiment treatments before mixing with *Spirulina platensis*

Chemical Parameters	Growth Medium Types	
	ZM	IMJ-2
pH	9.03	8.87
EC (μS/cm)	20,300	15,750
TDS (ppm)	10,200	7,880
Salinity (ppt)	11.6	8.72

The physical parameters were similar for both solutions including the appearance of Cloudy-Whitish Color. After mixing with *Spirulina platensis*, it had greenish color.

Table (4.8): Chemical Parameters for the fourth experiment treatments after mixing with *Spirulina platensis*

Chemical Parameters	Growth Medium Types	
	ZM	IMJ-2
pH	9.05	9.89
EC ($\mu\text{S}/\text{cm}$)	21,000	21,360
TDS (ppm)	10,500	11,630
Salinity (ppt)	12	11.78

4.1.4.2 Measurements

Zarrouk and IMJ-2 treatments started with similar concentrations, at a temperature of 35 °C. Both treatments increased with an average absorbance reading of 1.25 in similar way until day 10 of the experiment. In day 11 the growth of *Spirulina platensis* behaved differently as shown in figure (4.10). In day 14 both reached the highest growth level, ZM increased to reach absorbance of around 3.052 while IMJ-2 reached around 2.295. After that both were dropped down. (Appendix: Section D, table D.1)

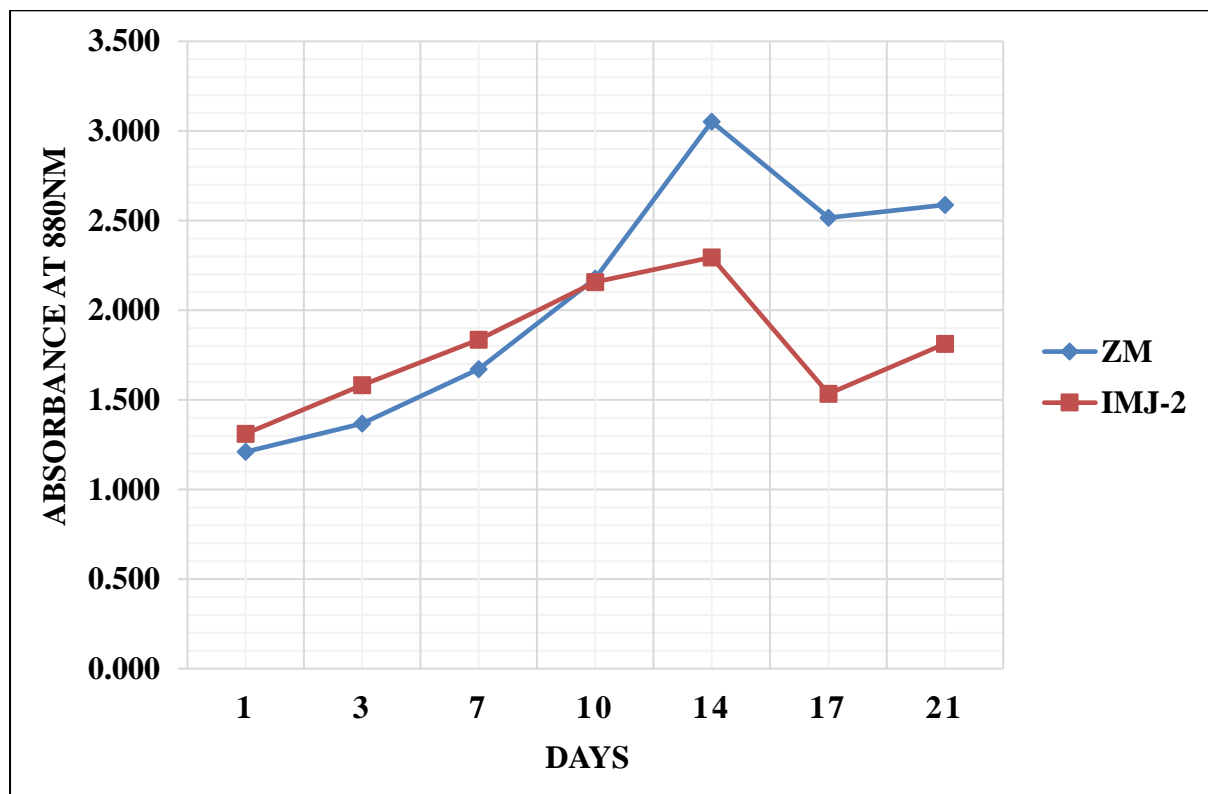


Figure (4.10): Measurements of Zarrouk medium and IMJ-2 at 880nm in the fourth experiment

4.1.4.3 Growth Rate (Percentage)

Growth rate performance was different for both medium types, ZM began with low growth rate in the first 3 days and then increased gradually, then decreased after day 10, while IMJ-2 began with a nearly constant rate then it was declined gradually after day 10 too, and both increased again after that as present in figure (4.11). (Appendix: Section D, table D.2)

As mentioned in section 4.1.1.3 before, growth rate percentage was calculated dependent of the absorbance data for its' own experiment, and it based on the following formula:

$$\text{Growth rate Percentage} = ((A2-A1)/A1)*100$$

So, ZM growth was 13% in the first 3 days, then it raised to 22%, 30%, and 40% respectively, then dropped down by -18%, and finally increased again by 3%, while IMJ-2 growth curve began with 21% in the first 3 days, then increased by 16%, 18%, and 6% respectively, then dropped by -33%, and finally increased by 18%.

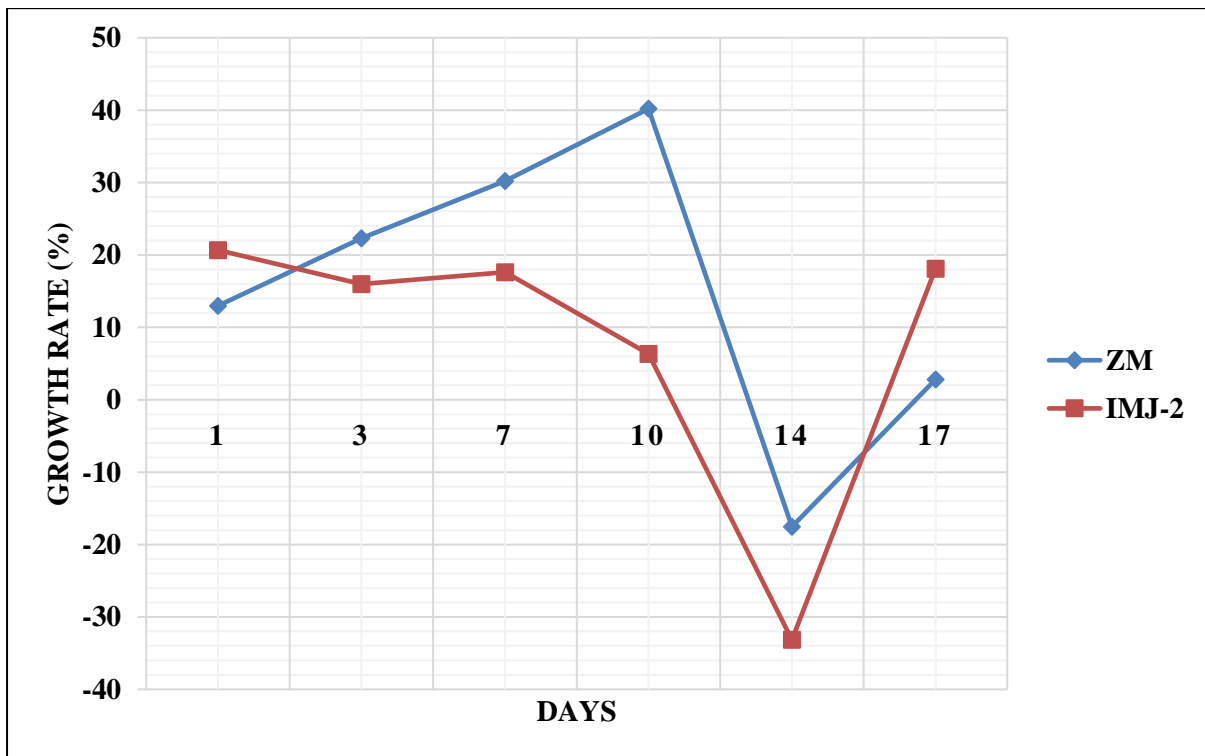


Figure (4.11): Growth rate (%) of the fourth experiment

4.1.4.4 Dry Weight (g/L)

Zarrouk medium and IMJ-2 medium had shown very good dry weight especially in the second week as shown in figure (4.12). (See Appendix: Section D, table D.3)

Dry weight in this experiment began with 16g/L for ZM and increased after one week to reach 21g/L, after that it got a huge increase to 59g/L and then decrease to 56g/L. While IMJ-2 treatment began with 16g/L too, and increased to reach 25g/L after one week and that was better than Zarrouk for this period, then it increased to be very close to ZM treatment and reach 52g/L and after that it dropped to 43g/L. According to dry weight data IMJ-2 treatment could be very effective and very competitive with Zarrouk medium with a cheaper price, which IMJ-2 price could be 1.38 ILS/L while Zarrouk still 8.9625 ILS/L.

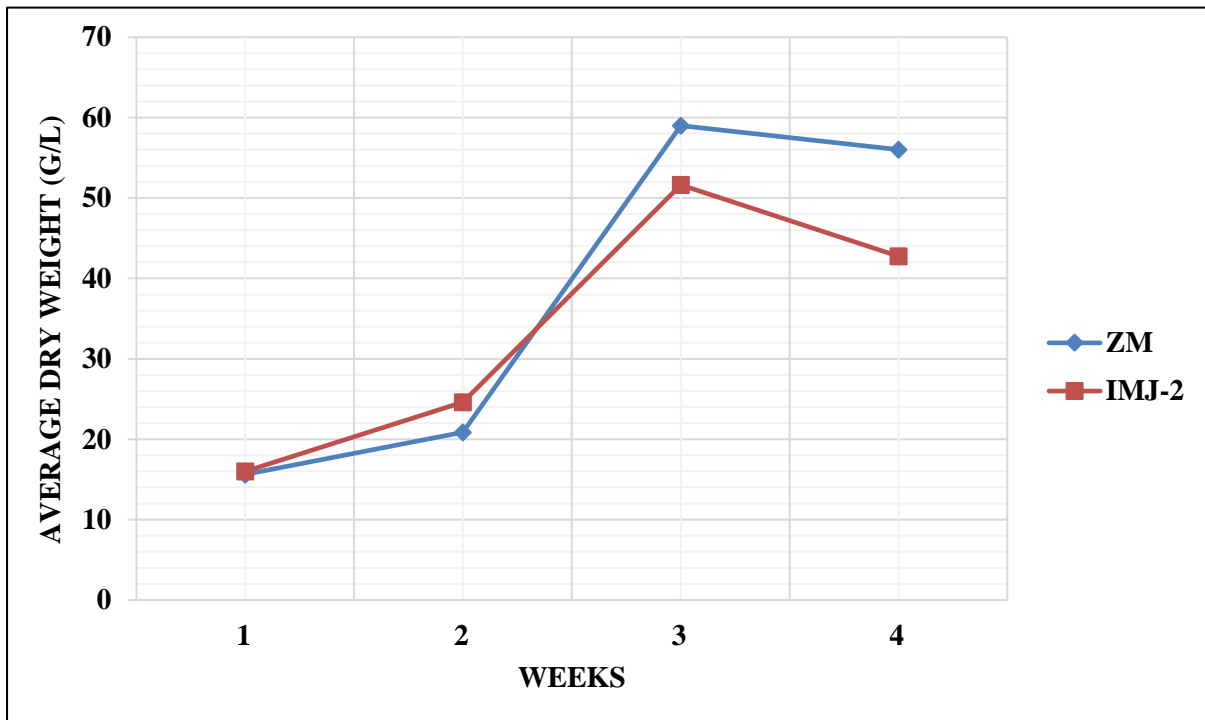


Figure (4.12): Dry weight (g/L) for the fourth experiment

4.1.5 Fifth Experiment

The fifth experiment was a comparison between ZM, FW, and the following types FW:BW (9:1), FW:MW (9:1), FW:PAS-3% (9:1), FW:IMJ-1 (9:1), and a mix composed of FW:(BW+MW+PAS-3%+IMJ-1) (9:1). In this experiment, pH values were between 8 and 9.30, while EC values varied between 9.8 and 21 mS/cm. All treatments began with moderate concentrations with absorbance around 1.25 at 880nm. ZM and Mix were the best types, while ZM, Mix, and FW:IMJ-1 took a similar behavior until day 10.

4.1.5.1 Physical and Chemical Parameters

The fifth Experiment was also done for 21 days, it was a comparison between Zarrouk medium as a reference medium with fish wastewater and varied types of fish wastewater and

plant ash solution 3%, brackish water, mining water, IMJ-1 medium, and mix of all. Table (4.9) below describes the chemical parameters of the solutions.

Table (4.9): Chemical Parameters for the fifth experiment before mixing with *Spirulina platensis*

Chemical Parameters	Growth Medium Types						
	ZM	FW	FW+BW	FW+MW	FW+PAS 3%	FW+IMJ-1	MIX
pH	9.03	7.08	7.91	7.96	7.95	7.92	7.89
EC ($\mu\text{S}/\text{cm}$)	20,300	6,890	9,020	7,166	7,828	7,614	7,613
TDS (ppm)	10,200	3,450	4,520	3,588	3,919	3,812	3,812
Salinity (ppt)	11.6	3.64	4.86	3.78	4.14	4.02	4.02

The resulting solutions after mixing with *Spirulina platensis* had greenish color with turbid appearance. The chemical parameters were described in Table 4.10.

Table (4.10): Chemical Parameters for the fifth experiment after mixing with *Spirulina platensis*

Chemical Parameters	Growth Medium Types						
	ZM	FW	FW+BW	FW+MW	FW+PAS 3%	FW+IMJ-1	MIX
pH	9.05	9.30	9.30	8.63	8.63	8.61	8.59
EC ($\mu\text{S}/\text{cm}$)	21,000	12500	14,630	9,833	10,164	10,057	10,057
TDS (ppm)	10,500	7200	8,270	5,394	5,560	5,506	5,506
Salinity (ppt)	12	6.7	7.92	5.24	5.42	5.36	5.36

4.1.5.2 Measurements

All types started with similar concentration (Figure 4.13) and increased during the first week with similar behavior. At the beginning of the second week all of them changed as follow, *Spirulina platensis* grown in Zarrouk medium, fish wastewater (including IMJ-1 medium), and mix solution increased the most and reached around 2.177, 2.198, and 2.173 respectively, while most of growth medium types increased by the end of week two and then decreased gradually. (See Appendix: Section E, E.1)

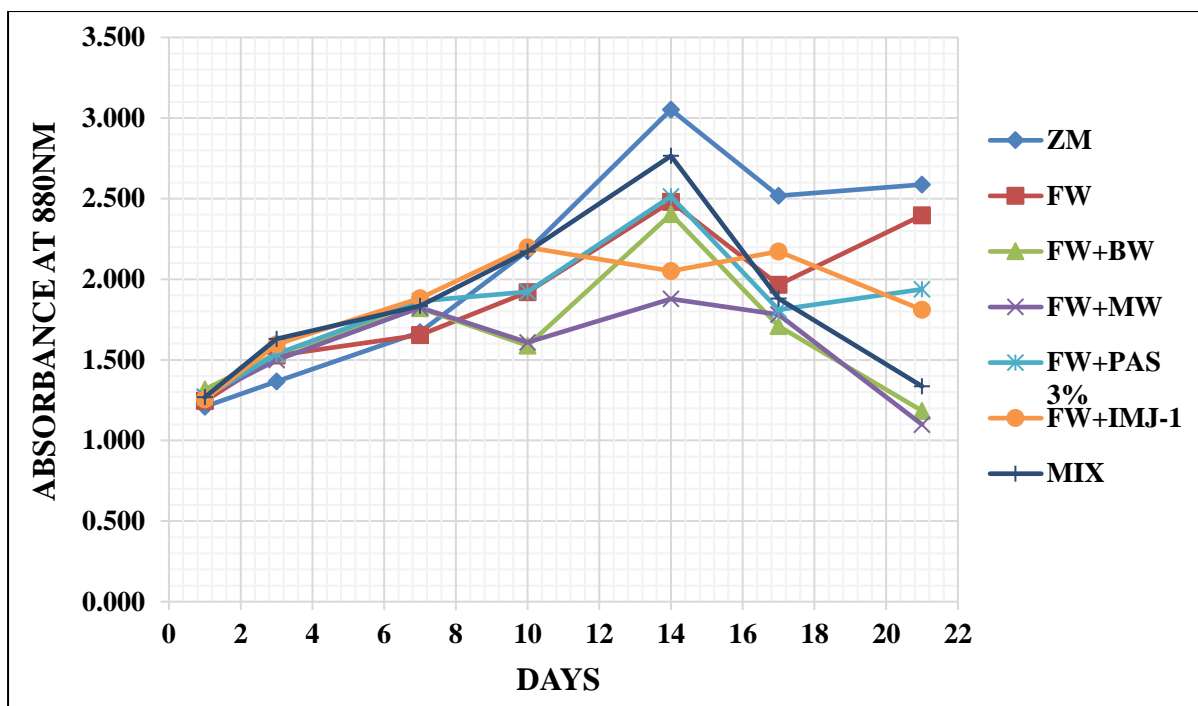


Figure (4.13): The fifth experiment absorbance results at 880nm.

4.1.5.3 Growth Rate (Percentage)

Growth rate measurements (Figure 4.14) were different according to the medium used. The best growth rate value during the first 3 days was recorded for mix solution with 29%, while the lowest one was for Zarrouk medium with 13%, but this changed with the end of the first week and till the end of the experiment. The overall and the best growth rate for 21 days was for Zarrouk medium with a growth rate of 90%, and close to it was fish wastewater with 77%, while fish wastewater (with PAS 3%), fish wastewater (with IMJ-1), fish wastewater (with Mix), fish wastewater (with BW), and fish wastewater (with MW) were 55%, 44%, 26%, 13%, and 2% respectively.

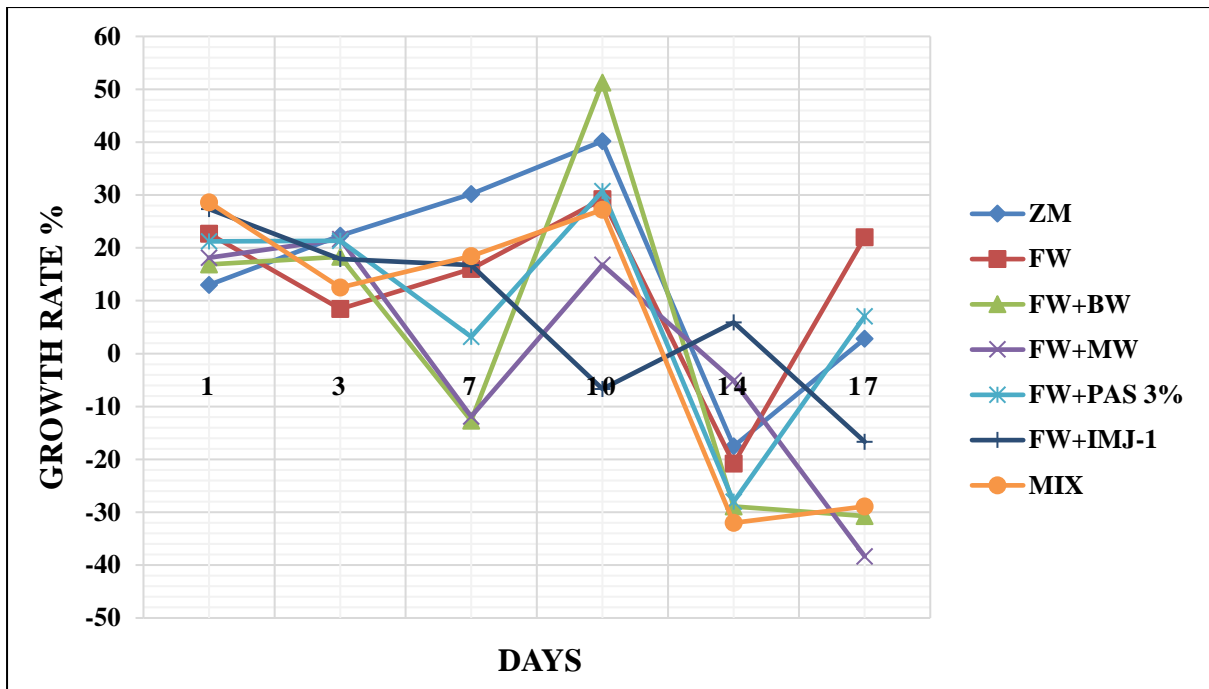


Figure (4.14): Growth rate (%) for experiment 5 growth medium types.

4.1.5.4 Dry Weight (g/L)

Spirulina platensis had a similar dry weight range from 13-16 g/L in day one in all growth medium types, while after one week and according to the behavior of *Spirulina* in each type of its' dry weight changed and increased in some types, in Zarrouk medium for example dry weight increased from 16 to 21 g/L, while in fish wastewater increased from 13 to 15 g/L, and fish wastewater and brackish water, *Spirulina platensis* dry weight does not change. It was around 14 g/L. The rest of growth medium types increased with lower rate than Zarrouk medium. While all of that changed after week two and most of them doubled their dry weight. The lowest one was fish wastewater with 17 g/L as increase from week one to week two, while most of them increase of around 20 g/L at the same period. Zarrouk medium still has the higher biomass with an increase of 38 g/L for the same period.

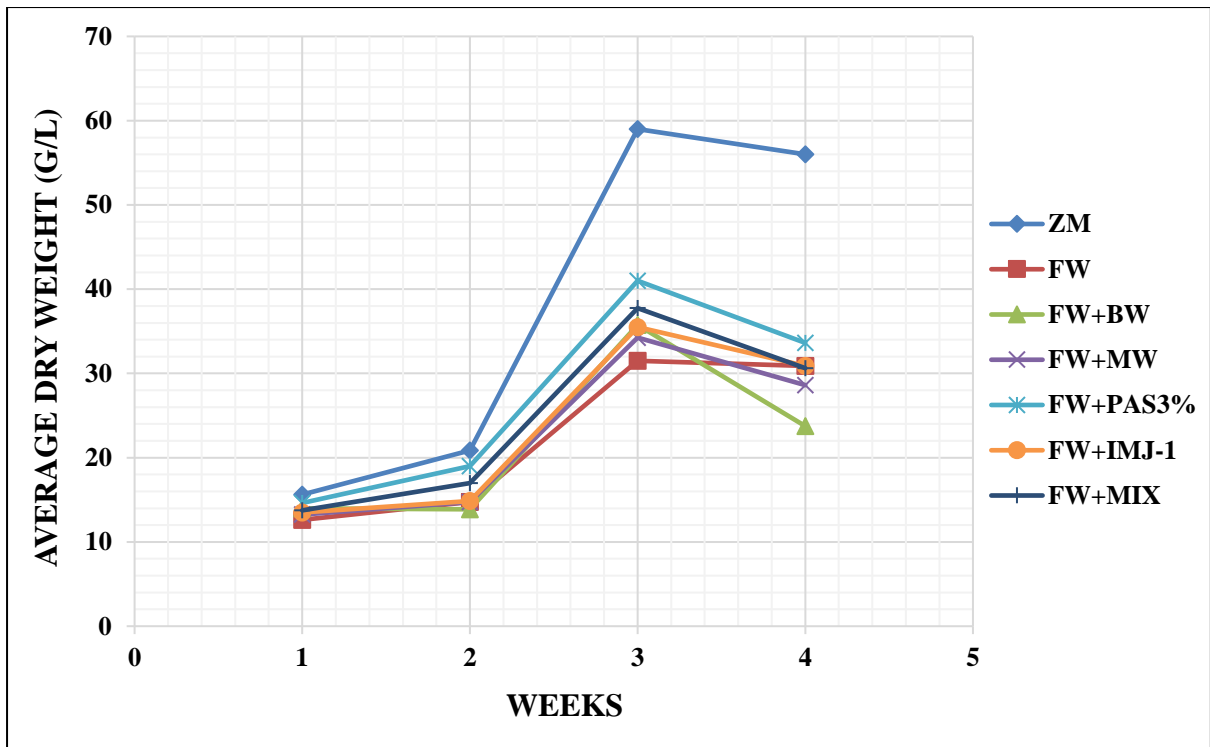


Figure (4.15): The fifth experiment dry weight (g/L)

4.2 Discussion

The result that was obtained from all the experiments in this study is that *Spirulina* was able to grow within a wide range of chemical parameters. The pH value of the medium that supports the growth of *Spirulina platensis* can range between 7.08 and 10.72 while the electrical conductivity can be between 6,600 and 21,500 $\mu\text{S}/\text{cm}$. The total dissolved solids range was between 3,300 and 10,200 ppm, and salinity ranges between 3.30 and 11.6 ppt. The optimum conditions were always achieved when the parameters move toward the higher values. The best chemical parameters after mixing of *Spirulina platensis* with the growth medium were range between 8.6 and 10.65 as pH value, and the best electrical conductivity ranges between 10,000 and 29,700 $\mu\text{S}/\text{cm}$, while the best total dissolved solids range between 5,500 and 19,500 ppm, and the best salinity ranges between 5.36 and 17.40 ppt. The best results were obtained with the higher values of alkaline and salty conditions.

Spirulina platensis grow rapidly during the first three to four days with a huge increase in growth rate because in the first three days low concentration of *Spirulina* cells will had enough nutrients and enough light saturation. The light will pass easily and reach all *Spirulina* trichomes while after three days the number of cells will increase to be more concentrated, and nutrients concentration will decrease; so the light cannot pass the water easily due to high numbers and the growth rate decrease due low nutrients especially nitrogen and less light. (Vonshak, 1997). Excessive light intensity may lead to photo-oxidation and inhibition, whereas low light levels will become growth-limiting. (Carvalho *et al.*, 2011)

From our practical experiences *Spirulina* growth can extend up to seven days depending mainly on light and nitrogen levels besides of other minor parameters. *Spirulina platensis* should be treated carefully like a baby and supported with nitrogen each four days if urea was used as a nitrogen source or each two weeks if sodium or potassium nitrate was used as nitrogen source. When *Spirulina* grown in bottles or in a transparent container, the inner wall of the container or the bottle should be cleaned at least once per week to allow light-pass and reach more *Spirulina* cells, because *Spirulina* movement in the container will make it stick to the wall surface especially if it was in straight shape and day by day, it will create a coat on the surface.

It was noted that dry weight in most treatments that used in this study got a little increase during the first week and got a huge increase after the second week and when compared it

with the absorbance, we found that dry weight is not always a good indicator and not represent the absorbance. Well, we tried another way and find that if dry weight measurements based on the change in growth rate and absorbance after taken the weight in the first day, it will give a related and corrected data.

Zarrouk medium was the best growth type, but it is not the cheaper. Fish wastewater with a price of 1.5 ILS/m³ was very good source to cultivate *Spirulina platensis* and needs to be controlled and tested always for its' elements content especially Nitrogen. From our practical experience fish wastewater even with filtration and autoclaving form a type of coating on the inner walls of transparent bottles that prevent light pass and led to *Spirulina* death. Plant ashes are coming from a natural source such as weed and grass that cause many problems for farmers and it is considered as a very good source to cultivate *Spirulina platensis* with a price of ~ 1.5 ILS/m³ and its' quality varied depending on the concentration used. Brackish and Mining water could be used in addition of other elements such nitrogen, phosphorous, and potassium mainly and it could be bought with around 1.5 ILS/m³. IMJ, IMJ-1, and IMJ-2 are very good type to cultivate *Spirulina platensis* for short periods with lower price than Zarrouk medium; also, all the types that composed of chemicals could be cheaper if it bought with large quantities.

Common notes could be shared between this study and the previous studies mentioned in chapter two. In comparison with Danesi et al. (2011), they found that the best results were achieved were at temperature 30 °C, and we tested *Spirulina* at 30 °C and 35 °C and got very good results, also they found that the best light intensity was on 60 $\mu\text{mol photons m}^{-2}.\text{s}^{-1}$ and this is close to around 5,000 Lux that intensity we used, also they got more cell productivity after using urea as nitrogen source and that was clear in the fourth experiment when dry weight compared between IMJ-2 with ZM. In this study we look for urea as a promising alternative as cheap nitrogen source and Soni and Sudhakar (2019) saw the same thing after their experiments, also they concluded that growth media based on organic nutrients produce more biomass yield, and we noticed that when comparing FW with ZM in most of our experiments. After doing the experiments in this study and from our experience we noticed that media used urea as nitrogen source sustain for 10 days at maximum and Sukumaran et al. (2018) noticed that too and recommended to add urea as pulse-fed throughout the cultivation period.

Chapter Five

Conclusions & Recommendations

- **5.1 Conclusions**
- **5.2 Recommendations**

Chapter Five: Conclusions & Recommendations

5.1 Conclusions

It is possible to introduce *Spirulina platensis* as a new economical agricultural activity to:

1. Palestine to overcome the malnutrition in Palestine and introduce a new economical industry that could be sold worldwide for the benefit of Palestinian economy and local people.
2. *Spirulina platensis* could be used as a healthy food for many people in Palestine. Production price of *Spirulina platensis* varied between countries around the world.
3. This study had proved that cultivation in Palestine is possible under low cost.
4. This is the first study in Palestine that investigate the possibility of *Spirulina* cultivation and production in the Jordan valley environment with lower cost than other international growth medium types and with a competitive quality.
5. The field is new in Palestine and need further research including testing the composition of different cultivation sources such as fish wastewater.

5.2 Recommendations

Results of this study revealed that it is possible to cultivate and produce *Spirulina platensis* in Palestinian environment, and the level of experience about *Spirulina* is very low compared to other *Spirulina* farmers and companies around the world. This study suggests the following recommendations:

1. Fish wastewater/effluents, plant ash, IMJ, IMJ-1, and IMJ-2 could be used as a good source for *Spirulina platensis* cultivation with more monitoring for its' components.
2. Because it is very hard to undo the negative effects of Mining stone companies in west bank, and with a pH over 8; it will be possible to use the extracted water from mining stone activities to help in *Spirulina platensis* cultivation.
3. *Spirulina* production using Fish wastewater reveals on the importance of Fish sector in Palestine, so this study recommends farmers to introduce fish such as Tilapia to their ponds that used in farms and use the water to cultivate *Spirulina*.
4. This study recommends decision-makers in Palestine to support microalgae sector especially *Spirulina* and introduce it to the Palestinian graduates to be *Spirulina* farmers.
5. This study recommends the production of *Spirulina* and transform it from raw material to be part of the food supplement and cosmetics sectors.

6. This study recommends the Palestinian standard institution to set Spirulina parameters to for Spirulina production in the Palestinian market.
7. To create Spirulina farm, a feasibility study should be prepared with creating a pilot system to adjust all growth parameters for Spirulina if it used for food supplements production.

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Appendixes

Section A: First Experiment

A.1 Measurement

Table (A.1): Description of the results in figure (4.1) to cultivate *Spirulina platensis*

Days	Growth Medium Types	
	Zarrouk Medium	Fish Wastewater
1	0.502	0.578
4	1.309	1.104
8	1.917	1.540
11	2.104	1.759
15	1.980	1.651
18	2.054	1.806
21	2.139	1.972

A.2 Growth Rate (Percentage)

Table (A.2): Description of the growth rate date (%) in figure (4.2).

Days	Growth Medium Types	
	Zarrouk Medium Growth Rate %	Fish Wastewater Growth Rate %
1	161	91
4	46	40
8	10	14
11	-6	-6
15	4	9
18	4	9

A.3 Dry Weight (g/L)

Table (A.3): Dry Weight (g/L) of Zarrouk medium vs. Fish Wastewater.

Weeks	Growth Medium Types Dry Weight (g/L)	
	Zarrouk Medium (g/L)	Fish Wastewater (g/L)
1	5.11	5.00
2	5.05	5.34
3	13.68	8.34
4	9.34	5.10

Section B: Second Experiment

B.1 Measurement

Table (B.1): Description of absorbance reading at 880nm for figure 4.4 in Second Experiment.

Days	Growth Medium Types				
	ZM	FW	BW 1:2	PAS 3%	MIX
0	0.636	0.709	0.563	0.712	0.720
4	1.007	1.160	1.328	1.160	1.289
7	1.198	1.454	1.540	1.528	1.560
11	1.491	1.626	1.634	1.757	1.817
14	1.673	1.733	1.740	2.026	1.935
18	1.390	1.811	1.940	2.006	1.695
21	1.561	2.035	1.819	2.413	1.923

B.2 Growth Rate (Percentage)

Table (B.2): Description of growth rate (%) for figure 4.5 in Second Experiment.

Days	Growth Medium Types				
	ZM	FW	BW 1:2	PAS 3%	MIX
0	58	64	167	63	79
4	19	26	16	32	21
7	25	12	6	15	16
11	13	6	7	15	7
14	-17	4	11	-1	-12
18	12	13	-6	19	13

B.3 Dry Weight (g/L)

Table (B.3): Description of dry weight (g/L) for figure 4.6 in Second Experiment

Days	Growth Medium Types Dry Weight (g/L)				
	ZM	FW	BW 1:2	PAS 3%	MIX
0	2.9	2.8	4.4	3.3	3.8
7	3.4	3.2	3.9	4.4	5.1
14	7.3	3.9	5.5	9.5	9.4
21	10.0	8.9	8.0	18.2	18.8

Section C: Third Experiment

C.1 Measurement

Table (C.1): Description of figure 4.7 measurement in Third Experiment.

Day	Growth Medium Types							
	ZM	FW	BW 1:2	MW	PAS 1.5%	MZ	FS	MIX
0	1.927	1.846	1.711	1.737	1.790	1.928	1.861	1.639
3	2.173	1.743	1.432	1.607	1.670	2.168	2.037	1.572
5	2.610	1.893	1.272	1.533	1.361	2.424	2.229	1.622
8	3.165	1.934	1.063	1.359	0.752	2.640	2.067	1.514
11	3.682	2.456	0.727	1.360	0.949	3.376	2.060	1.414
13	3.782	2.270	0.972	1.357	0.816	3.508	1.796	1.177
15	4.012	2.546	0.962	1.296	0.656	3.552	1.534	1.052
18	3.998	2.119	0.944	1.176	0.713	3.725	1.237	0.858
22	3.298	1.736	0.774	1.223	0.813	3.891	1.041	0.742

C.2 Growth Rate (Percentage)

Table (C.2): Description of figure 4.8 growth rate (%) results in Third Experiment.

Day	Growth Medium Types							
	ZM	FW	BW 1:2	MW	PAS 1.5%	MZ	FS	MIX
0	13	-6	-16	-7	-7	12	9	-4
3	20	9	-11	-5	-19	12	9	3
5	21	2	-16	-11	-45	9	-7	-7
8	16	27	-32	0	26	28	0	-7
11	3	-8	34	0	-14	4	-13	-17
13	6	12	-1	-4	-20	1	-15	-11
15	0	-17	-2	-9	9	5	-19	-18
18	-18	-18	-18	4	14	4	-16	-14

C.3 Dry Weight (g/L)

Table (C.3): Description of dry weight (g/L) in figure 4.9 for Third Experiment.

Week	Growth Medium Types Dry Weight (g/L)							
	ZM	FW	BW	MW	PAS 1.5%	MZ	FS	MIX
1	14.25	13.00	12.25	12.00	11.63	13.13	11.25	9.50
2	28.75	24.50	18.88	18.63	16.88	21.38	21.00	16.75
3	45.38	48.25	26.75	29.88	22.88	30.38	31.50	23.38
4	24.63	20.00	18.38	23.88	18.50	25.63	28.63	21.75

Section D: Fourth Experiment

D.1 Measurement

Table (D.1): Description of absorbance measurements at 880nm for figure 4.10 in the fourth experiment

Day Number	Growth Medium Types	
	ZM	IMJ-2
1	1.210	1.311
3	1.367	1.582
7	1.672	1.835
10	2.177	2.158
14	3.052	2.295
17	2.517	1.534
21	2.588	1.812

D.2 Growth Rate (Percentage)

Table (D.2): Description of figure 4.11 results.

Day Number	Growth Medium Types	
	ZM	IMJ-2
1	13	21
3	22	16
7	30	18
10	40	6
14	-18	-33
17	3	18

D.3 Dry Weight (g/L)

Table (D.3): Description of figure 4.12 dry weight (g/L) results.

Week	Growth Medium Types Dry Weight (g/L)	
	ZM	IMJ-2
1	16	16
2	21	25
3	59	52
4	56	43

Section E: Fifth Experiment

E.1 Measurement

Table (E.1): Description of figure 4.13 results.

Day Number	Growth Medium Types						
	ZM	FW	FW+BW	FW+MW	FW+PAS 3%	FW+IMJ-1	MIX
1	1.210	1.244	1.316	1.270	1.267	1.254	1.268
3	1.367	1.526	1.538	1.500	1.536	1.597	1.631
7	1.672	1.655	1.820	1.825	1.864	1.883	1.835
10	2.177	1.920	1.589	1.608	1.923	2.198	2.173
14	3.052	2.481	2.404	1.878	2.514	2.051	2.765
17	2.517	1.965	1.709	1.782	1.810	2.172	1.880
21	2.588	2.397	1.184	1.099	1.938	1.810	1.337

E.2 Growth Rate (Percentage)

Table (E.2): Description of figure 4.14 results.

Day Number	Growth Medium Types						
	ZM	FW	FW+BW	FW+MW	FW+PAS 3%	FW+IMJ-1	MIX
1	13	23	17	18	21	27	29
3	22	8	18	22	21	18	13
7	30	16	-13	-12	3	17	18
10	40	29	51	17	31	-7	27
14	-18	-21	-29	-5	-28	6	-32
17	3	22	-31	-38	7	-17	-29

E.3 Dry Weight (g/L)

Table (E.3): Description of figure 4.15 dry weight (g/L).

Week	Growth Medium Types Dry Weight (g/L)						
	ZM	FW	FW+BW	FW+MW	FW+PAS3%	FW+IMJ-1	FW+MIX
1	16	13	14	13	15	14	14
2	21	15	14	15	19	15	17
3	59	32	36	34	41	36	38
4	56	31	24	29	34	31	31