



Cultivating blue-green algae in wastewater for soil bio-fertilizers production and application on some vegetable crops in Egypt and Tunisia

By

Sayed Rashad Ahmed

B.Sc. Agric. Sci., Fac. of Agric., Ain Shams University (2003)

M.Sc. in African Studies, Natural Resources Dept., Faculty of African Postgraduate Studies

Cairo University (2012)

A Thesis Submitted in Partial Fulfillment of the Requirements for **Ph.D**

In

African Studies (Soil Resources)

Faculty of African Postgraduate Studies Cairo University EGYPT 2019

TABLE OF CONTENTS

LIST OF TABLES	Ι
LIST OF FIGURES	Π
ACKNOWLEDGMENTS	III
ABSTRACT	IV
INTRODUCTION	1
REVIEW OF LITERATURE	4
1. Water and Wastewater	4
1.1. Olive mill wastewater	7
1.2. Potatoes' processing wastewater	9
1.3. Olive and Potato in Egypt and Tunisia	10
2. Wastewater treatment	12
3. Microalgae	13
3.1. Blue- green algae (Cyanobacteria)	14
3.2. Useful applications of cyanobacteria	15
3.3. Cyanobacteria as Biofertilizers	16
4. Microalgae for wastewater treatment and	10
biofertilizer production	10
5. Effect of biofertilizers on celery and lettuce	25
plants	25
6. Effect of biofertilizers on sandy soil	28
properties	20
MATERIALS AND METHODS	31
1. Wastewater samples collection and analysis	31

1.1. Olive mill wastewater (OMW)	31
1.2. Potatoes industry wastewater (PIW)	31
2. Chemical analyses of wastewater	31
3. Cyanobacteria strains	34
4. Determination of microalgae growth in OMW	25
and PIW	55
5. Effect of cyanobacteria growth on total phenols	26
content of OMW	50
6. Soil samples	36
7. Pot experiment	37
7.1. Celery and lettuce plants	37
7.2. Chemical Fertilizer	38
7.3. Experiment 1	38
7.4. Experiment 2	39
8. Statistical analysis	39
RESULTS AND DISCUSSION	40
<u>PART 1:</u> Cultivation of cyanobacteria on olive mill wastewater and application of the bioformulated fertilizer on sandy soil for celery and lettuce cultivation	40
1- Cyanobacteria growth in olive mill wastewater (OMW)	40
2- Microscopic investigation results	46
3- Effect of cyanobacteria growth on phenols content of OMW	48
4- Characterization of cyanobacteria biofertlizers	49

5- Effect of OMW-biofertilizers on celery and lettuce	51
6- Effect of biofertilizers on NPK content of celery and lettuce	55
7- Effect of biofertilizers on some micronutrients in celery and lettuce	58
8- Effect of the OMW bio-formulated fertilizers on some properties of sandy soil after celery and lettuce harvesting	61
8-1-Effect of biofertilizers on some soil physical and chemical parameters	61
8-2-Effect of biofertilizers on availabe macronutrients in soil	63
<u>Part II:</u> Cultivation of cyanobacteria on Potato- industry wastewater and application of the bioformulated fertilizer on sandy soil for celery and lettuce cultivation	67
1- Microalgae growth in PIW	67
2- Microscopic investigation results	69
3- Specification of bio-formulated cyanobacteria biofertilizers	74
4- Effect of PIW- biofertilizers on celery and lettuce growth	75
5- Effect of biofertilizers on NPK content of celery and lettuce	76
6- Effect of biofertilizers on some micronutrients in celery and lettuce	81
7- Effect of the PIW- bio-formulated fertilizers on some properties of sandy soil after celery and lettuce harvesting	

7-1- Effect of biofertilizers on some soil physical and chemical properties	81
7-2- Effect of PIW- biofertilizers on available macronutrients of soil	84
CONCLUSION	87
SUMMARY	88
REFERENCES	94

LIST OF FIGURES

Figure no.		Page
Figure 1	pH variation of the media during cyanobacteria growth in OMW	45
Figure 2	Microscopic photos of (a) Anabaena, (b) Nostoc, (c) Spirulina and (d) mixed culture grown on OMW	47
Figure 3	Effect of <i>Spirulina</i> , <i>Anabena</i> and <i>Nostoc</i> cultivation on phenols removal	49
Figure 4	Variation of pH during cyanobacteria growth in PIW	72
Figure 5	Microscopic photos of (a) Anabaena, (b) Nostoc, (c) Spirulina and (d) mixed culture grown on PIW	73

LIST OF TABLES

Table no.		page
Table (1)	Chemical properties of OWW	32
Table (2)	Chemical properties of PIW	33
Table (3)	Experimental design for	34
	cyanobacteria cultivation in OMW	
Table (4)	Experimental design for	35
	cyanobacteria cultivation in PIW	
Table (5)	Physiochemical characteristics of the	37
	soil	
Table (6)	Experimental design for pot	38
	experiment 1	
Table (7)	Experimental design for pot	39
	experiment 2	
Table (8)	Chlorophyll content (µg/L) of cyanobacteria	42
	biomass grown on OMW	
Table (9)	Dry weight (g/L) of cyanobacteria biomass	43
	grown on OMW	
Table(10)	Optical density of cyanobacteria biomass	44
(10)	grown on OMW	
Table (11)	Composition of cyanobacteria	50
	biofertilizers	
Table	Effect of different fertilizers on celery	53
(12)	growth parameters	
Table (13)	Effect of different fertilizers on lettuce	54

	growth parameters	
Table	Effect of biofertilizers on macronutrients	
(14)	(%) content in celery leaves and roots	56
Table (15)	Effect of biofertilizers on macronutrients	57
	(%) content in lettuce leaves and roots	
Table	Effect of biofertilizers on	59
(16)	micronutrients (mg/Kg) in celery	
	leaves and roots	
Table	Effect of biofertilizers on	
(17)	micronutrients (mg/Kg) in lettuce	60
	leaves and roots	
Table (18)	Effect of OMW- biofertilizers on some soil properties	64
Table (19)	effect of OMW- biofertilizers on available	65
	macronutrients in soil	70
Table (20)	Chlorophyll content (µg/l) of cyanobacteria	70
	biomass grown on PIW	
Table	Dry weight (g/l) of cyanobacteria biomass	70
(21)	grown on PIW	
Table	Optical density of cyanobacteria biomass	71
(22)	grown on PIW	
Table	Composition of bio-formulated	74
(23)	cyanobacteria cultures	
Table	Effect of different fertilizers on celery	77
(24)	growth	
Table	Effect of different fertilizers on lettuce	78
(25)	growth	

Table	Effect of PIW-biofertilizers on	79
(26)	macronutrients content (%) in celery	
	leaves and roots	
Table (27)	Effect of PIW-biofertilizers on	80
	macronutrients content (%) in lettuce	
	leaves and roots	
Table	Effect of biofertilizers on	82
(28)	micronutrients (mg/Kg) in celery leaves	
	and roots	
Table	Effect of biofertilizers on	83
(29)	micronutrients (mg/Kg) in celery leaves	
	and roots	
Table (30)	Effect of PIW- biofertilizers on some soil	85
	properties	
Table (31)	Effect of PIW- biofertilizers on available	86
	macronutrients in soil	

Abbreviations list

ANOVA	Analysis of Variance
Bio-mix	Biofertilizer from cyanobacteria (<i>Spirulina</i> <i>platensis, Nostoc muscorum</i> and <i>Anabaena oryzae</i>) in olive mill wastewater
Bio-Spi	Biofertilizer from <i>Spirulina platensis</i> in olive mill wastewater
BSCs	biological soil crusts
Ca	Calcium
CSYE	cattle standing yard effluents
CEC	Cation exchange capacity
ст	Centimeter
CO ₂	Carbon dioxide
Cu	Copper
СМС	Chlorella mixed cultures
CH4	Methane
COF	commercial organic fertilizer
DE	dairy effluents
EC	electrical conductivity
Fe	Iron

FPW	fish processing wastewater
G	Gram
HRT	hydraulic retention time
К	Potassium
Kg	Kilogram
LSD	least significant difference
MPE	milking parlor effluents
m ³ /ton	Cubic meter per ton
Mg	Magnesium
mg/L	milligrams per liter
Mn	Manganese
Ν	Nitrogen
NPK	nitrogen, phosphorus and potassium
ОМ	Organic matter
OMW	Olive milling wastewater
OMWW	Olive milling wastewater
OD	optical density

PNSB	purple nonsulfur bacteria
Р	Phosphorus
PIW	Potato industry wastewater
PIW-Mix	Biofertilizer from cyanobacteria (<i>Spirulina platensis, Nostoc muscorum</i> and <i>Anabaena oryzae</i>) in potato industry wastewater
PIW- Spi	Biofertilizer from <i>Spirulina platensis</i> in potato industry wastewater
ppm	Part per million
RME	Rice mill effluent
S	Sulfur
SMC	Scenedesmus mixed cultures
тсор	total chemical oxygen demand
TWW	treated of wastewater
SWERI	Soils, Water and Environment Research institute
Zn	Zinc
%	Percentage
μg/g	Microgram per gram
μs/cm	Micro Siemens per centimeter
µg/L	Micro gram per liter

ABSTRACT

In the present study, the possibility of growing cyanobacteria in wastewaters from olive oil production (OMW) and potatoes industry (PIW) was investigated. Nostoc muscorum, Anabaena oryzae and Spirulina platensis were chosen as three blue-green algae for the present study. The three cyanobacteria were cultivated over 30 days period on olive effluent (OMW), diluted effluent (OMW 50%) and PIW. The growth of the three strains was achieved in the three media as indicated by their total chlorophyll, optical density and biomass dry weight. The initial phenols concentration in OMW wastewater was reduced by 50-60% in 15 days, and more than 80% of phenols were removed by day 30. The cyanobacteria grown on wastewater were formulated into biofertilizers and were applied on a sandy soil to grow celery and lettuce plants under different replacement levels (25, 50 and 75%) of the recommended chemical fertilizers, while the control did not receive any fertilizers in a greenhouse experiment. The results indicated that application of biofertilizers led to a significant (p<0.05) increase in the height of plant, root and stem lengths over the control group. The numbers of leaves per plant as well as chlorophyll content were highest in the treatments of biofertilizers (25 and 50%). Also, these treatments increased the total macro- and micro-nutrients of celery and lettuce plants. There was very remarkable enhancement in some recorded sandy soil properties after harvest i.e., pH, total organic matter, total nitrogen, phosphorus and potassium by the treatments of Bio-Mix with 25 and 50%.

The present study concluded that 1/4 or 1/2 of the recommended dose of NPK fertilizers could be saved for celery and lettuce growth by using biofertilizers produced from cyanobacteria grown on olive milling or potatoes industry wastewaters as promising eco-friendly bio-organic fertilizers.

Keywords: Cyanobacteria; *Anabaena oryzae*; *Nostoc muscorum*; *Spirulina platensis*; biofertilizer; celery; lettuce; agro-food wastewater.