

Mémoire

Auteur : Parmentier, Laurine

Promoteur(s) : Romain, Anne-Claude; 14823

Faculté : Faculté des Sciences

Diplôme : Master en sciences et gestion de l'environnement, à finalité spécialisée en surveillance de l'environnement

Année académique : 2020-2021

URI/URL : <http://hdl.handle.net/2268.2/13011>

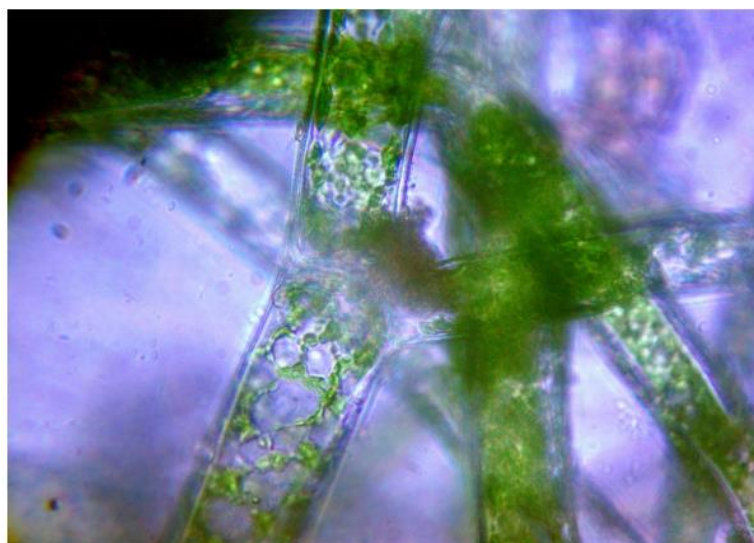
Avertissement à l'attention des usagers :

Tous les documents placés en accès ouvert sur le site le site MatheO sont protégés par le droit d'auteur. Conformément aux principes énoncés par la "Budapest Open Access Initiative"(BOAI, 2002), l'utilisateur du site peut lire, télécharger, copier, transmettre, imprimer, chercher ou faire un lien vers le texte intégral de ces documents, les disséquer pour les indexer, s'en servir de données pour un logiciel, ou s'en servir à toute autre fin légale (ou prévue par la réglementation relative au droit d'auteur). Toute utilisation du document à des fins commerciales est strictement interdite.

Par ailleurs, l'utilisateur s'engage à respecter les droits moraux de l'auteur, principalement le droit à l'intégrité de l'oeuvre et le droit de paternité et ce dans toute utilisation que l'utilisateur entreprend. Ainsi, à titre d'exemple, lorsqu'il reproduira un document par extrait ou dans son intégralité, l'utilisateur citera de manière complète les sources telles que mentionnées ci-dessus. Toute utilisation non explicitement autorisée ci-avant (telle que par exemple, la modification du document ou son résumé) nécessite l'autorisation préalable et expresse des auteurs ou de leurs ayants droit.

Sciences Institute

Environmental Sciences and Management Department



**Phytoremediation of eutrophicated coastal lagoon water using
macroalgae bioreactor: case study of the Il-Magħluq tal-Bahar
ta'Marsaskala Natura 2000 reserve**

Laurine PARMENTIER

**Submitted to the Environmental Sciences and Management Department in part
of the fulfilment of the requirements for the MSc in Environmental Sciences and
Management – Environmental Monitoring**

Tutors: Dr. Anne-Claude ROMAIN and James GABARRETTA

2020-2021

Reading comitee: Anne-Claude Romain, Claudia Falzone, Hugues Jupsin.

Copyright

Any reproduction of this document, by any means whatsoever, may only be made with the permission of the author and the academic authority* of the University of Liege.

*The academic authority is represented by the promoter(s) member(s) of the teaching staff of the University of Liege.

This document is the sole responsibility of its author.

Author of this document: Laurine Parmentier
(laurineparmentier@hotmail.com)

Copyright

Toute reproduction du présent document, par quelque procédé que ce soit, ne peut être réalisée qu'avec l'autorisation de l'auteur et de l'autorité académique* de l'Université de Liège.

*L'autorité académique est représentée par le(s) promoteur(s) membre(s) du personnel enseignant de l'Université de Liège.

Le présent document n'engage que son auteur.

Auteur du présent document : Laurine Parmentier
(laurineparmentier@hotmail.com)

1. ACKNOWLEDGEMENTS

The path for writing this thesis has been circuitous. I would like to express my sense of gratitude to all the people who challenged, supported and stuck with me along the way. I would like to thank all those people who contributed directly and indirectly to help me write my dissertation and the work that was described in it.

First and foremost, I thank my tutor from Malta, James Gabarretta, manager of the nature reserve in Marsaskala, for the assistance he provided during all the phases of my project and all the suggestions he made to improve it. His dedication and interest made it possible to complete my work.

I owe a deep sense of gratitude to Oriana Balzan, reserve manager at Nature Trust Malta, who helped me with great advice and suggestions throughout the writing my dissertation. Her guidance carried me through all the stages of my writing process.

I would also like to thank the supervisors I had in Belgium, Prof. Anne-Claude Romain and Claudia Falzone who helped me with the content and the analysis I needed. Their timely advice, scholarly guidance and scientific approach helped me to a great extent to be able to accomplish all of the tasks that come with a thesis.

I profusely thank Matthew Calleja, Shaun Darmanin, Luke Zammit and Prof. Lanfranco (University of Malta) who helped me in any way possible in gaining knowledge and information regarding this field. I greatly benefitted from their experience and their work in nature conservation and water analysis.

I am extremely thankful to the other interns and friends at Nature Trust Malta, Kelly Prisco, Eve Gangloff, Astrid Dubois, Kevin Normand, Maria Kleegrewe and Luis Ofenstein, who helped me setting up my project and who encouraged me when I encountered difficulties.

I would also like to thank Martine Vandenberghe, a close friend of my family, who went through those 82 pages to help me correct my English.

Finally, I am grateful to my friends and family for their continuous support and understanding while I was undertaking my project.

2. ABBREVIATIONS

BOD	Biochemical Oxygen Demand
COD	Chemical Oxygen Demand
CoGAP	Code of Good Agricultural Practice
DI water	Distilled water
DIY	Do It Yourself
DO	Dissolved Oxygen
ERA	Environment and Resources Authority
EU	European Union
EWA	Energy and Water Agency
FTW	Floating Treatment Wetland(s)
LR	Low Range
MEPA	Malta Environment and Planning Authority
MO	Management Objective
NGO	Non-Governmental Organization
nm	Nanometer
NTM-FEE	Nature Trust Malta - Foundation for the Environmental Education
NVZs	Nitrate Vulnerable Zones
OO	Operational Objective
SCA	Special Area of Conservation
SD	Standard Deviation
SIC	Site of Community Interest
SPA	Special Protection Areas
WCMP	Water Catchment Management Plan
WFD	Water Framework Directive
WSC	Water Services Corporation

3. TABLE OF CONTENT

1. Acknowledgements.....	ii
2. Abbreviations.....	iii
3. Table of content.....	iv
4. List of tables	vi
5. List of figures and graphs	viii
5.1. Figures.....	viii
5.2. Graphs.....	ix
6. Introduction.....	10
7. Context	12
7.1. Natura 2000 network.....	12
7.2. Presentation of the NGO Nature Trust Malta and the Il-Magħluq tal-Bahar ta'Marsaskala site.....	15
7.2.1 Marsaskala nature reserve and the algae epuration project	16
7.3. Threats to the coastal lagoon and management plan actions	19
7.4. Current water quality.....	22
7.4.1 Analysis of the water quality by Dale Bartolo	22
7.4.2 Analysis of the water quality by the Water Services Corporation	26
7.5. Water quality recommendations.....	27
7.6. Phytoepuration projects	27
7.6.1 Constructed wetland.....	28
7.6.2 Floating treatment wetland	28
7.6.3 Algae Bioreactor	29
7.7. Description of the algae	31
7.8. Previous studies with similar macroalgae	32
8. Methodology	34
8.1. Problems encountered before the installation of the system	34
8.2. Set-up of the algae bioreactor.....	34
8.3. Sampling plan	36
8.4. Parameters	36
8.4.1 Measurement of temperature, pH, conductivity, dissolved oxygen .	37
8.4.2 Analysis of nitrates.....	38
8.4.3 Analysis of nitrites.....	40
8.4.4 Analysis of phosphates.....	41
8.4.5 For an accurate measurement.....	42

8.4.6	Safety	42
8.4.7	Accuracy of the instruments.....	42
9.	Results	44
10.	Discussion.....	59
10.1.	Overview of the results.....	59
10.2.	Future perspectives.....	64
11.	Conclusion	66
13.	Bibliographie	68
14.	Annexes	72
14.1.	Annexe I: Results of the experimentation.....	72
14.2.	Annexe II : Box-plots of the main parameters for the different tanks ..	81
15.	Abstract.....	86

4. LIST OF TABLES

Table 1: Different designations for the site of Il-Maghluq tal-Bahar ta'Marsaskala.	17
Table 2: Visions, MO and OO corresponding to the maintenance of the habitat "Coastal Lagoon 1150" and having an impact on water quality monitoring.	21
Table 3: Overall results obtained after the one-year sampling period (Bartolo, 2019).....	23
Table 4: Laboratory analysis results for the month of February 2021 (Source: WSC).	26
Table 5: Laboratory analysis results for the month of March 2021 (Source: WSC).	26
Table 6: Objectives, issue and hypothesis of the study about the algae bioreactor.	31
Table 7: Tanks used during the experimentation and their characteristics.	35
Table 8: Parameters studied and the equipment used to measure them.	37
Table 9: Instruments used during the experimentation to determine the studied parameters and their accuracy.....	43
Table 10: Overall results obtained during the 13-day-sampling for the Control 1 tank (recalculated concentrations after evaporation of the water).	45
Table 11: Overall results obtained during the 13-day-sampling for the Control 2 tank (recalculated concentrations after evaporation of the water).	46
Table 12: Overall results obtained during the 13-day-sampling for the Ulva 1 tank (recalculated concentrations after evaporation of the water).....	47
Table 13: Overall results obtained during the 13-day-sampling for the Ulva 2 tank (recalculated concentrations after evaporation of the water).....	48
Table 14: Overall results obtained during the 13-day-sampling for the Cladophora 1 tank (recalculated concentrations after evaporation of the water).	49
Table 15: Overall results obtained during the 13-day-sampling for the Cladophora 2 tank (recalculated concentrations after evaporation of the water).	50
Table 16: Percentage of each parameter removed after the 13 days of experimentation for each tank.....	51
Table 17: Standard deviation of the dataset for the 3 main parameters and for the 3 types of tanks.....	53
Table 18: Results of the Mann-Whitney test and t-test for each day, each parameter and each tank.	54
Table 19: Results of the Spearman correlation test for different tanks and parameters.....	56
Table 20: Equations of the models for each parameters for the Cladophora tanks.	58
Table 21: Overall results obtained during the 13-day-sampling for the Control 1 tank (recalculated concentrations after evaporation of the water).	72
Table 22: Overall results obtained during the 13-day-sampling for the Control 2 tank (recalculated concentrations after evaporation of the water).	72
Table 23: Overall results obtained during the 13-day-sampling for the Ulva 1 tank (recalculated concentrations after evaporation of the water).....	73
Table 24: Overall results obtained during the 13-day-sampling for the Ulva 2 tank (recalculated concentrations after evaporation of the water).....	73

Table 25: Overall results obtained during the 13-day-sampling for the Cladophora 1 tank (recalculated concentrations after evaporation of the water).	74
Table 26: Overall results obtained during the 13-day-sampling for the Cladophora 2 tank (recalculated concentrations after evaporation of the water).	74
Table 27: Overall results obtained during the 13-day-sampling for the Control 1 tank (concentrations not recalculated to take the evaporation into account).	75
Table 28: Overall results obtained during the 13-day-sampling for the Control 2 tank (concentrations not recalculated to take the evaporation into account).	76
Table 29: Overall results obtained during the 13-day-sampling for the Ulva 1 tank (concentrations not recalculated to take the evaporation into account).	77
Table 30: Overall results obtained during the 13-day-sampling for the Ulva 2 tank (concentrations not recalculated to take the evaporation into account).	78
Table 31: Overall results obtained during the 13-day-sampling for the Cladophora 1 tank (concentrations not recalculated to take the evaporation into account).	79
Table 32: Overall results obtained during the 13-day-sampling for the Cladophora 2 tank (concentrations not recalculated to take the evaporation into account).	80

5. LIST OF FIGURES AND GRAPHS

5.1. FIGURES

Figure 1: Map of Natura 2000 land and marine areas in Malta (MEPA).	13
Figure 2: Map of the Mediterranean Biodiversity Hotspot (Source: iucn.org). ...	14
Figure 3: Map of the Maltese archipelago highlighting the different reserves managed by NTM-FEE, the Xrobb I-Ghagin center and the sea turtle rehabilitation center (San Lucjan) (Source: clipartmaps.com).	15
Figure 4: Map of the Marsaskala Special Area of Conservation and the government-owned part of the reserve (Source: MEPA)	17
Figure 5: Delimitation of the Ecologically and Scientifically Significant Area Level 1 and Ecologically Significant Area Level 3 of Il-Magħluq tal-Bahar ta' Marsaskala Reserve (Source: MEPA).	18
Figure 6: Habitat map of the part of the Il-Magħluq ta' Marsaskala site that Nature Trust Malta manages (Source: MEPA).	18
Figure 7: Topographic map of a part of Wied-Għajn in Marsaskala. The Natura 2000 reserve managed by NTM is circled in red (Source: topographic-map.com).	19
Figure 8: Topographic map of a part of Wied-Għajn in Marsaskala. The Natura2000 reserve managed by NTM is circled in red. Each red line represents a difference of 10 meters in altitude. (Source: http://geoserver.pa.org.mt/publicgeoserver)	19
Figure 9: Removal of Chaetomorpha algae in February 2021.	20
Figure 10: Mild eutrophication of the Marsakala pond with Cladophora algae in August 2021.	20
Figure 11: Sampling points selected during the study directed by Dale Bartolo.	23
Figure 12: Simplified representation of the Il Magħluq ta' Marsaskala site and the water treatment systems that will eventually be implemented after the various field experiments are carried out (Source: James Gabarretta for Nature Trust Malta).	28
Figure 13: Simplified isometric view of the site (Source: James Gabarretta for Nature Trust Malta).	28
Figure 14: Floating Treatment Wetland of Il-Magħluq tal-Bahar ta' Marsaskala Reserve.	29
Figure 15: Photography of <i>Ulva lactuca</i> (Source: researchgate.net)	31
Figure 16: Photography of the <i>Cladophora</i> sp. from the Marsaskala pond.	32
Figure 17: Set-up of the algae bioreactor at the back of the reserve in Marsaskala.	36
Figure 18: Multiparameter meter (Source: hannaservice.eu)	38
Figure 19: Hanna Marine Nitrate Low Range Checker (Source: hannaservice.eu).	39
Figure 20: Hanna Marine Nitrate Checker with the 3 nitrate reagents.	39
Figure 21: Cuvette with all the nitrate reagents becoming pink (concentration of 0,3 PPM) and the checker.	40
Figure 22: Hanna Multiparameter Aquaculture Photometer. (Source: hannainstruments.co.uk)	40
Figure 23: Cuvette with all the nitrite reagents becoming red (concentration of 43µg/L).	41

Figure 24: Cuvette with all the phosphate reagents becoming blue (concentration of 0,33, 0,35 and 0,85 PPM from left to right) and the checker.	41
Figure 25: Picture of a diatom and flagellates found in the water of the tanks...	60
Figure 26: Picture of one of the flagellates found in the water of the tanks.	60
Figure 27: Dragonfly on the air pump pipe of one of the tanks.	65

5.2. GRAPHS

Graph 1: Comparison of the results of the 2007 and 2013 assessments of the overall conservation status of habitats and species of Community importance (ERA). ..	14
Graph 2: Phosphate level compared to the limit of detection according to the WCMP (Bartolo, 2019).	25
Graph 3: Nitrate level compared to the limit of detection according to the WCMP (Bartolo, 2019).	25
Graph 4: Evolution of the nitrate concentration (mg/L) in each tank compared to the advised limit.	51
Graph 5: Evolution of the phosphate concentration (mg/L) in each tank compared to the advised limit.	52
Graph 6: Evolution of the nitrite concentration (mg/L) in the tanks.	52
Graph 7: Evolution of the nitrate, nitrite and phosphate in the control tanks 1 and 2.	54
Graph 8: Evolution of the nitrate, nitrite and phosphate in the Ulva tanks 1 and 2.	55
Graph 9: Evolution of the nitrate, nitrite and phosphate in the Cladophora tanks 1 and 2.	55
Graph 10: Regression for nitrate (mg/L) per day for the Cladophora tanks.	57
Graph 11: Regression for nitrite ($\mu\text{g/L}$) per day for the Cladophora tanks.	57
Graph 12: Regression for phosphate (mg/L) per day for the Cladophora tanks.	58
Graph 13: Levels of nitrites, nitrates and ammonium concentrations for each month in 2014 (AIS environmental, 2014).	61
Graph 14: Levels of phosphates concentration for each month in 2014 (AIS environmental, 2014).	61
Graph 15: Box-plots for the control tanks (nitrate concentration).	81
Graph 16: Box-plots for the control tanks (nitrite concentration).	81
Graph 17: Box-plots for the control tanks (phosphate concentration).	82
Graph 18: Box-plots for the Ulva tanks (nitrate concentration).	82
Graph 19: Box-plots for the Ulva tanks (nitrite concentration).	83
Graph 20: Box-plots for the Ulva tanks (phosphate concentration).	83
Graph 21: Box-plots for the Cladophora tanks (nitrate concentration).	84
Graph 22: Box-plots for the Cladophora tanks (nitrite concentration).	84
Graph 23: Box-plots for the Cladophora tanks (phosphate concentration).	85

6. INTRODUCTION

Everybody knows by now that one of the biggest environmental challenges that Humanity is facing nowadays is the loss of biodiversity and its multiple impacts. Land-use change, species overexploitation, socioeconomic demands, new technologies, governance, population growth, but most importantly, the food system are all direct and indirect drivers of global tragedies such as the biodiversity loss (Sharma, 2020). According to WWF, biodiversity is being destroyed by humans and their activities at a very high and dangerous rate for Humanity. The size of a species' population is important to monitor as it can be used to measure and determine the health of the ecosystems. The Living Planet Index pointed out an « *average of 68% decrease in the populations of mammals, birds, amphibians, reptiles and fish between 1970 and 2016* ». Other means and indicators show that biodiversity has been declining over the past decades. The lifestyle that humans have adopted in the 21st century did not help to cease the change, which is also emphasized by climate change (WWF, 2020). The biodiversity loss has and can have huge impacts on our human societies as it can alter our food system and food security, the quality of the ecosystems services, the economy...

Those trends are accentuated in the biodiversity hotspots where endemic flora and fauna are threatened and where conservation status is in crisis (International, s.d.). According to Conservation International, 36 zones can be qualified as biodiversity hotspots, including the Mediterranean region and therefore, the Maltese islands. The hotspots host as endemic more than 50% of the world's plant species and around 43% of bird, mammal, reptile and amphibian species.

One of the tools implemented to tackle the problem of fauna and flora loss was to create the Natura 2000 network via European directives that the Member States had to follow. This was initiated in response to the public concern about biodiversity. The aim of this European network is to protect the species, usually endemic and/or rare, and habitats within a site (Commission Européenne, 2009). Malta started to submit a number of sites on the archipelago in 2004. After collecting more data and with the assistance of experts, Malta declared 27 Special Areas of Conservation to be part of the Natura 2000 network, including the site of Il-Magħluq ta' Marsaskala (Environment & Resources Authority, s.d.)

The Natura 2000 site of Il-Magħluq tal-Bahar ta' Marsaskala is a saline marshland with a pond of brackish water, it hosts several salt-tolerant species which can be endangered and/or endemic, such as the Maltese killifish (*Aphanius fasciatus*). The site includes two habitats: the Mediterranean Salt Meadow (Habitat 1410) and the Coastal Lagoon (Habitat 1150). A management plan was implemented a few years ago to set out ways on how to protect the species, protect the environment and restore the habitats on site that has been altered by human activities. This plan was written thanks to the help of experts, previous studies about the site and fieldwork to assess the current state of the reserve and create Visions for the site as well as Management Objectives and Operational Objectives.

The brackish water from the pond within this Special Area of Conservation (SAC) is a result of freshwater runoffs coming from the surrounding fields and rainwater that mix with the seawater entering the site through the connection to the sea under the road (Environment and Resources Authority, 2014). In the management plan, ERA described

some of the threats that the habitats are facing at the moment: the most important being the pollution of the pond water because of the runoffs coming from the fields and surrounding streets. Indeed, nitrate, nitrite and phosphate levels have really high values and it can impact the biodiversity on site. As mentioned, the nature reserve hosts the killifish (*Aphanius fasciatus*) which is protected by the Species Protection Regulation (Bartolo, 2019) and only remains on a few sites in Malta. The killifish has also been declared as threatened by the Habitats Directive and the IUCN. Therefore, it is important to make sure that actions are being implemented to assure that the state of the Coastal Lagoon improves.

James Gabarretta, the manager of the site of Il-Maghluq ta' Marsaskala means, as a part of the MO and OO, to purify the water of the site thanks to a phytoremediation project which includes the Algae Bioreactor that is going to be discussed in this dissertation. The aim of the technique is to use the algae from the site to purify the water. This is an in-situ experiment which consists of setting up water tanks with algae directly on site and measuring the changes in the concentration of different parameters in the water. The two species used for the experimentation are *Ulva lactuca* and *Cladophora sp.*

This study will revolve around the concept of characterizing the changes in water quality over time with a combination of in-situ methods. Helping the water quality of the Coastal Lagoon habitat to improve is really important, especially in a Natura 2000 site with endemic species like the killifish. Improving the water quality onsite could help other species population to develop or be restored.

This thesis and the experimentation implemented have different objectives: to quantify the changes (if any) in phosphate, nitrates and nitrites in the water placed in the tanks and to determine which algae give the best results. Overall, this dissertation will answer (if possible) to the next question: is it possible to use the algae *Ulva lactuca* and *Cladophora sp.* in a bioreactor to remove excess nitrate and phosphate from the water in the tanks?

In the first section of the dissertation, I will explain the context of the study, including a presentation of the NGO and the site, the current water quality and the description of the project I carried out. I will then go through the methodology, the material and equipment I used for the experimentation. In the third part, I will spell the results out before finishing my dissertation with a discussion on the results and the future perspectives of the project.

7. CONTEXT

7.1. NATURA 2000 NETWORK

The Natura 2000 network is a European network of different nature conservation areas. The aim is to protect breeding and resting sites of rare and/or endangered species as well as some rare and protected habitats and to ensure their long-term survival (European Commission, n.d.). According to the European Commission, the Natura 2000 network protects 18% of European land and 8% of European marine territory in the 27 EU countries at the moment.

This European network was set up in response to the public concern about biodiversity loss and one of the objectives was to *"halt biodiversity loss by 2010 and sign an international agreement to significantly reduce biodiversity loss by that date"*. (European Commission, n.d.). It is important to emphasize that if an area is part of the Natura 2000 network, not all human and economic activities on the site should be stopped and prohibited: the network is based on the assumption that humans and nature can live together in harmony. However, sustainable activities on Natura 2000 sites are encouraged and a partnership between people and nature is advocated, taking into account social, cultural and economic requirements as well as conservation objectives (European Commission, n.d.) (Natura 2000, n.d.).

The Natura 2000 system aims to implement the Birds Directive 2009/147/EC and the Habitats Directive 92/43/EEC and is based on these two tools of EU legislation (European Commission, 2009). The list of habitats and species protected by the Natura 2000 network is included in the annexes of these two directives (Natura 2000, n.d.). These directives require from the State Members to create Areas of Community Interest (ACIs) which must be approved by the European Commission before joining those ACIs and the Natura 2000 network (Direction Départementale des Territoires, 2015). The ACIs will then be used to create two types of areas: Special Protection Areas (SPAs) under the Birds Directive and Special Areas of Conservation (SACs) under the Habitats Directive. These areas will then be used to form the Natura 2000 network.

Article 6.1 of the Habitats Directive 92/43/EEC states that where an area is considered a SAC, State Members must provide for the necessary conservation measures, which may include management plans or any other measures that meet the ecological requirements of the different habitat types. Articles 6.3 and 6.4 prevent the State Members from implementing projects that may significantly harm the protected species and habitats of the concerned area.

In Malta, 28.9% of the land of the archipelago is protected by one or more designations today¹. Of this (almost) 29%, 13.8% is classified as a Natura 2000 area, which represents 43.7 km² according to ERA². Just over 35% of Maltese waters are also

¹ These include "Bird Sanctuary", "National Park", "Nature Reserve", SAC of international or national importance, SPA, "Tree Protection Area" and "Special Area of Geological Importance". (Environment and Resources Authority, n.d.)

² The Environment and Resources Authority (ERA) is the public authority responsible for environmental matters in Malta. Its main aim is to safeguard the environment and to achieve sustainable living in Malta.

protected by a "Marine Protected Area" status, which correspond to 4,138km² of protected areas. The management objectives for these protected areas in Malta range from scientific research to tourism, maintenance of ecosystem services, recreation, education and sustainable use of sites and their resources. The various Natura 2000 terrestrial and marine sites in Malta are shown in figure 1 below.

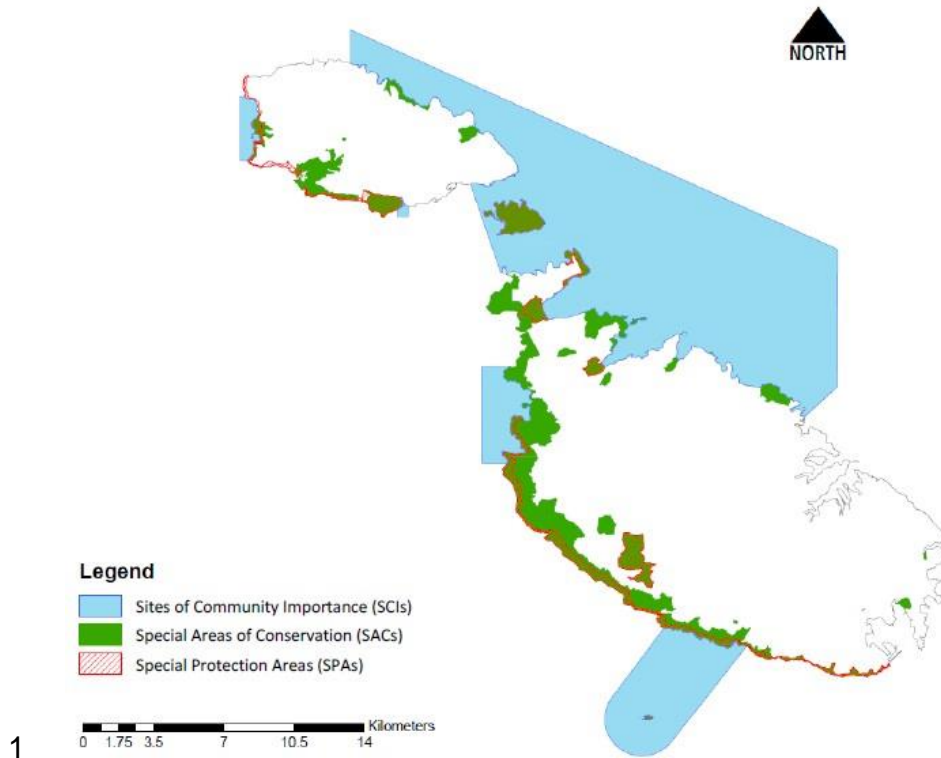


Figure 1: Map of Natura 2000 land and marine areas in Malta (MEPA).

It is particularly important to set up a strong Natura 2000 network in the Mediterranean Region as it is one of the world's biodiversity hotspots³. Indeed, it is a place of great biological interest but its biodiversity is threatened and faces enormous anthropic pressures. The Mediterranean basin stretches from Portugal to Jordan and includes various islands including the archipelago of Malta (see figure 2) (Derneđi, 2010).

The Authority ensures that environmental objectives are properly integrated at government and societal levels. It will, among other things, develop a system of plans and permits to monitor environmentally hazardous activities, be called upon to intervene in cases of damage or vandalism, ...

³ According to National Geographic and Conservation International, *biodiversity hot spots* are places rich in biodiversity (either fauna or flora or both) that are at risk of significant destruction. To be classified as such, these areas must have lost at least 70% of their vegetation due to human activities and host at least 1500 endemic vascular plants. These hotspots represent about 2% of the planet but host 44% of the world's plants and 35% of its terrestrial vertebrates.

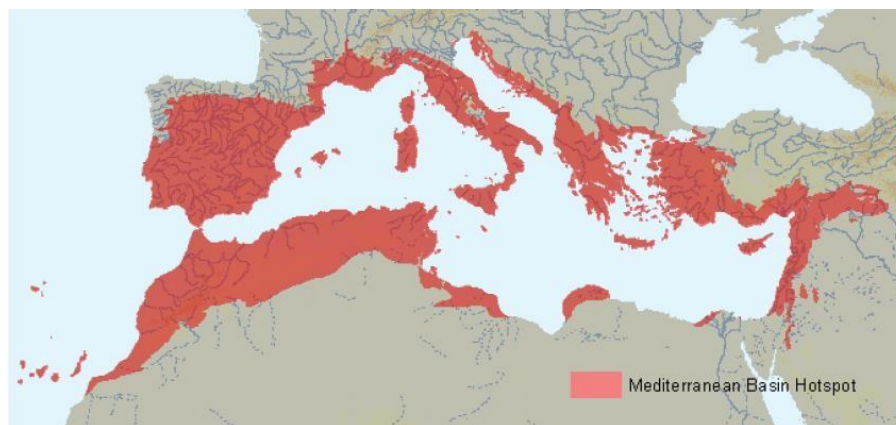
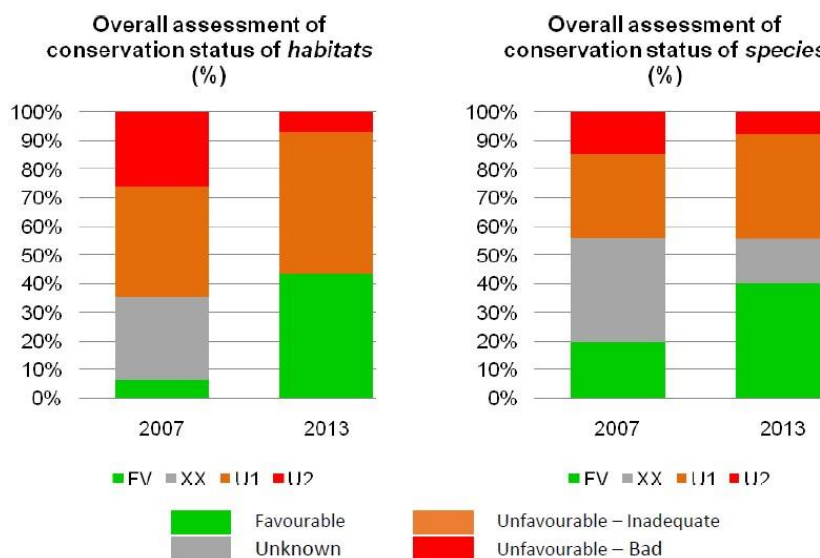


Figure 2: Map of the Mediterranean Biodiversity Hotspot (Source: iucn.org).

According to the IUCN, out of almost 6,000 species assessed in a 2018 study, 25% are threatened with extinction. This degradation in biodiversity is not new: already in 2008, the IUCN counted 19% of endangered species in the Mediterranean hotspot (Cuttlelod, Garcia, Malak, Temple, & Katariya, 2009).

In Malta, these trends are confirmed in the two assessments that were carried out by ERA in 2007 and 2013, in accordance with the requirements of the Habitats Directive and the Birds Directive. The aim was to verify the conservation of habitats and species and possible changes. Although the conservation status of species and habitats has improved slightly in recent years, 57% of the habitats assessed are still considered to have an unfavorable conservation status, as are 44% of the species of Community interest that were assessed (as shown in graph 1).



Graph 1: Comparison of the results of the 2007 and 2013 assessments of the overall conservation status of habitats and species of Community importance (ERA).

7.2. PRESENTATION OF THE NGO NATURE TRUST MALTA AND THE IL-MAGHLUQ TAL-BAHAR TA'MARSASKALA SITE

The organization for which the project is being carried out is called Nature Trust Malta - Foundation for Environmental Education (abbreviated to NTM or NTM-FEE). It is one of the largest and oldest environmental non-governmental organizations in the Maltese archipelago, having been founded in December 1962. It is strongly committed to the conservation of Malta's natural heritage by lobbying for protection status for certain species and habitats, as well as by implementing projects in various fields related to nature conservation. Indeed, the organization is very active in environmental education, wildlife rehabilitation and nature reserve management. (Nature Trust Malta - Foundation for Environmental Education, n.d.). NTM-FEE has a sustainable development center called Xrobb L-Ghagin center (in red in figure 1) which welcomes school groups and tourists to raise awareness on nature and its importance. This center also promotes ecotourism and has a hostel to welcome tourists.

Regarding the wildlife rehabilitation and protection, a rehabilitation center is being built to house all the injured animals that are protected on the Maltese islands⁴: the smaller animals are divided among the volunteers from the Rescue Team and the turtles are placed at the San Lucjan care center in Marsaxlokk (orange in figure 3). The NGO is responsible for the management of various reserves throughout the island of Malta and the island of Gozo: these include the reserves of Wied-Ghollieqa (in pink in figure 3), Pembroke (in light blue in figure 3), Marsaskala (in purple in figure 3), Marsaxlokk (in dark green in figure 3), Majjistral Park (in yellow in figure 3), Chadwick Lakes (in brown in figure 3) and Ramla Bay (in dark blue in figure 3). The main goal in those reserves is to achieve the restauration of habitats through actions such as removing alien species, planting of endemic species, raising awareness, organizing of open days and clean ups, the implementation of research project and so one.

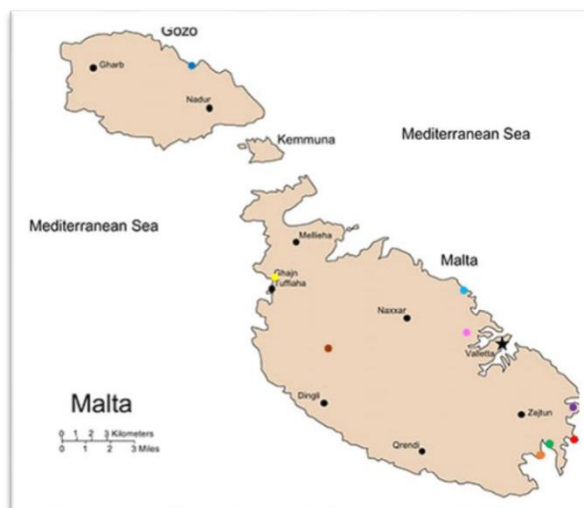


Figure 3: Map of the Maltese archipelago highlighting the different reserves managed by NTM-FEE, the Xrobb l-Ghagin center and the sea turtle rehabilitation center (San Lucjan) (Source: clipartmaps.com).

⁴ This includes hedgehogs, snakes, bats, dolphins, sea turtles, chameleons and ocellated lizards.

Several projects on a local and international scale have been implemented to protect the environment and the organization has been lobbying hard to get protection for endemic species on the Maltese islands (Nature Trust Malta - Foundation for Environmental Education, s.d.).

It is important to note that this organization is partly funded by the Maltese government and authorities such as ERA and EWA. It also receives funds from Europe and donations from individuals. Furthermore, the NGO receives some income by selling products from the reserves (such as capers, olive oil, honey etc.) and by allowing people to sponsor sea turtles from the San Lucjan centre.

This type of environmental NGO is essential in Malta as the environment is threatened by the expanding urban areas, tourism and atmospheric pollution. Indeed, according to a report from the European Commission from 10th of October 2019, Malta did not respect all the environmental laws and standards. The archipelago was asked to improve the water and waste treatment system as well as reduce the use of pesticides and implement actions to protect the Mediterranean coast.

7.2.1 MARSASKALA NATURE RESERVE AND THE ALGAE EPURATION PROJECT

The Algae Eparation project takes place in the Il-Maghluq tal-Bahar ta' Marsaskala⁵ Natura 2000 reserve (shown in purple in Figure 1) which is managed by James Gabarretta. The reserve is classified as a Special Area of Conservation and is situated in the locality of Marsaskala, in the south-east of the island of Malta. It is characterized by two interconnected brackish water ponds⁶ which have connections to the sea via a system of pipes running underneath the road which separates the site from the sea. This pond can have a maximum length of 230 meters and a maximum width of 30 meters depending on the level of water. The water in the pond comes from rain, sewage and run-off from the surrounding land and from the sea (Environment and Resources Authority, 2014).

As explained above, a Natura 2000 area can accommodate different types of land use and different types of activities despite its European protection status: this is why there are protected meadows as well as privately owned agricultural land next to the pond. According to ERA, agriculture is the predominant type of land use on the site, with 75% of the site being used for this activity.

NTM-FEE has access to and manages the part where the pond and meadows are located (in yellow in figure 4). This part of the reserve is government property while the agricultural areas within the site are private land. The entire Natura 2000 area concerned is shown in figure 4, circled in red.

⁵ The names "Il-Maghluq ta'Marsaskala" or "Marsaskala Reserve" are also used in this document and in the everyday life to refer to the site of Il-Maghluq tal-Bahar ta' Marsaskala.

⁶ The two interconnected ponds can be referred as one in this dissertation since it is one large body of water.



Figure 4: Map of the Marsaskala Special Area of Conservation and the government-owned part of the reserve (Source: MEPA)

The site is classified as a SAC of international importance as it is recognized as a Natura 2000 site. Parts of the site are also classified under other designations which give an additional and/or different protection to the areas concerned. Table 1 below shows the different statuses of the Marsaskala site and mentions the figures illustrating on a map to which part of the site the designations refer (Environment and Resources Authority, 2014):

Table 1: Different designations for the site of Il-Magħluq tal-Baħar ta' Marsaskala.

Designations	Part of the site / complete site	Reference figure
Special Area of Conservation of International Importance	Full site	Figure 4
Transitional Water Plan	Part of the site	/
Area of Ecological Importance and Site of Scientific Importance (Grade 1)	Part of the site	Figure 5
Area of Ecological Importance (Grade 3)	Part of the site	Figure 5

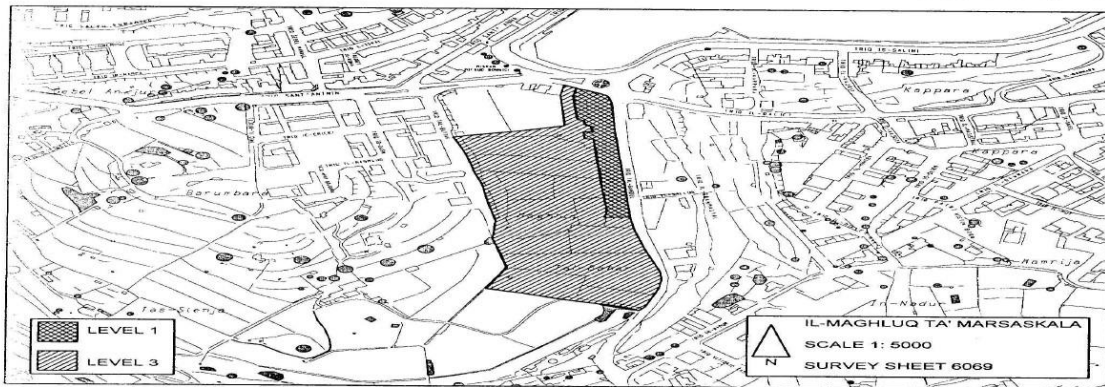


Figure 5: Delimitation of the Ecologically and Scientifically Significant Area Level 1 and Ecologically Significant Area Level 3 of Il-Magħluq tal-Baħar ta' Marsaskala Reserve (Source: MEPA).

One of the reasons why the site has many protection statuses is that it hosts endemic and/or protected species within its habitats, mainly on the site managed by Nature Trust Malta. According to the EUNIS classification, the Marsaskala site has two distinct habitats (shown in Figure 6):

- the coastal lagoon (1150), which corresponds to the pond within the site;
- the Medi terranean salt meadows (1410), which extend over the rest of the site.



Figure 6: Habitat map of the part of the Il-Magħluq ta' Marsaskala site that Nature Trust Malta manages (Source: MEPA).

The lagoon supports endemic plants on its banks such as *Ruppia sp.* and *Juncus Maritimus* but, above all, it provides a habitat for the endemic fish *Aphanius fasciatus* (also known as Corsican aphanius). This species is endangered and its populations are clearly declining on the Maltese islands which now only host this fish within 4 sites (Environment and Resources Authority, 2014) (Zammit-Mangion & Deidun, 2010). The Mediterranean salt meadows are dominated by *Juncus maritimus* but also contain other plants such as *Inula crithmoides* and *Limonium virgatum*. This habitat is found along the shores of the lagoon and is the largest and most diverse in terms of species on the site.

However, this document will mainly focus on the lagoon habitat and on improving its water quality.

7.3. THREATS TO THE COASTAL LAGOON AND MANAGEMENT PLAN ACTIONS

In the Management Plan drafted by ERA, it is said that despite its small surface area of 4.737m², the lagoon faces anthropogenic threats that affect the habitat. This can be explained by the location of the reserve which is in close proximity of roads, buildings, agricultural fields, the Marsaskala bus station, artificial beaches and other human activities.

According to Zammit-Mangon *et al.*, the effects mainly consist of waste that ends up on the site due to wind or is thrown on the site, as well as runoff of water coming from the surrounding streets and fields contaminated mostly by fertilizers and pesticides. This runoff, which is the main concern for the water quality of the pond, originates from the fields adjacent to the site and ends up in the pond and thus in habitat 1150. The runoff can be explained by the elevation of the reserve which is lower than the surrounding area, as shown in figures 7 and 8.

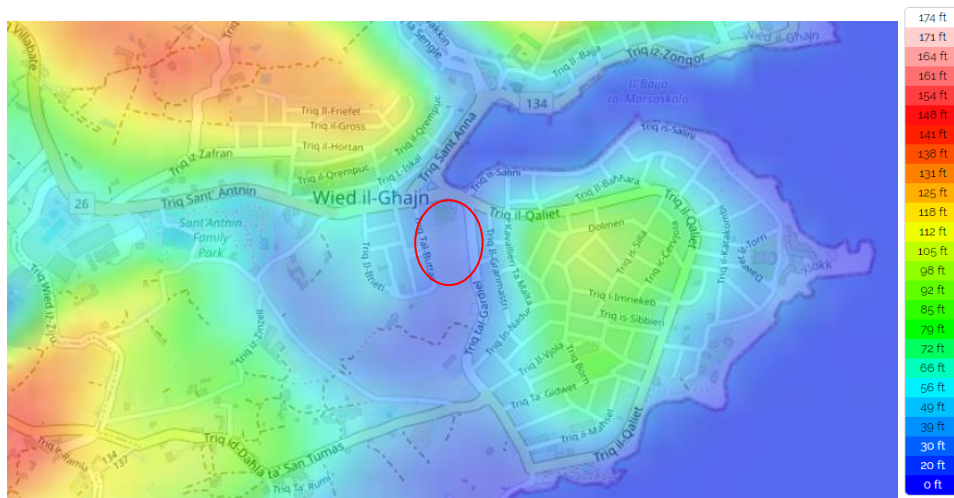


Figure 7: Topographic map of a part of Wied-Ghajn in Marsaskala. The Natura 2000 reserve managed by NTM is circled in red (Source: topographic-map.com).

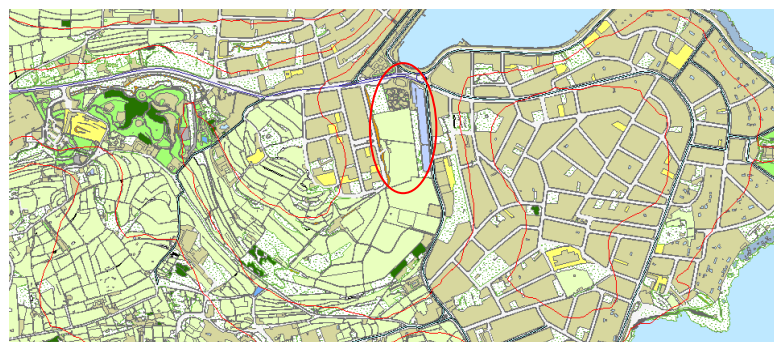


Figure 8: Topographic map of a part of Wied-Ghajn in Marsaskala. The Natura2000 reserve managed by NTM is circled in red. Each red line represents a difference of 10 meters in altitude. (Source: <http://geoserver.pa.org.mt/publicgeoserver>)

This increases the impact of the low dissolved oxygen level in the water during the summer period which is caused by the occasional eutrophication in the site and the currents in the water which sometimes become very weak (Environnement and Resources

Authority, 2014). So far this year, high to mild eutrophication has been observed from February until August in the second part of the pond (which is less deep). The eutrophication and the pollution of the pond can have repercussions on the population of killifish and their habitats. It is therefore important to try to stop or at least limitate or diminish the eutrophication, possibly by removing the extra nutrients from the water. Depending on the period of the year, the water of the pond can be highly to mildly eutrophicated. As it can be seen on the first picture hereunder (taken in February 2021), the eutrophication was so advanced that the manager of the site and the interns had to remove a part of the algae themselves (see figure 9). In August 2021, the pond was only mildly eutrophicated as shown in figure 10.



Figure 9: Removal of Chaetomorpha algae in February 2021.



Figure 10: Mild eutrophication of the Marsakala pond with Cladophora algae in August 2021.

This intrusion of water polluted with nitrates and phosphates is problematic as the whole Maltese archipelago is classified as a Nitrate Vulnerable Zone⁷ under the EU Nitrates Directive 91/676/EEC. Areas classified as such are polluted by direct or indirect

⁷ According to Annex I of the EU Directive 91/676/EEC, the Marsaskala reserve is considered an NVZ because it has suffered and is likely to suffer in the near future from eutrophication.

discharges of nitrates (or other substances that can be transformed into nitrates) of agricultural origin that threaten the water quality in the short term. According to Article 5 of the Directive, an action plan must be implemented to limit the most polluting activities. In Malta, a Code of Good Agricultural Practice has been set up in parallel with the National Nitrate Action Plan of 2011 (Environment & Resources Authority, n.d.). Some of the actions in the CoGAC must be carried out on a mandatory basis but the rest of the actions are implemented on a voluntary basis. This has not been enough to clean up the area sufficiently to avoid eutrophication episodes. Moreover, in the Management Plan that was written in 2014, ERA still mentions "*Negative agricultural practices within the site*".

In addition to this, ERA also states in the Management Plan that the reduction in the natural extent of the lagoon as well as the possible confinement of the lagoon to the sea (the pipe system below the road is not adequate to ensure and maintain the connection to the sea at all times) have resulted in a diminution in the resilience of the coastal lagoon.

As a result of these factors, the structure of the lagoon and its ecological functions are considered to be in poor condition and deteriorating, as are the future prospects of the habitat itself and of the killifish (*Aphanius fasciatus*) present on the site (Environment and Resources Authority, 2014). Therefore, different visions for the site and three Management Objectives and their corresponding Operational Objectives have been set up to improve lagoon habitat 1150 and its water quality. These are listed in the table below.

Table 2: Visions, MO and OO corresponding to the maintenance of the habitat "Coastal Lagoon 1150" and having an impact on water quality monitoring.

VISION 1: The Marsaskala wetland has expanded, its ecological value has improved and it has gained in naturalness and aesthetic value.	
MANAGEMENT OBJECTIVE (MO)	OPERATIONAL OBJECTIVES (OO)
1. Expand the area of lagoon habitat 1150.	1.1 Plan and implement a project for the expansion of habitat 1150. 1.2 Monitor the success of the lagoon expansion project in the first 5 years following its implementation.
VISION 2: All natural habitats, native flora and wildlife present at the SAC are sustained.	
2. Improve the structure and function of lagoon habitat 1150.	2.1. Ensure proper maintenance and, if possible, improve the lagoon-sea interface connection. 2.2. Ensure the prevention of excess silt accumulation at the sea interface and inside the lagoon.
3. Ensure the long term maintenance of the area, structure and function of lagoon habitat 1150.	3.1 Undertake seasonal inspections to monitor the size, structure and function of habitat 1150.

6. Increase the range occupied by <i>Aphanius fasciatus</i> on this site and the size of its population.	6.2. Monitor the range and population size of <i>Aphanius fasciatus</i> . 2.1. and 2.2 apply as well.
--	--

It is in this context of a will to improve the coastal lagoon and to safeguard the killies population that the algae water purification project is going to take place. It also falls under the various MOs in table 2.

7.4. CURRENT WATER QUALITY

7.4.1 ANALYSIS OF THE WATER QUALITY BY DALE BARTOLO

Between 2017 and 2018, Dale Bartolo conducted a one-year study to characterize the water quality of the pond within the Il-Magluq tal-Bahar ta'Marsaskala Natura 2000 reserve. The aim of the study was to determine the overall quality of the water but also to get an idea on the effects of anthropogenic activities on the area and the Coastal Lagoon as they could disturb the wildlife onsite.

To do so, Bartolo collected the following parameters directly on site once a month (except in January):

- Temperature,
- Total dissolved solids,
- Conductivity,
- pH,
- Dissolved oxygen.

Those parameters were collected directly onsite with a multi-parameter probe since they can change directly after the water is sampled from the pond.

Since the main concern of the site regarding the water pollution was the water runoff from agricultural lands, Bartolo analyzed the phosphates and nitrates level in a laboratory with a spectrophotometer.

Those parameters were checked in different sampling sites around the pond to represent the whole area (Bartolo, 2019) (see figure 11). One sampling point was in the first part of the pond, the other one at the limit between the first and the second part (where there is a small pebble wall) and one in the second part of the pond at the back of the

reserve. The samples were taken at the surface to avoid a high level of turbidity. Two replicate samplings were taken from each sampling point.



Figure 11: Sampling points selected during the study directed by Dale Bartolo.

After a year, Bartolo was able to create the following table with the results of his analysis:

Table 3: Overall results obtained after the one-year sampling period (Bartolo, 2019).

Point	Parameter						
	Temp (°C)	pH	DO (% sat)	Cond (m/s)	TDS	PO4(3-) (mg/l)	NO3- (g/l)
Nov point 1	20.8	8.11	66.52	1.00	0.00	0.01	0.03
Nov point 2	21.55	7.99	84.12	1.50	0.50	0.06	0.01
Nov point 3	22.00	7.95	62.13	3.50	2.00	0.00	0.03
Dec point 1	14.29	8.01	67.42	0.50	0.00	0.00	0.01
Dec point 2	14.52	7.95	61.11	1.50	1.00	0.00	0.01
Dec point 3	14.18	7.80	51.00	2.50	1.50	0.00	0.01
January	Did not sample						
Feb point 1	14.35	7.63	51.45	3999.00	2000.00	0.01	0.10
Feb point 2	16.60	7.99	51.40	3999.00	2000.00	0.00	0.01
Feb point 3	17.30	8.12	63.05	3999.00	2000.00	0.01	0.01
Mar point 1	15.30	8.88	41.71	2.00	1.50	0.01	0.01
Mar point 2	16.30	8.78	100.00	1.50	2.00	0.00	0.00

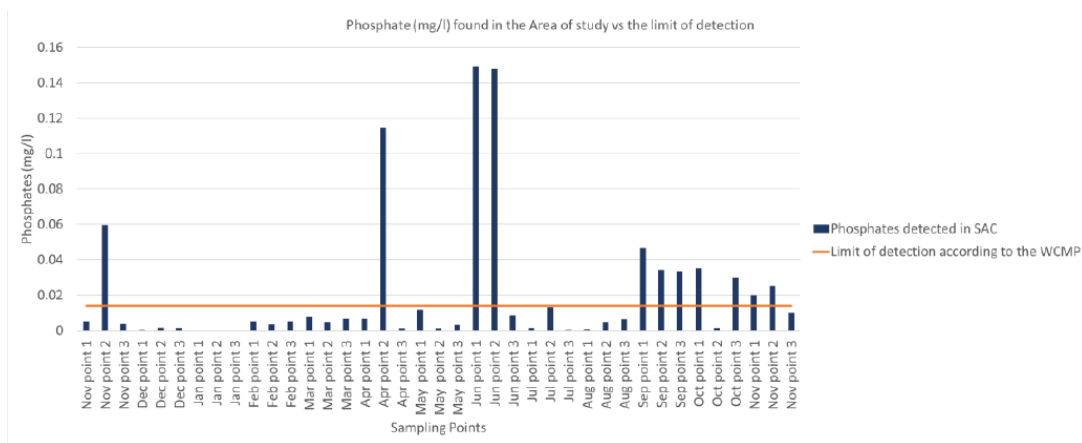
Mar point 3	16.60	8.24	95.55	1.00	2.00	0.01	0.00
Apr point 1	21.72	7.92	103.31	1.50	0.00	0.01	0.01
Apr point 2	21.76	7.61	71.00	2.50	1.50	0.11	0.01
Apr point 3	21.67	8.18	79.00	2.00	1.00	0.00	0.01
May point 1	27.95	6.98	61.12	3999.00	2000.00	0.01	0.00
May point 2	26.60	8.24	62.77	3999.00	2000.00	0.00	0.00
May point 3	28.05	8.10	104.21	3999.00	2000.00	0.00	0.01
Jun point 1	27.30	7.97	18.71	3999.00	2000.00	0.15	0.01
Jun point 2	26.10	8.21	107.00	3999.00	2000.00	0.15	0.02
Jun point 3	26.95	8.34	108.00	3999.00	2000.00	0.01	0.02
Jul point 1	29.85	8.29	83.00	3999.00	2000.00	0.00	0.02
Jul point 2	29.85	8.26	91.00	3999.00	2000.00	0.01	0.02
Jul point 3	29.20	8.07	16.00	3999.00	2000.00	0.00	0.02
Aug point 1	29.95	7.84	117.00	3999.00	2000.00	0.00	0.03
Aug point 2	29.70	7.75	85.86	3999.00	2000.00	0.00	0.15
Aug point 3	29.35	7.79	22.77	3999.00	2000.00	0.01	0.36
Sep point 1	28.75	7.99	65.00	3999.00	2000.00	0.05	1.35
Sep point 2	28.55	7.93	56.00	3999.00	2000.00	0.03	0.02
Sep point 3	28.20	7.93	5.00	3999.00	2000.00	0.04	0.01
Oct point 1	21.60	6.93	52.70	3999.00	2000.00	0.00	0.30
Oct point 2	21.45	7.28	47.65	3999.00	2000.00	0.03	0.12
Oct point 3	21.50	7.40	48.00	3999.00	2000.00	0.02	0.03
Nov point 1	21.35	7.95	132.79	3999.00	2000.00	0.03	0.06
Nov point 2	20.90	9.10	94.30	3999.00	2000.00	0.03	0.02
Nov point 3	20.35	8.08	80.50	3999.00	2000.00	0.01	0.14

The results showed the following variations in the parameters:

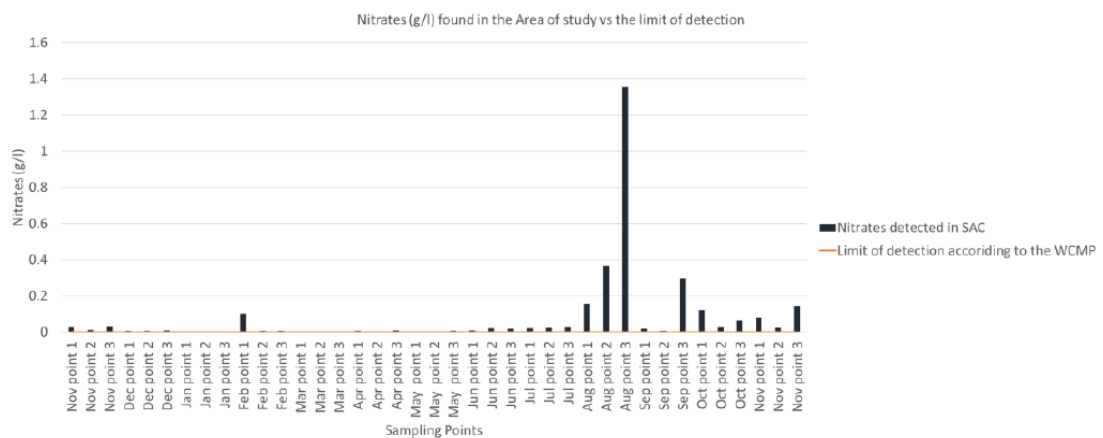
- 14.18 to 29.95 Celcius degrees for the temperature,
- 0 to 2000 g/L for the dissolved solids,
- 6.23 to 8.88 for the pH,

- 0.0007 to 0.143 g/L for the nitrates,
- 0.0003 mg/L to 0.15 mg/L for the phosphates,
- 5% to 132.79% of saturation for the dissolved oxygen.

The results that Bartolo got showed that the studied parameters were quite high compared to the standards used in the Second Water Catchment Management Plan for the Malta Water Catchment District (see section 5 “Water quality recommendations”) which was inspired by the European Water Framework Directive. It was shown after the study that seasonal variations and anthropogenic activities influence the water quality of the pond. However, the nitrates level was quite high and according to the WCMP it surpassed the limit of detection multiple times. The level of nitrates exceeded the limit of detection as well, all year long. Graphs 2 and 3 show that there was nitrates and phosphates pollution at that time.



Graph 2: Phosphate level compared to the limit of detection according to the WCMP (Bartolo, 2019).



Graph 3: Nitrate level compared to the limit of detection according to the WCMP (Bartolo, 2019).

The following conclusions were also reached after analyzing the results:

- There are huge seasonal variations for physical parameters such as temperature which directly influences the amount of dissolved oxygen in the water;

- The level of DO was low in average, with 49.43 to 80.82% depending on the sampling points. It was getting worse during the summer months.

All of these results and conclusions give an overall idea of how the parameters can change seasonally and what levels of nitrates and phosphates are expected to be found in the pond water.

7.4.2 ANALYSIS OF THE WATER QUALITY BY THE WATER SERVICES CORPORATION

More recently, the Water Services Corporation came to get the water tested in the two different basins of the pond in February and March. The results are shown in the following tables:

Table 4: Laboratory analysis results for the month of February 2021 (Source: WSC).

		LAB No.	21/01888	21/01889
		Sample Description	Shallow pond	Deep pond
Chemical Analysis				
Parameter	Method	Units	Result	Result
Turbidity	SOP/C/011B Nephelometry meter	N.T.U.	1.76	2.81
Conductivity	SOP/C/047 Meter	μScm^{-1}	3270	5010
pH	SOP/C/055 Meter	pH Units	8.51	8.14
Dissolved Oxygen*	SOP/C/WW022 Meter	%	95	83
Nitrates	SOP/C/UV001 Spectrophotometer	mg/L NO_3^-	4.64	3.48

Table 5: Laboratory analysis results for the month of March 2021 (Source: WSC).

		LAB No.	21/02758	21/02759
		Sample Description	Basin 1	Basin 2
Chemical Analysis				
Parameter	Method	Units	Result	Result
Turbidity	SOP/C/011B Nephelometry meter	N.T.U.	4.88	2.44
Conductivity	SOP/C/047 Meter	μScm^{-1}	51000	45000
pH	SOP/C/055 Meter	pH Units	7.76	8.21
Dissolved Oxygen*	SOP/C/WW022 Meter	%	92	80
Phosphates	SOP/C/010 Spot Test	mg/L	<0.1	<0.1
Nitrates	SOP/C/UV001 Spectrophotometer	mg/L NO_3^-	3.51	4.81

The level of phosphate was not analyzed at first but was under the lowest limit of detection by the laboratory equipment in March. Therefore, it is not possible to know if the phosphate level was surpassing the limit set by WCMP. Regarding the nitrate level, it was exceeding the WCMP limit by far.

Those two sample analysis show an important variation for the level of nitrates but since the sampling took place only twice, there is not enough data to reach to a conclusion. However, this gives an idea of the amount of nitrates that could be found in the water of the Marsaskala pond earlier this year.

7.5. WATER QUALITY RECOMMENDATIONS

The European WFD is one of the most important directives issued by the European Union since it is addressing and tackling water and water quality. It is intended to be one of the main drivers to lead to a sustainable management of the water within the EU.

Due to the issuing of the WFD, ERA and other governmental entities published “The 2nd Water Catchment Management Plan for the Malta Water Catchment District”. This document evaluated the previous plan and developed measures to achieve the environmental objectives that the Maltese government designed regarding the water management plan for 2021 (Sustainable Energy and Water Conservation Unit, 2015).

According to this plan and to previous studies of the water quality in Malta (Bartolo, 2019), the nitrate levels should not be higher than 1 mg/L (especially since Malta is in a NVZ) and the level of phosphates should ideally be less than 0.014 mg/L. Nothing was specified for the nitrites. It is important to remember that those limits are considered as recommendations.

7.6. PHYTOEPURATION PROJECTS

The Bioreactor project typically falls under Management Objectives 2 and 3, which affect the coastal lagoon habitat and which aim to improve the structure and functions of the lagoon and to ensure the long-term management of this habitat, its structure and functions. James Gabarretta, the site manager of Marsaskala, means to purify the water of the site through a phytoremediation project⁸ which includes different water purification techniques:

- Floating Treatment Wetlands (FTW),
- Constructed Wetland (CW),
- Algae bioreactor (or algae refugium on figures 12 and 13).

The three techniques will be tested separately over the following months to verify their effectiveness under field conditions. The most effective one(s) will be reused permanently on the site and possibly used in parallel as shown in figures 12 and 13.

⁸ Phytoremediation is a technique for cleaning up water or soil using plants, micro-organisms and their interactions.

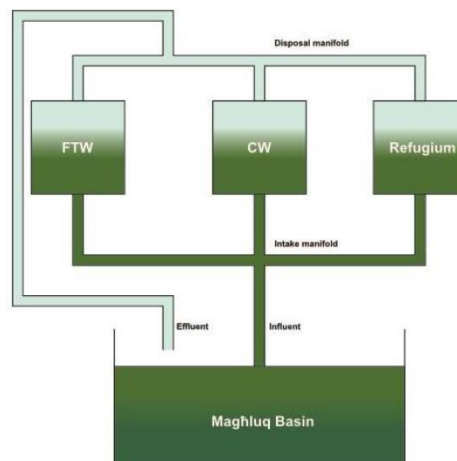


Figure 12: Simplified representation of the Il Maghluq ta' Marsaskala site and the water treatment systems that will eventually be implemented after the various field experiments are carried out (Source: James Gabarretta for Nature Trust Malta).

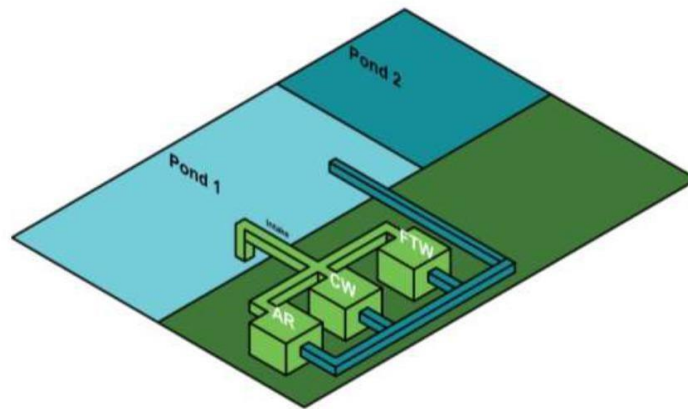


Figure 13: Simplified isometric view of the site (Source: James Gabarretta for Nature Trust Malta).

Although the three techniques all include water purification using plants growing on the site, they are all quite different.

7.6.1 CONSTRUCTED WETLAND

The creation of a CW aims to reproduce a wetland in an artificial way. The construction of a CW is based on the use of hydroponics, which allows the absorption of nutrients that pollute the water of a particular water body. This technique is relatively well known and used for water treatment (Gabarretta, 2021).

The construction and efficiency study of a CW are being carried out this year, along with the Bioreactor project studied in this thesis.

7.6.2 FLOATING TREATMENT WETLAND

FTWs are small wetlands that also use the principles of hydroponics by growing plants in a substrate that does not contain soil and allows the roots of the plants to access to water and therefore to the nutrients contained in it. The FTW structure is built with an inert material, resistant to water and to the weight of the plants and substrate. This material must also float on the surface of the water so that the roots of the plants can access to water and the plant itself remains on the surface (Gabarretta, 2021).

The FTWs built this year were made from a polyurethane board with holes drilled into it to put the substrate (perlite and clay). A wire mesh was placed underneath the structure to hold the substrate in place and allow roots to pass through the FTW. A jute cloth was sewn around the board to make it more aesthetically pleasing as shown in figure 14 below. The plants used in the Marsaskala Reserve FTW are *Juncus maritimus* and *Salsola sp.*



Figure 14: Floating Treatment Wetland of Il-Maghluq tal-Bahar ta' Marsaskala Reserve.

7.6.3 ALGAE BIOREACTOR

In general, the aim of the algae phytoepuration project is to control the level of phosphates and nitrates in the water of the pond as they are the two biggest pollutants present on the site. Other parameters have been analyzed as well during the experimentation period. The idea is to clean up the water with tanks containing polluted water from the site and algae on the surface. The algae will use the nutrients in the water to grow and clean the water at the same time.

As part of this project, I therefore carried out an in-situ experiment which consists of setting up water tanks with algae directly on the site and checking the changes in the concentration of different parameters in the water. This small-scale experiment is meant to reflect what actually happens in the pond and to purify the water thanks to the epuration process that happens naturally in the pond without creating eutrophication. As mentioned earlier, eutrophication is a common problem in this area and has been taking place for the whole year of 2021 so far.

Two types of macroalgae are present at the Marsaskala site and will be used in this experiment: *Ulva lactuca*. and *Cladophora sp.* The effectiveness of these algae for wastewater treatment has already been proven in laboratory conditions and in field experiments (See "Previous studies with similar macroalgae") (Sode, *et al.*, 2013) (Li, *et al.*, 2020) (Danielidis, Katsaros, & Dring, 2010) (Farahdiba, Hidayah, Asmar, & Myint, 2020).

Those two types of algae are both present in the pond water for most of the year. Unfortunately, due to the seasonal changes that occur throughout the year, the *Ulva lactuca* vanished from the pond when the experimentation started. The Malta Aquaculture Research Centre gave us some of the *Ulva lactuca* algae that they used for a similar phytoremediation project. When it comes to the *Cladophora* alga, it was identified by Pr. Sandro Lanfranco (University of Malta), Loïs Amelson and Léonie Falgous (interns at the

Department of Biology at the University of Malta) thanks to a sample taken in the reserve. The identification of the specimen to genus was carried out using the identification key in Rodríguez-Prieto, Ballesteros *et al.* (2013).

Bioreactor

The set-up is a form of algae bioreactor. Usually, this kind of bioreactor is used to produce and cultivate various species of algae. Even though the design of those reactors can be really diverse and differ widely, they are generally divided into two types: the open and the enclosed reactors (Borowitzka, 1999) (Lehr & Potsen, 2009). The one that was used in the set-up of this experimentation was an open bioreactor, which means that it was directly exposed to the air. They can be used for different purposes such as biomass production, fuel production, animal feed production or pollutants removal (Borowitzka, 1999) (Duan & Shi, 2014). In this case, the aim of the use of these algae bioreactors was to remove nitrates, nitrites and phosphates from the water or at least, diminish their concentrations.

The bioreactor used in this experimentation is close to an open plate bioreactor. This type of bioreactor is composed of different boxes or tanks, usually inclined to create movement in the water. They are frequently coupled (Wang, et al., 2021).

Nowadays, a lot of videos and articles on the internet can be found to create a DIY version of algae bioreactor. This was also an inspiration to create the set-up of the experimentation and the plate bioreactor with a low budget.

How it works

Nowadays, different types of algae (especially microalgae) are used during the treatment process of waste water in an algae bioreactor. Seaweed (and other vegetation) generally are able to accumulate and assimilate different substances in their cells such as plant nutrients, heavy metals, pesticides etc. The energy needed to go through this process comes from the photosynthesis. Bioreactors are usually based on photosynthesis and reactions that come with it.

Indeed, plants are able to assimilate the nitrogen contained in the water. The nitrates and nitrites will be converted into ammonium before being transformed into glutamine. Then, the glutamine will become organic matter and will be used by the plant (Olson, 2015). Nitrates is the major source of nitrogen for the plants and algae: it is essential for them to grow.

When it comes to phosphate, it is “*an essential nutrient for higher plants and algae*” (Cole, *et al.*, 2016). Plants are usually able to absorb phosphorus through the roots in the form of orthophosphate PO_4^{-3} or certain other forms of organic phosphorus. Orthophosphate is important for algal growth but also to feed life sustaining reactions such as energy transfer and activation of proteins. The plant or the algae will start using it after the absorption (Mikkelsen, 2013).

Objectives, issue and hypothesis of the Algae Bioreactor study

The study I conducted has different objectives. Indeed, I would like to quantify and measure the concentrations of phosphates, nitrites and nitrates and their possible changes during the first two weeks of my experimentation. Since I analyzed two different types of

algae, I would also like to determine which algae is the most appropriate and gives the best results. For the last two weeks of my experimentation, I would like to know if it is necessary to put an air stone in the tank and if the results are impacted by that. As mentioned before, NTM-FEE is an NGO with a restricted budget and it costs a certain amount of money to use the air stone all day long for several weeks.

The main issue of this dissertation is to know if it is actually possible to use the algae in those field conditions to purify the water. Knowing that, some positive and negative hypotheses were made, as stated in table 6 hereunder.

Table 6: Objectives, issue and hypothesis of the study about the algae bioreactor.

Objectives	<ul style="list-style-type: none"> a) Quantify the changes (if any) in nitrate, nitrite and phosphate in the bioreactor treatment system set up. b) Determine which algae give the best results.
Issue	Is it possible to use the algae <i>Ulva lactuca</i> and <i>Cladophora sp.</i> in a bioreactor system to remove excess nitrate, nitrite and phosphate from the water in the tanks?
Hypothesis	<ul style="list-style-type: none"> a) <ul style="list-style-type: none"> 1. The presence of algae in the system allows the levels of nitrate, nitrite and phosphate in the water to vary. 2. The presence of algae in the system does not cause the levels of nitrate and phosphate in the water to vary. b) <ul style="list-style-type: none"> 1. One of the algal species is more efficient in removing one of the nutrients studied (nitrate, nitrite or phosphate). 2. There is no species that is more efficient in the removal process of one of the nutrients studied.

7.7. DESCRIPTION OF THE ALGAE

Ulva lactuca is a marine green alga from the family Ulvaceae (see figure 15). This flat alga with a torn outline is considered as nitrophile (Burrows, 2013). It can reach a length of 30 centimeters but usually measures around 17 centimeters. The membrane is translucent, greenish, mushy and smooth. It is composed of two layers of cells organized irregularly with cup-shaped chloroplasts (Bates, s.d.)



Figure 15: Photography of *Ulva lactuca* (Source: researchgate.net)

It can be found worldwide and on the whole European continent, in particular where nutrients are abundant. Most of the time, this alga can be found in the littoral, sublittoral or on shore (Burrows, 2013). It is usually attached on rocks or other algae.

Cladophora sp. is a green alga from the family Cladophoraceae (see figure 16). This genus is multicellular, filamentous and consists of a lot of different species that are very hard to tell apart as a result of the variations occurring because of different factors such as habitat, age, environment... (De Souza Gestinari, Barreto Pereira, & Yocie, 2021) Usually, the filaments on the branches are not conjugated and have gametes with two flagella and spores with four flagella. Some species can be around 10 centimeters of diameter (Burrows, 2013) (Bryant & Irvine, 2016).

It is also one of the most common genus in the world and its species can be found all around the world, most commonly in ponds. They usually appear in form of thick blankets (Burrows, 2013).



Figure 16: Photography of the *Cladophora sp.* from the Marsaskala pond.

7.8. PREVIOUS STUDIES WITH SIMILAR MACROALGAE

The efficiency of some macroalgae to purify the water was proven before in several in-situ experimentations and laboratories. Several macroalgae were suggested for removing nitrogen and phosphates from wastewater (Cole, *et al.*, 2016). That is the reason why this experimentation is going to be tested onsite in Marsaskala. I will develop a few examples in the next paragraphs.

The *Cladophora* algae showed in the past that it had a great potential to treat wastewater (Whitton, 1970). Farahdiba *et al.* conducted an experimentation in 2020 about the removal of nitrogen and phosphorus by the macroalgae *Cladophora glomerata* with different nitrate concentrations. This alga was proved to be able to have a high removal efficiency of the nitrate and phosphate, especially in an environment with high nitrates concentrations.

In Greece, Danielidis *et al.* used *Ulva sp.* to treat the wastewater in a small sewage treatment plant. The experimentation showed that *Ulva sp.* could remove the phosphate

from the wastewater. Taking into account the results and the conditions on the Los island where the experiment was conducted, a biofiltering installation with *Ulva sp.* might be designed to remove the phosphate polluting the water.

A special plant column was built in India to treat the wastewater (Deviram, Pradeep, & Gyana Prasuna, 2011). To give a bit of context, getting clean water in some towns in India is arduous since small towns were constructed haphazardly within bigger cities rather than with a development plan. The untreated waste water could cause a lot of troubles to the environment and people's health. For the experiment, Deviram, Pradeep and Gyana Prasuna chose three algae species to use in the mats, including *Ulva sp.* It was showed that this species could remove a bit less than 50% of the BOD and the COD from the waste water after an incubation period of only 24 hours. After 72 hours of incubation, almost 100% was removed.

In the study called "*Bioremediation of reject water from anaerobically digested waste water sludge with macroalgae*" conducted by Sode *et al.*, *Ulva sp.* was used during the experimentation period. Even though the aim of the study was not only to purify the water, it was proven that *Ulva sp.* was able to remove N and P from the waste water.

All of those studies and many more gave James the idea to include a system with algae into the phytoremediation projects.

8. METHODOLOGY

8.1. PROBLEMS ENCOUNTERED BEFORE THE INSTALLATION OF THE SYSTEM

As mentioned before, this project of phytoepuration is implemented with the NGO Nature Trust Malta. I specifically wanted to emphasize that the algae bioreactor project and the CW project were conducted at the same time and under the same budget of 3200€. This implies that the budget for the different projects was very limited and that compromises had to be made between the money invested and the accuracy of the equipment that the NGO got. I had to consider this while defining the methodology, designing the set-up and making the list of the equipment needed. For both experiments, this budget allowed us to get all the material to set up the experiments (including the tanks, the air pumps, the plants etc.), the equipment to analyze the parameters and the reagents for the analysis.

When the order was placed, the equipment was supposed to arrive by the end of May or beginning of June. The delivery was delayed for a few weeks and the experimentation only started in July, which put both of the students conducting the experimentation in a rush.

Since the experiment took place in a protected zone, the set-up of the experiment had to be respectful of the nature and the fauna and flora species. At first, the tanks were supposed to be put into the water of the pond to get the same type of exposure as the water of the pond but it could have affected the killifish population and other species too much. The tanks were then put on land, very close to the pond.

8.2. SET-UP OF THE ALGAE BIOREACTOR

The algae bioreactor has been set up at the back of the Marsaskala nature reserve, as shown in figure 17, using the following equipment:

- 6 tanks (given by the Malta Aquaculture Research Centre),
- 6 air stones,
- 6 graduated tubes,
- 6 palettes,
- 1 kilo of algae per tank (except for the controls),
- 1 scale,
- Absorbent paper,
- Water from the pond.

It is essential to emphasize the fact that one of the most important goals of the experiment was to develop and implement a specific set-up and a new methodology based on other field studies and laboratory experimentations that could be adapted to the environment of the Marsaskala nature reserve. To set up the tanks and find the methodology of my project, I relied on the methodology of different experiments performed

in the laboratory and on the field (Danielidis, Katsaros, & Dring, Removal of phosphate by the green seaweed *Ulva lactuca*, 2010) (Li, *et al.*, 2020) (Sode, *et al.*, 2013) (Ge & Champagne, 2017) (World Health Organization, 2017) as well as on the Methodology and Sampling course given by Professor Anne-Claude Romain in the Faculty of Environmental Science at the University of Liège (Belgium).

Six identical tanks (made of non-hazardous plastic and inert to organisms) of 280 liters were used in this experiment and were filled with the same amount of water (Danielidis, Katsaros, & Dring, 2010) and were installed on palettes to level them. The water came from the Marsaskala pond and the macro elements were filtered through a piece of cloth.

Inside the tank, both types of algae will be placed on the surface after being collected directly from the site pond, which was the case for the *Cladophora sp.* The *Ulva lactuca* algae that were used during the experiment were given to Nature Trust Malta by the Malta Aquaculture Research Centre. Before placing them in the tank, they were rinsed with water and put to dry for 10 minutes. The algae, which are fairly easy to collect, were weighed to keep their weight at 1 kilogram in case they grow. A scale was used on site to measure the mass of the algae. The same amount of 1 kilogram of algae was placed in each of the different tanks (Sode, *et al.*, 2013). Each time the algae are weighed (once every 3 days), they were pre-dried in absorbent paper for 10 minutes before weighing.

Two of the tanks were filled with *Ulva sp.* algae and two others will be filled with *Cladophora sp.* The two remaining tanks were the controls so no algae were put into those tanks.

One air stone was installed in each of the tanks to allow sufficient aeration of the water (Sode, *et al.*, 2013) (Ge & Champagne, 2017) as well as to mimic the natural currents of the pond water. The algae have to be changed every two weeks according to their P and N uptake cycle (Sode, *et al.*, 2013) and also because the aim of the experiment is to have an efficient phytoepuration system that takes a short amount of time to purify the water.

The table 7 hereunder summarizes all the characteristics of the different tanks.

Table 7: Tanks used during the experimentation and their characteristics.

Tank number	Tank role	Algae used in the tank	Air pump	Amount of water (in liters)
1	Control 1	None	V	280
2	Control 2	None	V	280
3	Ulva 1	<i>Ulva lactuca</i>	V	280
4	Ulva 2	<i>Ulva lactuca</i>	V	280
5	Cladophora 1	<i>Cladophora sp.</i>	V	280
6	Cladophora 2	<i>Cladophora sp.</i>	V	280

The tanks were all placed on the ground next to the pond so that they can benefit from the same conditions of wind, sun, rain etc. as the pond (see figure 17). Unfortunately, it was not possible to place the tanks directly in the pond as this would damage the Coastal Lagoon habitat too much and would do more harm than good to the resident species.



Figure 17: Set-up of the algae bioreactor at the back of the reserve in Marsaskala.

8.3. SAMPLING PLAN

Samples were taken from the first day of the set-up (one sample per tank) and every day from the moment the experiment started. The samples were always taken at the same place in the tank and at the same time of day to change the parameters as little as possible.

The samples were taken every day onsite for a period of 13 days, starting on the first of July 2021 and ending on the 13 of July 2021.

The samples were analyzed directly on site as they can vary very quickly after sampling and as they should be analyzed within 24 hours (World Health Organization, 2017). The samples of water were taken and placed in a sample bottle. They were therefore checked directly onsite and were taken every day at 8 AM.

8.4. PARAMETERS

“Classical” parameters such as temperature, pH, conductivity, dissolved oxygen and salinity were taken with a Water Probe (see table 8).

Nitrate, phosphate and nitrite were measured using a Hanna Instruments optical photometer or checker (as shown in table 8). The Hanna photometer includes several methods pre-registered in the machine to measure the different parameters studied.

The data was then recorded and encoded in an Excel file before being analyzed.

Table 8: Parameters studied and the equipment used to measure them.

Parameter studied	Equipment used
Temperature of the water	Hanna Multiparameter Meter
pH	Hanna Multiparameter Meter
Conductivity	Hanna Multiparameter Meter
Dissolved oxygen	Hanna Multiparameter Meter
Salinity	Hanna Multiparameter Meter
Nitrates	Hanna Marine Nitrates Low Range Checker and Hannah marine nitrates reagents
Nitrites	Hanna Multiparameter Aquaculture Photometer and Hanna Nitrites LR reagents
Phosphates	Hanna Multiparameter Aquaculture Photometer and Hanna Phosphates reagents

During the experimental period, a bucket was installed right next to the pond in case of showers. The rain water might change the concentrations of nitrates, nitrites and phosphates of the water in the tank. This bucket would allow me to analyze the concentrations of the parameters studied in the rain water. The amount of nitrates, nitrites or phosphates added into the tanks would be known.

The evaporation of water will be measured thanks to the level of water rising or dropping in the reservoir as it can change the concentration levels as well.

8.4.1 MEASUREMENT OF TEMPERATURE, pH, CONDUCTIVITY, DISSOLVED OXYGEN

As mentioned before, the Hanna Multiparameter meter (see figure 18) was used to measure the temperature of the water (°C), the pH, the conductivity (mS/cm), the dissolved oxygen (%) and the salinity (PSU).



Figure 18: Multiparameter meter (Source: hannaservice.eu)

This probe is composed of 4 different sensors:

- pH sensor,
- pH/ORP sensor,
- Galvanic dissolved oxygen sensor,
- Four ring-electrode conductivity sensor.

The probe was calibrated every day before taking the measures with the Hanna Quick Cal solution. The probe was directly put in a calibration beaker 2/3 full with the said-solution and was calibrated automatically for the pH, conductivity and temperature after selecting the Quick Calibration option.

To calibrate the probe for the DO, the solution had to be removed from the calibration beaker and the probe needed to be shaken to remove every droplet of the Hanna Quick Cal solution. It is especially important not to have droplets on the DO sensor membrane. The probe is then put back in the empty beaker and the calibration will be done automatically.

After the calibration, a probe shield was screwed on the probe to protect the sensors. The probe was put at the bottom of the tank to take the measures after waiting for all the parameters to stabilize on the screen. A picture of the values was made for every measure taken during the experimentation.

Extra precaution was taken as the pH probe needs to stay in a solution of electrolytes when the probe is not used. Anyone using it put back a little cap with the provided Hanna solution.

8.4.2 ANALYSIS OF NITRATES

To analyze the nitrates in the water, the Hanna Marine Nitrate Low Range Checker was used (see figure 19) since the NaCl contained in the water can interfere with the methods used in the Hanna Multiparameter Environmental Analysis Photometer.



Figure 19: Hanna Marine Nitrate Low Range Checker (Source: hannaservice.eu).

Before being able to zero the checker, seven milliliters of the sample had to be taken with a syringe and put into the large mixing vial. Four milliliters of Marine Nitrate Reagent A were then added in the mixing vial (see figure 20 for the different reagents). The content of one packet of Marine Nitrate Reagent B (powder form) needed to be poured into the same vial before shaking it until the powder was completely dissolved. Those two reagents allow the checker to read the results without getting interferences due to the NaCl level in the water.

The content of the mixing vial was then drawn up in a syringe with a cover needle and filtered with a paper filter and a fennel before being put in a small 10mL cuvette until the “10 mL” mark was reached. In order to zero the checker, the cuvette was placed into the checker and the ON/OFF button was pressed twice until the “C2” message appeared on the display.

After zeroing the checker, the third reagent Marine Nitrate Reagent C (powder form) can be added into the cuvette before shaking it again until the powder is completely dissolved. To get the most accurate result, it is recommended to wait 8 minutes before reading the results. The higher the concentration in nitrates is, the pinker the sample is going to turn out as shown on figure 21.

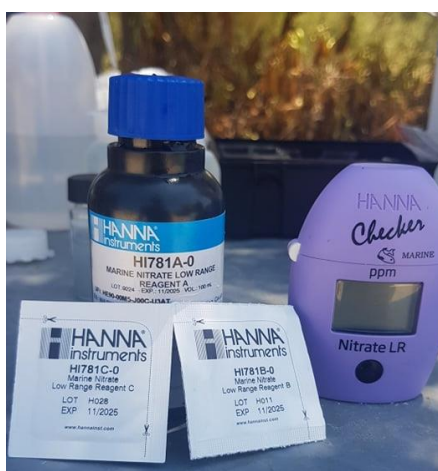


Figure 20: Hanna Marine Nitrate Checker with the 3 nitrate reagents.



Figure 21: Cuvette with all the nitrate reagents becoming pink (concentration of 0,3 PPM) and the checker.

After waiting those 8 minutes, the ON/OFF button was pressed and the instrument displayed the concentration of nitrates in ppm of NO₃⁻.

8.4.3 ANALYSIS OF NITRITES

To detect the concentration of nitrites, the equipment used was the Hanna Multiparameter Aquaculture Photometer (see figure 22). The light source in this photometer is a LED light and for this method, the wavelength used was 466 nm.



Figure 22: Hanna Multiparameter Aquaculture Photometer. (Source: hannainstruments.co.uk)

Once the method “Nitrite LR” method was selected on the display, the cuvette was filled up with 10 mL of the sample and placed into the holder. After closing the lid, the “Zero” key was pressed. When the display showed “-0.0-”, the meter has been zeroed and is ready for measurement.

After that, one packet of the Hanna Nitrite LR reagent was added to the cuvette. After being well shaken until the powder dissolved totally, the cuvette could be reinserted in the holder. The higher the concentration in nitrates is, the redder the sample is going to turn out as shown on figure 23.



Figure 23: Cuvette with all the nitrite reagents becoming red (concentration of $43\mu\text{g/L}$).

After waiting 15 minutes for the reagent to be fully effective, the button “Read” was pressed and the instrument displayed the concentration of nitrite (NO_2^-) in $\mu\text{g/L}$.

8.4.4 ANALYSIS OF PHOSPHATES

The phosphates were analyzed with the Hanna Multiparameter Aquaculture Photometer (see figure 22). There is normally a pre-set method on the photometer but because of some interferences that may occur during the reading of the results and with the reagent, the method selected on the photometer was “Absorbance Reading at 610 nm”. Indeed, the salt contained in the brackish water of the pond could have caused a variation in the results. The pre-set method “Phosphate LR” was made to use with fresh water only.

To read the absorption, a cuvette filled with 10 mL of the sample was put in the holder. After closing the lid, the “Zero” key was pressed. When the display showed “-0.0-”, the meter has been zeroed and is ready for measurement.

After zeroing the machine, one packet of Hanna Phosphate LR reagent was added into the cuvette. After shaking it well for a few minutes and after waiting 3 minutes for the reagent to settle, the “Read” button could be pressed. The instrument would display the absorbance results. The higher the concentration in nitrates is, the bluer the sample is going to turn out as shown on figure 24.

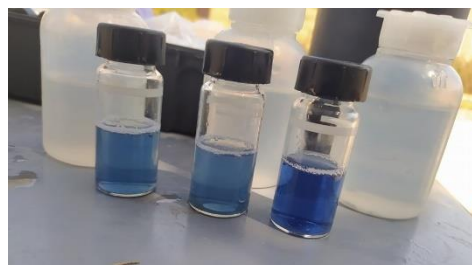


Figure 24: Cuvette with all the phosphate reagents becoming blue (concentration of 0,33, 0,35 and 0,85 PPM from left to right) and the checker.

To know the concentration thanks to the absorbance, a calibration curve was created. Several solutions with a known concentration of sodium phosphate were analyzed with the photometer to be able to create the curve and to determine the associated absorbance. Some NaCl was added to the solutions as well to replicate the water of the pond and get the best results possible. The amount of salt in the water of the pond in Marsaskala in June was 30 ppt, so 30 grams of NaCl were added in a liter of DI water.

Thanks to the calibration curve, a function linking the absorbance rate and the concentration levels in ppm was created. After getting this function, the concentration of phosphate could be determined with the absorbance.

8.4.5 FOR AN ACCURATE MEASUREMENT

To make sure the results were accurate and correctly measured and to limit the small mistakes that could have a big impact on them, I took the following precautions:

- The samples were free of debris;
- The cuvettes used to zero or read the results were dry and free of oil or fingerprints. If the cuvettes were dirty, they were rinsed with distilled water and wiped with a lint-free cloth;
- If they were some bubbles in the cuvettes, they were removed by gently tapping or swirling the cuvette;
- The sample mixed with the reagents was directly discarded after the analysis was completed to avoid getting permanent stains on the glass, as it could affect the results as well;
- Gloves were worn by anyone touching the cuvettes to avoid leaving fingerprints on the glass;
- The cuvettes, syringe and all the material used for the analysis were directly rinsed with DI water after use.

8.4.6 SAFETY

Some of the reagents used in the process of the analysis were not safe to use as it could cause some serious damage to the skin. Mask and gloves were provided to anyone who used the equipment and was doing the analysis as direct contact between those chemicals and the skin could be dangerous

8.4.7 ACCURACY OF THE INSTRUMENTS

The accuracy is the closeness of a measurement to the true value (Romain, 2020). Knowing the accuracy of the instruments allow to interpret the results better if, for instance, some results are erratic and could be credited to the lack of accuracy of an instrument.

For the Hanna Marine Nitrate Low Range Checker, the accuracy is ± 0.25 PPM and $\pm 2\%$ of the reading at 25 Celcius degrees according to the instruction manual and is ± 2.5 PPM and $\pm 5\%$ of the reading in case of using dilution⁹ (see table 9).

⁹ In this case, no dilution method was used to read the results as the concentration of nitrates in the sample never reached the maximum reading of 5PPM.

For the Hanna Aquaculture Photometer, the accuracy for the absorbance test that was used to measure the phosphate level is ± 0.003 Abs (at 1000 Abs). For the nitrite low range, the accuracy is $\pm 20 \mu\text{g/L}$ and $\pm 4\%$ of the reading at 25°C (see table 9).

In the Hanna Multiparameter Meter, all the sensors have different specifications and accuracy. The accuracy is $\pm 0,15$ Celcius degrees for the temperature. For the pH, the accuracy is ± 0.02 pH. When it comes to the DO, the accuracy is $\pm 1\%$ of the reading or $\pm 1.0\%$ whichever is greater for measurments between 0,0 and 300% and $\pm 3\%$ of the reading for measurements between 300% and 500%. The accuracy of the conductivity is $\pm 1\%$ of the reading or $1 \mu\text{S/cm}$ whichever is greater and for the salinity, the accuracy is $\pm 2\%$ of the reading or $\pm 1\text{PSU}$ whichever is greater (see table 9).

Table 9: Instruments used during the experimentation to determine the studied parameters and their accuracy.

Instrument	Accuracy
Hanna Marine Nitrates Low Range Checker	$\pm 0.25\text{PPM}$, $\pm 2\%$ of the reading at 25°C . $\pm 2.5 \text{ PPM}$, $\pm 5\%$ of the calculated reading using dilution
Hanna Aquaculture Photometer	<i>Phosphate</i> : ± 0.003 Abs (at 1000 Abs) <i>Nitrite</i> : $\pm 20 \mu\text{g/L}$ and $\pm 4\%$ of the reading at 25°C
Hanna Multiparameter Meter	<i>Temperature</i> : $\pm 0.15^\circ\text{C}$ <i>pH</i> : ± 0.02 pH <i>DO</i> : 0,0 to 300%: $\pm 1.5\%$ of the reading or $\pm 1\%$ whichever is greater 300% to 500%: $\pm 3\%$ of the reading <i>Conductivity</i> : $\pm 1\%$ of the reading or $1 \mu\text{S/cm}$ whichever is greater <i>Salinity</i> : $\pm 2\%$ of the reading or $\pm 1\text{PSU}$ whichever is greater.

9. RESULTS

From the 1 of July 2021 to the 15 of July 2021, a daily based sampling was undertaken in the tanks installed in the Il-Maghluq ta'Marsaskala. One sample was taken from each tank and all the parameters studied were analyzed on-site. Since the experimentation was conducted during the summer, the water in the tank evaporated and the concentrations had to be recalculated to get them without the water volume variations. The following formula was used to calculate the normalized volume:

$$C1.V1 = C2.V2$$

Where:

- C1 represents the concentration of the initial solution,
- V1 represents the volume of the initial solution,
- C2 represents the concentration of the final solution,
- V2 represents the volume of the final solution.

The results obtained for each tank during the experimentation (after recalculating the concentration to normalize the volume of water) can be seen on the tables hereunder. The r1, r2 and r2 correspond to the replicates of the measurements that were taken. The results I got on-site without taking account of the water volume variations can be seen in Annex I, as well as the tables for the extra parameters taken (pH, temperature, conductivity, DO salinity and liters of water in the tank) for the recalculated normalized volumes.

The results presented in this section were mostly obtained with the software Excel and XLStat and analyzed later thanks to the Analysis of Environmental Data course given by Claudia Falzone in the Faculty of Environmental Science at the University of Liège (Belgium). All the tests performed were done on the recalculated values, using the tables 10 to 15.

Table 10: Overall results obtained during the 13-day-sampling for the Control 1 tank (recalculated concentrations after evaporation of the water).

Day	NO_3^- (mg/L)				NO_2^- (μ g/L)				PO_4^{3-} (mg/L)			
	r1	r2	r3	Average	r1	r2	r3	Average	r1	r2	r3	Average
0	2.33	2.34	2.34	2.34	42.00	40.00	43.00	41.67	0.40	0.43	0.45	0.43
1	2.24	2.22	2.22	2.23	42.84	42.84	40.85	42.18	0.40	0.40	0.39	0.40
2	2.09	2.11	2.12	2.11	41.69	40.70	38.72	40.37	0.39	0.39	0.40	0.39
3	2.03	2.03	2.04	2.03	49.45	47.48	46.49	47.81	0.39	0.39	0.39	0.39
4	1.94	1.93	1.94	1.93	54.10	57.05	55.08	55.41	0.33	0.33	0.35	0.34
5	1.74	1.74	1.76	1.74	68.35	71.27	72.25	70.62	0.36	0.35	0.37	0.36
6	1.61	1.59	1.61	1.60	72.95	71.98	71.98	72.31	0.31	0.29	0.32	0.31
7	1.38	1.40	1.41	1.40	79.32	80.28	80.28	79.96	0.29	0.29	0.29	0.29
8	1.19	1.19	1.20	1.19	86.24	86.24	87.19	86.56	0.30	0.30	0.31	0.30
9	1.15	1.14	1.14	1.14	93.55	94.50	92.59	93.55	0.32	0.32	0.34	0.33
10	0.86	0.86	0.86	0.86	99.85	100.80	103.65	101.43	0.32	0.33	0.35	0.34
11	0.75	0.76	0.74	0.75	110.09	111.99	113.89	111.99	0.38	0.37	0.37	0.37
12	0.68	0.69	0.68	0.68	112.51	113.45	113.45	113.14	0.37	0.36	0.36	0.36

Table 11: Overall results obtained during the 13-day-sampling for the Control 2 tank (recalculated concentrations after evaporation of the water).

Day	NO_3^- (mg/L)				NO_2^- (μ g/L)				PO_4^{3-} (mg/L)			
	r1	r2	r3	Average	r1	r2	r3	Average	r1	r2	r3	Average
0	2.42	2.43	2.45	2.43	50.00	53.00	52.00	51.67	0.38	0.38	0.38	0.38
1	2.39	2.41	2.40	2.40	51.72	50.72	50.72	51.05	0.39	0.38	0.38	0.38
2	2.37	2.37	2.37	2.37	48.55	47.56	49.55	48.55	0.37	0.38	0.36	0.37
3	2.36	2.35	2.36	2.36	46.49	44.51	44.51	45.17	0.36	0.37	0.36	0.36
4	2.25	2.28	2.27	2.27	45.00	44.02	45.00	44.67	0.36	0.34	0.35	0.35
5	2.26	2.27	2.31	2.28	42.80	45.72	48.64	45.72	0.39	0.41	0.39	0.40
6	1.84	1.86	1.83	1.84	53.30	55.24	54.27	54.27	0.42	0.44	0.44	0.43
7	1.27	1.27	1.27	1.27	60.71	60.71	62.64	61.35	0.41	0.42	0.44	0.43
8	1.32	1.32	1.34	1.33	74.74	74.74	76.65	75.38	0.38	0.40	0.40	0.40
9	1.36	1.36	1.34	1.36	85.75	88.60	88.60	87.65	0.38	0.38	0.38	0.38
10	1.35	1.33	1.36	1.35	107.45	109.35	109.35	108.72	0.38	0.37	0.37	0.37
11	1.32	1.31	1.32	1.32	135.20	132.36	137.09	134.88	0.35	0.35	0.34	0.35
12	1.30	1.28	1.30	1.29	136.56	134.68	134.68	135.31	0.33	0.34	0.34	0.34

Table 12: Overall results obtained during the 13-day-sampling for the Ulva 1 tank (recalculated concentrations after evaporation of the water).

Day	NO_3^- (mg/L)				NO_2^- (μ g/L)				PO_4^{3-} (mg/L)			
	r1	r2	r3	Average	r1	r2	r3	Average	r1	r2	r3	Average
0	2.27	2.26	2.27	2.27	50.00	45.00	45.00	46.67	0.50	0.54	0.54	0.53
1	2.09	2.08	2.11	2.10	39.85	41.85	41.85	41.18	0.53	0.54	0.53	0.53
2	1.98	1.97	1.99	1.98	38.72	37.72	37.72	38.05	0.51	0.50	0.51	0.50
3	1.80	1.83	1.79	1.81	35.48	35.48	36.46	35.80	0.37	0.37	0.38	0.38
4	1.84	1.87	1.85	1.85	33.38	35.35	37.31	35.35	0.34	0.34	0.34	0.34
5	1.46	1.49	1.49	1.48	48.55	51.46	52.43	50.81	0.34	0.36	0.36	0.35
6	0.70	0.70	0.70	0.70	62.99	64.93	67.84	65.25	0.37	0.39	0.39	0.38
7	0.58	0.61	0.60	0.59	75.16	76.13	75.16	75.48	0.41	0.40	0.40	0.41
8	0.55	0.55	0.55	0.55	67.20	67.20	67.20	67.20	0.41	0.42	0.41	0.42
9	0.33	0.36	0.35	0.35	50.59	49.64	47.73	49.32	0.41	0.41	0.41	0.41
10	0.26	0.26	0.24	0.25	12.34	11.39	9.49	11.07	0.40	0.40	0.40	0.40
11	0.12	0.12	0.12	0.12	0.00	1.89	1.89	1.26	0.38	0.39	0.39	0.38
12	0.09	0.08	0.08	0.09	0.00	0.00	0.94	0.31	0.36	0.35	0.35	0.35

Table 13: Overall results obtained during the 13-day-sampling for the Ulva 2 tank (recalculated concentrations after evaporation of the water).

Day	NO_3^- (mg/L)				NO_2^- (μ g/L)				PO_4^{3-} (mg/L)			
	r1	r2	r3	Average	r1	r2	r3	Average	r1	r2	r3	Average
0	1.80	1.80	1.80	1.80	78.00	78.00	78.00	78.00	0.41	0.42	0.43	0.42
1	1.76	1.76	1.76	1.76	75.72	75.72	75.72	75.72	0.42	0.43	0.40	0.42
2	1.68	1.69	1.69	1.69	67.38	67.38	67.38	67.38	0.40	0.39	0.39	0.39
3	1.55	1.56	1.56	1.56	66.89	65.90	64.92	65.90	0.34	0.35	0.34	0.35
4	1.37	1.39	1.39	1.39	62.84	63.82	62.84	63.16	0.31	0.32	0.32	0.32
5	1.31	1.31	1.31	1.31	62.14	71.85	62.14	65.37	0.29	0.29	0.30	0.29
6	0.82	0.83	0.83	0.83	64.69	65.65	67.58	65.97	0.36	0.36	0.37	0.36
7	0.59	0.58	0.58	0.58	74.20	75.16	76.13	75.16	0.43	0.43	0.45	0.44
8	0.49	0.47	0.47	0.47	82.09	83.05	84.95	83.36	0.60	0.60	0.62	0.61
9	0.44	0.44	0.44	0.44	76.07	77.97	77.97	77.34	0.57	0.58	0.58	0.58
10	0.43	0.42	0.42	0.42	71.05	70.10	69.15	70.10	0.54	0.54	0.53	0.54
11	0.38	0.38	0.38	0.38	60.28	63.10	62.16	61.85	0.47	0.48	0.48	0.48
12	0.35	0.35	0.35	0.35	58.17	59.11	59.11	58.79	0.44	0.43	0.42	0.43

Table 14: Overall results obtained during the 13-day-sampling for the Cladophora 1 tank (recalculated concentrations after evaporation of the water).

Day	NO_3^- (mg/L)				NO_2^- (μ g/L)				PO_4^{3-} (mg/L)			
	r1	r2	r3	Average	r1	r2	r3	Average	r1	r2	r3	Average
0	2.14	2.14	2.14	2.14	49.00	49.00	50.00	49.33	0.45	0.49	0.46	0.47
1	1.94	1.95	1.95	1.94	47.91	48.91	48.91	48.58	0.45	0.45	0.44	0.45
2	1.71	1.71	1.71	1.71	44.75	44.75	44.75	44.75	0.44	0.46	0.43	0.44
3	1.48	1.51	1.51	1.50	42.53	42.53	39.56	41.54	0.40	0.41	0.40	0.40
4	1.34	1.32	1.32	1.33	40.40	41.39	39.42	40.40	0.41	0.41	0.39	0.41
5	0.83	0.83	0.83	0.83	34.11	34.11	36.06	34.76	0.39	0.39	0.38	0.39
6	0.57	0.57	0.57	0.57	38.84	36.89	33.98	36.57	0.39	0.36	0.36	0.37
7	0.36	0.36	0.36	0.36	31.92	32.89	31.92	32.24	0.36	0.35	0.34	0.35
8	0.27	0.28	0.28	0.28	25.92	26.88	26.88	26.56	0.34	0.35	0.34	0.34
9	0.23	0.24	0.24	0.24	22.00	22.00	22.95	22.32	0.33	0.35	0.35	0.35
10	0.15	0.15	0.15	0.15	19.09	20.05	21.95	20.36	0.32	0.32	0.33	0.32
11	0.11	0.11	0.11	0.11	16.20	16.20	17.15	16.51	0.30	0.30	0.32	0.31
12	0.08	0.07	0.07	0.07	14.21	15.16	15.16	14.84	0.28	0.27	0.27	0.27

Table 15: Overall results obtained during the 13-day-sampling for the Cladophora 2 tank (recalculated concentrations after evaporation of the water).

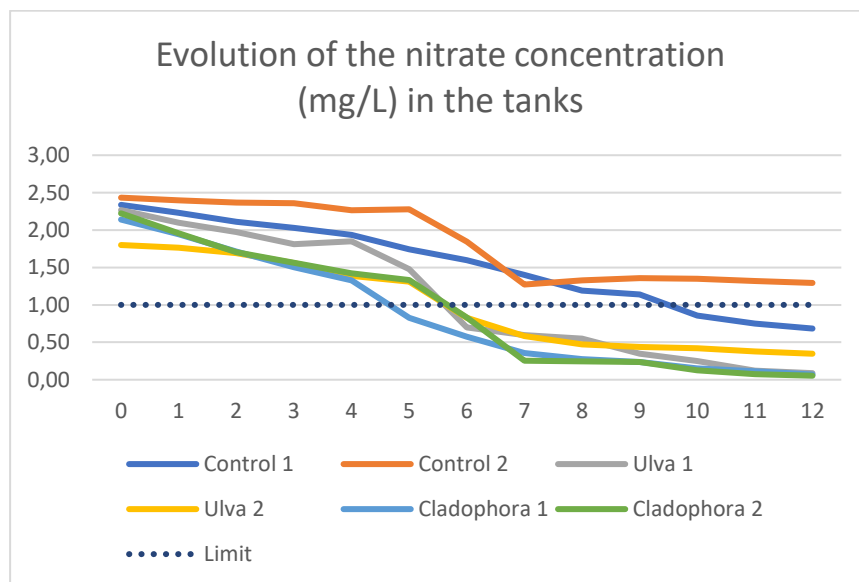
Day	NO_3^- (mg/L)				NO_2^- (μ g/L)				PO_4^{3-} (mg/L)			
	r1	r2	r3	Average	r1	r2	r3	Average	r1	r2	r3	Average
0	2.24	2.23	2.20	2.22	64.00	67.00	66.00	65.67	0.41	0.43	0.39	0.41
1	1.98	1.95	1.93	1.96	64.76	63.77	62.77	63.77	0.40	0.41	0.40	0.40
2	1.69	1.70	1.72	1.70	59.67	58.68	58.68	59.01	0.39	0.39	0.38	0.38
3	1.57	1.57	1.56	1.56	54.50	54.50	54.50	54.50	0.39	0.38	0.38	0.38
4	1.41	1.43	1.43	1.42	52.23	50.26	49.27	50.59	0.36	0.36	0.36	0.36
5	1.34	1.32	1.34	1.33	48.55	48.55	48.55	48.55	0.34	0.34	0.34	0.34
6	0.84	0.82	0.82	0.83	44.58	41.67	41.67	42.64	0.33	0.31	0.32	0.32
7	0.26	0.24	0.26	0.25	38.62	37.65	36.69	37.65	0.29	0.30	0.29	0.29
8	0.23	0.25	0.25	0.24	32.70	31.74	31.74	32.06	0.30	0.30	0.30	0.30
9	0.24	0.24	0.24	0.24	21.95	22.91	23.86	22.91	0.28	0.28	0.32	0.29
10	0.12	0.13	0.12	0.13	16.17	16.17	12.36	14.90	0.29	0.29	0.29	0.29
11	0.08	0.08	0.07	0.07	11.39	11.39	11.39	11.39	0.28	0.28	0.26	0.27
12	0.05	0.06	0.06	0.05	9.45	8.51	8.51	8.82	0.26	0.26	0.25	0.25

The previous tables helped to determine the percentage of the pollutant that was removed from the water after the 13 days as shown on the table 16. The worst result for each parameter was highlighted in red while the best result was highlighted in green and the second-best result was highlighted in yellow.

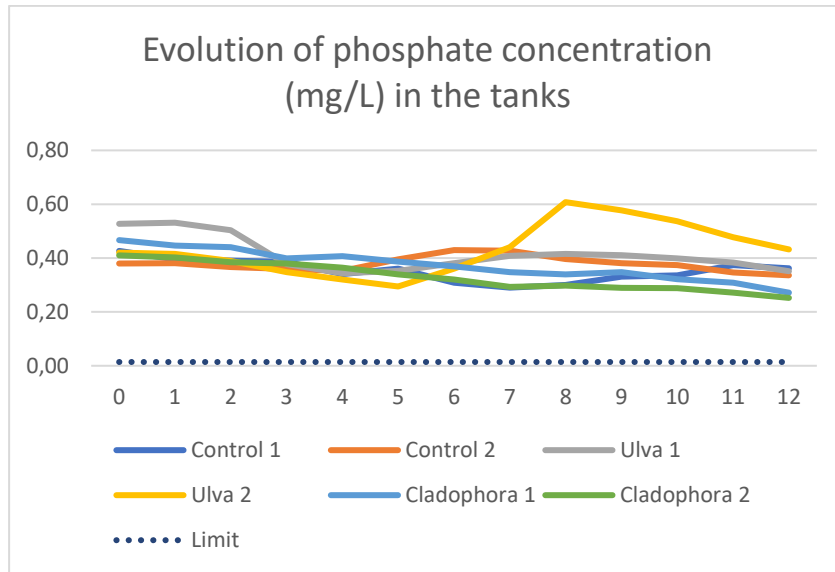
Table 16: Percentage of each parameter removed after the 13 days of experimentation for each tank.

	Nitrates	Nitrites	Phosphates
Control 1	-70.73%	171.53%	-15.06%
Control 2	-46.85%	161.89%	-11.60%
Ulva 1	-96.13%	-99.33%	-33.37%
Ulva 2	-80.72%	-24.62%	2.79%
Cladophora 1	-96.75%	-69.92%	-41.81%
Cladophora 2	-97.59%	-86.56%	-38.51%

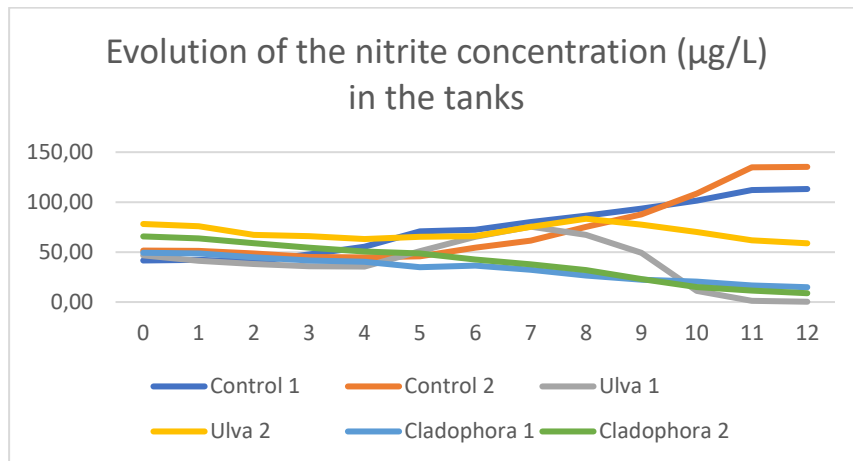
The graphs 4, 5 and 6 hereunder represent the evolution of the concentration of the 3 main parameters (nitrates, nitrites and phosphates) for all the tanks. To be more visual, the average of the 3 replicate samples taken every day was used to create this evolution graph. The recommendation limit was added on the graphs for the nitrate concentration and the phosphate concentration.



Graph 4: Evolution of the nitrate concentration (mg/L) in each tank compared to the advised limit.



Graph 5: Evolution of the phosphate concentration (mg/L) in each tank compared to the advised limit.



Graph 6: Evolution of the nitrite concentration (mg/L) in the tanks.

To determine the dispersion of the dataset around the mean and the way the data is spread out, the standard deviation was calculated with the 6 replicate samplings for the same parameter and the same type of tank (see table 17).

Table 17: Standard deviation of the dataset for the 3 main parameters and for the 3 types of tanks.

Day	NITRATES			NITRITES			PHOSPHATES		
	Control	Ulva	Cladophora	Control	Ulva	Cladophora	Control	Ulva	Cladophora
0	0.05	0.26	0.05	5.65	25.40	9.01	0.03	0.06	0.04
1	0.09	0.18	0.02	4.93	22.34	8.35	0.01	0.06	0.02
2	0.14	0.16	0.01	4.63	20.63	7.82	0.01	0.06	0.03
3	0.18	0.14	0.03	1.88	19.42	7.18	0.01	0.02	0.01
4	0.18	0.25	0.05	5.97	19.22	5.69	0.01	0.01	0.02
5	0.29	0.09	0.28	13.83	27.70	7.58	0.02	0.03	0.03
6	0.14	0.07	0.14	9.90	35.58	3.82	0.07	0.01	0.03
7	0.07	0.01	0.06	10.22	41.11	3.05	0.08	0.02	0.03
8	0.08	0.04	0.02	6.17	36.47	3.05	0.05	0.11	0.02
9	0.12	0.05	0.00	3.45	26.71	0.77	0.03	0.09	0.04
10	0.27	0.09	0.01	4.24	5.84	3.43	0.02	0.08	0.02
11	0.31	0.14	0.02	12.69	0.81	2.83	0.02	0.05	0.02
12	0.33	0.14	0.01	12.17	0.35	3.33	0.02	0.04	0.01

Before being able to undergo the statistic tests, the type of data distribution had to be determined for each day, each tank and each of the three main parameters. To define the distribution of the data for one type of tank, the 6 replicate samplings for the desired parameter were used. The distribution was nonnormal except for the following:

- **Control tanks:** nitrate concentration for day 11 and the phosphate concentration for day 1, day 11 and day 12;
- **Ulva tanks:** phosphate concentration for day 2 and nitrate concentration for day 11;
- **Cladophora tanks:** nitrates concentration for day 4 and day 12.

The boxplot graphs for the different type of tanks and for each main parameter can be found in annexe II.

Since the distribution was nonnormal, the Mann-Withney test was run to see if the tanks with the same characteristics could be merged together. For the days and parameters concerned by a normal distribution, a t-test was used. For both of the tests, the alpha value was 0.05 and the following hypotheses were used:

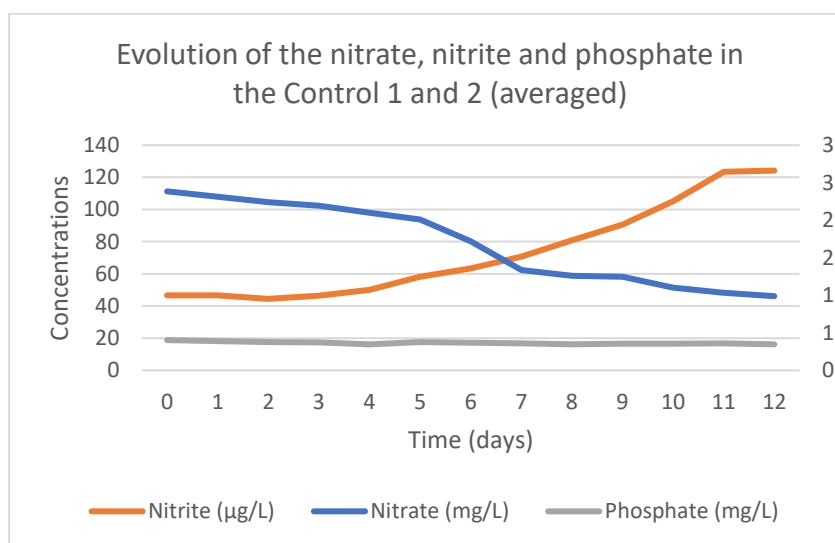
- H_0 : the difference between the means is equal to 0,
- H_a : the difference between the means is different from 0.

The results, which can be found in table 18, in bold are those with a p-value under 0.05 and when a t-test was used, the results were highlighted in yellow in the following table.

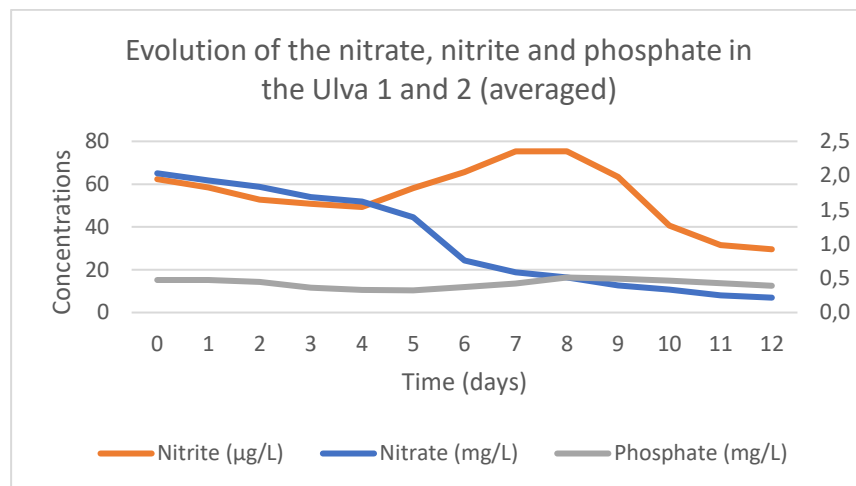
Table 18: Results of the Mann-Whitney test and t-test for each day, each parameter and each tank.

Day	Control			Ulva			Cladophora		
	Nitrate	Nitrite	Phosphates	Nitrate	Nitrite	Phosphates	Nitrate	Nitrite	Phosphates
0	0.077	0.100	0.064	0.059	0.077	0.077	0.064	0.077	0.100
1	0.077	0.072	0.041	0.064	0.077	0.077	0.600	0.077	0.072
2	0.064	0.100	0.077	0.077	0.072	0.000	0.700	0.059	0.077
3	0.072	0.200	0.059	0.077	0.072	0.072	0.072	0.059	0.072
4	0.077	0.077	0.300	0.077	0.077	0.059	0.001	0.100	0.059
5	0.077	0.100	0.077	0.059	0.077	0.072	0.059	0.059	0.059
6	0.077	0.077	0.077	0.059	0.077	0.072	0.059	0.077	0.077
7	0.064	0.072	0.064	0.400	0.072	0.072	0.059	0.077	0.077
8	0.072	0.072	0.072	0.059	0.059	0.072	0.072	0.072	0.059
9	0.072	0.077	0.059	0.064	0.077	0.059	0.400	1.000	0.072
10	0.064	0.077	0.077	0.072	0.077	0.059	0.059	0.077	0.072
11	1.000	0.100	0.004	0.000	0.600	0.072	0.059	0.059	0.100
12	0.072	0.072	0.004	0.059	0.600	0.077	0.072	0.072	0.072

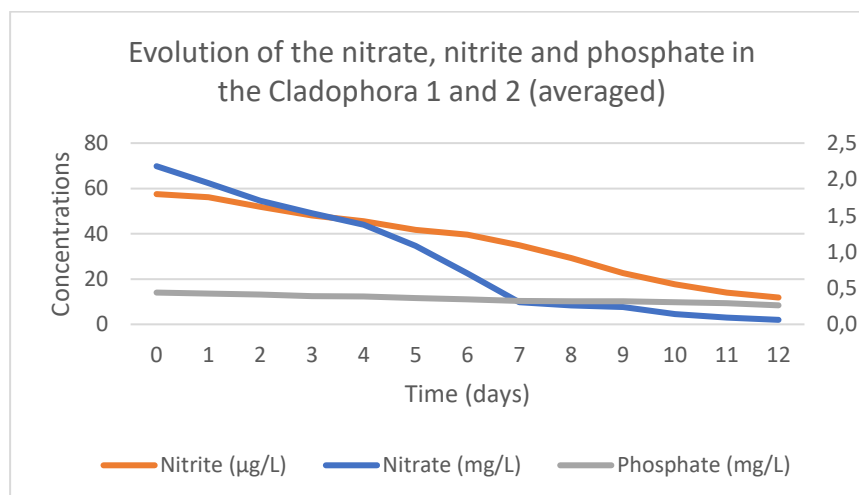
To get a better representation of how the concentrations varied through time for the different type of tanks, I chose to average the concentrations of the tanks with similar characteristics (see graphs 7, 8 and 9).



Graph 7: Evolution of the nitrate, nitrite and phosphate in the control tanks 1 and 2.



Graph 8: Evolution of the nitrate, nitrite and phosphate in the Ulva tanks 1 and 2.



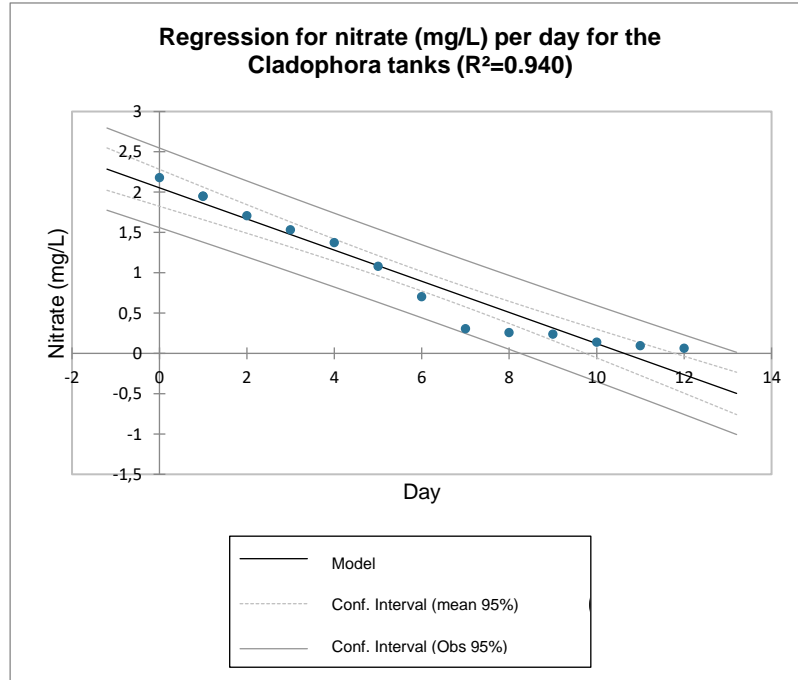
Graph 9: Evolution of the nitrate, nitrite and phosphate in the Cladophora tanks 1 and 2.

The Spearman correlation test was performed to determine if there was a correlation or not between the different types of tanks and between the control tanks and the algae tanks. The results are shown in table 19 hereunder.

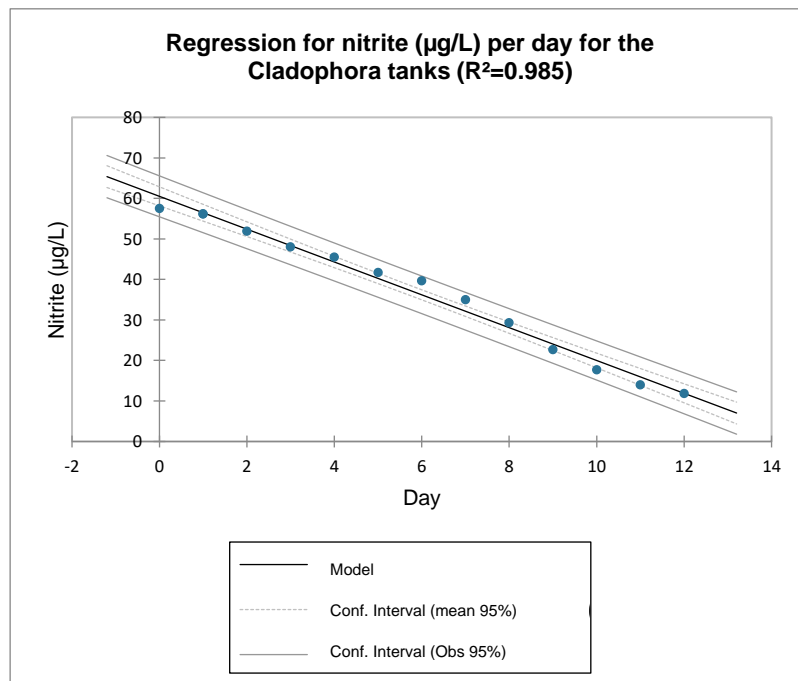
Table 19: Results of the Spearman correlation test for different tanks and parameters.

Tanks and parameters tested	Correlation	Significant ?
<i>CONTROL 1 & 2</i>		
Nitrates	0.895	Yes
Nitrites	0.840	Yes
Phosphates	-0.296	Yes
<i>ULVA 1 & 2</i>		
Nitrates	0.994	Yes
Nitrites	0.631	Yes
Phosphates	0.439	Yes
<i>CLADOPHORA 1 & 2</i>		
Nitrates	1	Yes
Nitrites	0.994	Yes
Phosphates	0.978	Yes
<i>ULVA & CONTROL</i>		
Nitrates	1	Yes
Nitrites	-0.219	Yes
Phosphates	0.038	Yes
<i>CLADOPHORA & CONTROL</i>		
Nitrates	1	Yes
Nitrites	-0.950	Yes
Phosphates	0.697	Yes
<i>ULVA & CLADOPHORA</i>		
Nitrates	1	Yes
Nitrites	0.291	Yes
Phosphates	-0.038	Yes
<i>ULVA 1 & 2 for the 7 first days</i>		
Nitrates	0.964	Yes
Nitrites	0.32	Yes
Phosphates	0.928	Yes

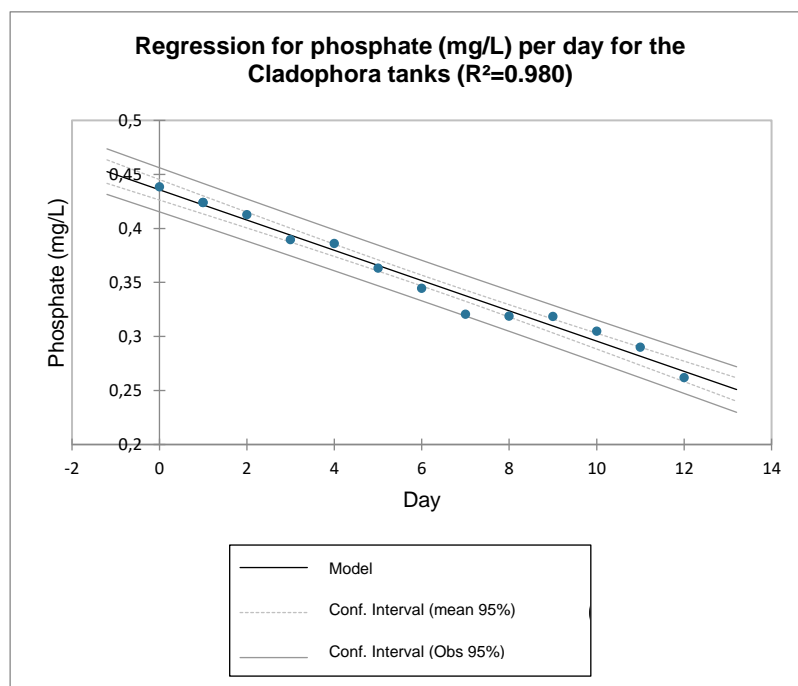
Thanks to the previous results, it was determined that the tanks with the same characteristics could be merged together. A regression curve and an equation of the model were created for the Cladophora tanks for each main parameter, as shown in the graphs 10, 11 and 12 and table 21 hereunder.



Graph 10: Regression for nitrate (mg/L) per day for the Cladophora tanks.



Graph 11: Regression for nitrite ($\mu\text{g/L}$) per day for the Cladophora tanks.



Graph 12: Regression for phosphate (mg/L) per day for the Cladophora tanks.

Parameter	Equation of the model
Nitrate (mg/L)	$=2.0535687978688 - 0.193207775557776 \times \text{Day}$
Nitrite ($\mu\text{g/L}$)	$=60.5114918414918 - 4.05174825174825 \times \text{Day}$
Phosphate (mg/L)	$=0.435761205461206 - 1.40065101565102\text{E-}02 \times \text{Day}$

Table 20: Equations of the models for each parameters for the Cladophora tanks.

10. DISCUSSION

10.1. OVERVIEW OF THE RESULTS

The main objectives of this in-situ experimentation were to quantify the changes in phosphate and nitrate in the tanks and to determine if one of the algae was more efficient than the other. Those two goals resulted in the formulation of the following question:

Is it possible to use the algae *Ulva lactuca* and *Cladophora sp.* in a bioreactor system to remove excess nitrate and phosphate from the water in the tanks?

After figuring out the objectives, the methodology was then created to answer this question, to possibly fulfil the objectives, to respect all the restrictions linked to the Natura 2000 status of the Il-Maghluq ta' Marsaskala site and the budget and time restriction. As mentioned before, the experimentation aims to improve the water quality of the pond in Marsaskala since it is directly polluted by the runoff coming from the fields (mostly) and the streets next to the study area. This research is part of a phytoepuration project with 3 different water purifying systems and it typically falls under the MO 2 and 3.

The experimentation was conducted during the month of July 2021 and the analysis of the parameters was done on-site. Those were considered to present a holistic outline of the evolution of the water quality within the tanks with the algae. The subsequent analysis and discussion will be done in light of the policies implemented in Malta to protect the different water bodies.

On the different evolution graphs (see graphs 4, 5 and 6), it is quite easy to see that the levels of nitrates, nitrites and phosphates on day 0 are not the same for all the tanks. It can be explained by the sediments contained in the water of the pond. Those sediments can release nitrates, nitrites and phosphates in the water and can « *sustain eutrophication for decades* » (Ma, *et al.*, 2021). Moreover, the dredging of the sediments in the pond started in March but had to be stopped not to harm the population of killifish. The water that was used in the tanks was coming from the pond and therefore, even if it was taken at the same place, it is possible to have different amount of sediments (especially since the water was moved for the dredging).

On these graphs, the levels of nitrates, nitrites and phosphates for the two control tanks are fluctuating. Usually and in a laboratory environment, the concentration of the control should not have varied that much. This can be explained by the microorganisms living in the pond water. Even though the water was filtered to remove any big particules, there were still living organisms in the water that could have influenced the results. After a few days into the experimentation and noticing the changes, I saw a weird looking « moss » growing in the tank. Not knowing what it was, I asked Matthew Calleja to help me analyze it with a microscope but after a few days, the moss disappeared. M. Calleja still proceeded to sample the water and check it with the microscope. He identified some flagellates and diatoms that can be seen on the figures 25 and 26 hereunder.



Figure 25: Picture of a diatom and flagellates found in the water of the tanks.



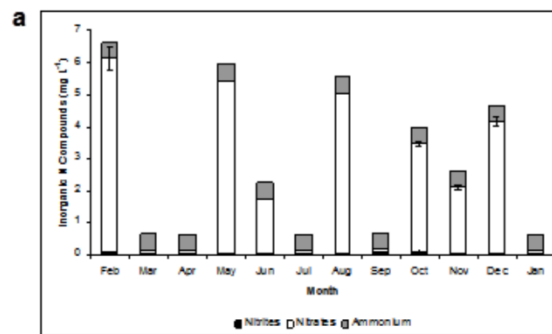
Figure 26: Picture of one of the flagellates found in the water of the tanks.

The diatoms are unicellular algae that are capable of doing photosynthesis. In certain cases, the flagellates can photosynthesize as well. Having those two organisms in the control tanks could have had a small influence on the results.

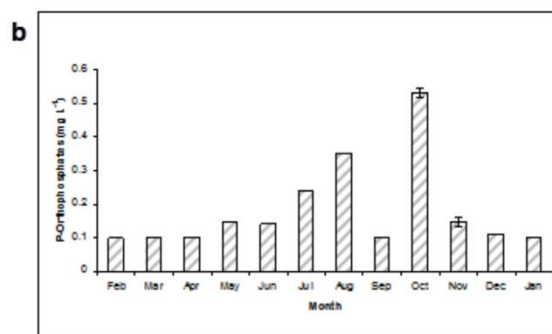
Moreover, air stones were placed in the tanks to mimic the currents. Those small stones were releasing tiny air bubbles into the tanks which might have had an effect on the purification of the water. Indeed, air bubbles are used in the wastewater treatment plant as a second treatment after decantation (AquaWal, s.d.). The oxygen freed in the tanks stimulates bacteria in the water to purify it from the pollutants. This could have affected the results as well and explain why the levels were still decreasing a bit.

Compared to a previous study about the water quality of transitional waters in Malta, the levels of concentration obtained on day 0 were expected (AIS environmental, 2014). The water from the pond faces seasonal changes according to AIS environmental and D. Bartolo as you can see on graphs 13 and 14. The levels of concentration are usually lower in June and July since there is less rain or no rain and therefore less runoff with contaminants to reach the pond.

Maghluq ta' Marsascale



Graph 13: Levels of nitrites, nitrates and ammonium concentrations for each month in 2014 (AIS environmental, 2014).



Graph 14: Levels of phosphates concentration for each month in 2014 (AIS environmental, 2014).

Still according to graphs 4, 5 and 6, the concentrations of the 3 main parameters globally decreased during the process. As it can be seen on table 16, between 40 and 97% of the nitrate were eliminated from the water depending on the type of tank. On graphs 4 and 5, the nitrate concentration limit was reached on day 5 by Cladophora 1, on day 6 by Cladophora 2, Ulva 1 and 2 and on day 10 by Control 1. The levels kept going down and by the end of the experimentation, the lowest levels were attained by the Cladophora tanks.

When it comes to the phosphate level, the recommended limit was reached by none of the tanks. The concentration in Ulva 2 tank actually increased by a bit more than 2% according to table 20. In the other tanks, between 11 and 41% of the phosphate were eliminated during the experimentation. The Cladophora tanks were the ones with the lowest level of phosphate by the end of the experimentation since the concentration dropped by approximately 40% for both tanks, still according to table 16.

Thanks to the color code of this table, it is easy to notice that the best and second best results can mostly be seen in the Cladophora tanks section. On the contrary, the worst results that were obtained could be found in the Control tank section of the table 16.

In graph 8, there is a peak of the phosphate concentration on day 7 to 9. This tallies with the dead algae found in the tanks Ulva 1 and Ulva 2 on day 9. Indeed, a part of the *Ulva lactuca* was completely discolored and sinking at the bottom of the tank. The dead algae were removed before continuing the experimentation. The dead algae could not have an effect on the purification of the water for obvious reason and could have increased the amount of phosphates present in the water by decomposing (OBVAJ, s.d.). The Ulva

algae were coming from another site with different environmental conditions which might have caused their death. Furthermore, during the experimentation, Malta experienced a heatwave with temperatures reaching up to 40 celcius degrees. That might have displeased the *Ulva lactuca* and resulted in its death.

Moreover, I personally think that I might have had a problem with the readings for the phosphate levels that could explain why the levels were not decreasing as it was expected. While doing the calibration curve to be able to link the absorbance with the phosphate concentrations, a solution with sodium phosphate was created. To mimic the water of the pond and since the sea salt can be a source of interference, NaCl was added in the water to reach the same NaCl concentration that was contained in the tanks. I did not think about the salt concentration changes caused by the evaporation of the water. This probably influenced the results and caused greater interferences as the concentration of NaCl was going up during the experimentation. I calculated the calibration curves with different NaCl concentrations, but I could not determine them all for all the salinity I obtained as I would have needed too much DI water and NaCl and I could not get more.

On graph 6, the concentration of nitrite is decreasing for both algae: between 25 and 99% of the nitrites were removed from the water (see table 16). In the *Ulva* tank, there is a peak that coincides with the dead algae found in the tank on day 9. That rise might be explained by the decomposition of that seaweed which released nitrites (OBVAJ, s.d.).

When it comes to the control, the concentration almost tripled. The levels were decreasing a bit until day 6 when the concentration started to go up. In the nitrogen cycle in the water, the ammonium and ammonia are transformed into nitrite and then eventually into nitrates thanks to bacteria. When too few bacteria are present in the water, it can result in a nitrite peak. No analysis for bacteria was conducted, so it is quite complicated to assume that it was the reason why the peak appeared.

The results obtained are quite different from the other studies about *Ulva lactuca* and *Cladophora sp.* That can be justified by the fact that most of the studies were done in laboratory conditions (see "Previous studies with similar macroalgae), which is quite different from the onsite conditions.

The standard deviation (see table 17) is quite low for all the parameters in general, especially the nitrates and the phosphates and all the parameters for the *Cladophora* algae. This means that the values from the dataset are close to the mean and are not too spread out. The cells that were highlighted in yellow in table 17 are the ones with a relatively high SD, meaning that the data is less concentrated around the mean. The SD was calculated with only 6 values: this might be the reason why it is high for some parameters and tanks on certain days.

The type of distribution was calculated and the results can be seen on table 18. For most of the days, the parameters and the type of tanks, the distribution was nonnormal. I decided to run a Mann-Whithney test to determine if the tanks with the same characteristics could be merged together. I considered running a t-test for the data with a normal distribution but since there is not a lot of data, I decided to apply a Mann-Withney test everywhere. Indeed, 6 values are not enough to establish that the distribution is normal. The p-values calculated are higher than the alpha significance level ($\alpha=0,05$) except for 6 of them which means that the null hypothesis H_0 (the difference between the

mean is equal to 0) cannot be rejected and that the differences between tanks with similar characteristics are not significant enough. Those tanks can be merged together.

The data from Control 1 and 2, Ulva 1 and 2 and Cladophora 1 and 2 have been averaged to create graphs 7, 8 and 9. It helps to visualize better what happened in the tanks during the experimentation. On all the graphs, the phosphates levels are not really evolving unlike the other parameters. The nitrate levels are diminishing in all the tanks. The peak of phosphates in the Ulva tanks and the peak of nitrites in the Control tanks are more obvious and discernible.

Since the distribution was nonnormal or considered as nonnormal, a Spearman correlation test was conducted for all the tanks and parameters (see table 19). The tests with a high correlation or high anticorrelation were highlighted in yellow. The closer the Spearman coefficient is to 1, the stronger the association between the tanks is.

The correlation is always very strong for the nitrates, as well as for all the parameters for the Cladophora 1 & 2. When the correlation is high, especially for all the parameters, it means that the experimentation is reproducible and the results are not too erratic. Moreover, the p-value were under 0,05 for all the tests which means that the results are significant.

Since some Ulva algae died during the process and influenced the results, I decided to run another test for the first 6 days of the experimentation. Indeed, at that moment there is a peak of nitrites and phosphates in the tanks (see graph 5). The results can be seen at the bottom of Table 23. The correlation for the nitrates and phosphates is quite high.

As mentioned before, all the high correlations have a p-value under the alpha significance level ($\alpha=0,05$) which means that there is less than 5% chance that the strength of the correlation happened by chance.

I decided to conduct a simple linear regression only on a part of the dataset as shown in the graphs 10, 11 and 12 and table 21. Indeed, the Cladophora seemed to be the best algae and therefore it is important to have an equation for the model to get an idea of how the concentrations of pollutants are going to evolve if the experimentation lasted for a few more days. The reasons why I did not perform the same statistics on the Ulva tanks are that this alga did not show good results and because a part of the algae died. This would have definitely influenced the equation of the model and therefore the real trends would have been misrepresented.

The R^2 (coefficient of determination) gives an idea of the percentage of the variability of nitrates, nitrites or phosphates which is explained by the variable "day". The closer to 1 the coefficient of determination is, the better the fit. In this case, 94% of the phosphate concentration evolution, 98.5% of the nitrite concentration evolution and 98% of the phosphate concentration evolution are explained by the variable "day". The dataset variances were tested statistically to determine if the variable "day" present significant information or not. According to the results of the test for all the main parameters, there is less than 0.01% chance of being wrong when assuming that the null hypothesis is wrong (no effect of the explanatory variable).

Given all that information, the *Cladophora sp.* seems to be the best algae to use after implementing this experimentation. Effectively, on the different graphs 4, 5, 6 and 9, the levels of concentration for all the parameters are dropping and have the lowest values (see graph 9). The data is not spread out and quite concentrated around the mean as table 17 shows. Moreover, the Spearman coefficient obtained showed a good correlation between the two *Cladophora* tanks. The experimentation is reproducible and significantly efficient for this alga. To predict the evolution of the concentrations of nitrate, nitrite and phosphate, the equation for the model of the linear regression can be used. According to those equations, the recommendation of 1mg/L of nitrate should be reached after 5,45 day and the recommendation of 0,014 should be reached after 30 days only.

Finally, it is important to keep in mind that the amount of data is quite low since the experimentation was ran only once. Moreover, there might be errors and uncertainty that could explain (partly or fully) some erratic data such as the peak in phosphate for the *Ulva*. Since a photometer and a probe were used during the experimentation, there might have been reading uncertainty due to the equipment. The water was not homogeneous from the beginning; the samples taken might not have been uniform as well and it might have created uncertainty. It is also a possibility that the methodology in itself created some uncertainty since the whole process was invented specifically for the experimentation. The environment itself, since the experimentation took place directly in the reserve, might have resulted in getting uncertainty as well because of the weather, the conditions of exposure etc. Some errors have probably been made during the 13 days. Indeed, the operator was unexperienced in laboratory procedures and the results (especially with the Hanna multiparameter probe) might have probably been read wrong.

10.2. FUTURE PERSPECTIVES

The methodology was created thanks to other methodologies used before in laboratories or on the field. It was also limited by the budget of the NGO that was used for this project and the CW project. In April, J. Gabarretta and the students conducting projects had to decide on which equipment would be the best for the analysis. After a month, the Hanna equipment was ordered but arrived a bit more than a month later than expected. That is the reason why the experimentation took place in July.

If I had had enough time, I would have done the experimentation twice in a row. I would have also done the same experimentation but with the best alga, with and without the air stones. Indeed, they need electricity to function, which costs money for the NGO. It could have been great to avoid this extra cost if the project was implemented on a larger scale. Different types of algae can be found in the pond depending on the season. Testing those other algae could be a great idea as well. Since I am staying in Malta for a bit longer, I offered J. Gabarretta to conduct the study linked to the air stones as it was planned before. It would be interesting to take out completely the air stones from the control tanks to reduce the removal of the contaminants and see the difference with the other tanks. Repeating the experimentation multiple times would allow the NGO to have more data

One thing that needs to be changed about the methodology is the procedure to fill up the tanks. As mentioned before, the water in the different tanks had different values because of the sediments. To simplify the analysis, it would have been ideal to sample the water in a huge tank of around 1680L (6x280). That would allow the sediments to mix and

get the same concentration before putting the water into the 6 smaller tanks. The water mix would be more homogeneous.

After all the different projects included in the phytoepuration proposal (the algae, the CW and the FTW projects) are analyzed, the algae project might be incorporated in a new large-scaled system of water purification with endemic and/or local plants. It will depend on the results of the other projects and their efficiency.

The experimentation should be repeated several times throughout the year. As mentioned previously, the seasonal changes are quite important (Bartolo, 2019) (AIS environmental, 2014). Moreover, it was hypothesized that the algae would respond differently depending on the photoperiod conditions: that could have an impact on the removal of nitrates (Farahdiba, Hidayah, Asmar, & Myint, 2020). It would be interesting to see how the results would be affected by them and what type of project would be the best to be implemented all year long. There might be different equations of model to predict how the system would react during the different seasons since the weather conditions are very different.

During the experimentation, a lot of insects and larvae from different species were found in all the tanks (an example is shown on the figure 27). On the other hand, four birds died in the water of the tanks. It could be interesting to investigate on the impacts that the tanks have on biodiversity with a small-scale and larger-scale experimentation. Nets will be added on top of the tanks to avoid having birds or larger animals drowning in the tanks.



Figure 27: Dragonfly on the air pump pipe of one of the tanks.

11. CONCLUSION

Il-Maghluq ta' Marsaskala is a Natura 2000 reserve with a brackish pond hosting multiple endemic and/or protected species including *Aphanius fasciatus*. The water ending up in the pond is a mix of seawater entering the site through pipes under the road, rainwater and fresh water from the surrounding fields. The runoff coming from the fields led to a phosphate and nitrate pollution on the site. To respect the management plan and be able to restore the habitats, a phytoepuration project was launched. The algae bioreactor project was part of it and the aim was to purify the water as much as possible within 2 weeks with *Cladophora sp.* and *Ulva lactuca*. Those two species can be found within the site. The study focused on 3 main parameters (nitrate, nitrite and phosphate concentration) and the analysis was done directly on site every day for 13 days in a row. The temperature, pH, conductivity, dissolved oxygen and salinity were taken as well to check if any changes could be explained by those last parameters.

The methodology used was based on other studies and adjusted to the budget of the organization and the limitations of the site. Indeed, since the reserve is classified as a Natura 2000 site, the methodology had to be modified to make sure that it respected all the laws and legislations applied on site. Unfortunately, the lack of time and some problems with the delivery of the equipment had an impact on the experimentation as the second part of it could not take place.

The results that were obtained demonstrated that the use of algae was definitely helpful to remove the pollutants from the water. Up to 40% of the phosphate was removed, as well as 96% of the nitrates and 99% of the nitrite contained in the water with algae, compared to up to 17% of phosphate removed, 70% of the nitrate removed and up to 171% of nitrites added with the control tanks. Even though some results were erratic, it was mostly explained by the environment and the methodology that was used to execute the experimentation.

After running a Spearman correlation test and given the outcome of the other statistics performed on the dataset, it conveys the impression that *Cladophora sp.* is the most effective and suitable alga of the two that were tested. *Cladophora sp.* tended to display the lowest results after the 13 days of experimentation. Moreover, the correlation obtained after the Spearman test proved that the experimentation was reproducible. This alga seemed to be the more efficient and therefore should be reused instead of *Ulva sp.* for other experimentations on the Marsaskala site. Thanks to the equation obtained with the linear regression, it was proved that the nitrates could be reached the recommendations after only 5 days for the nitrate concentration and after 30 days for the phosphate concentration.

To put it into a nutshell, the results obtained gave hope for the future of the phytoepuration project, in which the algae bioreactor experiment might be implemented if the CW and FTW show inferior results. The methodology needs to be revised before running the experiment again but further experimentation with different types of algae, with and without the air pumps and with different timing during the year should be conducted to make sure the best option is to use the *Cladophora sp.* as a mean to purify the pond water. This alga needs to be compared to other algae species found onsite and to the other phytoepuration projects (CW and FTW) to check which one is more efficient before

being implemented on a larger scale. Overall, all the results obtained convey the impression that the presence of the algae in the bioreactor allowed the levels of nitrate, nitrite and phosphate to vary.

Further experimentations still need to be implemented in the future to confirm all the results obtained, but they suggest that the water of the pond can be purified by algae (preferably by using *Cladophora sp.*) and remove the excess nitrate and phosphate.

13. BIBLIOGRAPHIE

- AIS environmental. (2014). *Baseline Surveys for Inland Surface and Transitional Waters - Hydromorphological, Physicochemical and Biological Quality Elements*. Malta: AIS environmental.
- AquaWal. (s.d.). *Comment l'eau est-elle épurée dans une station d'épuration?* Consulté le July 29, 2021, sur AquaWal: <https://www.aquawal.be/fr/comment-l-eau-est-elle-epuree-dans-une-step.html?IDC=587>
- Bartolo, D. (2019). *Water quality characterisation and its implications within il-Magħluq ta' Marsaskala (a Special Area of Conservation)*. Malta: MCAST Institute of Applied Sciences.
- Bates, C. (s.d.). *An introduction to the (macro)algae of British Columbia*. (U. o. Columbia, Éditeur, & Lab for Advanced Spatial Analysis, Department of Geography, University of British Columbia, Vancouver) Consulté le August 4, 2021, sur Electronic Atlas of the Flora of British Columbia: <https://ibis.geog.ubc.ca/biodiversity/eflora/algae.html>
- Borowitzka, M. A. (1999). Commercial production of microalgae: Ponds, tanks, and fermenters. *Marine Bioprocess Engineering, Proceedings of an International Symposium organized under auspices of the Working Party on Applied Biocatalysis of the European Federation of Biotechnology and the European Society for Marine Biotechnology. Progress in Indus(35)*, pp. 313-321.
- Bryant, J., & Irvine, L. (2016). Marimo, Cladophora, Posidonia and Other Plant Balls. *The Linnean*, 32, pp. 11-14.
- Burrows, E. M. (2013). *Seaweeds of the British Isles*. Pelagic Publishing.
- Cole, A., Neveux, J., Whelan, A., Morton, J., Vis, M., De Nys, R., & Paul, N. (2016). Adding value to the treatment of municipal wastewater through the intensive production of freshwater macroalgae. *Algal reseach(20)*, pp. 100-109.
- Commission Européenne. (2009). *Natura 2000*. Office des publications de la Comission Européenne.
- Conseil des Communautés Européennes. (1991, décembre 12). Directive du Conseil du 12 décembre 1991 concernant la protection de eaux contre la pollution par les nitrates à partir de sources agricoles (91/676/CEE). Journal officiel des Communautés Européennes.
- Conservation International. (s.d.). *Why are biodiversity hotspots important?* Consulté le July 9, 2021, sur Conservation International: <https://www.conservation.org/priorities/biodiversity-hotspots>
- Cuttlelod, A., Garcia, N., Malak, D. A., Temple, H., & Katariya, V. (2009). The Mediterranean: a biodiversity hotspot under threat. *Wildlife in a changing World - An analysis of the 2008 IUCN Red List of Threatened Species*, pp. 89 - 101.

- Danielidis, D., Katsaros, C., & Dring, M. (2010). Removal of phosphate by the green seaweed *Ulva lactuca*. *Journal of Applied Phycology*, 22(3), pp. 331-339.
- De Souza Gestinari, L., Barreto Pereira, S., & Yocie, Y.-V. (2021). Distribution of *Cladophora* Species (Cladophorales, Chlorophyta) along the Brazilian Coast. *Phytotaxa*, 14, pp. 22-42.
- Derneži, D. (2010). *Hotspot de la biodiversité du bassin méditerranéen*.
- Deviram, G., Pradeep, K., & Gyana Prasuna, R. (2011). Purification of waste water using Algal species. *European Journal of Experimental Biology*, 216-222.
- Direction Départementale des Territoires. (2015, novembre 19). *Sites d'Interet Communautaire (SIC) Natura 2000*. Récupéré sur data.gouv.fr: <https://www.data.gouv.fr/fr/datasets/sites-dinteret-communautaire-sic-natura-2000/>
- Duan, Y., & Shi, F. (2014, December). Bioreactor design for algal growth as a sustainable energy source. *Reactor and Process Design in Sustainable Energy Technology*, pp. 27-60.
- Environment & Resources Authority. (2014). *Natura 2000 Management Plan (SAC) - Il-Maghluq ta-Bahar ta' Marsaskala*. Environment and Resources Authority.
- Environment & Resources Authority. (s.d.). *Natura 2000 in Malta*. Consulté le July 9, 2021, sur ERA: <https://era.org.mt/topic/natura-2000-in-malta/>
- Environment & Resources Authority. (s.d.). *Nitrates Directive*. Consulté le avril 19, 2021, sur Environment & Resources Authority: <https://era.org.mt/topic/nitrates-directive/>
- European Commission. (2019). *The EU Environmental Implementation Review 2019, Country Report - MALTA*. Brussels: European Commission.
- European Commission. (s.d.). *Natura 2000*. Récupéré sur European Commission: https://ec.europa.eu/environment/nature/natura2000/index_en.htm
- European Environment Agency. (s.d.). *Habitat Types Search*. Consulté le avril 18, 2021, sur European Environment Agency: <https://eunis.eea.europa.eu/habitats.jsp>
- Falzone, C. (2020). *Analysis of Environmental Data*. Arlon: Université de Liège.
- Farahdiba, A., Hidayah, E., Asmar, G., & Myint, Y. (2020). Growth and Removal of Nitrogen and Phosphorus by a Macroalgae *Cladophora*. *Nature Environment and Pollution Technology*, 19(2), pp. 809-813.
- Gabarretta, J. (2021). *Phytoremediation techniques for il-Maghluq ta'Marsaskala*. Malta: Nature Trust Malta - Foundation for Environmental Education.
- IUCN. (2018). The Medirerranean, a global priority for conservation. *Infographic Posters*.
- Lehr, F., & Potsen, C. (2009, June). Closed photo-bioreactors as tools for biofuel production. *Current Opinion in Biotechnology*, 20(3), pp. 280-285.

- Ma, S.-N., Wang, H.-J., Wang, H.-Z., Zhang, M., Li, Y., Bian, S.-J., . . . Jeppesen, E. (2021). *Effects of nitrate on phosphorus release from lake sediments*. Wuhan: Elsevier.
- Mikkelsen, R. (2013). *A Closer Look at Phosphorus Uptake*. Merced: International Plant Nutrition Institute.
- Natura 2000. (s.d.). *Qu'est-ce que Natura 2000 ?* Consulté le mars 27, 2021, sur Site web du Centre de ressources Natura 2000: <https://www.natura2000.fr/natura-2000/qu-est-ce-que-natura-2000>
- Nature Trust Malta - Foundation for Environmental Education. (s.d.). *Who We Are*. Consulté le avril 13, 2021, sur Nature Trust Malta: <https://naturetrustmalta.org/who-we-are/>
- OBVAJ. (s.d.). *Le phosphore*. Consulté le July 28, 2021, sur OBVAJ: <http://obvaj.org/les-bonnes-pratiques/le-phosphore/#:~:text=Plusieurs%20effets%20secondaires%20surviennent%20suite,eau%20o%C3%B9%20elles%20seront%20d%C3%A9compos%C3%A9es.>
- Olson, G. (2015, November 05). *Cleaning up waste water through algae*. Consulté le June 27, 2021, sur Global Institute of Sustainability and Innovation: <https://sustainability-innovation.asu.edu/news/archive/11515-2/>
- Reich, P. B., Tilman, D., Isbell, F., Mueller, K., Hobbie, S. E., Flynn, D. F., & Eisenhauer, N. (2012, May 4). Impacts of Biodiversity Loss Escalate Through Time as Redundancy Fades. *Science*, 336, pp. 589-591.
- Romain, A.-C. (2020). *Methodology and Sampling course*. Arlon: Université de Liège.
- Sharma, S. (2020, September 18). *Reversing Biodiversity Loss & Safeguarding Our Future - Living Planet Report 2020*. Consulté le July 8, 2021, sur IUCN: <https://www.iucn.org/news/world-commission-environmental-law/202009/reversing-biodiversity-loss-safeguarding-our-future-living-planet-report-2020>
- Sode, S., Bruhn, A., Balsby, T., Larsen, M. M., Gotfredsen, A., & Rasmussen, M. B. (2013, October). Bioremediation of reject water from anaerobically digested waste water sludge with macroalgae (*Ulva lactuca*, Chlorophyta). *Bioresource Technology*, 146, pp. 426-435.
- Sustainable Energy and Water Conservation Unit. (2015). *The 2nd Water Catchment Management Plan for the Malta Water Catchment District*. Malta: Environment and Resources Authority.
- Wang, L., Wang, Q., Zhao, R., Tao, Y., Ying, K.-Z., & Mao, X.-Z. (2021, January 14). Novel Flat-Plate Photobioreactor with Inclined Baffles and Internal Structure Optimization to Improve Light Regime Performance. *ACS Sustainable Chem. Eng.*, 9(4), pp. 1550-1558.
- Whitton, B. (1970, July). Biology of *Cladophora* in freshwaters. *Water Research*, pp. 457-476.

- WWF. (2020). *Living Planet Report 2020 - Bending the curve of biodiversity loss*. Gland: WWF.
- Zammit-Mangion, M., & Deidun, A. (2010). *Management Recommendations for the Conservation of Threatened Aphanius fasciatus Nardo populations fr two wetlands in theMaltese Islands*. Societa italiana di Biologia Marina.
- Zammit-Mangion, M., Deidun, A., Vassallo-Agius, R., & Magri, M. (201). Management of threatened Aphanius fasciatus at Il-Magħluq, Malt. . *Proceedings of the Tenth International Conference on the Mediterranean Coastal Environment*. Rhodes: MEDCOAST.

14. ANNEXES

14.1. ANNEXE I: RESULTS OF THE EXPERIMENTATION

The first 6 tables (table 21 to 26) represent the overall results obtained during the sampling period for the general parameters with the recalculated concentrations after evaporation of the water.

Table 21: Overall results obtained during the 13-day-sampling for the Control 1 tank (recalculated concentrations after evaporation of the water).

pH	DO (%)	Conductivity (mS/cm)	Temperature (°C)	Salinity (PSU)	Water in the tank (L)
8.14	114.70	51.42	28.10	33.35	287.10
8.30	73.60	52.75	28.63	33.74	286.06
8.17	60.70	53.18	27.47	33.16	285.01
8.28	66.70	52.67	27.44	34.38	283.97
7.39	83.60	55.83	27.95	33.12	282.40
7.21	69.90	56.26	26.92	34.93	280.31
6.85	103.20	56.70	27.07	36.63	279.27
6.57	87.30	58.30	27.45	35.59	277.70
6.96	40.00	59.51	27.98	37.16	275.09
7.09	59.20	57.56	28.32	36.85	274.05
6.87	65.80	57.85	27.57	36.77	273.01
6.90	62.90	58.94	28.67	37.77	272.48
6.46	64.70	58.99	27.94	37.49	271.44

Table 22: Overall results obtained during the 13-day-sampling for the Control 2 tank (recalculated concentrations after evaporation of the water).

pH	DO (%)	Conductivity (mS/cm)	Temperature (°C)	Salinity (PSU)	Water in the tank (L)
8.16	109.80	51.39	28.28	33.61	287.10
7.56	70.30	53.67	27.75	33.49	285.53
7.25	65.60	53.18	27.53	33.95	284.49
8.01	67.40	54.36	27.49	34.16	283.97
7.74	63.80	54.82	28.02	34.82	280.84
7.24	56.90	55.68	27.56	34.82	279.27
6.16	59.00	56.33	26.97	36.24	278.23
6.56	55.30	57.24	27.37	36.30	276.66
6.80	54.30	58.04	27.50	36.03	275.09
7.33	65.40	58.79	27.48	37.70	273.53
7.22	60.70	62.24	27.35	39.89	273.01
7.03	57.80	62.03	27.45	38.05	271.44
7.00	56.00	63.46	37.65	37.70	270.40

Table 23: Overall results obtained during the 13-day-sampling for the Ulva 1 tank (recalculated concentrations after evaporation of the water).

pH	DO (%)	Conductivity (mS/cm)	Temperature (°C)	Salinity (PSU)	Water in the tank (L)
7.55	80.10	52.43	28.21	32.97	287.10
7.03	57.30	53.24	28.30	33.02	286.06
7.58	67.20	53.67	27.25	34.55	285.01
7.93	64.30	52.60	27.84	34.26	282.92
8.25	62.20	52.66	27.19	35.64	281.88
7.45	68.40	54.78	27.36	35.50	278.75
6.66	65.40	56.12	26.89	36.08	278.23
6.29	64.60	56.23	27.73	36.81	276.66
6.89	63.20	58.96	27.88	36.74	275.62
6.36	59.50	58.37	27.24	37.26	274.05
6.80	58.30	59.55	28.05	36.89	272.48
7.34	60.40	59.18	27.74	37.27	270.92
7.26	62.60	60.03	27.46	37.19	269.87

Table 24: Overall results obtained during the 13-day-sampling for the Ulva 2 tank (recalculated concentrations after evaporation of the water).

pH	DO (%)	Conductivity (mS/cm)	Temperature (°C)	Salinity (PSU)	Water in the tank (L)
8.24	74.90	51.37	28.58	33.50	287.10
7.39	63.50	53.80	27.36	33.53	286.06
7.01	67.30	50.24	27.18	33.56	284.49
7.54	61.60	56.72	27.99	34.77	282.40
6.87	50.70	53.16	28.10	36.22	281.88
6.36	42.00	55.94	27.71	35.98	278.75
6.35	65.10	56.58	27.56	36.33	277.18
6.82	71.50	57.63	27.94	35.91	276.66
7.17	59.00	61.56	27.86	36.18	274.05
7.39	60.80	62.45	27.39	37.09	273.01
7.42	55.70	60.32	27.85	36.70	271.96
6.78	43.20	60.06	28.24	37.33	270.40
7.32	50.20	60.34	27.87	37.12	269.35

Table 25: Overall results obtained during the 13-day-sampling for the Cladophora 1 tank (recalculated concentrations after evaporation of the water).

pH	DO (%)	Conductivity (mS/cm)	Temperature (°C)	Salinity (PSU)	Water in the tank (L)
8.42	71.70	51.36	28.75	33.27	287.10
7.35	43.60	52.54	28.45	33.29	286.58
7.26	57.20	50.56	27.14	33.35	285.53
7.95	52.70	53.25	27.24	32.76	283.97
7.89	28.80	53.44	27.45	32.30	282.92
6.26	48.50	56.45	27.29	33.68	279.79
6.78	65.90	57.28	26.96	37.00	278.75
6.74	50.20	58.45	26.89	37.00	277.70
7.38	56.60	59.33	27.93	36.95	275.62
6.93	65.20	59.35	27.45	36.99	274.57
7.25	54.80	59.16	27.62	37.10	274.05
7.78	58.20	60.30	28.03	37.19	273.53
7.57	60.40	59.79	27.94	36.98	271.96

Table 26: Overall results obtained during the 13-day-sampling for the Cladophora 2 tank (recalculated concentrations after evaporation of the water).

pH	DO (%)	Conductivity (mS/cm)	Temperature (°C)	Salinity (PSU)	Water in the tank (L)
8.41	69.00	44.21	28.60	33.25	287.10
8.24	60.40	42.45	27.53	33.52	286.06
7.45	40.70	48.60	27.24	34.07	285.53
7.27	53.60	51.56	27.64	34.71	284.49
6.98	56.30	53.74	27.01	35.23	282.92
6.68	38.10	55.57	27.66	35.72	278.75
7.32	40.30	55.99	27.82	35.32	278.23
7.19	52.90	57.53	27.99	35.68	277.18
7.36	55.60	58.45	27.50	36.23	276.14
7.70	50.60	61.85	27.32	37.74	274.05
7.24	48.45	61.56	27.42	37.59	273.01
7.30	42.00	62.22	27.77	37.75	272.48
7.43	46.50	61.30	27.63	37.63	271.44

The tables hereunder (tables 27 to 32) represent the overall results obtained during the sampling period for the main parameters without taking the evaporation into account.

Table 27: Overall results obtained during the 13-day-sampling for the Control 1 tank (concentrations not recalculated to take the evaporation into account).

Day	NO_3^- (mg/L)				NO_2^- (μ g/L)				PO_4^{3-} (mg/L)			
	r1	r2	r3	Average	r1	r2	r3	Average	r1	r2	r3	Average
0	2.33	2.34	2.34	2.34	42.00	40.00	43.00	41.67	0.40	0.43	0.45	0.43
1	2.25	2.23	2.23	2.24	43.00	43.00	41.00	42.33	0.40	0.40	0.39	0.40
2	2.11	2.13	2.14	2.13	42.00	41.00	39.00	40.67	0.39	0.39	0.40	0.39
3	2.05	2.05	2.06	2.05	50.00	48.00	47.00	48.33	0.39	0.39	0.39	0.39
4	1.97	1.96	1.97	1.97	55.00	58.00	56.00	56.33	0.34	0.34	0.36	0.35
5	1.78	1.78	1.80	1.79	70.00	73.00	74.00	72.33	0.37	0.36	0.38	0.37
6	1.65	1.63	1.65	1.64	75.00	74.00	74.00	74.33	0.32	0.30	0.33	0.32
7	1.43	1.45	1.46	1.45	82.00	83.00	83.00	82.67	0.30	0.30	0.30	0.30
8	1.24	1.24	1.25	1.24	90.00	90.00	91.00	90.33	0.31	0.31	0.32	0.31
9	1.20	1.19	1.19	1.19	98.00	99.00	97.00	98.00	0.34	0.34	0.36	0.35
10	0.90	0.90	0.90	0.90	105.00	106.00	109.00	106.67	0.34	0.35	0.37	0.35
11	0.79	0.80	0.78	0.79	116.00	118.00	120.00	118.00	0.40	0.39	0.39	0.39
12	0.72	0.73	0.72	0.72	119.00	120.00	120.00	119.67	0.39	0.38	0.38	0.38

Table 28: Overall results obtained during the 13-day-sampling for the Control 2 tank (concentrations not recalculated to take the evaporation into account).

Day	NO_3^- (mg/L)				NO_2^- (μ g/L)				PO_4^{3-} (mg/L)			
	r1	r2	r3	Average	r1	r2	r3	Average	r1	r2	r3	Average
0	2.42	2.43	2.45	2.43	50.00	53.00	52.00	51.67	0.38	0.38	0.38	0.38
1	2.40	2.42	2.41	2.41	52.00	51.00	51.00	51.33	0.39	0.38	0.38	0.38
2	2.39	2.39	2.39	2.39	49.00	48.00	50.00	49.00	0.37	0.38	0.36	0.37
3	2.39	2.38	2.39	2.39	47.00	45.00	45.00	45.67	0.36	0.37	0.36	0.36
4	2.30	2.33	2.32	2.32	46.00	45.00	46.00	45.67	0.37	0.35	0.36	0.36
5	2.32	2.33	2.37	2.34	44.00	47.00	50.00	47.00	0.40	0.42	0.40	0.41
6	1.90	1.92	1.89	1.90	55.00	57.00	56.00	56.00	0.43	0.45	0.45	0.44
7	1.32	1.32	1.32	1.32	63.00	63.00	65.00	63.67	0.43	0.44	0.46	0.44
8	1.38	1.38	1.40	1.39	78.00	78.00	80.00	78.67	0.40	0.42	0.42	0.41
9	1.43	1.43	1.41	1.42	90.00	93.00	93.00	92.00	0.40	0.40	0.40	0.40
10	1.42	1.40	1.43	1.42	113.00	115.00	115.00	114.33	0.40	0.39	0.39	0.39
11	1.40	1.39	1.40	1.40	143.00	140.00	145.00	142.67	0.37	0.37	0.36	0.37
12	1.38	1.36	1.38	1.37	145.00	143.00	143.00	143.67	0.35	0.36	0.36	0.36

Table 29: Overall results obtained during the 13-day-sampling for the Ulva 1 tank (concentrations not recalculated to take the evaporation into account).

Day	NO_3^- (mg/L)				NO_2^- (μ g/L)				PO_4^{3-} (mg/L)			
	r1	r2	r3	Average	r1	r2	r3	Average	r1	r2	r3	Average
0	2.27	2.26	2.27	2.27	50.00	45.00	45.00	46.67	0.50	0.54	0.54	0.53
1	2.10	2.09	2.12	2.10	40.00	42.00	42.00	41.33	0.53	0.54	0.53	0.53
2	1.99	1.98	2.00	1.99	39.00	38.00	38.00	38.33	0.51	0.50	0.51	0.51
3	1.83	1.86	1.82	1.84	36.00	36.00	37.00	36.33	0.38	0.38	0.39	0.38
4	1.87	1.90	1.88	1.88	34.00	36.00	38.00	36.00	0.35	0.35	0.35	0.35
5	1.50	1.53	1.53	1.52	50.00	53.00	54.00	52.33	0.35	0.37	0.37	0.36
6	0.72	0.72	0.72	0.72	65.00	67.00	70.00	67.33	0.38	0.40	0.40	0.39
7	0.60	0.63	0.62	0.62	78.00	79.00	78.00	78.33	0.43	0.42	0.42	0.42
8	0.57	0.57	0.57	0.57	70.00	70.00	70.00	70.00	0.43	0.44	0.43	0.43
9	0.35	0.38	0.37	0.37	53.00	52.00	50.00	51.67	0.43	0.43	0.43	0.43
10	0.27	0.27	0.25	0.26	13.00	12.00	10.00	11.67	0.42	0.42	0.42	0.42
11	0.13	0.13	0.13	0.13	0.00	2.00	2.00	1.33	0.40	0.41	0.41	0.41
12	0.10	0.09	0.09	0.09	0.00	0.00	1.00	0.33	0.38	0.37	0.37	0.37

Table 30: Overall results obtained during the 13-day-sampling for the Ulva 2 tank (concentrations not recalculated to take the evaporation into account).

Day	NO_3^- (mg/L)				NO_2^- (μ g/L)				PO_4^{3-} (mg/L)			
	r1	r2	r3	Average	r1	r2	r3	Average	r1	r2	r3	Average
0	1.80	1.80	1.81	1.80	78.00	78.00	78.00	78.00	0.41	0.42	0.43	0.42
1	1.77	1.77	1.76	1.77	76.00	76.00	76.00	76.00	0.42	0.43	0.40	0.42
2	1.70	1.71	1.65	1.69	68.00	68.00	68.00	68.00	0.40	0.39	0.39	0.39
3	1.58	1.59	1.60	1.59	68.00	67.00	66.00	67.00	0.35	0.36	0.35	0.35
4	1.40	1.42	1.40	1.41	64.00	65.00	64.00	64.33	0.32	0.33	0.33	0.33
5	1.35	1.35	1.35	1.35	64.00	74.00	64.00	67.33	0.30	0.30	0.31	0.30
6	0.85	0.86	0.86	0.86	67.00	68.00	70.00	68.33	0.37	0.37	0.38	0.37
7	0.61	0.60	0.62	0.61	77.00	78.00	79.00	78.00	0.45	0.45	0.47	0.46
8	0.51	0.49	0.48	0.49	86.00	87.00	89.00	87.33	0.63	0.63	0.65	0.64
9	0.46	0.46	0.46	0.46	80.00	82.00	82.00	81.33	0.60	0.61	0.61	0.61
10	0.45	0.44	0.45	0.45	75.00	74.00	73.00	74.00	0.57	0.57	0.56	0.57
11	0.40	0.40	0.40	0.40	64.00	67.00	66.00	65.67	0.50	0.51	0.51	0.51
12	0.37	0.37	0.36	0.37	62.00	63.00	63.00	62.67	0.47	0.46	0.45	0.46

Table 31: Overall results obtained during the 13-day-sampling for the Cladophora 1 tank (concentrations not recalculated to take the evaporation into account).

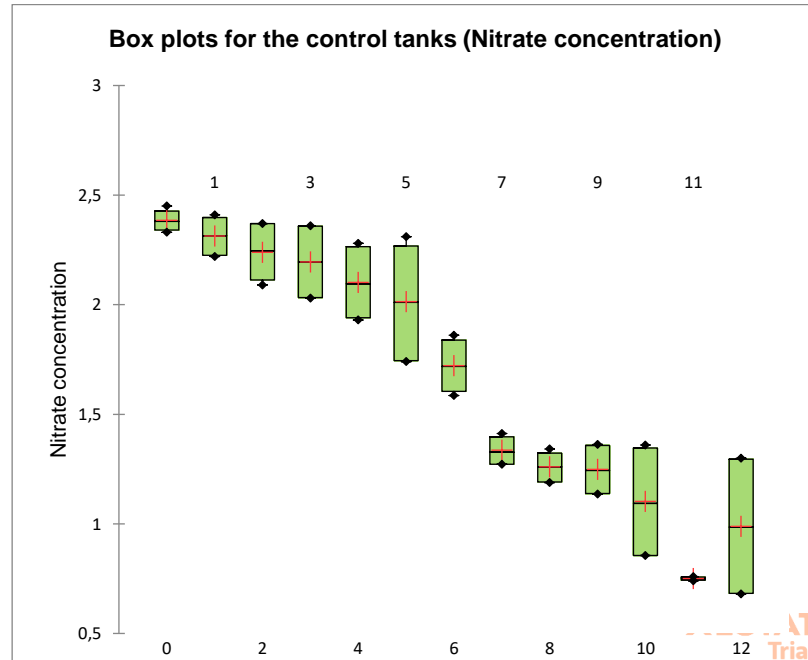
Day	NO_3^- (mg/L)				NO_2^- (μ g/L)				PO_4^{3-} (mg/L)			
	r1	r2	r3	Average	r1	r2	r3	Average	r1	r2	r3	Average
0	2.14	2.14	2.13	2.14	49.00	49.00	50.00	49.33	0.45	0.49	0.46	0.47
1	1.94	1.95	1.95	1.95	48.00	49.00	49.00	48.67	0.45	0.45	0.44	0.45
2	1.72	1.72	1.70	1.71	45.00	45.00	45.00	45.00	0.44	0.46	0.43	0.44
3	1.50	1.53	1.54	1.52	43.00	43.00	40.00	42.00	0.40	0.41	0.40	0.40
4	1.36	1.34	1.37	1.36	41.00	42.00	40.00	41.00	0.42	0.42	0.40	0.41
5	0.85	0.85	0.81	0.84	35.00	35.00	37.00	35.67	0.40	0.40	0.39	0.40
6	0.59	0.59	0.59	0.59	40.00	38.00	35.00	37.67	0.40	0.37	0.37	0.38
7	0.37	0.37	0.37	0.37	33.00	34.00	33.00	33.33	0.37	0.36	0.35	0.36
8	0.28	0.29	0.28	0.28	27.00	28.00	28.00	27.67	0.35	0.36	0.35	0.35
9	0.24	0.25	0.22	0.24	23.00	23.00	24.00	23.33	0.35	0.37	0.37	0.36
10	0.16	0.16	0.15	0.16	20.00	21.00	23.00	21.33	0.33	0.33	0.35	0.34
11	0.12	0.12	0.12	0.12	17.00	17.00	18.00	17.33	0.31	0.32	0.34	0.32
12	0.08	0.07	0.06	0.07	15.00	16.00	16.00	15.67	0.30	0.28	0.28	0.29

Table 32: Overall results obtained during the 13-day-sampling for the Cladophora 2 tank (concentrations not recalculated to take the evaporation into account).

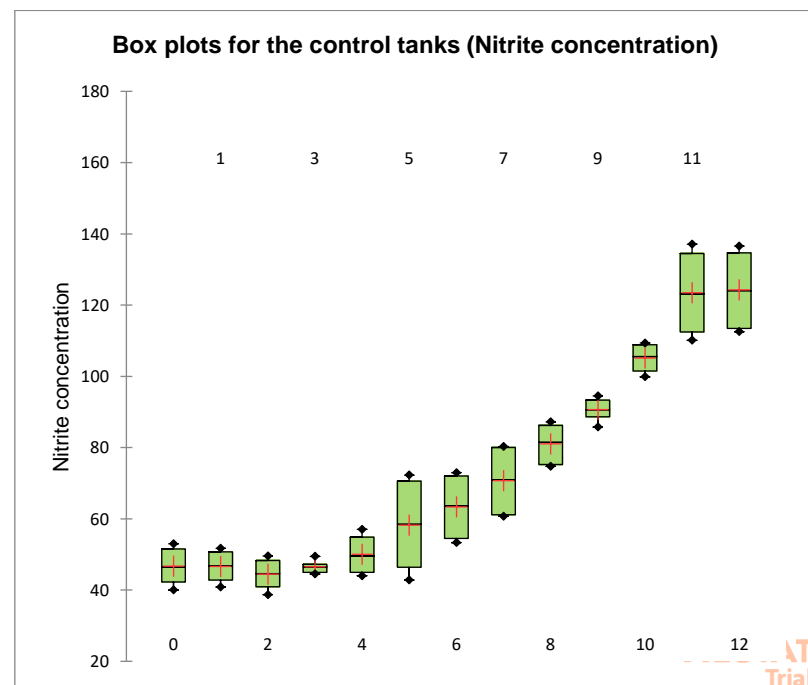
Day	NO_3^- (mg/L)				NO_2^- (μ g/L)				PO_4^{3-} (mg/L)			
	r1	r2	r3	Average	r1	r2	r3	Average	r1	r2	r3	Average
0	2.24	2.23	2.20	2.22	64.00	67.00	66.00	65.67	0.41	0.43	0.39	0.41
1	1.99	1.96	1.94	1.96	65.00	64.00	63.00	64.00	0.40	0.41	0.40	0.40
2	1.70	1.71	1.73	1.71	60.00	59.00	59.00	59.33	0.39	0.39	0.38	0.39
3	1.58	1.58	1.57	1.58	55.00	55.00	55.00	55.00	0.39	0.38	0.38	0.38
4	1.43	1.45	1.45	1.44	53.00	51.00	50.00	51.33	0.37	0.37	0.37	0.37
5	1.38	1.36	1.38	1.37	50.00	50.00	50.00	50.00	0.35	0.35	0.35	0.35
6	0.87	0.85	0.85	0.86	46.00	43.00	43.00	44.00	0.34	0.32	0.33	0.33
7	0.27	0.25	0.27	0.26	40.00	39.00	38.00	39.00	0.30	0.31	0.30	0.30
8	0.24	0.26	0.26	0.25	34.00	33.00	33.00	33.33	0.31	0.31	0.31	0.31
9	0.25	0.25	0.25	0.25	23.00	24.00	25.00	24.00	0.29	0.29	0.33	0.30
10	0.13	0.14	0.13	0.13	17.00	17.00	13.00	15.67	0.30	0.30	0.31	0.30
11	0.08	0.08	0.07	0.08	12.00	12.00	12.00	12.00	0.30	0.29	0.27	0.29
12	0.05	0.06	0.06	0.06	10.00	9.00	9.00	9.33	0.27	0.27	0.26	0.27

14.2. ANNEXE II : BOX-PLOTS OF THE MAIN PARAMETERS FOR THE DIFFERENT TANKS

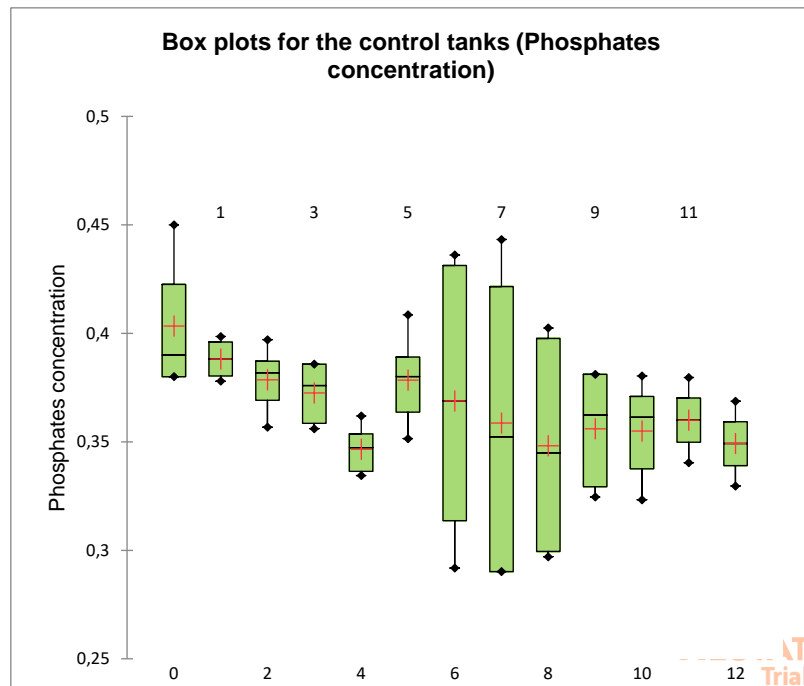
The different box-plots for the main parameters (nitrate, nitrite and phosphate concentrations) for the different type of tanks can be found in graphs 15 to 23 hereunder.



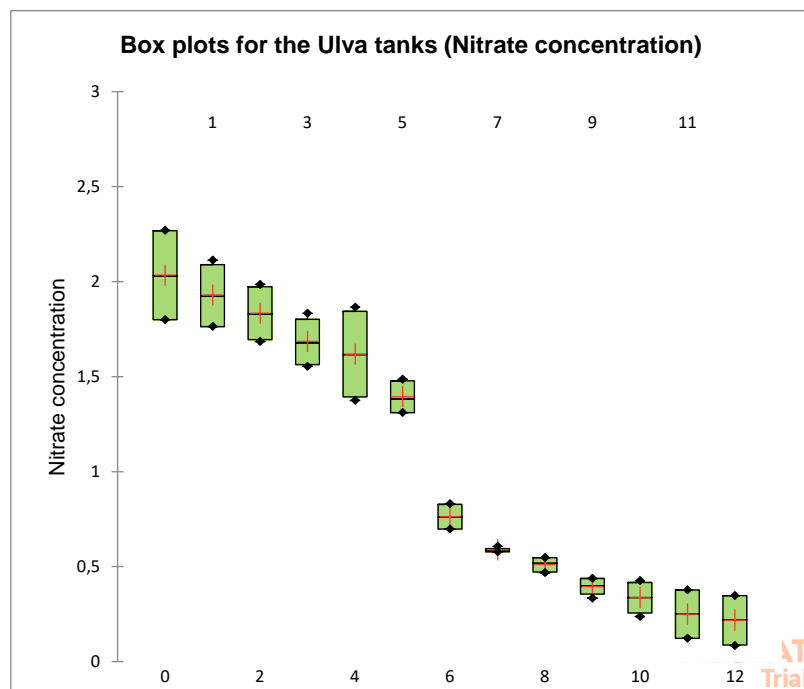
Graph 15: Box-plots for the control tanks (nitrate concentration).



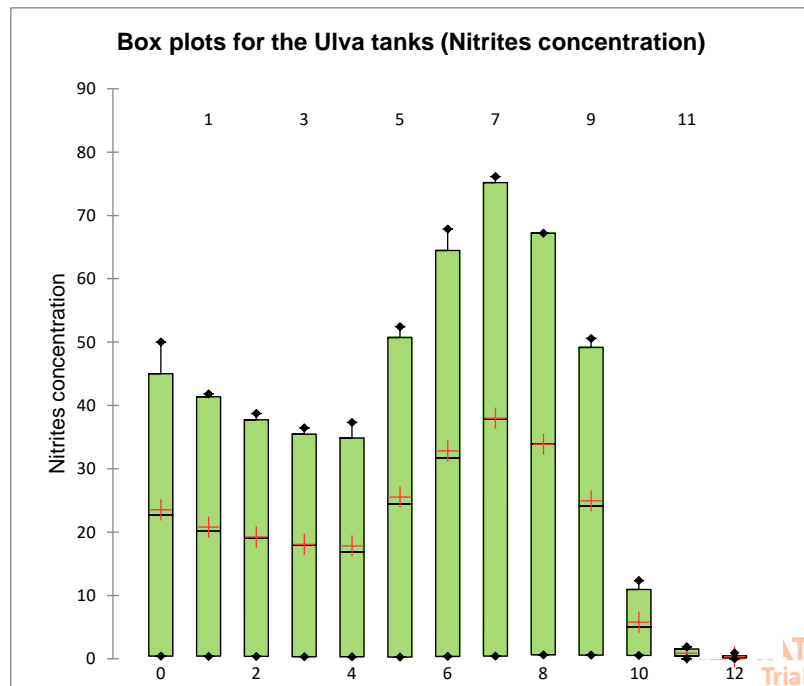
Graph 16: Box-plots for the control tanks (nitrite concentration).



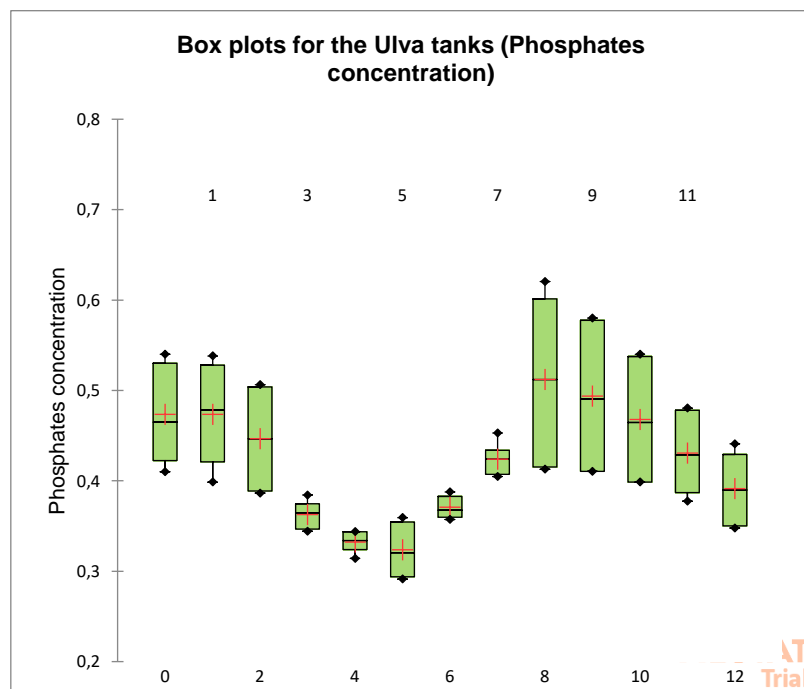
Graph 17: Box-plots for the control tanks (phosphate concentration).



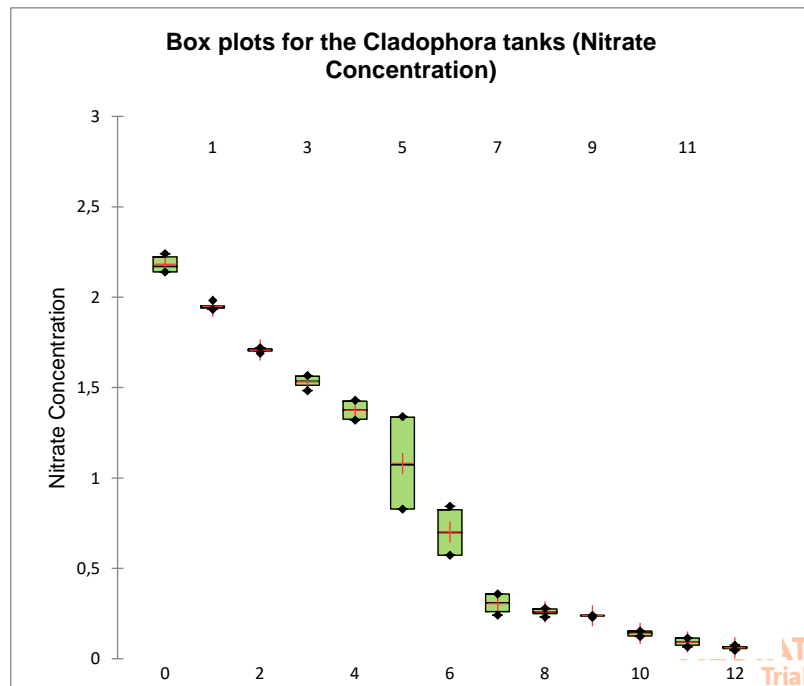
Graph 18: Box-plots for the Ulva tanks (nitrate concentration).



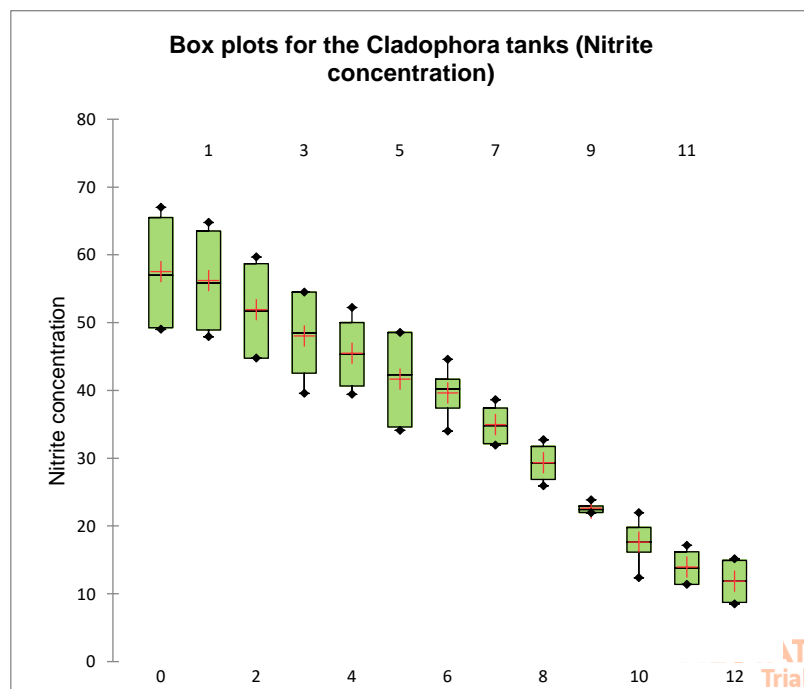
Graph 19: Box-plots for the Ulva tanks (nitrite concentration).



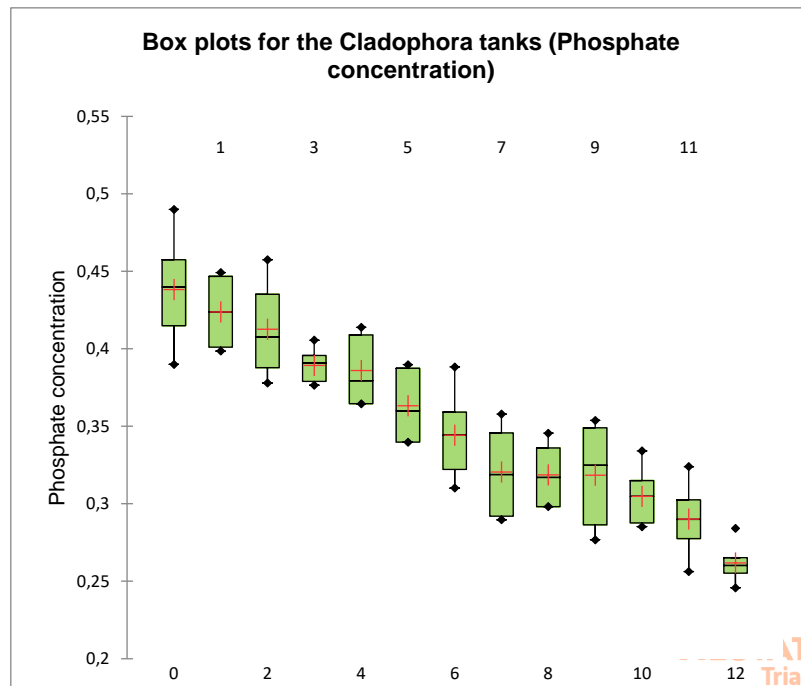
Graph 20: Box-plots for the Ulva tanks (phosphate concentration).



Graph 21: Box-plots for the Cladophora tanks (nitrate concentration).



Graph 22: Box-plots for the Cladophora tanks (nitrite concentration).



Graph 23: Box-plots for the Cladophora tanks (phosphate concentration).

15. ABSTRACT

Il-Maghluq ta' Marsaskala is a maltese Natura 2000 nature reserve that includes a small pond. The site hosts various endemic species that are important to protect: this is why the manager of the site, James Gabarretta, decided to implement a project of phytoremediation to purify the water of the pond with different water treatments. This project includes the algae bioreactor: two species from the site were used (*Ulva lactuca* and *Cladophora sp.*) to decontaminate the water. The objectives were to quantify the changes (if any) in the phosphate, nitrate and nitrite level in the water placed in the tanks and to determine which algae give the best results. The concentrations of the parameters in the control tanks were decreasing but less rapidly than in the tanks with the algae. The results obtained demonstrated that overall, up to 40% of the phosphate was removed, as well as 96% of the nitrates and 99% of the nitrite contained in the water with the algae. After running a Spearman correlation test and given the outcome of the other statistics performed on the dataset, it conveys the impression that *Cladophora sp.* is the most effective and suitable alga of the two that were tested. *Cladophora sp.* tended to display the lowest results after the 13 days of experimentation. Moreover, the correlation obtained after the Spearman test proved that the experimentation was reproducible. This alga seemed to be the more efficient and therefore should be reused instead of *Ulva sp.* for other experimentations in the Marsaskala site. Further experimentations have to be conducted to make sure that the algae can be used on a larger-scale project throughout the year.

Il-Maghluq ta' Marsaskala est une réserve maltaise classée Natura 2000 et est dotée d'un petit étang. Le site abrite diverses espèces endémiques qu'il est important de protéger: c'est pourquoi le gestionnaire du site, James Gabarretta, a décidé de mettre en œuvre un projet de phytoremédiation pour purifier l'eau de l'étang avec différents traitements de l'eau. Ce projet comprend le bioréacteur à algues: deux espèces du site ont été utilisées (*Ulva lactuca* et *Cladophora sp.*). Les objectifs étaient de quantifier les changements (s'il y en avait) du niveau de phosphate, nitrate et nitrite dans l'eau placée dans les réservoirs et de déterminer quelles algues donnent les meilleurs résultats. Les concentrations des paramètres dans les bassins de contrôle diminuaient, mais moins rapidement que dans les bassins avec les algues. Les résultats obtenus ont démontré que globalement jusqu'à 40% du phosphate a été éliminé, ainsi que 96% des nitrates et 99% des nitrites contenus dans l'eau avec les algues. Après avoir effectué un test de corrélation de Spearman et compte tenu des résultats des autres statistiques effectuées sur l'ensemble des données, cela donne l'impression que *Cladophora sp.* est l'algue la plus efficace et la plus appropriée des deux qui ont été testées. *Cladophora sp.* a eu tendance à afficher les résultats les plus faibles après les 13 jours d'expérimentation. De plus, la corrélation obtenue après le test de Spearman a prouvé que l'expérimentation était reproductible. Cette algue semble être la plus efficace et devrait donc être réutilisée à la place de *Ulva lactuca* pour d'autres expérimentations sur le site de Marsaskala. D'autres expériences doivent être menées pour s'assurer que les algues peuvent être utilisées dans un projet à plus grande échelle tout au long de l'année.

Nature Trust Malta contacts

Address:

Wied Ghollieqa Environment Centre,
Lower Level, Car Park 1,
University of Malta, Msida.

Postal Address:

Nature Trust (Malta)
PO Box 9,
Valletta. VLT 1000
MALTA

E-mail: info@naturetrustmalta.org

Phone Number: (+356) 21313150

Fax: (+356) 21320778

James Gabarretta:

(+356) 98898800

jamesg@naturetrustmalta.org