UNIVERSIDAD DE LAS PALMAS
DE GRAN CANARIA

CIHEAM
Instituto Agronómico
Mediterráneo de Zaragoza

IV. INTERNATIONAL MASTER IN MARINE AQUACULTURE

DIFFERENTIAL MACROALGAL PHYSIOLOGICAL PERFORMANCE IN A BIOFILTER SYSTEM: BIOMASS PRODUCTION, AMMONIUM UPTAKE AND HEAVY METALS CONTENTS

GIZEM TURKMEN

Las Palmas de Gran Canaria, December 2016

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Las Palmas de Gran Canaria, España

2014-2016

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Tesis presentada y públicamente defendida para la obtención del título de Máster Universitario en Cultivos Marinos

Las Palmas de Gran Canaria, 13 de diciembre de 2016

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Organizado conjuntamente por la Universidad de Las Palmas de Gran Canaria (ULPGC) y el Centro Internacional de Altos Estudios Agronómicos Mediterráneos (CIHEAM), a través del Instituto Agronómico Mediterráneo de Zaragoza (IAMZ)

DIFFERENTIAL MACROALGAL PHYSIOLOGICAL PERFORMANCE IN A BIOFILTER SYSTEM: BIOMASS PRODUCTION, AMMONIUM UPTAKE AND HEAVY METALS CONTENTS

GIZEM TURKMEN

Trabajo realizado en la Universidad de Las Palmas de Gran Canaria, España, bajo la dirección del Dr. Ricardo J. Haroun Tabraue del Instituto Universitario ECOAQUA de la Universidad de Las Palmas de Gran Canaria.

Presentado como requisito parcial para la obtención del Título oficial de Máster Universitario en Cultivos Marinos, otorgado por la Universidad de Las Palmas de Gran Canaria (ULPGC) y del Diploma de Master of Science en Acuicultura otorgado por el Centro Internacional de Altos Estudios Agronómicos Mediterráneos (CIHEAM).

Director	Autor
Dr. Ricardo J. **Haroun Tabraue**	Gizem **Turkmen**
Fdo:	Fdo: .....

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SUMMARY

As result of the photosynthetic production of macroalgal species and the input of nutrients from the effluents of an experimental aquaculture production site, an increase in algal biomass and indirectly a differential heavy metal uptake are expected. The biofilter system of the Scientific and Technological Marine Park of the University of Las Palmas de Gran Canaria (PCTM-ULPGC) was used as experimental model to assess the biomass production and heavy metal uptake of diverse macroalgal species. Monitoring of algal biomass was done during 12 months, with periodic sampling at three levels inside the biofiter system. In the case of the heavy metals, samples were taken from the same levels of the biofilter system in July. The algal samples were rinsed and concentrations of Cu, Mn, Fe, Zn, Cr, Ni, Pb, Cd were determined by using an Atomic Absorption Spectrophometer with a graphite tube atomizer (Analytik Jenna ContraAA 700). Three macroalgae species: *Ulva rigida* (C. Agardh, 1823), *Caulerpa racemose* Förskall (J. Agardh, 1873) and *Colpomenia sinuosa* (Mertens ex Roth Derbes & Solier, 1851) were cultured during 1 month in 9 tanks during 4 consecutive culture periods of 1 week. Ammonium (NH_4^+) uptake rates were calculated during first four hours in the beginning of each week.

Levels of the biofiltration system has no effect to heavy metal concentration. *Ulva rigida* showed faster NH_4^+ elimination from water, followed by *C. racemosa* and *C. sinuosa*. In the case of the nitrogen uptake, 180 gr. biomass of *U. rigida* was able to remove all NH_4^+ concentrations as high as 17.64±0.41 μmol/L after 2 hours while 3 hours were needed in the case of *C. racemosa*.

As conclusion, the biomass production was higher in *U. rigida* as well as it this specie showed better capacity for NH_4^+ removal from the water than the two species tested in this experiment. Regarding the heavy metals, in general *U. rigida* also showed higher concentrations when compared with the other two species. *C. sinuosa* and *C. racemosa* showed similar heavy metal concentrations at the end of the experiment.

RESUMEN EN ESPAÑOL

Como resultado de la producción fotosintética de especies de macroalgas y el flujo de nutrientes desde los efluentes de un sitio de producción acuícola experimental, se espera un aumento en la biomasa de algas y, indirectamente, una captación diferencial de metales pesados. El sistema de biofiltro del Parque Marino Científico y Tecnológico de la Universidad de Las Palmas de Gran Canaria fue utilizado como modelo experimental para evaluar la producción de biomasa y la captación de metales pesados de diversas especies de macroalgas. El monitoreo de la biomasa de algas se realizó durante 12 meses, con muestreo periódico en tres niveles dentro del sistema. Para la determinación de la concentración de metales, las muestras de algas fueron enjuagadas y las concentraciones de Cu, Mn, Fe, Zn, Cr, Ni, Pb, Cd fueron determinadas por una absorción atómica espectrómetro con un atomizador de tubo de grafito (Analytik Jenna ContraAA 700). Se cultivaron tres especies de macroalgas: *Ulva rigida* (C. Agardh, 1823), *Caulerpa racemosa* Förskall (J. Agardh, 1873) y *Colpomenia sinuosa* (Mertens ex Roth) Derbes & Solier, 1851, un mes en 9 tanques durante 4 períodos de cultivo consecutivos de 1 semana. Las tasas de absorción de amonio (NH_4^+) se calcularon durante las primeras cuatro horas al comienzo de cada semana.

Los niveles de muestreo dentro del sistema de biofiltración no mostraron diferencias respecto a la concentración de metales pesados. Por otra parte, *U. rigida* mostró una eliminación más rápida de NH_4^+ del agua, seguida de *C. racemosa* y *C. sinuosa*. Con respecto a la retención de nitrógeno, 180 gr. de biomasa de *U. rigida* fue capaz de eliminar todo el NH_4^+ tan alto como 17.64 ± 0.41 mol / L después de 2 horas mientras que en el caso de *C. racemosa* fueron necesarias 3 horas para ello.

En conclusión, la producción de biomasa fue mayor en *U. rigida*, así como una mayor capacidad de retención de NH_4^+ del agua. En cuanto a los metales pesados también *U. rigida* mostró mayores concentraciones de metales pesados en general, en comparación con *Colpomenia sinuosa*, mientras que no se encontraron diferencias con *C. racemosa* para la mayoría de los casos.

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LIST OF ABBREVIATIONS

ANOVA	Analysis of variance
Cu	Cobalt
Cr	Chromium
Cd	Cadmium
FAO	Food and Agriculture Organization of United Nations
Fe	Iron
GIA	Grupo de Investigación en Acuicultura
IMTA	Integrated Multi-Trophic Aquaculture
Mn	Manganese
nd	No difference
N	Nitrate
NH_4^+	Ammonium
Ni	Nikel
PAR	Photosynthetically Active Radiation
Pb	Lead
PCT	Parque CientÍfico Tecnológico de Taliarte
SD	Standard Deviation
SGR	Specific Growth Rate
SSAE	Soil Sciences of Agriculture Engineering
ULPGC	Universidad de Las Palmas de Gran Canaria
Zn	Zinc

ACKNOWLEDGEMENTS

Dedicated to,

My husband, **Serhat TURKMEN,** thank you for your HELP and FULL SUPPORT and also I'm very thankful to you for being around me whenever I need….
My family, thanks you for your support all the time…

Many thanks to,

Ricardo Haroun, for his support, concern, valuable suggestions and also friendly approach all the time;

Gercende Courtois de Viçose, for her support, important advices and concern;
Carmen María Hernández Cruz and Maria del Pino Viera Toledo, for their concern and support;

Bernardo Basurco and María Soledad Izquierdo López, for their special support,

and thanks to all **GIA TEAM** and **2014-2015 Term Master Friends**

and special thanks to,

O. Barış Derici, for his help in Heavy Metal Analysis and being a guide and a brother all the time;
Yolanda Garcia Fernandez, for her full support whenever I need and also being an advisor in every situation;
Juan Luis Pinchetti, for his concern and critical advises…

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This master thesis were partially supported by IAMZ-CIHEAM Mediterranean Agronomic Institute of Zaragoza through a scholarship given to Gizem TURKMEN.

Also the help provided on ammonium analytical process by the staff of the Spanish Bank of Algae is much appreciated.

Finally, the support of the Agriculture Engineering Faculty, Soil Laboratory, Adana, in Turkey to determine heavy metal concentrations is recognized.

1. INTRODUCTION

1.1. SEAWEEDS

1.1.1. Introduction to seaweeds

Seaweeds or macroalgae are marine photosynthetic organisms that are organised by biologists systematically into three main phyla, the green algae (Chlorophyta, Chloropphyceae), brown algae (Heterokontophyta, Phaeophyceae) and red algae (Rhodophyta, Bangiophyceae and Florideohyceae). Although, these multicellular organisms are variated in morphological and structural organisation due to differences among their tissues and anatomical features, they never reach to the specialization level of the vascular plant (Braune and Guiry, 2011).

Their natural habitats are between the top of the intertidal zone and the deepest zone where they can receive the sufficient light for their growth. They are by affected diverse environmental factors, such as light, temperature, salinity, water motion and nutrient availability in the water. They may interact with their epiphytic biota such as bacteria, fungi and sessile animals and also the interaction between the macroalgae and its epiflora in among the biological interactions. In addition to these interactions, definitely their development is controlled by internal factors such as their morphology (Lobban *et al.*, 1985).

1.1.2. Usage of Seaweeds

As the macroalgae are photosynthetic organisms, they play a key role in primary production of the ocean as constituent of the basis of marine food chain. Because of their potential nutritional benefits, they have been used as a novel food in the industry and medicine for various purposes in recent years (Shalaby, 2011). According to Wijesekara *et al.* (2011), some macroalgae are excellent

nutritional sources of vitamins (De Roeck-Holtzhauer *et al.*, 1991), carbohydrates (Paiva *et al.*, 2012) and minerals (Gupta and Abu-Ghannam, 2011). On the other hand, since most of the seaweeds involve high protein level and notable amino acid composition, they have been became to be an alternative potential source of proteins. In consequence the development of novel foods in Europe, such as functional foods could be a new possibility to use of seaweeds, especially the protein-rich species, in human (McHugh, 2003) and animal (Viera *et al.*, 2005) nutrition. Moreover, they have been used as a raw material for extraction of phycocolloids such as agar, carrageenan, which is already used in biotechnology applications, and also alginates from brown macroalgae (Armisen, 1995).

FAO (2014) remarked their massive production as 25-30 million tons worldwide which is mainly produced in Asian countries (Figure 1).

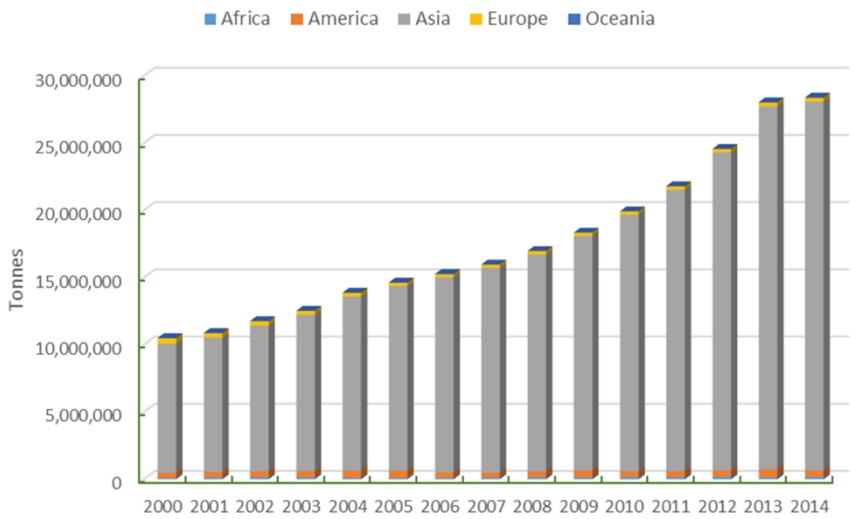

Figure 1. Annual worldwide plant production (taken from Capuzzo and McKie, 2016)

Their massive production not only to obtain alginate, agar and carrageenan from them but also to be used such as fertilizer, animal feed (e.g. fish feed), indirect source of fuel, cosmetic products, in integrated aquaculture for waste water treatment (FAO, 2014) (Figure 2).

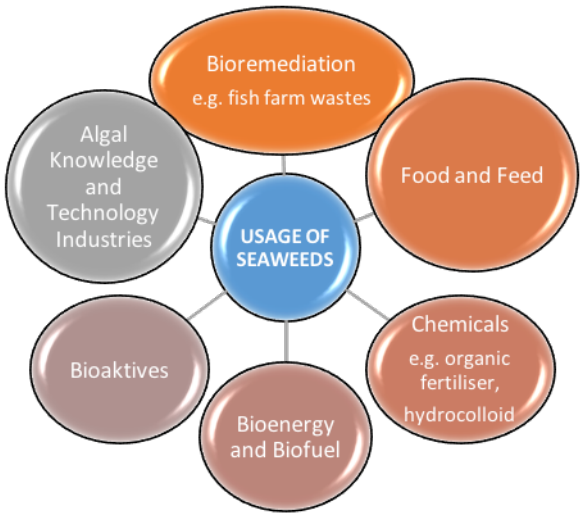

Figure 2. Worldwide main usage of seaweeds in industry and aquaculture. (taken from FAO (2014)).

According to McHugh (2003), especially in Asian countries such as China, Japan and Republic of Korea, 80 % of seaweed production is for human utilization; the rest of 20 % is used for extractions of phycocolloids, animal feed, fertilizer and water remediation in aquaculture. Moreover, as reported by Mouritsen *et al.* (2013), more than 500 species of seaweeds which belongs to 100 genera, have been used and collected. On the other hand, there are only 33 genera of seaweeds (mainly brown and red algae) harvested commercially worldwide (McHugh, 2003, Inniss *et al.*, 2016).

1.1.3. Nutrient requirements and uptake of seaweeds

In general, inorganic carbon, water, light and various mineral ions are the most important requirements in making the process of photosynthesis and

growth by seaweeds. In specific, C, H, O, P, Mg, Fe, Cu, Mn, Zn and Mo are also remarkably demanded by all algal groups. Indeed, S, K, and Ca are required by all algae groups, although they can be replaced by other elements. On the other hand, Na, Co, V, Si, Cl, B and I are demanded by only some algal groups. The values display, when the essential and non-essential elements are in available concentrations in water, seaweeds realize to accumulate them in their tissues so much more than their supply in water (Table I). Ion entry in seaweeds occur in three ways as 1) passive transport, 2) facilitated diffusion and 3) active transport (Lobban *et al.*, 1985). Among the requirements of the vitamins, vitamin $B_{12,}$ thiamine and biotin are the common ones for most of the seaweeds.

Features concerning nutrient such as nutrient uptake rates, nutrient assimilation, nutrient storage, critical tissue nutrient concentrations and growth rate should have known to realize their nutrient physiology. Nutrient uptake rates depend on some physical, chemical and biological factors. Among these factors, light, temperature, water motion, nutrient concentration, the form of the limiting nutrient, nutritional history of seaweed and life history (Harrison and Hurd, 2001) and also competitive ability, morphology of seaweeds, forms of nutrients and presence of the other nutrients are the most effective ones on nutrient uptake of seaweeds (Lobban *et al.*, 1985).

Table I. Concentrations of some essential elements in seawater and seaweeds (Lobban *et al.*, 1985).

Element	Mean concentration in sea water (μg/g)	Mean concentration in dry matter (μg/g)
H	105.00	49.500
Mg	1.290	7.300
S	905	19.400
K	406	41.100
Ca	412	14.300
C	27.3	274. 000
N	0.488	23.000
P	0.688	2.800
B	4.390	184
Zn	0.004	90
Fe	0.003	300
Cu	0.002	15
Mn	0.001	50

In addition to these factors, there are other very important factors, such as stocking density, tanks depth, water turnover-rate and also biomass harvesting frequency on nutrient uptake in seaweed cultures were described by Chopin *et al.* (2001). For instance, there are some studies on nutrient uptake by seaweeds with different temperatures (Pedersen *et al.*, 2004) and different water aeration levels that the uptake mechanism was significantly affected by both parameters (Msuya and Neori, 2008).

1.1.4. Description of the seaweeds used in the recent study

1.1.4.1. Ulva rigida (C. Agardh, 1823)

Ulva rigida is a native green alga belongs to the Phyllum Chlorophyta and the family Ulvaceae. It occurs throughout the world (Eastern Atlantic, Caribbean, Indian and Pacific Oceans). Its color differs from light green to dark green and gold color at margin when they are in reproductive season. *Ulva rigida* is generally found in nutrient rich areas (as eutrophic waters) and where wave

forces are low. Tufts of short blades with dark rhizoids are tips to identify *U. rigida*. They can tolerate stressful conditions. Optimum temperatures for *U. rigida* varies between approximately 4℃-27℃ and reasonable salinity value is 29-42 ppt (De Casabianca and Posada, 1998).

In general, due to their abundance throughout the world and their ability to adapt to various environmental stress conditions, *Ulva* species have been used in many biofiltering studies to treat wastewater from mariculture (Vandermeulen and Gordin, 1990, Cohen and Neori, 1991, Jimenez del Rio *et al.*, 1994, Hernández *et al.*, 2002, Hernández *et al.*, 2005) and also they are very valuable used as a novel food (Winberg *et al.*, 2009). In addition, as well as *Ulva sp.* are found edible for human, it was found as a suitable feed for abalone and sea urchins (Neori *et al.*, 2000, Neori *et al.*, 2004). For instance, Troell *et al.* (2006) demonstrated their faster growth feed with *Ulva sp.* then using pellet feed.

In terms of treatment the waste water, according to Jiménez del Rio et al., 1994, *Ulva sp.* is an excellent candidate for making the process of wastewater biofiltering due to their capacity to absorb and metabolize nitrogen rapidly with their remarkable growth rate. Indeed, they have been recommended as using in biofilters specially to recover large amounts of dissolved nitrogen (Fralick *et al.*, 1979, Vandermeulen and Gordin, 1990).

1.1.4.2. Caulerpa racemose (Förskall J. Agardh, 1873)

Caulerpa racemosa is a green macroalgae of the Phylum Chlorophyta and indeed in the family of Caulerpaceae. They are widely distributed in temperate and tropical seas such as Mediterranean (from Spain to Turkey) or Atlantic (Canary Islands). Because of their spherical ovate side-shoots branchets, they are

known as sea grapes in worldwide (Braune and Guiry, 2011). They can tolerate the temperature approximately from 8℃ to 28℃ and they need 30-40 ppt for optimum growth rate (Klein and Verlaque, 2008) .

There are many studies on growth conditions or ecological aspects for *Caulerpa sp*. (Carruthers *et al.*, 1993, Komatsu *et al.*, 1997) and their caulerpenyne levels (Dumay *et al.*, 2002), which are the most abundant cytotoxic, there are a very few number of studies on using them as biofilters for regulating water quality in recirculating culture systems (Chaitanawisuti *et al.*, 2011). Indeed, *C. racemosa* was used as a biofilter near nutrient-rich estuaries to avoid the eutrophication level and showed a high efficiency on both nitrogen and phosphorus uptake (Paulson and Kenosha, 2014).

1.1.4.3. Colpomenia sinuosa (Mertens ex Roth Derbes & Solier, 1851)

Colpomenia sinuoasa is a brown alga from the Phylum Ocrophyta and inside the family Scytophocaeae. This species is widely distributed in tropical to warm temperate seas all over the world. They have golden brown to light olive brown colour. Vandermeulen (1986) remarked in his early study that when the temperature levels differ from 5℃ to 20℃ effected significantly on growth rate (such as colder weather increase the growth rate), but when the salinity levels vary between 15 to 30 PSU, no significant effects were found on growth rates.

Notwithstanding this genus have essential structural role in many intertidal organisms and also widely occurrence, there are just a few of studies about their biology, history and growth rates (Wynne, 1976, Parsons, 1982, Vandermeulen and Dewreede, 1986, Toste *et al.*, 2003).

1.2. INTEGRATED MULTI-TROPHIC AQUACULTURE (IMTA) AND THE POINT OF VIEW OF SEAWEEDS IN IMTA

Chopin *et al.* (2001) stated that fed aquaculture (e.g. finfish, shrimp) requires integrated with organic and inorganic extractive aquaculture with respect of the maintain bioremediation capability, contribution of economic value by producing other marine corps and also increasing effect on cultivation unit in aquaculture industry. The obvious benefits of adopting integrated mariculture systems were emphasized by as well as Chopin *et al.* (2001). There are more findings from other studies showed the potential of the integrated systems in aquaculture (Troell *et al.*, 1999, Neori *et al.*, 2004). If extractive organisms such as seaweeds or commercial filter feeders as shellfish (Troell *et al.*, 2003) adopted in to the aquaculture, they can make the aquaculture production remain in other centuries (Neori, 2008). In addition, from the state point of long-term sustainability of aquaculture industry, the positive impacts of the integrated aquaculture were highlighted in many studies as well (Buschmann *et al.*, 1996, Troell *et al.*, 2003, Viera *et al.*, 2005).

This multi system provide re-used of the wastes as a fertilizer or food for the others (Chopin *et al.*, 2001) by dividing the multiple species into the parts (Figure 3) and integrate these multi-species in to the same system displays high production rates from semi-intensive culture systems (Neori *et al.*, 2004).

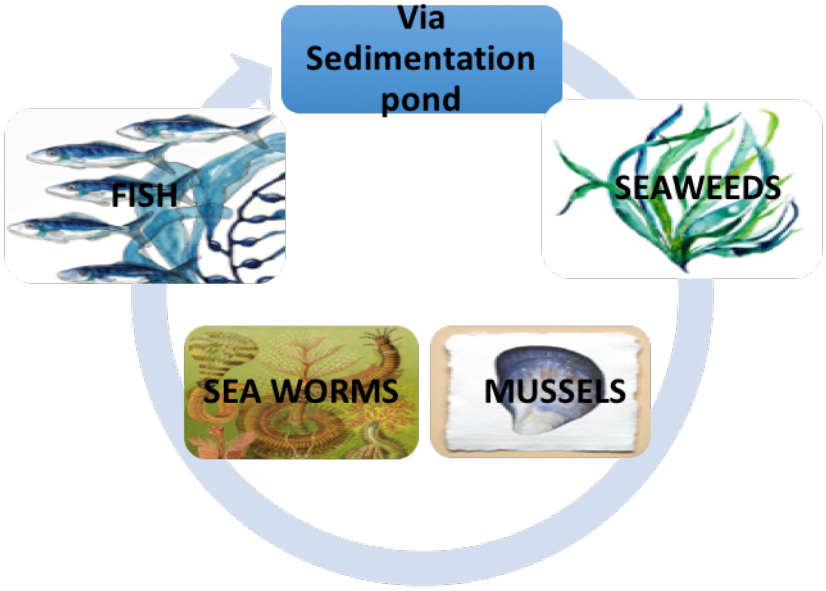

Figure 3. As an example of using multiple species in an Integrated Multi-trophic system consisting in algae, sea worms and molluscs cultivated in effluents from fish.

Waste quality and quantity are depending on the species (Figure 4), but in general, most of them released via feed into the environment (Troell *et al.*, 2003).

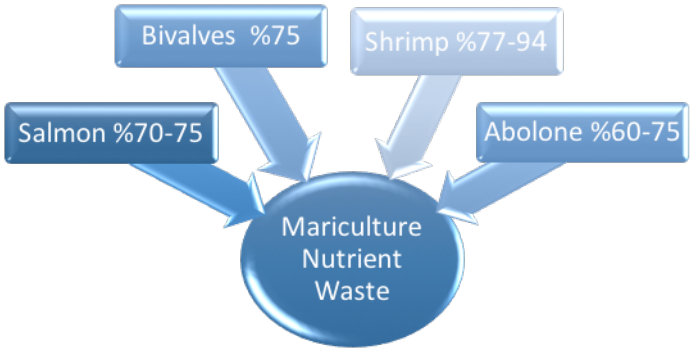

Figure 4. Percentages of nutrient discharge by different aquatic species through feeding (Troell *et al.*, 2003).

According to Lobban *et al.* (1985), seaweeds are particularly important due to their absorption of nutrients and own specific mechanisms for the storage of large amounts of nitrogen and phosphorus in their tissues. As mentioned

above (section 1.4), seaweeds are the best candidates to remove the nutrient due to the ability of their uptake and accumulation capacity in aquatic area.

Since seaweeds reduce the risk of eutrophication level effectually due to their high nutrient uptake capacity, they were suggested to be used in various industries particularly relative with the mariculture based in early studies (Ryther *et al.*, 1975, Cohen and Neori, 1991). Another study emphasized their low cost and they are energy saving when used as a water treatment (Abe and Ozaki, 1998). There have been several studies on production of seaweeds with fed aquaculture (Vandermeulen and Gordin, 1990, Neori *et al.*, 1991, Jimenez del Rio *et al.*, 1996, Troell *et al.*, 1999, Chow *et al.*, 2001, Hernández *et al.*, 2002, Neori *et al.*, 2004, Hernández *et al.*, 2005) and showed that their considerable benefits in aquaculture industry such as they can remain the clean and oxygen-rich recirculated water to be used by fishponds again.

Neori *et al.* (2004) showed that seaweeds to be used in IMTA, should have some basic criteria such as; high growth rate, tissue nitrogen content, ease cultivation, resistance to epiphytes and disease causing organisms, ease to get in to the growth environment, their market value. As an example of this, high level of nutrient willer algae, *Ulva sp.*, has been found several times as a great candidate (Vandermeulen and Gordin, 1990, Cohen and Neori, 1991, Hernández *et al.*, 2002, Hernández *et al.*, 2005) due to not only their efficiently removing up of the nutrient (e.g. N, P) but also their excellent growth rate in nutrient rich conditions. As well as some *Ulva* species, *Gracilaria* species are also recommended candidate not only since they are the main source of agar (Armisen, 1995), but also due to their high bioremediation capacity which was pointed out in several studies (Buschmann *et al.*, 1996, Viera *et al.*, 2005).

Indeed, Hernández *et al.* (2005), showed in their study which was with two macroalgae species (*Ulva rotundata*, *Gracilariopsis longissima*) using as biofilters for dissolved nutrients from *Sparus aurata* waste waters that they removed a greater percentage of phosphate and total dissolved inorganic nitrogen. Furthermore, apart from *Ulva* and *Gracilaria* species, many potential selected genera were also listed by Winberg *et al.* (2009) sort of, *Porphyra sp.*, *Asparogpsis sp*, *Grateloupia sp*., *Pterocladium sp*. *Ecklonia sp.* and *Sargassum sp.* due to their nutritional contents, fast growing, anti-viral properties and market value.

1.3. IMPORTANCE OF AMMONIUM FOR SEAWEEDS

The vital importance of nitrogen for seaweeds was discussed in earlies by Lobban *et al.* (1985), that N is major metabolic compound element of amino acids, purines, pyrimidines, porphyrins, amino sugars and amines. Moreover, this element was found to be the most limiting nutrient on algal growth in the sea. For instance, Pinchetti *et al.* (1998) reported that N availability clearly affected the specific composition of important biochemical parameters such as chlorophylls, saturated and unsaturated fatty acids, dietary fibres, ash and caloric contents, which were found directly correlated with C:N ratio dynamic.

According to Den Boer (1981), the form of the element can affect the nutrient uptake rates, as nitrogen in the form of ammonium can be taken more speedily than in the form of nitrate, urea and amino acids. From the standpoint of ammonium uptake, as well as physical factors (e.g. light, temperature, pH) there are many factors that affect the progress such as nutritional history of the plant (detailed in section 1.1.4). For instance, some seaweeds which are used to

grow under nitrogen-limiting conditions such as *Gracilaria foliifera* and *Agardhiella subulata* (D'Elia and DeBoer, 1978).

Furthermore, as reported by Lobban and Harrison (1994), renewing of the nitrogen element in the water column, in consequence of two main processes as reduction activities of bacteria and excretion by marine fauna, particularly by zooplankton community (Figure 5). As well as other studies (Foss *et al.*, 2004); according to early records, ammonium was found the primary dissolved nitrogenous metabolite form excreted by fishes (Vandermeulen and Gordin, 1990, Dosdat *et al.*, 1996, Lemarie *et al.*, 2004).

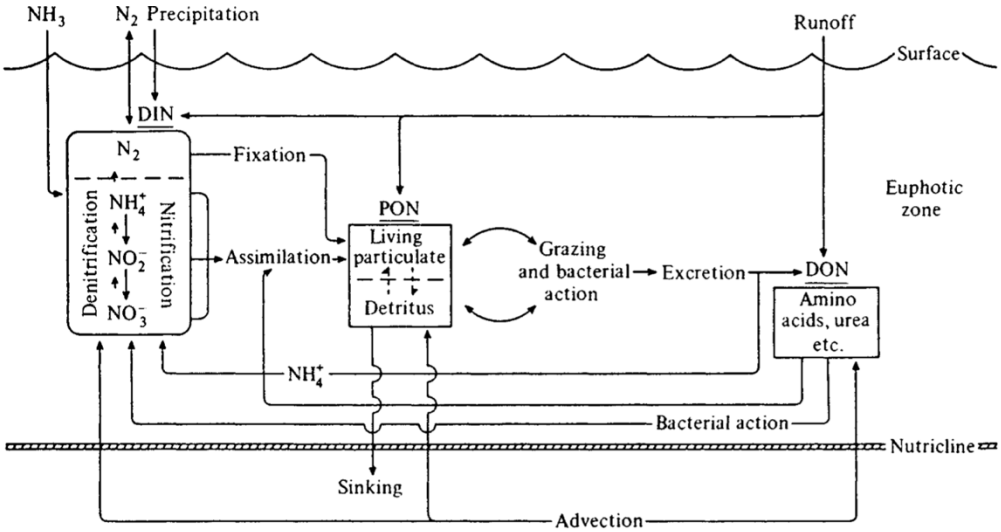

Figure 5. Nitrogen cycle in the sea regarding, PON=particulate organic nitrogen; DON=dissolved organic nitrogen; DIN= dissolved inorganic nitrogen (adopted from Lobban & Harrison, 1994)

1.4. WHY BIOFILTRATION OF AMMONIUM IS NECESSARY IN AQUACULTURE?

Since ammonium is a major metabolite from excretion of marine fauna and also their vital importance is clearly obvious for seaweeds, the biggest contrast on using seaweeds as biofilters under the mariculture effluents is placed

on NH_4 biofiltration (Cohen and Neori, 1991, Jimenez del Rio *et al.*, 1994). Thus, using seaweeds to recycle some dissolved nutrients, principally nitrogen in the form of ammonium due to it is a toxic to most commercial fishes produced by fish metabolite in aquatic environment and fed finfish/shrimp cultures (Cohen and Neori, 1991, Buschmann *et al.*, 1996, Neori *et al.*, 2004). For instance, Jones *et al.* (2001) found that how ammonium concentration (more than %95) reduce in two hours in an integrated treatment of mariculture effluents with seaweeds.

Treated water, in terms of ammonium, by using seaweeds especially with the commercial ones is becoming more importantly due to pollution risk via metabolites and uneaten feed not only in intensive fish farms, but also in marine offshore systems (Hernández *et al.*, 2002, Hernández *et al.*, 2005, Troell *et al.*, 2009).

1.5. HEAVY METAL CONCENTRATION LEVELS IN MACROALGAE SPECIES

1.5.1. Introduction to heavy metals and their relations with seaweeds

The term of 'Heavy metal' has been generally used due to those metals have atomic numbers higher than iron (59) or density higher than 5 mL^{-1} (Lobban *et al.*, 1985). According to Wood (1974), metals are classified in to three categories: 1) non critical; 2) toxic but very insoluble or rare 3) very toxic and relatively accessible (Table II) in terms of environmental pollution. Heavy metals such as Fe, Cu, Zn and Co are sampled as category three, however they are referred as essential nutrients and can limit the algal growth (see Table I in section 1.1.4). According to this underline, Cu is active in electron transport in photosynthesis, Co is a component of vitamin B_{12} and also Zn is found important for ribosome structure (Den Boer, 1981). Also metals, such as Hg or Pb are not

required for growth by seaweeds and also can be very toxic at very low (10-50 μg L^{-1}) concentrations (Lobban *et al.*, 1985).

Table II. Classification of elements due their toxicity and availability (adopted from Lobban *et al.* (1985))

Noncritical	Toxic but very insoluble or very rare	Very toxic and relatively accessible
Na, C, F, K, Mg, Fe, Rb, Ca, S, Sr, H, Cl, Al, O, Br, Si, N	Ti, Ga, Hf, La, Zr, Os, W, Rh, Nb, Ir, Ta, Ru, Re, Ba	Be, As, Au, Co, Se, Hg, Ni, Te, Tl, Cu, Pd, Pb, Zn, Ag, Sb, Sn, Cd, Bi, Pt

Metals exist in dissolved or particulate forms in an aquatic environment. Their forms as physical and chemical are controlled by environmental factors such as pH, redox potential, ionic strength, salinity, alkalinity, presence of organic and particulate matter.

Although there are many treatments on the removal of heavy metals from water and waste water, such as ion exchange, solvent extraction, reverse osmosis and adsorption they are relatively expensive and suitable for high concentrations (Babel and Kurniawan, 2003). Moreover, biosorption by living materials is one of the most effective technique to remove the toxic heavy metals from waste water (Pagnanelli *et al.*, 2000).

1.5.2. Heavy metal uptake and accumulation by seaweeds

Seaweed take up metals, when those are biologically available, both passively (an initial rapid) and actively (much slower). Some metals such as Pb and Sr can be adsorbed passively, on the other hand some such as Zn and Cd can be taken actively. Physical factors such as light and chemical factors such as existing of other nutrients can affect the metal uptake by seaweeds (Lobban *et*

al., 1985). Metal ions can be adsorbed by seaweeds onto cell surface within a short time (few seconds or in minutes) during the passive uptake. On the other hand, transporting of metal ions across the cell membrane into cytoplasm in relatively long time (Gadd, 1988).

There are several studies aimed to the removal of the metal ions from aquatic environments since the toxicity of heavy metals released into the environment (Kuyucak and Volesky, 1989, Fourest and Volesky, 1997, Bergasa *et al.*, 2007). Most of the studies focused on particularly seaweeds as a biologic material to remove the metals from industrial water containing heavy metals, since they are low cost and also have excellent absorbing capacity (Jin-Fen *et al.*, 2000). Due to the algae have genetically physiological tolerance to heavy metals (Lobban *et al.*, 1985), algae are advised for their use at recycle water, both in industries (Valdman and Leite, 2000) and as part of an Integrated Aquaculture; in case of they are able to be edible or not (Hernández *et al.*, 2005) and as a pollution index as well (Chardhry *et al.*, 2013). Indeed, there are many reports focused on particularly metal adsorption such as Co, Cd, Cr, Cu, Ni, Pb, Zn, not only by seaweeds (Vijayaraghavan *et al.*, 2005), but also by microalgae (Gardea-Torresday, 1988, Sandau *et al.*, 1996, Mohapatra and Gupta, 2005).

In addition, there are some reviews on heavy metals uptake by *Ulva rigida, Caulerpa racemosa* and *Colpomenia sinuosa* (Simeonova and Petkova, 2007, Dekhil *et al.*, 2011) which is one of the objectives of the present study. Those studies found that they are efficient biosorbent materials for heavy metal ions and suitable natural absorbents especially in wastewaters.

1.6. OBJECTIVES

Due to increasing aquaculture demand, sustainable approaches has been searched especially in last decade. Thus, Integrated Aquaculture is becoming more popular in recent years due to their positive environmental and socio-economic benefits for fed aquaculture industry. Since macroalgae have played an important role as a biofilter, they have been used in aquaculture farms, coastal areas with aquaculture effluents and also eutrophicated estuaries (Chopin *et al.*, 2001). Their usage is suggested influentially not only due to their high biofilter capacities of excess nutrient and pollutants, and also for their commercial values such as alginate, agar and carrageenan production, food, fertilizer, cosmetic products and also biofuels as well. Indeed, this system provides access to rich production using multi species simultaneously.

The present study concern the importance and main functions of macroalgae as well as how seaweeds may regulate ammonium uptake and heavy metal uptake, in the aquaculture systems and nature, since they are toxic to fish and other aquatic organism at elevated concentrations.

Therefore, the main objective is to provide a better understanding of uptake capabilities of three macroalgae species used: *Ulva rigida, Caulerpa racemose* and *Colpomenia sinuosa* inside a biofilter system located in the Scientific and Technological Marine Park of the ULPGC in Taliarte. The uptake capabilities measured were those of nitrogen in the form of ammonium as well as heavy metals (Cu, Mn, Fe, Zn, Cr, Ni, Pb, Cd). In addition, to determine their growth rates in the biofilter system of an experimental production unit, they were cultivated in outdoor conditions during four weeks.

Therefore, the complementary objectives of present study were;

1. To examine algae growth inside the aquaculture effluents and also in outdoor tank cultures in relation with environmental factors.
2. To access the capability of three algae species to biofilter ammonium in a specific time (three hours) and to determine which specie has the rapid ammonium removal after blocking of the nutrient income to the tanks,
3. To determine the heavy metal contents of the three macroalgae species which are growing in biofilter of PCTM,
4. To increase the knowledge about these three species, whether if they are suitable for using as a treatment of aquaculture waste water, not only scrubbing excess ammonium, but also as remediation tool of high heavy metal concentration.

2. MATERIALS & METHODS

2.1. EXPERIMENTAL SITE DESCRIPTION AND SETUP

The experimental set up was done at the integrated culture facilities of the Research Group in Aquaculture (GIA; ULPGC) and at the Marine Scientific and Technologic Park of University of Las Palmas de Gran Canaria at Taliarte (PCTM-ULPGC) that are located in the Eastern Side of the Island of Gran Canaria, (Telde, Spain.). The outdoor tanks (detailed below in 3.2.1) used for the experiment were located in the former Canarian Institute of Marine Science building. The enriched sea water was pumped directly to tanks from the Sedimentation tank (pond of 11 m^3), situated outside near the greenhouse in a complimentary building, where the excretory products from the mariculture and the uneaten feed increased the nutrient concentration in the water column. Feeding rate of the fishes was approximately 9,5 kg/day inside this facility. By the large, the feed ratio and feeding time (8 am to 10 am) was constant during the experiment. The biofilter facility situated outdoor at PCTM-ULPGC (PCTM) was used to select the algal culture and collecting the fresh algae for both algal experiment (described below in 3.2.1), which is designed as a staircase with 8 cascades, has 160 m^3 total volume approximately and 30 renovations a day. After passing through the algal biomasses, the water from the Biofilter system is pumped to a nearby discharge sea point.

2.2. ALGAL CULTURES

2.2.2. Species selection and tank designs

The three species: *U. rigida*, *C. racemosa* and *C. sinuosa* (detailed above in section 2.2.1) were used in all experiments (Figure 6). Thalli of *U. rigida* was collected from tanks which have been culturing under mariculture influents for years to use as feed for abalone in the old facility and thalli of *C. racemosa* and *C. sinuosa* were collected from biofilter facility in PCTM.

Figure 6. Displays the species of A) *Ulva rigida*, B) *Caulerpa racemosa* and C) *Colpomenia sinuosa* (27th of September, the very first sampling day)

Species were selected via direct observation about estimated amounts of all the algae occur in biofilter (from September 2015 to September 2016) to find out which species were dominant in the Biofilter system at PCTM. These three species (*U. rigida*, *C. racemosa*, *C. sinuosa*) were observed as dominant species in the Biofilter system all the year around. They were chosen particularly, since they existed in the Biofilter system in early Autumn much more than the rest of the year (Figure 7).

Thalli of *U. rigida* wasn't picked up from biofilter in PCTM considering they didn't exist in large biomass at September 2016 in biofilter, because they

were removed due to their massive abundance during the summer months of 2016. For that reason, *U. rigida* was collected from the tanks which are described above. The fresh and healthy thalli of each species were selected from their colonies when they were collected for the experiment.

Figure 7. Amount of the Algae in the Biofilter system at PCTM-ULPGC in Autumn, A) *Caulerpa* amount at 7/10/2015, B) *Colpomenia* amount at 7/10/2015, C) *Ulva* amount at 11/11/2015

All the experiments were conducted in 0,2 m^2 surface, 90 L, PVC tanks. All the species were unattached and kept suspended in water column by strong aeration system as a plastic tubes with holes that were located on the bottom of each tank. Via this aeration tubes, all the algae in each tank obtained the sun light periodically.

Each tank had a filter on the top of the tanks (10 cm deep) due to filter the water in the tanks from falling out organisms. The filters were connected with the draining tubes that threw out the drain water outside (Figure 8). The day before the experiment began, the tanks were cleaned with bleach and washed with fresh water, then they were washed with sea water and filled up.

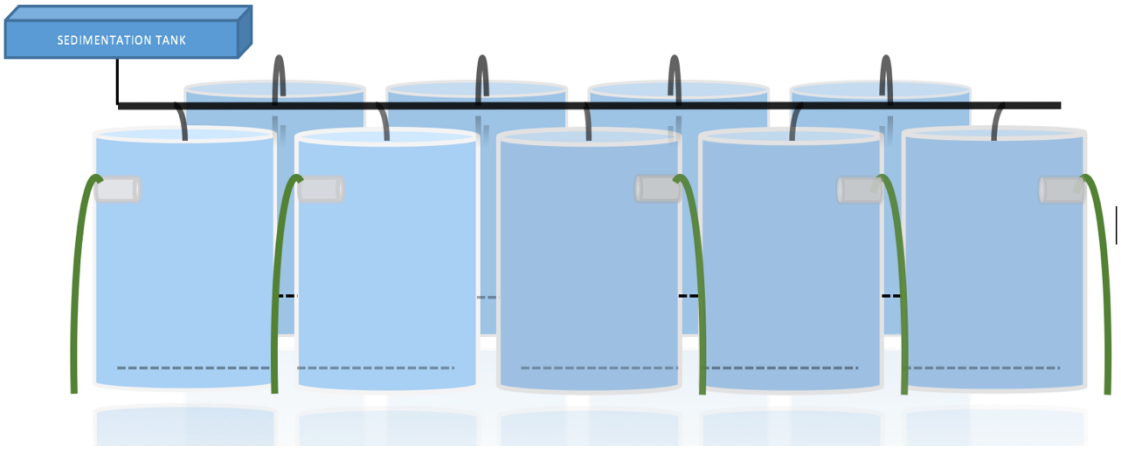

Figure 8. The outdoor tank design with water input coming from the sedimentation tank. The filters are on the top which are connected with draining tubes and the aeration tubes are on the bottom.

Figure 9. A) Water intake of the tanks, B) Tanks were covered in half by black nets to reduce strong light intensity.

Renovation of the tanks was set up as 8 volumes/day (turnover rate) for each tank. After stocking the algae to the tanks (detailed in 3.2.2), each tank was covered in half by black nets to prevent the algae from the excess sun light coming directly into the tanks (Figure 9).

2.2.3. Culturing techniques and maintenance

The four-week experiment was carried out in outdoor conditions from 28th of September 2016 to 26th of October 2016. Nine tanks were used in all the experiment. Algae were collected early in the morning at 27th of September. The collected algae were washed with clean sea water and the visible epiphytes and epifauna were removed by gently brushing and wiping in laboratory of GIA. At the same day, the tanks were stocked at wet weight in the tanks as 2 g/L for *U. rigida* and 3 g/L for *C. racemosa* and *C. sinuoasa*. Each species had triplicates and all the triplicates of each species had the same conditions in the tanks. Algae had been adopted to the tank conditions for 5 days before started to the experiment.

The algae were harvested weekly and the epiphytes were removed gently by hand and then they were placed in plastic baskets to drain the excess water on them, and then weighed their wet weights. The data were used to determine the yield and calculate the specific growth rates (d^{-1}) (Figure 10). After obtaining wet weights, they were replaced at the initial densities each week after harvesting. When they were overweight from original weights, the bad thalli of each species from triplicates were selected from the tanks and removed. On the other hand, when they were underweight from initial densities, the fresh thalli of each species were collected again as before (described above) to re-stocked to their initial densities.

Figure 10. A) Gain weight in harvesting (New fronds on *Caulerpa racemosa*), B) Algae were drained from excess water and then weighed

The tanks were cleaned by rinsing via freshwater after harvesting weekly and also the algae were separated from their epiphytes and epifauna gently at the same day. All the factors on the tanks were checked daily during the experiment weeks. Renovation per day of the tanks and aeration in the tanks were stabilized as much as possible during four-week experiment.

Water temperatures measurements were taken during weekdays, from the seawater, with Oxygurad, Handy Polaris (OxyGuard International A/S, Farum, Denmark). Sensivity of the pH meter was ±0.1. pH values measured during weekdays by taking 100 mL water sample from seawater. pH meter (pH-Meter Basic 20+, CRISON, Barcelona, Spain) calibrated before each use and measurements repeated until the values were stable. Sensitivity of the pH meter was ±0.01.

Photon flux density was obtained by LI-COR data logger during the experimental period, provided by the Spanish Bank of Algae (BEA).

2.2.4. Calculations of growth rate of algae

Growth rate was recorded weekly by measuring the wet weight gained of 3 triplicates of each specie (detailed above in 3.2.1). The specific growth rate (SGR) as percentage increase in wet weight for each species was calculated using the method described below by D'Elia and DeBoer (1978);

$$SGR\ (\%) = 100 * Ln(W_T/W_0)/t$$

Where, W_0=Initial biomass, W_T=Biomass at day t and t=days

2.3. AMMONIUM UPTAKE ANALYSIS FROM OUTDOOR TANK CULTURES

The biofiltering capacity for ammonium was basically determined by using the modified method based on disappearance of nutrient from medium measured calorimetrically (Harrison & Druehl, 1982). The day after harvesting algae, fifty ml of water samples were collected at every one hour intervals up to 3 hours from 9:30 am to 1:30 pm from each triplicated tanks of algae during the experimental period. First sampling was referred as 'time 0' at 9:30, were collected at inflow and outflow from the tanks. After the first sampling, the water valve was turned off for 3 hours and the samples were taken from the water columns of the tanks. Although, water exchange was stopped during the 3 hours, the aeration was kept constant in the tanks. In addition, for the estimation of the initial state of ammonium concentration, water samples were collected from sedimentation tank at the first sampling hour each week.

Pooled samples were stored in +4 ℃ in the laboratory of GIA till their analysis. The analyses were carried out in BEA at the same day after sampling period. Ammonium concentration was determined according to phenol-hypochlorite method by (Parsons *et al.*, 1984). This method basically relies on

the measurement of an indophenol colour (occurs green-blue) formed by ammonium in the presence of sodium nitroprusside after oxidation with hypochlorite and phenol reagents (Figure 11).

The uptake rates were calculated from week two to four for each species according to Cohen and Neori (1991)as below;

$$V = f\,(Si - So)$$

Where; V= Uptake rate (µmoles L^{-1} h^{-1}), f= Flow rate (number of exchanges h^{-1}) Si= Ammonia-N concentration at inflow

So= Ammonia-N concentration at outflow

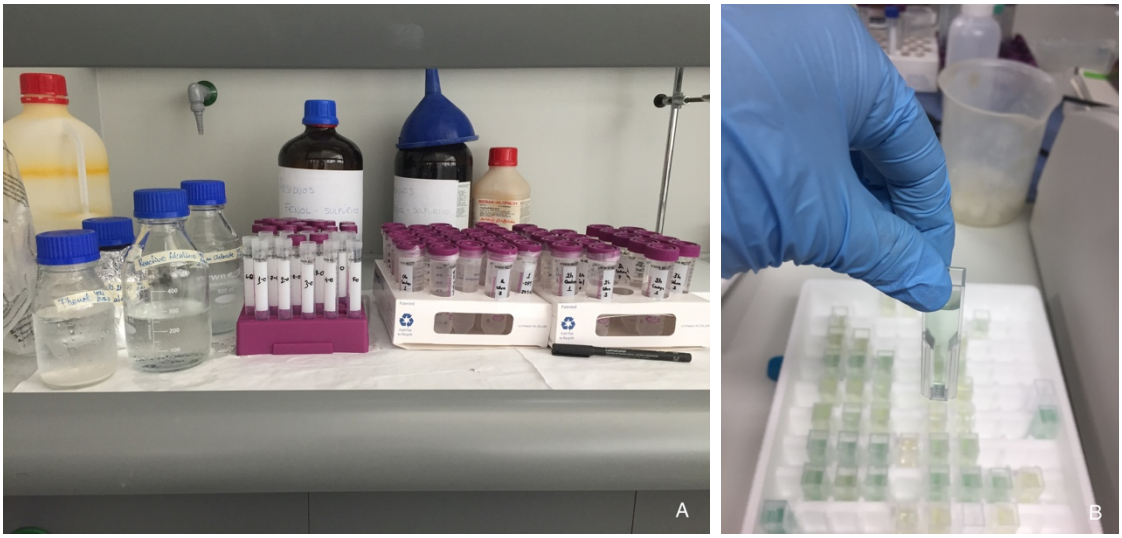

Figure 11. A) Samples from 0h to 3h B) Indophenol colour from the samples

2.4. HEAVY METAL CONCENTRATION LEVELS IN THE THREE MACROALGAE SPECIES FROM BIOLFILTER IN PCTM

2.4.1. Sampling from biofilter in PCTM and preparations of the macrolgae for the analysis

To measure the tissue content of heavy metals (Cu, Mn, Fe, Zn, Cr, Ni, Pb, and Cd) in the same algae (*U. rigida*, *C. racemosa* and *C. sinuosa*) samples were collected and, later measured in the Laboratory of Soil Sciences in Department of

Agriculture Engineering at Çukurova University (SSAE) located in Adana, Turkey. The sampling was carried out early in the morning at 24/07/2016 from the Biofilter system in PCTM-ULPGC. Triplicated samples were collected for each species from different sampling points as first samplings were from first column, the second ones were from the middle and the last ones were from last point on cascade type biofilter (detailed above in 3.1). Afterwards the algae were taken to the laboratory of GIA and the microorganisms were removed from thalli of each species and then washed with clean fresh water. Ensuing steps were weighing their wet weights and drying them for 24 h at 60 °C in oven-dry.

2.4.2. Heavy metal determinations in macroalgae

They were moved to SSAE to analyse the tissue heavy metal content at 02/08/2016. Before they were analysed, the drying samples were homogenized by grinding in an agate mortar. 0.2±0.3 g of dried samples were weighed and added with 7 ml of nitric acid (%65) to the Teflon tubes for predigesting overnight. After predigesting, 2 ml of hydrogen peroxide was added to the samples and the samples were treated in microwave-oven (CEM MarsXpress 240/50) at 200°C about 1 hour. After digestion, the solutions were diluted to 20 ml of final volume with Milli-Q water (Zarcinas *et al.*, 1987). Concentrations of Cu, Mn, Fe, Zn, Cr, Ni, Pb, Cd were determined by an Atomic Absorption Spectrophometer with a graphite tube atomizer (Analytik Jenna ContraAA 700) (Figure 12).

Figure 12 A) Weighing grinding of the dried algae B) Samples were treated in Microwave C) Atomic Absorption Spectrophometer ContraAA with the part of graphite tube atomizer

2.5. STATISTICAL ANALYSIS

All the data presented in tables and figures were given as a mean ± standard deviation. All data were analyzed for their normality and homogeneity using Levene's test and followed by One-way or Two-way ANOVA (heavy metals), to differentiate the effects of zones and species. Zones and species were also evaluated with One-way ANOVA tests to specify the differences between groups. Means were compared by post-hoc tests ($P<0.05$) using a SPSS software (IBM SPSS for Mac 21.0; SPSS Inc., Chicago, IL, USA).

3. RESULTS

3.1. ALGAL CULTURES

3.1.1. Growth and environmental factors

Ulva rigida average biomass was higher at the end of the cultivation periods (4 week) in comparison to *C. racemosa* and *C. sinuosa* (Table 3). The average biomass of *U. rigida*, *C. racemosa* and *C. sinuosa* were 429.9±142.9, 220.2±57.5 and 288±60.1, respectively (Table III and Figure 13).

Table III. Environmental and Growth parameters of *Ulva rigida*, *Caulerpa racemosa* and *Colpomenia sinuosa* after 4 consecutive 7-day cultivation period in tanks. Values are mean±SD (n=4). Different letters denote significant difference between average values.

	Total biomass (gr)					
Species	**Initial**	**1st week**	**2nd week**	**3rd week**	**4th week**	**Average**
U. rigida	180	583.3±72.3	490±60.8	398.3±45.4	248±27.8	429.9±142.9
C. racemosa	270	257.3±20.1	190±17.3	278.3±42.5	155±32.8	220.2±57.5
C. sinuosa	270	360±26.5	313.7±29.9	228.3±10.4	250±36.1	288±60.1
	SGR (%)					
U. rigida		16.7±1.8	14.2±1.7	11.3±1.7	4.5±1.6	12.4±5.3^{A}
C. racemosa		-0.7±1.1	-5.1±1.3	0.3±2.30	-8.2±3.1	-2.9±3.9^{B}
C. sinuosa		4.1±1.0	2.1±1.3	-2.4±0.6	-1.2±2.0	0.9±3.0^{B}
	Biomass increase (%)					
U. rigida		224	172	121	38	139
C. racemosa		-5	-30	3	-43	-18
C. sinuosa		33	16	-15	-7	7
	Temperature (°C)					
Weekly average		23.29±0.09	23.29±0.09	23.31±0.07	23.31±0.21	23.34±0.14
	PAR (µmol photons/m²s)					
Weekly average		82.87±3.2	70.12±7.63	69.62±13.49	71.5±5.97	72.92±9.91
Weekly cumulative		499.35	490.81	487.33	357.49	
	pH					
Weekly average		8.13±0.06	8.22±0.03	8.21±0.05	8.25±0.03	

Algal growth were equal to 139% biomass increase for *U. rigida,* -18% for *C. racemosa* and 7% for *C. sinuosa* in average of four weeks (Figure 13). *Ulva rigida* showed highest biomass increase by 224% in the first week while growth was 38% in the 4th week of the study and the biomass increased by different ratios through the experimental period (Table III).

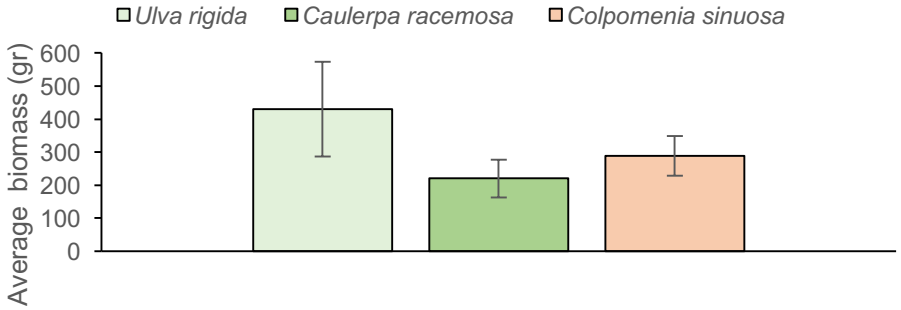

Figure 13. Average weights (gr) of *Ulva rigida*, *Caulerpa racemosa* and *Colpomenia sinuosa* after a week cultivation period. Values are means of SGR values from triplicates for 4 weeks (n=4). Error bars = SD.

Caulerpa racemosa biomass were increased only in the 3rd week by 0.3% and the rest of the experimental period showed decrease in average biomass (Table III). *Colpomenia sinuosa* biomass increased during the first (33%) and second weeks (16%) of the study however, biomass decreased in the last two weeks of the experiment by -2.4% and -1.2, respectively. As observed in average biomass, SGR of the *U. rigida* was the higher (12.4±5.) than *C. racemosa* (-2.9±3.9) and *C. sinuosa* (0.9±3.0) (P>0.05) (Figure 14).

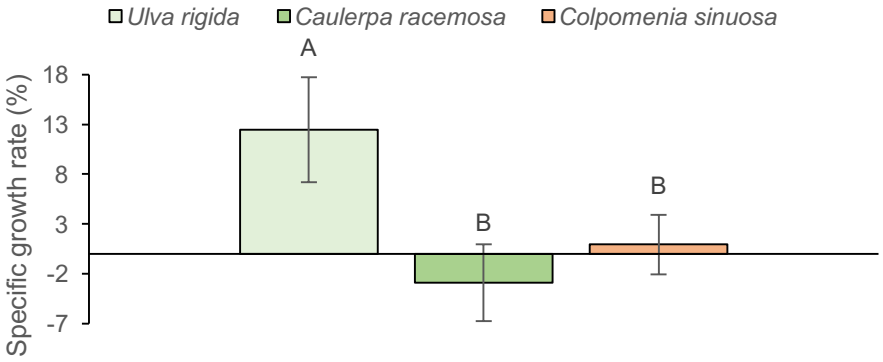

Figure 14. Average of Specific Growth Rate in *Ulva rigida*, *Caulerpa racemosa* and *Colpomenia sinuosa* after 4 consecutive periods of 7 days cultivation . Values are means of SGR values from triplicates for 4 weeks (n=4). Error bars = SD. Different letters denote significant difference between average values.

Water temperatures were 23.29±0.09, 23.29±0.09, 23.31±0.07 and 23.31±0.21 ^{o}C from week 1 to week 4, respectively (Table III). Avarage water temperature of four weeks was 23.34±0.14 ^{o}C. The PAR values were 82.87±3.2 μmol photons/m^2s at week 1, 70.12±7.63 μmol photons/m^2s at week 2, 69.62±13.49 at week 3 and 71.5±5.97 μmol photons/m^2s at week 4 (Table III). Cumulative PAR values showed decreasing trend from week 1 to week 4 and the cumulative PAR values were 499.35, 490.81, 487.33, 357.49 μmol photons/m^2s, respectively. The pH values of the seawater were almost similar during the experiment however, there was a tendency to increase from week 1 to 4. The highest pH value recorded at week 4 8.25±0.03 and the lowest was at week 1 by 8.13±0.06 (Table III).

3.1.2. Ammonium uptake of three macroalgae species

Ammonium concentration was measured during the four weeks at the same day, sampling procedure was explained in detail in materials and methods section. At the time of the sampling, biofilter water NH_4^+ concentrations were 17.64, 4.71, 7.28 and 5.14 μmol/L, respectively (Table 4).

Tank outlet of NH_4^+ concentrations at week 1 at 0 hour were similar for all three algae species however, there was a tendency to have higher concentration *C. sinuosa* water outlet (12.72±3.08) in comparison to *C. racemosa* (7.39±1.1 μmol/L) and *U. rigida* (5.76±4.77 μmol/L) ($P>0.05$). This tendency led significant differences after 1 hour *C. sinuosa* (10.58±2.88 μmol/L) had the highest concentration and there was no difference between *C. racemosa* and *U. rigida* ($P<0.01$) (Table 4). *U. rigida* could remove all NH_4^+ at hour 2. At the same sampling point *C. sinuosa* (7.21±2.59 μmol/L) had the higher NH_4^+ concentration

when compared with *C. racemosa* (2.28±0.97 µmol/L) (P<0.01). NH_4^+ concentration was dropped to 0 at hour 3 in *C. racemosa* tanks outlet (Table 4). Even at hour 3, *C. sinuosa* NH_4^+ concentration was 4.42±1.83 µmol/L. In summary for the first week 17.64 NH_4^+ concentration was cleared to 0 by *U. rigida* at hour 2, at hour 3 by *C. racemosa and C. sinuosa* could not remove NH_4^+ during the sampling times. At week 2, water NH_4^+ concentration (4.71±0.64 µmol/L) was the lowest of all 4-week experimental period. (Table IV).

Table IV. Nitrogen uptake of *Ulva rigida*, *Caulerpa racemosa* and *Colpomenia sinuosa* after 4 consecutive 7-day cultivation period in tanks. Values are mean±SD (n=4). Different letters denote significant difference between average values.

			U. rigida	***C. racemosa***	***C. sinuosa***	***ANOVA***
	Hour	**Water NH_4^+** (µmol/L)	**Tank outlet** (µmol/L)	**Tank outlet** (µmol/L)	**Tank outlet** (µmol/L)	
Week 1	**0**	17.64±0.41	5.76±4.77	7.39±1.1	12.72±3.08	n.d.
	1		^{B}0.65±0.29	^{B}3.59±1.32	^{A}10.58±2.88	*
	2		0	^{B}2.28±0.97	^{A}7.21±2.59	*
	3		0	0	4.42±1.83	-
Week 2	**0**	4.71±0.64	1.16±0.95	2.21±0.8	1.78±0.71	n.d.
	1		0	1.16±1.01	1.3±0.6	n.d.
	2		0	0	0	-
	3		0	0	0	-
Week 3	**0**	7.28±0.31	^{B}2.43±0.27	^{B}3.08±0.64	^{A}6.3±0.11	**
	1		^{B}1.52±0.11	^{B}1.88±0.31	^{A}4.24±0.22	**
	2		0	1.41±0	^{A}3.7±0.19	**
	3		0	0	2.83±0.06	-
Week 4	**0**	5.14±0.01	1.3±0.11	1.99±1.01	1.74±0.19	n.d.
	1		0	^{B}1.16±0.06	^{A}1.34±0.06	**
	2		0	0	1.05±0.13	-
	3		0	0	0	-

Thus, all three species could remove NH_4^+ from water. *U. rigida* used all the NH_4^+ from water at hour 1, *C. racemosa* at hour 2 and *C. sinuosa* at hour 3 (Table 4). No

differences found in NH_4^+ concentration of tank outlets at the week 2 of the experiment ($P>0.05$). At week 3, water NH_4^+ level was 7.28±0.31 μmol/L and significant differences were found among algal species from hour 0 to 2, the highest NH_4^+ concentration found at *C. sinuosa* ($P>0.05$) while *C. racemosa* and *U. rigida* were similar ($P<0.05$). Nevertheless, NH_4^+ decreased the 0 at hour 2 by *Ulva rigida* and hour 3 by *C. racemosa* (Table 4). Comparable trend observed at week 4 and week 2 and water NH_4^+ concentrations were similar. *U. rigida* used all the NH_4^+ from water at hour 1, *C. racemosa* at hour 2 and *C. sinuosa* at hour 3 (Table 4). Chances in NH_4^+ concentrations were also shown in 4 different graphs for all 4 weeks in figure 15.

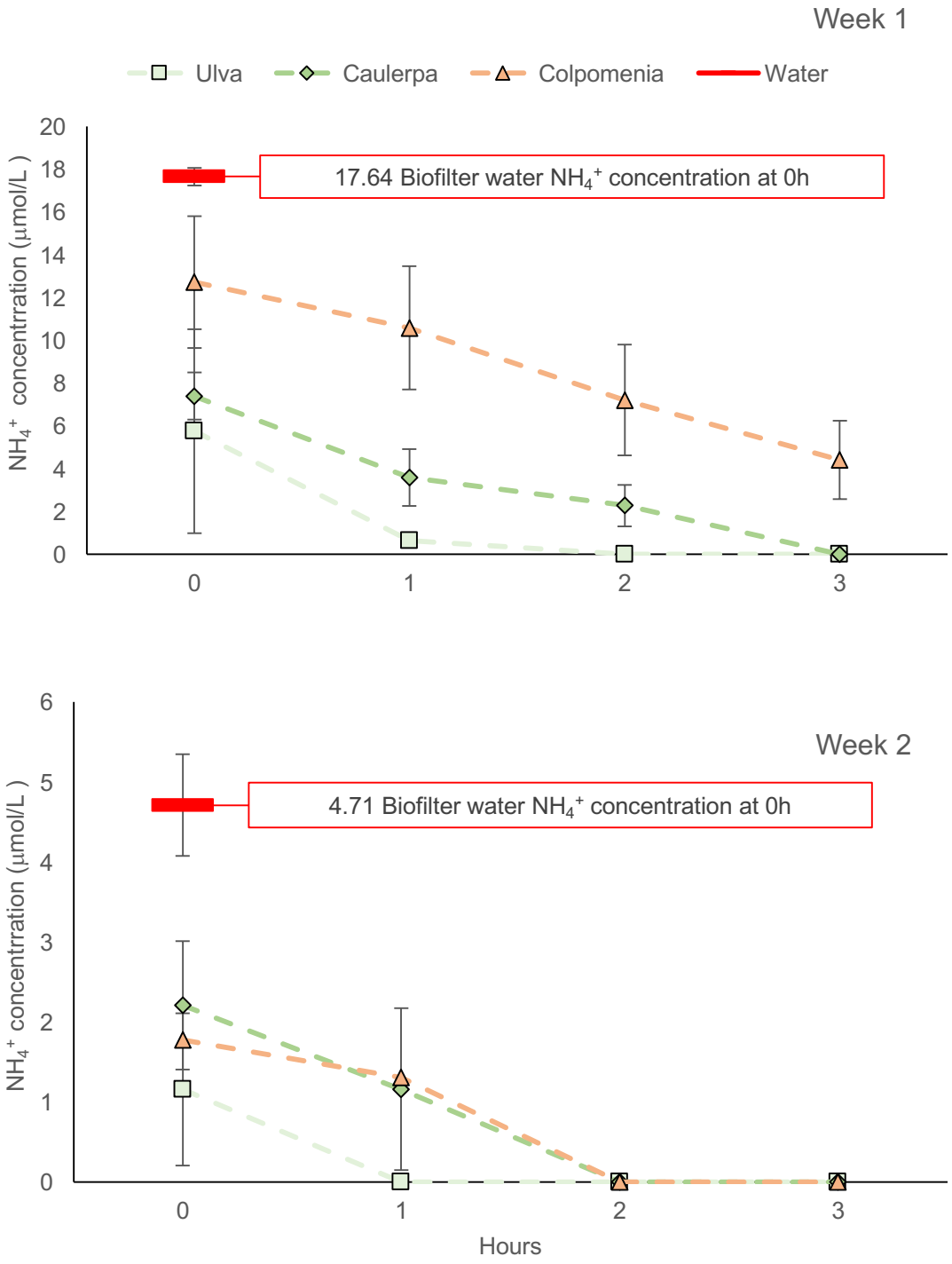

Figure 15. Water inlet NH_4^+ levels and utilization of NH_4^+ by three different macroalgae species (*Ulva rigida, Caulerpa racemosa and Colpomenia sinuosa*) during 3 hours.

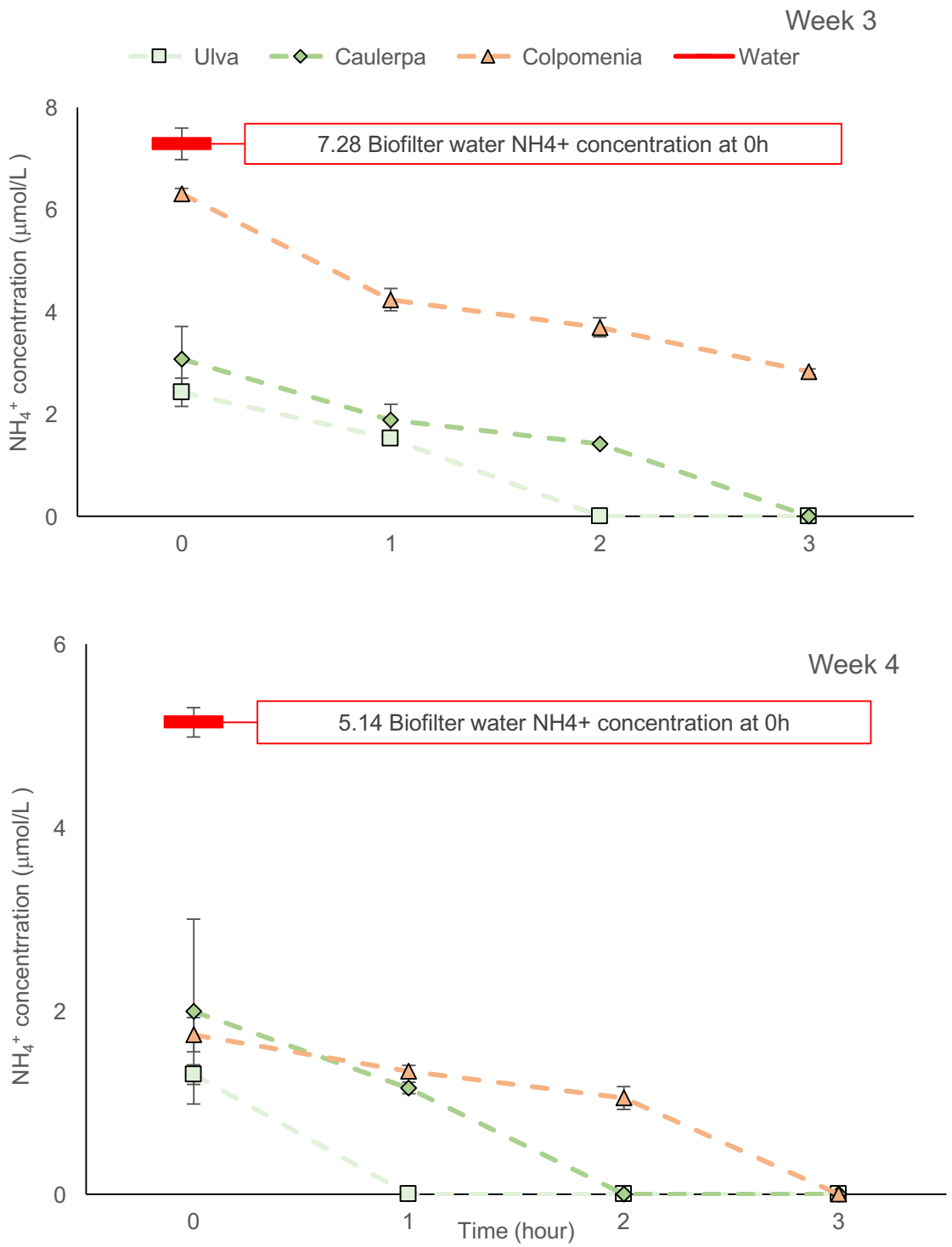

Figure 15. ***(Continued)*** Water inlet NH_4^+ levels and utilization of NH_4^+ by three different macroalgae species (*Ulva rigida, Caulerpa racemosa and Colpomenia sinuosa*) during 3 hours.

3.2. HEAVY METAL CONCENTRATIONS OF THREE MACROALGAE SPECIES

Heavy metal concentration were tested from the three different zones inside the Biofilter system: close to water inlet zone, middle zone and the end of the biofilter. Only Pb were significantly influenced by the biofilter zone whereas the rest of the heavy metals tested were similar among the zones. However, if we compared the heavy metal concentration and the macroalgal species, there were some differences for five heavy metals: Cu, Mn, Fn, Zr, Cb and Ni. Regardless of the zones when species were tested individually for 9 samples Cu concentration was higher in *U. rigida* then *C. racemosa* and *C. sinuosa* ($P<0.05$) (Table 5). *Colpomenia sinuosa* showed lower Mn values then the two macroalgae tested ($P<0.05$) (Table V), Fe was higher in *C. racemosa* than in *C. sinuosa* ($P<0.05$). On the other hand, Fe concentration was similar *U. rigida* if compared with the other two macroalgae specie ($P>0.05$) (Table 5). In the case of Zn, concentration were higher in *U. rigida* followed by *C. racemosa* and then *C. sinuosa* ($P>0.05$). Cr, Pb and Cd were similar in three algae species. And lastly, Ni concentration were lower in *C. sinuosa* ($P<0.05$) then *C. racemosa* and there was no difference with *U. rigida* ($P>0.05$) (Table 5). Chances in heavy metal concentrations were also shown in 8 different graphs for both species in figure 16.

Table V. Heavy metal concentration (mg/kg) in three different macroalgae species found at 3 zones inside the biofilter system of Scientific Marine Technology Park of University of Las Palmas de Gran Canaria (PCMT-ULPGC)(n=3).

	Ulva rigida (mg/kg)			***Caulerpa racemosa*** (mg/kg)			***Colpomenia sinuosa*** (mg/kg)			**2-way ANOVA**		
	1	2	3	1	2	3	1	2	3	**Zone (Z)**	**Specie (S)**	**ZxS**
Cu	12.7±0.1	14.3±1.5	13.8±2.5	2.9±0.1	4±0.8	11.5±1.4	8.2±2.7	6.1±0.7	9.2±4	nd	***	nd
Mn	40.8±4.6	29.7±0.3	36.3±1.6	16.4±0.8	34.3±4	82.8±9.3	16.1±0.5	8.9±0.1	11.1±0.8	nd	***	nd
Fe	1023.7±19.8	823.5±12.4	881.2±9.8	592.9±56.1	1137.2±15	2056.6±28.3	564.2±8.1	322.5±7.6	377.7±29.9	nd	nd	nd
Zn	145±1.1	153.2±1.1	160.6±0.1	82.8±4.1	97.2±1.7	117.8±7.1	31.2±5	26.5±4.7	23.9±6.9	nd	***	nd
Cr	5.9±0.1	4.1±0.3	5.3±0.8	5.2±0.3	6.1±0.9	35±0	3.4±0.4	2±0.2	2.7±0.6	nd	*	nd
Ni	9.9±0.6	9±0.2	8.5±0	6.1±0.4	7.8±0.8	32.5±1.5	4.3±0.5	3.1±0.5	4.5±0.6	nd	nd	nd
Pb	0.2±0.1	0.5±0.1	0.3±0.3	0.5±0.1	1±0.2	8.4±0.1	0.3±0	0.7±0.1	1±0.3	*	*	nd
Cd	0.1±0	0.1±0.1	0.1±0	0.1±0	0.3±0.3	0.1±0	0±0	0±0	0±0	nd	nd	nd

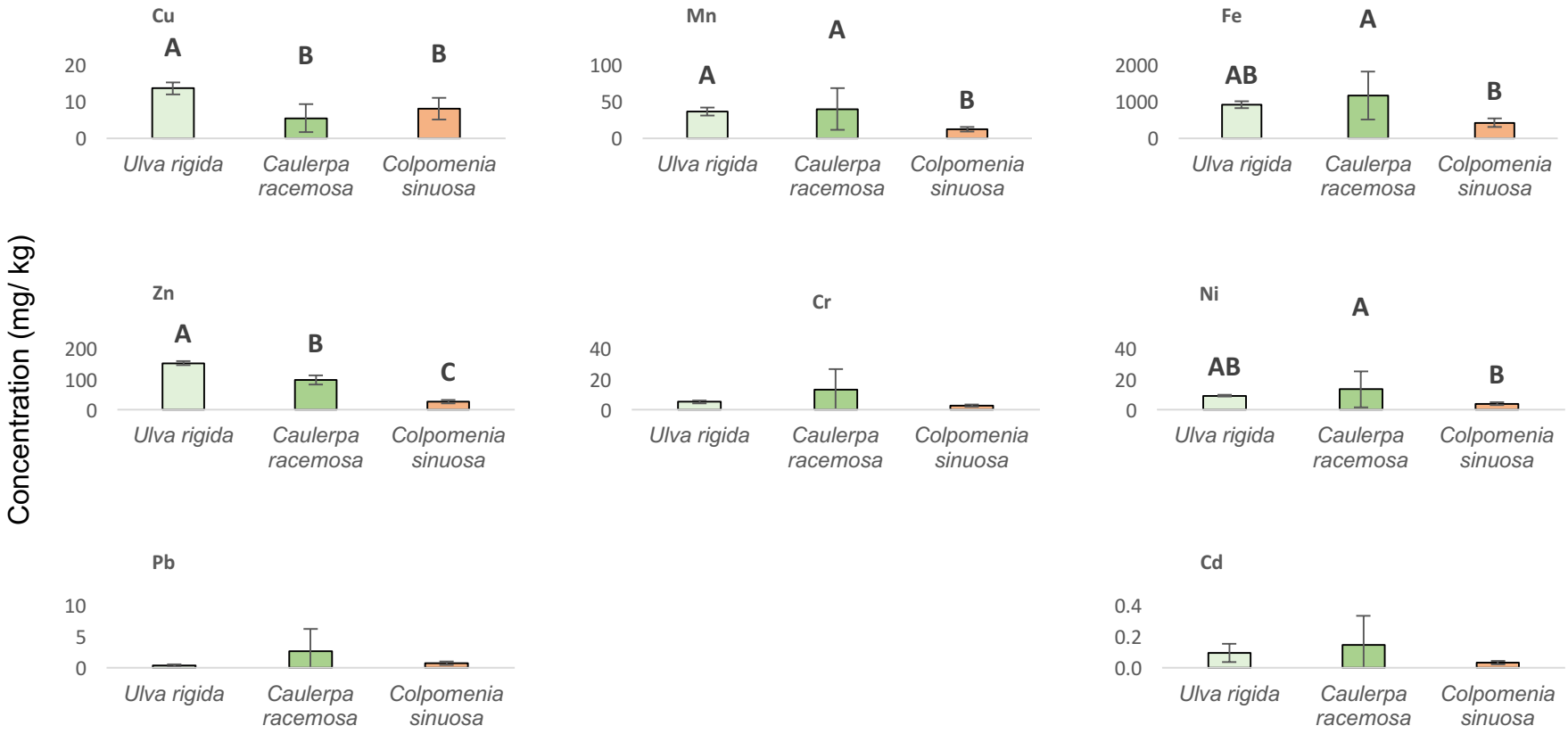

Figure 16. Heavy metal concentration in three different macroalgae species found in the Biofilter system of Scientific Marine Technology Park of University of Las Palmas de Gran Canaria (PCMT-ULPGC). Letters denote differences between the species at P>0.05 level (n=9).

4. DISCUSSION

4.1 ALGAL CULTURES

4.1.1 Macroalgae growth and environmental parameters

To increase the knowledge about the growth performance of three macroalgae species: *U. rigida*, *C. racemosa* and *C. sinuosa* under mariculture effluents in GIA, we tested their culture during 4 weeks in outdoor tank conditions. The results showed that the average biomass growth was higher for *U. rigida* then *C. racemosa* and *C. sinuosa* during the experimental period. Thus, while *U. rigida* showed a significant growth measured as SGR, *C. racemosa* and *C. sinuosa* did not show any significance for SGR during four weeks. Due to their fast growth rate, ease cultivation and tolerance to high temperature and irradiance range, *Ulva* species were used in several studies and eventually fulfilled the aims of the studies (Vandermeulen and Gordin, 1990, Cohen and Neori, 1991, Jimenez del Rio *et al.*, 1994, Hernández *et al.*, 2002, Hernández *et al.*, 2005). For instance, the results were showed resemblance when compared with other studies for *U. rigida* for SGR values, was found 12-16 $\%/d^{-1}$ in other study (Pinchetti *et al.* (1998) and found approximately 4-16 $\%/d^{-1}$ in recent study. And also it can be concluded that especially for the first week had the N riched conditions for the outdoor tanks culture experiment in recent study.

Although the green algae *Ulva sp*. was used in several studies, there are lack of studies on culturing of *C. racemosa* and *C. sinuosa*. Although, they had a massive abundant in biofilter at PCTM during Autumn months, they didn't show a significant growth performance in tank cultures.

The previous study on biofilter capacities in four macroalgae species, which was conducted by GIA in PCTM as well, demonstrated the negative growth

rate and also production for *C. sinuosa* in tank culture each week (Felaco, 2014), however, they were growing naturally at the Biofilter system in PCTM during the experiment period; it was found that for this particular species the problem is due to they could not adapt to free floating conditions (Felaco, 2014). In comparison with the present study, if their morphology is considered that should not culture them in tank conditions, surprisingly the negative growth rate was found at week three and week four. This result found in recent study gave rise to thought that *C. sinuosa* can be suggested to culture for short periods (<3 weeks) and also low renovation rate (8 vol/day) should have taken into account. In contrast to *C. sinuosa*, *C. racemosa* showed negative growth rate from the first week at culturing period. However, *C. racemosa* is a worldwide species and tolerate to wide range of environmental factors; a similar species (*C. lentillifera*) also require specific culturing technics such as sowing and tray methods (Rabia, 2016) instead of tank culture. In the light of this results, it is explicable that observation of increasing epiphyte biomass in the tanks and also deformed morphological structures of *C. racemosa* from the first week in the present study. However, Rabia (2016) demonstrate the positive growth for *Caulerpa sp*. with different culturing technics, they were not suggested to culture longer than 30 days as well. Moreover, *C. racemosa* had showed very high SGR (7 $\%/d^{-1}$) in tank based culture system in another study (Paul and de Nys, 2008) if it is compared with the recent study.

In all cases, *U. rigida* showed high performance for growth then *C. racemosa* and *C. sinuosa*. The values displayed that the decrease in average biomass and also specific growth rate when comparison between the weeks also (decrease in growth rate from week 1 to week 4). As mentioned above in first

section, emphasized that seaweed growth can be effected by several major physical factors such as light, temperature, salinity, water flow, limiting nutrient levels and pollution (Lobban *et al.*, 1985). Among the physical factors light was highlighted as the most important factor affecting plants due to providing the initial energy for photosynthesis. In recent study, the results showed that the PAR values decreased from week 1 to week 4 (especially in week 3). In agreement with Vergara *et al.* (1997) and Andría *et al.* (2001), an increase of saturation irradiance cause the increases in biomass of macroalgae as well. However, PAR estimations exhibited the decreasing; temperature and pH values, which were significant factors on seaweed growth (Lobban *et al.*, 1985), presented stable state approximately during the experiment period.

4.1.2 Ammonium biofiltration capacities of three macroalgae species

Present study aimed to obtain the remediate capabilities of *U. rigida*, *C. racemosa* and *C. sinuosa* with respect of ammonium retention in tank cultures and the results displayed that *U. rigida* was the fastest NH_4^+ scrubber among the selected species during the experiment period. In previous studies, *Ulva* species showed their capacity to utilize and also quick absorption different forms of nitrogen, mainly nitrate and ammonium (Vandermeulen and Gordin, 1990, Cohen and Neori, 1991, Jimenez del Rio *et al.*, 1996, Pinchetti *et al.*, 1998, Hernández *et al.*, 2002, Hernández *et al.*, 2005). However, the decrease in NH_4^+ concentrations in water from 0 hour was observed for both algae specie, the biggest and fastest consumption was found for *U. rigida* (%100 after 1 hour for all the weeks). On the other side, this data showed the higher amounts then ammonium biofiltration (up to %67) of *U. rotundata* reported by Hernández *et al.* (2005). According to Harrison and Hurd (2001), especially species with a high

growth rate like *Ulva* will require a very high N supply in order to prevent N limitation. This statement gave a rise to explain their faster consumption during the experiment period.

Furthermore, the difference was determined for *C. sinuosa* with *U. rigida* and *C. racemosa* and there were any significant difference found between *U. rigida* and *C. racemosa* for each week. This establishing showed the importance of *C. racemosa* for scrubbing NH_4^+ from water as well as *U. rigida.* Thus, this consequence supports the suggestions belongs to Chaitanawisuti *et al.* (2011) and Paulson and Kenosha (2014), of their potentially usage as a nutrient remover. *C. sinuosa* was found the slowest remover of NH_4^+ likewise the previous study on biofiltering capacities of 4 macroalgae species in GIA and PCTM as found by Felaco (2014). However, there is lack of studies on biolfiltering capacities of *C. sinuosa*, there are several studies suggested to use brown algae such as *Ecklonia sp* and *Sargassum sp.* due to their biofiltering capacity and also commercially importance (Winberg *et al.*, 2009).

In order to low concentrations found in especially week 2 and week 4 (4,71 µmol/L, 5,14 µmol/L), all NH_4^+ was wasted at hour three by each macroalgae. This result can support the theory of Lobban *et al.* (1985) and Harrison and Hurd (2001) as their rapid nutrient uptake under nutrient-limited conditions. In addition to this evaluation, there are several more factors can be effective on nutrient physiology of seaweeds. According to this factors; light, temperature, the stocking density, life history of seaweeds and also water exchange should be the most efficient ones in the present study. In addition, from the standpoint of decrease after first week in initial NH_4^+ concentrations of water came from sedimentation pond, it can be concluded with finishing the

zooplankton culturing in old facility after first week in experiment period since as mentioned before (in section 1.3), the regeneration of nitrogen in the water column occurs as a result of excretion by marine fauna particularly by zooplankton (Lobban *et al.* (1985).

According to Davis and Kris-Etherton (2003) and Davis *et al.* (2000), seaweeds were shown as important sorbents, due to their cell walls of especially in green and brown algae. In agreement with this statement, present study showed the removing capacities during the experiment period of three different algae which were belonging to green and brown algal groups.

4.2 HEAVY METAL CONCENTRATIONS IN THREE MACROALGAE SPECIES

Heavy metal concentrations of three macroalgae species experiment targeted to assess the heavy metals (Cu, Mn, Fe, Zn, Cr, Ni, Pb and Cd) uptake capabilities of *U. rigida*, *C. racemosa* and *C. sinuosa* used as macroalgae samples from biofilter at PCTM. In the light of this aim, the concentrations were determined and the results exhibited that *U. rigida*, *C. racemosa* and *C. sinuosa* showed they are great sorbers of metals as well as NH_4^+. When comparison between macroalgae used in present study, *U. rigida*, *C. racemosa* mostly showed bigger amounts of sorption capacities then *C. sinuosa* during the experiment. Due to owing the alginate composition in cell wall of brown algae (Phaeopyta), gives the them special sorption capacity (Fourest and Volesky, 1997). However, Phaeopyta was highlighted as great biosorbent due to their cell wall, the brown algae, *C. sinuosa* had bigger amount determined only of Cu than *C. racemosa* and also then *U. rigida* of Pb sorption in the present study. In addition, another study, which was studied Cd and Pb concentrations with some brown algae species

located in Canary Islands Lozano *et al.* (2003), showed lower than 1 μg/g concentrations for Cd levels in brown algae samples as similar as our study. On the other hand, most of the brown algae showed higher Pb levels (11,2 μg/g, mean) when compared with our study for Pb concentrations. As reported by Lobban *et al.* (1985), metals as, Pb, Cd, Zn, Ni, Cu can be very toxic in high concentrations for aquatic organisms. In present study, while highest amount of Cu and Zn, which are essential nutrients also for seaweeds (Den Boer, 1981) was occurred in *U. rigida* and the highest values of Cd, Pb, Zn and Ni was seen clearly in *C. racemosa.* This results support the previous studies for the excellent sorption capacities of these two macroalgae (Simeonova and Petkova, 2007, Dekhil *et al.*, 2011). Moreover, our results showed similarity as other study which was determined the highest heavy metal concentrations levels for Fe levels fallowed by Zn and Mn levels in *Ulva* and *Enteremorpha* species (Fytianos and Zarogiannis, 1999).

5. CONCLUSION

After reviewing the main data obtained in our experimental cases and compared them with relevant bibliographic results, the following conclusions can be drawn:

1. The biofiltration capacity of three macroalgae species are suitable for using them in biofilter system for land-based aquaculture production. Since the macroalgae diversity is very rich in Canarian Islands (Haroun *et al.*, 2002), new species can be explored that could suitable for various culture environments as well as better heavy metal remediate capability.

2. Hence, both species *U. rigida* and *C. racemosa* might be declared much rapid scrubbers, whereas *C. sinuosa* is considered as slow scrubber. As consequences, the two formers can be considered as potential candidates for bioremediation of domestic and industrial effluents which may cause environmental point pollution.
3. In general, all three macroalgae species used in the present study have ability to remove NH_4^+ and heavy metals from the water. Nevertheless, their stripping capacity varies for different metals.
4. Outdoor tank culture of three species showed shorter term period (2 weeks) of *C. sinuosa* is possible with increase in growth. However, *C. racemosa* showed negative growth during the experiment. *Ulva* seems to have better acclimation to the culture tanks and suitable for the tank cultivation.
5. Growth of the three macroalgal species was related to PAR, higher cumulative PAR resulted with higher algae yield especially for *U. rigida*.

6. It is important to analyse N concentrations in tissue of macroalgae species to better understanding in uptake mechanism for NH_4^+.

6. PREVIOUS STUDY

Previous study had two main objectives as;

1. Cultivation of *Graciliaria cervicornis* (from Rhodophyta Phylum) which is autonomous specie located only in Gran Canary Island among the Canary Islands (Haroun et al., 2009).
2. If the cultivation of *G. cervicornis* was succeeded in tank cultures, NH_4^+ biofiltration capacity of this algae were examined with other algae spesies as *Ulva rigida* (from Chlorophyta Phylum), *Caulerpa racemosa* (from Chlorophyta Phylum) and *Colpomenia sinuosa* (from Ocrophyta Phylum) which were presented in the biofilter system located in the Scientific and Technological Marine Park of the ULPGC in Taliarte and access the capability of four macroalgae species to biofilter ammonium in a specific time (three hours) and determine of which specie had the rapid ammonium removal after blocking of the nutrient income to the tanks.

The cultivation of *G. cervicornis* (Figure 17) cultivation experiment was carried out at GIA for 3 months from 26/10/2015 to 20/01/2016 in in 0,2 m^2 surface, 90 L, PVC tanks. *G. cervicornis* samples were collected from the north of Gran Canaria.. 90 L tanks filled with filtered seawater of 30 L. After 15 days, the water volume was reached to 90 L. Micronutrients such as iron, manganese, zinc, molybdenum, copper, cobalt, zinc, etc.) as 1.5 ml/L, and macronutrients (nitrogen, phosphorus, calcium, potassium, sodium, chloride, etc.) as 0.2 ml/L were added once in a week into the tanks. Algae were moved from the tanks and the epiphytes were removed kindly from the thalli and avoid the bad ones two times a week. After the tanks were cleaned and refilled with fresh filtered sea

water, algae, which were sorted out of the epiphytes, were replaced into the tanks.

However, new fronds were appeared after the third week of the experiment, biomass were decreased after two weeks during the experiment (Figure 17). The excessively epiphyte growth in the tanks made difficult to observe the clear biomass measurements of *G. cervicornis*. Indeed, the new colonies from nature could not collected again since they were not appeared sufficiently.

Figure 17. A) *Gracilaria cervicornis* from the very first day of the cultivation (26.10.2015) B) New fronds and epiphytes occur after the third week of the experiment

The culturing of *G. cervicornis* experiments was ended after 3 months since the experiment did not show succeeded. The possibilities such as low initial biomass to start the experiment (0,5 g/L), the tank conditions were conceivable why not to be succeeded in the previous experiment.

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