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Master thesis

Impact of light spectra on quality aspects of selected species of *Lemnaceae*

by

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Alla Silvana,
una donna di invidiabile forza e coraggio,
che direttamente o indirettamente mi ha insegnato tante cose
(anche solo mandandomi a “dar via i ciappi” quando ce n’è bisogno)
e da cui comunque non smetterò mai di imparare.

Alla mia mamma,
una donna spettacolare, che è molto di ciò che vorrei essere
e a cui guardo sempre come a un esempio.

E al mio papà,
un grande uomo che ammiro infinitamente
e che mi ha dimostrato come sia possibile migliorarsi sempre.

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° C	degrees Celsius
Al	aluminium
ATB	Leibniz-Institut für Agrartechnik und Bioökonomie e.V.
Au	silver
Ca	calcium
cm	centimeter
Cry	chryptochrome
Co	cobalt
CO_2	carbon dioxide
Cr	chromium
Cu	copper
DM	dry matter
DNA	Deoxyribonucleic Acid
E_x	extinction coefficient
FAO	Food and Agriculture Organization of the United Nations
Fe	iron
FM	fresh matter
g	gram
K	potassium
Kg	kilogram
L	liter
LED	light-emitting diode
Mn	manganese
Mg	magnesium
Mg	milligram
mL	milliliter
mm	millimeter
Na	sodium
N	nitrogen
NH_3-N	nitrogen-ammonia
nm	nanometer
NPK	Nitrogen Phosphorus Potassium
P	phosphorus
PAR	photosyntetically active radiation
Pb	lead
Pfr	phytochrome far red
Phy	phytochrome
PO_4-P	phosphorus-phosphate
Pr	phytochrome red
Phot	phototropin
Rpm	revolutions per minute
s	second
UV	ultraviolet
V	volume
Zn	zinc
ZTL	ZEITLUPE
µg	microgram
µmol	micromol

Chapter 1

Introduction

Duckweeds (*Lemnaceae*) are the smallest and structurally simplest flowering plants on Earth (Missouri Botanical Garden, 2015). They are spread worldwide and cover the surface of ponds and slow-moving water bodies. Several species are rich in crude protein and amino acids, and in secondary metabolites such as carotenoids; they generally have a low content of fats and a high amount of fibre (Leng et al., 1995; Leng, 1999). These characteristics make them totally suitable as an animal and human food source, indeed they have been traditionally consumed as human food and as animal feed in many Asian countries for generations (Bhanthumnavin & McGarry, 1971), and the interest towards them is nowadays increasing also in Western countries. Their capability to uptake and tolerate a high concentration of heavy metals (Leng, 1999) makes them also concretely suitable for phytoremediation aims.

The recent technology of light-emitting diodes proved to have a high potential in increasing yield and triggering the biosynthesis of nutritious compounds of many crops, being light involved in main biological processes such as photosynthesis, phototropism and photomorphogenesis of plant germination and growth (Taiz and Zeiger, 1998; Suruchi et al., 2012). LED technology is highly versatile, is safe and durable, and allows to actively manage the different light parameters affecting plants' processes, namely light quality, light intensity and duration (NASA, 2012; Olle and Viršile, 2013) in order to influence different metabolic pathways.

Therefore, growing duckweeds under LEDs is an interesting possibility to investigate how they react to the diverse light quality treatments, i.e. white, blue and red light which is the main objective of the present work. This type of study is of remarkable importance, because it could lead to the development of an optimum technique to grow in a short time a great biomass amount of *Lemnaceae* with a very high nutritional value, a method which could then be adapted to both the society of developed and developing countries.

Chapter 2

Literature Survey

2.1 Duckweeds

2.1.1 Botanical description and geographical distribution

Duckweeds, otherwise called water lens, are a family of annual aquatic monocots. They are the smallest and structurally simplest existing flowering plants, and they grow on or below the surface of still and slow-moving water bodies (Missouri Botanical Garden, 2015), while they can't survive in fast moving water. Duckweeds belong to the botanical family of *Lemnaceae,* a name coming from the Greek word "*limne*", which means "pond" (FAO, 2009). Duckweeds provide a shelter for many insects which live in association with them, and they are a source of food for fish (especially carps), snails, ducks, musk rats and flatworms (Leng, 1999). They don't show a differentiation in stem and leaves, as the plant body resembles to a thallus, called frond (although this term is not fully appropriate on a botanical point of view (Palomar College 2013)), with or without roots growing under the water. Duckweeds fronds can have one or many layers of aerenchyma, depending on the species, which make them float on or just below the water surface, and can have only one or several veins (Palomar College 2013). Even if they can potentially produce seeds and fruits, normally they reproduce vegetatively, as fronds contain many buds that can develop in new fronds, which can remain attached to the mother plant (Palomar College 2013). When it is produced, the flower is bisexual, with one or two stamens to form the androecium and a gynoecium with one single pistil. Along with temperature dropping, some species can produce a type of fronds filled with starch, called turions, that lay on the bottom of the water body and overwinter there, embedded in mud (Palomar College, 2013). Duckweeds usually form a packed and homogeneous clonal population covering the surface of water, where different species can cohabitate.

Duckweeds are spread worldwide: they have been found in all continents but Antarctica, since they cannot stand temperatures dropping below 0° C (optimum temperature for many species ranges between 17.5 and 30° C) (FAO, 2009). As

shown in Figure 1 (Landolt, 1986), in some areas *Lemnaceae* are not diffused due to a too wet or dry environment or to a too cold climate, while some other areas hadn't been explored enough at that time to determine whether duckweeds are present or not. In general, duckweeds grow better with a warm and sunny climate, but each species has a typical distribution according to its environmental requirements, for example *Lemna minor* is mainly distributed in Europe and Northern America, while *Lemna gibba* is found in the Mediterranean area and in Central and South America (Missouri Botanical Garden, 2006).

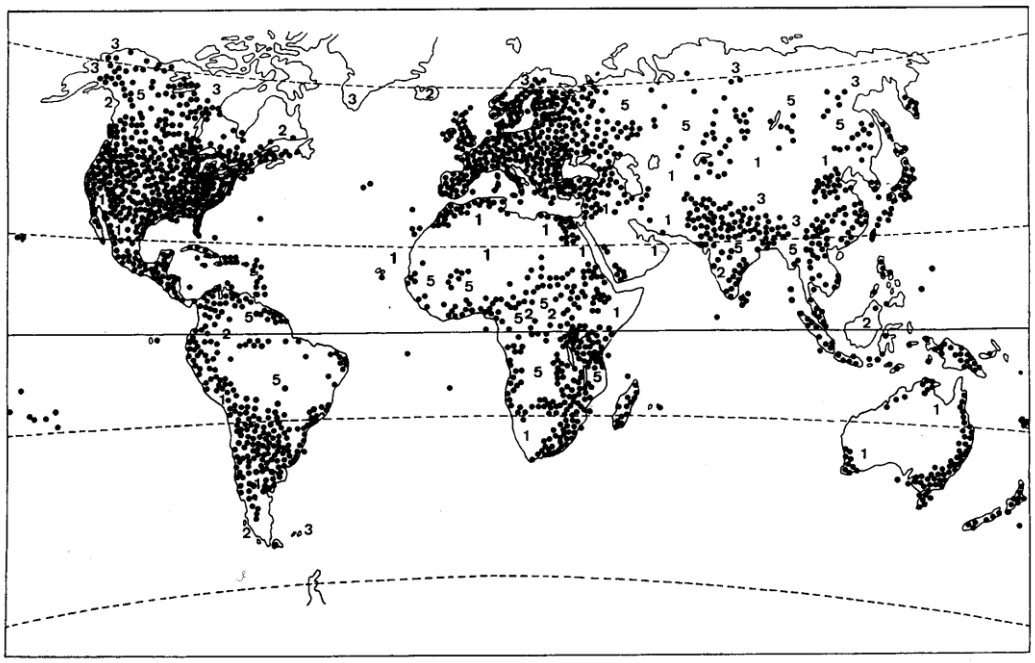


Figure 1: Geographical distribution of Lemnaceae *(Landolt, 1986). Numbers show why in that area duckweeds were not found: 1 = too dry, 2 = too wet, 3 = too cold, 5 = not sufficiently explored*

Spirodela polyrhiza

This species of duckweed is commonly called "common duckmeat", "greater duckweed", "common duckweed" and "duckmeat". Its slightly succulent thallus is 3-9 mm long and fronds have a diameter of 2.5-7 mm. Normally the upper part of it is green, while the side facing the water is coloured in purple/red (Figure 2). This duckweeds species is found world-wide and lives in lakes, ponds, slow-moving currents, sandy and not sandy wetlands. It lives in association with many insects, ducks, carps, muskrats, beavers and turtles (Illinois Wild Flowers, 2017).

Figure 2: Spirodela polyrhiza *(Illinois Wild Flowers, 2017).*

Wolffia arrhiza

This species of duckweed is commonly known as "spotless watermeal" and "rootless duckweed". It has very tiny green fronds, flat and with a diameter of ca. 1 mm, and it doesn't have roots (Figure 3). It is native to Europe, Africa, and parts of Asia, and it has also spread in other parts of the world. It is commonly found together with other species, such as *Wolfiella, Lemna minor* and *Pistia* (The IUCN Red List of Threatened Species, 2017).

Figure 3: Wolffia arrhiza *(Fair Dinkum Seeds, 2017)*

2.1.2 Taxonomy

Based on molecular phylogenetic studies *Lemnaceae* are monophyletic with the *Araceae* family (Cabrera et al., 2008), in fact in the beginning of the 21st century it has been proposed to place the duckweed species within the *Araceae*, due to some morphological similarities (Palomar College, 2013): in more recent years, molecular researches allowed to finally define *Lemnaceae* as a defined and independent group (Cusimano et al., 2011).

Since duckweeds rarely produce flowers and fruits, they are normally classified based on their vegetative characteristics such as presence or absence of roots, size, shape and anthocyanin pigmentation (Palomar College, 2013). According to their anatomic characteristics, duckweed species are divided in five genera: species with only one root belong to the *genus Lemna*, species with two or more roots belong to the *genera Spirodela* and *Landoltia*, species without roots with a flattened plant body belong to the *genus Wolffiella* and species with no roots and with oval-shaped plant body belong to the *genus Wolffia*. Also their size can be very different: *Spirodela* species are the largest ones and the *Wolffia* species are the smallest ones, with fronds that can be smaller than 1 mm (Missouri Botanical Garden, 2015) (Figure 4).

Figure 4: Two species of Lemnaceae *with very different size: the smallest one is* Wolffia arrhiza *and the other is* Spirodela polyrhiza (Saldarriaga, 2012).

Due to their very small size, it has been a serious challenge to investigate the systematic relationships of *Lemnaceae:* in fact, it is in some cases very difficult to discriminate among the different species according to the morphological traits. Cladograms have been produced to better show the phylogenetic relations among the *Lemnaceae* species, based not only on morphology but also on DNA sequences from chloroplast genes and introns and on the presence of secondary metabolites, such as flavonoids. In Figure 5 it is shown a cladogram adapted by Sree et al. (2016): it is based on previous studies of Les et al. (2002) and Tippery et al. (2015), who did their research on a combined analysis of flavonoids, allozymes, DNA sequences and morphological data. It represents the phylogenetic relationships among the different species, and clearly shows how *Landoltia, Lemna, Spirodela, Wolffia,* and *Wolffiella* are monophyletic. The two subfamilies *Lemnoideae* and *Wolffioideae* belong to *Araceae.*

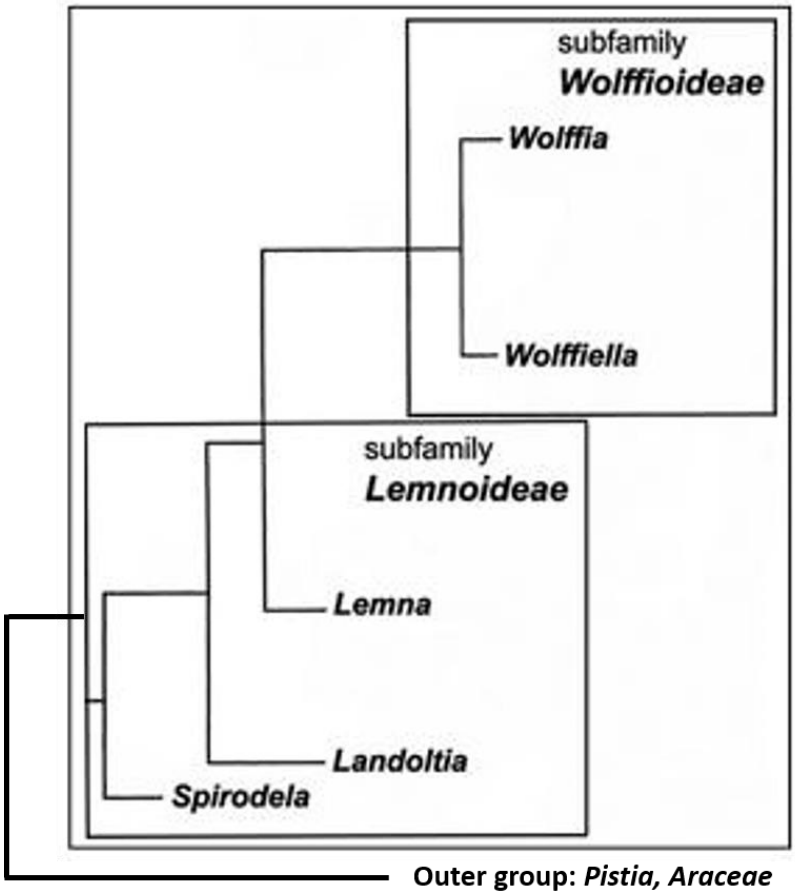


Figure 5: Cladogram adapted by Sree et al. (2016) showing the taxonomic relationship among the different genera of duckweeds.

2.1.3 Duckweeds production

Duckweeds farming requires a continuous care and an intensive management due to the quick reproduction of these plants. In fact, *Lemnaceae* growth rate is nearly exponential when pH and temperature are suitable and nutrients are available, therefore a frequent harvesting is necessary (Missouri Botanical Garden, 2013). Not only temperature and wetness are determinant for *Lemnaceae'*s growth, as many other factors such as light, pH and nutrient availability play a fundamental role. Many duckweed species can live well with a pH ranging from 5 to 9 (optimum pH is 6.5-7.5), but the tolerance is various from species to species (FAO, 2009). Phosphorus, potassium and nitrogen are the main limiting nutrients, although potassium is generally needed in low amounts. The main environmental requirements are found in Table 1, which is adapted from "Floating aquatic macrophytes – Duckweeds" (FAO, 2009).

Table 1: Important environmental factors for duckweeds growth. Table adapted from "Floating aquatic macrophytes – Duckweeds" (FAO, 2009)

Parameter	Minimum	Maximum	Optimum
Temperature (° C)	> 0	35.0	15.0-30.0
Ph	3	10.0	6.5-8.0
Nitrogen (mg/L NH_3-N)	Trace	375.0	7.0-12.0
Phosphorus (mg/L PO_4-P)	0.017	154.0	4.0-8.0

To get an optimum production, it is important that duckweeds form a homogeneous and compact layer on the water surface, in order to prevent light from penetrating into the water with a consequent development of algae and phytoplankton; when algae develop too much they produce a high amount of CO_2, which raises the pH to a value affecting duckweeds growth, while on the other hand phytoplankton asphyxiate the duckweeds' roots. Moreover, it's important to impede that duckweeds grow on multiple layer, because the ones in the bottom would die. Thus, it is generally recommended to harvest everyday 25% of the duckweeds in the pond. Wind also has a deleterious effect on the development of duckweeds, so that it is suggested to grow them in narrow ponds perpendicular to the direction of the common wind, or to divide the ponds in smaller areas, for example with bamboo canes or grids of polyethylene (FAO,

2009). Since *Lemnaceae* can grow on a water layer of a few millimeters up to three meters (Bartošová et al., 2015), the depth of water is not really relevant, although a depth between 20 and 50 cm is recommended to make it easier to harvest the plant and to reduce the stress factors (FAO, 2009). Temperature, pH and nutrient content should be always under control and maintained in an optimum range, to permit a good plant reproduction and avoid the development of algae and phytoplankton. Fertilizing and harvesting must be effectuated regularly.

Regarding the nutrient supply, the required fertilization depends on the source of the used water: for example, rainwater can be adjusted with NPK addition, while waters from housed animals are usually too high in ammonia and need a dilution before being used for duckweeds farming (Leng, 1999). All biodegradable organic material can be used as a fertilizer: “all kinds of animal manure, kitchen wastes, wastes from a wide range of food processing plants, biogas effluents, and slaughterhouse wastes”, but also solid materials mixed with water before being put into the ponds, such as “manure from livestock, night soil from villages, or food processing wastes” (FAO, 2009). Duckweeds are high in proteins and require much nitrogen, which is the most limiting nutrient in aquatic systems. Therefore, fertilizing with urea is usually the best way to provide the needed amount of this compound. In duckweeds farming is fundamental to obtain a nitrogen dilution which is not limiting growth and allows the plant to synthetize a high amount of crude proteins; a negative relationship has been found between the root length and the nitrogen applied with manure (Le Ha Chau, 1998). *Lemnaceae* don’t need to be provided with a very high amount of phosphorus, since they can uptake and accumulate it up to 1.5% of their dry weight, as a reserve for periods in which it could be a limiting nutrient. They don’t need a high quantity of potassium because normally the decaying plant materials present in the ponds are a sufficient source. Crude sea salt can be used as a source of trace minerals needed by duckweeds, such as sodium (Leng, 1999).

Duckweeds have a good capability to uptake and concentrate minerals, therefore they are capable to grow well under many different nutrient conditions (Leng, 1999). However, the grower should keep in mind that, to obtain an optimum

growth, the required amount of every nutrient needs to be adjusted for each particular growing situation and for each species.

Lemnaceae are mainly produced as animal food, and have always had an important role in developing countries, as part of aquaculture and integrated farming practices (Skillicorn et al. 1993; Leng, 1999) (Figure 6). In Vietnam and Bangladesh, duckweeds are commonly used to feed ducks and fish (e.g. tilapia), in Taiwan duckweed farmers also sell the plants to poultry and pig producers (Leng, 1999), which can feed the animals with an optimum mixture of duckweeds, cassava root and rice (Rodriguez & Preston, 2011). In fact, due to their nutrient composition, these plants are more suitable to feed animals than animal-derived foods or the commonly used legumes (e.g. alfalfa, soybeans), and they're often cultivated by small scale farmers in an integrated farming system, because livestock wastes can be used as a nutrient for duckweeds (Leng, 1999).

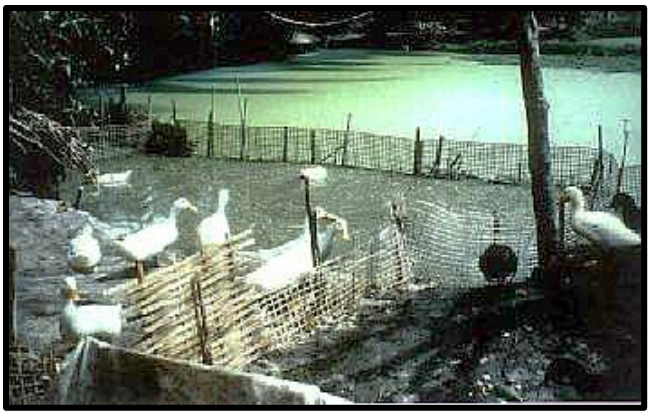


Figure 6: Integrated farming system in Vietnam, taking advantage of duckweed as duck and human food (Leng, 1999).

Some species of duckweed have been traditionally used as human food as well: for instance, *Wolffia arrhiza* has been eaten as a vegetable by many Burmese, Vietnamese, Latioan and Northern Thailand generations. In Thailand duckweeds are called "*khai-nam*", literally "eggs of water", recalling the shape of these plants, and have always been considered a food source for poor people (Bhanthumnavin & McGarry, 1971). Since a few years, also western countries started developing an increasing interest in duckweeds as human food, due mainly to their high

protein content. Especially in the U.S.A., some people started cultivating them in ponds or small swimming pools to consume them domestically; on the other hand, a few companies started producing duckweeds-derived product and to sell them as superfood: for example, a powder made by *Lemna* species is sold by Parabel™, U.S.A., with the name of "Lentein™ plus", as a highly nutrient food containing up to 65% protein and also rich in vitamins, minerals and micronutrients, carotenoids, flavonoids, and omega-3 fatty acids (www.parabel.com) (Figure 7).

Figure 7: Lentein™ plus, by Parabel™, U.S.A. (www.parabel.com)

2.1.5 Nutritional facts and phytoremediation use

Lemnaceae have been proved to be an optimum source of vitamins, especially A, B1, B2, B6, C, E and PP, and of essential amino acids, in particular cysteine, methionine and lysine, compared to the more traditionally consumed grains (Iqbal, 1999; Van den Berg et al., 2015; Appenroth et al., 2017). Therefore, they can be considered as a suitable food both for animals and humans. However, Appenroth et al. (2017) also reported that in some species of *Wolffiaideae* the amount of threonine, leucine and phenylalanine plus tyrosine was above the limits recommended by FAO. On the other hand, duckweeds contain a lower amount of fiber compared to other plants, because they don't need to support a stem or other similar structures (Leng et al., 1995). They generally have a very high crude protein content (the actual amount always depends upon the nitrogen content of the water where they grow and on the weather conditions), and they also

biosynthetize a remarkable amount of some other valuable compounds, such as phosphorus, potassium, carotene and xanthophylls, which confirm how duckweeds could be an optimum source of vitamin A and other important pigments (Leng et al., 1995; Leng, 1999; Ansal and Dhawan, 2007; Mwale and Gwaze, 2013). A recent study of Appenroth et al. (2017) reported that the protein content on a dry weight basis in six species of *Lemnaceae* of different genera was ranging between 20% and 35%, the fat content between 4% and 7%, and the starch content between 4% and 10%. Overall, duckweeds are a very positive promise as human food basing on their nutrient composition; the only concern in adopting them as human food is their sometimes too high content of polyphenols, the presence of crystallized oxalic acid, which can produce an unpleasant taste, and the difficulties in purifying the plant by associated pathogenic organisms (Iqbal, 1999).

Duckweeds also have a high capacity of assimilating macro elements (N, P, K, Ca, Na, Mg), micro elements (Fe, Mn, Zn, Cu, Co) and other trace elements (Pb, Al, Au, Cr) up to toxic levels. The majority of the required trace elements are often limiting in the livestock feed, so duckweeds would be, when grown in a proper and aware way, a good possibility to overcome this problem and feed animals with a really suitable product (Leng, 1999).

Due to this property, duckweeds have been recently studied also as a medium to absorb heavy metals from polluted water bodies or to clean effluents from the high amount of nitrogen and phosphorus dissolved in them. All species of *Lemnaceae* have been shown to easily accumulate a very high amount of heavy minerals, especially cadmium, lead and chromium (Leng, 1999). Thus, on one hand it is very important making sure to grow in a proper area the duckweeds for food purposes and, on the other, this property opens big possibilities as means of phytoremediation (e.g. of industrial or mining contaminated waters) or pollution level monitoring of water bodies. In this respect, Chiudioni et al. (2017) did a research in Italian watercourses, reporting the effectiveness of *Lemna minuta* in sequestering and tolerating cadmium up to 3,771 mg kg^{-1} on dry mass basis; *Lemna minor* and *Lemna gibba* have also been shown to be a very good way to remove uranium and thorium in highly polluted water bodies in Turkey, has they

are able to accumulate these trace elements in high amount as a function of time (Sasmaz et al., 2016).

Regarding duckweeds as a possible use to clean effluents, Goopy and Murray (2003) reported about many cases in which duckweeds have been used to clean primary and secondary effluents in U.S.A., India and Middle East countries, even if they specify that this capability to uptake phosphorus and nitrogen varies with the species, the season and the mineral concentration in the water.

2.2 Light

2.2.1 Role of light for plants

Light is fundamental for the primary metabolism of plants, since it is deeply involved in photosynthesis. In fact, photosynthesis is the only process that allows to harvest the energy derived from the sun, and to take advantage of it to synthetize organic compounds: photosynthetic organisms use this energy to oxidize water and reduce carbon dioxide into organic compounds, releasing oxygen during the process (Taiz and Zeiger, 1998). Besides the main role in photosynthesis, light is essentially involved in many other plant processes, such photoperiodism, phototropism and photomorphogenesis of plant germination and growth, and in the secondary metabolism (Suruchi et al., 2015).

Three parameters are of main importance to determine the effectiveness of light on plants' processes: light intensity, light duration and light quality. Light intensity is defined as the rate at which light energy is delivered to a unit of surface per unit time. The majority of vegetable and ornamental plants need a light intensity of 300 µmol m^{-2} s^{-1} for a suitable growth and, even if the optimum light intensity varies with the species, for many plants a value below 200 µmol m^{-2} s^{-1} can compromise growth and photosynthetic rate (Dole and Wilkins, 1999; Schwend, 2017). Nevertheless, a recent study by Yin et al. (2015), showed that generally the duckweed species *Lemna aequinoctialis* produces biomass and starch proportionally with increasing photoperiod and light intensity, with an optimum of 110 µmol m^{-2} s^{-1}. Light duration, or photoperiod, is defined as the number of continuous hours of light in each 24-hour period. Normally, some plants require a long period of darkness to flower and reproduce (the so called "short day plants" or "long night plants"), while others require a short night period, meaning at least

than 12 hours of light per day (the so called “long day plants”); then, there is a smaller group of plants which is not influenced by the photoperiod (“day neutral plants”) (Savonen, 2003). According to the previously mentioned study by Yin et al., the sample species of duckweed *Lemna aequinoctialis* responded the better to a longer photoperiod, namely 24 hours of light. Unfortunately, not many researches have been done in this direction on duckweeds and further studies are needed to have a better overview of this plants’ reaction to different light conditions. Light quality it is defined as the relative number of light particles at each wavelength, and it changes throughout the year due to the changing of the angle between the sun and the earth’s surface (Schwend, 2017). Since phytochromes react differently to the diverse wavelength, light quality has a very strong impact on the plants’ development. Light spectra can be generally considered as divided in three portions, according to the wavelength: the ultraviolet light section (UV), with wavelength ranging between ca. 10 and 390 nm, the visible light ranging between ca. 690 and 700 nm, and the infrared light portion, with wavelength values bigger than 700 nm. The visible light is also called PAR (Photosynthetically Active Radiation), as it’s the region of light usable for photosynthesis by biosynthetic organisms (Figure 8). In fact, photons at shorter wavelengths carry a very high energy, thus could damage cells and tissues (the majority is filtered by the ozone layer), while photons at longer wavelengths don’t carry a sufficient amount of energy for photosynthesis to take place.

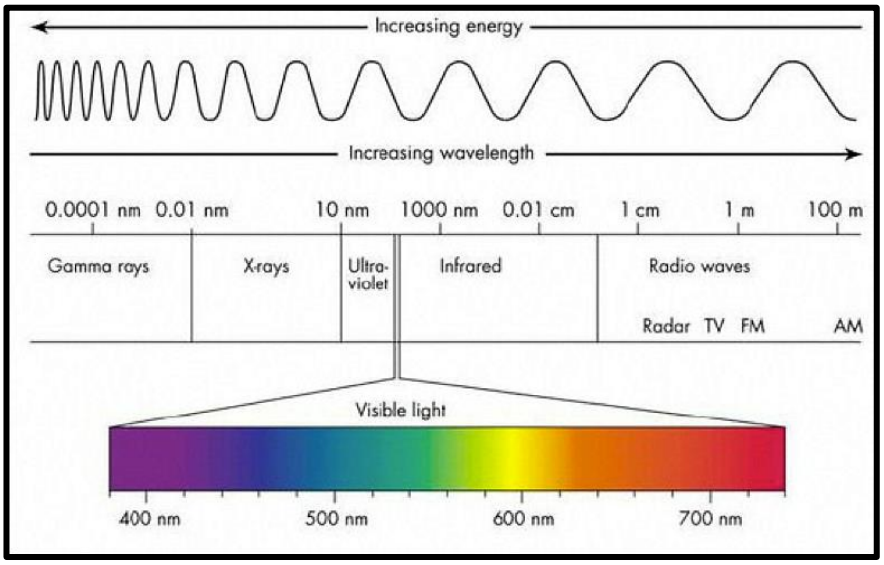


Figure 8: Light spectrum showing the different light regions, and the correlation between energy and wavelength (Datko, 2012).

2.2.2 Influence of light quality on pigments

The diverse plant photoreceptors, which are proteins containing light sensitive pigments are excited by different light spectra (Figure 9), so that different light wavelengths are capable to trigger diverse responses in the plant.

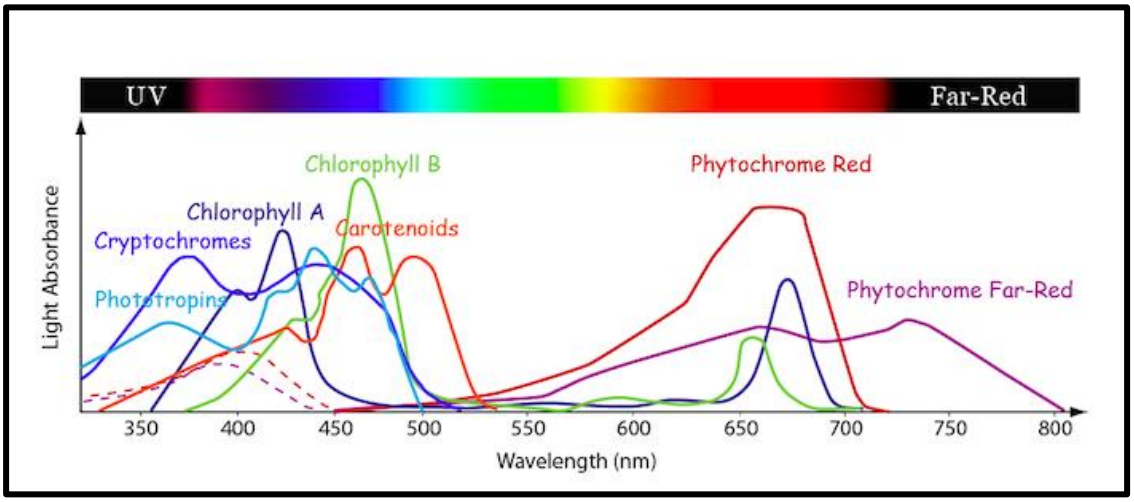


Figure 9: Curves of absorbance of different plant pigments (www.ledgrowlightshq.co.uk).

When a photon at a specific wavelength, and thus carrying a specific amount of energy, hits a compound, if the energy carried by the photon is sufficient the compound gets excited. The excitation consists in one of its electrons jumping from a bonding to a non-bonding orbital, triggering a specific reaction in the plant depending on the excited compound. Visible light can excite pigments involved in the primary metabolism and specifically in the biosynthesis processes, such as chlorophylls a and b, and compounds involved in other very important processes, such as germination al growth. Therefore, light quality is fundamental in determining the development, growth and surviving of plants (Rascio et al., 2012). One relevant group of pigments are the phytochromes (called phyA-F): these molecules are ubiquitous in land plants and are of main importance in regulating morphological ad physiological processes. These proteins contain a pigment called chromophore, and exist in two different photoreversible forms, with peaks of absorption respectively at 666 nm (red light) and at 730 nm (far red light) (Rascio et al., 2012). The so called Pr form absorbs at wavelengths between ca. 630 and 700 nm, and when it is excited converts to the so called Pfr form, which absorbs between ca. 700-800 nm and when excited gets back to the Pr form

(Figure 10). The Pfr form is the active one and can induce signalling cascades leading to a number of processes within the plant, which so strongly depend on the environmental light quality. Among them germination, inhibition of flowering, deetiolation, leaf senescence and abscission, production of plastidia, chloroplasts development and spatial orientation to avoid light stress (Taiz and Zeiger, 1998; Ruban, 2009; Rascio et al., 2012; Missouri Botanical Garden, 2013).

Another fundamental group of proteins is the one of cryptochromes (cry), also called blue light receptors: they also contain a chromophore and are involved in processes such as phototropism, flowering, root growth, apical dominance, programmed cell death, high-light stress response, plant height, photomorphogenesis, leaf photosynthetic functioning, deetiolation and opening of stomata (Taiz and Zeiger, 1998; Liu et al., 2011). They absorb and are activated by ultraviolet A light (315–400 nm), blue light and a small region of green light (ca. 400-500 nm), and they have two peaks of absorption, one in the visible blue light and one in the UV-A range; their activity is inhibited by yellow/green light (ca. 500-630 nm) (Figures 9 and 10).

Phototropins (phot) are the third group of photoreceptors, and they are also excited by UV-A and blue light, but in a different range than cryptochromes (ca. 340-495 nm). They are another example of how plants use light as an environmental signal (Taiz and Zeiger, 1998), as they are involved in processes aimed to optimize the photosynthetic efficiency of plants, such as phototropism, stomatal opening, chloroplast movements in response to changes in light intensity (Christie, 2007; Rascio et al., 2012) (Figure 10).

The protein ZEITLUPE (ZTL) is excited in the same wavelength range than phototropins, and has an important role in maintaining the circadian cycle (Kim at al., 2007) (Figure 10).

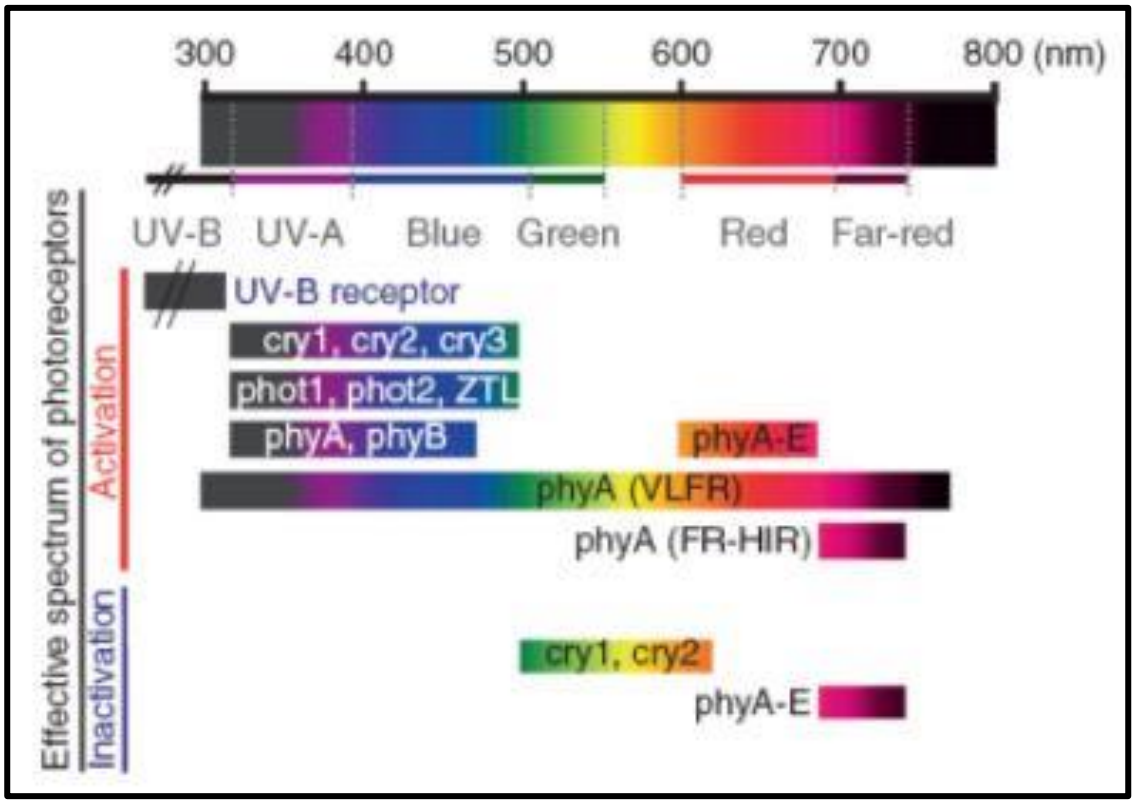


Figure 10: Phytochromes (phyA-E), phototropines (phot1-2, ZTL) and chriptocromes (cry1-3) activation and inactivation wavelength ranges (Rascio et al., 2012).

2.2.3 LED technology in plant production

A light-emitting diode (LED) is a semiconductor device able to emit light when electric current passes through it. Each LED is a combination of two different types of semiconductors: n-type and p-type (Hiskey, 2010). The n-type semiconductor has a higher number of electrons than of electron holes, while the p-type semiconductor has a higher number of electron holes. By applying an electric charge, the electrons and electron holes reach the junction of the semiconductors and there combine together. When they combine, the electrons lose energy because they pass from a higher orbital to a lower one: this released energy is in form of photons (Figure 11).

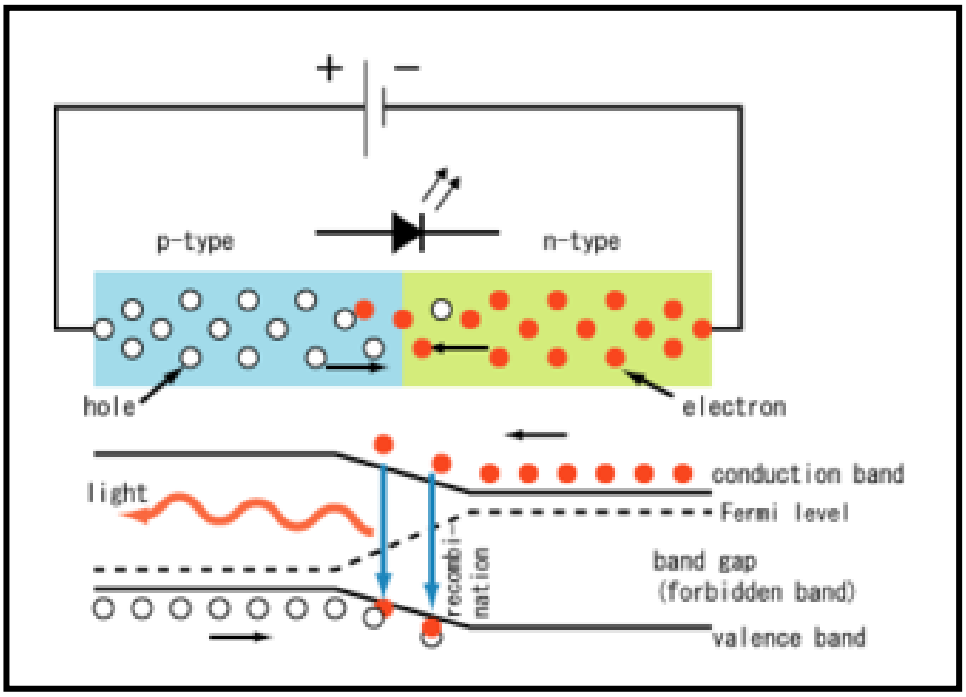


Figure 11: The n-type semiconductor's electrons are attracted by the p-type semiconductor's electron holes and meet in the junction in the middle. When they combine, they release energy in the form of light (Hiskey, 2010).

Whether the light is visible or not, depends on the amount of released energy and so on the wavelength of the emitted light. Basing on this principle, LEDs are produced in different materials and a different electric current is made pass through them, to produce light with a lower or higher energy depending on the aim (Hiskey, 2010). Normally, LEDs are made by metal alloys of indium, gallium, arsenic or by nitrogen and phosphor, because these can produce visible light (Schwend, 2017).

LEDs were invented in 1927 by a Russian inventor, Oleg Losev, but for many years it was not clear how to apply this idea in a practical way. After its discovery, several scientists worked on LED technology, until in 1961 Gary Pittman and Bob Biard from Texas Instruments discovered that a gallium-arsenide diode emits infrared light when connected to current, and in the same year they received patent for infrared LED (History of Lighting, 2017). Then in the 1980s, NASA scientists first worked on the concrete possibility of using LED technology as a mean of growing plants in a better way, and more specifically in the space environments, due to the efficiency and durability of light-emitting diodes (NASA, 2012).

LED can produce light of different spectra, from 240–360 nm (UV) to 660-900 nm (infra-red), therefore they can influence plants' growth in a wide range of aspects (Photonics, edu.photonics.com). Mizuno at el. (2011) reported that LED red light at 660 nm triggers the production of anthocyanins in red leaf cabbage; Li and Kubota (2009) studied that LED red light at 658 nm stimulates the biosynthesis of phenolic compounds in baby leaf lettuce; Lefsrud et al. (2008) showed how a pre-harvest treatment with red LEDs at 640 nm can increase the production of sinigrin and lutein in red leaf cabbage. Far red wavelengths are less effective in stimulating photosynthesis alone, but are way more useful when combined with other light: for examples, Stutte et al. (2009) reported that red and far red LEDs together can stimulate growth, biomass production, and leaf length in red leaf lettuce, while on the other hand they caused a decrease in chlorophylls, anthocyanins and carotenoids content (Olle and Viršile, 2013). Also blue LEDs were reported to influence plants growth and development: Johkan et al. (2010) reported that blue LEDs at 468 nm were capable to stimulate the roots' biomass accumulation and the accumulation of polyphenols in red leaf lettuce; Stutte et al. (2009) showed that at 440 nm, LEDs could increase the biosynthesis of anthocyanins and the leaf expansion rate in the same vegetable; Li and Kubota (2009) reported an increase in anthocyanins and carotenoids concentration after a treatment with blue LEDs at 476 nm in baby leaf lettuce. Green light then also proved to have an influence on plants: Samuolienè et al. (2012) studied cucumber, tomato and sweet pepper cultivars and reported that green light at 505 nm caused an increase in the leaf area, in the photosynthetic pigments amount an in the fresh and dry weight; in the same year, Novickovas at al. reported a similar behavior in cucumber plants, as when treated with LED light at 530 nm they responded with an increase in their leaves area and in fresh and dry weight. During the past decades, more and more studies confirmed the extremely high potential of LEDs in plant production in terms of both productivity and sustainability. They require a quiet high investment at first, but they have a high durability and the maintenance costs are affordable. LEDs have become extremely efficient in converting electric energy in photons, and they are almost completely environmentally safe, as they don't contain hazardous components. They are extremely versatile (different sizes, different semi-conductors materials and high specificity in wavelength) thus can be adapted to a wide range of aims

and situations, without almost any risk for the user, since they have a stable temperature and they don't reach high temperatures, which is also good in order to reduce heat stress in plants (Olle and Viršile, 2013). Due to all these characteristics, light-emitting diodes have a very high potential as a profitable resource in plant growing: they make it possible to precisely manage diverse plant metabolic and developing processes, not only in controlled growing chambers but also in greenhouses. Indeed, it's also a possible way toward sustainability of production: some companies (e.g. Cloudoponics and Leaf) already started a business by selling small growing chambers with LEDs directly to the consumer, to allow people to grow their own vegetables quickly at home.

2.2.4 Important compounds in duckweeds and LED impact

Plants can produce a wide range of organic compounds with several different functions, that are traditionally divided in the two groups "primary metabolites" and "secondary metabolites" (Rascio et al., 2012). The former contains compounds directly involved in essential processes as growth, development and reproduction: they are part of fundamental metabolic pathways such as the Krebs cycle, the glycolysis process and the Calvin cycle. Carbohydrates, chlorophylls, lipids, proteins, vitamins, organic acids, amino acids and nucleic acids are part of this group and they are widespread in the plant kingdom (Rascio et al., 2012). The latter includes all the organic compounds produced by the secondary metabolism of plants, that is composed by many biosynthetic pathways which are not leading to the fulfilment of fundamental physiological processes, differently form the primary metabolism. Therefore, the secondary metabolites are not essential for the basic growth of the plant, but they take part in a huge number of other processes mainly concerning the relationship between the plant and the surrounding environment (Demain and Fang, 2000). Secondary metabolites are differently distributed among species of the plant kingdom and their presence has a taxonomic relevance. These compounds can be classified in three main groups: terpenes (among them: carotenoids, essential oils, sterols, and phyretroids), phenolic compounds (such as coumarin, lignin and flavonoids) and nitrogen-containing secondary compounds (such as alkaloids, cyanogenetic glycosides and glucosinolates) (Taiz and Zeiger, 1998; Kabera et al., 2014).

Chlorophylls

Chlorophylls are a group of primary metabolites involved in the photosynthetic processes. They are the typical pigments of photosynthetic organisms, and in plants they are found in the chloroplast. They all are formed by four pyrrole rings (named by I to IV) bound into a tetrapyrrole ring with a magnesium atom in the center; this structure is connected with a long hydrophobic hydrocarbon tail, which binds the structure to the photosynthetic membrane; the diverse chlorophylls differ in the substituents around the ring (von Wettstein et al., 1995; Taiz and Zeiger, 1998). They function as an antenna which collects light energy and leads it to a reaction center, where the chemical reactions sing this energy can take place.

All chlorophylls are biosynthetized through a complex and long pathway, the 2-C-methyl-D-erythritol 4-phosphate pathway (Wang et al., 2016). The process starts with the amino acid glutamate, which is at first converted into 5-aminolevulinic acid by the activity of the enzyme glutamyl t-RNA reductase. Two molecules of this compound are then condensed to produce porphobilinogen, that then can form the pyrrole rings. Four molecules of porphobilinogen are condensed to produce the porphyrin structure of chlorophylls. Then, the enzyme magnesium chelatase adds an atom of magnesium to the structure, and as a final step the phytol tail is added by the activity of an enzyme called chlorophyll synthetase (Taiz and Zeiger, 1998). The chlorophylls content can vary much between living organisms: Chlorophyll a is the main antenna pigment, as it is present in almost all photosynthetic organisms (prokaryotes and eukaryotes) with two absorption peaks at 430 nm and 663 nm; the chlorophyll b is less represented, as it is present in the majority of eukaryotes (mosses, ferns, seed plants, green algae, euglenoids) but only in a group of prokaryotes (prochlorophytes), and has two peaks of absorption at 480 nm and 650 nm (Taiz and Zeiger, 1998). Shafi et al. (2015) calculated the amount of chlorophyll a and chlorophyll b in *Lemna minor* L., and reported them to be respectively 3.2 µg mL^{-1} and 1.9 µg mL^{-1}; another recent study by Mechora et al. (2014) reported in the same species a concentration of 2.58 mg g^{-1} of dry matter of chlorophyll a and a concentration of 2.14 mg g^{-1} of dry matter of chlorophyll b.

Carotenoids

Diverse types of carotenoids are found in the living organisms, and they are present in all the photosynthetic species, which is representative of their main role. However, they are involved not only in photosynthesis but also in photomorphogenic, photoprotective and developmental processes. They are lipophilic secondary metabolites, mostly C_{40} terpenoids, biosynthetized through the isoprenoid pathway: they derive from two isoprene isomers, isopentenyl diphosphate and dimethylallyl diphosphate, which are produced via the mevalonic acid pathway or from the methylerythrol 4-phosphate pathway (Nisar et al., 2015). They normally have an absorption range between 400 and 500 nm, therefore they appear coloured in orange, red and yellow. Carotenoids have two main roles in plants: they are accessory pigments and they are photoprotectants. In fact, they have a fundamental role in photosynthesis, as they absorb light and transfer its energy to chlorophylls (this is why they're called "accessory pigments"). On the other hand, they act as protectants against light: in some periods the light absorption rate of the chlorophylls is too high, and this amount of energy could eventually be released by them and react with molecular oxygen, producing the highly reactive singlet oxygen ($^1O_2$), that would deeply damage the photosynthetic membrane; so, when chlorophylls are too excited due to the high amount of energy absorbed, this energy is rapidly transferred to carotenoids, that release it as heat preventing a photo oxidative damage (Taiz and Zeiger, 1998). Lutein is a representative example of this class of carotenoids: it is the most abundant pigment in photosynthetic membranes, and it is fundamental in the complex-II structure functioning (Kim and Della Penna, 2006). Besides these main roles, carotenoids are also important in the pollination process, as due to their colours they attract pollinator insects. They are deeply involved in plant growth and development, as plants can biosynthetize stringolactones and abscisic acid (among the most important phytohormones) starting from carotenoids. Some carotenoids, such as lycopene, are pigments with a proved strong antioxidant activity, and others, such as ß – carotene (one of the most abundant carotenoids in plants), are precursors of vitamin A (Baranski and Cazzonelli, 2016). Duckweeds seem to be particularly rich in carotenoids: already back in the 1950s, Skillicorn et al. reported that "the total content of carotenoids in duckweed meal is 10 times higher than that in terrestrial plants", and more recently Appenroth at al. (2017) analysed five species of duckweeds and found

out that the predominant carotenoid was lutein (ca. 70 mg per 100 g dry weight), followed by ß – carotene (ca. 28 mg per 100 g dry weight).

Amino acids and proteins

Amino acids have a fundamental role both as biding blocks for proteins and as intermediates in many metabolic pathways. Their sequence in the protein determine its 3D structure, and consequentially its biological functions and activity (Alberts et al., 2002). They all contain an amino group (-NH2) and a carboxylic group (-COOH), and each different amino acid has a specific side chain (R group) with own properties. They can be neutral, positively or negatively charged, polar or not polar, and they are normally divided in aromatic amino acids, containing an aromatic ring (e.g. phenylalanine and tyrosine), and aliphatic amino acids, formed by an open carbon chain (e.g. valine and glycine) (www.aminoacidsguide.com).

The biosynthesis of amino acids requires carbon and nitrogen in the first place. Ammonia is the source of nitrogen for all the amino acids, while carbon derives from glucose through the glycolytic pathway, the pentose phosphate pathway, or the citric acid cycle. At different points of the pathway, the diverse amino acids can be synthetized: for example, histidine and serine can be synthetized via the pentose phosphate pathway, while the pyruvate produced via glycolysis can lead to the production of alanine, valine, isoleucine and leucine, and the citric acid cycle can produce aspartate and glutamate, which then can be converted into many other amino acids (Berg at al., 2002; bioinfo.org). All organisms use the amino acids to produce diverse proteins, and they can up- or down-regulate the protein biosynthesis depending on many and various factors. Humans are not able to produce themselves all the amino acids they require, therefore they rely on alimentation to uptake them: due to this, the crude protein content of food is of main relevance.

Duckweed are especially known to be particularly rich in proteins: already in 1980, Rusoff at al. reported that the crude protein content in different species of sun-dried duckweed ranged from 25.2 to 36.5% per dry weight; Leng at al. (1999) also reported that duckweed grown under ideal conditions and harvested regularly have a crude protein content of 35 to 43% per dry weight content. More recently, Li et al. (2016) found a protein content ranging from 32.63% to 36.20%

of dry weight in three different species of *Lemnaceae* grown at 25° C, and Appenroth et al. (2017) found that five analysed species of duckweed ranged from a 20% of protein content per dry weight to 35%. Regarding the amino acid composition, duckweeds are generally rich in leucine, threonine, valine, isoleucine and phenylalanine, while they contain a lower amount of cysteine, methionine, and tyrosine (Missouri Botanical Garden, 2011): Appenroth at al. (2017), in the previously mentioned research, found consistent results, and reported in addition a high content of alanine, aspartate and glutamate, and a very low content of histidine.

Heavy metals

Heavy metals are defined as metals with high densities, atomic weights or atomic numbers, even if there's not a universally accepted definition (chimica-online.it). Depending on the context and the purpose, different criteria are used to define an element as a heavy metal. Duckweed is known to be able to uptake and tolerate high amounts of toxic compounds, such as heavy metals like cadmium, copper, nickel and zinc, before showing symptoms. For instance, Verma and Suthar (2016) reported that the species *Lemna gibba* L. is capable to uptake up to 98.1% of lead and cadmium, when they are dissolved in the environment in a concentration of 10 mg/L at pH 7.

Influence of light on proteins, chlorophylls and carotenoids

Not many studies have been carried out regarding the influence of light quality on duckweeds' compounds. Nevertheless, many studies have been performed on different vegetables and ornamental plants. Muneer et al. (2014), studied the effects of blue LEDs (470 nm) at low and high intensity (80 and 238 $m^{-2}\,s^{-1}$) on *Lactuca sativa* L. and found out that blue LEDs at 238 $m^{-2}\,s^{-1}$ caused an increase in biomass and an overexpression of multiprotein complex proteins involved in photosynthesis, while blue LEDs at lower intensity decreased their expression. Amoozgar et al. (2017) found that a combination of 70% red + 30% blue LEDs was optimal to increase the carotenoids biosynthesis, while blue LEDs more than doubled the concentration of vitamin C in lettuce. Li and Kubota (2009) reported an increase in anthocyanins and carotenoids concentration after a treatment with blue LEDs at 476 nm in baby leaf lettuce, while Li et al. (2012) studied how blue

LED light increased the concentration of chlorophyll a, chlorophyll b and carotenoids in Chinese cabbage (Table 2).

Table 2: Summary of blue LED light impact on plant pigments and proteins.

Research	Light treatment	Species	Effect
Muneer et al. (2014)	▪ 238 $m^{-2}\,s^{-1}$ blue LED light (470 nm) ▪ 80 $m^{-2}\,s^{-1}$ blue LED light (470 nm)	*Lactuca sativa* L.	▪ 238 $m^{-2}\,s^{-1}$ blue LED light increased biomass and expression of multiprotein complex proteins ▪ 80 $m^{-2}\,s^{-1}$ blue LED light decreased the expression of multiprotein complex proteins
Amoozgar et al. (2017)	300 µmol $m^{-2}\,s^{-1}$ red LED light (650–665 nm) or/and blue LED light (460–475 nm)	*Lactuca sativa* L. cv. 'Grizzly'	Chlorophyll and carotenoid concentrations increased in the plants grown under 70% red + 30% blue LEDs
Li & Kubota (2009)	130 ± 10 µmol $m^{-2}\,s^{-1}$ blue LED light (400-500 nm)	*Lactuca sativa* L. cv. Red Cross	Carotenoid concentrations (xanthophylls and ß - carotene) increased by 6–8%
Li et al. (2012)	Various intensity of blue LED light (460 nm)	*Brassica campestris* L.	Concentrations of chlorophyll a, chlorophyll b, total chlorophyll and carotenoid increased

Li et al. (2012) also reported that red LED light increased the concentration of soluble protein in Chinese cabbage, and Lefsrud et al. (2008) studied also a species of Brassicaceae, and found out that the biosynthesis of chlorophyll a and chlorophyll b was mostrly triggered by red LED light. Ma et al. (2011) researched

on citrus (*Citrus unshiu* Marc) and reported that red LED light could enhance the carotenoids metabolism. On the contrary, Li and Kubota, in the same study mentioned before, established that red LED light at 734 nm decreased the concentration of chlorophylls and carotenoids in lettuce (Table 3).

Table 3: Summary of red LED light impact on plants pigments and proteins.

Research	Light treatment	Species	Effect
Li et al. (2012)	Various intensity of red LED light (660 nm)	*Brassica campestris* L.	Concentrations of soluble protein increased
Ma et al. (2011)	50 µmol m^{-2} s^{-1} red LED light (660 nm)	*Citrus unshiu* Marc.	Enhanced the carotenoids metabolism
Li & Kubota (2009)	160 µmol m^{-2} s^{-1} red LED light (734 nm)	*Lactuca sativa* L.	Concentration of chlorophylls and carotenoids decreased
Lefsrud et al. (2008)	253.3 µmol m^{-2} s^{-1} red LED light (640 nm)	*Brassica oleracea* var. *capitata* L.	Enhanced biosynthesis of chlorophyll a and chlorophyll b

White light also has an effect on plants: Zheng and van Labeke (2017) determined that white light at 100 µmol m^{-2} s^{-1} [7% blue (400–500 nm), 16% green (500–600 nm), 75% red (600–700 nm) and 2% far red (700–800 nm)] brought to the highest concentration of chlorophylls and carotenoids in *Chrysanthemum morifolium*, compared with sole red and blue light. Another research by Romero-Romero and Sanchez-Saavedra (2017) reported that white and yellow light increased the protein concentration compared with red, and blue light in Amphora sp., while yellow light was the most effective in increasing the carotenoid content (Table 4).

Table 4: Summary of white LED light impact on plants pigments and proteins.

Research	Light treatment	Species	Effect

Zheng & van Labeke (2017)	100 µmol m^{-2} s^{-1} white light	*Chrysanthemum morifolium*	Total leaf chlorophyll content was highest under white light compared with blue and red light
Romero-Romero & Sanchez-Saavedra (2017)	50 µmol m^{-2} s^{-1} white light (ca. 510 nm)	Amphora sp. (diatom)	Concentrations of proteins increased

Chapter 3

Material and methods

3.1 Cultivation of duckweeds

Five duckweed species were grown in growing chambers at the Albrecht Daniel Thaer-Institut für Agrar- und Gartenbauwissenschaften, Division Urban Plant Ecophysiology at Humboldt- Universität zu Berlin:

- *Wolffia columbiana* H. Karst. (1865)
- *Wolffia neglecta* Landolt
- *Wolffia arrhiza* (L.) Horkel ex Wimm.
- *Lemna trisulca* L.
- *Spirodela polyrhiza* (L.) Schleid. Teichlinse

The three species of *Wolffia* and *Lemna trisulca* L. were a courtesy of the Botanical Garden in Berlin-Dahlem, while *Spirodela polyrhiza* came from the Aquarium of Berlin. The plants were propagated in growing chambers (Adaptis, A1000, Conviron®, Canada), at 25° C for 12 hours (daytime) and 20° C for the other 12 hours (nighttime), and light intensity of 200 µmol m^{-2} s^{-1}. Each species was grown into a cell culture growing flask (volume of 225 cm^2, Costar®, U.S.A.), wrapped with aluminium foil on the sides so that plants receive light only from above, in a way preventing the development of algae (Figure 12).

Figure 12: Cell culture growing flasks (volume of 225 cm^2, by Costar®), wrapped with aluminium foil, in the growing chamber.

The cell culture growing flasks were filled with 100 mL of tap water and 100 mL of distilled water, plus some aquarium water to provide the plants with more nutrients. As soon as the plants grew, the content of a flask was splat in two and more for propagation purpose. After a couple weeks, some species were transferred into bigger trays (45 x 27 cm), covered with a film plastic to avoid excessive evaporation. The nutrient powder Universol Blue (ICL-Speciality Fertilizers, Netherlands) (Appendix A) was then added to the trays to allow a better growth: the amount of nutrient solution was adjusted from 1 g 3 L^{-1} of tap water to 0.5 g 3 L^{-1} of tap water, basing on the visual evaluation of the plants reactions (Figure 13). When a species filled one tray, half of it was moved into a new tray.

After 24 days, all the trays were moved into the greenhouse, at a nighttime temperature of 18° C for 12 hours and a daytime temperature of 24° C for the left 12 hours. The plants were propagated in the greenhouse for 26 days, refilling the trays with tap water containing Universol Blue (0.5 g 3 L^{-1} of tap water) and splitting the plant material in more trays when necessary (Figure 14).

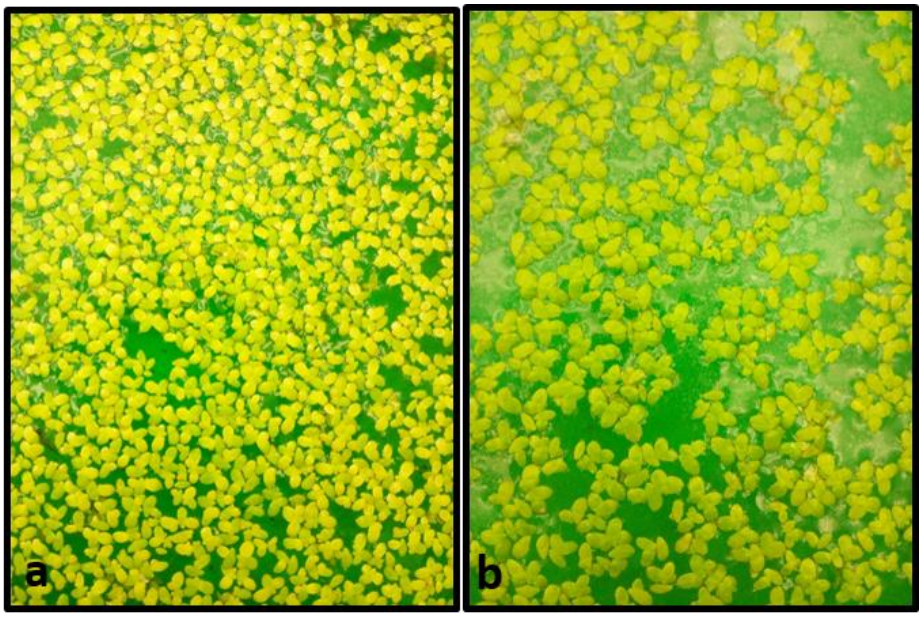


Figure 13: (a) Spirodela polyrhiza *showing yellowing symptoms; (b)* Spirodela polyrhiza *after the addition of Universol Blue.*

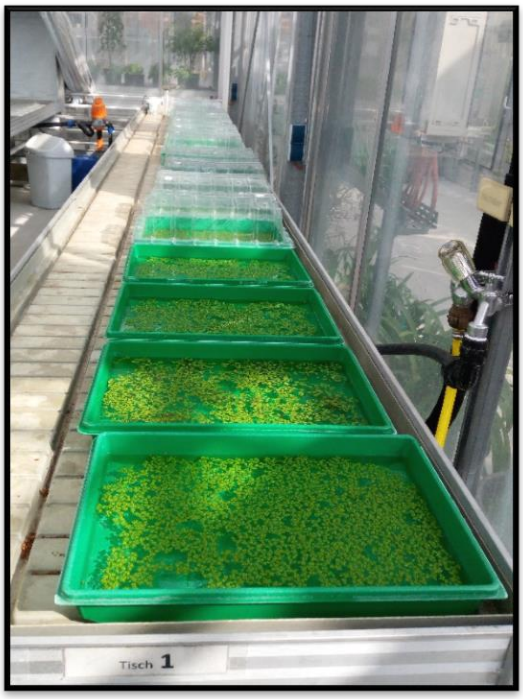


Figure 14: Trays containing duckweeds, propagated in the greenhouse at the Albrecht Daniel Thaer-Institut für Agrar- und Gartenbauwissenschaften

3.2 Light treatment

The two species which propagated best, i.e. *Wolffia arrhiza (*L.) Horkel ex Wimm. and *Spirodela polyrhiza* (L.) Schleid. were selected for the following light treatment. Four trays containing the plant material of each of the two species were transported to ATB (Leibniz-Institut für Agrartechnik und Bioökonomie e.V.) in Potsdam. All the trays were placed in a growing chamber (HGC 1014 V Fitotron, Vötsch Industrietechnik GmbH, Germany), at 18° C for 12 hours (nighttime) and 24° C for 12 hours (daytime), with a small tube pumping air in each tray to add oxygen to the duckweeds and keeping water in movement.

Two trays of *Wolffia arrhiza* and two trays of *Spirodela polyrhiza* were placed in the growing chamber under its white LED lamps (HQI-T Powerstar, 400 W) (130 ± 10 µmol m^{-2} s^{-1}) (Osram GmbH, Germany); one tray of each species was placed under LED panels (Photon Systems Instruments, Czech Republic) producing blue light (ca. 100 ± 10 µmol m^{-2} s^{-1}); and one tray of each species was placed under LED panels producing red light (30 ± 10 µmol m^{-2} s^{-1}) (Photon Systems Instruments, Czech Republic) (Figures 15 a and b).

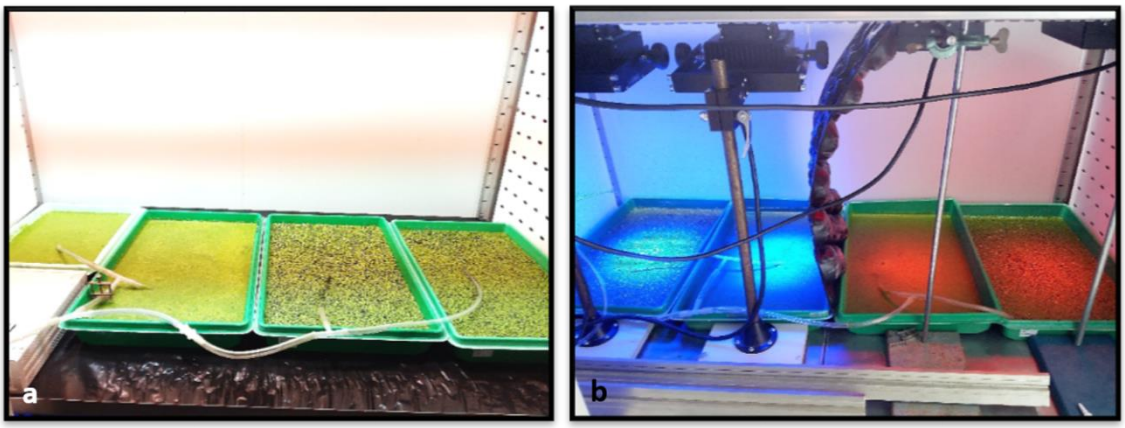


Figure 15: (a) Trays containing the two species Wolffia arrhiza *and* Spirodela polyrhiza*, developing in the growing chamber under white light (130 ± 10 µmol m^{-2} s^{-1}). (b) Trays containing the two species* Wolffia arrhiza *and* Spirodela polyrhiza*, in the growing chamber under blue light (ca. 100 ± 10 µmol m^{-2} s^{-1}) and red light (30 ± 10 µmol m^{-2} s^{-1}) LED panels.*

Every two days the plants were checked, and tap water containing Universol Blue (0.5 g 3 L^{-1} of tap water) was added to the trays if necessary. The experiment was performed twice, and samples were taken for analysis on the following days:

- Experiment 1: 5 and 9 days after the beginning of the light treatment.

- Experiment 2: 6 and 13 days after the beginning of the light treatment.

3.3 Fresh and dry matter determination

Around 20 g of each sample was used to determine the fresh and dry matter. The weight of the samples was firstly measured in aluminium tubes using an analytical balance (PB 2100S, Sartorius AG, Germany) to obtain the fresh weight value, and they were then dried overnight at 105° C in an oven (kelvitron®, Heraeus Instruments, Germany): after reaching a constant weight (the following day), tubes containing the dry material were weighted again to obtain the dry matter value. The percentage of dry matter out of the fresh matter was measured using the following formula (values for fresh and dry weight in Appendix B):

$$DM\ (\%) = \frac{DM \times 100}{FM}$$

3.4 Determination of carotenoids and chlorophylls

The determination of the carotenoids and chlorophylls content was performed according to Goodwin & Britton (1988). Around 4 g of each samples were stored at -20° C until further analysis. During the analysis samples were covered to prevent the degradation of the carotenoids. An amount of 0.50-0.54 g of the samples was weighted into 50 mL centrifugation tubes – for each sample four repetitions of the analysis were performed. Afterwards, the samples were homogenized with 15 mL of aceton-hexan (4:5) using an Ultra-Turrax T25 (IKA® Werke GmbH & Co. KG, Germany) at 13,500 min^{-1}. The homogenized plant material was then centrifuged at 4,000 rpm for 10 minutes (Heraeus Megafuge 8R, Thermo Fisher Scientific, Heraeus Holding GmbH, Germany). With a Pasteur pipette, the supernatant was transferred into 25 mL volumetric flasks, while the pellet was washed with 2 mL aceton-hexan (4:5) and then transferred in 10 mL centrifugation tubes and centrifuged at 4,000 rpm for 10 minutes. The supernatant was added to the 25 mL volumetric flasks, which were gently shaken to homogenize the content. Afterwards, the samples were transferred into glass cuvettes and the extinction values were measured with a spectrophotometer UVmini-1240 (Shimadzu, Germany) at the following wavelengths:

- 445 nm (lutein)

- 450 nm (total carotenoids)
- 453 nm (ß – carotene)
- 505 nm (lycopene)
- 645 nm (chlorophyll b)
- 663 nm (chlorophyll a)

The calculation of the carotenoids and chlorophylls content per fresh matter (FM) was then performed using the following formulas (extinction values and compounds' content in Appendix C), where V is the total volume of 25 mL and E_x is the extinction coefficient of that specific compound:

Total carotenoids

$$\mu g\ g^{-1}FM = \frac{E_{450} \times V \times 4}{Fresh\ Weight}$$

Lutein, ß – carotene & lycopene

$$\mu g\ g^{-1}FM = \frac{E_x \times V}{Fresh\ Weight}$$

Chlorophyll a

$$\mu g\ mL^{-1}(chl\ a) = 10{,}1 \times E_{663} - 1{,}01 \times E_{645}$$

$$\Downarrow$$

$$\mu g\ g^{-1}FM = \frac{chl\ (\mu g\ mL^{-1}) \times V}{Fresh\ Weight}$$

Chlorophyll b

$$\mu g\ mL^{-1}(chl\ b) = 16{,}4 \times E_{645} - 2{,}57 \times E_{663}$$

$$\Downarrow$$

$$\mu g\ g^{-1}FM = \frac{chl\ (\mu g\ mL^{-1}) \times V}{Fresh\ Weight}$$

Afterwards, it was possible to calculate the chlorophylls and carotenoids content per dry matter content using the formula:

$$\mu g\ g^{-1}DM = \frac{Fresh\ Weight\ (\mu g\ mL^{-1}) \times 100}{Dry\ Weight}$$

3.5 Lyophilization

The remaining material of each sample was lyophilized for three days with a freeze drier (Alpha 1-4 LSC plus, Martin Christ GmbH, Germany) combined with a LyoCube 4-8 LSC Plus (Martin Christ Gefriertrocknungsanlagen GmbH, Germany), and then milled with a MM 301 milling machine (RETSCH®, Germany) for one minute at 30 sec^{-1}. Afterwards the samples were stored in an exiccator until further analysis.

3.6 Analysis of protein content

The protein content of the samples treated with white light was calculated according to the Kjeldahl method. This method, firstly developed by Johan Kjeldahl (1883), allows to determine the nitrogen content in organic and inorganic substances. It has been modified and improved until now, but it always consists of three fundamental steps (McClements, 2007):

(1) Digestion – decomposition of nitrogen using a concentrated acid solution, to obtain ammonium sulphate solution

(2) Distillation – addition of base to the ammonium sulphate solution, to convert NH_4^+ to NH_3, and condensation of the NH_3 gas into a receiving solution

(3) Titration – quantification of the amount of NH_3 in the receiving solution

The NH_3 content value can then be converted in protein content value using a N factor of 6.25.

Chapter 4

Results

4.1 Effect of light on fresh and dry matter contents

Both fresh and dry matter showed considerably higher values in *Spirodela polyrhiza* than in *Wolffia arrhiza* during the whole treatment. Biomass did not linearly increase or decrease during the experiment (Figures 16 and 17). White light produced the highest amount of fresh matter, followed by blue light and by red light (Figure 16).

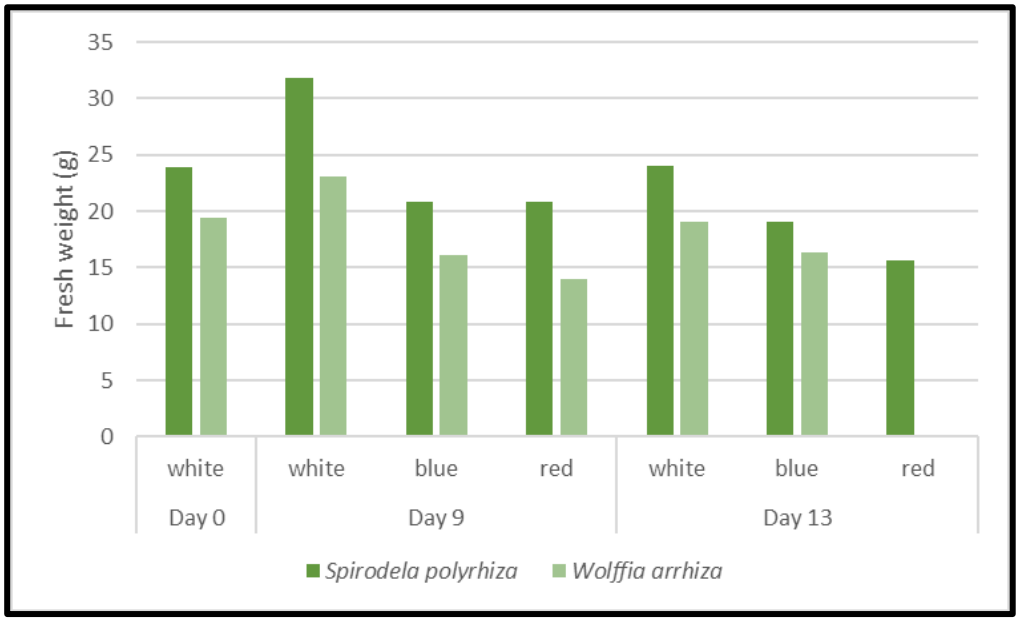


Figure 16: Fresh weight over time of Spirodela polyrhiza *and* Wolffia arrhiza *plants grown under blue, red and white light. Results of the two experiments are combined together.*

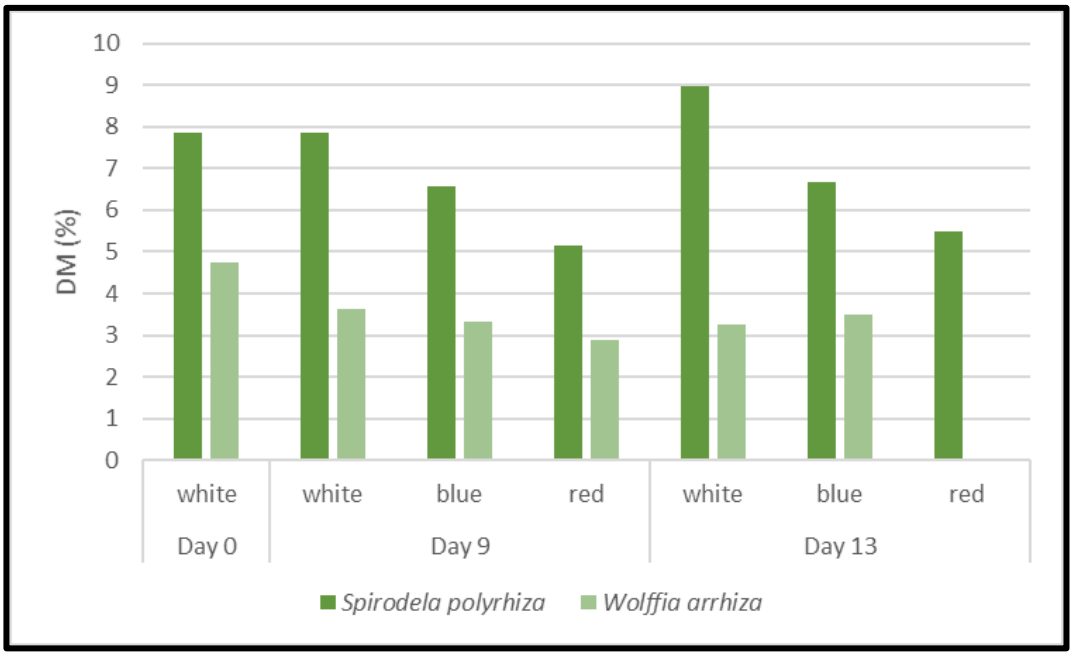
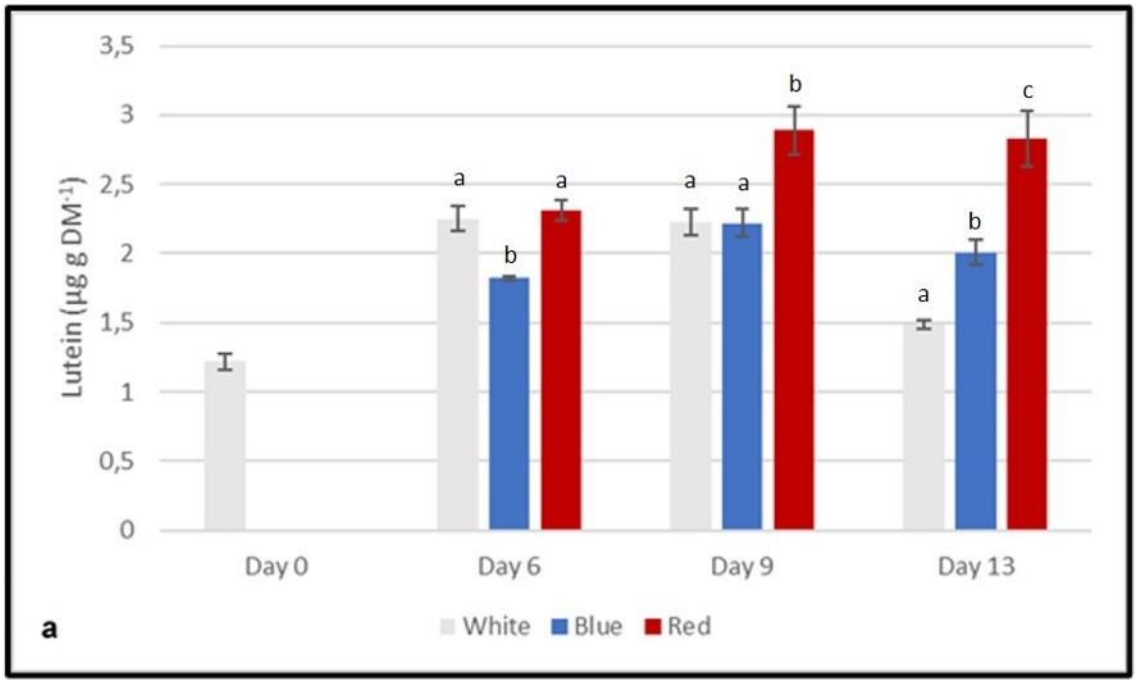


Figure 17: Average percentage of dry matter over time of Spirodela polyrhiza *and Wolffia arrhiza plants grown under blue, red and white light. Results of the two experiments are combined together.*

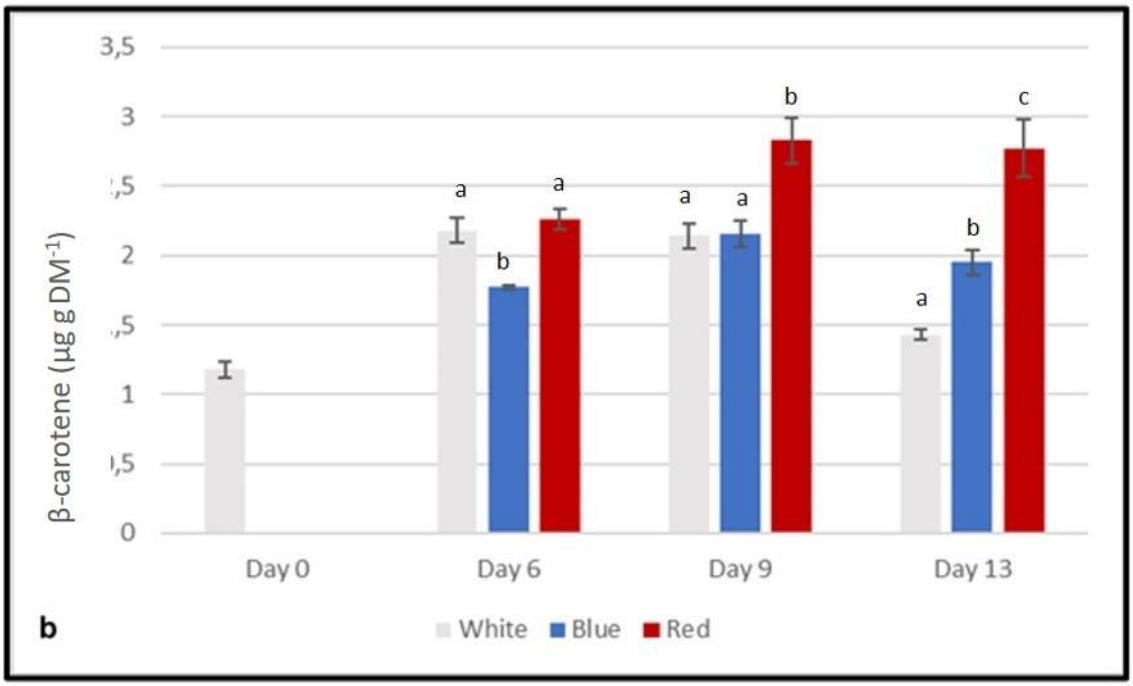
4.2 *Spirodela polyrhiza*

All *Spirodela polyrhiza* samples showed a similar pattern for the biosynthesis of lutein, β-carotene, lycopene, chlorophyll b and chlorophyll a: in general, all the three light treatments caused an increase in the production of these compounds compared to the control. In particular, the experiment showed significant differences regarding the amount of compounds synthetized under different light treatments on day 13: duckweeds produced more of these products when they grew under red light compared to white and blue light. This pattern was found when the compounds content was calculated both on dry matter and on fresh matter basis (graphs showing the compounds content on fresh matter basis in Appendix C). The only exception was the amount of lycopene per g FM: all the three treatments caused an increase in the production of lycopene too, but in this case the highest amount was synthetized by the plants grown under white light, however only tendentiously. A representative example is the content of lutein per g DM (Figure 18 a): the final amount of this compound on day 13 was 1.49 µg g^{-1} DM when grown under white light, 2.01 µg g^{-1} DM when grown under blue light and 2.83 µg g^{-1} DM when grown under red light. A similar pattern was found for all the other compounds except lycopene (Figure 18 c): in its case, red light was the most effective one as well (0.23 µg g^{-1} DM), but blue and white light caused

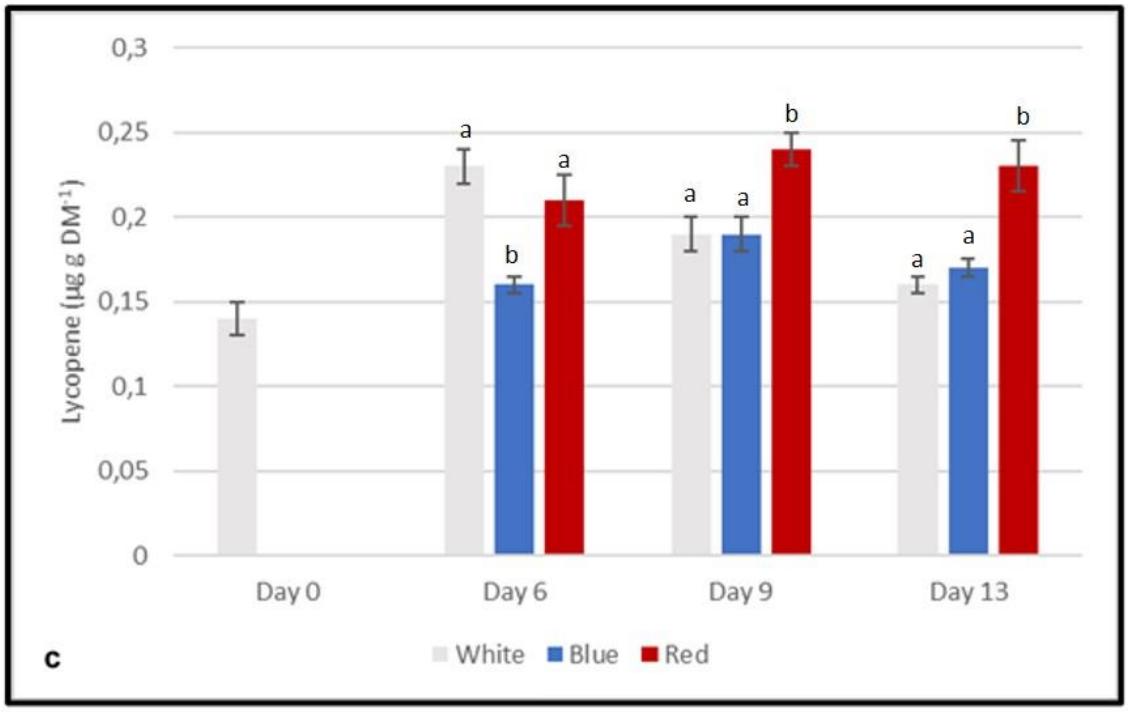
a comparable biosynthesis of this compound (0.16 µg g^{-1} DM and 0.17 µg g^{-1} DM respectively). The content of lutein, β-carotene, lycopene, chlorophyll b and chlorophyll a per DM is shown in Figure 18.

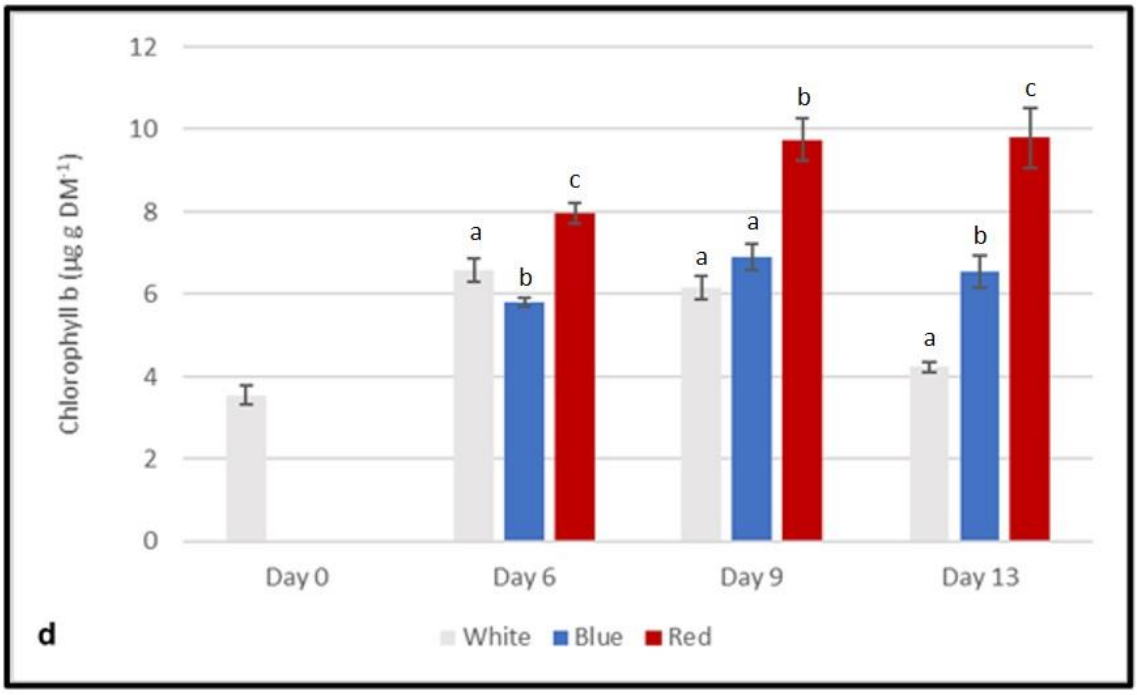


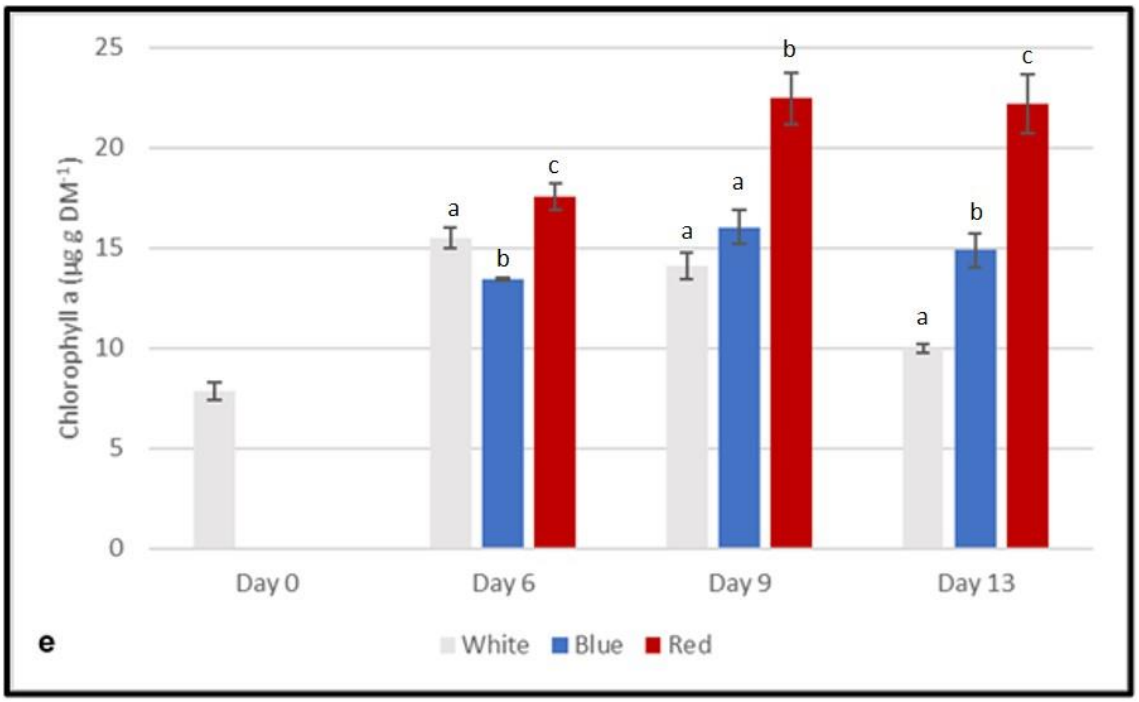
a



b


0,3
0,25
0,2
0,15
0,1
0,05
0
Lycopene (µg g DM-1)
a
b
a
a
a
b
a
a
b
a
a
b
Day 0
Day 6
Day 9
Day 13
White
Blue
Red
c


12
10
8
6
4
2
0
Chlorophyll b (µg g DM-1)
a
b
c
a
a
b
a
b
c
Day 0
Day 6
Day 9
Day 13
White
Blue
Red
d

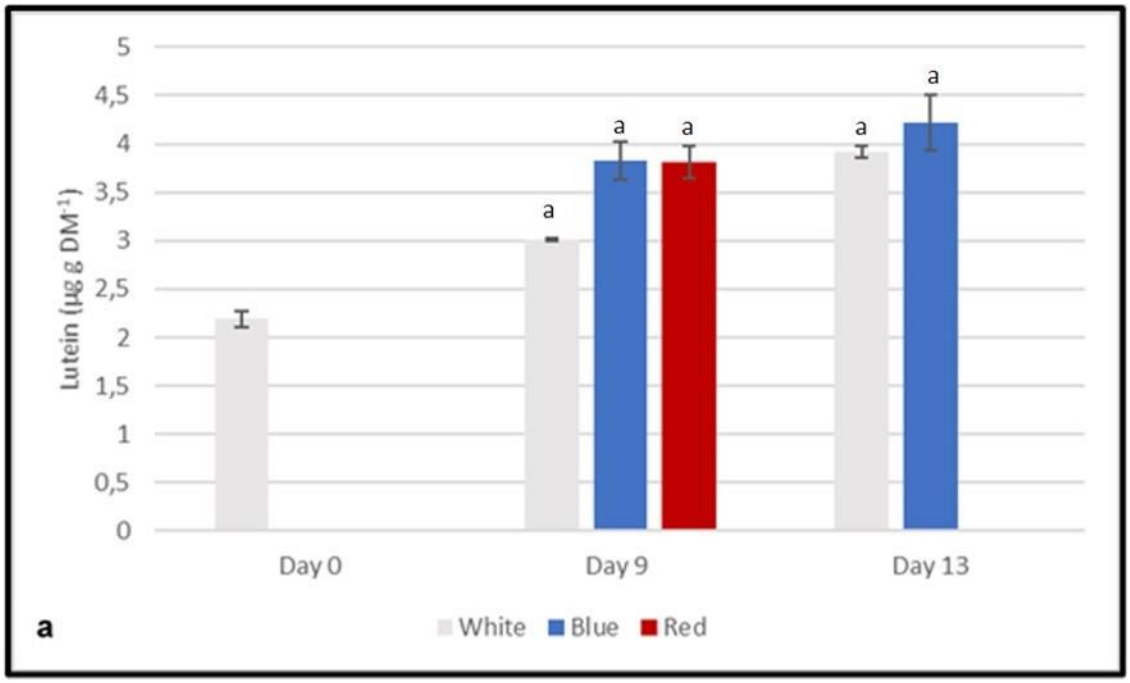


4.3 *Wolffia arrhiza*

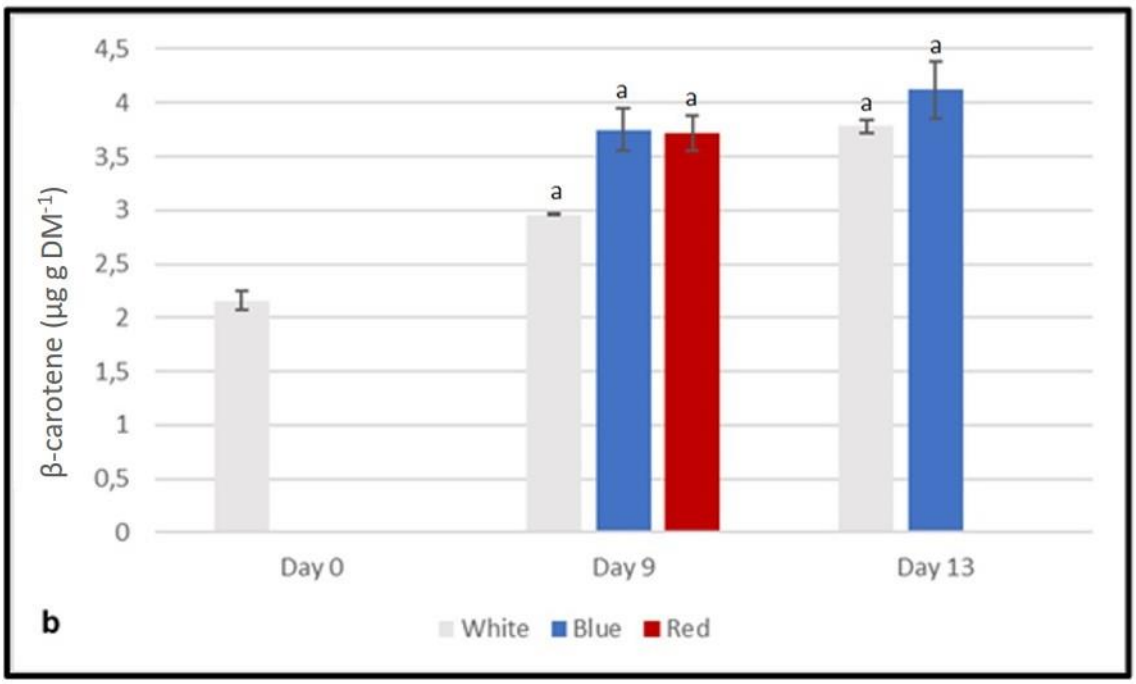
Almost all samples of *Wolffia arrhiza* showed a general increase of the compounds biosynthesis when measured on dry matter basis. Due to the lack of plant material, it was not possible to perform measurements per g DM on day 13 for the plants grown under red light, but the results of the measurements taken on day 9 showed that both blue and red light were more effective in triggering the biosynthesis of the compounds, however only tendentiously; red light was slightly more effective in triggering the biosynthesis of lycopene and chlorophyll a, while blue and red lights were comparably effective in stimulating the production of lutein, chlorophyll b and β-carotene. For instance, the chlorophyll a content on day 9 was 29.66 µg g^{-1} DM when grown under red light and 28.46 µg g^{-1} DM when grown under blue light (Figure 19 e), while the β-carotene content on the same day was 3.72 µg g^{-1} DM when grown under red light and 3.75 µg g^{-1} DM when grown under blue light (Figure 19 b).

Also when measured on fresh matter basis, the samples showed to be influenced by all the light treatments, in particular they showed an increased production of all compounds (graphs showing the compounds content on fresh matter basis in Appendix D). Only for lycopene, the final amount in plants grown under red light was lower than the control; in case of β-carotene, the detected increase in plants

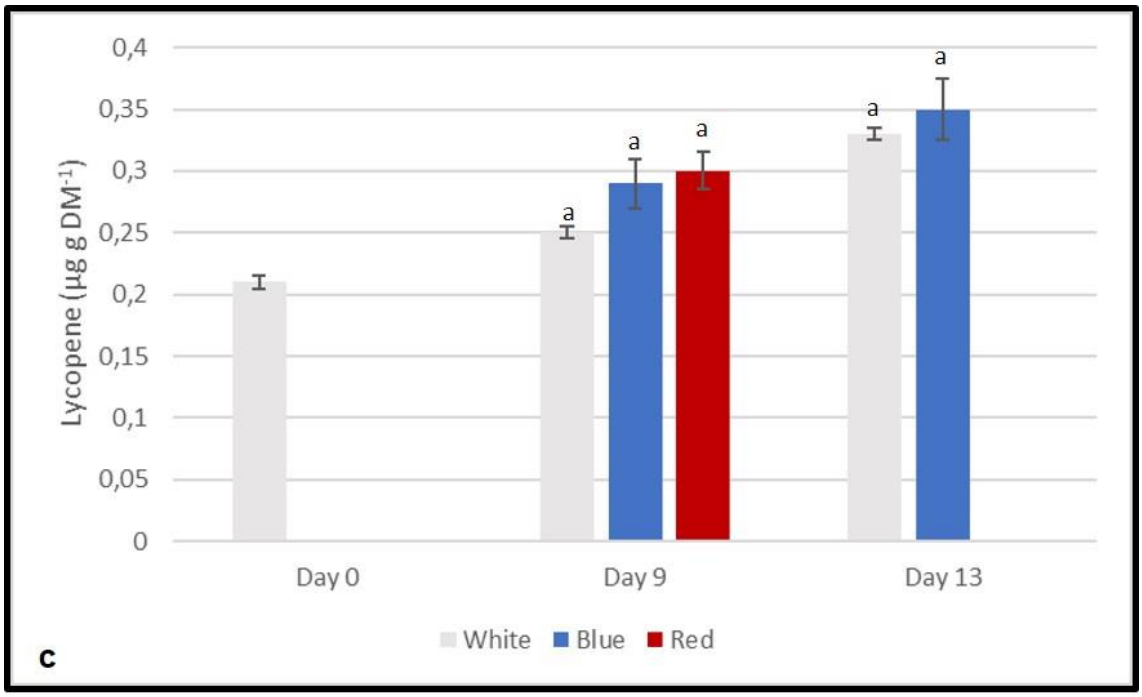
grown under red light was minimum. The blue light treatment showed to be significantly the most effective for all compounds but chlorophyll a, in fact it caused a higher biosynthesis rate for all the compounds compared with red and white light. The content of lutein, β-carotene, lycopene, chlorophyll b and chlorophyll a per DM is shown in Figure 19.

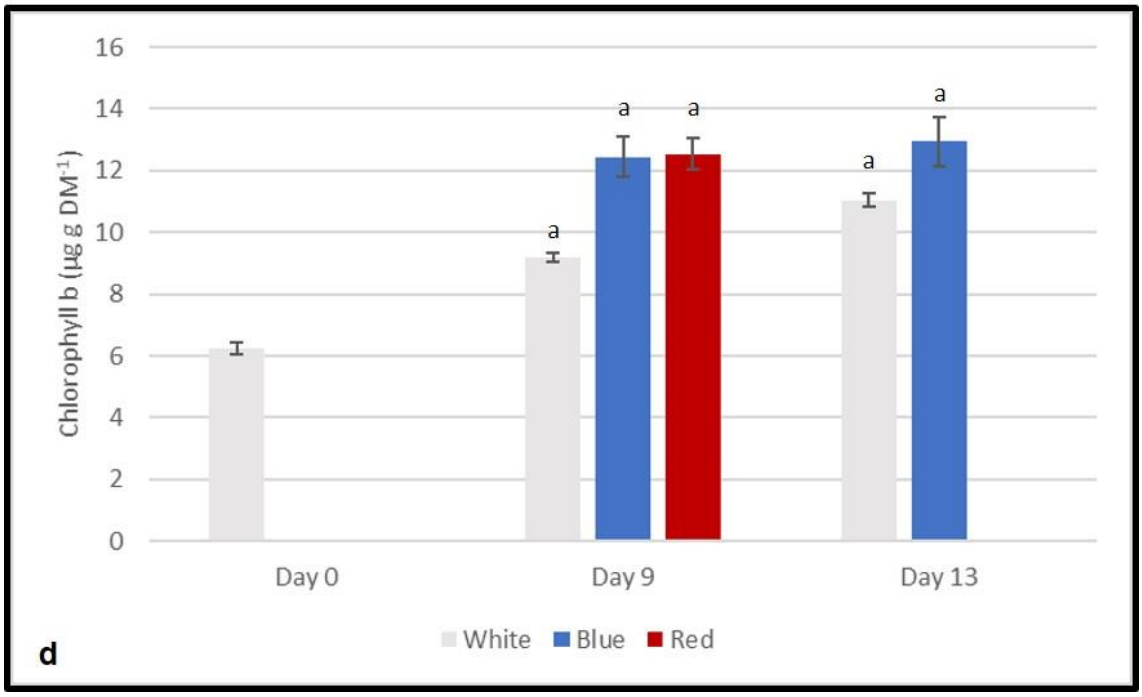


a



b


0,4
0,35
0,3
0,25
0,2
0,15
0,1
0,05
0
Lycopene (µg g DM-1)
a
a
a
a
a
Day 0
Day 9
Day 13
White
Blue
Red
c


16
14
12
10
8
6
4
2
0
Chlorophyll b (µg g DM-1)
a
a
a
a
Day 0
Day 9
Day 13
White
Blue
Red
d

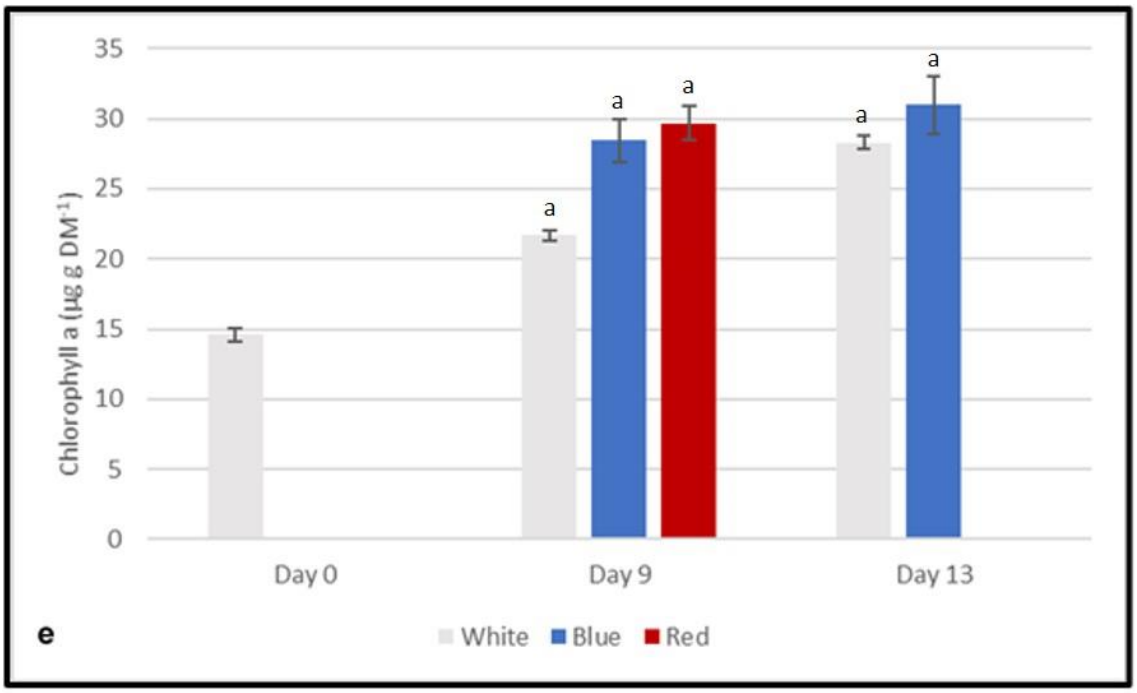
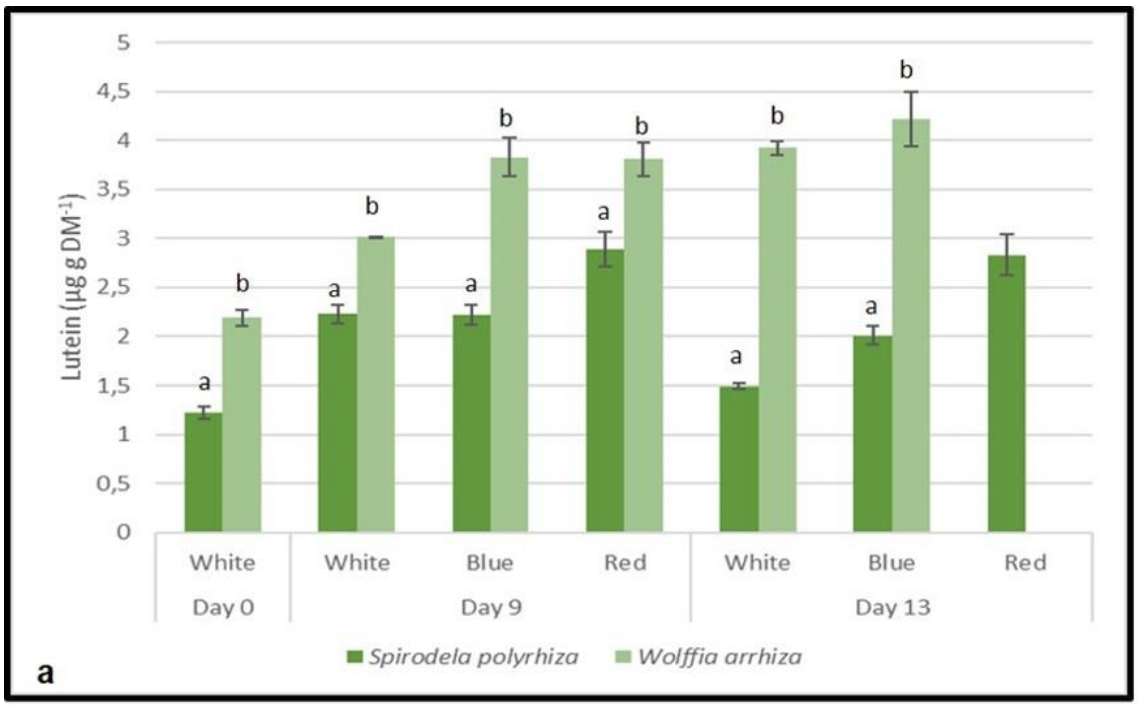
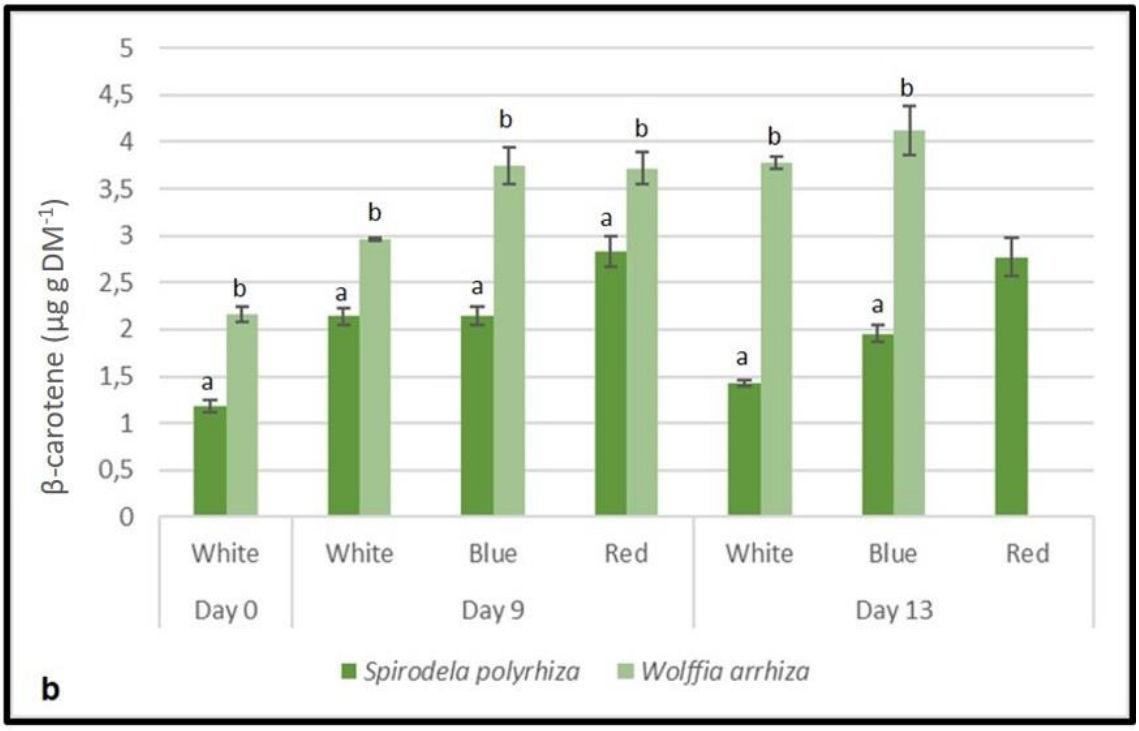


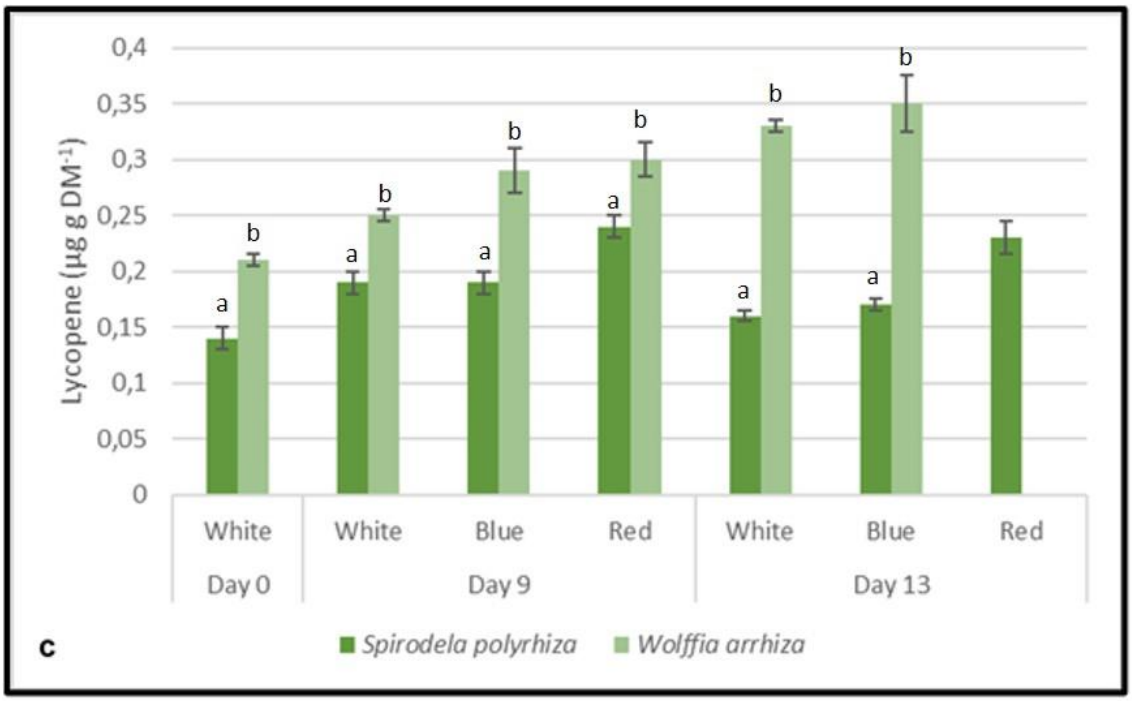
Figure 18: Content (µg g DM^{-1}) of (a) lutein, (b) β-carotene, (c) lycopene, (d) chlorophyll b, (e) chlorophyll a over time in Wolffia arrhiza *grown under blue, red and white light. Different letters indicate significant differences at p<0.05 (Anova test). Results of the two experiments are combined together.*

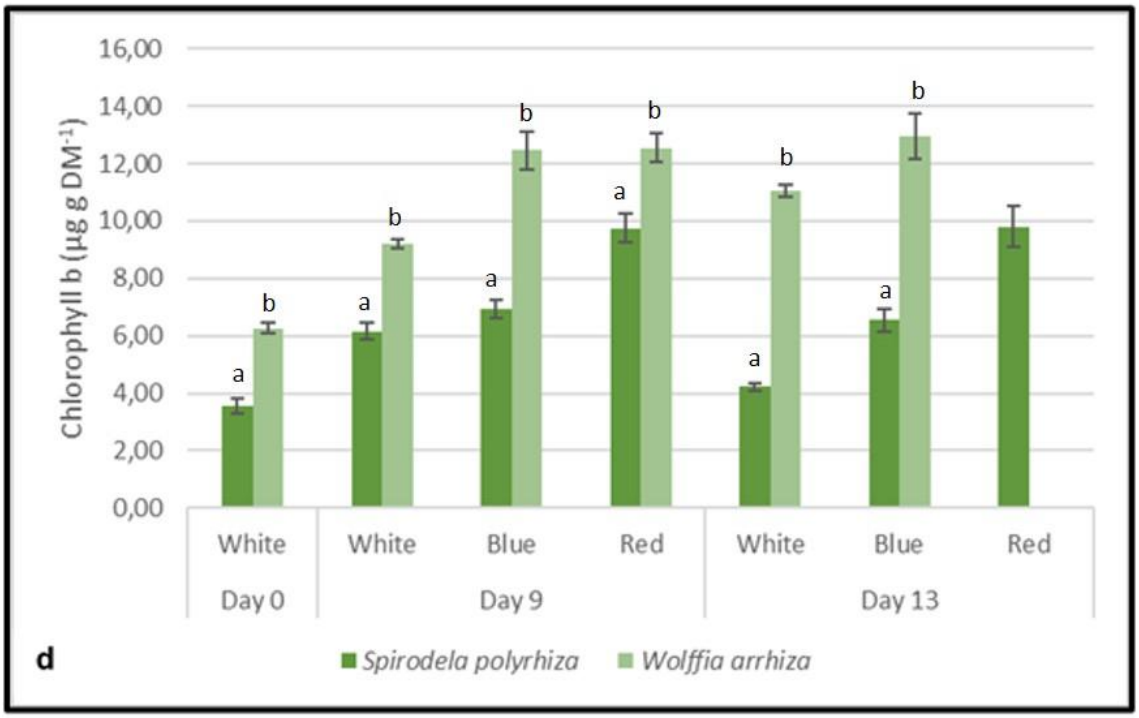
4.4 Comparison between *Spirodela polyrhiza* and *Wolffia arrhiza*

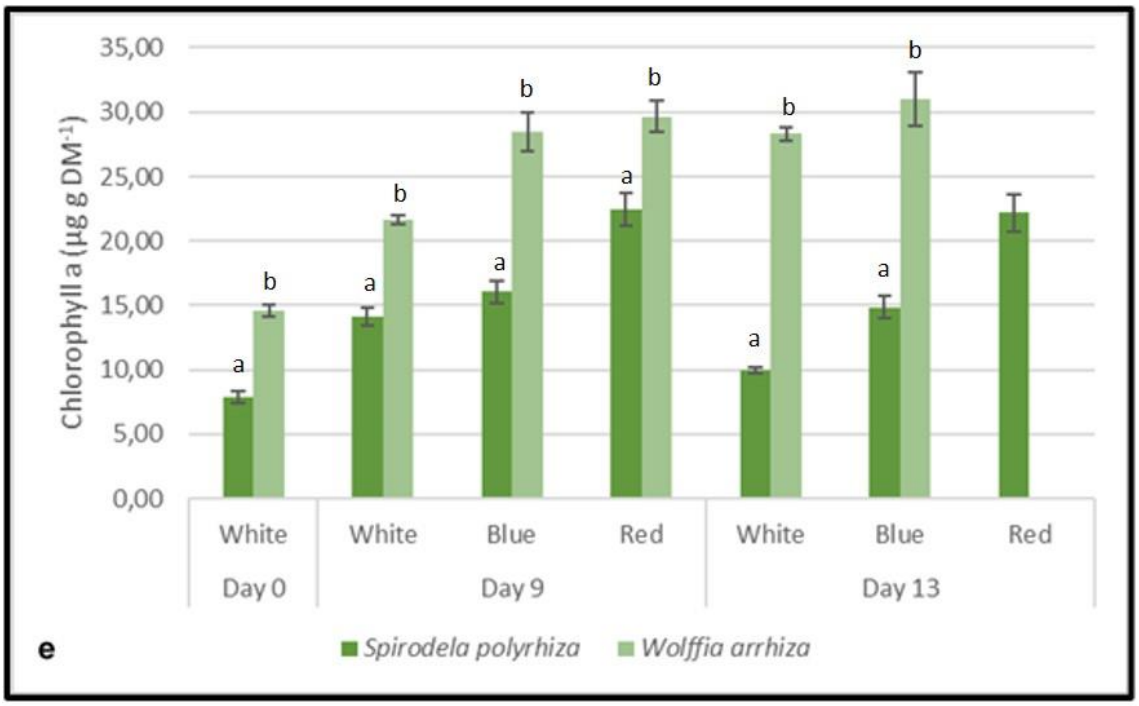
In general, the two species performed differently: *Spirodela polyrhiza* clearly showed to be more triggered to biosynthesize carotenoids and chlorophylls by red light, while *Wolffia arrhiza* was similarly influenced by blue and red light. The control samples of *Wolffia arrhiza* on day 0 synthetized a higher amount of all compounds compared with the ones of *Spirodela polyrhiza* when measured on dry matter basis (Figure 20); the same situation was found when the compounds content was measured on fresh matter basis, with the only exception of lycopene, that is slightly higher in *Spirodela polyrhiza* (graphs showing the compounds content over time on fresh matter basis in both species in Appendix E). During the whole treatment with all the three lights, *Wolffia arrhiza* biosynthetized a higher content of all compounds per g DM compared to *Spirodela polyrhiza,* while *Spirodela polyrhiza* produced a higher amount of all compounds per g FM. Only on day 13, a comparable amount of the compounds per g FM was found in the two species grown under blue light.


Lutein (µg g DM-1)
5
4,5
4
3,5
3
2,5
2
1,5
1
0,5
0
White
White
Blue
Red
White
Blue
Red
Day 0
Day 9
Day 13
Spirodela polyrhiza
Wolffia arrhiza
a


β-carotene (µg g DM-1)
5
4,5
4
3,5
3
2,5
2
1,5
1
0,5
0
White
White
Blue
Red
White
Blue
Red
Day 0
Day 9
Day 13
Spirodela polyrhiza
Wolffia arrhiza
b


0,4
0,35
0,3
0,25
0,2
0,15
0,1
0,05
0
Lycopene (µg g DM-1)
a
b
a
b
a
b
a
b
a
b
a
b
a
b
White
White
Blue
Red
White
Blue
Red
Day 0
Day 9
Day 13
c
Spirodela polyrhiza
Wolffia arrhiza


16,00
14,00
12,00
10,00
8,00
6,00
4,00
2,00
0,00
Chlorophyll b (µg g DM-1)
a
b
a
b
a
b
a
b
a
b
a
b
a
b
White
White
Blue
Red
White
Blue
Red
Day 0
Day 9
Day 13
d
Spirodela polyrhiza
Wolffia arrhiza



4.5 Impact of light treatment on protein content

The protein content varied between a minimum of 4.66% and a maximum of 19.76% of dry matter, corresponding respectively to *Wolffia arrhiza* on day 13 and *Spirodela polyrhiza* on day 6. On day 0 *Wolffia arrhiza* showed a significantly higher protein content compared to *Spirodela polyrhiza*, while on day 13 *Spirodela polyrhiza* had a significantly higher protein content (Figure 21).

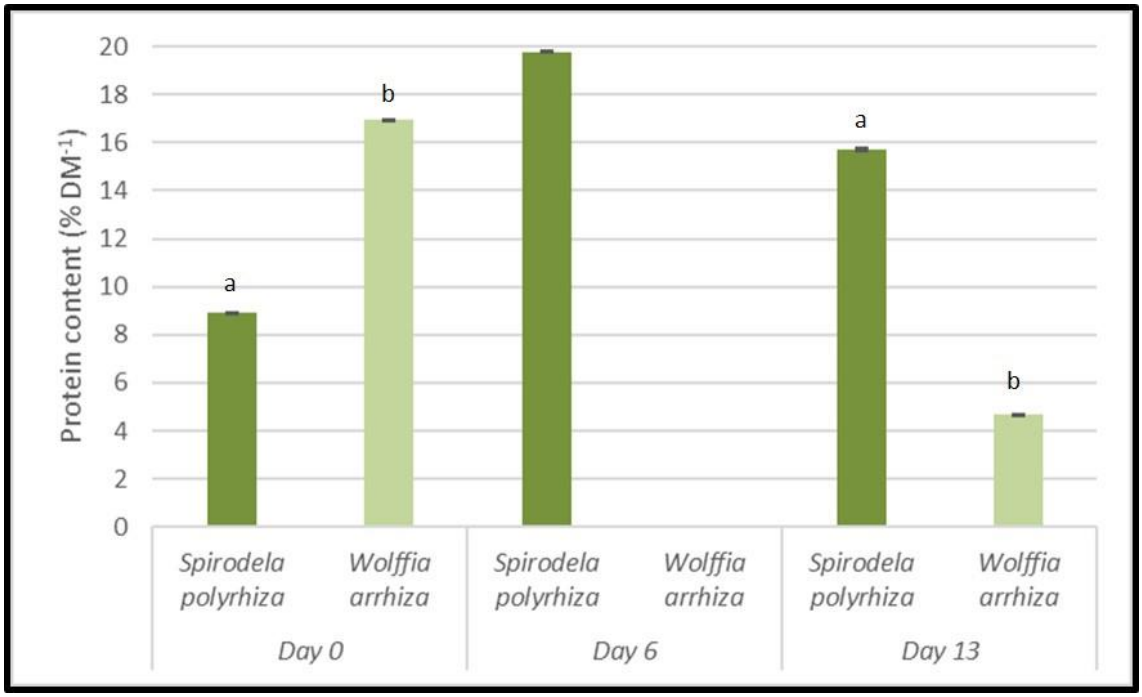


Figure 19: Protein content of Spirodela polyrhiza and Wolffia arrhiza grown under white light over time.

Chapter 5

Discussion

The general suitability of duckweeds as both human and animal food, especially in a context of integrated farming in developing countries, has already been stated (Skillicorn et al., 1993), and many studies proved duckweeds to truly be a fast growing and easy-to-cultivate source of nutritious compounds (Bhanthumnavin and McGarry, 1971; Leng et al., 1995; Goopy and Murray, 2003; Appenroth et al., 2017). Due to these characteristics, a raising interest towards *Lemnaceae* is currently developing, both on a business and social oriented point of view. Companies are looking at duckweeds as an appropriate plant to produce super food and functional food, and NGOs such as FAO strongly believe in their potential to be a proper quality food to feed poor people in developing countries (Leng, 1999; www.parabel.com).

With the consciousness of the beneficial effects of carotenoids and proteins for human health, one may consider the idea to actively stimulate their biosynthesis and accumulation, taking advantage of the available knowledge of the influence of light quality on them. Using LED lamps with this purpose would be indeed a promising, reliable and affordable via to reach this goal.

According to the results of this preparatory research, light quality influences all the evaluated parameters, namely production of biomass, biosynthesis of carotenoids, chlorophylls and proteins.

Biomass production

White light was the best one in triggering the biomass production, in both *Spirodela polyrhiza* and *Wolffia arrhiza*. This is consistent with the study of Hultberg et al. (2014) on the green microalga *Chlorella vulgaris*, which reported white light to be more effective than blue light to stimulate biomass production. On the contrary, a recent study by Rabara et al. (2017) reported that artichokes seedlings grown under blue and white LEDs had a production of biomass which was 67-76% lower compared to the ones grown under red light; also the study of Chen et al. (2017) confirmed red light to be more effective than white light on

biomass production in Savoy cabbage, even though the photochemical efficiency was best under white light. Furthermore, a study by Zhang et al. (2015), shows that red and white LEDs are better than blue ones in stimulating the biosynthesis of chlorophylls and the biomass accumulation in Romain lettuce (white LEDs are the most effective, followed by red and blue LEDs). Unfortunately, the literature regarding duckweeds is very scarce and doesn't allow to really estimate the reliability of these results in a broader sense. Nevertheless, there are proves that red and white light can trigger the biosynthesis of chlorophylls in some vegetables and ornamental plants (Lesfrud et al., 2008; Zhang et al., 2015; Zheng and van Labeke, 2017) and it's well known that chlorophylls are green pigment and mainly absorb red wavelengths (Rascio et al., 2012). So, red light can lead to a higher photosynthetic rate when there's enough CO_2 available, and likely consequently to an increased biomass production.

Synthesis of carotenoids and chlorophylls

Red LEDs clearly showed to be the most suitable to trigger the biosynthesis of carotenoids and chlorophylls in *Spirodela polyrhiza*, while in *Wolffia arrhiza* some differences among the light treatments were detected, even though they were not statistically different. Plants are known to produce more carotenoids to deal with light stress conditions, since these compounds play a primary role in protecting the photosynthesis apparatus from too intense light sources: it is likely that red LEDs enhanced the biosynthesis of carotenoids as a response. As reported by Ma et al. in 2011, red light at 660 nm enhanced the carotenoids metabolism in citrus fruits. Concerning chlorophylls, there are also evidences that light stress can trigger their production. Andersson et al. (2013) found out that in *Arabidopsis thaliana* a protein of the chlorophyll a/b-binding family was accumulated depending on light stress factors, and they supposed its biosynthesis to be a "photoprotective strategy induced within photosystem I in response to light stress". Lefsrud et al. (2008) reported that red LEDs at 640 nm could increase the amount of chlorophyll a and b in *Brassica oleracea.* On the other hand, Li and Kubota in 2009 found that red light at high wavelength (734 nm) decreased the concentration of both chlorophylls and carotenoids in *Lactuca sativa* L. Also in

case of carotenoids and chlorophylls, there are not specific researches regarding the influence of light quality on duckweeds.

Synthesis of proteins

Spirodela polyrhiza clearly showed a higher amount of proteins in the end of the white light treatment, compared with *Wolffia arrhiza.* In general, there is some evidence of white light triggering the protein synthesis in plants, for instance Romero-Romero & Sanchez-Saavedra reported in 2017 that white light at 540 nm highly triggered the protein synthesis in a diatom species, while on the other hand a study by Lin et al. (2013) showed that light quality didn't influence the soluble protein content in lettuce. Anything specific is found about duckweeds; these plants are confirmed to basically contain a high amount of crude proteins, nevertheless further studies are necessary to understand if light quality can have a specific role in determining the protein concentration. It is very important to bear in mind that an increase in protein content is not always a synonymous of better quality. Different light wavelengths and intensities can trigger the biosynthesis of diverse proteins: for instance, Muneer et al. (2014) reported blue LED light to lead to an increase of photosynthesis-related complex proteins in *Lactuca sativa* L., while Li et al. (2012) showed red LEDs to increase the concentration of soluble proteins in *Brassica campestris* L.. Light could also stimulate stress reactions, thus leading to the biosynthesis of enzymes involved in defence responses: for instance, red light triggers the production of protection pigments, and the increase of proteins involved in their biosynthesis is necessary and therefore likely to happen. In 2008, Xu et al. found out that an increase of light intensity could lead to an increase in the uptake of NH_3 by maize, and a following study by Ma et al. (2016) showed how *Brassica chinensis* L.'s capability of uptaking nitrogen can be influenced by light intensity and is best at 360 µmol m^{-2} s^{-1}. Thus, light can generally also affect the plants' nitrogen uptake, with a consequent influence on the proteins synthesis.

Without any doubt, duckweeds are highly promising plants, suitable for many and diverse aims, and it is worth investing on them. Nevertheless, there is still much to research to better understand the specific metabolism of *Lemnaceae* and how

they react to different stimuli, especially to light, in order to take advantage of this knowledge to harvest highly nutritious plants. This present Master study had the aim to evaluate if there is an actual possibility to proceed researching in this direction, and it indeed proved that duckweeds do response differently to different light spectra. It also showed how, depending on the purpose, the choice of the species is also of fundamental importance: *Spirodela polyrhiza* produced a higher amount of proteins, while *Wolffia arrhiza* showed a more intense production of carotenoids. Further studies are required to get a better understanding of the influence of light quality on the metabolic pathways of these plants.

Chapter 6

Summary

The present research was performed with the aim of better understanding the potential of LED lamps in increasing the proteins and pigments content of duckweeds, hopefully opening the way for further studies. *Lemnaceae* are already known to be a very good source of proteins and fibers, with a low fat content at the same time (Leng et al., 1995; Leng, 1999). *Spirodela polyrhiza* and *Wolffia arrhiza* were especially chosen as target species of this study, since in the growing chambers and greenhouse they performed better than other species in terms of biomass increase, quickness of propagation and low development of algae. The two target species were propagated into a growing chamber under white LED light (130 ± 10 µmol m^{-2} s^{-1}), red LED light (30 ± 10 µmol m^{-2} s^{-1}) and blue LED light (ca. 100 ± 10 µmol m^{-2} s^{-1}), and two repetitions of the experiment were performed. Unfortunately, during the second repetition there have been some issues concerning the red LED lamps, which made it difficult to precisely reproduce the experiment. Nevertheless, the evaluation of the content of protein and pigments (lutein, β-carotene, lycopene, chlorophyll b and chlorophyll a) was promising and showed that there is potential for further studies and applications of this technology: in *Spirodela polyrhiza*, red LEDs positively influenced the biosynthesis of carotenoids and chlorophylls compared with blue and white LEDs, and the protein content increased under white LEDs; on the contrary, white LEDs treatment decreased the protein content in *Wollfia arrhiza*, and its amount of chlorophylls and carotenoids was not deeply influenced by the different light treatments. The biomass production of both species was triggered when duckweeds were grown under white LEDs. The experiment should be reproduced to provide a more precise overview of the potential of LEDs, but the present results are already a valuable starting point for other researchers who may be interested in deepening this knowledge, as they clearly show that LEDs can influence in a positive way the quality attributes of duckweed, hopefully leading to the possibility of farming more nutritious duckweeds.

Declaration of Authorship

I hereby declare that the present thesis has not been submitted as a part of any examination procedure and has been independently written. All passages, including those from the internet, which were used directly on in modified form, especially those sources using text, graphs, charts or picture, are indicated as such. I realize that an infringement of these principles which would amount to either an attempt of deception or deceit will lead to the institution of proceedings against myself.

Berlin, August 2017

Silvia Torri

Acknowledgements

During my master's thesis research I learned much, not only on a scientific point of view but also about myself. It has been a stimulating, interesting and sometimes challenging path, which was the last step of a wonderful Master program spent in three among the best European Universities.

Therefore, I would like to thank all who accompanied me during this time. First of all, a huge thank to Ms. Susanne Meier, who taught me how to perform the carotenoid analysis and was always present and patient. I would also like to thank Mr. Stefan Irrgang for his help in propagating different species of duckweeds and for being always available to answer my questions and to support my work, Mrs. Linda Nagler for working with me to carry out the duckweeds propagation in the greenhouse, and Prof. Werner Herppich from ATB for allowing me to use their growing chamber and LED lamps.
I also would like to especially thank my co-supervisor at HU-Berlin, Prof. Susanne Huyskens-Keil, as she offered me such an interesting research topic, and always supported me during the project by answering my questions, gathering information, and giving pieces of advice and precious feedback. Then, I would love to thank my main supervisor from BOKU, Prof. Anna Keutgen: together with Prof. Huyskens-Keil, she read and evaluated my thesis, and also was totally available to help me with all the bureaucratic issues I went through to be eligible to get a double degree (and it was definitely much!), I really appreciated that because it has been very strenuous for me.
A last big thank to Dr. Christian Bruhn, to be the donor of my Deutschlandstipendium and so contributing to allow me to stay in Berlin and do my master's thesis research at Humboldt University.

Appendix

A) Universol Blue (ICL-Speciality Fertilizers, Netherlands) (Table 5)

Table 5: Mineral content of Universol Blue (ICL-Speciality Fertilizers, Netherlands) (https://icl-sf.com/ie-en/products/ornamental_horticulture/2041-universol-blue/)

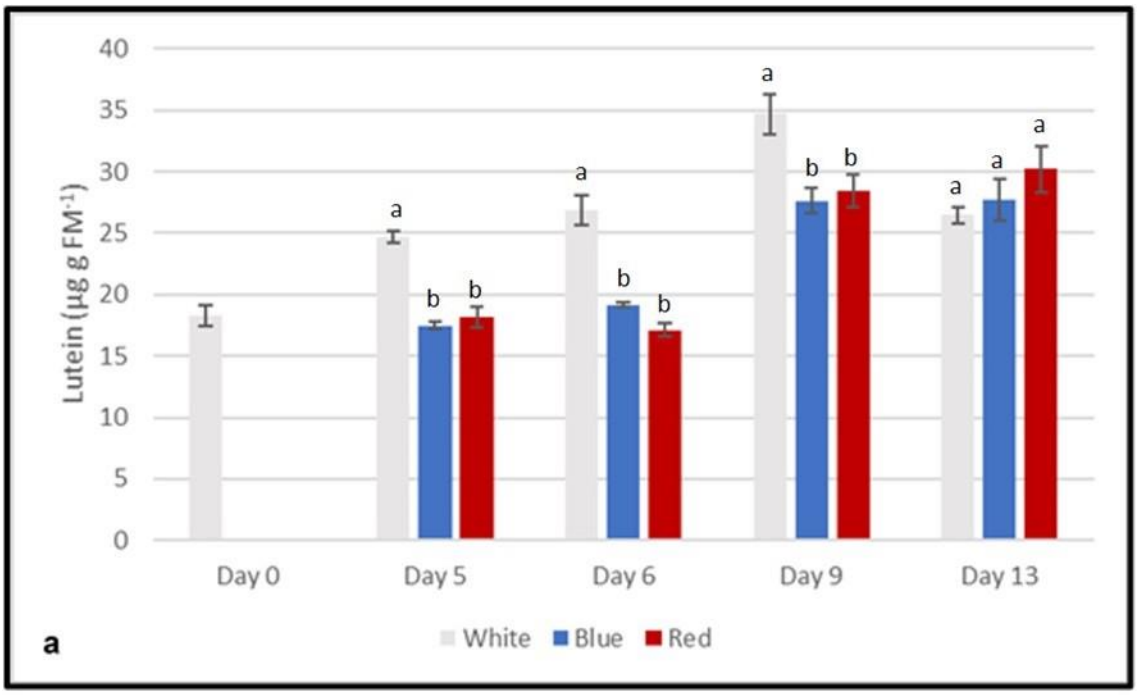
Nitrogen	Total	18%
	Nitrate nitrogen (NO3-N)	10%
	Ammoniacal nitrogen (NH4-N)	7,70%
Phosphorus	Total	4,80%
	Water soluble	4,80%
Potassium	Total	14,90%
	Water soluble	14,90%
Magnesium	Total	1,50%
	Water soluble	1,50%
Iron	Total	0,10%
	Water soluble	0,10%
	Chelated by EDTA	0,10%
Manganese	Total	0,04%
	Water soluble	0,04%
	Chelated by EDTA	0,04%
Boron	Tota	0,01%
	Water soluble	0,01%
Copper	Total	0,01%
	Water soluble	0,01%
	Chelated by EDTA	0,01%
Molybdenum	Total	0,00%
	Water soluble	0,00%
Zinc	Total	0,01%
	Water soluble	0,01%
	Chelated by EDTA	0,01%

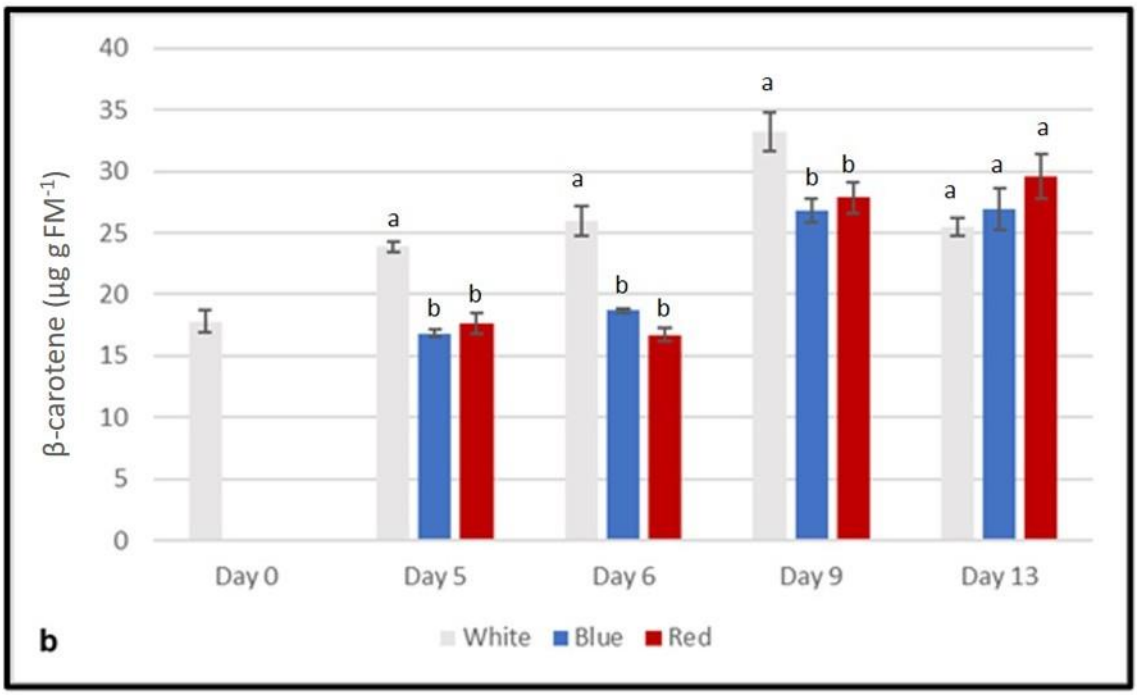
B) Fresh and dry matter values present the final results (%) in the chapter results (Table 6)

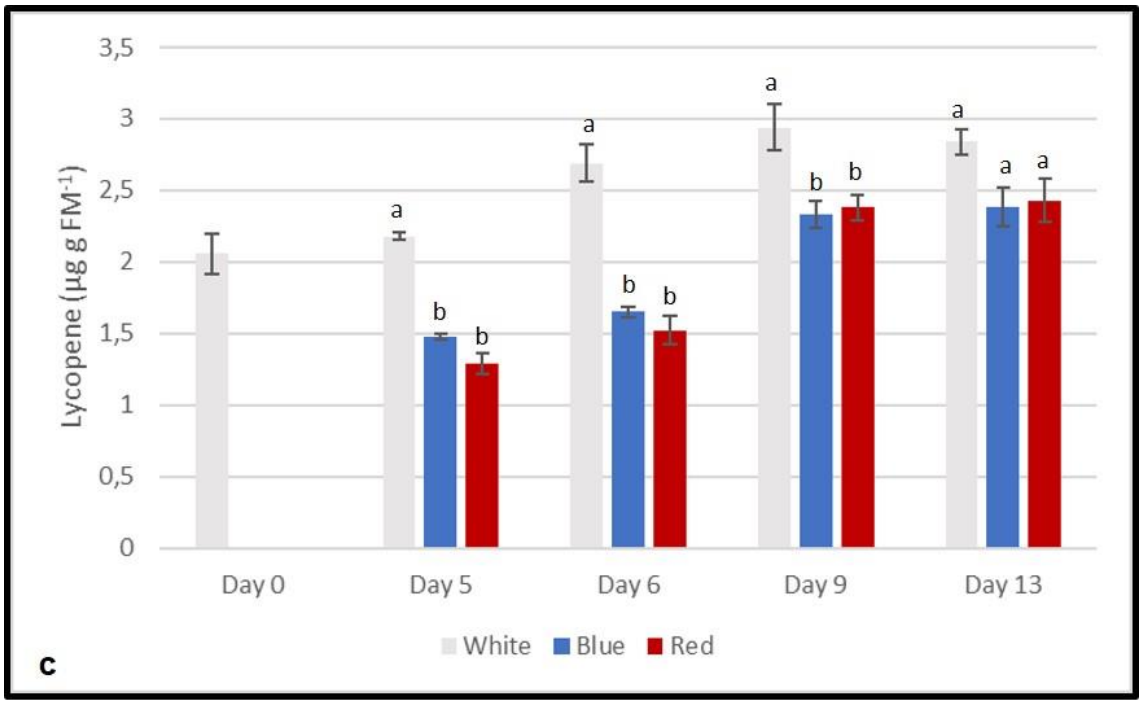
Table 6: Values of the fresh matter (FW) and dry matter (DW) of the first and second experiment

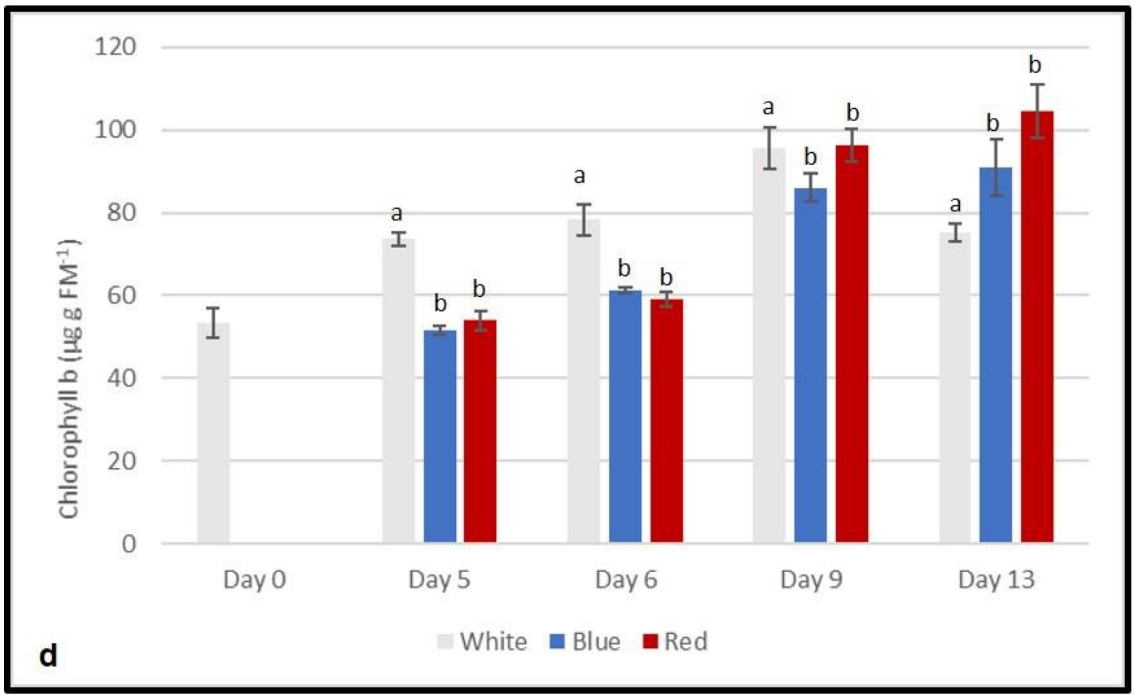
1st experiment					
Species	**Light treatment**	**Day**	**FW (g)**	**DW (g)**	**DW (%)**
Spirodela polyrhiza	White	5	-	-	-
Spirodela polyrhiza	Blue	5	-	-	-
Spirodela polyrhiza	Red	5	-	-	-
Wolffia arrhiza	White	9	28,68	1,05	3,66
Wolffia arrhiza	White	9	34,85	1,25	3,59
Wolffia arrhiza	Blue	9	20,83	0,69	3,31
Wolffia arrhiza	Red	9	20,83	0,6	2,88
Spirodela polyrhiza	White	9	23,11	1,97	8,52
Spirodela polyrhiza	White	9	23,17	1,66	7,16
Spirodela polyrhiza	Blue	9	16,21	1,17	7,22
Spirodela polyrhiza	Blue	9	16,02	0,95	5,93
Spirodela polyrhiza	Red	9	12,03	0,63	5,24
Spirodela polyrhiza	Red	9	13,96	0,71	5,09
2nd experiment					
Sample	**Light treatment**	**Day**	**FW (g)**	**DW (g)**	**DW (%)**
Spirodela polyrhiza	Control	0	22,19	1,73	7,80
Spirodela polyrhiza	Control	0	25,66	2,03	7,91
Wolffia arrhiza	Control	0	19,38	0,92	4,75
Spirodela polyrhiza	White	6	21,02	1,28	6,09
Spirodela polyrhiza	White	6	18,13	1,18	6,51
Spirodela polyrhiza	Blue	6	18,36	1,02	5,56
Spirodela polyrhiza	Red	6	17,16	0,68	3,96
Spirodela polyrhiza	White	13	19,98	1,43	7,16
Spirodela polyrhiza	White	13	27,95	3,02	10,81
Spirodela polyrhiza	Blue	13	19,08	1,27	6,66
Spirodela polyrhiza	Red	13	15,7	0,86	5,48
Wolffia arrhiza	White	13	19,05	0,62	3,25
Wolffia arrhiza	Blue	13	16,37	0,57	3,48
Wolffia arrhiza	Red	13	-	-	-

C) Graphs showing the content of lutein, β-carotene, lycopene, chlorophyll b and chlorophyll a on fresh matter basis in *Spirodela polyrhiza* (Figure 22)






3,5
3
2,5
2
1,5
1
0,5
0
Lycopene (µg g FM-1)
a
b
b
a
b
b
a
b
b
a
b
b
a
a
a
Day 0
Day 5
Day 6
Day 9
Day 13
White
Blue
Red
c


120
100
80
60
40
20
0
Chlorophyll b (µg g FM-1)
a
b
b
a
b
b
a
b
b
a
b
b
a
b
b
Day 0
Day 5
Day 6
Day 9
Day 13
White
Blue
Red
d

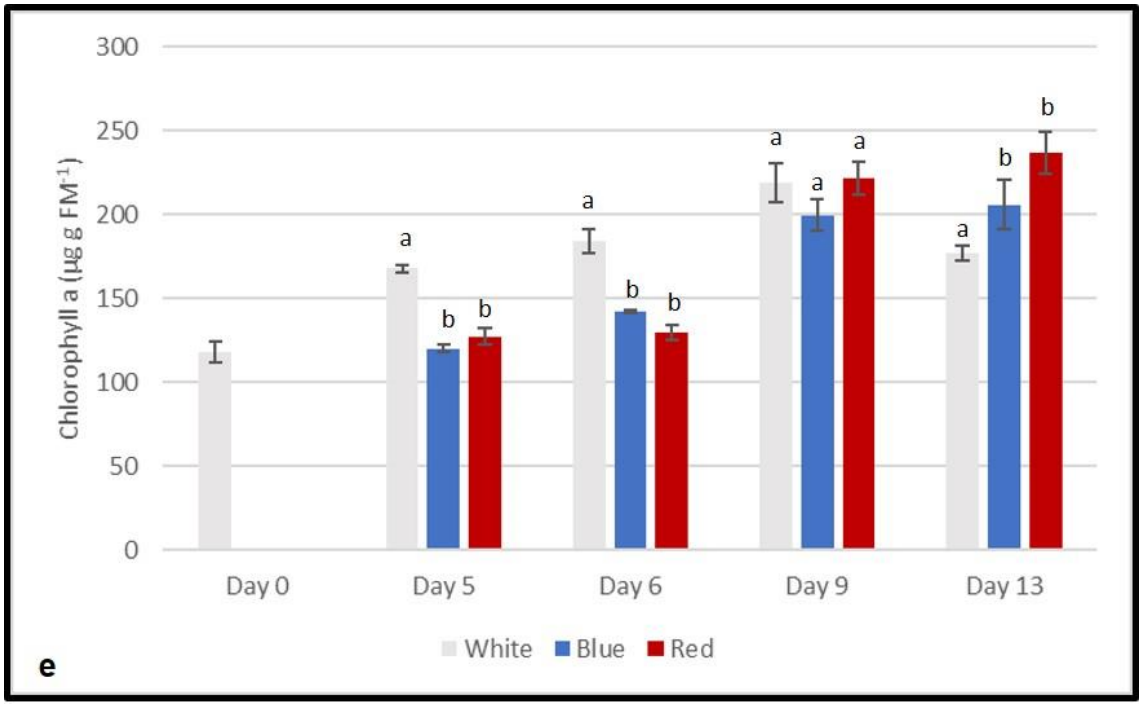
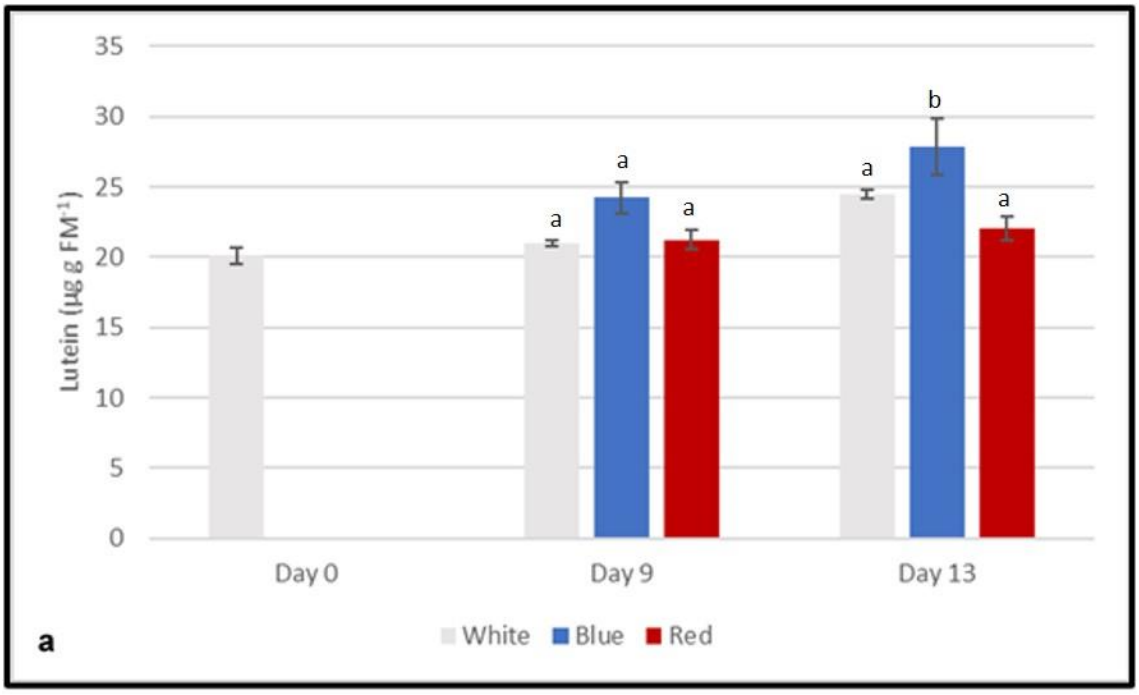
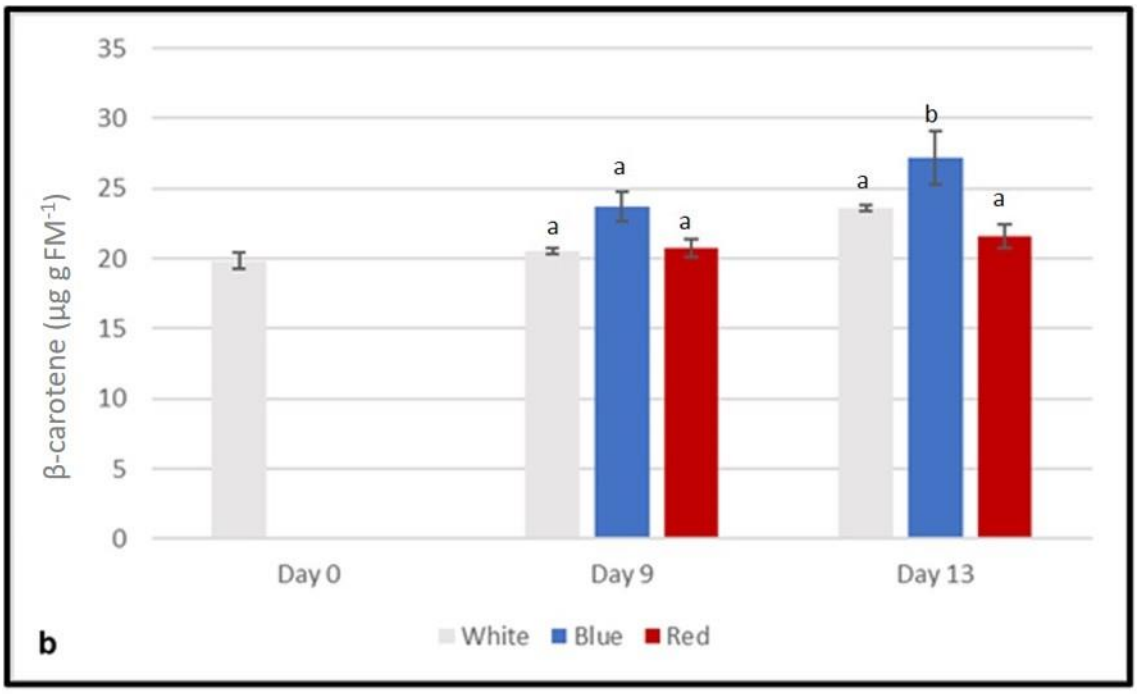
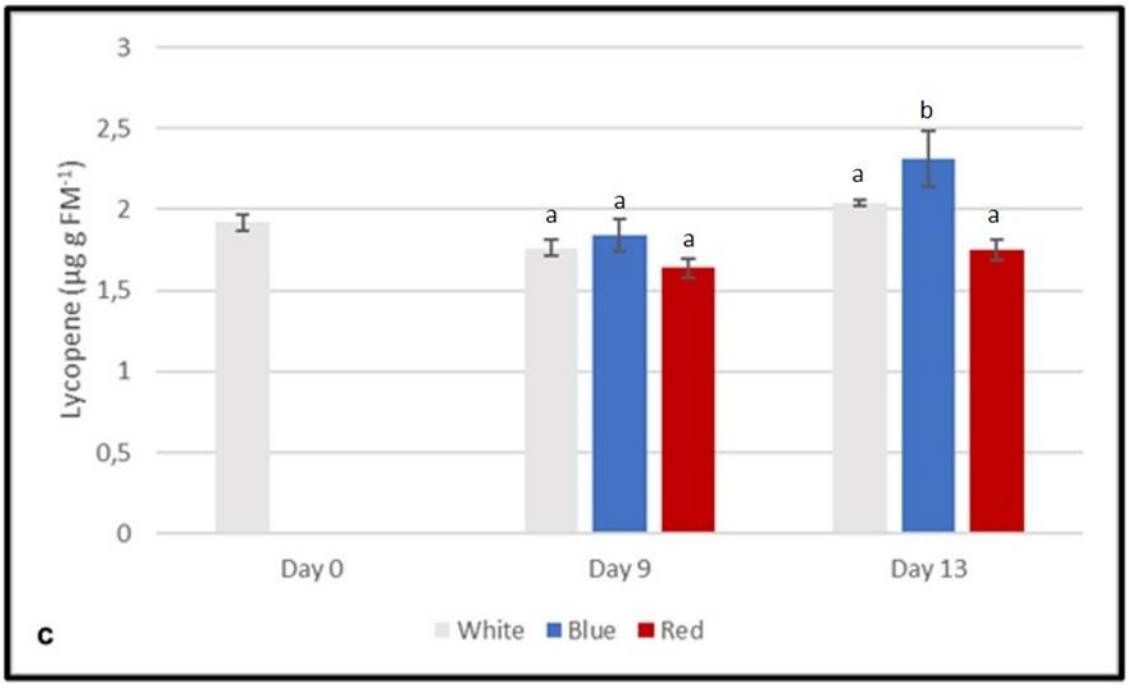


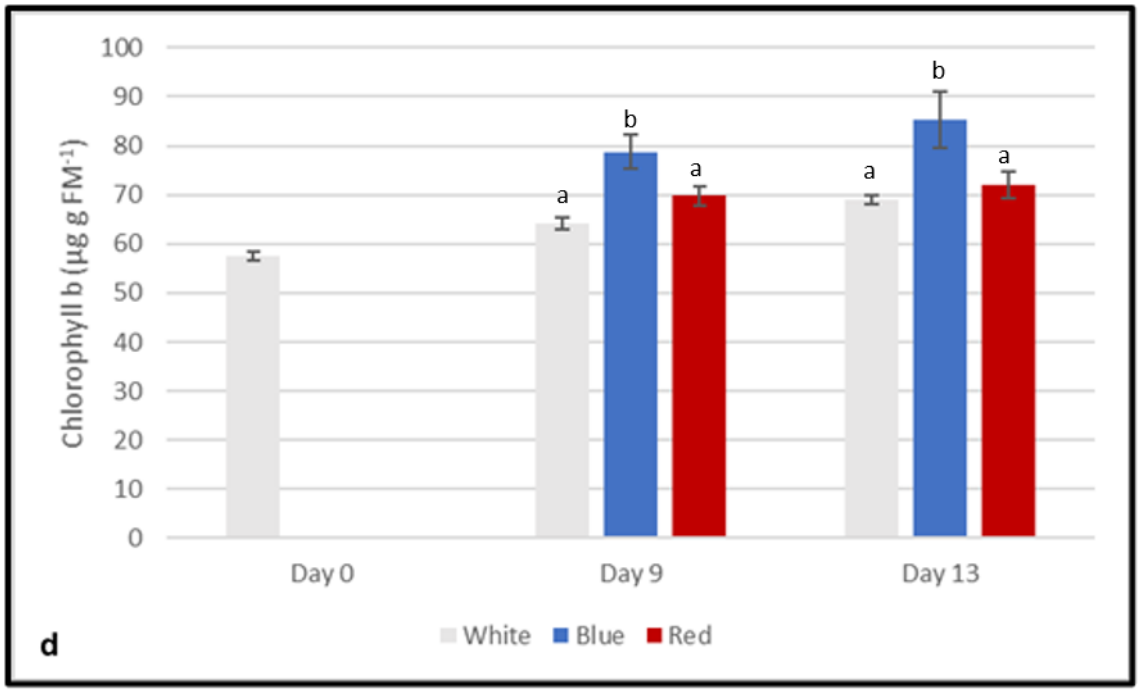
Figure 20: Content (µg g FM^{-1}) of (a) lutein, (b) β-carotene, (c) lycopene, (d) chlorophyll b, (e) chlorophyll a over time in Spirodela polyrhiza *grown under blue, red and white light. Different letters indicate significant differences at p<0.05 (Anova test). Results of the two experiments are combined together.*

E) Graphs showing the content of lutein, β-carotene, lycopene, chlorophyll b and chlorophyll a on fresh matter basis in *Wolffia arrhiza* (Figure 23)




35
30
25
20
15
10
5
0
β-carotene (µg g FM-1)
a
a
a
a
b
a
Day 0
Day 9
Day 13
White
Blue
Red
b


3
2,5
2
1,5
1
0,5
0
Lycopene (µg g FM-1)
a
a
a
a
b
a
Day 0
Day 9
Day 13
White
Blue
Red
c



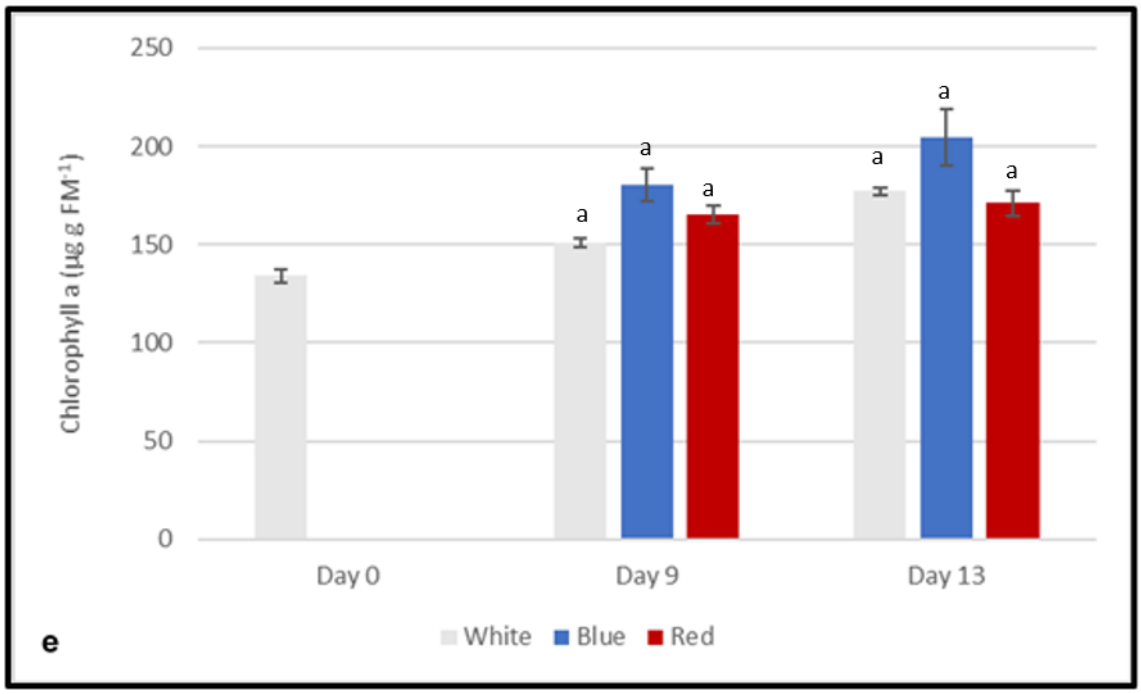
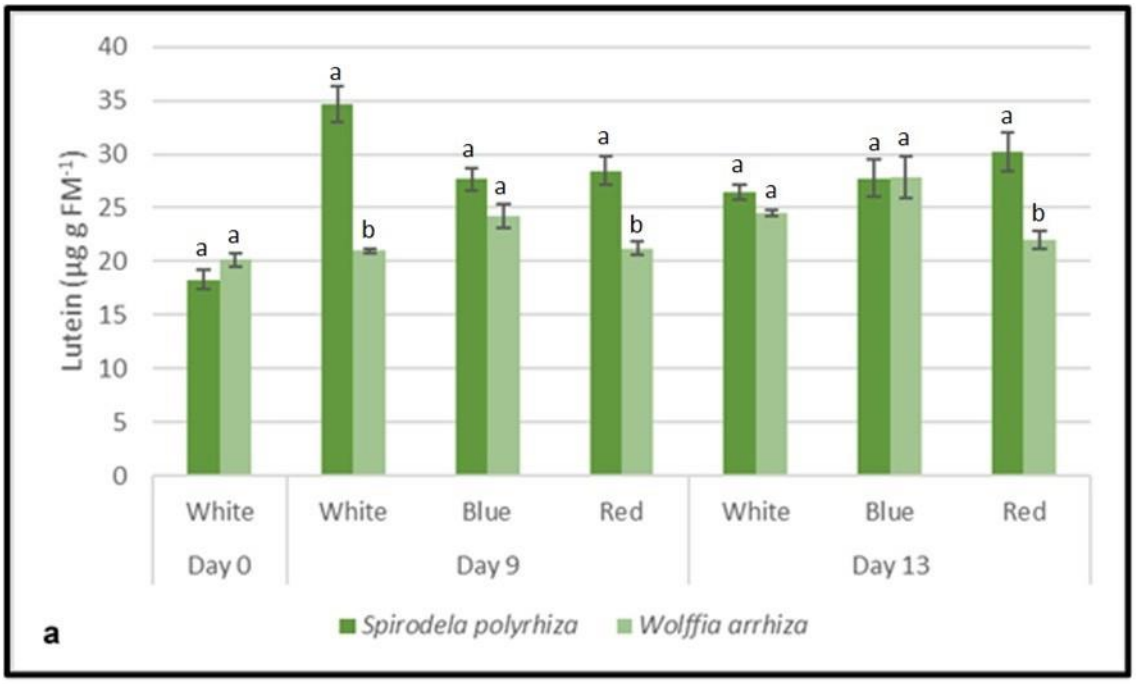
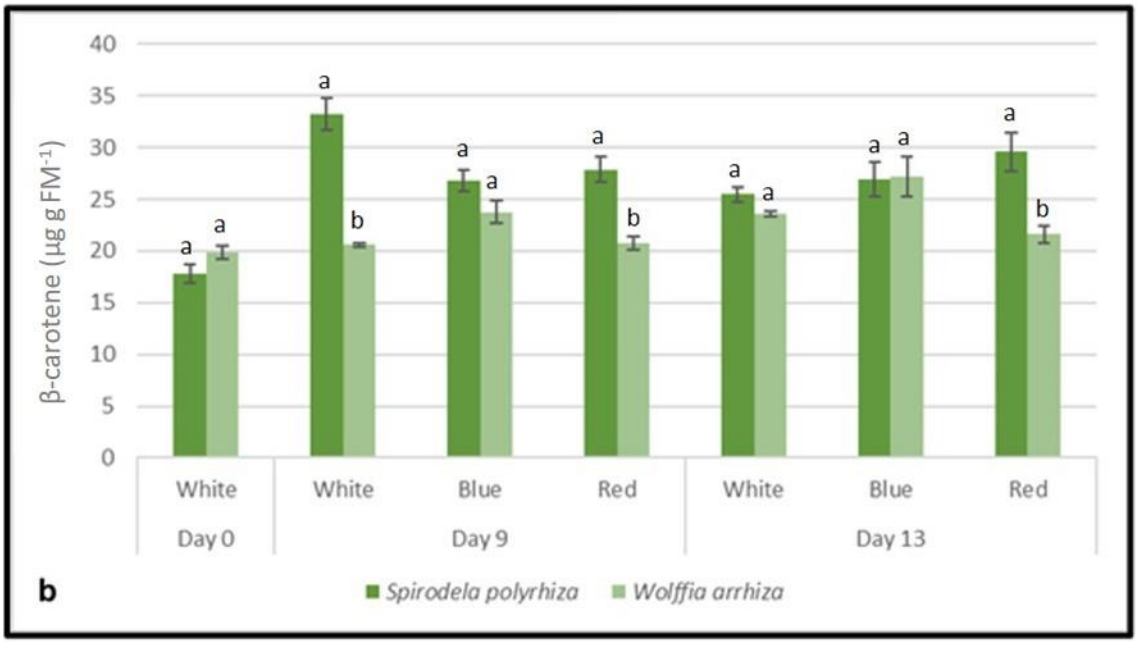
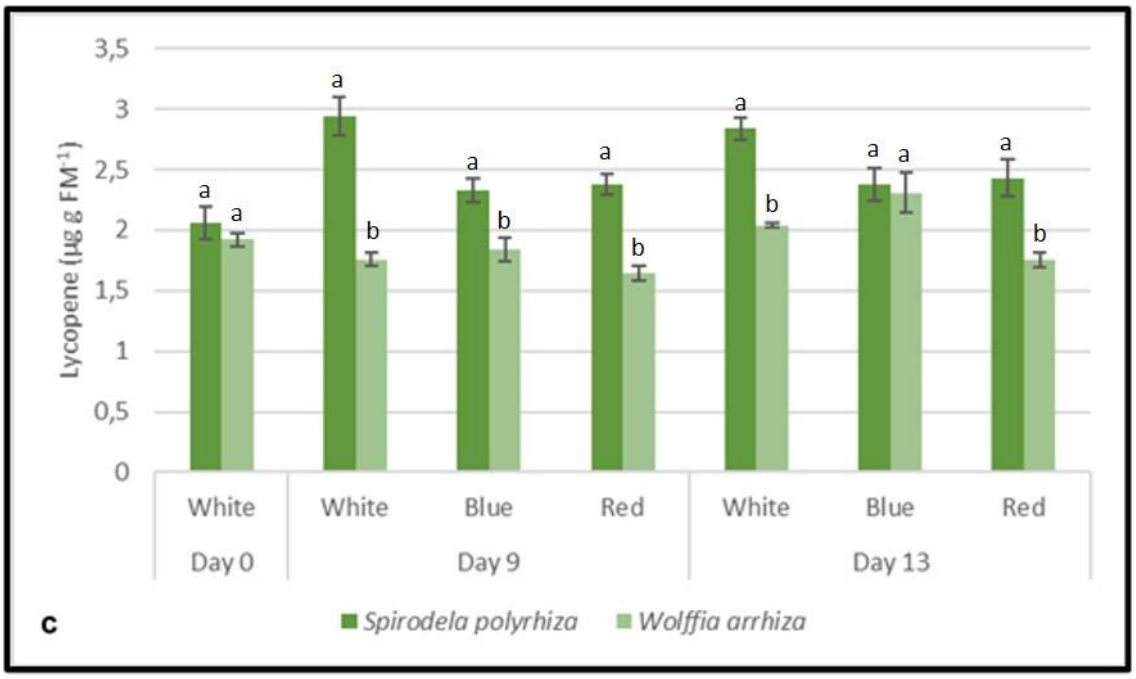


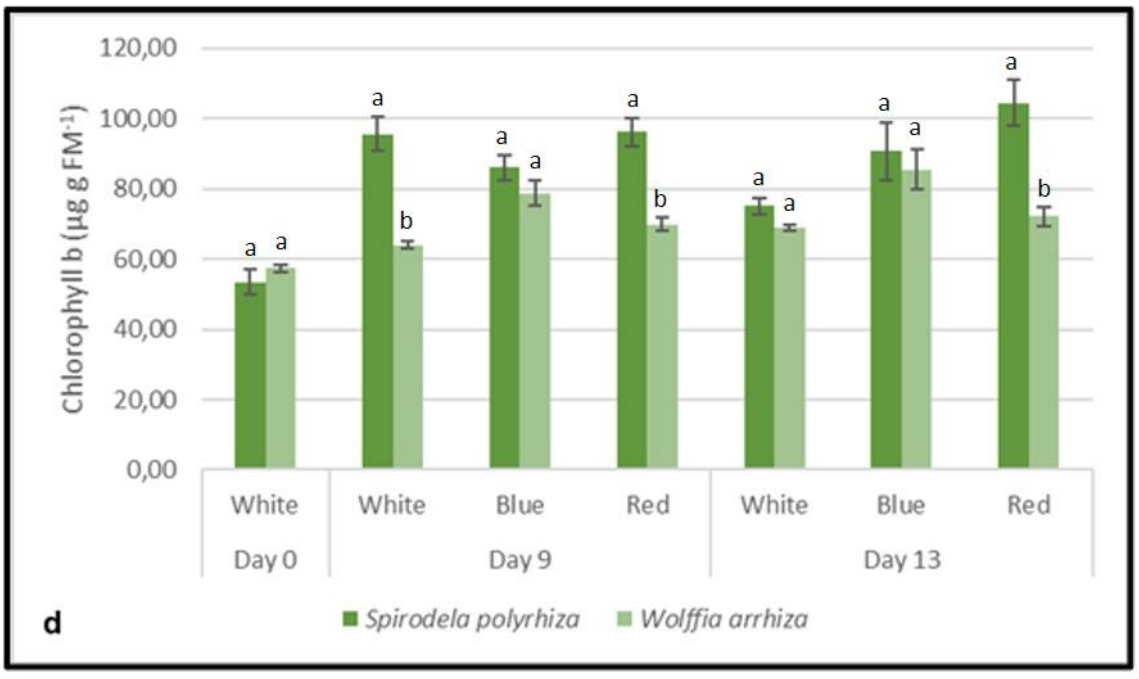
Figure 21: Content (µg g FM^{-1}) of (a) lutein, (b) β-carotene, (c) lycopene, (d) chlorophyll b, (e) chlorophyll a over time in Wolffia arrhiza *grown under blue, red and white light. Different letters indicate significant differences at p<0.05 (Anova test). Results of the two experiments are combined together.*

F) Graphs comparing the content of lutein, β-carotene, lycopene, chlorophyll b and chlorophyll a on fresh matter basis over time in *Spirodela polyrhiza* and *Wolffia arrhiza* (Figure 24)






3,5
3
2,5
2
1,5
1
0,5
0
Lycopene (µg g FM-1)
a a a b a b a a b a b a a b
White White Blue Red White Blue Red
Day 0 Day 9 Day 13
c
Spirodela polyrhiza
Wolffia arrhiza


120,00
100,00
80,00
60,00
40,00
20,00
0,00
Chlorophyll b (µg g FM-1)
a a a b a a b a b a a b a a b
White White Blue Red White Blue Red
Day 0 Day 9 Day 13
d
Spirodela polyrhiza
Wolffia arrhiza

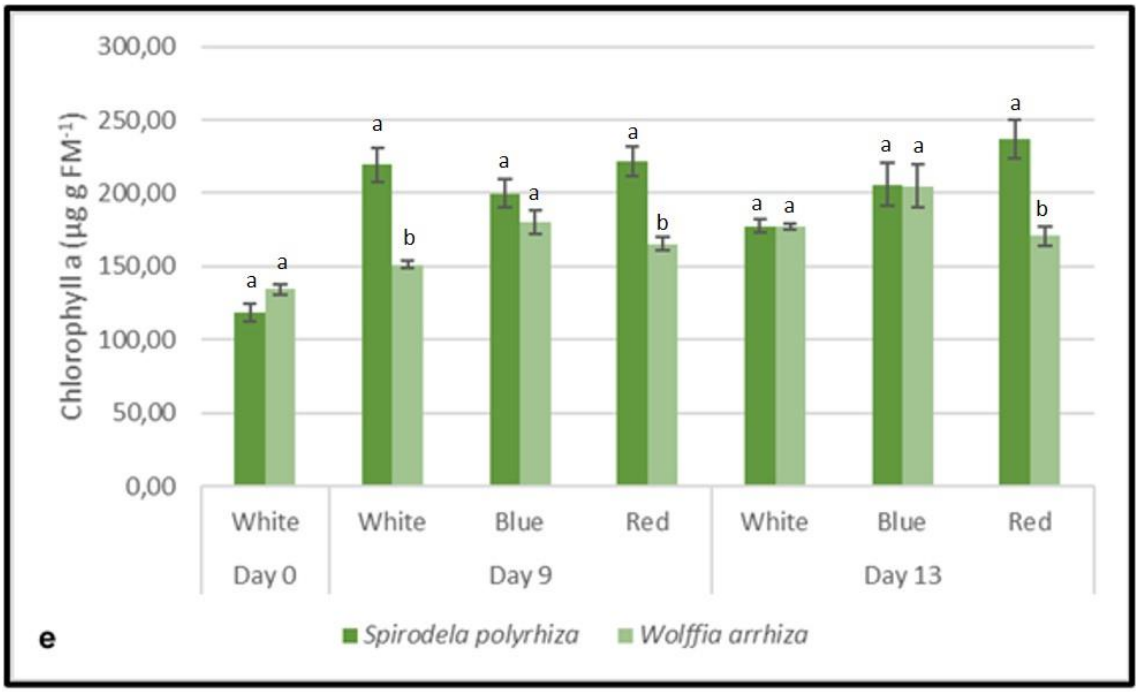


Figure 22: Content (µg g FM^{-1}) of (a) lutein, (b) β-carotene, (c) lycopene, (d) chlorophyll b, (e) chlorophyll a over time in Spirodela polyrhiza *and* Wolffia arrhiza *grown under blue, red and white light. Different letters indicate significant differences at p<0.05 (Anova test). Results of the two experiments are combined together.*

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