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The LED's spectrophotometry in precision agriculture: A brief survey

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ABSTRACT
Precision agriculture is one of the research fields with the greatest development in recent years. Its target is to reduce the waste with the help of seeking methods that allow fruit and vegetables to be grown in a sustainable way by reducing the environmental impact of fertilizers, pesticides, water leaks and so on. To do that, many agricultural companies experiment specific scientific optical techniques as spectroscopy or fluorescence measurements. This permit to get interesting data on the health status of a plant or fruit/vegetable. In spectrophotometry, the use of LEDs has the advantage of using a light source at a certain wavelength which corresponds to the color of the light emitted and this can have various experimental advantages, which allow for rapid diagnostic analysis of the target without carrying out invasive measurements.

Section: REVIEW PAPER

Keywords: LED spectrophotometry; precision agriculture; chlorophyll; measurement; spectroscopy

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1. INTRODUCTION

Chlorophyll is a fundamental organic molecule for plant life since its interaction with sunlight is the beginning of a process, known as chlorophyll photosynthesis, which generates ATP energy, complex sugars and oxygen. The Chlorophyll molecule has a ring structure with a magnesium atom in the centre, this "ring" is linked to the thylakoid of the plant cell through a hydrocarbon filament [1] (Figure 1, [2]).

Although we generally speak of chlorophyll in the singular, there are two types of chlorophyll in plants, which are catalogued as chlorophyll ***a*** and chlorophyll ***b*** (in literature it's also possible to find other types of chlorophyll catalogued with the letters ***c-d-e-f*** [3] which are typical of algae and cyanobacteria). At the chemical level, as shown in Figure 1, both molecules have the same structure with the difference that chlorophyll ***a*** has a CH_3 group while chlorophyll ***b*** has a CHO group [1]. From a spectroscopic point of view, both the chlorophylls, in the visible spectrum (VIS), absorb red and blue light and reflect green radiation which gives the classic colour observed in a leaf. Chlorophyll ***a*** has an absorption peak at 700 nm and it is called chlorophyll P_{700}, while chlorophyll ***b*** or P_{690}, has a peak at 680 nm and therefore it requires slightly greater energy to be excited and to give rise the photosynthetic processes. Furthermore, the 78 % of the incident radiation on a leaf is absorbed, the 20 % is dissipated as heat and only the 2 % is emitted in the form of fluorescence [4].

Now, if only chlorophyll operates in the light phase of photosynthesis, the rest of the VIS spectra would not be used

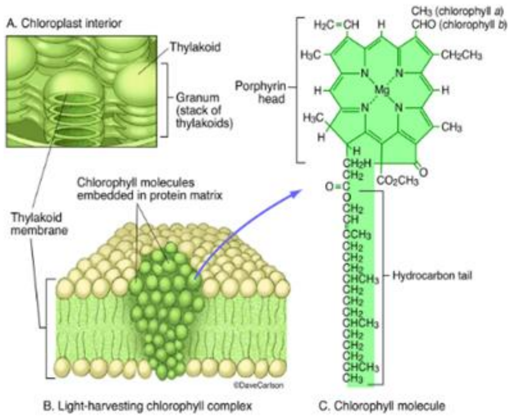


Figure 1. Graphic representation of the chlorophyll molecule. The chemical difference between chlorophyll ***a*** and chlorophyll ***b*** can be observed on C. These molecules, responsible for starting the photosynthetic process of a plant, are found within a protein matrix (B) present in the thylakoids of the plant cell (A) [2].

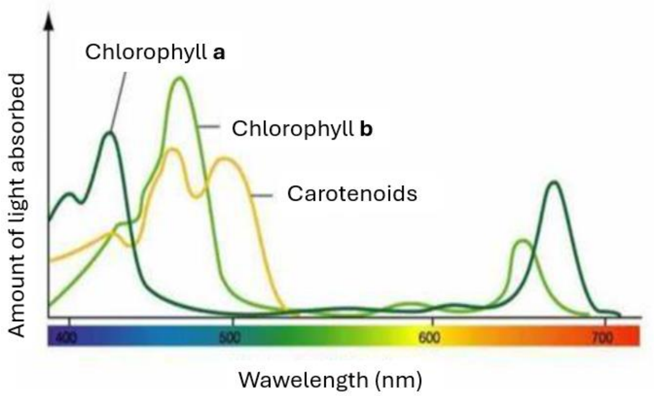


Figure 2. Absorption spectrum of chlorophyll ***a*** and ***b*** and accessory pigments (carotenoids) [6].

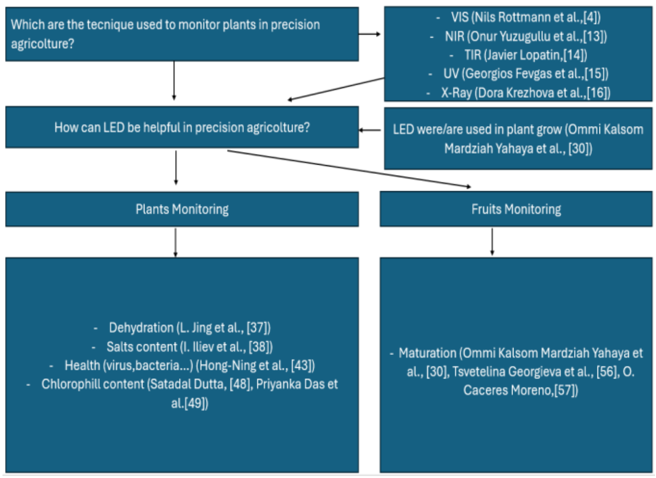


Figure 3. Conceptual map of the arguments analysed in this review.

by plants; therefore, to absorb more light in the visible spectra, on leave's surface the latter has developed accessory pigments that absorb photons with intermediate wavelengths between red and blue and then they transfer the energy to the chlorophyll itself and permit the beginning of photosynthesis. The most common accessory pigments present on leaves are carotenoids [5], which have an intense yellow colour that can be observed when the leaf cells stop synthesizing chlorophyll and therefore it begins to take on a yellowish colour [1].

As can be observed in Figure 2, the plant is thus able to exploit a large part of the visible spectra by using chlorophyll ***a*** to absorb blue and red radiation, chlorophyll ***b*** to absorb orange and violet and carotenoids for green radiation [6].
Another type of accessory pigments are the phycobilins which are presented in red algae [7] that live in shallow seabed and absorb wavelengths around yellow-green and orange.

In light of what has been said so far, it is clear the link between the chlorophylls (and the other chemical components present in the plants) with the VIS spectrum and easily born the question whether it was possible to monitor a plant by carrying out a spectroscopic observation and if so, what were the most suitable methodologies for this study.

2. PAPER STRUCTURE

Now sensors and microsensors can be applied to different applications ranging from security [8], [9], environment [10], until quantum physics applications [11]. These little instruments can make measurements which can be combined to machine learning algorithms to monitor for example the plants growth [12], traffic [10], air environment [10], water [13], [14], etc.

The point of view that this review wants to offer aims to analyse the use of Light Emitting Diode (LED) sensors in the fields of precision agriculture, and so the questions that came to light during the drafting phase were:

A) If LEDs were used, if so, which ones and in what way? Can they facilitate the development and growth of a plant? What can be the advantages and applications?

B) Are there parameters that can give indications of the state of the plant as the chlorophyll content in the leaf varies? For instance, how does the fluorescence of the leaf vary as the chlorophyll content varies? Does the leaf spectrum also depend on the source-target distance?

C) Using LEDs as a source, what are the chemical-physical parameters that can be analysed by a simple spectroscopic measurement? Is it possible to monitor the water or salt content on the leaf? Is it possible to observe the possible onset of a leaf disease simply by observing a variation in the spectrum?

D) In addition to agricultural applications, can the use of LEDs also be extended to the field of monitoring the growth rate and therefore ripening of a fruit?

To do that, we have analysed several papers, and we have selected about 60 publications which we have used to write this review. The goal of this work is to summarize a series of techniques, which use LED as light source. Since in precision agriculture it is important to detect the health of plants and the maturation of fruits, in Figure 3, it is shown which are the main points of our work.

The literature search of the 60 articles was conducted using different sites such as Scopus, ResearchGate, IEEE Xplore Acta IMEKO, etc. Furthermore, I used the following keywords as a search engine: LED spectroscopy (reflection, absorption, fluorescence), chlorophyll, fruit and vegetable monitoring.

As seen in Figure 3, the discussion of this review began with a brief summary of the spectroscopic techniques suitable for monitoring the health status of plants. After that, the discussion focused on the development of LED technology in the fields of precision agriculture: at first, we talked about the use of these technology for the growth of plants for indoor cultivation, then we talked about the techniques of monitoring plants using LED sensors that can be used to evaluate any lack of water, salts, etc. Finally, we discussed how these techniques can also be used in monitoring the state of ripeness of fruit and vegetables with the aim of having a high-quality product while minimizing waste.

3. METHODS USED IN MONITORING THE STATUS OF A PLANT

The technologies most used in monitoring the health and growth status of a plant concern spectroscopy techniques ranging from visible (VIS) [4], to X-rays [15], to near infrared (NIR) [16], to thermal infrared (TIR) [17] and to ultraviolet (UV) [18]. These spectroscopical methods exploit different forms of radiation-matter interaction ranging from the scattering produced by the leaf [4], to the reflection of the incident radiation [19], to the emission of thermal waves (TIR) [17], to fluorescence [20] generated by a stimulus induced by an external radiation (Figure 4). These methodologies listed before can be applied through different experimental approaches which can include, or measurements carried out remotely with specific databases and satellite measurements [21], or measurements carried out in situ. These last operations can be done on drones [22] or portable instruments such as spectrometers and fluorometers [23] or using electronic sensors such as LEDs [4], lasers [24] or antennas [25] as a source, which are capable of generate a signal which interact with a certain target and it produces radiation (reflected, emitted, etc.), which is then captured by a photodetector [19]. These instruments, based on the wavelength of the received signal, will produce an electrical signal whose content can be analysed by obtaining the frequency spectrum. In order to allow the monitoring of large fields, often many measurement nodes are simultaneously employed and the time synchronization is a need to have consistent measurements [26]. Afterwards, from the spectroscopic analysis, it is possible to trace the chemical nature of the analysed target and through a Wi-Fi, Bluetooth or ZigBee data collection systems, it is possible to have a statistic of the measurements' distribution. This communication can also be done using a LEDs system that communicate with drones through a binary system [27]. With the data collected, using machine learning techniques [4], [28], it is possible to obtain useful information for monitoring a plant or fruit. At this point, it could be interesting to try to analyse the different techniques used so far by analysing some studies already carried out, trying to focus attention on any advantages and disadvantages.

In particular, the discussion will be focused on the spectroscopic techniques that use LEDs light as source. This method has the advantage that it permits to send radiation at a specific wavelength from UV, to VIS, to NIR.

LEDs have many applications in precision agriculture since they can be used both for strictly diagnostic purposes (related to monitoring the health status of a plant [19] or the state of ripeness of a fruit [29]) and to improve the growth status of indoor crops [30]. This last factor has a significant historical weight since in the second half of the 1980s the very first prototypes of indoor culture were carried out using a LED system as light source for the growth of the plant [31].

3.1. LEDs in plant growth

The choice to exploit a light with a particular combination of wavelengths is due to the fact that chlorophyll absorbs red and blue [1] and therefore the use of a LED light source that combines these colours (Figure 5) allows for greater efficiency in photosynthetic processes and consequently better plant growth [32]. Furthermore, greenhouse cultures have the

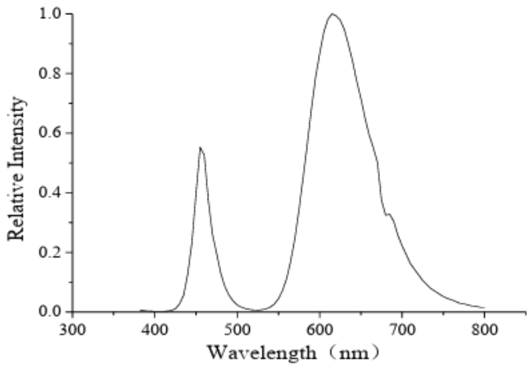


Figure 5. Emission spectrum of two LEDs, one green and one red, used in the study [33] for the growth process of a plant.

advantage of creating the best climatic conditions to allow the plant to grow healthily and quickly, since just like in a laboratory, some fundamental parameters such as temperature, humidity, air quality can be controlled and therefore create an environment that can reduce the occurrence of some plant diseases caused by external pollutants. Reducing the environmental impact and controlling various factors useful for the well-being of the plant can have greater economic profits. For example, it is possible to reduce the growth time of the plant itself, or you can reduce the quantity of pesticides and fertilizers using specific doses that can permit to keep the plant healthy without polluting the neighbouring soils.

Considering what has been said, the indoor culture technique that uses LED light as a light source for photosynthesis is certainly very interesting and it reduces the environmental impact and can accelerate the growth process of the plant [32]. Despite the advantages just discussed, the limitation of this type of culture is linked to the fact that in greenhouses it is usually possible to grow modest-sized plants such as flowers, vegetables (peas in the case of [33]) and small-sized fruits such as strawberries. In the case of floriculture, Figure 6 can be an example to underline how the use of LEDs can reduce the growth times of a plant. In particular, the study [34] has used LEDs whose emission wavelength overlapped with the absorption spectrum of chlorophyll and by increasing the intensity of the light sent over the weeks, a more rapid growth of the flower was observed [34] compared to a seedling grown in an external environment.

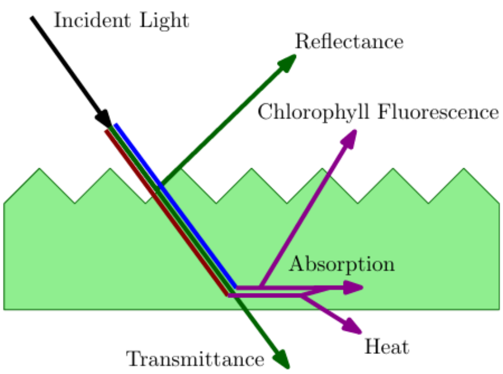


Figure 4. Graphical schematization of the radiation-matter interaction. The light incident on the surface of the leaf is reflected in green frequencies, while they are absorbed in blue and red. The absorbed light is partly transmitted, absorbed in the form of heat and only 2 % is re-emitted in the form of fluorescence [4].

Figure 6. Macroscopic effects of the growth process of a plant using a LED light as source. The photo was taken by the studio [34] after growing the plant for 50 days under a light generated by LEDs. As can be seen, this process is in a more advanced state than a seedling grown with natural light alone (image on the right).

3.2. LEDs Agri-food applications for the ISS

The use of LEDs for growing plants and vegetables also has many applications in the space sector [35]. Thinking of growing vegetables inside a spacecraft can improve the quality of life of astronauts who can benefit from good quality and practically zero-kilometre vegetables. This would also imply a reduction in mission expenses, and it will permit to make the crew autonomous from an agri-food point of view. Obviously, these technologies are being studied on Earth with the aim of applying these techniques on the current International Space Station (ISS) [36], [37] and even a hypothetical future mission to Mars [35].

Furthermore, since photosynthesis takes in carbon dioxide and releases oxygen, growing small plants in space can be an important support in the production of oxygen inside spacecraft as well as an effective technique to reduce the concentration of carbon dioxide [36].

As discussed in introduction, chlorophyll is a biomolecule that absorbs blue and red light but what happens if radiation is used whose emission peak is different from the emission peaks of chlorophyll? Does photosynthesis have the same efficiency in that case?

To answer these questions, it's interesting to cite the study [38] in which it is exploited the property of LEDs in emitting light at a particular wavelength (Figure 7) to understand what implications may have when, for example, a plant is illuminated with a different light from red and blue. As seen in [1], chlorophyll, and the way in which it interacts with sunlight leads to variations in the photosynthesis process and therefore a different development if this is exposed to light with specific characteristics that only LEDs can provide us [39]. The study [38] has analysed several tomato plants by illuminating them with LEDs of different colours for a period of 30 days. During this time lap, the chlorophyll concentration of the plant was analysed using fluorescence spectroscopy, observing, for example, how the latter varies as the light used varies.

As can be seen, the photosynthetic rate (Figure 8) is greater if LEDs used emit wavelengths equal to the absorption peaks of the chlorophyll and this at a macroscopic level can be seen with a different growth rate of the plant.

Obviously, it turns out that if we use red and blue LEDs simultaneously, the growth of the plant is more rapid then others (Figure 9). In spacecraft, therefore, to grow plants quickly and efficiently, it is necessary to use a LED light whose wavelengths are absorbed by chlorophyll **a** and **b** to start the photosynthesis processes [38].

3.3. Diagnostic techniques using LEDs

Now, can LEDs also be useful for the plant monitoring? How can incident light interact on a physical level with the

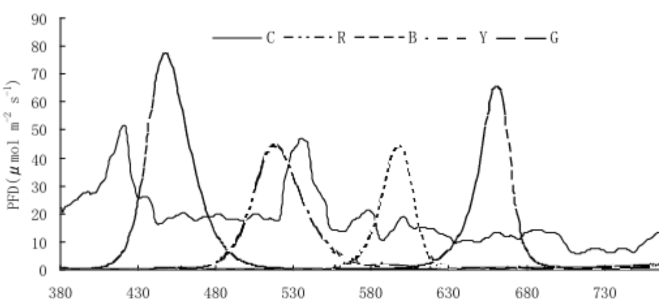


Figure 7. Emission spectrum of the LEDs used in the study [38].

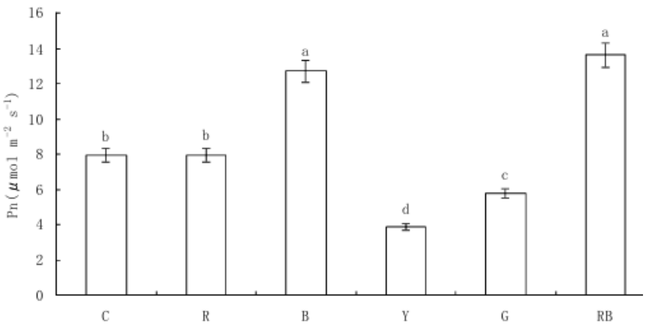


Figure 8. Photosynthetic rate of different plants grown using different LEDs; as can be seen, using colours with wavelengths that correspond to blue and red facilitate growth due to the fact that chlorophyll absorbs those specific wavelengths [38].

surface of the plant and what notions can be useful for precision agriculture?

First of all, it should be underlined that from an economic level LEDs are inexpensive, and the instrumentation required for the analyses presents many interesting practical aspects such as the low time required for data acquisition, the easy transportability of the devices and above all the low environmental impact. This is certainly one of the most important factors since non-invasive measurements do not alter in any way the growth of the plants, or the ripening process of the fruit can be carried out. Now, at a spectroscopic level, chlorophyll, in addition to absorbing blue-red radiation and reflecting green light, the 2 % of the radiation is subject to fluorescence, which can be analysed using a spectroscope [4]. To accentuate the effect of fluorescence, in the case of chlorophyll, it is necessary to use a blue or a UV LED, which excites the chlorophyll, and the outermost electrons are subjected to a transition towards a higher energy level. Once this first step is completed, electrons lose energy and release energy in the form of heat. After that, there is a subsequent lowering of the energy with three main processes: one always due to heat, one due to photosynthesis and one to fluorescence [40].

This process just described in Figure 10, it can be reproduced with a red LED but here the energy jump of the electrons is lower and there is a direct return to the non-excited state through the three processes listed above (fluorescence, photosynthesis, heat release). So, if we bring the discussion back

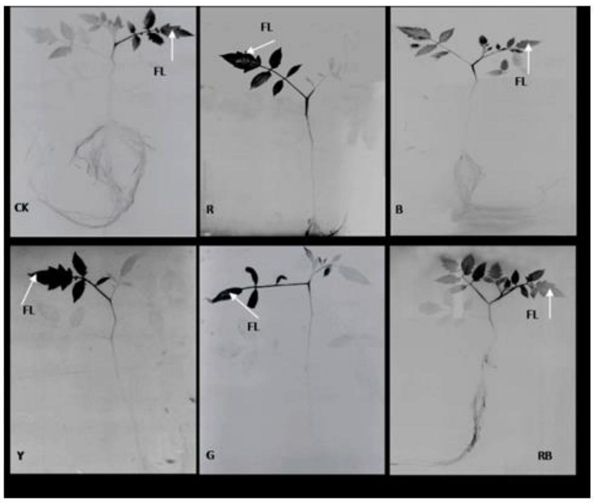


Figure 9. Macroscopic effects on the growth of a plant using different colour LEDs [38].

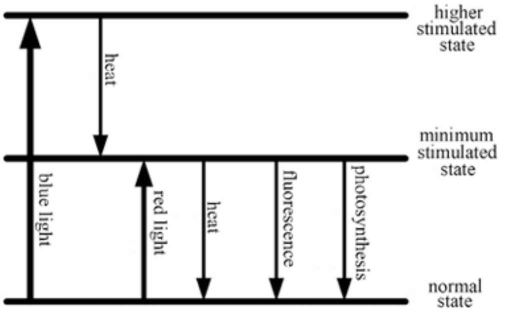


Figure 10. Schematization of the energy jumps of electrons subjected to LED radiation and two specific wavelengths, blue and red [40].

to a spectral level, the fluorescence of the plant due to chlorophyll presents a peak in correspondence of red colour at a wavelength of approximately 669 nm [41] (Figure 11).

The usefulness of analysing chlorophyll fluorescence has several points of application for plant monitoring since the intensity of the signal does not depend on the incident radiation but it is influenced by the chlorophyll content present in the leaf, which implicate a possible water shortage [42], nutrients [43], temperature [44] and growth [45]. Some measurements carried out in the study [40] can also give us information on the spatial distribution of the stress to which the leaf is subjected over its entire surface.

As can be observed in Figure 12 while there is an increase of the time in which the plant doesn't take a sufficient quantity of water, the average fluorescence F slowly tends to decrease and the areas of the leaf that are further away from the "nutrient source" will show a change in F first as seen in Figure 11. At a quantitative level, the study [40] carried out fluorescence measurements as time varied, calculating the relative fluorescence defined as F_{740}/F_{685} and obtaining the results highlighted in Table 1.

As can be observed in Figure 13, the relative fluorescence tends to decrease with the time and in the last spectrum we can see a relative maximum at 740 nm which underline the presence of the carotenoids (Figure 14).

Now, the presence of stress on the leaves of a plant can be induced by many factors such as water [40], temperature [44] or the presence of an excess of salts present in the soil [41]. These are present in the soil either in the form of ions (Ca^{2+}, Mg^{2+}, K^{+}) or dissolved in an aqueous solution [1] in different concentrations based on the chemical-physical characteristics of these and the climatic conditions of the surrounding environment. The salts dissolved in the soil are absorbed by the

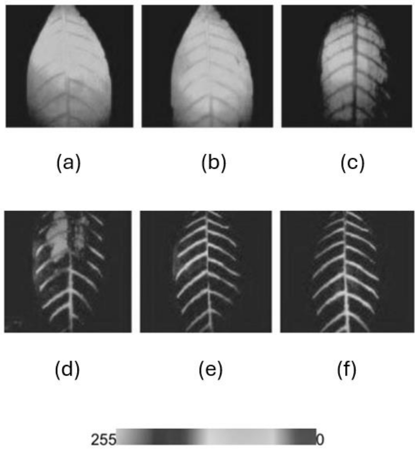


Figure 12. Fluorescence of the leaf of a plant subjected to stress induced by a lack of water as time varies (the initial time is state (a) and the final time is state (f)). As the water concentration decreases, the fluorescence becomes concentrated in certain regions of the plant [40].

Table 1. Fluorescence values corresponding to the peaks at 740 nm and 685 nm and the F_{740}/F_{685} ratio which tends to increase as the water stress of the plant increases as the peak corresponding to the wavelength equal to 745 nm tends to decrease due to the lack of water present on the surface of the leaf [40].

Time (s)	F_{740} (Raw counts)	F_{685} (Raw counts)	$\frac{F_{740}}{F_{685}}$
75	977.4264	1899.058	0.514690
150	678.1801	1195.924	0.567076
225	600.0380	903.8670	0.663857
300	486.0697	819.5421	0.593099
375	472.8344	716.9846	0.659476
450	447.3566	686.4860	0.651662
525	468.7231	616.1900	0.760679
600	438.0380	571.0887	0.767023
675	412.2950	513.4824	0.802939
750	405.9910	505.9204	0.802480

plant by roots and then it is transported through the trunk to the individual branches of the leaves. Here, to avoid the dehydration of the leaf, the stomata tend to open and close according with the concentration of salt removed from the soil. In the study [41], the variation in the fluorescence of the plant was analysed as the NaCl present varied. In particular, the study [41] has introduced 40 mM and 80 mM of NaCl and the spectral analysis were carried out using LED sources, which sent LED radiation with a peak at 470 nm against the leaves and the

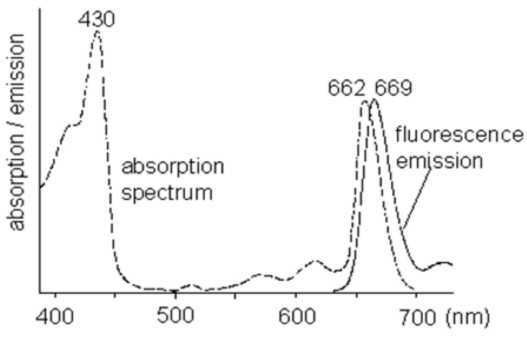


Figure 11. Absorption spectrum of chlorophyll and its fluorescence as the frequency varies. As can be seen, the fluorescence peak is found in the red at 669 nm while the absorption peaks are at 430 nm, in the blue, and at 662 nm, in the red [41].

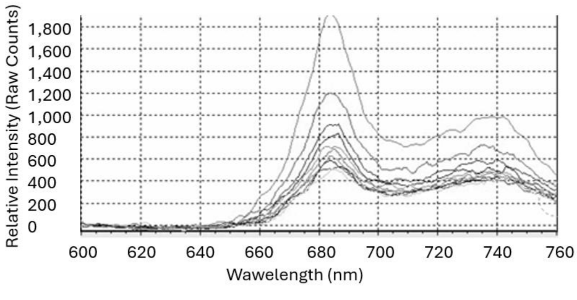


Figure 13. Spectral trend of leaf fluorescence as time varies and therefore as the water stress to which the plant is subjected increases [40]. As can be seen, the intensity of the fluorescence tends to decrease due to the decrease in the chlorophyll content present on the surface of the leaf.

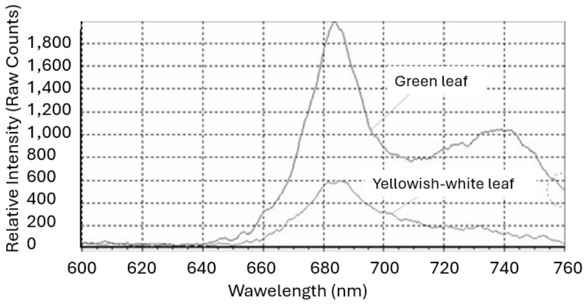


Figure 14. Fluorescence of a green leaf where chlorophyll is present in abundance and of a yellow leaf where carotenoids dominate. As can be seen, only the red peak remains [40].

Figure 16. Macroscopic observations of the effects of a concentration of salt in the leaves of a plant, in particular, the photo presents 80 mM of NaCl and the effect can be observed at a macroscopic level [41].

fluorescence was measured through a spectrometer working in the VIS and in the NIR.

As can be seen in Figure 15, as the concentration of salts increases, there is a notable variation in relative fluorescence and at a macroscopic level the leafs after 14 days report the following anomalies on the surface (Figure 16) which were highlighted in particular for the plant with 80 mM of NaCl. With the analysis of the fluorescence of the leaves can permit to make observations on any microscopic disturbances caused by various factors (temperature, salts, etc.) that are not visible to the naked eye. This result has the advantage of carrying out targeted interventions aimed at safeguarding the chemical-physical conditions of the soil which contribute to the well-being of the plant.

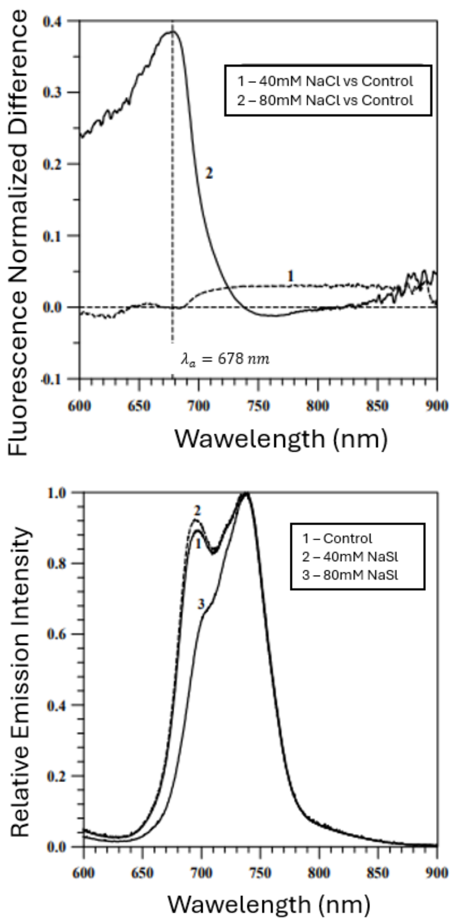


Figure 15. Emission spectrum of a plant with different concentrations of NaCl present in the leaves (40 mM, 80 mM); on the bottom there is the relative fluorescence at the same concentrations of NaCl [41].

These spectral analyses on the surface of a plant also have wide application for the diagnosis of diseases. This can be highlighted both with initial microscopic effects not visible to the naked eye and with evident variations due to a different colour of part of the leaf. This change at the spectral level involves a different fluorescence due to a variation in the chlorophyll content [46]. This effect just described can be observed in Figure 17 where the spectrum of a healthy leaf is much weaker than that of a diseased leaf. This intensity appears to be well marked in correspondence with an emission peak in the NIR (700 nm - 750 nm).

The application just discussed was taken from a study [47] where the following experimental setup (Figure 18) was used to analyse the leaves of a cucumber plant.

Even LEDs were not used for this study [46], since the radiation transmitted to the software was filtered through 16 filters that selected 16 distinct wavelengths (Figure 20), the future idea [47] could be to use LEDs light as source. This new method has the advantage of having only a small range of wavelengths emitted and therefore from the fluorescence of the leaf it could be possible to reconstruct its state of health and try to identify any diseases even before macroscopic effects are observed on its surface. Obviously, the limitation of this analysis [46], [47] is linked to the fact that the biotic factors, that can induce health problems in a plant, are numerous and range from bacteria, viruses, fungi, etc. Therefore, to be able to trace the explicit cause that has caused the change in fluorescence of the leaf, it is necessary to make use of specific biological

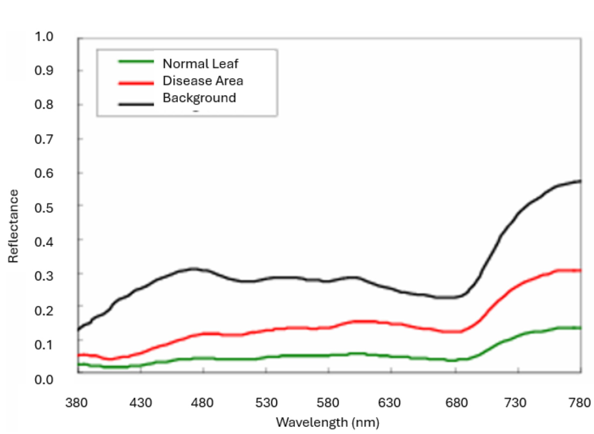


Figure 17. Spectral variations in the fluorescence of a plant with diseased leaves compared to a healthy one [47].

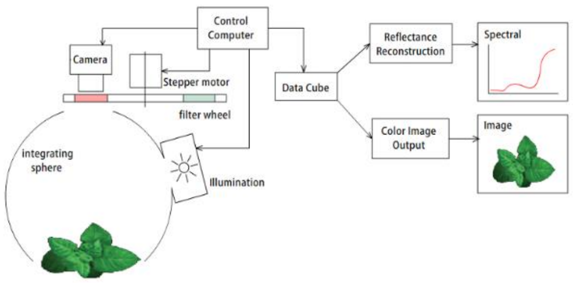


Figure 18. Experimental setup to measure the spectrum of a plant whose leaves may present biotic variations caused by bacteria and viruses that can cause changes to the spectrum. The setup involves the use of a light source that hits a target, a leaf, and the radiation produced by the fluorescence is filtered by a filter that selects certain wavelengths [46].

investigations. The advantage of this technique is linked to the fact that having knowledge of the state of health of the plant at a microscopic level through a simple investigation of the spectrum is certainly a very interesting starting point.

Given that the signal produced by fluorescence is very low (2 % of the total radiation) and the lifetime of this signal is in the order of nanoseconds [4], is it possible to use an experimental technique capable of increasing the effect? To do this, it is necessary to use specific sources such as blue LEDs [40], which increase the effect of the leaf's fluorescence. To avoid interference with the sent signal, this signal can be collected by a phototransistor in the NIR and to prevent it from having a sort of "contamination" with Sun's light [4], the LED signal can be modulated at a certain frequency, which in the case of [4] is equal to $\mathrm{f}_{\mathrm{LED}} = 35\ \mathrm{kHz}$. Furthermore, to understand the health status of the plant through the chlorophyll content present on the surface of the leaf, it is possible to send discrete signals with a period of

$$t_{\text{pulse}} = \frac{10}{457} \cdots \frac{100}{457} \frac{1}{f_{\text{LED}}} . \quad (1)$$

The signal sent once it interacts with the chlorophyll present, for example, in the seedlings of a lawn, as in study [4], the fluorescence signal is collected by a phototransistor, as shown in Figure 19, and once filtered it can be analysed by a microprocessor [48], [49].

This study [4] was carried out in nine gardens [46], which have nine different areal distribution of grass. The objective of [4] was to analyse the chlorophyll present in the various grass seedlings and based on the spatial distribution of the latter it was possible to provide specific data to automatic lawnmowers [47]

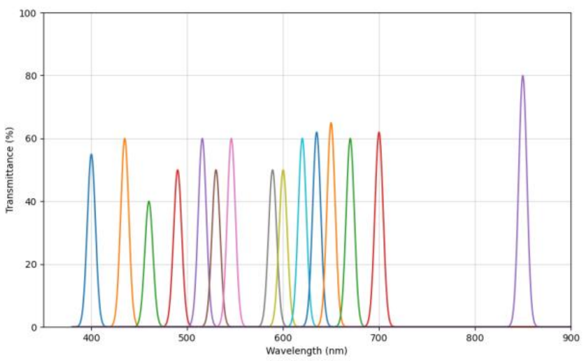


Figure 20. Spectrum of the filtered radiation. As can be seen, all the spectra have only one emission peak and therefore in the future we could think of modifying the experimental setup by introducing LEDs as a light source [47].

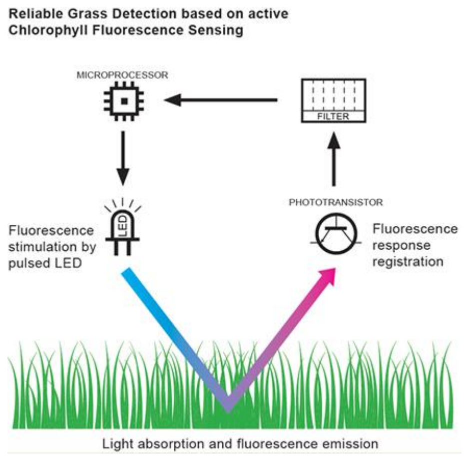


Figure 19. Experimental setup for the measurement of the chlorophyll content through the fluorescence of the grass of a lawn, which is stimulated by an LED source placed on a drone and the signal coming from the lawn is analysed by a phototransistor and after being filtered, the spectral content it is sent into a microprocessor which estimates its chlorophyll content [49].

built with special sensors capable to carry out a targeted cut based on the spectroscopy data provided by sensors placed at a given height h above the ground.

As can be seen in Figure 21, the instrument that carries out fluorescence measurements can also be inserted on board of a drone, which carries out measurements on a particular sector of a hypothetical garden. Then, the data collected can be transmitted to the lawnmower, which proceeds with a targeted cut in areas where chlorophyll has, for example, reduced fluorescence compared to average.

Now when we carry out spectroscopy measurements, we tend to set the source and the target at a given distance d, but the question to ask is how does the fluorescence spectrum vary in the case of chlorophyll as the distance d varies?

To answer this question, it may be interesting to cite a study carried out on the spectroscopy of algae [50]. These organisms, which live mainly in rivers, lakes, shallow seas, etc., exploit photosynthesis for their metabolism and therefore at a chemical level contain both chlorophyll [7] and other substances sensitive to light such as phycobilin which are typical of red algae [1]. The study [50] had different objectives from those of this review; however, it offers an interesting insight into the trend of chlorophyll a reflectance as the LED source varies (Figure 23).

As can be observed in Figure 22.b, the reflectance (at 500 nm) tends to decrease as the source-target's distance increases following a linear law. This means that even in the field of precision agriculture, the data collected for a hypothetical plant must always take into consideration the variable height or distance source-target.

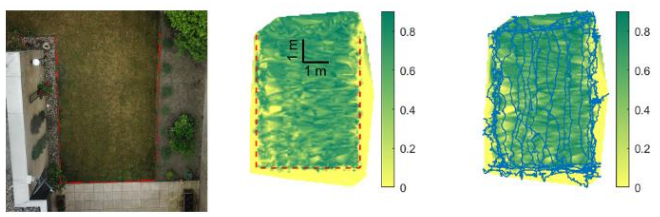
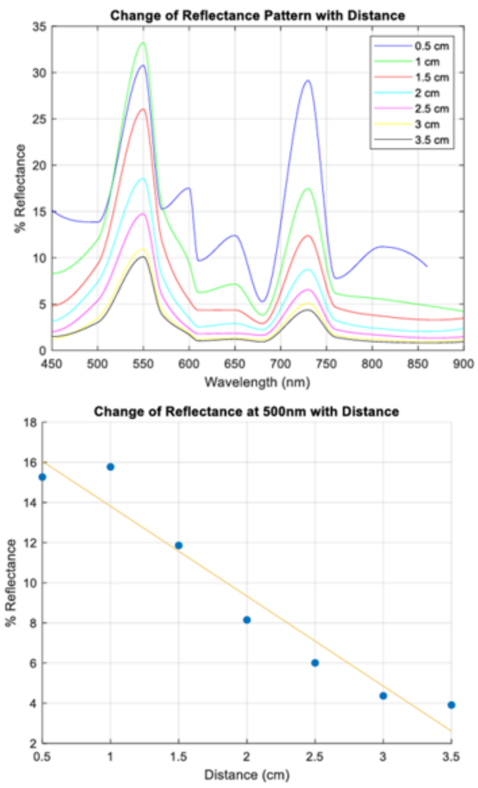


Figure 21. Example of a measurement set carried out on a meadow. The image on the left depicts the soil being analysed taken by a drone, in the centre there is a plot of the lawn's fluorescence based on the chlorophyll content and on the right the movements of the lawnmower have been inserted [48].



a)
b)

Figure 23. Reflectance of a solution containing algae as the source-target distance varies. As can be observed, fluorescence tends to decrease as the source-target distance increases [50].

After having made some considerations on the variations in the fluorescence and reflectance of plants, it is now necessary to analyse the concept of absorbance, which, in the case of chlorophyll [1], presents two peaks corresponding to red and blue with values that vary depending on the species being analysed [6]. The study [51] analyses the absorption of chlorophyll, Figure 22, through an experimental setup in which LEDs are used which send light radiation at a specific wavelength (1000 nm-1270 nm) on a sample containing chlorophyll and glycerol.

At a spectral level, the latter has a very reduced spectral response in the wavelengths used for the purposes of the experiment but at a chemical level it "picks up" the chlorophyll

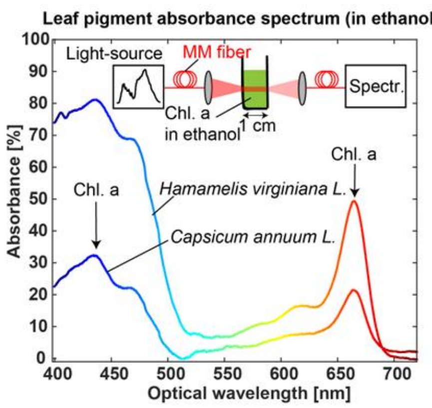


Figure 22. Chlorophyll absorption spectrum for two specific seedlings. The experimental setup is composed of a LED source that transmits light into a solution containing ethanol and chlorophyll. The latter is present inside a leaf that is inserted into the ethanol solution. Once the signal penetrates the solution it is then received by a phototransistor [51].

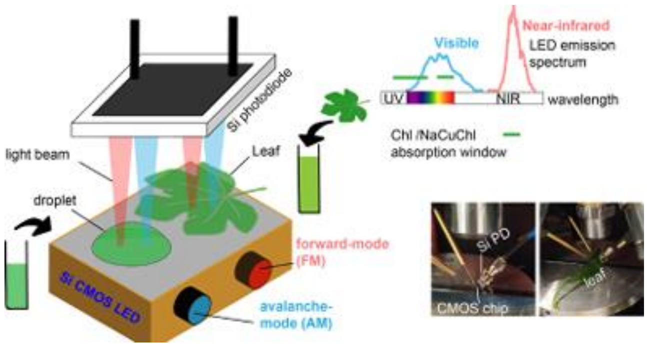


Figure 24. Experimental Set Up [51] already discussed in Figure 22.

contained within the leaf, slowly increasing its concentration within the solution.

As can be seen in Figure 22 and Figure 24, the light radiation sent by the LED is passed through the solution and a part of the radiation is absorbed according to the Beer-Lambert's law:

$$\alpha_{C1}(\lambda) = -\frac{\ln\left(1 - A_{C1}(\lambda)\right)}{L_{\mathrm{opt}}}, \tag{2}$$

where L_{opt} is the optical thickness and $A_{C1}(\lambda)$ is the absorbance of the solution and it is measured by the spectrophotometer. From (2), it is possible to calculate the molar absorption coefficient through the following relationship:

$$\alpha_{\mathrm{M}}(\lambda) = \left(\frac{c_{\mathrm{ref}}}{c_1}\right) \cdot \left(\frac{\alpha_{\mathrm{c1}}(\lambda)}{\alpha_{\mathrm{c1}}(626\ \mathrm{nm})}\right) \cdot 7192\,, \tag{3}$$

where the molar absorption coefficient of NaCuCl, used for the calibration, at the wavelength of 626 nm is equal to 7192 cm^{-1} mol^{-1} L. Before measuring the molar absorption coefficient of a particular substance, the $\alpha_{\mathrm{M}}^{\mathrm{LED}}(\lambda)$ was measured using that defined integral:

$$\alpha_{\mathrm{M}}^{\mathrm{LED}}(\lambda) = \int_{\lambda} \alpha_{\mathrm{M}}(\lambda) \cdot \epsilon(\lambda) \cdot \,\mathrm{d}\lambda\,, \tag{4}$$

where the emittance ε is calculated by:

$$\epsilon(\lambda) = \frac{E(\lambda)}{\int_{\lambda} E(\lambda)\,\mathrm{d}\lambda}, \tag{5}$$

where E is the intensity of the electroluminescence signal and the two integrals of (4) and (5) are calculated between the wavelengths of 400 nm and 900 nm.

As can be seen in Figure 25, in correspondence with the absorption peak of chlorophyll at 660 nm, the absorbance of the solution increases as the content of the latter in the solution dissolved in glycerol increases for both samples analysed.

Given that fluorescence is proportional to the chlorophyll content present in a leaf and the spectrum varies depending on the species being analysed, it is possible to exploit this data also in the agri-food sector. For example, the study [52] carried out in India proposes a very simple setup in which LEDs and a simple smartphone are used to analyse the chlorophyll content inside tea leaves to obtain the type of tea and the quality of the product itself [53]. This study carried out measurements at 4 different wavelengths using 4 blue LEDs (450 nm, 460 nm, 470 nm and 475 nm) observing a spectral trend with a relative maximum at 680 nm with an increase in intensity as the incident radiation increased (Figure 26).

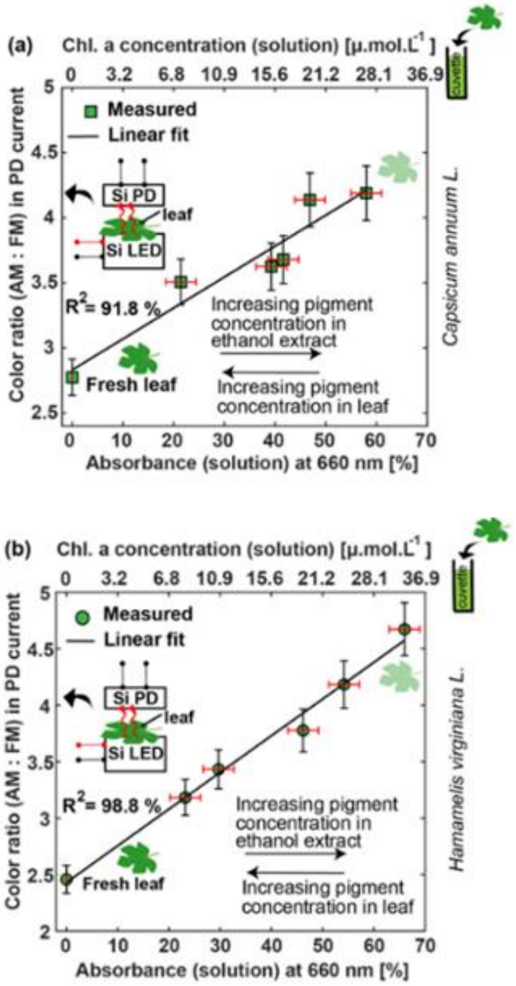


Figure 25. Inside the solution composed of leaf and ethanol the chlorophyll tends to dissolve inside the ethanol solution and therefore as time increases the chlorophyll tends to concentrate more in the solution, impoverishing the leaf. In the graphs, we can observe the linear growth of absorbance as the chlorophyll dissolved in ethanol increases [51].

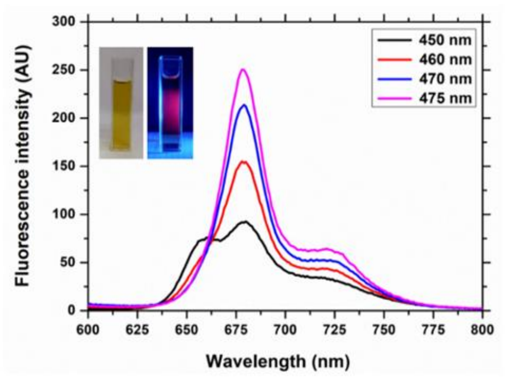


Figure 26. Fluorescence of a solution containing ethanol and chlorophyll as the wavelength of the sent signal varies [52].

This intensity tends to increase linearly as the chlorophyll content present in the leaf increases as can be observed in the following graphs (Figure 27).

The chlorophyll content presents in Figure 27.b was calculated using a SPAD-502 and a linear trend of F was observed compared to the maximum peak of the spectrum observed in Figure 26. Through the linear fit [47], a theoretical

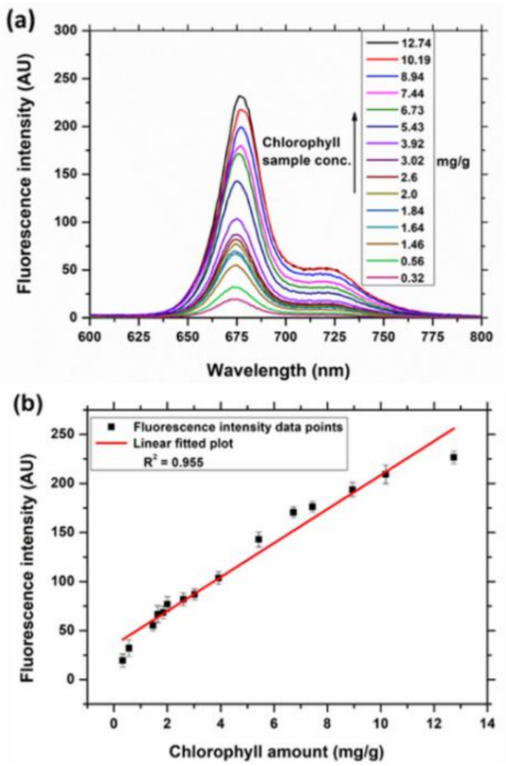


Figure 27. The fluorescence of a solution containing chlorophyll + ethanol tends to increase linearly as the chlorophyll content increases following the following spectral trend (a) with a peak that tends to increase linearly (b) [52].

model has been proposed that exploits the linearity of b with which it is possible to calculate the trend of the spectrum as normalized fluorescence (*N.F*) varies:

$$Ch\,C. = \frac{N.F - 0.19683}{0.06803}. \qquad (6)$$

On a practical level, this methodology has obtained interesting results in comparison with measurements carried out using devices capable of measuring the chlorophyll content and fluorescence techniques as can be seen in the following graph. The goal of this study [50] was to study the spectroscopic differences of eight different types of tea, which can be easily discriminated due to the different chlorophyll content (Figure 28).

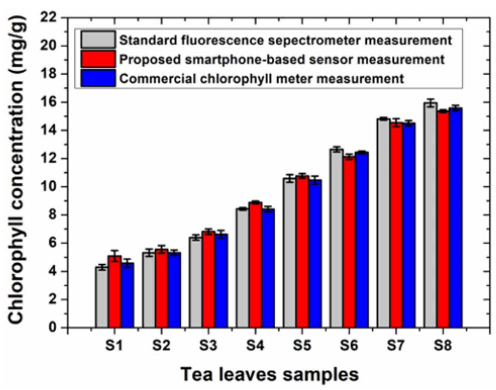


Figure 28. Chlorophyll content of eight different types of tea, this value was estimated using chlorophyll fluorescence and using three different measurement methods, which had the aim of validating a smart fluorescence calculation system using LEDs and a simple smartphone [52].

3.4. Diagnostic methods applied to fruit using LEDs

Up to now, the discussion has been focused on the diagnostic methods for monitoring the health status of plants and to do that we have seen how you can use LEDs to control fundamental parameters for its fitness such as water content [40], salts [41] and biotic factors [47]. We have also seen that we can monitor those simply reasoning about any changes in the chlorophyll spectrum present on the surface of the leaf. Now, can these techniques be somehow applied to monitor the state of ripeness of fruits? Can this procedure lead to interesting agri-food benefits such as better product quality and therefore derive economic benefits?

Also in this case there are many diagnostic techniques (from VIS [54], to NIR [55], to ultrasound [56], etc.) but the discussion will be focused exclusively on spectroscopy and fluorescence which has LEDs as its source [57] and we will analyse some interesting studies for precision agriculture.

The use of LEDs, which emit in UV and VIS can provide us with interesting data on the ripening rate of a fruit such as grapes from a vineyard [53]. This is interesting because if we know before the state of ripeness of a bunch of grapes, it can lead to get better a product with a superior quality, or we can reduce the environmental impact of fertilizers. This has an economic reflex; in fact, it can be possible to make greater economic profit during difficult seasons caused, for example, from climatic conditions such as drought and high temperatures. The study [58] carried out on the Magliano farm in Tuscany allowed the analysis of vineyards on bunches of almost ripe grapes (Figure 29). To do this, the fluorescence of the fruit was analysed when it is excited by a light radiation produced by LEDs in the UV (375 nm) and in the VIS (450 nm, 515 nm, 630 nm). Combining the response in the red and near infrared allows the analysis of the chlorophyll content present inside the fruit as well as the growth rate of the plant for both red grapes and white grapes. In particular, in the case of the latter, the colour of the various bunches is given by the anthocyanin's content which is also present in ripe peaches and apples. The importance of flavanols whose content can be calculated via spectroscopy is closely linked to the protection of the plant. The ratio between chlorophyll and flavanols is called the Nitrogen Balanced Index NBI and it is an index relating to the vigour of the plant. To observe better fluorescence of flavanols it is necessary to use LEDs in the UV in such a way as to minimize the effects of chlorophyll, which absorbs very little radiation in that spectral regime.

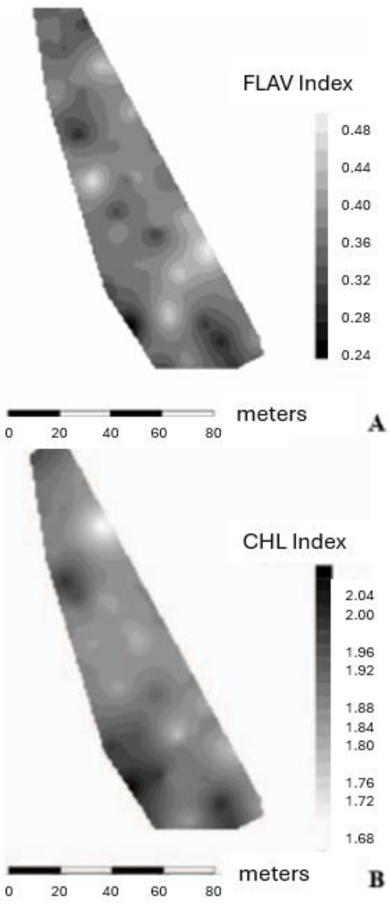


Figure 29. Aerial image of a vineyard on which fluorescence measurements were carried out to understand the growth rate of the plant from the chlorophyll content (B) and the content of flavanols (A) for grape ripening [58].

Since using LED to monitor fruit's fluorescence it is a non-invasive technique, this is certainly one of the best methods in diagnosing and monitoring the ripeness of fruit, vegetables and olives at the moment of ripening have a chemical change that will give them a different colour and flavour. During this phase, variations in chlorophyll concentration are observed and other chemical species appear such as flavones, anthocyanins, etc., the study [59] uses LEDs that emit ultraviolet light (375 nm) and in the VIS (450 nm, 520 nm, 630 nm) (also called RGB LEDs). The fluorescence of the plant in the red RF and in the far red RFR allows us to carry out interesting chemical analysis such as evaluating the content of flavanols through the following relationship:

$$FLAV = \log\left(\frac{FRF_{\mathrm{red}}}{FRF_{\mathrm{UV}}}\right), \tag{7}$$

or the content of chlorophyll

$$CHL = \frac{FRF_{\mathrm{red}}}{RF_{\mathrm{red}}}, \tag{8}$$

or the anthocyanin's content

$$\mathrm{ANTHRG} = \log\left(\frac{FRF_{\mathrm{red}}}{FRF_{\mathrm{green}}}\right). \tag{9}$$

These analyses were carried out to study the ripening of kiwis under different meteorological conditions (sunny, partially cloudy, and cloudy skies).

As can be seen in Figure 30, the chlorophyll content decreases in the absence of sun, which is fundamental for triggering photosynthetic processes, and it is based on the

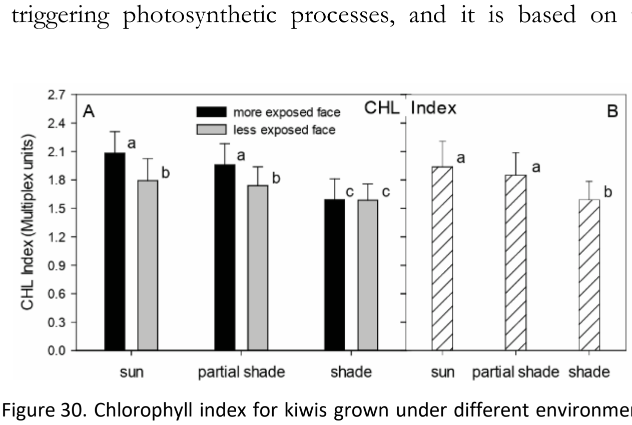


Figure 30. Chlorophyll index for kiwis grown under different environmental conditions [59].

exposure of the fruit itself. Therefore, using LED measurement technique can be useful for monitoring the plant and knowing the exact moment to do the harvest. These can be difficult to do if there are different climatic conditions that imply a variation in the maturation time of fruits since photosynthesis is strictly linked to exposure in sunlight.

To monitor fruits, given that they take on different colours based on their chemistry when they ripen, it is necessary to analyse the spectrum of the predominant molecules on their peel. For example, in the case of plums [59] at the time of ripening they develop a greater concentration of flavanols, and anthocyanins as can be observed in the following Figure 31.

These factors are fundamental to establish the state of ripeness of the fruit in accordance with the content of phenolics present. Fluorescence can be one of the best methodologies for monitoring the ripening of fruit and vegetables before harvesting since it is a rapid technique and, at the same time, it is minimally invasive since it is not necessary to pick the fruit to analyse its spectrum.

Therefore, using RGB LEDs can be an inexpensive and advantageous way to analyse the quality and ripeness of a fruit. So much so that several studies have been carried out in which this methodology was used such as in the study [33] in which we worked by analysing a mango plantation through the following experimental setup shown in Figure 32. The light source was three LEDs (red, green and blue) which sent a light signal onto the surface of the fruit by proceeding both with single-color measurements and by combining the light of two or three LEDs together. This last setup, as seen in Table 2, leads to greater accuracy in the data acquisition phase.

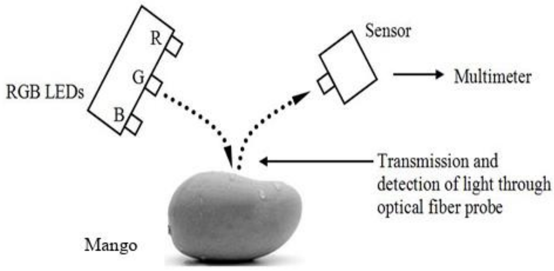


Figure 32. Experimental setup to observe the reflectance spectra of the surface of a mango as the colour of the fruit varies. The source used are three LEDs (red, green, blue) and the target of the measurements [33] is to understand the state of ripeness of a fruit by observing microscopic variations in the colour of the peel.

The advantage of this technique is that based on the colour of the peel, it is possible to carry out a simple spectroscopy measurement to evaluate the quality of the fruit. In any case, in future this technique should be implemented by, for example, increasing the number of LEDs with which to carry out the measurements. This choice will have the advantages of improving the accuracy of the measurements to cover a large part of the VIS spectrum and of having an agricultural industry capable of marketing better quality goods from the point of view of nutrients and flavour. LED investigations on colorimetry can also provide objective data on maturation, therefore going beyond the qualitative and subjective concept that can be done with a human eye. So much so that for the ripening of a fruit, such as for mango, Table 3, each shade and shade of colour corresponds to a different level of ripeness.

The study of the peel of a fruit is certainly a fundamental element for the analysis of the maturation and state of conservation of a fruit. The reasons why these types of analysis are carried out are linked to the fact that the final intent is to

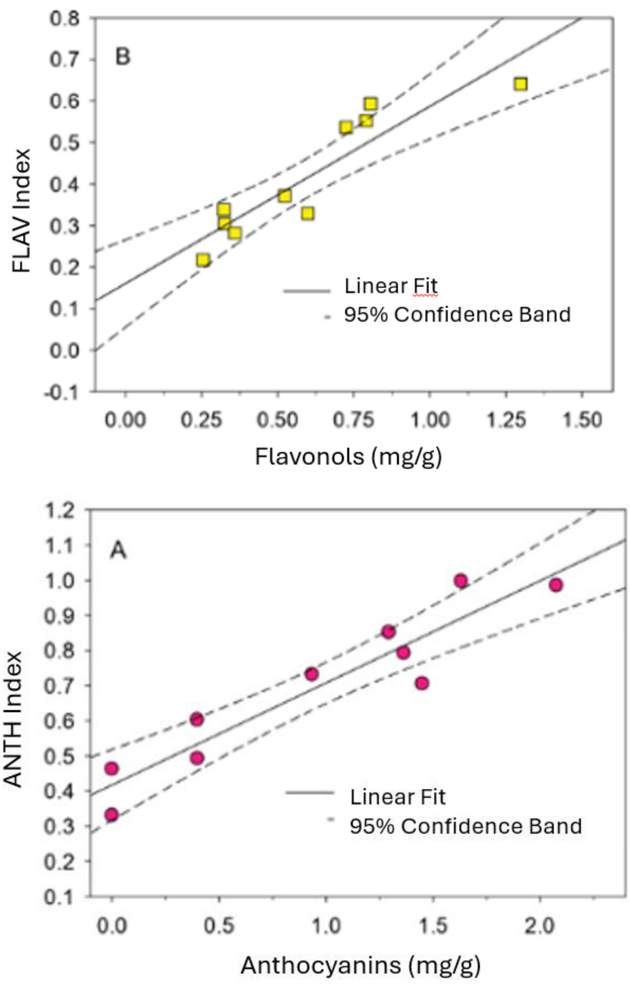


Figure 31. Concentration of flavonoids and anthocyanins during the ripening phase of a fruit [59].

Table 2. Accuracy index of the measurements carried out via the three LEDs on the mango peel [33].

Optical System	Wavelength	Index Accuracy (R^2)
LED	635 nm, 525 nm, 470 nm	0.870
	635 nm, 525 nm	0.869
	635 nm, 470 nm	0.872
	525 nm, 470 nm	0.351
	635nm	0.795
	525 nm	0.150
	470 nm	0.279

Table 3. Specifics of the ripeness state of a mango based on the colour of the peel [33].

Index	Specifications Colour	Specifications Ripeness
1	Green and dull in color	Immature
2	Light green with shiny skin	Mature
3	Yellowish green	Mature
4	Yellow with a little green	Almost Ripe
5	Fully yellow in color	Ripe
6	Yellow with a little orange	Overripe

provide high quality goods and therefore obtain the greatest economic profit from them. Obviously, in the agricultural sector the products that can provide greater revenues, in addition to fruit and vegetables, are grapes and olives from which high-quality wines and oils are obtained. In particular, the study of olives can have interesting opportunities for producing an oil with a pH and a quantity of peroxides such that the oil produced can be sold cheaply. To do this, it could be interesting to cite the study [60], which analysed the quality of olives, harvested both on the ground and directly from the tree using RGB LEDs, IR lamps through the following experimental setup, and a background light placed underneath the sample of olives being analysed.

Through the setup of Figure 33, a series of high-quality images were taken, highlighting the optical differences between the olives harvested on the ground and those present on the tree at the time of harvesting.

In general, it has been observed that the colour of the olives on the tree is lighter and tends towards green than those harvested on the ground (Figure 34). This has repercussions on a chemical level as the olives on the ground have an average acidity and a much higher peroxide index than the olives harvested from the tree as the former have completed the ripening process. In fact, once they fall, they are subjected to degradation's processes due to a series of factors induced by contact with the soil. Obviously, during harvest we tend to use both types of olives and therefore to estimate the parameters listed in Table 4 they can be carried out by carrying out a simple average between the parameters of the olives harvested on the ground and those harvested on the tree.

This study [60] can be taken as a starting point for monitoring the rate of maturation of the olives with the aim of carrying out the harvest when the fruit can bring the greatest profit on the quality of the oil produced [61]. However, this last step can also be done after milling through a fluorescence measurement [56].

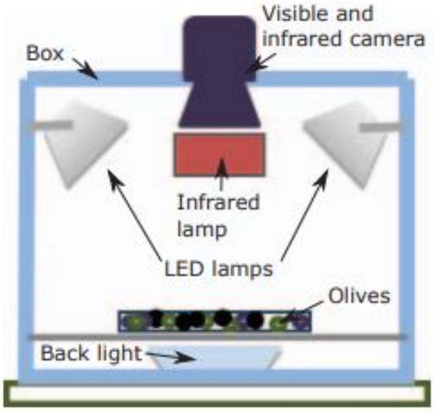


Figure 33. Experimental setup for the study of freshly harvested olives using RGB LEDs and IR lamps [60]

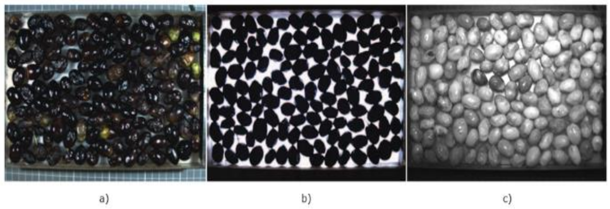


Figure 34. Image of olive sample using RGB LED (a), background light (b), IR light (c) [60].

Table 4. Average acidity and index of peroxides present in olives harvested on the ground and in those present on the tree. Here are summarized the quality parameters obtained by the accredited laboratory [60].

Olive Fruit Sample	Parameter	Average Value	Min. Value	Max. Value
Ground Fruit Lots	Acidity	7.96	2.18	14.48
	Peroxides Index	36.04	15.50	74.80
Tree Fruit Lots	Acidity	0.46	0.20	1.79
	Peroxides Index	4.62	0.20	9.10
All Fruit Lots	Acidity	4.31	0.20	14.48
	Peroxides Index	20.73	0.20	74.80

4. CONCLUSIONS

The discussion just carried out on the diagnostic techniques for monitoring the health status of a plant can provide us with interesting insights into their usefulness in the fields of precision agriculture. The objective is to reduce costs linked to any waste (for non-marketable vegetables and fruits) and to use sustainable agriculture techniques in line with the environment. This motivation pushes us to exploit the technologies available to carry out accurate and minimally invasive investigations. Many of these techniques make use of measurements carried out remotely via satellites and drones [62]-[64], which allow spectroscopy measurements to be carried out on the surface of a leaf or fruit from which the chemistry can be traced. The acquired data can then be processed using machine learning software that builds models capable of tracing the state of health of the plant or the state of ripeness of a fruit or vegetable. These applications are very attractive, for example, in the analysis of vineyards, where the aim is to produce high quality wines which on an economic level are an excellent profit for producers, or in the analysis of fruit ripening, where tends to provide high quality goods both in terms of nutrition and flavour. Furthermore, understanding the state of health of a plant can be useful for diagnosing stress related to water shortages or diseases which, at first, cannot be observed with the naked eye and therefore interventions can be carried out aimed at safeguarding its state of health by introducing quantities precise quantities of water and fertilizers with the aim of minimizing chemical-environmental impacts.

In recent years, new technologies include the use of LEDs as light source, since those methods permit to have a peak emission ranging from UV to VIS to IR. These techniques have the advantage of reducing the environmental impacts, costs and times with which to carry out targeted monitoring which are essential in the field of precision agriculture. Having data in a relatively short time allows you to carry out targeted interventions even before having macroscopic effects by carrying out, for example, the administration of a certain quantity of water, choosing targeted dosages of fertilizers, etc.

However, in the context of fruit monitoring, using LEDs as a source, it allows to monitor the chemistry of the fruit and therefore various factors associated with ripening and above all to carry out non-invasive measurements that do not require any sampling from the tree. This has several advantages such as reducing crop waste and at the same time making the greatest economic profit by putting better quality goods on the market.

In the future, the use of LED in spectrophotometry, had to be implemented with the use of new technology such as drones [62]-[64]. These new automated systems will be able to carry out very rapid measurements on leaves, fruits, etc., carrying out a very rapid diagnosis based on machine learning techniques that are able to recognize any problems of dehydration, excess salts, etc. The goal will be to improve the quality of products while reducing waste (water consumption, fertilizers, pesticides, etc.) and crop losses.

The use of LEDs as diagnostic sensors of the health of the plant presents many environmental advantages related to the reduction of soil and water pollution. The targeted use of fertilizers and pesticides, for example, can reduce the environmental impact on the soil by reducing not only the pollution of these but also the contamination of groundwater. The use of diagnostic techniques that quantify any water shortages in the leaf imply a reduced consumption of this during irrigation, reducing any critical issues related to the supply of water resources especially during the summer months.

AUTHORS' CONTRIBUTION

Conceptualization, F.F. and F.L.; methodology, F.L.; validation, F.L; formal analysis, F.F.; investigation, F.F.; resources, F.L; data curation, F.F.; writing - original draft preparation, F.F.; writing - review and editing, F.F. and F.L.; supervision, F.L. All authors have read and agreed to the published version of the manuscript.

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