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COLORIMETRIC ANALYSIS OF THE LEAVES OF PLANTS EXPOSED TO CADMIUM

Análisis colorimétrico de hojas de plantas expuestas a cadmio

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Abstract

Cadmium is a toxic heavy metal that is uptake and translocate in plants in its divalent form Cd^{2+}. The presence of cadmium in plant tissues at different concentrations can generate phenotypic changes of great significance in plants and are initially expressed by the leaves. In this study cell phone photographs of control plants and plants with cadmium at 100 μg/L were taken, with the objective of identifying morphological changes and significant differences in colorimetric parameters of chroma, hue and brightness in leaves of Basil (*Ocimum Basilicum*), Peppermint (*Mentha spicata*), Lettuce (*Lactuca sativa*) and Spinach (*Spinacia oleracea*), which were grown under the floating root method. In addition, an analysis of variance was carried out for the colorimetric parameters and the results obtained indicate representative variations for a very small period of exposure. The methodology of this work is a first approach to the characterization of images of plants contaminated with heavy metals among other contaminants, as a detection and analysis strategy that can support the understanding of issues of bioaccumulation of contaminants in plants and contribute significantly to the problem of food safety through identification and monitoring.

Keywords*:* *cadmium, colorimetry parameters, foliar tissue.*

Resumen

El cadmio es un metal pesado tóxico que se absorbe, internaliza y se transloca en las plantas en su forma divalente Cd^{2+}. La presencia de cadmio en tejidos vegetales a diferentes concentraciones puede generar cambios fenotípicos de gran significancia en las plantas y son expresados inicialmente por las hojas. En este estudio se tomaron fotografías con celular a plantas control y plantas con cadmio a 100 μg/L, con el objetivo de identificar cambios morfológicos y diferencias significativas en parámetros colorimétricos de croma, tono y brillo en hojas de plantas de Albahaca (*Ocimum Basilicum*), Hierbabuena (*Mentha spicata*), Lechuga (*Lactuca sativa*) y Espinaca (*Spinacia oleracea*), que fueron cultivadas bajo el método de raíz flotante. En el estudio se realizó un análisis de varianza para los parámetros colorimétricos y resultados obtenidos indican variaciones representativas para un periodo muy pequeño de exposición. La metodología de este trabajo es un primer acercamiento a la caracterización de imágenes de plantas contaminadas con metales pesados entre otros contaminantes, como una estrategia de detección y análisis que puede apoyar la comprensión en temas de bioacumulación de contaminantes en plantas y aportar significativamente a la problemática de seguridad alimentaria mediante la identificación y el seguimiento.

Palabras clave: *cadmio, parámetros de color, tejido foliar.*

1. INTRODUCTION

Cadmium (Cd) contamination is an issue of great concern in water systems, specifically in irrigation water for agricultural activities [1]. Global and local studies highlight the accumulation of Cd concentrations in plant species for human consumption [2–4]. In Colombia, specifically, traces of cadmium contamination have been reported that exceed the limits established according to the European Pharmacopoeia (0.05 µg/g), FAO (0.02 µg/g), World Health Organization (0.03 µg/g), and Colombian regulations [5–9].

The presence of Cd in vegetal species for human consumption represents a significant health hazard and threatens the food safety of the country [10, 11]. Cadmium is a toxic heavy metal widely dispersed in the environment. According to the World Health Organization, Cd is one of the 10 compounds of greatest public health concern [12]. This element enters the atmosphere as a result of atmospheric particle deposition from the combustion of fossil fuels, volcanic eruptions, and other natural processes. It is subsequently deposited in water systems that interact with plants and is translocated through biochemical mechanisms in its divalent form, Cd^{2+}. After the accumulation process, the contaminated species is integrated into the trophic chain, where it enters the human body through the food chain [13,14].

Cadmium uptakes biological systems due to its chemical similarity to zinc (Zn). Cd and Zn belong to the same group in the periodic table, which means that they have a similar electronic configuration [15]. For this reason, Cd can easily replace Zn in biological and enzymatic processes, which can have negative effects on human health [15]. However, molecular mechanisms and processes associated with the internalization and translocation of heavy metals in plants are under study [16].

Cd toxicity interferes negatively in plants at high concentrations because it influences growth inhibition, activation or inhibition of enzymes, reduction of transpiration rate and water content, and interferes in the entry, transport, and utilization of essential elements (Ca, Mg, P, and K) and water, causing severe nutritional and water imbalances in the plant [16–19]. According to Rizwan et al. (2017 [20], high concentrations of Cd in vegetables increase the number of ROS (reactive oxygen species), cause genotoxicity, and generate damage to the DNA.

The phytotoxic effects are different depending on the metal, although some metals have some similarities, such as Cd and As. Cd in plants shows visible effects such as inhibition of growth, photosynthesis, and chlorophyll fluorescence; dark spots on root tips; yellow pigments on leaves; drying of leaves; chlorosis; and necrosis [21–23].

The morphological changes that plants show, mainly in the leaf tissue, are the first indication of heavy metal toxicity [24, 25]. Colorimetry is a method used to evaluate colorimetric changes in the aerial vegetative tissues of plants using parameters such as hue, chroma, and brightness.

Colorimetry is a method used to identify color variations in aqueous solutions or solid substances associated with the concentration of an analyte of interest [26, 27]. Concerning cadmium, this method has been used to evaluate color changes in sensors with fluorescent responses for the detection of Cd2+ in rice samples [28]. It has also been used to identify wavelengths in heavy metal diagnostics by analyzing reflectance differences in RGB images [29]. Colorimetry has been used to evaluate quality indices in plant products; however, there are no significant reports that establish significant color differences with phenotypic changes in plants in response to a contaminant of interest.

The objective of this work is to identify significant changes using a two-factor analysis of variance in the colorimetric parameters of chroma, hue, and gloss of leaves of different plant species: basil (*Ocimum basilicum*), peppermint (*Mentha spicata*), lettuce (*Lactuca sativa*), and spinach (*Spinacia oleracea*) as a result of exposure to cadmium (100 µg/l) during the cultivation time.

2. MATERIALS AND METHODS

2.1. Seedling culture and image capture

In the experimental development of this work, seedlings of basil (*Ocimum basilicum*), peppermint (*Mentha spicata*), lettuce (*Lactuca sativa*), and spinach (*Spinacia oleracea*), obtained from the Biosystems Center of the Jorge Tadeo Lozano University in Bogotá, Colombia, were used.

The experimental design consisted of randomized blocks with three replications, as shown in Figure 1. For each block, 15 control seedlings and 15 root seedlings exposed to cadmium at 100 µg/L were used. The solution was composed of water fertilizer (10 ml/L) and cadmium at concentrations of 100 µg/L, respectively, for a period of seven days. The fertilizer "Crecilizer" was obtained from a local store, and the cadmium was purchased from Sigma Aldrich.

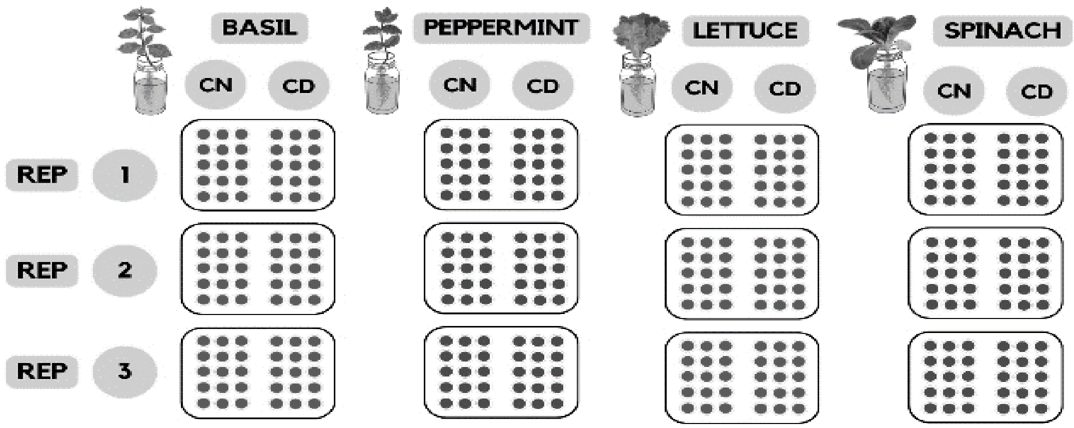

Figure 1. Randomized block experimental design with three replications, with control seedlings (CN) and seedlings exposed to cadmium (CD).

2.2 Image capture and calculations of chroma, tone, and brightness.

Photographic monitoring was performed during the 7 days of the experiment. For each replicate, 2 control seedlings and 2 seedlings per treatment were randomly chosen from each replicate, for a total of 48 samples photographed per day.

The photographs were captured using the camera of a Huawei P30 mobile device, model MAR-LX3A, with a resolution of 24 megapixels. The set-up for the photographic monitoring consisted of a tripod for mobile devices and a sheet of white foamy paper (see Figure 2) to establish a suitable background for greater distinction in the morphological changes of the seedlings and to establish conditions in which it is possible to normalize the information captured in the photographs. The distance between the camera and seedlings is no greater than 50 cm.

For the colorimetric analysis, the ENVI program was used. For each image, five different areas of 60 x 60 pixels were delimited on the leaves of the sample of each species to subsequently express the information of these areas in an average in the RGB color model, thus giving a general and representative sweep of the color state in the 5 areas of the leaves of each of the seedlings analyzed. On the other hand, a random area was taken, both in location and dimensions, of the white background where the samples of the seedlings were photographed to establish a reference point to normalize the values obtained with the ENVI program of the selected areas and thus achieve homogenization of the information obtained, independent of the information obtained and independent of the variable light conditions that were experienced during the photographic monitoring. With the information obtained and the averages in the RGB color model of the 5 selected areas of the leaves and the reference area, equations 1-3 were used to normalize the values of the RGB color model for R equation (1), for G equation (2), and finally for B equation (3).

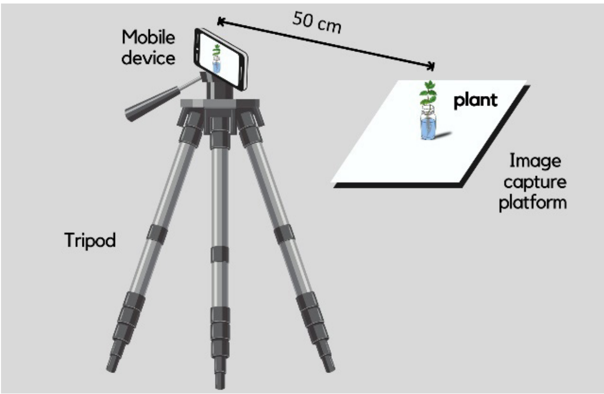

Figure 2. Set up for daily photographic monitoring of the selected samples.

$$X_{RN} \frac{X_R * 255}{X_R} \quad (1)$$

$$X_{GN} \frac{X_G * 255}{X_G} \quad (2)$$

$$X_{BN} \frac{X_B * 255}{X_B} \quad (3)$$

After the normalization of the RGB values, using the ColorMINe.org website, these values were taken to the CIE-L*ab color model, which describes color in terms of lightness or brightness (L), red-green intensity (a), and yellow-blue intensity (b), to subsequently determine other color properties such as chroma (C) obtained by equation (4) and tone (h) obtained by equation (5).

$$C = \sqrt{a^2 + b^2} \quad (4)$$

$$h = arctan\left(\frac{b}{a}\right) * \frac{180}{\pi} \quad (5)$$

a. Statistical analysis

The data were treated statistically using a two-factor analysis of variance with a significance level of 95%. Tukey's multiple comparisons test was used.

3. RESULTS AND DISCUSSION

In this study, a morphological analysis of the four plant species (basil, spearmint, lettuce, and spinach) during seven days of cadmium exposure is performed. On the other hand, significant differences between chroma, hue, and brightness parameters are evaluated for the different species.

3.1 Phenotypic changes in plant species and effects due to cadmium exposure

Cadmium is a non-essential element that interferes with plant metabolism and alters cell function, as shown in Figure 3. Plants express various symptoms, such as necrosis in the form of blotches (3A), chlorosis and loss of pigmentation (3B), and loss of turgor (3C).

Necrosis is the death of plant tissue and manifests itself on the leaves as brown or black spots. Rots appear in all plant tissues (leaves, stems, roots) and flowers. Chlorosis refers to the decrease in chlorophyll in the leaves and manifests itself as a yellowish color in the leaves. Turgor loss is the loss of water pressure in plant cells and can cause leaves and stems to wilt and fall.

The symptoms that cadmium produces in plants can vary depending on the species and the conditions of exposure; however, the most representative effects for each species are still a current topic of study.

Plants that were exposed to cadmium showed effects of chlorosis, loss of turgor, and a modified color from green to brown, as seen in Figure 3. Concerning necrosis, species behaved from most affected to least affected as follows: Lettuce > Basil > Mint > Spinach. This is probably due to differences in the way different plant species metabolize cadmium, and thus their cellular functions are affected.

The peppermint, unlike the others, did not show changes between the control sample and the treatment sample. It could be understood that regardless of whether this species is exposed or not to cadmium, it always presents necrosis, either due to its morphology or specific physiology. In addition, since this is a symptom directly associated with the plant cells, it is the most noticeable symptom in the study.

During the days of exposure, a modified color was observed for the lettuce seedlings from day 5. Regarding the loss of turgor for the different species, affectation was observed for all species, mainly in spinach, which showed symptoms from the first day. Chlorosis was the least representative change. This work focuses on identifying representative changes in the color of plant leaves by analyzing parameters such as chroma, hue, and brightness.

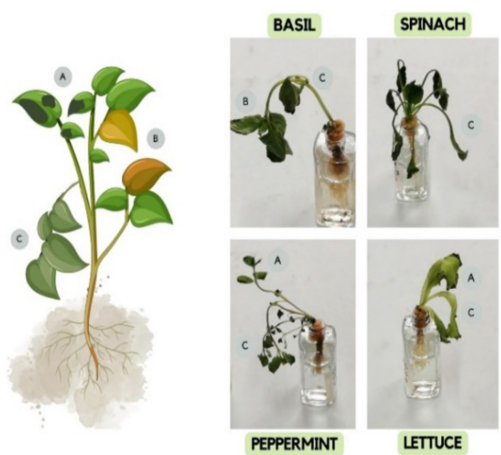

Figure 3. Phenotypic effects due to cadmium exposure in plant species.

3.2 Qualitative analysis of color variations

The color variations in the RGB model from day 1 (D1) until day 7 (D7) for control (CN) and cadmium (CD) plants of lettuce (L), spinach (S), basil (B), and peppermint (P) are shown in Figure 4. To acquire the colors, the photographs taken by the Huawei P30 mobile handset, model MAR-LX3A, and camera were analyzed using the RGB color space. The three primary components of the image—red (R), green (G), and blue (B)—are the basis for this approach. It is possible to get a numerical representation of the observed color by combining the intensities of these three colors to represent each pixel in the image.

In particular, the RGB channel values were retrieved for every plant examined and for every point in time of exposition to cadmium. This provided information about the chromatic composition of the leaves of basil, peppermint, lettuce, and spinach. The lettuce presents a greater color change in its leaves due to a low tolerance to cadmium because of stress. Other species presented a lower modified color variation. It is important to highlight that color differences are not very significant within the RGB color model, so it is necessary to move to the CIE-L*ab model and analyze other color properties.

It is also important to highlight the time of the experiment, which indicates that during a complete germination process, the color change is more evident for each species. The range of pigments found in each species, including chlorophyll, which gives plants their green color, is the reason for this difference in green hues. Different species have different relative concentrations of chlorophyll and other photosynthetic pigments, which affects how intense and what shade of green their leaves are. In addition, variations in pigment expression and, consequently, in the color hues detected in color analysis may be attributed to genetic variables and environmental circumstances unique to individual plants. But to the unaided eye, there are no appreciable alterations upon exposure to cadmium.

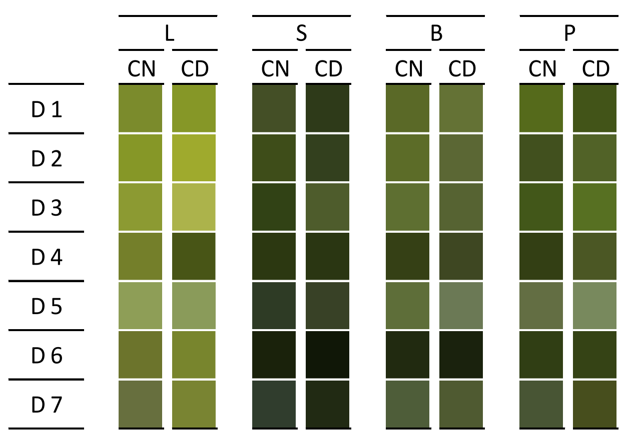

Figure 4. Evolution of the average color of lettuce (L), spinach (S), basil (B), and peppermint (P) leaves during the 7 days of the experiment.

3.3 Colorimetric analysis of leaf tissue

For the colorimetric analysis, chroma, hue, and brightness parameters were determined. Chroma indicates the saturation and intensity of the color; brightness gives us information about the luminosity of the analyzed color; and finally, hue gives us information about which colors are present in the analyzed samples.

3.3.1. Chroma analysis of the study species

The Chroma values for each species, lettuce (L), spinach (S), basil (B), and peppermint (P), are presented in Table 1, and the factor comparisons for each day are shown in Figure 5.

The chroma values obtained for the different species range from 55.155% to 14.058%. The greatest chroma values in this instance are found in lettuce, indicating more intensity and purity of color in the leaves. Conversely, as compared to the other species under consideration, spinach has the lowest chroma values, suggesting a lower saturation and potentially a duller color tone.

Table 1. Chroma values for each species.

	DAY	1	2	3	4	5	6	7
BASIL	CN	35.587	35.479	37.369	27.155	26.896	19.417	19.693
	CD	34.175	33.720	33.245	25.771	22.941	17.104	27.702
PEPPER-MINT	CN	36.169	32.936	35.798	28.818	21.724	26.404	23.717
	CD	35.136	38.859	40.458	34.027	24.903	26.919	28.301
LETTUCE	CN	48.981	47.762	50.603	50.772	35.426	37.057	35.175
	CD	53.564	54.041	55.156	47.566	37.013	38.730	39.645
SPINACH	CN	25.100	31.474	27.806	24.178	18.435	16.097	14.058
	CD	23.685	27.901	27.473	22.124	16.402	14.251	17.447

The chroma values are within a range of 23 to 48. The control plants and the plants exposed to cadmium indicate a decrease in the initial chroma values from day 4, except for lettuce, because the values increase on days 3 and 4 and then decrease. These results indicate that color intensity decreases in plants under stress conditions. This suggests improving cultivation conditions to obtain a better analysis of the chroma changes that plants may present under exposition to pollutants.

Figure 5 shows significant differences among species; this is related to the different phenotypic changes in leaf tissue color. During the first 3 days, no differences were found for lettuce, basil, or peppermint. On days 6 and 7, lettuce and spinach show significant differences. Spinach is the species that shows the greatest variation compared to other species. These changes can be due to natural causes, such as a lack of nutrients or oxygen, as a response to stressful conditions of the environment on the plants.

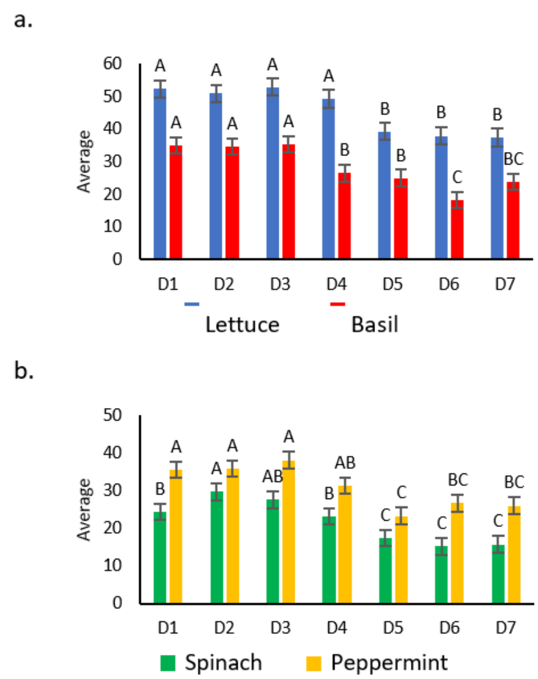

Figure 5. Graph of means for the chroma of (a) lettuce and basil, (b) spinach and peppermint with results of day factor comparisons.

On the other hand, as for the cultivation factor, the species that presented significant differences was peppermint. The response of this factor and the lower influence on the other 3 species can be related to the tolerance of each one to the presence of cadmium. This can also be connected with the diseases of necrosis and loss of turgor presented in Figure 4 for peppermint, where the one that was exposed to cadmium presented these symptoms earlier compared to the one that was in the control crop.

These results demonstrated that a colorimetric study for the analysis of phenotypic changes in chroma is possible.

The time factor is an important parameter to evaluate the response in the leaf tissue. In the experiment, cadmium concentrations were not varied; therefore, it is suggested to make variations to find significant differences for each species depending on the element.

3.3.2. Leaf tissue brightness of the study species

The brightness values for lettuce, spinach, basil, and peppermint are presented in Table 2, and the factor comparison for day 1 (D1) until day 7 (D7) is shown in Figure 6. The brightness values obtained for the different species range from 67.913% to 13.853%. Lettuce is the species with the highest values, while spinach has the lowest values. The variance in glossiness values observed across the examined species may be a sign of variations in the surface composition and leaf structure. With greater gloss values than spinach, lettuce may have a smoother surface or more reflective trichomes, which might have an impact on light reflection and the gloss values that are measured.

Table 2. Brightness values for each species.

	DAY	1	2	3	4	5	6	7
BASIL	CN	36.180	38.087	42.227	25.037	41.570	15.950	31.750
	CD	39.370	36.433	36.243	24.737	52.713	15.933	32.250
PEPPER-MINT	CN	35.097	35.787	33.687	26.313	44.397	22.560	37.427
	CD	31.160	39.147	42.027	33.853	49.847	25.267	31.250
LETTUCE	CN	55.353	48.640	58.467	53.037	67.913	41.817	51.960
	CD	54.977	58.313	67.543	50.890	66.763	40.923	49.490
SPINACH	CN	26.457	34.143	33.110	20.620	37.993	13.853	26.627
	CD	25.750	28.630	32.047	19.813	38.827	14.167	18.170

The brightness values are in the range of 25 to 40 for day 1. In the first three days, the brightness of basil and mint increases. Lettuce and spinach show a variation of values that increase and decrease during the duration of the experiment. On day 6, the lowest value is observed for all plants, including control plants and plants with cadmium. By day 7, the brightness values decreased for basil, lettuce, and spinach. Peppermint increases the brightness of control plants and preserves the brightness of cadmium plants. Brightness is a representative parameter that denotes differences between control plants and plants with cadmium because the values show that if the plant is in optimal condition, the brightness increases.

The presence of cadmium reduced the brightness of spinach, lettuce, and basil. This is an interesting result because it indicates a possible correlation between brightness and the classification of plants in terms of bioaccumulation, such as retainers, accumulators, and hyperaccumulators. However, more information is still required on the molecular and physicochemical processes involved in metal bioaccumulation processes in plants and their physical effects.

The results for brightness presented significant differences for the four species, as can be observed in Figure 6, with which it is possible to establish the possibility of carrying out a colorimetric study to evaluate the phenotypic changes in brightness, and a study should be carried out to establish the levels of the factors cadmium concentration and analysis time to achieve a greater significance of these factors in the dependent variable brightness.

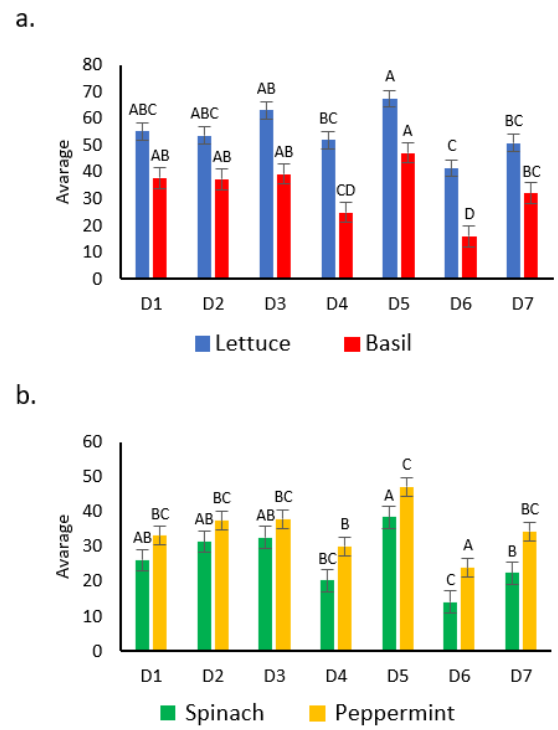

Figure 6. Graphic for the brightness of (a) lettuce and basil and (b) spinach and peppermint, resulting in day factor comparisons.

3.3.3. Leaf tissue hue of the study species

The hue for each species is presented in Table 3, and the factor comparison for day is shown in Figure 7. The tone values obtained for the different species range from 134,335° to 108,832°. Spinach is the species with the highest values, while lettuce has the lowest values. When it comes to color, tone is defined as a color's location within the chromatic spectrum. In this context, tone is represented in the CIE-L*ab color space and is expressed in degrees. With the greatest hue values, lettuce seems more light-colored or luminescent, whereas spinach has the lowest values and may have deeper or more vivid colors.

Table 3. Tone values for each species.

	DAY	1	2	3	4	5	6	7
BASIL	CN	117.422	117.436	117.678	117.342	120.962	121.105	127.409
	CD	116.749	117.464	119.369	118.999	122.060	121.775	117.738
PEPPER-MINT	CN	118.378	118.422	120.955	118.209	123.209	121.572	124.843
	CD	120.075	118.275	119.203	117.018	120.989	119.183	113.396
LETTUCE	CN	112.370	114.345	112.184	109.793	116.583	111.564	114.467
	CD	111.950	109.900	108.832	110.543	117.397	111.609	112.904
SPINACH	CN	120.147	116.939	119.720	118.411	124.366	122.235	134.335
	CD	121.181	119.199	120.285	119.611	124.149	124.272	122.101

Hue values increased for control plants between day 1 and day 7. For spinach, lettuce, and basil plants exposed to cadmium, a very small increase in hue is observed between day 1 and day 7. While peppermint plants reduce their tone.

Regarding the tone variable, for spinach and mint, it was necessary to apply the non-parametric analysis of variance, Kruskal-Wallis, and when evaluating the effect of the individual factors, there is no response of the tone variable to a combined factor, as shown in Figure 7.

For the day factor, mint does not present significant differences and shows similar shades to each other during the cultivation time. While the other species denote a different response in form and intensity. The modified color affects the intensity or saturation of the colors and does not influence the appearance of new shades in the leaf tissue as a function of time.

Regarding the cultivation factor, peppermint presents significant differences due to the possible aspects mentioned above in the chroma regarding this factor. Finally, for the combined effect, basil is the only one that presents considerable significance for this factor, as shown in Table 3, which indicates that both the time and the concentration to which it was exposed are sufficient to identify the effect. of cadmium on the phenotypic changes in tone for this species.

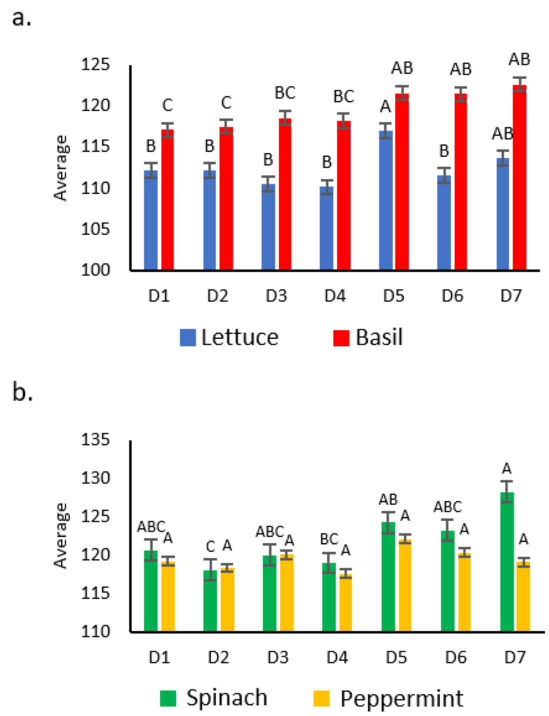

Figure 7. Graph of means for the tone of (a) lettuce and basil and (b) spinach and mint, with the result of comparisons of the day factor.

4. CONCLUSIONS

The results obtained in this study indicated variations in the chroma, tone, and brightness parameters of plants exposed to cadmium during the exposure time. The analysis of the different parameters indicated variations for each species as a function of cadmium. The exposure time also indicated that colorimetric parameters in plants present changes from the first day, which motivates the deepening and development of new heavy metal detection techniques through the use of photographs from mobile devices and the analysis of data.

To thoroughly evaluate the results obtained, it is proposed to evaluate the changes in color parameters during the entire germination process of a plant exposed to a contaminant of interest because it allows greater data and information collection. Furthermore, the characterization of images with colorimetric parameters is projected as a low-cost and easily accessible method. However, it is important to evaluate different variables such as time, element, vegetative species, element concentration, and image capture conditions.

Although there are many parameters to evaluate, image characterization could significantly contribute to the early detection of cadmium, among other elements of interest, mainly in plants of great interest for human consumption.

Image characterization could be an important contribution to bioaccumulation studies because it could help identify retainer, accumulator, and hyperaccumulator plants of heavy metals, among other contaminants of interest. It is also a method that requires coupling machine learning techniques for data analysis, which would allow results in real time. However, this technique is recent and has not yet been used for the detection of specific metals in foods for human consumption.

In addition, image analysis can significantly contribute to the problem of food safety and environmental monitoring of crops to guarantee the safety of products for human consumption.

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CONFLICT OF INTEREST

The authors do not have any type of conflict of interest to declare.

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