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Alteration induced in the absorption of a photon by Pb in the chloroplast center of leaves of *Vigna radiata* L

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Abstract
Vigna radiata L. is included in a dietary meal worldwide, especially in Asian countries, and is also a part of herbal medicine. It was selected to monitor the photon absorption under Pb metal on the chloroplasts center. The seedlings of *Vigna radiata* L. were cultured in nutrient solution for 15 days at several concentrations of Pb viz., 0, 50, 100, 150, and 200 ppm. Then harvested, washed, homogenized in ethanol, centrifuged and the supernatants were subjected to spectrophotometric analysis. Results showed that a hypsochromic shift in wavelength was observed under Pb stress which was discussed as an alteration in the absorption of photon wavelength of different pigments observed in the early growing stages. It was observed that the alteration in photon absorption consequently appears in photosynthetic activities, followed by the formation of the excited state of the chlorophyll and the release of energy. Moreover, the shift in wavelength in the UV region showed the synthesis of secondary metabolites. It was concluded the effects of Pb on photon absorption of chloroplast center was strongly reliant on the distribution of pigment concentrations and their absorbance with the formation of compounds that absorb radiation in UV region.
Keywords: Pb, photon, chloroplast center, secondary metabolites

1. INTRODUCTION

Among heavy metals, the Pb, part of sewage, paints and gasoline, has no biological function in plants and humans and is widely distributed in the environment. Heavy metals are persistent and cannot be degraded. Therefore, their presence in soil seriously threatens the soil where crops are cultivated. It also threatens in the future by reducing the production yield as they induce alteration in the plant's photochemical and photobiological reaction [1]. Zhou et al. [2] reported that as the concentration of Pb increases, the growth of plants decreases, followed by a decrease in photosynthetic activity. It was also established that a decrease in chloroplast pigments results in photoinactivation of the photosynthetic system. Fatoba et al. [3] observed that heavy metals triggered nearly mutilation of the chloroplasts of the plant where an alteration in the green colour of chlorophyll from bright to light and then yellow-green to brown appeared as the bright green colours changed light green, yellowish green or brown. An adverse effect of Pb accumulation on the overall growth of the plants was reported by Hu et al. [4]. They reported that increasing the level of Pb decreases the chlorophyll with serious damages in leaf cells, including swelling of chloroplast, interruption, and vanishing of chloroplast covers with other deformation [5]. Paunov et al. [6] reported the inhibition in relative growth rate and photosystem II under heavy metals Cd (50 µM) and Zn (600 µM) in wheat seedlings grown in the nutrient solution. Ashraf et al. [7] reported the impact of Pb on the growth rate and productivity of rice cultivars, i.e., Meixiangzhan-2 (MXZ-2), Xiangyaxiangzhan (XYXZ), Guixiangzhan (GXZ), Basmati-385 (B-385), and Nongxiang-18 (NX-18). The germination of seeds is the first step in plant life, but if the soil is contaminated by heavy metals like Pb, then interference in growth rate with decreasing cell enlargement and root elongation is observed [8,9]. Lead disturbs the cell metabolism process due to its easy accumulation in plants. These include inhibiting photosynthesis and mineral elements, which are ultimately related to growth. Plants exposed to heavy metal stress showed survival strategies in which the synthesis of phenolic compounds helps protect plants from oxidative stress [10-14]. Phenolic compounds are involved in plants' growth rate and are tangled in plant responses to environmental stresses, including wounding, pathogen attack, mineral deficiencies, and heavy metals stress . Biotic and abiotic stresses increase secondary metabolites, antioxidant activity, flavonoids, and phenolic content [15-18]. The current article explores the alteration in the absorption of a photon in seedlings of *Vigna radiata* exposed to Pb heavy metal in a nutrient solution applied in various concentrations. This study examined different wavelengths of a photon of light related to the different pigments and compounds produced during the stress in leaves.

2. MATERIAL AND METHODS

The effect of Pb on seedlings of the *Vigna radita* was investigated by subjecting them to water culture for a period of 15 d. Pb has been added as PbCl2 at concentrations viz., 0, 50, 100, 150 and 200 ppm to the Hoagland nutrient solution. After 15 d of exposure to the treatments, the leaves were harvested, washed, homogenized in ethanol, and centrifuged. The supernatants were collected and analyzed by Schimadzu spectrophotometer, which allows programmed automatic recording and printing out of the proper wavelengths and absorbency values. The absorbance of pigments in the 700-200 nm wavelength was scanned. The characteristic absorbance wavelength of the pigment and compounds were analyzed [5].

3. RESULT AND DISCUSSION

The spectrophotometric technique is applied for measuring the alteration in wavelength of pigments under Pb stress by observing the absorbance that appears at that specific wavelength. The present investigation discussed the impact of heavy metal Pb by monitoring variations in wavelengths to explore the pigments functions and initial primary and secondary reactions. Chlorophyll is considered a central pigment of photosynthesis for the absorption of solar radiation, and two types one is chlorophyll a and b. The excitation of this pigment after photo absorption is important for photosynthesis activation. Analysis showed that hypsochromic shift in wavelength under Pb stress was observed, which was discussed as an alteration in the absorption of photon wavelength of different pigments observed during 15 days of monitoring in the early stages of growth. Results are compared with control plants as standard and discussed related to the alteration in wavelength absorption. Spectral analysis of the ethanolic extract of control plants pigments showed wavelengths at 663 and 432, which showed photon absorption in the Visible region of 400-700nm wavelengths signifying that the absorption of a photon by chlorophyll, xanthophyll and carotenoids. These pigments utilize the energy of these wavelengths to initiate excitation reactions. It also reflects the green region and absorption of red wavelength, indicating normal photosynthesis where maximum absorption at 663 nm (blue region of 600-700 nm) and 432 nm (red range 425-450nm) 0.732 and 1.980 were observed, respectively. Chlorophyll usually transfers its excitation energy to other molecules in the chloroplast center to assemble CO2 and release oxygen and water as by-products. When plants were subjected to 50ppm of Pb concentration, eight wavelengths were observed compared to the control plant, where six were observed with slight shift in wavelength from 663 to 665 (0.608) and 432 to 434(1.056) with a decrease in absorbance, indicates the effect of Pb on chloroplast center which may indicate that the photoinactivation started under Pb stress. The presence of new peaks at a wavelength of 322 and 212nm from the Visible region to UV region showed the presence of new compounds under metal stress. They reflected the synthesis of compounds whose absorbance is in UV region in table 1.

Table 1. Spectral regions of absorption maxima of pigments and secondary metabolites of Vigna radiata in ethanolic extract under Pb stress at different concentration.

Absorption maxima	Absorbance of compounds in UV/ Visible region	Compounds absorbing in UV region	Compounds in visible region	Literature Reports	References
Control plants					
663	0.7320	-	Chlorophyll pigments	400nm-700nm	https://openlab.citytech.cuny.edu/bio-oer/photosynthesis/absorbance-spectra-of-photosynthetic-pigments/
617	0.287	-			
585	0.156	-			
535	0.128	-			
456	0.937	-			
432	1.98	-			
50ppm					
665	0.608	-	Chlorophyll pigments	400nm-700nm	https://openlab.citytech.cuny.edu/bio-oer/photosynthesis/absorbance-spectra-of-photosynthetic-pigments/
606	0.123	-			
535	0.187	-			
506	0.191	-			
434	1.056	-			
411	1.831	-			
322	2.262	Stilbenes 300–310, 320–330			Cerovic [18] Harborne [20] Jurd [21] Mabry[22]
212	0.091				

100ppm					
663	0.748	-	Chlorophyll pigments	400nm-700nm	https://openlab.citytech.cuny.edu/bio-oer/photosynthesis/absorbance-spectra-of-photosynthetic-pigments/
615	0.219	-			
579	0.193	-			
533	0.191	-			
456	1.076	-			
432	1.615	-			
376	1.222	Chalcones	-	365–390	Cerovic [18] Harborne [20] Jurd [21] Mabry[22]
320	2.558	stilbenes	-	320–330	
293	1.397	Phenolic acids	-	235–305	
271	1.258	Simple phenol	-	266-295	
235	1.772	Sinapic acid	- - - - -	235	
150ppm					
663	0.336	-	Chlorophyll pigments	400nm-700nm	https://openlab.citytech.cuny.edu/bio-oer/photosynthesis/absorbance-spectra-of-photosynthetic-pigments/
612	0.079	-			
574	0.073	-			
533	0.086	-			
457	0.624	-			
431	0.88	-			
374	0.731	Chalcones		3-65–390	Cerovic [18] Harborne [20] Jurd [21] Mabry[22]
321	1.958	stilbenes		**32-0–330**	
270	0.146	Simple phenoles		-	
200ppm					
323	0.459	Hydroxycinnamic acids	-	310-332	Cerovic [18] Harborne [20] Jurd [21] Mabry[22]
294	1.945	Phenolic acids	-	235-305	
259	0.659	Chalcones	-	240-260	
238	0.158	Sinapic acid	-	235	

The spectral analysis at 100ppm reported that there are 11 wavelengths observed which showed the harmful effect of Pb at higher concentrations with a decrease in absorbance of chlorophyll contents [8]. In contrast, an increase in absorbance at 432 nm compared to 50 ppm and lowered compared to control showed an increase in carotenoid pigments to protect plants under stress. The hypsochromic shift of the wavelength in UV region (UV absorption started at 320-400nm) also showed that under Pb stress, some molecules or part of them that absorb light strongly in the UV are produced (376,320,293,271, and 235nm) referred to as chromophores which may be due to the substitution of some elements or breaking of some molecules [11]. Carotenoids in the chloroplasts center provide help in absorbing the excess energy and dissipating it as heat. According to the earlier reports, carotenoids play a critical role in the photosynthetic system of microalgae, such as by protecting the photosynthetic apparatus against photooxidative damage [12-19]. The peaks in the UV region were 376,320,293,271, and 235, where absorbance was 1.222,2.558,1.397,1.258 and 1.772. The shift in the UV region suggested that secondary metabolites like Chalcones, stilbenes, Phenolic acids, Simple phenol and Sinapic acid were synthesized for the survival of the plants under stress [20-22].

Analysis of the plants at 150ppm reports 9 wavelengths which showed the lethal effect of Pb at higher concentrations with a decrease in photon absorption by significant pigments and the disappearance of some wavelengths. The absorbance observed at 374,321 and 270 was 0.731,1.958 and 0.1146, respectively, which was less than 100 ppm. The analysis at 200 ppm showed no radiation absorption in the visible region with a shift in the UV region where no peak of essential pigments like chlorophyll, xanthophyll and carotenoids was observed. It indicated that Pb diminished the chloroplast center. An alternative pathway of secondary metabolites was operated at 200ppm with a reduced growth rate of the plants, and plants at 100-200ppm observed immediate death. Only four wavelengths at 200ppm in the UV region at 323,294,259, and 238 showed a reverse metabolic pathway. A detailed literature search showed wavelength from 320-340 nm showed the synthesis of isoflavones and flavanones [13-22], which were activated through a shift in wavelength from visible to violet region for photoprotection through UV screening effect and by quenching the reactive oxygen [14]. Results established that a shift in wavelength from visible to UV region under Pb stress inactivates the photosynthesis of the plants, due to which plants were unable to survive.

Photosynthesis is essential for all plants related to their growth. It is an indispensable photochemical reaction on which the earth's life depends as oxygen is released through this process. There are two major pigments in the plants, chlorophylls: a, b, c, d and carotenoids. Photosynthetically-active photons absorb by pigments in normal plants in the wavelength range of 700 nm to 400 nm. Each pigment in the center of the chlorophyll has a typical absorption wavelength which generates electrons to power photosynthesis. Additional pigments like chlorophyll b and beta carotene absorb energy that chlorophyll a does not absorb. Moreover, another common class of pigments, namely xanthophyll, is yellow and absorbs the most at 435 nm (blue).

Pb affects the processes of photosynthesis, where at low concentration (50ppm) of Pb, slight variation in the visible range of wavelength suggest the slow inactivation of converting solar energy into chemical energy for the synthesis of carbohydrates with the release of oxygen and water as a by-product.

Analysis of the extract showed that the chloroplast center was affected very severely, where chlorophyll contents were diminished at higher concentrations (200 ppm)[18], and no chlorophyll peak was observed. Results established that photosynthetic apparatus was the first target of the heavy metal, and measurements of the wavelengths and absorbance are a good accurate and easy tool to detect the impact of metal [15, 16]. The results suggest that the primary process required for the growth development is affected under Pb accumulation where altered photon absorption inhibited the formation of an excited state and appearance of new wavelength related to the secondary compounds like phenols observed. Yang et al. **[17]** also reported that net photosynthetic rate decreases with an increase in the concentration of Pb in *Davidia involucrate.* It was also suggested that the alliterated wavelength in the UV region might be responsible for increasing heat in photosynthetic apparatus due to which plants experience rapid death [18,5]. Photoinactivation started in the wavelength range between 320 and 335 nm with a maximum above 350 nm. The current results are similar to the earlier report of Zhou et al.,[2] in which they disclose that at higher concentrations of Pb, the growth of plants decreases, followed by the reduction in photosynthetic activity due to a decrease in chlorophyll a and b linked with lessening in absorbance of a photon.

CONCLUSION

It was concluded that the inhibition in photosynthesis of the plants was due to the photoinactivation of photosynthetic pigments under Pb accumulation in leaves. The appearance of the new wavelength in pigment extract reflects an alteration in the metabolic pathway that may be for the survival of the plants, which failed at a higher concentration of Pb where no pigment in the visible region was reported. However, more research is required regarding the photochemical reaction of plants under heavy metal stress.

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