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GRADUATE RESEARCH ARTICLE

Effect of Gamma-Irradiated Ceramic Wastewater Sludge on Photosynthetic Pigments of Phaseolus Vulgaris L. (Cv. Valentino and Cv. Nebraska) Plants

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ABSTRACT

The common bean plant is a highly polymorphic species, annual herb, erect and bushy, 20–60 cm tall, or twining with stems 2–3 m long, with a taproot and nitrogen nodules which the bacteria could convert nitrogen gas and stores it in the common bean plant roots. Leaves are alternate, green, or purple, trifoliolate, stipulate, petiolate, markedly pulvinus at base; leaflets ovate, entire; acuminate, 6–15 cm long, 3–11 cm wide. Many soils have been contaminated with several pollutants, mostly arising from wastes of human activities such as the use of pesticides, sewer sludge, mining and smelting sources, radioactive substances, and industrial dischargers. These different contaminants can degrade the soil and cause a negative impact on the plant due to disturbing nutrient cycling within ecosystems and subsequently human health. This study aimed to elucidate the mechanism by which the plant may cope with heavy metals stress represented in this study in the form of industrial ceramic wastewater sludge. And, evaluating the role of gamma radiation on ceramic wastewater sludge, could reduce the percentage of the endogenous levels of heavy metals in ceramic sludge. This study investigates the influence of Photosynthetic pigments of the two common bean cultivars Valentino and Nebraska which are grown in light soil clay/sand (1:1) incorporated with ceramic-wastewater sludge (CWWS) non-irradiated (1% and 2%) and gamma-irradiated (2.5 kGy and 10 kGy) for 30 days. The application of non-irradiated CWWS induced an elevation in the contents of chlorophylls a, b, and carotenoids in the two cultivars while a reverse trend was observed in the leaves of both cultivars treated with gamma irradiated CWWS.

Keywords: Ceramic- wastewater sludge, Common bean, Gamma-irradiated.

1 Introduction

1.1 Plant growth and yield

The edible common bean (*Phaseolus vulgaris L.)* is an annual plant, grown worldwide for both dry seeds and as a green bean. Its leaf is also occasionally used as a vegetable. Generally, Plant growth and development depend on environmental conditions that mainly include the availability of micro-and macro-elements in the soil [1]. The seventeen most important nutrients for plants are up taken by plants from their growing medium [2]. Macronutrients represent nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), sulfur (S), magnesium (Mg), carbon (C), oxygen (O), / and hydrogen (H). Micronutrients (or trace minerals) are iron (Fe), boron (B), chlorine (Cl), manganese (Mn), zinc (Zn), copper (Cu), molybdenum (Mo), nickel (Ni). Plants consume these elements as ions, where macronutrients are required in larger quantities than micronutrients. Hydrogen, oxygen, nitrogen, and carbon contribute to over 95% of a plant's entire biomass on a dry matter weight basis. Micronutrients are present in plant tissue in quantities measured in parts per million, ranging from 0.1 to 200 ppm, or less than 0.02% dry weight [3]. In this case, soil amended with these nutrients strongly affects the photosynthetic apparatus structure and functions, plant growth, and development [4].

1.2 Ceramic-wastewater sludge

The ceramic industry in the world showed tremendous development in the last two decades [5]. The composition of these ceramic industry wastewaters varies widely and includes high concentrations of both suspended & dissolved solids and electrolytes of very different nature [6], as well as significant amounts of organic substances that mostly come from the additives used in decorating the tiles [7]. Many studies show that Sewage sludge amendments with the different concentrations of Ni, Zn, Mn, Pb, Cr, Cd, and Cu significantly increased the contents of chlorophyll a, b, total, and carotenoids in rice (Oryza sativa L.) plants [8, 9]. A similar result was obtained by [10], who found that the increment in pigment content was in parallel with raising the sludge content in soil up to 50% in wheat leaves and jews mallow plants. Moreover, the increment in photosynthetic pigments (chlorophylls a, b, and carotenoids) content in sunflower (Helianthus annuus) plants grown under sewage sludge was reported by [11]. A positive increase in chlorophylls a, b, and carotenoid content was also recorded in spinach leaf (Spinacia oleracea), fenugreek (Trigonella corniculata) treated with biochar-amended sewage-irrigated contaminated soil [12], and different types of biochars used to mitigate Cd stress on sunflower (Helianthus; L.) growth in agricultural soil irrigated with wastewater [13, 14]. [15] reported that cadmium has been reported to cause inhibition of PSII activity in vivo transplanted R. lacera lichen at 20 different sites, due to interaction with the water-splitting enzyme, possibly by substitution of the Mn ions [16].

1.3 Gamma Radiation

On the other hand, Radiation processing such as gamma-ray (symbol γ) are measured in units called kilo Grays (kGy) has been considered as a promising technology for the treatment of wastewater or sludge has been discussed by [17, 18]. kill microbes by direct and indirect hits and gamma-ray irradiation as a pre-treatment process has been studied to release soluble carbohydrates from activated sludge [19], and electron beam irradiation has been applied for the wastewater sludge volume reduction [20]. However, the feasibility of the solubilized sludge carbon source by gamma-ray irradiation on biological nitrogen removal has seldom been investigated. The application of irradiated sewage sludge led to a positive effect in leaf chlorophyll index of Ocimum basilicum L. over the respective control [21, 22], and a non-significantly increase in chlorophyll content of mature fennel leaves [23]. This data was in accordance with the results obtained by [24] who indicated also a non-significant effect in chlorophyll content of methi (Trigonella foenum-graecum L.) plants grown in irradiated sludge.

2 Materials and Methods

2.1 Materials

2.1.1 Time course experiment

All the experiments have been carried out in the greenhouse at the Botanic Garden, Faculty of Science, Ain Shams University, Cairo, Egypt. The relative humidity ranged between 24 and 6%. The maximum day temperature was 38 ºC, while the minimum temperature was 18ºC. All pots were applied with sufficient irrigation. Different analyses were done in the laboratory of plant physiology on the same campus except for gamma radiation experiments that were held in the Nuclear Research Center, Atomic Energy Authority, Cairo. All the chemicals used in this study were of high purity, purchased from Sigma-Aldrich Chemical Co., Germany, and all organic solvents were of AR grade.

2.1.2 Plant material

Pure lots of seeds of common bean (*Phaseolus vulgaris L.* cv. Valentino and Nebraska) were obtained from the Department of Vegetable Crops Production and Technology, Horticulture Research Institute, Agriculture Research Centre, Giza, Egypt. Ceramic-wastewater sludge was collected from a drainage outlet of a ceramic industrial factory in Abou-Zaabal, Egypt. Virgin sandy soil was obtained from Belbeis desert, Sharquia Governorate Northern East Cairo.

2.1.3 Ceramic-wastewater sludge and the soil used

An analysis of some physical and chemical properties of the soil used in this study was done as described by [25]. The pH, electric conductivity, soluble cations, and anions were determined in the soil paste extract. Sodium, potassium, calcium, magnesium, chloride, sulfates, carbonates as well as bicarbonates were determined by analytical technique called atomic absorption spectrometer GBC (SavantAA) which it depends on the absorption of different radiation wavelengths comes from light source to determine different atoms in clay/sandy soil. Organic matter was determined using the method of [26]. (Table 1). Non-gamma-irradiated ceramic and gamma-irradiated ceramic wastewater sludge was analyzed by an atomic absorption apparatus and the data was recorded in Table 2 [27] and Table 3 respectively.

Table 1: *Physical and chemical analysis of clay/sandy soil.*

Properties	**Value**
pH	7.3
EC (dS m^{-1})	2.18
Soluble anions (%)	
Na^+	0.019
HCO_3	Not detected
Cl^-	0.010
SO_4	0.015
Soluble cations (%)	
Ca^{++}	0.015
Mg^{++}	0.124
K^+	0.014
Organic carbon (g/100g soil)	2.34
Physical analysis	
Gravels (%)	4.6
Coarse sand (%)	14.3
Medium sand (%)	9.62
Fine sand (%)	55.32
Very fine sand (%)	8.82
Silt and Clay	7.34
Texture	Sandy soil

Table 2: *Analysis of the ceramic wastewater sludge (%).*

Mineral ion	Concentration (% W/W)
Iron (Fe)	1.77
Zinc (Zn)	0.65
Lead (Pb)	0.04
Calcium (Ca)	1.93
Magnesium (Mg)	0.25
Sodium (Na)	1.59
Potassium (K)	1.01
Aluminum (Al)	8.33
Silicon (Si)	29.47

Table 3: *Analysis of the gamma-irradiated ceramic wastewater sludge (%)*

Mineral ion	Concentration (% W/W)
Iron (Fe)	1.16
Zinc (Zn)	0.45
Lead (Pb)	0.02
Calcium (Ca)	0.095
Magnesium (Mg)	0.025
Sodium (Na)	1.45
Potassium (K)	1.12
Aluminum (Al)	2.77
Silicon (Si)	5.55

2.1.4 The yield experiments

The planting pots were divided into 2 groups; the first group was used for cv. Valentino and the second one for cv. Nebraska. The sterilized common bean seeds were sown in pots (30×18 cm) filled with homogenous clay/sandy (1:1) soil (8 Kg). The physical and chemical analysis of soil properties of the field experiment is given in (Table 2). The used clay/sandy soil was divided into 7 series for each cultivar which was incorporated by (non-radiated and gamma-irradiated) ceramic wastewater sludge at levels (0%, 1%, 1% of 2.5 kGy, 1% of 10 kGy, 2%, 2% of 2.5 kGy, 2% of 10 kGy) w/w and the first one without ceramic sludge (control). A randomized complete block design was used with three replicates for each treatment.

2.2 Methods

2.2.1 Extract and estimation of photosynthetic pigments

Extraction

A known fresh weight of leaves was homogenized in 85% aqueous acetone for 5 minutes. The homogenate was centrifuged, and the supernatant was made up to known volume with 85% aqueous acetone. The spectrophotometric method recommended by [28]was used.

Estimation

The absorbance was measured against a blank of pure 85% aqueous acetone at 3 wavelengths of 663, 644, and 452.5 nm using a spectrophotometer (Spectronic 601, Milton Roy Company). It was possible to determine the concentrations of the pigment fractions (chlorophyll a, chlorophyll b, and carotenoids) as µg/ml using the following equations [29]:

$$\text{Chlorophyll a} = 10.3\ E_{663} - 0.918\ E_{644} \quad (1)$$

$$\text{Chlorophyll b} = 19.7\ E_{644} - 3.870\ E_{663} \quad (2)$$

$$\text{Carotenoids} = 4.2\ E_{452.5} - (0.0264\ \text{chlorophyll a} + 0.426\ \text{chlorophyll b}) \quad (3)$$

Finally, the pigment contents were calculated as µg/g fresh weight of leaves.

2.2.2 Statistical Analysis

The data were expressed as mean ± standard error (SE) often replicated and statistical analysis was performed by one-way analysis of variance ANOVA, followed by SPSS Statistics for Windows, Version 21. P<0.05 for the means compared [30], using the least significant difference (LSD at 0.05% level).

3 Results

The application of ceramic wastewater sludge significantly increased chlorophyll a, b and carotenoid contents in leaves of the two investigated *Phaseolus vulgaris* cultivars (Valentino & Nebraska) and the percentage of increase was 23.2% and 43% above the control value in 2% concentrations at 2.5 kGy and 10 kGy, respectively, as compared with the control plants grown in ceramic sludge-free soil (Table 4 and Figures 1, 2).

Table 4: *Effect of different levels (w/w) of ceramic wastewater sludge (CW-WS) at 1% and 2% on photosynthetic pigments of Phaseolus vulgaris L. (cv. Valentino and cv. Nebraska) plants (30-day-old) grown in clay/sandy soil irradiated and non-irradiated with relatively low and high rates of gamma rays (2.5 and 10 R, respectively). Results are means of three replicates ±SE.*

Cultivar	Treatments \ Parameters	Chlorophyll a (µg/g FW)	Chlorophyll b	Carotenoids
Valentino	**0.0 %**	155.2±3.3	52.1±0.33	76.9±0.03
	1.0 %	182.5±3.33	87.8±0.33	92.8±0.33
	2.0 %	175.5±3.3	63.9± 0.33	81.3±0.33
	1.0 % + 2.5 R	107.1±3.3	31.7±0.03	45.4±0.3
	1.0 % + 10 R	33.1±0.34	17.5±0.033	18.1±0.3
	2.0 % + 2.5 R	101.7±3.33	31.4±0.33	51.2±0.33
	2.0 % + 10 R	110.4±3.3	31.0±0.33	42.5±0.3
LSD at 0.05%		26.83	12.66	14.10
Nebraska	**0.0 %**	114.6±3.4	55.6±3.3	79.9±0.033
	1.0 %	165.3±3.33	83.3±3.3	86.1±0.033
	2.0 %	150.6±3.3	60.6±3.33	82.1±0.033
	1.0 % + 2.5 R	161.6±3.3	48.3±3.33	70.6±0.33
	1.0 % + 10 R	162.3±3.3	31.6±3.33	90.8±0.3
	2.0 % + 2.5 R	88.0±0.33	26.0±0.03	98.5±0.3
	2.0 % + 10 R	103.0±3.3	30.8±0.033	114.4±3.3
LSD at 0.05%		16.84	10.74	7.31

*R=kGy.

The maximum increases in all previous parameters of *P. vulgaris* leaves were obtained at 1% concentration. However, the irradiation treatments at two doses used (2.5 kGy, and 10 kGy) significantly decreased the contents of chlorophylls a, b, and carotenoids in the leaves of *P. vulgaris* cv. Valentino, the reduction was higher at 10 kGy. The carotenoid contents exhibited reverse manners in the leaves of *P. vulgaris* cv. Nebraska.

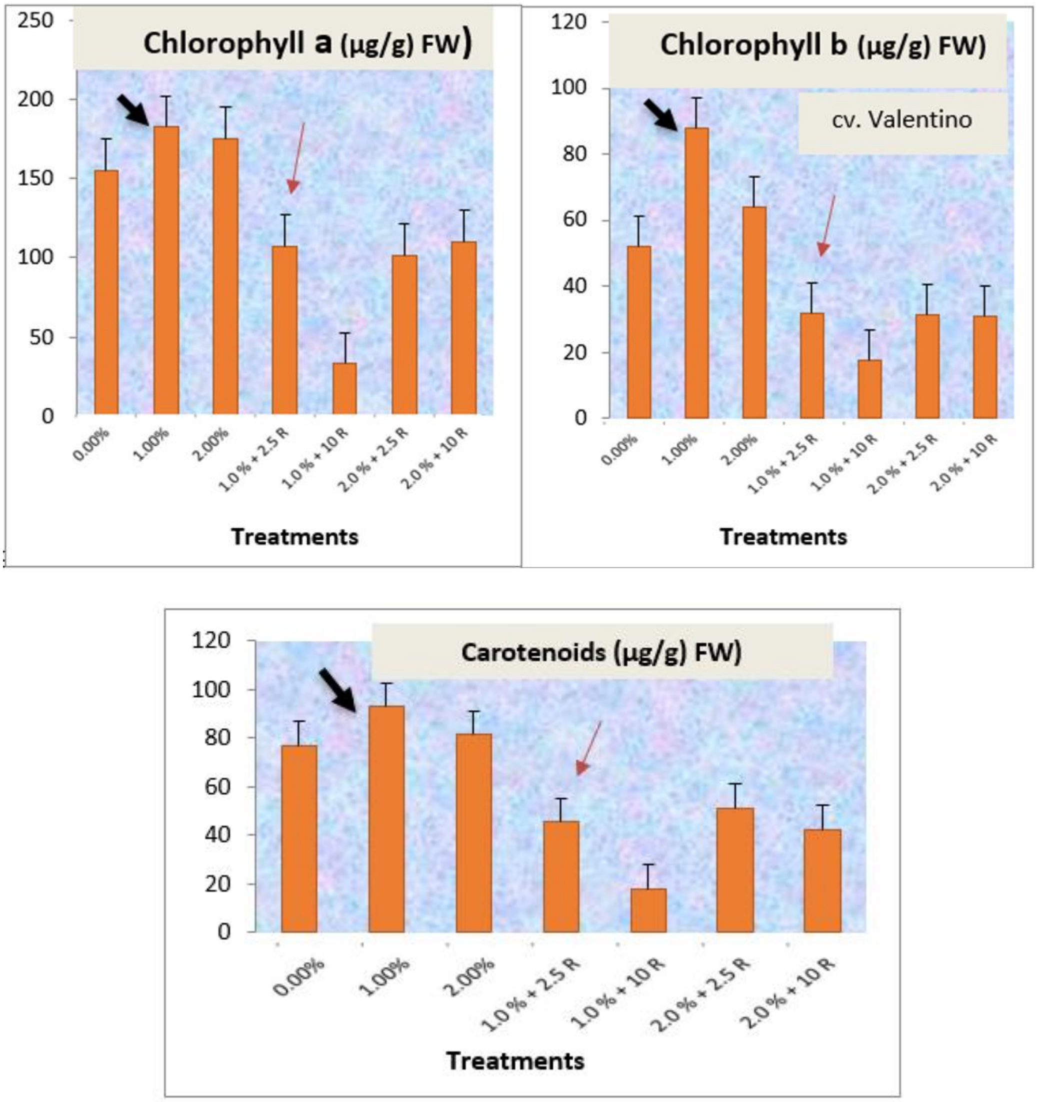

Figure 1: *Effect of different levels (w/w) of ceramic wastewater sludge (CWWS) at 1% and 2% on photosynthetic pigments of Phaseolus vulgaris L. (cv. Valentino) plants (30-day-old) grown in clay/sandy soil irradiated and non-irradiated with relatively low and high rates of gamma rays (2.5 and 10 R, respectively). Results are means of three replicates ±SE. The maximum increases in all previous parameters of P. vulgaris leaves were obtained at 1% concentration (black arrows), and at 1% concentration, 2.5 kGy (red arrows).*

**R=kGy.*

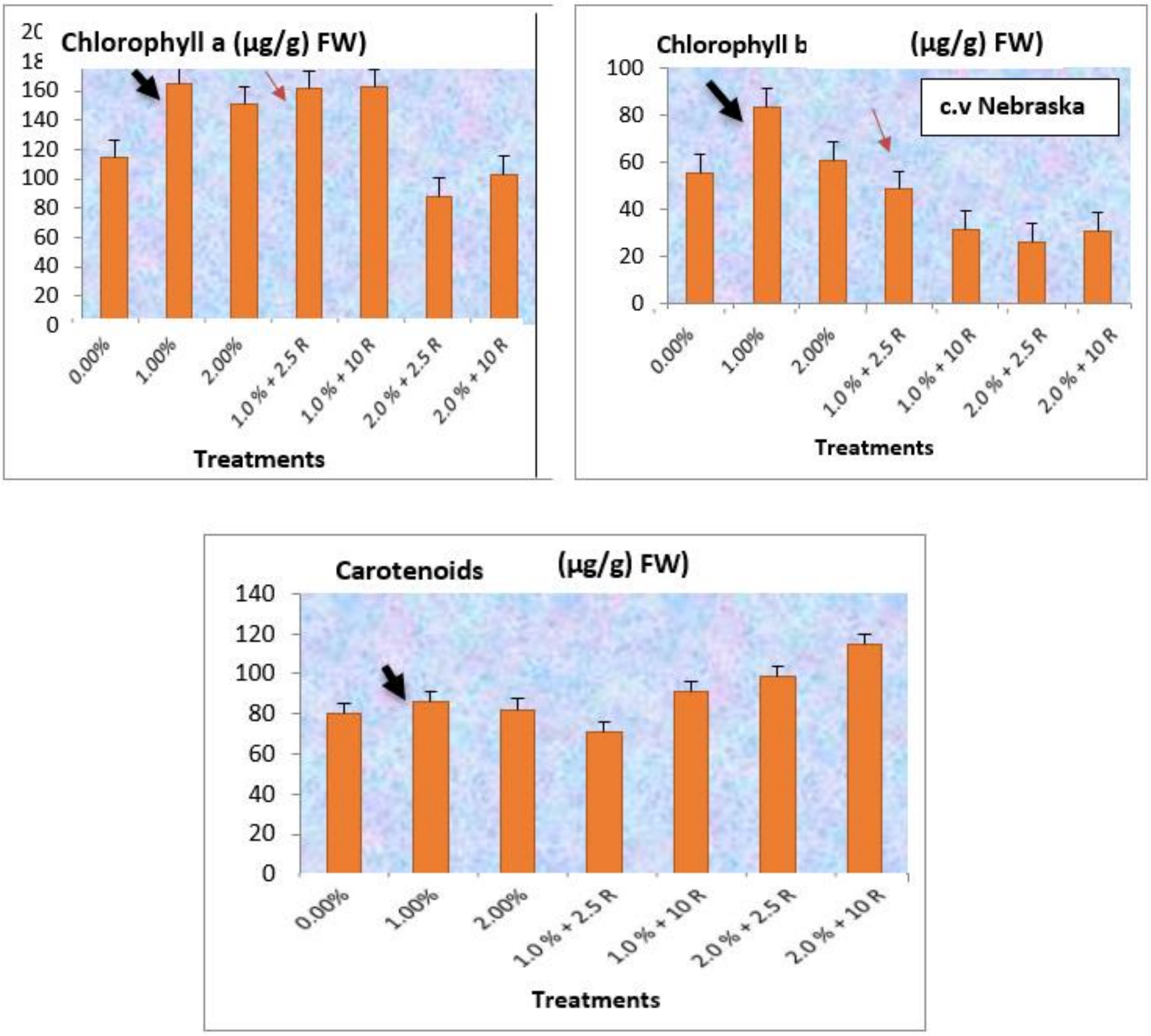

Figure 2: *Effect of different levels (w/w) of ceramic wastewater sludge (CWWS) at 1% and 2% on photosynthetic pigments of Phaseolus vulgaris L. (cv. Nebraska) plants (30-day-old) grown in clay/sandy soil irradiated and non-irradiated with relatively low and high rates of gamma rays (2.5 and 10 R, respectively). Results are means of three replicates ±SE. The maximum increases in all previous parameters of P. vulgaris leaves were obtained at 1% concentration (black arrows), and at 1% concentration, 2.5 kGy for chlorophyll a and b (red arrows) and increase carotenoid content in (c.v Nebraska) at concentration 1,2% with different radiation (2.5 and 10kGy).*

**R=kGy.*

4 Discussion

In this study, a remarkable reduction of chlorophylls a and b were recorded in the two investigated *P. vulgaris* cultivars (cv. Valentino & cv. Nebraska) cultivated under irradiated ceramic wastewater sludge (Table 4, **Error! Reference source not found. Error! Reference source not found.**). This reduction might be attributed to the high accumulation of heavy metals that inhibits chlorophyll synthesis or destructs the chloroplasts, reducing the number of chloroplasts of the leaves and enhancing their degradation [31, 32]. The results of the present work also agreed with those of [33-35] who found that chlorophylls a and b were reduced with increasing Ni levels in different cultivars of radish which indicated inhibition of chlorophyll biosynthesis. Also, summer squash was found to accumulate higher concentrations of Ni, Cr, Co, and Zn which were found to be responsible for the reduction in chlorophylls a and b and total pigment content [36]. It is interesting to note that heavy metals inhibit the enzymes responsible for chlorophyll biosynthesis.

Thus, cadmium was reported to inhibit the key enzymes protochlorophyllide reductase and aminolevulinic acid dehydratase (ALA) synthesis, which is involved in the reduction of protochlorophyll to chlorophyll [37, 38]. Moreover, heavy metals inhibit the photosynthesis ability and redox imbalance in the plant [39]. The inhibition of photosynthesis function was attributed to inhibition of the Calvin cycle [40]. Reducing CO_2 fixation, reduced aggregation of pigment-protein complexes of the photosystems and damage of chloroplasts induced by ROS are other effects caused by heavy metals [41, 42].
Carotenoids act as light-harvesting pigments, essential non-enzymatic antioxidant components of the photosynthetic apparatus, which play an essential role in the protection of chlorophyll and membrane destruction by quenching triplet chlorophyll and preventing photo-oxidative damage of the photosynthetic apparatus by detoxifying ROS [43]. Metals can enhance or reduce carotenoid production depending on metal types [44, 45]. Several studies have reported an increase [46, 47], a decrease [48, 49], or no difference [50] affect the heavy metal stress on the content of carotenoids in plants. These results are in agreement with the results of this study (Table 4 and Figures 1 &2). [51] reported that the highest concentration of heavy metals significantly decreased carotenoid contents in the two mangrove species. This reduction would lead to a diminished capacity to protect photosystems against photo-oxidation [52]. Besides, the bleaching of carotenoids in the reaction center of PSII destabilizes PSII structure and triggers the degradation of the D1 protein [52].

5 Conclusion

The common bean is a member of the family Fabaceae and contributes a high nutritional value to the human diet as well as representing a primary source of protein. The aim of the present work is to elucidate the mechanism by which the plant may cope with heavy metals stress represented in this study in the form of industrial ceramic wastewater sludge, this was evaluated by studying the physiological changes in response to heavy metals stress and tried to enhance heavy metals stress in the two investigated common bean cultivars (cv. Valentino and cv. Nebraska) treated with either non-radiated and gamma-irradiated ceramic wastewater sludge by selection the permissible levels of ceramic wastewater sludge which have a positive or beneficial effect on common bean plants. On the other hand, to evaluate the role of gamma radiation on ceramic wastewater sludge to induce the physiological and biochemical responses of common bean and reduce the percentage of the endogenous levels of heavy metals in ceramic sludge incorporated with clay/sandy (1:1) soil. The data revealed that heavy metals of ceramic wastewater sludge at 1% and 2% levels (non-irradiated and gamma-irradiated at 2.5 kGy and 10 kGy) incorporated with clay/sandy (1:1) soil led to changes in most physiological and biochemical processes of two common bean cultivars under study (cv. Valentino and cv. Nebraska). It could be recommended that the use of low and permissible levels of ceramic wastewater sludge (1%) be irradiated with low doses of gamma rays (2.5 kGy) to improve the chlorophyll pigments in both cultivars. This had a positive effect on the productivity of this common bean cultivar which enhanced the yield quality and quantity of common bean plants as well as the desired nutritional value of edible Phaseolus vulgaris L. (cv. Valentino and Nebraska) plants.

6 Declarations

6.1 Competing Interests

The authors declared that there is no conflict of interest.

6.2 Publisher's Note

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