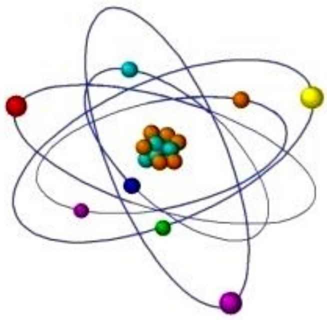
EFEECTS OF RADIATION ON THE OPTICAL PROPERTIES OF SPIRULINA PLATENSIS AFTER REPLACING POTASSIUM IONS WITH CESIUM IONS IN NUTRITION MEDIUM



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ABSTRACT: *The optical properties of Spirulina platensis were studied under varying ratios of cesium and potassium ions, followed by gamma radiation exposure. The study highlights the critical role of potassium ions in the post-irradiation recovery process of irradiated cells, regardless of the presence of cesium ions. Analysis of the obtained spectra revealed a high level of stability in the optical properties of Spirulina platensis under ionizing radiation, when not observed difference in terms of the constituents of spirulina. These results indicate the low chemical toxicity of cesium ions. Additionally, the paper explores a possible mechanism for the inhibition of the post-irradiation recovery process, suggesting that it may be due to the replacement of potassium ions by cesium ions—an essential factor for the growth and development of Spirulina platensis cell cultures.*

Key words: *Spirulina platensis*, Cesium ions, Gamma irradiation

INTRODUCTION

Cyanobacteria, or blue-green algae, are among the oldest photosynthetic prokaryotes. Their broad range of physiological adaptations allows them to be utilized in various remediation methods for cleaning ecosystems affected by technogenic pollution, including radionuclides. Radioactive pollutants released during any accident or incident at a nuclear power facility include cesium-137. Radiocaesium is the most problematic element among radionuclides due to its high specific radioactivity, long half-life, and ability to accumulate in living organisms [1]. It is well known that algae exhibit high sensitivity to heavy metals. Heavy metals can interfere with photosynthesis and enzymatic metabolism in algae, leading to growth inhibition and further irreversible adverse effects. Therefore, algae can serve as a test model for assessing freshwater quality and pollutant toxicity.

Blue-green microalgae are rich in nutritional and bioactive compounds that promote various biological functions in the human body. One of the most practically significant properties of algae, particularly spirulina, is their potential efficiency in adsorption, which can be used as a cost-effective, effective, and non-toxic means for removing heavy metals from the body

[2]. The discovery of new technologies has drawn attention to algae-based adsorbents, which can remove radionuclides from the body. Spirulina has long been associated with detoxification, particularly the detoxification of heavy metals. It is highly effective in binding and removing toxic metals such as lead, cadmium, chromium, mercury, strontium, and thallium [3]. These properties have been the basis for numerous studies on pollution elimination through adsorption [4,5]. Most remediation methods are based on the interactions of various environmental elements with radionuclides, influencing their migration into living organisms. These interactions also involve cesium and potassium ions [6, 7]. This raises an important question: how significant is the presence of cesium and potassium ions in forming a radiobiological effect, as expressed by changes in the optical properties of *Spirulina platensis* culture?

MATERIALS AND METHODS

The research subject was a culture of blue-green algae, *Spirulina platensis*, cultivated on Zarrouk's medium. This medium is widely recognized as a standard growing medium for this organism [8]. Over time, many modified media have been developed to address various scientific and practical challenges [9,10]. The *Spirulina platensis* strain IPPAS B-256 was cultivated in Zarrouk's modified alkaline water-salt medium [8] under the following conditions: a temperature of 28°C, illumination of approximately 5000 lux, constant stirring, and periodic re-cultivation. Before the experiments, the cells were cultured for 7 days in standard Zarrouk' s medium. Subsequently, 175 mL of modified Zarrouk' s medium was added to the *Spirulina* culture grown in 25 mL of standard Zarrouk' s medium, and the culture was re-cultivated. However, under these conditions, *Spirulina* growth was not observed. To address this, 200 mL of the solution in cesium medium was retained, 200 mL of standard medium was added to it, and the mixture was set up for cultivation. Unlike the earlier setup, where the cesium concentration was 0.3 g/200 mL, the modified medium in this instance contained 0.15 g/200 mL cesium (a reduced composition). Control cultures were grown in Zarrouk's modified medium containing Cs(I) ions at a concentration of 0.15 g/200 mL. *Spirulina platensis* grown in a modified medium containing 0.15 g/200 mL of cesium was removed from cultivation after 2 week and subjected to prolonged gamma irradiation at a dose of 150 kGy. The irradiation was performed using a gamma installation with a ^{137}Cs radioisotope as the radiation source, operating at a dose rate of 1.1 Gy per minute. After irradiation spirulina was re-cultivated in standard Zarrouk's medium. The compositions of both standard Zarrouk's and modified Zarrouk's media are presented in Table 1.

Growth was measured by monitoring changes in absorbance at 560 nm using a Cintra 10e UV-Vis spectrometer. *Spirulina platensis* grows optimally within a pH range of 9÷11. This suspension of spirulina cells at pH 10.9 was used to record absorption spectra from 400 to 800 nm. The concentration of *Spirulina platensis* was determined based on instrumental data. Solutions of metal ions were prepared in deionized water using appropriate inorganic salts. The $CsCl_2$ reagent used was of analytical grade.

Table 1. Compositions of Zarrouk's media

Macronutrient	Standard Zar rouk's media (g/l)	Modified Zar rouk's media (g/l)
NaCl	1.0	1.0
CaCl2	0.04	0.08
NaNO3	2.5	2.5
FeEDTA	0.0131	0.0131
EDTA (Na)	0.08	0.08
K2SO4	1.0	-
MgSO4x7H2O	0.2	0.2
NaHCO3	16.8	16.8
K2HPO4	1	-
CsCl	-	2
A5 micronutrient H_3BO_3, $MnCl_2x4H_2O$, $ZnSO_4x7H_2O$, $CuSO_4x5H_2O$, MoO3.	1ml	1ml
The second solution NH_4VO_3, $K_2Cr_2(SO4)_4x24H_2O$ $NiSO_4x7H_2O$ $Na_2WO_4x2H_2O$, $Co(NO_3)_4x6H_2O$, TiO_2	1ml	1ml

RESULTS AND DISCUSSIONS

The main goal of the experiment was to study the optical properties of *Spirulina platensis* after replacing potassium ions with cesium ions in nutrition medium, followed by irradiation with gamma radiation. Figure 1 illustrates the absorption spectra of *Spirulina platensis* grown in a standard Zarrouk medium, where potassium (K^+) ions were substituted with cesium (Cs^+) ions. Two conditions were tested: in the first case, the Cs^+ concentration was 0.3 g/200 mL (1), and in the second case, the Cs^+ concentration was 0.15 g/200 mL (2). As observed in Figure 1, no growth or development of the culture was recorded at the higher Cs^+ concentration (0.3 g/200 mL). However, at the lower Cs^+ concentration (0.15 g/200 mL), the absorption spectra closely resembled those of the control cells.

The next stage of the study focused on examining the effect of radiation exposure on the culture grown in a modified Zarrouk medium containing Cs^+ ions at a concentration of 0.15

g/200 mL. These cells were exposed to gamma radiation at a total dose of 150 kGy, followed by the transfer of the irradiated culture to a standard Zarrouk medium. The results are presented in Figure 1. It can be observed that the absorption spectrum of *Spirulina platensis* after cultivation in the modified medium closely matches the spectrum of *Spirulina platensis* that was irradiated after cultivation in the modified medium (2) and subsequently re-cultured in the standard medium (3). The absorption spectra shown in the figure did not reveal any differences like the spectral composition; the only variation observed was in the concentration levels.

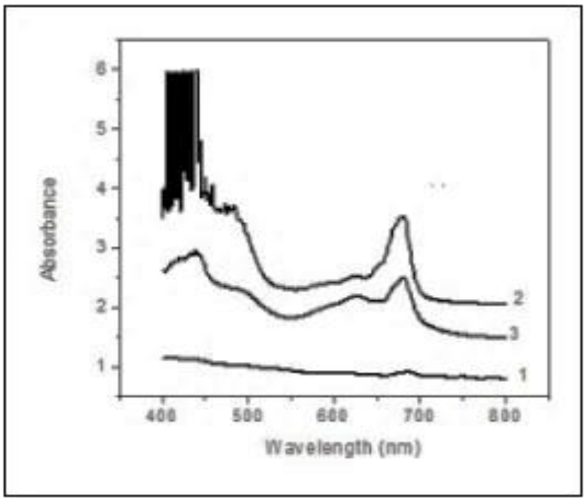

Figure 1. Effect of gamma radiation on the absorption spectra of *Spirulina platensis* at different K^+ and Cs^+ ion ratios

1 – Spirulina in the modified nutrient medium with a high concentration of Cs^+ ions (not irradiated). 2 – Spirulina in the modified nutrient medium with a balanced concentration of K^+ and Cs^+ ions before irradiation. 3 – Spirulina in standard Zarrouk nutrient medium used for post-irradiation cultivation of spirulina cells exposed to a gamma radiation after case 2.

Figure 2 presents the absorption spectrum characteristics of *Spirulina platensis*. The absorption peak at 681 nm corresponds to chlorophyll a (Chl a), while the peak at 621 nm is associated with phycocyanin (PC). Additionally, the peak at 440 nm is attributed to the Soret band of Chl a. The data presented in Figure 2 demonstrate that an increase in the metal concentration in the nutrient solution leads to a decrease in absorption intensity in both control (spirulina as control cultivated is in Zarrouk's modified medium containing Cs(I) ions at a concentration of 0.15 g/200 mL) and irradiated cells. Additionally, the effect of cesium ions on the same cellular components of *Spirulina platensis* was examined at the same gamma irradiation dose. Figure 3 illustrates the effect of cesium ions on the absorption intensity maxima on the constituents of spirulina at wavelengths of 440 nm, 621 nm, and 681 nm. Cases 1 and 2 in Figure 3 indicate that changes in the absorption intensity of *Spirulina platensis* components (chlorophyll a, phycocyanin, and carotenoids) follow a consistent pattern across all components. Specifically, as the cesium concentration increases, the absorption intensity decreases. This reduction occurs uniformly in both control cells and cells that were irradiated and subsequently recultivated.

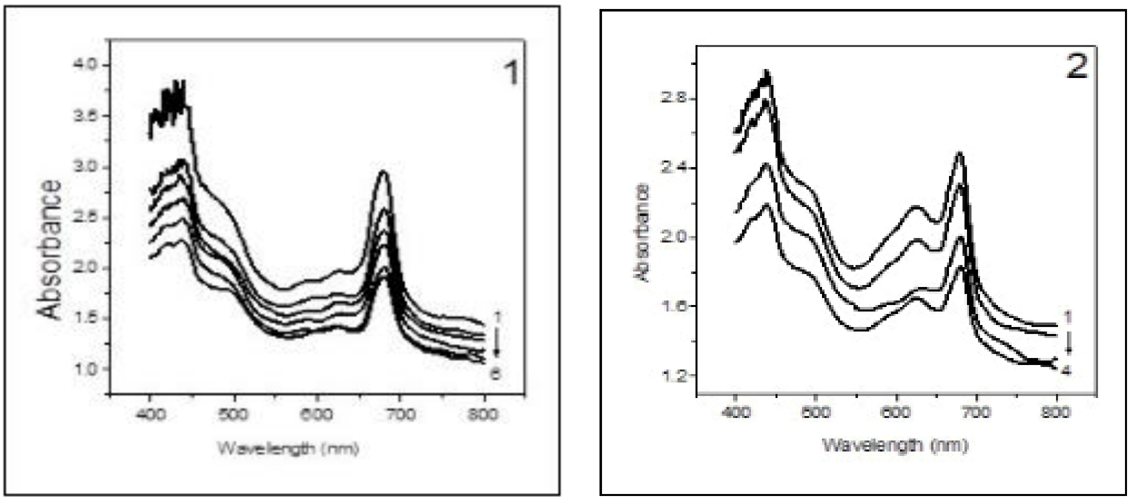

Figure 2. Effect of cesium ions ([Cs^+]1à6= (0; 4 ÷ 8 mM) on the absorption spectra of control (1) and irradiated and re-cultivated *Spirulina platensis* cells (2) [Cs^+]1à4= (0; 4 ÷ 6 mM).

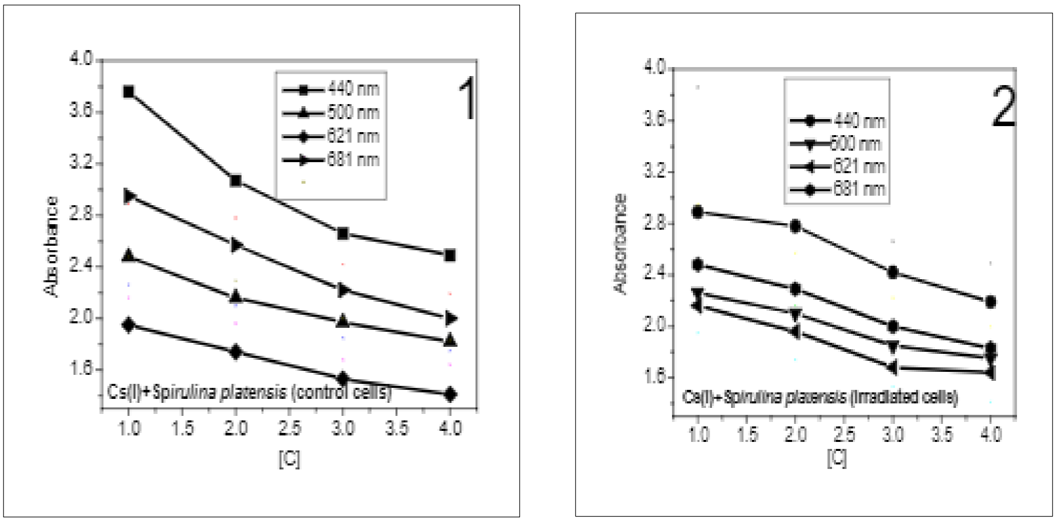

Figure 3. Effect of gamma radiation on changes in the spectral characteristics of *Spirulina platensis* components as control (1), as irradiated cells (2) after re-cultivation. (440 nm – Soret band of chlorophyll a.; 621 nm – Phycocyanin (basic protein); 681 nm – Chlorophyll a; 500 nm – Carotenoids).

By comparing the results in the figures, it is evident that the change between the maximum and minimum absorption values for the control culture at 440 nm is 33%, while for irradiated Spirulina platensis, it is 25%. At 500 nm, the changes are 27% for the control and 22% for the irradiated cells. At 621 nm, the changes are 28% and 24%, respectively, and at 681 nm, the values are 32% and 26%. The largest difference is observed at 440 nm, with an 8% variation. A similar effect has been reported in other publications [11-14]. In work [11], the analysis and identification of irradiated spirulina powder were conducted using three-stage infrared macro-fingerprint spectroscopy. The study demonstrated that the saccharides in the spirulina powder exhibited higher thermal stability than the proteins. However, the autopeaks of the irradiated samples showed distinct differences compared to the non-irradiated sample. The extracted biomasses of four cyanobacteria (*Nostoc carneum, Nostoc insulare, Oscillatoria geminata, and Spirulina laxissima*) grown in axenic mass culture were tested for their ability to adsorb four radionuclides (^{134}Cs, ^{85}Sr, ^{226}Ra, and ^{241}Am) under various pH regimes. Additionally, two of the cyanobacterial biomasses *(N. carneum* and *O. geminata*) were phosphorylated before testing their capacity as radionuclide adsorbers. Non-phosphorylated cyanobacterial biomass showed very low adsorption of ^{134}Cs [9]. The accident at the Fukushima-1 nuclear power plant in March 2011 resulted in the release of exceptionally high levels of radionuclides into the environment, primarily ^{137}Cs, ^{90}Sr, and ^{131}I. Since these radionuclides are biophilic, they pose a significant risk due to their biological uptake and subsequent food chain contamination within the ecosystem. In a study [10], researchers selected microalgae and aquatic plants capable of effectively removing radionuclides from the environment. They demonstrated that these organisms have the highest ability to eliminate radioactive **^{137}Cs** through cellular accumulation. Recent studies have emphasized that adsorption is critical in removing **^{137}Cs** from wastewater for environmental remediation. Over the past decade, significant research has focused on new adsorbents, such as Prussian blue, graphene oxide, hydrogels, and Nano adsorbents, due to their remarkable cesium adsorption capacity [14]. However, microalgae remain one of the primary adsorbents.

Despite the practical importance of cesium uptake by cells, its physiological role has not been sufficiently studied. However, since ^{137}Cs is a close analog of potassium, cells tend to utilize it for similar functions. Nevertheless, cesium differs significantly from potassium. Experimental studies have shown that during passive transport, the permeability of cesium ions through cell membranes is approximately six times lower than that of potassium ions [15]. Additionally, cesium blocks potassium channels. Replacing potassium ions with cesium ions likely leads to inhibition, even when the proportion of cesium is no more than 2% of the total potassium content [16]. This may indicate the physiological distinctiveness of these elements. The arrangement of ions likely results from their specificity in passing through potassium channels. The sizes of the cations in question are such that potassium ions (**K^+**) exhibit the highest permeability. The other ions, due to their larger sizes and different capacities to lose hydration shells, face a slower rate of crossing cell membranes. Cesium ions, in particular, can block potassium channels, preventing the passage of potassium ions through them. These factors may explain the specificity of cesium ion action when exposed to the irradiated culture of *Spirulina platensis*.

Thus, the optical properties of *Spirulina platensis* grown in modified Zarrouk & apos;s medium with Cs^+ ions (0.15 g/00 mL), irradiated with ^{137}Cs gamma radiation (150 kGy), and subsequently re-grown in standard Zarrouk's medium do not show significant differences. Additionally, there are no observed differences in the content of Cs^+ ions in the same cellular components of *Spirulina platensis* between control cells and irradiated cells.

Based on the data obtained, we can conclude the following:

1. Cesium ions are not chemically toxic when affecting the optical properties of *Spirulina platensis*. Moreover, a high level of radioresistance can allow for the absorption of high concentrations of radioactive cesium (^{137}Cs) without significantly impacting the growth

and development of the culture, as well as the intensity of post-radiation recovery at high doses of ionizing radiation. It is possible, that the process of post-radiation recovery in *Spirulina platensis* culture completely halts in the absence of potassium ions in the nutrient medium.

2. The primary mechanism on post-radiation growth and development of irradiated cells is the partial replacement of potassium ions, which are essential for cellular life and in this case was not observed the negative effect of cesium ions.

REFERENCES

[1]. Iwamoto, Koji, and Yoshihiro Shiraiwa, 2017, Accumulation of cesium by aquatic plants and algae. Impact of cesium on plants and the environment, 171-185.

[2]. Yadav Mahendra, Kumar Vivek, Jain Shelly, Sandal, Nidhi Chauhan, Meenakshi, 2022/04/09, Assessment of Adsorption and Removal Efficacy of Spirulina Powder for Strontium and Thallium, Preprint, DOI:10.21203/rs.3.rs-1539864/v1.

[3]. Mahendra Yadav, Komal Rani, Nidhi Sandal, Meenakshi Kanwar Chauhan, 2022, An approach towards safe and sustainable use of the green alga Chlorella for removal of radionuclides and heavy metal ions, Journal of Applied Phycology, 24, 2117-2133.

[4]. Xindai Li, Guangming Xu, Meng Xia, Xinyao Liu, Fuqiang Fan, Junfeng Dou. Research on the remediation of cesium pollution by adsorption: Insights from bibliometric analysis Chemosphere, Volume 308, Part 2, 2022, 136445.

[5]. R. Jalali-Rad, H. Ghafourian, Y. Asef, S.T. Dalir, M.H. Sahafipour, B.M. Gharanjik Biosorption of cesium by native and chemically modified biomass of marine algae: introduce the new biosorbents for biotechnology applications. Journal of Hazardous Materials. Volume 116, Issues 1–2, 10, 2004, Pages 125-134.

[6]. Anna Burger, Irene Lichtscheidl. Stable and radioactive cesium: A review about distribution in the environment, uptake and translocation in plants, plant reactions and plants' potential for bioremediation. Science of The Total Environment. Volume 618, 15, 2018, Pages 1459-1485.

[7]. Shahjalal Khandaker, Megumu Fujibayashi, Takahiro Kuba. Innovative potassium hexacyanoferrate intercalated into layered double hydroxide adsorbent for efficient cesium removal from seawater. Separation and Purification Technology. Vol. 354, Part 5, 2025, 128984.

[8]. C Zarrouk. 1966, Contribution a l'etude d'une Cyanobacterie: Influence de Divers Facteurs Physiques et Chimiques sur la Croissanceet la Photosynthese de *Spirulina maxima* (Setchell et Gardner) Geitler. Ph. D. Thesis. University of Paris, France.

[9]. P Dalgaard and K Koutsoumanis.2001, Comparison of maximum specific growth rates and lag times estimated from absorbance and viable count data by different mathematical models. J. Microbiol. Meth., 43, 183-94.

[10]. PR Pai, A Manasa, T Kalaivani, CPM Ajeesh, C Rajasekaran and BN Prasad, 2008, Simplified Cost-Effective Media Variants for the Rapid Culture of *Spirulina platensis*. Recent Advances in Biotechnology, Excel India Publishers, New Delhi, 2008, 1-129.

[11]. Hai Jing Liu, Chang Hua Xu, Qun Zhou, Feng Wang, Wei Ming Li, Yi Ming Ha, Su Qin Sun, 2013, Analysis and identification of irradiated Spirulina powder by a Three - step infrared macro-fingerprint spectroscopy, Radiation Physics and Chemistry,85, 210-217.

[12]. Pohl, P., Schimmack, W., 2006, Adsorption of Radionuclides (^{134}Cs, ^{85}Sr, ^{226}Ra, ^{241}Am) by Extracted Biomasses of Cyanobacteria (Nostoc Carneum, N. Insulare, Oscillatoria Geminata and Spirulina Laxis-Sima) and Phaeophyceae (Laminaria Digitata and L. Japonica; Waste Products from Alginate Production) at Different pH. J Appl Phycol. 18, 135–143.

[13]. Fukuda, Sy., Iwamoto, K., Atsumi, M. et al.,2014, Global searches for microalgae and aquatic plants that can eliminate radioactive cesium, iodine and strontium from the radio-polluted aquatic environment: a bioremediation strategy. J Plant Res.127,79–89.

[14]. Muhammad Yaqub, Ladawan Mee-Ngern, Wontae Lee, 2024, Cesium adsorption from an aqueous medium for environmental remediation: A comprehensive analysis of adsorbents, sources, factors, models, challenges, and opportunities, Science of The Total Environment, 950, 175368.

[15]. Yurin V.M., Sokolik A.I., Kudryashov A.P. Regulation of Ion Transport Through Plant Cell Membranes. Minsk: Navuka i Tehnika, 1991. 272 pp. (in Russian).

[16]. Yudintseva E.V., Gulyakin I.V. Agrochemistry of Radioactive Isotopes of Strontium and Cesium. Moscow: Atomizdat, 1968. 472 pp. (in Russian).