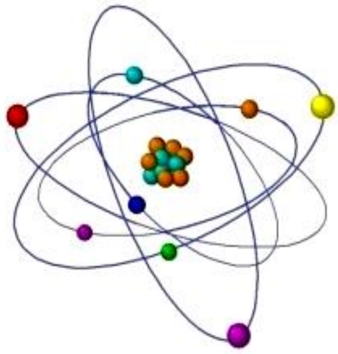
INFLUENCE OF Mn(II) IONS ON FROZEN AND GAMMA IRRADIATED SPIRULINA PLATENSIS



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ABSTRACT: *The influence of Mn(II) ions on frozen Spirulina platensis at -80°C and -20°C and high-dose irradiated ^{137}Cs (400 kGy) was studied using a UV–Visible spectrometer. The absorption process was relatively fast in small concentrations of Mn(II) in the case of interaction with irradiated Spirulina platensis. However, for frozen Spirulina platensis, the absorption decrease was regarded as very slow. It was shown that the effect of Mn(II) ions on Spirulina platensis constituents (chlorophyll a, phycocyanin, carotenoids) under various conditions is different.*

Key words: *Spirulina platensis*, Mn(II) ions, gamma-irradiation

INTRODUCTION

Spirulina platensis (filamentous cyanobacteria) is a blue-green microalga with a worldwide distribution, being easily cultivated and controlled, growing naturally in marshes, reservoirs, waterways, and paddy fields. *Spirulina* has been used for food since time immemorial by tribes living around Lake Chad in Africa. The chemical composition of *Spirulina* includes protein (55-70%), carbohydrates (15-25%), essential fatty acids (18%), vitamins, and minerals. *Spirulina* also contains many photosynthetic pigments, such as chlorophylls, carotene, and phycocyanins. Chlorophyll is the most visible pigment in *Spirulina*. It releases ions when struck by the energy of sunlight. These free ions proceed to stimulate the biochemical reactions that form proteins, vitamins, and sugars in *Spirulina* cultures [1]. Carotenoids are generally responsible for the red and yellow hues seen in nature. Beta-carotene accounts for 80% of the carotenoids present in *Spirulina*, which is convertible into vitamin A [2,3]. Phycobiliproteins are a small group of highly conserved chromoproteins that constitute the phycobilisomes, a macromolecular protein complex. The most common classes of phycobiliproteins are C-phycocyanin (CPC), allophycocyanin (APC), and phycoerythrin (PE), which comprise about 20% of the cellular protein. Phycocyanins are an important source of blue pigment for use in food coloring. The photosynthetic energy transfer in the phycobiliproteins (PBPs) from phycocyanin (PC) to chlorophyll a can be influenced by different types of heavy metal ions, such as Hg [4] and Cr [5]. In cyanobacteria, metals exert toxic action mostly by damaging the chloroplast and disturbing photosynthesis [6]. In previous works [7-9], we studied the biosorption and accumulation of different metal ions in this alga and its main protein phycocyanin in vitro and in vivo under various conditions. In this work, using UV-visible spectrometry, we studied the effect of Mn(II) ions on *Spirulina platensis* frozen at -80°C and -20°C and irradiated with high doses (400 kGy) of ^{137}Cs, and then recultivated.

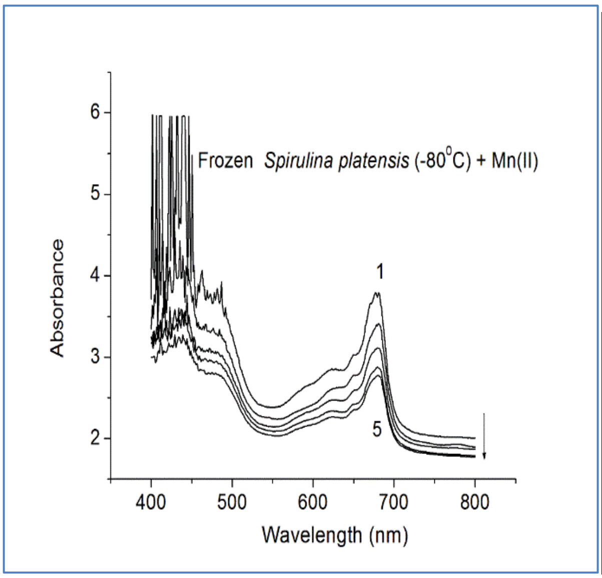
MATERIALS AND METHODS

Spirulina platensis IPPAS B-256 strain was cultivated in a standard Zarrouk [10] alkaline water-salt medium at 34°C, illumination ~5000 lux, at constant mixing in batch cultures [11]. The cultivation of the *Spirulina platensis* intact cells was conducted for 14 days. The growth was measured by optical density by monitoring changes in absorption at a wavelength of 560 nm using the UV-Visible spectrometer Cintra 10e. *Spirulina* grows optimally in a pH range of 9-11. The intact cell suspension of *Spirulina platensis* at pH 10.1 in the nutrient medium was used for scanning the absorption spectra from 400 to 800 nm. The concentration of *Spirulina platensis* was determined by the instrumental data. *Spirulina platensis* suspension (100 ml) was irradiated with 400 kGy gamma radiation from April to January over 10 months using ^{137}Cs as a gamma radiation source at the Applied Research Center, E. Andronikashvili Institute of Physics (Dose rate -1.1 Gy per minute). After 14 days of cultivation, *Spirulina platensis* was frozen by stepwise freezing at 23°C, 4°C, and then separately at -20°C in one case and -80°C in another case for 48 hours. In the first case, the suspension (100 ml) after irradiation (400 kGy) and freezing (after melting at room temperature) was filled up with Zarrouk medium and then recultivated. The solutions of metal ions were prepared in deionized water with appropriate amounts of inorganic salt. The reagents MnCl2·4H2O were of analytical grade.

RESULTS AND DISCUSSIONS

The influence of Mn(II) ions on *Spirulina platensis* frozen at -80°C and -20°C and high-dose irradiated C^{137s}, and then recultivated, was studied as a function of metal concentration. **Figure 1.** shows the absorption characteristics after irradiation, freezing, and recultivation of *Spirulina platensis* cells. The peak at 681 nm is due to the absorption of chlorophyll a (Chl a). The peak at 621 nm is due to the absorption of phycocyanin (PC). The peak at 500 nm is due to the absorption of carotenoids. A peak at 440 nm is due to the Soret band of Chl a [12]. In **Figure 1.** (A, B, C) the effect of Mn(II) ions on the absorption of irradiated (400 kGy), frozen at -80°C and -20°C, thawed, and recultivated *Spirulina platensis* cells is shown. It is seen from **Figure 1.** that with increasing metal concentrations, absorption intensity decreased for all cases. As can be seen from **Figure 1.** the absorption process was relatively fast at small concentrations of Mn(II) in the case of interaction with irradiated *Spirulina platensis*, but for frozen *Spirulina platensis* the decrease is regarded as very slow. The influence of Mn(II) ions after irradiation (400 kGy) and freezing at -20°C and -80°C, and then recultivation of *Spirulina platensis* constituents (chlorophyll a (Chl a), phycocyanin (PC), carotenoids) are shown in **Figures. 2-5**. It is clear that the effect of Mn(II) ions on the absorption intensity maxima for wavelengths 440 nm, 500 nm, 621 nm, and 681 nm of *Spirulina platensis* constituents is different. In particular, at 440 nm (changes to the Soret band of chlorophyll a), with increasing Mn(II) concentration, the intensity of absorption decreases at a high dose of radiation, decreases less after freezing at

-20°C, but almost does not change at -80°C. Analogous results were received for carotenoids at 500 nm wavelengths. For the change in absorption intensity at 621 nm, which is the peak of absorption of the major protein phycocyanin in *Spirulina platensis*, the absorption intensity very efficiently decreases at a high dose of radiation but almost does not change after freezing at -20°C and -80°C. The influence of Mn(II) ions after irradiation and freezing, and then recultivation of *Spirulina platensis* at 681 nm wavelengths (changes in chlorophyll a) is similar. In the review [13], the stability of phycocyanin (pH, temperature, and light) is discussed, considering the physicochemical parameters of kinetic modeling. The optimal working pH range for phycocyanin is between 5.5 and 6.0, and it remains stable up to 45°C. However, exposure to relatively high temperatures or acidic pH decreases its half-life and increases the



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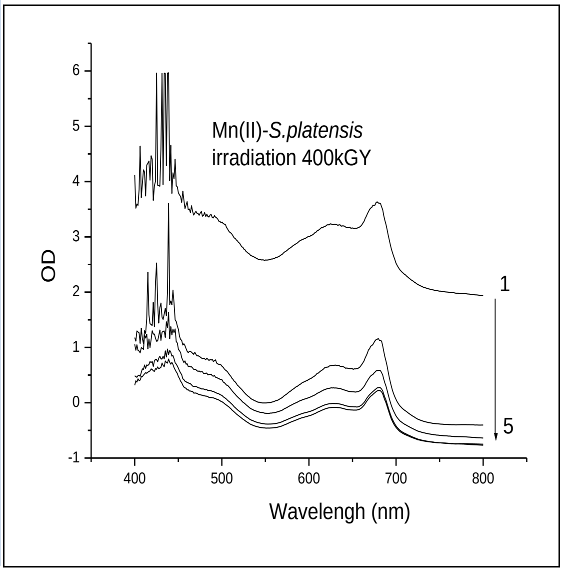

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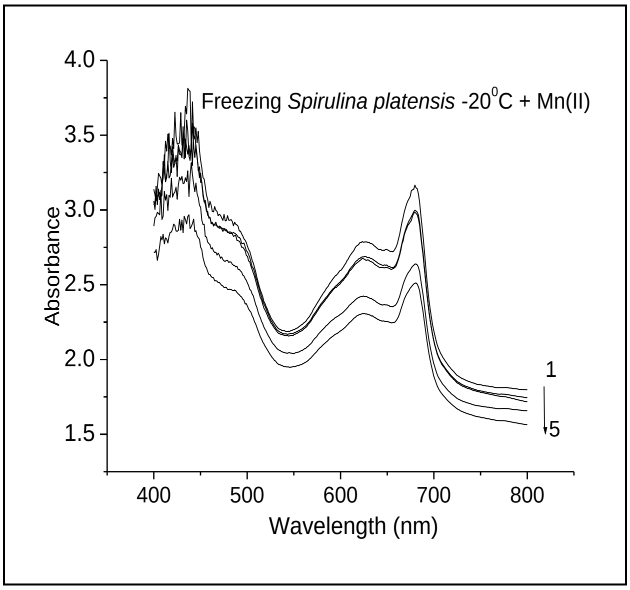
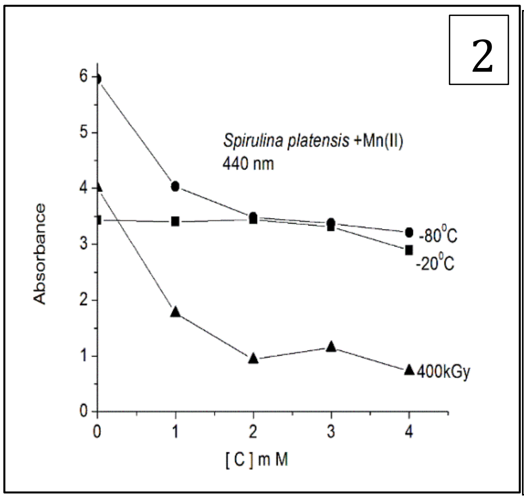


C

Fig.1. (A,B,C) Absorption spectra of Spirulina platensis after frozen at -800C, at -200C, at irradiated and re-cultivation-(1) + Mn(II) ions (2-5).



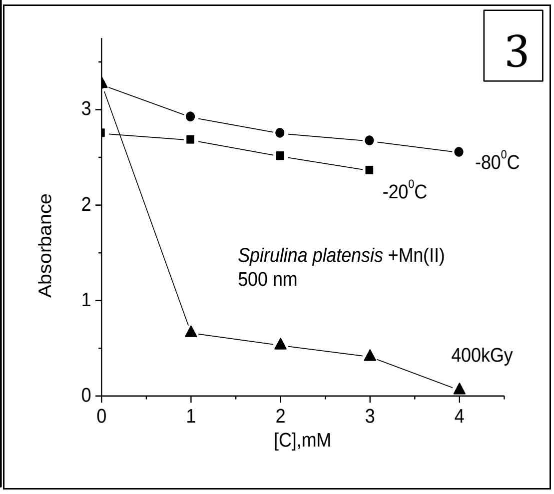
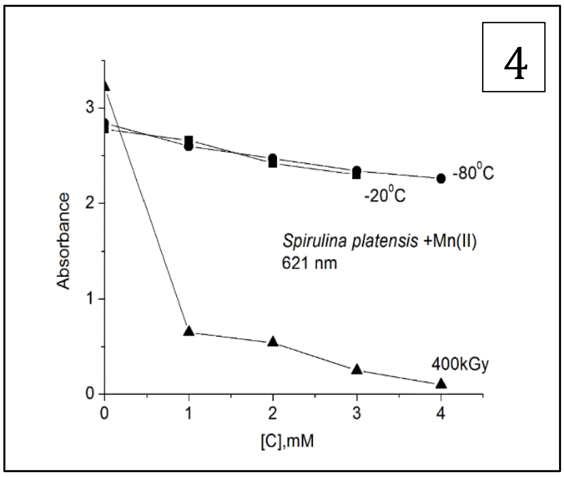


Figure 2. Influence of Mn(II) ions after irradiation (400kGy) and after frozen -20^{0}C and -80^{0}C on temperature and then recultivation of *Spirulina platensis* (changes to the constituent- soret band into the chlorophyll a)

Figure 3. Influence of Mn(II) ions after irradiation (400kGy) and after frozen -20^{0}C and -80^{0}C on temperature and irradiated and then recultivation of *Spirulina platensis* (changes in the carotenoids)



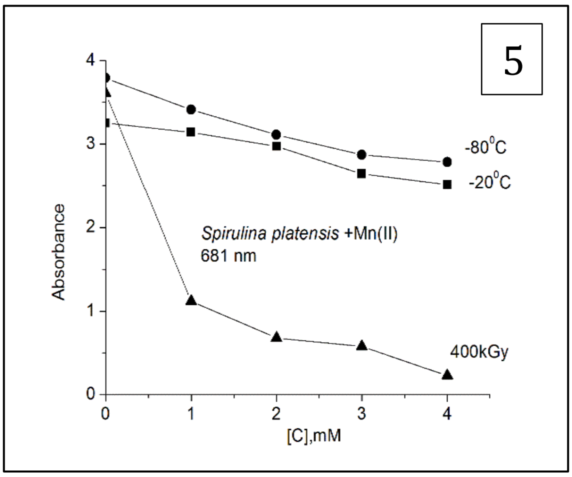


Figure 4. Influence of Mn(II) ions after irradiation (400kGy) and after frozen -20^{0}C and -80^{0}C on temperature and irradiated and then recultivation of *Spirulina platensis* (changes in the basic protein -the phycocyanin)

Figure 5. Influence of Mn(II) ions after irradiation (400kGy) and after frozen -20^{0}C and -80^{0}C on temperature and irradiated and then recultivation of *Spirulina platensis* (changes in the chlorophyll a).

Maximum biosorption capacity of *A. platensis*, determined from the Langmuir equation, was 44.3 mg/g for Mn(II) ions [14]. The raw and enriched microalgal biomass was examined by ICP-OES to determine its multielemental analysis before and after biosorption, FTIR to indicate functional groups that participated in biosorption, and SEM-EDX to illustrate the binding of metal ions on the surface of algal biomass. FTIR spectroscopy evidenced that biosorption of metal ions was mainly due to carboxylate groups present on the microalgal cell wall. *Arthrospira platensis* turned out to be a good biosorbent of metal ions [14].

Using optical and DSC studies, it was shown that *Spirulina platensis* irradiated with 137Cs γ 400 kGy and then stored under anaerobic conditions in the dark at 4°C remained viable despite 96.9% denaturation of its whole biomass. The DSC data showed that PBSc in the recultivated samples, which were preliminarily irradiated with 10-400 kGy doses, melted cooperatively [15]. Combined effects of ^{137}Cs gamma irradiation and heavy metal ions Ni(II), Zn(II), and Ag(I) on *Spirulina platensis* cells using UV-VIS spectrometry after three times irradiation and recultivation were discussed [16].

It was shown that the possible use of gamma irradiation together with Ni(II) and Zn(II) ions does not change the nature of the interaction of these metal ions with *Spirulina platensis*. Whereas in contrast to the ions Ni(II) and Zn(II), for silver ions, an increase in intensity is observed in both the irradiated and non-irradiated states. The combined effects of ionizing radiation and other stressors such as silver ions on *Spirulina platensis* exhibit synergistic effects for C-phycocyanin as a stimulatory agent to raise its content [16]. FTIR spectroscopy evidenced that biosorption of metal ions was mainly due to carboxylate groups present on the microalgal cell wall. SEM analysis clearly showed that biosorption occurred. While the constituents are distinct among different algal strains, it is necessary to screen the algae with high adsorption capacities for heavy metal ions by analyzing the algal components [17]. The results of field-emission scanning electron microscopy, Fourier-transform infrared, and X-ray photoelectron spectroscopy analyses of the N-containing functional groups indicated that *Spirulina platensis* was successfully immobilized on the alginate matrix [17,18].

Chronic kidney disease is a significant health problem. In work [19], the protective mechanism of *Spirulina platensis* against γ-irradiation (R) and/or thioacetamide (TAA)-induced nephrotoxicity in rats was investigated. Rats intoxicated with R or TAA showed alterations in kidney function markers, antioxidant enzymes, and several inflammatory markers. Rats also acquired apoptosis, evidenced by high caspase-3 activity [19]. The high subzero preservation of *Spirulina*: *Spirulina platensis* CMU2 and *Spirulina platensis* GD1 was performed by stepwise freezing at 25°C, 4°C, and -20°C for 30 min. Then they were preserved at -80°C for seven months with four cryoprotectants: dimethyl sulfoxide, horse serum, calf serum, and glycerol. The viability of these two strains and cell concentration was not significantly different. The survival of *Spirulina platensis* depended on the type and concentration of cryoprotectant and thawing temperature [20]. This apparent protective effect of *SP* is mediated by the regulation of miR-1 and miR-146a gene expression, preventing the release of ROS, inflammation, apoptosis, and autophagy via the AMPK/mTOR pathway. Subsequently, administration of *SP* could be a convenient food supplement for protection against R and/or TAA-induced nephrotoxicity.

Thus, as of today, the investigation of irradiated and frozen *Spirulina platensis* and the effect of metal ions on it is very relevant, both for medical purposes and as promising biosorbents for the removal of toxic metals from industrial effluents due to the presence of various free functional groups.

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