INFLUENCE OF METAL IONS AFTER ONE YEAR REPEATED IRRADIATION OF SPIRULINA PLATENSIS

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ABSTRACT: *Combined effects of Cs^{137}gamma irradiation and heavy metal ions Ni(II), Zn(II), Cu(II), Mg(II), Mn(II), Cr(III), Cr(VI), Co(II),Pb(II),Cd(II) on Spirulina platensis cells using UV-VIS spectrometryafter one year again repeated cultivation, irradiation and recultivation were discussed.*

It was shown, that possible use of gamma irradiation together with Ni(II),Cr(III), Cu(II),Pb(II), Cd(II),Co(II) and Zn(II) ions does not change nature of interaction of these metal ions on Spirulinaplatensis.In the case combined effects of ionizing radiation and Mg(II) ions for Spirulina platensis exhibit synergetic effects for all components of Spirulina platensis as a stimulatory agent to raise the contents of it. This trend is seen to continue for all constituents of Spirulina platensis when these metal ions were added in vitro after one year irradiation and recultivation. Chromium (VI) does not contribute to a decrease in absorption intensity unlike Cr(III) and Cu(II). Influence of these metal ions on the constituent (Chl a, Phycocyanin, soret band of Chl a) of cyanobacterium Spirulina platensis differs from its effectiveness.

Key words: Spirulina platensis, metal ions, gamma irradiation

INTRODUCTION

All biological material has affinity for metals and radionuclides, some biomolecules function specifically to bind metals. The bioaccumulative properties of marine organisms towards radionuclides may be very useful for potential application in monitoring and assessment procedures of the marine environment as such and especially in monitoring nuclear facility waste sites. Radionuclides can be used as radiotracers in studies of heavy metal and organic pollutant behaviour in marine flora [1,2]. Because heavy metals can have different influences on marine algae, it is important to recognize bioaccumulation as a means of assessing the potential risk arising from the presence of heavy metals in the environment. Once in the environment, metals may undergo transformation, either into various mobile forms in an environmental. Biological activity accounts for a large number of the environmental for heavy and toxic metals whether derived from natural or anthropogenic sources. Metal ions can be accumulated by microorganisms by non-specific physico-chemical interactionsas well as specific mechanisms of sequestration or transport [3].

Spirulina is one of multicellular unbranched non-heterocystous filamentous microalgae which are recognizable by the unique open left-handed helix along the entire length of the filament. Compared to other foods, Spirulina shows extraordinary nutritional value as it provides high levels of many essential nutrients and minerals. Spirulina is the most highly consumed microalga [4], which has been widely used as human healthy food centuries ago and it is currently produced commercially as healthy food and valued additives in a hugely profitable business. In our works [5,6] influence of 7.2 kGy Cs^{137} gamma irradiation have been studied with optical and differential scanning microcalorimetry (DSC) methods for cyanobacterium Spirulina platensis intact cells in the suspension, wet mass, and dry mass samples and also simultaneous effects of Cd(II), Pb(II) ions and γ-irradiation on stability of Spirulinaplatensisintact cells after 7.2 kGy Cs^{137} gamma irradiation and without irradiation.

In this paper, discussed combined effects of Cs^{137} gamma irradiation and heavy metal ions same concentrations on Spirulina platensis cells using UV-VIS spectrometry ,when after one year the same Spirulina platensis (which was irradiated with 7.2 kGy one year ahead) again irradiated and recultivated 2-times with 10kGy gamma irradiation.

MATERIALS AND METHODS

Spirulina platensis IPPAS B–256 strain was cultivated in a standard Zarrouk [7] alkaline water–salt medium at 34ºC, illumination ~5000lux, at constant mixing in batch cultures [8]. The cultivation of the Spirulinaplatensiscells was conducted for 14 days. The growth was measured by optical density by monitoring of changes in absorption at wave length 560nm using the UV–Visible spectrometer Cintra 10e. The intact cells suspension of Spirulina platensisat pH 9.1 in the nutrition medium was used for scanning the absorption spectra from 400 to 800nm.

The concentration of Spirulinaplatensis was determined by the instrumental data [9,10]. In this work Spirulina platensiswhich was irradiated with 7.2 kGy, then recultivated and re-irradiated with the same dose as studied by us in the papers [5,6]. After one year this irradiated and cultivated Spirulina platensis stood up for re-cultivation. 100 ml of this was added to 100 ml a standard Zarrouk medium. After 2 weeks of incubation the reseived mass was irradiated again.Spirulinaplatensissuspension after 14 days of cultivation have been irradiated with 10kGy gamma radiation for 14 days using Cs^{137} as a gamma radiation source at the Applied Research Center, E. Andronikashvili Institute of Physics.

Dose rate - 0.017Gy/sec. In this case after 2-times repeited irradiation and recultivation were studied effect of metal ions on Spirulina platensis. The dry weight and the concentration of different compounds were estimated at late exponential phase.

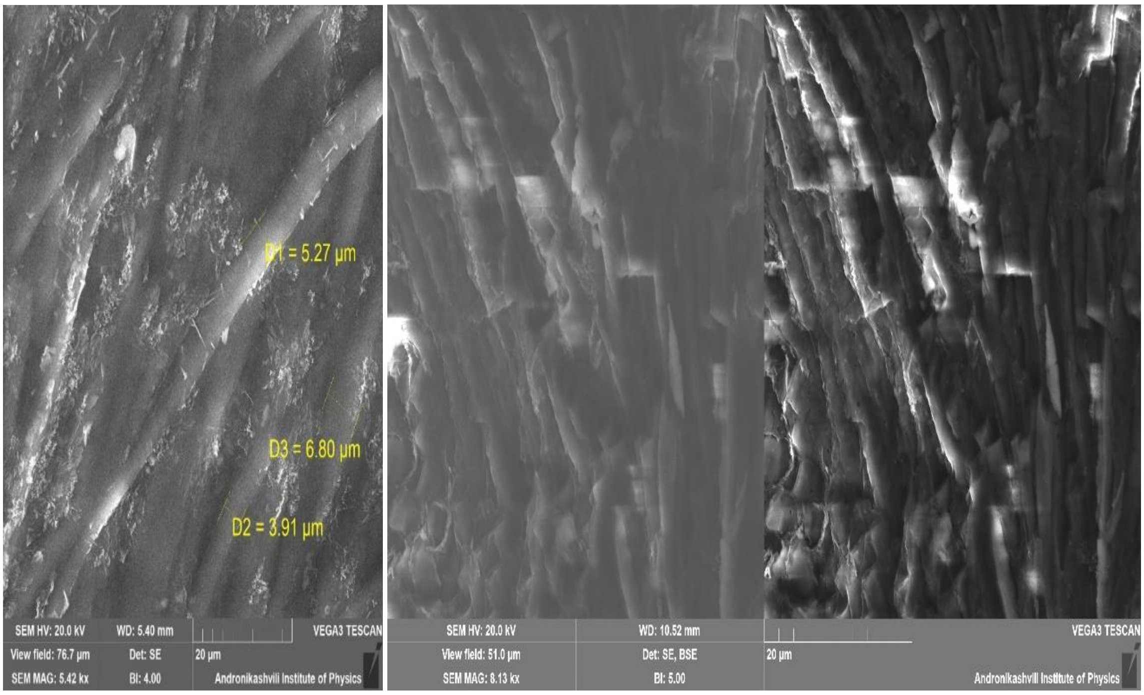
In addition, Spirulina platensis image was taken under an electron microscope after 1 year the first incubation and 10 kGy after irradiation and recultivation. Reagents: $CuCl_2 \cdot 6H_2O$, $Pb(NO_3)_2$, $CdS0_4$, $MgCl_2 \cdot 6H_2O$, K_2CrO_4, $CrCl_3$,$MnCl_2 \cdot 4H_20$, ZnSO4, $CoCl_2$, $NiCl_2$, $AgNO_3$ were of analytical grade.

RESULTS AND DISCUSSIONS

In fig.1 are presented electron microscope images of intact cells of Spirulina platensis and cells after irradiation. It is clear, that in the case after radiation the structure of cells of Spirulina platensis is ,,broken”.

Fig.2 shows the absorption spectra of after two times irradiation (every case irradiation dose 10 kGy) and recultivation of Spirulina platensis. The peak at 681 nm is due to the absorption of Chl a peak. At 621 nm is due to the absorption of phycocyanin (PC). A peak at 440 nm is due to soret band of Chl a [11].

Absorbance peaks of all components (Chl a peak, PC, soret band of Chl a) of Spirulina platensis are observed in the same wavelengths, as in the case intact cells of Spirulina platensis. In fig.3 also are shown effect of Cu(II), Mg(II) ions same concentrations on the absorption of the intact cells after irradiation and recultivation of Spirulina platensis. For illustration only some absorption spectra of metal ions are shown on Fig.3 (as an example Mg(II) and Cu(II))), for disregard pictures rebooting .It is seen from figures, that with increasing metal concentrations absorption intensity decreased for Cu(II) metal ions and increased for Mg(II) ions.



1 **2** **3**

Fig.1. Images of intact cells of Spirulina platensis (1) and cells after irradiation (2,3) using electron microscope

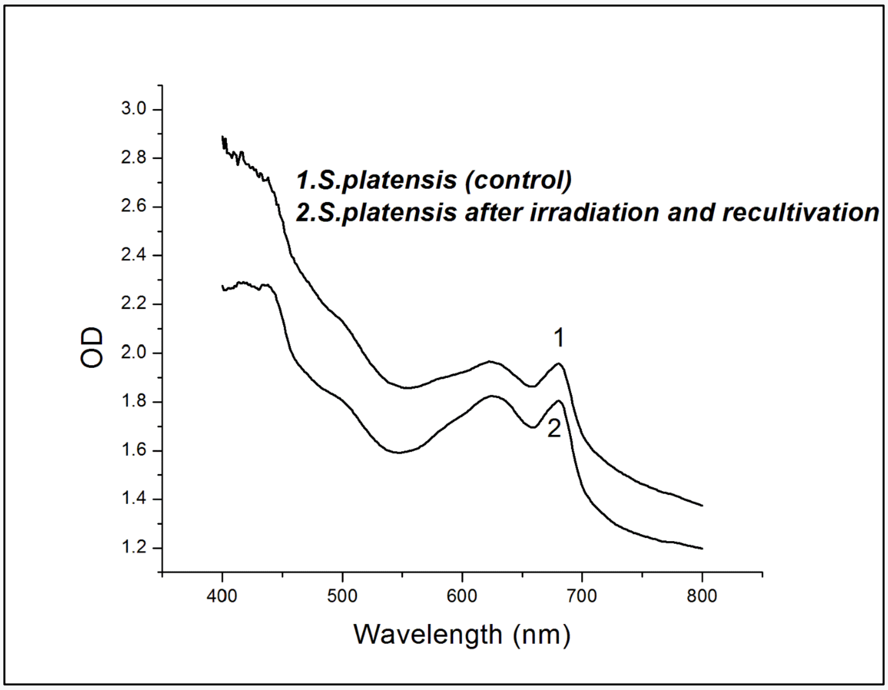


Fig.2. The absorption spectra of the cells of Spirulina platensis recorded after cultivation

1 – Control after incubation 7 days

2 – Suspension after 2- times with 10 kGy gamma-irradiation and after incubation for 14 days

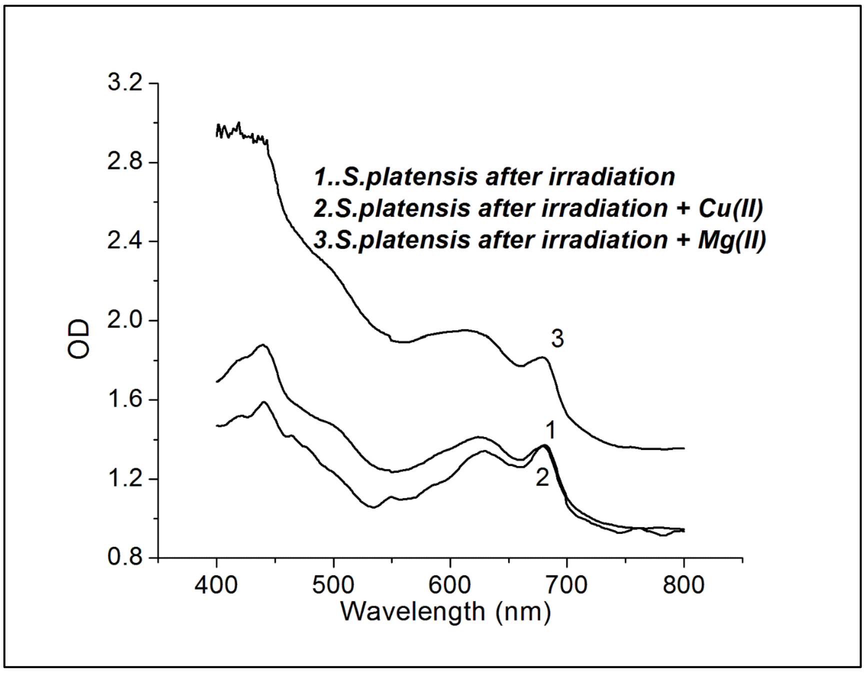


Fig.3. Effect of Mg(II) and Cu(II) ions on the absorption of irradiated suspension of Spirulina platensisafter 2- times irradiation and recultivation

By us influence of the same metal ions on the same cellular components of Spirulina platensis the same irradiation dose was also investigated. Effect of metal ions on the absorption intensity maximums for wavelengths 440nm, 621 nm and 681nm are shown in fig.4. It is evident, that effect of metal ions to components of Spirulina platensis (Chl a, Phycocyanin, soret band of Chl a) strongly depend upon the metal and influence is arranged in the descending order as follows:

1.For soret band of Chl a →440 nm : Co(II) > Ni(II) > Zn(II)>Pb(II) > Cu(II) > Cr(III)>Cd(II) >Mn(II)
2. For Phycocyanin→621 nm : Co (II)> Zn(II)>Cr(III) >Ni(II) > Cd(II) > Cu(II) > Pb(II) >Mn(II).
3. For Chl a →681 nm: Ni(II)> Cd(II)> Cr(III) > Zn(II)> Cu(II)>Co(II)>Pb(II)>Mn(II).

It is clear, that the efficiency for different metal ions is arranged in not the same sequence for soret band of Chl a , for phycocyanin and for Chl a. In particular, in the case of soret band of Chl a more effective isfor Co(II) and not effective Mn(II). For phycocyanin Co(II) and Mn(II), for Chl a Ni(II) and Mn(II) respectively. Though the efficiency Co(II) and Mn(II) ions in the case soret band of Chl a and phycocyanin are in good agreement , but efficiency for other metal ions are difference. This study show that possible use of gamma irradiation together with Co(II) and Zn(II) ions does not change nature of interaction of these metal ions on Spirulina platensis after irradiation, because without irradiation effect was the same[13]. In the case combined effects of ionizing radiation and other stressor such is Mg(II) ions for Spirulina platensis exhibit synergetic effects for all components of Spirulina platensis as a stimulatory agent to raise the contents of it. It is known that the standard Zarrouk medium contains Mg(II) ions since they contribute to the growth of Spirulina. This trend is seen to continue for all constituents of Spirulina platensis when these metal ions were added in vitro after irradiation and recultivation.

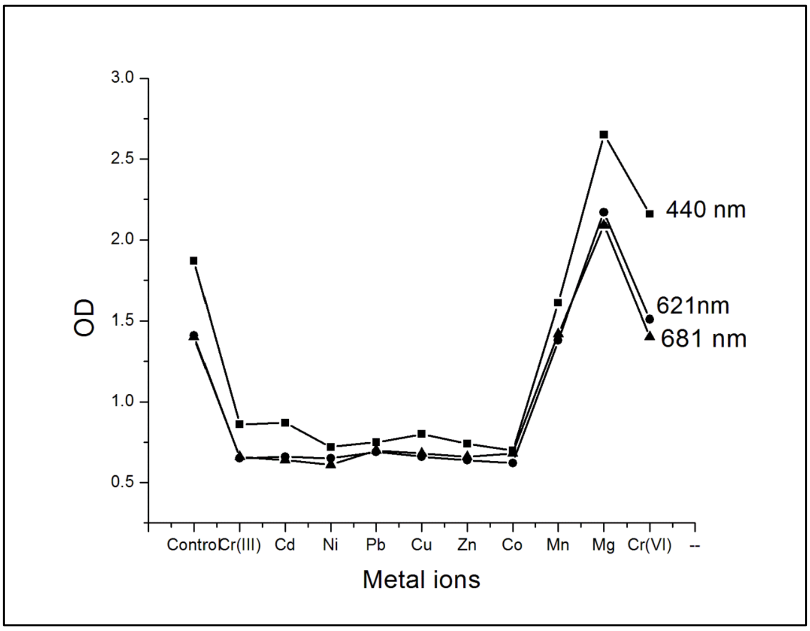


Fig.4 Changes in the absorption of components (soret band of Chl a→ 440 nm, C-phycocyanin→621 nm, Chl a →681 nm) of Spirulina platensis under various metal ions after 2-times irradiation

The absorption intensity of intact cells of Spirulina platensisdecreases, when Cr (III), Cr (VI), Cu (II) ions are added [14]. Significant difference between the absorption intensity for Cu (II) – Spirulina platensisand Cr (VI), Cr (III) Spirulina platensis were observed. Cu (II) was more effectively adsorbed by cyanobacterium than Cr (VI) and Cr (III) [14]. As shown in Figure 4, those ratios are destroyed after irradiation in this case, chromium (VI) leads in a completely different way, namely chromium (VI) does not contribute to a decrease in intensity unlike Cr (III) and Cu (II). Due to the repulsive electrostatic interactions, Cr (VI) anion species are generally poorly adsorbed by the negatively charged soil particles and can move freely in the aqueous environments. In contrast, Cr (III) species normally carry positive electric charges and therefore can be easily adsorbed on the negatively charged soil particles [15]. Apparently this difference is more noticeable after irradiation.

REFERENCES

1. Kleinschmidt R., 2009, Uptake and depuration of ^{131}I by the macroalgae Catenellanipae – Potential use as an environmental monitor for radiophar- maceutical waste, Mar. Pollut. Bull., 58 (10), 1539–1543,doi:10.1016/ j.marpolbul.2009.05.011.

2. Strezov A., Nonova T., 2009, Influence of macroalgal diversity on accumulation of radionuclides and heavy metals in Bulgarian Black Sea ecosystems, J. Environ. Radioactiv., 100 (2), 144–150, doi:10.1016/j.jenvrad.2008.09.007.

3. Misra, T. P., 1992, Heavy metals, bacterial resistance. In: Encyclopedia of Microbiology,Academic Press, San Diego, CA, 2, 549-560.

4. Bleakley S, Hayes M., 2017, Algal proteins: Extraction, application, and challenges concerning production. Foods 6, 33,doi.org/10.3390/foods6050033

5. Monaselidze J., Gelagutashvili E., Bagdavadze N., Gongadze A., Gogebashvili M., Ivanishvili N., Gorgoshidze M., 2019, J.Pharm. Appl.Chem. 5((2), 75-80, doi: 10.18576/jpac/050205

6. Monaselidze J., Gelagutashvili E., Bagdavadze N., GorgoshidzeM. and LomidzeE., 2019, Europ.Chem.Bull., 8(2), 39-43. doi: 10.17628/ecb.2019.8.38-43
7. Zarrouk C., 1966, Contribution to the cyanophyceae study: influence various physical and chemical factors on growth and photosynthesis of Spirulina maxima(Setch et Gardner) Geitler extract. Doctorate Thesis, Faculty of Sciences. University of Paris,France.p.146.
8. Mosulishvili L., Belokobilsky A., Gelagutashvili E., Rcheulishvili A., Tsibakhashvili N., 1997, Proceedings of the Georgian Acad. of Sciences, Biol. series, 23(1-6), 105-113.
9. Bennet A., Bogorad L., 1973, J. Cell Biol., 58, 419,doi.org/10.1083/jcb.58.2.419.
10. Patel, A., Mishra, S. M., Pawar, R., Ghosh, P., 2005, Protein Express. Purif.,40, 248. doi.org/10.1016/j.pep.2004.10.028
11. Lau, R.H., M.M.MacKenzie and W.F. Doolitle., 1979, J. Bacteriol. 132,771-778.
12.GelagutashviliE., BagdavadzeN., 2018,Influence of metal ions (Ag(I), Co(II), Ni(II), Zn(II), Mn(II)) on the constituent of the intact cells ofSpirulinaplatensis,Nanostudies, 17/18,57-62.
13.GelagutashviliE., BagdavadzeN., RcheulishviliA., 2017,Biosorption of Cr(III), Cr(VI), Cu(II) ions by intact cells of Spirulina platensis,E-printarXiv: 1922850Physics ; Biological Physics.Cornell University.
14. Silva B., Figueiredo H., Quintelas C., NevesI. C. and Tavares T., 2008,Zeolites as supports for the biorecovery of hexavalent and trivalent chromium, Microporous and Mesoporous Materials, 116,555-560. doi:10.1016/j.micromeso.2008.05.015