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ORIGINAL ARTICLE

Impact Of Nuclear Radiation On Plants Photosynthesis And Chlorophyll Content After Bombing With U^{328} Enriched Bombs

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Branislav Radomir Jovanić, Božidar Radenković, Marijana Despotović-Zrakić, Zorica Bogdanović and Bratimir Panić: Impact Of Nuclear Radiation On Plants Photosynthesis And Chlorophyll Content After Bombing With U^{328} Enriched Bombs

ABSTRACT

Effect of nuclear radiation during eleven years after bombing on six plants species physiological activities was observed. It was shown that significant change in photosynthetic activities, photosynthesis pigments and abilities the plant to converse absorbed solar energy in to photosynthetic product was change. Also it was shown that chlorophyll contents was significant change in to several plants.

Key words: ^{238}U uranium, gamma radiation, plants photosynthesis, photosynthesis pigments contents, physiological activities.

Introduction

This paper is an extension of researching the effect of induced radioactivity on photosynthesis in plants (Jovanić *et al*., 2003). The research results show that photosynthesis in plants is affected by components of induced radioactivity. After the NATO bombing of Serbia, an increase of soil radiation has been detected. From the scientific point of view, it is interesting to find out how this increased soil radioactivity caused by bombing with U^{328} enriched uranium bombs has influenced photosynthesis in plants over a long period of time. This paper presents the results of research conducted for 11 years. We believe that in this period of time it is possible to determine if there are any permanent effects of increased soil radioactivity. In the authors' opinion, this research is of high importance for preservation of environment where humans, animals and plants live.

Material

The leaves from six different plant species were research: Ribwort Plantain (Herba Plantaginis lanceolatae); Stinging nettle or bull nettle (Urtica dioica L. – Urticaceae); Small-leaved Lime (Tilia cordata); Strawberry (Fragariae herba); Maple (Acer palmatum); Common Polypody (Polypodium vulgare). All plants species were grown in the Belgrade area before, during and after NATO bombing.

Methods

It was shown that chlorophyll fluorescence can be use as power tool for monitoring change in photosynthesis and some physiological parameter (Jovanić *et al*., 2003; Ndao *et al*., 2005; Buah-Bassuah *et al*., 2008). The fluorescence ratios blue/red (F440/F690) and blue/far-red (F440/F740) proved to be very sensitive early stress and strain indicators of plants. Also the fluorescence ratio ref/far red F690/F740 is an indicator of the in situ Chl content (Stober *et al*., 1993; Buschmann *et al*., 1998). Taking account mentioned above all research of the effect of γ–radiations of the soil after NATO bombing the plant photosynthesis and relative change of photosynthesis pigments (Chlorophyll a,b) and carotenoides) was based on fluorescence measurement.

In order to simplify consideration we considered relative change of the pigments content (Jovanić *et al*., 2002; Jovanić *et al*., 2003; Jovanić *et al*., 2004). Subscript Chll and Car denote Chlorophyll(a,b) and carotenides pigments, respectively. So, one have:

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$$\Delta Chl(a,b) = 100 \times [FIR_{Chl}^{Rad}] \times [FIR_{Chl}^{Cont}]^{-1} \qquad Eq.(1)$$

and

$$\Delta Car = 100 \times [FIR_{Car}^{Rad}] \times [FIR_{Car}^{Cont}]^{-1} \qquad Eq.(2)$$

in agreement with literature data in Eq.(1) FIR_{Chl}^{Rad} , FIR_{Chl}^{Cont} , FIR_{Car}^{Rad} and FIR_{Car}^{Rad} are red/far-red (F[690]/F[735]) fluorescence intensity and in Eq.(2) are blue/red blue/red (F[440]/F[690]) fluorescence intensity (Takacs *et al*., 2000).

Also, using literature data we were able to determine the amount of energy, W_{ph} which may be potentially stored through primary products of photosynthesis using fluorescence spectra (Jovanić *et al*., 2003; Mudrik *et al*., 2000).

The amount of energy, which may be potentially stored through primary products of photosynthesis for the time interval Δt, in literature is called as photosynthesis energy of radiation, W_{ph} (Mudrik *et al*., 2000):

$$W_{PH} = \alpha_{\max} \int_{t1}^{t2} I_{NPAR} dt \qquad Eq.(3)$$

were t_1 and t_2 are the beginning and the end of the radiation energy input, α_{max} is the coefficient of energy use by efficiency by plants for photosynthesis at a wavelength of 680nm. It was assumed that Δt is 1h, which is similar to the time period used in literature (Mudrik *et al*., 2000; Woodword, 1983).

I_{NPAR} is measured irradiance which can be expressed as:

$$I_{NPAR} = \int_{\lambda_1}^{\lambda_2} K(\lambda)_{ph} I(\lambda) d\lambda \qquad Eq.(4)$$

were λ_1 = 400nm and λ_2 = 800nm, $K(\lambda)_{ph}$ is the coefficient which is determined from the action spectrum of photosynthesis as the ratio of the rates of photosynthesis at wavelength λ and at wavelength 680nm, at which the rate of photosynthesis is maximal. The deviation from the mean values, obtained of 66 experimental action spectra (Mudrik *et al*., 2000) for different plants, at a coefficient $K(\lambda)_{ph}$ between 400nm-800nmm is about 5%. Finally combining Eq.(3) and Eq(4) we obtained:

$$W_{ph} = \alpha_{\max} \int_{0}^{1} \left[\int_{400}^{800} K(\lambda)_{ph} I(\lambda) d\lambda \right] dt \qquad Eq.(5)$$

If (W_{ph}^{cont}) and ($W_{ph}^{\gamma-rad}$) are the amount of energy, which may be potentially stored through primary products of photosynthesis for control and the plant exposed to the γ–radiations we are able to determinate rate of change due stress, in our case due radiation. Integral in Eq.(5) is equal to the area under the fluorescence curve in given wavelength range. Assuming that the amount of energy stored through primary products of photosynthesis is 100% in control plant (not expose to the γ–radiations) (W_{ph}^{cont}) than for plant exposed to the γ–radiations ($W_{ph}^{\gamma-rad}$) it can be x%. The loss of the ability of the plant to stored absorbed energy through primary products of photosynthesis, can be express as a relative change in the amount of energy which may be potentially stored through primary products of photosynthesis :

$$\Delta_{ph}[\%] = 100 - 100 \times \left(W_{ph}^{\gamma\text{-rad}}\right) \times .\left(W_{ph}^{cont}\right)^{-1} \qquad Eq.(6)$$

Using fluorescence spectra and equation (6) we are able to determinate the rate of change in photosynthesis apparatus efficiency induced by stress.

Fluorescence measurement were performed with now well known and in literature described methods (Jovanić *et al*., 2003; Ndao *et al*., 2005; Buah-Bassuah *et al*., 2008). A 405nm high-power light emitted diode (LED) with power output of 400mW was us used as the excitation source. In front of the LED was the places interference filter 405nm (±10nm). The entrance aperture of the fiber was placed 10mm away from the leaf and directed to the center of the illuminated area at 90° of the leaf axis. Fluorescence emitted radiation from intact leaf was collected and directed through an optical fiber (N.A. of 0.22 and 1000μm diameter) that was coupled to the portable 2048-element CCD spectrometer (AVANTES 1000 PC). The fluorescence light was observed at 90° to the light axis. Optical resolution was 0.15nm. Data collection and spectrum processing were conducted in real time with microcomputer and commercially software OOI Base (AVANTES Inc.). For the evaluation of middle value and standard deviation of some physical occurrences which depend on one stochastic argument, with the precision better that 1%, thirty samples are necessary (Bardou *et al*., 2002). Therefore in this work over 20 measurements were performed for each group which satisfies the mentioned criteria.

Results and Discussions

As it is known that intensity of radioactivity decrease with distance we have plant leaves at three different distances from the soil. Therefore considered plats species one can classified in three group depending who far (approximate in m) from soil the leaves are placed: 1) 3m and above (maple, small-leaved lime), 2) 1-1.5m (common polypody, stinging nettle) and 3) 0.5m or close to the soil (strawberry, ribwort plantain).

a) Maple tree (Acer pseudoplatanus L).:

On the Figure 1 it is present fluorescence spectra of the maple tree. It is clear that significant change in fluorescence spectra occurred. This is in agreement with literature data that in plant γ-irradiation induced dramatic change in chlorophyll fluorescence spectra's (Al-Salhi *et al*., 2004). There are three well defined peaks: first at about 550nm, second at about 690nm and third at about 740nm. Exact maximums positions are given in Table 1. First correspond to the carotenoides pigment, second and third correspond to the chlorophyll (a,b).There positions are present in the Table 1. The positions of the first and second maximums are practically unchanged bath the position of the third maximum varied comparing with control plants (plants before NATO bombing).

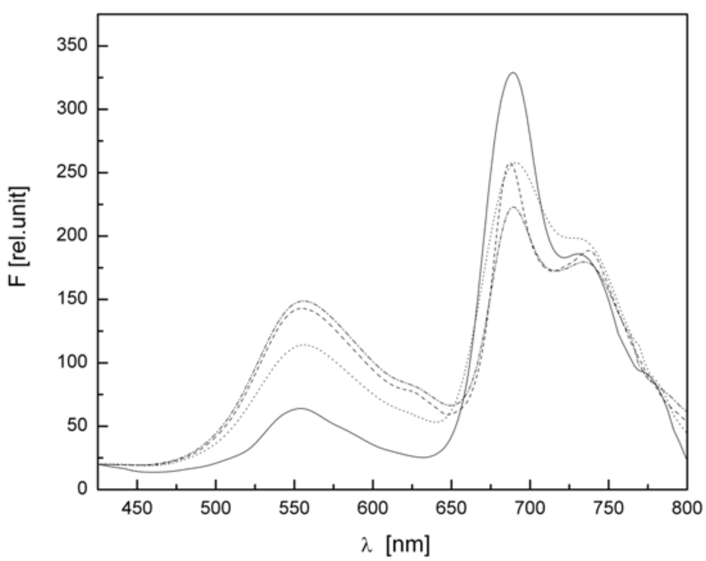

Fig. 1: Fluorescence spectras for maple tree (*Acer pseudoplatanus* L). during eleven years after NATO bombing. (——) control, (----) 2 years, (····) 5 years, (·—·) 11 years.

In the Table 1 are present results for area under the fluorescence curve, the position of the fluorescence for chlorophyll first and second maxima and carotenoides maxima, Δ_{ph}, and relative change of the content for chlorophyll(a,b) $\Delta_{Chl(a,b)}$ and carotenoides Δ_{car}.

On the fluorescence spectra presented on Figure 1 there are three well defined peaks. From the Figure 1one can see that intensity of the fluorescence peaks decrease as the time increase. The leaves were examined by measuring chlorophyll fluorescence before and 2 years, 5 years, 11 years after NATO bombing. It is undoubtedly found that the value of fluorescence intensity ratio (FIR) at 690 nm and 735 nm (F(690)/F(735)) depends upon the ionizing radiation dose. There are several examples of mentioned effect of γ-irradiation on plant leaf fluorescence. So, the similar effect of γ-irradiation on pumpkin (*Cucurbita pepo*) and bean (*Phaseolus vulgaris L*) was observed (Jovanić *et al*., 2002; Jovanić *et al*., 2004). The spectra difference was manifested by decrease of FIR due to changed chlorophyll luminescence, the possible reason for which could be increase of chlorophyll concentration during the recovery process of the plant. Also, the similar effect was observed on corn leafs exposed to the high γ-irradiation doses (Al-Salhi *et al*., 2004). Mentioned data have shown that high doses of γ-irradiation have significant effect on leafs fluorescence spectra as direct consequence of change in biophysical and morphological properties. This explanation is in agreement with experimental confirm fact that gamma radiation act on chlorophyll synthesis (Bogdanović *et al*., 1990). On the other hand netphotosynthesis can reduce due increasing the chlorophyll concentrations (Chouliaras *et al*., 2004). Gamma ray inhibit the formation of chlorophyll in potato tubers (Schwimmer S*et al*., 1958). Also, irradiation decreased the chlorophyll content of the plants Plantago ovata Forsk (Sahaa *et al*., 2010). Therefore taking account mentioned above possible explanation of fluorescence spectra can be due change in photosynthesis pigment concentration (chlorophyll a,b).

Table 1. Maple tree (*Acer pseudoplatanus* L). W_{ph}- area under the fluorescence spectra, Δ_{ph} – relative change W_{ph}, fluorescence maxima, FIR-fluorescence intensity ratio, $\Delta_{Chl(a,b)}$- relative change of the chlorophyll(a,b) content, Δ_{car} -relative change of the carotenoides content.

Time [years]	0	2	5	11
W_{ph} [rel.unit]	162874	157262	131616	107599
Δ_{ph} [%]	0	3.45	19.19	33.93
λ[nm]	549.28 686.33 740.58	556.02 689.37 734.28	554.95 687.34 737.27	549.28 686.33 738.26
$FIR_{[690/740]}$ $FIR_{[550/690]}$	1.921 0.132	1.033 0.360	1.495 0.658	1.649 0.505
$\Delta_{Chl(a,b)}$ [%] Δ_{car} [%]	0 0	46.23 277	22.17 498	14.16 382

Leaves are the major sites of photosynthesis in most plants. In leaves there are about half a million chloroplasts per mm^2 of leaf surface. Chloroplasts convert the light energy absorbed by chlorophyll to chemical energy. Chlorophylls located in the chloroplasts are responsible for photosynthesis. Most studies indicate that photosynthesis, which is the vital process by which green plants prepare their food, is highly sensitive to radiation (Bassam *et al*., 1978; Al-Salhi *et al*., 2004.). The loss of the ability of the plant to store absorbed energy through primary products of photosynthesis Δ_{ph} decrease with time (Table 1). The similar effect of gamma irradiation was observed on fluorescence spectra for bean and pumpkin (Jovanić *et al*., 2002; Jovanić *et al*., 2004). The possible explanation can be connect with γ-irradiation induce change in plant.

Effect of the γ-irradiation on second important pigment necessary for photosynthesis was obtained from observed blue/green FIR because direct correlation between carotenoides concentration and blue/green FIR exist (Stober *et al*., 1993). It was obtained that γ-irradiation induce dramatically change other pigment (carotenoids) from 277% for second year, 498% for fifth year and 382% for eleventh years comparing to the control sample (sample before NATO bombing). This is in agreement with literature data that γ-irradiation generally resulted in the inhibition of carotenogenesis (Villegas *et al*., 1972) and have inhibitory on carotenoides content (Burns *et al*., 1957)

b) Small-leaved Lime (Tilia cordata):

Fluorescence spectra of small-leaved Lime (*Tilia cordata*) during eleven years after NATO bombing are present on Figure 2. Exact maximums positions are given in Table 2.

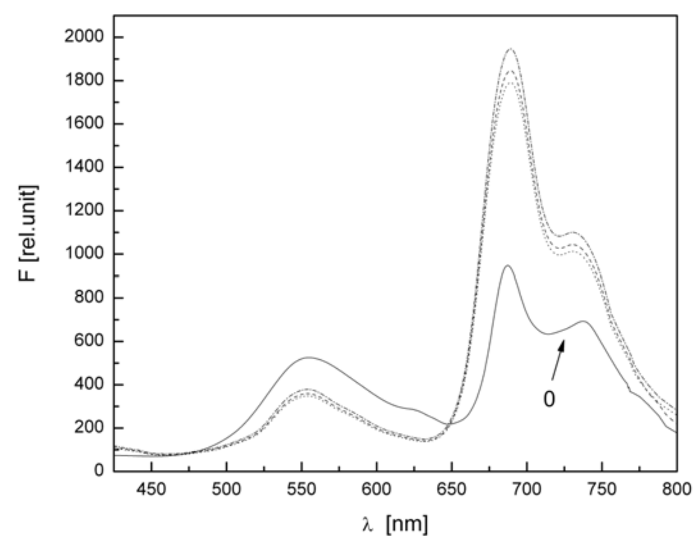

Fig. 2: Fluorescence spectras for small-leaved Lime (*Tilia cordata*) during eleven years after NATO bombing. (——) control, (----) 2 years, (····) 5 years, (·—·) 11 years.

Two fluorescence peaks, at about 550nm and 740nm, are well defined. Third peaks are overlapped with second one but using computer software we were able to resolve it. Area under the fluorescence curve W_{ph} significantly decreases during the time. Placing in Eq.(6) obtained W_{ph} we have calculated Δ_{ph} [%]. It is clear that the amount of energy, W_{ph} which may be potentially stored through primary products of photosynthesis decrease and after eleven years and become different 30.7% comparing with control plants. $FIR_{[550/690]}$ change very small and have maximum between first and fifth years (~ 10%) after that it decrease at about ~ 3% .

Table 2: Fluorescence spectra of the small-leaved Lime (*Tilia cordata*), W_{ph}- area under the fluorescence spectra, Δ_{ph} – relative change W_{ph}, fluorescence maximas, FIR-fluorescence intensity ratio, $\Delta_{Chl(a,b)}$- relative change of the chlorophyll(a,b) content, Δ_{car} -relative change of the carotenoides content.

Time [years]	0	1	5	11
W_{ph} [rel.unit]	55681	41174	39468	38598
Δ_{ph} [%]	0	26	29.1	30.7
λ[nm]	550.34 681.00 739.53	553.89 681.58 748.07	553.89 680.91 740.138	554.25 681.58 748.07
$FIR_{[690/740]}$ $FIR_{[550/690]}$	0.142 0.367	0.138 0.407	0.138 0.407	0.138 0.407
$\Delta_{Chl(a,b)}$ [%] Δ_{car} [%]	0 0	2.82 10.90	2.82 10.90	4.22 2.72

Explanation can be that plants found way to survive and has adapted to new living condition. On the other hand $FIR_{[690/740]}$ is practically unchanged and this mean the chlorophyll pigments content is at practically unchanged. Obtained results lead on conclusion that chlorophyll pigments are insensitive on soil nuclear radiation. This is in collision with literature data. Namely it was found that gamma-ray irradiation induced a significant increase of the chlorophyll contents in maize leaves (Singh, 1971). Also gamma ray dosages of the order of magnitude of 10 kilorads inhibit the formation of chlorophyll in illuminated potato tubers (Schwimmer *et al*., 1958). The irradiation decreased the chlorophyll content of the plants Plantago ovate Forks (Sahaa *et al*., 2010). Obtained discrepancy can be explained due the fact that mentioned plants species are not the threes.

c) Bull nettle (Urtica dioica L. – Urticaceae)

Fluorescence spectra's of bull nettle (*Urtica dioica L. – Urticaceae*) during eleven years after NATO bombing are present on Figure 3. At the first look one can see that significant change in fluorescence spectra occurred. This is in agreement with literature data which have shown in plant γ-irradiation induced dramatic change in chlorophyll fluorescence spectra's (Bogdanović *et al*., 1990). Three fluorescence peaks are presented: two well defined fluorescence peaks at about 550nm and 740nm, and one third which is not well resolved at about 690nm. Exact maximums positions are given in Table 3.

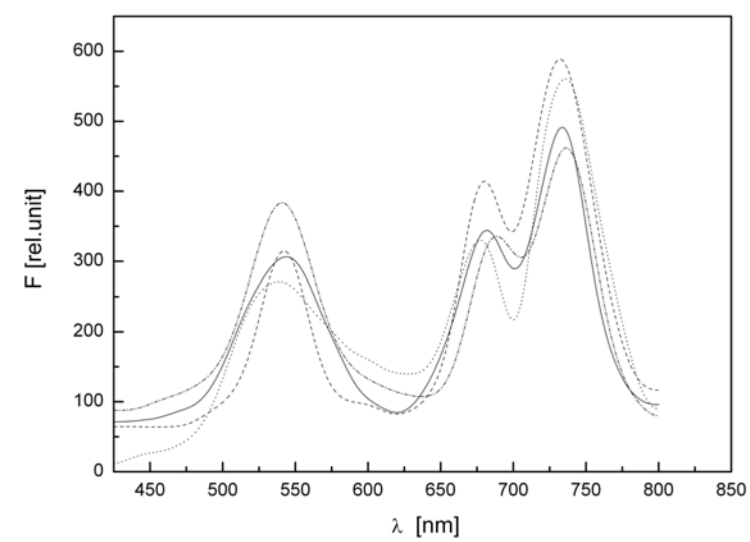

Fig. 3: Fluorescence spectras for bull nettle (*Urtica dioica L. – Urticaceae*) during eleven years after NATO bombing. (──) control, (----) 2 years, (···) 5 years, (·─·) 11 years.

The third one is best resolved in control plants but with time it is overlapping with peaks at 740nm. Area under the curve W_{ph} decrease in second and fifth years but small increase in ninth years occurs. As in previously described results placing in Eq.(6) obtained W_{ph} we have calculated Δ_{ph} [%]. Analyzing obtained values for Δ_{ph} one can see that relative change of the amount of energy, W_{ph} which may be potentially stored through primary products of photosynthesis increase in second and fifth and decrease in ninth years. $FIR_{[550/690]}$ increase from 0.620 for control plants and 0.653 for second years, 0.886 for fifth years and 1.231 for ninth years.

Using Eq.(2) relative change of the of the carotenoides contents ΔCar were calculated. One ca sees that ΔCar increase dramatically so at ninth years it is 98.54%. Similar dramatic effect of nuclear radiation was obtained for maple described mentioned above. This is in agreement with literature data which have shown that nuclear radiation dramatically affect on carotenoides pigments (Stober *et al*., 1993). Possible explanation can be connect with inhibition of carotenoides activity and is in agreement with literature data that γ-irradiation generally resulted in the inhibition of carotenogenesis (Villegas *et al*., 1972). On the other hand $FIR_{[690/740]}$ decrease shove form 0.265 for control to 0.235 for ninth years. Using Eq.(1) relative change $\Delta_{Chl(a,b)}$ of chlorophyll content change non linearly and is 14.24% in second years and have maximum change in fifth years 15.72%. In eleventh years $\Delta_{Chl(a,b)}$ decrease to 12.77%. This decreasing in chlorophyll content in ninth years may be due that process of plant adaptations to the nuclear radiation of the soil. Obtained change in chlorophyll content is for expectation taking account literature data about nuclear radiation on chlorophyll pigments. Namely mentioned data have shown that the irradiation have significant effect on chlorophyll content of the plants leaves (Jovanić *et al*., 2002; Jovanić *et al*., 2004) and in some cases inhibit the formation of chlorophyll (Schwimmer *et al*., 1958). This is in collision with some literature data in which was found that gamma-ray irradiation induced a significant increase of the chlorophyll contents in maize leaves (Singh *et al*., 1971).

Table 3: Fluorescence spectra of the stinging nettle or bull nettle (*Urtica dioica L. – Urticaceae*) , W_{ph}- area under the fluorescence spectra, Δ_{ph} – relative change W_{ph}, fluorescence maxima, FIR-fluorescence intensity ratio, $\Delta_{Chl(a,b)}$- relative change of the chlorophyll (a,b) content, Δ_{car} -relative change of the carotenoides content.

Time [years]	0	2	5	11
W_{ph} [rel.unit]	60186	54683	43181	52919
Δ_{ph} [%]	0	9.14	28.25	12.07
λ[nm]	560.98 681.58 734.95	561.33 681.58 738.48	560.98 681.58 744.89	561.68 681.70 741.75
$FIR_{[690/740]}$ $FIR_{[550/690]}$	0.265 0.620	0.232 0.653	0.229 0.886	0.235 1.231
$\Delta_{Chl(a,b)}$ [%] Δ_{car} [%]	0 0	14.24 5.05	15.72 42.90	12.77 98.54

d) Common Polypody (Polypodium vulgare):

On Figure 4 are presented the fluorescence spectrums for Common Polypody (*Polypodium vulgare*) eleven years after NATO bombing. Fluorescence spectrums have two well resolved fluorescence maximums at about 690nm and 740nm. Maximum in shorter wavelength at about 550nm do not exist. Areas under the curve W_{ph} varied very small with time (see Table 4).

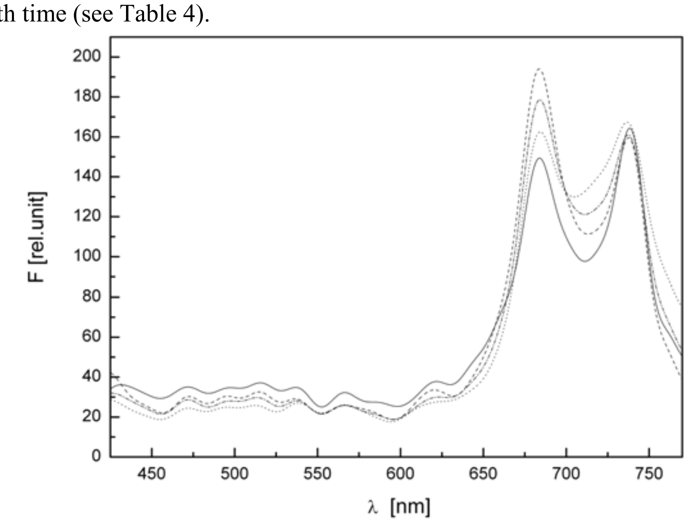

Fig. 4: Fluorescence spectras for Common Polypody (*Polypodium vulgare*) during eleven years after NATO bombing. (——) control, (----) 2 years, (····) 5 years, (·—·) 11 years.

As in mentioned above described procedure we have calculated Δ_{ph} using considered plants fluorescence spectrums,Eq.(6) and obtained W_{ph}. Obtained data are presented in Table 4.

Table 4: Fluorescence spectra of the Common Polypody (*Polypodium vulgare*), W_{ph}- area under the fluorescence spectra, Δ_{ph} – relative change W_{ph}, fluorescence maximas, FIR-fluorescence intensity ratio, $\Delta_{Chl(a,b)}$- relative change of the chlorophyll(a,b) content, Δ_{car} - relative change of the carotenoides content.

Time [years]	0	2	5	11
W_{ph} [rel.unit]	20807	20659	20701	20686
Δ_{ph} [%]	0	0.71	0.509	0.581
λ[nm]	535.45 683.73 738.21	537.54 683.68 737.63	535.50 684.04 736.37	536.94 683.91 737.20
$FIR_{[690/740]}$ $FIR_{[550/690]}$	0.935 ---------------	1.244 ----------------	1.070 ------------------	1.221 -------------
$\Delta_{Chl(a,b)}$ [%] Δ_{car} [%]	0 ---------------	24.86 ----------------	14.41 ------------------	30.61 -------------

Analyzing obtained Δ_{ph} one can see that the amount of energy, W_{ph} which may be potentially stored through primary products of photosynthesis decrease and after eleven years change is small comparing to values for control plants. It leads on conclusion that nuclear soil nuclear radiations due NATO bombing did not have effect on Δ_{ph}. $FIR_{[690/740]}$ similar Δ_{ph} did not change a lot during the time. Obtained calculated values for relative chlorophyll change $\Delta_{Chl(a,b)}$ change none linearly with time. In fist year $\Delta_{Chl(a,b)}$ increase to about 24.86%, in fifth year decrease to the 14.42% and finally in eleventh years come up maximum value at about 30.61%. This data are in agreement with the results given in literatures data where chlorophyll contents were found to be lower in irradiated plants than in unirradiated ones (Dale *et al*., 1997;Ling *et al*., 2008). Possible explanation can be that in this plant species metabolic process connected with chlorophyll molecules are insensitive on the effect of soil nuclear radiations.

d) Strawberry (Fragariae herba)

On the Figure 5 is present fluorescence spectrums of the strawberry during eleven years after NATO bombing. The fluorescence spectra are different with well define two peaks, similar as for small-leaved Lime: first at about 550nm and second at about 740nm. Exact maximums positions are given in Table 5.

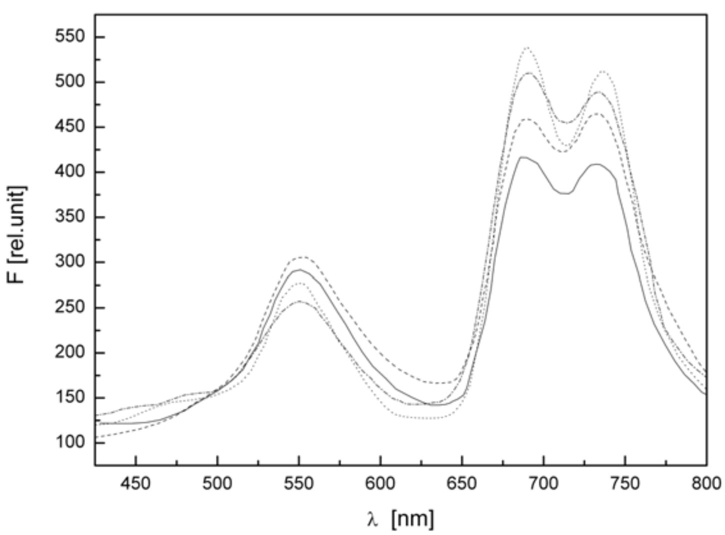

Fig. 5: Fluorescence spectra for Strawberry (*Fragariae herba*) during eleven years after NATO bombing. (——) control, (----) 2 years, (····) 5 years, (·—·) 11 years.

Third fluorescence peaks is not well resolved but it was no problem to determine its positions using computer software. Area under the fluorescence spectrums significantly decrease with time. Calculated Δ_{ph} using obtained W_{ph} is was obtained similar changes as for small-leaved Lime. It means that the amount of energy, W_{ph} which may be potentially stored through primary products of photosynthesis decrease and after eleven years and become different 30.7% and comparing with control plants. $FIR_{[690/740]}$ increase in first year, in fifth year decrease comparing to the value for fifth years. Finally in eleven year it become close to the value for control plants. In other words, chlorophyll content dramatically change in first years after bombing and have maximum (47.97%) then chlorophyll content change decrease but still is higher than for control plants (22.30%). This is in agreement with literature data about effect of gamma radiation on chlorophyll in plant leaves. Namely, a number of researchers investigated the effect of gamma radiation on chlorophyll content (Dale *et al*., 1997; Griffiths *et al*., 1997; Byun *et al*., 2002; Ling *et al*., 2008; Kiong *et al*., 2008). Some data have shown that the irradiation significant decreased the chlorophyll content of the plants leaves (Sahaa *et al*., 2010). Also there are several examples in which is found that gamma-ray-induced chlorophyll mutations in the seeds of Triticum vulgare, Avena sativa (monosomic plants) and A. strigosa which were irradiated with gamma-rays Chlorophyll mutations was found (Nishiyama *et al*., 1961; Reddya *et al*., 1978; Akbar *et al*., 2003).

Table 5: Fluorescence spectra of the Strawberry (*Fragariae herba*), W_{ph}- area under the fluorescence spectra, Δ_{ph} – relative change W_{ph}, fluorescence maxima, FIR-fluorescence intensity ratio, $\Delta_{Chl(a,b)}$- relative change of the chlorophyll (a,b) content, Δ_{car} -relative change of the carotenoides content.

Time [years]	0	2	5	11
W_{ph} [rel.unit]	46900	43429	36580	32648
Δ_{ph} [%]	0	7.40	22.00	30.39
λ[nm]	559.09 685.58 742.06	559.23 684.30 740.96	559.19 683.66 743.51	560.51 683.58 742.56
$FIR_{[690/740]}$ $FIR_{[550/690]}$	0.296 0.909	0.438 1.091	0.197 0.781	0.230 0.829
$\Delta_{Chl(a,b)}$ [%] Δ_{car} [%]	0 0	47.97 11.11	33.44 14.08	22.30 8.82

Obtained great change in chlorophyll content can be explained in miner that gamma-ray nuclear radiation have significant effect on it (Schwimmer *et al*., 1958; Singh, 1971; Sahaa *et al*., 2010) and induced chlorophyll mutations as it were found in literature dads (Nishiyama *et al*., 1961;Reddya *et al*., 1978; Akbar *et al*., 2003). Contents of the carotenodes pigments change in different manner and in smaller magnitude. So change of the crotenoides content increased in first and fifth year to 11.11% and 14.08%, respectively. In ninth years crotenoides content change smaller than in first and fifth years (8.82%). It can be explained with assumption that plant slowly adapted to the new conditions. Obtained results are in agreement with literature data which have shown that nuclear irradiation results in inhibition of carotenoides (Villegas *et al*., 1972). Also it was shown that that γ-radiation have great effect in the total carotenoids of tropical Indian fruits. So for the same radiation dose of 1.0 Mrad, carotenoids of orange juice are destroyed to the extent of 38 per cent, while those of mango pulp undergo a destruction by only 24 per cent induced great differ widely in their sensitivity to gamma radiation (Sawanta *et al*., 1970).

e) Ribwort plantain (Herba Plantaginis lanceolatae)

Finally on the Figure 6 it is present fluorescence spectra of the ribwort plantain (*Herba Plantaginis lanceolatae*). Obtained results of the effect of γ-radiations presented in Table 6.

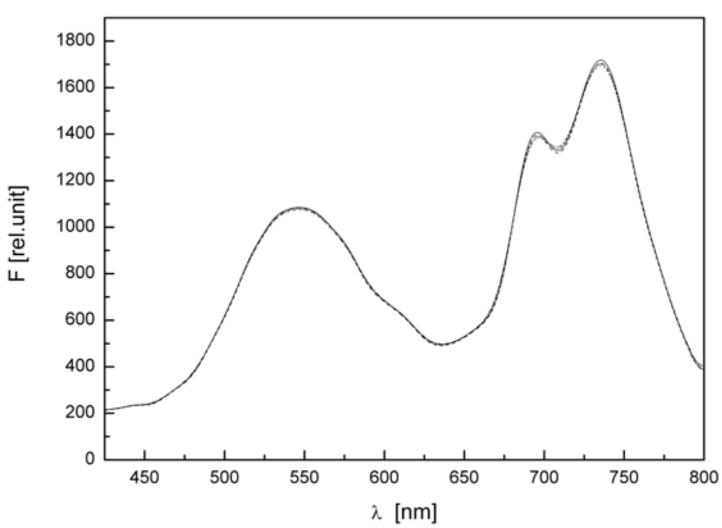

Fig. 6: Fluorescence spectra for Ribwort Plantain (Herba Plantaginis lanceolatae) during eleven years after NATO bombing. (——) control, (----) 2 years, (····) 5 years, (·—·) 11 years.

The difference between the areas under the curve are very small, the biggest is about 4.59%. As in mentioned above five plan in second year after bombing chlorophyll(a,b) relative change increase up to about 13.13% and after that it increase to about 22.79% and finally in eleventh decrease to 13.13% . For carotenoides obtained results un quite different comparing with Chlorophyll (a,b). Namely, relative change of the carotenoides content is very small and practically constant during eleven years (~1.1%). This results lead us on assumptions that carotenoides molecules us insensitive on γ-radiations. Also, obtained results for APFW lead on concision that in ribwort plantain ability to stored absorbed solar energy through the primary products of photosynthesis is practically unchanged during eleven years after bombing. It varied between 0.05% in second years to in fifth 4.395% . After that Δ_{ph} decreased to 3.965% in eleventh years.

Table 6: Ribwort Plantain (*Herba Plantaginis lanceolatae*) W_{ph} - area under the fluorescence spectra, Δ_{ph} – relative change W_{ph}, fluorescence maxima, FIR-fluorescence intensity ratio, $\Delta_{Chl(a,b)}$- relative change of the chlorophyll(a,b) content, Δ_{car} -relative change of the carotenoides content.

Generation	0	2	5	11
W_{ph} [rel.unit]	64032	63992	61217	61493
Δ_{ph} [%]	0	0.062	4.396	3.965
λ[nm]	561.07 681.58 739.28	562.08 681.92 739.92	561.32 682.08 739.26	545.86 682.26 739.92
$FIR_{[690/740]}$ $FIR_{[550/690]}$	0.373 1.063	0.324 1.076	0.288 1.172	0.324 1.076
$\Delta_{Chl(a,b)}$ [%] Δ_{car} [%]	0 0	13.13 1.12	22.79 10.25	13.13 1.12

Taking account results presented in Table 6 one can concluded that γ-radiations presented practically have no effects on ribwort plantain. Or in the other words Δ_{ph} and carotenoides content did not change during eleven years after NATO bombing. Changing in chlorophyll content is in agreement with literature data. The irradiation decreased the chlorophyll content of the plants Plantago ovata Forsk (Sahaa *et al*. 2010). Obtained carotenoides insensitivity on γ-radiations is quite unexpected comparing with literature data. Namely it was shown γ-radiation have great effect in the total carotenoids of tropical Indian fruits. So for the same radiation dose of 1·0 Mrad, carotenoids of orange juice are destroyed to the extent of 38 per cent, while those of mango pulp undergo a destruction by only 24 percent (Sawanta *et al*., 1970). Small sensitivity of the carotenoids on gamma irradiations is in good agreement with literature date. Gamma irradiation did not affect the formation of carotenoids (Thomas *et al*., 2006). On some plant gamma irradiation had no significant effects on the carotene content (Mitchell *et al*., 2006). Possible explanation is that this plant species has great resistibility and great ability for adaptation on unfavorable environment condition. What is the reason(s) for this we don't understand and have not idea to explain it. But this is unexpected, comparing with previously described effect for five species.

All mentioned presented results are not unexpected and that they are similar to the effect on plants induced by Chernobyl accident. There are numerous examples that nuclear radiation cause significant change in plant. It was shown that chronically nuclear radiation cause change of enzyme activity in corn (Zivalyuk *et al*., 1994). Some authors have found that nuclear radiation from Chernobyl cause many abnormally morphology in plants like change in need length, change in DNA structure, enzyme activity (Zelena *et al*., 2005). Also, It was shown that γ irradiation caused a massive of photogenic change and mortality of pine after Chernobyl incident (Arkhirov *et al*., 1994). Other authors have shown (Davis *et al*., 2003; Jin-Hong Kim *et al*., 2004) that nuclear radiation induces, beside change in concentration of the photosynthetic pigment (chlorophyll a and b, carotenoides), dramatic change in rate of photosynthesis, leaf size, thickness and structure. The irradiation of oats and triticale plants caused the disorganization of the cardinal physiological processes (Stoeva *et al*., 2007).

Conclusion:

Nuclear radiation caused by bombing induced significant change in plant photosynthetic activity, ability to converse light absorbed energy in to photosynthetic products and chlorophyll content.

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