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Full Length Research Paper

Sunflower chlorophyll levels after magnetic nanoparticle supply

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This study reported the results of an experimental investigation regarding the chlorophyll contents in sunflower seedlings supplied with low concentrations of magnetic nanoparticles. Iron and iron-cobalt oxides were prepared in the form of colloidal nanoparticles for the administration in the culture medium of young plant seedlings during their very early ontogenetic stages - as possible basis of new biotechnological tool in plant growth controlling. The changes in the contents of chlorophyll A, chlorophyll B and carotene like pigments were evidenced by spectral measurements. Magnetite nanoparticles influenced negatively the photosynthetic pigment biosynthesis by diminishing chlorophyll content with up to 50% while slighter effect was evidenced in the case of cobalt ferrite nanoparticles that induced only up to 28% chlorophyll level decreasing. The influence upon photosynthetic system LHC II was revealed as consistent with the higher diminution of chlorophyll ratio in the case of cobalt ferrite supplied seedlings than for magnetite supplied ones sustaining the hypothesis of photosynthesis sensitivity to the presence of magnetic nanoparticles stress.

Key words: Sunflower seedlings, photosynthetic pigments, nanosized metal oxides.

INTRODUCTION

Scientist interest in heavy metals impact on photosynthesis processes may be considered one of the hallmarks of applied plant biochemistry as well as of plant environmental pollution research. Plant cultivation efficiency is more and more investigated with focus on the environment gradients effects – one of them being the result of nanometric metal oxides generated from both natural and artificial sources - that could be also used in controlled cultivation of agricultural plants.

Granick (1957) was one of the firsts that evidenced the role of iron mixed oxide, the magnetite, in the redox processes from the chloroplast membrane –with possible implications in photosynthesis mechanisms clarification and even for its artificial modeling. In the frame of the studies dedicated to photosynthesis modeling, Jiao and Frei (2009) focused on cobalt oxides with nanometric size as inorganic catalysts able of water photooxidation and chlorophyll synthesis influence.

In recent decades, Sala was one of the firsts who evidenced the effects of magnetic nanoparticles in plants, that is the increase of chlorophyll levels and photosynthesis rate in seven days old beans seedlings following the addition of 0.1% magnetite based magnetic fluid in the culture medium (Sala, 1999).

Considering the magnetic features of nanoparticulate matter composed by magnetic metal oxides, some considerations regarding the low magnetic field influence on plant growth need to be mentioned. For example, sugar beat plants exhibited slight increase of chlorophyll levels in their leaves after magnetic exposure to low flux density magnetic field (Rochalska, 2005); *in vitro* tissue cultures of soybean (Atak et al., 2003) as well as of *Pawlovnia* explants (Celik et al., 2008) presented increased chlorophyll levels for relatively short time exposure to low intensity magnetic field while for longer exposure time, the diminution of both chlorophyll types was observed; different responses of chlorophyll levels in maize (diminution) and sunflower (increasing) were evidenced after magnetic exposure to continuous static magnetic field (Temirci et al., 2007).

In the next, we present comparatively the response of young sunflower plantlets to magnetite and cobalt ferrite nanoparticle suspensions added in the culture medium,

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Abbreviations: SO, Sodium oleate; **NP,** nanoparticle; **LHC,** light harvesting complex.

based on chlorophyll content spectral measurement.

MATERIALS AND METHODS

Magnetic nanoparticles (NP)

Ultrafine particles of magnetite and cobalt ferrite were prepared by chemical coprecipitation method (Massart, 1981). Hot solution of 25% NaOH was added under constant stirring for 30s in the mixtures of $FeCl_3$ and $FeCl_2$ - or $CoCl_2$ solutions in their respective stoichiometry (1:2) at 80°C. The magnetic particles were separated from the reaction medium in magnetic field gradient, being washed several times with distilled water at room temperature. Sodium oleate solution (SO) was added to the ferrophase as the surfactant that allows the final dispersion of magnetic nanoparticles (NP) in distilled water under stirring for more 2 h at about 80°C. Sodium oleate was chosen to coat the magnetic NP as being the best water soluble substitute of oleic acid (known for its suitability for oily stabilized NP suspensions) - the SO biocompatibility being evidenced in the literature dedicated to magnetic NP bio-applications (Sun et al., 2007).

Magnetization curves of ferrite powders using a vibrating sample magnetometer (VSM) Quantum Design, model 6000 were recorded, their mathematical processing providing the average magnetic diameter and saturation magnetization of the two types of magnetic core/non-magnetic shell systems used for further experimental investigation of metal oxide impact on chlorophyll synthesis.

Vegetal material and magnetic nanoparticle treatment

Sunflower (*Helianthus annuus* L.) was chosen as biological material considering its economic importance for agriculture and food industry. The seeds were harvested from a single plant with vigorous biological features from an experimental micropopulation (to limit as much as possible the genophond variations). Seeds were surface sterilized by immersion in 70% ethanol for about one minute, then washed several times with distilled water.

Their germination was conducted on watered porous paper support in Petri dishes with 25 seeds each (two dishes, that is 50 seeds per experimental variant), in darkness at the temperature of 22.0 ± 0.5°C (in Incucell device). Daily supply with about 10 ml magnetic nanoparticles suspension per dish (concentrations of 20, 40, 60, 80 and 100 µl/L) was carried out during 12 days, plant growth being conducted in controlled conditions of temperature (24.0 ± 0.5°C), illumination (dark/light cycle: 14 h/10 h) and 90% humidity, into a climate room (Angelantoni Scientifica).

The assay of chlorophyll A, chlorophyll B and total carotenoid pigments, selectively extracted in 90% acetone was accomplished in 12 days old seedlings based on Meyer-Berthenrath's method modified by Stirban (Stirban, 1985), the pigment level (in mg per g of fresh substance mass) being assessed according to the formulae:

$$ChlA = [[12.3E(663)-0.86E(645)]*v]/1000dw \quad (1)$$

$$ChlB = [[19.3E(645)-3.6E(663)]*v]/1000dw \quad (2)$$

$$TC = [10E(472)*v]/1000dw \quad (3)$$

where, ChlA(B) represents the chlorophyll A(B) content while T.C. has the meaning of total carotene content; $E(\lambda)$ is the light extinction to the wavelength λ; *d* is the quartz cell width (of 1cm); *v* is the 90% acetone extract volume (ml) and *w* is the fresh tissue weight (g). The spectral device was a Shimadzu UV1700 spectrophotometer working in the UV-VIS range.

Statistical analysis

Three repetitions of the whole experiment were carried out for all experimental variants. The main control seedlings were supplied only with distilled water. The SO-control was also arranged by supplying the corresponding seedlings with the sodium oleate aliquot (SO) calculated for the highest magnetic suspension concentration (100 µl/l). Average values and standard deviations were calculated; *t*-test was applied to assess the statistical significance of the changes revealed between control seedlings and the magnetic nanoparticle treated ones.

RESULTS AND DISCUSSION

Magnetic nanoparticles prepared by chemical co-precipitation and coated in sodium oleate hydrosoluble shell exhibit similar values of saturation magnetization, of about 60 emu/g and magnetic core diameter - of about 8.5 nm - as resulted from VSM investigation.

The concentration of NP suspensions were chosen in the range 20 ÷ 100 µl/l - corresponding to 10^{15} - 10^{16} nanopartic/cm^3 accordingly to other studies dedicated to colloidal magnetic nanoparticle influence on vegetal organisms (Racuciu and Creanga, 2009).

The percentage of sunflower germinated seed (Photo 1) was over 92% in all samples according to Table 1. No specific correlation between the NP suspension type or concentration and the germinative level could be revealed.

The graphs from Figure 1 present the photosynthetic pigment contents normalized to the control sample (taken equal to the unit). General inhibitory effect was found in the case of Fe_3O_4 NP with rather similar decreasing of all tree types of photosynthetic pigment to the increase of the NP suspension concentration.

The differences between the control seedlings (supplied only with distilled water) and those grown in the presence of sodium oleate aliquots were practically missing while for the Fe_3O_4 NP supplied seedlings all the differences recorded comparatively to the control were statistically significant - related to the significance threshold $p = 0.05$.

In Figures 2a and b (where the absolute values of the pigment contents were taken into account), good linear correlations between chlorophyll A and B - respectively carotene pigment content are shown, with correlation coefficient over 0.9 - suggesting that Fe_3O_4 NPs affected in similar ways the biosynthesis of main photosynthetic pigments.

The supplying with $CoFe_2O_4$ NPs resulted also in the diminution of chlorophylls and carotenes contents (Figure 3) – however, with quantitatively different responses than in the case of Fe_3O_4 NPs.

The level of chlorophyll A appeared to be diminished – with up to 30% compared to the control- while chlorophyll

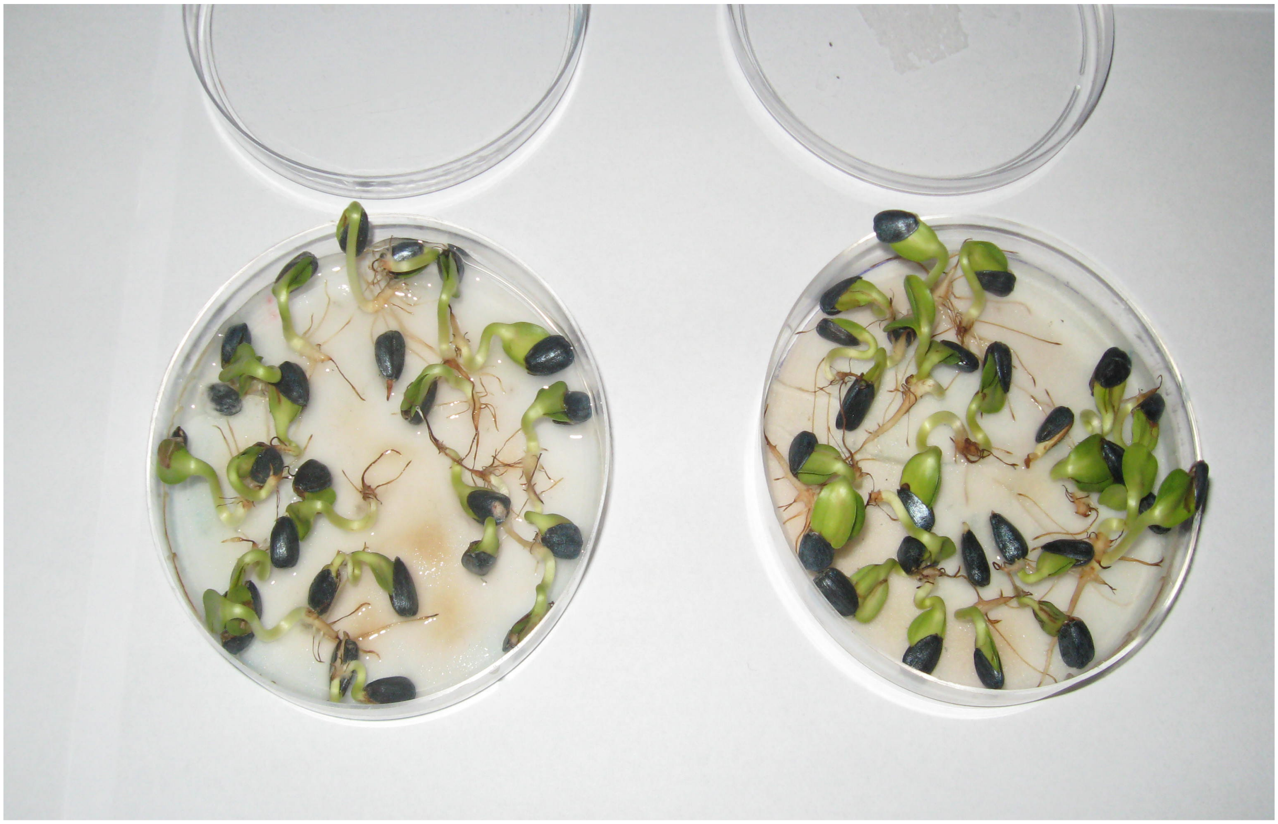

Photo 1. Petri dishes with sunflower germinated seeds.

Table 1. Germination percentage in sunflower seeds.

Concentration (µl/l) / NPs	0	0_SO	20	40	60	80	100
Fe_3O_4	96	94	94	92	94	96	94
$CoFe_2O_4$	94	94	96	94	92	94	96

B presented statistically significant diminutions only for the higher two NP concentrations – with 25 to 30%. It seems that the presence of cobalt ions in the $CoFe_2O_4$ NPs is concordant with some literature reports that mentioned the slight stimulatory effect of cobalt ions of chlorophyll biosynthesis (Czerpack et al., 1994; Sheekh et al., 2003) or the stimulatory effect of slight exposure to low magnetic field on sunflower seedlings (Temirci et al., 2007); in these views could be understood the slighter effect consisting in chlorophyll biosynthesis reduction by $CoFe_2O_4$ NPs than by Fe_3O_4 NPs.

Unlike the case of Fe_3O_4 NPs, no mathematical correlation could be revealed in the case of $CoFe_2O_4$ NPs between the photosynthetic pigment levels – suggesting that cobalt presence affects their biosynthesis by putative different biochemical/biophysical pathways.

The calculation of derived physiological parameters was further accomplished for the result discussion and interpretation (Figures 4 and 5).

From Figures 4 and 5, it is visible that chlorophyll ratio was non-significantly decreased by the Fe_3O_4 NP suspension supply in the seedlings culture medium (except for the lowest concentration of 20 µl/l) while for $CoFe_2O_4$ NP suspension significant diminution was evident for the concentrations of 20-40-60 µl/l ($p<0.05$).

It seems that the redox activity of LHC II (Light Harvesting Complex II) components from the photosynthetic system II exhibits lower sensitivity to the Fe_3O_4 NPs - than to $CoFe_2O_4$ NPs, at least for the sunflower plantlets studied inhere. In other studies, the response of *Zea mays* seedlings to the administration of the same range of Fe_3O_4 NP suspension concentration resulted in the diminution of chlorophyll ratio while *Cucurbita pepo* seedlings exhibited a slight increase of chlorophyll contents (Racuciu et al., 2009).

The ratio of chlorophylls sum to the all pigment sum was not significantly changed neither for Fe_3O_4 NPs nor for $CoFe_2O_4$ NPs (Figures 4 and 5); as known the total

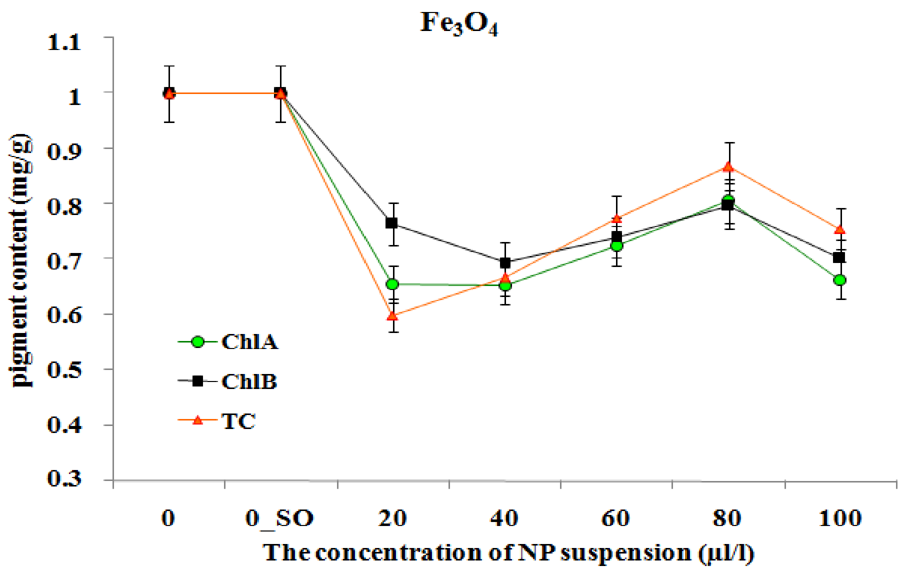

Figure 1. Chlorophylls and carotenes contents in the seedlings supplied with Fe_3O_4 NP suspension.

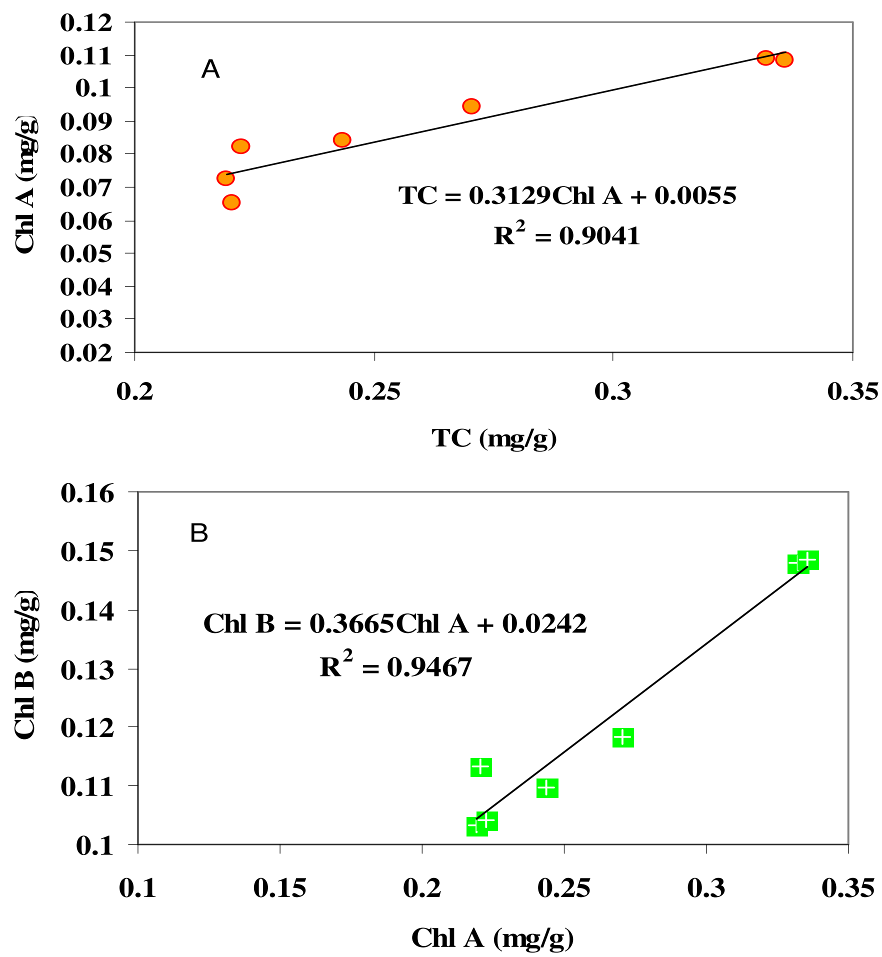

Figure 2a and b. Correlations between the photosynthetic pigments in the case of Fe_3O_4 NP suspension (R- linear correlation coefficient)

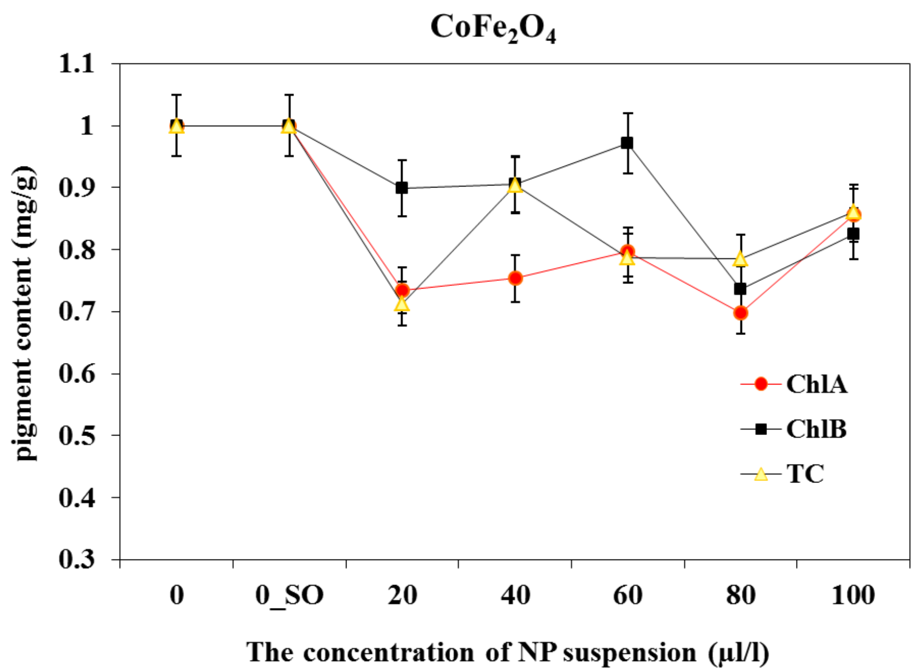

Figure 3. Chlorophylls and carotenes contents in the seedlings supplied with $CoFe_2O_4$ NP suspension.

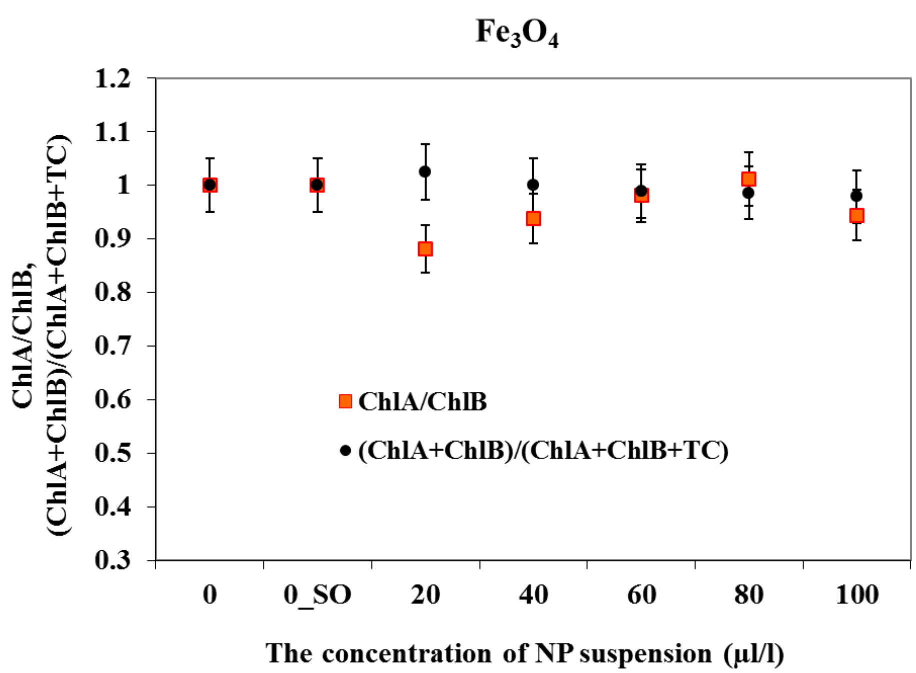

Figure 4. The chlorophyll ratio and the ratio of chlorophyll sum and all pigment sum in the case of the seedlings supplied with Fe_3O_4 NP suspension.

content of chlorophyll A and B can be considered as an index of the total amount of light-harvesting and electron transport components from the chloroplast membranes (Terry, 1983) so that one could suppose that the entire enzyme aggregate remained actually unaffected in the presence of magnetic NP suspensions administrated to sunflower seedlings.

As mentioned earlier, to get certain qualitative insight in

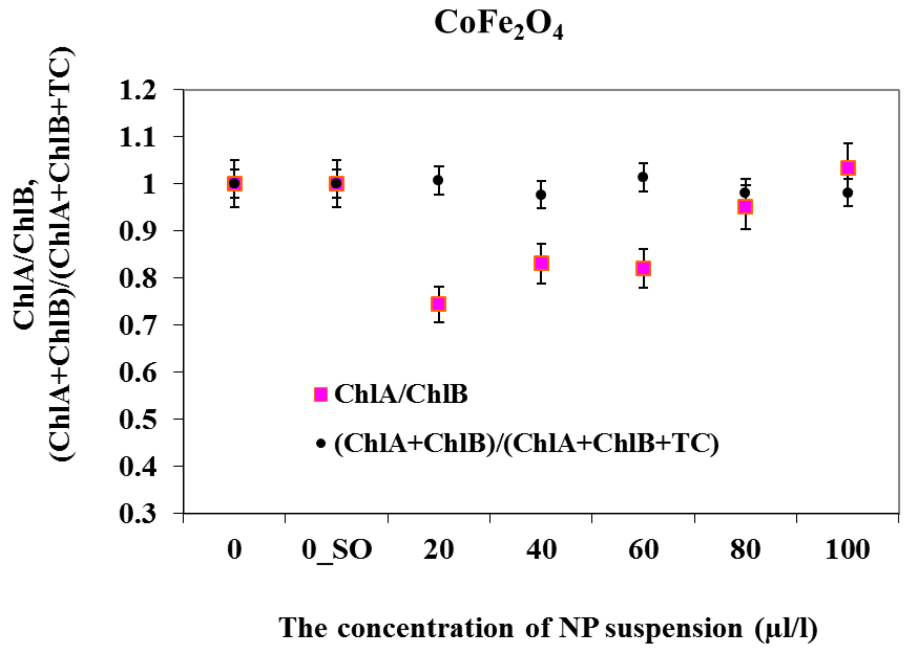

Figure 5. The chlorophyll ratio and the ratio of chlorophyll sum and all pigment sum in the case of the seedlings supplied with $CoFe_2O_4$ NP suspensions.

the magnetic NP suspensions effects upon the cellular and molecular bases of chloroplast biochemistry we might presume that both chemical and magnetic influences need to be taken into account.

As known, iron is taken up by plants as ferrous ions serving as an activator for several essential biochemical processes like photosynthesis, respiration and symbiotic nitrogen fixation while iron deficiency can induce serious troubles in plant metabolism even with lethal consequences.

The complex role of iron in photosynthesis has focused the attention of some researchers like Geider and Roche (1994) that emphasized the increase of certain complex molecules levels from phytoplankton green tissue in response to iron limitation in the environment or Terry (1983) that showed that iron deficiency-mediated changes in chlorophyll content does not necessarily affect dark respiration rate or the CO_2 transformation pathway; or like Nenova (2009) that found that in pea seedlings the supply of iron resulted in no change of maximum activity of photosynthetic system II.

Despite of iron which is the fourth abundant element in the Earth crust, the natural sources of cobalt in the environment (soil, dust, seawater, volcanic eruptions and forest fires) release smaller amounts of metal ions and combinations, these being also supplied from burning coal and oil, from car exhausts, and from industrial processes. The lower natural abundance of cobalt compared to iron has also counted for our experimental project design so that the mixed iron and cobalt oxide was utilized in parallel to the magnetite.

Both stimulatory and inhibitory effects of cobalt ions were evidenced in various laboratory investigations. For example, the aforementioned Czerpak et al. (1994) shows that 10^{-5}-10^{-6} M concentrations of Co^{2+} ions have stimulatory effects on chlorophyll biosynthesis in *Chlorella* (increasing with 45 and 65% the chlorophyll levels) while Sheekh et al. (2003) found enhanced chlorophyll content for relatively low cobalt levels but also diminished chlorophyll amounts to the increase of cobalt ions concentration - in two exposed green algae species.

From the viewpoint of NP magnetic effects not the iron or cobalt ions but the whole nonosized metal oxide structure is going to be considered; it was calculated that the magnetic moment of nanosized magnetic particles found in living organisms is $10^7 \div 10^9$ times higher than the elementary magnetic moment known in physics (Binhi, 2007) so that the corresponding magnetic field energy is higher than the thermal reference, k_BT (k_B – Boltzmann's constant; T- absolute temperature). In this respect, the description of biological effects of low magnetic fields at cellular level can be better shaped since, for instance, the magnetic field generated by magnetic NPs that penetrated plant vascular system and remained attached to cellular membranes could influence the conductivity of mechanosensitive ion channels - resulting further in cell biochemistry modulation (Huges et al., 2010). So, the complexity of the biological effects revealed in the frame of the studies focused on young plant seedlings exposed to chemical and magnetic action of Fe_3O_4 or $CoFe_2O_4$ NPs still needs further phenomenological clarification, possibly focused on stress enzyme analysis– which is consistent with our next stage of research plan in this field.

Conclusions

Magnetic nanoparticles of Fe_3O_4 induced similar and statistically significant diminution of chlorophylls and carotene levels in sunflower seedlings but with general slight effect on the photosynthesis global indicator – the chlorophyll ratio. Iron partial substitution with cobalt ions in nanometric magnetic particles of $CoFe_2O_4$ resulted in more accentuated inhibitory effect upon Light Harvesting Complex II from chloroplast membranes of sunflower seedlings with possible different influences on the biochemical mechanisms underlying chlorophylls and respectively carotene biosynthesis. The phenomenological background of the observed effects need to be related not only to the iron and cobalt ions impact on the cell metabolism but also to the magnetic influence exerted by nanometric metal ion oxides attached to some biological structures.

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