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BRIEF COMMUNICATIONS

Influence of Fe^0 Nanoparticles, Magnetite Fe_3O_4 Nanoparticles, and Iron (II) Sulfate ($FeSO_4$) Solutions on the Content of Photosynthetic Pigments in *Triticum vulgare*

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Abstract—Seeds and seedlings of soft wheat (*Triticum vulgare* Vill.) were used to study seed germination, leaf elongation, and the content of photosynthetic pigments (chlorophylls *a, b* and carotenoids) as affected by five concentrations of iron-containing nanoparticles (NP): spherical Fe^0 NP with the diameter of 80 ± 5 nm and the magnetite Fe_3O_4 NP measuring 50–80 nm in width and 4–10 nm in height. The effects of $FeSO_4$ solutions were also tested for comparison. The parameters examined varied as a function of the exogenous agent applied, the agent concentration, and the exposure duration. The highest sensitivity of seedlings was observed in the presence of increasing concentrations of iron (II) sulfate in the nutrient medium. This was evident from the decrease in seed germination percentage, inhibition of leaf growth, and the diminished content of photosynthetic pigments. The apparent toxicity of iron nanoforms varied depending on the parameter examined. (1) The strongest inhibition of germination was exerted by Fe^0 NP (toxicity assessed from germination percentage was 3.3% higher with Fe^0 NP than with magnetite NP); (2) the inhibition of leaf elongation on the 4th day after germination was most evident in the presence of Fe^0 NP (a 12% stronger inhibition in the presence of Fe^0 NP than in the presence of magnetite NP), whereas on the 7th day the inhibition was most pronounced with magnetite NP (a 9% stronger inhibition in the presence of Fe_3SO_4 NP than in the presence of Fe^0 NP); (3) the lowest total content of photosynthetic pigments on the 4th day of seedling growth was noted in the presence of magnetite NP (8% lower in the presence of Fe_3SO_4 NP than in the presence of Fe^0 NP), whereas on the 7th day the lowest pigment pool was observed in the presence Fe^0 NP (a 3% reduction compared to that in the presence of magnetite NP). The highest content of photosynthetic pigments was recorded in the presence of 0.125 and 0.001 g/L of Fe^0 NP, 0.5 g/L and 1 μg/L of Fe_3O_4 NP, and 1 mg/L $FeSO_4$.

Keywords: Triticum vulgare, nanoparticles, iron, magnetite, content of photosynthetic pigments, chlorophylls *a* and *b*, carotenoids, seed germination; seedlings, leaf length

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INTRODUCTION

Iron is an essential nutrient that constitutes the largest portion in the total content of metal microelements in plant cells. The average content of this element in plants is 20–80 mg/kg dry wt. Iron is a cofactor for about 140 plant enzyme systems (peroxidases, catalases, ferredoxin, cytochrome, etc.) [1–3]. Iron plays a significant role in the formation of chloroplast thylakoids. Each unit of photosystem I and II contains about 20 iron atoms [3–5]. Since iron transport in plants is hampered because of iron binding with citrate, the element deficiency in young tissues may appear in the form of interveinal chlorosis [3, 4]. Iron deficiency inhibits primarily photosystems I and II and lowers the content of photosynthetic pigments, especially of the total chlorophyll content. The decrease in the amount of green pigments is mainly due to the retarded synthesis of δ-aminolevulinic acid, a precursor of chlorophyll and porphyrins. The stress related to iron deficiency generates reactive oxygen species (ROS) that modify pigments and initiate lipid peroxidation and membrane destruction [3]. However, some plant species, for example from the family Poacea, respond to iron deficiency by the "strategy II" mechanism: they release low-molecular chelate substances, phytosiderophores. These substances produce stable complexes with Fe^{3+}, thus promoting the transfer of a poorly accessible form Fe^{3+} across the plasmalemma to the cytoplasm by means of a highly specific transport system [6].

In the last few years the unique properties of iron-containing nanomaterials and derived nanoclusters are widely used in various areas [7]. Small sizes of these particles (from 1 to 100 nm) and a large surface area determine specific physicochemical properties of nanoparticles (NP) that differ from properties of the respective macroscopic forms [8, 9]. For example,

Abbreviations: Chl *a* and Chl *b*—chlorophylls *a* and *b*; NP—nanoparticles.

metal NP smaller than 10 nm are highly reactive owing to energy excess; the NP with dimensions of about 1 nm tend to aggregate and react with other chemical substances [10]. It is known that metabolic permeation of NP into the plant cell is restricted by the diameter of cell wall pores (from 5 to 20 nm) [11, 12]. However, the permeation of NP can also occur by means of endocytosis, transporter proteins, as well as through ion channels. After entering the cytoplasm, NP can bind to various organelles, thus altering physiological and intracellular plant functions [13].

The occurrence of NP in a metastable non-equilibrium state allows researchers to study novel and hardly predictable physiological and biochemical processes in plants. Scarce data suggest that presowing treatment of seeds with iron NP has a positive influence on germination energy at NP concentration of 0.001% and exerts negative influence at NP concentrations up to 0.01% [14]. Some authors revealed the stimulatory effect of metal NP on chlorophyll content and plant growth [15–17]. Other researchers noted chromosomal aberrations in juvenile plants treated with magnetic NP; the treatment with magnetite led to the elevation and inhibition of chlorophyll content at low and high concentrations, respectively [18, 19]. It was also found that the excess of magnetic NP in living plant tissues results in oxidative stress and inhibits photosynthesis [19].

The existence of such phenomena indicates that the impact of NP-related stress on plant pigments at a wide range of NP concentrations is quite an actual problem. Therefore, the aim of this study was to examine the influence of iron NP, magnetite NP, and iron (II) sulfate on germination of *Triticum vulgare* seeds, as well as on seedling growth and the content of photosynthetic pigments in shoots of seedlings grown under laboratory conditions.

MATERIALS AND METHODS

Experiments were conducted using laboratory facilities of the Institute of Bioelementology, Orenburg State University. The Fe^0 nanoparticles were obtained by the method of high-temperature condensation with a MiGen laboratory system (Institute of Energy Problems in Chemical Physics, Russian Academy of Sciences). Preliminary investigation of NP morphology with a JSM-7401F electron scanning microscope (JEOL, Japan) revealed that these particles are spheres with a diameter of 80 ± 5 nm (data not shown). For comparison we also used iron (Fe^0) spherical microparticles measuring 10 μm (Alfa Aesar, Germany) and iron (II) sulfate solutions ($FeSO_4 \cdot 7H_2O$, laboratory grade, Lenreaktiv, Russia). The Fe_3O_4 nanoparticles (magnetite, $FeO \cdot Fe_2O_3$) measuring 50–80 nm in width and 4–10 nm in height were prepared by the method of chemical interaction of ammonia hydrate with $FeCl_2$ and $Fe_2(SO_4)_3$ [20]. Subsequent procedures involving magnetic dispersion of NP were conducted in several steps according to a previously described method [21].

The seeds and shoots of soft wheat (*Triticum vulgare* Vill.) seedlings were used to analyze the action of iron compounds on plants under laboratory conditions. Seeds were surface-sterilized with 0.01% $KMnO_4$ solution for 5 min, triply washed with distilled water, and placed on filter paper into plastic containers (30 seeds per dish). Next, a 5-mL volume of freshly prepared solutions of iron NP, magnetite NP, and iron (II) sulfate was added to each sample. Solutions were prepared in distilled water at concentrations (iron content) of 2.0, 0.5, 0.125, 0.001, and 10^{-6} g/L. Solutions were prepared by placing weighed amounts of substances into glass flasks with distilled water and by subsequent 15-min ultrasound treatment at a frequency of 35 kHz in a bath-type sonicator system (Sapfir TTTs, ZAO PKF Sapfir, Russia).

Untreated (control group) plants were grown on distilled water. The treated and untreated seedlings were grown for 7 days under natural illumination at air temperature of 23 ± 1°C. In order to prevent drying, all plant samples were watered every other day with 5 mL of distilled water. We did not use nutrient solutions to avoid the risk of possible redox status changes in tested plants.

The percentage of seed germination was determined on the 3rd day of experiment according to GOST state standards [22]. The content of chlorophyll *a* (Chl *a*), Chl *b*, and carotenoids was determined on the 4th and 7th days of experiment by measuring the absorbance of ethanol extracts. Measurements were made with a KFK-3 photometer (Russia) according to Shlyk [23]. The ethanol extracts were prepared from averaged samples of plant material. Ten plants were selected in each sample to excise one leaf per plant. The collected fresh material was weighed on an analytical balance VL-210 (Russia) with the precision of 0.1 mg and then ground in a porcelain mortar in the presence of $CaCO_3$ (to neutralize the acidic content of the cell sap), the calcined quartz sand (to improve grinding), and 3 mL of 96% ethanol. The homogenate was filtered repeatedly until complete extraction of photosynthetic pigments; the extract in a volumetric flask was adjusted to the volume of 25 mL with the solvent, and then the absorbance was measured in 1-cm^3 optic cells.

All experiments were performed in three replicates with three assays per replicate. The results were treated statistically using Microsoft Excel and were presented as mean values and standard deviations. The statistically significant differences in parameters between treated and untreated samples were revealed with the Student's *t*-test at $p < 0.95$.

RESULTS

The percentage of seed germination is a basic indicator of phytotoxicity for a wide range of xenobiotics.

Effect of various concentrations of iron compounds on seed germination and leaf length in *Triticum vulgare* seedlings

Treatment	Concentration, g/L	Germination capacity, %	Leaf length, mm	
			4th day	7th day
NP Fe^0	2.0	88.89 ± 1.93	4.67 ± 1.05	12.52 ± 1.28
	0.5	93.67 ± 1.64	5.08 ± 0.72	12.62 ± 1.04
	0.125	96.17 ± 0.16	5.62 ± 0.56	13.53 ± 0.49
	1×10^{-3}	98.89 ± 1.11	7.52 ± 0.84	14.47 ± 1.07
	1×10^{-6}	97.78 ± 2.22	11.63 ± 0.64	13.08 ± 0.41
NP Fe_3O_4	2.0	74.44 ± 9.49	7.22 ± 0.57	8.15 ± 0.55
	0.5	95.55 ± 2.22	9.58 ± 0.87	13.62 ± 1.29
	0.125	95.56 ± 1.11	10.30 ± 1.01	15.95 ± 1.32
	1×10^{-3}	97.74 ± 1.13	11.68 ± 0.63	17.18 ± 1.34
	1×10^{-6}	98.89 ± 1.13	11.80 ± 0.73	17.43 ± 0.65
NP $FeSO_4$	0.5	7.78 ± 4.01	–	–
	0.125	9.72 ± 2.26	–	–
	1×10^{-3}	85.32 ± 4.33	7.27 ± 1.82	15.38 ± 1.28
	1×10^{-6}	88.81 ± 2.14	11.72 ± 0.77	17.70 ± 1.72
Untreated		95.84 ± 0.96	11.97 ± 0.57	19.23 ± 0.36

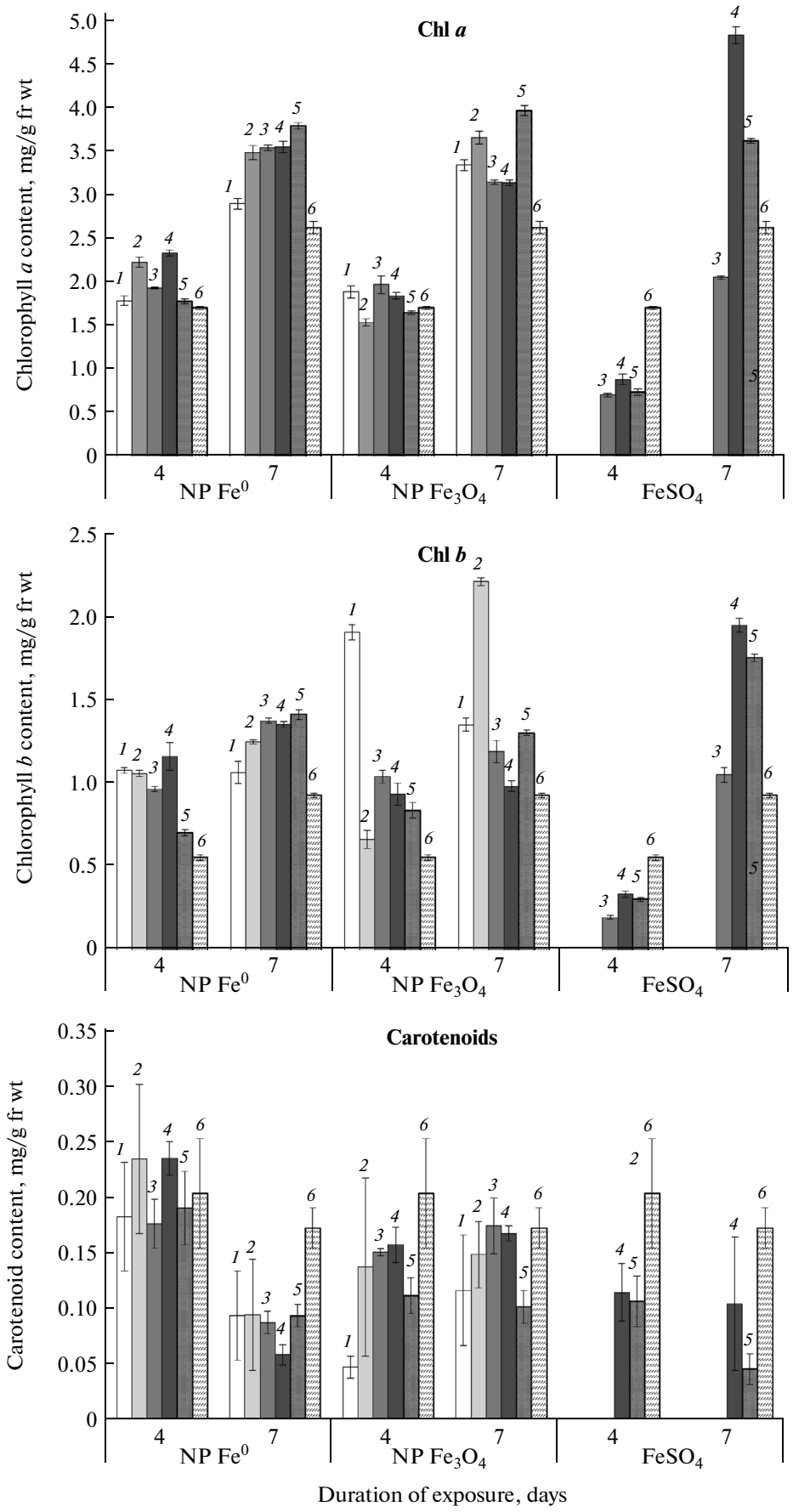
As shown in the table, the germination percentage of wheat seeds depended on the concentration and the colloid condition of the chemical agent added. Our analysis revealed that the sulfate form of iron had the strongest inhibitory action on seed germination. This was particularly evident at increasing concentrations of the salt added into the test system. For example, at maximal $FeSO_4$ concentrations of 2.0, 0.5, and 0.125 g/L, the seed germination percentage reduced by 100, 92, and 90%, respectively. On the other hand, a very slight stimulating action on seed germination was observed in the presence of 1 mg/L of Fe^0 NP and 1 μg/L of magnetite (Fe_3O_4) NP; in both treatments the germination percentage was 3% higher than in untreated samples (table). By comparing the effects of iron nanoforms on seed germination, we found that the average germination percentage was higher for seeds treated with Fe_3O_4 NP (95%) than for seeds treated with Fe^0 NP (92%).

Measurements of leaf lengths revealed the dynamics similar to that of seed germination. Specifically, the increase in iron concentration impeded shoot growth (table). The treatment of plant samples with nanoparticles and ionic iron forms had never stimulating effect on leaf elongation; on the contrary, these treatments always reduced the leaf length both on the 4th and 7th days of the experiment (table). It should be recalled that the inclusion into the medium of 1 mg/L Fe^0 NP and 1 μg/L Fe^0 NP ensured the highest seed germination percentage (99%) among the all treatments tested. The incubation of wheat seeds in the presence of 0.5 and 0.125 g/L $FeSO_4$ resulted in the plant death on the 4th day of the experiment. By contrast, solutions containing equivalent concentrations of magnetite NP promoted leaf elongation by 89% and 100% compared to solutions of iron NP and iron (II) sulfate, respectively (table). Comparative analysis of iron nanoforms revealed that the inhibitory impact of iron NP on the 4th day was 12% stronger than that of magnetite NP, whereas the impact of iron NP on the 7th day was weaker by 9% than that of magnetite NP.

The analysis of photosynthetic pigment content in leaves of *T. vulgare* seedlings showed that the dynamics of Chl *a* and Chl *b* content was positive and more stable under the treatments with iron nanoforms compared to treatments with iron (II) sulfate. For example, the content of Chl *a* and *b* increased above the control level in the presence of Fe^0 NP (by 16–26 and 19–30%, respectively) and in the presence of magnetite NP (by 7–20 and 22–38%, respectively) (figure).

A clear increase in chlorophyll content was observed after 7-day contact of plants with solutions containing 1 μg/L iron NP (44%) and magnetite NP (51%). By contrast, the presence in solution of iron sulfate (Fe_3O_4) diminished the average content of Chl *a* and *b* by 56 and 52%, respectively. Despite the discovered negative influence of iron sulfate, it should

Content of photosynthetic pigments in leaves of *T. vulgare* after 4- and 7-day exposure of seedlings on solutions with various concentrations of iron compounds.
1—2.0 g/L, *2*—0.5 g/L, *3*—0.125 g/L, *4*—1 mg/L, *5*—1 μg/L, *6*—untreated.


Chl a
Chlorophyll a content, mg/g fr wt
Chl b
Chlorophyll b content, mg/g fr wt
Carotenoids
Carotenoid content, mg/g fr wt
NP Fe0
NP Fe3O4
FeSO4
Duration of exposure, days

be noted that the chlorophyll content in shoots of seedlings treated with 1 mg/L $FeSO_4$ increased on the 7th day of treatment to 4.8 mg/g fr wt, which was 84% higher than the control value and 36 and 55% higher than in treatments with iron NP and magnetite NP, respectively (figure).

Our data on the dynamics of yellow pigments showed that carotenoid content in untreated shoots was 0.1–0.5 mg/g fr wt and decreased in seedlings treated with Fe^0 NP, Fe_3O_4 NP, and $FeSO_4$ by 10–52%, 28–37%, and 45–49%, respectively. The strongest inhibition was observed in the treatments with magnetite NP (Fe_3O_4, 2 g/L) and $FeSO_4$ (at concentrations above 0.125 g/L) (figure).

DISCUSSION

Complex assessment of physiological parameters of *T. vulgare* seedlings grown in the presence of different forms of iron nanoparticles led us to conclude that the plant organism is an intricate flexible system readily reacting to the presence of iron NP in the medium. This was manifested in changes of seed germination, growth rate, and the content of photosynthetic pigments in leaves. The highest sensitivity of wheat seedlings was observed in solutions with increasing $FeSO_4$ concentrations. This was evident from the inhibition of seed germination (by 59.9% with respect to germination percentage of untreated seeds), leaf elongation (by 68.27% and 65.6% on the 4th and the 7th days, respectively), and biosyntheses of photosynthetic pigments.

The apparent toxicity of iron nanoforms varied depending on the parameter examined. (1) The strongest inhibition of germination was exerted by Fe^0 NP (the toxicity was 3.3% higher with Fe^0 NP than with magnetite NP); (2) the inhibition of leaf length on the 4th day after germination was most evident in the presence of Fe^0 NP (a 12% stronger inhibition in the presence of Fe^0 NP than in the presence of magnetite NP), whereas on the 7th day the inhibition was most pronounced with magnetite NP (a 9% stronger inhibition in the presence of Fe_3SO_4 than in the presence of Fe^0 NP); (3) the lowest total content of photosynthetic pigments on the 4th day of seedling growth was noted in the presence of magnetite NP (8% lower than in the presence of Fe^0 NP), whereas on the 7th day it was observed in the presence Fe^0 NP (3% reduction compared to pigment content in the presence of magnetite NP).

The stimulating effect of iron compounds on examined parameters was observed at low iron concentrations: 0.125 and 0.001 g/L Fe^0, 0.5 g/L and 1 μg/L Fe_3O_4, and 1 mg/L $FeSO_4$. This effect might be due to the high reaction capacity of NP. The nanoparticles may be oxidized to Fe^{2+}, $Fe(OH)_2$, or $Fe(OH)_3$, and these forms would be absorbed on the oxide film around NP [8, 24–26]; they may produce agglomerates inaccessible for the root system and may exert prolonged action on the tissue level. On the whole, the content of Chl *a* and *b* showed the trend to the increase that was concurrent with the decrease in carotenoid content. One may assume that the treatment with iron compounds facilitated the retention of chlorophyll pool owing to maintenance of carotenoid content at a certain level. This role of carotenoids is likely since carotenoids are able to quench chlorophyll excitations and prevent the production of singlet oxygen and other ROS [24, 25].

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