KSÜ Mühendislik Bilimleri Dergisi, 25(1), 2022 KSU J Eng Sci, 25(1), 2022
Araştırma Makalesi Research Article

Kahramanmaras Sutcu Imam University
Journal of Engineering Sciences

Geliş Tarihi: 23.10.2021 Received Date : 23.10.2021
Kabul Tarihi: 10.12.2021 Accepted Date : 10.12.2021

THE ECOTOXICOLOGICAL EVALUATIONS OF FE_3O_4, HAp, AND FE_3O_4-HAp NANOCOMPOSITE ON WHEAT: IMPACT ON CHLOROPHYLL CONTENT

FE_3O_4, HAp, VE FE_3O_4-HAp NANOKOMPOZİTİNİN BUĞDAY ÜZERİNDEKİ EKOTOKSİKOLOJİK DEĞERLENDİRMESİ: KLOROFİL İÇERİĞİNE ETKİSİ

Z. Görkem DOĞAROĞLU[1] (ORCID: 0000-0002-6566-5244)
Yağmur UYSAL[1]* (ORCID: 0000-0002-7217-8217)

[1] Mersin Üniversitesi, Çevre Mühendisliği Bölümü, Mersin, Türkiye

*Sorumlu Yazar / Corresponding Author: Yağmur UYSAL, yuysal@mersin.edu.tr

ABSTRACT

Nanoparticles have an increasing accumulation and effect as day to day in aquatic, terrestrial and atmospheric environments, and one of the most basic factors determining these effects is their sizes. As the material size decreases, the distribution and accumulation of particles are facilitated and accelerated. In this study, the possible nanotoxicological effects of nanomagnetite (Fe_3O_4 NPs), hydroxyapatite (HAp) (synthesized by the recovery of waste eggshells) and Fe_3O_4-HAp nanocomposite on wheat germination percentage and chlorophyll production were evaluated. It was determined with SEM images that the Fe_3O_4 nanoparticles was in the size of 22-30 nm, while the Fe_3O_4-HAp was 90-350 nm. The presence of HAp particles caused a decreasing in the germination percentage compared to presence of only Fe_3O_4 nanoparticles, as like in the root elongation (20-27%). The chlorophyll content was determined in the both aged and young leaves on second, third and fourth weeks of wheat plants. Results showed that wheat plants were sensitive in the early stage of plant growth (second week) to the all test chemicals. The presence of HAp in the growth media decreased the chlorophyll content of wheat because of their sizes. The maximum decreasing of chlorophyll content in wheat was observed at the 40 mg L^{-1} HAp treatment as 86%. All the test chemicals used in this study uses in many areas, thus it should be detailed evaluated ecotoxicological aspect.

Keywords: Chlorophyll, hydroxyapatite, nanoparticles, magnetite, phytotoxicity

ÖZET

Nanopartiküller sucul, karasal ve atmosferik ortamlarda gün geçtikçe artan bir birikim ve etkiye sahiptirler ve bu etkileri belirleyen en temel faktörlerden bir tanesi boyutlarıdır. Malzeme boyutu azaldıkça partiküllerin doğadaki dağılım ve birikimleri kolaylaşır ve hızlanır. Bu çalışmada, nanomanyetit (Fe_3O_4 NPs), hidroksiapatit (HAp) (atık yumurta kabuğunun geri kazanımı ile sentezlenen) ve Fe_3O_4-HAp nanokompozitinin olası nanotoksikolojik etkileri, buğday çimlenme yüzdesindeki değişiklikler ve klorofil üretimindeki etkileri olarak değerlendirilmiştir. SEM görüntüleri incelendiğinde Fe_3O_4 nanopartikülünün boyutlarının 22-30 nm, Fe_3O_4-HAp nanokompozitinin ise 90-350 nm aralığında olduğu tespit edilmiştir. HAp partiküllerinin varlığı sadece Fe_3O_4 nanopartiküllerinin varlığına kıyasla kök uzamada olduğu gibi (%20-27), çimlenme yüzdesinde de bir azalmaya neden olmuştur. Buğday bitkisinin klorofil içerikleri hem genç hem de yaşlı yapraklarda olmak üzere ikinci, üçüncü ve dördüncü haftalarda ölçülmüştür. Sonuçlar, buğdayın büyümesinin erken aşamasında (ikinci hafta) tüm test kimyasallarına daha duyarlı olduğunu göstermiştir. Bitki büyütme ortamındaki HAp varlığı, boyutlarından ve demir kullanımını inhibe ettiğinden buğdayın klorofil üretimi azaltmıştır. Klorofil içeriğindeki maksimum azalma 40 mg L^{-1} HAp uygulamasında %86 olarak bulunmuştur. Bu çalışmada kullanılan tüm test kimyasalları birçok alanda kullanılmaktadır, bu nedenle ekotoksikolojik yönüyle ayrıntılı olarak değerlendirilmelidir.

Anahtar Kelimeler: Fitotoksisite, hidroksiapatit, klorofil, nanopartikül, manyetit

ToCite: DOĞAROĞLU, Z.G., & UYSAL, Y., (2022). THE ECOTOXICOLOGICAL EVALUATIONS OF FE_3O_4, HAp, AND FE_3O_4-HAp NANOCOMPOSITE ON WHEAT: IMPACT ON CHLOROPHYLL CONTENT. *Kahramanmaraş Sütçü İmam Üniversitesi Mühendislik Bilimleri Dergisi*, 25(1), 7-16.

INTRODUCTION

Plants are one of the most important components of human and animal's life. They can be face to face with different toxic or non-toxic chemicals at any stage of their life, due to some human activities. In recent years, new chemicals, known as nanoparticles, with some unique properties have started to be produced as a result of developing and changing technologies. These particles have special properties, such as reduction in size, increasing surface/volume ratio, some physical, optical, and mechanical properties. The increasing production and use and thus disposal of these particles in every field, caused releasing into the environmental media (aquatic, terrestrial, and atmospheric). For this reason, it is important to determine the effects of nanoparticles on plants, especially plants in food chain. Plants can internalize, accumulate, and/or give some different (enzymatic or non-enzymatic) reaction to these special particles.

One of the most affected plant structures by nanoparticles is photosynthetic pigments. Photosynthetic pigments are chemical elements which responsible for absorbing the light that necessary for the photosynthesis process to occur. Chlorophyll is a type of photosynthetic pigments, plays a critical role in the photosynthesize process (Mezacasa et al., 2020; Borhan et al., 2017). The chlorophyll measurement is made to determine plants health, photosynthetic performance, and chloroplast development (Ji et al., 2020). The chlorophyll concentration generally is determined via spectrophotometric methods; however, these methods need some toxic and flammable chemicals. Nowadays, with the development of new technological devices, chlorophyll content can be easily determined. Thus, with these devices fast, on-site, without destroying the plant tissues, and simple measurements that can also be performed by farmers, can be realized (Hawkins, Gardiner, and Comer, 2008). The chlorophyll meter (SPAD-502) is a small spectrophotometer that measures the absorbance of light at a wavelength of 650 nm, making it easy to monitor physiological changes in plants and does not require any chemicals (Hawkins et al., 2008). The use of this chlorophyll meter has been applied in many scientific studies (Mezacasa et al., 2020; Borhan et al., 2017; Dray Jr., Center, and Mattison, 2012).

Iron is an essential element for plants, animals and humans and is a micronutrient that needed in small amounts (Bolat and Kara, 2017). Plants use iron (Fe(II) and Fe(III)) for many cellular and metabolic activity such as photosynthesis, nitrogen fixation, DNA synthesis (Ghafariyan et al. 2013). Today, there are many types of iron-based nanoparticles (magnetite, maghemite, superparamagnetic iron oxide etc.) that are used in many different fields. Iron dissolved from these nanoparticles may be potential nutrient source for plants. Magnetite particles have both Fe (II) and Fe (III) ionic forms in the structure, thus it has different physico-chemical properties from other iron oxide forms (Su, 2017). Studies related to the effects of magnetite (Fe_3O_4) nanoparticles on germination, plant growth, and antioxidative enzymes of wheat and other plants, are included in the literature (De Souza et al. 2021; Iannone et al., 2016). De Souza et al. (2019) showed that the Fe_3O_4 nanoparticles significantly increased the soil nutrient content and Iannone et al. (2016) also indicated that the Fe_3O_4 nanoparticles (~ 11 nm) can be used in agricultural applications because of its non-toxic feature for wheat plants. Although hydroxyapatite, obtained from eggshell, is generally used in many fields, such as biomedicals as bone substitute, regenerative dentistry, ceramic production, and water and air treatment as absorbent (Girelli, Astolfi, and Scuto, 2020), studies about the application of hydroxyapatite (HAp) on agriculture is very limited (Rop et al., 2018). However, HAp obtained from eggshells is a valuable waste material that needs to be recycled, due to the nutrients it contains.

There are many studies in the literature related to magnetite nanoparticles, but according to our knowledge there are not any study about the individual effects of these two particles, and the effect of magnetite-hydroxyapatite nanocomposite on the chlorophyll content of wheat plants. In this study, the effects of Fe_3O_4, HAp and Fe_3O_4-HAp nanocomposite particles on the chlorophyll content of wheat plants were evaluated.

MATERIALS AND METHOD

Fe_3O_4 Nanoparticles, Hydroxyapatite and Nanocomposite Preparation

In this study, magnetite particles were synthesized according to Petcharoen and Sirivat (2012). Briefly, Fe_3O_4 solution was prepared by adding 6.1278 g of solid $FeCl_3.4H_2O$ and 3.0121 g of solid $FeCl_2.6H_2O$ into 100 mL of deionized water under N_2 medium. When the solution temperature reached 85 ^oC, 25 mL of ammonia (NH_3, 25% purity) solution was added and solution stirred for 30 minutes to be a homogeneous mixture. Then this solution cooled at room temperature and a black precipitate formed. The formed Fe_3O_4 nanoparticles were washed several times with distilled water and separated with a neodymium magnet.

Eggshells (ESs), used in this study, were collected from Mersin University-Turkey main cafeteria. Firstly, waste ESs washed three times with tap water to remove the organic substance and then washed with hexane and distilled water for disinfection. The membrane layer was manually peeled off in the ESs and dried in the oven overnight at 80 °C. Finally, the dried eggshells were ground and sieved with a mesh of 212 μm.

To prepared Fe_3O_4-HAp nanocomposite, magnetite solution was prepared according to mentioned before, but the solution stirred for 2 minutes to be a homogeneous mixture. 3 g ESs powder was added in this solution and stirred out in nitrogen during 30 min and cooled at room temperature. The black precipitate formed in the solution. The obtained Fe_3O_4-HAp nanocomposite were washed several times with distilled water and separated with a neodymium magnet (Petcharoen and Sirivat, 2012).

Seed Germination and Root-Shoot Elongation

The wheat seeds (*Triticum aestivum* - İkizce 96) were purchased from Mersin Province, Turkey, kept at 4-5 ^{o}C for 3 days before the germination processes. The seeds in uniform size were sterilized 3 % sodium hypochlorite for 10 min after the treatment of 70% ethanol for 30 s. Seeds were washed several times with deionized water to remove surface residues. To determine the seed germination and seedling vigor index, 10 seeds in uniform size were placed in petri dishes (100 x 15 mm, glass petri) included double layer filter paper. 5 mL test chemicals (Fe_3O_4, HAp, and Fe_3O_4-HAp nanocomposite) at different concentrations were added into the petri dishes and incubated 7 days at 25 ^{o}C in the dark. After the germination process, the number of germination seeds were counted in every dish and the seedling vigor index was calculated according to Doğaroğlu, Eren, and Baran (2019) and the root and shoot elongation was measured using millimetric paper. Then the germinated plants were transferred to the pots included 50 g turf (pH 6–8, total N: 0.2–0.45%, water holding capacity: 300–450%) and watered with tap water every second days. After the sowing the seedlings, 10 mL test chemicals were added to pots and the plants were grown for 28 days. All steps were realized in three replications.

Chlorophyll Content

Chlorophyll content was measured every week, after the planting. The measurements were done for three weeks. The chlorophyll content was measured at the fully expanded aged and young leaves using SPAD-502 chlorophyll meter (Konica-Minolta, Osaka, Japan 0.06 cm^2 measurement area and its accuracy is ±1.0 SPAD units) (Lin et al., 2010). The measurements were realized every week, between 13:00-16:00 h at the middle of same leaf three times. The mean of three recordings data were given as SPAD value.

Statistical Analyzes

The statistical analysis of chlorophyll content was performed using one-way analysis of variance (ANOVA) with LSD test. SPSS Statistic program version 20 was used. The significance of difference for all measured were calculated and the mean was compared by the least significant difference, LSD, $p<0.05$.

RESULTS AND DISCUSSION

Characterization of Test Chemicals

The size and morphology of synthesized Fe_3O_4 NPs, HAp, and Fe_3O_4-HAp nanocomposite particles were realized by field emission scanning electron microscopy (FE-SEM). It was observed that Fe_3O_4-NPs have a regular and spherical grain size in the range of 22-30 nm (Fig. 1a) while, HAp particles have porous, nano and/or microscale irregular sizes (Fig. 1b). It has been determined that the structure of HAp has a planar shape and that there are pores of different sizes (90-350 nm) on it. In the SEM images of the Fe_3O_4-HAp nanocomposite, it was seen that Fe_3O_4-NPs were placed inside the HAp pores and on the surface of the composite (Fig. 1c).

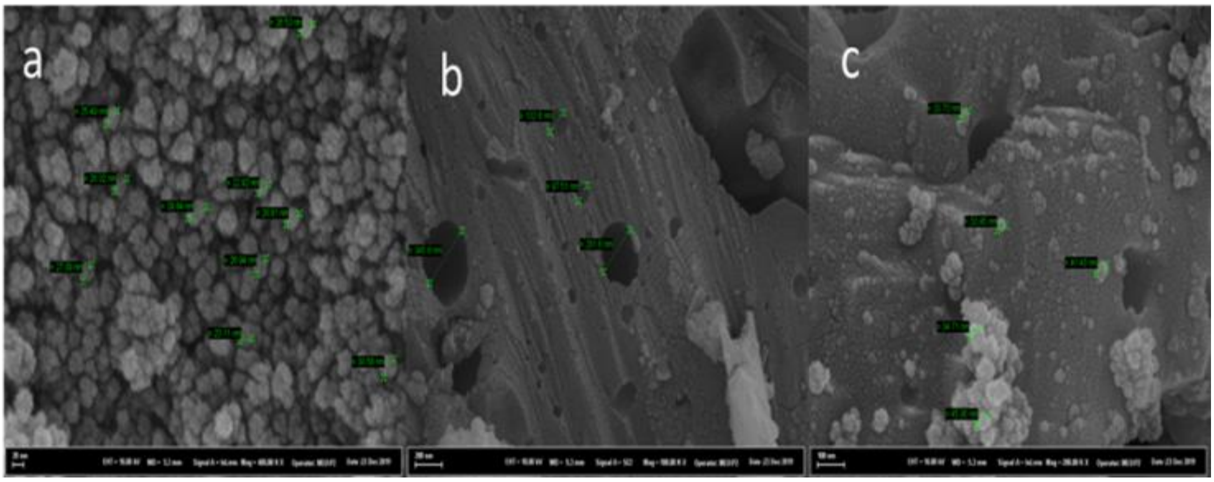


Figure 1. FE-SEM images of a) Fe_3O_4 NPs, b) HAp, and c) Fe_3O_4-HAp nanocomposite

Seed Germination and Root-Shoot Elongation

The effects of Fe_3O_4 NPs, HAp, and Fe_3O_4-HAp nanocomposite on seed germination were evaluated firstly. The minimum germination percentage was determined at the treatment of nanocomposites. The average germination percentage of wheat was determined as 99% for Fe_3O_4 NPs, 97.5% for HAp, and 96% for Fe_3O_4-HAp nanocomposite. However, the changes in the germination percentage was not significant compared to their own control group ($p>0.05$). Similar results are also included in the literature. For example, Iannone et al. (2016) and Iannone et al. (2021) reported that the citrate coated-Fe_3O_4 NPs, in the size of 10 nm, and 14 nm was not affected the seed germination of wheat, and soybean and alfalfa plants, respectively. Nevertheless, the Fe_3O_4 NPs, HAp, and Fe_3O_4-HAp nanocomposite treatment negatively affected the root elongation. The presence of hydroxyapatite in the test media caused an inhibition of root growth and development, compared to control. The root elongation was inhibited with increasing HAp concentrations and the minimum root length was determined in the HAp treatment at 640 mg L^{-1} concentration (Fig.2a). The wheat plants exhibited a decrease of 16.77% at the Fe_3O_4 NPs (320 mg L^{-1}), 27.1% at the HAp (640 mg L^{-1}), and 20.44% at the Fe_3O_4-HAp nanocomposite (40 mg L^{-1}) treatments in root length, compared to control. In contrast to root elongation, the shoot elongation was affected positively (Fig.2b) because the roots have more sensitivity than shoots to external factors. In the germination phase, the roots (radicle) are out of the seed coat earlier than shoots, so they are more exposed to external factors compared to the shoots. In addition, in order to ensure plant growth and development, it causes the roots to be more sensitive to external pollutants or nutrient supplements due to the desire to reach nutrients. The shoot elongation of wheat plants exhibited an increase of 28 % at the Fe_3O_4-NPs (40 mg L^{-1}), 21% at the HAp (40 mg L^{-1}), and 31% at the Fe_3O_4-HAp nanocomposite (640 mg L^{-1}), compared to control. The similar result has been reported by De Souza-Torres et al. (2021). The authors indicated that in very small size (6.7 nm) of 2000 mg L^{-1} Fe_3O_4-NPs caused an increase of 27.5% in shoot length of common bean plants. The seedling vigor index depended on germination percentage and root-shoot length. Seedling vigor index depended on germination percentage and root-shoot length. Thus, it showed that why the SVI decreased in the concentration of 40 mg L^{-1} (due to the root length) and 160 mg L^{-1} (due to root length-germination percentage) Fe_3O_4-HAp nanocomposite. Also, it decreased in the concentration of 640 mg L^{-1} HAp treatments largely originated from low root length (Fig. 2c).

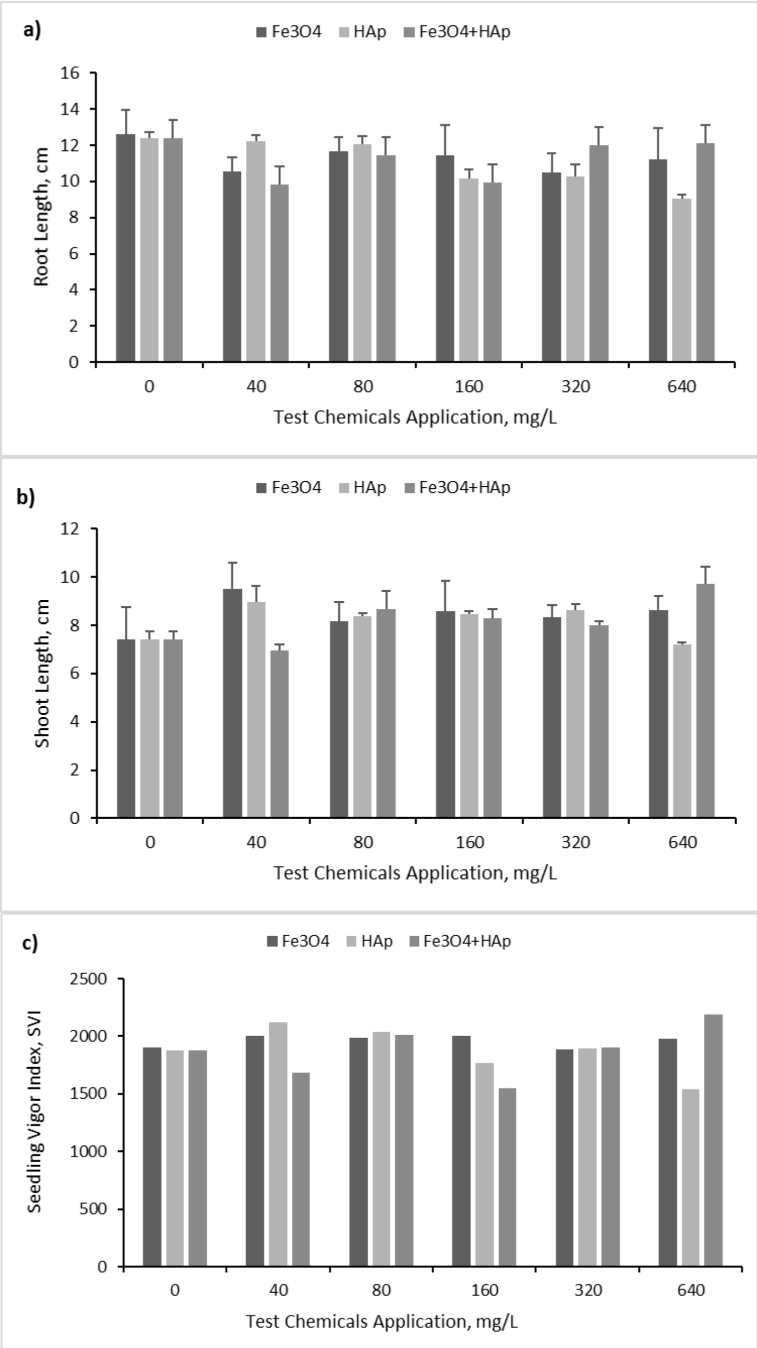


Figure 2. Effects of Magnetite Nanoparticles, Hydroxyapatite, and Magnetite-Hydroxyapatite Nanocomposites on a) Root Elongation, b) Shoot Elongation, and c) Seedling Vigor Index of Wheat

Chlorophyll Content of Wheat

The results showed that the chlorophyll content of aged leaves of wheat exposed to magnetite nanoparticles decreased compared to the results of other weeks (Fig.3a). In the second week of aged leaves, the plants expend energy to produce chlorophyll, due to need photosynthetic activity to grow. Iron is one of the major factors in physiological developments of living organisms and also, it is important to chlorophyll production in plants (Shankramma et al., 2016). It is used as an enzyme cofactor for photosynthetic reactions (Al Amri et al., 2020). In generally, iron-based nanomaterials, used as nano-fertilizers, can be used iron supplier for plants, especially in Fe-deficient soils. The results of our study for Fe_3O_4-NPs in chlorophyll content of aged leaves in second week showed significantly changes at the 40 mg L^{-1} (12.9% decrease) and 160 mg L^{-1} (9.8% increase) concentrations, compared to control ($p<0.05$). In the third and fourth weeks, the chlorophyll contents of aged leaves were decreased compared to both control plants and second week, and there were statistically significant within the concentration groups ($p<0.05$). On the other hand, an increasing trend was observed in young leaves on third week and in the next week the chlorophyll content showed no differences compared to control (Fig.3b) ($p>0.05$). Similarly, the authors showed that the chlorophyll content of muskmelon exposed to Fe_3O_4-NPs were lower than control in the second week, it increased in the third week and in the fourth week the chlorophyll content has no change compared to control Wang et al. (2019).

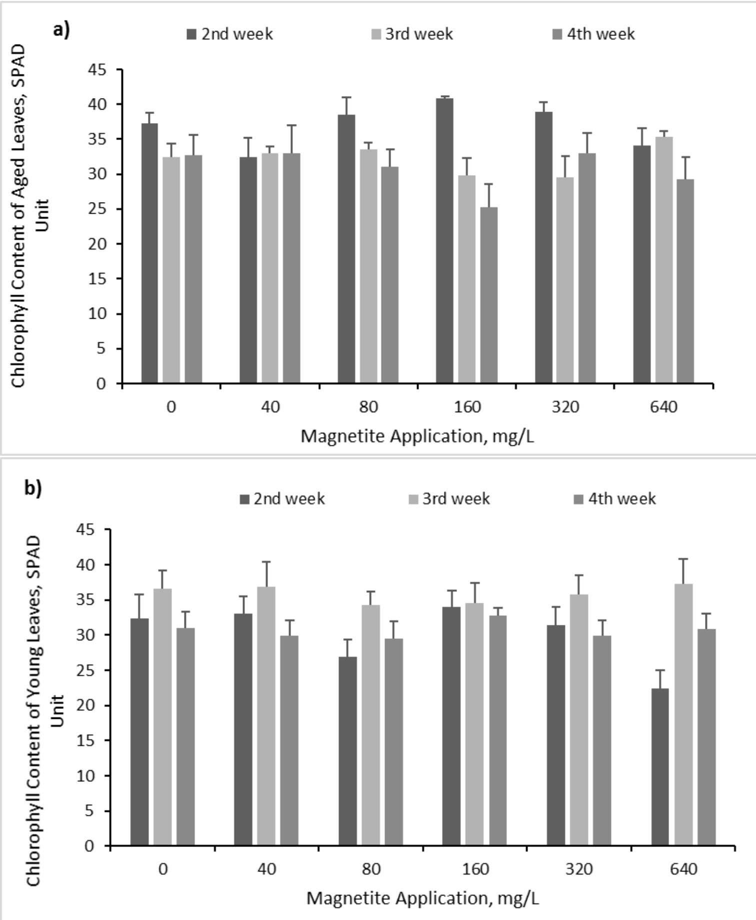
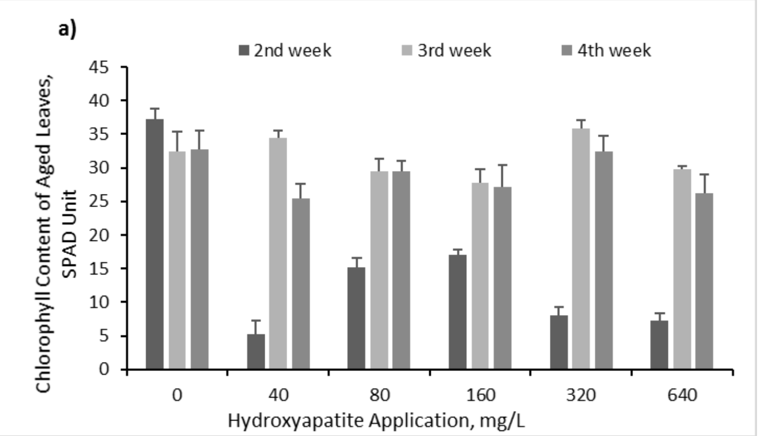


Figure 3. Effects of Magnetite Nanoparticles on Chlorophyll Content of a) Aged Leaves and b) Young Leaves of Wheat

Although hydroxyapatite particles have the potential to be evaluated as agricultural nutrients sources, the application areas are focused on biomedical areas (Rop et al., 2018). The main source of phosphorus in the soil is rocks and minerals (apatite) and also organic materials include organic phosphorus. However, the large part of the phosphorus in the soil is in the form of which plants cannot benefit because phosphate ions are kept tight in the soil (Bolat and Kara, 2017). Plants affect from the phosphorus in the soil since it effects root growth and development, plant maturation, early seed formation, fertilization and increases the resistance to diseases (Bilen ve Sezen, 1993). Feng et al. (2021) showed that there were positive effects of HAp on winter wheat. The authors reported that the plants were well grow, healthy, and greenish. On the other hand, if the phosphorus is too much in the soil, the deficiency of micronutrient elements such as zinc and iron likely to occur (Bolat and Kara, 2017). In this study, it was observed that the first leaves (aged leaves), the chlorophyll formation was inhibited by the presence of HAp compared to control, especially in the second week ($p<0.05$). It is supposed that the second week was the adaptation period for plants to HAp, because in the next weeks the chlorophyll content increased but was not as much as the control (Fig. 4a). The young leaves did not showed sensitivity to HAp as much as aged leaves, since the adaptation period was finished in second week (Fig.4b). The dose-depend-chlorophyll content observation showed that the HAp concentration was not statistically significant in third week (except 40 and 160 mg L^{-1}) and fourth week (except 40 mg L^{-1}), compared to control.



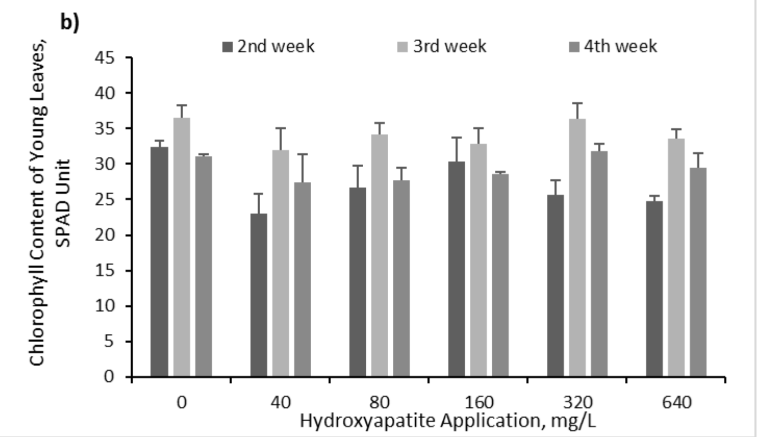


Figure 4. Effects of Hydroxyapatite on Chlorophyll Content of a) Aged Leaves and b) Young Leaves of Wheat

Although the magnetic nanoparticles have low toxicity and their biologically compatible (Yang, Gong, and Zhang, 2010), the aggregation process makes magnetite nanoparticles difficult to internalized to plant cell (Dağlioğlu and Yilmaz, 2018). Hydroxyapatite particles is the best inorganic auxiliary material that prevent aggregation of Fe_3O_4 (Kermanian, Naghibi, and Sadighian, 2020). However, the chlorophyll content of aged leaves treated with magnetite-hydroxyapatite nanocomposite have shown an opposite tendency compared to treated with magnetite nanoparticles. It was assumed that the particles size is the main factor in this tendency. The chlorophyll content increased in the second week at 40 mg L^{-1} nanocomposite concentration, while it decreased at the increasing nanocomposite concentrations ($p<0.05$). The minimum and the maximum decreases in second week were calculated as 20.4% and 39.5% at the 640 mg L^{-1} and 80 mg L^{-1} nanocomposite concentrations, respectively. The significantly decreases were observed in the third week at the increasing nanocomposite concentration (302 and 640 mg L^{-1}, $p<0.05$), while were not observed significantly changes in chlorophyll content of aged leaves in fourth week ($p>0.05$), compared to control (Fig. 5a). According to Bolat and Kara (2017) the symptoms of iron deficiencies appear on young leaves as decrease in plant growth and production of chlorophyll. Similarly, in this study, it was observed that the chlorophyll content decreased in young leaves, since the uptake of iron from Fe_3O_4-HAp nanocomposite was inhibited due to their sizes (Fig. 5b). Especially, the chlorophyll content of in the early stage of plant growth (second week) was very low ($p<0.05$), except 80 mg L^{-1}. The minimum and the maximum decreases of chlorophyll content was determined in second week as 18.5 % and 66.7 % at the 640 and 320 mg L^{-1} nanocomposite concentrations, respectively. Compared to chlorophyll content in second week, it increased in third and fourth weeks but was not as much as the control. In the third- and fourth weeks, the minimum chlorophyll content of young leaves was observed at the 640 and 320 mg L^{-1} nanocomposite concentration ($p<0.05$), respectively.

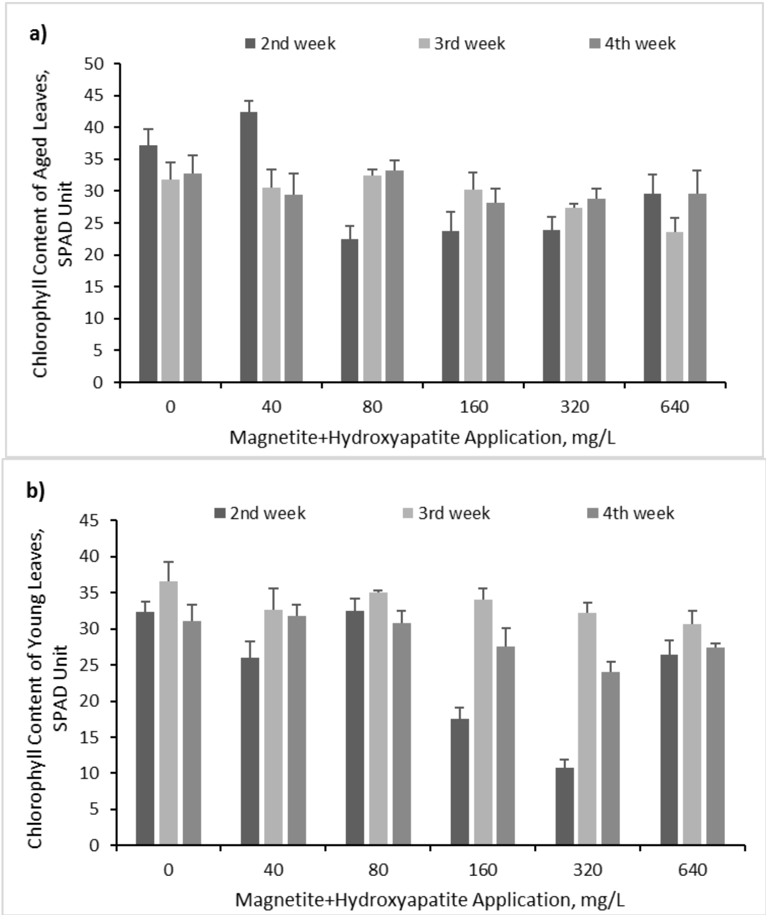


Figure 5. Effects of Magnetite-Hydroxyapatite Nanocomposite on Chlorophyll Content of a) Aged Leaves and b) Young Leaves of Wheat

CONCLUSION

In this study, ecotoxicological evaluation of Fe_3O_4, HAp and Fe3O4-HAp nanocomposites was made based on wheat plant. In the results obtained, it was determined that the materials used did not have a negative effect on the germination of wheat, the root elongation of the wheat was negatively affected by the test chemicals, while the shoot elongation was positively affected. It has been observed that Fe_3O_4 nanoparticles adversely affect the chlorophyll content in both young and old leaves of wheat at increasing concentrations during the early growth period, as in HAp application. However, chlorophyll production was lower in plants treated with HAp than with other test chemicals. It was determined that the use of iron in chlorophyll production was inhibited by the presence of HAp in the growing medium.

ACKNOWLEDGEMENTS

This work was supported by the Mersin University Scientific Research Projects Unit (Project Number:2019-1-AP4-3494). Authors are grateful to MEU-BAP for supports.

REFERENCES

Al-Amri, N., Tombuloglu, H., Slimani, Y., Akhtar, S., Barghouthi, M., Almessiere, M., Alshammari, T., Baykal, A., Sabit, H., Ercan, I. and Ozcelik, S. (2020). Size effect of iron (III) oxide nanomaterials on the growth, and their uptake and translocation in common wheat (*Triticum aestivum* L.). *Ecotoxicology and Environmental Safety*, 194, 110377. doi:10.1016/j.ecoenv.2020.110377

Bilen, S. ve Sezen, Y. (1993). Toprak reaksiyonunun bitki besin elementleri elverişliliği üzerine etkisi. *Atatürk Üniversitesi Ziraat Fakültesi Dergisi*, 24(2), 156-166.

Bolat, İ. and Kara, Ö. (2017). Plant nutrients: Sources, functions, deficiencies and redundancy. *J of Bartin Faculty of Forestry*, 19(1), 218-228. doi: 10.24011/barofd.251313

Borhan, M.S., Panigrahi, S., Satter, M.A. and Gu, H. (2017). Evaluation of computer imaging technique for predicting the SPAD readings in potato leaves. *Information Processing in Agriculture*, 4, 275-282. doi:10.1016/j.inpa.2017.07.005

Dağlioğlu, Y. and Yilmaz, H.Ö. (2018). Nanoparticle characterization methods and its importance in ecotoxicity experiments. *Marmara Fen Bilimleri Dergisi*,1, 1-17. doi: 10.7240/marufbd.346547

De Souza, A., Govea‑Alcaide, E., Masunaga, S.H., Fajardo‑Rosabal, L., Effenberger, F., Rossi, L.M. and Jardim R.F. (2019). Impact of Fe_3O_4 nanoparticle on nutrient accumulation in common bean plants grown in soil. *SN Applied Sciences*, 1, 308. doi:10.1007/s42452-019-0321-y

De Souza-Torres, A., Govea-Alcaide, E., G´omez-Padilla, E., Masunaga, S.H., Effenberger, F.B., Rossi, L.M., L´opez-S´anchez, R. and Jardim, R.F. (2021). Fe_3O_4 nanoparticles and Rhizobium inoculation enhance nodulation, nitrogen fixation and growth of common bean plants grown in soil. *Rhizosphere*, 17, 100275. doi:10.1016/j.rhisph.2020.100275

Doğaroğlu, Z.G., Eren, A. ve Baran, M.F. (2019). Effects of ZnO nanoparticles and ethylenediamine-N,N′-disuccinic acid on seed germination of four different plants. *Global Challenges*, 1800111. doi: 10.1002/gch2.201800111

Dray Jr., F.A., Center, T.D. and Mattison, E.D. (2012). In situ estimates of waterhyacinth leaf tissue nitrogen using a SPAD-502 chlorophyll meter. *Aquatic Botany*, 100, 72-75. doi:10.1016/j.aquabot.2012.03.005

Feng, Y., Yang, J., Liu, W., Yan, Y. and Wang, Y. (2021). Hydroxyapatite as a passivator for safe wheat production and its impacts on soil microbial communities in a Cd-contaminated alkaline soil. *Journal of Hazardous Materials*, 404, 124005. doi:10.1016/j.jhazmat.2020.124005

Ghafariyan, M.H., Malakouti, M.J., Dadpour, M.R., Stroeve, P. and Mahmoudi, M. (2013). Effects of magnetite nanoparticles on soybean chlorophyll. *Environmental Science and Technology*, 47, 10645−10652. doi:10.1021/es402249b

Girelli, A.M., Astolfi, M.L. and Scuto, F.R. (2020). Agro-industrial wastes as potential carriers for enzyme immobilization: A review. *Chemosphere*, 244, 125368. doi:10.1016/j.chemosphere.2019.125368

Hawkins, T. S., Gardiner, E. S. and Comer, G.S. (2009). Modeling the relationship between extractable chlorophyll and SPAD-502 readings for endangered plant species research. *Journal for Nature Conservation*, 17, 123-127. doi:10.1016/j.jnc.2008.12.007

Iannone, M.F., Groppa, M.D., de Sousa, M.E., van Raap, M.B.F. and Benavides, M.P. (2016). Impact of magnetite iron oxide nanoparticles on wheat (*Triticum aestivum* L.) development: Evaluation of oxidative damage. *Environmental and Experimental Botany*, 131, 77–88. doi:10.1016/j.envexpbot.2016.07.004

Iannone, M.F., Groppa, M.D., Zawoznik, M.S., Coral, D.F., van Raap, M.B.F. and Benavides, M.P. (2021). Magnetite nanoparticles coated with citric acid are not phytotoxic and stimulate soybean and alfalfa growth. *Ecotoxicology and Environmental Safety*, 211, 111942. doi:10.1016/j.ecoenv.2021.111942

Ji, R., Shi, W., Wang, Y., Zhang, H. and Min, J. (2020). Nondestructive estimation of bok choy nitrogen status with an active canopy sensor in comparison to a chlorophyll meter. *Pedosphere*, 30(6), 769–777. doi:10.1016/S1002-0160(20)60037-6

Kermanian, M., Naghibi, M. and Sadighian, S. (2020). One-pot hydrothermal synthesis of a magnetic hydroxyapatite nanocomposite for MR imaging and pH-Sensitive drug delivery applications. *Heliyon*, 6:e04928. doi:10.1016/j.heliyon.2020.e04928

Lin, F.F., Qiu, L.F., Deng, J.S., Shi, Y.Y., Chen, L.S. and Wang, K. (2010). Investigation of SPAD meter-based indices for estimating rice nitrogen status. *Computers and Electronics in Agriculture*, 71S, 60–65. doi:10.1016/j.compag.2009.09.006

Mezacasa, A.V., Queiroz, A.M., Graciano, D.E., Pontes, M.S., Santiago, E.F., Oliveira, I.P., Lopez, A.J., Casagrande, G.A., Scherer, M.D., dos Reis, D.D., Oliveira, S.L. and Caires, A.R.L. (2020). Effects of gold nanoparticles on photophysical behaviour of chlorophyll and pheophytin. *Journal of Photochemistry and Photobiology A: Chemistry*, 389, 1-8. doi:10.1016/j.jphotochem.2019.112252

Petcharoen, K. and Sirivat, A. (2012). Synthesis and characterization of magnetite nanoparticles via the chemical co-precipitation method. *Mat. Sci. and Eng.: B.*,177(5), 421-427. doi:10.1016/j.mseb.2012.01.003

Rop, K., Karuku, G.N., Mbui, D., Michira, I. and Njomo, N. (2018). Formulation of slow release NPK fertilizer (cellulose-graft-poly (acrylamide)/nano-hydroxyapatite /soluble fertilizer) composite and evaluating its N mineralization potential. *Annals of Agricultural Sciences*, 63, 163–172. doi:10.1016/j.aoas.2018.11.001

Shankramma, K., Yallappa, S., Shivanna, M.B. and Manjanna, J. (2016). Fe_2O_3 magnetic nanoparticles to enhance *S. lycopersicum* (tomato) plant growth and their biomineralization. *Applied Nanoscience*, 6, 983–990. doi:10.1007/s13204-015-0510-y

Su, C. (2017). Environmental implications and applications of engineered nanoscale magnetite and its hybrid nanocomposites: A review of recent literature. *Journal of Hazardous Materials*, 322, 48-84. doi:10.1016/j.jhazmat.2016.06.060

Wang, Y., Wang, S., Xu, M., Xiao, L., Dai, Z. and Li, J. (2019). The impacts of γ-Fe_2O_3 and Fe_3O_4 nanoparticles on the physiology and fruit quality of muskmelon (*Cucumis melo*) plants. *Environmental Pollution*, 249,1011-1018. doi:10.1016/j.envpol.2019.03.119

Yang, Z., Gong, X. and Zhang, C. (2010). Recyclable Fe_3O_4/hydroxyapatite composite nanoparticles for photocatalytic applications. *Chemical Engineering Journal*,165, 117–121. doi:10.1016/j.cej.2010.09.001

Zhi-Hui, M., Lei, D., Fu-Zhou, D., Xiao-Juan, L. and Dan-Yu, Q. (2020). Angle effects of vegetation indices and the influence on prediction of SPAD values in soybean and maize. *International Journal of Applied Earth Observation Geoinformation*, 93, 102198. doi:10.1016/j.jag.2020.102198