Populus ciliata conjugated of iron oxide nanoparticles and their potential antibacterial activities against human bacterial pathogens

M. Hafeez[a], R. Shaheen[a], S. Ali[b,*], H. A. Shakir[c], M. Irfan[d], T. A. Mughal[b], A. Hassan[b], M. A. Khan[e], S. Mumtaz[b]
[a]*Department of Chemistry, University of Azad Jammu & Kashmir, Muzaffarabad, Azad Kashmir, Pakistan*
[b]*Applied Entomology and Medical Toxicology Laboratory, Government College University, Lahore, Pakistan*
[c]*Institute of Zoology, The University of the Punjab, Lahore, Pakistan*
[d]*Department of Biotechnology, University of Sargodha, Sargodha, Pakistan*
[e]*Green Hills Science College Muzaffarabad AJK, Pakistan*

Green synthesis is gaining huge significance because of its environmentally harmonious nature and low cost. This is an important technique to synthesize metal oxide nanoparticles. In the current study, iron oxide nanoparticles (Fe_2O_3-NPs) were formulated by using *Populus ciliata* leaf extract and ferrous sulphate ($FeSO_4.7H_2O$). These NPs were analyzed by X-ray Diffraction (XRD), Scanning Electron Microscopy (SEM), Transmission Electron Microscopy (TEM), Fourier Transform Spectroscopy (FT-IR), and Energy Dispersive X-ray (EDX). The synthesized NPs were used against Gram positive and negative bacteria to find their bactericidal potential. These NPs were found active against *Klebsiella pneumonia*, *Escherichia coli*, *Bacillus (B) licheniformis* and *B. subtilis. B. licheniformis* showed the highest antibacterial activity (zone of inhibition) up to 29.1±0.5 mm at 8 mg/mL concentration. This study concludes that *Populus ciliata* conjugated Iron oxides NPs could be used a potential antibacterial agent.

(Received May 2, 2021; Accepted July 28, 2021)

Keywords: Populu sciliata, Fe_2O_3-NPs, Green synthesis, Antibacterial studies

1. Introduction

Nanotechnology is a technique that encompasses the manipulation of matter through specific physical or chemical processes to synthesize various materials. Materials synthesized via this technique have unique properties and possess several particular applications [1]. Through nanometer length scale fabrication, this technique covers the formation and utilization of materials, different systems and devices [2, 3]. Generally, it is concerned with the synthesis of NPs having variable morphology, size, chemical composition, controlled disparity and their potential applications for human welfare. Nanoparticles have unique thermal, optical, physical and chemical properties [4] and hence find several applications in chemistry, medicine, energy, environment, agriculture, heavy industry, consumer goods and information/ communications sectors [5].

Different chemical as well as physical methods can be used to fabricate nanoparticles. Some of these methods are pyrolysis [6], chemical reduction [7], template-assisted synthesis [8], electrochemical [9], hydrothermal synthesis [10], vapor-solid growth techniques [11], sol-gel process [12] and chemical co-precipitation [13]. Though these methods can synthesize nanomaterials successfully, certain drawbacks are also associated with these procedures. For instance, more time is required for the synthesis, defective surface formation, large energy requirements and high manufacturing cost [14]. Similarly, chemical methods of NPs synthesis are not environmentally friendly, for example, chemical reduction techniques and sol-gel method involve the usage of toxic chemicals, contamination problems and hazardous by-products formation [14]. Hence, it is a growing need to develop those methods that are less toxic, clean and ecofriendly for the synthesis of NPs. Green synthesis is a preferred method for the synthesis of

* Corresponding author: dr.shaukatali@gcu.edu.pk

NPs because of many associated benefits [15]. In this method, higher pressure and more energy are not required and hence is an energy-efficient process [16].

Fe_2O_3-NPs find significant applications in various fields. These NPs are biocompatible, biodegradable and non-toxic to human beings [17, 18]. Fe_2O_3-NPs are inert and are also present in living systems [19]. Furthermore, these are super paramagnetic, too [20]. Owing to these unique properties, Fe_2O_3-NPs are being extensively used for various applications including magnetic storage media [21], catalysis [22, 23], magnetic resonance imaging[24, 25], biosensors [26], targeted drug delivery [27, 28] and cancer treatment [29]. These NPs also play a critical role in environmental remediation cycles, for instance in the removal of inorganic and organic pollutants from wastewater [30].

Now a days, there is a growing interest to synthesize Fe_2O_3-NPs through biosynthesis. In this method, the NPs are synthesized using microbes or plant extracts. The plants' extracts mediated synthesis of NPs is preferable over microbial synthesis due to the disadvantages (longer synthesis time, the requirement of aseptic conditions and low yields) associated with the latter. Recently, there are several reports for the synthesis of Fe_2O_3-NPs using different plants based materials such as *Musa ornata* [31], *Linum usitatissimu* [32], *Jatropha gosspifolia*[33], *Platanus orientalis*[34], *Lagenaria siceraria* [35], pomegranate seeds extract[36], *Artemisia vulgaris*[37], tea leaves extracts, *Sorghum* bran, Plantain peel, Banana peel, *Dodonaea viscosa*, *Tridax procumbens*, Pomegranate, mulberry, cherry, Vine leaves, grape marc, *Terminalia chebula*, *Eucalyptus tereticornis*, *Melaleuca nesophila* and *Rosmarinus officinalis* [38].

This study has reported the synthesis of Fe_2O_3-NPs using *Populus ciliate* (family: *Silicaceae)* leaf extract. This is the first report of *Populus ciliate* mediated Fe_2O_3-NPs synthesis. This plant is mainly found in Indo-Pak and Bhutan. The wood of the plant is used for making different articles but its leaves remain as bio-waste. Such waste could be beneficially used for the synthesis of NPs.

2. Material and methods

2.1. Materials

The ferrous sulphate heptahydrate ($FeSO_4.7H_2O$) was purchased from MERK (city and country). Fresh and healthy leaves of *Populus ciliate* were collected from the garden of University of Azad Jammu and Kashmir, Muzaffarabad, Pakistan.

2.2. Preparation of leaves' extract

The leaves' extract was prepared according to a reported procedure [39, 40]. The leaves were cleaned with tap water followed by distilled water and dried under air at room temperature. The chopped leaves (10 g) were dissolved distilled water (100 mL) heated to boiling for 2 hours. The heating was stopped and solution color change was noted from colorless to light brown. The solution was filtered and kept in refrigerator for further use.

2.3. Synthesis of Fe_2O_3-NPs

The Fe_2O_3-NPs using plant extract was carried out by a reported procedure [40].

Both plant extract and metal salt solution were mixed in a conical flask. This solution was kept for 3 hours at 80^oC on hot plate. The change in color (light brown to dark brown) confirmed the synthesis of stable nanoparticles. The sediment was washed with double distilled water twice by repeated centrifugation (2000 rpm) for 15 minutes. The sediment was heated at 100^oC for 8 hours in an oven. The material was cooled and stored for further spectral studies.

2.4. Characterization

The synthesized Fe_2O_3-NPs were characterized by different techniques including FT-IR, XRD, SEM, TEM and EDX. The functional groups, types of bonds and composition of different compounds were found by using an FTIR spectrophotometer (Shimadzu 8400). KBr pellets were used as a standard to record the spectrum in the range of 4000-400 nm. The absorption of different IR frequencies was recorded by placing the samples in the path of the IR radiation source. To

confirm the formation of Fe_2O_3-NPs, XRD analysis was performed on the instrument (Bruker-D8 Advance -X-ray diffractometer) by using Cu-Kα radiation (λ= 1.5418 Å). Morphology and size of prepared Fe_2O_3-NPs werealso analyzed by using electron microscopy techniques. SEM analysis was done by using an instrument (FEINOVA nano SEM 450) and TEM analysis was carried out with HITACHI H-7700TEM at 200 Kv.

2.5. Anti-bacterial Activities

The antibacterial assay has been performed according to our previous studies published elsewhere [39-40]. Briefly, different concentrations (2, 4, 8 mg/mL) of Fe_2O_3-NPs were prepared in distilled water. The Petri plates were prepared by pouring nutrient agar in plates and then spreading the bacterial pathogens. Wells were made in solidified plates to pour the 50 µL of each concentration of Fe_2O_3-NPs solution, control solution (water) and bacitracin solution (positive control). After overnight incubation at 37°C, bacterial growth inhibition zones showed by each solution were measured using the graduated scale. The procedure was repeated in triplicates. The mean and standard errors of the mean of zones measurement were calculated.

2.6. Statistical data analysis

Data were presented as mean±SEM. The data were analyzed statistically by one-way ANOVA, followed by "Dunnett's Multiple Comparison Test", to find any significant differences By GraphPad Prism version 5.0 for windows (GraphPad Software, San Diego, CA, USA). Values of $P \leq 0.05$ were considered as significant.

3. Results and discussion

3.1. FTIR spectroscopy

The important functional groups present in the leaves' extract of *Populus ciliata* were identified using FTIR spectroscopy. Fig. 1 depicted the FTIR spectrum of synthesized Fe_2O_3-NPs. The prominent and important peaks observed in the FTIR spectrum were 3432, 2918, 1639, 551 cm^{-1}. The peak at 3432 cm^{-1} represented the alcohols' or phenols' hydroxyl group. The peak at 2918 cm^{-1} can be accredited to the -CH stretching vibrations of-CH_3 and -CH_2groups.The peak at 1639 cm^{-1} represented the C=O group of tertiary amides. Another peek at 551 cm^{-1} can be attributed to the Fe-O bond in iron Fe_2O_3-NPs [43]. The reduction of iron salt by plant biomolecules under mild conditions resulted in the formation of Fe_2O_3-NPs.

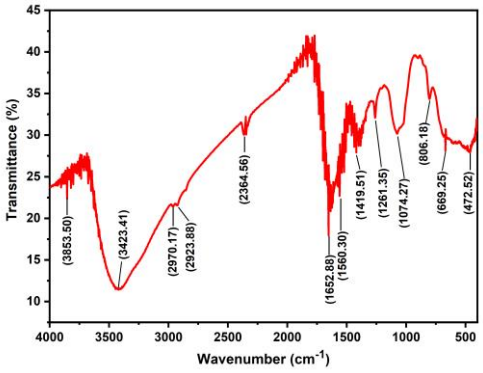

Fig. 1. FTIR spectra of Fe_2O_3-NPs.

3.2. X-ray diffraction (XRD)

The XRD analysis of Fe_2O_3-NPs was performed to find out the phase and composition of the synthesized NPs. The XRD spectrum of Fe_2O_3 nanoparticles showed diffraction peaks at 2θ values of 24.16°, 33.12°, 35.66°, 40.63°, 49.48°, 54.08°, 57.42°, 63.94° and 64.80° corresponding to 012, 104, 110, 113, 024, 116, 122, 214 and 300 diffraction planes (Fig. 2). The XRD data confirmed the hexagonal phase of the hematite. These observed peaks were found in good

agreement with JCPD card no84-0306 which indicated the presence of only Fe_2O_3 nanoparticles. Moreover, the presence of sharp peaks in the XRD spectrum showed that the synthesized material is very pure. The XRD data was found to be in close agreement with the reported literature [44-46].

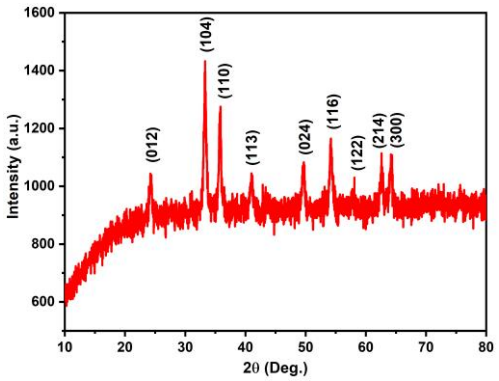

Fig. 2. XRD spectra of Fe_2O_3-NPs.

3.3. Electron Microscopy (EM)

Electron microscopy techniques were employed to analyze the structural features of F_2O_3-NPs. The surface morphology was investigated by SEM. Fig. 3a and b depicted the SEM micrographs. SEM image in fig. 3a showed a few groups of aggregated particles and an image at a higher resolution (Fig. 3b) showed the presence of several free-standing elongated particles of uniform size and shape. The aggregation of particles was plausibly due to the agglomeration of particles to reduce the surface energies. The TEM image (Fig. 3c) confirmed the uniform sized and shaped elongated NPs as indicated by SEM images. Based on these observations, it was assumed that the synthesized NPs were freestanding which became aggregated upon sample preparation. The diameter of the nanoparticles estimated from microscopy images lies in the range of 20-25 nm.

3.4. Energy dispersive X-ray (EDX)

F_2O_3-NPs were also analyzed by energy-dispersive X-ray (EDX) analysis. The EDX spectrum (Fig. 3d) contained the intense peaks of Fe and oxygen which implies the formation of oxide in the synthesized sample.The atomic percentage obtained from EDX quantification was 31.10% oxygen, 68.11% iron. These EDX results indicated the crystalline purity of the synthesized F_2O_3 nanoparticles. Also, the absence of peaks of other elements indicated the crystalline purity of the synthesized material. Similar EDX results had already been reported for F_2O_3-NPs [47].

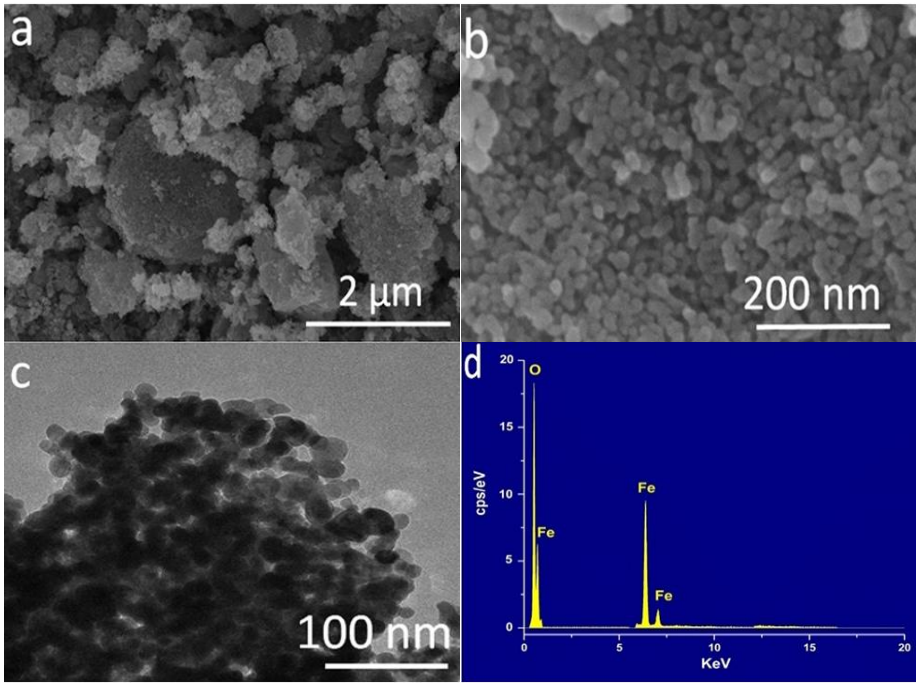

Fig. 3. (a,b) SEM images at different magnifications (c) TEM image and (d) EDX spectra of F_2O_3-NPs.

3.5. Antibacterial Assay

In the reported literature of the Fe_2O_3-NPs, most of the applications are catalytic. The biological applications of these nanoparticles have been less explored.Sravanthi*et al* studied the antibacterial activities of FeO-NPs against Gram-negative (*E.coli*) and Gram-positive bacteria (*S.aureus).* They found good results of FeO-NPs activity against bacteria and observed the zone of inhibition (19 mm) against *E. coli* and (15 mm) against *S. aureus*[47]. Another study revealed that the growth of *E.coli* was completely inhibited by ferric oxide-NPs. The authors concluded thatFe_2O_3-NPs generated reactive oxygen species (ROS) in the bacterial cell which caused damage to protein and DNA of bacteria [48].S. Kanagasubbulakshmi and K. kadirvelu synthesized the Fe_3O_4-NPs using *Lagenariasiceraria (leaves extract) and* the zone of inhibition was found to be 10 mm for*Escherichia coli,* and 8 mm for *Staphylococcus aureus.* Naseem and Farrukh also studied Fe-NPs synthesized by using different plants such as *Azadirachtaindica, Lawsoniainermis, Camellia sinensis,Gardenia jasminoides*and *Cinnamon zeylanicum* against Gram-negative*(Proteus mirabilis, E. coli and Salmonella Enterica*) and Gram-positive bacterium (*Staphylococcus aureus*). It was concluded that FeO-NPs showed effective results against these bacterial strains. FeO-NPs obtained from *Gardenia jasminoides*were more active against *Staphylococcus aureus* with an inhibition zone (16mm), whereas for another plant *Lawsoniainermis,* the inhibition zone (15 mm) was observed. The FeO-NPs obtained from*Gardenia jasminoides*and*Lawsoniainermis* leaves extracts were also analyzed against three bacteria *(E.coli, S. enteric* and*P.mirabilis*) [49]. Fe_3O_4-NPs synthesized from ethanolic extract of *Argemonemexicana* leaves were also studied against *P. mirabilis and E. coli,* the inhibition zones of 18 mm and 13 mm were observed. Against *B.subtills,* low activity was observed [49]. So, the inhibitory action of these NPs was due to the synergic inhibitory action of ROS and phytochemicals generation [49]. Another study revealed that the antimicrobial activity of Fe-NPs synthesized by using flower sheath extract of *Musa ornate* extract against multidrug-resistant bacteria and common food borne pathogens such as *E.coli, S.aureus, S.agalacitiae* and *S. enteric* by well diffusion method. They concluded that FeO-NPs have maximum antimicrobial activity against *S. aureus* (32 mm) and *S. agalactiae* (28 mm). The activity was dependent upon the concentration used [30].S. Saqib*et al* have used Fe_3O_4- NPs against *S. dysentery E. coli, ,* and *S. aureus* and observed lower activities of these NPs.

In our study, maximum activity (29.1± 0.5mm) was observed against *B. licheniformis* when a higher concentration (8 mg/mL) of Fe_2O_3-NPs was used (Table 1 and Fig. 4). This activity

is higher in comparison to activity (24.2±1.8) of bacitracin for a similar concentration of NPs. The maximum activity (17.3±0.5 mm) of Fe_2O_3-NPs was observed against *E. coli* when a higher concentration (8 mg/mL) was used. However, a similar concentration of bacitracin against *E. coli* was bit more effective with antibacterial activity (23.5±1.7mm). These activity results are quite satisfactory in comparison to previous reported results of Fe_2O_3/Fe-NPs [30, 47-49].

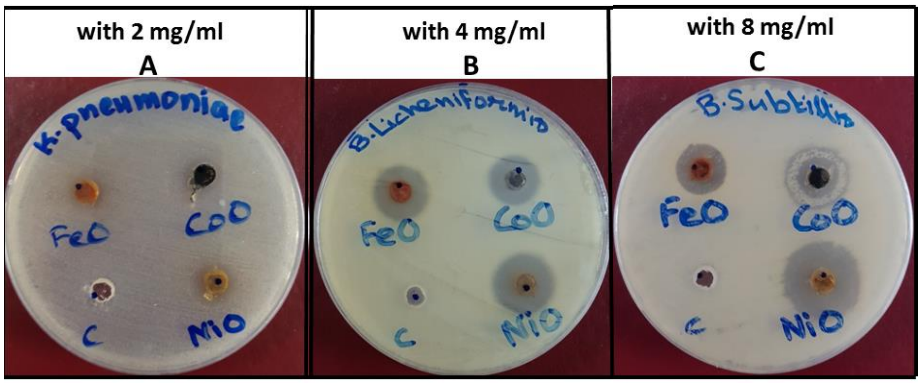

Fig. 4. Antibacterial activity of silver nanoparticles against different microbes; (a). Klebsiella pneumonia, (b). Bacillus licheniformis, (c). Bacillus subtilis.

Table 1. Antibacterial activity of F_2O_3-NPs.

		Zones of inhibition by control (water) (mm)	Zones of inhibition by bacitracin (mm)				Zones of inhibition by *Populus ciliata* conjugated Iron oxide nanoparticles Activity of Fe_2O_3 NPs (mm)			
Bacterial strains			**2 mgmL^{-1}**	**4 mgmL^{-1}**	**8 mgmL^{-1}**	**Mean activity of bacitracin (mm)**	**2 mgmL^{-1}**	**4 mgmL^{-1}**	**8 mgmL^{-1}**	**Mean activity of FeO NPs (mm)**
Gram-negative bacteria	***Escherichia coli***	0±0	14.5±0.5	19.9±0.8	23.5±1.7	19.3±1.0**	9.1±0.5	13.2±0.3	17.3±0.5	13.3±0.4
	Klebsiella pneumonia	0±0	12.2±0.2	24.9±1.3	27.2±2.1	21.4±1.2***	8.1±0.2	14.2±0.7	11.6.2±0.8	11.3±0.6
Gram-positive bacteria	***Bacillus subtilis***	0±0	9.3±0.3	21.1±1.1	25.3±2.0	18.6±1.1	11.4±0.3	17.6±0.6	21.3±0.9	16.8±0.6
	Bacillus licheniformis	0±0	13.1±0.2	21.2±1.5	24.2±1.8	19.5±1.2	19.3±0.3	24.2±0.5	29.1±0.5	24.2±0.4***

Note: Each value represents the mean ±SEM of four replicates with 95 % CI. A positive control is a commercial bacitracin. Statistical icon: *** = $p \leq 0.001$.

4. Conclusion

This research work has reported the green method of synthesis of Fe_2O_3-NPs.Leaves extracts of *Populusciliata* were utilized for the synthesis of these NPs. Various techniques such as FT-IR, XRD, SEM, TEM and EDX were used to analyze the synthesized Fe_2O_3-NPS. FTIR studies helped in identifying the functional groups involved in the capping and reduction of the synthesized NPs. The XRD analysis confirmed the formation of Fe_2O_3nanoparticles. SEM and TEM disclosed the surface and internal morphology of the nanoparticles, respectively. EDX confirmed the presence of only iron and oxygen in the synthesized product.

Antibacterial activities of synthesized Fe_2O_3-NPs were also performed against Gram negative and Gram-positive bacteria which proved that synthesized NPs showed inhibitory effect against both bacterial strains. Results showed that by increasing the concentration of Fe_2O_3-NPs,

antibacterial activity was enhanced. Hence in conclusion the Fe_2O_3-NPs had good antibacterial activities and could be used as a disinfectant for water purification and other biomedical applications.

Acknowledgments

Authors are grateful to Dr. Muhammad Zaheer, Assistant Professor, Department of Chemistry and Chemical Engineering, LUMS, Lahore for providing access to Central Analysis Laboratory, LUMS, Lahore.

References

[1] D. Drobne, Arh Hig Rada Toksikol. **58**, 471 (2007).
[2] D. T. Chiu, Anal. and bioanal. chem. **397**, 3179 (2010).
[3] M. B. Dixon, C. Falconet, L. Ho, C. W. Chow, B. K. O'Neill, G. Newcombe, J. Hazard. Mater. **188**, 288 (2011).
[4] J. G. Parsons, J. R., Peralta-Videa, K. M. Dokken, J. L. Gardea- Torresdey, Nanotechnologies for the Life Sciences: Online. (2007).
[5] S. Sahoo, IEEE Conf. Nanotechnol., 10th, p. 1205, IEEE, (2010).
[6] M. Worden, L. Bergquist & T. Hegmann, RSC adv. **5**, 100384 (2015).
[7] Y. C. Zhu, J. Z. Zhao, B. Zhou, X. Zhao, Z. C. Wang, Chem. Res. Chin. Univ. **10**, 24 008).
[8] D. Mishra, H. Zabel, S. V. Ulyanov, V. P. Romanov, V. M. Uzdin, J. Appl. Phys. **115**, 054104 (2014).
[9] I. Karimzadeh, M. Aghazadeh, T. Doroudi, M. R. Ganjali, P. H. Kolivand, Mater. Res. Innov. **22**, 352 (2018)
[10] B. Palagiri, R. Chintaparty, R. R. Nagireddy, V. S. R. Imma Reddy, Phase Transit. **90**, 578 017).
[11] S. I. Futko, B. G. Shulitskii, V. A. Labunov & E. M. Ermolaeva, J. Eng. Phys. Thermophys. **88**, 1432 (2015).
[12] Z. N. Kayani, S. Arshad, S. Riaz, S. Naseem, IEEE Trans. Magn. **50**, (2014).
[13] A. Taufiq, S. Pratapa, M. Zainuri, Mater. Sci. Forum. **827**, 229 (2015).
[14] K. N. Thakkar, S. S. Mhatre, R. Y. Parikh, Nanomedicine: NBM. **6**, 257 (2010).
[15] S. Senapati, A. Ahmad, M. I. Khan, M., Sastry, R. Kumar, Small **1**, 517 (2005).
[16] V. Bansal, D. Rautaray, A. Ahmad, M. Sastry, J. Mater. Chem. **14**, 3303 (2004)
[17] F. Q. Hu, L. Wei, Z. Zhou, Y. L. Ran, Z. Li, M. Y. Gao, Adv. Mater. **18**, 2553 (2006).
[18] L. Zhang, W. F. Dong, H. B. Sun, Nanoscale **5**, 7664 (2013).
[19] R. M. Cornell, U. Schwertmann, JWS (2003).
[20] A. R. Mahdavian, M. A. S. Mirrahimi, Chem. Eng. J. **159**, 264 (2010).
[21] B. D. Terris, T. Thomson, J. Phys. D Appl. Phys. **38**, R199. (2005).
[22] M. B. Gawande, P. S. Branco, R. S. Varma, Chem. Soc. Rev. **42**, 3371 (2013).
[23] S. N. Shelke, S. R. Bankar, G. R. Mhaske, S. S. Kadam, D. K. Murade, S. B. Bhorkade, R. Zboril, Chem. Eng. **2**, 1699 (2014).
[24] C. Y. Haw, F. Mohamed, C. H. Chia, S. Radiman, S. Zakaria, N. Huang, H. Lim, Ceram. Int. **36**, 1417 (2010).
[25] R. Qiao, C. Yang, M. Gao, J. Mater. Chem. **19**, 6274 (2009)
[26] A. L. Kavitha, H. G. Prabu, S. A. Babu, S. K. Suja, J. Nanosci. **13**, 98 (2013)
[27] M. Salem, Y. Xia, A. Allan, S. Rohani, E. R. Gillies, RSC Adv. **5**, 37521 (2015)
[28] K. D. Wani, B. S. Kadu, P. Mansara, P. Gupta, A. V. Deore, R. C. Chikate, R. Kaul-Ghanekar, PloS ONE **9**, e107315 (2014).
[29] A. Jordan, R. Scholz, P. Wust, H. Fähling, R. Felix, J. Magn. Magn. Mater. **201**, 413 (1999).
[30] P. Xu, G. M. Zeng, D. L. Huang, C. L. Feng, S. Hu, M. H. Zhao, G. X. Xie, Sci. Total Environ. **424**, 1 (2012).
[31] S. Saranya, K. Vijayarani, S. Pavithra, J. Pharm. Sci. **79**, 688 (2017).

[32] G. Karunakaran, M. Jagathambal, N. Van Minh, E. Kolesnikov, A. Gusev, O. V. Zakharova, D. Kuznetsov, IJBB **11**, 289 (2017).
[33] Z. Karkuzhali, A. Yogamoorthi, IJNN **6**, 47 (2015).
[34] H. S. Devi, M. A. Boda, M. A. Shah, S. Parveen, A. H. Wani, Green Process Synth. **8**, 38 2019).
[35] S. Kanagasubbulakshmi, K. Kadirvelu, Def. Life. Sci. J. **2**, 422 (2017).
[36] I. Ismat Bibi, N. Nazar, S. Ata, M. Sultan, A. Ali, A. Abbas, F. Jalal, J. Mater. Res. Technol. **8**, 6115 (2019).
[37] M. A. J. Kouhbanani, N. Beheshtkhoo, A. M. Amani, S. Taghizadeh, V. Beigi, A. Z. Bazmandeh, N. Khalaf, Mater. Res. Express. **5**, 115013 (2018).
[38] M. Herlekar, S. Barve, R. Kumar, J. Nanomater. **2014**, (2014).
[39] M. Hafeez, R. Arshad, M. U. Hameed, B. Akram, M. N. Ahmed, S. A. Kazmi, S. Ali, Mater. Res. Express. **6**, 075064 (2019).
[40] M. Hafeez, R. Arshad, J. Khan, B. Akram, M. N. Ahmad, M. U. Hameed, S. Haq, Mater. Res. Express **6**, 055043 (2019).
[41] M. A. Bhosale, D. Ummineni, T. Sasaki, D. Nishio-Hamane, B. M. Bhanage, J Mol Catal A Chem. **404**, 8 (2015).
[42] H. I. Abdulah, A. M. Farhan, A. J. Ali, J. Chem. Pharm. Res. **7**, 588 (2015).
[43] S. Karthikeyeni,T. Sivavijayakumar, J. Acad. indus. Res. **1**, 649 (2013).
[44] T. Shahwan , S. Abusirriah, Chem. Eng. J. **172**, 258 (2011).
[45] M. Sravanthi, D. M. Kumar, M. Ravichandra, G. Vasu, K. Hemalatha, Review. Int. J. Curr. Res. Aca. Rev. **4**, 30 (2016).
[46] B. Shahmoradi, I. A. Ibrahim, N. Sakamoto, S. Ananda, R. Somashekar, T. N. G. Row, K. Byrappa, J. Environ. Sci. Health A **45**, 1248 (2010).
[47] T. Naseem, M. A. Farrukh, J. Chem., (2015).
[48] J. Taylor, T. Rabe, L. McGaw, A. Jäger, Van Staden, J. Plant growth regul. **34**, 23 (2001).
[49] S. Arora, BIOLOGIX 70.