



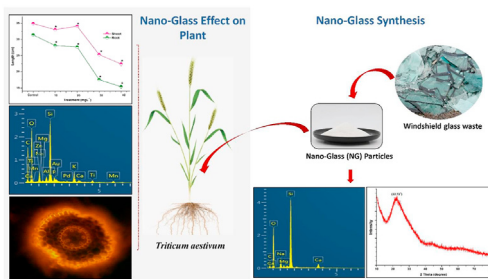
Research article

Impact of nano-glass (NG) particles on seed germination and its accumulation in plant parts of wheat (*Triticum aestivum* L.)Swati Agarwal^{a,1}, Sonu Kumari^{b,1}, Nidhi Sharma^a, Suphiya Khan^{a,*}^a Department of Bioscience and Biotechnology, Banasthali Vidyapith, Rajasthan, India^b Department of Biotechnology, Faculty of Engineering and Technology, Rama University, G.T. Road, Kanpur, India

HIGHLIGHTS

- Nano-glass (NG) particles synthesized from waste windshield glass.
- NG inhibits the seed germination with root and shoot length.
- The effect of NG on chlorophyll shows higher reduction.
- NG localization was observed through FESEM, EDX and fluorescence microscopy.

GRAPHICAL ABSTRACT



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ABSTRACT

Micro/nano-contaminants have been the focal pollutants in environmental science, which includes several nanomaterials, nanocomposites, fibers, glass, plastics etc. Micro/nano size pollutants are more harmful than macro pollutants due to their size. Therefore, there is an urgent need for research on the possible fate of glass particles in the environment, especially in plant and soil systems. Here, the synthesized nano-glass (NG) from the waste windshield and analyzed its uptake and effect on the wheat (*Triticum aestivum* L.) plant system in a hydroponic solution. The findings provided direct evidence that NG reduced the germination % with increasing NG concentrations as 100, 96, 92, and 92% for 10, 20, 30, and 40 mg L⁻¹. The lowest root and shoot height (15.40 and 22.42 cm) was achieved in the 40 mg L⁻¹ NG treatment. Decrement in fresh and dry wt. with a maximum reduction of chlorophyll a, b and total content (6.19, 4.98, and 11.17 mg g⁻¹ fresh wt.) was obtained at 40 mg L⁻¹ at 21 days. Rhodamine B was used for fluorescence imaging in seedlings to detect NG movement, Results showed that NG moves via xylem tissues of root part to other parts of the plant. Based on the currently limited or no data and uncertainty regarding the actual impact of NG on soil and plant systems, suggested considerations to address key knowledge gaps are delineated. Further studies are required as a flora build-up of NG can have both environmental influence and consequences on agronomic sustainability and food safety.

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1. Introduction

Micro/nano-contaminants have been the focal pollutants in environmental science, which includes several nanomaterials, nanocomposites, fibers, glass, plastics etc. Micro/nano size pollutants are more harmful than macro pollutants due to their size (Waring et al., 2018). Therefore, there is an urgent need for research on the possible fate of glass particles in the environment, especially in plant and soil systems. As the size of the contaminant gets smaller in size it can easily be mixed with the soil system and further translocate in plants. According to the literature materials having a size less than 300 nm can be easily up taken by in different human cells also (Garnett and Kallinteri, 2006) and materials less than 70 nm can even be taken up by a cell's nuclei (Geiser et al., 2005; Chen and von Mikecz, 2005).

Environmental pollution caused by micro/nano glass particles didn't get attention until now. Day-by-day production of glass gets increased and as result, their breakage and disposal also get increased. Glass has endlessly recyclable properties and it never loses its purity (Luczak, 2020) but the broken pieces of glass do not get easily recovered and recycled (Meyer et al., 2001). Other than its good recycling property it has several problems such as its complicated manufacturing process, and tedious and careful transport requirements. Starting with its manufacturing process, glass required a special kind of sand, which is particularly found in riverbeds and seabed. Desert sand can't be used for its manufacturing, which ultimately leads to shore communities being open to flooding and erosion (Gavriletea, 2017). Previous literature showed that every year 50 billion tons of sea/river sand is used for the manufacturing of glass (Peduzzi, 2014). From its raw material harvesting to transport and making glass the overall process requires huge labor and energy cost.

There is no previous data available on the buildup and influence of nano-glass (NG) on plant systems. Flora contains a basic living constituent of terrestrial ecosystems and is an essential source of human nourishment (Beschta and Ripple, 2009; Sá et al., 2020). Therefore, analyzing the reaction between the NG and flora is critical. Given the fundamental function of agroecosystems in food manufacturing and the close-fitting assembly with soil biodiversity, the buildup of NG in the terrestrial setting offers numerous possible lanes for the exposure of humans and biota to NG and can cause severe effects on organisms (together with food harvests) and human wellbeing. Considering the ubiquity of NG particles in water systems and soil it is imperative to assess their mobility in crops.

There are several studies conducted on different metal nanoparticles and their effect on plant systems (Rizwan et al., 2017; Tombuloglu et al., 2018, 2019a, 2019b, 2019c, 2019d; Chen, 2018). Silica or silicates, the major glass components and their effect on plants (Ismail et al., 2022; Shivaraj et al., 2022), marine animals (Neff et al., 2000; Ali et al., 2021) and higher animals (Kusaka et al., 2014; Mahdipour et al., 2022) were also studied. But there is no literature available on research conducted for nano-glass particles (NGPs). Thus, in-depth research is essential to determine the presence of NG in soil and plant systems. A study showed improvement in plant growth indicators and photosynthesis after Si NPs treatment (Khan et al., 2020). Another study reported the similar positive effect of Si NPs on the growth and chlorophyll contents of the wheat plant (Hussain et al., 2019).

Wheat (*Triticum aestivum* L.) is the prime cereal harvest and a staple nutrition basis for more than 50% of the globe's inhabitants (Al-Amri et al., 2020; Kumar and Pareek, 2022). 762 million metric tons of wheat are produced yearly worldwide (Sher et al., 2021). And there is no prior research reported on the influence of NG on wheat or any other plant growth performance have been conducted, the data concerning the harmfulness of NG to plants are still not known.

The present article is the first-ever manuscript presenting the effect of NG on plant systems and will contribute to a novel understanding of the possible health hazards and environmental influences of NG on edible flora. Authors have chosen Wheat (*Triticum aestivum* L.) as an

experimental crop to (1) the influence of NG on seed germination and root/shoot length; (2) the effect of NG on wheat seedlings, particularly at aerial plant parts; (3) translocation of NG from roots to aerial plant parts through FESEM and EDX localization of NG through fluorescence microscopy.

2. Materials and methods

2.1. Chemicals and plant material

Chemicals consumed in the present research were of analytical grade. Millipore ultrapure water was utilized throughout the experiment. *Triticum aestivum* seeds were collected from KVK, Banasthali Vidyapith, India. Windshield waste glass materials used in the experiment were collected from local accident sites and roads.

2.2. Nano-size glass preparation

Windshield glass samples were collected from roads and accident sites in Tonk district, Rajasthan (India). These glasses were cleaned and dried for further micro and nano-size glass synthesis using a mortar pestle. Further, these micro-sized glass particles were converted into nano-size particles through ball milling. Micro-sized glass particles were ball milled for 4–12 h by a conventional planetary ball mill (DG Power and Industrial Solutions Limited, Model: VSS23 2 p5 CEB) with stainless steel vials and zirconia balls (50 balls 12 mm). The ratio of the ball and micro-sized glass particles weight was 50:1 and the rotating speed was set to 300 rpm. The preparation of nano-sized glass particles by using sonication (PCI Analytics, Model: PCI 750FM) and ball milling techniques is illustrated schematically (Figure 1).

2.3. Nanoglass (NG) characterization

Prepared nano-size glass particles were further characterized for their size, structure, and purity. The surface structure of prepared NGs was observed by field emission scanning electron microscopy (FESEM) (MIRA3 TESCAN). The elemental configuration of NGs was recognized by energy-dispersive X-ray (EDX). Phase identification and crystal structures of the NGs were characterized through an X-ray diffractometer (XRD Bruker D8Discover). Transmission electron microscopy (TEM) images for the samples were recorded.

2.4. Plant seed germination

Wheat seeds of nearly even dimensions were chosen and surface sterilized through reaction with NaOCl (Li et al., 2020). Seeds were rinsed three times with Millipore water to eradicate the remaining NaOCl matter. Germinating studies were conducted similarly to the procedure of Lin and Xing (2007). Seeds were later soaked in NG suspensions as 10, 20, 30, 40, and 50 mg L⁻¹ for around 2 h. Treatment suspensions were synthesized through NG stock suspension and then sonicated through ultrasonic vibration (100 W, 25 kHz). Accordingly, 25 seeds were relocated onto a Petri dish with a test medium comprising one portion of qualitative filter paper. Later, incubated for 5 days in a growth chamber under a controlled environment, the germination of seeds was documented. Seed germination was analyzed through Eq. (1):

$$\text{Relative seed germination (RSG)} = \frac{\text{Seeds germinated with NG}}{\text{Seeds germinated with control}} \times 100 \quad (1)$$

2.5. Growth parameters

Seeds were placed on cotton gauze soaked in Millipore water for germination in the growth chamber for 5 days. Seedlings of even development were transported to Hoagland suspension (Hoagland and

Arnon, 1950). Subsequently, even seedlings were shifted to 250 mL glasses with the dissimilar quantity of NG suspensions as 10, 20, 30, 40, and 50 mg L⁻¹ dispersed in Hoagland. Individual test experiments were conducted in 3 sets. Hoagland containers were cautiously shielded by utilizing aluminum foil to dodge algae growth and water loss. All seedling containers were grown at room temperature for 21 days with a photoperiod of 14 h. The nutrient suspension was provided after 3 days interval to overcome water shortage due to evaporation or plant transpiration. During the harvest period, the seedlings were rinsed and washed 4 times with Millipore water. Shoot-root tissues were detached; their dimensions and weights were reported. Dry biomass was analyzed after freeze-drying for 48 h.

2.6. Microscopic observation

For the localization of NGs, only the highest concentration (50 mg L⁻¹) of NG was chosen in a separate test. Later 21 days of treatment, the accumulation of NGs in root and shoot parts were analyzed through FESEM and EDX (MIRA3 TESCAN). Seedlings grown in Hoagland suspension with no NG were depicted as controls.

The root and leaf samples were synthesized as depicted by Zhao et al. (2017). Further, root and shoot tissues were prefixed with phosphate (pH 7.2) buffered with 2.5% glutaraldehyde and then fixed in 1% osmium tetroxide for 1 h. Finally, the dehydrated sections were analyzed via FESEM and EDX.

2.7. Chlorophyll and protein concentrations

Chlorophyll content of seedlings [fresh mass (FM)] was analyzed (Arnon, 1949). For this, 21 days old seedlings leaves were crushed with chilled acetone and incubated overnight. Chlorophyll concentrations were detected as below Eqs. (2), (3), and (4):

$$\text{Chl a (mg L}^{-1}\text{)} = 12.72 (\text{A663}) - 2.59 (\text{A645}) \quad (2)$$

$$\text{Chl b (mg L}^{-1}\text{)} = 22.88 (\text{A645}) - 4.67 (\text{A663}) \quad (3)$$

$$\text{Total Chlorophyll content (mg L}^{-1}\text{)} = \text{Chl a} + \text{Chl b} \quad (4)$$

Protein concentration was analyzed through the procedure of Liang et al. (2008) utilizing bovine serum albumin as the standard. Both chlorophyll and protein content were determined using a spectrophotometer (Systronics, PC Based double beam spectrometer 2202).

2.8. Synthesis of rhodamine B-doped NG

Rhodamine B-doped NG was synthesized utilizing a one-step procedure in control conditions. Rhodamine B (0.2 g) was added to 100 mL of ethanol to synthesize a stock mixture with a concentration of 0.01 mol L⁻¹. Ethanol (8 mL) and deionized water (5 mL) were combined in a 25 mL round-bottom flask and 0.0157 g of CTAB was poured. An aliquot of rhodamine B stock mixture was added to this solution under stirring. Ammonium hydroxide (665 μL) and NG (80 μL) were then added. After stirring for 4 h, NG was procured from the suspension through centrifugation and rinsed with Millipore water numerous times to eliminate surfactants and superfluous dye molecules (Mu et al., 2011). The attained NG was dried in a vacuum for 24 h and kept in closed containers.

To further confirm the localization of NGs, only the maximum concentration (50 mg L⁻¹) of NG was selected in individual experiments. Seedlings exposed to rhodamine B doped NGs were grown with a similar method as mentioned earlier. After 21 days' exposure, the location of NGs was examined using fluorescence microscopy. Seedlings grown in a Hoagland mixture that has no NG were applied as controls.

2.9. Statistical analysis

Statistically entire data was analyzed using SPSS-16.0 statistical software (SPSS Inc., Chicago, IL, USA). The data of various analyses were presented as mean ± standard errors (SE). Results obtained from various treatments at different time intervals were compared with ANOVA i.e. analysis of variance. Statistical significance was observed at $p < 0.05$.

3. Results and discussion

3.1. NG characterization

The external structure and elemental arrangement of prepared NG were characterized through FESEM, EDX spectrum, and XRD analysis. Figure 2a and b clearly indicates the formation of NG in the study. FESEM image illustrates irregular shapes of NG synthesized from windshield glass waste (Figure 2a). The configuration of NG was studied through the EDX spectrum. EDX confirmed the presence of O (59.10%), Si (29.70%), Na (7%), Ca (3%), and Mg (1.3%) in NG sample (Figure 2b). EDX illustration established the existence of each O and Si with other elements in the NG matter.

Phase purity and crystallinity of the prepared NG were detected via XRD peaks. The main diffraction peaks of synthesized NG were obtained at $2\theta = 22.72^\circ$ (Figure 2c). The reported peaks were parallel to the standard files of JCPDS file no: 01-076-0941, which showed the much higher degree of crystallinity of NG (Hu et al., 2016). The normal crystalline dimensions of NPs can be obtained through the Debye-Scherrer equation (Homaieghar and Elbahri, 2014). The Debye-Scherrer equation Eq. (5):

$$D = k\lambda \quad (5)$$

$$\beta_{hkl} \cos\theta_{hkl}$$

Where D is the crystallite size, k is Scherrer constant (0.9), λ is the X-ray wavelength of radiation for Cu K α (0.154 nm), β_{hkl} is the full-width at half maximum (FWHM) and θ_{hkl} is the diffraction angle. The obtained average size of NG was 2 ± 12 nm. Figure 2d shows the HRTEM image of the NG and confirmed its crystalline nature with size.

3.2. Impact on seed germination

The action route of NG on seed germination, growth, and development is yet not studied. It is known that seed germination offers an appropriate basis for plant growth and development. In the current study, seed germination of wheat was altered when treated with all quantities of NG (Figure 3b). In all the treatments, NG has reduced seed germination with increasing concentrations. The maximum germination percentage value was reported for 10 mg L⁻¹ NG concentration as 100%. Other than this, germination percentage was decreased with elevating NG concentrations to 96, 92, and 92% for 20, 30, and 40 mg L⁻¹ NG concentrations. This showed a dose-dependent effect of NG on seed germination of wheat. The seed covering of angiosperm seeds defends it against hostile ecological circumstances and manages germination (Debeaujon et al., 2000). This might be affected by the reduced germination rate of wheat with NG applications. Matter covering the exterior of the pores can minimize water movement, as reported in soybean seeds (Calero et al., 1981). This proposes that blockage of the pores with NG might hinder water movement and therefore postpone germination. These outcomes are dissimilar to the experiments of Alsaeedi et al. (2019), which observed that Si NPs suspensions (0, 100, 200, 300, and 400 mg L⁻¹) significantly increased the final germination% up to 28.7%. However, these outcomes are in arrangement with experiments of Azimi et al. (2014) with a reduction in seed germination after application of silica NPs (SiO₂) concentration as 80 mg⁻¹ on tall wheatgrass (*Agropyron elongatum* L.).

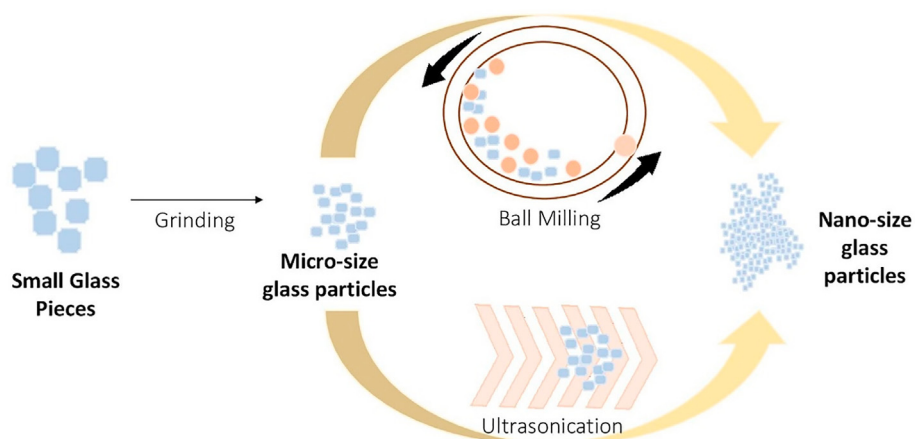


Figure 1. Small pieces of waste windshield glass with its nano-size particle formation using ball-milling and ultrasonication.

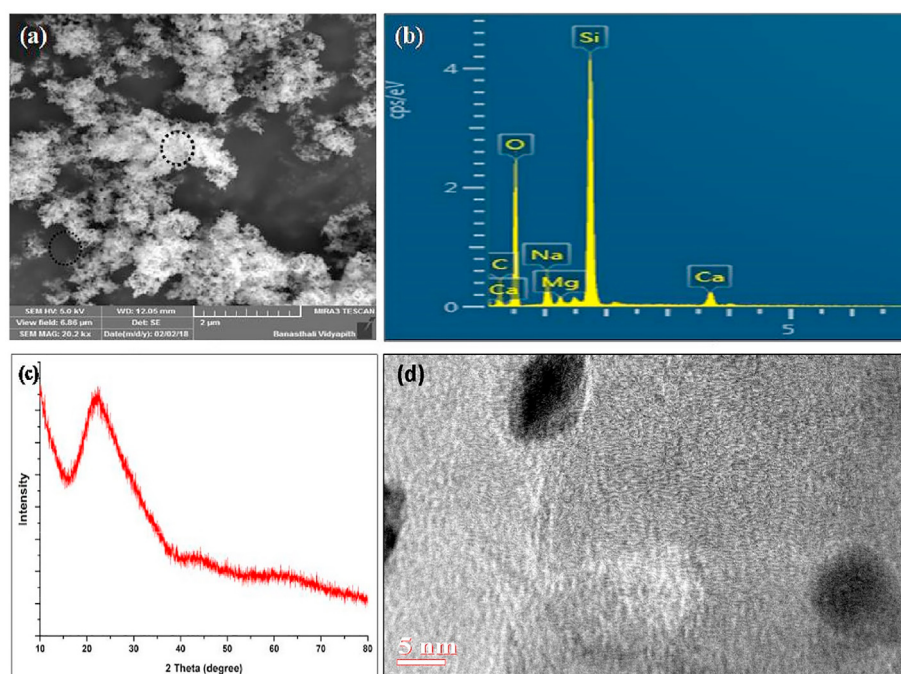


Figure 2. NG characterization through (a) FESEM image, (b) EDX spectrum, (c) XRD and (d) HRTEM.

3.3. Impact on growth parameters

NG exposure resulted in a sharp growth parameters decrement in root and shoot length of wheat seedlings. Results demonstrated that the maximum root length and shoot length (28.13 and 34.11 cm) was observed in 20 mg L⁻¹ NG treated wheat plants under control conditions. On the contrary, the lowest root and shoot height (15.40 and 22.42 cm) was achieved in the 40 mg L⁻¹ NG treatment (Figure 3c). The mechanism of NG phytotoxicity remains unclear. A similar significant decreasing trend for root and shoot length was also recorded at maximum concentrations of Si NPs. A similar study was reported with the inhibition of the growth of faba bean roots at 400 mg L⁻¹ Si NPs concentration (Thabet et al., 2021). NG was possibly aggregated at this quantity, the outcome in the hindrance of the air and water source via the seed coat that needs more study. In the part of genotoxicity, nevertheless, chromosomal aberrations were reported in *Vicia faba* plants treated with Si NPs at considerable minimum content (Galal et al., 2020). The reported

influence of Si NPs on seedling development can be constructive, non-significant, or harmful (Thabet et al., 2021). Alterations in the influences of Si NPs might be because of the other parameters such as variations in flora, the nature of growing media, NP property, together with treatment period and technique of NPs (Rui et al., 2014).

The effects of NG concentrations on the root-shoot fresh wt. and root-shoot dry wt. were analyzed. It is understood from Figure 4a, that there was considerable variation ($p < 0.05$) among control and NG treated plants' fresh and dry wt. In addition, both the root-shoot fresh and dry wt. of wheat seedlings declined with the amplified NG treatments. After 21 days of treatment with NG, the highest fresh and dry root (0.08 and 0.014 g) and shoot wt. (0.18 and 0.025 g) was obtained at 10 mg L⁻¹ (Figure 4a). Fresh-dry wt. might decrease due to the reduced growth parameters as root-shoot length with increased NG concentrations. Likewise, the Bt-transgenic root fresh biomass was reduced with increasing SiO₂ NPs treatment, respectively (Rui et al., 2014). Present outcomes are favored by earlier experiments by Rao and Shekhawat

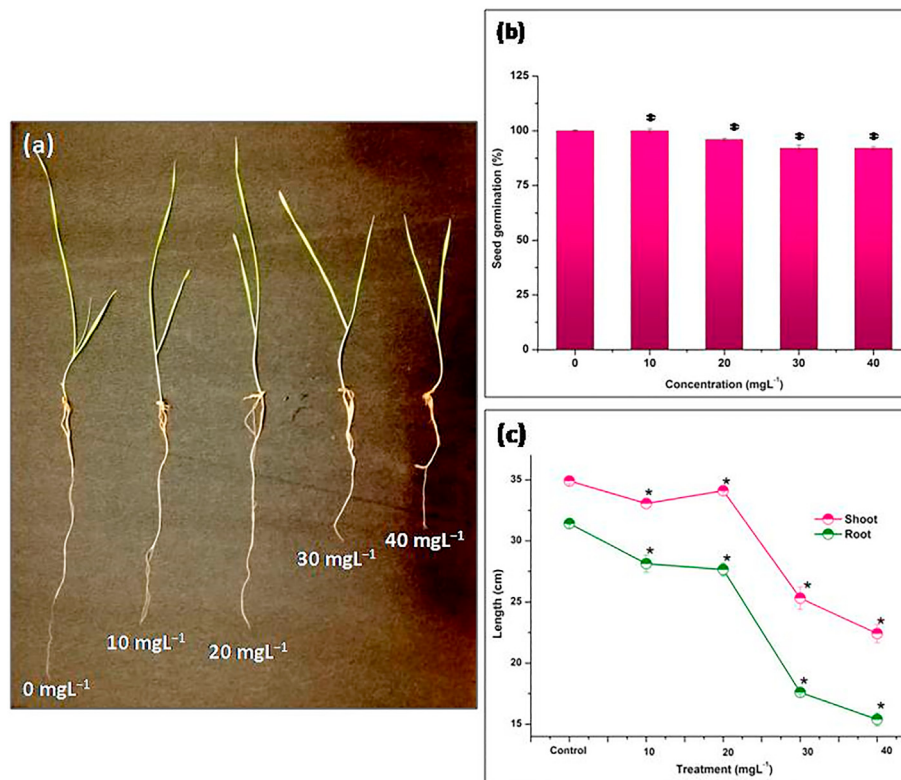


Figure 3. Effect of NG on *Triticum aestivum* plant (a) growth parameters, (b) seed germination, and (c) root-shoot length at 21 days period.

(2014). The shoot biomass of non-transgenic and Bt-transgenic cotton reduced with the rise of SiO₂ NPs concentrations.

3.4. Impact on chlorophyll and protein content

The effect of NG on chlorophyll content (chl a, chl b, and total chl.) contents is shown in Figure 4b. It was obvious that NG concentrations significantly affected the chlorophyll contents in wheat seedlings. The highest chlorophyll a, b, and total content (8.60, 6.08, and 14.68 mg g⁻¹ fresh wt.) were obtained at 10 mg L⁻¹. Maximum reduction of chlorophyll a, b and total content (6.19, 4.98, and 11.17 mg g⁻¹ fresh wt.) was

obtained at 40 mg L⁻¹ (Figure 4b). These results are in agreement with the previous study by Wei et al. (2010), exposure concentration of 50 mg L⁻¹ SiO₂ NPs decreased the chlorophyll content especially chl-a significantly (Wei et al., 2010). Chl-a plays an important role in the light-harvesting complex and PSII reaction center as an electron donor to the photosynthetic electron transport chain (Geoffroy et al., 2004). Therefore, especially chl-a could serve as a more sensitive parameter in plant growth inhibition and can be used for early detection of Si NPs exposure.

A significant decrease in the protein content of the wheat plant sample was observed (Figure 5a). As the concentration of NG gets

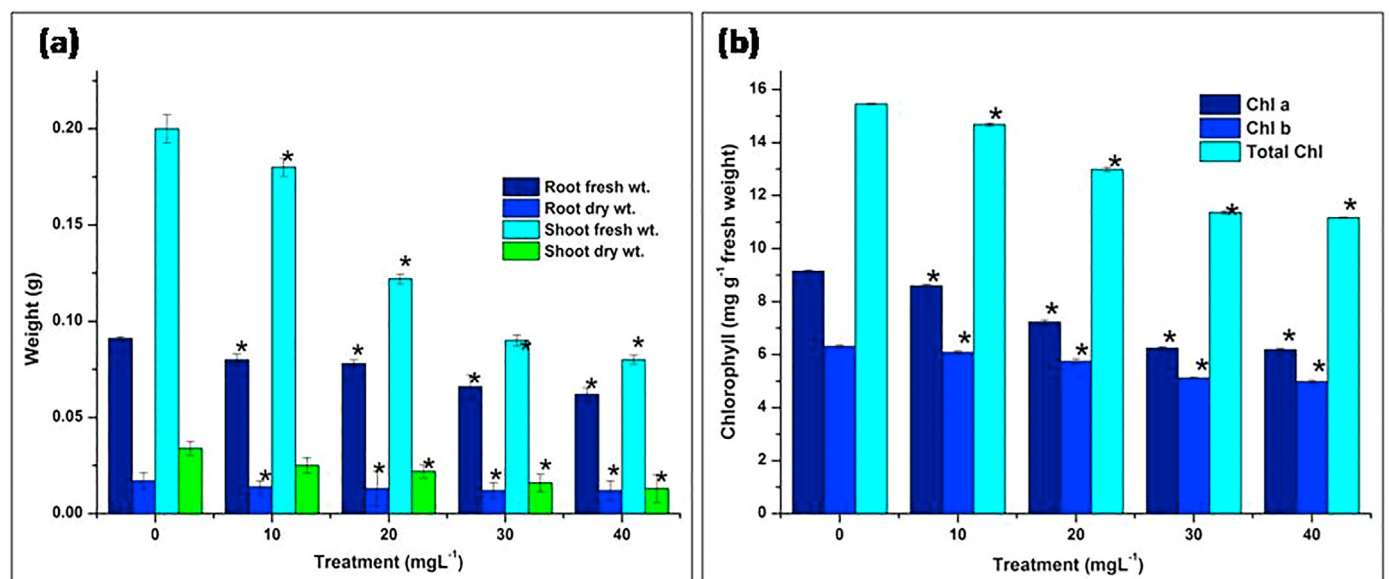


Figure 4. Effect of NG on *Triticum aestivum* plant (a) Root-shoot fresh and dry wt. and (b) Chl a, Chl b and total chl. at 21 days period.

increases (10, 20, 30, and 40 mg L⁻¹) caused a decrease in protein content over 21 days period. At 40 mg L⁻¹ concentration, the maximum significant decrease in protein was obtained as 7.02 mg g⁻¹ fw over control. The decrease in protein content at all NG concentrations suggests it's a toxic effect on wheat seedlings. A similar reduction in protein content was reported with an increase in polystyrene nano plastic concentrations (Li et al., 2021). However, in-depth studies are required for understanding the mechanism behind it.

3.5. Microscopic studies

In addition, the accumulated NG in leaves, shoot, and root tissues were observed through FESEM and EDX studies (Fig. 6a-i). FESEM and EDX spectrum of leaves, shoot, and root tissues showed the presence of Si elements and confirmed the Si accumulation in plant tissues. Elemental analysis of the whole plant showed that the application of 40 mg L⁻¹ NG was optimal for the maximal accumulation of Si in this study. EDX analysis confirmed that the leaves sample has O (65.80%), Si (1.80%), Na (10.20%), Cl (6.80%), and P (2.90%) (Figure 6c). Shoot tissue EDX confirmed the presence of O (73.70%), Si (2.20%), Mn (9.30%), K (4.40%) and P (5.20%) (Figure 6f). Finally, root tissue elemental analysis

confirmed the presence of O (59.60%), Si (19.40%), Na (4.20%), K (5.0%), and Mn (1.90%) at 40 mg L⁻¹ NG treatment at 21 days (Figure 6i). From EDX analysis it is confirmed high accumulation of Si in leaf, shoot and root tissues after NG treatment. Among all, root tissues were reported with a maximum Si% of 19.40. This shows the high accumulation in the root part with low translocation of Si in shoot and leaf parts.

3.6. Rhodamine B coated NG uptake in wheat seedlings

In previous studies, rhodamine B was found to be well suited for fluorescence imaging in seedlings. NG doped rhodamine B fluorescence was observed in root tissues of wheat seedlings (Figure 7). The epidermis and exodermis layer of the root had maximum visible rhodamine B fluorescence than the other layers, and the fluorescence was concentrated in the outermost epidermis area (Figure 7). Other than this, rhodamine B fluorescence was greater in the inner part of the endodermis. Among the vascular bundles, maximum fluorescence was observed in xylem tissues. This presence of NG in vascular tissue might imply that NG moves via xylem tissues from the root part into deeper tissue for travel to another part of plants.

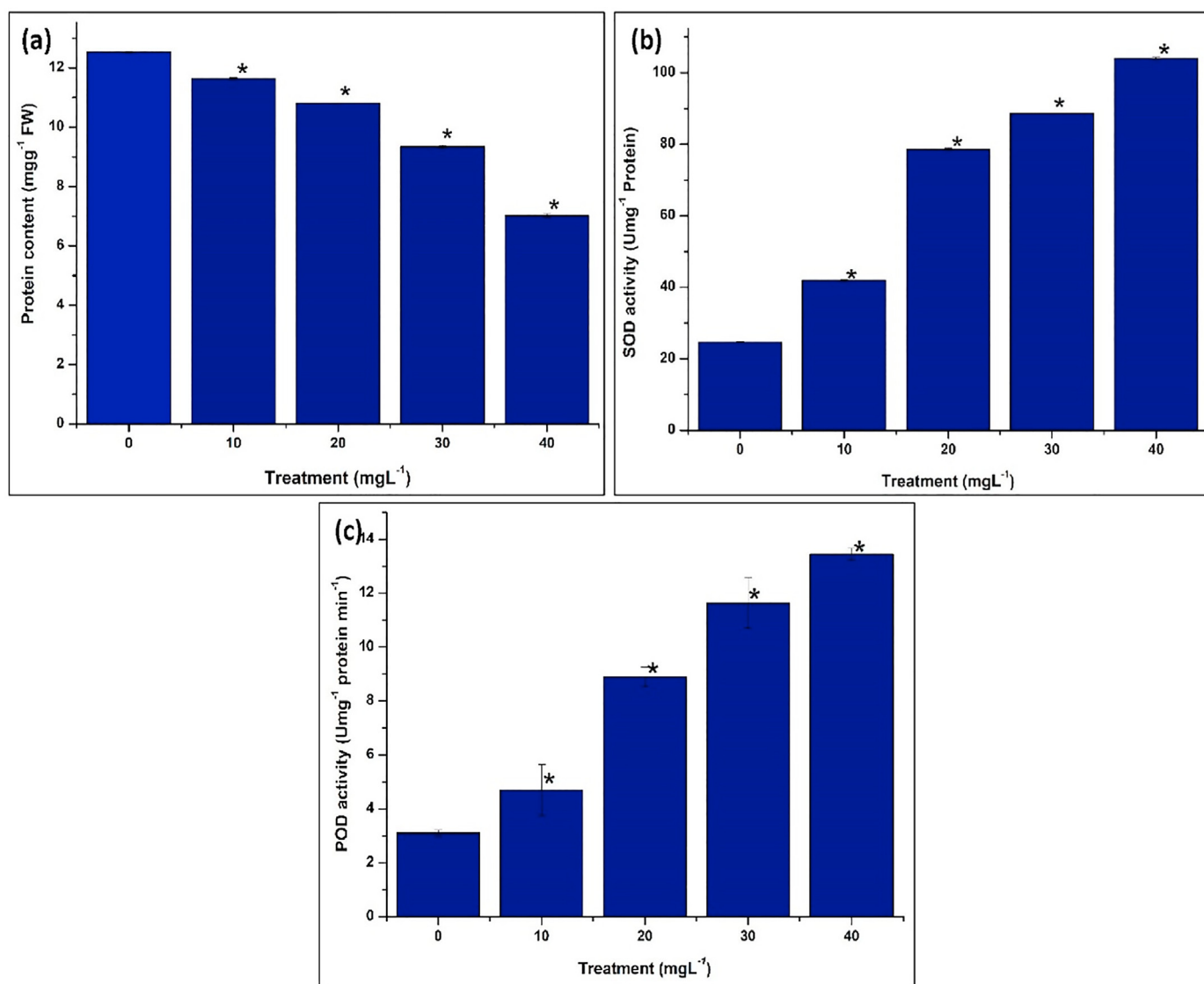


Figure 5. (a) Protein content in leaves of wheat plants treated with NG concentrations (0, 10, 20, 30, and 40 mg L⁻¹), (b) SOD activity of wheat seedlings, and (c) POD activity of *Triticum aestivum* seedlings at 21 days period.

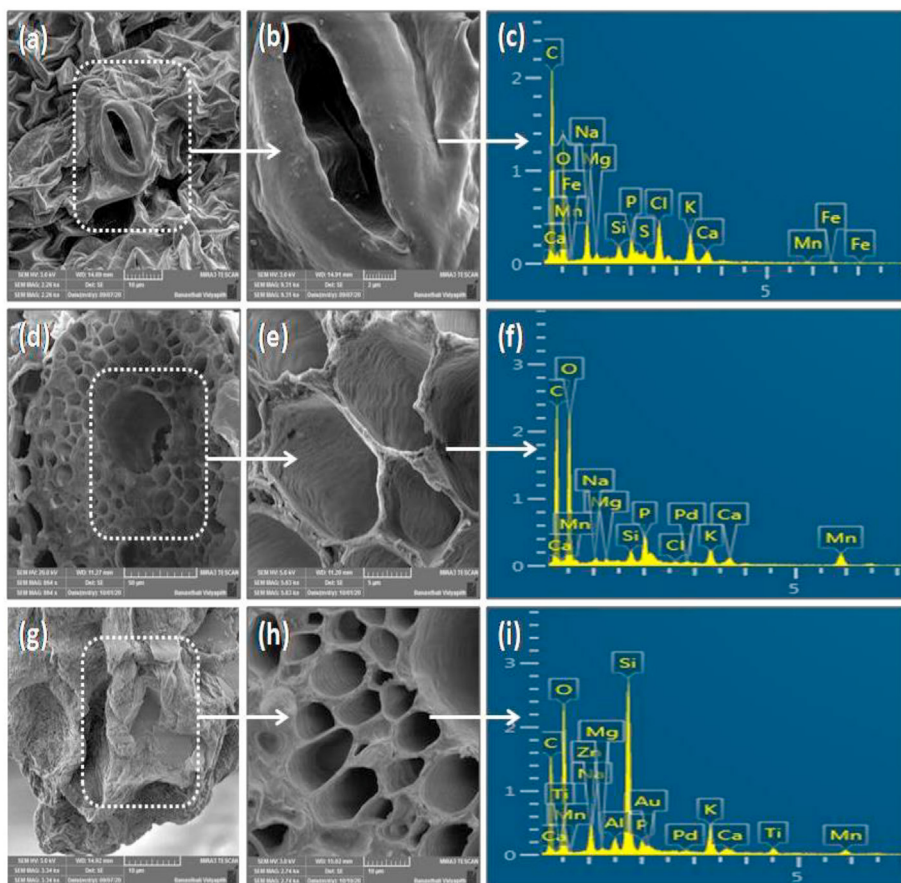


Figure 6. FESEM images of transversal cut of *Triticum aestivum* (a–c) leaves, (d–f) shoot, and (g–i) root tissues at 21 days period.

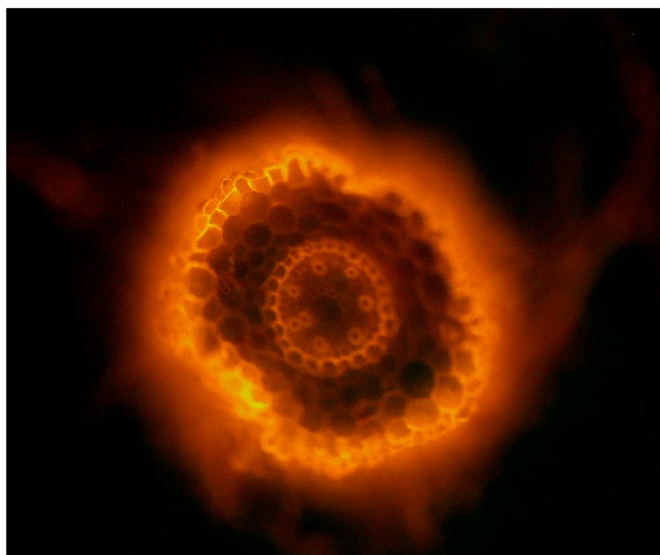


Figure 7. Imaging of Fluorescent dye (Rhodamine B) doped NG uptake through *Triticum aestivum* root.

Rhodamine B a fluorescent dye was used for the tracking of NG in plant cells. It is very sensitive and reliable method for the detection and localization of NPs in living cells (Hussain et al., 2013). There are several reports published on the detection of NPs in plant cells through the

fluorescence method (Cifuentes et al., 2010; Lee et al., 2012). As expected, there was fluorescence observed in the cross-sections of root cells treated with rhodamine B, mainly in epidermis and associated cells around the Casparian band of the wheat root.

4. Conclusion

In this work, the effect of Nanoglass was studied first time in the wheat plant system. This study investigated the effect of waste windshield NG on wheat seedlings at 21 days intervals. NG synthesis, characterization, and its effect on germination %, growth parameters, and chlorophyll content were conducted. Our results indicate a high accumulation of NG in the root part with low translocation of Si in the shoot and leaf parts. Plant growth was also get affected by the increase in NG dose in the plant. For the very first time, this study provided proof of the NG effect on crop plants; further investigations are required for in-depth knowledge.

Declarations

Author contribution statement

Swati Agarwal: Conceived and designed the experiments; Performed the experiments; Wrote the paper.

Sonu Kumari: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data.

Nidhi Sharma: Performed the experiments.

Suphiya Khan: Contributed reagents, materials, analysis tools or data.

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Data availability statement

Data included in article/supp. material/referenced in article.

Declaration of interest's statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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