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Toxic effects of graphene on the growth and nutritional levels of wheat (*Triticum aestivum* L.): short- and long-term exposure studies

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HIGHLIGHTS

- Graphene induced root elongation, whereas no changes were observed in leaf growth.
- Graphene caused roots oxidative damage and impairment of root hair production.
- Graphene adversely affected photosynthesis and shoot biomass production.
- Imbalance in nutrient homeostasis was observed after 30 days of graphene exposure.

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ABSTRACT

Increased use of graphene materials might lead to their release into the environment. However, only a few studies have investigated the impact of graphene-based materials on green plants. In the present study, effects of graphene on plant roots and shoots after 48 h or 30 days of hydroponic culture were evaluated to determine its phytotoxicity. Results showed that although exposure to graphene (250, 500, 1000 and 1500 mg L^{-1}) significantly improved root elongation, root hair production was impaired. These observations might be associated with graphene induced-oxidative stress (indicated by nitroblue tetrazolium (NBT) and Evans blue staining, malondialdehyde (MDA) estimation, and antioxidant enzyme activity assay). After 30 days of graphene exposure, shoot biomass, chlorophyll content, PSII activity and levels of several nutrient elements (N, K, Ca, Mg, Fe, Zn and Cu) were reduced, indicating that graphene inhibited plant growth and photosynthesis, and caused an imbalance of nutrient homeostasis. Based on these findings, we conclude that graphene has growth-limiting effects on plants, including root hair reduction, oxidative burst, photosynthesis inhibition, and nutritional disorder.

1. Introduction

Graphene is a class of 2D carbon nanoparticles, and owing to its unique properties, it has shown great promise in a wide range of applications, including energy storage, nanoelectronic devices, batteries, transparent conductors, and environmental protection [1,2]. Recent studies suggest that graphene materials are stable and can spread over long distances in the environment [3]. Therefore, owing to their widespread use, it is important to assess the potential risks associated with their release into the environment and the consequent interactions with organisms.

Recently, accumulating evidence from *in vitro* and *in vivo* studies has confirmed that graphene materials have hazardous effects on human and animal cells [4,5], microorganisms [6], and plants [7]. Although the health risks posed by graphene materials have been documented, existing toxicological data are still fragmentary, and the risks to ecosystems remain largely unexplored. In particular, information on the impact of graphene materials on plants, the key components of ecological systems, remains scarce. To date, to our knowledge, only five studies have attempted to determine the interactions of graphene materials (graphene and graphene oxide (GO)) with green plants [7–11]. Begum [7] suggested the negative effects of graphene, wherein the growth of

Abbreviations: GO, graphene oxide; MDA, malondialdehyde; SOD, superoxide dismutase; POD, peroxidase; TBA, thiobarbituric acid; TCA, trichloroacetic acid; NBT, nitroblue tetrazolium; $O_2^{\cdot-}$, superoxide; N, nitrogen; P, phosphorus; K, potassium; Ca, calcium; Na, sodium; Mg, magnesium; Fe, iron; Cu, copper; Zn, zinc; PSII, Photosystem II; F_0, minimum fluorescence; F_m, maximum fluorescence yield; F_v/F_m, maximum quantum efficiency; Y(II), yield of photochemical quantum; qP, photochemical quenching coefficient; ETR, electron transport ratio.

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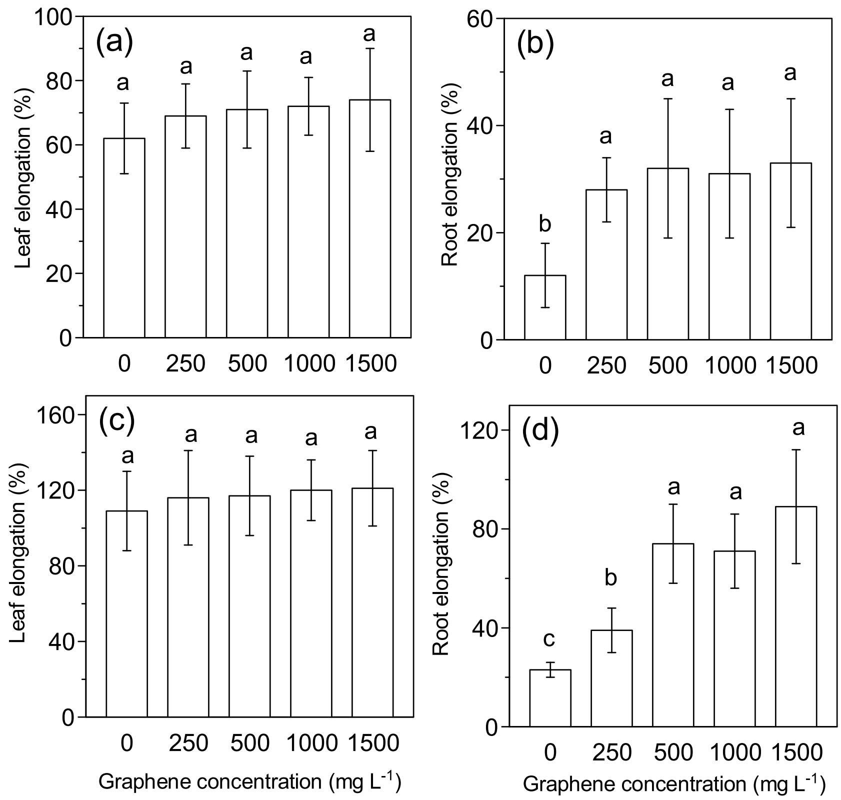

Fig. 1. Effects of different graphene levels (0, 250, 500, 1000 and 1500 mg L^{-1}) on the leaf (a, c) and root (b, d) elongations of wheat (*T. aestivum* L.). Plants were measured 24 h (a, b) and 48 h (c, d) after treatment. Error bars represent the standard error of means (*n* = *12*). Means with different letters are significantly different at *P* < *0.05*.

leaves of cabbage, tomato, and red spinach plants was inhibited after 20 days of treatment with 500–2000 mg L^{-1} graphene. Decreasing the concentration of graphene (200 mg L^{-1}) for 5 days did not have significant adverse effects on seed germination, root length, or fresh weight of wheat plants [8]. Wang [9] and Zhao [10] showed that GO exposure in the μg L^{-1} range for 8 days did not alter germination, seed development, or shoot and root growth in *Arabidopsis* plants. Increasing the GO concentration to 100 mg L^{-1} for 10 days significantly decreased root growth [11]. These findings indicate the importance of investigating the impacts of graphene materials on plants. However, owing to the limited information on this subject as mentioned above, the potential toxicity of graphene-based materials for plants is unclear, and is sometimes contradictory with the knowledge of their toxicity to animals [12].

Therefore, in light of these concerns, the present study aimed to investigate the short- and long-term phytotoxicity of graphene against wheat (*Triticum aestivum* L.), the model crop plant in the guidelines for chemical testing proposed by the US Environmental Protection Agency [13]. Alterations in leaf and root growth after treatment with graphene for hours and weeks were systematically examined. To the best of our knowledge, this study is the first to report the impact of graphene-based materials on mineral nutrient levels and photosystem II (PSII) activity in plants.

2. Materials and methods

2.1. Graphene properties and characterization

Graphene (Purity >99 wt%; Thickness: 0.5–3.0 nm; OD: 0.2–5 μm; Layers: 1–2; SSA: 1000–1217 $m^2 g^{-1}$; Appearance: black powder; resistance rate: 0.001 mΩ·cm) were purchased from Hengqiu Graphene Technology Co., Ltd (Suzhou, China). As an emergent nanomaterial, although the related applications of graphene are rapidly increasing, the current or future concentrations of graphene in the environment have not been determined. Hence, to be comparable with the doses of graphene used in previous phytotoxicity studies [7,8] and combined according to the US EPA toxicity studies on the food crops which commonly carried out at high concentrations (2000–5000 mg L^{-1}) of the nanoparticles [14,15], the exposed graphene was prepared at 0–1500 mg L^{-1} in present study.

2.2. Plant cultivation

Wheat Seeds (*Triticum aestivum* L.) were rinsed thoroughly in distilled water and then germinated at 25 °C in the dark. The germinated seeds were transferred to a net tray floated on a container filled with 0.5 mM $CaSO_4$. For the dose-response experiment, uniformly seedlings with seminal roots of 2–3 cm length were exposed to 0.5 mM $CaSO_4$ solution containing 0, 250, 500, 1000 and 1500 mg L^{-1} graphene. The lengths of the root and leaf were measured after 0, 24 and 48 h of exposure. The root and leaf elongations were calculated as follows: (final length – initial length)/initial length. Values presented here were the means of twelve replicated experiments. Thereafter, the roots of the seedlings were subjected to the measurements of oxidative stress indices described below. In addition, as shown in Fig. 1, the concentrations of 500 mg L^{-1} graphene at which significant effect was observed in root elongation measure were chosen for root hairs observation and staining tests. Root hairs were observed from 2 to 4 mm from the tip of the primary root [16]. The images were acquired using a Motic microscope (Beijin, China). All the experiments were conducted in a growth room with an average temperature of 28/23 °C, relative humidity of 50/80%, and photo-cycle of 14 h/10 h (day/night), and