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Graphene oxide and its viability as a constituent in nanofertilizers

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The use of chemical fertilizers and phytochemicals is a common practice in major crop production, promoting increased production per hectare but also representing a growing environmental concern. In response to this problem, this work carried out an ecotoxicity study and characterized the changes in soil properties resulting from the use of graphene oxide (GO), a component used in the formulation of new nanofertilizer and nanobiostimulant pellets for agriculture due to its effective carrier properties and previously reported non-toxicity in other areas. *Lepidium sativum* L. petri dish and pot trials were performed according to the EN 16086-2 European Standard and OECD guidelines to evaluate germination, vitality, and root development. Soil parameters such as pH, electrical conductivity (EC), total organic carbon (TOC), and water holding capacity (WHC) were also monitored. Although no significant phytotoxic effects were observed at most concentrations, higher doses (2.00 mg mL^{-1}) exhibited deviations in plant behavior and TOC levels. These findings help define the preliminary safe-use thresholds for GO in agricultural applications.

KEYWORDS

ecotoxicity, nanobiostimulant, nanoparticles, nanosafety, nanotechnology, soil properties

1 Introduction

Graphene is a single atom-thick layer of graphite, with sp2 carbon atoms bonded in a two-dimensional tightly packed hexagon structure (Zhang et al., 2005). Graphene oxide (GO) is becoming a popular composite in several areas due to its lower production costs and physical and chemical properties such as high solubility, biocompatibility, electrical conductivity, and low-to-no toxicity (Lee et al., 2011; Moradi et al., 2021; Basak et al., 2024). Nanocarriers for agricultural use are classified into three main categories: metallic, polymeric, and carbon-based nanomaterials. Compared to others, GO stands out for its ease of synthesis and superior adsorbing capabilities (Li et al., 2024; Zhang et al., 2025). It is an oxidized form of graphene and reveals a high density of oxygen functional groups in the carbon matrix, which allows interactions with several materials, leading to different functionalization (Loh et al., 2010; De et al., 2011; Perreault et al., 2015), has antibacterial and antifungal activity (Chen et al., 2014), and can be functionalized and used as a new nanocarrier for various substances (Liu et al., 2013). In addition, it acts as a

FIGURE 1
Image processing in the germination tests.

barrier agent in slow-release composites (Machado et al., 2019; Saraiva et al., 2022) and can be used in several layers to meet the required release time. These properties make GO an advantageous candidate to be included in new nanofertilizer and/or nanobiostimulant production since it can carry different compounds and control their release over time, thus reducing inputs and enhancing the productivity of crops while controlling soil nutrient imbalances (Saraiva et al., 2023).

However, nanotechnology and the use of GO in agriculture raise toxicity concerns and require more research as it enters the food chain (Fatima et al., 2021), although GO nanotoxicity can be reduced by the use of some substances such as amines, according to Lee et al. (2011). In addition, the interaction with soil/plant systems and the effect of plant root exudates can promote alterations in the dynamics of GO transport and assimilation, as described by Junjie et al. (2015), who discovered that root exudates alter some GO

TABLE 1 Characterization of pH and EC of GO dispersions.

Petri dish trial	pH (Sorensen)	EC (μS/cm)
Control	6.0	2.4
0.25 mg.mL^{-1}	3.5	129
0.50 mg.mL^{-1}	3.4	223
1.00 mg.mL^{-1}	3.1	601
2.00 mg.mL^{-1}	2.8	1,076

properties, such as the size and C:O ratio, which can also alter its potential toxicity effect.

To contribute to a more comprehensive understanding of the effects of GO in agriculture, the aim of this work was to investigate the ecotoxicity effect in *Lepidium sativum* L. as a model organism that represents an intermediate level of the terrestrial food chain and characterize some of the alterations that occur in soil properties by the use of GO for its incorporation in the development of nanofertilizing pellets. These components could be a sustainable option for the design of pellets that are capable of accurately and efficiently nourishing and stimulating crop resistance to diseases, reducing the need for phytopharmaceuticals, provided that they are proven safe and have defined limits of use.

2 Materials and methods

To perform the assays, commercially available non-functionalized dispersions of graphene oxide (GO) (0.5 mg.mL^{-1} and 4 mg.mL^{-1}, Graphenea, monolayer content >95%) were used to prepare a range of solutions from 0.25 mg.mL^{-1} to 2.00 mg.mL^{-1} by diffusing the dispersion in ultrapure water and stirring for 24 h.

2.1 Characterization of dispersions

For the characterization of dispersions, the pH and EC were measured using the Professional Bench Meter PC 80 Pro DHS Kit with the pH electrode and conductivity cell.

2.2 Phytotoxicity study—Petri dish

For the bioassays to determine the ecotoxicity of GO, we followed the OECD guidelines and the European Standard (EN 16086-2, 2012). These two methods were chosen because they both target *Lepidium sativum*, commonly named the garden cress, which is a dicotyledonous plant from the Brassicaceae (Cruciferae) family (OECD, 2006) that represents an intermediate level of the terrestrial food chain, reflecting effects from lower levels and having significance for higher trophic levels. To evaluate the possible effect of GO concentration in the soil matrix, seed germination tests were performed according to the European standard EN 16086-2. No sterile conditions were used. The root length was measured using ImageJ, and the germination (G) and the Munoo-Liisa vitality index (MLV%) were calculated using Equations 1 and 2, respectively, allowing the phytotoxicity level to be qualified. The treatments used were control (UP water) and 0.25 mg.mL^{-1}, 0.50 mg.mL^{-1}, 1.00 mg.mL^{-1}, and 2.00 mg.mL^{-1} dispersions.

$$\mathbf{G}\ (\%) = \frac{\boldsymbol{ANGS}}{\boldsymbol{TS}}, \qquad (1)$$

where ANGS represents the average number of germinated seeds and TS represents the total number of seeds.

$$\mathbf{MLV}\ (\%) = \frac{(\boldsymbol{GR1RL1}) + (\boldsymbol{GR2RL2}) + (\boldsymbol{GR3RL3})}{\mathbf{3}(\boldsymbol{GRCRLC})} \times \mathbf{100}, \qquad (2)$$

where GR represents germination in each dish, RL represents the root length on each dish, (s1) represents the first dish, (s2) represents the second dish, (s3) represents the third dish, GRC represents germination in the control, and RLC represents the average root length in the control.

The Petri dish tests followed EN 16086-2, with the necessary adaptations for the dispersion test. For each test, 10 seeds were placed on a filter paper in 9-cm-diameter Petri dishes in quadruplicate. In addition, 3 mL of each treatment was added. The Petri dishes were incubated at 25 °C ± 2 °C for 72 h, after which the images were processed using ImageJ software (Figure 1).

2.3 Pot trial

Loamy sandy soil that was sieved to 2 mm, with an apparent soil density of 1.57 g mL^{-1} and a field capacity of 0.48 mL water.mL soil^{-1}, was used. The treatments were as follows: control, 0.25 mg.g^{-1} soil, 0.50 mg.g^{-1} soil, 1.00 mg.g^{-1} soil, and 2.00 mg.g^{-1} soil. This test lasted for 15 days since, according to the OECD guideline number 208, the test ends at 14 days after control germination.

Germination, the number of leaves 5 days after germination, and the plant's final fresh weight were determined. At the end of the trial, the fresh weight of the plants was determined after cutting roots to minimize interference from soil residues.

The incubator conditions, temperature (T) and relative humidity (RH), were monitored using a Vanguard Hydroponics combined meter, and a 16-h photoperiod was applied.

2.4 Soil characterization and monitoring

To study the impact of GO on soil properties, electrical conductivity (EC), pH, total organic carbon (TOC), and water holding capacity (WHC) were determined. EC and pH were determined in a 10:25 soil–water suspension (10 g of soil/25 mL water) after 2 h of stirring. EC was measured using an Orion Star™ A212 Conductivity Benchtop Meter and Orion™ DuraProbe™ 013005MD, while pH was determined using a Thermo Fisher Scientific™ Orion™ 3-Star Benchtop pH Meter and Orion™ ROSS Ultra™ Refillable pH/ATC Triode™ 8157BNUMD electrode. TOC was determined in 650 ± 70 mg of soil using a fully automated solid sampler multi EA 4000 solid analyzer (Analytik Jena), and water holding capacity was determined

TABLE 2 Petri-dish germination test results.

Petri dish trial	Germinated seeds 72 h [%]	Root length 72 h [cm]	Munoo-Liisa index [%]
Control	98%[a]	1.20 ± 0.23[a]	100%
0.25 mg.mL^{-1}	98%[a]	2.07 ± 0.15[a]	178%
0.50 mg.mL^{-1}	95%[a]	1.91 ± 0.03[a]	160%
1.00 mg.mL^{-1}	98%[a]	1.35 ± 0.19[a]	116%
2.00 mg.mL^{-1}	98%[a]	0.81 ± 0.01[a]	69%

Different letters represent means with significant differences for $p < 0.05$.

using Equation 3 after maintaining the soil at the field capacity, which is the amount of water that a soil sample can hold after being saturated and allowed to drain. Four concentrations were studied: 0.25 mg.g^{-1}, 0.5 mg.g^{-1}, 1.0 mg.g^{-1}, and 2.0 mg.g^{-1} of soil, and all determinations were compared with the control, which was loamy sandy soil.

$$\boldsymbol{WHC} = \frac{\boldsymbol{WL}}{\boldsymbol{SW}} \times \mathbf{100}, \quad (3)$$

where WL represents the weight loss after drying 105 ˚C and SW represents the soil weight after drying 105 ˚C.

2.5 Statistical analysis

All variables measured in this work were recorded in quadruplicate, and mean values and standard deviations were determined, allowing the parametric test of analysis of simple variances (one-way ANOVA). When the F-test of ANOVA was significant, Tukey's honest significant difference (HSD) *post hoc* test was used for multiple comparisons of means. The level of significance considered was 5% ($p < 0.05$) in order to verify the existence of significant differences between the modalities.

3 Results

3.1 Characterization of dispersions

The pH of the several dispersions fell within the range of 3.5 to 2.8, reducing as the GO concentration increased, and the EC had the expected reverse result, increasing along with the concentration, in the range of 2.4 µS/cm for the control to 129 µS/cm for 0.25 mg.mL^{-1} to 1,076 µS/cm for 2.00 mg.mL^{-1} (Table 1).

3.2 Phytotoxicity study

The results obtained in Petri dishes are shown in Table 2. A single-factor analysis of variance was carried out, and the difference in means was obtained using the Tukey test ($p < 0.05$). In addition, although germination and average root length remained statistically unchanged, subtle behavioral shifts at higher concentrations indicate potential physiological stress.

In terms of MLV %, seeds treated with 2.00 mg.mL^{-1} here the only ones below the control threshold.

3.3 Pot trial

3.3.1 Incubation conditions

The incubator chamber conditions were monitored and are presented in Figure 2 and Table 3. The temperature oscillated between 20.1 ˚C and 26.8 ˚C, with a mean value of 23.5 ˚C. The relative humidity oscillated between 52% and 80%, with a mean value of 66%.

3.3.2 Plant parameters

Table 4 presents the results obtained for pot trial monitoring, and Figure 3 illustrates *L. sativum* plants per treatment at the end of the test, before the fresh weight assessment.

Despite the mortality of 1 plant per repetition in the 2 mg.g soil^{-1} treatment, none of the parameters observed showed significant differences for $p = 0.05$.

3.4 Soil results

The results revealed no significant differences in soil pH, EC, and WHC (Table 5), while TOC determination revealed significant differences in 2.00 mg.g^{-1} treatment ($p = 0.05$).

4 Discussion

4.1 Characterization of dispersions

Depending on the conditions of application, the acidic pH determined for the several dispersions can be an important factor when incorporated in the soil/plant systems since it impacts diverse soil functions and can lead to damage in plants (Long et al., 2017), which must be accounted for in their utilization as a nanofertilizer component.

4.2 Phytotoxicity study

In accordance with the results obtained by Chen et al. (2018) in wheat for 24 h and 72 h tests, the results reveal no significant differences ($p < 0.05$) in germination or in average root length compared to the control, even indicating a stimulating effect in the root growth at lower concentrations. Low concentrations of GO are reported to have positive effects in several studies: raspberry, 2 mg/L; aloe vera, 10–100 mg/L; maize, 50 mg/L; and wheat, 100 mg/L (Chen

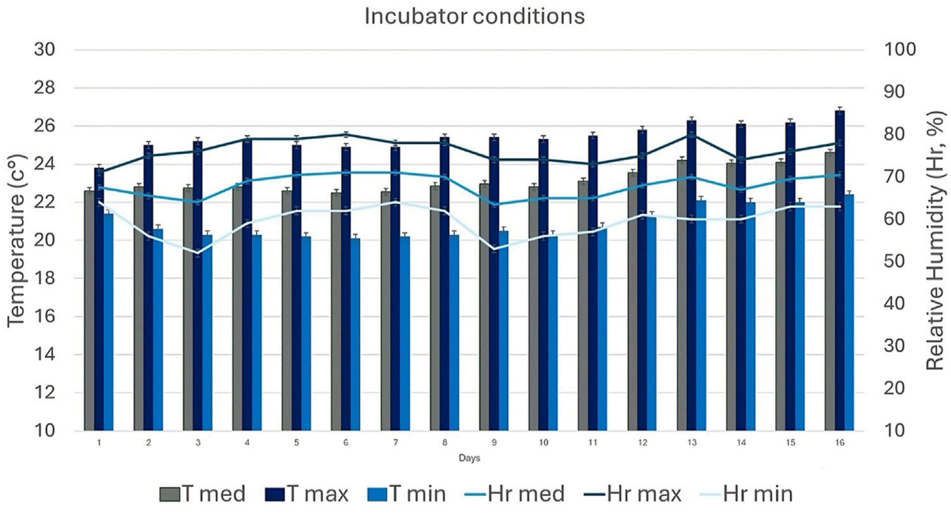

FIGURE 2
Temperature and relative humidity conditions during the pot trial.

TABLE 3 Pot trial conditions.

Incubator conditions	
Sandy loamy soil	✓
Temperature (°C)	23.5 ± 4.7
RH (%)	66.0 ± 19.8
Photoperiod	16 h

et al., 2018; Hu et al., 2019; Ren et al., 2020; Chen et al., 2021; Zhang et al., 2021).

The MLV % compares seed germination and average root length of the seeds under different treatments to those of the control, and although no significant differences ($p < 0.05$) in germination or average root length were found, this index clearly shows that the seeds treated with 2.00 mg.mL^{-1} presented a different behavior and were the only ones under the control threshold due to the values of root length.

4.3 Pot trial

4.3.1 Incubation conditions

The results from monitoring the incubation chamber showed that the required conditions of the test were fulfilled according to the OECD 208 guidelines, validating the test.

4.3.2 Plant parameters

The results obtained are consistent with Hammerschmiedt et al. (2023), where no significant differences in plant weight above ground were found by the use of GO, while other studies point to the improved biomass achieved by graphene nanomaterials. Wang et al. (2023) found that graphene boosted nutrient absorption by maize plants, inducing improved nutrient uptake with increasing application, leading to higher plant biomass (aboveground fresh and dry weight, plant height, and stalk thickness), and Zhou et al. (2023) observed this effect in *Iris pseudacorus*. For the 2 mg.g soil^{-1} treatment, once again, a different behavior was observed. One plant per repetition perished during the test; however, the fresh weight and number of leaves of the remaining plants were consistent with the those observed in plants from other treatments, thus indicating the viability of germination and growth under this treatment.

4.3.3 Soil

Although using loamy sandy soil, the results are in accordance with other studies in different types of soil (silty clay loam and potting soil loam/peat/perlite, 1:1:1), which showed no significant differences in soil pH, EC, and WHC (Zhao et al., 2020; Hammerschmiedt et al., 2023; Peña-Álvarez et al., 2024). In different soil texture types, the results may vary, as higher ionic strength, surface charge, and surface area lead to stronger interactions with GO (Lu et al., 2019).

Zhao et al. (2020) also obtained no change in the soil pH parameter but observed that GO refrained water from evaporating in a soil sample treated daily with 100 μg/mL water, and the possible relationship with WHC should be explored further. As expected, TOC determination showed significant differences in 2.00 mg.g^{-1} treatment due to GO's carbon contribution. In many areas with low organic C and organic matter content in the soil, an increase in TOC levels could represent an opportunity to increase soil productivity by improving soil texture and, consequently, key soil functions such as nutrient and water holding capacity (He et al., 2018; Andelkovic et al., 2018; Saraiva et al., 2023). Peña-Álvarez et al. (2024) reported that GO (20 mg.g^{-1}) had a significant impact on soil pH, redox potential (Eh), and EC in higher concentrations.

5 Conclusion

Biological systems are vast and complex, so the interactions between nanofertilizers, carriers, and ecosystems, which

TABLE 4 Pot trial monitoring results.

Graphene oxide concentration [mg/g soil]	Mean germination [%]	Mean number of leaves	Fresh weight [g/plant]
Control	96%[a]	9.40 ± 2.06[a]	0.042 ± 0.009[a]
0.25 mg/g	92%[a]	11.0 ± 2.37[a]	0.057 ± 0.004[a]
0.50 mg/g	92%[a]	8.80 ± 2.99[a]	0.053 ± 0.011[a]
1.00 mg/g	96%[a]	10.40 ± 1.74[a]	0.066 ± 0.003[a]
2.00 mg/g	92%[a]	8.60 ± 2.50[a]	0.062 ± 0.007[a]

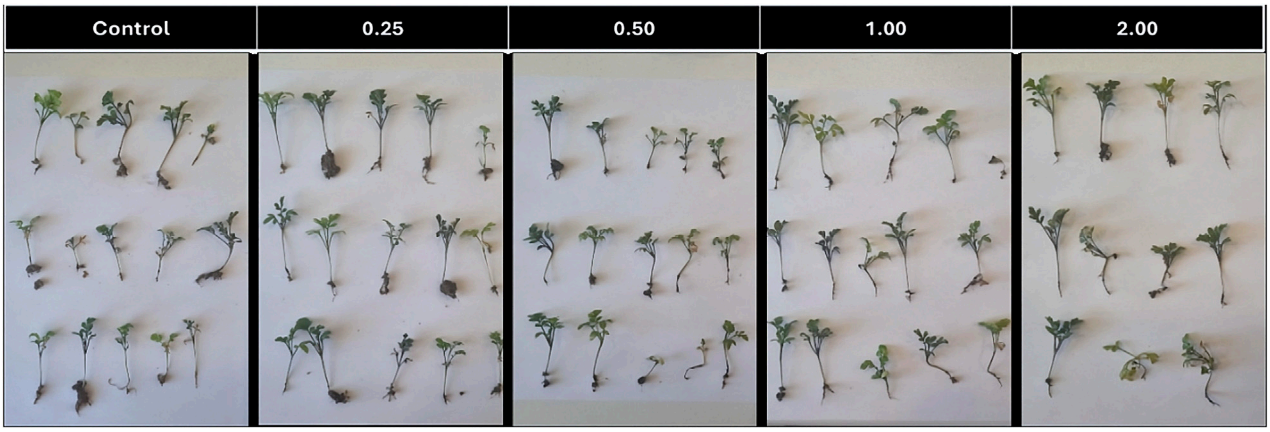

FIGURE 3
Lepidium sativum plants at the end of the pot trial.

TABLE 5 Evaluation of soil parameters.

	TOC (%)	pH	EC (μS/cm)	WHC (%)
Control	0.517 ± 0.029[a]	5.513 ± 0.148[a]	1,484.5 ± 27.5[a]	26.6 ± 0.42[a]
0.25 mg/g	0.507 ± 0.005[a]	5.350 ± 0.127[a]	1,507.5 ± 253.5[a]	25.9 ± 1.34[a]
0.50 mg/g	0.505 ± 0.015[a]	5.387 ± 0.037[a]	1,359.5 ± 63.5[a]	26.5 ± 1.05[a]
1.00 mg/g	0.587 ± 0.029 [ab]	5.467 ± 0.041[a]	1,690.0 ± 213.6[a]	26.6 ± 0.08[a]
2.00 mg/g	0.650 ± 0.022[b]	5.407 ± 0.076[a]	1720.0 ± 244.2[a]	24.0 ± 0.25[a]

include several compartments and biotic factors, are also complex.

This study addresses the need for the risk assessment of nanomaterials. Although the final product and the design of the complete nanofertilizer must undergo this process and be tested again, this is an important stage in the development of new GO-based nanofertilizers. The results showed concentration-dependent alterations in dispersion characterization, but overall, all the results showed no significant differences between GO treatments and control.

In conclusion, the study confirms that graphene oxide, within concentrations of up to 1.00 mg/mL, can be safely applied without significant phytotoxic effects or disruption to soil function. However, early signs of biological deviation at 2.00 mg/mL suggest a critical threshold for safe usage. These findings support the need for cautious use of GO in nanofertilizer formulations and highlight the need for long-term environmental studies.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Author contributions

RS: Conceptualization, Data curation, Funding acquisition, Investigation, Methodology, Writing – original draft. QF: Conceptualization, Resources, Writing – review and editing. GR: Resources, Writing – review and editing. MO: Conceptualization, Funding acquisition, Methodology, Resources, Writing – review and editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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