The Effects of Titanium Dioxide Anatase Nanopowder on the Common Bean Plant

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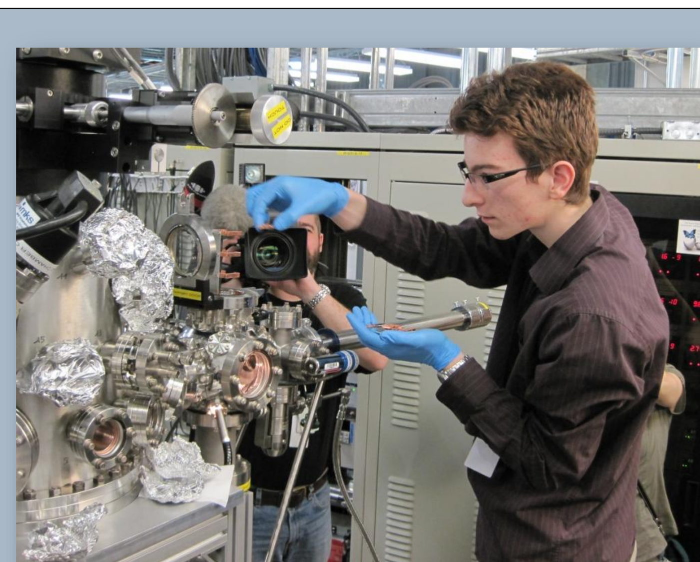
Abstract

Since the introduction of nanoscience as a research field there has been a great deal of study into the physical properties of nanoparticles and into the development of new nanomaterials. However, there has been limited research into the environmental implications of nanoparticle use. This project aims to contribute to current theories and to promote further research in this line of study. Existing research on the impact of nanoparticles on plant growth shows conflicting results. Dr. Baoshan Xing et al. found that zinc and zinc oxide nanoparticles inhibit root growth in rapeseed[1]. Conversely, it has been found that the activity of Rubisco activase, a crucial enzyme in carbon fixation, was increased in spinach plants grown in the presence of titanium dioxide nanoparticles[2]. Clearly there are many factors that influence the interaction between nanoparticles and plants. In order to gain a comprehensive understanding of these interactions, multiple studies must be conducted on a range of nanoparticles and plant species.

The project focuses on the effects of titanium dioxide anatase nanopowder on the common bean (Phaseolus vulgaris); specifically, it aims to analyze the growth rate, mineral absorption, and the intake and transport of nanoparticles. Titanium dioxide was selected because of its photo-catalytic potential, and its widespread usage in commercial products. The bean plants are subjected to varying concentrations of titanium dioxide nanoparticles in different stages of plant life, and quantitative observations of their growth are taken on location. Using the Canadian Light Source's SGM beamline, the concentrations of different elements within the plant roots are determined. The results suggest possible environmental considerations of nanoparticle use, and provide a basis for further research in the field of nanoscience.

Yongjian Lu transplanting bean plants into nanoparticle-contaminated soil.



Eric Langlois placing sample slides into the SGM beamline sample chamber while being filmed by the CBC crew.

Method

General Method

Beans were sprouted in water and planted in growth medium containing varying concentrations of nanoparticles. Two batches of plants were made, along with necessary controls. Batch 1 consisted of 80 plants grown in nanosoil (growth medium containing nanoparticles). 10 bins of plants were grown, with 8 plants in each bin. Each bin of plants was exposed to a different concentration of nanosoil, ranging from 1 ppm to 25000 ppm. Plants grown in normal growth medium (no exposure to nanoparticles) served as the negative controls for batch one. Batch 2 consisted of 80 plants grown in normal soil, which were transplanted into nanosoil of varying concentrations (same concentrations as in batch 1; nanosoil was made separately) once the plants reached maturity. Plants grown in normal growth medium which were transplanted into normal growth medium served as the negative controls for batch two. After 5 weeks of growth, all plants were extracted from growth medium, and from each plant, one leaf, one segment of the main stem, and one main branch of the roots were isolated, dried, crushed to a fine powder, and placed in separate sealed glass tubes.

A batch of the growing bean plants.

Soil Concentration of TiO_2 (ppm)	Number of Plants (Normal+Transplant)
Control	10 + 10
1	8+7
2.5	8+7
5	8+7
50	8+7
250	8+7
500	8+7
1000	8+7
2500	8+7
5000	8+7
25000	8+7

The beams just after sprouting.

Bean sprouting

Approximately 200 common beans (*Phaseolus vulgaris*) were sprouted in a porcelain plate. Normal tap water was added to the plate so that each bean had direct contact with the water. Humidity was preserved by covering the plate with a damp paper towel. The plate was placed in a warm environment for the beans to sprout. Sprouting took approximately five days.

Growth Medium Preparation

Nanosoil (growth medium + nanoparticles + tap water): For higher concentrations of nanosoil, the appropriate mass of nanoparticles was placed directly into a plastic cup. 40g of growth medium was then poured into the cup. 100mL of water was aliquoted into each cup. The contents of the cup were then mixed thoroughly. Ex. For 25000 ppm nanosoil, 1g nanosoil + 40g growth medium + 100mL water. For lower concentrations of nanosoil, a concentrated suspension of nanowater (nanoparticles + water, with frequent agitation to maintain suspension) was made, then diluted to the necessary concentration. Finally, water was added to make a final volume of 100 mL. Ex. For 1 ppm nanosoil, the concentrated suspension was diluted to 40µg/10mL. 10mL of diluted suspension + 90mL of water + 40g of growth medium were added to the cup. The contents were then mixed thoroughly.

Tim Xu creating a diluted suspension of Titanium Dioxide nanoparticles.

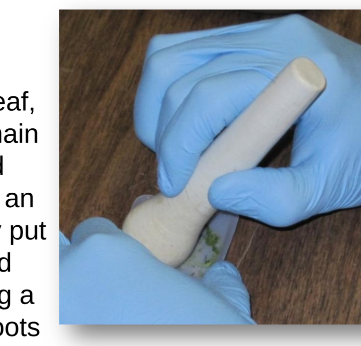
Normal soil (growth medium + tap water): For all controls and plants that were to be grown to maturity in normal soil, then transplanted into nanosoil (nanosoil used for transplants was prepared in the same way as nanosoil mentioned above), 40g of growth medium + 100mL of water were added to each cup and mixed thoroughly.

Plant Care and Monitoring

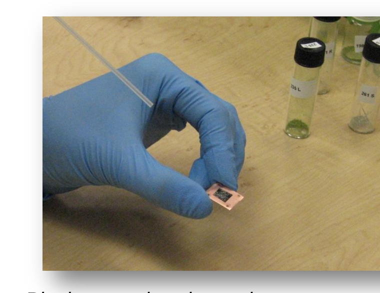
Plants were watered three times a week (Monday, Wednesday, Friday), and measurements of overall plant height and leaf length were taken weekly. The amount of water added was determined by the humidity of the soil in each plant's pot ensuring all plants were exposed to similar water levels in the soil. Measurements of the height of the main stem and the length of the longest leaf on the plant were taken with a ruler.

Sample Preparation

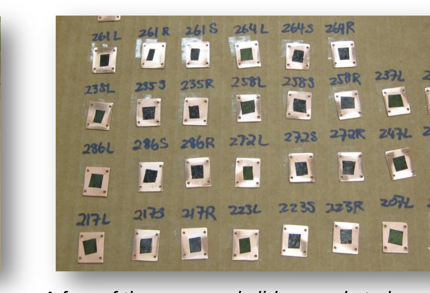
From each plant, a part of the leaf, a segment of the stem, and a main branch of the root were severed with clean scissors and dried in an incubator. Leaves were directly put into the glass sample holder and crushed into a fine powder using a glass stirring rod. Stems and roots were crushed using mortar and pestle and then put into glass sample holders. All samples were taken to Saskatoon, where a select few were placed on SGM beamline copper slides using carbon tape.



Crushing a leaf sample with a pestle.



Placing powdered samples on copper slides.



A few of the prepared slides ready to be scanned.

Discussion

Initial results come from the physical measurements taken as the plants were growing. Measurements of the plant height (Fig 1) and leaf length (not shown) were taken on a weekly basis throughout the growth period of the plants. This graph shows the average stem height of the plants at each soil concentration. The plants were divided into four main groups: Non-transplanted controls, transplanted controls (transplanted from uncontaminated soil to uncontaminated soil to account for the stresses involved), plants grown to maturity in uncontaminated soil then transplanted to nanosoil, and plants grown entirely in nanosoil. The mature group was transplanted between weeks two and three. As the graph shows, the nanosoil contaminated plants experienced visibly stunted growth in the first few weeks of development and never fully caught up to the other plants, although their growth rates eventually did. Furthermore, all but one of the plant groups exposed to nanoparticles grew less than the control groups. The act of transplanting the mature group did not have an immediate impact on the growth rate however before the transplant the mature plants were keeping pace with the controls but afterwards fell behind the control transplant group. (Note: The stem heights on the last measurement are sometimes shorter than the week before because the plants began collapsing under their own weight.) From these preliminary measurements it appears the presence of titanium dioxide nanoparticles in the soil inhibited the growth of the bean plants to some degree.

The SGM beamline scans of the crushed plant samples show a number of surprising results. Despite the very small size of the TiO2 nanoparticles there was no detectable presence of titanium within either the leaves or the roots of the plants grown in the highest soil concentration – 25000ppm – of the nanoparticles (Fig 2). Any detectable levels of titanium dioxide within the samples would appear as a peak in intensity around 460 eV. Therefore, it appears that the bean plants used in this experiment do not absorb titanium dioxide nanoparticles into their roots from the soil even when the particles are present in very high concentrations.

Despite not having absorbed the titanium dioxide nanoparticles, the plants grown in contaminated soil nevertheless appear to have been affected by their presence. Figure 3 compares the levels of calcium within a control sample root and root samples grown in 5000ppm and 25000ppm nanosoil. A low resolution makes analysis difficult however the peaks for the contaminated root samples are shorter than the calcium edges of the control sample. This suggests that the presence of TiO2 nanoparticles in the soil may have impeded the uptake of calcium into the roots of the plant. A deficit of calcium in plants has been linked to stunted plant growth under stress in previous studies[3], so this could explain the difference in growth observed between the plants grown in contaminated soil as opposed to those grown in regular soil. Figure 4 shows a similar occurrence for nitrogen. The nitrogen edge is smaller for the plant grown in contaminated soil than it is for the control plant. However, a combination of the small difference in peak heights, large amount of noise, and small number of samples samples makes any predictions concerning the effect of TiO2 presence on nitrogen uptake inconclusive. The most drastic results are visible in the sodium concentration scan (Fig. 5) The control root sample has a well-defined peak at the sodium edge but in both the 5000ppm and 25000ppm root sample any concentration of sodium in the roots is insignificant compared to the background noise. Therefore, although the titanium dioxide nanoparticles were not absorbed by the plants, the presence of titanium dioxide nanoparticles within the soil at high concentrations significantly decreased the amount of sodium present in the plants' roots as well as potentially having reduced the concentrations of calcium and nitrogen in the roots.

Observations and Results

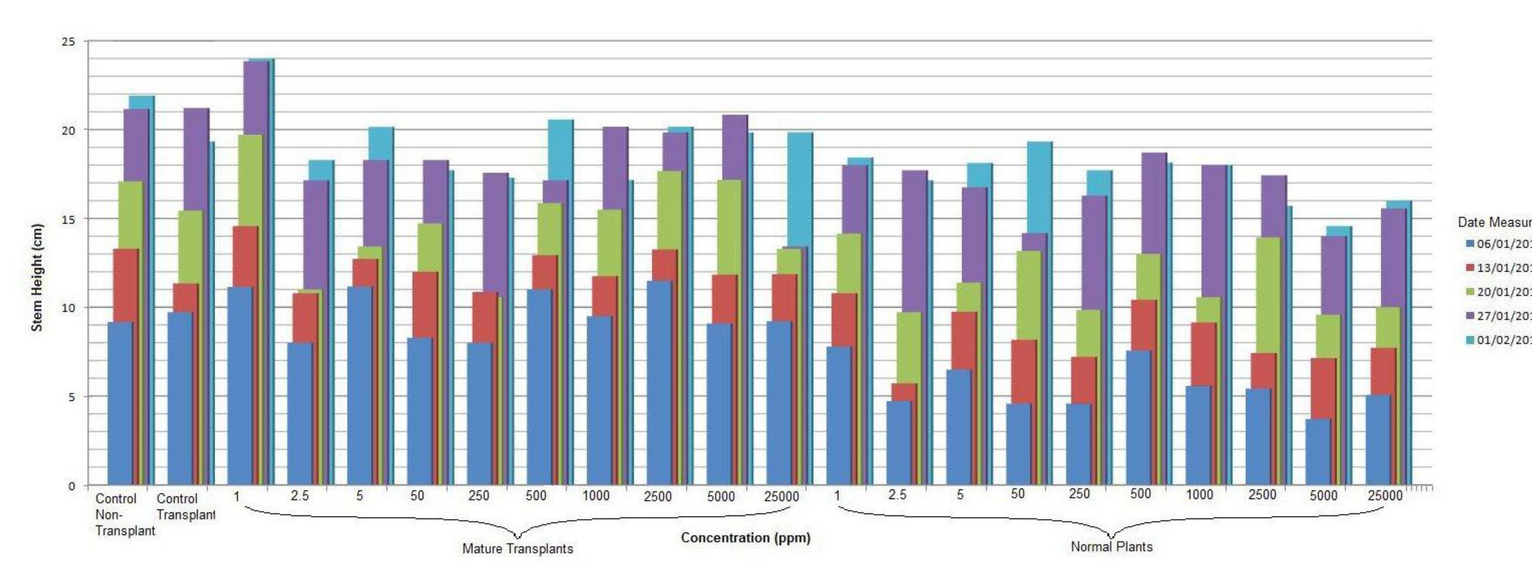


Fig 1: Average bean plant stem heights by concentration over time.

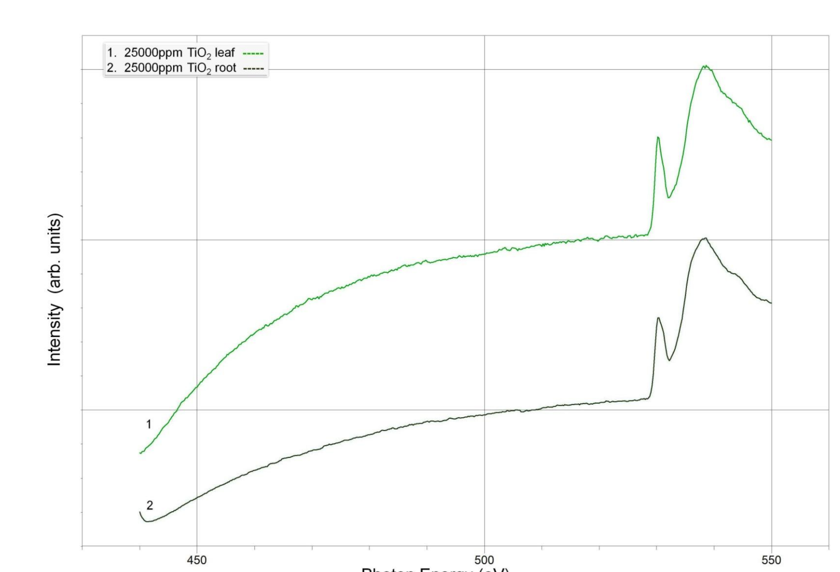


Fig 2: SGM Beamline scan of the titanium and oxygen K edges in TiO_2-contaminated root and leaf samples. Oxygen peak from 530 – 550 eV used to normalize all scans. No visible titanium peak at 460 eV.

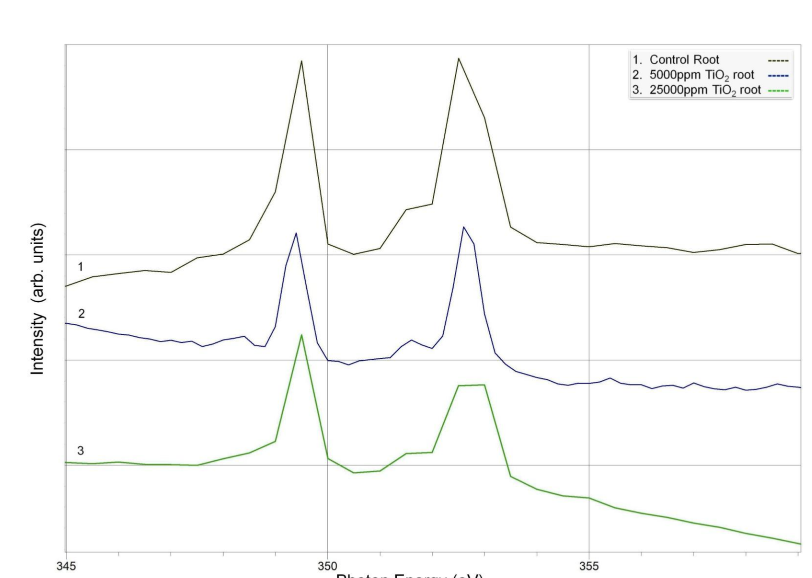


Fig 3: SGM Beamline scan of the calcium edge in control and contaminated plant root samples. Diminished peak size in contaminated samples compared to the control.

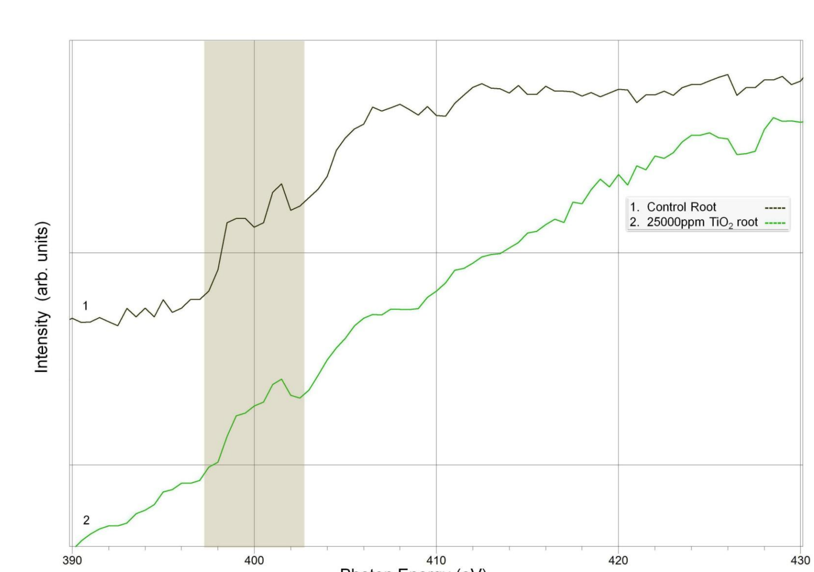


Fig 4: SGM Beamline scan of the nitrogen edge (highlighted) in control and contaminated plant root samples. Slightly diminished peak size in contaminated sample compared to the control.

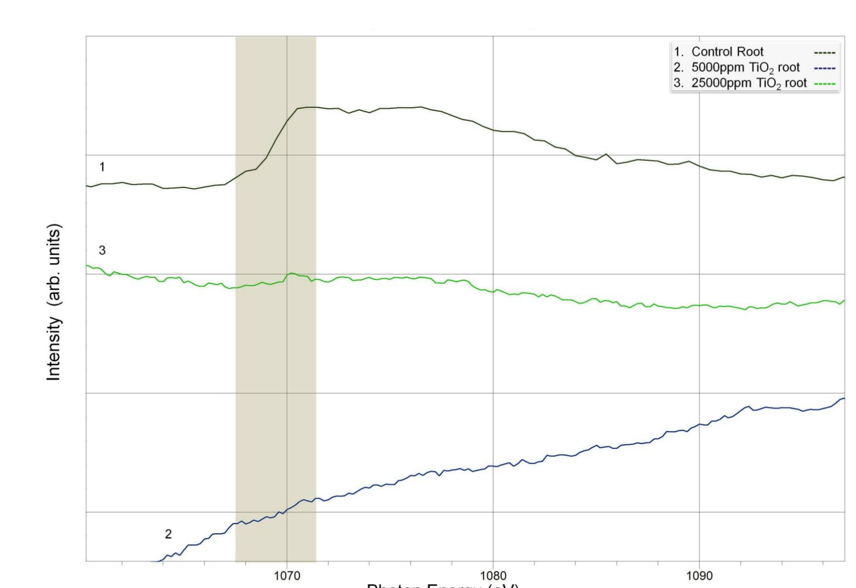


Fig 5: SGM Beamline scan of the sodium edge (highlighted) in control and contaminated plant root samples. Peak is insignificant compared to the noise in the contaminated samples.

Getting results on the SGM beamline. Counter clockwise from top right: Dr.Robert Blyth, David Chevrier, Nicole Sidebottom, Tracy Walker, Victor Malkov, Eric Langlois, Sherry Chu, Tim Xu, and Yongjian Lu.

Conclusion

The presence of titanium dioxide anatase nanopowder in the growth medium of bean plants appears to inhibit the absorption of essential nutrients and minerals, although the particles were not taken up by the plants in levels that we could observe. Specifically, the analysis revealed a visible absence of sodium in the roots of plants exposed to the nanoparticles and a notable decrease in calcium and nitrogen concentrations compared to the control plants. In previous studies, the mineral calcium was also shown to have a direct effect on plant growth[3]. The decreased levels of these elements could account for the initial stunted growth observed in plants grown in nanoparticle-contaminated soil.

Based on these preliminary findings, further research could be conducted to yield more conclusive results. Future experiments could confirm or disprove the direct influence of titanium dioxide nanoparticles on nutrient and mineral absorption in the bean plant, and potentially link stunted growth to the lack of these essential nutrients and minerals. The correlation between titanium concentrations in the growth medium and calcium or sodium concentrations in the plant could also be examined more closely.

Thank You

The BSYNC team thanks Ms. Tracy Walker and Dr. Robert Blyth for helping us throughout the project and for sharing their knowledge of the synchrotron and the scientific research process. We would also like to thank the CLS for donating beamtime for our project, without which we could not have done what we did, and NSERC for sponsoring our project.

References

[1] Xing, Baoshan, and Daohui Lin. "Phytotoxicity of Nanoparticles: Inhibition of Seed Germination and Root Growth." *Environmental Pollution* 150.2 (2007): 243-50. [2] Ma, Linglan, Chao Liu, Chunxiang Qu, Sitao Yin, Jie Liu, Fengqing Gao, and Fashui Hong. "Rubisco Activase MRNA Expression in Spinach: Modulation by Nanoanatase Treatment." *Biological Trace Element Research* 122.2 (2008): 168-78. [3] White, Philip J., and Martin R. Broadley. "Calcium in Plants." *Annals of Botany* 92 (2003): 487-511.