# PLANT RESPONSE TO NANO METAL OXIDES AND THEIR COMPOSITES: CHLOROPHYLL CONTENT AS A STRESS INDICATOR

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**Abstract**

Interaction between engineered nanoparticles and plants is still a widely unexplored area. While there is a growing body of research on the toxicity of nanoparticles to plants, their stress-inducing capabilities are less known. Determination of chlorophyll content in plant leaves is a relatively easy way of real-time assessment of the physiological state of the plant. Cauliflower (*Brassica oleracea* convar. Botrytis) seedlings were subjected to the $TiO_2$ nanoparticles either by being planted in the nano-$TiO_2$ enriched soil (1g/kg) or by exposure of its leaves to nano-$TiO_2$ suspension (1g/L). Chlorophyll content in the shoots (mmol/m$^2$) was measured both before and following the exposure in set interval; shoots were then harvested and prepared for Ti content analysis. While no significant decrease of chlorophyll content was observed in the unexposed plants, chlorophyll content significantly decreased after four days in the seedlings growing in the nanoparticle-enriched soil and after twelve days in the seedlings subjected to the suspension. All the seedlings appeared vital, yet the decrease in chlorophyll content indicates nanoparticle-induced stress that may seriously affect the yield from cultivated crops when these are exposed to nano-metal oxides or their composites as well as raise health concerns due to their bioaccumulation in the plant tissues.

**Keywords:** Nano metal oxides, chlorophyll, exposure, stress

## 1. INTRODUCTION

Rapid development of nanoparticles (NPs) production and manufacturing results in increase their release to all parts of the environment. Since the plants are essential constituent of the ecosystems - standing at the beginning of the food chain - investigation of their interaction with nanoparticles (NPs) is important to avoid to potential ecological risk as well as negative effect on human health [1]. NPs are known to negatively affect plant growth, cell structure, as well as the plant physiology (reduction in seed germination, suppression of root elongation, reduction of biomass and leaf production) [2] biochemical functions and, finally, can cause the plant death [3]. Regarding the impact of metal oxide nanoparticles (including nano-$TiO_2$, one of the most frequently used nanomaterials) on photosynthesis, both positive and negative effects were reported [4] [5] [6].

Since chlorophyll (namely chlorophyll a) is the most sensitive to photodegradation from all the other photosynthetic pigments, its content in shoots is a valuable indicator of the impact the exposure to nanoparticles has on the plant [3]. Several studies on photosynthetic organisms proved that before any detrimental effect on post-exposure viability or growth was observed, the chlorophyll levels already decreased noticeably [4] [5]. Chlorophyll content was also found in plants when no signs of toxicity were apparent using the conventional toxicological methods [6]. In addition, non-destructive methods using a method based on radiation transmittance allow measurements in vivo and monitoring of the response of an individual plant over the course of the experiment [7]

The aim of this pilot study was to assess whether, in plants exposed to metal oxide ($TiO_2$) nanoparticles either via root system or leaves, the chlorophyll content determined *in vivo* -- can be used for NP-exposure-induced physiological stress.

## 2. MATERIALS AND METHODS

Store-bought young cauliflower plants (*Brassica oleracea* convar. Botrytis, n = 9) were transferred to the laboratory and, after three days of acclimatization, divided into three equally sized groups. The first group of plants was planted in pots containing a mixture of sowing and propagation soil substrate (AGRO) and nano-$TiO_2$ powder (1 g/kg) prepared by mixing both components together in a rotational shaker for 24 hours. The second group was planted in the same, yet uncontaminated, substrate and their leaves were sprayed, biweekly, with 5 ml of 1g/L water suspension of nano-$TiO_2$. The last group was only planted to the substrate and subjected to no treatment (control). During the experiment, all plants were watered with tap water when necessary (dry substrate).

$TiO_2$ NPs for the experiments were prepared from titanyl sulfate (Precheza a.s.) precursor containing 102 g of $TiO_2$ per 1 $dm^3$ of suspension. The suspension was hydrolysed and, consequently continuously stirred for 5 hours at 100°C. The solid phase was then separated by decantation, washed several times with distilled water until the conductivity of the filtrate was lower than 100 $\mu S \cdot cm^{-1}$, and dried at 105 °C for 24 h.

The substrates used - both enriched and original - were leached and the leachate was analysed for Ti content. Atomic emission spectroscopy with inductively coupled plasma (ISP-AES) showed no titanium leaching from the original substrate (<0.01 mg/L) and 0.028 mg/L leaching from the contaminated substrate. The chlorophyll content measurement was performed *in vivo* using MC-100 Chlorophyll Concentration Meter (Apogee) in ten replications per measurement. All the leaves available for the measurement of the plant were targeted. The measurement was performed immediately after planting, after 24 hours, 4 days and 12 days from the planting. Measured chlorophyll content was expressed in $\mu mol\ m^{-2}$.

After the exposure period, the above-ground parts of plants were harvested and dried to constant weight at 45 °C; due to the small yield, the samples were merged according to the treatment, homogenized and pulverized. After thermal digestion in a mix of chemicals ($HF+HNO_3+H_2O_2$), the samples were analysed for the content of Ti (as well as Ca, K, Mg and Na, essential mineral elements) using ICP-AES (SPECTRO CIROS VISION, Spectro).

Statistical analysis of the measured data as well as their graphical representation using the R [8] One-way analysis of variance (ANOVA) was used to assess the differences in chlorophyll content among the treatments, post hoc Tukey HSD test was used for the pair-wise comparison and identification of the particular differences.

## 3. RESULTS AND DISCUSSION

The resulting content of the selected elements in the shoots (after 12 days of experiment) showed marginal difference in the content of essential elements among the treatments (**Figure 1**). However, the titanium content was significantly higher in the above-ground cauliflower biomass of the plants subjected to the nano-$TiO_2$ exposure via leaves (in suspension) - while the difference between the control plants and plants grown on NP-enriched soil was negligible.

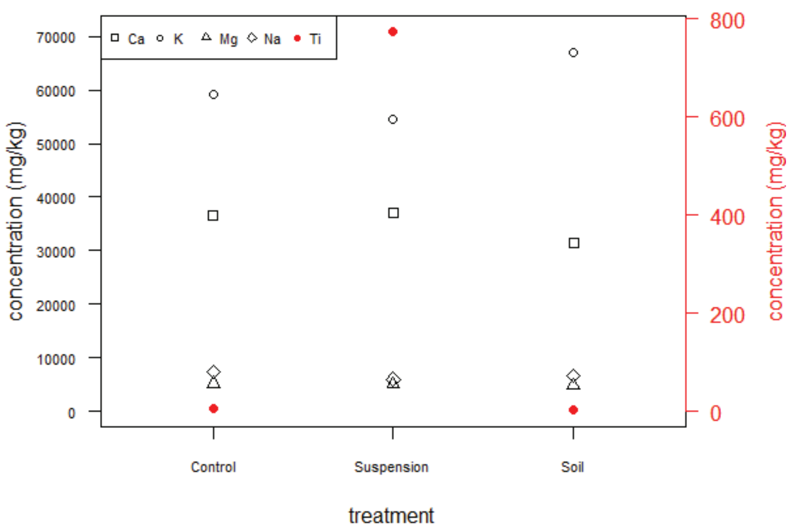


**Figure 1** Content of selected elements in the plants depending on the treatment

In the control plants, a slight decrease in chlorophyll content was observed at the end of the experimental period, however, it was not found to be significant by ANOVA (**Figure 2**). It is, indeed, conceivable that the stress of replanting had some effect on the experimental plants - expressed in chlorophyll decrease - nevertheless, the experimental period was too short to fully address this issue.

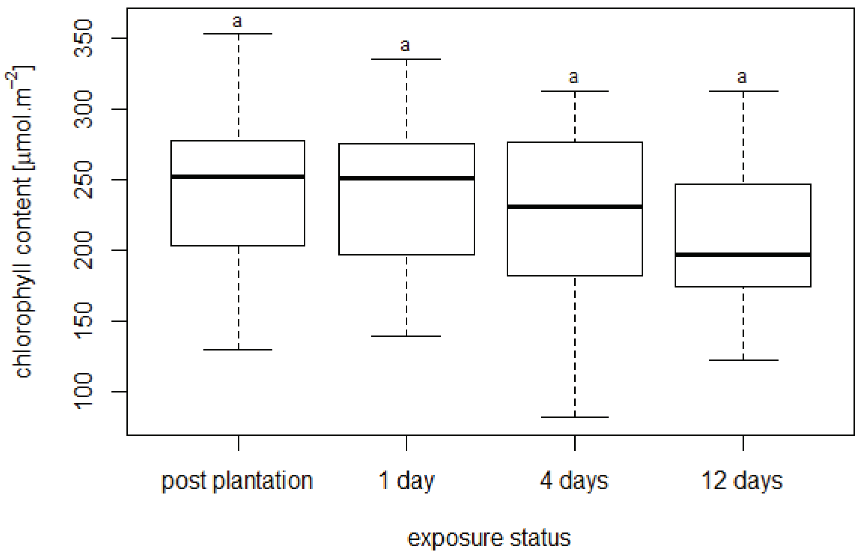


**Figure 2** Chlorophyll content in the unexposed (control) plants. Identical letters above the boxplots denote the non-significant difference between the measurements.

In the plants subjected to the nano-$TiO_2$ suspension sprayed on their leaves, the decrease in chlorophyll was much more prominent around the 12th day (**Figure 3**). ANOVA confirmed this decrease as significant ($p = 2.02 \cdot 10^{-9}$), Tukey HSD test specified that the difference is between the 12-day measurements and all the other measurements while the rest of the measurements did not significantly differ from each other.

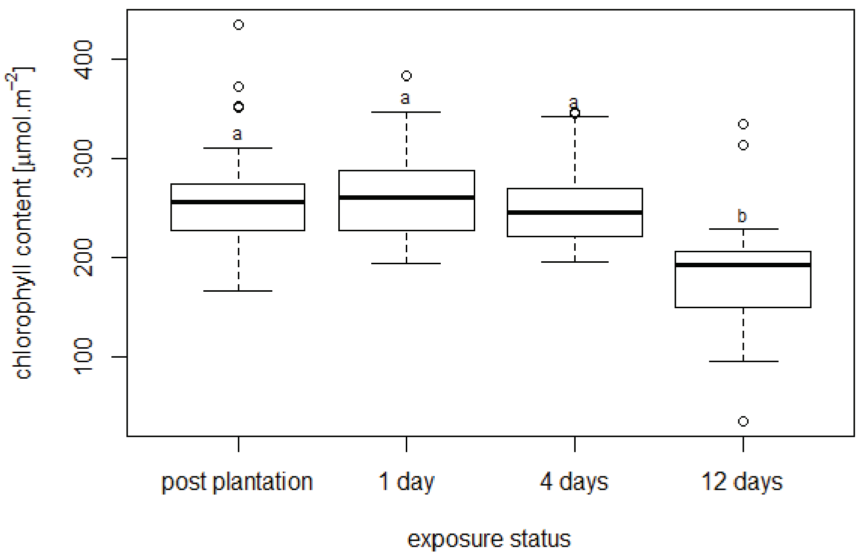


**Figure 3** Chlorophyll content in the plants exposed to the 1g/L nano-$TiO_2$ suspension. Identical letters above the boxplots denote the non-significant difference between the measurements.

Chlorophyll content in the plants grown in the substrate enriched with nano-$TiO_2$ showed the most distinct decrease - this time, it was as early as at the 4-day mark. Further, a steep decrease in chlorophyll content was observed after 12 days from replanting. Both of these decreases were found to be significant by ANOVA ($p = 3.62 \cdot 10^{-11}$), Tukey HSD test further confirmed significant difference not only between the particular decreases and post-plantation state but also between each of the decreases.

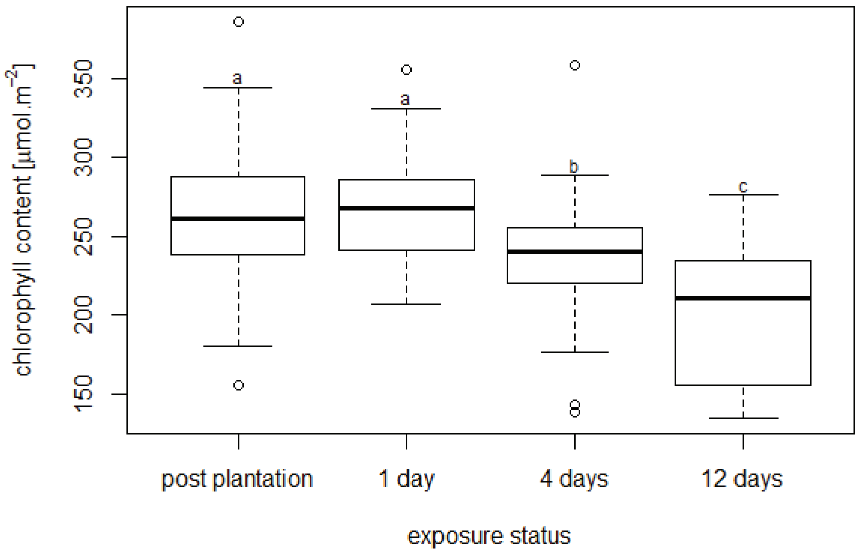


**Figure 4** Chlorophyll content in the plants planted on in the nano-$TiO_2$ - enriched soil. Identical letters above the boxplots denote the non-significant difference between the measurements.

It is apparent that both treatments considerably affected the chlorophyll content in the assessed plants while the exposure via roots (growing on the artificially contaminated substrate) proved to have a more damaging impact on the chlorophyll content than the exposure via leaves. In addition, chlorophyll content - measured using the chosen non-invasive method - was confirmed to be an indicator of the stress induced by the nano-$TiO_2$ exposure suitable for a real-life assessment of the physiological state of the exposed plant even in such a short period of exposure time.

The significant decrease in chlorophyll content in the plants grown on the NP-enriched soil (even in comparison with plants exposed to nano-$TiO_2$ via leaves) in combination with the fact that no titanium was found in the above-ground parts of these plants is noteworthy. It indicates that while the plants absorbed enough nanoparticles to negatively affect their photosynthesis, the translocation of the accumulated NPs was low to none. This is of great importance since modelling of environmental concentrations of engineered $TiO_2$ nanoparticles suggests the soils to be the greatest source of nanoparticles available to plants [9]. It is also consistent with the findings of Asli and Neumann [10] who found that the nanoparticles enter the root hairs of maize (*Zea mays* L.) and adhere to their cell walls, which causes the physical clogging of the root system leading to reduced water and nutrient uptake. Due to the environmentally rather unreasonable concentration of NPs in the substrate, this may be easily the case, however, the specific mechanism of the nano-$TiO_2$ penetration and accumulation in the roots of cauliflower is yet to be uncovered.

## 4. CONCLUSION

Nanoparticles of $TiO_2$ were found to affect the chlorophyll content in the cauliflower plants regardless of whether they have been exposed via roots or leaves. Leaf exposure lead to accumulation of the titanium in the shoots, while root exposure affected the plant physiology both quicker and more severely. On the other hand, transport of the nano-$TiO_2$ from the roots to the above-ground parts of the plants was not confirmed; hence, the alteration of the plant physiology must take place immediately in the plant root system. Chlorophyll content measured using non-invasive method was proved to be a valuable indicator of the plant stress levels following the exposure to the $TiO_2$ nanoparticles in both assessed ways of exposure.

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