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Impact of Titanium Dioxide Nanoparticles in Irrigation Water on Potato Growth and Yield

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Abstract
A 2-year (2017 and 2018) field lysimeter study was carried out to examine the effect of titanium dioxide nanoparticles (TiO_2 NPs) in irrigation wastewater on soil characteristics and potato (*Solanum tuberosum* L.) yield. Potatoes were planted in lysimeters (1.00 m × 0.45 m) in sandy soil and subjected to four treatments: freshwater (FW), wastewater (WW), freshwater + TiO_2 NPs (FW + NP) and wastewater + TiO_2 NPs (WW + NP), in triplicate. Potato tubers were harvested at maturity (120 days after planting). Both the TiO_2 NPs (with/without 1 mg L^{-1} TiO_2 NPs) and irrigation treatments (FW *vs.* WW) had a significant effect ($p \leq 0.05$) on chlorophyll content; however, they had little or no effect on soil physicochemical parameters (cation exchange capacity (CEC), pH and soil organic matter (SOM)), plant growth parameters (plant height, above-ground and root fresh weight) or yield (tuber weight, number of tubers and tuber grading). For both years, the total nitrogen content of the leaves increased consistently together with leaf chlorophyll content. Furthermore, tuber yield under FW, WW and WW + NP treatments were higher in the first year than in the second, likely due to higher growing season temperatures in the second year. This study furthers the knowledge on the impact of TiO_2 NPs on plant growth by showing that at 1 mg L^{-1}, irrigation water can increase greenness without inhibiting plant growth and yield. In addition, the potato plants, irrigated with water containing TiO_2 NPs, did not become infected with early and late blight diseases either year.

Keywords Crop productivity · Sandy soil · *Solanum tuberosum* L · TiO_2 NPs

Introduction

With population growth and increased industrialization and urbanization, global water usage has been increasing. Limited availability of freshwater has led to water scarcity; by 2025, about 1.8 billion people are expected to be living under acute water shortages (Rizzo et al. 2020). As irrigated agriculture is one of the primary

Extended author information available on the last page of the article uses of freshwater, the increasing food demands of a growing world population will lead to greater use of freshwater in agriculture (DESA 2014). Although only 20% of arable lands are irrigated, they account for 40% of global food production (IBRD-IDA 2020).

It is estimated that by 2050, 70% of the world's population will be concentrated in urban areas, producing large volumes of wastewater (DESA 2014). In developing countries, over 95% of industrial and municipal wastewater effluents are released into the environment untreated or only partially treated, therefore, threatening the health of vital ecosystems and downstream human populations (UN-Water 2017). Accordingly, more efficient use and effective treatments of wastewater are necessary.

Wastewater irrigation has the potential to increase agricultural food production, promote freshwater conservation and limit the harmful practice of openly discharging untreated wastewater into the environment (Libutti et al. 2018; Qadir et al. 2010). Wastewater irrigation can also help combat the global decline in soil fertility by increasing soil organic carbon and by increasing the availability of soil nitrogen, phosphorus and potassium (Marofi et al. 2015). Irrigation with wastewater can help confront the major issues of water scarcity and decline in soil fertility (Becerra-Castro et al. 2015).

Nonetheless, wastewater irrigation also poses major risks. Without adequate ex situ or in situ treatment, wastewater irrigation can introduce contaminants into the surrounding environment and human food systems (Helmecke et al. 2020). Wastewater may contain toxic combinations of inorganic and organic contaminants, including endocrine disruptors, as well as carcinogenic, mutagenic and teratogenic substances (Shakir et al. 2017; Xing et al. 2007), which can be toxic to humans and wildlife (Lopes et al. 2015). Soil contamination and potential adverse impacts on crop production depend on the contaminant's physicochemical nature, concentration, toxicity, solubility, degradability and the rate and frequency of its application (Elgallal et al. 2016).

Sunscreens, cosmetics and paints contain nanoparticles, materials with dimensions less than 100 nm, which can make their way into wastewater (Cai et al. 2017; Mahdavi et al. 2015). NPs interact with the soil–water system and may affect the movement and translocation of several elements and chemicals. In addition, they may promote the germination and development of plants (Khot et al. 2012). For example, certain plant species can take up, accumulate and translocate TiO_2 NPs (Cai et al. 2017). Hong et al. (2005) showed that spinach (*Spinacia oleracea* L.) growth was accelerated in the presence of soil treated with 0.25% TiO_2 NPs. This reflected a threefold enhancement in photosynthesis and a 42% greater Rubisco activity in spinach (Gao et al. 2008; Nair et al. 2010). Similarly, Yang et al. (2006) showed that soils amended with TiO_2 NPs improved spinach plant growth, leading to increased nitrogen metabolism, stimulated nitrate absorption and increased the transformation of inorganic nitrogen into organic nitrogen in the soil, thereby, boosting spinach both fresh and dry weights. Moreover, studies have suggested that there may be a NP concentration threshold of 5 mg L^{-1} in water for growth stimulation, beyond which TiO_2 NPs may inhibit growth (Song et al. 2013).

While some studies have investigated the effects of NPs on plant germination and development with the intent of promoting their use in agricultural applications

(Khot et al. 2012), other studies showed that NPs can induce phytotoxicity and have a negative effect on plant seed germination and growth. Thus, at present consensus on use of soil amendments with NPs is that they can have both a positive and a negative impact on plant growth and yield; this is likely related to the dose and plant species.

The impact of NP-bearing irrigation water on plant growth and yield of potatoes is not fully known. Furthermore, the impact of TiO_2 NPs when in a complex matrix such as wastewater is unknown. To fill this gap in knowledge, this study investigates the effect of TiO_2 NPs in wastewater on the growth and yield of potatoes. A 2-year experiment was carried out to compare fresh or wastewater irrigation, in the presence or absence of TiO_2 NPs. The concentration of TiO_2 NPs that were added to the wastewater (1 mg L^{-1}) was similar to the concentrations in raw sewage (Westerhoff et al. 2011), and lower than the concentration threshold above which plant growth is inhibited (Song et al. 2013). We hypothesize that TiO_2 NPs in wastewater may have a positive impact on plant growth, in comparison to wastewater irrigation without TiO_2 NPs. To the best of our knowledge, this is the first study to compare the effect of TiO_2 NPs, present in freshwater and wastewater, on plant (potato) growth and yield.

Materials and Methods

Experiment Setup

A 2-year experiment was conducted on the Macdonald Campus of McGill University, Sainte-Anne-de-Bellevue, Quebec, Canada (45°24′48.6″ N latitude and 73°56′28.1″ W longitude) in the summers of 2017 and 2018. A total of 12 PVC lysimeters (1 m height × 0.45 m inner diameter), were filled to a bulk density of 1.35 Mg/m^3 with sandy soil, obtained from the Macdonald Campus Farm. Further detailed description of the pre-experimental soil used in this study has been documented in Mawof et al. (2021). Two treatment factors (type of irrigation water and presence/absence of nanoparticles) were factorially combined, resulting in four treatments: freshwater alone (FW), wastewater alone (WW), FW with nanoparticles (FW + NP) and WW with nanoparticles (WW + NP). The four triplicated treatments were randomly allocated to the 12 lysimeters. A canvas tent was set up over the lysimeters to prevent rainwater from entering, thereby allowing the desired volume of irrigation water to be applied manually. An array of ten LED bulbs (60 W each) were installed above the lysimeter array to supplement the natural light blocked due to the tent. A MQ-200 Quantum Flux sensor (Apogee Instruments Inc., Logan, Utah) was used to determine the light intensity under the tent. All lysimeters were brought to field capacity by applying freshwater 1 day before planting. Following local guidelines, SENCOR® 75 F (active ingredient: metribuzin, 4-amino-6-*tert*-butyl-3-methylsulfanyl-1,2,4-triazin-5-one), a herbicide approved for use on potatoes in Canada, was applied to the soil at the rate of 2.25 L ha^{-1}, prior to planting (OMAFRA 2019).

Potato seed tubers (*Solanum tuberosum* L., cv. Russet Burbank), on average weighing 100 g each, were purchased from Global Agri. Services Inc. (New Brunswick, Canada). The seed tubers were stored at 8–10 °C, then placed in a cardboard box at room temperature, 2 weeks prior to planting, to encourage sprouting. On the day of planting, one tuber was planted 0.10 m deep in the centre of each lysimeter. Urea, triple super phosphate (TSP) and potassium chloride (KCl) were applied at the locally recommended fertilization rate for potatoes. Nitrogen was applied at a rate of 180 kg N ha^{-1} (Parent and Gagné 2010); 30% each on Days 0 and 31 post-planting, and the remaining 40% in four equal parts on Days 46, 53, 60 and 67 post-planting (Stark et al. 2004). Potassium (280 kg K ha^{-1}) and phosphorus (44 kg P ha^{-1}) were applied on Day 0 (Parent and Gagné 2010). After planting (Day 0), each lysimeter was irrigated with 11.5 L (~72 mm) every 10 days; there were eight irrigation events in each season. The irrigation volume was determined based on the potato plants' water requirements and the growing season duration for 'Russet Burbank' (120 days) (FAO 2015). Weather data (daily mean relative humidity and temperature) were collected from an Environment Canada weather station, located at Sainte-Anne-de-Bellevue, QC (45°25′38.000″N, 73°55′45.000″W) and averaged for each month of interest (Environment Canada 2021). After the 2017 growing season, lysimeters were covered with plastic bags for the off-season, and the soil in each lysimeter was left undisturbed for re-use in 2018.

Preparation of Synthetic Wastewater and TiO_2 NPs for Irrigation

The concentrations of various components in the synthetic wastewater (WW) were prepared in the lab immediately before each irrigation; these components have been documented elsewhere (Mawof et al. 2022). The organic contaminants and metal concentrations were formulated to represent a 'worst-case scenario' of WWs reported in the literature. All the constituents of the synthetic wastewater were purchased from Sigma Aldrich (St. Louis, MO, USA) or Fisher Scientific (Waltham, MA, USA).

TiO_2 NPs (21 nm particle diameter, 99.5% purity and CAS No. 13463–67-7) were procured from Sigma-Aldrich. Nanoparticles can agglomerate and must therefore be dispersed before mixing them into the irrigation water. In this case, very-low viscosity sodium alginate, obtained from VWR (Ville Mont-Royal, QC), was used to disperse the nanoparticles in water. A 1000 mg L^{-1} stock solution of TiO_2 NPs was prepared 1 h before irrigation by adding 100 mg of sodium alginate to 90 mL of deionized water, heating to 80 °C, then adding 100 mg of TiO_2 NPs and deionized water to bring the total volume to 100 mL. This 1000 mg L^{-1} stock solution was vortexed for 30 s and sonicated for 10 min to ensure particle dispersion and homogenization. Prior to irrigation, 40 mL of the stock solution was added to 40 L of irrigation water. The TiO_2 NPs treatment, resulting in a final concentration of 1 mg L^{-1} of TiO_2 NPs, was similar to a concentration previously detected in wastewater (Westerhoff et al. 2011).

TiO_2 NPs were characterized using transmission electron microscopy (Fig. 1) (Talos F200X G2 STEM). The zeta potential + 12.9 mV (± 0.4) of the particles was

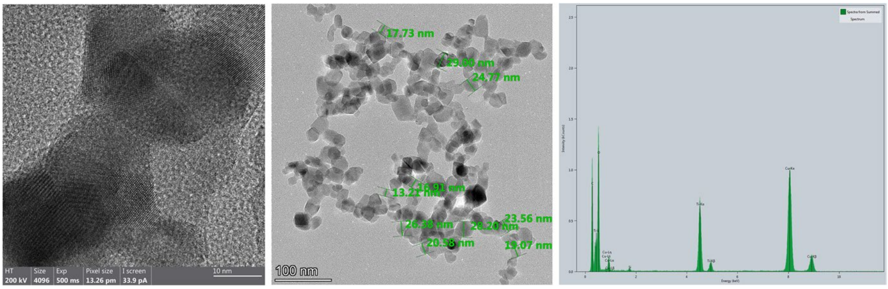


Fig. 1 TEM images of TiO_2 NPs were obtained after atmospheric drying of particle suspensions (100 ppm) on 200-mesh Cu TEM grids with carbon film. These images were acquired using a Talos F200X G2 STEM

determined using dynamic light scattering (DLS, NanoBrook OMNI Instrument, USA).

Soil Physicochemical Properties

At the end of each season, soil samples were collected at the surface and at a 0.1 m depth for the determination of cation exchange capacity (CEC), pH and soil organic matter (SOM). The CEC was measured using the $BaCl_2$ method (Hendershot et al. 1993). For pH measurements, soil samples (10 g of dried prior to measurement) were mixed with water (20 mL) for 30 min (Rayment and Higginson 1992), and the pH was measured with an electrode (Accumet pH metre model AB15, Fisher Scientific, USA). SOM was quantified by loss-on-ignition (Schulte et al. 1991).

Plant Physiological Parameters

Plant physiological parameters, namely chlorophyll content, photosynthetic activity, light reflectance, transpiration rate and stomatal conductance, were measured during both crop seasons. A chlorophyll meter (SPAD-502 Plus; Konica Minolta, Japan) was used to estimate relative chlorophyll content, 2 days before each irrigation and 5 days after each irrigation, in both growth periods. In the second year only, plant photosynthetic activity, transpiration rate, and stomatal conductance were measured, 5 days after each irrigation using a Licor 6400 (LI-COR, Nebraska, USA). Crop vigour, quantified by multispectral reflectance (normalized difference vegetation index (NDVI)), was also measured, 5 days after irrigation, using an active crop canopy sensor (Crop Circle ACS-470; Holland Scientific Inc., Nebraska, USA).

Plant Yield

In both years, the potatoes in each lysimeter were harvested 120 days after planting, as per local growing season recommendations for 'Russet Burbank' potatoes. Above-ground biomass was separated into stems and leaves. The fresh weight of the above-ground biomass and the height of the shoots were measured. The underground biomass was also harvested, and the roots and tubers were separated and weighed. Tubers were counted and graded using the scale as previously reported (Shiri-e-Ja et al. 2009; USDA 1983). The total organic carbon (TOC) and total nitrogen (TN) content of potato flesh and leaves were determined using an NC Analyzer (Thermo Finnigan Flash EA-1112, Thermo Fisher Scientific Inc., MA, USA).

Data Analysis

Physiological parameters were analyzed by considering treatments and measurement times as factors. For soil parameters, plant growth and yield, treatment was considered as the only factor; therefore, the data was analyzed using one-way analysis of variance (ANOVA). The data for each year were tested separately. A least significant difference test was used for a pair-wise comparison, and differences were considered significant when $p \leq 0.05$. All analyses was performed using IBM SPSS® V.24 (Copyright © IBM Crop, 2016 Armonk, NY, USA).

Results and Discussion

The addition of TiO_2 NPs to wastewater (WW) or freshwater (FW) had no impact on the soil physicochemical, plant growth parameters, crop vigour or potato yields, indicating that the presence of 1 mg L^{-1} TiO_2 NPs in irrigation water did not have an effect. On the other hand, significant effects were observed with regards to some physiological parameters, especially chlorophyll content (in both years), nitrogen content, photosynthetic rate, transpiration rate, and stomatal conductance in the second year. Furthermore, there were significant effects depending on water type (FW versus WW) on soil parameters and some plant parameters. Finally, time had a significant effect on the plant's physiological parameters. The impact of the treatments on the soil physicochemical properties, followed by plant physiological properties and growth parameters, is discussed below.

Impact on Soil Physicochemical Properties

The application of wastewater or freshwater significantly affected the soil's physicochemical properties; however, the application of TiO_2 NPs did not have any impact (Table 1). The CEC of the surface soil was significantly higher ($p < 0.05$) under FW irrigation than with either WW or WW + NP irrigation; however, no significant differences in soil CEC were observed at other depths. SOM following the FW and FW + NP treatments was roughly unchanged from measurements prior to any irrigation regime, but SOM in the WW and WW + NP was higher than that in the initial soil. At both depths (surface and 0.10 m), SOM was significantly greater ($p \leq 0.05$) under WW and WW + NP treatments as opposed to the FW and FW + NP treatments. Soil pH at the soil surface was significantly lower ($p \leq 0.05$) following WW

Table 1 Effects of freshwater (FW), wastewater (WW), freshwater with TiO_2 NPs (FW+NP) and wastewater with TiO_2 NPs (WW+NP) on soil cation exchange capacity (CEC), soil organic matter (SOM) and pH

Treatments	CEC ($cmol_c\ kg^{-1}$)		SOM (%)		pH	
	Surface	0.10 m	Surface	0.10 m	Surface	0.10 m
FW	3.14 ± 0.50^{a}	3.19 ± 0.33^{a}	1.74 ± 0.05^{b}	1.74 ± 0.05^{b}	5.76 ± 0.11^{a}	5.70 ± 0.20^{a}
WW	1.79 ± 0.29^{c}	2.62 ± 1.24^{a}	2.80 ± 0.64^{a}	2.72 ± 0.19^{a}	5.00 ± 0.10^{b}	5.26 ± 0.15^{a}
FW+NP	2.85 ± 0.30^{ab}	2.77 ± 0.10^{a}	1.79 ± 0.11^{b}	1.79 ± 0.11^{b}	5.76 ± 0.11^{a}	5.76 ± 0.11^{a}
WW+NP	2.33 ± 0.31^{bc}	3.44 ± 1.81^{a}	2.60 ± 0.55^{a}	2.69 ± 0.06^{a}	5.08 ± 0.07^{b}	5.30 ± 0.30^{a}

The different letters in each column represent a significant difference at $p \leq 0.05$; values are mean ± standard error of three replicates

and WW+NP treatments than for the FW and FW+NP treatments. However, pH was similar in all treatments at 0.1 m depth.

Soil is a complex matrix. When NPs enter the soil, they are either physically retained or chemically adsorbed onto the surfaces of soil particles (Reddy et al. 2016). Depending on the chemical and physical properties of the soil and its texture, such interactions could either reduce or increase the bioavailability and mobility of the NPs. Furthermore, properties such as SOM, salinity, ionic strength, pH, clay content, and microbial community are expected to influence the behaviour of the TiO_2 NPs in the soil (Thiagarajan and Ramasubbu 2021).

Plant Physiological Parameters

While no significant differences existed among treatments for NDVI, photosynthetic rate, transpiration rate or stomatal conductance, there was a tendency for these parameters to be greater under the WW (*vs.* FW) treatments. This trend concurs with that of the observed above-ground biomass. However, the presence of TiO_2 NPs had a significant impact on the relative chlorophyll content (reflected in SPAD readings) in both years. Nonetheless, overall, NPs did not have any marked effect on plant physiological parameters.

In 2018, LI-COR measurements of photosynthetic and transpiration rates, along with stomatal conductance (Table 2), showed a significant response ($p \leq 0.05$) to time towards the end of the season. Only minor treatment differences, if any, were found on Days 45, 65, 85 and 95. The photosynthetic rate was significantly less ($p < 0.05$) for FW+NP than for WW+NP on Days 85 and 95. Moreover, the photosynthetic rate for the FW+NP treatment was lower than that obtained for the WW on days 45 and 85. Treatments had no effect on photosynthetic rate on Days 55, 65 and 75.

Transpiration rate was greater under WW as opposed to FW+NP on Day 45 and greater under WW+NP when compared to FW+NP on Day 65, while on Day 95, the transpiration rate under FW was greater than under FW+NP. Treatments had no effect on the transpiration rate on Days 55, 75 and 85.

Table 2 Effect of freshwater (FW), wastewater (WW), freshwater with TiO_2 NPs (FW + NP) and wastewater with TiO_2 NPs (WW + NP) on photosynthetic rate, transpiration rate and stomatal conductance of potato plants in 2018

	Day 45	Day 55	Day 65	Day 75	Day 85	Day 95
Photosynthesis rate (µmol CO_2 m^{-2} s^{-1})						
FW	9.62 ± 1.38^{ab}	10.43 ± 4.02^{a}	10.12 ± 2.07^{a}	8.36 ± 1.24^{a}	4.77 ± 2.76^{b}	5.39 ± 1.92^{a}
WW	12.46 ± 1.53^{a}	13.20 ± 1.05^{a}	10.82 ± 1.06^{a}	8.36 ± 4.16^{a}	10.05 ± 1.85^{a}	4.81 ± 1.21^{ab}
FW + NP	8.58 ± 1.10^{b}	9.88 ± 0.91^{a}	9.79 ± 3.10^{a}	7.62 ± 1.57^{a}	5.55 ± 1.87^{b}	1.58 ± 2.13^{b}
WW + NP	10.75 ± 2.52^{ab}	11.96 ± 1.65^{a}	12.44 ± 3.07^{a}	7.98 ± 0.88^{a}	9.64 ± 0.40^{a}	5.43 ± 0.31^{a}
Transpiration rate (mmol H_2O m^{-2} s^{-1})						
FW	0.65 ± 0.18^{ab}	3.11 ± 1.67^{a}	3.27 ± 0.56^{ab}	2.62 ± 1.11^{a}	0.95 ± 0.56^{a}	2.00 ± 0.55^{a}
WW	0.88 ± 0.33^{a}	4.32 ± 1.04^{a}	3.62 ± 0.37^{ab}	2.28 ± 1.94^{a}	1.49 ± 0.11^{a}	1.01 ± 0.37^{ab}
FW + NP	0.31 ± 0.07^{b}	3.69 ± 0.47^{a}	2.70 ± 0.60^{b}	1.96 ± 0.48^{a}	1.08 ± 0.51^{a}	0.84 ± 0.71^{b}
WW + NP	0.61 ± 0.19^{ab}	3.34 ± 0.97^{a}	3.79 ± 0.69^{a}	1.58 ± 0.37^{a}	1.32 ± 0.27^{a}	1.46 ± 0.05^{ab}
Stomatal conductance (mol H_2O m^{-2} s^{-1})						
FW	0.08 ± 0.02^{ab}	0.27 ± 0.26^{a}	0.37 ± 0.14^{a}	0.21 ± 0.15^{a}	0.12 ± 0.08^{a}	0.12 ± 0.06^{a}
WW	0.12 ± 0.05^{a}	0.36 ± 0.17^{a}	0.42 ± 0.07^{a}	0.18 ± 0.19^{a}	0.20 ± 0.02^{a}	0.05 ± 0.02^{ab}
FW + NP	0.04 ± 0.01^{b}	0.23 ± 0.04^{a}	0.28 ± 0.08^{a}	0.12 ± 0.03^{a}	0.13 ± 0.07^{a}	0.04 ± 0.03^{b}
WW + NP	0.08 ± 0.03^{ab}	0.21 ± 0.09^{a}	0.44 ± 0.13^{a}	0.10 ± 0.03^{a}	0.17 ± 0.04^{a}	0.07 ± 0.01^{ab}

The different letters in each column represent a significant difference at $p \leq 0.05$; values are mean ± standard error of three replicates

The stomatal conductance was greater under WW than under FW + NP on day 45 and greater under FW as compared to WW + NP on Day 95. Treatments had no effect on stomatal conductance on Days 55, 65, 75 and 85.

Studies have found that the presence of TiO_2 NPs increased the rate of photosynthesis. For example, in a pot study, Li et al. (2015) found that exposure to TiO_2 NPs at concentrations of 0.500, 2.500, and 400 mg L^{-1} improved the morphological and physiological parameters (photosynthetic, chlorophyll content) of *Brassica napus* L. Moreover, the photosynthetic rate and chlorophyll content showed a significant but gradual increase with TiO_2 NPs concentration, indicating that there could be an impact of the presence of TiO_2 NPs on crop growth and physiological parameters for high TiO_2 NPs concentrations. But as the concentration of TiO_2 NPs in our experiment was 1 mg L^{-1}, it was likely to have had only a minimal effect. In contrast to this study, Ji et al. (2017) found the chlorophyll content and photosynthetic rate of rice plants (*Oryza sativa* L.) exposed to TiO_2 NPs increased in the presence of 1000 mg L^{-1} of TiO_2 (21 nm). Qi et al. (2013) also reported that the application of TiO_2 NPs improved the net photosynthetic rate, transpiration rate and stomatal conductance of tomato (*Solanum lycopersicum* L.) leaves. Thus, the plant-species-dependent effect of TiO_2 NPs cannot be ignored, and further studies on potatoes and other crops are warranted. Results of prior studies, along with those of the present study, indicate that TiO_2 NPs may enhance photosynthesis in some species of plants, though this is likely dependent on the type of plant and the size/nature of the TiO_2 NPs.

Crop vigour (NDVI) measurements showed no significant difference ($p > 0.05$) between treatments in either growing season, indicating that the treatments did not have an impact on the above-ground biomass (Table 3). NDVI values for all treatments ranged from 0.82 (Day 55) to 0.75 (Day 95), showing a decreasing trend with time. However, there was no treatment effect, suggesting that wastewater or freshwater irrigation, alone or mixed with TiO_2 NPs, had a similar impact on potatoes' vigour.

The effects of the irrigation treatments on the relative chlorophyll content (SPAD), during the early and mid-season, were inconsistent in both years (Fig. 2). During the late season (Days 97, 105), however, chlorophyll content was higher ($p \leq 0.05$) in the WW + NP as compared to other treatments in 2017 and, in 2018, higher under WW + NP as opposed to WW or FW regimes. Treatments with the TiO_2 NPs increased the leaf chlorophyll content in the latter portion of the season. Nonetheless, the presence of TiO_2 NPs had no impact on the TOC of the plant but did increase total nitrogen in potato tubers and leaves. However, the significant impact of the TiO_2 NPs on nitrogen corresponded with an increase in plant leaf chlorophyll content.

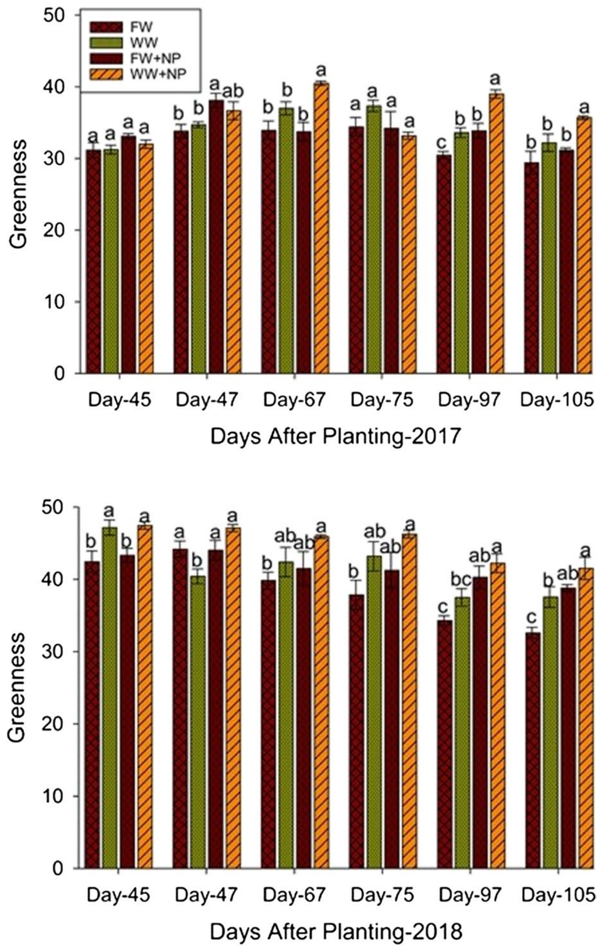
The increased chlorophyll content of the leaves under the application of the TiO_2 NPs, especially when applied with the wastewater, suggests that TiO_2 NPs may enhance the plant to uptake nutrients. This was clearly shown by the SPAD readings (WW + NP for both years and FW + NP in the second year); however, no effect was observed on photosynthetic rate. The effects on chlorophyll content seem to be linked to an improvement of nitrogen assimilation. Yang et al. (2007) similarly established that a soil amendment of TiO_2 NPs favoured the growth of spinach, accelerated nitrogen assimilation and enhanced chlorophyll content. TiO_2 NPs helped plants absorb nitrate and favour the conversion of inorganic nitrogen to organic nitrogen, and into protein and chlorophyll (Mishra et al. 2014; Yang et al. 2007). Similar findings were also observed by Morteza et al. (2013), who found that the application of TiO_2 NPs significantly increased chlorophyll, carotenoids and

Table 3 Effect of freshwater (FW), wastewater (WW), freshwater with TiO_2 NPs (FW + NP) and wastewater with TiO_2 NPs (WW + NP) on normalized difference vegetation index (NDVI) readings on potato plants in 2017 and 2018

	Day 55	Day 65	Day 75	Day 85	Day 95
2017					
FW	0.85 ± 0.05^{a}	0.84 ± 0.01^{a}	0.76 ± 0.05^{a}	0.67 ± 0.09^{a}	0.62 ± 0.16^{b}
WW	0.88 ± 0.02^{a}	0.80 ± 0.12^{a}	0.76 ± 0.10^{a}	0.65 ± 0.15^{a}	0.75 ± 0.10^{ab}
FW + NP	0.68 ± 0.14^{b}	0.76 ± 0.16^{a}	0.82 ± 0.04^{a}	0.84 ± 0.02^{a}	0.84 ± 0.07^{a}
WW + NP	0.86 ± 0.04^{a}	0.79 ± 0.08^{a}	0.82 ± 0.04^{a}	0.78 ± 0.18^{a}	0.82 ± 0.08^{ab}
2018					
FW	0.79 ± 0.05^{a}	0.82 ± 0.03^{a}	0.85 ± 0.02^{ab}	0.78 ± 0.03^{a}	0.83 ± 0.03^{a}
WW	0.77 ± 0.02^{a}	0.79 ± 0.14^{a}	0.85 ± 0.02^{a}	0.79 ± 0.05^{a}	0.82 ± 0.05^{a}
FW + NP	0.75 ± 0.11^{a}	0.80 ± 0.09^{a}	0.79 ± 0.03^{b}	0.74 ± 0.11^{a}	0.84 ± 0.01^{a}
WW + NP	0.80 ± 0.06^{a}	0.73 ± 0.22^{a}	0.74 ± 0.04^{c}	0.81 ± 0.08^{a}	0.79 ± 0.14^{a}

The different letters in each column represent a significant difference at $p \leq 0.05$; values are mean ± standard error of three replicates

Fig. 2 Effect of freshwater (FW), wastewater (WW), freshwater with TiO_2 NPs (FW + NP) and wastewater with TiO_2 NPs (WW + NP) irrigation on potato plant greenness in 2017 and 2018. The different letters on the bars in each column represent significant difference at $p \leq 0.05$ (mean ± SD, $n = 3$)



anthocyanins in maize (*Zea mays* L.). SPAD readings in the second season revealed a slight increase in greenness from that of the first season, potentially due to the accumulation of TiO_2 NPs in the soil over 2 years, or due to the warmer weather in 2018 that could have helped the plant take up nutrients into the above-ground biomass, because of enhanced greenness. Meanwhile, Tan et al. (2017) found that hydrophilic TiO_2 particles (coated with aluminium oxide and glycerol) reduced relative chlorophyll content in a study on the impact of unmodified, hydrophobic and hydrophilic TiO_2 NPs on field-grown basil (*Ocimum basilicum* L.).

Overall, these studies show that TiO_2 NPs likely have a stimulating impact on chlorophyll production in plants, and, in some cases, this translates to increased plant yield. This was evident from the higher SPAD readings in the nano-particle treatments in the present study. Similarly, Rui et al. (2016) found that iron oxide nanoparticles increased the chlorophyll content of peanut (*Arachis hypogaea* L.) crop leaves and promoted plant growth by regulating antioxidant activity and phytohormone contents. Studies by Servin et al. (2012, 2013) found that TiO_2 NPs applied to cucumber (*Cucumis sativus* L.) increased the chlorophyll content of the leaves, as well as increasing both the potassium and phosphorus content of the cucumber fruit. However, despite an increase of chlorophyll content found in this study and those cited above, the presence or absence of TiO_2 NPs did not affect crop growth.

Plant Growth Parameters

The plant growth parameters (plant height, above-ground and root fresh weight) for both years are shown in Fig. 3. The above-ground biomass weight in 2018 was significantly greater ($p \leq 0.05$) in the WW treatments as opposed to the FW treatments, irrespective of the presence or absence of NPs. In 2017, differences in the above-ground biomass were not statistically significant, even though they were numerically higher for the WW treatments. It is likely that the additional nutrients supplied in the WW had a positive impact on vegetative growth (and countered the effects of any additional contaminants). Shoot height remained unaffected by the treatments in both years. In 2017, but not 2018, root weight was greater in the WW treatment as compared with the other treatments. However, an increase in above-ground biomass from 2017 and 2018 occurred in all treatments. For example, the mean weight of the above-ground biomass for WW was 0.9 kg in 2017 and 1.45 kg in 2018, while for FW + NP, they were 0.47 kg in 2017 and 0.68 in 2018. Furthermore, in 2018, for FW and FW + NP, the mean weights for the above-ground biomass were 0.69 and 0.68 kg, respectively, while for WW and WW + NP, it was 1.45 and 1.35 kg. Such results indicate that the WW increased the above-ground biomass, while TiO_2 NPs did not have an impact. Similarly, the mean shoot height for WW treatment was 997 mm in 2017 and 1121 mm in 2018. Comparatively, for the FW, the mean shoot height was 887 mm in 2017 and 995 mm in 2018. The greater shoot weight and decreased root weight in the second season, in comparison to the first season, were attributable to warmer growing season (May–August) temperatures in 2018 (Mawof et al. 2021).

Like what was observed in our study, TiO_2 NPs did not affect germination and or root elongation for wheat (*Triticum æstivum* L.), oilseed rape (*Brassica napus* L.) and *Arabidopsis thaliana* L. (Larue et al. 2011), as well as lettuce (*Lactuca sativa* L.), radish (*Raphanus sativus* L.) and cucumber (Wu et al. 2012). Several studies, with conflicting findings, have documented the effect of TiO_2 NPs on plant growth and physiology. Larue et al. (2012a, b) reported a size-dependent distribution of TiO_2 NPs in wheat plants. The accumulation of NPs has also been reported to have no impact on seed germination or on the plants' total biomass. The impact of TiO_2 NPs on wheat and rapeseed plantlets, grown under hydroponic conditions, was also studied by the same group (Larue et al. 2012a, b), and they showed that there was a treatment effect on germination, evapotranspiration and total plant biomass. In contrast, Jaberzadeh et al. (2013) found that TiO_2 NPs application at 0.01%, 0.02% and 0.03% improved almost all agronomic parameters for wheat, especially at the rate of

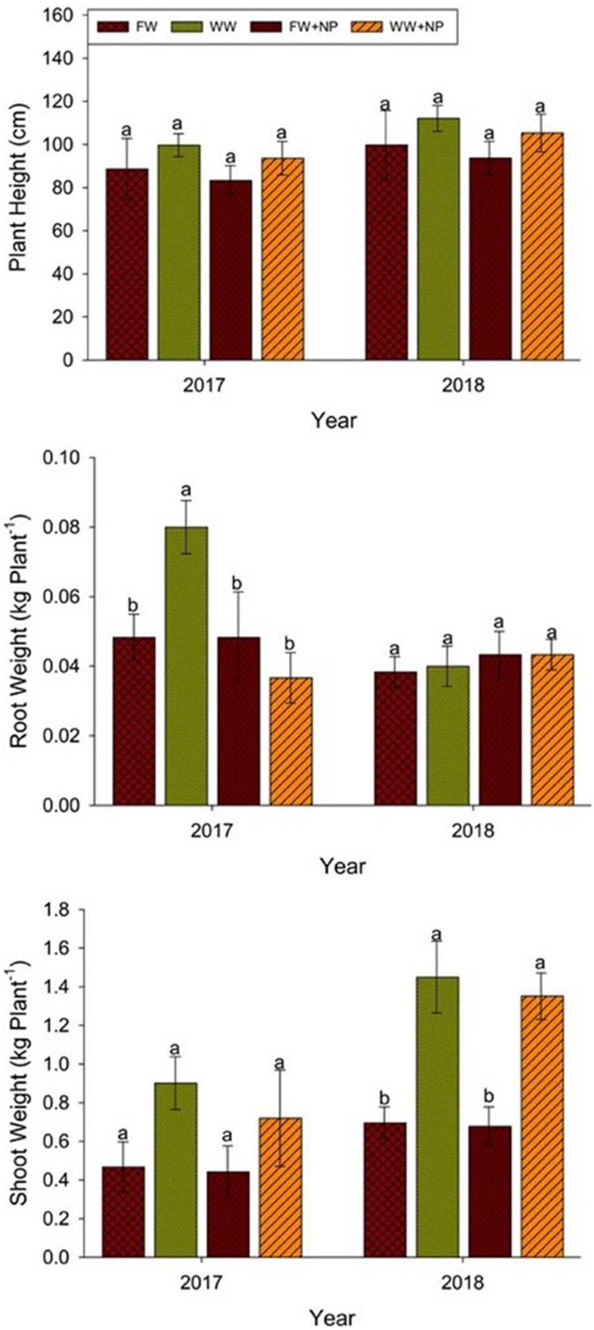


Fig. 3 Effects of freshwater (FW), wastewater (WW), freshwater with TiO_2 NPs (FW + NP) and wastewater with TiO_2 NPs (WW + NP) on potato plant height, root weight and shoot weight in 2017 and 2018. The different letters on the bars in each column represent significant difference at $p \leq 0.05$ (mean ± SD, $n = 3$)

0.02%, as compared to water-stressed plants. This impact may be dose dependent to a certain threshold. Rafique et al. (2014) found that TiO_2 NPs increased wheat root length, shoot length and biomass up to an application rate of 60 mg kg^{-1}. However, application of TiO_2 NPs at a greater concentration (*i.e.* 80–100 mg kg^{-1}) inhibited root and shoot length, reduced plant biomass as well as being toxic to plants. Likewise, Song et al. (2012) found that TiO_2 NPs enhanced elongation (more than 2.5-fold) and fresh weight (twofold) of duckweed (*Lemna minor* L.) up to a concentration of 0.5 g L^{-1}, while at greater concentrations, the plants sustained significant damage. In contrast to the current study, Rui et al. (2016) found that NPs increased root length, biomass and plant height for peanut plants. However, peanut plants are prone to iron deficiency, which may be why iron oxide NPs had such an impact on plant growth (Rui et al. 2016). While the presence of the low exposure concentration of TiO_2 NPs did not impact plant growth parameters in this study, the impact of TiO_2 NPs on crop yield was inconsistent.

Yield Components

Treatments had no effect on tuber weight and number of tubers in 2018, or on marketable tuber yield, in either year (Table 4). However, tuber weight was less in the FW + NP treatment, compared with the other treatments in 2017. Similarly, the number of tubers was lower in the FW + NP treatment, as compared to FW and WW in 2017, but no significant differences were observed between WW and WW + NP in either year. Average tuber weights ranged between 0.32 kg (FW + NP) and 0.89 kg (WW) in 2017 and between 0.35 kg (FW + NP) and 0.64 kg (WW and WW + NP) in 2018.

As shown above, the application of TiO_2 NPs resulted in inconsistent effects on yields. However, in most cases, no effect on yield was observed in the presence or absence of TiO_2 NPs. The influence of NPs on the plants, their toxicity and plant translocation should be investigated to better understand the impact of NPs on plant yield.

The effects of NPs on plants differ depending on their concentrations, size and even the plant species and surrounding environment (Rico et al. 2011). The presence of NPs in irrigation water could be both detrimental or positive, depending on the plant species and the properties and concentration of the NPs.

Khater (2015) investigated the foliar spray application of TiO_2 nanoparticles to coriander at concentrations of 2, 4 and 6 mg L^{-1}, finding a positive relationship between the dose and an increase in plant height, shoot length, number of branches

Table 4 Effect of freshwater (FW), wastewater (WW), freshwater with TiO_2 NPs (FW + NP) and wastewater with TiO_2 NPs (WW + NP) on potato tuber weight, number of tubers and tuber grading in 2017 and 2018

	Tuber weight (kg)		No. of tubers		% Tuber grading (> 50 mm)	
	2017	2018	2017	2018	2017	2018
FW	0.86 ± 0.06[a]	0.58 ± 0.20[a]	17.33 ± 4.51[a]	6.66 ± 3.05[a]	19.61 ± 13.54[a]	48.33 ± 44.81[a]
WW	0.89 ± 0.11[a]	0.64 ± 0.30[a]	15.33 ± 3.51[a]	6.66 ± 2.31[a]	39.88 ± 17.77[a]	54.16 ± 7.21[a]
FW + NP	0.32 ± 0.06[b]	0.35 ± 0.16[a]	7.33 ± 2.88[b]	6.33 ± 1.15[a]	43.52 ± 27.82[a]	47.62 ± 45.92[a]
WW + NP	0.77 ± 0.38[a]	0.64 ± 0.11[a]	10.66 ± 6.43[ab]	7.66 ± 3.05[a]	38.89 ± 9.62[a]	40.34 ± 21.02[a]

The different letters in each column represent a significant difference at $p \leq 0.05$; values are mean ± standard error of three replicates and plant yield. It was also observed by Owolade and Ogunleti (2008) that the seed yield of cowpea (*Vigna unguiculata* L.) was increased by foliar application of nano TiO_2, which may be attributed to the increased rate of photosynthesis. However, the mechanism behind the effects of the TiO_2 NPs is still unclear. In addition, the role of the interaction of the TiO_2 NPs and metals and other organic contaminants on plant yield should be given further consideration. Like our findings, Moll et al. (2017) and Larue et al. (2018) reported that exposure to TiO_2 NPs did not affect the biomass, biomass of seedlings or chlorophyll content of wheat. Dai et al. (2019) found that 1000 mg/L of TiO_2 NPs could increase root length for wheat and reduce Cd^{2+} toxicity in wheat seedlings. Thiagarajan and Ramasubbu (2021) summarized that the toxic effects of TiO_2 NPs in food crops were triggered only at very high exposure concentrations (> 1000 mg L^{-1}).

Total Organic Carbon (TOC) and Total Nitrogen (TN) Content in Potatoes

Treatments had no effect on the TOC content in tuber flesh, in either year (Table 5). The TOC content in potato leaf was significantly higher ($p \leq 0.05$) in FW + NP as compared to WW in 2017 and WW + NP in 2018. The addition of TiO_2 NPs through irrigation also had no impact on the TOC in the plant but increased total nitrogen in the potato tubers and leaves. The TN content in the tuber flesh was significantly higher ($p \leq 0.05$) in WW + NP than FW in both years. Similar trends were observed in leaf TN. Several studies have proved that TiO_2 NPs can impact the enzymes that regulate nitrogen metabolism, potentially favouring the conversion of inorganic nitrogen into chlorophyll and proteins. For example, Zheng et al. (2005) found that the spray application of TiO_2 NPs on spinach leaves increased the activity of enzymes that promote nitrate adsorption, accelerating the transformation of inorganic nitrogen into organic nitrogen. The significant impact of TiO_2 NPs on nitrogen

Table 5 Effect of freshwater (FW), wastewater (WW), freshwater with TiO_2 NPs (FW + NP) and wastewater with TiO_2 NPs (WW + NP) on total content of carbon and nitrogen in potato flesh and leaf in 2017 and 2018

Treatment	C-Flesh	C-Leaf	N-Flesh	N-Leaf
2017				
FW	40.23 ± 0.22^{a}	41.13 ± 0.28^{ab}	1.04 ± 0.27^{c}	2.60 ± 0.56^{b}
FW + NP	39.98 ± 0.95^{a}	42.21 ± 1.01^{a}	1.65 ± 0.07^{ab}	3.96 ± 0.13^{a}
WW	40.84 ± 0.17^{a}	39.96 ± 1.52^{b}	1.47 ± 0.08^{bc}	4.01 ± 0.28^{a}
WW + NP	40.90 ± 0.95^{a}	41.75 ± 0.45^{ab}	1.99 ± 0.46^{a}	4.04 ± 0.38^{a}
2018				
FW	40.24 ± 0.21^{a}	43.80 ± 0.47^{a}	1.50 ± 0.18^{b}	3.54 ± 0.51^{b}
FW + NP	40.28 ± 0.07^{a}	43.89 ± 0.33^{a}	1.63 ± 0.09^{ab}	4.60 ± 0.84^{ab}
WW	40.46 ± 0.34^{a}	42.96 ± 0.44^{ab}	1.57 ± 0.18^{ab}	4.48 ± 0.92^{ab}
WW + NP	40.55 ± 0.19^{a}	42.75 ± 0.79^{b}	1.82 ± 0.20^{a}	4.84 ± 0.28^{a}

The different letters in each column represent a significant difference at $p \leq 0.05$; values are mean ± standard deviation of three replicates coincided with an increase in plant leaf chlorophyll content, although this did not result in any significant impact on yield.

Conclusions

TiO_2 NPs (1 mg L^{-1}) did not have a negative impact on potato yield when the potato plants were irrigated with freshwater or wastewater. The study also showed that the presence of TiO_2 NPs in both freshwater or wastewater significantly increased the chlorophyll content of the potato leaves. When the potato plants were irrigated with freshwater or wastewater containing TiO_2 NPs, they did not get infected with early and late blight diseases, in either year in this study. Nonetheless, more research is required to elucidate the exact mechanisms of these effects and the possible impact of TiO_2 NPs on the nutritional quality of potatoes and the potential effects on humans.

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Data Availability The data that support the findings of this study are not openly available due to reasons of sensitivity and are available from the corresponding author upon reasonable request.

Declarations

Conflict of Interest The authors declare no competing interests.

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