Assessing the impacts of sewage sludge amendment containing nano-TiO_2 on tomato plants: A life cycle study

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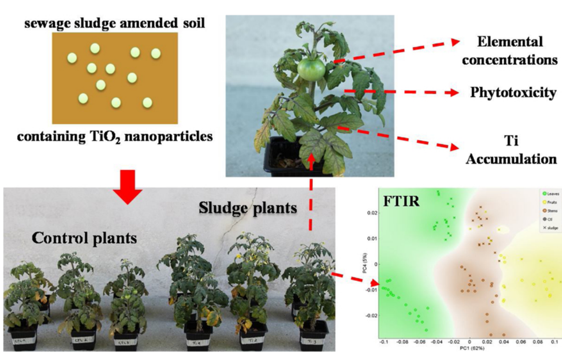
GRAPHICAL ABSTRACT



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ABSTRACT

Increasing evidence indicates the presence of engineered nanoparticles (ENPs) in sewage sludge derived from wastewater treatment. Land application of sewage sludge is, therefore, considered as an important pathway for ENP transfer to the environment. The aim of this work was to understand the effects of sewage sludge containing nano-TiO_2 on plants (tomato) when used as an amendment in agricultural soil. We assessed developmental parameters for the entire plant life cycle along with metabolic and bio-macromolecule changes and titanium accumulation in plants. The results suggest that the sewage sludge amendment containing nano-TiO_2 increased plant growth (142% leaf biomass, 102% fruit yield), without causing changes in biochemical responses, except for a 43% decrease in leaf tannin concentration. Changes in elemental concentrations (mainly Fe, B, P, Na, and Mn) of plant stem, leaves and, to a lesser extent fruits were observed. Fourier-transformed infrared analysis showed maximum changes in plant leaves (decrease in tannins and lignins and increase in carbohydrates) but no

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change in fruits. No significant Ti enrichment was detected in tomato fruits. In conclusion, we evidenced no acute toxicity to plants and no major implication for food safety after one plant life cycle exposure.

1. Introduction

Engineered nanoparticles (ENPs) have many industrial applications [1,2]. Among all ENPs, nano-TiO_2 are one of the most produced [3]. Their photocatalytic activity, high stability and anticorrosion properties make them suitable for a wide range of applications such as cosmetics, food, paints or water treatment processes [4–7]. As bulk material (possibly containing a fraction of ENPs), TiO_2 is widely used as a pigment [4]. With a constant rise in their production, ENPs will unavoidably make their way to the environment. Agricultural soil, in particular, is a very sensitive compartment and the main sink for ENPs [3]. ENPs accumulate *via* sewage sludge application as fertilizer in agriculture [8,9]. Many studies have already evidenced ENP presence in wastewater treatment plants [10,11] and further in biosolids [7]. Secondly, the use of ENPs in plant protection products also leads to their intentional input in soil [12–15]. A probabilistic material flow analysis predicted nano-TiO_2, among other ENPs, to be present in the highest concentrations in the environment with up to 61 mg Ti/kg for sludge amended soil [3]. The concentrations of nano-TiO_2 in biosolids and treated soils would have increased 30–40 times between 2005 and 2012 [3].

Sewage sludge is widely used in agricultural fields as it represents a highly sustainable strategy to address two important global challenges: efficient agriculture production thanks to inputs in micro and macronutrients and organic matter, as well as disposal of large quantities of "waste". However, this entails the risk of toxicity and food chain contamination due to the presence of heavy metals and organic pollutants in sewage sludge [16]. The European Union (Directive 86/278/EEC) and the U.S.A. (Title 40 Code of Federal Regulations, Part 503) have legislations defining permissible limits for heavy metal in sewage sludge used for land application [17,18]. While ENPs are emerging as possible pollutant with unknown risks, no regulation exists so far for governing their release in the environment [19–21].

Despite the risk of food chain transfer of ENPs and the wide use of sewage sludge as amendments, there is limited research on the impact of ENPs on plants through application of sewage sludge amended soil. Recently, several studies have investigated the phytotoxicity of pristine nano-TiO_2 on plants [6,22–26]. Du et al. [27] observed that nano-TiO_2 decreased plant biomass in wheat and inhibited soil enzyme activity. Servin et al. [28] reported root-to-fruit translocation of nano-TiO_2 in cucumber grown on a spiked soil. More recently, we observed no acute phytotoxicity on wheat plants grown in nano-TiO_2 spiked soil, but a significant Ti uptake in sandy soils [29]. However, studies investigating the environmental impacts of nano-TiO_2 resulting from sewage sludge applications are very few [30–32].

The objective of the present study was to develop a realistic exposure scenario simulating the growth of edible plants on agricultural soil treated with sewage sludge containing nano-TiO_2 allowing investigating the implications for food safety of such practice. Monitoring the entire life cycle of tomato plants grown on this sewage sludge amended soil, we investigated the impact on (i) plant development (ii) biochemical responses (iii) changes in biomacromolecules using Fourier Transformed Infra-Red spectroscopy (FTIR) (iv) effect on plant elemental concentrations and (v) Ti accumulation by inductively coupled plasma-optical emission spectrometry (ICP-OES).

2. Materials and methods

2.1. Soil and sewage sludge description

The sewage sludge was produced in a pilot wastewater treatment plant at the Swiss Federal Institute of Aquatic Science and Technology (EAWAG, Dubendorf, Switzerland) [33]. Nano X-ray fluorescence (XRF) evidenced the presence of micro- and nano-TiO_2 in the sludge (26% ENPs) [34]. X ray absorption spectroscopy (XANES) showed that Ti was present in the sludge as 55 ± 1% anatase and 45 ± 2% rutile. Other metals, such as Zn and Cu, were also detected however, according to previous analyses, they were mainly present in the micrometric range, associated with sulfur or organic matter which are responsible for their stabilization in soils [35]. The top layer (0 − 30 cm) of a loamy soil (38% sand, 42% silt and 20% clay) was collected at La Côte Saint-André (Isère, France) from a field under permanent pasture to serve as “control soil”. Control soil contained 37 ± 2% anatase, 49 ± 7% rutile and 14 ± 6% amorphous TiO_2 [34]. Both sludge and soil were previously characterized [35] and the main physico-chemical properties are given in supplementary information (SI). A mixture of soil and sewage sludge (w/w) was prepared in the ratio of 1:10 (sludge: soil) and considered as “sludge amended soil”. In this condition, 20% of the Ti was < 100 nm and about 50% in the 100–300 nm range [34].

2.2. Plant exposure

Twelve experimental units were filled with 200 g of sludge amended soil or control soil (six biological replicates per condition). Seeds of tomato plants (*Solanum lycopersicum*, var. Red Robin, provider: Germinance) were sown and grown until the ripening of tomato fruits with watering *ad libitum*. All experiments were carried out in a growth chamber under controlled conditions: day/night photoperiod (16/8 h), 100 µmol/m^2/s, day/night temperature (24/20 ± 1 °C), and day/night relative humidity (70/75%). The development parameters (number of leaves, flowers, fruits, plant height) were monitored throughout the whole plant life cycle. Chlorophyll level was assessed everyday (SPAD-502 chlorophyll meter, Minolta Camera Co., Japan). After 4 months (120 days), plants were harvested, fresh biomass was recorded and plants were split in three. Fresh leaves for biochemical analyses were weighed, immediately frozen in liquid nitrogen and stored at −80 °C until analysis. The remaining leaves along with stems and fruits were dried for determination of elemental concentrations by ICP-OES and further ground (Fast Prep® grinder) for the assessment of biomacromolecular changes by FTIR.

2.3. Analysis of biochemical responses

Three biochemical responses were investigated in tomato leaves: oxidative stress (lipid peroxidation [36]), photosynthesis (chlorophylls a and b [37]) and secondary metabolites (phenolic compounds [38], tannins [39] and flavonoids [40]). Detailed methods are described in SI.

2.4. FTIR analysis

FTIR was used to probe molecular vibrations in plant samples and thus gain information on the biochemical composition (lipids, carbohydrates, proteins). Each powdered sample was analysed in ATR-mode over a range of 4000 to 400 cm^{-1} using a diamond crystal (Thermo Nicolet, Nexus, Smart Orbit). Three independent technical replicates from each sample were acquired. The full experimental setup for FTIR acquisition is given in SI. OMNIC software was used to export experimental spectra.

Data treatment was performed using Orange software [41]. Briefly, data were pre-processed which implies selection of the region of interest (here 1755 – 455 cm^{-1} range which contained most of the variability by looking at the average and standard deviation of the absorbance), vector normalization and smoothing by Savitzky-Golay filter. Principal component analyses (PCA) were performed to visualize the data in a new coordinate space optimized for detection of differences between groups. Second derivative was performed to highlight spectral differences detected using PCA.

2.5. Elemental concentration in soil, sewage sludge and plants

ICP-OES was used to measure Ti and other micro and macro-elements in plant leaves, stems and fruits as well as in soils [29]. Soil exchangeable and extractable Ti concentrations were determined through $BaCl_2$ and HCl extractions, respectively (see SI for more information). Blank (only chemicals) and standard reference material (NIST 1573a, tomato leaves) were used as controls in the digestion process. Analyses were performed on an ICP-OES Spectro Arcos (Ametek).

2.6. Statistical analysis

Data reported were averages of six biological replicates ± standard deviations (SD). Data were checked for normality (Shapiro's test) and homoscedacity (Bartlett's test). When assumptions were met for parametric analyses, a Student *t*-test was used. Otherwise, a Wilcoxon test was applied. Statistical significance was based on a p value < 0.05. Additionally, ICP-OES results were analysed using PCA to identify different profiles. All statistical analyses were performed using the R statistical software (version 3.1.3) [42] and vegan package [43] for PCA.

3. Results and discussion

3.1. Titanium concentration in soil

Titanium concentration for the sludge amended soil was 819 ± 62 mg/kg and for control soil 801 ± 102 mg/kg (Figure S1 for other elemental concentrations). The exchangeable Ti concentration for control soil and sludge amended soil was 0.004% and 0.006%, respectively. The extractable soil fraction for both soils was 0.08%. No significant difference was found between the control and sludge amended soil for the total, extractable and exchangeable Ti ($p > 0.05$).

Despite the fact that both soils had similar Ti concentrations, Ti origin was different. While the control soil had geogenic Ti concentration from natural soil, the sludge amended soil had 9/10 natural Ti and 1/10 coming from the sludge which was most probably engineered TiO_2. Those two Ti forms might have different behaviour in the environment and different toxicity potentials. Indeed, natural TiO_2 had a rough surface and was included in organo-mineral soil aggregates (with Si, Al, Fe), whereas TiO_2 observed in the sludge was composed of homo and heteroaggregates dominated by organic matter [34].

Properties such as organic matter content and clay content have been cited as important parameters controlling ENP behaviour in soil, influencing their mobility [44]. The presence of high organic matter content in the soil (8.8%) and in the sludge amended soil (10.9%) thus is a probable explanation for low exchangeable and extractable Ti. The agricultural soil was also rich in clay (20%) which further elucidates our results. Indeed, in our recent work investigating TiO_2 ENP fate in 4 different types of soil, the soil with highest clay content (42%) displayed no ENP mobility compared to sand and silty sand soils [29]. Likewise, Gogos et al. [45] reported that leached Ti amount in a brown earth soil (sandy loamy to loamy fine fraction) was found to be 10^{-4}% of the initial spiked Ti amount even at the highest concentration of 1000 mg/kg.

3.2. Plant growth and developmental parameters

Plants grown in sewage sludge amended soil significantly increased biomass production for all organs (stem: +70%, leaves: + 142% and fruits: + 102%, $p < 0.050$) as well as the number of leaves (20 *vs.* 15 per plant for amended and control soil respectively: +33%, $p = 0.047$) and flowers (23 per plant in amended soil *vs.* 10 for control soil: +130%, $p = 0.012$) compared to plants grown in the control soil (Fig. 1A, B). Fruit yield was also significantly increased with an average of 4.4 fruits per plant amounting for 21.50 g in the sludge amended soil and 2.2 fruits per plant for a total weight of 10.63 g in the control soil ($p = 0.010$ for fruit number and $p = 0.005$ for fruit biomass). The average plant height for sludge amended soil was 20.68 ± 1.42 cm which was 43% higher than plants grown in the control soil (14.46 ± 0.45 cm, $p = 0.012$, data not shown). The monitoring of developmental parameters through the entire plant life cycle showed that after an average of 50 days of experiment, plants in the amended soil started exhibiting greater development compared to the control plants (Figure S2 A, B, C).

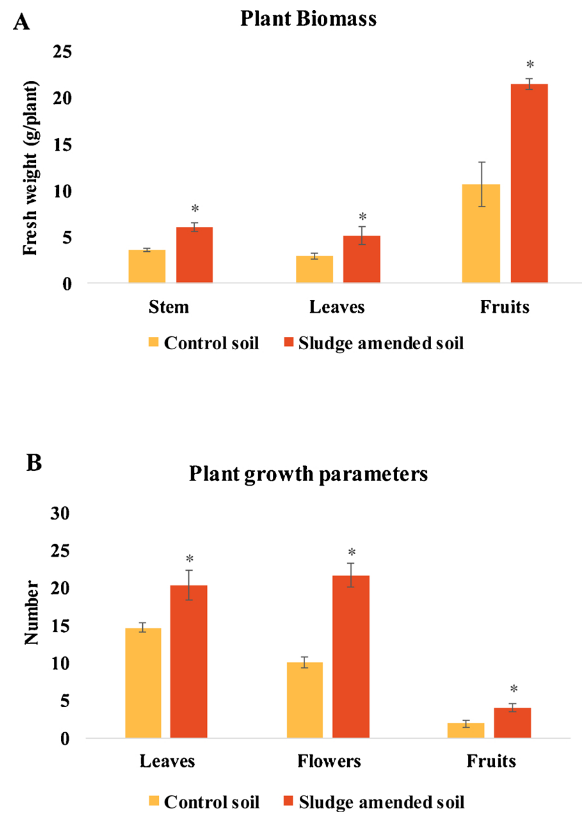

Fig. 1. (A) Plant fresh biomass for stem, leaves and fruits and (B) number of leaves, flowers and fruits for tomato plants grown for 120 days on control soil and sludge amended soil. Asterisks represent significant difference ($p < 0.05$) as indicated by Student *t*-test ($n = 6$).

In this study, we found that sewage sludge amendment containing nano-TiO_2 stimulated plant growth and tomato yield. Sewage sludge was rich in organic matter and contained substantial amount of nutrients. Total carbon, nitrogen, phosphorus and sulphur concentrations in sludge amended soil were multiplied by 3.4, 6.8, 1.8 and 23.6, respectively in comparison with control soil, thereby contributing to the fertilizer effect. The fact that sewage sludge application is an effective mean for improvement of plant vegetative growth has been widely reported [46–48].

Some studies in the literature have explored the phytotoxicity of nano-TiO_2 using developmental parameters with contrasting results. Nano-TiO_2 (750 mg/L) were found to induce longer roots in cucumber plants [28] as well as increased germination rates in *Arabidopsis thaliana* seedlings (500 mg/L) [49]. However, Song et al. found no effects of nano-TiO_2 treatment (up to 5000 mg/L) on oilseed rape, lettuce and kidney bean [50]. More recently, we evidenced no impact on wheat grown on different types of soils after exposure to 500 mg/kg [29]. Finally, in a sewage sludge study, Josko and Oleszczuk (2013) reported that inclusion of 10 mg/kg TiO_2 ENPs in sewage sludge amended sandy soil inhibited root growth in plants *Lepidium sativum* and *Sinapis alba* [16]. Still it is unclear how TiO_2 ENPs affect plants; it seems that in most studies there was no clear dose-response effects and that those effects were modulated by soil properties. However, bulk Ti has been considered as a biostimulant for a long time and has been used in commercial foliar fertilizers for improving plant growth and development [51]. For instance, use of Tytanit®, a Ti based foliar fertilizer, significantly improved the fruit yield of tomato at 960 g Ti /ha [52]. In our study we concluded that the overall improved yield of tomato plants after sewage sludge amendment was attributed to the nutritive value of sludge, while we cannot be certain on the positive or negative effects of nano-TiO_2.

3.3. Biochemical responses

No significant difference was observed in leaves for all the biomarkers related with secondary metabolites except tannins (Table 1, Table S2). Leaf tannins significantly decreased of 42.7% in plants exposed to sewage sludge ($p = 0.022$). Overall, phenolic compound, flavonoid, chlorophyll *a* and *b* and malondialdehyde (MDA) concentrations were not significantly modified.

Among secondary metabolites, phenolic compounds (such as tannins and flavonoids) are widely distributed in plants, and play a prominent role in general defence strategies (against herbivores, microbes, or competing plants), as well as contributing to food quality [53]. Usually, a high tannin concentration favours plant growth in poor soil, in which the same plant species with a low tannin concentration could not grow [54,55]. A decrease in secondary metabolites might weaken the defence system of the plants and be a threat to plant survival in a more competitive environment (*vs.* in growth chamber). So far a very limited number of studies have investigated the impact of ENPs on secondary metabolites.

For photosynthesis assessment, SPAD chlorophyll meter measurements recorded along plant growth revealed no major difference in chlorophyll content (data not shown). This result was further confirmed by assessing photosynthetic pigment concentrations in leaves at the end of exposure: the average concentration of chlorophyll *a* and *b* increased of 18.3 and 34.8%, respectively but this evolution was not significant ($p = 0.520$ and $p = 0.623$, respectively). Previous studies have also reported similar results where chlorophyll content in wheat [56] and tomato [57] was not impacted by TiO_2 ENP treatment.

Finally, oxidative stress was assessed through lipid peroxidation: leaf malondialdehyde (MDA) concentration did not change significantly ($p = 0.572$) with values from 3.84 ± 2.43 in control plants to 4.86 ± 1.64 nmol/g f.wt in plants grown on sludge amended soil. Likewise, Koce et al. [58] found no significant effect on anti-oxidative enzymes and lipid peroxidation levels in *Allium cepa* in a 24 h study using a wide range of nano-TiO_2 concentration (0.1–1000 mg/L). MDA is the ultimate product of lipid peroxidation damage caused by generation of free radicals. Flavonoids and tannins are reported to exert inhibition of lipid peroxidation and scavenging of oxygen radicals [54]. Tomato plants might thus have gone through a moderate oxidative stress during growth as suggested by the significant decreased in tannin

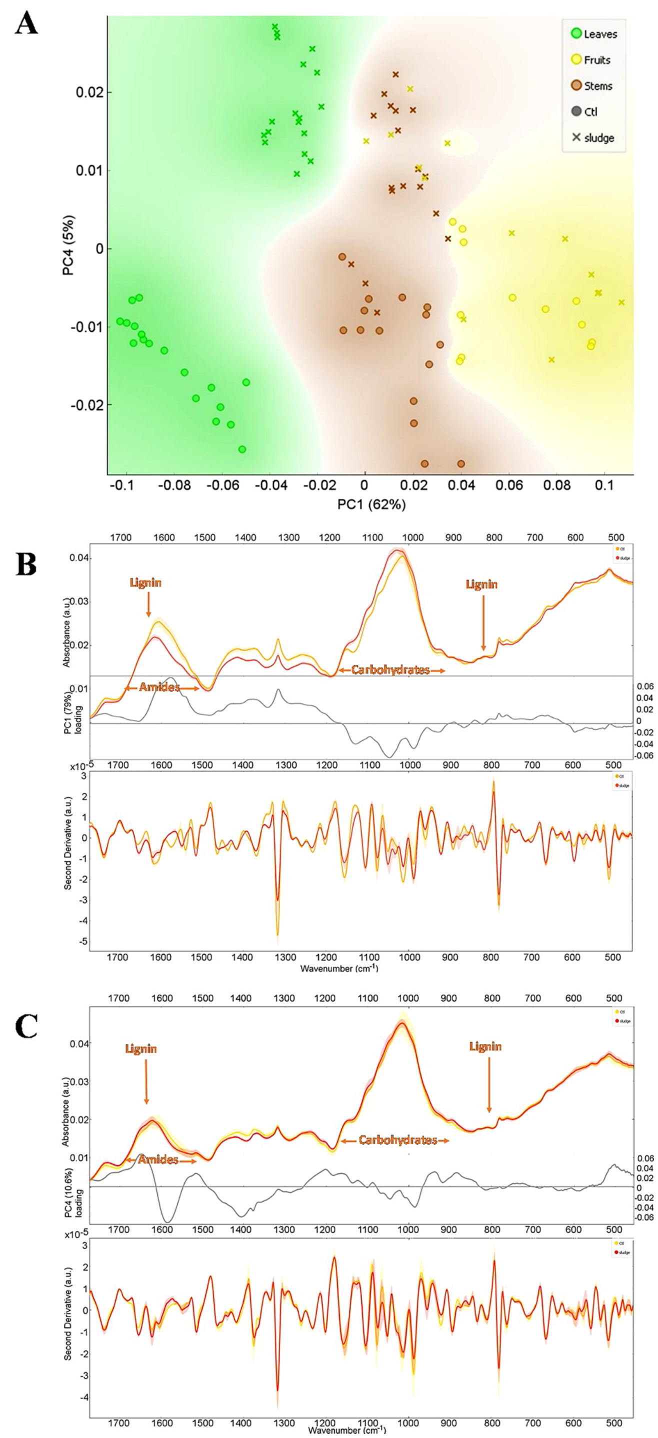

Fig. 2. (A) PCA including FTIR spectra from all samples (leaves, stems, fruits) of tomato plants grown for 120 days on control soil and sludge amended soil. FTIR absorbance (upper panel), loadings (in black upper panel) and second derivative (lower panel) spectra of (B) leaves and (C) stems. The shading of the spectra represents the standard deviation.

Table 1
Variation relative to the control for flavonoids, phenolic compounds, tannins, chlorophylls a, b and malondialdehyde (MDA) in leaves of tomato plants grown for 120 days on sludge amended soils. Asterisks represent significant difference ($p < 0.05$) as indicated by Student *t*-test ($n = 6$).

Biomarker	Phenolic compounds	Flavonoids	Tannins	Chlorophyll a	Chlorophyll b	MDA
Variation relative to control	+ 2.4%	−9.2%	−42.7%*	+18.3%	+34.8%	+26.6%

concentration and trend in increased MDA content (+26.6%, however, not significant upon harvest). The lack of biochemical response might also be associated with the duration of exposure. Indeed, a longer contact duration has been found to favour interactions between ENPs and sewage sludge components (*e.g.* organic matter) thereby reducing their bioavailability (and toxicity) through adsorption or aggregation [16,59]. Also it is possible that initially plants might have experienced stress but later adapted to it such that no significant difference was observed later. Dosing of reactive oxygen species (ROS) and anti-oxidative enzymes during exposure would be interesting to further understand implied processes.

3.4. Impact on plant biomacromolecules: FTIR analysis

In this study, FTIR was used to identify changes in specific functional groups in tomato plants (Fig. 2A, B, C and Figure S3). During data pre-processing, on the absorbance spectra, it was noted that most of the variability observed in the average spectra of each group arose from the carbohydrate/protein/aromatic/saccharide region (1755-455 cm^{-1}), while the PCA on the lipid region (between 3000 and 2800 cm^{-1}) failed to separate the groups in this region and the region in-between (2800-1755 cm^{-1}) was mainly background noise.

We thus selected the range from 1755 to 455 cm^{-1}, also known as fingerprint region, for further processing. After PCA treatment, PC1 (62%) separated clusters corresponding to the stems, leaves and fruits, while on PC4 (5%) data segregated clearly in two groups (control *vs.* sludge amended soil) for leaf samples, to a lesser extent for stem samples but not for fruits (Fig. 2A). These results highlight thus no impact of the sewage sludge treatment on the biomacromolecules of the edible part of tomato (Figure S3). Excluding the fruits from the analysis, leaves and stems were further processed to identify the main spectral differences.

As previously mentioned, the major difference between control and sludge was found in the leaves. These differences were highlighted with PC1 explaining 79% of the variance between leaf samples, and changes were evidenced in the second derivative (Fig. 2B). The majority of the differences were associated with spectral bands of carbohydrate (900-1200 cm^{-1}) attributed to C–O stretching vibrations and OH deformation vibrations. Differences were also detected in the region 1480-1180 cm^{-1} where CH_2 wagging vibrations occur that may correspond to aromatic and ring vibrations, followed by changes in the amide, phenolic and aromatic band contributions (1700-1500 cm^{-1}) where compounds such as lignin or tannin (that are mainly aromatic) could be highly contributing [60]. Indeed, the band at 1515 cm^{-1} is quite characteristic of lignin [61].

In particular in leaves, the relative intensities of bands 1029, 1050, 1075 and 1144 cm^{-1} mainly characteristic of carbohydrates were increased in sludge plants while bands for lignin (666, 760, 780, 1260, 1315, 1370, 1416, 1515, 1540, 1606 cm^{-1}) were stronger in control plants. The spectral region 1200-1610 cm^{-1} contains many contributions that are characteristic of tannins [62] and lignin [63]. This result is in agreement with the decrease of tannins observed when measuring biochemical biomarkers.

In stems, the differences were significantly smaller (Fig. 2C), to separate the control from the sludge condition, it was necessary to look into the loadings of the PC4 explaining 10.6% of the variance of the samples. The major differences occurred in the amide, aromatic regions (1680-1500 cm^{-1}) and in the 1500-1300 cm^{-1} region, typical of polysaccharides and cellulose. A decrease at 1417 and 1372 cm^{-1} and a slight shift from 1614 to 1620 cm^{-1} was observed in the sludge condition. This decrease may also be reflecting the changes in concentration of tannin and lignin but in a less evident manner. Indeed, when looking at the band at 1515 cm^{-1}, no apparent change was observed.

With all these observations in mind, we can state that fruits were not affected by the exposure to the sewage sludge treatment, while some changes were observable in the stems and leaves. Changes are in the same spectral regions, but the differences are greater in the leaves. Those results imply that the impact of sludge containing TiO_2 ENP on food nutritional value of leafy crop should be investigated more closely. Additionally, impact on other fruits cannot be excluded since a study from Servin et al. [61] evidenced changes in cucumber fruits upon TiO_2 ENP exposure.

3.5. Effect on plant elemental concentrations

Plant macro (S, Ca, Mg, P, K) and microelements (Al, B, Cu, Fe, Mn, Na, Si, Zn) were determined in the different organs to assess the impact of sewage sludge amendment on plant ionome (Figure S4A, B, C). Tomato leaves were again found to be the most impacted plant organ which is in line with the above discussed FTIR results.

Sewage sludge amendment significantly increased the translocation (concentration in the leaves/concentration in the soil) of Fe, B, and Na in all plant organs (stem, leaves, fruits) while S, P increased only in leaves and stem (Fig. 3A, B, C). Mn and Si translocation was also consistently lower in sewage sludge exposed plants. Those elements are of primary importance for plant development. For instance, B is an essential micronutrient influencing good seed set and fruit development as it plays a vital role in growth of the pollen tube during flower pollination [64]. Na is a functional nutrient regulating stomatal opening and closing [65]. Sulfur is also a key component of defense compounds cysteine, glutathione and phytochelatins where it appears in the sulfhydryl (-SH or thiol) group. These thiol compounds synthesised in plants under metal stress represent a very important detoxification pathways for plant tolerance and survival [66]. Also, changes in nutrient content can impact the overall plant nutritional quality and taste of crops [67].

Studies in the literature report both sewage sludge [68] and Ti as stimulants of increased nutrient absorptions in plants [52]. While it is likely that the increase in many elements in plants was primarily due to the effect of the sludge (more bioavailable elements), it might partly also be associated with the presence of TiO_2 functioning as promoter of plant nutrition.

In particular, TiO_2 ENPs have been reported to increase P uptake in several studies on different plant species (lettuce [69], wheat [70], tomato [71]). Likewise, Ti has also been associated with enhancement of Fe uptake [51]. In a hydroponic study of two weeks, nano-TiO_2 (concentration of 0.5–2 g/L) also significantly increased S, Mg and Fe contents in tomato leaves and roots [71]. Similarly, application of Ti in the form of Tytanit® foliar fertilizer increased N, Ca, and Mg contents of tomato plants [52]. The decrease in Mn concentration after TiO_2 ENP application has also been evidenced before [72].

More studies are needed to better distinguish the sludge effect from the ENP effect, for example by spiking different concentrations of TiO_2 ENPs in the waste water before treatment.

3.6. Presence of Ti in plant parts

Ti concentrations were 1.19 ± 0.51 and 1.03 ± 0.35 mg/kg in stems, 2.59 ± 1.62 and 1.14 ± 0.31 mg/kg in leaves, and 2.31 ± 1.67 and 0.88 ± 0.30 mg/kg in fruits for plants grown on control soil and sludge amended soil, respectively (Fig. 4). While Ti concentration was similar for both soils Ti uptake was reduced in plant organs in sludge amended soil with significant reduction in leaves ($p = 0.032$). Further µXRF analysis was performed to investigate Ti distribution in leaf cross sections (Figure S5). However, Ti was below the detection limit in both samples (description of the experimental set-up and results in SI).

Our results are in agreement with the literature that has reported low uptake of Ti based ENPs from soil. Du et al. [27] in their study of 7 months on wheat found no significant difference in the Ti content of wheat grain between nano-TiO_2 treated soil (10 g/kg TiO_2) and control soil classified as loamy clay. Burke et al. [73] also found very low levels

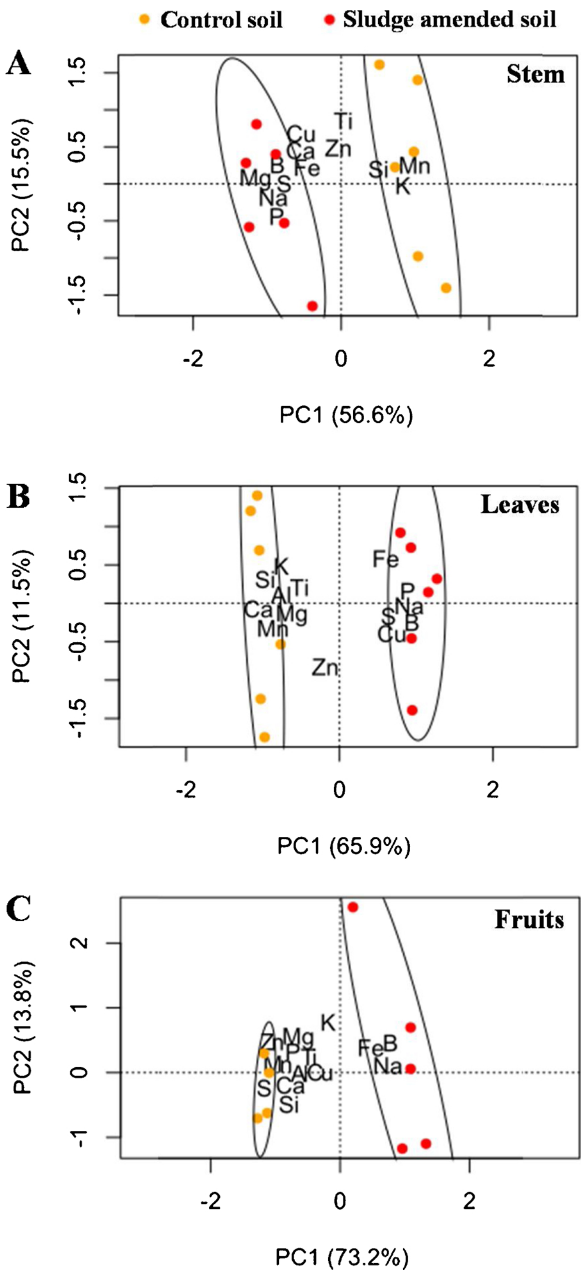

Fig. 3. Principal component analysis (PCA) based on elemental translocation factors (concentration in the plant organ/concentration in the soil) in (A) stem (B) leaves and (C) fruits for tomato plants grown for 120 days on control soil and sludge amended soil, as determined by ICP-OES.

of Ti ($<$ 2.5 mg/kg) in the above ground tissue of soybean and maize plants after a six week exposure to 200 mg/kg TiO_2 ENPs.

The lower translocation of Ti in the leaves of plants grown on sewage sludge amended soil might be due to the presence of a significantly higher amount of organic matter (+24%) consisting of functional groups that readily form complexes with ENPs, compared to the control soil, thereby possibly, reducing the bioavailability of Ti. Indeed, studies have reported that pH and organic matter directly influence the bioavailability of ENPs [74]. Similarly, we previously evidenced that Ti exhibited no significant root internalization and no upward translocation to leaves in wheat plants grown in soils with organic matter content above 1.5%.

So far there is no defined range for deficiency, sufficiency or toxicity for Ti in plants [75]. However, Kabata-Pendias and Pendias (2001) suggested that Ti content ranging from 50 to 200 mg kg^{-1} in mature

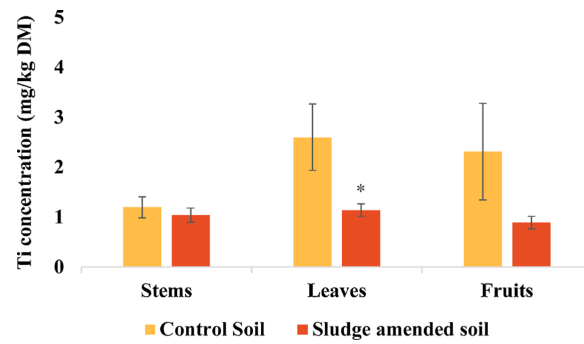

Fig. 4. Ti concentrations in the stem, leaves and fruits for tomato plants grown for 120 days on control soil and sludge amended soil. Asterisks indicate significant difference ($p < 0.05$) as indicated by Student *t*-test ($n = 6$).

leaves of plants could be excessive or toxic [76] while Lyu et al. proposed a Ti threshold of 50 mg kg^{-1} in leaf tissues [51]. So, based on our results, Ti concentrations in plant parts were far lower than these proposed concentrations, thus suggesting no risk of toxicity.

Another issue to consider is that nano-TiO_2 exhibit a high potential to adsorb toxic metals on their surfaces as well as nutrients [7,77,78]. Over the years, repeated applications of nano-TiO_2 through sewage sludge might lead to the release of metals sorbed on ENP surface into the environment becoming readily available for crop plants [7] and the decreased bioavailability of nutrients such as Ca and Fe [79]. According to Hartmann and Baun (2010) the interaction between ENPs and toxic compounds in environmental mixtures can either amplify or alleviate toxicity [80]. But it is still unclear whether the co-contamination of ENPs and metals would lead to an enhance toxicity of the mixture (Trojan horse effect) or rather a decreased one.

Our results indicate that sewage sludge amendment containing nano-TiO_2 improved the plant yield due to its high organic matter and nutrient content and did not lead to significant changes in the edible part of tomato. However, over time nano-TiO_2 in sewage sludge is expected to increase while organic matter content may decrease if the sludge application stops. This may affect Ti availability in the long run. Another aspect to take into consideration is the impact of such amendment on soil bacterial communities along time.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.jhazmat.2019.02.036.

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