Hindawi Publishing Corporation
International Journal of Photoenergy
Volume 2016, Article ID 8060847, 14 pages
http://dx.doi.org/10.1155/2016/8060847

Research Article

Evaluation of the Antimicrobial Activity of Nanostructured Materials of Titanium Dioxide Doped with Silver and/or Copper and Their Effects on *Arabidopsis thaliana*

Cristina Garcidueñas-Piña,[1] Iliana E. Medina-Ramírez,[1] Plinio Guzmán,[2] Roberto Rico-Martínez,[1] José Francisco Morales-Domínguez,[1] and Isidoro Rubio-Franchini[3]

[1]*Departamento de Química, Universidad Autónoma de Aguascalientes, Avenida Universidad No. 940, Ciudad Universitaria, 20131 Aguascalientes, AGS, Mexico*
[2]*Departamento de Ingeniería Genética, Centro de Investigación y Estudios Avanzados (CINVESTAV), Unidad Irapuato, Km 9.6 Libramiento Norte Carretera Irapuato-León, 36821 Irapuato, GTO, Mexico*
[3]*Laboratorio Estatal de Salud Pública, Departamento de Control Ambiental, Instituto de Servicios de Salud del Estado de Aguascalientes (ISSEA), Avenida Siglo XXI No. 105, Ciudad Satélite Morelos, 20298 Aguascalientes, AGS, Mexico*

Correspondence should be addressed to Iliana E. Medina-Ramírez; iemedina@correo.uaa.mx

Received 23 October 2015; Revised 14 February 2016; Accepted 30 March 2016

Academic Editor: Patrick S. M. Dunlop

Nanostructured materials (NSMs) of silver ($Ag@TiO_2$) and copper (TiO_2-Cu^{2+}) doped titanium dioxide were synthesized, fully characterized, and evaluated for their antimicrobial efficiency and effects on *Arabidopsis thaliana*. The NSMs were prepared using an environmentally benign route. The physicochemical properties of the materials were determined with analytical techniques. These materials are active under visible light, exhibit a small size (10–12 nm), are crystalline (anatase), and liberate metal ions (Ag^+ and Cu^{2+}) in solution. Microbicide activity was observed in *E. coli* C600 and *S. cerevisiae* W303 strains treated with several concentrations of $Ag@TiO_2$ and TiO_2-Cu^{2+}, radiated and nonradiated, and after different times. Higher inactivation was achieved with $Ag@TiO_2$ in *E. coli*, with value of log inactivation of 2.2 with 0.5 mg/mL after 4 h, than in *S. cerevisiae*, with a log inactivation of 2.6 with 10 mg/mL after 24 h. The impact of these NSMs in plants was evaluated in *Arabidopsis thaliana* Col-0 strain exposed to such materials at different conditions and concentrations, and physical and biochemical effects were analyzed. Seeds exposed to NSMs did not show effects on germination and growth. However, seedlings treated with these materials modified their growth and their total chlorophyll content.

1. Introduction

Nanostructured materials (NSMs) have become the focus of numerous investigations due to their unique physicochemical properties which are different from the same materials with larger dimensions [1]; these properties have been exploited for the development of several new products with different technological and medical applications [2, 3]. Lately, engineered NSMs can be produced with specific physicochemical properties for advanced applications. Some of these NSMs have demonstrated microbicide activity and are being used as disinfectants for water purification, with several advantages over the traditional water disinfection technologies. Researchers have a special interest in metal/semiconductor oxide composites since they have been shown to be efficient bifunctional materials, which can be used in numerous physical, biological, biomedical, and pharmaceutical applications [4]. Among the various materials, titanium dioxide (TiO_2) has been widely studied because of its chemical stability, low toxicity, abundance, and excellent photocatalytic activity [5].

It has also been demonstrated that coupling TiO_2 with noble metals renders visible light active materials with increased photocatalytic and photoelectrochemical responses of the composite material by reducing the fast recombination of the photogenerated charge carriers [6, 7]. It has been demonstrated that Ag@TiO_2 efficiently removes several pathogenic microorganisms from water, air, and surfaces [8–11]; however, the ecological impact of this material has been scarcely studied [12–14]. Although to a lesser extent than Ag@TiO_2 composites, the bactericidal effect of TiO_2-Cu^{2+} materials has been lately investigated [15–17]. We have been working on the implementation of green routes for the synthesis of metal doped NSMs with enhanced activity [18–20]. Ag@TiO_2 and/or TiO_2-Cu^{2+} synthetized by that methodology need a systematic study of characterization, photocatalytic activity, antimicrobial activity, and toxicological impact. The study of the antimicrobial activity in model organisms has the advantage of obtaining results in a reproducible, easy, cheap, and fast way. *Escherichia coli* C600 strain is a harmless gram negative bacterium of easy handling in the laboratory. Furthermore, *Saccharomyces cerevisiae* W303a strain is a fungus unicellular yeast with a comparable lifespan to that of *E. coli*. Therefore, both microorganisms were selected to study antimicrobial activity of Ag@TiO_2 and TiO_2-Cu^{2+} to determine the range of effective antimicrobial concentrations. The seeds disinfection like potential use of these materials was considered, so that the antimicrobial activity was evaluated in seeds and in fungal contaminants seeds too.

The impact of interaction of plants with NSMs is becoming a priority since plants can serve as reservoirs of NSMs, and plants are directly exposed to soil, water, and air, where NSMs could be dispersed. As a result of that interaction, uptake and accumulation of NSMs is likely to take place giving rise to deleterious effects in plants or they may become one of the main routes of exposure to NSMs for higher species [21]. Despite the preliminary efforts to determine the potential hazard of NSMs in plants, currently published results are contradictory regarding phytotoxicity of NSMs. For instance, Shaw and Hossain [22] reported that the interaction of NSMs (CuO) with plants affects their development and productivity, whereas Li and coworkers [23] indicate that the interaction of NSMs (Fe_3O_4) with watermelon seedlings is beneficial, since increased accumulation of chlorophyll is observed. In the same direction, Kole and coworkers [24] reported on the effects of seed treatment with fullerol NSMs that resulted in increasing plant biomass, fruit yield, and phytomedicine content in bitter melon; thus, the authors suggest the use of fullerol for crop improvement. In view of the contradictory findings described in the above-mentioned reports and due to the lack of standards and guidance for NSMs toxicity evaluation, it is evident that many technical challenges must be surpassed to achieve objective information regarding toxicity evaluation of NSMs. *Arabidopsis thaliana* is being used as model organism, because of its small size, little space requirements, and short life cycle. Its small seed size results in a relatively large surface area to volume ratio, which is conducive to higher sensitivity to toxicants. *A. thaliana* is also the first plant to have its genome sequenced, which facilitates future work on its molecular response to NSMs [25]. Therefore, the goals of this work are as follows: (a) to report on the synthesis and characterization of TiO_2-Cu^{2+}, (b) to evaluate the antimicrobial activity of Ag@TiO_2 and TiO_2-Cu^{2+} in two model organisms: *E. coli* and *S. cerevisiae* and in fungi isolated from seeds, and (c) to analyze the effects of the interaction of Ag@TiO_2 and TiO_2-Cu^{2+} on *A. thaliana*.

2. Materials and Methods

2.1. Synthesis and Characterization of Ag@TiO_2 and TiO_2-Cu^{2+}

2.1.1. Synthesis of NSMs. Ag@TiO_2 were synthesized according to previously reported protocol by our research group [18]. TiO_2-Cu^{2+} were synthesized following the similar procedure for Ag@TiO_2 materials, with slight modifications: in a flask, copper sulfate ($CuSO_4$) was dissolved in acetic acid ($C_2H_4O_2$) and titanium isopropoxide $Ti(OCH(CH_3)_2)_4$ was added slowly and stirred continuously. Arabic gum was added and the mixture was dried in a heating mantle and then thermally treated in a furnace at 350°C for 3 h. The resulting powders were washed with a mixture of H_2O : ethanol (3 : 1) and allowed to dry.

2.1.2. Characterization of NSMs. The TiO_2-Cu^{2+} were characterized by X-ray diffraction (XRD), scanning electron microscopy-energy dispersive spectrometry (SEM-EDS), dynamic light scattering, and atomic absorption spectroscopy (AAS). The powder XRD patterns of the samples were recorded in a Brucker D Advance Diffractometer using Cu K_α radiation (λ = 1.5418 Å) at a scanning rate of 0.05°/min for 2θ ranging from 10° to 85°. The XRD patterns were analyzed using standard ICDD files. The average particle size distribution in the samples was measured by dynamic light scattering using a Malvern particle size analyzer. The particle surface charge of the samples was analyzed by measuring the ζ potential of the samples.

2.2. Evaluation of Antimicrobial Activity of Ag@TiO_2 and TiO_2-Cu^{2+}. The antimicrobial activity of Ag@TiO_2 and TiO_2-Cu^{2+} was investigated in the bacterium *E. coli* strain C600 (F-tonA21 thi-1 thr-1 leuB6 lacY1 glnV44 rfbC1 fhuA1 λ-) and the yeast *S. cerevisiae* strain W303 (MATa/MATα {leu2-3,112 trp1-1 can1-100 ura3-1 ade2-1 his3-11,15} [phi+]), both laboratory strains and model organisms. They were cultivated using standard microbiological techniques. *E. coli* C600 was cultured on LB (Luria-Bertani) and *S. cerevisiae* W303 in PD (Potato-Dextrose) solid media. A colony was taken and inoculated in 5 mL of liquid medium and incubated for 24 h under stirring at 37°C for *E. coli* and 28°C for *S. cerevisiae*. Then, a volume of 1.5 mL of the culture was placed in a microcentrifuge tube and centrifuged at 12,000 rpm for 5 min; the supernatant was removed and the pellet was suspended in sterile potassium phosphate buffer 0.05 M pH 7. This microbial suspension was diluted to obtain a title of 10^6 CFU/mL (colony forming units per milliliter) for *E. coli* C600 and 10^4 CFU/mL for *S. cerevisiae* W303. A volume of 1 mL of

Table 1: Treatments of exposition of *A. thaliana* to NSM.

Treatment	*A. thaliana*	Exposure medium	Exposure time	Parameter
1	Seeds	H_2O distilled Phosphate buffer pH 5.7	24 h	Germination and development
2	Seeds-seedlings	H_2O distilled MS liquid	1 week	Elongation of seedling
3	Two-day-old seedlings	H_2O distilled	24 h	Elongation of seedling

Exposure media were supplemented with 0, 0.5, 1, 2.5, 5, and 10 mg/mL of NSM ($Ag@TiO_2$ or TiO_2-Cu^{2+}), 0.015 mM Ag^0, or 1 mM $CuSO_4$.

the microorganism suspension was placed in test tube and NSMs were added to have concentrations of 0.00, 0.5, 1, 2.5, 5, and 10 mg/mL. Colloidal silver BacDyn Plus® 0.015 mM, a commercial disinfectant (Ag^0), and $CuSO_4$ (1 mM) were used as controls. The suspensions were mixed and incubated at 25°C with shaking at 150 rpm. The inactivation of microorganisms (MOs) was measured at different time intervals (2, 4, 6, 8, and 24 h). To evaluate the antimicrobial effect of metal (Ag^0 or Cu^{2+}), some suspensions were incubated in the dark; to demonstrate the synergistic effect of NSMs (metal + photocatalysis), other suspensions were incubated under radiation (8 ft., 75-Watt cool white linear fluorescent light bulb). After incubation, aliquots of suspensions were inoculated in solid medium (LB for *E. coli* C600 and PD for *S. cerevisiae* W303) and after incubation the number of colonies was counted to calculate the surviving number of CFU/mL. The log inactivation was calculated as $\log_{10}(N/N_0)$, where N_0 represent the initial CFU/mL and N the CFU/mL surviving to treatment. Experiments were performed in triplicate and sterile conditions were maintained.

2.3. Evaluation of the Antimicrobial Action of $Ag@TiO_2$ and TiO_2-Cu^{2+} for Crop Protection. To evaluate the efficiency of NSMs ($Ag@TiO_2$ and TiO_2-Cu^{2+}) for microbial control in crop protection, treatment of fungal pathogens *in vitro* and disinfection of *A. thaliana* seeds were examined. The efficiency of NSMs for disinfection of *A. thaliana* seeds was investigated with different conditions. The biological material (50 seeds) was exposed to NSMs (10 mg/mL of $Ag@TiO_2$ or TiO_2-Cu^{2+}) and the effect of the pretreatment of the seeds (previous exposition to NSMs) with ethanol (70% v/v) solution or Tween (0.15% w/v) was also evaluated. Treatment with sodium hypochlorite and Tween 20 (common disinfection agents) was used as positive control, and sterile H_2O was used as negative control. Experiments were performed in triplicate and sterile conditions were maintained.

An *in vitro* assay was conducted to assess the capacity for the treatment of plant pathogens of the NSMs under study; thus, microorganisms were isolated from *A. thaliana* seeds and exposed to disinfection agent at different concentrations using a sterile potassium phosphate buffer (0.05 M, pH 7). The suspensions were incubated at 25°C in a shaker incubator under visible light for 24 h. After that, 100 μL of the suspension was spread in PD agar and incubated at 25°C for 3 days.

Antifungal activity of NSMs was evaluated using two fungal species (isolated microorganisms from nondisinfected seeds of *A. thaliana* cultured *in vitro*): *Aspergillus* spp. and *Fusarium* spp. fungi (1000 spores/mL) and NSMs (10 mg/mL of $Ag@TiO_2$ or TiO_2-Cu^{2+}) were incubated under visible light for 24 h at 25°C. After that, 10 μL aliquot of the suspensions was placed in PD agar medium and incubated at 25°C for 3 days. Growth inhibition was measured by calculating radial inhibition against control group. Experiments were conducted in triplicate. Radial inhibition (RI%) was calculated in accordance with the following equation [26]:

$$\text{RI\%} = \frac{(D - d) \times 100}{D}, \tag{1}$$

where D is diameter of fungal growth (control group) and d is diameter of fungal growth (exposed to NSMs).

2.4. Evaluation of Effects on A. thaliana Exposed to $Ag@TiO_2$ and TiO_2-Cu^{2+}. Seeds of *A. thaliana* were disinfected using traditional techniques (sodium hypochlorite and Tween 20), incubated at 4°C for 3 days, and exposed to NSMs under several conditions: (a) exposition of seeds to NSMs under visible light for a day to analyze germination in filter paper and development in Murashige and Skoog medium (MS) [27]; (b) exposition of seeds-seedlings to NSMs in distilled water or MS liquid medium for a period of a week to analyze elongation of seedlings; (c) exposition of two-day-old seedlings to NSMs in distilled water under visible light and/or dark conditions for a day to analyze elongation of seedling after 4 days. Media were supplemented with 0, 0.5, 1, 2.5, 5, and 10 mg/mL of NSMs ($Ag@TiO_2$ or TiO_2-Cu^{2+}), 0.015 mM of Ag^0, or 1 mM of $CuSO_4$ (Table 1). The length of seedlings was measured with image analysis using the software Motic Images Plus 2.0.

2.5. Quantification of Chlorophyll and Anthocyanin. Total chlorophyll content was determined by using a modified method described by Nair and Chung [28], with slight modifications: seedlings treated with NSMs both, for a day and for a week, were incubated in 96% (v/v) ethanol (50 mg of tissue for 10 mL of ethanol) under dark conditions at 4°C for 3 days. The absorbance of the supernatant was measured at wavelengths of 665 (A_{665}) and 649 (A_{649}) nm using a spectrophotometer (Thermo Scientific Genesys 10S UV-Vis).

Total chlorophyll contents were determined by using the following formula:

$$\begin{aligned}&\text{Chlorophyll a (Chla)}\\&\quad = (13.95)\left(A_{665}\right) - (6.88)\left(A_{649}\right),\\&\text{Chlorophyll b (Chlb)}\\&\quad = (24.96)\left(A_{649}\right) - (7.32)\left(A_{665}\right),\\&\text{Total chlorophyll} = (\text{Chla} + \text{Chlb}).\end{aligned} \tag{2}$$

The anthocyanin content was determined as described by Nair and Chung [28] with slight modifications: seedlings were incubated in a 99 : 1 methanol : HCl (v/v) solution at 4°C overnight. Supernatant was separated and light absorption was measured at 530 and 657 nm (A_{530} and A_{657}) using a UV-Vis spectrophotometer (Thermo Scientific Genesys 10S). The relative units of anthocyanin (RUA) levels were determined with the following equation:

$$\text{RUA/g} = \left[A_{530} - \left(0.25 \times A_{657}\right)\right]. \tag{3}$$

2.6. Quantification of Silver and Copper by Atomic Absorption Spectrophotometry. Seedlings were washed with either sterile distilled H_2O and/or 10 mM EDTA solution (with a pH of 6) to remove metal adhered on the plants surface; they were dried at 75°C for 48 h, digested with concentrated HNO_3 at 115°C for 1 h, and diluted with sterile distilled H_2O to obtain 1 mg of tissue/mL. The processed seedlings and the exposure mediums were filtered and acidified with HNO_3 and analyzed using AAS (Analyst AS-90Plus).

2.7. Statistical Analysis. Results were compared by one-way analysis of variance (ANOVA) followed by Duncan's test for comparison and lineal regression analysis. All data were expressed as mean ± one standard deviation. A value of $p < 0.05$ was considered statistically significant.

3. Results and Discussion

3.1. Synthesis and Characterization of Ag@TiO_2 and TiO_2-Cu^{2+}. The synthesis of Ag@TiO_2 and TiO_2-Cu^{2+} nanostructured materials was achieved using an environmentally friendly sol-gel procedure. The amount of silver or copper into the materials was formulated to be 1% (mol/mol; Ag/Ti or Cu^{2+}/Ti). Results of analysis of Ag@TiO_2 composites by SEM-EDX indicate that the TiO_2 is spherical with a mean size diameter of ~10 nm (Figure 1(a)). Silver nanoparticles show a mean diameter smaller than 10 nm; thus, it was not possible to determine their mean size by SEM analysis. EDX analysis demonstrates the existence of Ti, O, and Ag in accordance with the molar ratio of the formulation (Figure 1(b)). SEM analysis of TiO_2-Cu^{2+} also indicates a spherical shape and mean size of ~10 nm (Figure 1(c)). The presence of Ti, O, and Cu in the appropriate mole ratio was demonstrated by EDX data (Figure 1(d)). The materials were obtained as crystalline nanostructured powders after thermal annealing at 350°C. The materials exhibit anatase crystalline structure.

The aggregation state of NSMs is a critical factor for experimentally assessing their toxic activity. It is well known that independent of the primary particle size (as solid state materials) NSMs tend to agglomerate when suspended in different media. The biological components present in the culture media and electrolytes may interact with NSMs to change their physicochemical properties (i.e., modification of surface properties) and aggregation state. The stability of these materials was evaluated using TiO_2 (Sigma-Aldrich) as control. Dynamic light scattering analysis of the materials suspended in distilled water or Physiological Saline Solution (PSS) was performed and these results indicate that the materials form bigger aggregates in saline solution than in distilled water. The hydrodynamic radio of the materials is 477 nm (TiO_2), 467 nm (Ag@TiO_2), and 360 nm (TiO_2-Cu^{2+}) when suspended in distilled water. For the suspensions in PSS, the agglomerates exhibit a mean size of 774 nm (TiO_2), 776 nm (Ag@TiO_2), and 750 nm (TiO_2-Cu^{2+}). Zeta potential of the suspension was also evaluated. For distilled water suspensions, the following zeta potential values were encountered: +10.39 mV (TiO_2), −27.83 mV (Ag@TiO_2), and +14.73 mV (TiO_2-Cu^{2+}). In the case of PSS suspensions, the following results were encountered: +48.01 mV (TiO_2), +81.94 mV (Ag@TiO_2), and +6.85 mV (TiO_2-Cu^{2+}). The studied materials present a positive surface charge when suspended in PSS. It has been reported that positively charged nanoparticles interact more strongly with biomolecules due to favored electrostatic interactions since at physiological pH value biomolecules are negatively charged, thus facilitating interaction of NSMs and biomolecules.

3.2. Antimicrobial Activity of Ag@TiO_2 and TiO_2-Cu^{2+} in E. coli and S. cerevisiae. Two sets of inactivation kinetics assays (under visible light and in the dark) were conducted to evaluate the *E. coli* inactivation rate (Figures 2(a) and 2(b)). The results obtained indicate that Ag@TiO_2 exerted strong bacterial inactivation (3 U log) in a short time period (2 h with 10 mg/mL of NSMs). It was also observed that lower amounts of NSMs exhibit longer inhibition times: 2.2 U log with 0.5 mg/mL at 4 h and 2.9 U log with 1 mg/mL at 4 h (Figure 2(a)). There are no significant differences for inactivation rate and extent of inactivation when assays are performed under radiation or in the dark (Figures 2(a) and 2(b)), which indicates that the principal mechanism for inactivation is the release of Ag^+ ions from Ag@TiO_2 materials [8, 29]. There is no inactivation of *E. coli* growth as a consequence of exposure to visible light radiation. Colloidal silver inhibited bacteria growth with 6 U log at 2 h (Figures 2(a) and 2(b)). Although the inactivation rate of colloidal silver is higher than the one observed for Ag@TiO_2 composites, both materials exerted efficient bactericidal effect.

In general, *S. cerevisiae* was more resistant than *E. coli* to colloidal silver and Ag@TiO_2 NSMs microbicidal activity (Figures 2(c) and 2(d)). Longer exposure times at high concentrations of NSMs were necessary to achieve a good inhibition of *S. cerevisiae* growth, with a maximum inactivation of 2.6 U log with 10 mg/mL under visible light after 24 h (Figure 2(c)). The tolerance observed by yeast to the

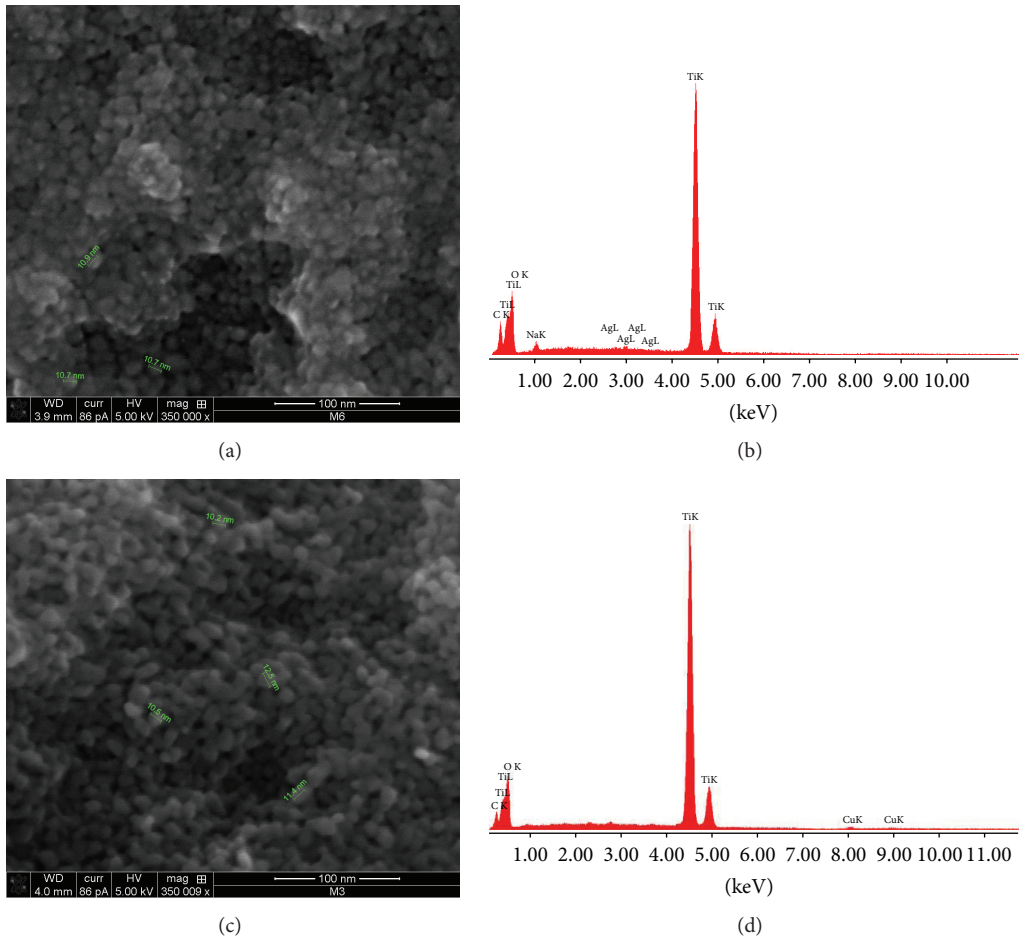


Figure 1: Characterization of NSMs. (a) SEM image of Ag@TiO_2. (b) Elemental composition of Ag@TiO_2 (EDX). (c) SEM image of TiO_2-Cu^{2+}. (d) Elemental composition of TiO_2-Cu^{2+} (EDX).

microbicidal activity of Ag NSMs is due to the more complex structure (the presence of a cell wall, composed of beta-glucans, chitin, and mannoproteins, limits the passage of Ag^+ ion) of these microorganisms [30]. When the variable under study was light radiation, slight differences were observed for the inhibition of *S. cerevisiae* with Ag@TiO_2, with 1.9 U log with 10 mg/mL, in dark after 24 h (Figure 2(d)). For *E. coli*, an inhibition growth mechanism based on Ag^+ activity was suggested since no enhancement in the rate or extent of inactivation was observed when the assay was conducted under visible light radiation. In accordance with several previously published reports, metal doped TiO_2 NSMs, when activated with visible light, generate reactive oxygen species (ROS) that are associated with cell damage in microorganisms [7, 31]. For the growth inhibition of *S. cerevisiae*, the effect of ROS is more significant since the components of the cell wall can be susceptible to oxidation.

The TiO_2-Cu^2 antimicrobial activity was evaluated under the same conditions reported for Ag@TiO_2 materials. Regarding high *E. coli* densities (10^6 CFU/mL), TiO_2-Cu^{2+} NSMs did not show bactericidal activity even at the highest concentration (10 mg/mL) (Figure 2(e)). As observed for silver based materials, the activity of TiO_2-Cu^{2+} against *E. coli* depends on the amount of metal ions liberated into solution; however, since the microbicidal activity of silver is higher in comparison to copper, larger amounts of Cu^{2+} must be released to achieve bacterial inactivation [8, 12]. By comparing the effect of colloidal silver and copper sulfate solution, inactivation rate and extent of inactivation are lower for copper sulfate, corroborating that higher concentrations of this ion are required to achieve inactivation of elevated microbial densities (10^6 CFU/mL). Nonradiated samples did not show inhibitory effect.

In the evaluation of the growth inhibition for *S. cerevisiae* using TiO_2-Cu^{2+} materials, results indicate similar behavior to Ag@TiO_2; the viability of *S. cerevisiae* diminished as a function of material concentration, time of exposure, and visible light radiation. The maximum inactivation was of 1.9 U log with 10 mg/mL after 24 h (Figure 2(f)).

It has been postulated that the microbicidal activity of Ag@TiO_2 is proportional to the amount of silver ions released into the medium [8, 29]. To correlate the activity of the NSMs under investigation to their liberation of metal ions into solution, supernatants of the exposition medium were analyzed by AAS. Reported results indicate the presence of silver at a concentration of 0.16 ppm in the exposure medium

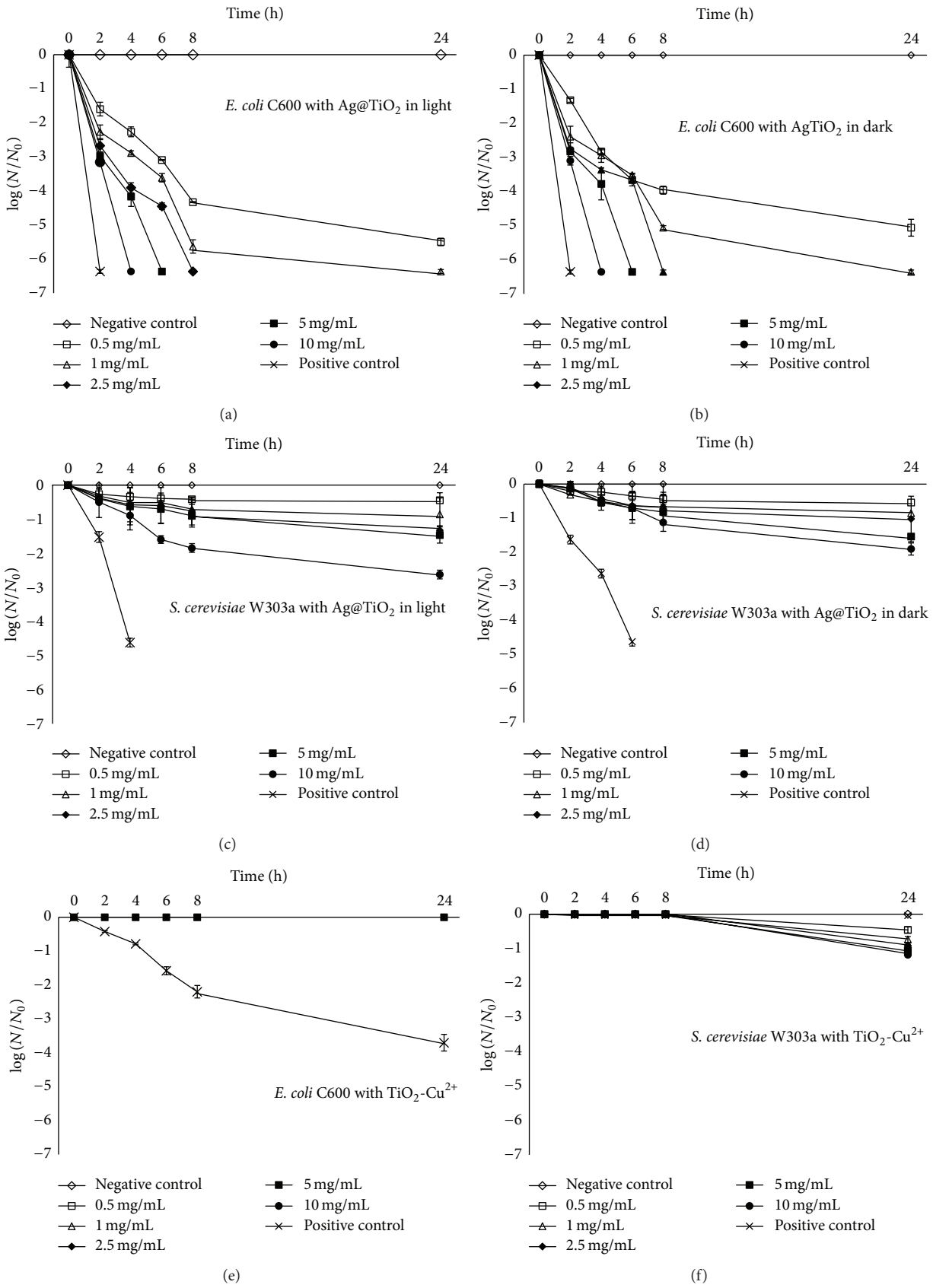
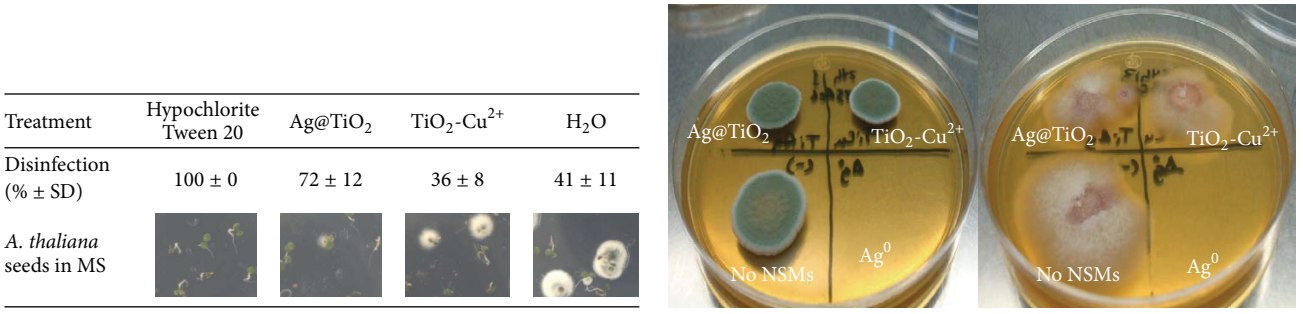


Figure 2: Inactivation of microorganisms by NSMs: *E. coli* with $Ag@TiO_2$ (a) under visible light and (b) in dark; *S. cerevisiae* with $Ag@TiO_2$ (c) under visible light and (d) in dark; (e) *E. coli* with TiO_2-Cu^{2+}; (f) *S. cerevisiae* with TiO_2-Cu^{2+}. As positive control, Ag^0 for $Ag@TiO_2$ and $CuSO_4$ for TiO_2-Cu^{2+}. Bars represent standard deviation; each treatment was performed in triplicate.

Table 2: Determination of copper and silver by atomic absorption spectrophotometry.

	Seedling treated in MS for a week[a]		Seedling treated in H_2O for 24 h[a]	
NSMs	(Ag) mg/g	(Cu) mg/g	(Ag) mg/g	(Cu) mg/g
$Ag@TiO_2$	0.58 ± 0.03	—	0.23 ± 0.23	—
TiO_2-Cu^{2+}	—	0.29 ± 0.06	—	0.31 ± 0.03
Ag^0	0.2 ± 0.05	—	0.12 ± 0.01	—
Control (−)	0	0.12 ± 0.016	0	0.13 ± 0.01
	MS medium after a week[b]		H_2O after a week[b]	
NSMs	(Ag) ppm	(Cu) ppm	(Ag) ppm	(Cu) ppm
$Ag@TiO_2$	0.17 ± 0.01	—	0	—
TiO_2-Cu^{2+}	—	3.17 ± 0.148	—	0.08 ± 0.02
Ag^0	0.085 ± 0.03	—	0.47 ± 0.01	—
Control (−)	0	0.11 ± 0.05	0	0.04 ± 0.002
	H_2O after 24 h[b]		Phosphate buffer pH 5.7 after 24 h[b]	
NSMs	(Ag) ppm	(Cu) ppm	(Ag) ppm	(Cu) ppm
$Ag@TiO_2$	0.06 ± 0.003	—	0.1 ± 0.01	—
TiO_2-Cu^{2+}	—	0.25 ± 0.03	—	0.47 ± 0.07
Ag^0	0.63 ± 0.02	—	0.25 ± 0.02	—
Control (−)	0	0.11 ± 0.05	0	0.03 ± 0.001
	Phosphate buffer pH 7 after 24 h[c]			
NSMs	(Ag) ppm	(Cu) ppm		
$Ag@TiO_2$	0.16 ± 0.02	—		
TiO_2-Cu^{2+}	—	0.5 ± 0.01		
Ag^0	0.2 ± 0.04	—		
Control (−)	0	0.1 ± 0.03		

[a]Seedling treated, [b]exposure medium of seedlings, and [c]exposure medium of microorganisms with 1 mg/mL of $Ag@TiO_2$, 1 mg/mL of TiO_2-Cu^{2+}, and 0.015 mM Ag^0.



(a) (b)

Figure 3: Seeds and their contaminants treated with $Ag@TiO_2$ and TiO_2-Cu^{2+}. (a) Treatment of *A. thaliana* seeds with 10 mg/mL of NSMs prior to cultivation *in vitro*; (b) fungal contaminants seeds exposed to 10 mg/mL of NSMs; each treatment was performed in triplicate.

(phosphate buffer pH 7; Table 2). Copper was also found in the exposition medium (Table 2) at very low concentration (0.5 ppm). Although the amount of Cu^{2+} released into the medium is higher in comparison to released Ag^+, better performance of silver based materials is achieved due to enhanced microbicidal activity of Ag^+.

3.3. Activity of $Ag@TiO_2$ and TiO_2-Cu^{2+} in Seed Disinfection. Several experiments were conducted to assess $Ag@TiO_2$ and TiO_2-Cu^{2+} use as disinfectants for axenic conditions. *A. thaliana* seeds were treated with 10 mg/mL of NSMs for 24 h and then cultured in MS agar medium. Results are summarized in Figure 3(a) and indicate that total seed disinfection was not observed with this treatment. Although inhibition of microbial growth was observed for the samples treated with NSMs, the activity of the materials is insufficient for *in vitro* culture of seeds ($Ag@TiO_2$: 72% inhibition; TiO_2-Cu^{2+}: 36% inhibition; control: 41% inhibition). It is important to remark that the materials show good microbicidal activity when they are evaluated using a single microorganisms species (i.e., *E.*

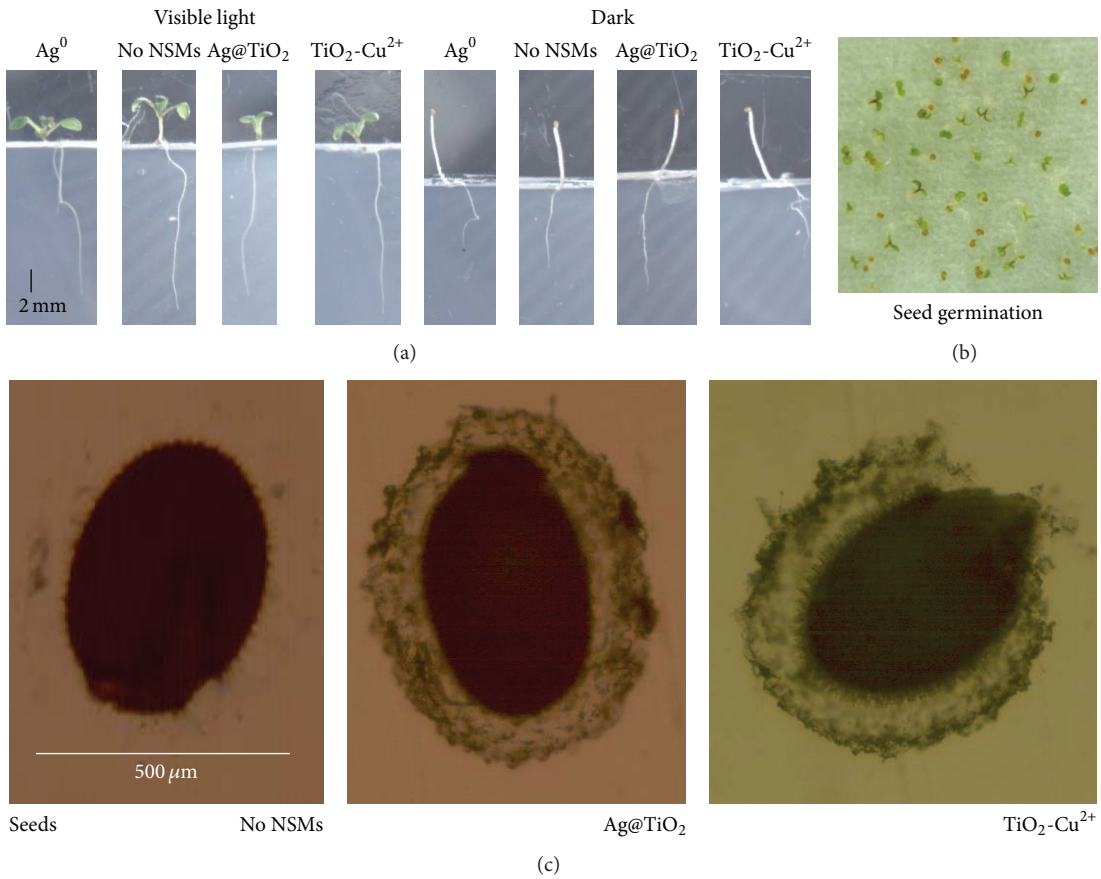


FIGURE 4: Seeds of *A. thaliana* exposed to $Ag@TiO_2$ and TiO_2-Cu^{2+}. (a) Seedlings grown in MS agar medium, $n = 10$; (b) germinated seeds, $n = 90$; (c) NSMs adhered on seeds.

coli or *S. cerevisiae*); however, when the materials are exposed to microorganisms consortiums, the activity of the materials seems to be diminished.

In a different experiment, two fungi species (*Aspergillus* spp. and *Fusarium* spp.) isolated from *A. thaliana* seeds (without disinfection) cultivated were treated with 10 mg/mL of $Ag@TiO_2$ and 10 mg/mL of TiO_2-Cu^{2+} under visible light for 24 h. After that, 10 μL was inoculated and incubated in PD agar. Results indicate radial inhibition of fungal growth in the groups treated with NSMs (Figure 3(b)). The inhibition was calculated measuring diameters of colonies and expressed as radial inhibition percentage (RI%). In the case of *Aspergillus* spp., RI% was as follows: 35% ($Ag@TiO_2$) and 45% (TiO_2-Cu^{2+}). For *Fusarium* spp., the values encountered for RI% were as follows: 17% ($Ag@TiO_2$) and 34% (TiO_2-Cu^{2+}). We observed that copper doped materials show better antimicrobial activity against the fungal species under study, with *Aspergillus* spp. being more sensible to the treatment. Kim and coworkers [26] reported the activity of silver NSMs against several plant pathogenic fungi; an RI% of 100% was observed which is ascribed to metal toxicity. It is important to remark that, in the last decade, caution has been suggested regarding the industrial application of nanosilver formulations, since USEPA (2008) has classified all products containing nanostructured silver as pesticides and also recommended the analysis of their potential risks to human and environmental health [3]. Although the efficiency of these materials is lower regarding growth inhibition of fungi, as previously mentioned, it is worth remarking that these materials are doped with very low amounts of the metal impurity (1 mol%). Thus, it is predicted that TiO_2-Cu^{2+} or $Ag@TiO_2$ at higher dopant concentration should be considered as potential candidates for the treatment of plant pathogenic microorganisms.

3.4. Effects on A. thaliana by Exposure to $Ag@TiO_2$ and TiO_2-Cu^{2+}. This work aims to elucidate the impact that metal doped NSMs might pose on plants. *A. thaliana* seeds or seedlings were exposed to metal doped NSMs ($Ag@TiO_2$ or TiO_2-Cu^{2+}). After interaction with NSMs, evaluation of plant growth and development of *A. thaliana* was investigated. Different sets of experiments were conducted to achieve this goal. First, seeds were exposed for 24 h to different NSMs or metal ions ($Ag@TiO_2$, TiO_2-Cu^{2+}, Ag^0, and Cu^{2+}) using different media (distilled water or potassium phosphate buffer pH 5.7). Germination and development of seeds was evaluated. The interaction of NSMs with seeds of *A. thaliana* does not affect germination (100%) or their development (Figures 4(a) and 4(b)). A more detailed microscopic analysis of the seeds indicates that $Ag@TiO_2$ and TiO_2-Cu^{2+} are

adhered to the seed surface (Figure 4(c)). However, this physical interaction did not interrupt the flow of water inside the seed. Also, it can be inferred that the seed coat acts like a membrane with selective permeability, which protects the embryo from dangerous substances (present in the surrounding medium), preventing the entry of these materials; for that reason, the germination or development of seedlings was not affected. Germination of *A. thaliana* seeds was not affected by the exposition media (distilled water or potassium phosphate buffer pH 5.7) used for evaluation of interaction of NSMs with plants.

Nanosilver formulations and copper salts are currently being used as insecticides or fungicides for plant protection applications. However, the risk assessment of these formulations has been poorly investigated [12]. In this work, the activity of colloidal silver and Cu^{2+} ions was evaluated since these metals are used as dopants and lately they have been associated with deleterious effects or bioaccumulation in plants [3]. Thus, seeds of *A. thaliana* were exposed to commercial formulation of colloidal silver (0.015 mM Ag^0) or to a solution of $CuSO_4$ 1 mM. The interaction of *A. thaliana* with colloidal silver did not affect the germination of seeds and/or the growth of the seedlings. Metal ions are associated with the toxic effects exerted by metallic nanoparticles (MNPs), since MNPs are insoluble in water and also have a tendency to agglomerate in this medium, which limits the flow of these materials into the plant. However, MNPs are reactive and prone to oxidation, giving rise to the liberation of metal ions into solution. In this study, the concentration of free Ag^+ ions was not sufficient to induce changes in germination and/or growth of *A. thaliana* (Figures 4(a) and 4(b)). On the other hand, the seeds exposed to 1 mM $CuSO_4$ solution exhibited a total inhibition of germination. The AAS results showed that although exposure medium contains metal ions (Table 2) dissociated from NSMs, H_2O (silver 0.06 ± 0.003 mg/L; copper 0.24 ± 0.03 mg/L), and phosphate buffer pH 5.7 (silver 0.1±0.008 mg/L; copper 0.47± 0.067 mg/L), such amount of metal ions did not produce toxicity in *A. thaliana* seeds. Meanwhile, seeds exposed to 0.015 mM Ag^0 had normal germination and growth (Figures 4(a) and 4(b)), although silver found in the exposure medium with Ag^0 was ten times more concentrated (0.6 ± 0.02 mg/L) than exposure medium with Ag@TiO_2 (Table 2). The results reported in this work agree with previous reports that describe inhibition of germination due to the exposure of seeds to high concentrations of Cu^{2+}. For instance, Ivanova and coworkers [32] found that the germination of *Brassica napus* L. can be inhibited in the presence of $CuSO_4$ 0.3 mM. Later, Sethy and Ghosh [33] suggested that copper prevents water ingress to the seed and affects sucrose metabolism, inhibiting germination.

There are more reports on the evaluation of acute toxicity than on the evaluation of chronic toxicity of NSMs towards diverse living organisms. In this work, we evaluated the effect of time of exposure of plants to NSMs. Seeds of *A. thaliana* were exposed to NSMs for a time period of a week; in general, seedlings exhibited growth inhibition as a result of interaction with NSMs; also, differences were encountered regarding growth inhibition, depending on the exposition medium (distilled water or MS) of *A. thaliana* to NSMs. Results are schematically represented in Figures 5 and 6. Growth inhibition of the treated seedlings is easily observed when compared to control group. Significant differences (with respect to control group) are observed at NSMs concentrations equal to or higher than 2.5 mg/mL, independent of the chemical composition of the material (Ag@TiO_2 or TiO_2-Cu^{2+}) when the exposition medium is MS (Figures 5(a), 5(b), 6(a), and 6(b)). If exposure medium is distilled water, growth inhibition is dependent on the chemical composition of the material; for instance, TiO_2-Cu^{2+} exert toxic effect at lower concentration (0.5 mg/mL) than Ag@TiO_2 (2.5 mg/mL) (Figures 5(c), 5(d), 6(a), and 6(b)). To justify the differences in the toxicity exerted for NSMs under study, AAS was employed to determine the translocation of silver and/or copper into the seedlings. Results are summarized in Table 2. When seedlings were exposed to NSMs for a period of a week using MS as exposure medium, it was observed that seedlings exposed to Ag@TiO_2 accumulate higher amount of silver (0.58 mg/g of tissue) in comparison to control group (0 mg/g of tissue) or the group exposed to colloidal silver (0.2 mg/g of tissue). Although the total amount of silver in the colloidal solution is higher than the one encountered in Ag@TiO_2 NSMs, it is likely that the particle size of colloidal solution is bigger; also, particles might agglomerate or precipitate from the medium, reducing the bioavailability of the metal. In the case of Ag@TiO_2 materials, silver nanoparticles are very small but are supported on the TiO_2 matrix; thus, the leaching of Ag^+ ions is controlled, resulting in a higher bioavailability. From Figure 6(a), we can see that seedlings exposed to Ag@TiO_2 (2.5 mg of NSMs/mL) showed an elongation of 0.45 cm versus the elongation observed for the seedlings exposed to colloidal silver which was encountered to be 0.61 cm. From here we can see that toxicity (growth inhibition) of NSMs is directly related to the amount of free Ag^+ ions, in accordance with results published in literature [34].

As previously discussed, copper is an essential element in plants but the redox properties of this element also contribute to its inherent toxicity. The amount of copper in seedlings exposed to 1 mg/mL of TiO_2-Cu^{2+} for a week in MS was determined by AAS, and it was found to be 0.29 mg/g of tissue, while the average content of Cu in plant tissue has been reported as 6 μg/g dry weight (Table 2) [35]. Leaching of copper ions from the material was also determined by means of AAS, after interaction with biological samples. It was observed that the amount of residual Cu^{2+} is higher in MS medium than the amount of Cu^{2+} encountered in distilled water. On the contrary, biological samples exposed to NSMs in MS medium contain less amount of copper than samples exposed to NSMs using distilled water. The bioavailability of Cu^{2+} ion in the presence of EDTA contained in MS medium is reduced due to the formation of metal complexes. EDTA is added to MS medium in order to form Fe^{2+}-EDTA complexes ($\log K_f = 14.3$) and avoid iron precipitation [36]. Since EDTA is a good metal chelator, it can also form complexes with Cu^{2+} ($\log K_f = 18.8$); thus, as Cu^{2+} ion leaches from TiO_2-Cu^{2+} material, it is complexed and thereby

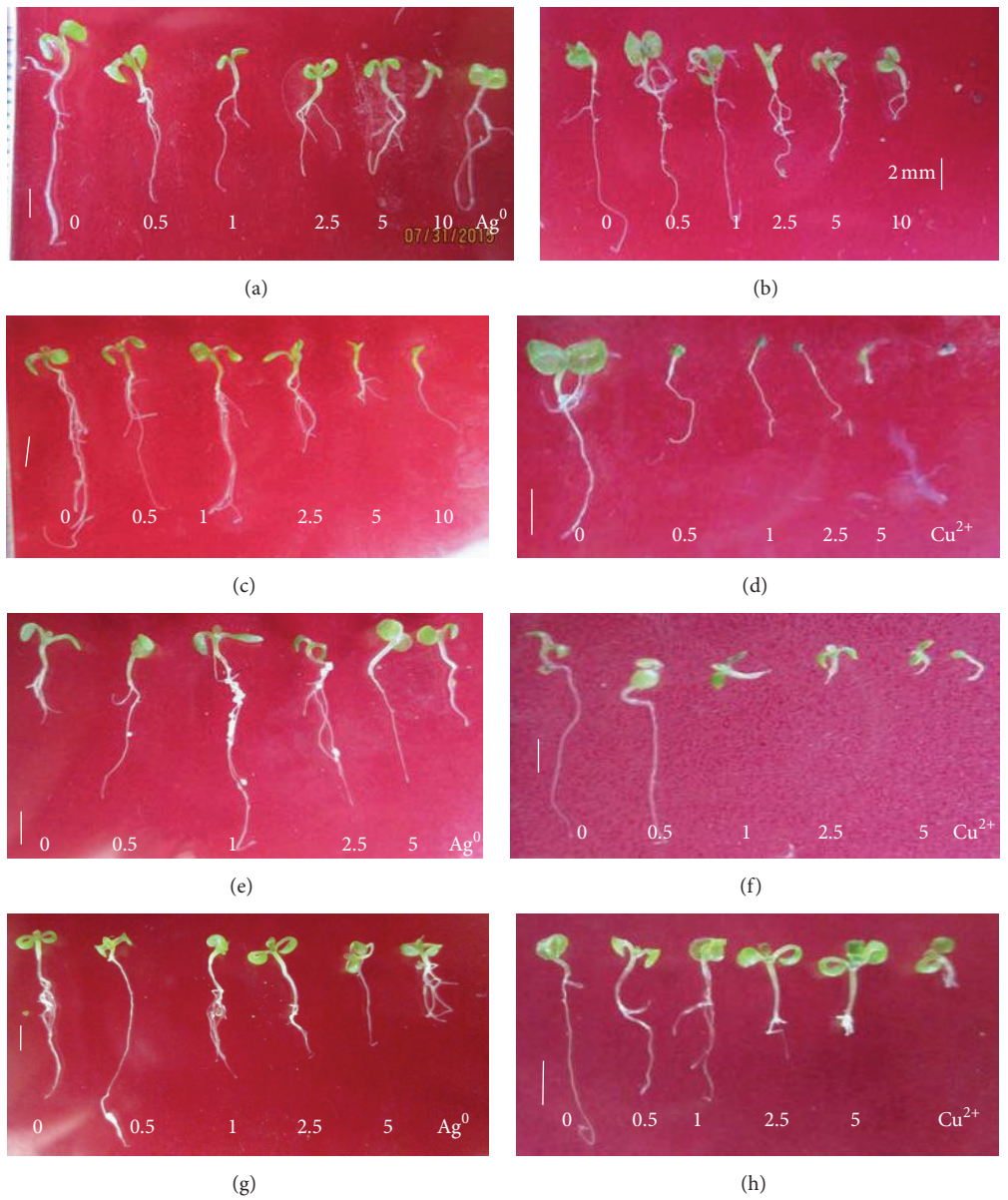


FIGURE 5: Seedlings of *A. thaliana* exposed to NSMs. (a) Ag@TiO_2 and (b) TiO_2-Cu^{2+} in MS broth medium for a week; (c) Ag@TiO_2 and (d) TiO_2-Cu^{2+} in H_2O for a week; (e) Ag@TiO_2 and (f) TiO_2-Cu^{2+} in H_2O, radiated for 24 h; (g) Ag@TiO_2 and (h) TiO_2-Cu^{2+} in H_2O, nonradiated for 24 h. Numbers indicate NSMs concentrations (0, 0.5 1, 2.5, 5, and 10 mg/mL).

its bioavailability reduced. In summary, for seedlings exposed to NSMs using water as medium for exposition, the amount of copper translocated into the plant was higher than the one encountered for seedlings exposed in MS medium (Table 2). Also, the toxicity of Cu^{2+} ions (growth inhibition) is clearly observed when plants are exposed using distilled water as exposure medium (Figure 6(b)).

In a different experiment, two-day-old seedlings were exposed to NSMs suspended in distilled water (Treatment 3, Table 1) for a day. Results are summarized and schematically represented in Figures 5(e), 5(f), 5(g), 5(h), and 6. Dose-response experiments indicate that the interaction of *A. thaliana* seedlings with Ag@TiO_2 favors seedling growth at concentrations $\leq$ 2.5 mg/mL under visible light for a period of 24 h (Figures 5(e) and 6(a)). Treated seedlings under these conditions showed larger growth (with a length of 2 ± 0.5 cm) than seedlings without interaction with NSMs (with a length of 1.3 ± 0.3 cm) (Figures 5(e) and 6(a)). Beneficial effects due to the interaction of plants with silver NSMs and/or TiO_2 materials have been previously reported. For instance, Shams and coworkers [37] reported an improvement in the development of cucumber due to its interaction with Ag NSMs. Silver doped titanium dioxide can be activated under visible light radiation generating e^-/h^+ reactive species that interact with molecular oxygen and/or water to produce reactive oxygen species (ROS), particularly the hydroxyl

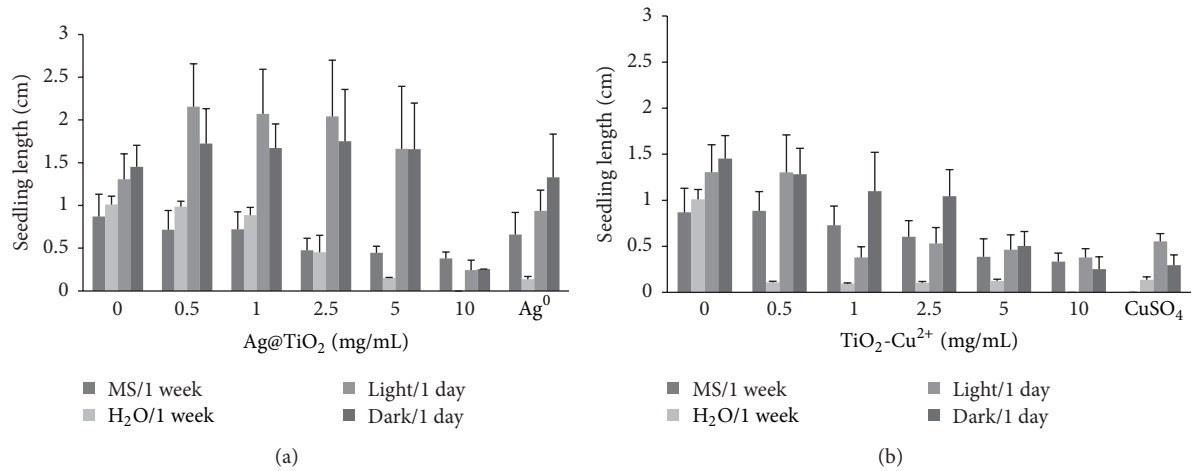


Figure 6: Seedlings elongation after exposure to NSMs: (a) $Ag@TiO_2$ and (b) TiO_2-Cu^{2+}. Columns represent the mean and the bars represent ± one standard deviation; $n = 10$.

radical (HO·), a very strong oxidizing agent that has been associated with participating in the loosening of the plant cell wall, resulting in root elongation [38]. On the other hand, deleterious effects due to the interaction of Ag NSMs with plants have been reported. For instance, Wang and coworkers [34] reported stimulatory effect on root elongation, fresh weight, and evapotranspiration of poplars and *A. thaliana* when exposed to low concentrations of Ag NSMs, whereas Ag NSMs were phytotoxic above specific concentrations. Phytostimulation (seedling elongation) of *A. thaliana* due to the interaction with $Ag@TiO_2$ at low concentrations (0.5, 1, and 2.5 mg/mL) was observed. However, at higher concentrations (≥5 mg/mL) stimulatory effect was not observed (Figures 5(e) and 6(a)). The synergistic activity of the composite material was demonstrated, since there are no significant differences in elongation (in comparison to control group) of *A. thaliana* when exposed to $Ag@TiO_2$ without radiation (Figures 5(g) and 6(a)).

In a related experiment, two-day-old seedlings exposed to TiO_2-Cu^{2+} for 24 h (Treatment 3, Table 1) were measured 4 days after exposure. Seedlings exposed to NSMs showed lower elongation than control group seedlings (Figures 5(f), 5(h), and 6(b)). The difference of elongation was significant at concentrations higher than 0.5 mg/mL under visible light and 5 mg/mL in the dark (Figure 6(b)). Those results showed that visible light potentiates TiO_2-Cu^{2+} effect on *A. thaliana*, probably because plant stress is higher in those conditions. This result may be due to the photocatalytic activity of the material that generates ROS which are responsible for oxidative stress in diverse living organisms. Also, liberation of Cu^{2+} ions into solution is favored as a result of the photoactivation of the catalyst [39]. Previous reports associate stress with a greater production of ethylene, which limits root elongation [35]. Seedlings treated with $CuSO_4$ showed less elongation too (Figures 5(f), 5(h), and 6(b)); that effect has been known for some time; although copper has important functions (e.g., cofactor of cytochrome oxidase enzyme), copper rapidly becomes toxic in liquid plant cultures [35]. Copper has affinity for sulfhydryl groups in membranes which induce lipoperoxidation, producing damage in cell membrane or free radicals, and lower permeability of root cells [40]. To determine the amount of leached copper, exposure media and plants exposed to TiO_2-Cu^{2+} were analyzed by atomic absorption spectrometry. Copper was found in water medium exposed for 24 h to TiO_2-Cu^{2+} (0.2 ± 0.03 mg/L); in water medium without NSMs, the amount of copper was 0.1 ± 0.05 mg/L (Table 2), which indicates that copper was dissociated from NSMs. Copper content in seedlings was higher in those exposed to TiO_2-Cu^{2+} (0.3 ± 0.03 mg/g of tissue) than in those that were not exposed (0.1 ± 0.01 mg/g) (Table 2), which suggests that copper participates in the observed deleterious effects (Figures 5(f) and 5(h)).

3.5. Total Chlorophyll and Anthocyanin Content. Total chlorophyll content was measured to analyze the effect of NSMs on the production of photosynthetic pigment in seedlings of *A. thaliana*. Seedlings exposed to 1 mg/mL of NSMs in MS for a week showed increased production of chlorophyll; the values were 1.8 ± 0.3 mg/g of tissue for $Ag@TiO_2$, 1.6 ± 0.08 mg/g for TiO_2-Cu^{2+}, and 1 ± 0.2 mg/g for negative control (seedlings that were not exposed to NSMs) (Figure 7(a)). However, seedlings exposed to 2.5 mg/mL of NSMs for a week suffer a reduction to 0.3 ± 0.02 mg/g of total chlorophyll for both NSMs (Figures 7(a) and 7(b)). Regarding positive controls, the chlorophyll content of seedlings exposed for a week to Ag^0 was 0.7 ± 0.15 mg/g and $CuSO_4$ 0.01 ± 0.001 mg/g. In another experiment, seedlings exposed for 24 h did not show significant differences in the total chlorophyll content of the seedlings in some conditions: (a) 1 mg/mL of $Ag@TiO_2$ (1.4 ± 0.36 mg/g), (b) 1 mg/mL of TiO_2-Cu^{2+} (1.4 ± 0.37 mg/g), (c) Ag^0 (1.3 ± 0.24 mg/g), and (d) negative control without NSMs (1.2 ± 0.35 mg/g) (Figures 7(a) and 7(b)). However, in different conditions the total chlorophyll content was lower than in negative control, for example, in seedlings exposed to 5 mg/mg of $Ag@TiO_2$ (0.5 ± 0.07 mg/g

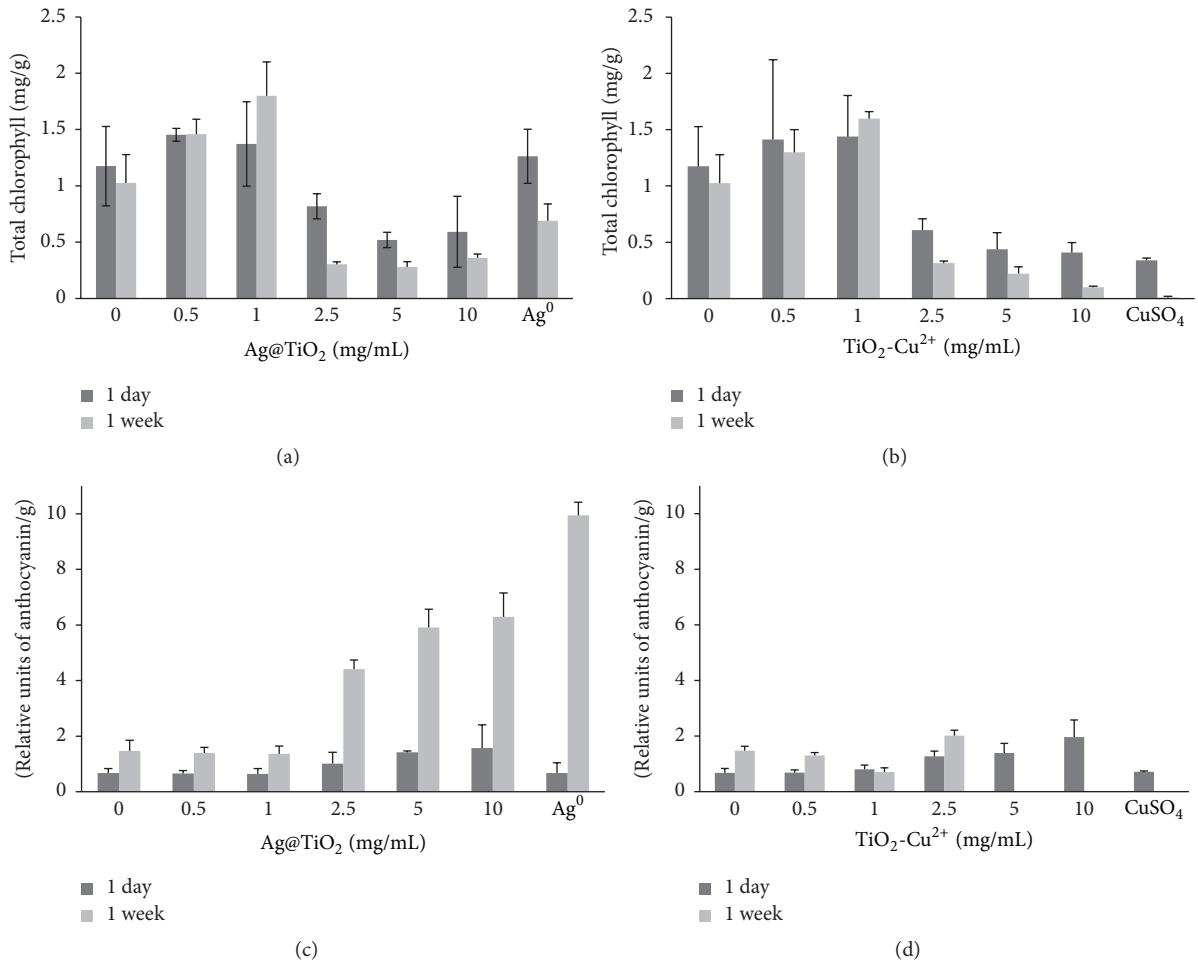


Figure 7: Total chlorophyll and anthocyanin content. Total chlorophyll content (mg/g of tissue) in seedling exposed to (a) $Ag@TiO_2$ and (b) TiO_2-Cu^{2+}; anthocyanin content (relative units of anthocyanin (RUA)/g of tissue) in seedlings exposed to (c) $Ag@TiO_2$ and (d) TiO_2-Cu^{2+}; columns represent the mean and the bars represent ± one standard deviation; $n = 3$.

of tissue), 5 mg/mL of TiO_2-Cu^{2+} (0.4 ± 0.14 mg/g of tissue), and $CuSO_4$ (0.3 ± 0.02 mg/g of tissue) (Figures 7(a) and 7(b)).

There are some reports that indicate an increase in the amount of chlorophyll in plants upon exposition to TiO_2 NSMs; for instance, Morteza and coworkers [41] suggested that exposition of *Zea mays* L., in reproductive stage, to TiO_2 NSMs contributes to stabilizing the integrity of the chloroplast membrane, protecting chlorophyll. Although other studies report a decrease of chlorophyll content, for example, Nair and Chung suggest that seedlings of *Oryza sativa* L. exposed to silver NSMs [28] and seedlings of *Brassica juncea* L. exposed to copper oxide NSMs [42] showed a reduction of total chlorophyll content due to damage caused to chloroplast membranes as a result of lipid peroxidation by oxidative stress produced by the excess of metal or as result of depletion of iron (Fe) by copper or due to reduced biomass.

To examine the impact of NSM in *A. thaliana*, the anthocyanin content was measured. Anthocyanins are flavonoids that are responsible for coloration in flowers and fruits of higher plants. They are pigments with protective functions against UV radiation; ROS, microorganisms, and the total amount of anthocyanin in plants can change due to some type of stress [43]. Seedling exposed to lower concentrations of NSMs in MS for a week does not show significant differences into the anthocyanin with respect to control group without NSMs (1.4 ± 0.4 relative units of anthocyanin per gram of tissue (RUA/g)), for example, (a) 0.5 mg/mL of TiO_2-Cu^{2+} (1.3 ± 0.1 RUA/g) and (b) 1 mg/mL of $Ag@TiO_2$ (1.3 ± 0.3 RUA/g) (Figures 7(c) and 7(d)); but the anthocyanin content was increased in seedling exposed to 2.5 mg/mL and higher concentration of $Ag@TiO_2$ (4.4 ± 0.3 RUA/g). The anthocyanin content in seedlings exposed for 24 h was significantly higher with respect to negative control (0.67 ± 0.14 RUA/g) in some conditions, for example, in seedlings exposed to 5 mg/mL of $Ag@TiO_2$ (1.4 ± 0.05 RUA/g) and 2.5 mg/mL of TiO_2-Cu^{2+} (1.2 ± 0.2 RUA/g) (Figures 7(c) and 7(d)). Syu and coworkers [44] suggest that the increase of anthocyanin content in *A. thaliana* is due to oxidative stress produced by exposition to Ag NSM and NSMs studied in the present work produce ROS and metal (Ag and Cu) is leached by the materials, which could cause oxidative stress stimulating the anthocyanin production.

4. Conclusions

The synthesis of TiO_2-Cu^{2+} and Ag@TiO_2 was achieved using an environmentally friendly sol-gel procedure. The nanometric nature, crystallinity, and purity of the materials were demonstrated. Ag@TiO_2 and TiO_2-Cu^{2+} showed antimicrobial activity against *E. coli* and *S. cerevisiae*, due to either the photocatalytic property or the metals toxicity. Ag@TiO_2 was more active than TiO_2-Cu^{2+}, while *E. coli* was more sensitive to Ag@TiO_2 than *S. cerevisiae*. These NSMs showed moderate inhibitory activity against filamentous fungi. The interaction of Ag@TiO_2 with *A. thaliana* showed the following: (a) germination was not affected; (b) seedlings exposed to low concentrations and under light for 24 h had higher elongation; (c) seedling exposed for a week grew less; and (d) total chlorophyll content decreased and anthocyanin increased in seedlings exposed at high concentrations for a week, while seedlings exposed to TiO_2-Cu^{2+} showed the following: (a) no effect on germination; (b) lower elongation, depending on concentration; and (c) lower total chlorophyll content in seedlings exposed at higher concentrations.

Competing Interests

The authors declare that they have no competing interests.

References

[1] H. Shi, R. Magaye, V. Castranova, and J. Zhao, "Titanium dioxide nanoparticles: a review of current toxicological data," *Particle and Fibre Toxicology*, vol. 10, article 15, 2013.

[2] U. Song, H. Jun, B. Waldman et al., "Functional analyses of nanoparticle toxicity: a comparative study of the effects of TiO_2 and Ag on tomatoes (*Lycopersicon esculentum*)," *Ecotoxicology and Environmental Safety*, vol. 93, pp. 60–67, 2013.

[3] N. A. Anjum, S. S. Gill, A. C. Duarte, E. Pereira, and I. Ahmad, "Silver nanoparticles in soil-plant systems," *Journal of Nanoparticle Research*, vol. 15, article 1896, 2013.

[4] J. Thiel, L. Pakstis, S. Buzby et al., "Antibacterial properties of silver-doped titania," *Small*, vol. 3, no. 5, pp. 799–803, 2007.

[5] A. N. Banerjee, "The design, fabrication, and photocatalytic utility of nanostructured semiconductors: focus on TiO_2-based nanostructures," *Nanotechnology, Science and Applications*, vol. 4, no. 1, pp. 35–65, 2011.

[6] P. D. Cozzoli, R. Comparelli, E. Fanizza, M. L. Curri, A. Agostiano, and D. Laub, "Photocatalytic synthesis of silver nanoparticles stabilized by TiO_2 nanorods: a semiconductor/metal nanocomposite in homogeneous nonpolar solution," *Journal of the American Chemical Society*, vol. 126, no. 12, pp. 3868–3879, 2004.

[7] M. B. Wilker, K. J. Schnitzenbaumer, and G. Dukovic, "Recent progress in photocatalysis mediated by colloidal II-VI nanocrystals," *Israel Journal of Chemistry*, vol. 52, no. 11-12, pp. 1002–1015, 2012.

[8] X. H. Yang, H. T. Fu, X. C. Wang, J. L. Yang, X. C. Jiang, and A. B. Yu, "Synthesis of silver-titanium dioxide nanocomposites for antimicrobial applications," *Journal of Nanoparticle Research*, vol. 16, article 2526, 2014.

[9] Y. Paz, "Application of TiO_2 photocatalysis for air treatment: Patents' overview," *Applied Catalysis B: Environmental*, vol. 99, no. 3-4, pp. 448–460, 2010.

[10] M. V. Liga, E. L. Bryant, V. L. Colvin, and Q. Li, "Virus inactivation by silver doped titanium dioxide nanoparticles for drinking water treatment," *Water Research*, vol. 45, no. 2, pp. 535–544, 2011.

[11] L. Zhao, P. K. Chu, Y. Zhang, and Z. Wu, "Antibacterial coatings on titanium implants," *Journal of Biomedical Materials Research Part B: Applied Biomaterials*, vol. 91, no. 1, pp. 470–480, 2009.

[12] A. Gogos, K. Knauer, and T. D. Bucheli, "Nanomaterials in plant protection and fertilization: current state, foreseen applications, and research priorities," *Journal of Agricultural and Food Chemistry*, vol. 60, no. 39, pp. 9781–9792, 2012.

[13] S. G. Royce, D. Mukherjee, T. Cai et al., "Modeling population exposures to silver nanoparticles present in consumer products," *Journal of Nanoparticle Research*, vol. 16, no. 11, pp. 2724–2749, 2014.

[14] P. Miralles, E. Johnson, T. L. Church, and A. T. Harris, "Multiwalled carbon nanotubes in alfalfa and wheat: toxicology and uptake," *Journal of the Royal Society Interface*, vol. 9, no. 77, pp. 3514–3527, 2012.

[15] M. B. Fisher, D. A. Keane, P. Fernández-Ibáñez et al., "Nitrogen and copper doped solar light active TiO_2 photocatalysts for water decontamination," *Applied Catalysis B: Environmental*, vol. 130-131, pp. 8–13, 2013.

[16] T.-D. Pham and B.-K. Lee, "Cu doped TiO_2/GF for photocatalytic disinfection of *Escherichia coli* in bioaerosols under visible light irradiation: application and mechanism," *Applied Surface Science*, vol. 296, pp. 15–23, 2014.

[17] H. M. Yadav, S. V. Otari, V. B. Koli et al., "Preparation and characterization of copper-doped anatase TiO_2 nanoparticles with visible light photocatalytic antibacterial activity," *Journal of Photochemistry and Photobiology A: Chemistry*, vol. 280, pp. 32–38, 2014.

[18] X. Pan, I. Medina-Ramirez, R. Mernaugh, and J. Liu, "Nanocharacterization and bactericidal performance of silver modified titania photocatalyst," *Colloids and Surfaces B: Biointerfaces*, vol. 77, no. 1, pp. 82–89, 2010.

[19] I. Medina-Ramirez, Z. Luo, S. Bashir, R. Mernaugh, and J. L. Liu, "Facile design and nanostructural evaluation of silver-modified titania used as disinfectant," *Dalton Transactions*, vol. 40, no. 5, pp. 1047–1054, 2011.

[20] I. Medina-Ramírez, J. L. Liu, A. Hernández-Ramírez et al., "Synthesis, characterization, photocatalytic evaluation, and toxicity studies of TiO_2-Fe^{3+} nanocatalyst," *Journal of Materials Science*, vol. 49, no. 15, pp. 5309–5323, 2014.

[21] X. Ma, J. Geiser-Lee, Y. Deng, and A. Kolmakov, "Interactions between engineered nanoparticles (ENPs) and plants: phytotoxicity, uptake and accumulation," *Science of the Total Environment*, vol. 408, no. 16, pp. 3053–3061, 2010.

[22] A. K. Shaw and Z. Hossain, "Impact of nano-CuO stress on rice (*Oryza sativa* L.) seedlings," *Chemosphere*, vol. 93, no. 6, pp. 906–915, 2013.

[23] J. Li, P. R. Chang, J. Huang, Y. Wang, H. Yuan, and H. Ren, "Physiological effects of magnetic iron oxide nanoparticles towards watermelon," *Journal of Nanoscience and Nanotechnology*, vol. 13, no. 8, pp. 5561–5567, 2013.

[24] C. Kole, P. Kole, K. M. Randunu et al., "Nanobiotechnology can boost crop production and quality: first evidence from increased plant biomass, fruit yield and phytomedicine content

in bitter melon (*Momordica charantia*)," *BMC Biotechnology*, vol. 13, article 37, 2013.

[25] C. W. Lee, S. Mahendra, K. Zodrow et al., "Developmental phytotoxicity of metal oxide nanoparticles to *Arabidopsis thaliana*," *Environmental Toxicology and Chemistry*, vol. 29, no. 3, pp. 669–675, 2010.

[26] S. W. Kim, J. H. Jung, K. Lamsal, Y. S. Kim, J. S. Min, and Y. S. Lee, "Antifungal effects of silver nanoparticles (AgNPs) against various plant pathogenic fungi," *Mycobiology*, vol. 40, no. 1, pp. 53–58, 2012.

[27] T. Murashige and F. Skoog, "A revised medium for rapid growth and bio assays with tobacco tissue cultures," *Physiologia Plantarum*, vol. 15, no. 3, pp. 473–497, 1962.

[28] P. M. G. Nair and I. M. Chung, "Assessment of silver nanoparticle-induced physiological and molecular changes in Arabidopsis thaliana," *Environmental Science and Pollution Research*, vol. 21, no. 14, pp. 8858–8869, 2014.

[29] Z.-M. Xiu, Q.-B. Zhang, H. L. Puppala, V. L. Colvin, and P. J. J. Alvarez, "Negligible particle-specific antibacterial activity of silver nanoparticles," *Nano Letters*, vol. 12, no. 8, pp. 4271–4275, 2012.

[30] L. S. Dorobantu, C. Fallone, A. J. Noble et al., "Toxicity of silver nanoparticles against bacteria, yeast, and algae," *Journal of Nanoparticle Research*, vol. 17, no. 4, 2015.

[31] K. Gupta, R. P. Singh, A. Pandey, and A. Pandey, "Photocatalytic antibacterial performance of TiO_2 and Ag-doped TiO_2 against *S. aureus*. *P. aeruginosa* and *E. coli*," *Beilstein Journal of Nanotechnology*, vol. 4, no. 1, pp. 345–351, 2013.

[32] E. M. Ivanova, V. P. Kholodova, and V. I. V. Kuznetsov, "Biological effects of high copper and zinc concentrations and their interaction in rapeseed plants," *Russian Journal of Plant Physiology*, vol. 57, no. 6, pp. 806–814, 2010.

[33] S. K. Sethy and S. Ghosh, "Effect of heavy metals on germination of seeds," *Journal of Natural Science, Biology and Medicine*, vol. 4, no. 2, pp. 272–275, 2013.

[34] J. Wang, Y. Koo, A. Alexander et al., "Phytostimulation of poplars and *Arabidopsis* exposed to silver nanoparticles and Ag^+ at sublethal concentrations," *Environmental Science and Technology*, vol. 47, no. 10, pp. 5442–5449, 2013.

[35] F. B. Salisbury and C. V. Ross, *Fisiología vegetal*, Grupo Editorial Iberoamerica, Salomon, Mexico, 1994.

[36] T. Lincoln and E. Zeiger, *Plant Physiology*, Sinauer Associates, Sunderland, Mass, USA, 5th edition, 2010.

[37] G. Shams, M. Ranjbar, and A. Amiri, "Effect of silver nanoparticles on concentration of silver heavy element and growth indexes in cucumber (*Cucumis sativus* L. negeen)," *Journal of Nanoparticle Research*, vol. 15, article 1630, 2013.

[38] J.-H. Kim, Y. Lee, E.-J. Kim et al., "Exposure of iron nanoparticles to *Arabidopsis thaliana* enhances root elongation by triggering cell wall loosening," *Environmental Science and Technology*, vol. 48, no. 6, pp. 3477–3485, 2014.

[39] J. G. McEvoy and Z. Zhang, "Antimicrobial and photocatalytic disinfection mechanisms in silver-modified photocatalysts under dark and light conditions," *Journal of Photochemistry and Photobiology C: Photochemistry Reviews*, vol. 19, no. 1, pp. 62–75, 2014.

[40] A. Emamverdian, Y. Ding, F. Mokhberdoran, and Y. Xie, "Heavy metal stress and some mechanisms of plant defense response," *The Scientific World Journal*, vol. 2015, Article ID 756120, 18 pages, 2015.

[41] E. Morteza, P. Moaveni, H. A. Farahani, and M. Kiyani, "Study of photosynthetic pigments changes of maize (*Zea mays* L.) under nano TiO_2 spraying at various growth stages," *SpringerPlus*, vol. 2, no. 1, article 247, 2013.

[42] P. M. G. Nair and I. M. Chung, "Study on the correlation between copper oxide nanoparticles induced growth suppression and enhanced lignification in Indian mustard (*Brassica juncea* L.)," *Ecotoxicology and Environmental Safety*, vol. 113, pp. 302–313, 2015.

[43] D. N. Feyissa, T. Løvdal, K. M. Olsen, R. Slimestad, and C. Lillo, "The endogenous *GL3*, but not *EGL3*, gene is necessary for anthocyanin accumulation as induced by nitrogen depletion in arabidopsis rosette stage leaves," *Planta*, vol. 230, no. 4, pp. 747–754, 2009.

[44] Y.-Y. Syu, J.-H. Hung, J.-C. Chen, and H.-W. Chuang, "Impacts of size and shape of silver nanoparticles on *Arabidopsis* plant growth and gene expression," *Plant Physiology and Biochemistry*, vol. 83, pp. 57–64, 2014.