Research Square

Preprints are preliminary reports that have not undergone peer review.
They should not be considered conclusive, used to inform clinical practice,
or referenced by the media as validated information.

Phytoremediation of heavy element contaminated water using Eichhornia crassipes (water hyacinth) injected with multiwalled carbon nanotubes and titanium dioxide nanotubes – a clean environmental nanotechnology

Iris Eunice Serrato-Mireles
University of Michoacán, Universidad Michoacana de San Nicolás de Hidalgo (UMSNH))
Vivechana Agarwal
Autonomous University of the State of Morelos (Universidad Autónoma del Estado de Morelos (UAEM))
Ezequiel Arredondo-Vargas
University of Michoacán, Universidad Michoacana de San Nicolás de Hidalgo (UMSNH))
Ma. Guadalupe Garnica Romo
University of Michoacán, Universidad Michoacana de San Nicolás de Hidalgo (UMSNH))
Rodolfo López-Gómez
Institute of Chemical and Biological Research (IIQB), UMSNH
Nabanita Dasgupta Schubert
nabanita.schubert@umich.mx

University of Michoacán, Universidad Michoacana de San Nicolás de Hidalgo (UMSNH))

Research Article

Keywords: phytoremediation, heavy elements, Eichornia crassipes (water hyacinth), carbon nanotubes, titanium dioxide nanotubes

Posted Date: April 1st, 2025

DOI: https://doi.org/10.21203/rs.3.rs-6331778/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License. Read Full License

Additional Declarations: No competing interests reported.

Abstract

The aquatic plant *E. crassipes*, injected with multi-walled carbon nanotubes (MWCNT) and titanium dioxide nanotubes (TiNT) in its aerial parts, was used for the phytoremediation of dissolved heavy-element contaminants in the water of the lake “Laguna Jovita” (Mexico). The objective was to increase plant biomass and heavy element tolerance while internally anchoring the nanoparticle to prevent secondary environmental contamination by its dispersal. The hydrophobic nanoparticles were dispersed by a natural plant surfactant (*S. saponaria*). A comprehensive experimental analysis was carried out that consisted of the physico-chemical characteristics of the water before, during and after the 12 day period of phytoremediation, the plant´s physiological characteristics, the physicochemical quantification of the plant´s cellular extracts taken from the root and the injected and non-injected aerial parts and the elemental analysis of macronutrients, micronutrients and toxic heavy-elements of these extracts using the high sensitivity technique of Total Reflection Xray Fluorescence spectrometry. The results show a notable decrease in the water´s pollutions indices, an increase of plant biomass and chlorophyll and overall, the highest micronutrient and toxic heavy-element yields of the cellular extracts from the nanoparticle injected aerial plant part. The results are discussed in terms of the adsorbability of the nanoparticles, ROS scavenging by the nanoparticles, TiNT´s photocatalytic role and the possible synergistic action of the nanoparticle in the plant´s biochemistry of heavy-element detoxification wherein low nanoparticle concentrations act as catalyst and higher ones as inhibitors. Summarising, injected nanoparticles, especially TiNTs, increase the phytoextraction efficacy of *E.crassipes*, demonstrating the phytoremediative power of this technology.

1. INTRODUCTION

Water is one of the most important natural resources for human life and a crucial factor in sustaining various economic and alimentary activities, such as fishing, agriculture, livestock and almost all types of industry. These activities, in addition to excessive water use and urban and industrial waste-water discharges, have threatened water quality with the release of organic and inorganic pollutants, such as heavy metals and metalloids. Although many of these heavy elements are micronutrients necessary for the development of living organisms, exceeding the recommended intake limits is harmful to health. The lack of biological mechanisms for their elimination, together with their integration into the food chain, leads to bioaccumulation (Narváez-Valderrama et al. 2012).

Although physical, chemical and biological technologies are available for water treatment, these, especially the first two, possess the disadvantages of being expensive, in addition to causing unwanted secondary effects on the environment. Therefore, there is an interest in the use of natural technologies, such as phytoremediation, as a sustainable, clean, cost-effective and aesthetically pleasing alternative for the restoration of polluted environments. Phytoremediation is a nature-friendly environmental biotechnology that uses plants that have the ability to extract, contain, remove or neutralize organic and inorganic compounds including heavy elements (HE), from contaminated environments (Pedraza-Guevara, 2021). Phytoextraction is one of the most important ways plants use to remediate HE contaminated substrates (Garbisu and Alkorta, 2001). It consists of the uptake of contaminating elements by plant roots and their accumulation in stems and leaves. Therefore, with this natural process, the use of plants for contamination removal can be repeated indefinitely until the residual concentration of the elements is within the limits considered acceptable (Delgadillo et al. 2011). The mass of phytoextracted HE is the product of the HE concentration in the plant and the plant´s biomass (Dasgupta Schubert et al. 2011). Per harvest, the mass of HE that can be phytoextracted is limited by the reduction in biomass due to the toxicity of the higher concentrations of the HE in plant tissue, thus imposing a natural limit to the efficiency of phytoremediation.

NB We direct attention to the use of the term “heavy elements” rather than the more conventional term “heavy metals” because the contaminants of high atomic number are not just metallic but also semi and non-metallic. In this work the term “heavy elements” encompasses dissolved ions and neutrals as well as suspended species of chemicals containing an element of the fourth period or beyond, in the water.

For the last few decades the common water weed, the water hyacinth (*Eichhornia crassipes*), has been recognized as a very effective decontaminant of polluted water bodies and has been used for waste-water treatment (Gupta, 1980). The plant is a relatively high-biomass aquatic species, has a prolific growth-rate, a ravenous appetite for both organic and inorganic aqueous contaminants, has a short life cycle and can be harvested easily - in essence making it an efficient phytoextractor of toxic HEs (Rezania et al. 2015). The model phytoremediant chosen for this work therefore is the naturally occurring wild-type *E. crassipes*.

Studies involving the phytoremediation of HEs often involve artificially constructed polluted substrates through the appropriate spiking of the pure substrate. The results obtained from such experiments may not adequately transfer to large water bodies that not only present the problems of scale but that are also subject to different climatic and geochemical variables. To adequately transfer the knowledge gained from this work to real-life problems concerning polluted water bodies, all substrates used in this work have been taken from an actual polluted lake, the "Laguna Jovita" in the municipality of Naranja de Tapia, Michoacán, Mexico as detailed in section 2.

Since the last decade there has been a surge in the application of nanotechnology to the question of environmental decontamination. Studies have shown success in water treatments using adsorbent nanomaterials (NMs), membranes impregnated with nanoparticles (NPs), photocatalytic NMs and other types of nano substances. Their efficacy depends on their chemical composition, homogeneity and texture, as well as on the method of their preparation (Roy et al. 2011). The distinguishing feature of enhanced reactivity at the nano scale allows the alteration of the physico-chemical property of the pollutant permitting its facile removal. In this regard, carbon nanotubes (CNT) as both their single-walled (SWCNT) or multi-walled (MWCNT) variants, have been recognized as versatile and effective NPs for bioremediation. Their excellent physical and chemical properties, which include their high surface adsorptivity, their function as redox catalysts, combined with their excellent mechanical and electrical properties, have been successfully harnessed for bioremediation and other end-uses (Mukherjee et al. 2016). Titanium dioxide nanotubes (TiNT) also possess beneficial mechanical properties. Additionally, they are good photocatalysts,

oxidants and are biologically and chemically inert. All these properties have been utilised for the removal of organic and inorganic pollutants from water (Roy et al. 2011).

The external application of MWCNTs to plants has been shown to favourably influence plant biomass development and the delivery of water and micronutrients to seeds and plants (Tiwari et al. 2014, Matthew et al. 2020). Likewise, the application of Titanium dioxide NPs ($nTiO_2$) has demonstrated plant growth promotion (Mattiello et al. 2018). However, at high concentrations, MWCNTs become toxic inducing oxidative damage and the retardation of photosynthesis (Wani et al. 2024), whilst high concentrations of $nTiO_2$ have been seen to inhibit plant growth by harming plant growth promoting bacteria in soil (Chavan et al. 2020). In fact, with the proliferation of NPs in the environment, serious concerns are beginning to be voiced over their transport and eventual entry into the human body (Reis et al. 2023).

The objective of this work is to leverage the aforesaid beneficial properties of MWCNTs and TiNTs with the intrinsic phytoextractive property of *E. crassipes*, towards the (hypothesized) resultant of a higher plant biomass that would increase the phytoextracted HE yield of *E. crassipes*. However, the administration of the NPs to the aqueous substrate is precluded because given the large bodies of water this technology seeks to address, an inordinately large mass of NPs would be required even for the low NP concentrations deemed beneficial, and this in turn would cause unacceptable damage to the very environment that the technology seeks to clean, in addition to being prohibitively costly. To circumvent this inherent dichotomy, we have directly administered the NPs by injection at concentrations far below toxic levels to the plant aerenchyma and leaf with the hope of achieving the aforesaid synergy whilst at the same time anchoring the NP and preventing its dispersal to the wider environment. Although the injection of NPs into plant cells for the direct delivery of genetic material or pesticides has been reported (Sembada and Lengoro, 2024) the injection of NPs into an organ of the living plant as a means to affect its HE uptake, as described in this work, is new.

Finally, a truly sustainable technology is one that operates in a closed cycle, *i.e.* the residual material is gainfully utilized in other ways. We have attempted to achieve that goal by utilizing the biomass of *E. crassipes* after phytoextraction to synthesize other nano allotropes of Carbon, the details of which are to be published separately.

2. MATERIALS AND METHODS

Materials and instruments used

MWCNT (Sigma-Aldrich® Cat. No. 724769, CAS No. 308068-56-6) ≥ 95% carbon basis, O.D. × I.D. × L (10 ± 1)nm × (4.5 ± 0.5)nm × (3- ~6)μm, avg. no. of layers 6 - 8; TiNT (Sigma-Aldrich® Cat. No.799289, CAS No. 13463-67-7) powder material of nanotubes with average diameter of 25nm; Soap nuts (*Sapindus saponaria*) as commercially sold; For quantitative analysis all reagents were of Analytical Grade purity; All glassware were borosilicate; Solutions utilized in the experiments were constituted in distilled water (DW) or tri-distilled water (tDW). The make and model of the instruments in the measurements have been cited as per occurrence in the description that follows.

2.1 E. crassipes collection and cultivation

E. crassipes plants were collected from the lake "Jovita" in the town of Naranja de Tapia, Zacapu, Mexico, GPS: 19.7667–101.75 (Fig. 1). The plants were collected from an area of 1 m^2 of the lake`s surface, hydrated with lake water and transported to the Biophysical Chemistry and Radiation Studies Laboratory (LBER) of the University of Michoacán (UMSNH), for further cultivation.

The water hyacinth plants were washed with a 3% household bleach solution and rinsed with tap water to clean debris and remove insect larvae. They were kept under observation for 3 days in a tank filled with lake water in order to select the healthiest plants. Thereafter 4 litre rectangular plastic recipients containing lake water were each fitted with 3 plants and transferred to a laboratory growth chamber whose operational conditions are described below.

2.2 Phytoremediation of water contaminated with heavy elements

Both MWCNT and TiNT are hydrophobic NPs. To disperse them in water and render them bio-available, a surfactant would be needed. However, no synthetic surfactant such as Triton-X that is generally used in nanotechnology, can be used because the living cell membranes would then disintegrate. We have used the extract of the soap nut (*Sapindus saponaria*) - a purely natural plant-derived surfactant - as an NP dispersant. NP suspensions at the concentrations of 2 and 3 mg/l of the MWCNT and 2, 3, 5 mg/l of the TiNT were made in aqueous media containing *S. saponaria* and hydrated water hyacinth extracts as described below. These concentrations were arrived at after a trial study that found concentrations ≥ 5mg/l to cause necrosis and < 1mg/l to produce no discernible effect.

These suspensions were used for the determination of the NP concentration dependent phytoremediative capacity of NP injected *E. crassipes*, which forms the central theme of this study.

Hydrated E. crassipes extract:

The aerial part of the plant was dried in an oven (Ecoshel™ 9023A, FCD-3000) at 60°C for 48 hours and then ground to a powder using a Moulinex™ DPA2 chopper-grinder. 1 g of the obtained water Hyacinth powder was added to 100 ml of DW then shaken first manually (30s), then on a Fisher Scientific 2500 rpm "speed 8" vortex mixer (60s), left to stand for 60 min then vacuum filtered using a Whatman grade 41 filter paper. This constituted the primary solution of the *E. crassipes* extract. The mass of 1 g of dry biomass powder was chosen after heuristically establishing it as the maximum in the primary extract that caused no damage to the living plant.

Preparation of surfactant and NP suspensions:

2.7 g of *S. saponaria* nuts were added to a beaker containing 50 ml of DW and allowed to simmer on a hot plate (Thermo-Scientific®) at 90ºC for 20 min. The mixture was allowed to cool to room temperature and vacuum filtered through a Whatman filter grade 41 filter paper. The water hyacinth primary extract and *S. saponaria* extract solutions were sterilized by autoclaving at 1 atm pressure and 120°C for 30min. Once the solutions had reached room temperature, a 100 ml solution, termed "base", was prepared in a laminar flow hood by mixing the *E. crassipes* and the *S. saponaria* extract solutions at a respective volume ratio of (95:5)%. Using the base solution, stock solutions at the concentration of 50 mg/l of the MWCNT and TiNT were realized which included the step of homogenization by 10 minutes of ultrasonication followed by 1 minute of pulsed vortex shaking. Subsequently, appropriate dilutions were carried out to yield the working concentrations (Table 1). All volumetric operations were carried out in a laminar flow hood.
Table 1. Concentrations of the different treatments for the evaluation of MWCNT and TiNT NPs for the phytoremediation of polluted water by *E. crassipes*. The blank was plain DW.

Treatment	Surfactant stock solution	Multiwalled carbon nanotubes (mg/l)*	Titania nanotubes (mg/l)*
Blank	No	-	-
Control	Yes	-	-
MWCNT_2	Yes	2	-
MWCNT_3	Yes	3	-
TiNT_2	Yes	-	2
TiNT_3	Yes	-	3
TiNT_5	Yes	-	5

*The number corresponds to the concentration in mg/l of each nanoparticle.

Injection of E. crassipes with the NP suspensions:

The water hyacinths were gently patted dry with paper towels, weighed and brought to the sterile environment of a laminar flow hood. Selected regions of the aerenchyma, the bulbous petiole of the plant - henceforth termed just the bulb - and the underside of the leaf, were sterilized with 70% alcohol via cotton swabs. A sterile insulin syringe of 1 ml volume was used to inject 0.2 ml of the particular treatment (Table 1) to the bulb and 0.04 ml to the underside of the leaf (Fig. 2). The bulb acts as the plant´s "buoy" but is otherwise not central to the plant´s metabolism and so the larger injected volume there was perceived to be minimally invasive. The injected suspension then disperses into the plant tissue.
To avoid contamination by microorganisms, "Campeche wax" was placed over each hole made by the needle. After completing the full set of treatments to the plants, they were accommodated in rectangular plastic buckets each of 4l capacity at a density of 3 plants to each bucket and placed inside a growth chamber that had an average temperature of 25°C, an average humidity of 40% and a photoperiod of 14 h at 2000 lux light intensity. Within the plastic bucket, each plant was passed through a 2 cm^2 hole in a biodegradable Styrofoam lid in such a way that the roots remained submerged in water whilst the aerial part including the bulb were supported by the lid and separated from water to prevent the premature decomposition of the bulb. Polluted lake water from the "Jovita" Laguna de Naranja de Tapia lake (Fig. 1) was used as the phytoremediation medium. An aeration system for fish tanks was installed in the growth chamber that operated between 08:00 to 19:00 h, daily. Each treatment of Table 1 was applied to 3 plants, bringing the total for all treatments to 21 plants, with some extras.

2.2.1 Variables measured during the period of water phytoremediation

The period of phytoremediation by the different water hyacinth - NP composites (Table 1) was 12 days during which daily measurements of the water variables pH, conductivity, Total Dissolved Solids (TDS), were carried out using the BLE-C600™ water quality tester. Six measurements using a 25 ml syringe for water withdrawal, were made for each treatment type, at the 4 extremities and the centre of the tank, and one very close to the roots to ascertain a possible efflux of ions, including H^+ ions, by the root. After the measurements, the root-zone water was returned to its plastic bucket corresponding to the particular treatment.

Given the large volume of the bulk water as compared to the root zone water, little discernible variation in the magnitudes of the variables between the two is expected, especially in the case of the pH were the logarithmic relation to the H^+ ion concentration decreases the sensitivity. Such ions as may be exuded would soon diffuse into the bulk where the largeness of the latter would serve to dampen any observable difference in concentration. However, what one might plausibly expect is the fluctuation of the variable (e.g. the pH) at the root zone to register a discernible difference from the bulk values given the small volume defining the root zone and its closeness to the solid root surface. In analytical chemistry, statistical fluctuations of a variable or its precision, are commonly measured as the % relative standard deviation (RSD) (https://demo.bco-dmo.org/parameter/1952, last accessed 03/Dec/2024)). Accordingly, the grand average of the variable`s value and its standard deviation calculated by averaging over all replicates over all days of the phytoremediation process, for each treatment type, was obtained for (a) the bulk water and (b) the root zone water. Thereafter the % fluctuation of the variable for the cases (a) and (b) were computed as the %RSD using these grand averages and their standard deviations.

2.2.2. Harvesting of the *E. crassipes* plants

After 12 days, the plants were harvested, which consisted of removing the plant from the phytoremediation medium, placing the plant on a mesh to remove excess water and then leaving the plant on paper towels to free the roots of water. The whole plant was then weighed on an analytical balance (Ohaus® PA224). The root and aerial parts were then separated and weighed. Further, the aerial part was sectioned into the part containing the bulb and leaf where the NPs had been injected - the "injected part" - and the "non-injected part" constituted by the new bulbs and shoots that had emerged during the 12-day period. Each separate section was weighed. All these weights were recorded as fresh weights of the respective parts.

2.2.2.1 Chlorophyll a and b

The measurement of the chlorophyll content was carried out according to the method proposed by Hansmann (1973). The aerial part of *E. crassipes* was macerated for 10 mins in 10 ml of 90% acetone and subsequently further pulverized for 60 mins in an ultrasonic bath at 40 kHz (SRA™ digital ultrasonic cleaner) in order to extract the maximum amount of pigment. Thereafter, the samples were rested for 24 h at a temperature of 4°C in complete darkness (to prevent the photodegradation of chlorophyll). The supernatant was obtained working in low light and the absorbance was measured using 90% acetone as a blank, by UV/Vis spectrophotometry (VELAB® VE-5600UVPC) at the wavelengths of 750, 663, 647 and 630 nm. The chlorophyll **a** and **b** concentrations, C**a** and C**b** in mg/l, were calculated (Lichtenthaler, 1987) as,

$$\boldsymbol{Ca} = (12.7\ x\ A663) - (2.69\ x\ A645)$$

$$\boldsymbol{Cb} = (22.9\ x\ A645) - (4.68\ x\ A663)$$

Using these values, the mass-concentrations of the chlorophylls ***a*** and ***b*** in the samples were determined as,

$$Chlorophyll\ \boldsymbol{a}\ (\frac{mg}{\text{kg}}) = \frac{\boldsymbol{Ca}\ \text{x}\ v}{w}$$

$$Chlorophyll\ \boldsymbol{b}\ (\frac{mg}{\text{kg}}) = \frac{\boldsymbol{Cb}\ \text{x}\ v}{w}$$

where *v* (l) is the volume of acetone used to extract the pigments; and *w* (kg) is the dry biomass of the sample.

2.2.2.2 Measurements on the plant extracts, the dry biomass yield and the plant water content.

The fresh parts of each plant of each treatment type corresponding to the root, the injected and non-injected bulbs and the leaves were separately ground in a blender (OSTER™) with 25 ml of tri-DW, then centrifuged at 12,500 rpm (DYNAMICA™ Velocity 14R) for 20 mins. The supernatant was extracted and its pH, conductivity and TDS were measured using the water quality tester BLE-C600®.

Thereafter, the whole fresh sample mass was extracted from the centrifuge and was dried in the oven at 60°C until constant weights were obtained. These weights constituted the dry biomasses. The relevant % moisture content was determined as,

$$\%\ moisture = \left(\frac{FW - DW}{FW}\right) * 100$$

where FW and DW are the fresh (section 2.2.2) and dry weights respectively.

2.3 Elemental analysis by Total Reflection X-ray Fluorescence (TXRF) spectrometry.

TXRF is a relatively new variant of the x-ray fluorescence (XRF) elemental analysis technique. In TXRF spectrometry, the exciting x-ray beam hits the sample - deposited as a very thin homogeneous layer atop an optically polished quartz or acrylic sample carrier disc - at angles below the critical angle that results in the total external reflection of the incident beam. Scattering and matrix effects that normally diminish the sensitivity of XRF analyses are substantially reduced, amongst other benefits, making TXRF a valuable micro-trace analytical technique (Rodríguez Alejandre et al. 2023) The TXRF spectrometer used in this work was the S2 Picofox® of Bruker GmbH.

The recommended sample preparation protocol defined in the S2 Picofox User´s Manual for liquid samples was used. This necessitated making the sample side of the acrylic disc hydrophobic by the deposition of 10 µl of silicone fluid (SERVA™) at the disc centre and subsequently drying it by heating to 35°C for 60 mins. For each treatment type (Table 1) 990 µl of the plant extracts of the root, and the bulb + leaves (aerial part) obtained as described above were taken, to which 10 µl of a Ga internal standard (Sigma-Aldrich™ 1000 mg/l) were added to yield 10 mg/l of the standard. The solution was ultrasonicated for 15 mins to homogenize the solution and remove any clumping as might have existed. Subsequently the solution was vortexed for 1 min in a pulsed mode and immediately thereafter 10 µl of this sample solution was deposited onto the centre of the previously hydrophobisized acrylic disc. The sample was then dried in the oven at 35°C for 30 min. The low heating temperature of 35°C was chosen in this work to reduce the chance of loss of volatile species. The sample discs were stored at room temperature in closed sterile plastic containers to guard against any contamination. Finally, multi-elemental analysis for macronutrients, micronutrients and toxic HEs was performed using the TXRF spectrometer.

2.4. Quality of the phytoremediated water.

The initial and final characterizations of the water used for phytoremediation with the NP treated water hyacinths, were carried out. For the analyzed variables of hardness and alkalinity, phosphorus, and nitrogen, the methods described in "Standard methods for the examination for water and wastewater 23rd edition" APHA-AWWA-WEF were used.

- Alkalinity	2320 B. Titration Method
- Calcium hardness	2340 C. EDTA Titrimetric Method
- Total hardness	2340 C. EDTA Titrimetric Method
- Total phosphorus	4500-P. Manual Digestion for Total Phosphurus
- Ortophosphates	4500-P. Ascorbic Acid Method
- Nitrates	4500-NO 3. Cadmium Reduction Method
- Nitrites	4500-NO 2. Colorimetric Method
- Amonium	4500-NH 3. Phenate Method

Colorimetric techniques were used for the turbidity and suspended solids variables, which were determined using the Hach® DR/820 colorimetric instrument, following the analytical procedure described in the USEPA-approved DR/800 Colorimetric Series Manual, Method 365.2, and Standard Method 4500-PE for Wastewater.

3. RESULTS

Averages and their standard errors of the measured parameters were calculated for each set of replicates for each treatment type. The statistical analysis through the one factor ANOVA was done using the Tukey test (0.05), via the IBM SPSS Statistics 23 program.

3.1 Phytoremediation of water contaminated with heavy elements.

In this section, the results for the determination of the physico-chemical characteristics of the water that underwent phytoremediation, are presented.

3.1.2 Variables measured during the water phytoremediation period (pH, conductivity and TDS).

Table 2
Hydrogen ion potential (pH) of water phytoremediated by *E. crassipes* injected with multi-walled carbon nanotubes (MWCNT) at concentrations of 2 and 3 mg/l and Titania nanotubes (TiNT) at concentrations of 2, 3 and 5 mg/l, indicated in the table as MWCNT_x and TiNT_y where x = 2,3 and y = 2,3,5. R = Water near the root. G = water of the bulk (general) medium. Tukey test (0.05), equal letters do not indicate a significant difference.

Treatments		Day_1	Day_5	Day_6	Day_7	Day_8	Day_11	Day_12
Blank	**R**	(7.61±0.15)^{a}	(7.74±0.24)^{a}	(7.57±0.18)^{a}	(7.90±0.34)^{a}	(7.41±0.11)^{d}	(7.79±0.08)^{abc}	(7.68±0.16)^{bc}
	G	(7.78±0.25)^{a}	(7.74±0.31)^{b}	(7.56±0.13)^{bc}	(7.80±0.26)^{ab}	(7.44±0.17)^{d}	(7.55±0.25)^{d}	(7.72±0.23)^{a}
Control	**R**	(7.61±0.15)^{a}	(7.61±0.17)^{a}	(7.71±0.07)^{a}	(7.77±0.06)^{a}	(7.80±0.08)^{ab}	(7.85±0.08)^{ab}	(7.70±0.04)^{bc}
	G	(7.78±0.25)^{a}	(7.68±0.14)^{b}	(7.62±0.12)^{abc}	(7.74±0.07)^{abc}	(7.60±0.23)^{abc}	(7.71±0.20)^{cd}	(7.70±0.14)^{a}
MWCNT_2	**R**	(7.61±0.15)^{a}	(7.73±0.18)^{a}	(7.54±0.20)^{a}	(7.72±0.09)^{a}	(7.83±0.03)^{a}	(7.97±0.04)^{a}	(7.81±0.05)^{abc}
	G	(7.78±0.25)^{a}	(7.84±0.05)^{b}	(7.49±0.05)^{abc}	(7.58±0.14)^{c}	(7.62±0.09)^{abcd}	(7.95±0.06)^{ab}	(7.85±0.19)^{a}
MWCNT_3	**R**	(7.61±0.15)^{a}	(7.74±0.20)^{a}	(7.69±0.07)^{a}	(7.68±0.13)^{a}	(7.75±0.06)^{ab}	(7.96±0.07)^{a}	(7.89±0.04)^{ab}
	G	(7.78±0.25)^{a}	(8.38±0.94)^{a}	(7.65±0.07)^{abc}	(7.67±0.19)^{bc}	(7.78±0.06)^{ab}	(8.06±0.09)^{a}	(7.88±0.25)^{a}
TiNT_2	**R**	(7.61±0.15)^{a}	(7.79±0.15)^{a}	(7.61±0.16)^{a}	(7.92±0.18)^{a}	(7.73±0.06)^{abc}	(7.80±0.11)^{abc}	(7.91±0.09)^{a}
	G	(7.78±0.25)^{a}	(7.79±0.07)^{b}	(7.74±0.10)^{ab}	(7.91±0.10)^{a}	(7.58±0.04)^{cd}	(7.49±0.11)^{d}	(7.80±0.30)^{a}
TiNT_3	**R**	(7.61±0.15)^{a}	(7.68±0.15)^{a}	(7.57±0.24)^{a}	(7.74±0.09)^{a}	(7.73±0.05)^{abc}	(7.78±0.19)^{abc}	(7.90±0.02)^{a}
	G	(7.78±0.25)^{a}	(7.78±0.06)^{b}	(7.60±0.25)^{abc}	(7.77±0.11)^{abc}	(7.64±0.09)^{abc}	(7.26±0.23)^{e}	(7.84±0.24)^{a}
TiNT_5	**R**	(7.61±0.15)^{a}	(7.71±0.17)^{a}	(7.77±0.04)^{a}	(7.74±0.07)^{a}	(7.67±0.04)^{ab}	(7.73±0.07)^{bc}	(7.75±0.18)^{bc}
	G	(7.78±0.25)^{a}	(7.83±0.03)^{b}	(7.61±0.03)^{abc}	(7.59±0.05)^{c}	(7.60±0.04)^{abcd}	(7.60±0.07)^{cd}	(7.81±0.07)^{a}

3.3.2 Plant variables measured after harvest

Table 3
Fresh biomass of the water hyacinth plants at the beginning and end of the phytoremediation process. 'Initial weight' corresponds to the plant without treatments; 'final weight' corresponds to the weight after 12 days; Refer to Table 2 for the abbreviations and symbols.

Tratment	Initial weight of the whole plant (g)	Final weight of the whole plant (g)	Growth difference after 12 days of phytoremediation (g)
Blank	(17.63±0.55)^{b}	(15.79±0.26)^{a}	(-1.84±0.31)^{a}
Control	(48.65±8.86)^{a}	(29.33±11.53)^{a}	(-19.31±2.72)^{b}
MWCNT_2	(28.47±11.50)^{ab}	(27.99±9.96)^{a}	(-0.37±1.62)^{a}
MWCNT_3	(28.90±10.63)^{ab}	(30.68±6.20)^{a}	(1.78±4.99)^{a}
TiNT_2	(25.16±7.08)^{ab}	(29.11±5.99)^{a}	(3.99±1.38)^{a}
TiNT_3	(32.37±11.84)^{ab}	(38.95±11.65)^{a}	(6.58±1.80)^{a}
TiNT_5	(23.14±5.60)^{b}	(27.66±9.36)^{a}	(4.52±5.31)^{a}

3.4 Analysis of the plant cellular extract for micronutrients, HEs and macronutrients by TXRF spectrometry.

3.5. Water quality

4. DISCUSSION

4.1 Physicochemical variables of the HE polluted water undergoing phytoremediation by water hyacinths with/without NP treatment.

The results for the variables of pH, conductivity and TDS shown in Table 2 and Figs. 4–6 show that the pH is rather uniform maintaining a value between 7.41 to 8.38 during the 12 days of the remediation process for all treatments. Overall, there was little difference between the pHs of the root-zone and the bulk water. This is to be expected because the highly mobile small ion H^+ even if it were to be effluxed out of the roots would quickly diffuse into the bulk media. Wegner et al. 2021 state that dissolved ions are taken up by the root through the plasma membrane potential supplied by the H^+ pump upon H^+ efflux. However, the water hyacinth root is completely submerged in the water so dissolved ion uptake can more easily occur without the H^+ pump being actuated (or less so), simply by water inflow through the transpiration stream. The pH in both the root-zone and bulk water was maintained slightly above 7 which is considered as optimal for freshwater organisms that inhabit or ingest it (Betancourt & Labaut, 2013). Figure 6 reveals that the root-zone water for all treatments suffered less fluctuations in pH than the bulk water. Large fluctuations in a system occur when it is far from stability. Our results corroborate the observations made by some (Rodriguez-Lara et al, 2022) that water hyacinth stabilizes the pH of water.

The water conductivity (Fig. 4) was nearly constant for the Blank and decreased slightly but erratically over the 12-day period of the phytoremediation for the Control. For all the other cases, *i.e.* the plants injected with the 2 NP types at different concentrations, the water conductivity showed a clear decline over the 12-day period. The conductivity rose slightly towards the end (day-12). It was due to the commencement of decomposition of the plant roots that released cellular ions into the water. The statistical difference of the conductivities between the NP treated plants and the Blank and Control became significant after day-7 or day-8, clearly demonstrating the successive progress of dissolved ion removal from the water by the plant. Overall, the mean conductivities of the root-zone water were slightly lower than the mean conductivity of the bulk. The most effective was the plant with the TiNT_3 treatment that registered a decrease in the root-zone conductivity from the start value of 649. 83 µS to 553.33 µS on day-11. The fact that the NP treated plants become capable of a higher efficiency of ion removal may rest on the two occurrences of (a) ion adsorption at the active sites of the dispersed NPs in the plant (Roy *et al.*, 2011; Margulis and Sagan, 2008) and (b) a synergistic enhancement of the plant's physiological and biochemical processes of ion absorption by the NP, in particular by the TiNT_3. The reason to include (b) is because if the mechanism were only passive adsorption, then the degree of reduction of water conductivity would scale by the NP concentrations of the treatments, which it does not.

The TDS refers to all the dissolved species present in the water and comprises of ions, dissolved compounds and colloidal particles. Figure 5 shows the results, where again the steady decline in TDS concentrations with time will be noted except near the end of the 12-day experimental period where a slightly greater TDS concentration occurs as compared to the end-period conductivity increase in Fig. 4. The gamut of cellular components which include all the species defined within TDS, released by the decomposing plant parts as against only ions, near the end of the phytoremediation period, accounts for this relative increase. The root-zone water had overall slightly higher TDS values relative to the bulk water which is indicative of their higher load of root exudates in addition to those in the bulk water. However, the most interesting aspect of Fig. 5 is the significantly larger reduction of TDS over the phytoremediation period by the plants injected with the NPs. Of these the most effective again was the treatment with TiNT_3 where the TDS was reduced from its start value of 452.83 mg/l to 386.83 mg/l, representing a reduction by 66 mg/l. The NP injected plants therefore are good absorbers not only of ions but also of all dissolved species. This could be a consequence of the higher throughput of water through the plant, for example, as it occurs in the event of a higher transpiration rate. This will be addressed later in connection with the moisture content of the plants. As noted before, the capacity of the treated plant to decrease the water's TDS is apparently not correlated with the NP's concentration within the plant which would have been the case if the mechanism were simple adsorption on the surfaces of the NPs.

Water hyacinths have been used as phytoremediators of polluted waters (Fahruddin, 2020) to absorb and/or filter out molecular and ionic pollutants through their extensive root systems. This work shows that this function can be enhanced by the localization of NPs within the plant by injection at suitable anatomical sites. The great advantage is that this technique restricts the dispersal of the NP to the environment at large, which could have deleterious secondary consequences such as the entry into animal and human food chains. NPs have been extensively used for the removal of pollutants from wastewater (Mehta et al. 2024). One technique is to introduce the NP at minimal concentrations into the wastewater, so that it adsorbs and precipitates out the HE as a sediment. However, there remains the possibility of the desorption and detachment of the HE as well as the NP from the sediment by changes in the water chemistry, geophysical upheavals and other processes. Additionally, even though the NP concentrations are small, even for moderate scale sedimentation of the HE, substantial quantities of the NP would have to be used, which makes the technology cost prohibitive.

In a previous work (González-Alejandre et al. 2021) we had estimated the time taken by the MWCNTs of the type used in this work to diffuse through the tortuous pathways inside the seed-coat and cell-wall, as 314d and 21 min respectively. Considering the much larger distances between the aerial part where the NPs were injected to the root-water interface, it is reasonable to assume that the MWCNT and its morphologically similar TiNT would take a time far exceeding the 12 days of the current experiment to diffuse out of the plant root into the water, passing as they would have to, through many inter-cellular orifices. It would require the unusual case of substantial phloem pressure to mechanically extrude the NPs out of the root to the water, considering the same limitation of NP sizes, to obtain any measurable expulsion of the NP. Even if that were to happen the masses expelled would be miniscule given that the highest mass introduced into the plant was the TiNT_5 at 1.2 µg per plant. Hence it is reasonable to suppose that the NPs remain well localised within the plant while performing their function. Besides, this technique exploits the availability of a very large number of HE sequestration sites within the plant in addition to adsorption on the NP surface alone, so that it requires a modest outlay of investment in NPs. Thus, the scheme presented in this work offers a viable technique for the efficient and effective phytoremediation of polluted water without the danger of collateral damage.

The results of this experiment show (a) the capacity of the plant to absorb dissolved ions and dissolved solids in a progressive way during its lifespan (b) the clear involvement of root absorption in the reduction of ionic concentrations of the polluted water (c) the reduction of TDS by root absorption but also of its slight increment by root exudates as compared to the bulk water (c) the possible synergistic enhancement of the plant's intrinsic mechanisms of ion and dissolved solid absorption by small quantities of the MWCNT and TiNT introduced *in vivo*.

4.2 Harvest

The biomass measurements of the harvested water hyacinths (Table 3) showed that the post-harvest weights in the sets Blank, Control and MWCNT_2 were lower than their initial weights. This was due to a blight caused by a species of aquatic snails that had attacked all the treatments. However, for the treatment with the NPs, the snails avoided consuming the injected aerial parts, possibly because of a greater degree of HE localization there (see Fig. 12). The HE may act similarly as the secondary metabolites that plants form to protect themselves from pests (Jiménez et al. 2003). Thus, as Table 3 shows, the NP injected plants registered post-harvest weight increases as compared to their initial weights.

Figures 7 and 8 concern the post-harvest fresh and dry biomasses where for the aerial part no statistically significant differences are seen although they are manifested for the root between the Blank and the TiNT_3 treatment, amongst others. Nonetheless, the figures show that the highest fresh and dry aerial part biomasses were achieved with the TiNT @ 3mg/l. Relatively very few studies exist of the use of MWCNT and TiNT NPs for phytoremediation although there are abundant studies of the use of these NPs in plant biological research such as their use in improving plant tolerance to environmental stress. The TiO_2 based formulation "Tytanit" is commonly used in low doses as a fertilizer (Kleiber and Markiewicz, 2013). Our aforesaid results with TiNT confirm the expectation of the benefits of low dose Ti.

The percentage of moisture in the plants is also affected by the internal presence of the NPs (Fig. 9). For the Blank the quantities of moisture in the root and aerial parts were 97.02% and 93.08% respectively. The treatment with NPs caused a general decrease: the root content was (95.35–95.85)% whereas the aerial part showed a greater variability being higher (94.71%) than the Blank for the MWCNT_2 treatments but very close to the value for the Control (94.78%), with the lowest (92.53%) for the TiNT_2 treatments. Moisture content in the plant is the nett result of water absorption and transpiration. Transpiration plays a pivotal role in adult plants and may change the water balance. González-García et al, 2021 show that CNT + Al reduce transpiration whereas the increase of transpiration with the corresponding reduction in moisture content, occurs upon treatment with TiO_2 NPs (Qi et al. 2013). Altogether, the results of Fig. 9 conform to the expectation of relatively lower and higher moisture contents for the TiNT and MWCNT treatments respectively.

Figure 10 shows that whilst statistically there is no significant difference of chlorophyll **a** between the treatments, the treatment with TiNT_2 showed a noticeable increase in chlorophyll **b** as compared to the rest. Chlorophyll is an important plant pigment crucial for the process of photosynthesis that governs plant growth and development and in turn the plant's ability to absorb water and thereby external substances - nutrients and pollutants alike. Levels of chlorophyll in a plant vary with the degree of stress induced by pollutants and exogenous agents (Putra et al. 2016). The fact of the near constancy of the chlorophyll levels across all treatments investigated in this work, indicates that the injected NPs did not induce stress on the plants, allowing them their normal photosynthetic function essential to plant health (Waghmode et al. 2019).

From Fig. 11 it is seen that the pH of the extracts from the aerial part injected with the MWCNT and TiNT or with just the surfactant (the 'Control'), is slightly lower than that from the extract of the non-injected aerial part at all concentrations. CNTs are known to depress the pH of aqueous media (Leonavicius et al. 2017). TiNTs are well-known photoelectrochemical catalysts where the intermediate half reaction in the overall water-splitting reaction, generates an H^+ ion. CNT based electrocatalysts are known to split water molecules thereby releasing the H^+ ion in a parallel manner. However, all these observations have been made *in vitro* where the substrates were not the living plant cells. This work shows that the CNTs and TiNTs tend to preserve their tendency to liberate protons even within the complex matrix of cells of the living plant. Physiologically, the plant´s internal pH is regulated by pectins and enzymes, which are involved in ionic balance and act as recognition molecules for the symbiosis of organisms, as well as for protection against insects and/or pathogens

(Azcón-Bieto and Talón 2008). So, the ‘recognition’ of the NPs as well as the surfactant fluid (see section 2.2) as ‘foreign entities’ might also have had a role to play in the small relative decrease in pH of the extracts taken from the injected aerial part of the water hyacinth plant.

The Conductivity and TDS results of Fig. 11 are nearly identical in trend. For all cases, these indices were higher for the treated plants than for the non-treated ones and within the former, the values were higher for the injected aerial parts, except for the Control where the injected and non-injected parts showed about the same ionic conductivity. The sets MWCNT_2 and TiNT_2 registered the highest values.

The aforesaid observations suggest the following to have occurred (a) adsorption of ions by the NP surfaces and the synergistic action of the NPs on the physiological chemistry of ion and solubilized compound acquisition by the plant (b) localization of the NPs largely to the environs of the injection site (c) non-localization of the surfactant extract (see section 2.2.2). The first ensues from the greater values of the indices for NP treatments, the second because of the clear difference in magnitude between the injected and non-injected parts and the last because for the Control, which was injected only with the surfactant, the difference between the two parts is practically non-existent indicating a free dispersal of the surfactant throughout the plant. The fact that the injected NPs more or less preserve their intrinsic nano-surface properties (good adsorbancy) and their hydrophobicity (non-rapid dispersal throughout the plant) while not producing any deleterious physiological effects indicates that they are well-tolerated and are biocompatible NPs (Roy et al. 2011).

4.3 Biomass extract analysis for micronutrient and heavy elements by TXRF spectrometry.

Figure 12 shows the heavy element concentrations both for the toxic as well as the micronutrients summed over all treatments so as to facilitate a comparison between the two, followed by the graphs for the concentration of each micronutrient and toxic HE with Fig. 13 for the micronutrients and Fig. 14 for the toxic HEs. The elemental concentrations measured are those of the dissolved species in the cellular fluid and those that had desorbed out from the solid surfaces (cellular components and the NPs) by the extraction procedure. The residual species that remained strongly adsorbed on, or attached to, the said solid surfaces were not measured. Given that plants store excess HE as chelated (dissolved) species within cellular compartments (Viehweger, 2014), often the vacuole, the fraction of this unretrieved HE, is likely to be small.

Consulting Fig. 12, micronutrient elements are found to be more concentrated in the aerial parts of the water hyacinth with the parts injected with the NPs showing the highest concentrations – around 3000 mg/l for the treatments with MWCNT_2, TiNT_2 and TiNT_3. Micronutrient concentrations in the root are also higher for the NP treated plants as compared to the Control, reinforcing our previously stated contention of the synergistic effect of the NPs on the whole plant´s biochemistry. The fact that the aerial part records the greatest increase in micronutrient concentration is expected given the importance of these micronutrient elements for the photosynthetic apparatus of the plant and for its cellular respiration (Taiz and Zeiger, 1991). Both CNTs and TiNTs display a high degree of surface adsorption of ions and polar species (Alguacil et al. 2014; Selmani et al. 2023). Therefore, adsorption could account for the higher micronutrient concentrations in the injected aerial part, in which case they would scale with higher NP concentrations, but that is not the case. An optimal NP concentration is shown to exist wherein the maximum micronutrient concentration is manifest. For the MWCNT it is at 2 mg/l and for the TiNT, at 3 mg/l while higher values show a reduction of micronutrient concentration. This diminution at high concentrations but beneficence at lower ones, could stem from the fact that both types of NPs can act as scavengers of reactive oxygen species (ROS) as well as its producer (Fenoglio et al, 2006; Hsieh et al. 2014; Li et al. 2014; Canillas et al. 2020). At low or high concentrations one or the other would dominate, affecting the outcome of the desired process. A similar concentration dependence and the associated reversal from beneficence to toxicity of the MWCNT has been seen by us in previous works (Tiwari et al. 2014; González-Alejandre et al. 2021)

In the case of the toxic HEs, the same trend can be discerned between the NP treatments and the single non-NP treatment (the Control). Toxic HE concentrations were generally higher for the NP treated plants where the aerial part showed a greater concentration than the root. This is in contrast to most HE accumulating land plants where relatively few allow the transport of the toxic HE to the aerial part where the sensitive photosynthetic apparatus is located. The higher concentration of the toxic HE in the aerial part of the water hyacinth can only mean that the HE is carried up largely by the simple inflow of water by the transpiration stream. Once again, the injected part registers a greater toxic HE concentration than the non-injected part and this difference is manifest only for the NP treatments. Also, this difference is more marked than for the case of the micronutrients - the NP injected part retaining almost double the concentration of the non-injected part, in some cases. It is clear then that the small concentrations of the NPs have a relatively strong influence on the biochemical redox reactions involved in toxic HE detoxification and sequestration, in addition to their adsorptive retention of the toxic HE on their surfaces. In contact with plant cellular matter, these HE promptly produce ROS which become the vectors of the HE´s toxicity (Berni et al. 2019). However, if the MWCNT and TiNT are in the vicinity, they can scavenge out these ROS, thereby detoxifying the HE, which can then be sequestered in the vacuoles by the appropriate proteins (phytochelatins, metallochaperones etc.). However, at the highest TiNT concentration of 5 mg/l (TiNT_5), the aerial HE concentration drops to a level lower than the Control’s and the difference between the injected and non-injected parts almost disappears. If at higher concentrations, the NPs begin to produce the ROS themselves, they no longer aid the HE detoxification process and themselves become an agent of stress. Irshad et al. (2021) have pointed out the occurrence of synergistic and antagonistic effects of NPs with respect to HE accumulation and plant growth, that depend also on the environmental conditions the plant is exposed to.

The results for each micronutrient and toxic HE, are described below. The overall mechanism of the functional association of the MWCNT and TiNT with the said elements has been described in the preceding paragraphs.

Cl

It is an essential micronutrient, however, at high concentrations it becomes toxic, causing damage to root and leaf cells, that affects photosynthesis and water balance (Taiz and Zeiger 1991). Figure 13a shows that the aerial part injected with the NP retains the highest amount of Cl for all treatments. The highest level of 3741.47 mg/l, was attained by the MWCNT_2 treatment.

Mn

Mn is an essential element for aquatic plants. It is involved in photosynthesis, enzyme activity and oxidative stress resistance. In this work the cellular extracts of the injected aerial parts showed the highest Mn content with the highest concentration of 2.81 mg/l being in the MWCNT_3 treatment. For this treatment set, new shoots were found to have grown during the phytoremediation period on the side of the plant that had not been injected with the NP. Its chlorophyll **a** content too (Fig. 10) was the highest. Both these features could be triggered by the high Mn content of this plant which helped ameliorate oxidative stress allowing new growth and the promotion of photosynthesis.

Fe

Root absorption and retention was competitive, so much so that for the NP treatments, it exceeded the Fe contents in the non-injected aerial parts. By and large the aerial part injected with the NP still contained the higher concentration, with the highest (0.72 mg/l) reached for the TiNT_3 treatment. The non-injected aerial part for the NP set, showed low retentions with values that were even lower than the Control´s. One mechanism plants utilize to get rid of excess HE is by root exudation (Viehweger, 2014). Amongst other things, the high root concentrations of Fe might be such an indication.

Ni

Although nickel (Ni) is required in small amounts, it plays an important role in plants because it is required for the assimilation of nitrogen (N) which in turn is crucial for plant biomass development. To not have excess Ni absorption affect plant growth adversely, Ni in the substrate must be at low concentrations. In this work the concentration of Ni in water was less than 0.01 mg/L, which allowed adequate growth of the plant. The highest concentration of Ni (Fig. 13d) was found with the MWCNT _2 treatment in the aerial part injected with nanoparticles (0.0934 mg/L) and lower in the root (0.0116 mg/L). Because of its association with N assimilation and thereby to plant biomass, a comparison between Fig. 8 (*E. crassipes* biomass) and Fig. 13d might be instructive. We note the inverse correlation of the Ni concentrations in *E.crassipes* to the plant biomass, the lowest biomass achieved being for the MWCNT_2 treatment and for the Control where the cellular Ni concentrations were relatively the highest. For all other treatments where the Ni concentrations were low as required of its trace status, the biomasses were relatively higher.

Cu

Figure 13e shows that the highest concentration of Cu was found in the Control (0.58 g/L), followed by the MWCNT _2 treatment (0.51 mg/L), both for the non-injected aerial part. For the rest of the NP treatments, the tissue Cu concentrations were low. Unlike the previous elements presented, the injected aerial parts containing the NPs accumulated less Cu, whereas the non-injected parts which included new shoots, accumulated more. The root also shows sizable accumulation. The Cu homeostasis mechanism in plants regulates Cu to lie in a small range of low concentrations because of its potent toxicity to the photosynthetic apparatus, for which reason even in Cu tolerant plants, Cu is mostly localized in the roots (Dasgupta-Schubert et al, 2011). The same mechanism may be operative in the present case. Root to shoot ion transport mechanisms may favour the transport of other metal ions over Cu, leaving the root relatively enriched in Cu (Jin et al. 2021; Irshad et al. 2021). The Cu that does enter the shoot faces a different environment in the NP injected part. The MWCNT and TiNT are redox catalysts as mentioned previously in connection with Fig. 12, so it is plausible that in their vicinity such properties could interfere in the redox reactions involved in Cu assimilation and homeostasis locally, leaving the Cu ions to traverse other parts, including possibly exudation at the root.

Zn

Zinc is another essential micronutrient for plant growth and development. Its function is aimed at the formation of various enzymes involved in protein synthesis. This element is also associated with the regulation of stress in the plant, strengthening cell membranes and helping cells to maintain their structure and function in unfavourable environmental conditions (González et al. 2018). It is also important for the synthesis of chlorophyll. Figure 13f shows the variation of the Zn concentration across treatment types in the plant. Except for the TiNT_5 treatment, Zn values were approximately equal in all treatments with no apparent preference for accumulation in the injected aerial part. Li et al. (2016) mention that Zn, is found in greater proportion in the roots, but in this work only the treatment with TiNT_2 showed a higher level in the root (1.71 mg/l); for all other treatments the preferred location was the aerial part, indicating that the solubilized Zn species ascended the plant unimpeded through water inflow. The highest content was shown in the injected aerial part of TiNT_5 where it reached the high value of 4.51 mg/l. The chlorophyll levels (Fig. 10) were apparently uncorrelated with the Zn concentration profile, except in the case of TiNT_5 where the relatively high content of Zn in the injected aerial part might have impeded chlorophyll development. Figure 10 shows that for TiNT_5 the chlorophyll level fell below even that of the Blank plant. Cherif et al report that high Zn concentrations depress total chlorophyll content (Cherif et al 2010).

The toxic HE concentrations are shown in Fig. 14. These elements have no known physiological usefulness. Consequently, plants have a low tolerance threshold for them. Plants limit the activity of these HE by several strategies which include restricting their uptake and transport to the sensitive aerial parts. However, once within the plant tissue, they are sequestered in cellular vacuoles aided by proteins such as phytochelatins, metallothioneins, secondary metabolites etc. (Riyazuddin et al, 2021). The goal of the phytoremediation of polluted environments is to select plants that either have the innate ability to accumulate such HE or engineer suitable plants to achieve that end. In the following we examine how the NP treated *E. crassipes* plant performs vis à vis the accumulation of certain known HE offenders.

Cr

The most stable oxidation states of Cr are Cr(VI) and Cr(III). Their bioavailability in soil and their translocation and toxicity within the plant vary (Prieto-Méndez et al. 2009) but Cr(VI) is generally considered as the more toxic of the two. Because of its toxicity, plants tend to limit the translocation of Cr to the

aerial parts with the result that Cr in plants is localised in the root system (López-Velásquez, 2022). In this work, one finds the aerial part of the plant, particularly the one injected with the NP, to show consistently higher concentrations than the rest of the plant parts. The injected parts for the treatments with TiNT_2 and TiNT_3 showed the highest values at 0.27 and 0.28mg/l respectively which were more than double that of the Control. Furthermore, Cr in the plant root of the TiNT_3 treatment was below the detection limit. Despite the storage of Cr in the sensitive aerial parts, all plants remained healthy producing adequate biomass (Figs. 7 and 8). This likely means that the plant spends little of its ATP energy currency to take up and transport Cr to the aerial parts, suggesting that the element simply travels up by water inflow through apoplastic vias. This phytoextractive ability of the NP engineered *E.crassipes* for Cr, is remarkable given the rarity of Cr phytoextracting plants. It strongly suggests leveraging this plant-NP synergism for phytoextraction towards water pollution control. So far NPs have been limited largely to use in synthetic membranes for water treatment (Chávez-Lizarraga 2018).

As

In plants, this element causes growth inhibition, a decrease in photosynthesis, adversely affects metabolic processes and even causes a decrease in seed germination (Prieto-Méndez et al. 2009). As Fig. 14b demonstrates, As concentrations overall were relatively low. This is to be expected because environmentally As is largely present as the anions arsenate $(AsO_4)^{3-}$ or arsenite $(AsO_3)^{3-}$ in water bodies where it precipitates to the sediment as insoluble species when bound to some dissolved cations like Ca^{2+}. In this work for all the NP treatments, As was poorly transported to the aerial parts as compared to the Control. In fact for the treatments TiNT_2 and TiNT_3, As was more concentrated in the roots than in the aerial parts. The exception was the treatment TiNT_5 where the non-injected aerial part registered an unusually large concentration. Nonetheless the biomasses for this treatment (Figs. 7 and 8), although suffering a decrement as compared to the other NP treatments, were still comparable to those of the Control, revealing the restorative effect of this photocatalytic NP for the whole plant (Roy et al. 2011).

Cd

The presence of this element especially in the aerial parts is known to negatively impact plant growth and development by interfering in physiological/biochemical processes (Bonanno & Lo Giudice 2010; Prasad & Maiti, 2016). Figure 14c indicates that Cd was localised more in the injected aerial parts except for the TiNT_5 treatment where the concentration profile was more akin to the Control's. The concentration in the injected part for the TiNT_3 treatment was the highest. Cd possesses no physiological importance hence the relatively higher concentration in the aerial parts arises from the same mechanism of passive water borne transport alluded to earlier for other toxic HE. Li et al. (2016) mention that high Cd content of the water where *E. crassipes* grows, leads to decreased plant growth, as well as chlorosis. The high Cd concentration in the aforesaid plant parts as well as the high Cd content of the polluted water (see section 4.4) however did not affect the plant biomasses (Figs. 7and 8) or the chlorophyll contents (Fig. 10) which again reinforces the idea that the TiNT could be adsorbing this HE on its active sites as well as aiding the plant´s biochemistry for the management of HE toxicity.

Br

Bromine is considered as a non-essential and non-damage-causing element to plants. It is used as an additive in pesticides, dyes, medicines, among other chemicals. Consequently, with the increase of the world´s population and industrial output, this element is likely to become a future burden to the environment (Leri et al. 2024). Figure 14d shows that water hyacinth takes up more Br upon treatment with the NP and localizes it preferentially in the injected aerial parts. The concentrations peak with the TiNT_2 and TiNT_3 treatments. Thus, this notable phytoextractive ability could serve as a guideline for future phytoremediative technologies for the clean-up of Br contaminated waters.

The goal of phytoremediation is the removal of toxic HE from the environment by having plants take-up and store the element preferably in its aerial parts where it can be easily removed. The total toxic HE yields, expressed as mg of the total toxic HE mass per Kg dry plant biomass are shown in Fig. 15 where the highest yields were found to occur of the NP treated plants and in particular for the injected aerial part, so much so that the average yield for the aerial part for the NP treatments surpassed the same for the Control by ~ 33 mg/Kg. The highest yield was found for the TiNT_3 treatment and comparatively the least for the TiNT_5 treatment. The greater efficacy of low concentrations of TiNT in general over the MWCNT indicates that the former´s photocatalytic property augments the anti-ROS properties of these nanomaterials alluded to earlier but that at higher concentrations the toxicity of the TiNT comes to bear. It must be borne in mind that the NPs were injected into shallow layers of the plant tissue where the UV component of the ambient light of the growth chamber could penetrate and induce photocatalytic activity. The high yield for the TiNT_3 treatment is expected to reduce the chlorophyll level given that this is a well-known effect of toxic HE absorption (Hadad et al 2022). Figure 10 bears out this expectation, but the reduction was not severe enough to affect biomass development – in fact the latter was the highest for the TiNT_3 treatment (section 4.2)

The water hyacinth cellular extracts were also analysed for macronutrients P, S, K and Ca. (Fig. 16). P is an essential nutrient for aquatic plants as it is directly related to important biological processes, including energy transfer within cells, photosynthesis, and root formation and growth (Taiz and Zeiger 1991). The P concentration was the highest in the aerial part of the Control injected with the plant surfactant (section 2.2), whereas the NP treated plants showed no marked preference for the element (Fig. 16a). MWCNT and TiNT possess redox properties where the redox potential aids the photocatalytic property of the TiNT (Roy et al. 2011; Mukherjee et al. 2016). This might possibly have transformed the dissolved P species into poorly soluble forms that would tend to precipitate out intra or extra cellularly. It has to be stressed that the plant-based surfactant used for the dispersion of the NPs (section 2.2) might not have been strong enough (as against commercial surfactants such as Triton-X, for instance) to detach and disperse the P into the cellular extract, so the low P concentrations in the NP treatments could be a consequence of both these occurrences. The "invisible" presence of P in all treatments however, was assured by the robust biomass development demonstrated by the plant (Fig. 8).

Sulphur (S) is another essential element and its main function is in the formation of proteins and enzymes. Although sulphur is not directly related to photosynthesis, this element is present in some enzymes that facilitate the conversion of energy in the cells, making the process more efficient (Taiz and

Zeiger 1991). Figure 16b illustrates that S concentrations are rather varied amongst the treatments, but in general the NP treated plants registered the overall higher whole-plant S contents.

The concentration of potassium (K) was higher in the aerial parts, particularly in the injected aerial parts, and sizably lower in the roots, for all treatments (Fig. 16c). The concentration reached the maximum for the TiNT_3 treatment but fell off for the highest NP concentration used – the TiNT_5. K is one of the most important nutrients for plants. It is considered responsible for triggering enzymes that regulate the opening of stomata for water balance, and favors the development and growth of the plant (Taiz and Zeiger 1991). We note that the biomass pattern (Figs. 7 & 8) roughly concurs with the K concentration profile of Fig. 16c.

Calcium (Ca) is a key element for biological and structural processes, being the main component of the cell wall. This element is involved in the regulation of water balance and its deficiency causes abnormal root development and therefore poor plant growth (Taiz and Zeiger 1991). The trends for the Ca concentrations (Fig. 16d) followed the by-now familiar tendency of the injected aerial parts in the NP treated plants to store relatively the greatest amounts of the element, with the exception of TiNT_5 which showed a relative Ca distribution among its parts similar to the Control´s, except higher in magnitude.

4.4 Water quality before and after phytoremediation

The initial characterization of the water of "Laguna Jovita" was carried out and then compared with the results obtained after the 12 day period of the phytoremediation process. The HE content of the water was only determined initially. The purpose was to ascertain the suitability of the water for the cultivation of the water hyacinth, to assure a low stress environment in order to observe exclusively whether or not the concentrations of nanoparticles (MWCNT and TiNT) were suitable, *i.e.* did not cause stress in, or death of, the plants. The results obtained (Fig. 17) for As, Cr and Cd show that only Cd was outside the maximum permissible limit for reservoirs, lakes and lagoons as stipulated by the Mexican government's norm NOM-001-SAGARPA-2021. The monthly average according to this standard for As, Cr and Cd is 0.1 mg/l, 0.5 mg/l and 0.1mg/l, respectively; the values obtained in this work for these elements were 0.005 mg/l, 0.165 mg/l and 1.55 mg/l respectively, demonstrating that whilst As and Cr were well within the limits, Cd exceeded it by more than an order of magnitude. This makes Cd an element of great interest for the decontamination of the water of the lake because of Cd`s known toxicity to aquatic life. This work offers a low-cost solution towards HE clean-up of such a body of water through the effective coupling of NPs with an efficient phytoremediating plant for maximising the benefit of the technology.

Figure 18a and 18b shows that the lake water (initial_water) has a relatively high degree of alkalinity and hardness which leads one to infer that its ionic content is high. The different treatments reduce the alkalinity with the highest reduction occurring for the treatment with TiNT_3. Water alkalinity largely results from the relative balance of ions containing the (CO_3) species - a preponderance of the bicarbonate form $(HCO_3)^-$ favours alkalinity whereas an excess of the carbonate $(CO_3)^{2-}$ moves the scale towards an acidic pH (Islam and Majumder, 2020). Calcium and magnesium are the main contributors to water hardness which is why total water hardness is expressed as calcium carbonate in mg/l, where the ranges 76–150 mg/l and 151–300 mg/l classify the water as semi-hard and hard respectively (Department of Health, 2024). Figure 18b shows that the lake water (initial _water) can be classified as hard whereas all the NP phytoremediation treatments result in a reduction of total hardness of the water, with levels that allow it to be classified as semi-hard. The optimal range of total hardness for the flourishing of aquatic life is 20–180 mg/l (Echiegu et al. 2018). The treatments with MWCNT and TiNT leave the water with a hardness within this optimal range, demonstrating that they produce no deleterious effects on the larger ecosystem.

The characterization of the initial_water for P shows a concentration of 95.2 µg/L for total phosphorus and 52. 44 µg/L for orthophosphates (Fig. 18c). The concentrations for both species increased in the water phytoremediated by the water hyacinth. This could be due to the decomposition of organic matter, which is directly related to orthophosphates. The NOM-001-SAGARPA-2021 does not specify maximum permissible values for orthophosphates. This P species however, is implicitly within the total P content, for which the aforesaid norm stipulates a maximum monthly average of 5 mg/L. As Fig. 18c shows, all treatments resulted in values below this limit. Thus, we note that no treatment caused alterations in the water that could promote eutrophication (Khan & Ansari 2005).

For nitrogen (N), three of the forms in which it can be found in water bodies were determined, *viz*. the nitrate $(NO_3)^-$, nitrite $(NO_2)^-$ and ammonium ions $(NH_4)^+$ (Fig. 18d). Free ammonia (NH_3) is not likely to have been the form measured, because it is a gas and readily dissolves in water to form the ammonium ion. The nitrate ion is the maximally oxidized and the most stable of the three forms. Free ammonia, followed by nitrite are considered to be the most toxic whereas the least toxic ions are the nitrate and ammonium ions (Camargo et al, 2005). In water bodies the ammonium and nitrite ions are both oxidized to the nitrate by aerobic chemoautotrophic bacteria (Camargo et al 2005), so a preponderance of nitrate is likely to be observed. This is evident in the results for the initial_water (lake water). However, plants tend to take up nitrates preferentially leaving its rhizosphere enriched in ammonia and thereby incrementing its pH (Canna, 2017; Guo et al, 2022. This is clearly seen in Fig. 18d where all plant-bearing water samples demonstrated a higher ammonium ion content and pH > 7 values (Table. 2). However, if this were the only mechanism the levels of ammonium and nitrate in the water would have been approximately inversely correlated. However, they are not, suggesting thereby that ammonium efflux from the plant may also be occurring and at a rate that depends on the treatment type. We say this because the decaying organic matter and the nitrate/nitrite ammonification by anaerobic bacteria in the water are likely to be approximately a constant for all treatments, leaving only the plant treatment as the significant variable. Therefore, the particular NP treatment may have a significant role to play since prominent variability of the ammonium levels is shown by the NP treatments. Low concentrations of both the MWCNT and TiNT produce higher ammonium content. The plant-bearing water also had relatively larger contents of the nitrite ion, even though in absolute terms their concentrations were low. The rhizopheric environments of these waters contained decaying organic matter and therefore possessed nitrifying bacteria. Even though N intermediates such as NO_2^- form during the decomposition, they rarely accumulate in the water because of consumption by the bacteria (Wetzel 1983; Yang et al. 2008). The norm NOM-001-SAGARPA-2021 gives the maximum permissible limit for total nitrogen as 15 mg/L. Figure 18d shows that even for the highest value of 86.88 µg/L corresponding to the MWCNT_3 treatment, this limit was not exceeded.

For the case of suspended solids (Fig. 18e), there was a slight increase in concentration in all treatments compared to the baseline water, which may be due to root hairs suspended in the medium. The high value for the Blank, might have been a happenstance. Suspended solids are solid particles that do not dissolve in water and can be organic or inorganic, contributing to the eutrophication of water bodies and increasing its turbidity. Aquatic plants have been known to reduce turbidity (Austin et al, 2017). The results for water turbidity (Fig. 18f) showed a noticeable decrease of this quantity with the MWCNT_3 and TiNT treatments of the water hyacinth plant, especially with the TiNT_5 treatment. Thus, colloidal particles too may have been removed by certain phytoabsorptive processes of the water hyacinth which were enhanced by the NP treatments.

4.5 Some general observations

In practically all the measurements where both the Blank and Control were measured, the Control showed a different behavioural pattern than the Blank, in most cases registering an increase of the measured parameter. The Control had only the small volume of the plant-based surfactant injected into it, that was predominantly the *E.crassipes* cellular extract itself (section 2.2). This stimulatory effect of the extract on the Control could then possibly be a hormetic response to the injection perceived by the plant as an abnormal biotic entrant.

Remarkably in this work, the predominant location of the HE species whether as micronutrient or as toxic element, was in the aerial part, which is contrary to the findings of most phytoremediation studies where the vast majority of plants are not phytoextractors but rather phytostabilizers, affixing the HE to the root, in order to spare the sensitive photosynthetic organs. There are obvious technological advantages with phytoextraction such as HE removal through harvesting. Metal hyperaccumulators are a class of phytoextractors that display high aerial concentrations, but the plant also becomes stressed in the process, resulting in poor biomasses, which reduce the nett yield of the HE. None of the treatments used in this work resulted in the lowering of biomass due to stress and neither was chlorophyll production inhibited (Figs. 8 and 10). Resultingly relatively high HE yields were obtained from the aerial parts, particularly the part that had been injected (Fig. 15). This points to the utility of the synergy in function between the injected NP and the plants physiological chemistry for the purpose of HE uptake and sequestration when minuscule concentrations of the NP are injected into the plant. The process is very sensitive to concentration, small increases having the capacity to reverse the process. Also, the TiNT nanoparticles are photoreactive, hence the net exposure time to a given light energy *i.e.* the photon dose, would also set a limit to the duration of the experiment. In the present case the total time was restricted to 12 days that set a limit to the dose of ambient light as well as restricted the contribution by the natural die-off of the plant. It is important to avoid the latter, because cell lysis upon death leads to the back-release of the contaminants taken up by the plant

5. Summary

The efficacy of the phytoremediation of polluted lake water from the Laguna Jovita Michoacan, Mexico using the aquatic plant *E. crassipes*, was investigated. Its aerial parts were injected with the NPs, MWCNT and TiNT. These NPs were chosen because of their redox properties, adsorbability and additionally for TiNT, for its photocatalytic property and biocompatibility. Initial trial studies established their plant-tolerated concentrations as 2, 3 mg/l for the MWCNT and 2,3, 5 mg/l for the TiNT.

The NPs were injected into the plant rather than dispersed into the aquatic media in order to immobilize them within the plant instead of potentially having them secondarily contaminate the environment. The clear difference in the results between the injected aerial part of the plant and the rest of the plant parts indicates that the NPs remained largely localised with practically no diffusional migration as anticipated from our previous calculations. Whilst NPs have been used within plants in other ways, this is possibly the first time they are being inserted into the live plant to transform it into a more efficient phytoremediator.

The PR experiment was carried out for 12 days. The initial and final water quality was tested and during the 12 day period, the physico-chemical properties of the root-zone and bulk waters of the containers in which the plants were grown, were measured. Plant cellular extracts from the root, the injected and non-injected aerial parts were analysed for their physico-chemical properties as well as their concentrations of the macro and micronutrient elements and the toxic HE taken up from the water.

The water characterization results indicated that after 12 days, pollution levels in the water had mostly decreased. In almost all cases, the aerial part, in particular the injected aerial part, was found to possess relatively the greatest concentrations of the micronutrient elements and the toxic HE.

The plant-water system showed a high sensitivity to the NP concentrations. In general, low concentrations produced more favourable results for PR. Given that these NPs are both ROS scavengers and generators, the present results suggest that their scavenging of the ROS produced by the toxic HE dominates when their concentrations are low. Overall, the treatment with TiNT at a concentration of 3 mg/l produced the best results.

The highest relative efficiency of the NP injected aerial part with respect to HE concentration indicates that surface adsorption of the HE by the NPs, ROS scavenging and the synergistic effect of the NPs on the redox processes of the plant's biochemical mechanisms of HE detoxification and sequestration, could possibly be involved, with photocatalytic action by the TiNTs producing an additional beneficial contribution.

6. Conclusion

This work establishes *E. crassipes* (water hyacinth) injected with low concentrations of MWCNT and TiNT, but especially the latter, as an efficient phytoextractor of HE and other species producing undesirable effects such as hardness and turbidity in polluted waters, without affecting the aquatic system. With effective management, this nanotechnology aided phytoremediation scheme might serve as a model for the clean-up of polluted water efficiently, cost-effectively and without the generation of secondary pollutants.

Declarations

Acknowledgement

This work was carried out with the support of SECIHTI of Mexico that funded the doctoral study of IES-M (scholarship recipient number 764570) and the Coordination of Scientific Research (CIC) of the University of Michoacan (UMSNH). The authors are grateful for the collaboration of M.C. Luis María Suarez Rodríguez of UMSNH, Dr. David García Hernández of the Technical University of Morelia (UTM) and Biol. Claudio Osuna Paredes of the Regional Center for Aquatic and Fisheries Research (CRIAP), Mexican Institute of Research in Fishery and Sustainable Agriculture (IMIPAS).

Conflict of interest:

There was no conflict of interest involved in this work.

Role of authors:

IES-M: assembly of resources and execution of experiment, writing of the draft manuscript

VA, EA-V, MGGR, RL-G: consultations; supply of materials and some specific laboratory tools and assistance with their implementation.

NDS: conceptualization and planning of the work, guidance on execution and data interpretation, final manuscript edition and writing

Data availability:

Data of this work are available upon request.

Author Contribution

Role of authors: I.E. S-M.: assembly of resources and execution of experiment, writing of the draft manuscriptV.A., E.A-V., Ma. G. G.R., R. L-G.: consultations; supply of materials and some specific laboratory tools and assistance with their implementation.N. D.S.: conceptualization and planning of the work, guidance on execution and data interpretation, final manuscript edition and writing .All authors reviewed the manuscript.

References

1. Abinaya S, Saraswathi R, Rajamohan S, Mohammed SA (2018) Phyto-remediation of total dissolved solids (TDS) by Eichhornia Crassipes, Pistia Stratiotes and Chrysopogon Zizanioides from second stage RO-Brine solution. Res. J. Chem. Environ, 22(5):36-41. https://www.researchgate.net/publication/324774342_Phyto-remediation_of_total_dissolved_solids_TDS_by_Eichhornia_Crassipes_Pistia_Stratiotes_and_Chrysopogon_Zizanioides_from_second_stage_RO-Brine_solution
2. Alguacil FJ, Cerpa A, Lado I, López Gómez FA (2014) Technologies for the 21st century: carbon nanotubes as adsorbents of metals. https://doi.org/10.3989/revmetalm.025
3. Austin ÅN, Hansen JP, Donadi S, Eklöf JS (2017) Relationships between aquatic vegetation and water turbidity: A field survey across seasons and spatial scales. PLOS one, 12(8), e0181419. https://doi.org/10.1371/journal.pone.0181419
4. Azcón-Bieto J, Talón M (2008) Fundamentals of plant physiology. 2nd edn McGraw-Hill-Interamericana. (in Spanish)
5. Barceló J & Poschenrieder C (2003) Phytoremediation: principles and perspectives. Contributions to science, 2(3), 333-344. https://raco.cat/index.php/Contributions/article/view/14273/351950
6. Barceló J, Poschenrieder C, Gunsé B (1987) The impact of chromium on the environment. II. Chromium in living organisms. Cir Far, 293, 31-48. (in Spanish) https://www.researchgate.net/profile/Charlotte-Poschenrieder/publication/276955606_El_impacto_del_cromo_en_el_medio_ambiente_II_El_Cr_en_los_organismos_vivos/links/555c923408ae8c0cab2a impacto-del-cromo-en-el-medio-ambiente-II-El-Cr-en-los-organism
7. Benavides-Mendoza A & Francisco-Francisco N (2022) Recent applications of chlorophyll fluorescence in plant crops. Epistemus (Sonora), 16(33), 106-114. (in Spanish). https://doi.org/10.36790/epistemus.v16i33.285
8. Berni R, Luyckx M, Xu X, Legay S, Sergeant K, Hausman JF, Luts S, Cai G, Guerriero G (2019) Reactive oxygen species and heavy metal stress in plants: Impact on the cell wall and secondary metabolism. Environmental and Experimental Botany, 161, 98-106. https://doi.org/10.1016/j.envexpbot.2018.10.017
9. Betancourt C & Labaut Y (2013) The physicochemical quality of water in reservoirs, main variables to be considered. Revista Científica Agroecosistemas, 1(1). http://rid.unrn.edu.ar/handle/20.500.12049/8257 (Accesed 26 November 2024)
10. Bonanno G, Giudice RL (2010) Heavy metal bioaccumulation by the organs of Phragmites australis (common reed) and their potential use as contamination indicators. Ecological indicators, 10(3), 639-645. https://doi.org/10.1016/j.ecolind.2009.11.002
11. Bruker (2013) Sample types and preparation TXRF. Germany. https://www.bruker.com/applications/environmental.html (Accesed 26 November 2024)
12. Camargo JA, Alonso A, Salamanca A (2005) Nitrate toxicity to aquatic animals: a review with new data for freshwater invertebrates. Chemosphere, 58(9), 1255-1267. https://doi.org/10.1016/j.chemosphere.2004.10.044
13. Canillas M, Chinarro E, Freitas M, Pêgo AP, Moreno B (2020) Titanium dioxide catalytic activity contributes to the process of free radical scavenging. Journal of Catalysis, 381, 186-192. https://doi.org/10.1016/j.jcat.2019.09.030

14. Canna (2017) The solution for growth and bloom. How the ammonium-nitrate ratio affects your plants. https://www.cannagardening.com/articles/how-ammonium-nitrate-ratio-affects-your-plants (Accessed 22/Feb/2025)
15. Carrión C, Ponce-de León C, Cram S, Sommer I, Hernández M, Vanegas C (2012) Potential use of water lily (Eichhornia crassipes) in Xochimilco for phytoremediation of metals. Agrociencia, 46(6), 609-620. (in Spanish) https://www.scielo.org.mx/pdf/agro/v46n6/v46n6a7.pdf
16. Chavan S, Sarangdhar V, Nadanathangam V (2020) Toxicological effects of TiO2 nanoparticles on plant growth promoting soil bacteria. Emerging Contaminants, 6, 87-92. https://doi.org/10.1016/j.emcon.2020.01.003
17. Chávez-Lizárraga GA (2018) Nanotechnology as an Alternative for Wastewater Treatment: Advances, Advantages and Disadvantages. Journal of the Selva Andina Research Society, 9(1), 52-61. (in Spanish) http://www.scielo.org.bo/scielo.php?pid=S2072-92942018000100005&script=sci_arttext
18. Chen G, Qiu J, Liu Y, Jiang R, Cai S, Liu Y, Ouyang G (2015) Carbon Nanotubes Act as Contaminant Carriers and Translocate within Plants. Scientific Reports, 5: 15682. https://doi.org/10.1038/srep15682
19. Cherif J, Derbel N, Nakkach M, Von Bergmann H, Jemal F, Lakhdar ZB (2010) Analysis of in vivo chlorophyll fluorescence spectra to monitor physiological state of tomato plants growing under zinc stress. Journal of Photochemistry and Photobiology B: Biology, 101(3), 332-339. https://doi.org/10.1016/j.jphotobiol.2010.08.005
20. Dasgupta-Schubert N, Barrera MG, Alvarado CJ, Castillo OS, Zaragoza EM, Alexander S, Landsberger S, Robinson S (2011) The uptake of copper by Aldama dentata: ecophysiological response, its modeling, and the implication for phytoremediation. Water, Air, & Soil Pollution, 220, 37-55. https://doi.org/10.1007/s11270-010-0733-1
21. Delgadillo-López AE, González-Ramírez CA, Prieto-García F, Villagómez-Ibarra JR, Acevedo-Sandoval O (2011) Phytoremediation: an alternative for eliminating pollution. Tropical and subtropical agroecosystems, 14(2), 597-612. https://www.scielo.org.mx/scielo.php?pid=S1870-04622011000200002&script=sci_abstract&tlng=pt
22. Department of Health (2024) Hardness in drinking water. Vermont, USA. https://www.healthvermont.gov/environment/drinking-water/hardness-drinking-water (Accessed 20 Feb 2025).
23. Dickinson NM, Baker AJ, Doronila A, Laidlaw S, Reeves RD (2009) Phytoremediation of inorganics: realism and synergies. International Journal of Phytoremediation, 11(2), 97-114. https://doi.org/10.1080/15226510802378368
24. Echiegu EA, Ezeugwu LI, Ugwu SN (2018) Effects of water hyacinth (Eichhornia crassipes) on the physicochemical properties of fishpond water and growth of African catfish. African Journal of Agricultural Research, 13(2), 54-66. https://academicjournals.org/journal/AJAR/article-full-text-pdf/9B5356F55627
25. Eid EM, Shaltout KH, Moghanm FS, Youssef MSG, El-Mohsnawy E, Haroun SA (2019) Bioaccumulation and translocation of nine heavy metals by Eichhornia crassipes in Nile Delta, Egypt: perspectives for phytoremediation. International journal of phytoremediation, 21(8), 821–830. https://doi.org/10.1080/15226514.2019.1566885
26. Fahruddin F (2020) Absorption of heavy metal lead (Pb) by water hyacinth (Eichhornia crassipes) and its influence to total dissolved solids of groundwater in phytoremediation. Jurnal Akta Kimia Indonesia (Indonesia Chimica Acta), 10-15. https://doi.org/10.20956/ica.v13i1.9977
27. Fenoglio I, Tomatis M, Lison D, Muller J, Fonseca A, Nagy JB, Fubini B (2006) Reactivity of carbon nanotubes: free radical generation or scavenging activity?. Free Radical Biology and Medicine, 40(7), 1227-1233. https://doi.org/10.1016/j.freeradbiomed.2005.11.010
28. Garbisu C, Alkorta I (2001) Phytoextraction: a cost-effective plant-based technology for the removal of metals from the environment. Bioresource technology, 77(3), 229-236. https://doi.org/10.1016/S0960-8524(00)00108-5
29. González Alejandre M, Agarwal V, Martínez Trujillo M, González Cortes J. C, Dasgupta-Schubert N (2021) Nanomaterial-aided seed regeneration in the global warming scenario: multiwalled carbon nanotubes, gold nanoparticles and heat-aged maize seeds. Applied Nanoscience, 11, 1531-1547. https://doi.org/10.1007/s13204-021-01804-9
30. González-García Y, Cadenas-Pliego G, Alpuche-Solís ÁG, Cabrera RI, Juárez-Maldonado A (2021). Carbon nanotubes decrease the negative impact of Alternaria solani in tomato crop. Nanomaterials, 11(5), 1080. https://doi.org/10.3390/nano11051080
31. González CI, Maine MA, Cazenave J, Hadad HR, Benavides MP (2015) Ni accumulation and its effects on physiological and biochemical parameters of Eichhornia crassipes. Environmental and Experimental Botany, 117, 20-27. https://doi.org/10.1016/j.envexpbot.2015.04.006
32. González CI, Maine MA, Hadad HR, Sanchez GC, Benavides MP, Campagnoli MA (2018) Effects on Eichhornia crassipes under Zn stress. Environmental Science and Pollution Research, 25, 26957-26964. https://doi.org/10.1007/s11356-018-2741-4
33. Gupta GC (1980) Use of water hyacinths in wastewater treatment (a brief literature review). Journal of Environmental health, 80-82. https://www.jstor.org/stable/44537520 (Accessed 28 November 2024)
34. Hadad HR, Maine MA, Mufarrege MM, Del Sastre MV, Di Luca GA (2011) Bioaccumulation kinetics and toxic effects of Cr, Ni and Zn on Eichhornia crassipes. Journal of hazardous materials, 190(1-3), 1016-1022. https://doi.org/10.1016/j.jhazmat.2011.04.044
35. Hsieh HS, Wu R, Jafvert CT (2014) Light-independent reactive oxygen species (ROS) formation through electron transfer from carboxylated single-walled carbon nanotubes in water. Environmental science & technology, 48(19), 11330-11336. https://doi.org/10.1021/es503163w
36. Irshad MA, Nawaz R, Zia ur Rehman M, Adrees M, Rizwan M, Ali S, Ahmad S, Tasleem S (2021) Synthesis, characterization and advanced sustainable applications of titanium dioxide nanoparticles: A review. Ecotoxicology and environmental safety, 212, 111978. https://doi.org/10.1016/j.ecoenv.2021.111978
37. Islam MS & Majumder SMMH (2020) Alkalinity and hardness of natural waters in Chittagong City of Bangladesh. International Journal of Science and Business, 4(1), 137-150. https://doi.org/10.5281/zenodo.3606945

38. Jiménez GS, Ducoing HP, Sosa MR (2003) Involvement of secondary metabolites in plant defence. Revista mexicana de fitopatología, 21(3), 355-363. (in Spanish) https://www.researchgate.net/publication/242671743_La_Participacion_de_los_Metabolitos_Secundarios_en_la_Defensa_de_las_Plantas
39. Jin MF, You MX, Lan QQ, Cai LY, Lin MZ (2021) Effect of copper on the photosynthesis and growth of Eichhornia crassipes. Plant Biology, 23(5), 777–784. https://doi.org/10.1111/plb.13281
40. Khan FA, Ansari AA (2005) Eutrophication: an ecological vision. The botanical review. 71(4), 449-482. https://doi.org/10.1663/0006-8101(2005)071[0449:EAEV]2.0.CO;2
41. Kleiber T, Markiewicz B (2013) Application of "Tytanit" in greenhouse tomato growing. Acta Scientiarum Polonorum. Hortorum Cultus, 12(3), 117-126. DOI: 10.24425/jwld.2023.143738
42. Leonavičius D, Pundienė I, Girskas G, Pranckevičienė J, Kligys M, Sinica M (2017) The influence of carbon nanotubes on the properties of water solutions and fresh cement pastes. In IOP Conference Series: Materials Science and Engineering (Vol. 251, No. 1, p. 012023). IOP Publishing. DOI 10.1088/1757-899X/251/1/012023
43. Leri AC, Hettithanthri O, Bolan S, Zhang T, Unrine J, Myneni S, Nachman DR, Tran HT, Phillips AJ, Hou D, Wang Y, Vithanage M, Padhye LP, Zad TJ, Heitz A, Siddique KHM, Wang H, Rinklebe J, Kirkham MB, Bolan N (2024) Bromine contamination and risk management in terrestrial and aquatic ecosystems. Journal of Hazardous Materials, 133881. https://doi.org/10.1016/j.jhazmat.2024.133881
44. Li M, Yin JJ, Wamer WG, Lo YM (2014) Mechanistic characterization of titanium dioxide nanoparticle-induced toxicity using electron spin resonance. Journal of Food and Drug Analysis, 22(1), 76-85. https://doi.org/10.1016/j.jfda.2014.01.006
45. Li Q, Zhan J, Chen B, Meng X, Pan X (2016) Removal of Pb, Zn, Cu, and Cd by two types of Eichhornia crassipes. Environmental Engineering Science, 33(2), 88-97. https://doi.org/10.1089/ees.2015.0091
46. Lichtenthaler HK (1987) Chlorophylls and carotenoids: pigments of photosynthetic biomembranes. In Methods in enzymology. 148, 350-382 https://doi.org/10.1016/0076-6879(87)48036-1
47. López Velásquez A (2022) Efficiency of phytoextraction of heavy metals from waters of the Prieto River, Santa Bárbara Almoloya, Puebla. Dissertation, Benemérita Universidad Autónoma de Puebla. https://repositorioinstitucional.buap.mx/items/a05046ce-1537-4fe6-bf86-41c8bb806b48 (Accessed 26 November 2026)
48. Margulis L, & Sagan D (2008). The process of nutrition in plants. Fundamentals of plant physiology. 242-258. (in Spanish) https://www.mheducation.es/bcv/guide/capitulo/8448180895.pdf
49. Mathew S, Tiwari DK, Tripathi D (2021) Interaction of carbon nanotubes with plant system: A review. Carbon Letters, 31(2), 167-176. https://doi.org/10.1007/s42823-020-00195-1
50. Mattiello A, Lizzi D, Marchiol L (2018) Influence of titanium dioxide nanoparticles (nTiO2) on crop plants: a systematic overview. Nanomaterials in Plants, Algae, and Microorganisms, 277-296. https://doi.org/10.1016/B978-0-12-811487-2.00012-8
51. Mehta J, Ali GA, Singh GP, Althobiti RA, Bauddh K (2024) Phytoremediation of metal nanoparticles from the contaminated water bodies: A review. Egyptian Journal of Chemistry. DOI: 10.21608/ejchem.2024.233361.8612
52. Mukherjee A, Majumdar S, Servin AD, Pagano L, Dhankher OP, White JC (2016) Carbon nanomaterials in agriculture. Frontiers in plant science, 7: 172. https://doi.org/10.3389/fpls.2016.00172
53. Narváez Valderrama JF, Palacio Baena JA, Molina Pérez FJ (2012) Persistence of pesticides in the environment and their ecotoxicity: A review of natural degradation processes. Gestión y ambiente, 15(3), 27-37. (in Spanish) https://www.redalyc.org/pdf/1694/169424893002.pdf
54. NORMA Oficial Mexicana NOM-001-SEMARNAT-2021, Que establece los límites permisibles de contaminantes en las descargas de aguas residuales en cuerpos receptores propiedad de la nación. https://www.dof.gob.mx/nota_detalle.php?codigo=5645374&fecha=11/03/2022#gsc.tab=0 (Accesed 25 November 2025)
55. Pedraza-Guevara MS (2021) Fitorremediación en cuerpos de agua contaminados por metales pesados. Innova Biology Sciences, 1(1), 61-78. https://dialnet.unirioja.es/servlet/articulo?codigo=8600053
56. Prasad B, & Maiti D (2016) Comparative study of metal uptake by Eichhornia crassipes growing in ponds from mining and nonmining areas—a field study. Bioremediation Journal, 20(2), 144–152. https://doi.org/10.1080/10889868.2015.1113924
57. Prieto-Méndez J, Gutierrez-Ramírez CA, Román-Gutiérrez AD, Prieto-García F (2009) Contamination and phytotoxicity of plants by heavy metals from soils and water. Tropical and subtropical Agroecosystems, 10(1),29-44. https://www.redalyc.org/pdf/939/93911243003.pdf
58. Putra RS, Novarita D, and Cahyana F (2016) Remediation of lead (Pb) and copper (Cu) using water hyacinth (Eichornia crassipes (Mart.) Solms) with electro-assisted phytoremediation (EAPR). In AIP Conference Proceedings (Vol. 1744, No. 1). AIP Publishing. https://doi.org/10.1063/1.4953526
59. Qi M, Liu Y, Li T (2013) Nano-TiO 2 improve the photosynthesis of tomato leaves under mild heat stress. Biological trace element research, 156, 323-328. https://doi.org/10.1007/s12011-013-9833-2
60. Reis AT, Costa C, Fraga S (2023) Editorial of Special Issue: The Toxicity of Nanomaterials and Legacy Contaminants: Risks to the Environment and Human Health. International Journal of Molecular Sciences, 24(14), 11723. https://doi.org/10.3390/ijms241411723
61. Rezania S, Ponraj M, Talaiekhozani A, Mohamad SE, Din MFM, Taib SM, Farzaneh S, Sairan FM (2015) Perspectives of phytoremediation using water hyacinth for removal of heavy metals, organic and inorganic pollutants in wastewater. Journal of environmental management, 163, 125-133. https://doi.org/10.1016/j.jenvman.2015.08.018
62. Riyazuddin R, Nisha N, Ejaz B, Khan MIR, Kumar M, Ramteke PW, Gupta R (2021) A comprehensive review on the heavy metal toxicity and sequestration in plants. Biomolecules, 12(1), 43. doi: 10.3390/biom12010043

63. Rodríguez Alejandre JL, Vijay RS, Acosta Sánchez LA, Dasgupta-Schubert N (2023). Total Reflection Xray Fluorescence (TXRF) spectrometry as a powerful and broad-spectrum analytical tool in the nuclear sciences. In Journal of Physics: Conference Series (Vol. 2619, No. 1, p. 012014). IOP Publishing. doi:10.1088/1742-6596/2619/1/012014
64. Rodríguez-Lara JW, Cervantes-Ortiz F, Arámbula-Villa G, Mariscal-Amaro LA, Aguirre-Mancilla CL, Andrio-Enríquez E (2022) Water lily (Eichhornia crassipes): a review. Agronomía Mesoamericana, 44201-44201. (in Spanish) https://www.scielo.sa.cr/pdf/am/v33n1/2215-3608-am-33-01-00024.pdf
65. Roy P, Berger S, Schmuki P (2011) TiO2 nanotubes: synthesis and applications. Angewandte Chemie International Edition, 50(13), 2904-2939. https://doi.org/10.1002/anie.201001374
66. Rusydi AF (2018) Correlation between conductivity and total dissolved solid in various type of water: A review. In IOP conference series: earth and environmental science. 118, 012019. IOP publishing. https://doi.org/10.1088/1755-1315/118/1/012019
67. Šebesta M, Kolenčík M, Sunil BR, Illa R, Mosnáček J, Ingle AP, Urík M (2021) Field application of ZnO and TiO2 nanoparticles on agricultural plants. Agronomy, 11(11), 2281. https://doi.org/10.3390/agronomy11112281
68. Selmani A, Siboulet B, Špadina M, Foucaud Y, Dražić G, Radatović B, Korade K, Nemet I, Kovačević D, Dufrêche JF, Bohinc K (2023) Cation Adsorption in TiO2 Nanotubes: Implication for Water Decontamination. ACS applied nano materials, 6(14), 12711-12725. https://doi.org/10.1021/acsanm.3c00916
69. Sembada AA, Lenggoro IW (2024) Transport of nanoparticles into plants and their detection methods. Nanomaterials, 14(2), 131. https://doi.org/10.3390/nano14020131
70. Taiz L, Zeiger E (1991) Plant Physiology. 2nd Ed. Benjamin/Cummings Publishing Company, Inc.
71. Teuchies J, Jacobs S, Oosterlee L, Bervoets L, Meire P (2013) Role of plants in metal cycling in a tidal wetland: Implications for phytoremidiation. Science of the Total Environment, 445, 146-154. https://doi.org/10.1016/j.scitotenv.2012.11.088
72. Tiwari DK, Dasgupta-Schubert N, Villaseñor-Cendejas LM, Villegas J, Carreto-Montoya L, Borjas-García SE (2014) Interfacing carbon nanotubes (CNT) with plants: enhancement of growth, water and ionic nutrient uptake in maize (Zea mays) and implications for nanoagriculture. Applied Nanoscience, 4, 577-591. https://doi.org/10.1007/s13204-013-0236-7
73. Viehweger K (2014). How plants cope with heavy metals. Botanical Studies, 55, 1-12. https://doi.org/10.1186/1999-3110-55-35
74. Waghmode MS, Gunjal AB, Mulla JA, Patil NN, Nawani NN (2019) Studies on the titanium dioxide nanoparticles: Biosynthesis, applications and remediation. SN Applied Sciences, 1(4), 310. https://doi.org/10.1007/s42452-019-0337-3
75. Wani AK, Khan Z, Sena S, Akhtar N, Alreshdi MA, Yadav KK, Alkahtani AM, Waheed WA, Rahayu F, Tafakresnanto C, Latifah E, Hariyono B, Arifin Z, Eltayeb LB (2024) Carbon nanotubes in plant dynamics: unravelling multifaceted roles and phytotoxic implications. Plant Physiology and Biochemistry, 108628. https://doi.org/10.1016/j.plaphy.2024.108628
76. Wegner LH, Li X, Zhang J, Yu M, Shabala S, Hao Z (2021) Biochemical and biophysical pH clamp controlling Net H+ efflux across the plasma membrane of plant cells. New Phytologist, 230(2), 408-415. https://doi.org/10.1111/nph.17176
77. Yang XE, Wu X, Hao HL, He ZL (2008) Mechanisms and assessment of water eutrophication. Journal of Zhejiang University Science B, 9, 197-209. https://doi.org/10.1631/jzus.B0710626
78. Zhou JM, Jiang ZC, Qin XQ, Zhang LK, Huang QB, Xu GL, Dionysiou DD (2020) Efficiency of Pb, Zn, Cd, and Mn removal from karst water by Eichhornia crassipes. International Journal of Environmental Research and Public Health, 17(15), 5329. https://doi.org/10.3390/ijerph17155329
79. Zhou W, Zhu D, Tan L, Liao S, Hu Z, Hamilton D (2007) Extraction and retrieval of potassium from water hyacinth (Eichhornia crassipes). Bioresource technology, 98(1), 226-231. https://doi.org/10.1016/j.biortech.2005.11.011

Figures

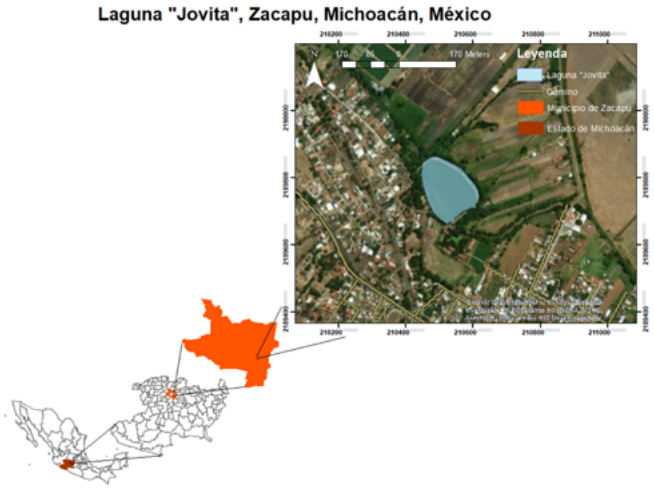


Figure 1

Map of the lake "Jovita", Laguna de Naranja de Tapia, Zacapu municipality, Mich., Mexico.

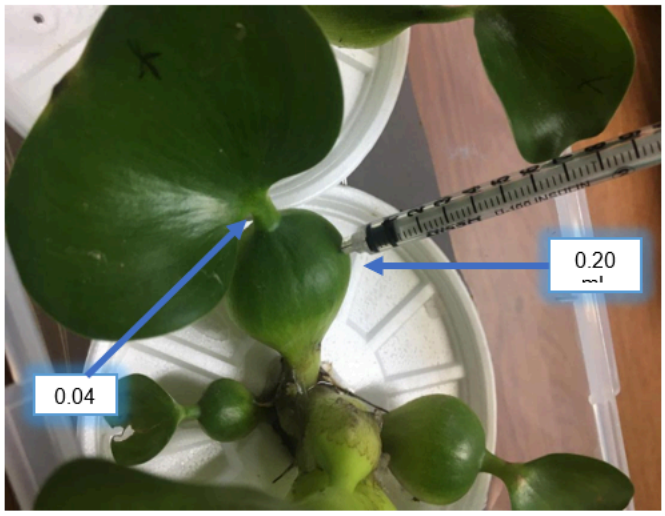


Figure 2

The process of the injection of suspensions pertaining to a specific treatment (see Table 1) into the bulb and the underside of the leaf.



Figure 3

Three plant replicates for each treatment type accommodated in a plastic bucket which separation of the root and aerial parts by biodegradable styrofoam lids.

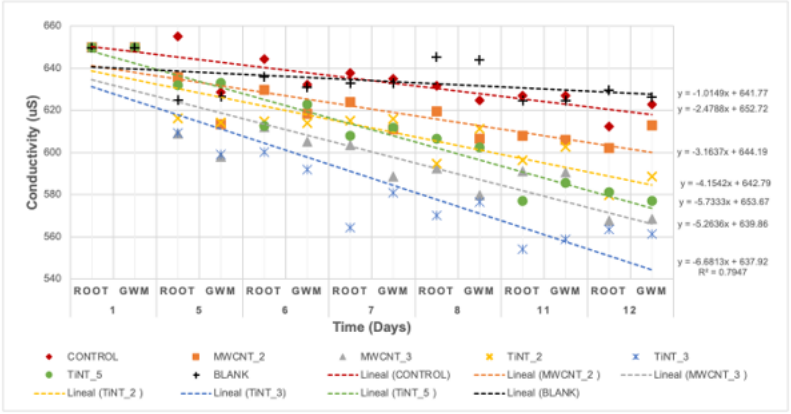


Figure 4

Water conductivity of the bulk (general) and near-root media in which the water hyacinths treated with the MWCNT and TiNT at the concentrations cited in Table 2, were grown. Trend lines and their corresponding equations are shown in the graph.

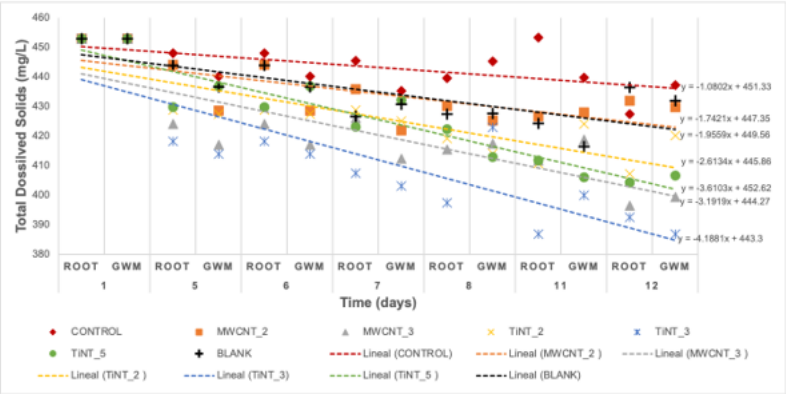


Figure 5

Data, trend lines and their corresponding equations for the total dissolved solids (TDS) variable. The rest are the same as for fig. 4.

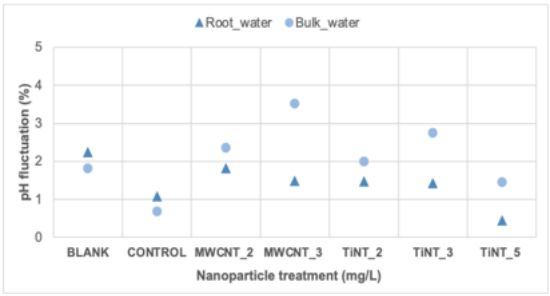


Figure 6

Percentage fluctuation of the pH of water from the phytoremediation process during the 12-day period (refer to section 2.2.1).

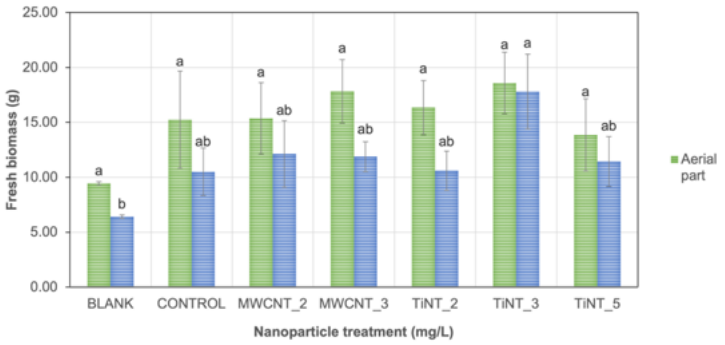


Figure 7

Fresh water hyacinth biomass after harvest (refer to section 2.2.2). The abbreviations and symbols have been described earlier (Table 2).

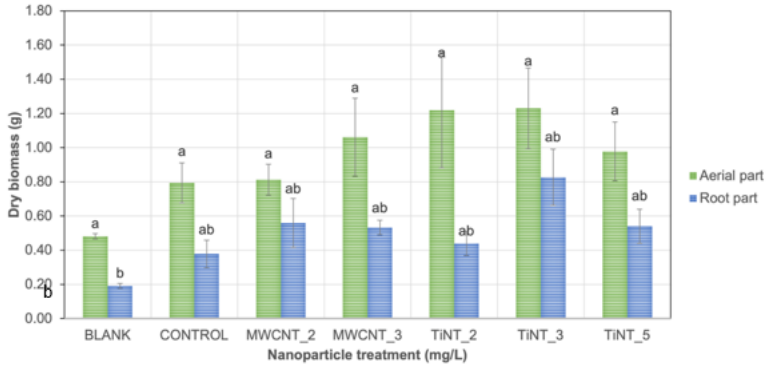


Figure 8

Dry biomass of water hyacinth after harvest (section 2.2.2.2). The abbreviations and symbols are as before.

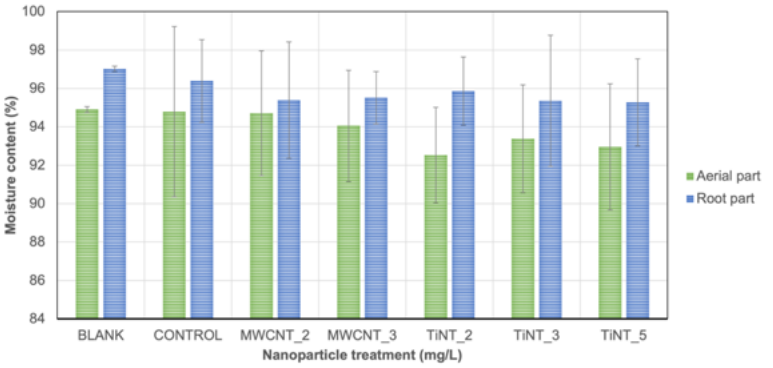


Figure 9

Moisture content of the aerial part and root of the water hyacinths after harvest (section 2.2.2.2). The abbreviations and symbols are as before.

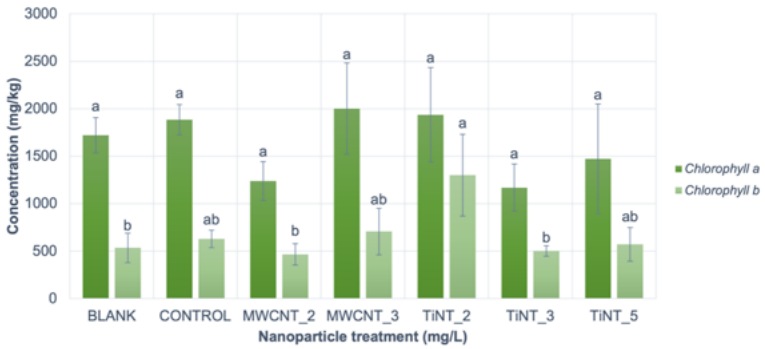


Figure 10

Chlorophyll **a**and **b** in aerial parts of water hyacinth. The abbreviations and symbols are as before.

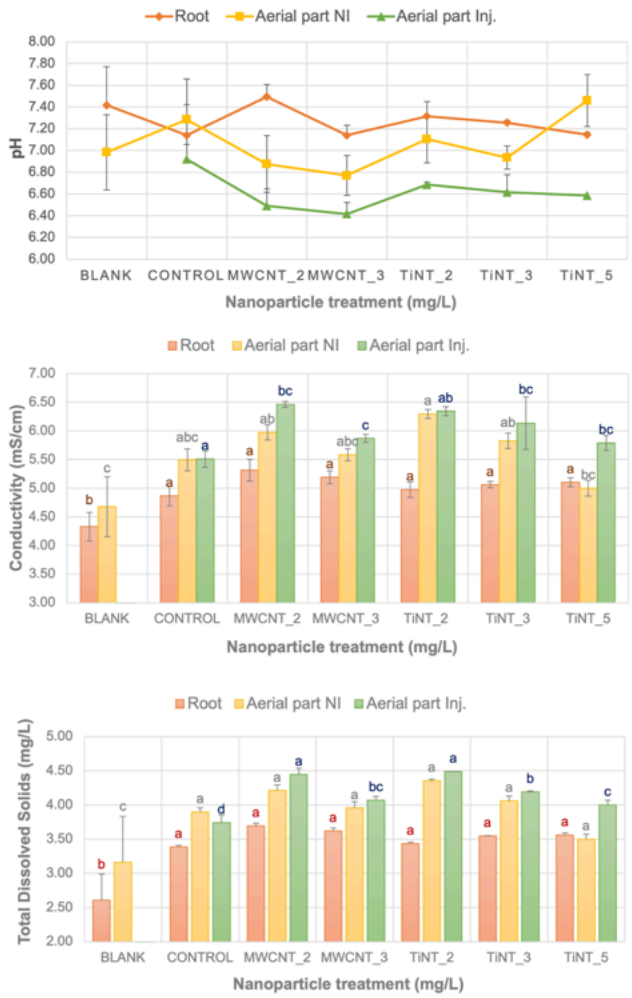


Figure 11

pH, conductivity, and total dissolved solids of the cellular extracts of the water hyacinth plant. Root: water hyacinth root, NI: aerial part not injected, Inj: aerial part injected with nanoparticles. The abbreviations and symbols have their usual meaning.

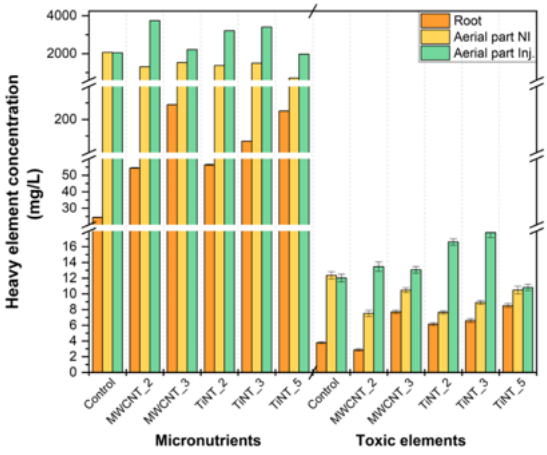


Figure 12

Total HE concentration in the cellular extract of the water hyacinth plant used in aquatic phytoremediation. The legends and abbreviations have their usual meaning.

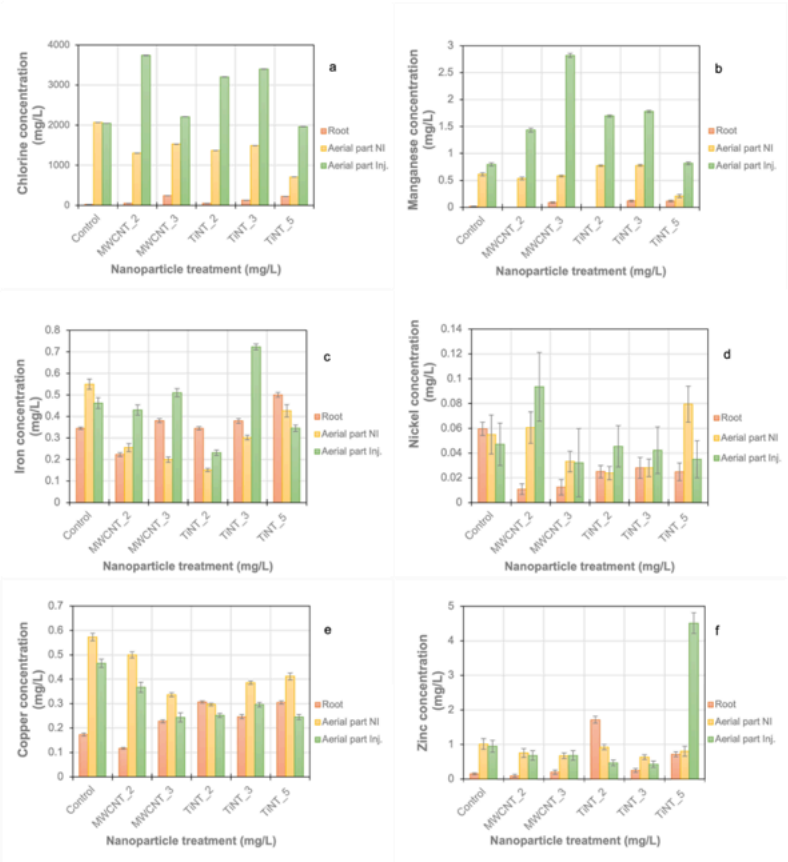


Figure 13

Analysis of the water hyacinth cellular extract for the determination of the micronutrients, chlorine (Cl), manganese (Mn), iron (Fe), nickel (Ni), copper (Cu) and zinc (Zn). The rest of the entries have their usual meanings.

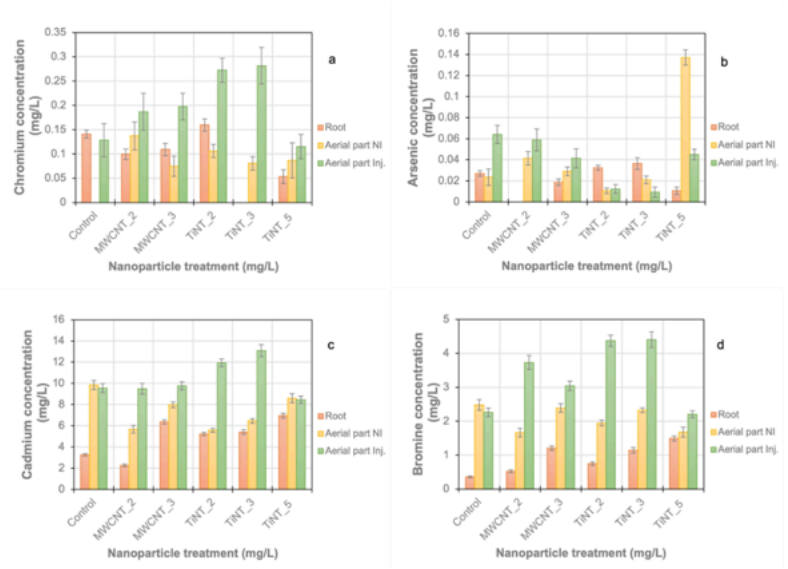


Figure 14

Analysis of water hyacinth cellular extracts for the determination of the toxic elements, arsenic (As), chromium (Cr), cadmium (Cd) and bromine (Br). The abbreviations have their usual meaning.

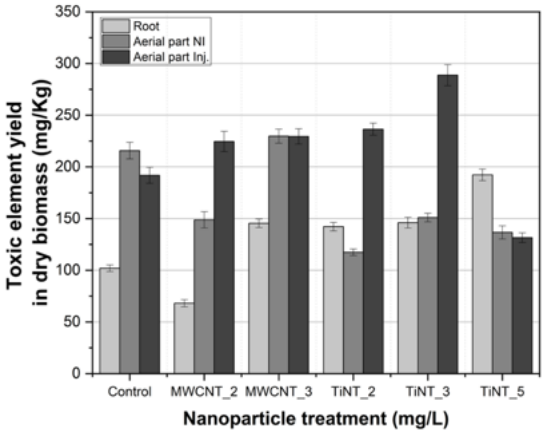


Figure 15

Yield of the sum of (solubilized) toxic elements in the dry biomass of water hyacinth.

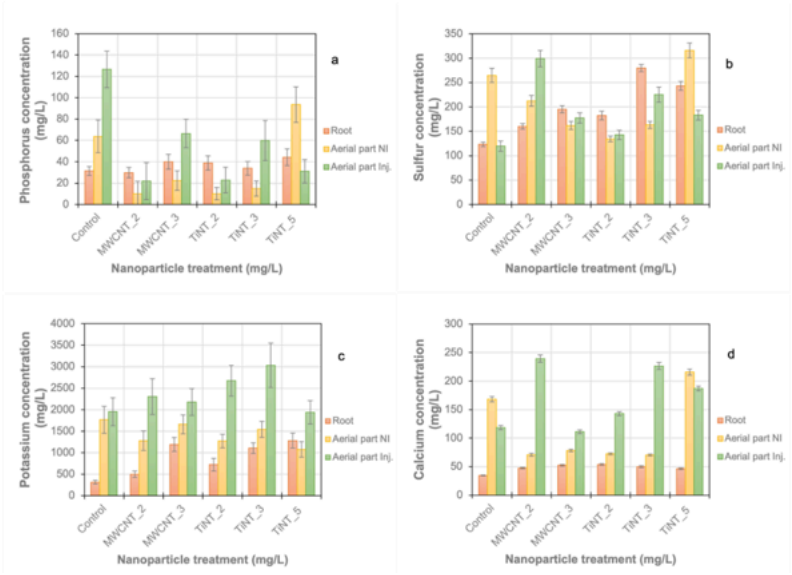


Figure 16

Analysis of water hyacinth cellular extracts for the determination of the macronutrients, phosphorus (P), sulphur (S), potassium (K) and calcium (Ca).

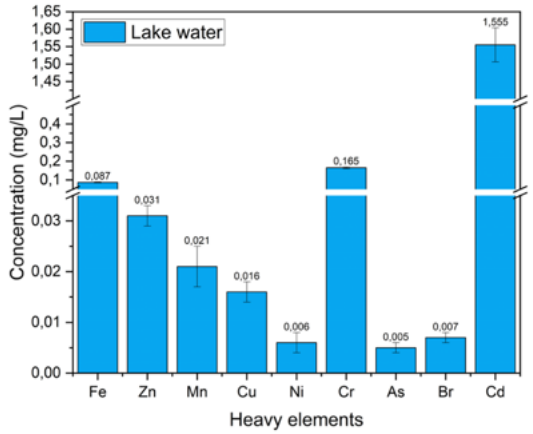


Figure 17

Analysis of heavy elements in the water of “Laguna Jovita, Naranja de Tapia, Michoacán”.

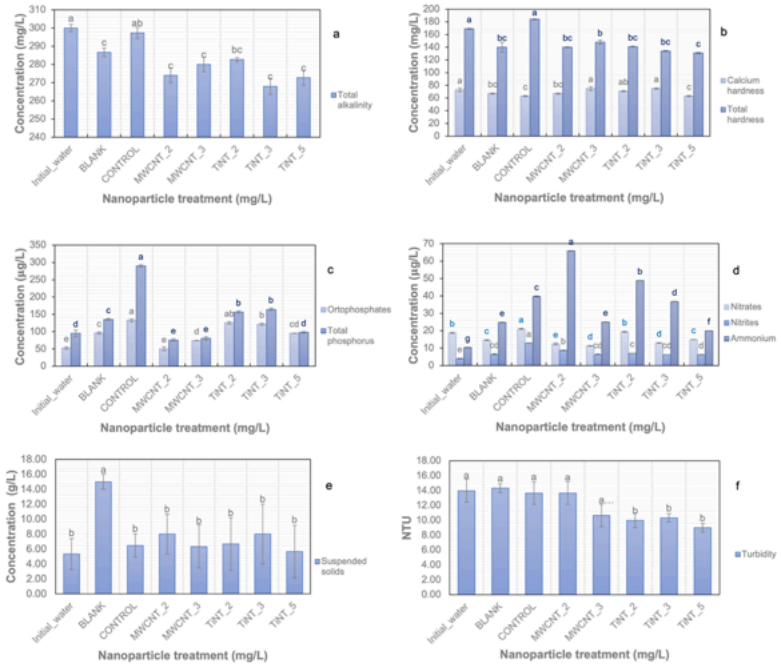


Figure 18

Physico-chemical characterization of the water used for phytoremediation by the water hyacinth plant. NTU is nephelometric turbidity unit.