# ANNEXES

# INDEX

**Annex I**_____**i**
**Annex II**_____**iii**
**Annex III**_____**iv**
**Annex IV**_____**v**
**Annex V**_____**vi**
**Annex VI**_____**vii**
**Annex VII**_____**viii**

# ANNEX I

*Chlamydomonas reinhardtii* is about 5–10 µm unicellular green alga with multiple mitochondria, two anterior flagella for motility and mating and a chloroplast that houses the pigments, the photosynthetic apparatus and critical metabolic pathways [44].

Figure I.1 shows the structure of the algal cells, where it is possible to distinguish the cell wall, chloroplast and flagellas.

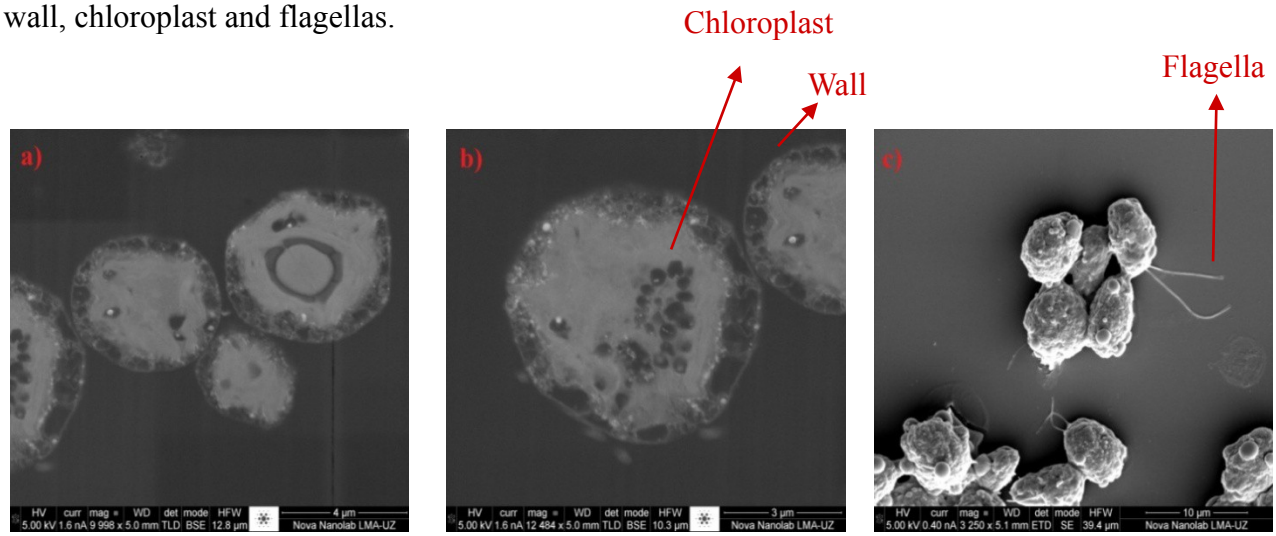



**Figure I.1. SEM images of internal (a and b) and external structure (c) of *Chlamydomonas reinhardtii.***

Figure I.2 shows a typical exponential growth phase of the algal cells in Talaquil media. From 24 to 72 h, there is a linear growth phase. For the experiments, algal cells have been synchronized and recollected after 72 h, before stationary phase starts. After 96 hours the nutrients are finished and algae stop growing to be in a stationary stage. At this point, either they have more nutrients or they will die.

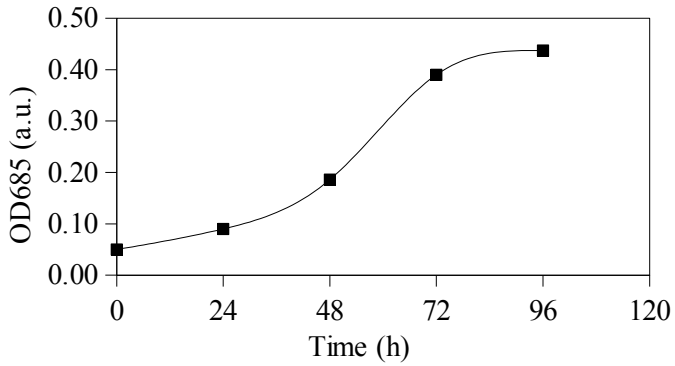



**Figure I.2. Typical growth of *Chlamydomonas reinhardtii* in Talaquil media in terms of $OD_{685}$**

Talaquil growth medium was prepared with the following concentrations: $5 \cdot 10^{-4}$ M $CaCl_2 \cdot 2H_2O$; $1.2 \cdot 10^{-3}$ M $NaHCO_3$; $5 \cdot 10^{-5}$ M $K_2HPO_4 \cdot 3H_2O$; $1 \cdot 10^{-3}$ M $NH_4Cl$; $5 \cdot 10^{-5}$ M $H_3BO_3$; buffered with 10 mM of MOPS (3-morpholine propanesulfonic acid) to pH 7.5.

In order to make sure the fluorescence of the labelled nanoparticles does not interfere with the one of the algae, they both were measured exciting at 448 nm. In Figure I.3 is observed one peak for NPs at 595 nm and other at 696 nm for the algae.

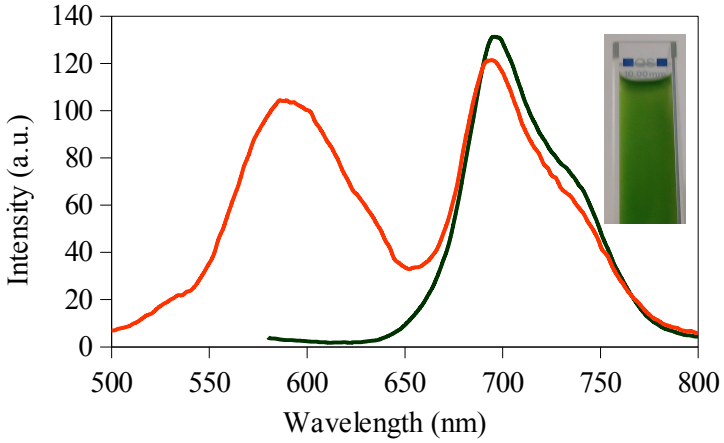



**Figure I.3. Fluorescence interference of labelled NPs and green alga. In orange, fluorescence of algae mixed with the NPs. In green, fluorescence of just alga. The inset shows an image of the algal cells in a suspension of 10 mM MOPS.**

**[44]** S.S. Merchant. *Science. 2007;318(5848):245-50.*

# ANNEX II

Physicochemical analysis of Ebro River freshwater, extracted in Zaragoza on 23/06/2017, is shown in Table II.1; conductance = 2456 µS/cm and pH 7.9.

**Table II.1. Parameter measured in a physicochemical analysis of Ebro River freshwater.**

| *Parameters* | *mg/L* |
|---|---|
| *TSS* | 6 |
| *Organic matter* | 3.2 |
| *SDT* | 1688 |
| $F^-$ | 0.11 |
| $Cl^-$ | 178.11 |
| $NO_2^-$ | 0.07 |
| $Br^-$ | 0.1 |
| $NO_3^-$ | 6.36 |
| $PO_4^{3-}$ | 0 |
| $SO_4^{2-}$ | 220.34 |
| *Total Salinity* | 279.62 |
| $Na^+$ | 150.86 |
| $NH_3^-$ | 0.05 |
| $K^+$ | 2.9 |
| $Ca^{2+}$ | 110.62 |
| $Mg^{2+}$ | 24.89 |
| *COT* | 14.87 |
| *NT* | 4.42 |

# ANNEX III

The amount of $Ru(phen)_3:SiO_2$ nanopowder deposited on each coverslip can be determined by spectrofluorimetry. The fluorescence intensity of the resuspended $Ru(phen)_3:SiO_2$ NPs in water was related to the labelled NPs concentration, through the following calibration curve (Figure III.1).

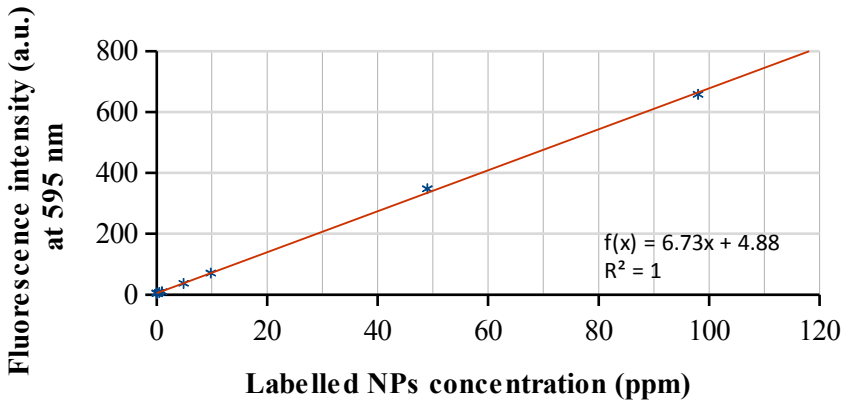



**Figure III.1. Calibration curve for fluorescence intensity of $Ru(phen)_3:SiO_2$ NPs.**

# ANNEX IV

Solvent selection used in the surface sampling procedure; water was selected as solvent to resuspend the $Ru(phen)_3$:$SiO_2$ NPs deposited in the coverslips, due to a higher fluorescent signal (Figure IV.1).

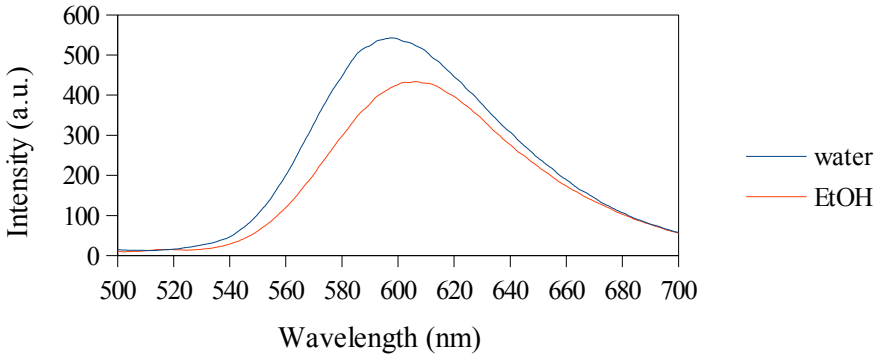



**Figure IV.1. Fluorescence intensity of 50 ppm of nanoparticles suspended in water and ethanol. Emission was excited at 448 nm.**

Table IV.1 shows the results of the experiments carried out in order to obtain the degree of nanoparticles detachment. An initial amount of powder nanoparticles was deposited over coverslips. Then, those NPs were resuspended in 5 mL of water and measured by spectrofluorimetry. This fluorescence signal was converted into ppm of NPs thanks to a calibration curve (*Annex III*). The difference between the NPs recovered and the initial concentration provides the percent of NPs recovered. As a result, an average of 96.2 ± 4.5 % of NPs was recovered.

**Table IV.1. Percent of nanoparticles recovered from an initial amount.**

| ***Samples*** | ***Initial mass (mg)*** | ***Initial concentration (ppm)*** | ***Fluorescence intensity (a.u.)*** | ***SD (%)*** | ***Concentration NPs recovered (ppm)*** | ***Recovered NPs (%)*** |
|---|---|---|---|---|---|---|
| *Sample 1* | 0.11 | 21.6 | 153.1 | 1.6 | 22 | 101.9 |
| *Sample 2* | 0.1 | 20.6 | 143.5 | 1.2 | 20.6 | 100 |
| *Sample 3* | 0.1 | 20 | 132.1 | 0.9 | 18.9 | 94.5 |
| *Sample 4* | 0.12 | 23.4 | 150.9 | 0.4 | 21.7 | 92.7 |
| *Sample 5* | 0.14 | 27.8 | 176.7 | 0.7 | 25.5 | 91.8 |

# ANNEX V

Table V.1 and V.2 shows the signal-to-noise (S/N) ratio from fluorimetry analysis of the $Ru(phen)_3$:$SiO_2$ NPs recovered after the pouring and transferring experiments. A S/N ratio of 3 is generally considered acceptable when estimating the detection limit [24].

**Table V.1. Signal-to-noise ratio (S/N) from fluorimetry analysis of the $Ru(phen)_3$:$SiO_2$ NPs recovered after pouring experiment.**

| S/N ratio | 1 | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|
| A | 6.3 ± 0.3 | | | | | |
| B | 9.1 ± 1.1 | 63.5 ± 2.3 | 11 ± 0.6 | 5.1 ± 0.4 | 11.6 ± 0.5 | 1.6 ± 0.5 |
| C | 1 ± 0.2 | 2.1 ± 0 | 2.7 ± 0.4 | 4.8 ± 0.2 | 11.7 ± 0.6 | 1.3 ± 0.1 |
| D | 1 ± 0.2 | 4 ± 0.5 | 2 ± 0.7 | 1 ± 0.2 | 1.2 ± 0.1 | 1 ± 0.3 |
| E | 1.2 ± 0.6 | 1.7 ± 0.7 | 1.2 ± 1 | 2.3 ± 1.4 | 1.5 ± 0.8 | 1.2 ± 1.2 |
| F | 1.7 ± 1.2 | 1.9 ± 0.7 | 1.1 ± 0.2 | 1.3 ± 0.4 | | |
| G | 1.9 ± 0.8 | 1.4 ± 0.6 | | | | |

**Table V.2. Signal-to-noise ratio (S/N) from fluorimetry analysis of the $Ru(phen)_3$:$SiO_2$ NPs recovered after transferring experiment.**

| S/N ratio | 1 | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|
| A | 123.6 ± 4.6 | | | | | |
| B | 1.3 ± 0.1 | 1 | 6.4 ± 1.1 | 4.1 ± 1.5 | 1.9 ± 1.2 | 1.2 ± 0.2 |
| C | 1 | 1.4 ± 0.1 | 1 | 2.3 ± 0.4 | 1.2 ± 0.1 | 1 |
| D | 1 | 1.3 ± 0.3 | 1 | 1.1 ± 0.1 | 1.3 ± 0.9 | 1 |
| E | 1 | 1 | 1 | 1 | 1 | 1 |
| F | 1 | 1 | 1 | 1 | | |
| G | 1 | 1 | | | | |

# ANNEX VI

Photosynthetic yield values of *Chlamydomonas reinhardtii* obtained for long-term exposure experiments (72 h) are shown in Table VI.1. The values correspond to Control (algal cells in absence of NPs) and algal cell incubated with NPs.

**Table VI.1 Photosynthetic yield of control and algal cell exposed to different NP concentration during 72 h.**

| *NPs concentration (ppm)* | *Yield Control* | *Yield Algae with NPs* |
|---|---|---|
| *25* | 0.40 ± 0.02 | 0.41 ± 0.01 |
| *50* | 0.31 ± 0.02 | 0.21 ± 0.03 |
| *100* | 0.52 ± 0.06 | 0.53 ± 0.08 |
| *400* | 0.54 ± 0.12 | 0.49 ± 0.1 |

# ANNEX VII

SEM images of pristine and aged $Ru(phen)_3$:$SiO_2$ NPs in different scenarios.

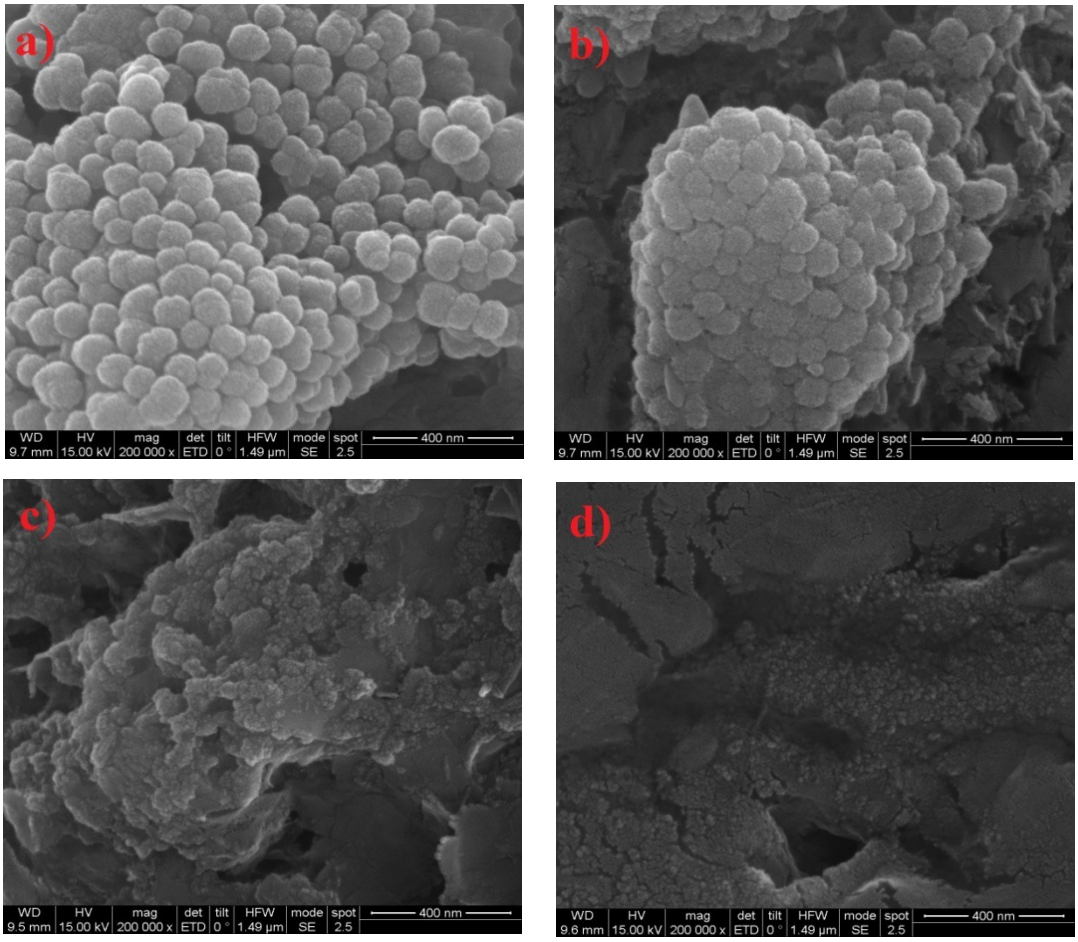
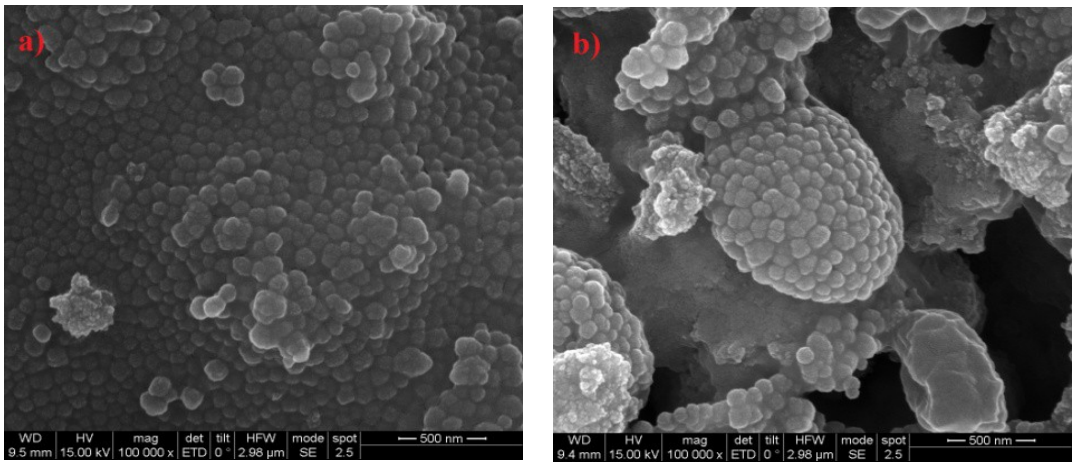



**Figure VII.1. SEM images of (a) pristine and aged $Ru(phen)_3$:$SiO_2$ NPs considering the geogenic source ($SO_4^{2-}$, $Ca^{2+}$, $Na^+$): exposure under (b) UV light, (c) PAR light and (d) darkness.**



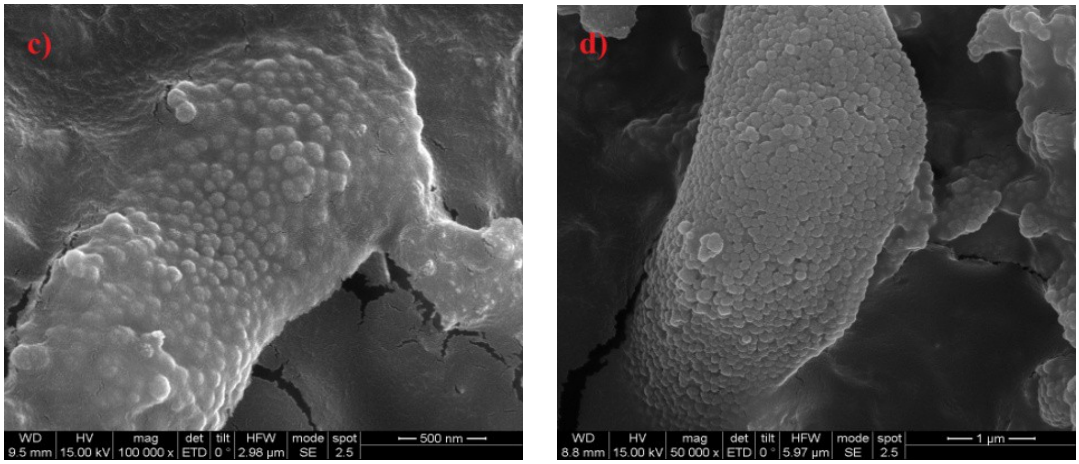



**Figure VII.2. SEM images of (a) pristine and aged Ru(phen)$_3$:SiO$_2$ NPs considering the turbidity source (TSS): exposure under (b) UV light, (c) PAR light and (d) darkness.**

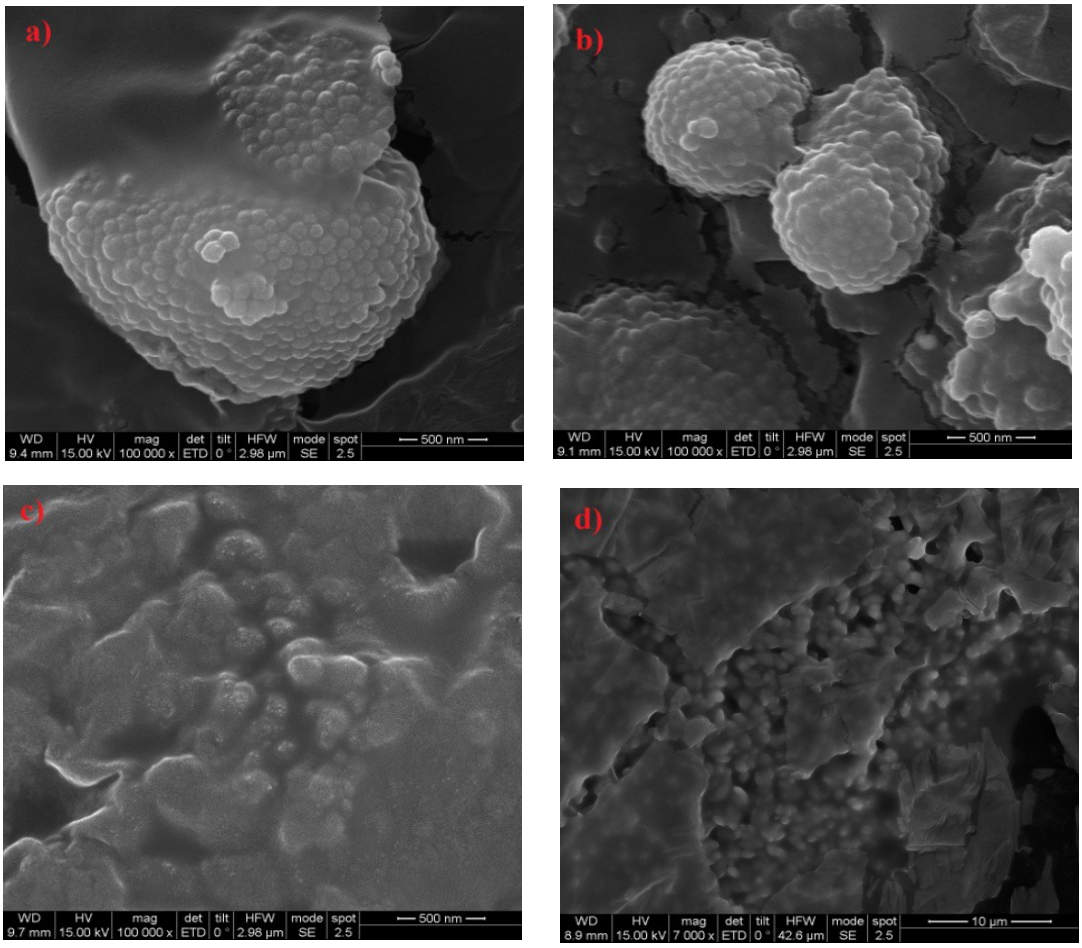



**Figure VII.3. SEM images of (a) pristine and aged Ru(phen)$_3$:SiO$_2$ NPs considering the anthropogenic source ($NO_3^-$): exposure under (b) UV light, (c) PAR light and (d) darkness.**