Supporting Information

Study of the toxicity of ZnO nanoparticles to *Chlorella sorokiniana* under the influence of phosphate: spectroscopic quantification, photosynthetic efficiency and gene expression analysis

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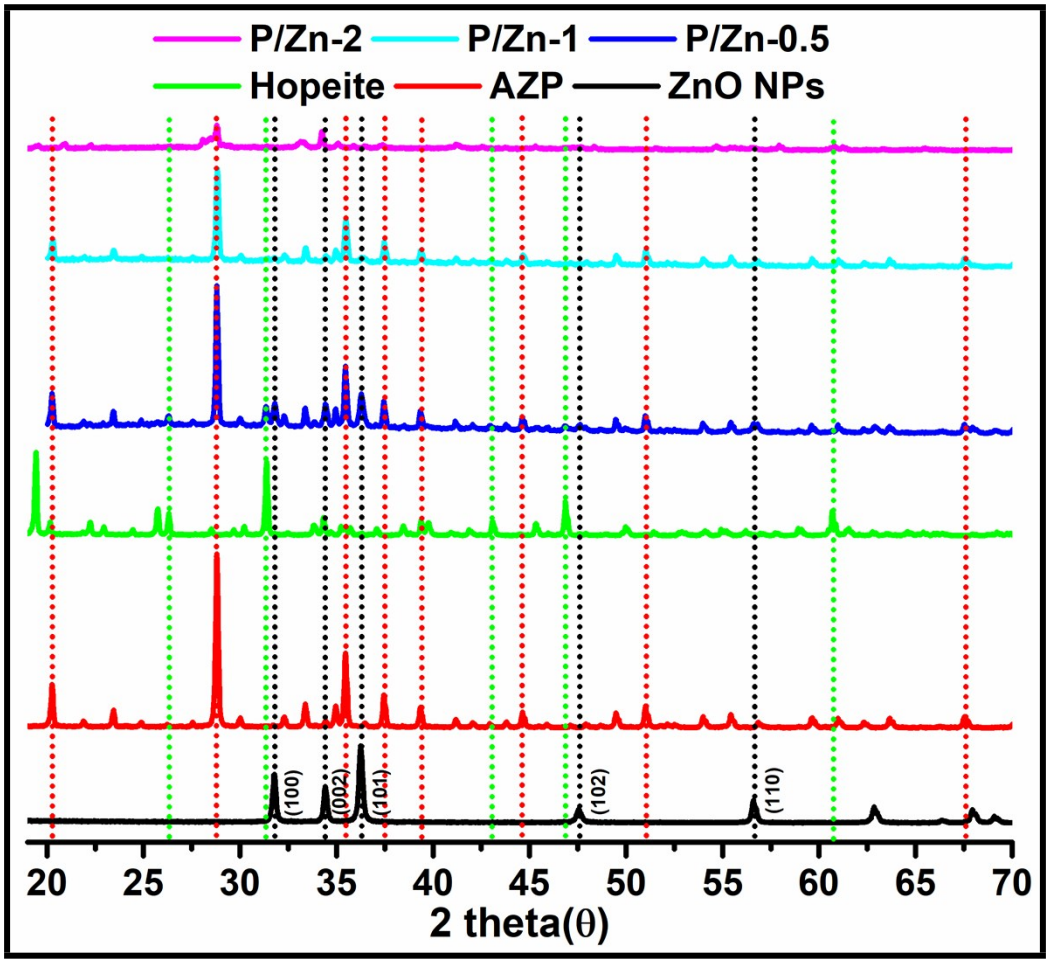

Fig. S1. X-ray diffraction (XRD) patterns of ZnO NPs and transformed ZnO NPs.

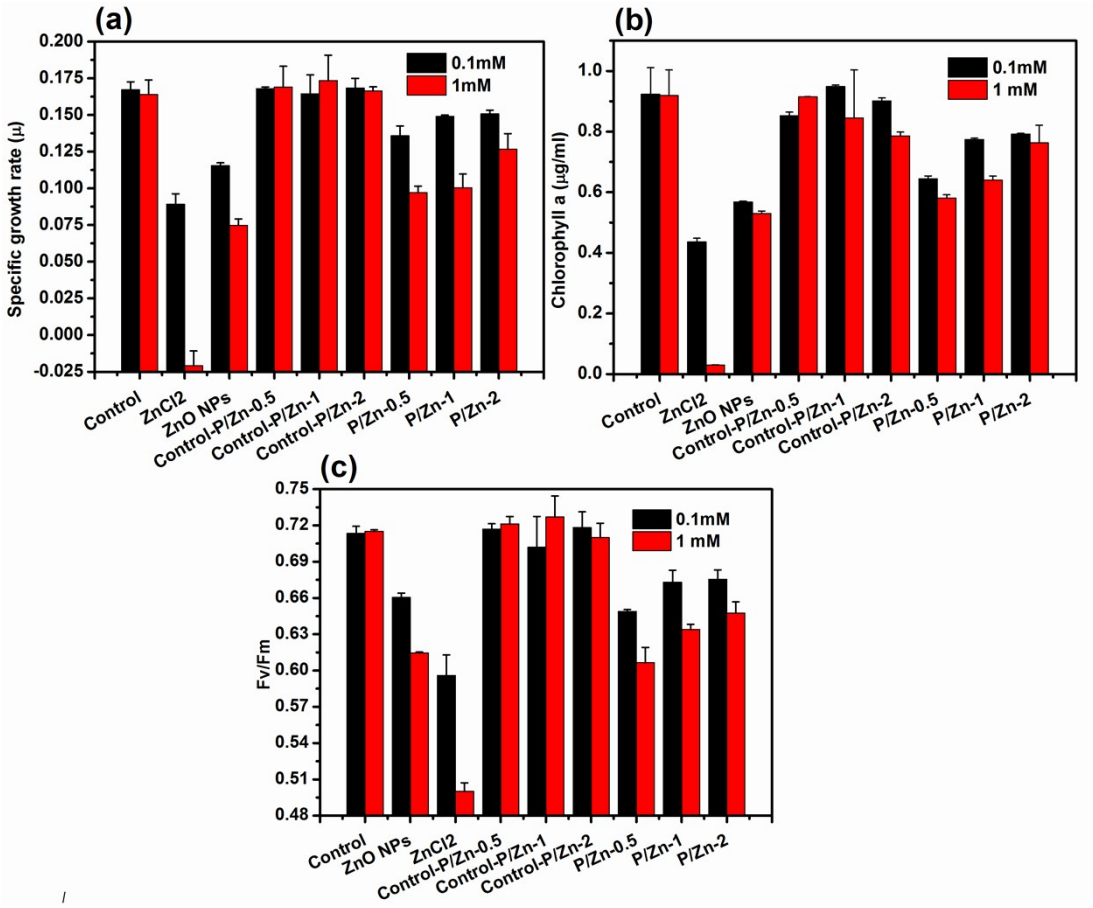

Fig. S2. Effects of the pristine (Control), the blank phosphate control (Control-P/Zn-0.5; 1 and 2) and transformed ZnO nanoparticles (P/Zn=0.5; 1 and 2) with different concentrations (0.1 and 1mM) on *C. sorokiniana* after treated for 7 days(a: specific growth rate (μ); b: chlorophyll a; c:Fv/Fm).

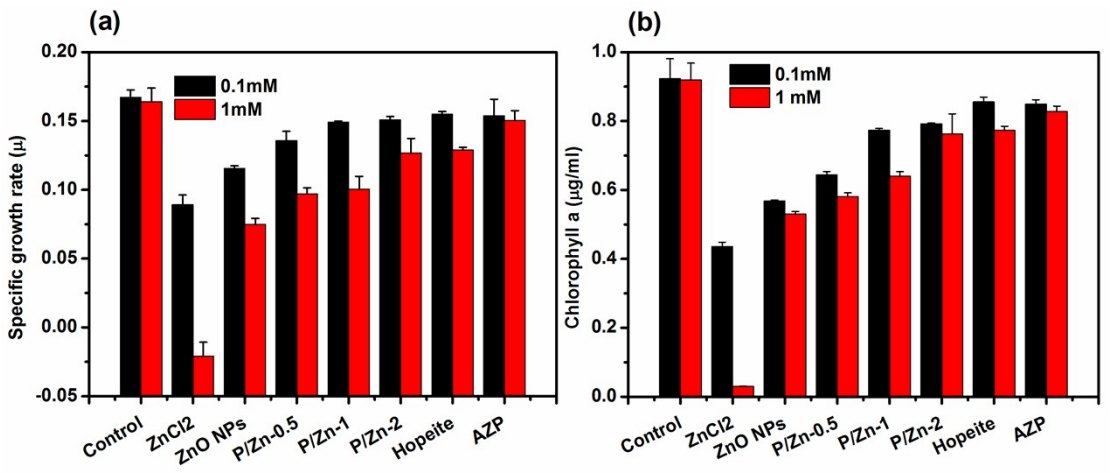

Fig. S3. Effects of the pristine ZnO NPs (Control), the Hopeite, AZP controls and transformed ZnO nanoparticles (P/Zn=0.5, 1 and 2) with different concentrations (0.1 and 1mM) on *C. sorokiniana* after treated for 7 days (a: specific growth rate (μ); b: chlorophyll a).

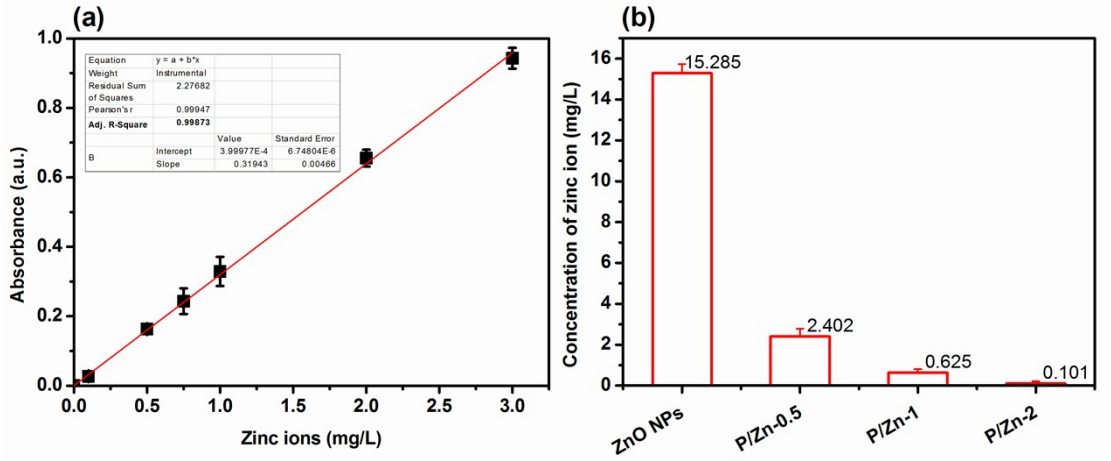

Fig.S4. (a) Standard curve for determination of zinc ion by the zincon colorimetric method. **(b)** Concentration of zinc ion released from ZnO NPs transformed in different P/Zn molar ratios for 20 days at pH 7.0.

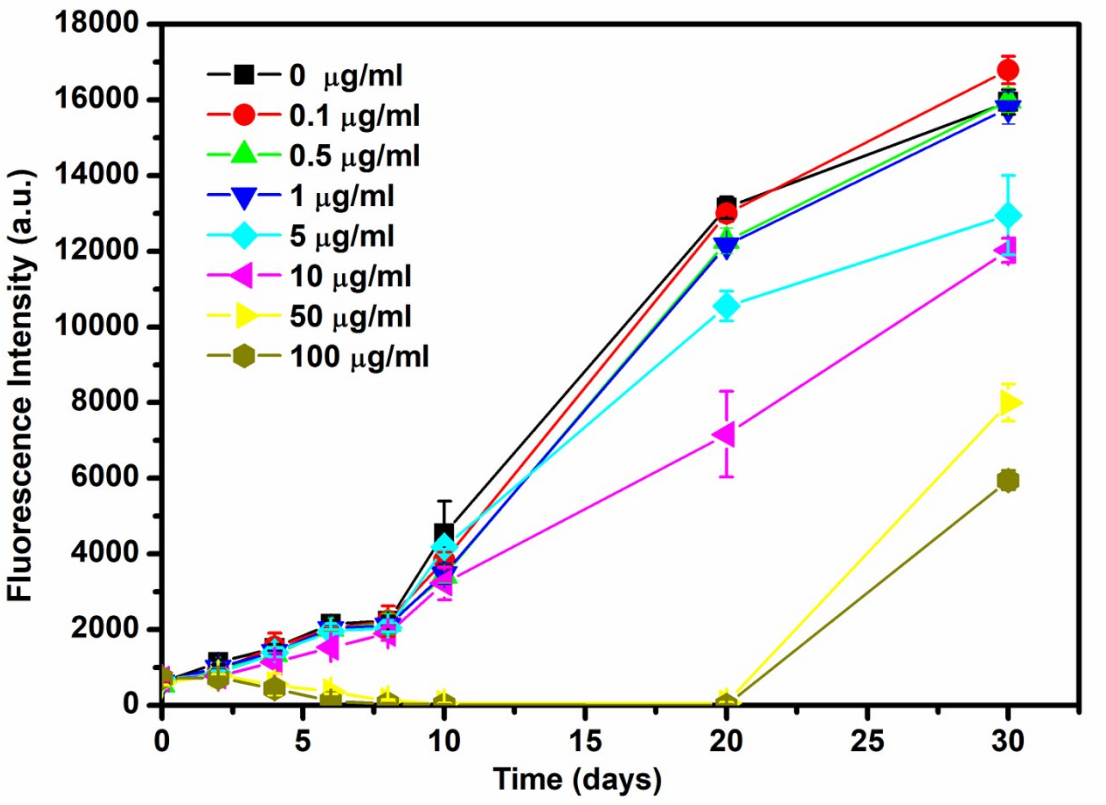

Fig.S5. Toxicity of dissolved zinc ions on green algae *C. sorokiniana* at different concentration

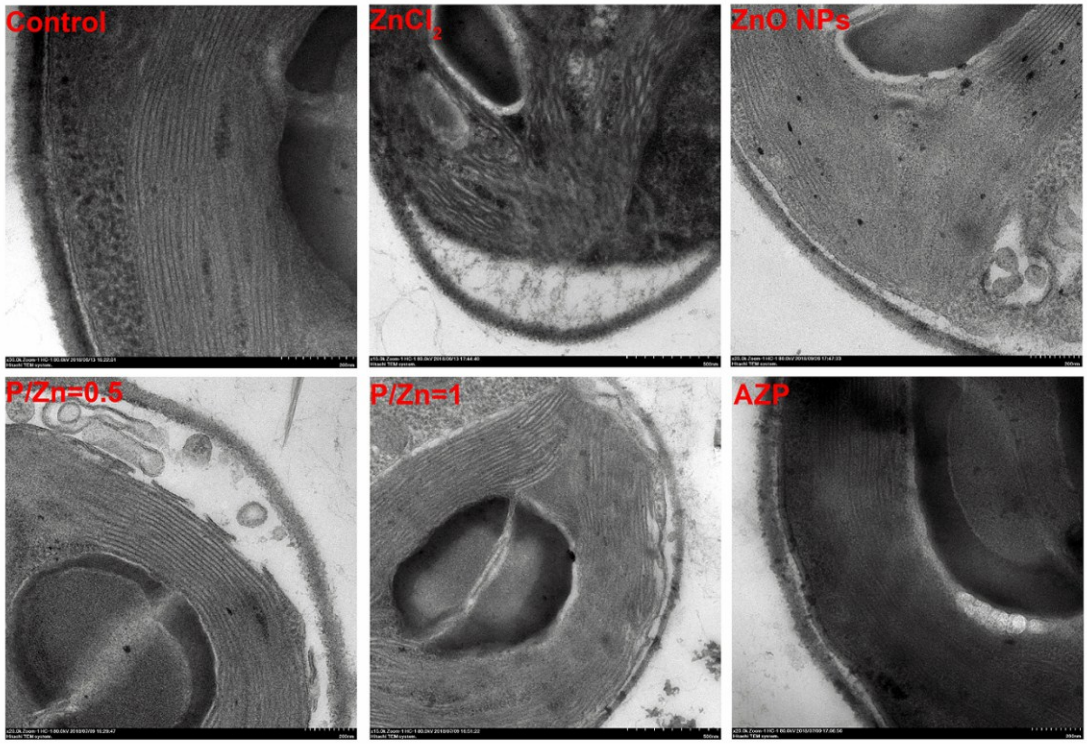

Fig.S6. TEM images of *C. sorokiniana* cells treated by zinc ion ($ZnCl_2$), the pristine and transformed ZnO NPs and AZP.

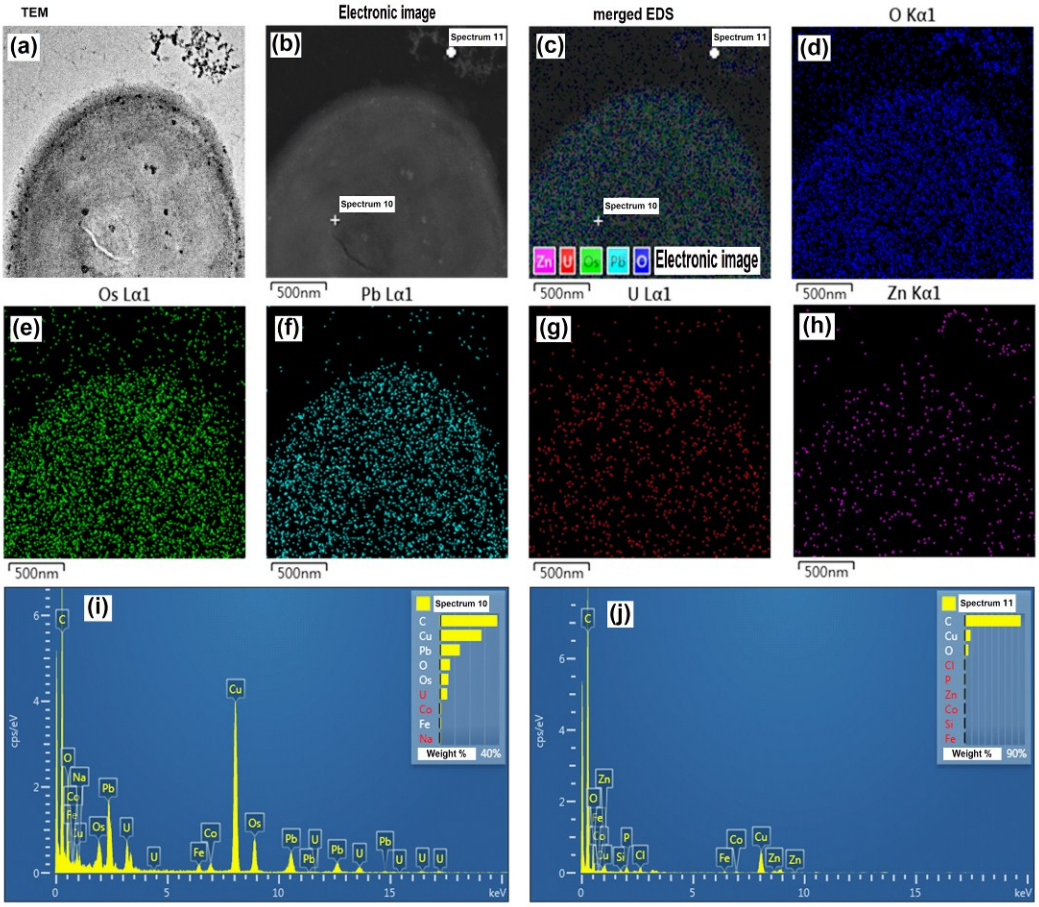

Fig.S7. TEM images and elemental mapping images of algal cells treated by the ZnO NPs (a: TEM; b:Electronic image/ BF image; c: merged elemental mapping images; d~h: elemental mapping images corresponding to elements (d) O, (e) Os, (f) Pb, (g) U, (h) Zn; i: EDS spectrum of black dots inside the algal cell in spot 10; j: EDS spectrum of particle beside the algal cell in spot 11).

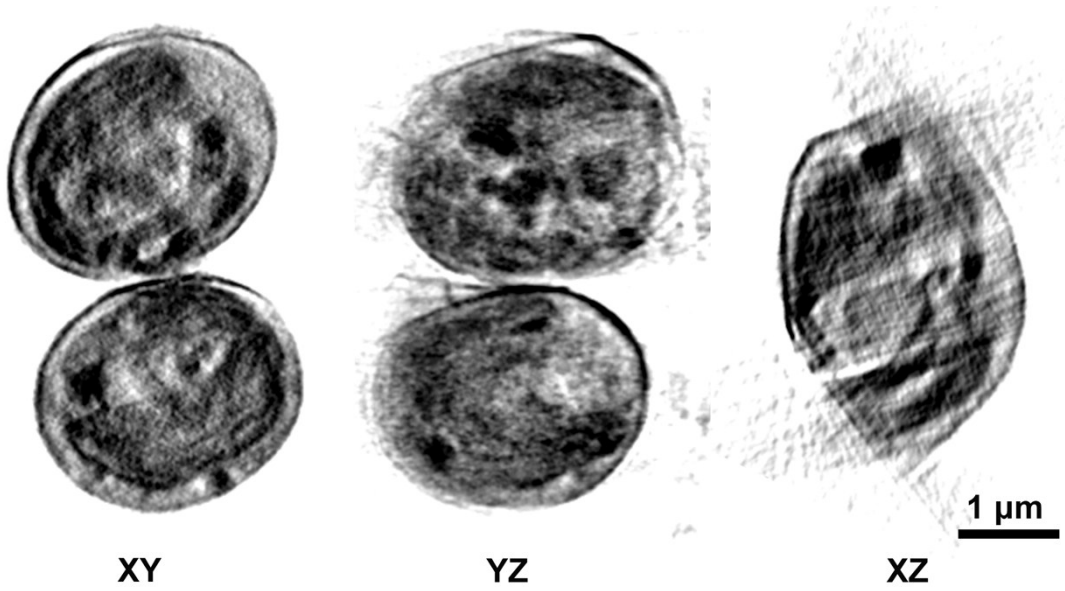

Fig.S8. Soft X-ray imaging of algal cell treated by the pristine ZnO NPs (The X-ray Nano-CT experiment was performed on BL07W beamline at the National Synchrotron Radiation Laboratory (NSRL，Hefei, China). Grids with algal cell were mounted in the homemade plunge freezer, and rapidly put into the liquid nitrogen in the movable cryo-preserving container for cooling down to liquid nitrogen temperature, and transferred into the soft X-ray imaging vacuum cryogenic chamber for imaging. Soft X-ray beam was focused onto the sample, and received on the CCD detector, and then the sample was imaged. As shown in Fig.S8, we found ZnO NPs attached around/on the algal surface and few ZnO NPs entered into algal cells at different axial locations).

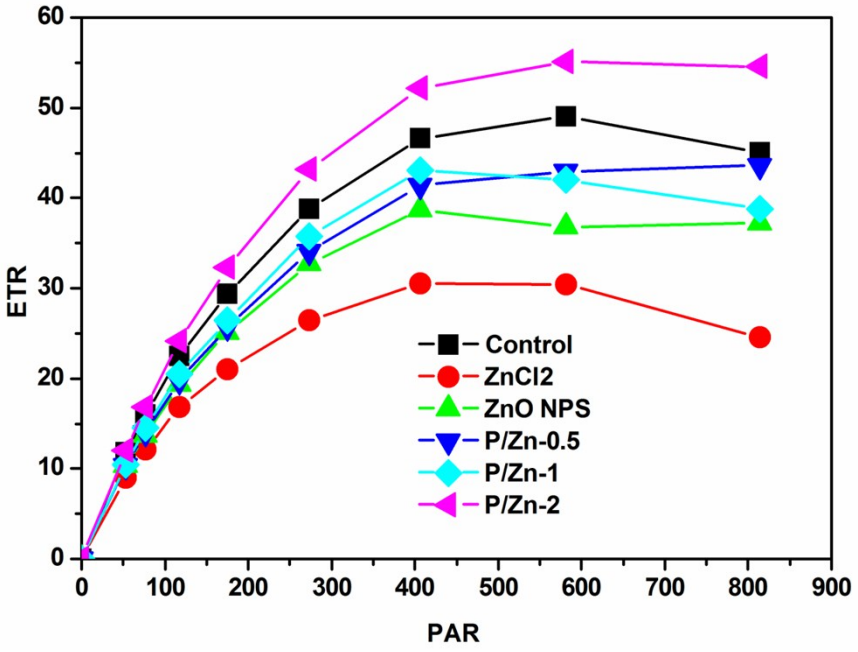

Fig. S9. The electron transport rates (ETR) of *C. sorokiniana* treated by the pristine and transformed ZnO NPs (P/Zn-0.5, P/Zn-1 and P/Zn-2) at 1 mM for 7 days.

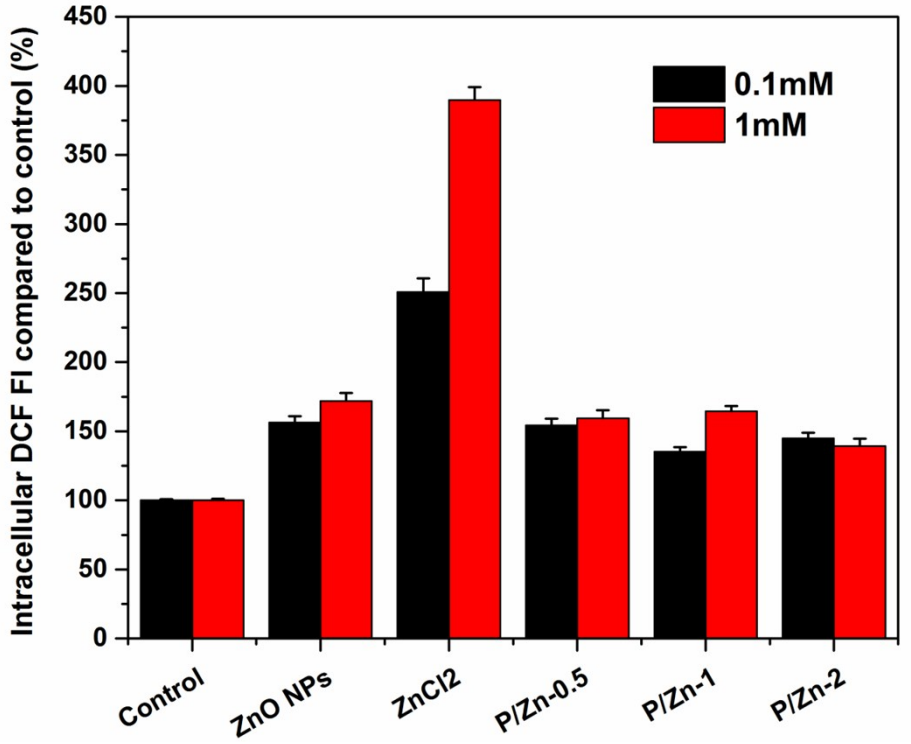

Fig. S10. The relative intracellular ROS contents of *C. sorokiniana* treated by the pristine and transformed ZnO NPs (P/Zn-0.5, P/Zn-1 and P/Zn-2) for 7days.

Table S1 Composition of BG11 Medium

Component	Amount
$K_2HPO_4 \cdot 3H_2O$	0.04 g
$MgSO_4 \cdot 7H_2O$	0.075 g
$CaCl_2 \cdot 2H_2O$	0.036 g
Citric acid	0.006 g
Ferric ammonium citrate	0.006 g
EDTA (disodium salt)	0.001 g
Na_2CO_3	0.02 g
Trace metal mix A_5	1.0 ml
Agar (if needed)	10.0 g
Distilled water	1.0 L
Trace metal mix A5	
H_3BO_3	2.86 g
$MnCl_2 \cdot 4H_2O$	1.81 g
$ZnSO_4 \cdot 7H_2O$	0.222 g
$NaMoO_4 \cdot 2H_2O$	0.39 g
$CuSO_4 \cdot 5H_2O$	0.079 g
$Co(NO_3)_2 \cdot 6H_2O$	49.4 mg
Distilled water	1.0 L

The pH should be 7.1 after sterilization

Table S2 Primers designed for qRT-PCR

Gene name	Sequence (5' to 3')	Base number
***pet*A- Forward**	GCTTCAGCAAGTCAGGCTTATC	22
***pet*A- Reverse**	CACATACAATACGACCATTCGC	22
***pet*B- Forward**	AGGAATAGCGTCAGGAACAC	20
***pet*B- Reverse**	CGTGAACTTACTTGGGTTACTG	22
***atp*B- Forward**	CATTGTGTGCGTGCTGTAGC	20
***atp*B- Reverse**	GGTGCTGAACGGTGAATAGG	20
***psa*A- Forward**	GGTCCATTTACAGGCGAAGG	20
***psa*A- Reverse**	CACCAGCACCGACAATACAG	20
***psa*A- Forward**	AGAAGTTACAAGTGAACCGTGC	22
***psa*A- Reverse**	CTTTCCGTTTAGGTATGCGTC	20
***psb*A1- Forward**	GCAACTGGTGCTGAGTAAGC	20
***psb*A1- Reverse**	TTCCTACATCTAACGCTATCGG	22
***psb*D1- Forward**	TCAACTCCAGCGAATAGTATGG	22
***psb*D1- Reverse**	AAAGACCCGTGAAGAGCAAC	20
18sRNA- Forward	CATCTAAGGGCATCACAGAC	20
18sRNA- Reverse	TGATTCCGGTAACGAACGAG	20