

SUPPLEMENTARY MATERIALS

The sulphidation of ZnO nanoparticles enhances zinc recovery in the Zn-starved barley (*Hordeum vulgare* L.): The interplay of metal acquisition and cellular homeostasis

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Materials and Methods

Tab. S1. Initial Concentrations of nZnO and related S/ZnO ratio.

Sample	S-/ZnO	c ZnO (mol·dm ⁻³)	ZnS efficiency (wt%)
1	0.002	0.500	82.70
2	0.010	0.100	78.95
3	0.020	0.050	87.65
4	0.040	0.025	77.53
5	0.100	0.010	56.79

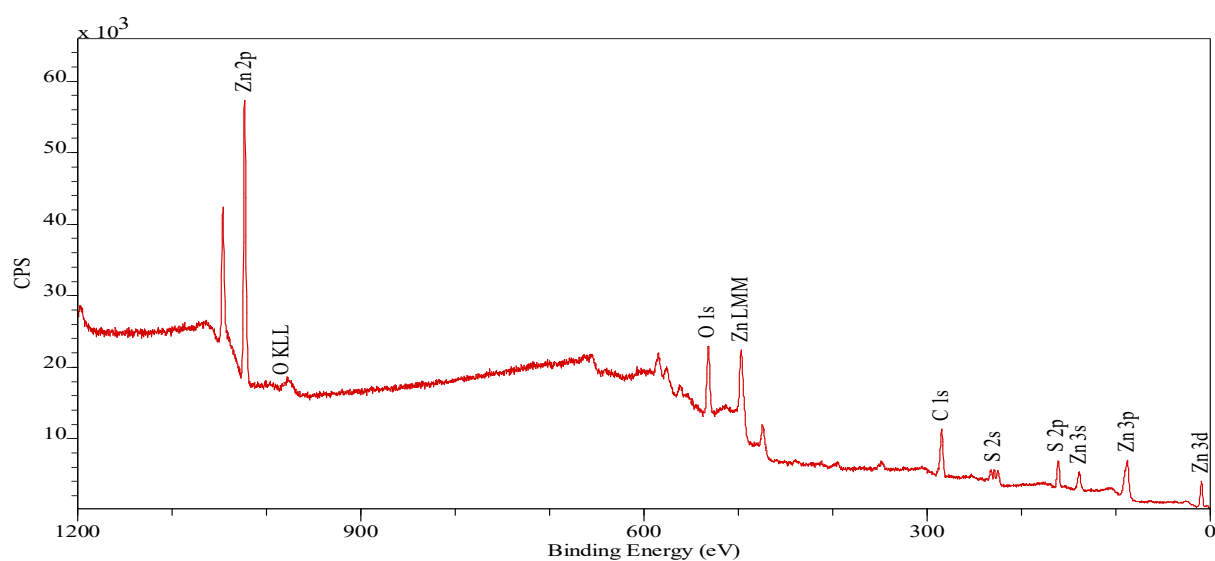


Fig. S1. XPS pattern of sulph-nZnO.

Tab. S2. The properties of the pristine and sulphidized ZnO ENPs.

ENPs	S _{BET}	V _p	V _m	R _p	D _A	ζ
nZnO	10.1 ± 0.7	0.029 ± 0.001	1.41 ± 0.04	114.4 ± 3.3	1646 ± 27	+30.1
sulph-nZnO	29.2 ± 0.9	0.231 ± 0.007	1.53 ± 0.04	316.5 ± 9.3	2117 ± 115	+3.83

S_{BET} - BET surface area (m²/g), V_p - pore volume (cm³/g), V_m - micropore volume (0.10⁻³ cm³/g), R_p - pore radius (Å), D_A - mean aggregates diameter (nm), ζ - zeta potential (mV).

Tab. S3. The list of treatments.

Name	Medium	Treatments*
control (-)	Zn ion-free Hoagland solution	no additives
control (+)	Hoagland solution (with ZnSO ₄)	no additives
nZnO s	Zn ion-free Hoagland solution	nZnO (0.5 mg Zn/L)
sulph-nZnO	Zn ion-free Hoagland solution	sulph-nZnO (0.5 mg Zn/L)
ZnSO ₄	Zn ion-free Hoagland solution	ZnSO ₄ (0.5 mg Zn/L)

The additives (ENPs or metal salt) were added to the solution at a tillering stage of barley growth (BBCH:21).

Gene	Primer	Primer (5' → 3') sequence	NCBI/ Phytozome Acc. number	Localization (UniProt)
ZIP1	F	TTCATCTATGTCGCCATCAACCACCTC	IBSC contig _2164938	Cell membrane
	R	CTATGCCAGTGAGCACACCGAAGAAC		
ZIP3	F	GTGAGGCCATCCACCATCAA	FJ208991	
	R	CACCCAAGCCTATGCCTTCA		
ZIP6	F	GCGAGGAAGAAGCAGAAGATGG	AK369168	
	R	AAGACCTGGTGGAAAGGAGAGAGC		
ZIP8	F	CGCTCTTCTTCTCACTCACC	FJ208993	
	R	GGAGAGGTTTACGATGACCTG		
ZIP10	F	GACCTCATTGCTGCTGATTT	AK363919	
	R	AGCTAGGCAACAGGTCGTAAGT		
ZIP14	F	CACAGATGCACGATCAGAGAA	AK249958	
	R	CCACAATATCCACGGAACTCA		
MTP1	F	ACAGCCATAATTATCACCAG	AM286795	Vacuole membrane
	R	CGAGGTGTTACCGGCAT		
Cu/Zn SOD	F	GGTGACACGACTAATGGAT	U69536.1	Cytosol, chloroplast, peroxisome. mitochondria
	R	TGACGGACTTCATCTTCTG		
ADP	F	AGGTTCTTGATGCTGATGTG	EF405961.1	
	R	CGGAGTCCAACACTACTGAATG		
ACT	F	GGACGCACAACAGGTATC	GQ339780.1	
	R	CGAGGTCAAGACGAAGGA		

Tab. S4. Sequences of primers and internal reference used for RT-PCR.

Results

Tab. S5. The properties of ENPs in Hoagland solutions before plant exposure. Different letters indicate significant differences among the treatments (Tukey test, $p < 0.05$).

ENPs	ζ -potential [mV]	Average aggregate size [nm]
nZnO	-8.8 ± 0.9^a	350.1 ± 82
sulph-nZnO	-10.9 ± 0.2^b	OOR

OOR - the result was out of measuring range (0.3nm - 15 μ m).

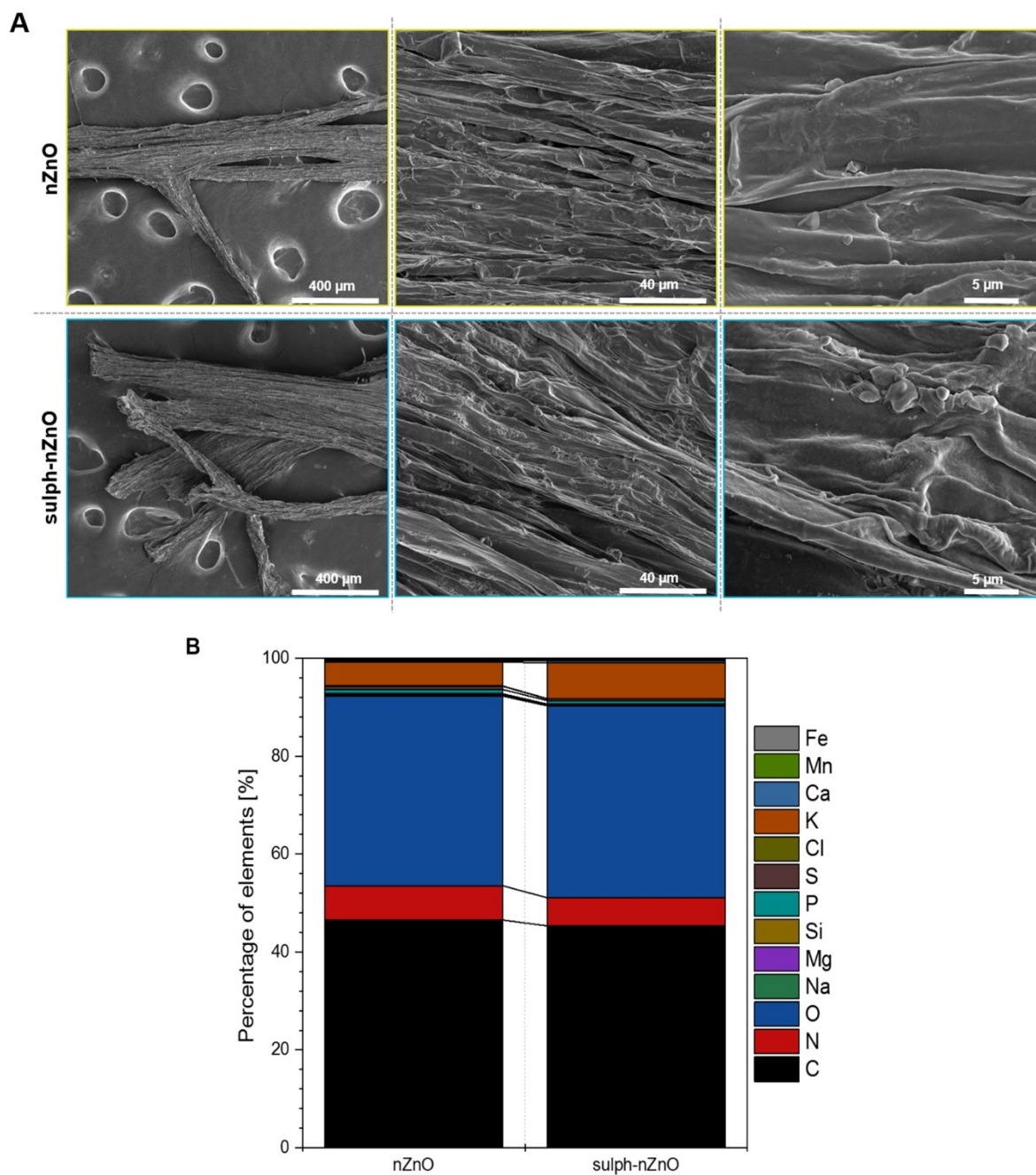


Fig. S2. The microscopic images (A) and elemental composition (B) of tip roots exposed to nZnO and sulph-ZnO for 1 day. The images and elemental analysis were performed with Scanning Electron Microscopy integrated with Energy Dispersive Spectroscopy (Quanta™ 3D FEG, FEI with EDAX SDD Apollo detector). The percentage of elements is the average content of elements collected from 6 scans of EDAX.

Tab. S6. The ratios of DOC or TOC (measured in HGL medium after plant exposure) to the dry weight of barley roots. The control (-) and the control (+) mean the untreated plants grown in HGL solutions without or with Zn ions (as ZnSO₄), respectively. The treatments of ENPs or ZnSO₄ at the rate of 0.5 mg Zn/L were performed.

Treatments	DOC/DW _{roots}		TOC/DW _{roots}	
	1 day	7 days	1 day	7 days
control (-)	10.2	12.2	10.9	13.1
control (+)	8.3	6.9	8.8	7.3
nZnO	7.5	8.0	7.8	8.3
sulph-nZnO	8.7	8.7	9.2	9.3
ZnSO ₄	8.6	10.3	8.9	11.1

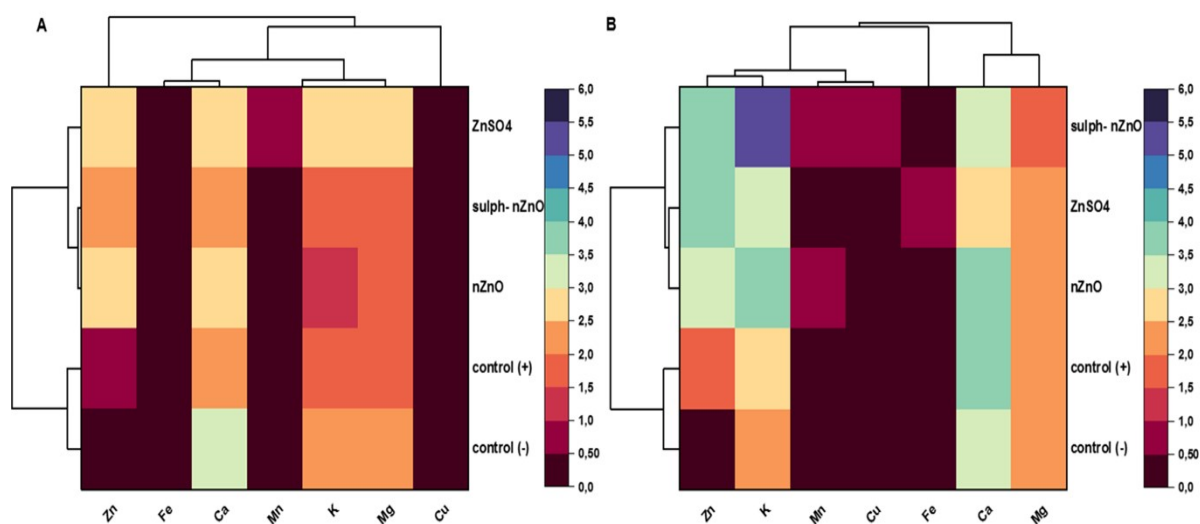


Fig. S3. The heatmaps present the translocation factors of metals in plants exposed to Zn compounds for 1 day (A) and 7 days (B). The control (-) and the control (+) mean the untreated plants grown in HGL solutions without or with Zn ions (as ZnSO₄), respectively. The dendrograms were performed based on the Pearson factors.