

Supplementary Information

Table S1. Instrument operating conditions (A) Agilent ICP-MS; (B) Agilent LC-QQQ.

A

ICP Parameters

Nebulizer Type

MicroMist

Isotopes Monitored

²³Na, ²⁴Mg, ³⁹K, ⁵⁵Mn, ⁵⁶Fe, ⁵⁹Co, ⁶³Cu, ⁶⁶Zn, ¹¹³Cd, ²⁰⁸Pb, ⁷²Ge, ¹⁰³Rh, ¹⁹¹Ir

Forward Power

1500 W

Plasma Gas Flow

14.97 L/min

Auxilliary Gas Flow

0.90 L/min

Carrier Gas Flow

1.07 L/min

B

UPLC Parameters

Colum

Zorbax Plus C18 (100 x 4.5mm, 3.5 μm)

Column Temperature

40 °C

Mobile Phase

Ultrapure water with 0.1% formic acid (A), Methanol with 0.1% formic acid (B)

Flow Rate

0.5 mL/min

Injection Volume

10 μL

Elution Gradient

Initial equilibration at 2% B for 3 min, followed by step gradient from 2-20% and 20-70% B at 2 min each. Flush with 70% B for 2 min, before re-equilibrating to initial column condition for 3 min. (Total run time is 12 min)

QQQ Parameter

Ion source

Agilent Jetstream electrospray ionization (ESI)

Ionization Mode

Positive

iFunnel

160 V (High Pressure), 100 V (Low Pressure)

Gas Temperature

Gas Flow Rate

260 °C

15 L/min

Sheath Gas Temperature

Sheath Gas Flow Rate

300 °C

11 L/min

Nebulizer

45 psi

Table S2. Source parameters for analysis of thiol peptide standards on Agilent LC-QQQ.

	Precursor Ion (Da)	Qual. Product Ion (Da)	Quan. Product Ion (Da)	Collision Energy (eV)
Reduced Glutathione (GSH)	308.1	162.0	233.0	12
Oxidized Glutathione (GSSG)	613.2	355.2	484.1	22
Phytochelatin2 (PC2)	540.3	232.9	336.0	20
Phytochelatin3 (PC3)	772.3	232.8	465.1	10
Phytochelatin4 (PC4)	1004.4	464.6	540.0	42
Phytochelatin5 (PC5)	1236.5	697.2	771.9	53
N-acetylcysteine (NAC)	164.2	75.9	59.0	11

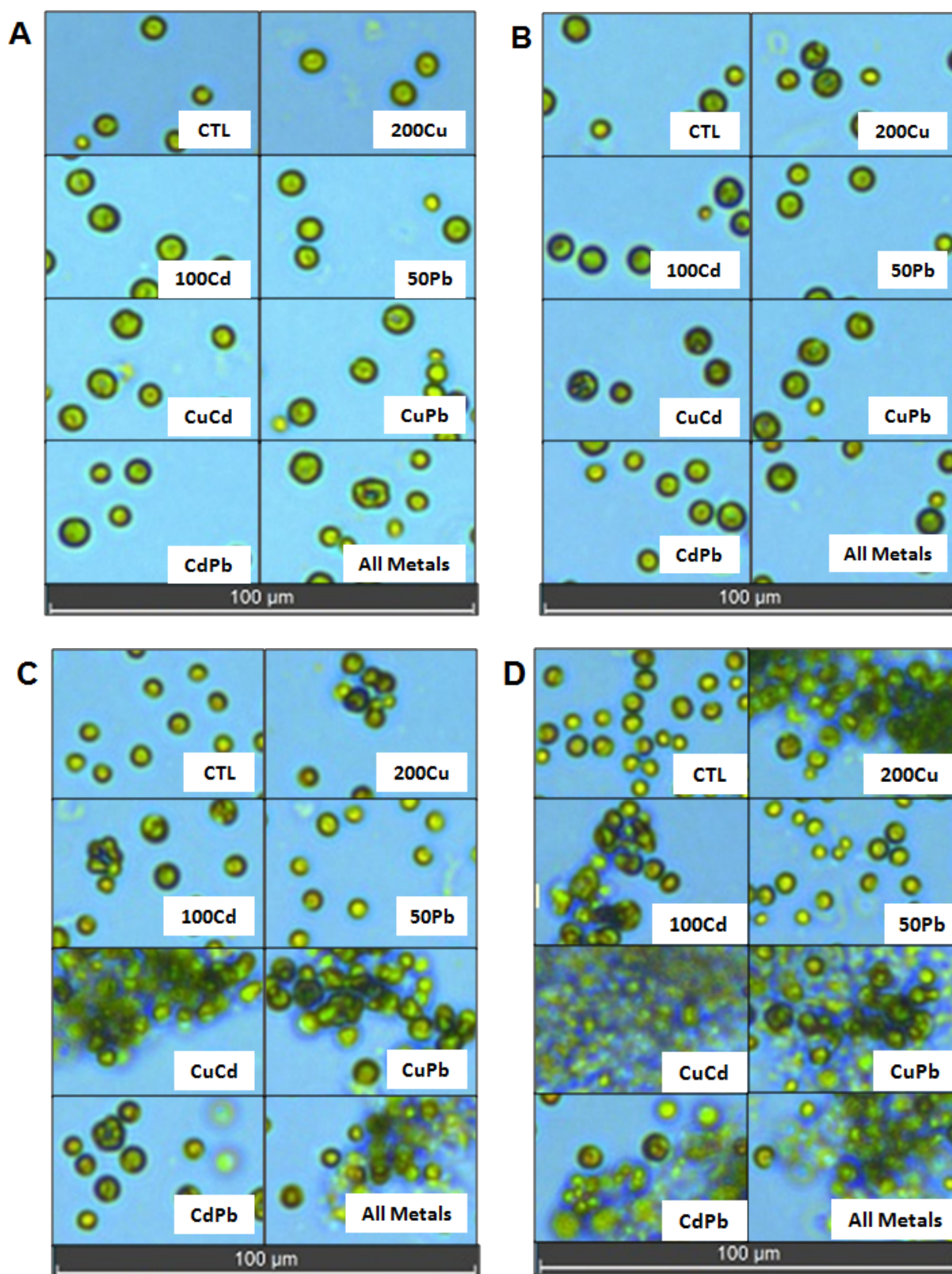
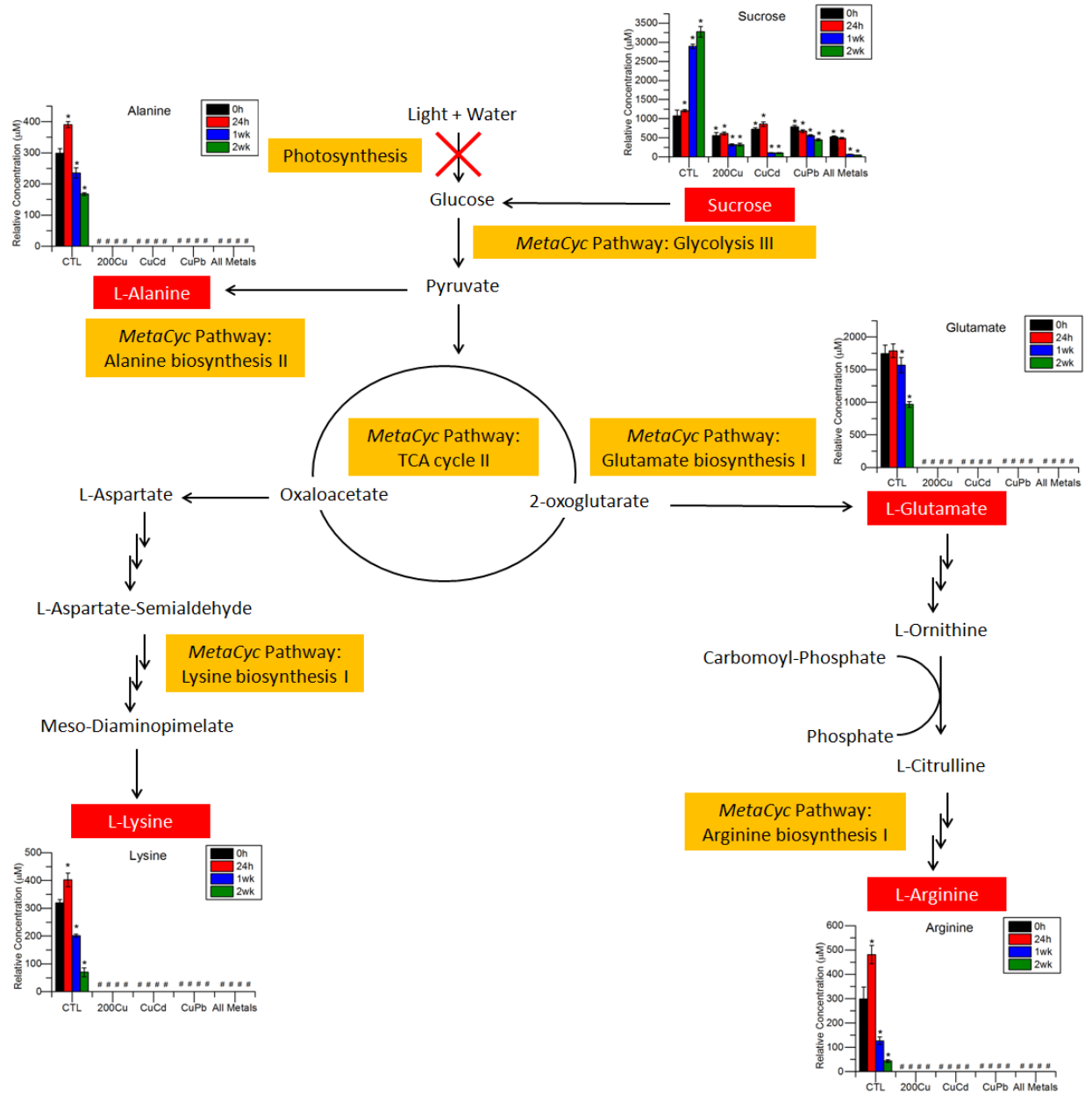


Figure S1. Optical micrographs of *Chlorella sp.* cells treated with various metal combinations at different exposure durations. Magnification at 40 x. A) 0h; B) 24h; C) 1 week; D) 2 weeks.

A



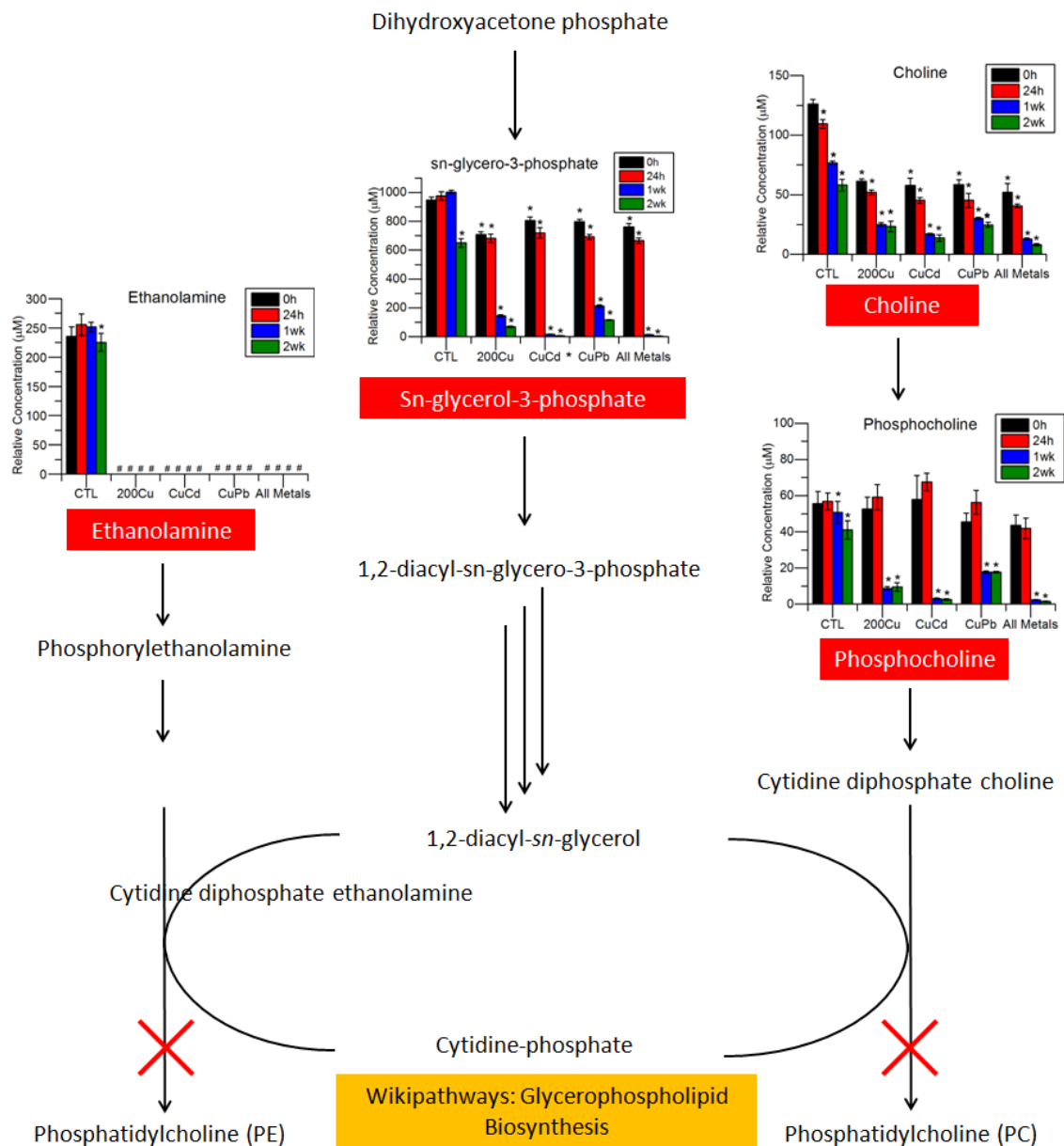
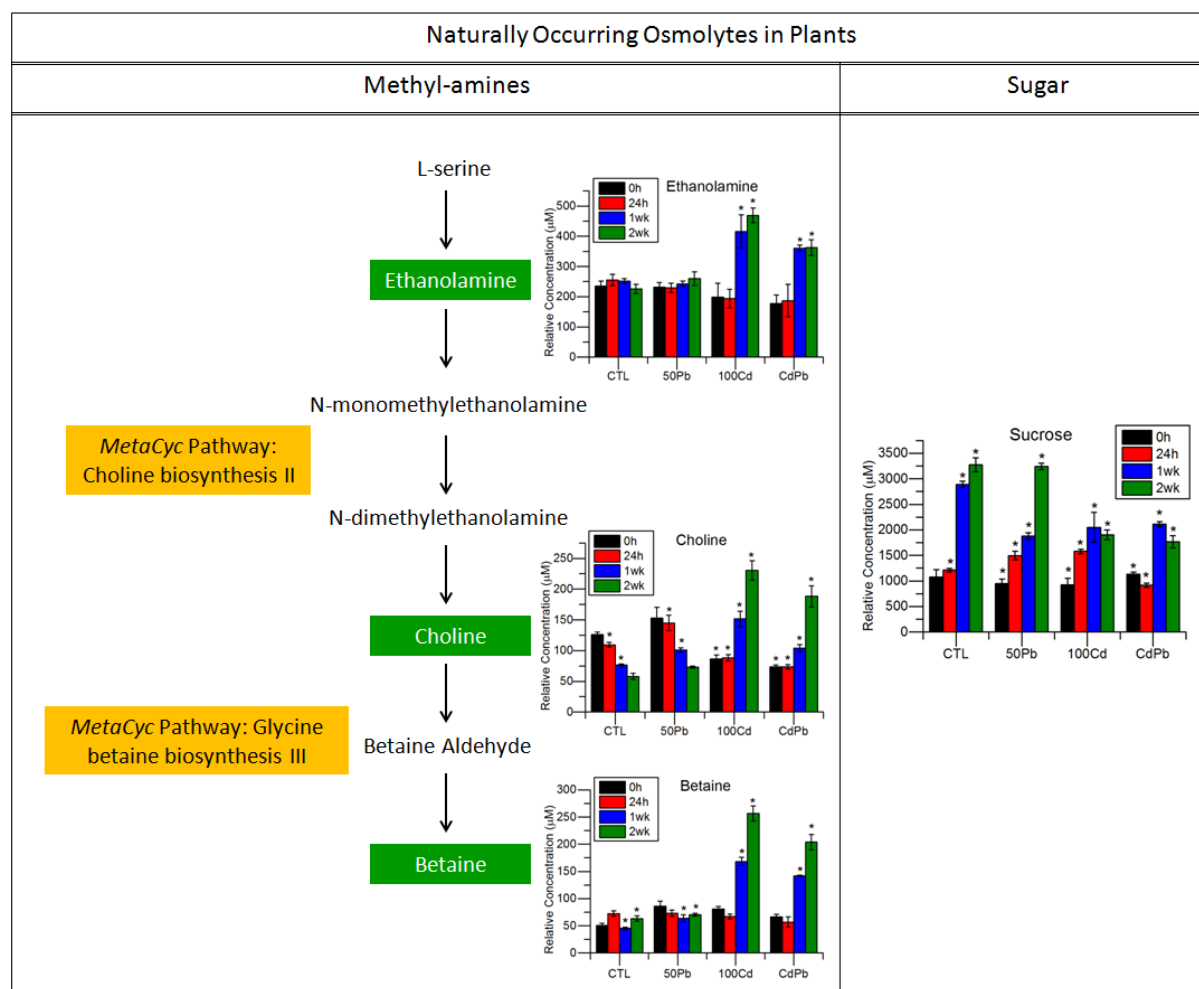
B

Figure S2. Proposed metabolic changes in *Chlorella sp.* acutely and chronically exposed to Cu. (A) Photosynthesis impairment arise from Cu-induced stress impacts carbohydrate metabolism and amino acid biosynthesis. (B) Cu-induced oxidative stress affects phospholipids biosynthesis. Key metabolic pathways are highlighted in orange, and key metabolites that decrease significantly ($p < 0.001$) upon exposure to Cu are highlighted in red. #Absence of metabolites in *Chlorella sp.*



Fi

figure S3. Accumulation of naturally occurring osmolytes in *Chlorella sp.* after prolong Cd exposure. Key metabolic pathways are highlighted in orange, and key metabolites that increase significantly ($p < 0.001$) over time are highlighted in green.