

Uptake, accumulation, translocation and subcellular distribution of perchlorate in tea (*Camellia sinensis L.*) plants

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MATERIALS AND METHODS

Instruments.

Quantitative analysis of perchlorate was determined by a Waters ACQUITY UPLC H-Class coupled to a Waters Xevo TQ-S Micro triple-quadrupole mass spectrometer.

Perchlorate was separated using a Thermo Hypercarb column (2.1×100 mm, $5 \mu\text{m}$) with the column temperature at 35°C . The mobile phase consisted of acetonitrile (A) and 10 mM ammonium acetate in water (B) was applied at a flow rate of 0.3 mL/min with the following gradient program: 0-2 min at 50%A, 2-2.5 min at 50-90%A, 2.5-3.5 min at 90%A, 3.5-4 min at 90-50%A, 4-5 min at 50%A. The injection volume was 5 μL .

Waters Xevo TQ-S Micro was operated in negative ionization mode (ESI $^-$). The source temperature was 150°C . Capillary voltage and cone voltage were 0.2 kV and 10 V, respectively. The desolvation temperature was 350°C . Source gas (N_2 , 99.5%) was at the flow rate of 650 L/H for desolvation and 50 L/H for cone. In the multiple reaction monitoring (MRM) mode, the dwell time was 0.082 s. Collision gas (Ar, 99.995%) was at the flow rate of 0.25 mL/min. Cone voltage and collision voltage were 16 V and 18 V, respectively.

Table S1. Nutrient solution composition.

Nutrient	Concentration (mmol/L)	Nutrient	Concentration (μmol/L)
$(\text{NH}_4)_2\text{SO}_4$	0.60	EDTA Na_2Fe	4.20
$\text{Ca}(\text{NO}_3)_2$	0.30	H_3BO_3	7.00
KH_2PO_4	0.07	MnSO_4	1.00
K_2SO_4	0.30	ZnSO_4	0.67
MgSO_4	0.67	CuSO_4	0.13
CaCl_2	0.23	$(\text{NH}_4)_6\text{Mo}_7\text{O}_{24}$	0.047
$\text{Al}_2(\text{SO}_4)_3$	0.035		

Table S2. Spiked levels, recoveries, relative standard deviations (RSDs).

matrix	spiked level (mg/kg)	Mean recovery (%, n=5)	RSD (%, n=5)
Cell walls (F1)	0.004	87.9	8.0
	0.1	86.0	7.5
	0.5	84.0	5.6
	5	92.4	3.4
Organelles (F2)	0.004	98.6	15.1
	0.1	97.0	2.9
	0.5	83.4	8.8
	5	108.8	3.1
Soluble fractions (F3)	0.01	92.0	6.1
	1	104.9	4.9
	10	100.3	6.8

Table S3. Linear regression, R^2 , MEs.

Matrix	Linearity range (mg/L)	Regression eq	R^2	MEs
Solvent	0.005-1	$y = 195.2x - 0.200$	0.9999	-
Cell walls	0.005-1	$y = 143.5x - 0.127$	0.9997	0.74
Cell organelles	0.005-1	$y = 144.7x + 1.094$	0.9971	0.74
Soluble fractions	0.005-1	$y = 219.2x + 0.521$	0.9998	1.12

Table S4. Linear regression of tissue perchlorate concentration (21st day) and exposure concentration.

Cultivar	Tissue	Regression eq	R ²
Fuding Dabai	Tender leaf	y = 3.508x - 0.308	0.9995
	Mature leaf	y = 3.166x + 0.168	0.9999
Wuniuzao	Root	y = 0.405x + 0.064	0.9997
	Tender leaf	y = 3.846x + 1.340	0.9967
	Mature leaf	y = 3.052x + 1.175	0.9978

Table S5. The bioconcentration factor (BCF) of field samples.

Province	Soil (mg/kg)	Fresh leaves (mg/kg)	BCF
Zhejiang	0.041	0.912	22.50
	0.036	0.077	2.12
	0.008	0.016	1.88
	0.013	0.034	2.52
	0.009	0.018	1.88
	0.022	0.947	43.82
	0.007	0.012	1.73
	0.008	0.209	24.75
	0.009	0.199	21.22
	0.011	0.133	12.41
	0.019	0.081	4.37
	0.007	0.147	22.13
	0.004	0.019	4.87
	0.014	0.035	2.50
	0.001	0.034	24.29
	0.004	0.036	10.29
Yunnan	0.013	0.001	0.10
	0.013	0.007	0.52
Hunan	0.028	0.225	7.94
	0.019	0.104	5.42
	0.021	0.109	5.14
	0.020	0.094	4.64
	0.020	0.174	8.58
	0.020	0.063	3.11
	0.020	0.125	6.17
	0.020	0.056	2.79

	0.020	0.088	4.39
Fujian	0.016	0.108	6.90
	0.016	0.260	16.59
	0.016	0.083	5.31
	0.015	0.569	38.29
	0.010	0.233	23.92
	0.010	0.204	20.94
	0.010	0.194	19.96
	0.007	0.195	28.03
	0.007	0.232	33.41
	0.006	0.161	26.22
	0.006	0.170	27.59
	0.006	0.238	38.63
	0.006	0.227	36.92
	0.006	0.144	23.32
	0.006	0.250	40.53

Table S6. The BCFs of perchlorate in plant leaves.

Plant	Exposure concentration (mg/L)	BCF
Snap bean	0.01-10	55-70
Willow	10	10
	20	5.7
Smartweed	0.1	8.2
Lettuce	1-2	4.8-13.2
	0.001-0.01	2-40
	0.026-0.181	62-292

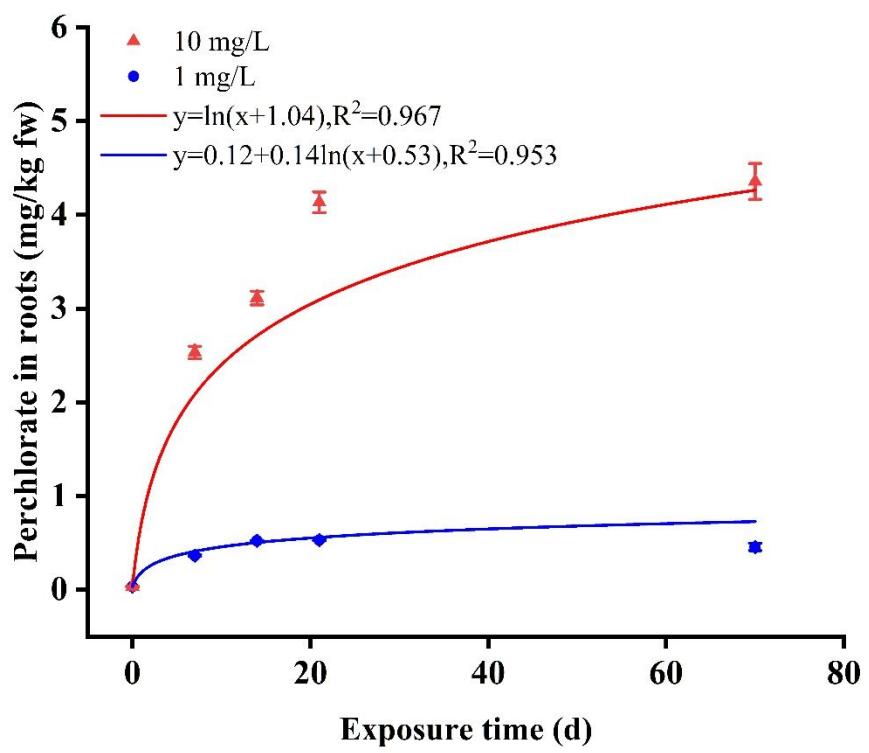


Figure S1. Perchlorate concentration in root of Wuniuzao under 1 and 10mg/L perchlorate treatment as a function of exposure time. (n=3)

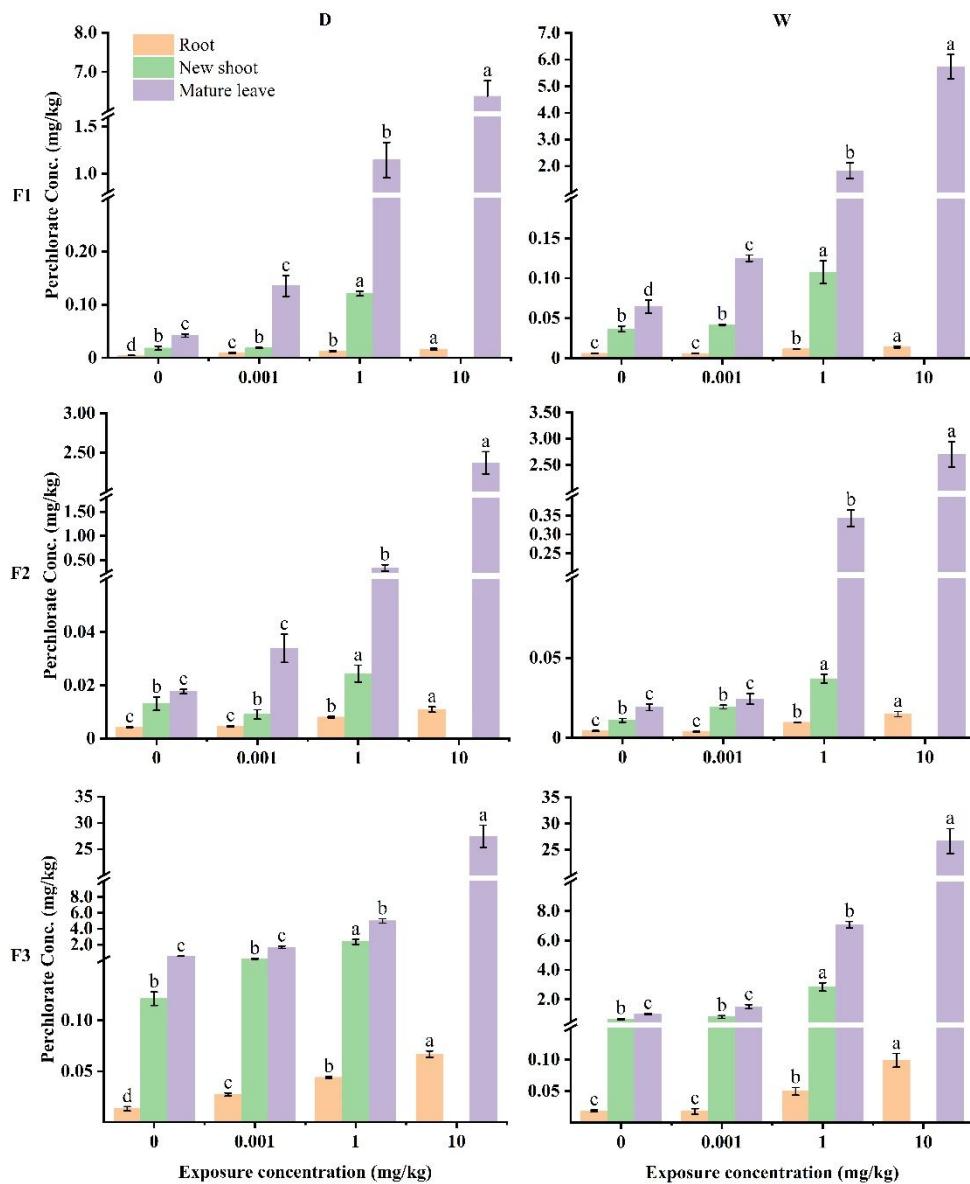


Figure S2. Perchlorate concentration of different tissues in each subcellular fraction. Roots(R), new shoots(B), mature leaves(M). Fuding Dabaicha(D), Wuniuzao(W). Subcellular fraction: cell wall(F1), cell organelles(F2) and soluble fractions(F3), (n=3). Different letters indicate significant differences ($p<0.05$) among exposure concentrations.