Arsenate toxicity and metabolism in the halotolerant microalga *Dunaliella salina* under various phosphate regimes

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Supplementary Figures

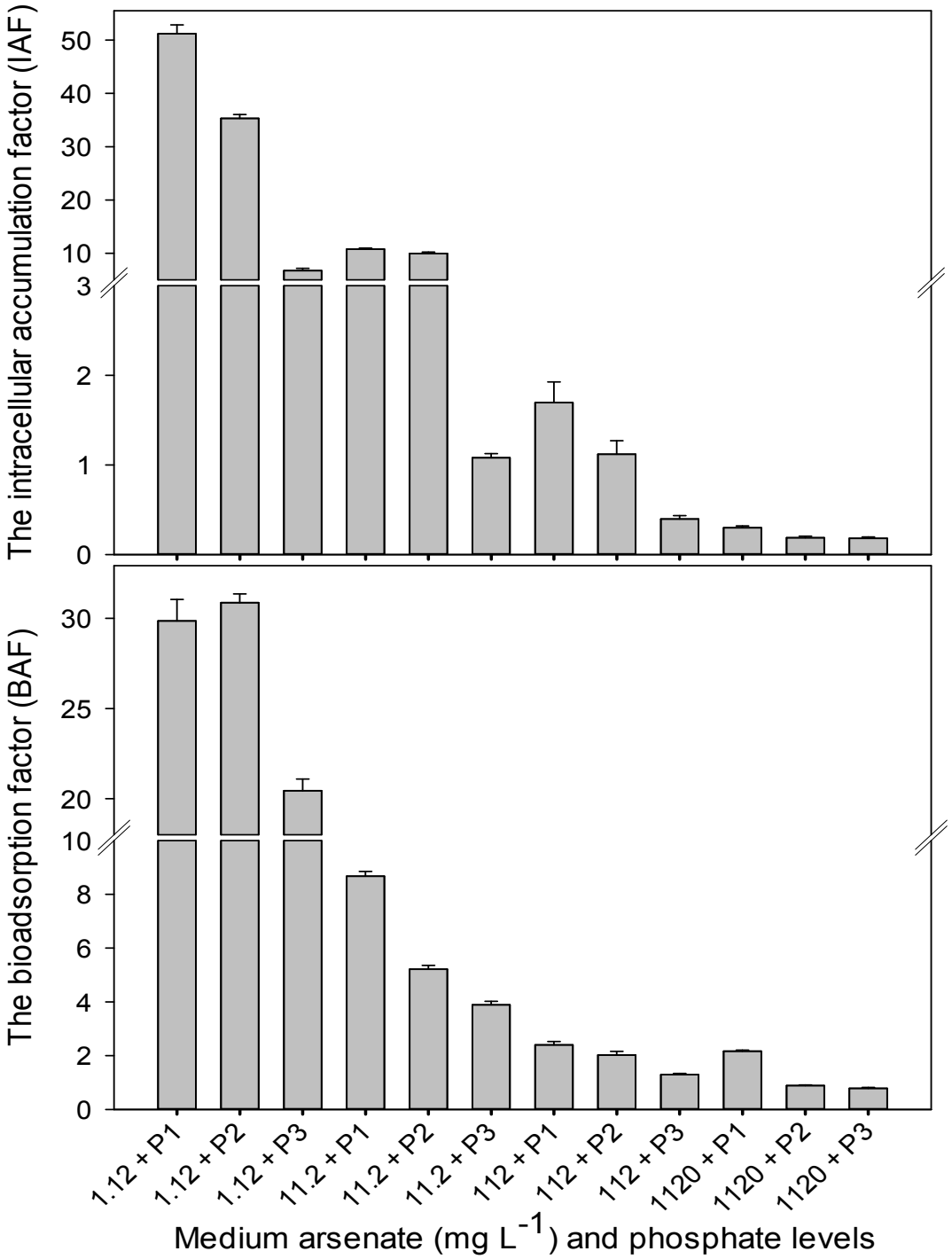

Fig. S1 The bioadsorption factor (BAF) and intracellular accumulation factor (IAF) of *D. salina* after exposure to various indicated concentrations (1.12, 11.2, 112, 1120 mg L^{-1}) of As(V) in P_1, P_2 and P_3 growth medium for 24 h. P_1, P_2 and P_3 denote 0.112, 1.12 and 11.2 mg L^{-1} phosphate, respectively. Data are means ± SE (n=3)

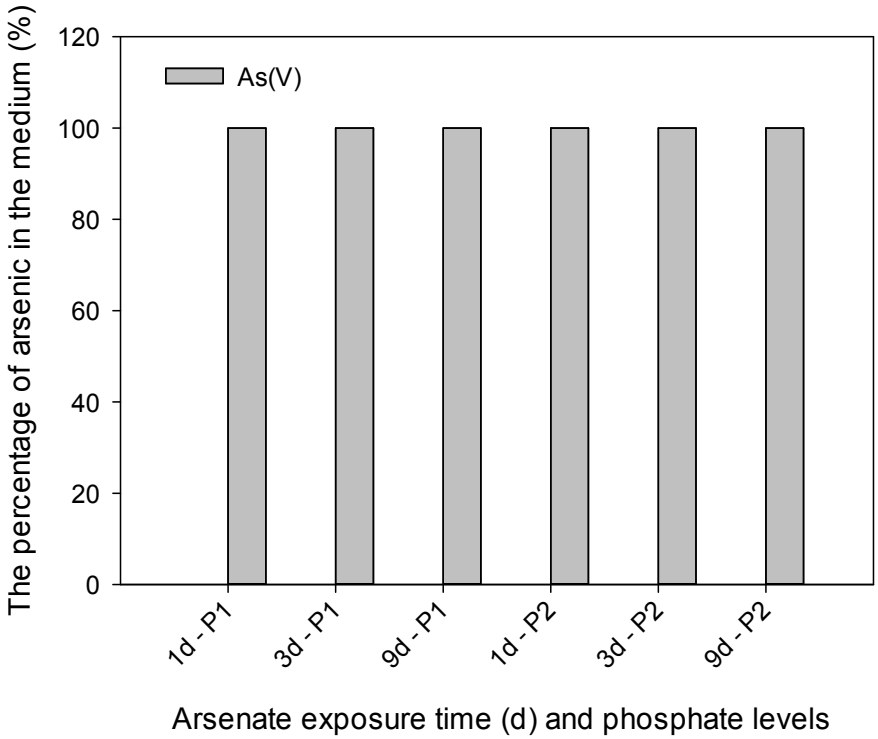

Fig. S2 Transformation of the added As(V) (1.12 mg L^{-1}) in P_1 and P_2 media in which *D. salina* was grown for 3 days and removed by centrifugation and filtration. P_1 and P_2 denote 0.112 and 1.12 mg L^{-1} phosphate, respectively. Data are means ± SE (n=3)

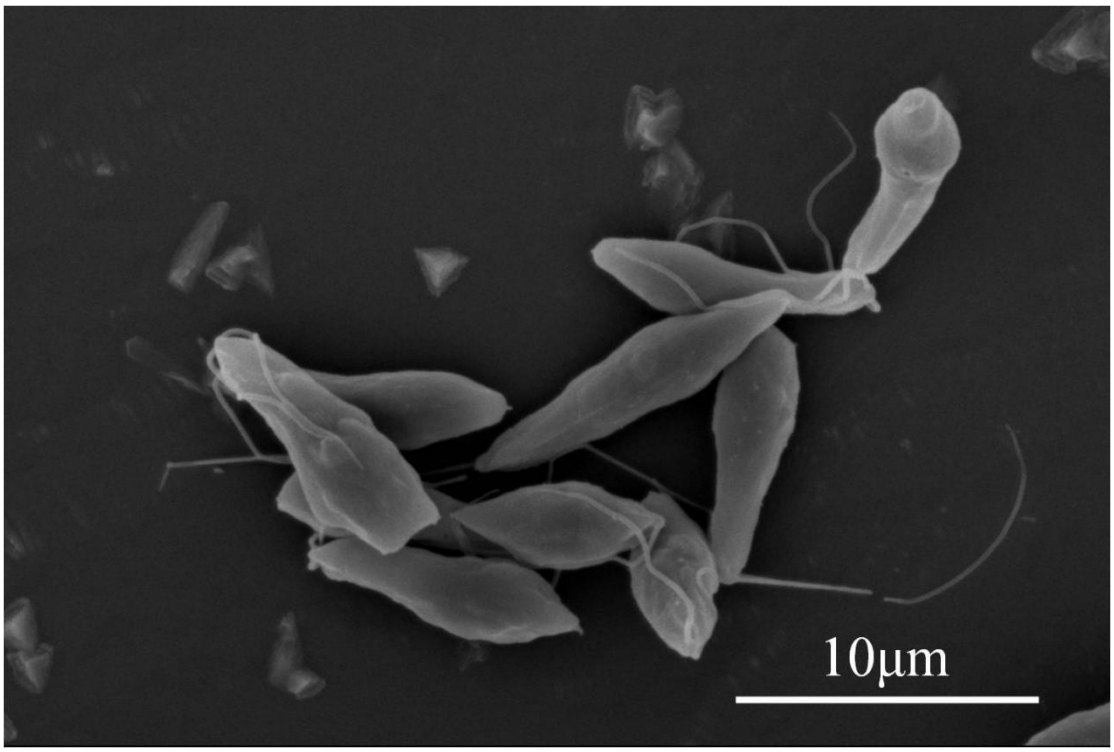

Fig. S3 Scanning electron micrograph of axenic *Dunaliella salina*. Scale=10 μm

Supplementary Tables

Table S1 The component information of synthetic sea salt from Qingdao Sea-Salt Aquarium Technology Company (China).

Item	Components of sea salt (S=35‰)
d(t=24°C)	1.024-1.026
pH	8.10-8.50
Na^{+}(mg L^{-1})	9300-9700
Mg^{2+}(mg L^{-1})	1320-1400
K^{+}(mg L^{-1})	350-400
Ca^{2+}(mg L^{-1})	380-430
Sr^{2+}(mg L^{-1})	9.0-10.50
Rb^{2+}(mg L^{-1})	0.11-0.13
Fe(mg L^{-1})	0.06-0.20
Li^{+}(mg L^{-1})	0.12-0.15
Cl^{-}(mg L^{-1})	17300-17800
SO_4^{2-}(mg L^{-1})	2260-2500
Br^{-}(mg L^{-1})	20-40
F^{-}(mg L^{-1})	0.6-1.1
B(mg L^{-1})	4.0-6.0

Table S2 As(V) concentration (mg L^{-1}) and the growth inhibition (GI) rate in different treatments (A-J) of the three toxicity experiments for *D. salina* under P_1 (0.112 mg L^{-1}), P_2 (1.12 mg L^{-1}) and P_3 (11.2 mg L^{-1}) conditions, respectively.

As(V) treatments (mg L^{-1})	Phosphate levels and GI (% of control)					
	P_1	GI_1	P_2	GI_2	P_3	GI_3
A	0		0		0	
B	0.0112	3.2 ± 0.2%	0.56	0.0 ± 0.0%	1.12	0.0 ± 0.0%
C	0.056	8.1 ± 0.5%	1.12	0.0 ± 0.0%	11.2	0.0 ± 0.0%
D	0.112	15.2 ± 0.9%	5.6	5.7 ± 0.3%	56	11.3 ± 0.7%
E	0.56	33.4 ± 2.1%	11.2	24.3 ± 1.4%	112	32.4 ± 2.0%
F	1.12	66.3 ± 4.4%	56	41.4 ± 2.4%	336	67.3 ± 4.2%
G	5.6	82.1 ± 5.1%	112	84.2 ± 5.0%	560	74.2 ± 8.7%
H	11.2	90.6 ± 5.7%	336	90.6 ± 6.1%	1120	89.9 ± 5.6%
I	112	100 ± 0.0%	560	98.4 ± 10.2%	1680	91.6 ± 6.8%
J	1120	100 ± 0.0%	1120	99.2 ± 13.4%	2240	99.0 ± 6.2%

Data are means ± standard deviation (n = 3).

Table S3 Average phosphate depletion rates of the 6 media during the 9-d cultivation.

Time (h/d)	Rate (μg L^{-1} h^{-1})					
	P_2	P_2+As_1	P_2+ As_2	P_3	P_3+ As_1	P_3+ As_2
0-2h	80.9 ± 2.0	78.3 ± 2.8	70.4 ± 1.7	256 ± 6.5	247 ± 9.6	233 ± 3.6
3-4h	52.7 ± 1.8	48.6 ± 1.7	9.4 ± 1.5	25.1 ± 1.4	23.8 ± 1.9	29.3 ± 3.1
5-8h	55.8 ± 2.3	52.7 ± 2.7	38.1 ± 4.3	61.6 ± 3.6	53.3 ± 3.0	52.2 ± 2.7
9-12h	33.3 ± 4.5	35.7 ± 1.5	24.1 ± 3.6	17.4 ± 1.5	23.9 ± 1.2	24.6 ± 1.3
13-24h	29.3 ± 2.3	28.8 ± 1.0	22.1 ± 1.9	5.4 ± 0.3	4.3 ± 0.8	1.4 ± 0.2
1-2d	3.9 ± 0.2	4.9 ± 0.2	13 ± 0.8	26.4 ± 1.2	25.8 ± 1.6	15.3 ± 2.8
2-3d	n.d.	n.d.	3.4 ± 0.4	22.5 ± 0.6	20.3 ± 0.9	5.6 ± 1.1
3-4d	n.d.	n.d.	n.d.	16.7 ± 1.6	18.7 ± 2.0	13.3 ± 2.3
4-5d	n.d.	n.d.	n.d.	17.1 ± 1.8	15.7 ± 2.3	22.3 ± 2.5
5-6d	n.d.	n.d.	n.d.	17.4 ± 1.5	20.0 ± 1.8	12.8 ± 2.1
6-7d	n.d.	n.d.	n.d.	16.3 ± 1.0	18.4 ± 2.4	20.3 ± 2.5
7-8d	n.d.	n.d.	n.d.	26.3 ± 1.3	25.3 ± 2.3	18.5 ± 2.2
8-9d	n.d.	n.d.	n.d.	19.2 ± 1.3	16.7 ± 2.7	24.3 ± 2.8

h and d denote hour and day, n.d. denote not detected, As_1 and As_2 denote 11.2 and 1120 μg L^{-1} As(V), respectively. P_2 and P_3 denote 1.12 and 11.2 mg L^{-1} phosphate, respectively. Data are means ± standard deviation (n = 3).

Table S4 Two-way ANOVA of the effects of As(V), phosphate and their interactions on the total As and P contents in *D. salina* cells for 9-d As(V) incubation (F values).

	Adsorbed As	Absorbed As	P
As(V)	16088.197**	2047.160**	52.370**
phosphate	56.316**	461.602**	12751.770**
As(V)× phosphate	48.274**	441.361**	16.734**

** represents significant differences between two treatments (p<0.01).

Table S5 Average arsenate depletion rates of the 6 media during the 9-d cultivation.

Time (h/d)	Rate (μg L^{-1} h^{-1})					
	As_1+P_1	As_1+P_2	As_1+P_3	As_2+P_1	As_2+P_2	As_2+P_3
0-4h	0.11 ± 0.04	0.05 ± 0.04	0.01 ± 0.03	1.0 ± 0.15	0.82 ± 0.23	0.34 ± 0.28
5-24h	0.15 ± 0.00	0.09 ± 0.01	0.01 ± 0.00	1.3 ± 0.26	1.8 ± 0.20	1.1 ± 1.3
1-2d	0.20 ± 0.01	0.13 ± 0.00	0.01 ± 0.00	0.30 ± 0.10	0.46 ± 0.27	0.15 ± 0.08
2-3d	0.10 ± 0.00	0.12 ± 0.00	0.00 ± 0.00	0.77 ± 0.26	0.47 ± 0.13	1.1 ± 0.06
3-4d	0.01 ± 0.00	0.07 ± 0.00	0.00 ± 0.00	1.6 ± 0.21	0.68 ± 0.38	-1.15 ± 0.66
4-5d	0.00 ± 0.00	0.06 ± 0.00	0.00 ± 0.00	1.0 ± 1.0	-0.15 ± 0.47	0.81 ± 0.73
5-6d	0.00 ± 0.00	0.01 ± 0.00	0.00 ± 0.00	-0.19 ± 0.15	-0.05 ± 0.07	-0.03 ± 0.08
6-7d	0.00 ± 0.00	0.00 ± 0.00	0.01 ± 0.00	-0.44 ± 0.35	-0.36 ± 0.10	-0.47 ± 0.41
7-8d	0.00 ± 0.00	0.00 ± 0.00	0.01 ± 0.00	-1.0 ± 1.0	0.87 ± 0.48	-0.38 ± 0.44
8-9d	0.00 ± 0.00	0.00 ± 0.00	0.02 ± 0.00	0.70 ± 0.14	1.9 ± 0.43	-0.18 ± 0.26

h and d denote hour and day. n.d. denote not detected. As_1 and As_2 denote 11.2 and 1120 μg L^{-1} As(V), respectively. P_1, P_2 and P_3 denote 0.112, 1.12 and 11.2 mg L^{-1} phosphate, respectively. Data are means ± standard deviation (n = 3).