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Supplementary Information

Short-term exposure of positively charged polystyrene nanoparticles causes oxidative stress and membrane destruction in cyanobacteria

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Summary

Total 13 pages

7 figures

5 tables

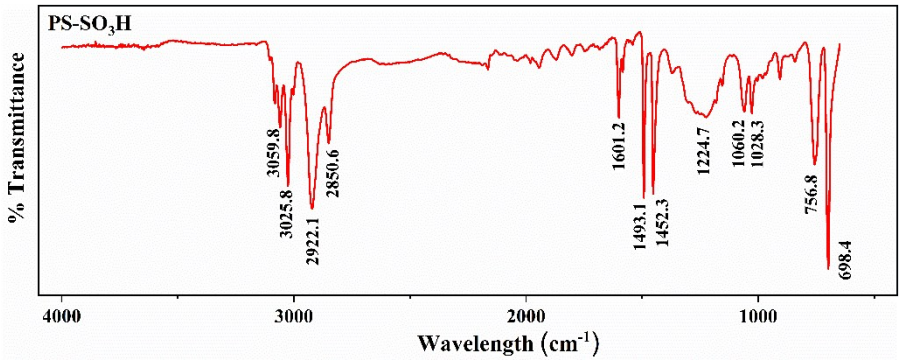

Figure S1 FTIR spectra of PS-SO_3H after exposure to deionized water a for two days. A series of strong peaks at 3026, 2921, 1601, 1493, 1452, 757 and 698 cm^{-1} correspond to the characteristic peaks of benzene in nanoplastics [1].

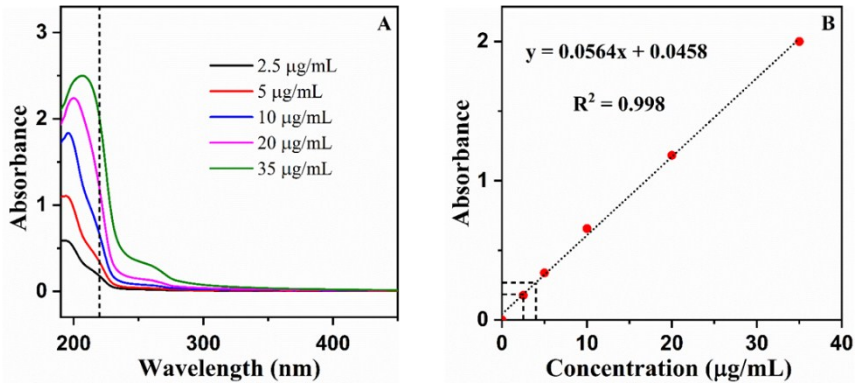

Figure S2 The concentration of PS-NH_2 was detected via UV–Vis spectra. UV–Vis spectra of PS-NH_2 with different concentrations in deionized water (A). The absorbance of PS-NH_2 at 220 nm is fitted to its concentration in deionized water (B).

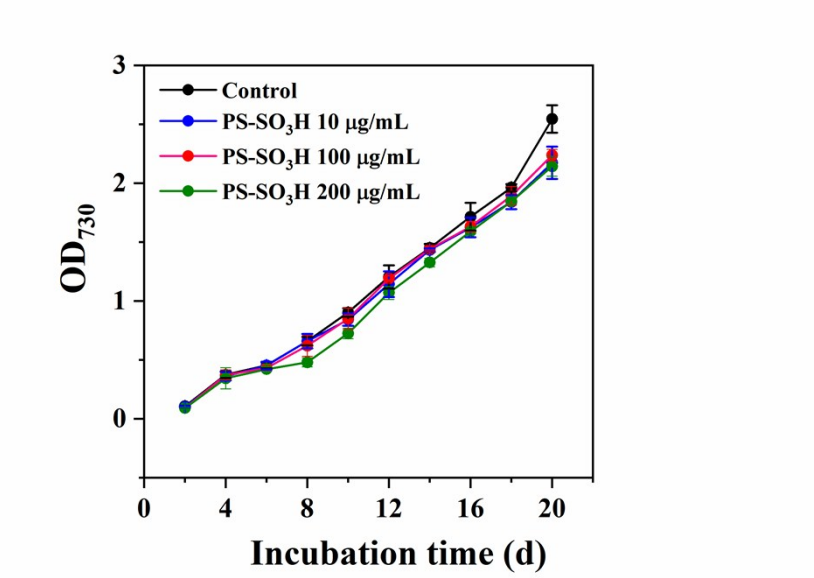

Figure S3 Effects of different concentrations of PS-SO_3H on the cell density of *S. elongatus* PCC 7942.

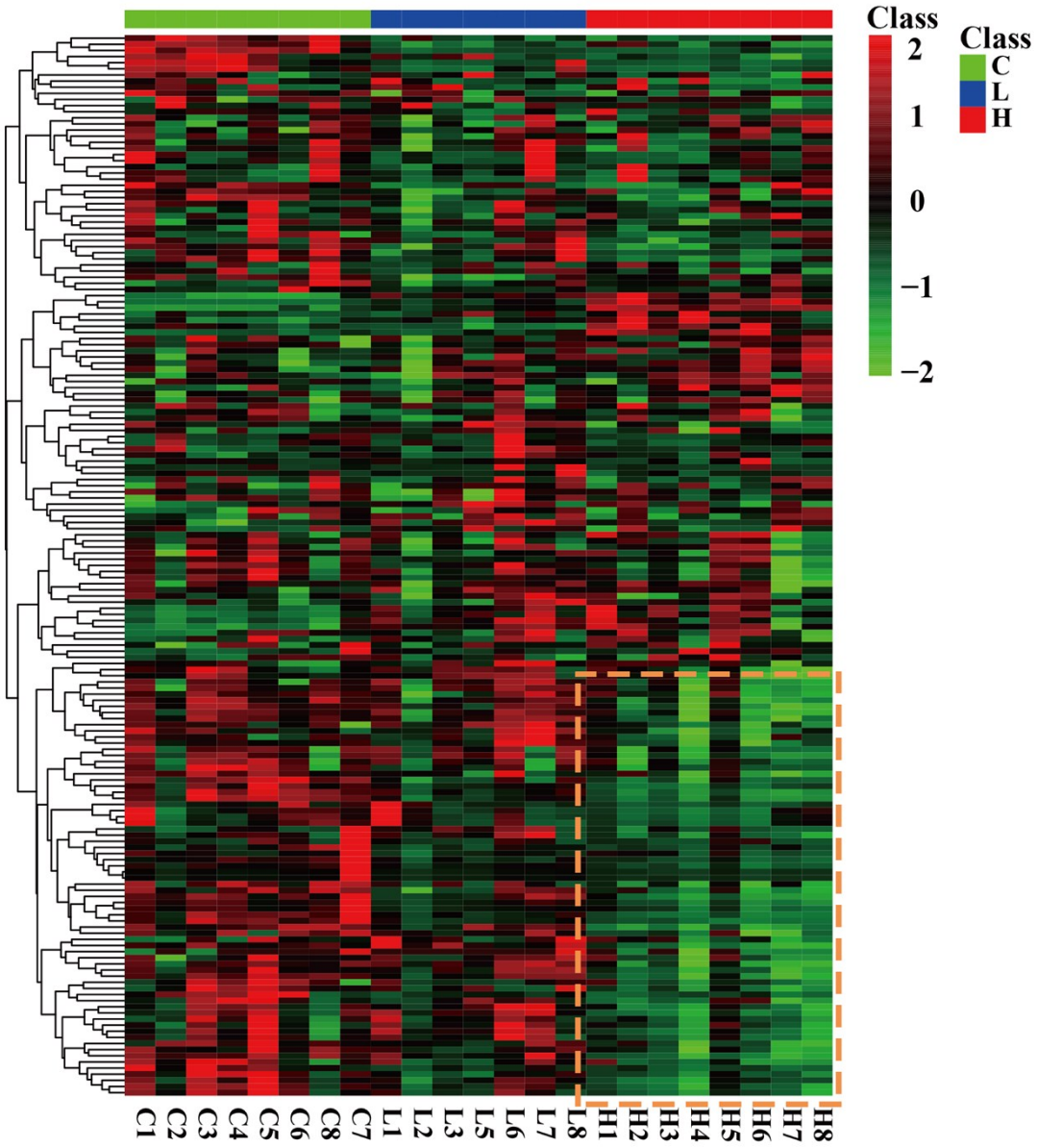

Figure S4 The hierarchical clustering heat map presents a global view of metabolite changes in *Synechococcus* exposed to different concentrations of PS-NH_2 and control. Significantly inhibited metabolites are circled by a dotted orange box. "C" refers to the control group. "L" and "H" refer to low and high concentrations of PS-NH_2, respectively.

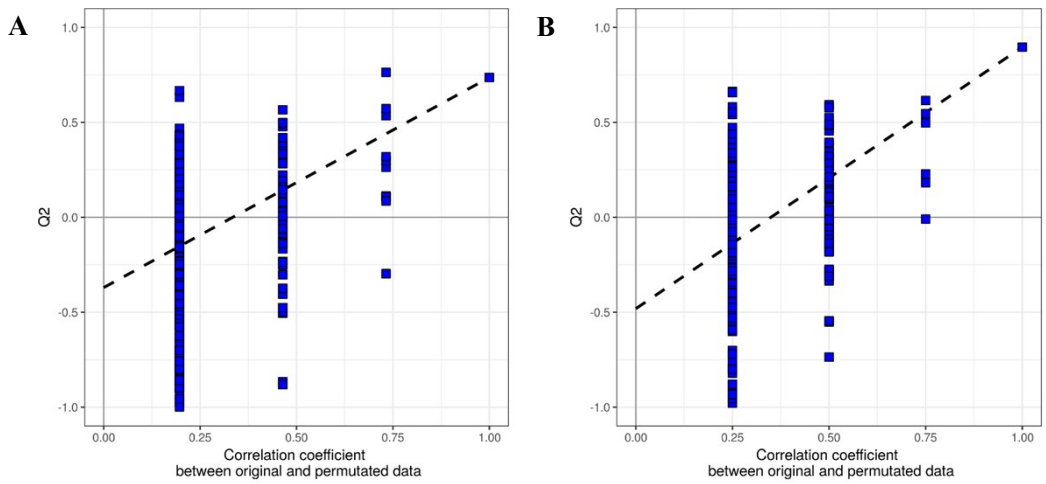

Figure S5 The permutation testing was employed to assess validation of the classification mode. "A" for assessing OPLS-DA model of low concentration group vs control group, "B" for assessing OPLS-DA model of high concentration group vs control group. The intercept of Q_2Y with a threshold less than zero indicates a valid model.

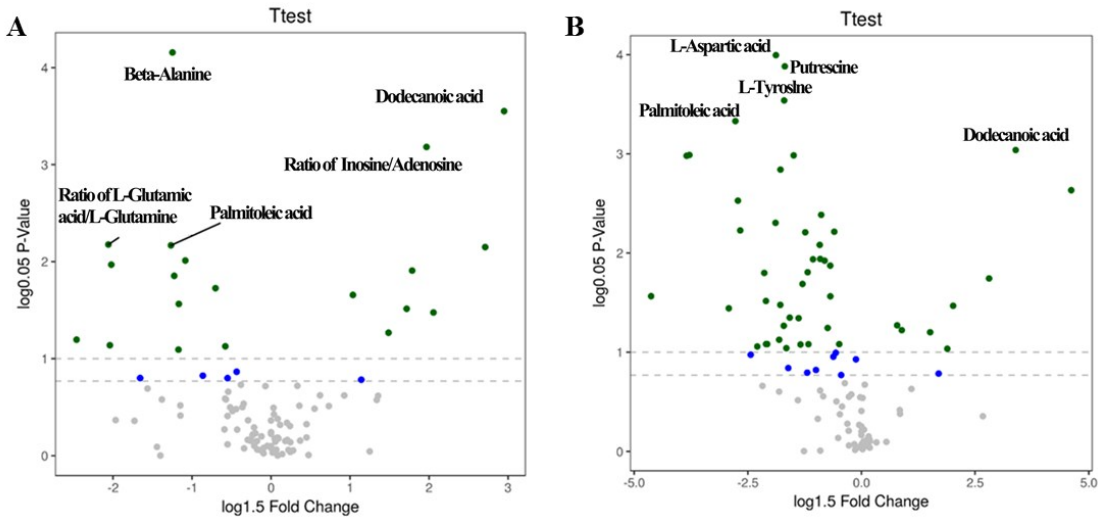

Figure S6 An enhanced volcano plot showed the differential metabolites selected with the multicriteria assessment. The differential metabolites were obtained using univariate statistical analysis, student T-test. "A" for low concentration group vs control group, "B" for high concentration group vs control group. The p-value together with log 1.5 (fold change) are introduced with a cutoff value of 0.05, 0.01 for p value and 1.5 for log1.5 FC, respectively.

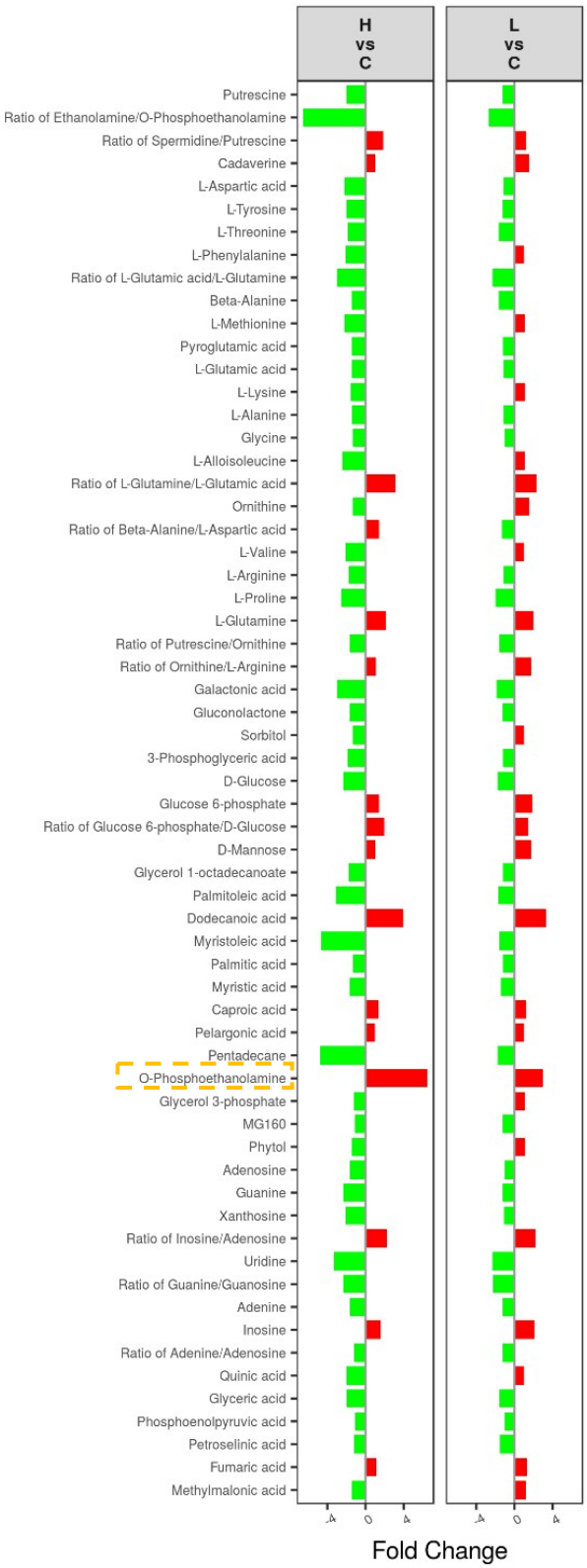

Figure S7 Fold changes of differential metabolites in low- (L) and high-concentration (H) group vs control group (C). "C" refers to the control group. "L" and "H" refer to low and high concentrations of PS-NH_2, respectively.

Table S1 The strains and plasmids used in this study.

Strain	**Relevant genotypes**	**Reference**
PCC7942	Wild-type	
JW11	Same as wild-type, but *⊿gp*	This work
JW12	*Ptrc*: *pssA* integrated at NSI	This work
Plasmid	Genotypes	
pUC18	Amp^R; broad host range vector	Takara
PSHG299	Kan^R; plasmid containing kanamycin resistance	Takara
pAM2991	$Spec^R$; NSI targeting vector; Ptrc	
pGP1	Amp^R; *gp* knockout vector	This work
pJW1	$Spec^R$; NSI targeting vector; Ptrc::*pssA*	This work

Table S2 Primers for construction of the mutant strains used in this study.

Primers	**Sequences (5' - 3')**
GP1	GCCTGCAGGTCGACTCTAGAGGATCCAACCACGACTGGCCTGA GGGCTACTTCGAA
GP2	GACGTTTCCCGTTGAATATGGCTCATGTAACCGCAGTAGGAGG CCACGTTGACAATC
GP3	TCATTTGATGCTCGATGAGTTTTTCTAAGTCTTAGCCCGCTTCA AGAGTGGCGTTGC
GP4	GACCATGATTACGAATTCGAGCTCGGTATCAGGTTCGCCAACA CGGATTACCGTCGAT
GP5	GATTGTCAACGTGGCCTCCTACTGCGGTTACATGAGCCATATTC AACGGGAAACGTC
GP6	GCAACGCCACTCTTGAAGCGGGCTAAGACTTAGAAAAACTCAT CGAGCATCAAATGA
GP7	ATCGACGGTAATCCGTGTTGGCGAACCTGATACCGAGCTCGAA TTCGTAATCATGGTC
GP8	TTCGAAGTAGCCCTCAGGCCAGTCGTGGTTGGATCCTCTAGAG TCGACCTGCAGGC
YZ05	CGATCGCTTTTACAGCAATGACAT
YZ06	ACCTCCTCAAAATTGTTTGCGCCTATC
JW2	CGGTACCCGGGGATCCATGTTGTCAAAATTTAAGCGTAAT
JW3	CGACTCTAGAGGATCCTTACAGGATGCGGCTAATTA

Table S3 Metabolite annotation results for the study samples.

Terms	Counts
Detected	153
Annotated	82
Metabolite Ratios	20
Unknowns	71

Table S4 Differential metabolites between the low-concentration PS-NH_2 group (2.50 μg mL^{-1}) and the control group using univariate statistical analysis.

Class	Name	HMDBID	KeggID	P	FC
Alkylamines	Cadaverine	HMDB02322	C01672	7.00E-03	1.522
	Ratio of Ethanolamine/O-Phosphoethanolamine	HMDB00149/HMDB00224	C00189/C00346	2.80E-02	0.369
Amino Acid	Beta-Alanine	HMDB00056	C00099	3.90E-06	0.603
	Ratio of L-Glutamic acid/L-Glutamine	HMDB00148/HMDB00641	C00025/C00064	1.50E-03	0.434
	L-Threonine	HMDB00167	C00188	3.90E-03	0.609
	Ratio of Beta-Alanine/L-Aspartic acid	HMDB00056/HMDB00191	C00099/C00049	5.70E-03	0.752
	L-Glutamine	HMDB00641	C00064	1.10E-02	2.005
	Ratio of L-Glutamine/L-Glutamic acid	HMDB00641/HMDB00148	C00064/C00025	1.20E-02	2.301
	Ratio of Putrescine/Ornithine	HMDB01414/HMDB00214	C00134/C00077	3.80E-02	0.622
	Pyroglutamic acid	HMDB00267	C01879	0.075	0.838
	L-Proline	HMDB00162	C00148	0.091	0.511
	Ornithine	HMDB00214	C00077	0.096	1.589
Carbohydrates	Glucose 6-phosphate	HMDB01401	C00092	2.20E-02	1.827
Fatty Acids	Dodecanoic acid	HMDB00638	C02679	2.40E-05	3.306
	Palmitoleic acid	HMDB03229	C08362	1.50E-03	0.598
	Myristoleic acid	HMDB02000	C08322	9.20E-03	0.623
	Myristic acid	HMDB00806	C06424	0.085	0.705
Lipids	O-Phosphoethanolamine	HMDB00224	C00346	1.60E-03	3.000
	MG160	HMDB11564	NA	3.40E-02	0.791
Nucleotide	Ratio of Inosine/Adenosine	HMDB00195/HMDB00050	C00294/C00212	7.30E-05	2.220
	Ratio of Guanine/Guanosine	HMDB00132/HMDB00133	C00242/C00387	2.70E-03	0.441
	Inosine	HMDB00195	C00294	3.30E-03	2.062
	Uridine	HMDB00296	C00299	3.30E-02	0.438
	Guanine	HMDB00132	C00242	0.091	0.800
Organic Acids	Petroselinic acid	HMDB02080	C08363	2.40E-03	0.644

P	Fold Change	
p≤0.05	significantly decreased	significant difference between the group. FC<1
p≤0.05	significantly increased	significant difference between the group. FC>1
0.05<p≤0.1	decreased	significant difference between the group. FC<1
0.05<p≤0.1	increased	significant difference between the group. FC>1

Table S5 Differential metabolites between the high-concentration PS-NH_2 group (4.00 μg mL^{-1}) and the control group using univariate statistical analysis.

Class	Name	HMDBID	KeggID	p	FC
Alkylamines	Putrescine	HMDB01414	C00134	8.90E-06	0.505
	Ratio of Ethanolamine/O-Phosphoethanolamine	HMDB00149/HMDB00224	C00189/C00346	9.20E-03	0.153
	Ratio of Spermidine/Putrescine	HMDB01257/HMDB01414	C00315/C00134	2.70E-02	1.846
Amino Acid	L-Aspartic acid	HMDB00191	C00049	6.30E-06	0.466
	L-Tyrosine	HMDB00158	C00082	2.50E-05	0.502
	L-Threonine	HMDB00167	C00188	1.30E-04	0.546
	L-Phenylalanine	HMDB00159	C00079	2.00E-04	0.486
	Ratio of L-Glutamic acid/L-Glutamine	HMDB00148/HMDB00641	C00025/C00064	5.10E-04	0.332
	Beta-Alanine	HMDB00056	C00099	7.90E-04	0.698
	L-Methionine	HMDB00696	C00073	1.00E-03	0.465
	Pyroglutamic acid	HMDB00267	C01879	1.90E-03	0.689
	L-Glutamic acid	HMDB00148	C00025	3.00E-03	0.692
	L-Lysine	HMDB00182	C00047	3.00E-03	0.649
	L-Alanine	HMDB00161	C00041	3.10E-03	0.720
	Glycine	HMDB00123	C00037	3.70E-03	0.757
	L-Alloisoleucine	HMDB00557	NA	4.60E-03	0.420
	Ratio of L-Glutamine/L-Glutamic acid	HMDB00641/HMDB00148	C00064/C00025	5.40E-03	3.122
	Ornithine	HMDB00214	C00077	2.40E-02	0.740
	Ratio of Beta-Alanine/L-Aspartic acid	HMDB00056/HMDB00191	C00099/C00049	2.60E-02	1.433
	L-Valine	HMDB00883	C00183	3.40E-02	0.480
	L-Arginine	HMDB00517	C00062	4.00E-02	0.581
	L-Proline	HMDB00162	C00148	4.20E-02	0.395
	L-Glutamine	HMDB00641	C00064	4.50E-02	2.151
	L-Serine	HMDB00187	C00065	0.057	0.778
	L-Leucine	HMDB00687	C00123	0.08	0.521
	L-Asparagine	HMDB00168	C00152	0.092	0.617
Carbohydrates	Galactonic acid	HMDB00565	C00880	1.30E-03	0.339
	Gluconolactone	HMDB00150	C00198	6.30E-03	0.591
	Sorbitol	HMDB00247	C00794	9.20E-03	0.758
	3-Phosphoglyceric acid	HMDB00807	C00597	1.80E-02	0.527
	D-Glucose	HMDB00122	C00031	3.90E-02	0.432
	D-Galactose	HMDB00143	C00984	0.051	0.795
	Ratio of D-Glucose/Sucrose	HMDB00122/HMDB00258	C00031/C00089	0.054	0.372
	Ratio of Glucose 6-phosphate/D-Glucose	HMDB01401/HMDB00122	C00092/C00031	0.095	1.991
Esters	Glycerol 1-octadecanoate	HMDB31075	NA	1.80E-02	0.571
Fatty Acids	Palmitoleic acid	HMDB03229	C08362	4.60E-05	0.325
	Dodecanoic acid	HMDB00638	C02679	1.10E-04	3.959
	Myristoleic acid	HMDB02000	C08322	1.30E-04	0.215
	Palmitic acid	HMDB00220	C00249	1.30E-03	0.785
	Myristic acid	HMDB00806	C06424	1.30E-03	0.606
	Caproic acid	HMDB00535	C01585	2.20E-02	1.375
	Stearic acid	HMDB00827	C01530	0.099	0.834
Hydrocarbons	Pentadecane	HMDB59886	C08388	1.30E-04	0.210
Lipids	O-Phosphoethanolamine	HMDB00224	C00346	3.70E-04	6.500
	Glycerol 3-phosphate	HMDB00126	C00093	3.90E-02	0.820
	Decanoylcarnitine	HMDB00651	NA	0.085	0.667
Nucleotide	Adenosine	HMDB00050	C00212	4.50E-03	0.619
	Guanine	HMDB00132	C00242	1.10E-02	0.427
	Xanthosine	HMDB00299	C01762	1.20E-02	0.485
	Ratio of Inosine/Adenosine	HMDB00195/HMDB00050	C00294/C00212	1.20E-02	2.266
	Uridine	HMDB00296	C00299	1.30E-02	0.306
	Ratio of Guanine/Guanosine	HMDB00132/HMDB00133	C00242/C00387	3.90E-02	0.427
	Adenine	HMDB00034	C00147	3.90E-02	0.623
Organic Acids	Quinic acid	HMDB03072	C06746	2.20E-02	0.500
	Glyceric acid	HMDB00139	C00258	4.40E-02	0.512
	Phosphoenolpyruvic acid	HMDB00263	C00074	0.062	0.952

p	Fold Change	
p≤0.05	significantly decreased	significant difference between the group. FC<1
p≤0.05	significantly increased	significant difference between the group. FC>1
0.05<p≤0.1	decreased	significant difference between the group. FC<1
0.05<p≤0.1	increased	significant difference between the group. FC>1

Reference

1. Z. Dong, W. Zhang, Y. Qiu, Z. Yang, J. Wang and Y. Zhang, Cotransport of nanoplastics (NPs) with fullerene (C60) in saturated sand: Effect of NPs/C60 ratio and seawater salinity, *Water Res*., 2018, **148**, 469-478.