International Journal of
Environmental Research and Public Health

MDPI

Supplementary Material

Article

Response of the Intertidal Microbial Community Structure and Metabolic Profiles to Zinc Oxide Nanoparticle Exposure

Yinghai Wu [1,2,3], Xinyu Rong [1], Cuiya Zhang [1], Renduo Zhang [3], Tao He [2], Yunjun Yu [2], Zhuangming Zhao [2], Jing Yang [2,*] and Rui Han [1,4,*]

[1] Dalian Ocean University, Dalian 116023, China; wuyinghai@dlou.edu.cn (Y.W.); r0807xy@163.com (X.R.); zhangcuiya@dlou.edu.cn (C.Z.)
[2] South China Institute of Environmental Science, Ministry of Ecology and Environment, Guangzhou 510655, China; hetao@scies.org (T.H.); yuyunjun@scies.org (Y.Y.); zhaozhuangming@scies.org (Z.Z.)
[3] Guangdong Provincial Key Laboratory of Environmental Pollution Control and Remediation Technology, Sun Yat-sen University, Guangzhou 510275, China; zhangrd@mail.sysu.edu.cn
[4] Key Laboratory of Environment Controlled Aquaculture, Ministry of Education, Dalian 116023, China
* Correspondence: yangjing@scies.org (J.Y.); hanrui@dlou.edu.cn (R.H.); Tel.: +86-20-29119533 (J.Y.); +86-411-84763257 (R.H.)

Received: 22 February 2020; Accepted: 24 March 2020; Published:

International Journal of
Environmental Research and Public Health

Table S1. Characterization of ZnO NPs used in the study. Product No.: Z112847, CAS No.: 1314-13-2.

Test	Specification		Target value
	Min	**MAX**	
Appearance	white powder		
Solubility	low solubility in water or ethanol; soluble in acid, ammonia, and alkali hydroxide		
Shape	spheroidal		
Average Particle Size	20 nm	40 nm	40 nm
Purity (Based on Trace Metal Analysis)	49075	2808	100%
Total Metallic Impurities	55385	2931	1000 ppm
Zn (Complexocetric Titration)	79.1%	81.5%	81.5%

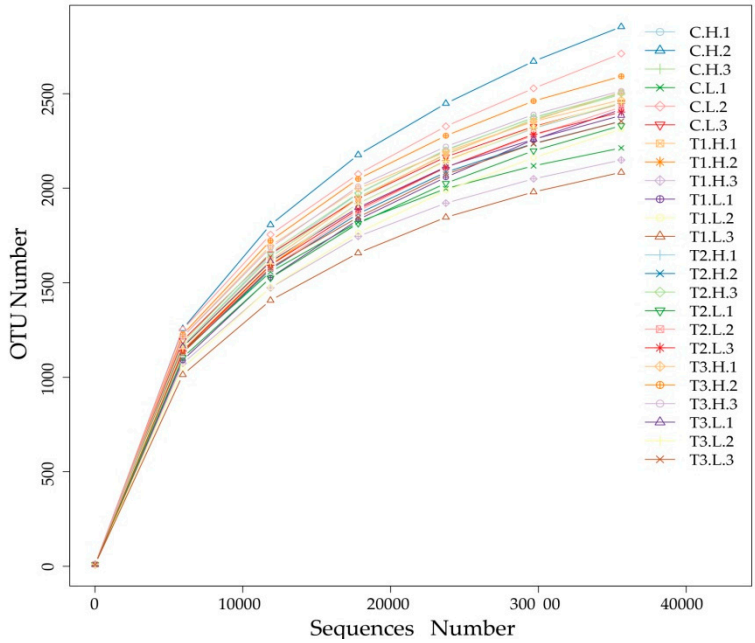

Figure S1. Rarefaction curves for operational taxonomic unit (OTU) comparison. The curves for OTU number were calculated using Mothur (v1.27.0) using a 0.03 distance for OTUs. In the group name description, the letter C means CK; H and L indicate intermittently submerged and submerged, respectively; T1, T2, and T3 are the three treatments of ZnO NP concentrations, 40 mg·L^{-1}, 80 mg·L^{-1}, 120 mg·L^{-1}. Three duplicates in each group are numbered 1, 2, and 3.

Table S2. Summary of 16S RNA Miseq sequences, operational taxonomic units (OTUs), and microbial diversity of sediment samples. The samples numbers are shown in Figure S1. Microbial alpha diversity (e.g., Chao1, PD_whole_tree, and Simpson) was estimated based on 97% OTU (operational taxonomic units) clusters.

Sample ID	No. of Sequences	No. of OTUs	Chao1	Observed_Species	ACE	PD_Whole_Tree	Simpson
C.H.1	49754	2990	2943.87	5118	3075.694	208.269	0.992
C.H.2	61973	3413	3863.134	4726	3903.579	233.031	0.99
C.H.3	54311	2997	2886.589	4746	3119.597	204.739	0.992
C.L.1	43538	2607	2499.968	4890	2550.179	184.158	0.993
C.L.2	40283	2712	5648.304	5145	3903.982	224.832	0.995
C.L.3	51614	2909	2914.188	5058	2999.113	201.823	0.994
T1.H.1	57537	2948	3287.002	4604	3328.091	197.646	0.993
T1.H.2	55959	2894	2851.574	3795	3067.816	198.726	0.993
T1.H.3	49773	2529	2442.684	4827	2524.119	174.231	0.993
T1.L.1	66323	2963	3288.002	4767	3447.206	191.244	0.992
T1.L.2	62148	2834	3073.965	4771	3249.89	186.63	0.992
T1.L.3	50328	2467	2465.394	4849	2563.407	173.119	0.991
T2.H.1	55494	2905	2857.686	4545	3089.622	197.039	0.994
T2.H.2	50922	2799	2768.633	4763	2893.564	188.957	0.993
T2.H.3	55120	2986	2903.529	4613	3084.858	204.178	0.994
T2.L.1	56173	2782	2978.811	4516	3049.136	185.414	0.994
T2.L.2	57264	2926	3152.252	4675	3242.271	200.459	0.995
T2.L.3	53422	2865	2758.373	4109	2960.304	195.006	0.995
T3.H.1	44028	2843	2827.177	4069	2905.582	200.313	0.995
T3.H.2	53871	3100	2965.041	4325	3171.151	206.781	0.995
T3.H.3	49389	2970	2899.376	4480	3025.368	202.651	0.994
T3.L.1	49075	2808	2850.673	4701	2926.071	195.298	0.994
T3.L.2	55385	2931	2818.876	4019	3069.817	201.309	0.994
T3.L.3	51099	2805	2739.564	4751	2922.207	193.599	0.994

Table S3. Carbon sources in the Biolog Ecoplates

Carbohydrates	Carboxylic Acids	Polymers	Amino Acids
α-D-lactose	α-Ketobutyric acid	α-Cyclodextrin	L-Arginine
β-Methyl D-glucoside	D-Galacturonic acid	Glycogen	L-Asparagine
D-Cellobiose	D-Glucosaminic acid	Tween 40	L-Phenylalanine
D-Mannitol	D-Malic acid	Tween 80	L-Serine
I-Erythritol	Itaconic acid		L-Threonine
Glucose-1-phosphate	γ-Hydroxybutyric acid	Phenolic compounds	Glycyl-L-glutamic acid
D-Galactonic acid-γ-lactone	Pyruvic acid methyl ester	2-Hydroxy benzoic acid	
D,L-α-Glycerol phosphate		4-Hydroxy benzoic acid	Amines
N-Acetyl-D-glucosamine			Phenyl ethylamine
D,L-α-Glycerol phosphate			Putrecine