Supplementary Information

Temperature and salinity jointly drive the toxicity of zinc oxide nanoparticles: A challenge to environmental risk assessment under global climate change

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Appendix A1. Determination of test temperature and salinity

Temperature and salinity in the experiment were selected based on the realistic environmental conditions in Hong Kong seawaters, where the test animals, *T. japonicus*, were obtained[1] (Fig. S1). Temperature in rock pools during the summer of Hong Kong could be between 26 to 32 °C while it was between 15 to 18 °C during winter[2]. Comparatively, the average salinity at the sea surface of Hong Kong could range from 12.5 to 33.8 PSU during the wet and dry seasons[1]. In extreme cases, the salinity could even drop to 1 PSU in intertidal zone after heavy precipitation[3]. Influence of global climate change is evident in Hong Kong. For example, an increase of about 1.25 °C and a continuous upward trend of extreme hot weather was observed in Hong Kong throughout the last century[4,5]. Likewise, dramatic rainfalls have been recorded in the past and predicted to become more frequent[6,7], which could draw the salinity to further downward extremes. Therefore, an increase of 4.8 °C in temperature, as predicted by IPCC[8], was also considered as a worst-case scenario (Fig. S1).

Appendix A2. Response Surface Model

The response surface model (RSM) was constructed using a two-factor multilevel factorial design by Design-Expert (v11., Stat-Ease Inc., U.S.A.), following a five-tiered approach: (1) the initial fitting model was proposed by the software based on a basket of factors, including model *p*-value, lack-of-fit *p*-value, adjusted and predicted r^2; (2) components within the initial model were further screened based on their *p*-value, Akaike information criterion (AIC) and hierarchical structure of the model; (3) the final model was confirmed by the final model *p*-value, lack-of-fit *p*-value, adjusted r^2 and predicted r^2 using ANOVA analysis, as well as the variance inflation factor (VIF) of each component; (4) diagnosis of the final model on the fitted data was further performed using normal probability plot and residual plot, and (5) model that required the least transformation of data and met all above criteria was selected.

Following is a basic form of the model equation:

$$Y(x_A, x_B) = k + a_A x_A + a_B x_B + a_{AB} x_A x_B + b_A x_A^2 + b_B x_B^2 \cdots + n_A x_A^n + n_B x_B^n \cdots$$

RSM equation where Y is the response of the rotifer while x_A and x_B are doses of the two chemicals in the mixture; k and a, b…n… are the coefficients of the regression equation.

The results suggested that there was a differential interaction between temperature and salinity on the toxicity of the chemicals (Table S4**)**. For ZnO-NPs, only temperature had a significant effect on their toxicity and there was a significant interaction of the temperature and salinity at the secondary level. For ZnO-BKs, both temperature and salinity had a significant effect on the toxicity but the two factors interacted only at tertiary level. For Zn-IONs, both factors had a significant effect on their toxicity and they interacted at secondary and tertiary levels. Meanwhile, over-interpretation on these interaction terms should be avoided as they were just describing the changes of calculated LC_{50} along with temperature and salinity, and could be subjected to experimental variations[9,10]. However, given the distinct difference in monitored physicochemical properties of the three chemicals and their observed toxicity, it should be conceivable that these three chemicals had different interactions with temperature and salinity.

Appendix A3. Current marine water quality criteria

Lai et al.[11] found a large deficiency in the current regulations of products containing engineered nanoparticles. Although a case-by-case risk evaluation has been adopted for the products in some commercial sectors, most of the reviewed regulations did not distinguish engineered nanoparticles from their bulk- and ionic counterparts[11]. This has raised a concern over the potential influences of engineered nanoparticles on the environment and human health[12,13].

Given the limited information about nano-specific water quality criteria, the predicted toxicity values of ZnO-NPs and the associated chemicals in this study were only compared to the existing water quality criteria for Zn ions (Table S1). The range of current predicted environmental concentrations of ZnO-NPs in the surface water is between around 0.0001 μg/L and 76 μg/L[14-16], which could be comparable to current criteria for Zn ions (Table S1) and the predicted LC_{10} of ZnO-NPs in this study (Fig. S5). Nevertheless, the predicted environmental concentrations of ZnO-NPs from the same research group have been increased by 10 times within 5 years, implying their increased contamination and potential negative impact in the environment[14,15]. Therefore, the results of the prediction of the toxicity of ZnO-NPs in different salinity and temperature, as demonstrated in this study, could provide useful information for developing site-species water quality criteria of ZnO-NPs that will provide better protection to aquatic ecosystems around the world.

Temperature and salinity in Hong Kong

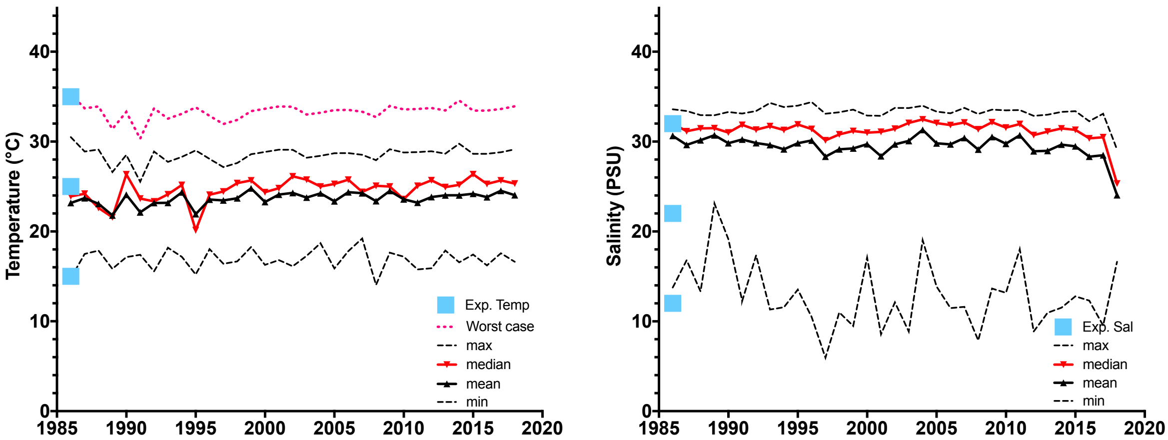

Fig. S1 Variation of temperature and salinity over the years in Hong Kong waters (Mirs Bay, Southern, North Western and Victoria Harbour), superimposed with selected experimental conditions. Line of the worst case in temperature implies the increment predicted by IPCC under the highest emission scenario.

Morphology and size of the particles

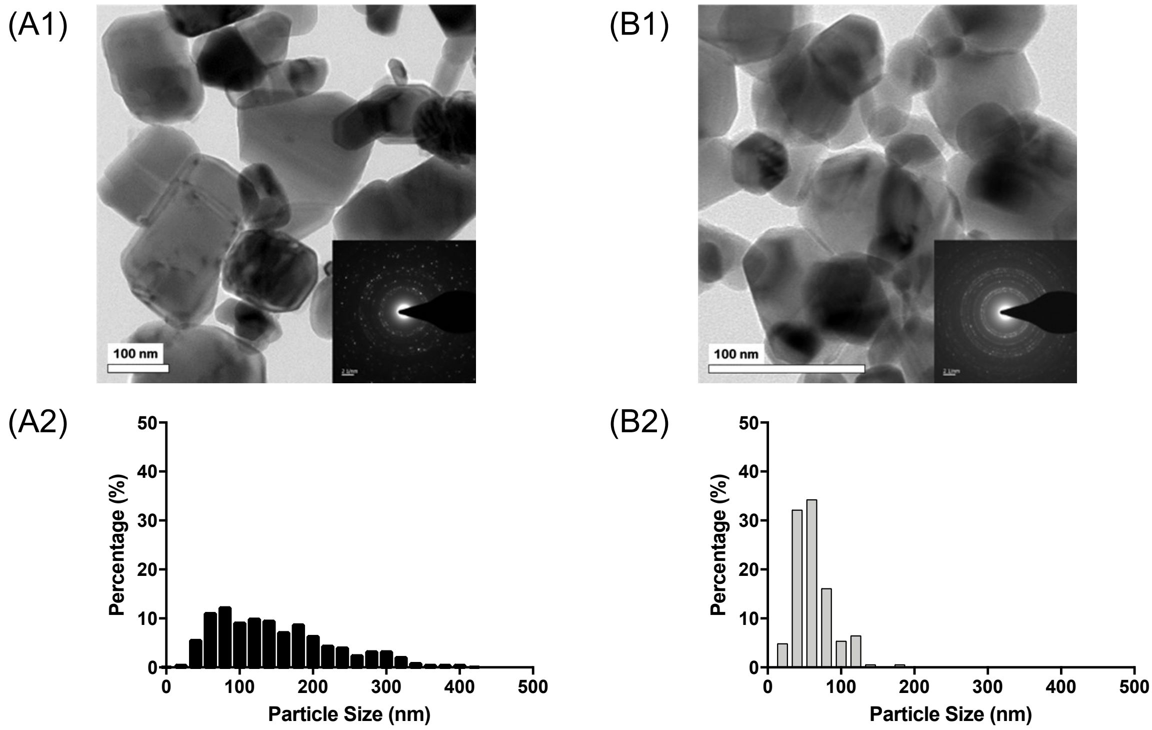

Fig. S2 (1) TEM image and (2) size distribution of dry powder of (A) ZnO-BKs and (B) ZnO-NPs. Inset at the bottom right corner of A1 and B1 are the SAED patterns of the particles; the central diffraction spot is masked by a shield in order to record the pattern.

Crystal phase of the particles

The X-Ray Diffraction analysis confirmed that both ZnO-BKs and ZnO-NPs share a comparable crystal phase, i.e. hexagonal wurtzite[17,18].

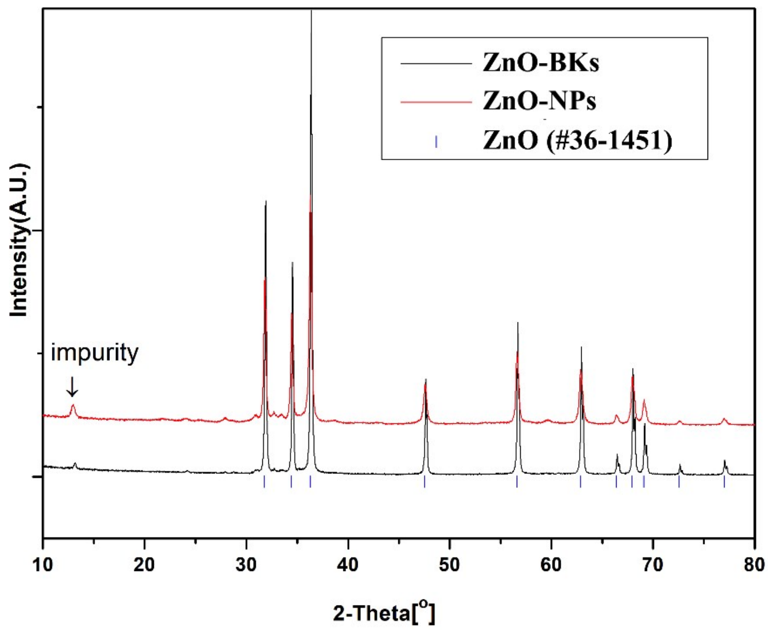

Fig. S3 Crystal phase of ZnO-BKs and ZnO-NPs.

ROS signal of the chemicals

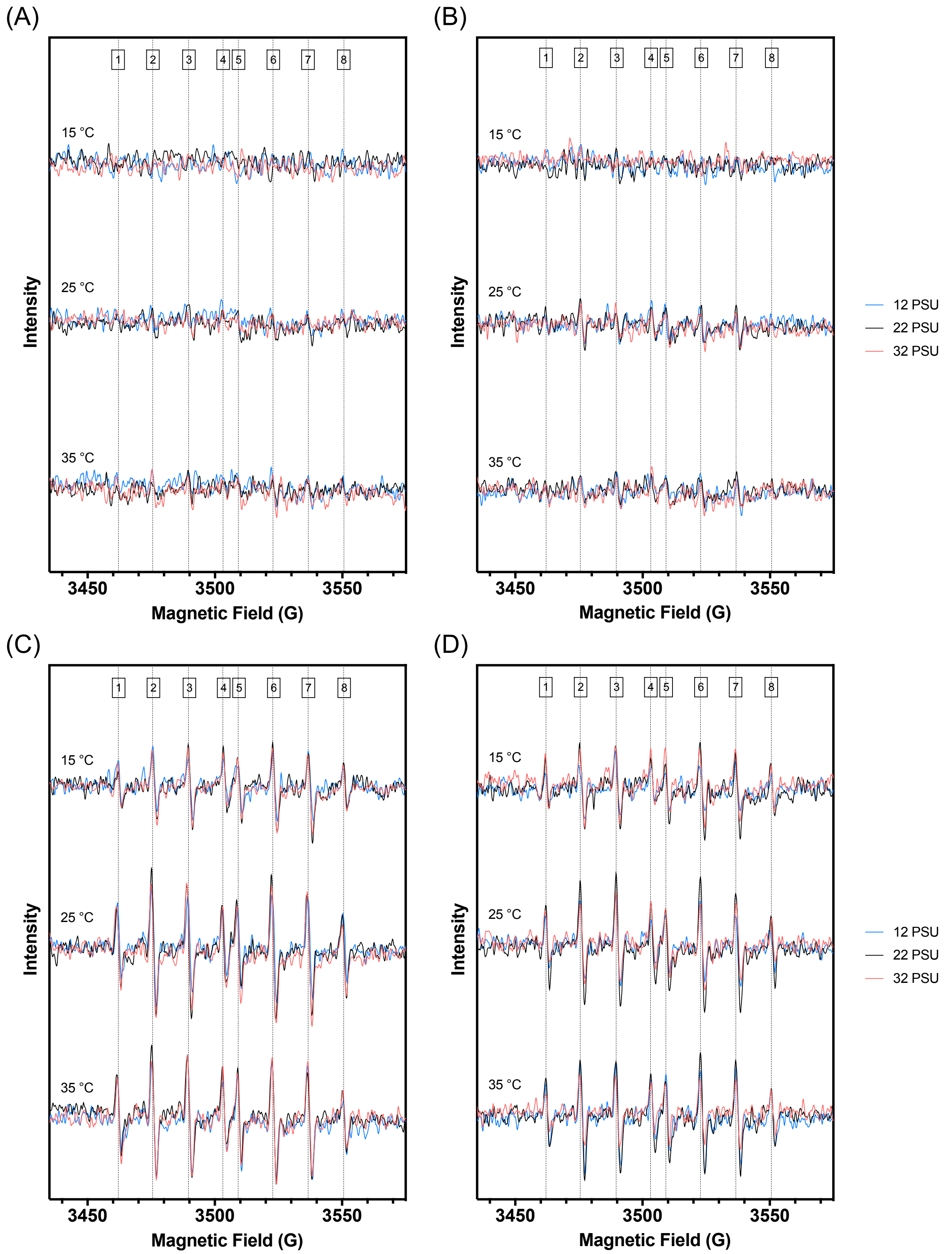

Fig. S4 ROS intensity in the solutions of (A) FASW, (B) Zn-IONs, (C) ZnO-NPs and (D) ZnO-BKs under the influence of different temperatures and salinities.

RSM results

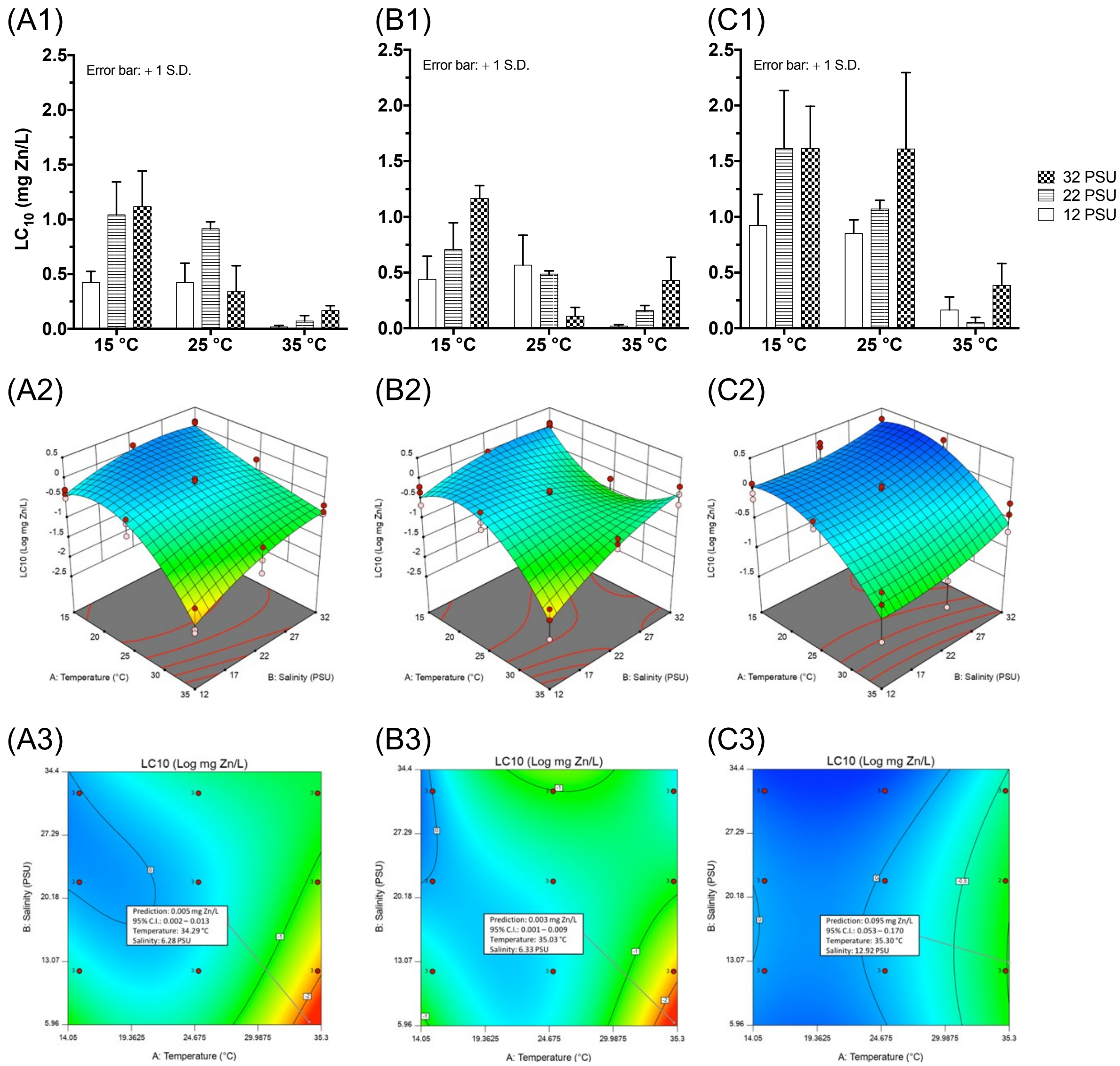

Fig. S5 LC_{10} of (A) ZnO-NPs, (B) ZnO-BKs and (C) Zn-IONs under the influence of different temperatures and salinities.

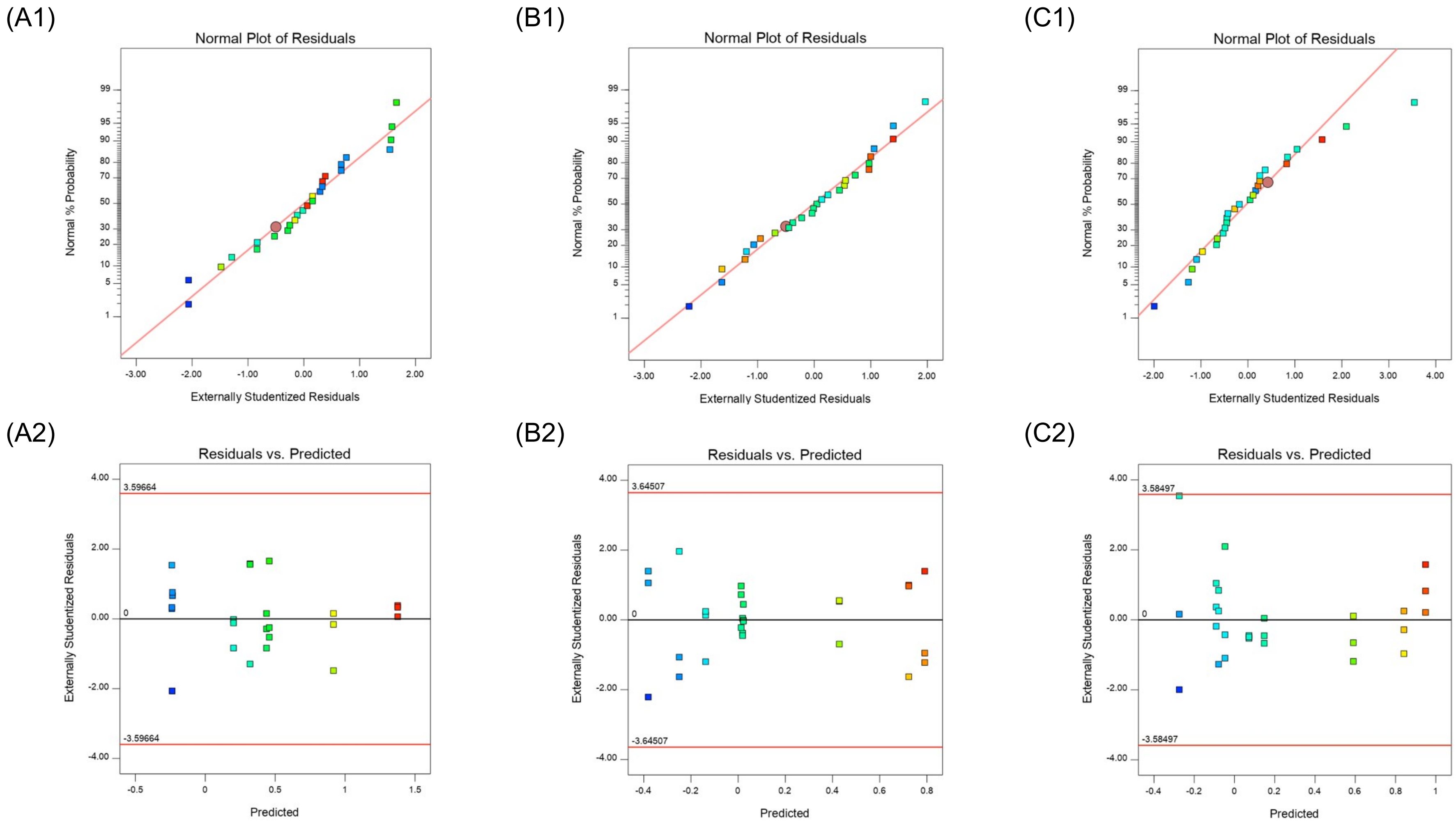

Fig. S6 (1) Normal probability plot and (2) Residual plot for the response surface model of (A) Zn-IONs, (B) ZnO-BKs and (C) ZnO-NPs under the influence of different temperatures and salinities.

Marine water quality criteria of zinc in different countries

Marine water quality criteria of zinc in different countries were surveyed. Some countries have applied different water quality criteria for water zones with different beneficial uses. The strictest criteria (the smallest value) and the loosest criteria (the largest value) are indicated in Fig. 8.

Table S1 Marine water quality criteria of zinc in different countries for water zones with different beneficial uses.

Countries		Values (μg/L)	Sources
Viet Nam	National Technical Regulation on Coastal Water Quality	Ocean areas: 20 Sea areas: 50 Coastal aquaculture: 500 Coastal beaches and recreation area: 1000 Other coastal areas: 2000	19
Philippines	Water Quality Guidelines and General Effluent Standards	Protected waters (Class SA): 40 Fishery and recreation (Class SB): 50 Fishery and recreation (Class SC): 800 Navigable waters (Class SD): 1500	19
China	Sea water quality standard (GB 3097-1997)	Marine protection rea (Type I): 20 Aquaculture area and beaches (Type II): 50 General industrial and recreation area (Type III): 100 Harbour area (Type IV): 500	20
Korea	Environmental Standards for Water Quality and Aquatic Ecosystem	Coastal waters: 100	19
USA	National Recommended Aquatic Life Criteria table	Saltwater Criterion Continuous Concentration: 81 Saltwater Criterion Maximum Concentration: 90	21
Indonesia	Standard Quality of Seawater	Marine ecosystem: 50 Marine recreation: 95 Ports and harbours: 100	19
Malaysia	Marine Water Quality Criteria and Standard	Marine Protected Areas (Class I): 15 Recreational and Mariculture (Class 2): 50 Ports, Oil & Gas Fields (Class 3): 100 Estuarine (Class 4): 50	19
Australia	Toxicant default guideline values for water quality in aquatic ecosystems (Marine Water)	99% of species protection: 7 95% of species protection: 15 99% of species protection: 23 99% of species protection: 43	22
UK	Guidelines for managing water quality impacts within UK European marine sites	40	23
Japan	Environmental Quality Standards for Water Pollution	Coastal waters: 10 – 20	19

19. WEPA, 2017; 20. Ministry of Ecology and Environment of China (GB 3097-1997), 1998; 21. USEPA, 1995; 22. Australian Government Initiative, 2019; 23. Cole et. Al., 2010.

ANOVA results

Table S2
Abbreviations: Temp: temperature; Sal: salinity; chem: chemical

(A) Hydrodynamic size					
Source	**Type III Sum of Squares**	**df**	**Mean Square**	**F-value**	***p*-value**
temp	34594.04	2	17297.02	19.99	< 0.001*
sal	14759.70	2	7379.85	8.53	0.001*
chem	12696.00	1	12696.00	14.68	< 0.001*
temp * sal	8116.41	4	2029.10	2.35	0.073
temp * chem	4773.44	2	2386.72	2.76	0.077
sal * chem	14983.11	2	7491.56	8.66	0.001*
temp * sal * chem	919.44	4	229.86	0.27	0.898
Error	31144.00	36	865.11		
Total	32206382.00	54			
Corrected Total	121986.15	53		7491.5555	

(B) Zeta potential					
Source	**Type III Sum of Squares**	**df**	**Mean Square**	**F-value**	***p*-value**
temp	314.87	2	157.44	15.32	< 0.001*
sal	4.39	2	2.20	0.21	0.809
chem	9.71	1	9.71	0.94	0.338
temp * sal	33.65	4	8.41	0.82	0.522
temp * chem	23.80	2	11.90	1.16	0.326
sal * chem	48.36	2	24.18	2.35	0.110
temp * sal * chem	33.70	4	8.43	0.82	0.521
Error	370.08	36	10.28		
Total	4373.13	54			
Corrected Total	838.57	53			

(C) Ion dissolution					
Source	**Type III Sum of Squares**	**df**	**Mean Square**	**F-value**	***p*-value**
Temp	965.27	2	482.63	127.02	< 0.001*
sal	470.46	2	235.23	61.91	< 0.001*
chem	64.65	1	64.65	17.02	< 0.001*
temp * sal	74.28	4	18.57	4.89	0.002*
temp * chem	214.61	2	107.30	28.24	< 0.001*
sal * chem	93.11	2	46.55	12.25	< 0.001*
tem * sal * chem	70.82	4	17.70	4.66	0.003*
Error	205.18	54	3.80		

Total	9441.72	72			
Corrected Total	2158.38	71			

(D) LC$_{50}$					
Source	**Type III Sum of Squares**	**df**	**Mean Square**	**F-value**	***p*-value**
temp	13.34	2	6.67	692.86	< 0.001*
sal	0.60	2	0.30	30.96	< 0.001*
chem	0.47	2	0.23	24.36	< 0.001*
temp * sal	0.86	4	0.21	22.24	< 0.001*
temp * chem	0.81	4	0.20	21.10	< 0.001*
sal * chem	0.72	4	0.18	18.73	< 0.001*
temp * sal * chem	0.61	8	0.08	7.87	< 0.001*
Error	0.52	54	0.01		
Total	22.24	81			
Corrected Total	17.92	80			

(E) GST					
Source	**Type III Sum of Squares**	**df**	**Mean Square**	**F-value**	***p*-value**
temp	0.06	2	0.028	31.62	< 0.001*
sal	0.05	2	0.024	26.58	< 0.001*
chem	0.05	2	0.022	24.80	< 0.001*
temp * sal	0.00	4	0.001	1.22	0.313
temp * chem	0.03	4	0.007	7.32	< 0.001*
sal * chem	0.01	4	0.001	1.45	0.230
temp * sal * chem	0.01	8	0.002	1.84	0.089
Error	0.05	54	0.001		
Total	349.21	81			
Corrected Total	0.25	80			

(F) SOD					
Source	**Type III Sum of Squares**	**df**	**Mean Square**	**F-value**	***p*-value**
temp	0.07	2	0.036	39.53	< 0.001*
sal	0.06	2	0.032	35.14	< 0.001*
chem	0.00	2	0.002	1.82	0.172
temp * sal	0.02	4	0.004	4.15	0.005*
temp * chem	0.01	4	0.003	3.00	0.026*
sal * chem	0.01	4	0.003	3.20	0.020*
temp * sal * chem	0.02	8	0.002	2.23	0.039*
Error	0.05	54	0.001		
Total	335.86	81			

Corrected Total	0.24	80

Table S3 Toxicity of the test chemicals under different temperatures and salinities.

		LC_{10} ± 1 S.D. (mg Zn/L)	LC_{50} ± 1 S.D. (mg Zn/L)
Temperature: 15 °C			
12 PSU	Zn-IONs	0.92 ± 0.28	3.05 ± 0.66
	ZnO-BKs	0.44 ± 0.21	2.75 ± 0.30
	ZnO-NPs	0.42 ± 0.10	3.43 ± 0.53
22 PSU	Zn-IONs	1.61 ± 0.52	7.58 ± 1.21
	ZnO-BKs	0.71 ± 0.24	6.03 ± 1.41
	ZnO-NPs	1.04 ± 0.30	11.12 ± 1.82
32 PSU	Zn-IONs	1.61 ± 0.38	24.94 ± 0.78
	ZnO-BKs	1.17 ± 0.12	5.49 ± 1.15
	ZnO-NPs	1.12 ± 0.33	6.46 ± 0.93
Temperature: 25 °C			
12 PSU	Zn-IONs	0.85 ± 0.12	1.51 ± 0.12
	ZnO-BKs	0.57 ± 0.27	1.00 ± 0.04
	ZnO-NPs	0.42 ± 0.18	0.91 ± 0.14
22 PSU	Zn-IONs	1.05 ± 0.07	2.42 ± 0.69
	ZnO-BKs	0.49 ± 0.03	1.12 ± 0.12
	ZnO-NPs	0.91 ± 0.06	1.29 ± 0.12
32 PSU	Zn-IONs	1.61 ± 0.68	2.60 ± 0.23
	ZnO-BKs	0.11 ± 0.08	0.56 ± 0.17
	ZnO-NPs	0.35 ± 0.23	0.84 ± 0.21
Temperature: 35 °C			
12 PSU	Zn-IONs	0.16 ± 0.12	0.66 ± 0.08
	ZnO-BKs	0.02 ± 0.01	0.44 ± 0.11
	ZnO-NPs	0.02 ± 0.01	0.97 ± 0.39
22 PSU	Zn-IONs	0.06 ± 0.07	0.34 ± 0.13
	ZnO-BKs	0.17 ± 0.03	0.70 ± 0.08
	ZnO-NPs	0.07 ± 0.05	1.04 ± 0.01
32 PSU	Zn-IONs	0.39 ± 0.20	0.75 ± 0.16
	ZnO-BKs	0.43 ± 0.21	1.08 ± 0.05
	ZnO-NPs	0.17 ± 0.04	0.65 ± 0.36

Table S4 RSM model for the effect of temperature and salinity on chemicals: (A) Zn-IONs, (B) ZnO-BKs and (C) Zn-NPs. *p*-value with an asterisk represents the significant term at α = 0.05.

(A) Zn-IONs					
Source	**Sum of Squares**	**df**	**Mean Square**	**F-value**	**p-value**
Model	7.010	5	1.400	170.280	< 0.0001*
A-Temperature	5.640	1	5.640	685.410	< 0.0001*
B-Salinity	0.084	1	0.084	10.180	0.0046*
AB	0.627	1	0.627	76.220	< 0.0001*
A^2	0.002	1	0.002	0.290	0.5962
A^2B	0.051	1	0.051	6.140	0.0223*
Residual	0.165	20	0.008		
Lack of Fit	0.089	3	0.030	6.720	0.0034*
Pure Error	0.075	17	0.004		
Cor Total	7.170	25			

Model equation in terms of coded factors:

$$Y(A,B) = 0.320 - 0.577A + 0.118B - 0.229AB + 0.020A^2 + 0.112A^2B$$

Model equation in terms of actual factors:

$$Y(A,B) = -1.174 + 0.106A + 0.139B - 0.008AB - 0.002A^2 + 0.0001A^2B$$

(B) ZnO-BKs					
Source	**Sum of Squares**	**df**	**Mean Square**	**F-value**	**p-value**
Model	4.180	7	0.597	89.840	< 0.0001*
A-Temperature	1.290	1	1.290	194.790	< 0.0001*
B-Salinity	0.108	1	0.108	16.280	0.0007*
AB	0.009	1	0.009	1.330	0.2624
A^2	0.591	1	0.591	88.930	< 0.0001*
B^2	0.099	1	0.099	14.860	0.0011*
A^2B	0.381	1	0.381	57.370	< 0.0001*
AB^2	0.030	1	0.030	4.520	0.0468*
Residual	0.126	19	0.007		
Lack of Fit	0.009	1	0.009	1.360	0.2582
Pure Error	0.117	18	0.007		
Cor Total	4.300	26			

Model equation in terms of coded factors:

$$Y(A,B) = 0.013 - 0.464A - 0.134B + 0.027AB + 0.314A^2 - 0.128B^2 + 0.309A^2B + 0.087AB^2$$

Model equation in terms of actual factors:

$$Y(A,B) = -2.334 + 0.172A + 0.324B - 0.019AB - 0.004A^2 - 0.003B^2 + 0.0003A^2B + 0.0001AB^2$$

(C) ZnO-NPs					
Source	**Sum of Squares**	**df**	**Mean Square**	**F-value**	**p-value**
Model	4.750	5	0.950	65.500	< 0.0001*
A-Temperature	3.460	1	3.460	238.780	< 0.0001*
B-Salinity	0.001	1	0.001	0.045	0.8347
AB	0.171	1	0.171	11.820	0.0025*
A^2	0.789	1	0.789	54.440	< 0.0001*
B^2	0.325	1	0.325	22.420	0.0001*
Residual	0.305	21	0.015		
Lack of Fit	0.065	3	0.022	1.630	0.2183
Pure Error	0.240	18	0.013		
Cor Total	5.050	26			

Model equation in terms of coded factors:

$$Y(A,B) = 0.148 - 0.439A + 0.006B - 0.120AB + 3.363A^2 - 0.233B^2$$

Model equation in terms of actual factors:

$$Y(A,B) = -1.714 - 0.199A + 0.132B - 0.001AB + 0.004A^2 - 0.002B^2$$

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