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Supporting Information (SI)

Key component group of environment nanoparticles and corresponding contribution to oxidative stress of Escherichia coli in water

Chunlei Jiao[a,b], Tong Sun[a,b], Min Chen[a,b], Yiwei Cai[a,b], Guiying Li[a,b], Taicheng An[a,b,*]

[a] *Guangdong-Hong Kong-Macao Joint Laboratory for Contaminants Exposure and Health, Guangdong Key Laboratory of Environmental Catalysis and Health Risk Control, Institute of Environmental Health and Pollution control, Guangdong University of Technology, Guangzhou 510006, China*

[b] *Guangzhou Key Laboratory of Environmental Catalysis and Pollution Control, Guangdong Basic Research Center of Excellence for Ecological Security and Green Development, School of Environmental Science and Engineering, Guangdong University of Technology, Guangzhou 510006, China*

Corresponding author: Prof. Taicheng An. E-mail: antc99@gdut.edu.cn;

Text S1. Extraction and separation of environmental NPs

The operation process is as follows: **I.** Installation of the ultrafiltration membrane capsule (10 kDa, Omega^{TM}, PULL, USA). **II.** Extraction and concentration. The sample in the feedstock tank was injected into the TFF system, the filtrate was drained and collected, and the reflux was re-injected into the in the feedstock tank until the concentration ratio (1:500) was reached. **III.** Demineralization. Add the same volume of ultrapure water to the feedstock tank and repeat the previous step (II) 5 times to obtain the origin environmental NP suspensions (≥10 kDa) for characterization and subsequent separation. **IV.** Separation and concentration. The ultrafiltration membrane capsule was replaced with 1,000 kDa, 100 kDa a()nd 10 kDa successively, and the above steps (I/II/III) were repeated to extract the collected filtrate, that is, environmental NP suspensions in different molecular weight ranges were obtained. Thus, the molecular weights of environmental NPs used in subsequent bacterial exposure experiments ranged from 10–100 kDa, 100–1000 kDa, and ≥1,000 kDa, named as, sample A, sample B, and sample C, respectively.

Text S2. Digestion of environmental nanoparticles

Take 100 µL samples into a 25 mL conical flask, add 4 mL aqua regia, and mix well. After covering the surface dish, heat it on the heating plate to 160 ℃ and keep it for 2.5 h. After the digestion was complete, the temperature of the heating plate was raised to 200 ℃ for acid removal. The solution in the conical flask was evaporated to nearly dry, then 1 mL aqua regia was added and evaporated to nearly dry again. The residual liquid was transferred to the volumetric bottle, and the conical bottle was cleaned several times with 2% HNO_3. The cleaning liquid was transferred to the volumetric bottle.

Text S3. Detection of bacterial physiological and biochemical properties

Growth and cell activity assays: Bacterial concentrations after exposure and in controls were assessed using the LB-agar plate counting method.[3] Briefly, for each obtained bacterial sample, an appropriate gradient dilution was conducted with sterilized normal saline and 0.1 mL diluted sample was uniformly spread on nutrient agar plates, and then the nutrient agar plates were incubated for 24 h at 37 °C. The number of single colonies formed was counted to

quantify the viable and cultivable bacterial cells.

The intracellular adenosine-triphosphate (ATP) concentration of bacterial cell suspension in each sample was measured using the ATP Assay Kit (S0026, Beyotime Biotechnology, China).[15] That is, the intracellular dehydrogenase content was measured using the microbial activity assay kit-WST (M439, DOJINDO, Japan). The kit uses WST®-8 as a colorimetric indicator, which is reduced to a water-soluble orange formazan dye by intracellular dehydrogenase. In addition, the amount of formazan produced is proportional to the number of living cells. The staining solution was prepared by mixing the WST solution and the electron carrier solution in a 9:1 ratio. An appropriate concentration (2-3×10^8 CFU/mL) of bacterial cells suspension was prepared, and 190 μL of the suspension was inoculated in a 96-well plate. Add 10 μL of staining solution, and incubate the suspension for 30 min at 37 °C. The absorbance at 450 nm was measured by microplate reader (Varioskan LUX, Thermo Scientific, USA).

The cellular ATP concentration of bacterial cell suspension in each sample was measured using ATP Assay Kit (S0026, Beyotime Biotechnology, China). Take an appropriate amount (about 3×10^6 CFU/mL) of bacterial cells, add 200 μL of lysate, and shake well. After lysis, centrifuge the suspension at 4 ºC, 12,000g for 5 min and take supernatant for subsequent determination. The ATP standard solution was diluted with ATP assay lysate to appropriate concentration gradients (0.01, 0.03, 0.1, 0.3, 1, 3, and 10 μM) to determine the standard curve. ATP assay solution was diluted with ATP assay diluent at 1:9 ratio to prepare ATP assay working solution. Add 100 μL of ATP assay working solution to a black 96-well plate and place it at room temperature for 3-5 minutes to consume about all background ATP. Add 20 μL of sample or standard to each test hole, quickly mix well, and determine the RLU value by microplate reader (Varioskan LUX, Thermo Scientific, USA) with chemiluminescence mode.

Intracellular ROS levels. The DCFH-DA dye (S0033S, Beyotime, China) was used to stain cells and measure intracellular ROS generation after exposure to environmental NPs. In brief, bacterial cultures (1×10^6–2×10^7 CFU/mL) were incubated with DCFH-DA (10 μM) for 20 min at 37 °C. The samples were gently shaken every 3–5 min, and after treatment were washed three times in phosphate buffer saline (PBS) to remove unbound DCFH-DA. A total 250 μL sample was transferred into a 96-well plate to measure the fluorescence intensity (excitation 488 nm, emission 525 nm) with a

microplate reader (Varioskan LUX, Thermo Scientific, USA). All samples were analysed in triplicate.

Enzymatic activity assays. Extraction and concentration determination of bacterial protein: 2 mL of *E. coli* suspension after the treatment of environmental NPs was taken out and centrifuged at 8,000 rpm for 10 min to harvest the cells. The cell was lysed with bacterial protein extraction kit (C600596, Sangon Biotech, China). The harvested cells were resuspended in 400 μL 1×cell lysis buffer, mixed with 4 μL PMSF and 8 μL lysozyme, and incubated on a shaker for 40 min at 37 °C. The suspension was added with 20 μL DNase/RNase and continued to incubated on a shaker for 10 min at 37 °C. The lysate was centrifuged at 5,000 rpm, 4 °C for 30 min, and the supernatant was ready for determination of protein concentration and catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) activity assays.

Concentration determination of bacterial protein: All enzymatic activities were normalized by protein concentration, which was detected by BCA protein assay kit (P0012S, Beyotime, China). BCA working solution was prepared with BCA reagent A and B according to the determined volume ratio (50:1). The standard diluent was used in the protein standard to 20 μL and the final concentrations were 0, 0.025, 0.05, 0.1, 0.2, 0.3, 0.4, 0.5 mg/mL, respectively. Add 20 μL protein sample to the sample holes of the 96-well plate, then add 200 μL BCA working solution and leave for 30 min at 37ºC. The absorbance at 562 nm was measured by microplate reader (Varioskan LUX, Thermo Scientific, USA).

Enzymatic activity assays: The of CAT activity was assayed using a catalase assay kit (S0051, Beyotime, China). Prepare 250 mM and 5 mM H_2O_2 solutions according to the actual concentration of H_2O_2 provided by the kit. The working solution of color development was prepared by diluting peroxidase with color developing substrate at 1:1000 ratio. The catalase assay buffer was used in the 5 mM H_2O_2 solution to 100 μL and the final concentrations were 0, 0.625, 1.25, 2.5 and 3.75 mM, respectively. Take 20 μL of bacterial protein samples into 1.5 mL plastic tube, add 20 μL of catalase assay buffer, and mix well. Add 10 μL of 250 mM H_2O_2 solution, mix quickly, and leave for 3 min at 25ºC. Add 450 μL catalase termination solution and mix well to terminate above reaction. Add 40 μL of catalase assay buffer and 10 μL of the above terminated reaction solution in another plastic tube and mix well. Add 10 μL diluted reaction solution and 20 μL H_2O_2 solution to the sample holes of the 96-well plate, then add

200 μL working solution of color development and leave for 15 min at 25ºC. The absorbance at 520 nm was measured by microplate reader. The of SOD activity was assayed using a total superoxide dismutase assay kit with WST-8 (S0101S, Beyotime, China). 160 μL WST-8/enzyme working solution can be prepared by evenly mixing 151 μL SOD assay buffer, 8 μL WST-8 and 1 μL enzyme solution. Reaction initiating working solution was prepared by adding 39 μL of SOD assay buffer to 1μL reaction initiating solution (40X). 160 μL WST-8/enzyme working solution and 20 μL reaction initiating working solution were added to 20 μL bacterial protein sample. The blank control 1 was obtained by adding 160 μL WST-8/enzyme working solution and 20 μL reaction initiating working solution to 20 μL SOD assay buffer. The blank control 2 was obtained by adding 160 μL WST-8/enzyme working solution to 40 μL SOD assay buffer. All reaction solutions were incubated at 37 °C for 30 min. The absorbance at 450 nm was measured by microplate reader. The SOD activity was calculated as follows:

$$\text{Inhibition rate (\%)} = (A_{\text{blank control 1}} - A_{\text{sample}})/(A_{\text{blank control 1}} - A_{\text{blank control 2}}) \times 100\%$$

SOD activity unit in the sample = Inhibition rate/(1-Inhibition rate) units

Where the A value is the absorbance of reaction solution at 450 nm.

The of GSH-Px activity was assayed using a total glutathione peroxidase assay kit with NADPH (S0058, Beyotime, China). The 62.5mM NADPH solution was prepared by adding 220 μL of Mili-Q water to 11.5 mg NADPH. The 75mM GSH solution was prepared by adding 433 μL of Mili-Q water to 10 mg GSH. The GPx assay working solution was prepared by adding 35 μL GPx assay buffer, 2 μL 62.5mM NADPH solution, 2 μL 75mM GSH solution and 1 μL glutathione reductase. The 30 mM peroxide reagent solution was prepared by adding 5 mL of Mili-Q water to 21.5 mg Cum-OOH. 30 μL GPx assay buffer, 20 μL bacterial protein sample and 40 μL GPx assay solution were added into the sample holes of the 96-well plate successively, mixed well, and incubated at room temperature for 15 min to consume the GSSG in the sample. Add 10 μL of 30 mM peroxide reagent solution to above reaction solution and mix well. The absorbance at 340 nm was measured continuously for 20 min by microplate reader. The GSH-Px activity was calculated as follows:

$$\Delta A_{340\ (\text{blank})} = A_{340\ (\text{blank})\ (\text{Time 0})} - A_{340\ (\text{blank})\ (\text{Time n})}$$

$$\Delta A_{340\ (\text{sample})} = A_{340\ (\text{sample})\ (\text{Time 0})} - A_{340\ (\text{sample})\ (\text{Time n})}$$

$$\Delta A_{340} = \Delta A_{340\ (\text{sample})} - \Delta A_{340\ (\text{blank})}$$

$\Delta A_{340}/min = \Delta A_{340}/n$

GSH-Px activity in the sample = $\Delta A_{340}/min/(\varepsilon^{\mu M} \times L)$/Protein concentration

Where the $\varepsilon^{\mu M}$ is the molar extinction coefficient, which of NADPH in A_{340} is 0.00622 $\mu M^{-1} cm^{-1}$. L (cm) is the path length for measuring absorbance. The path length of 100 μL sample in 96-well plate is about 0.276 cm.

Text S4. Assessment of bacterial morphology under environmental NP stress

SEM assays. The bacterial cell structure was observed by SEM (Sigma300, Zeiss, Germany) under different treatments. Briefly, the bacterial cells were firstly fixed on a single crystal silicon with 2.5% glutaraldehyde solution for 2 h. After washing with sterilized PBS three times, the samples were dehydrated with a graded series of ethanol (30%, 50%, 70%, 90%, and 100%) for 10 min, respectively. Finally, the dehydrated samples were dried by vacuum freeze drying, coated with gold foil, and observed under SEM.

TEM assess. The adsorption of NPs on the bacterial cell surface and cellular damage was detected by a transmission electron microscope (TEM) (HT7700, Hitachi, Japan) as follows[16]: The bacterial cells were firstly fixed with 2.5% glutaraldehyde solution for 12 h and then fixed again with 1% osmic acid for 1 h. Rinse the sample three times with 0.1 M phosphate buffer solution (PB) (PH=7.4) for 15 min each time. The samples were dehydrated with a graded series of acetone (30%, 50%, 70%, 80%, 95% and 100%) for 10 min, respectively. After dehydration, specimens were infiltrated in a series of acetone-embedding agents (SPI) (acetone: embedding agent =3:1, 1:1, 1:3) for 6 h, embedded in pure embedding agent overnight, and polymerized in a 60 ℃ oven for 48 h. Ultrathin sections of specimens measuring nearly 70–90 nm were prepared by an ultramicrotome (UC7, Leica, Germany) using a diamond knife (Ultra 45°, Diatome, Switzerland). Sections were stained with uranyl acetate (2%; Sigma-Aldrich, USA) followed by counter-staining with lead citrate (2%; Sigma-Aldrich, USA). The sections were mounted on carbon-coated Cu grids and examined under TEM.

Text S5. Detection of mRNA expression of stress response-coding genes

Total bacterial RNA was extracted from the cell pellet using the column bacterial total RNA extraction and purification kit (B518655, Sangon Biotech, China). Next, RNA was

transcribed to cDNA by reverse transcription polymerase chain reaction (RT-PCR) with a reverse transcription kit (B639252, Sangon Biotech, China). Real-time PCR was carried out using SYBR Green I (TaKaRa, Japan). The absolute quantification method was used to quantify the oxidative stress regulatory genes (*rpoS*, *soxR*, *ompR*, *osmC*, *osmY*), DNA repair genes (*radA*, *recJ*, *rpoD*, *rpoH*, *ruvB*), outer membrane functional protein genes (*ompA*, *ponB*), folate/DNA/RNA synthesis genes (*folA*, *gyrA*, *rpoB*), quorum sensing genes (*lsrk*). 16S rRNA was used as an internal control.[1] The primers were designed specifically for this study using the Oligo 6.0 software. Primer sequences are listed Table S1. These primers and the 16S rRNA primers were synthesized by Tsingke Biotechnology Co., Ltd., China. The real-time PCR mixtures consisted of 10 μL of 2 × SGExcel FastSYBR Mixture (B532955, Sangon Biotech, China), 0.4 μL of forward and reverse primer (10 μM final concentrations), 1 μL of cDNA template, and 8.2 μL of RNase-Free ddH_2O. And, the total volume was 20 μL. The thermocycling profile for the amplifications was 95 °C for 3 min, followed by 40 cycles of 95 °C for 5 s, 60 °C for 20 s, followed by a melting curve stage to verify specificity. Each reaction was run in triplicate. The real-time PCR equipment used was Bio-Rad CFX 96 Touch Real-Time PCR System (USA), and the software was Bio-Rad CFX Manager 3.0. In addition, the mRNA expression levels were calculated using the $2^{-\Delta\Delta CT}$ method[2] as follows:

$$\Delta Ct = Ct_{\text{Functional genes}} - Ct_{\text{16S rRNA gene}}$$

$$\Delta\Delta Ct = \Delta Ct_{\text{Expose}} - \Delta Ct_{\text{Control}}$$

$$\text{FC(fold change)} = 2^{-\Delta\Delta Ct}$$

where the Ct value is the cycle threshold. The functional genes are the ones mentioned above. No environmental NPs was added in the control group.

Table S1 Gene information and primers for qPCR

	Gene	Primer	Sequence (5' to 3')	TM (°C)	Length of products (bp)
Internal control	*16S rRNA*	*16S rRNA*-FP	TGCATCTGATACTGGCAAGC	55.3	131
		16S rRNA-RP	ACCTGAGCGTCAGTCTTCGT	58.7	
Oxidative stress regulatory	*rpoS*	*rpoS*-FP	GTTATGGCAATCGTGGTCTG	53.8	122
		rpoS-RP	CCAGGTTGCGTATGTTGAGA	54.9	
	soxR	*soxR*-FP	TGCTCAGCGTATTGGCATTC	55.8	123
		soxR-RP	TCCAACTCTTCTCGCCATTG	54.9	
	ompR	*ompR*-FP	CGCTGACGACTACATTCCAA	54.8	119
		ompR-RP	TTACCGCCTCTTCCTGTGAC	57.1	
	osmC	*osmC*-FP	GTGCTGAACCAACAGCCGTA	58.3	82
		osmC-RP	CTGCGCCAATCAGTTCTTCA	55.7	
	osmY	*osmY*-FP	ACGCGCAGACTACCAATGAA	56.9	138
		osmY-RP	CGGTGCTCTTGATGTTGTCA	55.4	
DNA repair	*radA*	*radA*-FP	GTGCAAGGAAGTGCCATTCT	58.3	120
		radA-RP	CTCTTCGCCGGTGACATACA	57.3	
	recJ	*recJ*-FP	ATACAACTTCGTCGCCGTGA	56.5	121
		recJ-RP	CACTGCGTTCCAGTTCTTGC	57.3	
	rpoD	*rpoD*-FP	TCCGGAAGCGATCACCTATC	56.7	123
		rpoD-RP	GCGGTAGGTGCCAGATCTTC	58.7	
	rpoH	*rpoH*-FP	AACAAGTCCACGTTGCAGGA	57.1	111
		rpoH-RP	TTACGCTTCAATGGCAGCAC	56.2	
	ruvB	*ruvB*-FP	CGCAGGTTCGTTCACAGATG	56.8	123
		ruvB-RP	CGACAATGTTGGCAAGCGTA	56.1	
Outer membrane functional protein	*ompA*	*ompA*-FP	TGAGCCTGGGTGTTTCCTA	54.6	167
		ompA-RP	CAGAGCAGCCTGACCTTCC	55.0	
	ponB	*ponB*-FP	GGCATTCCGGCACTGAAGAA	57.5	97
		ponB-RP	CGACCATCGCACGAACTTCA	57.5	
Folate/DNA/RNA synthesis	*folA*	*folA-FP*	TGACGCATATCGACGCAGAAGTG	59.6	142
		folA-RP	CCGCCGCTCCAGAATCTCAAAG	61.4	
	gyrA	*gyrA-FP*	CGAGTTCAACCGTCTGCGTACC	61.4	121
		gyrA-RP	ACCTTCAGCGGAGAACAGCATTAC	59.6	
	rpoB	*rpoB-FP*	TCTGTCTCTGGGCGATCTGGATAC	61.3	98
		rpoB-RP	GCTGGCTGGAACCGAAGAACTC	61.4	
Quorum sensing	*lsrk*	*lsrk*-FP	CTTTAAGTGCCATCCCCAGA	60.1	250
		lsrk-RP	AATGTGCCGGTTTCTTTGAC	60	

Table S2 The top 9 element concentration of environmental NP samples (mg/L)

	Sn	Si	Sb	Fe	Zn	Bi	Al	Mo
Sample A	0.259	0.209	0.169	0.086	0.058	0.049	0.068	0.050
Sample B	0.194	0.118	0.178	0.082	0.064	0.060	0.031	0.034
Sample C	0.220	0.157	0.060	0.106	0.075	0.056	0.037	0.050

Table S3 Molecular weight range, hydrodynamic size, and component-peak position and corresponding integral concentration of key elements of samples A, B and C

	Molecular weight range[a] (kDa)	Hydrodynamic size[b] (nm)	The integral concentration of key elements for each NP component[c] (μg/L)						
			Residence time[d] (min)	845	1039	1241	1489	1918	3795
			Size[e] (nm)	15.62	17.50	20.27	25.34	41.50	843.83
Sample A	10-100	894±108	Al	130.56	11.15	14.60	6.24	11.37	520.50
			Si	23.39	17.58	13.54	11.99	31.79	139.55
			Fe	61.11	5.91	6.30	1.30	11.37	196.15
			Zn	0.00	118.09	2.90	3.62	23.47	0.00
Sample B	100-1000	891±68	Al	172.80	87.38	16.68	49.85	25.86	383.60
			Si	62.29	25.85	12.18	17.49	0.00	120.48
			Fe	82.46	42.04	17.24	9.26	1.27	251.41
			Zn	0.00	125.30	2.96	1.43	2.20	0.00
Sample C	≥1000	1047±59	Al	103.05	0.00	0.00	31.25	1.30	470.78
			Si	46.17	5.28	3.93	3.37	7.60	107.34
			Fe	73.52	1.14	3.71	13.48	1.68	173.18
			Zn	0.00	126.34	2.44	1.42	1.29	0.00

[a] The molecular weight of the sample was determined by the diameter of the ultrafiltration membrane configured by the tangential flow filtration (TFF).

[b] The hydrodynamic size of each sample was determined by dynamic light scattering (DLS).

[c] Peak baseline correction and peak area integration for ICP-MS spectra were performed in Origin 2018.

[d] Asymmetric flow field flow fractionation (AF4) depended on the different hydrodynamic activity properties (generally referring to particle size) of the different NP components in the sample to achieve the purpose of separation, thus each NPs component (peak) had corresponding residence times.

[e] The size of each NPs component was obtained from the fitting curve interpolation of the size of standard latex microspheres and their residence time (see **Fig. S7** for the calculation process).

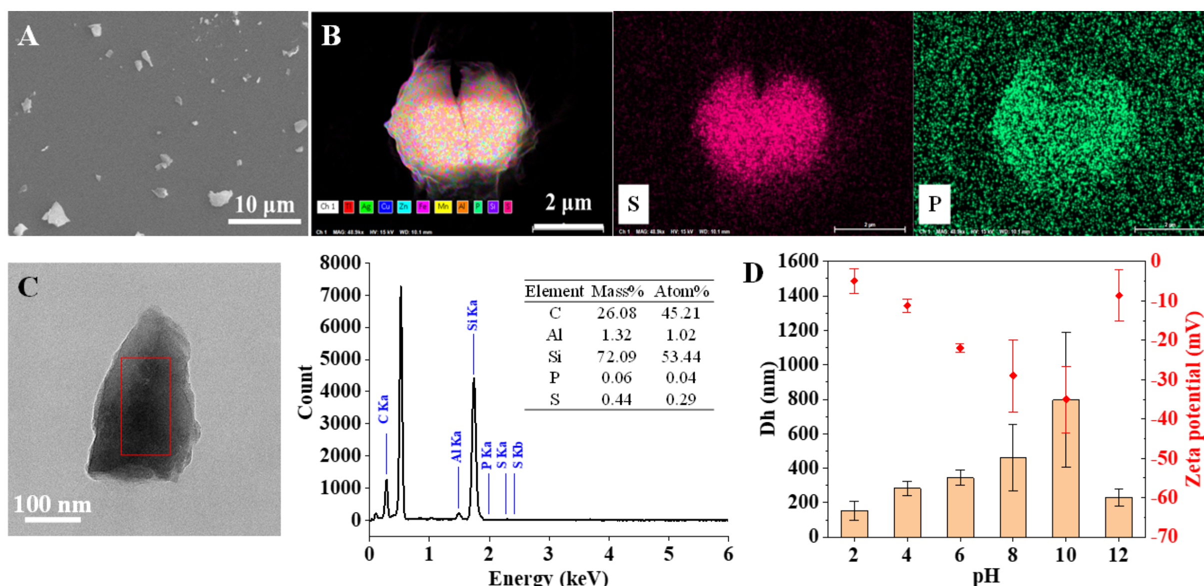

Fig. S1 (**A**) Origin environmental NP micromorphology (≥10 kDa); (**B**) EDS elemental (i.e., S, P) mapping on the surface of environmental NPs; (**C**) EDS energy spectrum and semi-quantitative content of the main elements on the environmental NPs; (**D**) Zeta potential and hydrodynamic size of environmental NPs.

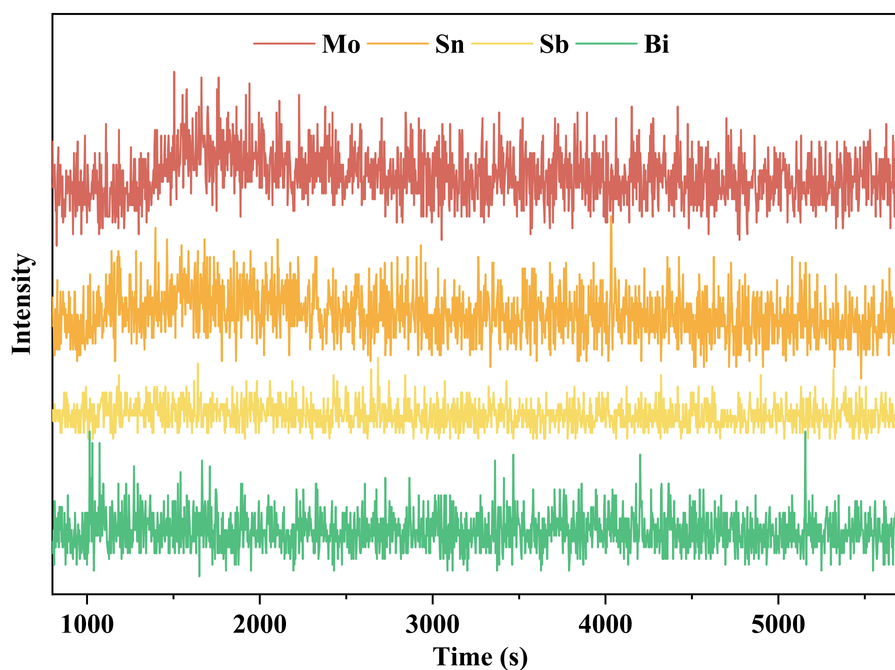

Fig. S2 ICP-MS spectra of elements not included in NP analysis due to excessive noise or no significant NP signal.

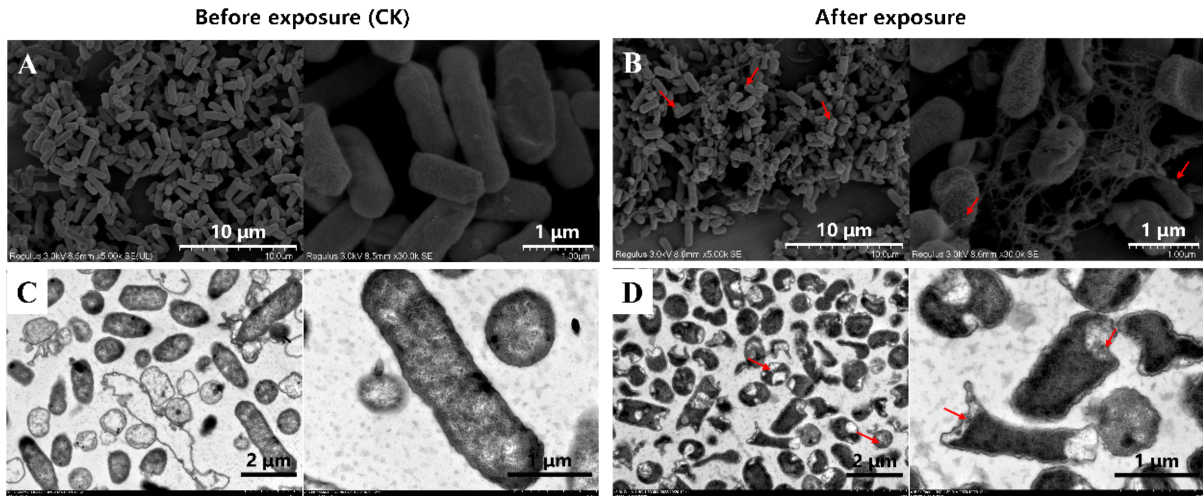

Fig. S3 The micromorphology of *E. coli* before (**A, C**) and after (**B, D**) exposure to 10 mg/L sample A for 24 h. (**A, B**) SEM; (**C, D**) TEM. The red arrows indicate highly suspected NPs located on the surface and inside the bacterial cells.

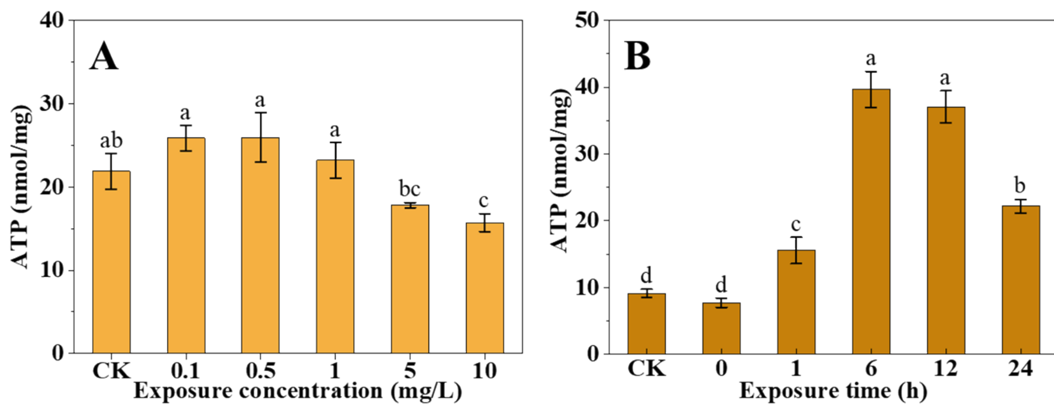

Fig. S4 Cellular ATP of *E. coli* treated with 0–10 mg/L sample A for 24 h (**A**) or 10 mg/L sample A for 0–24 h (**B**). Data are average of three replicates ± standard deviations. Different letters stand for statistical differences between treatments at $p \leq 0.05$.

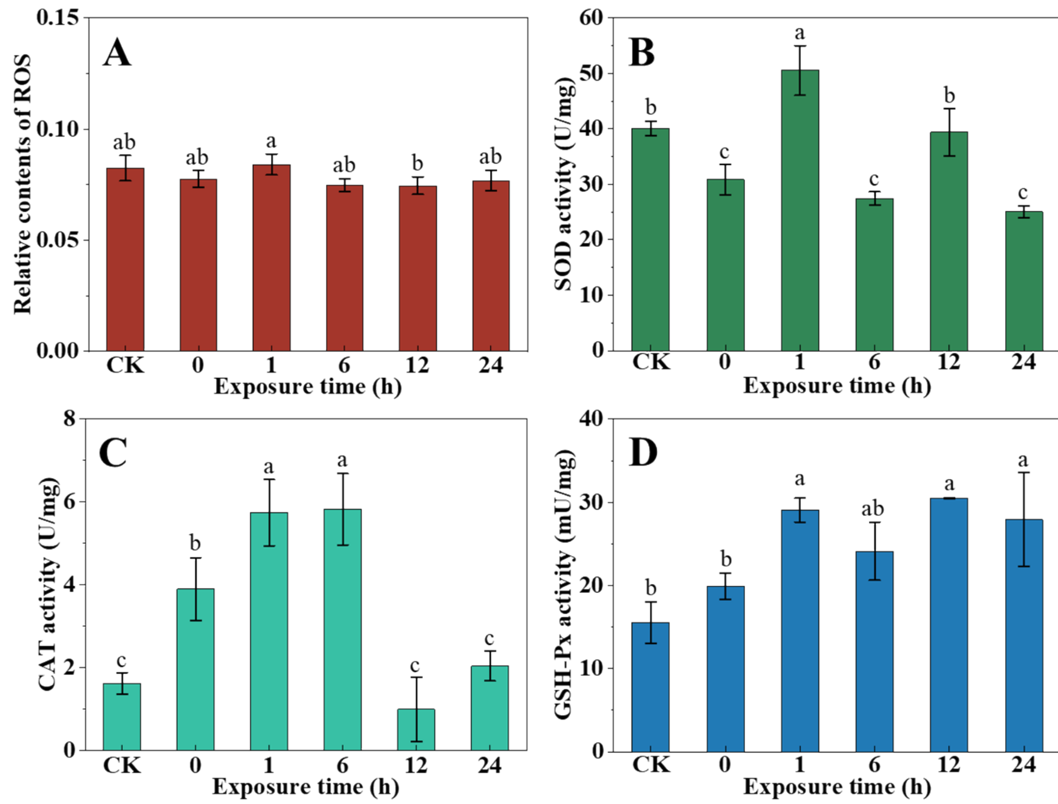

Fig. S5 (**A**) Intracellular ROS, (**B**) SOD, (**C**) CAT, and (**D**) GSH-Px activities of *E. coli* treated with 10 mg/L sample A for 0–24 h. Data are average of three replicates ± standard deviations. Different letters stand for statistical differences between treatments at $p \leq 0.05$.

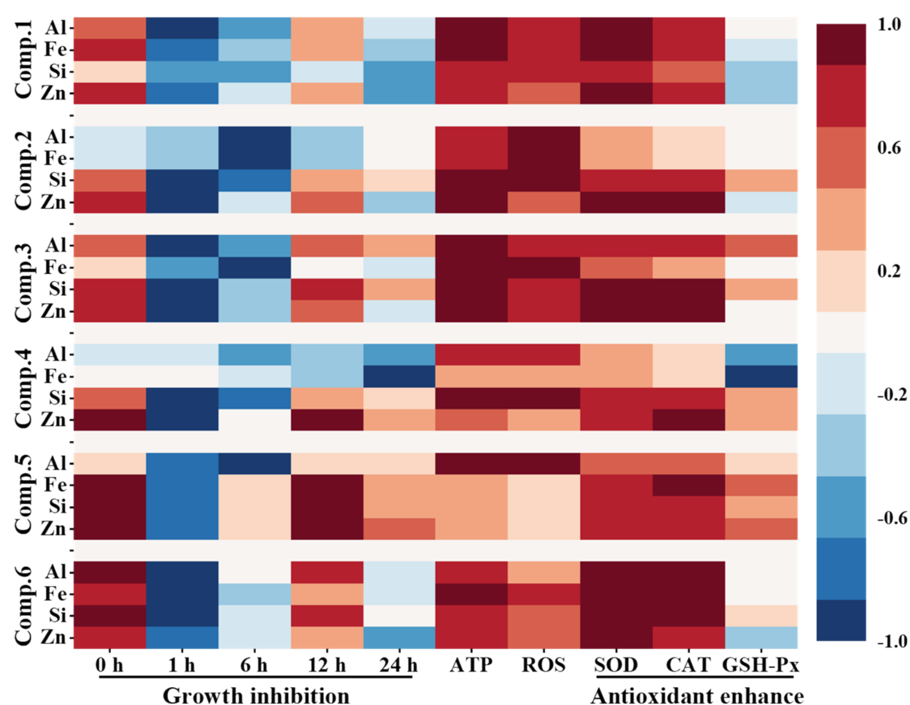

Fig. S6 Correlation heatmap of element abundance in each component of environmental NPs and bacterial physiological and biochemical indicators. Red spectrum color indicates a positive correlation, and blue spectrum color indicated a negative correlation. The correlation between the structural features of environmental NPs (i.e., NP component and its key element abundance) and the indicators of oxidative stress in bacteria were obtained by the Pearson's correlation analysis using Python's Pandas library. r≥0.8 means there is a strong correlation between the two comparison indicators.

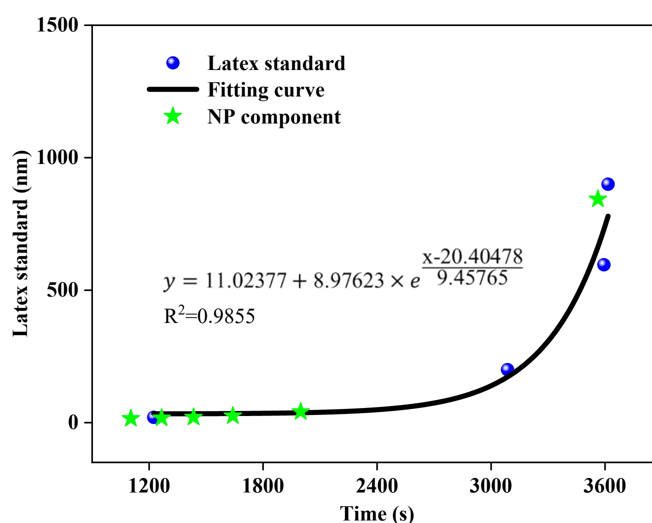

Fig. S7 The fitting curve of NP-hydrodynamic size and residence time for AF4 separation. It was obtained using latex standards with sizes of 20, 200, 596, and 900 nm (from Postnova Analytics). The hydrodynamic sizes of 6 environmental NP components were obtained by inter- or extrapolation on the fitting curves.

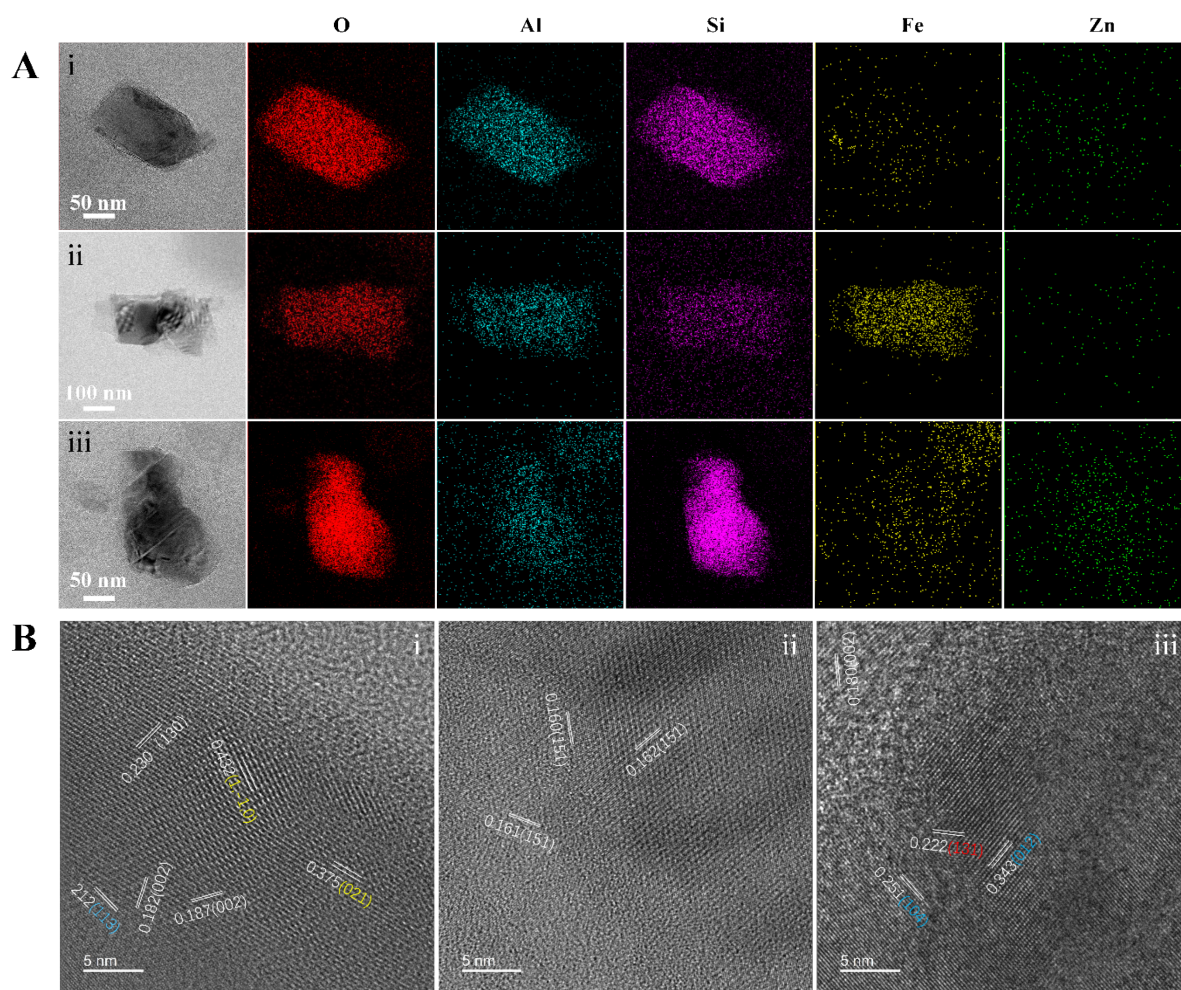

Fig. S8 (**A**) Key elemental (i.e., O, Al, Si, Fe, and Zn) mapping of Al-containing components in environmental NP. Among them, the content of O is 54.61%, 58.42% and 54.94%, respectively. (**B**) The lattice spacing and crystal plane parameters of Al species crystals in Al-containing components. Among them, the characteristic crystal planes of α-Al_2O_3, γ-Al_2O_3, $Al(OH)_3$, and halloysite are labeled in blue, white, red, and yellow, respectively.

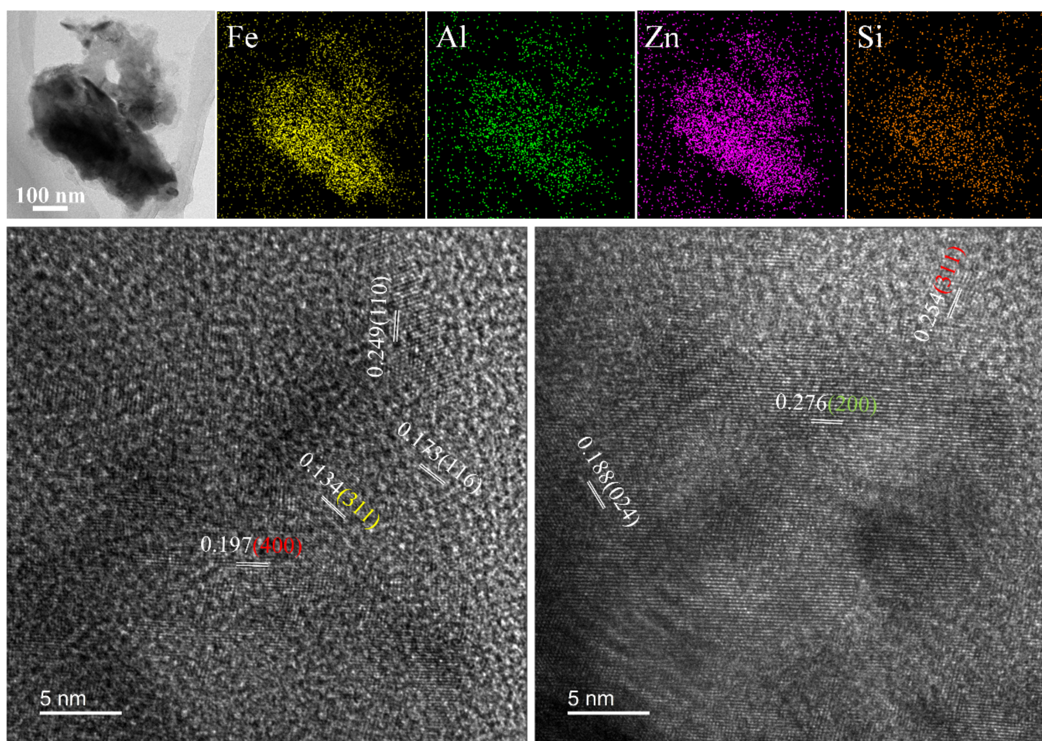

Fig. S9 Key elemental (i.e., Fe, Al, Zn, and Si) mapping and lattice spacing of Fe species crystals of Fe-containing components in environmental NP. Among them, the characteristic crystal planes of Fe_2O_3, Fe_3O_4, FeO, and FeS_2 are labeled in white, red, yellow, and green, respectively.

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