Electronic Supplementary Material (ESI) for Environmental Science: Nano.

Electronic Supplementary Information

Comparative toxicity of bimetallic Fe nanoparticles toward *Escherichia coli*: mechanism and environmental implications

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1. Experimental details

1) Preparation of bimetallic Fe NPs. Bimetallic Fe NPs were synthesized via displacement plating of Fe NPs with each metal additive. First, Fe NPs were prepared by reducing 0.5 M $FeCl_3$ with 0.8 M $NaBH_4$. Then, the Fe NPs were immersed in 1 mM solutions of the respective metal salts, and the mixture was allowed to react for 30 min on a rolling mixer. The resulting suspension was centrifuged and rinsed several times with degassed, deionized water by pursing with N_2 for 1 h.

2) Characterization of NPs. High-resolution transmission electron microscopy (HRTEM) and energy-filtered TEM (EFTEM) analysis of the NPs were carried out using a JEM-2200FS microscope (JEOL) with Cs-correction at the operating voltage of 200 kV. X-ray photoelectron spectroscopy (XPS) spectra were obtained by a VGESCALAB 250 (Thermo VG Scientific) with a monochromatic Al Kα excitation source (1486.6 eV). The hydrodynamic size and zeta potential measurements were performed in a dilute aqueous NP suspension (20 mg/L) with a Zetasizer ELSZ-1000 (OTSUKA).

3) Antibacterial tests. *Escherichia coli* DH5α was used as a test organism. *E. coli* was grown aerobically at 37 °C for 12−16 h in Luria-Bertani (LB) medium. The saturated cultures were diluted in ultrapure water to $OD_{600} = 0.05$. The cells were incubated with different concentrations of NPs (10, 100, and 500 mg/L) at 37 °C under gentle stirring (160 rpm). Untreated cells were employed as a control. After 10 h incubation, aliquots were taken, and serially diluted. The dilutions were plated in triplicate on LB agar plates and incubated overnight at 37 °C, and colonies were counted.

4) Analysis of intracellular reactive oxygen species (ROS). Intracellular ROS assays were performed using the ROS-sensitive fluorescent probe, 2,7-dichlorodihydrofluorescein diacetate (DCF-DA). The cells were exposed to 100 mg/L of NPs for 3 h, and then further incubated in water containing 10 μM of DCF-DA for 30 min in a dark. The fluorescence intensity was analyzed by a Shimadzu RF-5301 spectrofluorometer and a confocal Zeiss LSM 510 Meta scanning microscope with excitation at 480 nm and emission at 530 nm.

5) Glutathione peroxidase (GPx) assay. GPx activity was determined spectrophotometrically by monitoring the oxidation of NADPH in the presence of glutathione, glutathione reductase,

and cell homogenate at 340 nm with a commercial kit supplied by BioVision. The enzyme units were expressed as nmol NADPH consumption/min/mg protein.

6) FTIR sample preparation and analysis. *E. coli* suspensions treated by 100 mg/L NPs were centrifuged, and the precipitates were dried at 60 °C overnight. Specimens for FTIR were made by compressing the mixture of 1 mg bacterial sample and 100 mg KBR into pellets using a hydraulic press. FTIR spectra were recorded on a Bomen MB-104 FTIR spectrometer in the 4000−500 cm^{-1} range.

7) TEM analysis of NP-treated cells. Harvested cells were fixed with 1% formaldehyde and 1% glutaraldehyde, dehydrated in ethanol, and embedded in EMBed 812 and propylene oxide before being sectioned by an ultra-tome. The bacterial sections were analyzed with a Hitachi H-7600 TEM.

8) Chemical analysis. The deposited metal content (wt %) was determined by an inductively coupled plasma-atomic emission spectrometer (ICP-AES, IRIS-AP) after digesting the particles with 0.2 M HCl. The concentration of dissolved iron was also quantified using an ICP-AES. The anions were measured by an ion chromatograph (IC, Dionex DX-120) that was equipped with a conductivity detector and an AS-14 (4 mm × 25 mm) column.

Table S1. Physical and chemical properties of bare and bimetallic Fe NPs.

Particle	Deposited metal content (wt%)	Primary particle size (nm)[a]	Hydrodynamic particle size (nm)	Zeta potential (mV)
Fe	–	50 − 70	866.4 ± 130.5	26.3 ± 1.23
Fe/Pd	2.8		1312.3 ± 233.1	22.4 ± 0.89
Fe/Ni	2.0		810.7 ± 187.5	27.5 ± 3.13
Fe/Cu	1.9		691.1 ± 156.3	33.9 ± 2.17
Fe/Pt	3.2		991.2 ± 270.6	16.5 ± 1.15

[a] From TEM images (n=100).

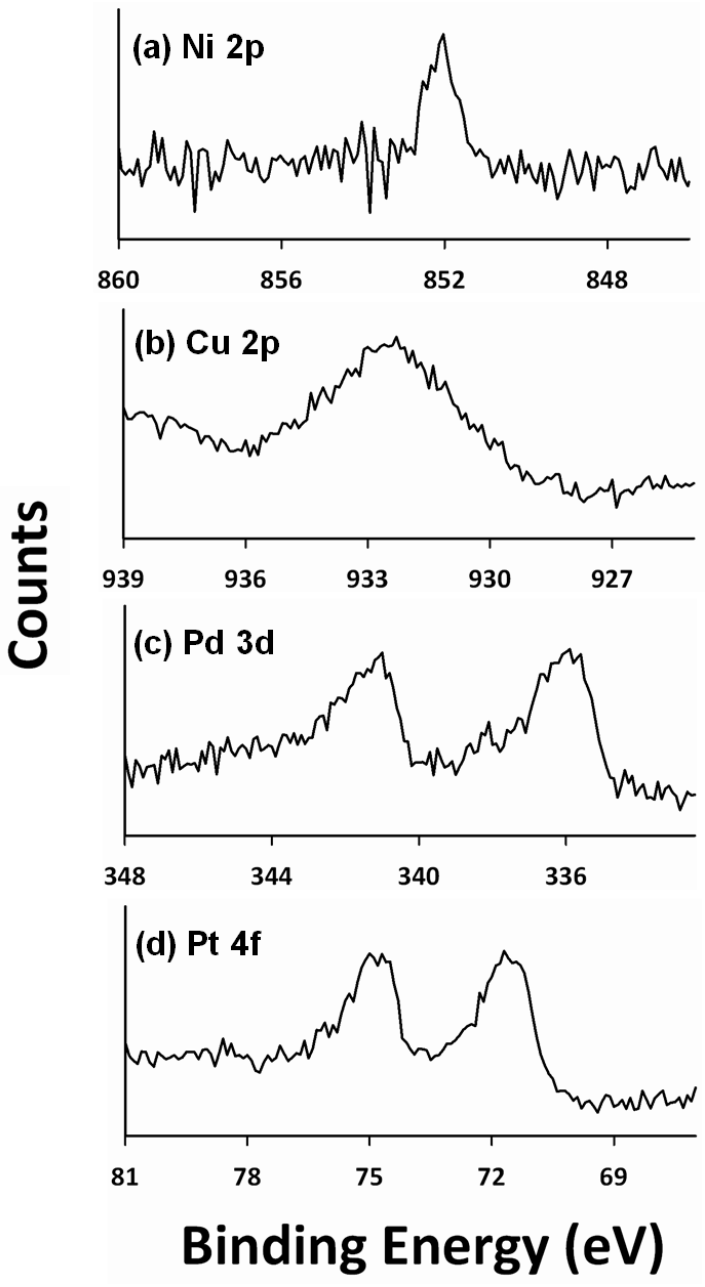

Fig. S1 XPS spectra obtained from (a) Fe/Ni, (b) Fe/Cu, (c) Fe/Pd, and (d) Fe/Pt.

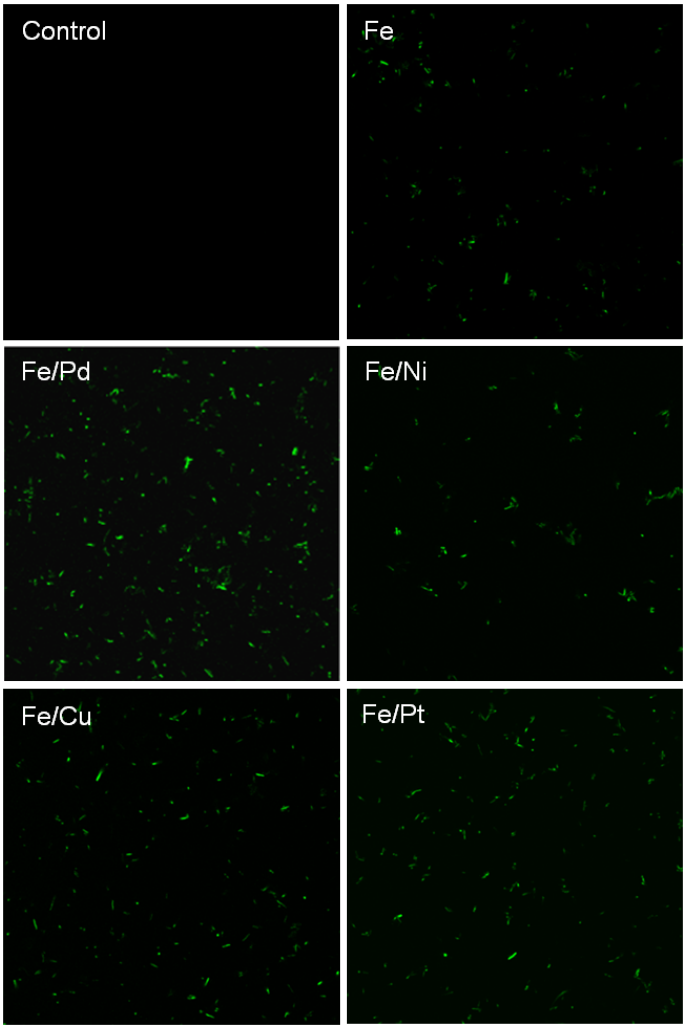

Fig. S2 Confocal microscopic images of native and NPs (100 mg/L)-treated cells for 3 h subjected to DCF-DA staining.

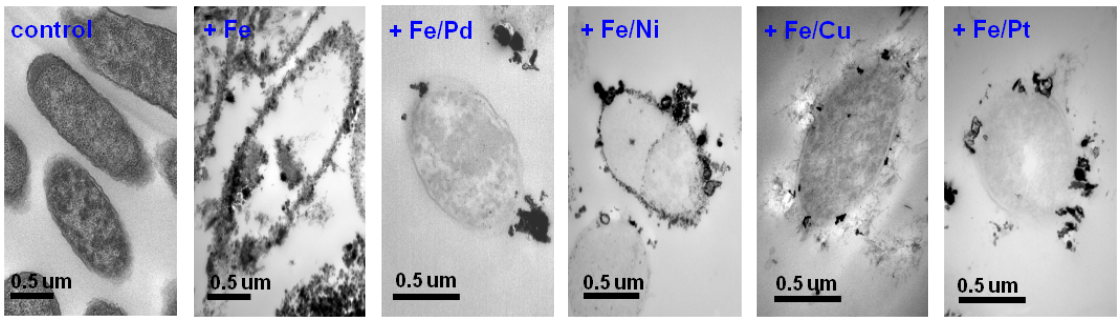

Fig. S3 TEM images of native and NPs-treated cells. *E. coli* cells were exposed to 100 mg/L NPs for 10 h.

Table S2. Summary of toxicological responses induced by NPs exposure.

Particle	DCF-DA fluorescence	GPx activity (mU/mL)	Dissolved Fe conc.[a] (μM)	IR intensity			Anion conc. (μM)		
				C−O/ C−O−C	PO_2−	−CH_3	Cl^-	PO_3^{2-}	SO_4^{2-}
None	15.5 ± 0.95	0.010 ± 0.004	0.00	strong	strong	strong	2.75 ± 1.06	0.00	0.00
Fe	53.4 ± 5.32	0.292 ± 0.009	9.08 ± 0.11	weak	weak	weak	30.7 ± 1.02	64.1 ± 1.48	0.00
Fe/Pd	117.6 ± 1.60	0.173 ± 0.014	1.54 ± 0.09	strong	strong	strong	14.9 ± 0.14	52.2 ± 2.19	1.75 ± 0.35
Fe/Ni	72.5 ± 3.03	0.464 ± 0.045	5.22 ± 0.12	strong	strong	weak	10.4 ± 0.78	62.7 ± 2.05	38.9 ± 1.90
Fe/Cu	98.3 ± 4.53	0.190 ± 0.017	2.77 ± 0.08	weak	weak	weak	16.3 ± 0.57	68.9 ± 2.69	0.00
Fe/Pt	114.6 ± 4.71	0.181 ± 0.003	2.16 ± 0.22	strong	strong	strong	17.9 ± 0.14	38.7 ± 1.84	2.45 ± 0.92

[a] Data after 10 h exposure