

Supplementary Materials

Methods

Text S1 Details of artificial wastewater

The compositions of synthetic wastewater simulating the influent of WWTPs were shown as following: one hundred liters of synthetic wastewater contained 264 ml concentrated feed, 72.6 ml phosphorus stock solution, 10 g ammonium chloride, 2 ml trace element feed and 10 ml acetic acid. The concentrated feed contained (g/L): 12.89 peptone, 4 yeast extract, 34 $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$, 20 $\text{MgSO}_4 \cdot 7 \text{H}_2\text{O}$ and 9 $\text{CaCl}_2 \cdot 2 \text{H}_2\text{O}$. The phosphorus stock solution contained (g/L): 33 K_2HPO_4 and 29 KH_2PO_4 . The trace element feed contained (g/L): 1.50 $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$, 0.03 $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, 0.12 $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$, 0.06 $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$, 0.12 $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, 0.15 $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$, 0.18 KI, 0.15 H_3BO_3 and 10 ethylenediamine tetraacetic acid. The synthetic wastewater concentration is about 200 mg/L COD, 45 mg/L total nitrogen (TN), 35 mg/L $\text{NH}_4^+\text{-N}$, and 10 mg/L total phosphate (TP).

Text S2 Antioxidant enzyme activities and Malonaldehyde content in leaves

Superoxide dismutase (SOD) (EC 1.15.1.1) was determined by measuring the ability of SOD enzyme to depress the photochemical reduction of nitro blue tetrazolium (NBT), using a method developed by Giannopolitis and Ries (1977). The reaction solution (3 mL) was illuminated for 15 min under fluorescent lamps (15W), which contained K-phosphate buffer (pH 7.8), 75 nM NBT, 2 nM riboflavin, 13 mM methionine, 0.1 mM EDTA and 100 μL of enzyme extract. One unit of enzyme activity was regarded as the amount of SOD enzyme that inhibited the NBT in the photochemical reduction by 50% at 560 nm.

Catalase (CAT) (EC1.11.1.6) activity was measured based on the decomposition of H_2O_2 ($\epsilon = 39.4 \text{ mM}^{-1} \text{ cm}^{-1}$) at 240 nm for three minutes, using a method developed by Aebi (1984). Each 3 mL reaction solution contained 10 mM H_2O_2 , 50 mM K-phosphate buffer (pH = 7.0), 100 μL of enzyme extract. One unit of enzymatic activity was regarded as the amount of CAT that decomposed 1 μmol of H_2O_2 per minute.

Peroxidase (POD) (EC 1.11.1.7) activity was assayed by monitoring the oxidation rate of guaiacol to tetraguaiacol (extinction coefficient: $26.6 \text{ mM}^{-1} \text{ cm}^{-1}$) in a total volume of 3 mL, using a method developed by Kenten and Mann (1954). The reaction mixture included 50 mM potassium phosphate buffer (pH = 7.0), 0.4% H_2O_2 , 1% guaiacol and 100 μL of enzyme extract. The increase in absorbance was determined at 470 nm after the augment of H_2O_2 for 10 minutes.

Malonaldehyde (MDA), a final product of lipid peroxidation, was calculated by measuring the concentration of the thiobarbituric acid reactive substances (TBARS), using a method developed by Hodges et al. (1999). 0.3 g leaf tissues were ground and homogenized with 5 mL 10% trichloroacetic acid (TCA), and then transferred into a 10 mL centrifuge tube. After centrifugation (6000 rpm), 2 mL of supernatant was added to 0.6% thiobarbituric acid (TBA). After heating on the boiling water bath for 30 minutes, the compound was immediately cooled in ice water, and then centrifuged (10000 rpm) for 10 min. The TBARS content was computed according to the following equation:

$$\text{TBARS}(\text{nmol } g^{-1} \text{ FW}) = [6.452 \times (A_{532} - A_{600}) - (0.559 \times A_{450})] \quad (1)$$

Text S3 Measure of photosynthesis parameters

Gas exchange parameters, including net photosynthesis rate, stomatal conductance, intercellular CO_2 concentration and transpiration rate were measured simultaneously using a portable infra-red (IR) gas exchange analyzer (6400 XT, Licor, Lincoln, NE, USA), which was equipped with a clamp-on leaf chamber (6 cm^2) and a red/blue light source (6400-02B). The carbon dioxide (CO_2) concentration and light intensity were fixed at a constant level of $400 \mu\text{molCO}_2 \text{ mol}^{-1}$ and $425 \mu\text{mol m}^{-2} \text{ s}^{-1}$, respectively.

Text S4 Measure of root activity

Root activity was measured according to 2,3,5- triphenyl tetrazolium chloride (TTC) reduction as described previously (Hu et al., 2016; Tan et al., 2017). About 0.5 g of trends root samples were cut into 0.5-1cm sections and

incubated in a mixture solution of 5 ml 1% (w/v) TTC and 5 ml 0.1M phosphate buffer (pH=7.5) at 37°C for 1 h. The incubation was terminated by adding 1ml 1M sulfuric acid to the mixture. For triphenyl formazan (TF, red product) extraction, the roots were removed and blotted up on filter paper and grinded in the mortar containing 3-5 ml ethyl acetate and a little bit SiO₂ powder. The liquid phase was transferred to a 10 ml stoppered colorimetric tube. Ethyl acetate was added up to the 10 ml level, and the released TF was quantified spectrophotometrically at 485 nm. The concentration of TF reduction was calculated and determine the root vigor as $\mu\text{g TF}\cdot\text{g}^{-1}\text{ root}\cdot\text{h}^{-1}$.

Text S5 Measure of P element content in plants and ENPs distribution in wetland microcosms

In order to monitor the contents of P element and ENPs in the plant, plant samples were harvested from each CW and washed thoroughly with deionized water after the 60-day exposure experiments. Roots, stems and leaves were separated and dried off and weighed. The dry samples were ground to fine powders and screened with 100 mesh, and 5 g powder samples were digested with a mixture of HNO₃ and HClO₃ (1:1) at 120 °C for 3 h on a heating plate, and the digestion solution after filtrating volume to 20 ml. The contents of P element and ENPs within the filtrate was analyzed by inductively coupled plasma-optical mass spectrometry (ICP-MS) (Novozamsky et al., 1983; Zhang et al., 2012). The contents of P element and ENPs within every tissue were calculated by the concentrations of P element and ENPs and the weight of powder samples.

The ENPs mass within influent was calculated as the following equation:

$$m_{in} = \sum_{i=1}^{12} C_{in} \cdot V_{in} \quad (1)$$

The ENPs mass within effluent was calculated as the following equation:

$$m_{out} = \sum_{i=1}^{12} C_{out} \cdot V_{out} \quad (2)$$

The ENPs mass within the substrate was calculated as the following equation:

$$m_{substrate} = m_{in} - m_{out} - m_{plant} \quad (3)$$

Where m_{in} , m_{out} , m_{plant} , and $m_{substrate}$ are the contents of ENPs within the total influent, effluent, plants and substrate, respectively. C_{in} , C_{out} , V_{in} , and V_{out} are the concentrations of ENPs within the influent and

effluent and the volume of ENPs within the influent and effluent, respectively.

Text S6 Measurements of elemental analysis

In order to monitor the content of C, N, H and S in the plant of CWs, plant samples were harvested from each constructed wetland and washed thoroughly with deionized water after the 60-day exposure experiments. Plants were dried off and weighed. The dry samples were ground to fine powders and screened with 100 mesh (Chen et al., 2014), then weight 5mg to measure by elemental analyzer (Vario EL cube, Germany).

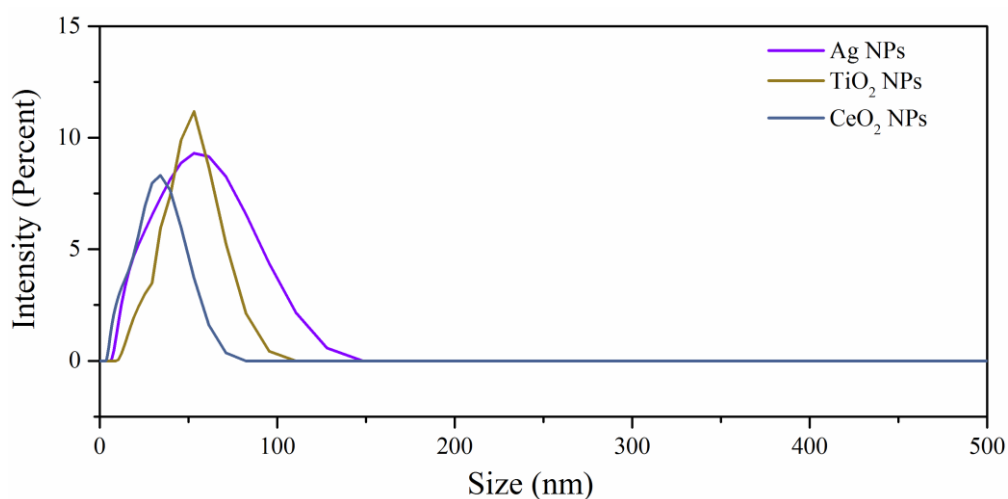


Fig. S1. Size distribution image of Ag, TiO₂, and CeO₂ nanoparticles in synthetic wastewater.

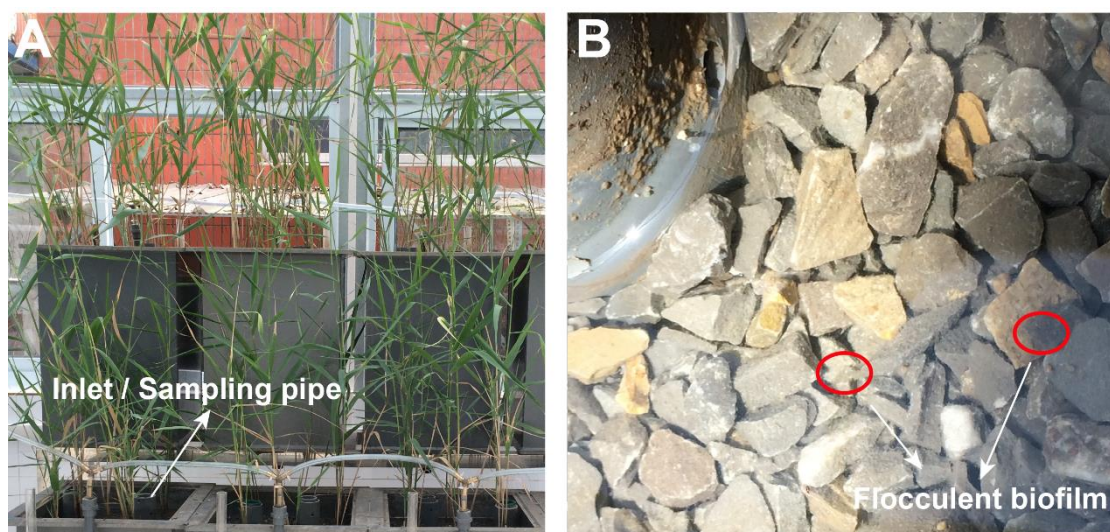


Fig. S2. The condition of plant (A) and biofilm (B) in CW microcosms after the 4-month pre-incubation period.

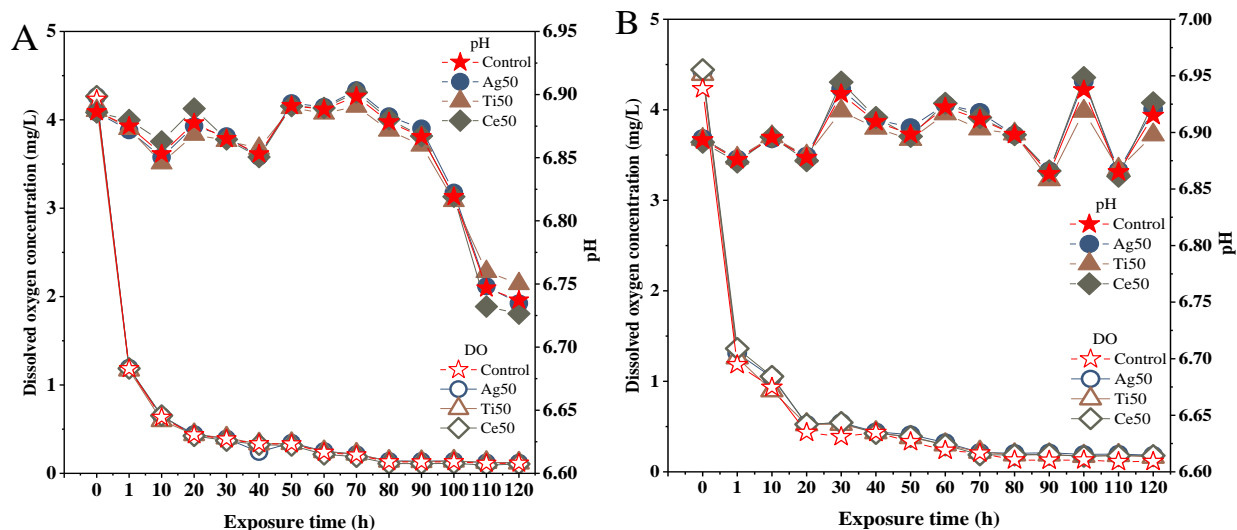


Fig. S3. Variations in DO and pH of interstitial water during day 1-5 (A) and day 61-65 (B) exposure to ENPs at different concentrations.

Table S1. Variations in nutrient removal efficiency (%) during day 61-65 exposure to ENPs at different concentrations (data presented as the mean \pm standard deviation, n = 3).

	Control	Ag NPs		TiO ₂ NPs		CeO ₂ NPs	
		1 mg/L	50 mg/L	1 mg/L	50 mg/L	1 mg/L	50 mg/L
COD	88.3 \pm 1.5	85.1 \pm 5.2	45.5 \pm 2.3	85.1 \pm 4.5	78.3 \pm 4.4	81.7 \pm 2.9	73.5 \pm 3.6
NH ₄ ⁺ -N	77.4 \pm 2.3	33.5 \pm 2.1	10.2 \pm 1.1	57.5 \pm 3.7	67.1 \pm 3.8	60.6 \pm 3.3	54.3 \pm 1.8
TN	77.8 \pm 1.6	37.9 \pm 4.5	13.8 \pm 2.3	48.5 \pm 5.3	51.0 \pm 2.7	61.3 \pm 2.4	49.6 \pm 1.4
TP	3.2 \pm 0.3	1.3 \pm 0.2	0.5 \pm 0.1	3.8 \pm 1.2	3.7 \pm 0.8	3.1 \pm 0.1	3.2 \pm 0.6

Note: The concentrations of nutrient in effluent under ENPs feeding were respective 29 - 109 mg/L for COD, 11.5-31.4 mg/L for NH₄⁺-N, 17.4 - 38.8 mg/L for TN and 9.6-10 mg/L for TP after 60-day exposure experiment.

References

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