Supporting information

Parental exposure to TiO_2 NPs promotes the mutilgenerational reproductive toxicity of cadmium in *Caenorhabditis elegans* via accumulation of Cd in germ cells

Jingjing Wang,[a,c] Yaguang Nie,[a,b] Hui Dai,[a] Mudi Wang,[a] Lei Cheng,[a,c] Zhen Yang,[a] Shaopeng Chen,[a] Guoping Zhao,[a] Lijun Wu,[a,b] Shouhong Guang,[d] and An Xu*[,a,b]

[a]Key Laboratory of High Magnetic Field and Ion Beam Physical Biology, Chinese Academy of Sciences; Anhui Province Key Laboratory of Environmental Toxicology and Pollution Control Technology, Hefei Institutes of Physical Science, Chinese Academy of Sciences, Hefei, Anhui 230031, P. R. China

[b]Institutes of Physical Science and Information Technology, An Hui University, Hefei, Anhui, 230601, P. R. China

[c]School of Environmental Science and Optoelectronic Technology, University of Science and Technology of China, Hefei, Anhui 230026, P. R. China

[d]School of Life Sciences, University of Science and Technology of China, Hefei, Anhui 230027, P. R. China

*Corresponding author:

An Xu, phD

Institute of Technical Biology & Agriculture Engineering

Hefei Institutes of Physical Science, Chinese Academy of Sciences

Hefei 230031, Anhui, China P.O. Box 1138

Tel: 86-551-65593336. Fax: 86-551-65595670

E-mail address: anxu@ipp.ac.cn

Number of pages in Supporting information (including cover and reference): 9

Number of tables in supporting information: 1

Number of figures in supporting information: 6

Number of texts in supporting information: 1

Experimental design

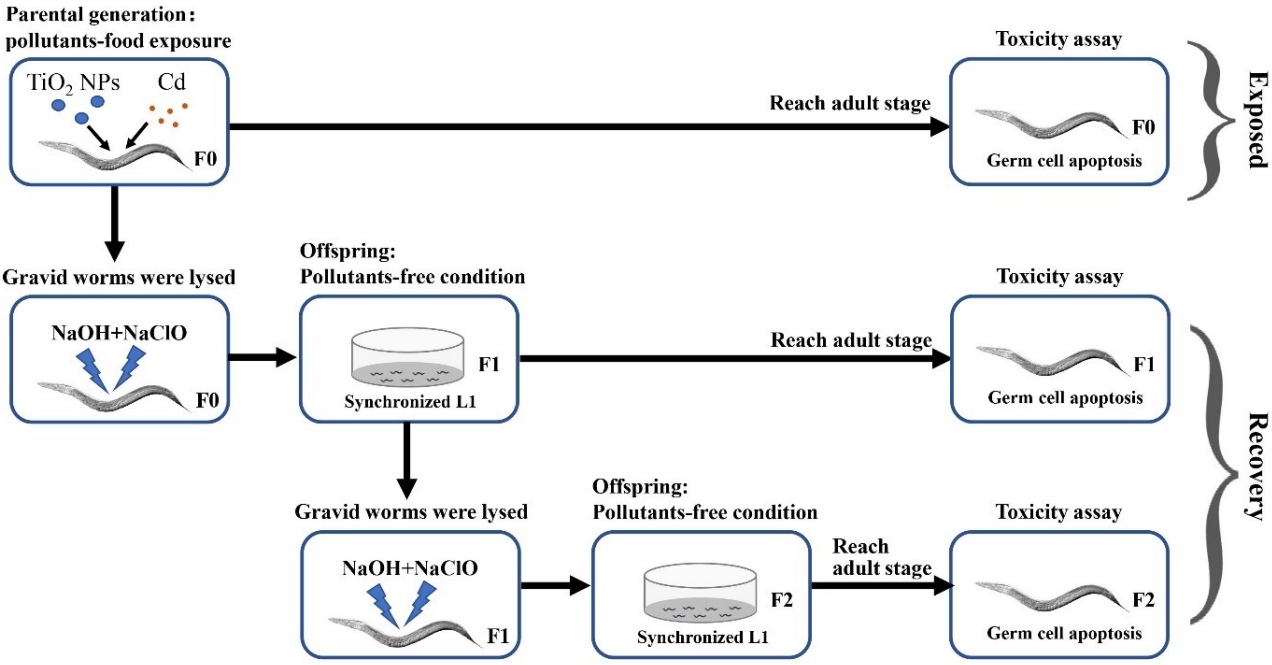

Figure S1. Experimental design for parental and filial generations of *C. elegans*.

Primer sequences for PCR

Table S1. Primers sequences used for the qRT-PCR analyses

Gene	Fw 5'-3'	Rev 5'-3'	Product length	Annealing T ℃	NCBI Reference Sequence
mtl-2	TTCCTGCAACACCGGAACTA	GTTGGCACACTTGCATCCTC	130	59	NM_073418.5
act-1	GGTTGCCGCTCTTGTTGTAG	TCTCCGACGTACGAGTCCTT	156	60	NM_074081.5

Text S1: The Details regarding operation conditions for LA-ICP-MS

Bioimaging tests were performed at the Ore Deposit and Exploration Centre (ODEC), using a laser ablation system, coupled to a quadrupole-based inductively coupled plasma-mass spectrometer (ICP-MS) (Agilent 7900). The ICP-MS system was optimized daily to maximize sensitivity to isotopes of the mass range of interest, while maintaining the lowest possible production of molecular oxide species (i.e., 232Th16O/232Th), usually < 0.2%. Element maps were created by ablating sets of parallel line rasters in a grid across the sample. A beam size of 8 μm and scan speed of 8 μm/s were chosen in this study. A laser repetition of 10 Hz was selected at a constant energy output of 80 mJ, resulting in an energy density of ~2 J/cm^2 at the target. A 20-s background acquisition was acquired at the beginning of scanning, and a delay of 20-s was used after ablation to allow for cell wash-out, gas stabilization, and computer processing. Reference materials NIST-610 or GSE-1G at the start and end of each mapping were analyzed for data calibration. Images were compiled and processed using the program LaIcpMsSoftWare2.2 (in-house designed mapping reduction software based on Matlab). For each raster and every element, the average background was subtracted from its corresponding raster, and the rasters were compiled into a 2-D image displaying the combined background/drift corrected intensity for each element.

Workflow for Bioimaging

I. Sample preparation. After exposure, the worms were rinsed with pure water, and then transferred to microscopic slides and dried in an oven at 60 °C to obtain single, separated worms.

II. LA-ICP-MS measurement. Bioimaging tests were performed at the ODEC of Hefei University of Technology, using a laser ablation system coupled to a quadrupole-based ICP-MS.

III. Generation of images and quantification. Images were compiled and processed using the program LaIcpMsSoftWare2.2.

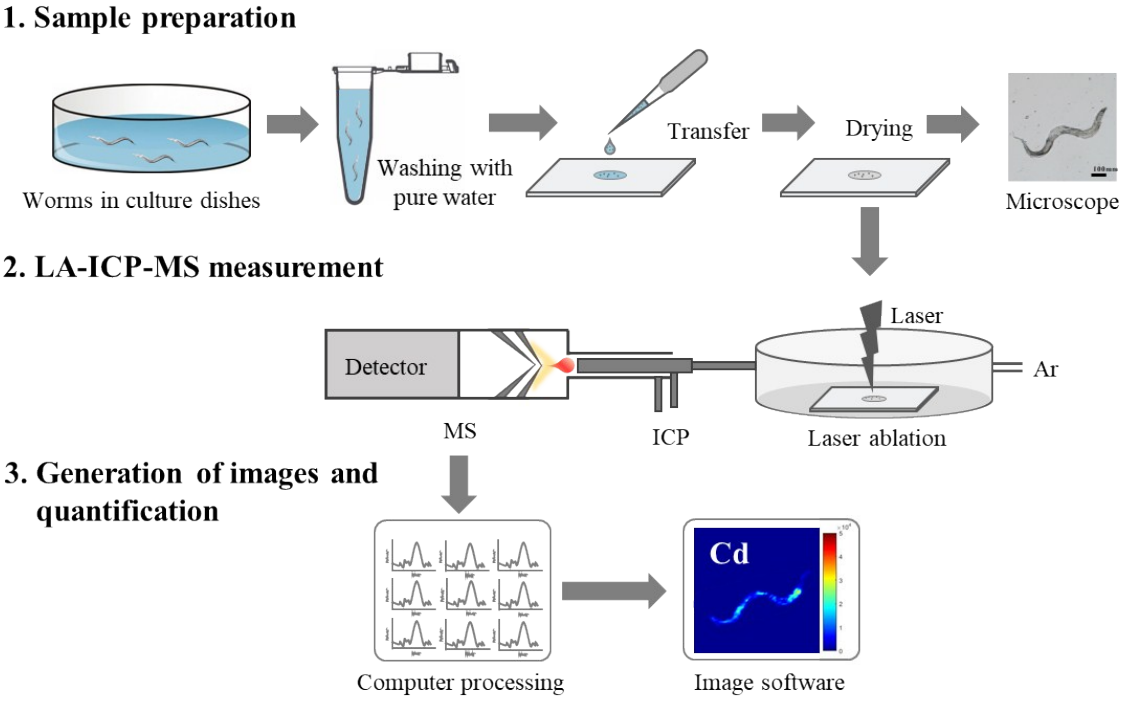

Figure S2. Overview of the workflow of the sample preparation and analysis process of LA-ICP-MS measurement.

Acquisition of Intact Gonads of Nematodes

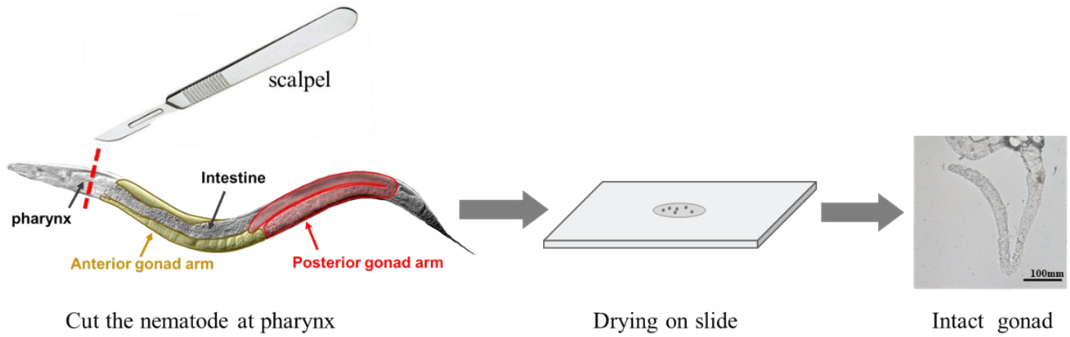

Figure S3. The method of obtaining the intact gonads of the *C. elegans*.

Reproductive Toxicity of Cd Associated with TiO_2 NPs in *C. elegans*

The effect on reproduction is a basic parameter for evaluating the ecotoxicity of environmental pollutants. Synchronized L4 adult stage *C. elegans* were exposed to the mixture of 2 μg/mL TiO_2 and Cd (1 μg/mL) for 12 h to investigate the influence of TiO_2 NPs on Cd-induced.

Fluorescence microscope pictures showed that the presence of 5 nm TiO_2 promoted Cd-induced apoptosis in the meiosis region of nematode gonad (Figure S4).

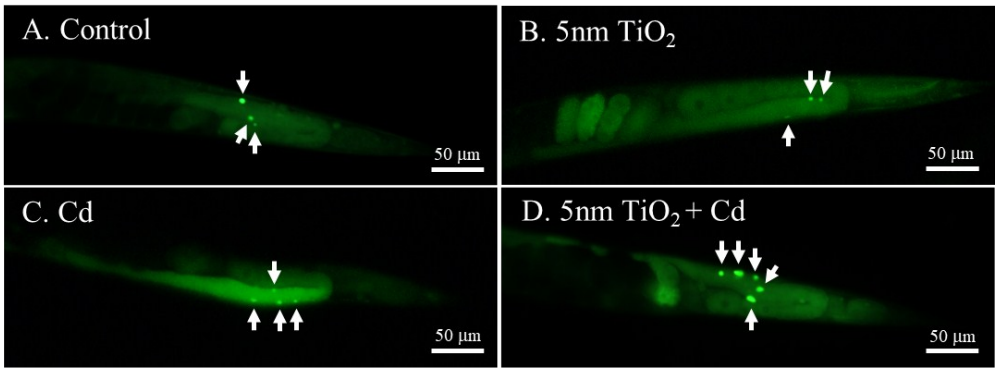

Figure S4. Representative fluorescence microscopy images of the germline cell apoptosis in *C. elegans* treated with (A) KM (control), (B) 5nm TiO_2, (C) Cd, and (D) TiO_2 NPs + Cd. Apoptotic cells are indicated by white arrows.

ζ potential of TiO_2 NPs

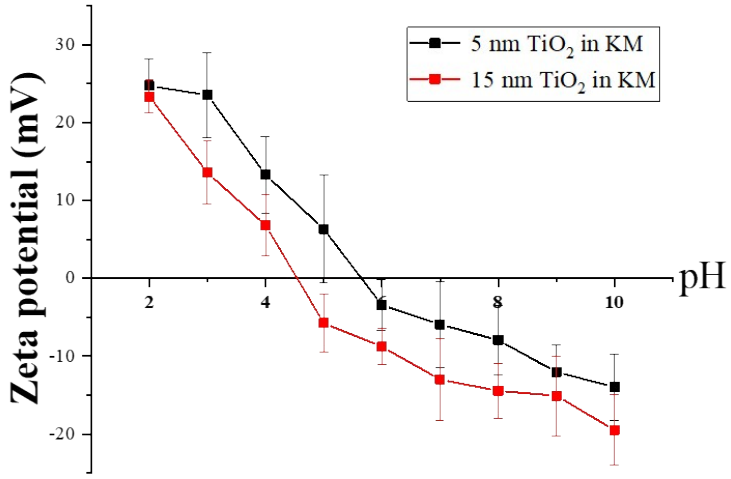

Figure S5. ζ potential of 5 and 15 nm TiO_2 suspensions at different pH in KM.

Spatial Distribution of Cd and Ti in *C. elegans*

Tissue-specific distribution of nanoparticles with co-contaminants could aid in our understanding of internal processes related to the accumulation of co-contaminants and thus further our understanding of their combined toxicity.

The lateral distribution of Cd and Ti in Figure S6A indicated that TiO_2 NPs were mainly concentrated in the gut, however, a considerable amount of Cd was detected in other parts of *C. elegans*, which was consisted with our speculations on the part of efflux experiment (Figure 3). Figure S6B showed that a large amount of TiO_2 NPs accumulated in pharyngeal pump, vulva and anus (Figure S6B).

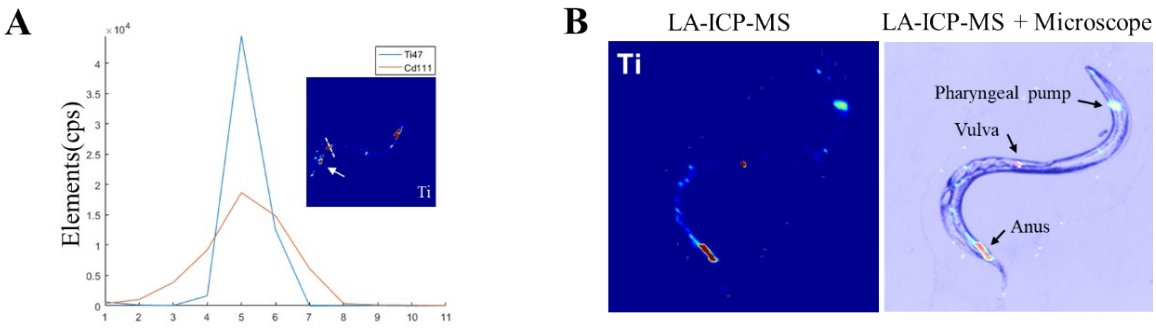

Figure S6. (A) Horizontal distribution of Ti and Cd in *C. elegans* under the co-exposure of 5nm TiO_2 and Cd. The white dotted lines were the transverse inspection line. The white arrowhead indicated the pharynx at the head region of nematodes. (B) The main accumulating organs in the body of the TiO_2 NPs.