Electronic supplementary information

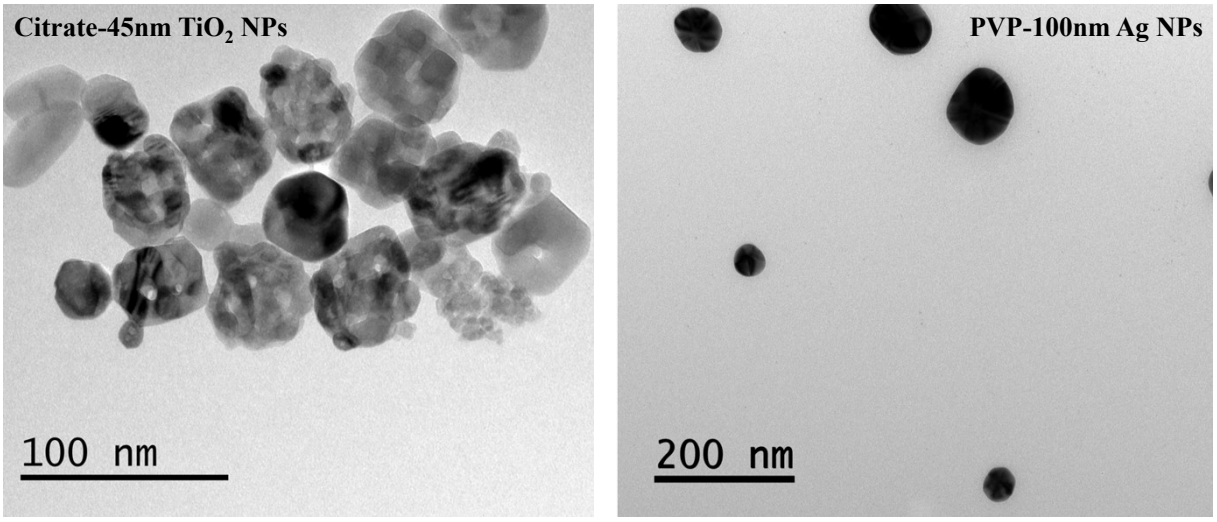
Bioaccumulation and human risk assessment of inorganic nanoparticles in aquaculture species

Cristian Suárez-Oubiña, Paloma Herbello-Hermelo, Natalia Mallo, María Vázquez, Santiago Cabaleiro, Raquel Domínguez-González, Antonio Moreda-Piñeiro, Pilar Bermejo-Barrera

Sea bream, sea bass, and Japanese carpet shell exposure trials and sample preparation

Commercial fish feed pellets (Biomar Iberia, S.A., Palencia, Spain) were used for feeding seabreams and seabass along the bioaccumulation assay. Titanium dioxide NPs [pristine 45 nm TiO_2 NPs (99.5% purity, mixture of rutile and anatase; Sigma-Aldrich)] and Ag NPs [polyvinylpyrrolidone (PVP)-coated Ag NPs (15 nm diameter, 25% wt silver and 75% wt PVP) from SSNano (Houston, TX, USA) were incorporated to food following the method described in previous investigations [1]: first, a premixture was prepared by combining micronized calcium carbonated (C.T.S. España S.L., Madrid, Spain) and TiO_2 NPs or Ag NPs NPs at an equivalent of 5% of the weight of pellets. The premixture was then added to commercial fish feed pellets for pellet coating until achieving 0.25, 0.75, and 1.5 mg kg^{-1} of fish per day. These low exposure doses were selected based on the low NPs levels expected in the environment (and hence in the treated seawater and feed) and also for avoiding changes on the ingestion ratio as consequence of a different palatability of the fish feed containing NPs. Previously, commercial 15 nm (PVP)-coated Ag NPs (15 nm) were dispersed in ultrapure water (1.0 g L^{-1}) for 15 min using a bath sonicator (37 kHz, 100 %), whereas 45 nm TiO_2 NPs were stabilized with trisodium citrate dehydrate aqueous solution reaching a weight ratio of 1:1.5 TiO_2:citrate [dispersion with an ultrasonic probe (Branson Disintegrator Ultrasonic Mod. 450) with 30 s pulse on / 5 s pulse off, and 50 % amplitude for 30 min]. The final concentration of citrate-coated TiO_2 NPs was 1.0 g L^{-1}. Feed used in the control group (unexposed specimens) was also coated with micronized $CaCO_3$ but without NPs. Nanoparticles characterization was performed by transmission electronic microscopy (TEM), see TEM images as follows.

TEM images of Citrate-45nm TiO_2 NPs and PVP-100nm Ag NPs. TEM images of the rest of NPs were acquired using JEOL JEM 1010 transmission electron microscope operating at 100 kV.



Sea bass specimens (fifty individuals in each tank, average initial weight of 121.6 g) were kept in open circuit 400 L open circuit tanks and exposed to 100 nm Ag NPs (PVP coating) for 90 days. Similarly, sea bream (one hundred and twenty individuals in each tank, average initial weight of 7.7 g) were kept in 300 L f and were exposed to 45 nm TiO_2 NPs (citrate coating) for 90 days. Sampling was performed each 15 days obtaining exposure times of 0 (experiment beginning), 15, 30, 45, 60, 75, and 90 days. Different exposure NPs concentrations were tested for both cultured species: 0 (control tanks), and 0.25, 0.75 and 1.5 mg kg^{-1} (concentration referred as the mg of Ag NPs or TiO_2 NPs per kg of fish). Fish were fed at a daily feeding rate of 0.7-1% for sea bass and of 2.5-3% for sea bream. Each NPs concentration condition was replicated three times (three different tanks for control and each tested concentration). At the end of the experiment (after 90 days), the specimens were fasted for one day before being killed with an overdose of anaesthetic and exsanguination. Finally, the specimens were dissected and divided in muscle+skin, liver, and kidney, obtaining three replicates (three tanks) for each NPs concentration exposure and time. The samples were frozen and preserved at -20ºC until their analysis. Data regarding weight and shell length for Japanese carpet throughout the TiO_2 NPs exposure trial are listed in Table S4 and S5 (ESI), respectively, whereas Table S6 and S5 (ESI) show the weight and shell length for experiments with Ag NPs. Statistically significant differences (95% confidence level) for the mean weight and shell length after Ag NPs exposure were not found.

Japanese carpet shell specimens (forty individuals in each tank) were kept in 50 L close circuit tanks (50% water renewal on Monday and Friday, and 100% on Wednesday) and exposed to 100 nm Ag NPs (PVP coating) or 45 nm TiO_2 NPs (citrate coating) for 28 days. Carpet shells were fed with microalgae mixture (*Isochrysis galbana* (T-ISO) and *Phaeodactylum tricornutum* (50:50, v/v)) on Monday, Wednesday and Friday and NPs were added together with the feed on Wednesday. Different exposure NPs concentrations were tested: 0 (control tanks), and 0.10 and 1.0 mg L^{-1} (each NPs concentration condition was replicated three times (three different tanks for control and each tested concentration). Sampling was performed each 7 days (on Wednesday) obtaining exposure times of 0 (experiment beginning), 7, 14, 21, and 28 days. Data regarding weight, as well as shell length for Japanese carpet shells throughout the exposure trial are included in ESI.

Water parameters (pH, temperature, salinity and O_2 content) were daily monitored using hand probes (Standard probe for O_2 content, pH/redox probe for pH, temperature probe for temperature control, and salinity probe for salinity measurements) from Oxyguard (Farum, Denmark). Salinity (35.52±0.19 ‰), pH (8.04±0.8) and O_2 (8.54±0.08) values were very stable and remained constant throughout the trials. Temperature changes are showed in ESI Figures (Fig. S2-S7).

Microwave assisted acid digestion

Muscle+skin samples, approximately 1.000 g of homogenised tissue (manually homogenized before storing) were subjected to microwave assisted acid digestion with an Ethos Easy Advanced Microwave Digestion System (Milestone, Sorisole, Italy) in triplicate, whereas only one replicate of liver and kidney tissues (0.1500 g) per specimen was used for digestion (small sample size). Regarding clams, also 1.000 g of homogenised wet tissue was subjected to the digestion procedure in triplicate (three carpet shells from each tank and exposure condition). Therefore, total Ag and Ti concentrations were referred to nine replicates for sea bass and sea bream muscle+skin, and for Japanese carpet shell (three subsamples from each specimen, and three specimens – three tanks) and to three replicates for sea bass and sea bream liver and kidney (one subsample from each specimen, and three specimens – three tanks). Digestion was performed with 4.0 mL of ultrapure water (18.2 MΩ cm of resistivity from a Milli-Q® IQ 7003 purification device system, Millipore Co., Bedford, MA, USA), 3.0 mL of 69% (w/v) HNO_3

(Panreac, Barcelona, Spain) and 1.0 mL of 33% (w/v) H_2O_2 (Panreac), and with a microwave program of four stages operating at 1800 W: a first heating ramp from room temperature to 100°C for 5 min, a second heating ramp from 100 to 170°C for 10 min, a third heating ramp from 170 to 220°C for 10 min, and a final heating stage at 220°C for 10 min. Two blanks were obtained in each microwave set of samples. After cold-down, acid digests were made up to 25 mL with ultrapure water, and they were kept in clean plastic tubes at room temperature before ICP-MS measurements.

Enzymatic hydrolysis for Ag NPs and TiO_2 NPs extraction

Enzymatic hydrolysis procedures were based on previous developments [2,3] with slight modifications. The procedure consists of weighting approximately 1.00 g of homogenized seafood tissue and adding 7.5 mL of a daily prepared enzymatic solution containing pancreatin from porcine pancreas (Sigma Aldrich, Osterode, Germany) and lipase from *Candida rugose* (Sigma-Aldrich) at 3.0 g L^{-1} each one for Ag NPs isolation, and 8.0 g L^{-1} each one for TiO_2 NPs isolation, dissolved in a mixture of 0.2 M NaH_2PO_4 (Merck, Darmstadt, Germany) and 0.2 M NaOH (Merck) at pH 7.4 [pH measurements by using an pH50+ pH-meter (XS Instruments, Carpi Mo, Italy)]. The mixtures were then stirred (orbital-horizontal shaking) at 37°C and 150 rpm (Ag NPs isolation) and 200 rpm (TiO_2 NPs isolation) for 12 h using a Boxcult temperature-controlled chamber (Stuart Scientific, Surrey, UK) with a Rotabit orbital-rocking platform shaker (J.P. Selecta, Barcelona, Spain). After enzymatic hydrolysis, the mixtures were passed through 5.0 µm pore size Minisart™ NML syringe filters (Sartorius, Goettingen, Germany) and the filtrate were made up to 10 mL with ultrapure water. Three blanks were performed for each set of sample preparation. The enzymatic extracts were stored at 4°C and they were analysed in the same day.

***In vitro* digestion procedure: bio-accessibility assays**

An *in vitro* digestion approach that replicated the environment of the stomach and intestines in two phases was used to model the human gastrointestinal process [4]. The homogenized seafood tissue (0.50 g of raw or cocked sample) was mixed with 20 mL of ultrapure water in an Erlenmeyer flask and after a few minutes of stabilization the pH was adjusted at 2.0 (gastric pH) by adding dropwise 0.1 M hydrochloric acid (prepared from 37% hydrochloric acid, Merck, Darmstadt, Germany). Then, 0.15 g of gastric solution [16 g of pepsin (from porcine gastric mucosa (Sigma-Aldrich) in 100 mL of 0.1 M hydrochloric acid] was added, and the flask was covered with Parafilm® and placed in the Boxcult temperature-controlled chamber for gastric digestion (37 °C and orbital-horizontal shaking at 150 rpm for 2.0 h).

The enzymatic activity in the gastric digest was stopped by immersing the flask in an ice-water bath, and the pH of the mixture was then adjusted to 7.0. (0.1 M sodium hydroxide dropwise) followed by adding the intestinal solution [4.0 g pancreatin and 25 g bile salts (Sigma-Aldrich) in 1.0 L of 0.1 M sodium dihydrogen carbonate (solution prepared from sodium hydrogen carbonate, Panreac)]. The intestinal digestion was then performed at 37 °C and orbital-horizontal shaking at 150 rpm for 2.0 h. The enzymatic digests were allowed to cold down in an ice bath. The bio-accessible fraction was obtained after enzymatic digest filtration (5.0 µm filters), and they were stored in polyethylene tubes at -20 °C until analysis. Each fish sample was subjected to the enzymatic procedure in duplicate and one reagent blank was also prepared.

Caco-2 monolayer development

Experiment regarding Caco-2 cellular transport were performed inside an SMH-100 laminar flow cupboard from Telstar (Tarrasa, Spain).

Caco-2 cells (Sigma-Aldrich) were maintained in Dulbecco's Modified Eagle Medium – DMEM – (Corning, New York, USA) containing 10% (v/v) fetal bovine serum, 2 mM L-glutamine, 1% (v/v) penicillin/streptomycin, 1 mM sodium pyruvate, and 1 mM non-essential amino acids (NEAA) at 37 °C in a controlled environment with 95% relative humidity and a 5% CO_2 flow [icoMed incubator (Memmert, Schwabach, Germany)]. The medium was changed every two or three days until 80% confluence was reached. A solution containing trypsin (Corning) at 0.5 g L^{-1} was then used to detach the cells before resuspension in DMEM. Caco-2 cells were seeded (7.5×10^4 cells cm^{-2}) on polyester membrane inserts in six-well Transwell® (24 mm diameter, 0.4 µm pore size polyester membrane, from Corning). The insert divides the well into two compartments: the bottom (basolateral chamber) which represents the serous cavity, and the top (apical chamber) which represents the intestinal lumen. Resuspended cells (1.5 mL) were introduced in the apical chamber whereas 2.0 mL of DMEM were placed in the basolateral chamber. The Transwell plates are then placed in a temperature-controlled environment (37 °C, 95% relative humidity, and 5% CO_2 flow, medium changing every three days) to generate a Caco-2 monolayer. A transepithelial electrical resistance (TEER) value of 250 Ω cm^2, required for a proper Caco-2 monolayer development, was measured with a Millicell ERS-2 (Millipore Co.).

Cellular transport

The cellular transport has been described elsewhere [34]. Before bio-accessible fraction loading on the developed Caco-2 monolayer, 7.5 mL of the bio-accessible fraction (section 2.7.) was heated at 100 °C for 10 min in a water bath (2000209 oven from J.P. Selecta) to denature the remaining gastrointestinal enzymes and the osmolarity of the solution was adjusted to 280-300 mΩ cm^2 with a K-7400S freezing point osmometer (Knauer, Berlin, Germany) by adding 1.0 g L^{-1} of glucose (solution prepared from D-(+)-glucose, Sigma Aldrich) and 10 mM sodium chloride (solution prepared sodium chloride, Sigma Aldrich) dropwise. A volume of 1.5 mL of treated bio-accessible fraction and Lucifer Yellow (Sigma Aldrich) were added to the apical chamber and 2.0 mL of Hanks' Balanced Salt Solution (HBSS) (Sigma Aldrich) was added to the basolateral chamber for cellular transport assay. Identical temperature, humidity, and CO_2 conditions than those used for Caco-2 monolayer development were set, and cellular transport was performed for 2.0 h. The basolateral and apical solutions were carefully removed and kept for analysis. Each bio-accessible fraction was subjected to the cellular transport procedure in triplicate which allows six independent measurements (two bio-accessible fractions per sample). At least two blanks were subjected to the same process in each set of samples.

Caco-2 monolayer integrity after cellular transport assay was checked with a FLUOstar OPTIMA fluorimeter Microplate reader (BMG Labtech, Leicester, UK) using Sterilin black 96-well microtiter plates (400 µL) were from Thermo Fisher (Dublin, Irland).

ICP-MS measurements

Total Ag and Ti measurements were performed with a NexION 2000 ICP-MS (PerkinElmer, Waltham, MA, USA)]. The instrument was equipped with a triple nickel cone interface, a concentric Meinhard™ type nebulizer coupled to a cyclonic spray chamber (Glass Expansion, Inc., Melbourne, Australia) and attached to a quartz torch with a 2.5 mm i.d. quartz injector tube. Data acquisition and management were performed with Syngistix™ Nano Application 2.5 version software (PerkinElmer). Daily performance was assessed by monitoring and verifying intensities of Be, In, U, and Ce as well as a background (mass-to-charge ratio of 202), and Ce^{++}/Ce and CeO/Ce ratios using a NexION Setup Solution (Perkin Elmer).

Internal standards such as rhodium and scandium were from Fluka (Steinheim, Switzerland), whereas 99.999% helium (Nippon Gases, Madrid, Spain) as a collision gas, and 99.999% ammonia (Nippon Gases) as a reaction gas were used for silver and titanium determination,

respectively. Silver and titanium standards for calibration were prepared from mono-elemental 1000 mg L^{-1} titanium [$(NH_4)_2TiF_6$] and silver ($AgNO_3$) from Merck.

Single particle-ICP-MS measurements

Particle number concentrations and size distributions of Ag NPs and TiO_2 NPs were assessed by spICP-MS using a PerkinElmer NexION 2000 ICP-MS. Since an exact mass-to-charge ratio is not isolated with the quadrupole ion deflector (axial field voltage, AFT at 350 V), RPa (high-mass cut-off) and RPq (low-mass cut-off) rejection parameters must be fixed at optimized values previously studied for better focusing of the mass-to-charge ratio of interest in DRC work-mode for titanium determination (ammonia cluster at m/z 131). Daily performance was assessed by monitoring Be, In, U, Ce (Ce^{++}/ Ce and CeO/Ce ratios) and background and verifying intensities as well as ICP-MS measurements. Transport efficiency (TE%) was assessed by the particle frequency method, which implies the assessment of the sample flow rate, an aqueous ionic Au calibration (within the 0.5–3.0 µg L^{-1}, prepared from Au standard from Merck), and the measurement of an Au NPs suspension (1.0×10^5 particles mL^{-1}) prepared from an Au NPs certified reference [N8151035 (49.6 nm by TEM, 12.4 ng mL^{-1}, 9.89×10^6 NPs mL^{-1}, in 1.0 mM aqueous citrate) from nanoComposix (San Diego, CA, USA)]. Therefore, the sample flow rate was established by aspirating ultrapure water and weighing the solution after and before aspiration at the selected pump conditions (sample flow rates were between 0.19 and 0.21 mL min^{-1}). Transport efficiency (TE%) values (close to 8.0%) were automatically calculated by Syngistix ™ Nano Application.

Calibrations were performed using ultrapure water and 1.0% (v/v) glycerol (prepared from 99.5% glycerol, Sigma-Aldrich) covering ionic Ti and Ag concentrations within the 0.1–10 µg L^{-1} range. Several reagent blanks were also analysed throughout the work. Extracts containing TiO_2 NPs and/or Ag NPs were dispersed before analysis with an USC-TH ultrasound water bath (45 Hz, 80 W) from VWR International Eurolab S.L (Barcelona, Spain). The limit of detection (number concentration and size) and quantification (number concentration) of the method are listed in Table S1 (ESI).

Table S1. Sensitivity: Limits of detection obtained for sea bream and sea bass measurements.

Fish tissue (analyte)	spICP-MS (NPs assessment)			ICP-MS (total Ag/Ti assessment)	
	LOD_{size} (nm)[a]	$LOD_{number\ concentration}$ (particles g^{-1})[b]	$LOQ_{number\ concentration}$ (particles g^{-1})[c]	LOD (ng g^{-1})[b]	LOQ (ng g^{-1})[c]
Sea Bass (Ag)	6	2.27×10^{6}	7.57×10^{6}	7.60	25.3
Sea Bream (Ti)	27	2.09×10^{5}	6.97×10^{5}	83.2	277

(a) LOD_{size} given by Syngistix™ Nano Application 2.5 version routine based on the 5σ (5×baseline standard deviation) criterion after measuring a reagent blank (diluted enzymatic solution)

(b) Based on $LOD = \frac{3\,\sigma}{m}$; σ the standard deviation of eleven measurements of a blank (acid digest) and m the slope of the calibration (standard addition method)

(c) Based on $LOD = \frac{10\,\sigma}{m}$; σ the standard deviation of eleven measurements of a blank (enzymatic extract) and m the slope of the calibration

Table S2. Growth parameters for sea bream trial after 90 days of TiO_2 NPs bioaccumulation assay.

Sea bream Parameters	TiO_2 nanoparticle dose (mg/kg)			
	0	0.25	0.75	1.5
IBW (g)	7.7±1.5	7.16±1.81	7.61±1.3	7.31±1.1
FBW (g)	28.4±3.1	28.7±2.1	28.5±3.2	29.3±3.7
WG%)	73±2.8	73±1.2	73±1.5	74±1.9
FCR	1.9±0.3	1.9±0.2	2±0.2	2.1±0.2
SGR (%)	1.4±0.1	1.5±0.1	1.5±0.1	1.5±0.1

- Results are presented as mean ± SD (Initial and final weight: n=12; WG (%), FCR and SGR (%): n = 3). No significant differences between any weight within the same row were observed ($p < 0.05$).
- IBW (Initial body weight, g) = body weight at start of experiment (t=0 day); FBW (Final body weight, g) = Body weight at end of experiment (t=90 day); WG (Weight gain, %) = ((FBG (g) – IBW (g))/FBW (g)) x 100. Feed conversion ratio (FCR) = dry feed intake (g)/Weight gain (g); SGR (Specific growth rate/day, %) = 100x (ln FBW - ln IBW/ number of days).

Table S3. Growth parameters for sea bass trial after 90 days of Ag NPs bioaccumulation assay.

Sea bass Parameters	TiO_2 nanoparticle dose (mg/kg)			
	0	0.25	0.75	1.5
IBW (g)	118.5±21.3	127.9±27.1	118.2±22.7	117.5±27.8
FBW (g)	180.0±20.8	180.9±20.1	192.37±21.1	197.3±27.5
WG%)	32.6±4.0	32.8±2.1	36.76±2.6	38.3±5.0
FCR	2.4±0.3	2.4±0.3	2.9±0.3	2.8±0.5
SGR (%)	0.44±0.1	0.44±0.1	0.51±0.1	0.54±0.1

- Results are presented as mean ± SD (Initial and final weight: n=12; WG (%), FCR and SGR (%): n = 3). No significant differences between any weight within the same row were observed ($p < 0.05$)
- IBW (Initial body weight, g) = body weight at start of experiment (t=0 day); FBW (Final body weight, g) = Body weight at end of experiment (t=90 day); WG (Weight gain, %) = ((FBG (g) – IBW (g))/FBW (g)) x 100. Feed conversion ratio (FCR) = dry feed intake (g)/Weight gain (g); SGR (Specific growth rate/day, %) = 100x (ln FBW - ln IBW/ number of days).

Table S4. Weight evolution in Japanese carpet shell throughout 45 nm TiO_2 NPs bioaccumulation assay.

Time (days)	TiO_2 nanoparticle dose (mg/L)		
	0	0.1	1.0
0	19.9±2.3	19.9±2.3	19.9±2.3
7	18.2±1.8	18.5±2.2	18.5±1.7
14	18.2±1.2	19.3±2.0	19.1±2.4
21	18.8±1.0	18.8±2.5	19.7±1.7
28	19.1±1.8	18.8±2.3	18.8±3.4

Results are presented as mean ± SD (n = 12). No significant differences between any weight within the same row were observed.

Table S5 Shell length evolution in Japanese carpet shell throughout 45 nm TiO_2 NPs bioaccumulation assay.

Time (days)	TiO_2 nanoparticle dose (mg/L)		
	0	0.1	1.0
0	4.2±0.3^{a}	4.2±0.3^{a}	4.2±0.3^{a}
7	4.2±0.3^{a}	4.3±0.2^{ab}	4.0±0.2^{b}
14	4.4±0.2^{a}	4.5±0.3^{b}	4.2±0.2^{b}
21	4.5±0.2^{a}	4.5±0.3^{a}	4.4±0.3^{a}
28	4.6±0.3^{a}	4.7±0.3^{a}	4.7±0.3^{a}

Results are presented as mean ± SD (n = 12). Values within the same row not sharing a common superscript letter are significantly different ($p < 0.05$).

Table S6. Weight evolution in Japanese carpet shell throughout 100 nm Ag NPs bioaccumulation assay

Time (days)	Ag nanoparticle dose (mg/L)		
	0	0.1	1.0
0	23.3±3.4	23.3±3.3	23.3±3.3
7	24.0±5.2	22.0±6.5	21.6±4.5
14	22.4±3.8	20.1±2.4	20.8±1.9
21	20.6±4.5	21.1±3.9	20.2±3.0
28	18.3±3.3	18.3±3.3	19.4±3.8

Results are presented as mean ± SD (n = 12). No significant differences between any weight within the same row were observed ($p < 0.05$).

Table S7. Shell length evolution in Japanese carpet shell throughout 100 nm Ag NPs bioaccumulation assay

Time (days)	Ag nanoparticle dose (mg/L)		
	0	0.1	1.0
0	4.7±0.2	4.7±0.2	4.7±0.2
7	4.8±0.3	4.7±0.5	4.5±0.4
14	4.5±0.3	4.3±0.2	4.5±0.2
21	4.6±0.3	4.6±0.4	4.7±0.3
28	4.5±0.3	4.8±0.4	4.6±0.3

Results are presented as mean ± SD (n = 12). No significant differences between any weight within the same row were observed ($p < 0.05$).

Table S8. Mean sizes of Ag NPs and TiO_2 NPs during the exposure assays for sea bass and seabream specimens

Sea bass	ID sample	Ag NPs mean size (nm)	Seabream	ID sample	TiO_2 NPs mean size (nm)
kidney	0.75 mg kg^{-1} (15 days)	----[a]	kidney	0.75 mg kg^{-1} (15 days)	----[a]
	1.50 mg kg^{-1} (15 days)	24±2		1.50 mg kg^{-1} (15 days)	----[a]
	0.75 mg kg^{-1} (30 days)	----[a]		0.75 mg kg^{-1} (30 days)	----[a]
	1.50 mg kg^{-1} (30 days)	23±2		1.50 mg kg^{-1} (30 days)	----[a]
	0.75 mg kg^{-1} (45 days)	23±1		0.75 mg kg^{-1} (45 days)	----[a]
	1.50 mg kg^{-1} (45 days)	----[a]		1.50 mg kg^{-1} (45 days)	----[a]
	0.75 mg kg^{-1} (60 days)	25±1		0.75 mg kg^{-1} (60 days)	132±7
	1.50 mg kg^{-1} (60 days)	24±1		1.50 mg kg^{-1} (60 days)	132±4
	0.75 mg kg^{-1} (75 days)	19±1		0.75 mg kg^{-1} (75 days)	----[a]
	1.50 mg kg^{-1} (75 days)	23±2		1.50 mg kg^{-1} (75 days)	----[a]
	0.75 mg kg^{-1} (90 days)	24±4		0.75 mg kg^{-1} (90 days)	141±3
	1.50 mg kg^{-1} (90 days)	23±1		1.50 mg kg^{-1} (90 days)	111±4
liver	0.75 mg kg^{-1} (15 days)	41±1	liver	0.75 mg kg^{-1} (15 days)	148±5
	1.50 mg kg^{-1} (15 days)	32±1		1.50 mg kg^{-1} (15 days)	122±5
	0.75 mg kg^{-1} (30 days)	37±5		0.75 mg kg^{-1} (30 days)	161±6
	1.50 mg kg^{-1} (30 days)	28±5		1.50 mg kg^{-1} (30 days)	106±5
	0.75 mg kg^{-1} (45	32±5		0.75 mg kg^{-1} (45	116±9

	days)			days)	
	1.50 mg kg^{-1} (45 days)	39±2		1.50 mg kg^{-1} (45 days)	184±4
	0.75 mg kg^{-1} (60 days)	39±3		0.75 mg kg^{-1} (60 days)	116±5
	1.50 mg kg^{-1} (60 days)	30±3		1.50 mg kg^{-1} (60 days)	134±7
	0.75 mg kg^{-1} (75 days)	34±7		0.75 mg kg^{-1} (75 days)	142±4
	1.50 mg kg^{-1} (75 days)	34±1		1.50 mg kg^{-1} (75 days)	103±3
	0.75 mg kg^{-1} (90 days)	24±1		0.75 mg kg^{-1} (90 days)	134±4
	1.50 mg kg^{-1} (90 days)	24±1		1.50 mg kg^{-1} (90 days)	117±5
muscle + skin			muscle + skin	1.50 mg kg^{-1} (45 days)	121±6
				1.50 mg kg^{-1} (75 days)	135±7

Table S9. Mean sizes of Ag NPs and TiO_2 NPs during the exposure assays for Japanese carpet shell specimens

ID sample	Mean size (nm)	
	Ag NPs	TiO_2 NPs
0.10 mg kg^{-1} (7 days)	34±7	234±20
1.0 mg kg^{-1} (7 days)	26±1	194±12
0.10 mg kg^{-1} (14 days)	37±15	181±21
1.0 mg kg^{-1} (14 days)	32±3	156±17
0.10 mg kg^{-1} (21 days)	37±18	206±7
1.0 mg kg^{-1} (21 days)	31±8	190±9
0.10 mg kg^{-1} (28 days)	30±1	225±5
1.0 mg kg^{-1} (28 days)	24±5	165±2

Table S10. Moisture content of Japanese carpet shell and sea bream samples

Sample ID	Moisture (%)
Japanese carpet shell (Ag)	
CA0.1_28 Raw	75
CA0.1_28 Grilled	50
CA1.0_28 Raw	68
CA1.0_28 Grilled	55
Japanese carpet shell (Ti)	
CT1.0_21 Raw	70
CT1.0_21 Grilled	58
CT1.0_28 Raw	72
CT1.0_28 Grilled	59
Sea bream (Ti)	
SBr1.5_90 Raw	73
SBr1.5_90 Grilled	45
SBr1.5_90 Boiled	65

Table S11. Mean sizes of Ag NPs and TiO2 NPs in the bio-accessible and basolateral fractions (n=3)

	Sample identification	Mean size (nm)	
Ag NPs (Japanese carpet shell)		Bio-accessible fraction	Basolateral fraction
	CA0.1_28 Raw	31±6	27±3
	CA0.1_28 Grilled	35±5	33±4
	CA1.0_28 Raw	30±3	21±3
	CA1.0_28 Grilled	37±4	33±3
TiO_2 NPs (Japanese carpet shell)			
	CT1.0_21 Raw	73±5	144±7
	CT1.0_21 Grilled	85±9	110±8
	CT1.0_28 Raw	74±5	142±11
	CT1.0_28 Grilled	121±7	127±10
TiO_2 NPs (Sea bream)			
	SBr1.5_90 Raw	102±6	147±12
	SBr1.5_90 Grilled	122±9	147±9

Fig. S1. Silver concentrations in sea bass kidney (A), liver (B), and muscle plus skin (C) after several exposure conditions.

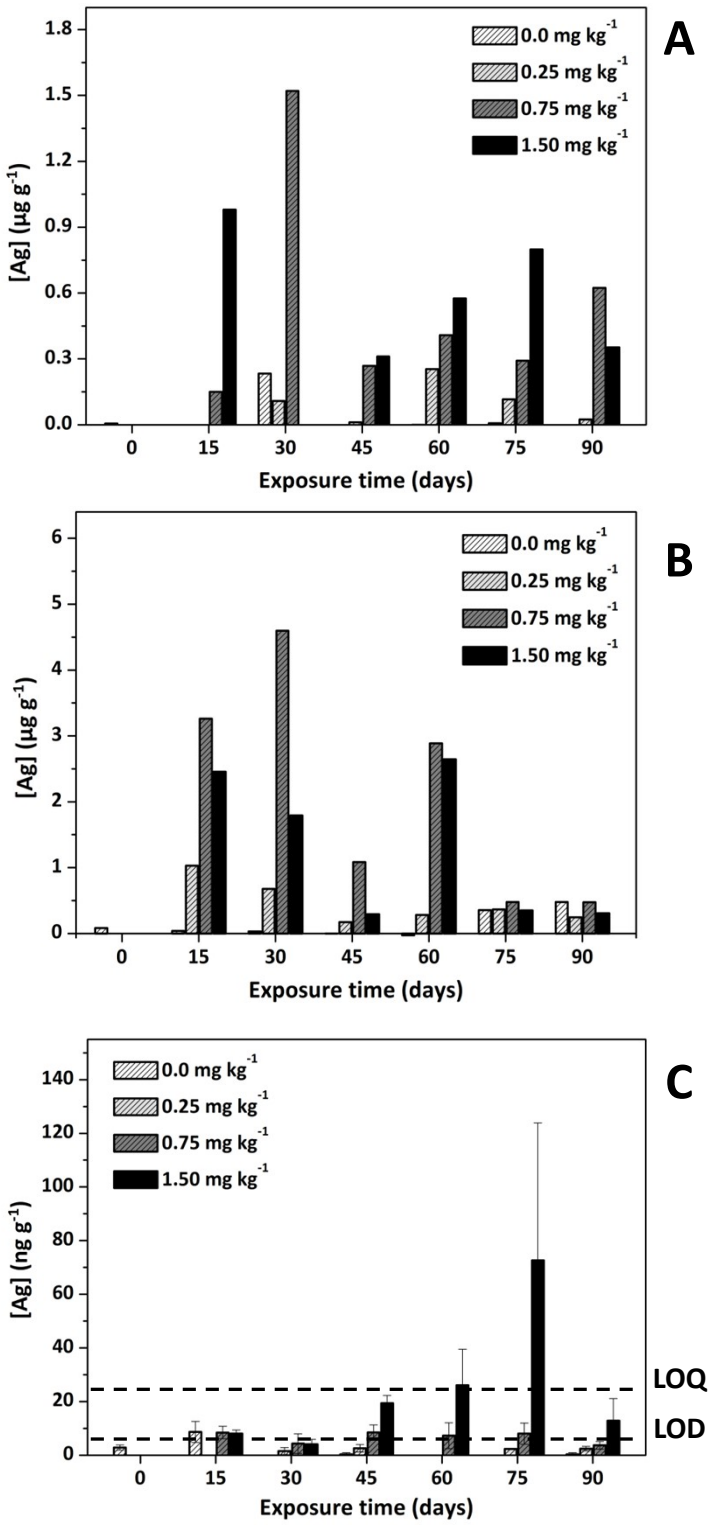


Fig. S2. Evolution of weight and temperature in sea bream throughout 45 nm TiO_2 NPs bioaccumulation assay.

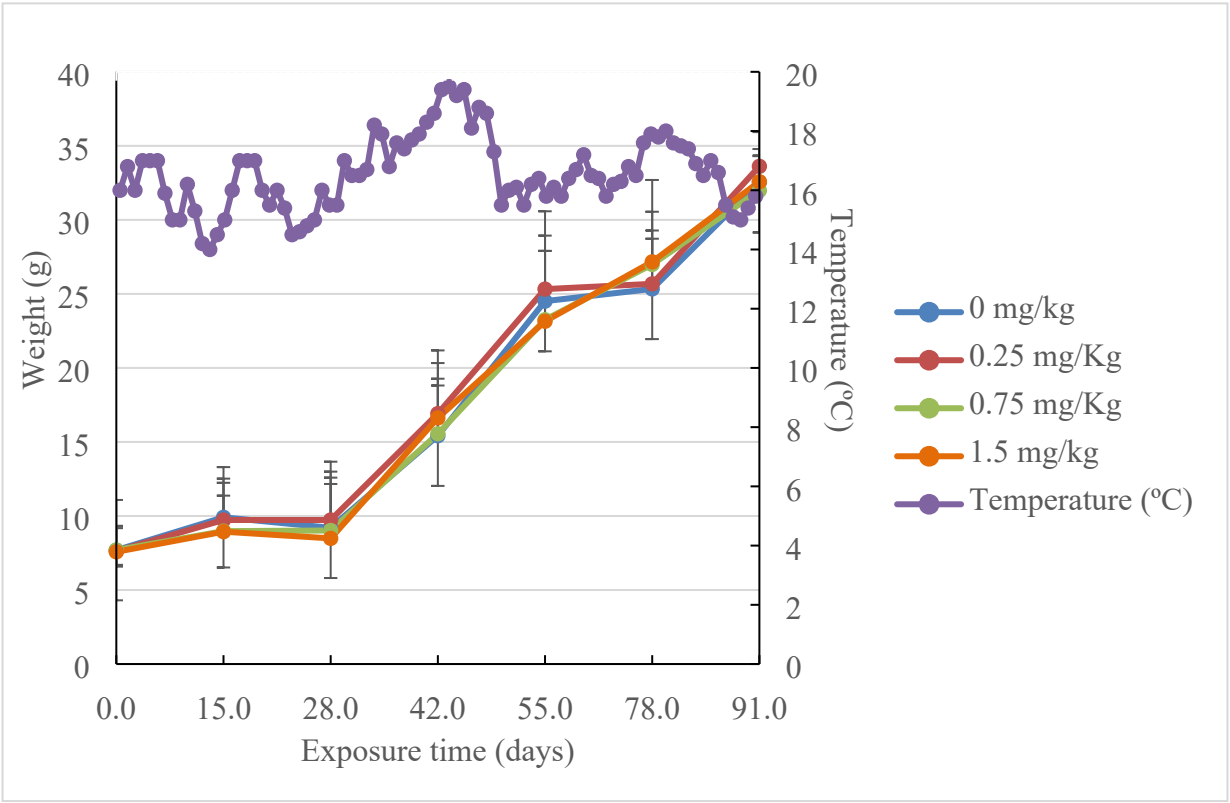


Fig. S3. Evolution of weight and temperature in sea bass throughout 100 nm Ag NPs bioaccumulation assay.

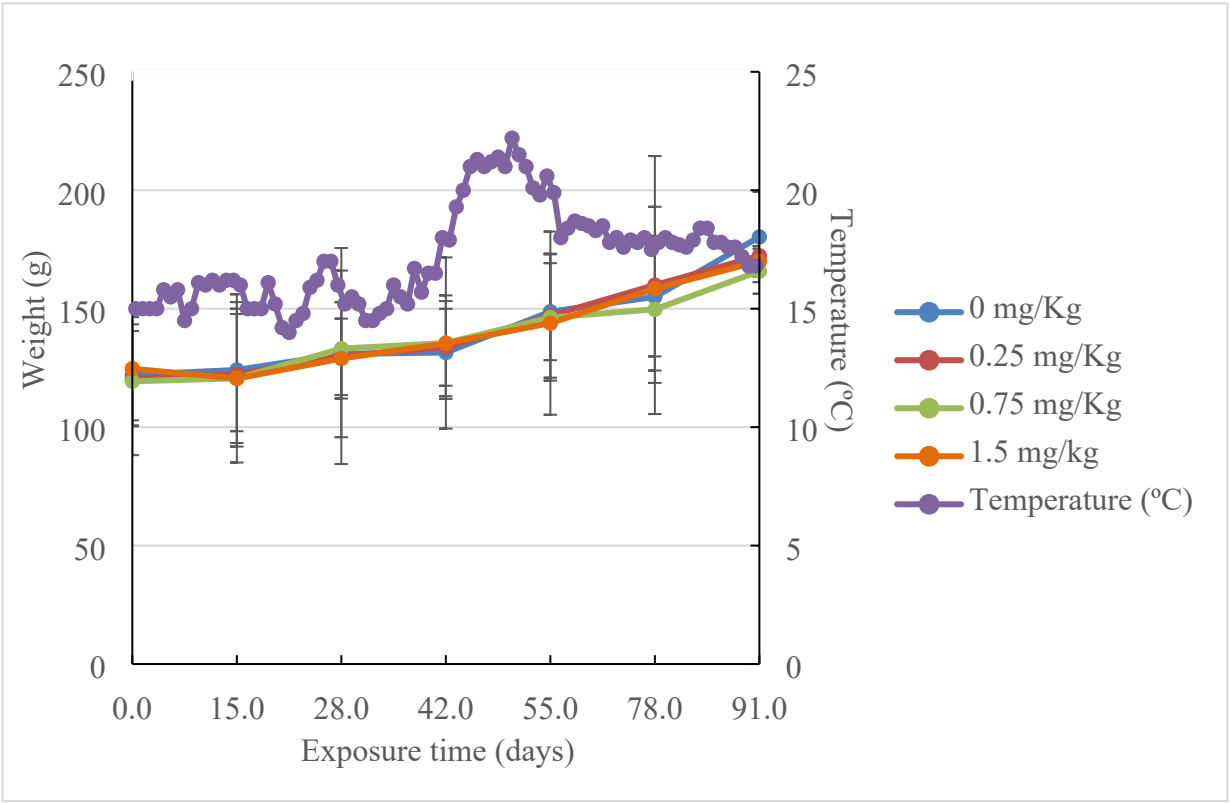


Fig. S4. Evolution of weight and temperature in Japanese carpet shell throughout 45 nm TiO_2 NPs bioaccumulation assay

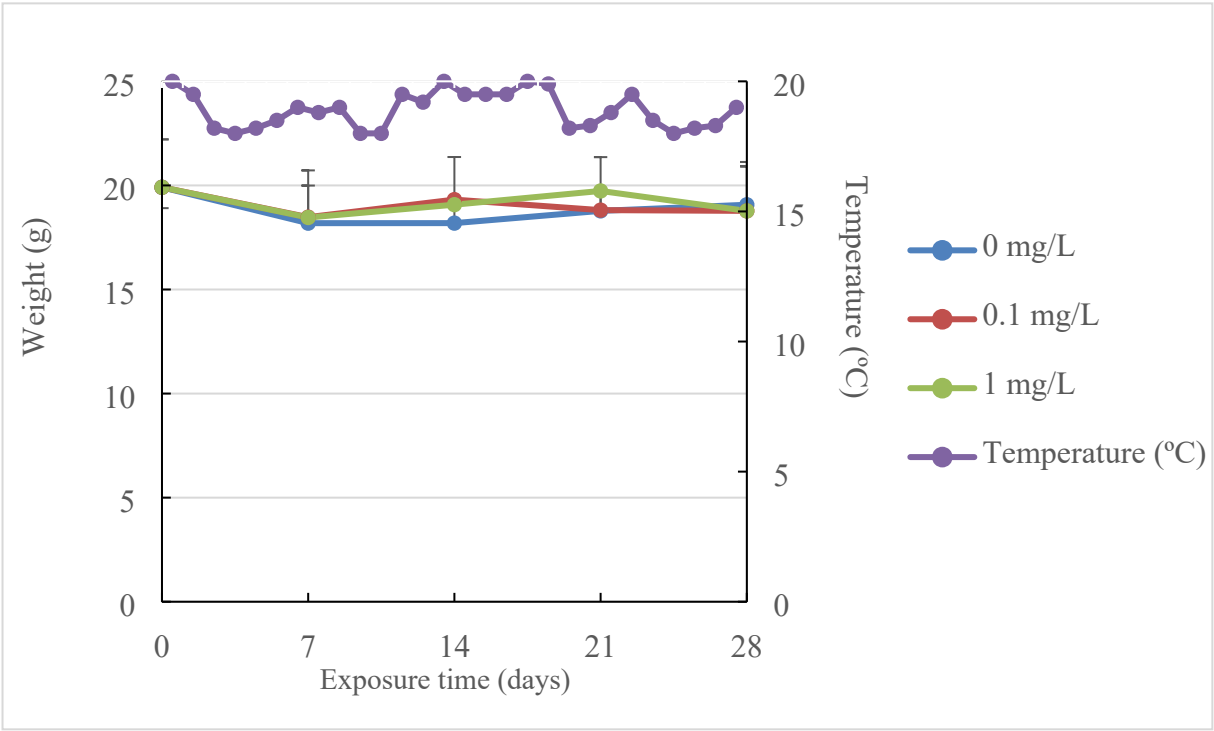


Fig. S5. Evolution of shell length and temperature in Japanese carpet shell throughout 45 nm TiO_2 NPs bioaccumulation assay

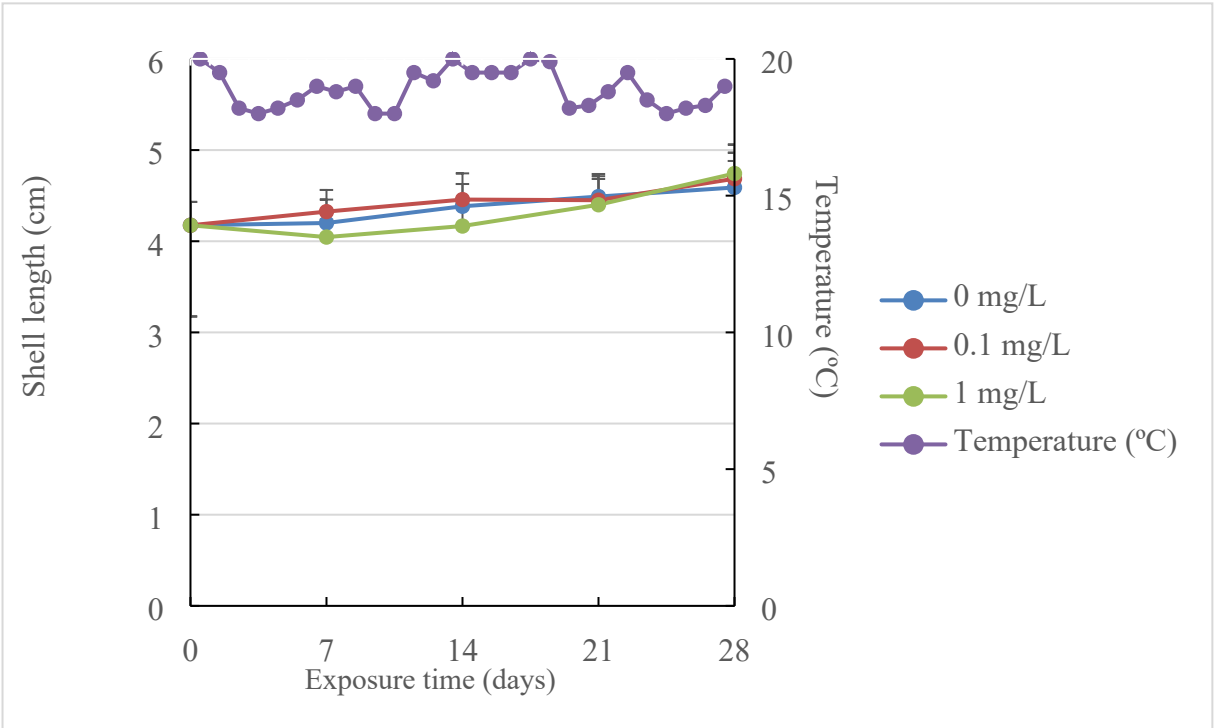


Fig. S6. Evolution of weight and temperature in Japanese carpet shell throughout 100 nm Ag NPs bioaccumulation assay

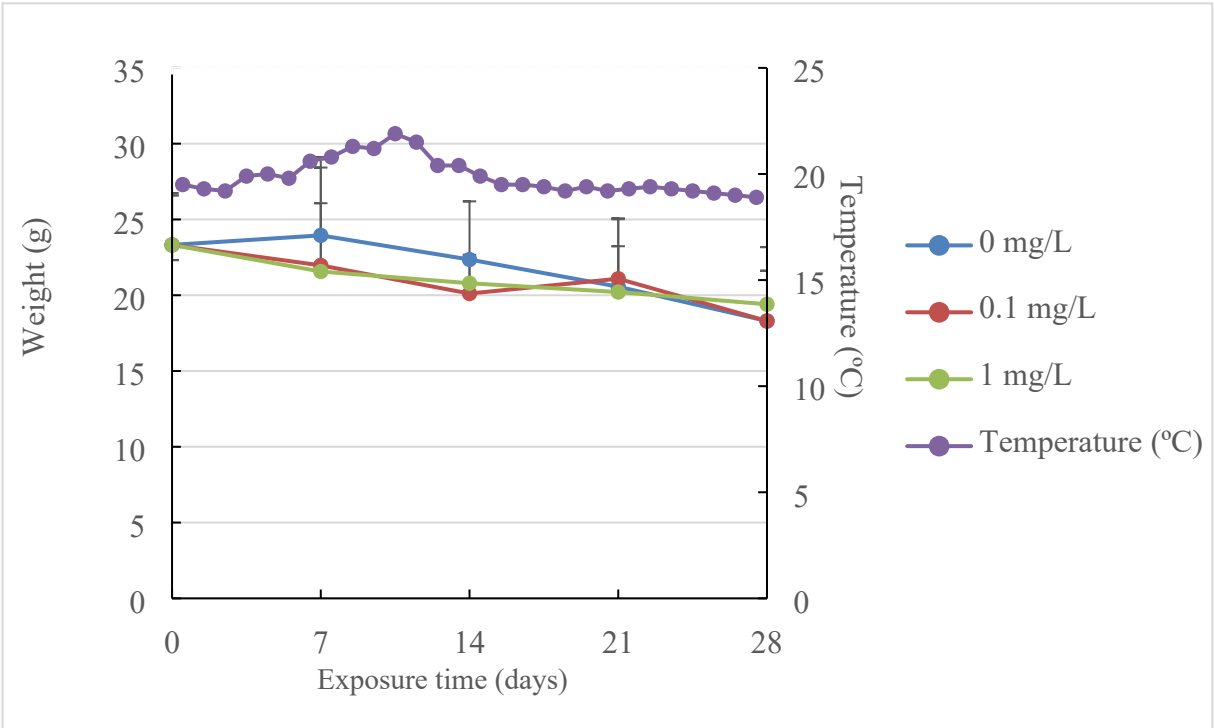


Fig. S7. Evolution of shell length and temperature in Japanese carpet shell throughout 100 nm Ag NPs bioaccumulation assay.

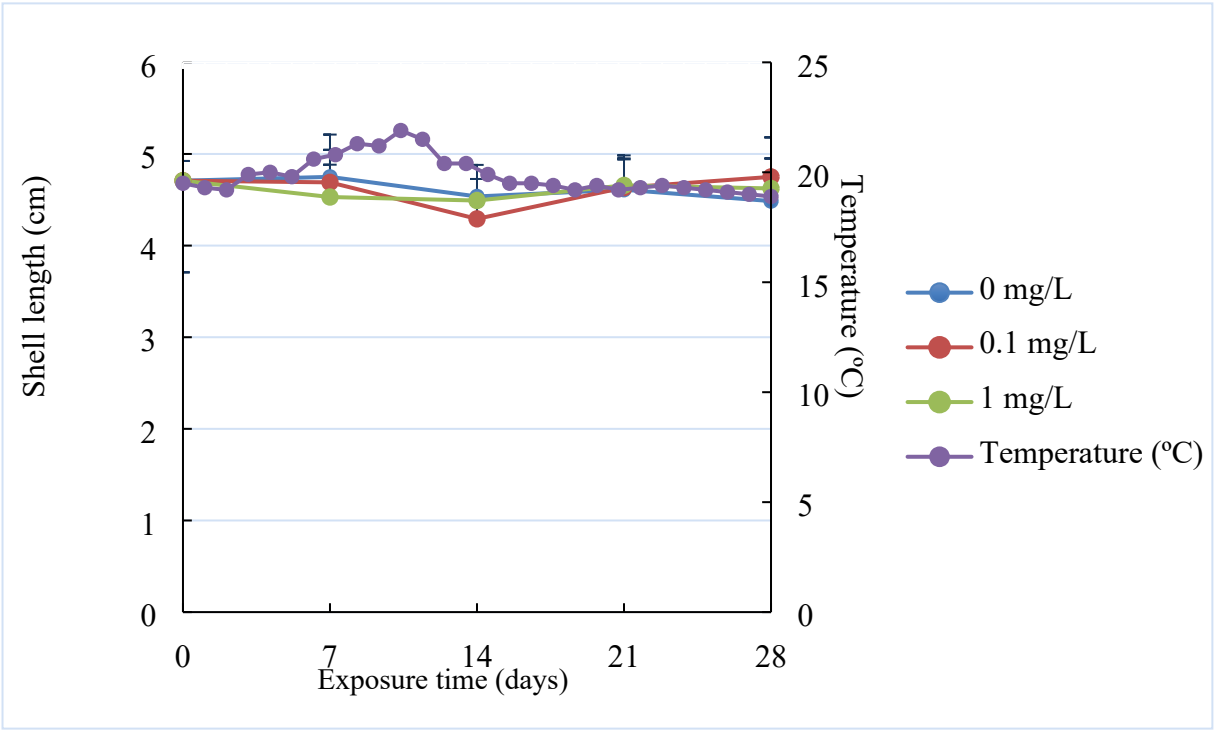


Fig. S8. Ag NPs concentrations (expressed as Ag mass) in sea bass kidney (A), and liver (B) after several exposure conditions

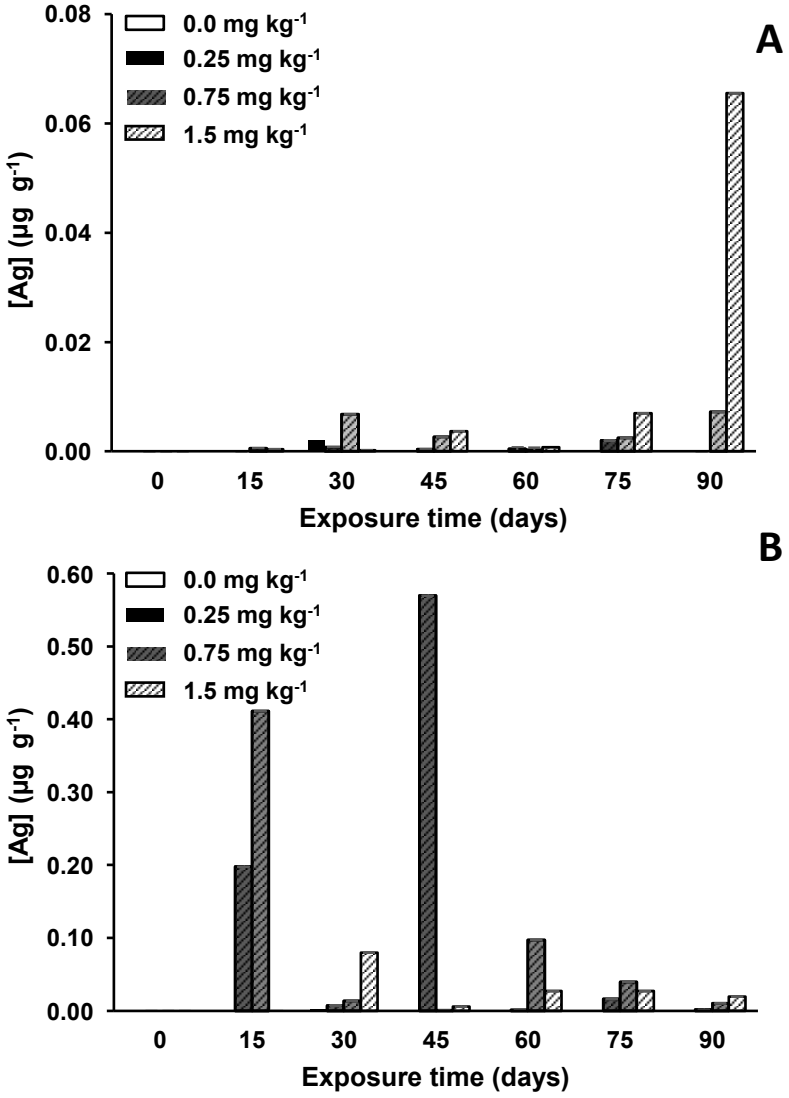


Fig. S9. Titanium (A) and TiO_2 NPs (expressed as number concentration) (B) contents in raw and cooked (grilling and boiling) pooled sea bream's flesh, and Ti (A) and TiO_2 NPs (B) contents in the bio-accessible fractions from raw and cooked (grilling and boiling) sea bream's muscle

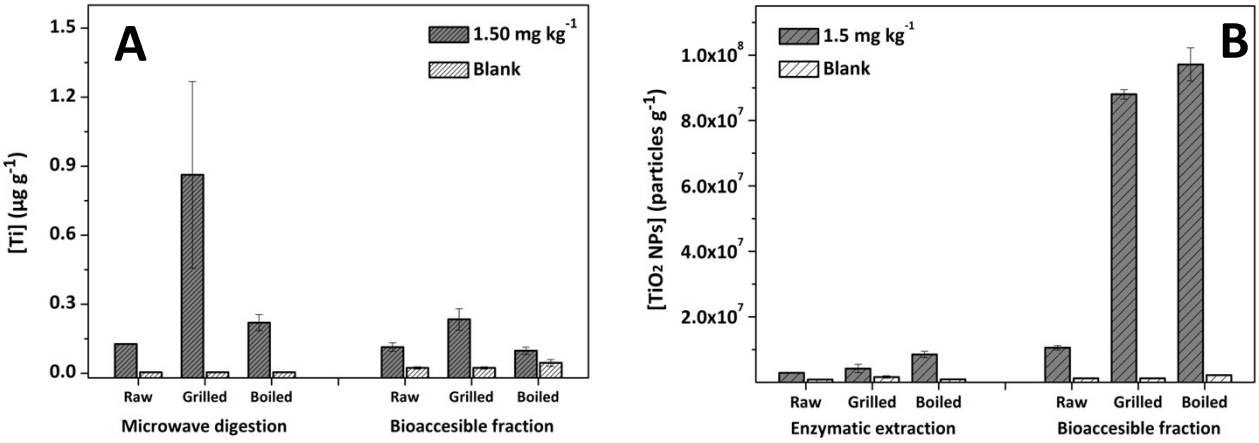
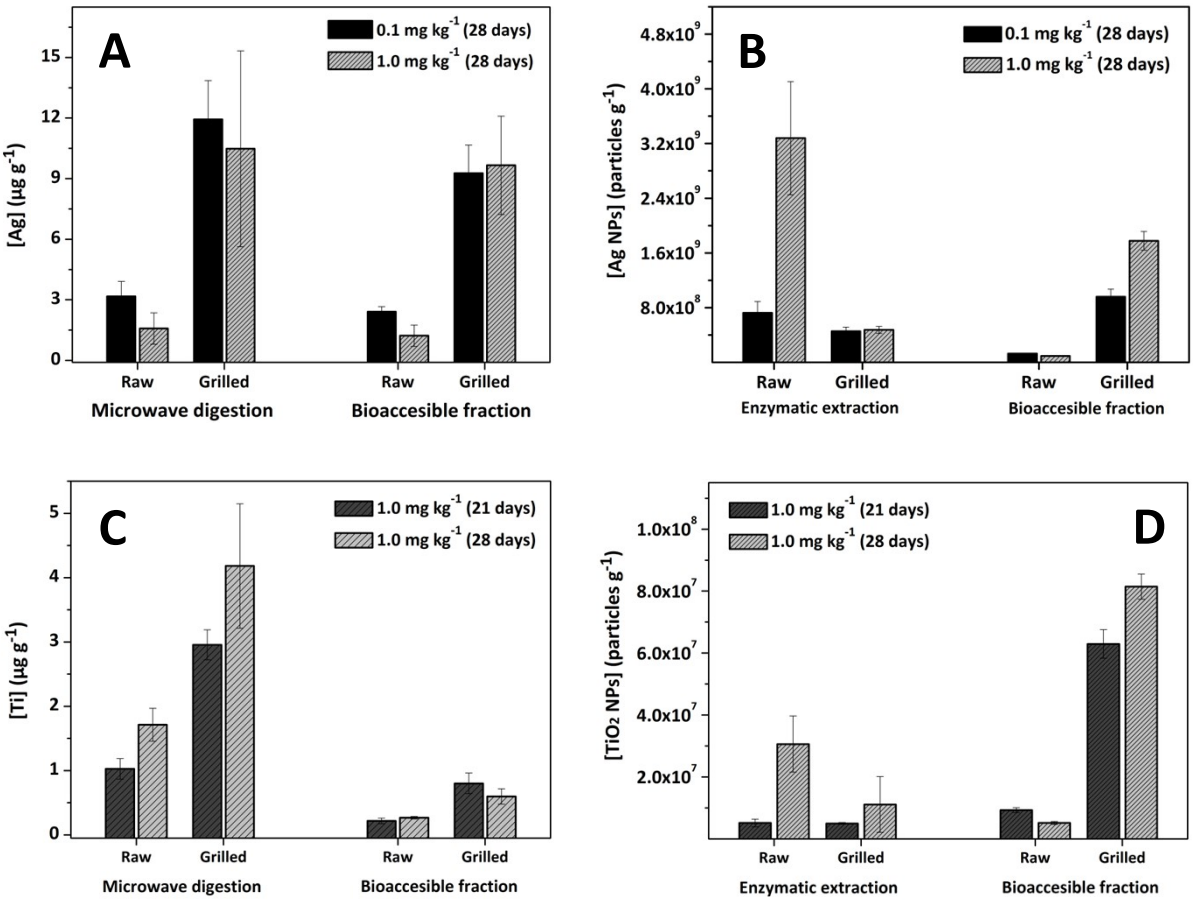


Fig. S10. Silver (A), Ag NPs (expressed as number concentration) (B), Ti (C), and TiO_2 NPs (expressed as number concentration) (D) contents in raw and grilled pooled Japanese carpet shell tissues, and Ag (A), Ag NPs (expressed as number concentration) (B), Ti (C), and TiO_2 NPs (expressed as number concentration) (D) contents in the bio-accessible fractions from raw and grilled Japanese carpet shell tissues.



References

[1] M. J. Araújo, M. L. Sousa, E. Fonseca, A. B. Felpeto, J. C. Martins, M. Vázquez, N. Mallo, L. Rodriguez-Lorenzo, M. Quarato, I. Pinheiro, M. V. Turkina, J. J. López-Mayán, E. Peña-Vázquez, M. C. Barciela-Alonso, M. Spuch-Calvar, M. Oliveira, P. Bermejo-Barrera, S. Cabaleiro, B. Espiña, V. Vasconcelos and A. Campos, *Chemosphere*, 2022, **308**, 136110.

[2] M. V. Taboada-López, N. Alonso-Seijo, P. Herbello-Hermelo, P. Bermejo-Barrera and A. Moreda-Piñeiro, *Microchem. J.*, 2019, **148**, 652–660.

[3] M. V. Taboada-López, P. Herbello-Hermelo, R. Domínguez-González, P. Bermejo-Barrera and A. Moreda-Piñeiro, *Talanta*, 2019, **195**, 23–32.

[4] M. V. Taboada-López, B. H. Leal-Martínez, R. Domínguez-González, P. Bermejo-Barrera, P. Taboada-Antelo and A. Moreda-Piñeiro, *Talanta*, 2021, **233**, 1–32.