读书报告

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Assessment of silver nanoparticle toxicity for common carp (*Cyprinus carpio*) fish embryos using a novel method controlling the agglomeration in the aquatic media

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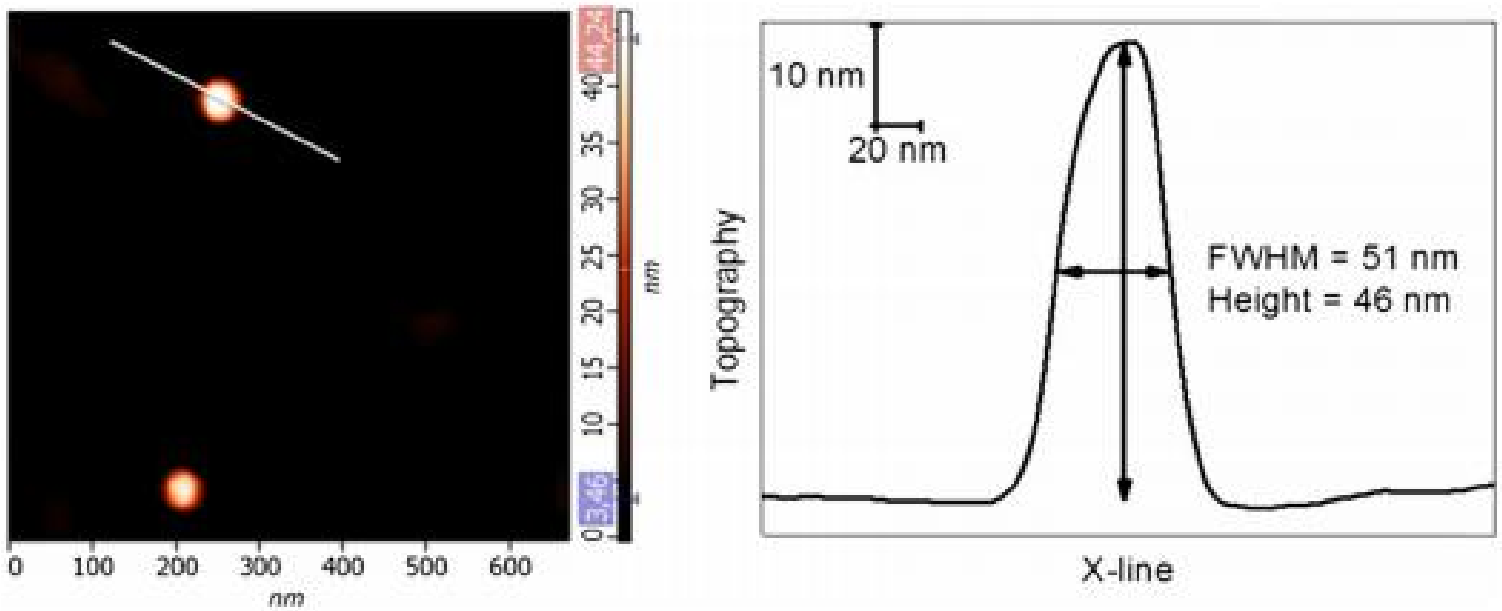
Fig. 1 Atomic force microscopy (AFM) image and profile of tested silver nanoparticles



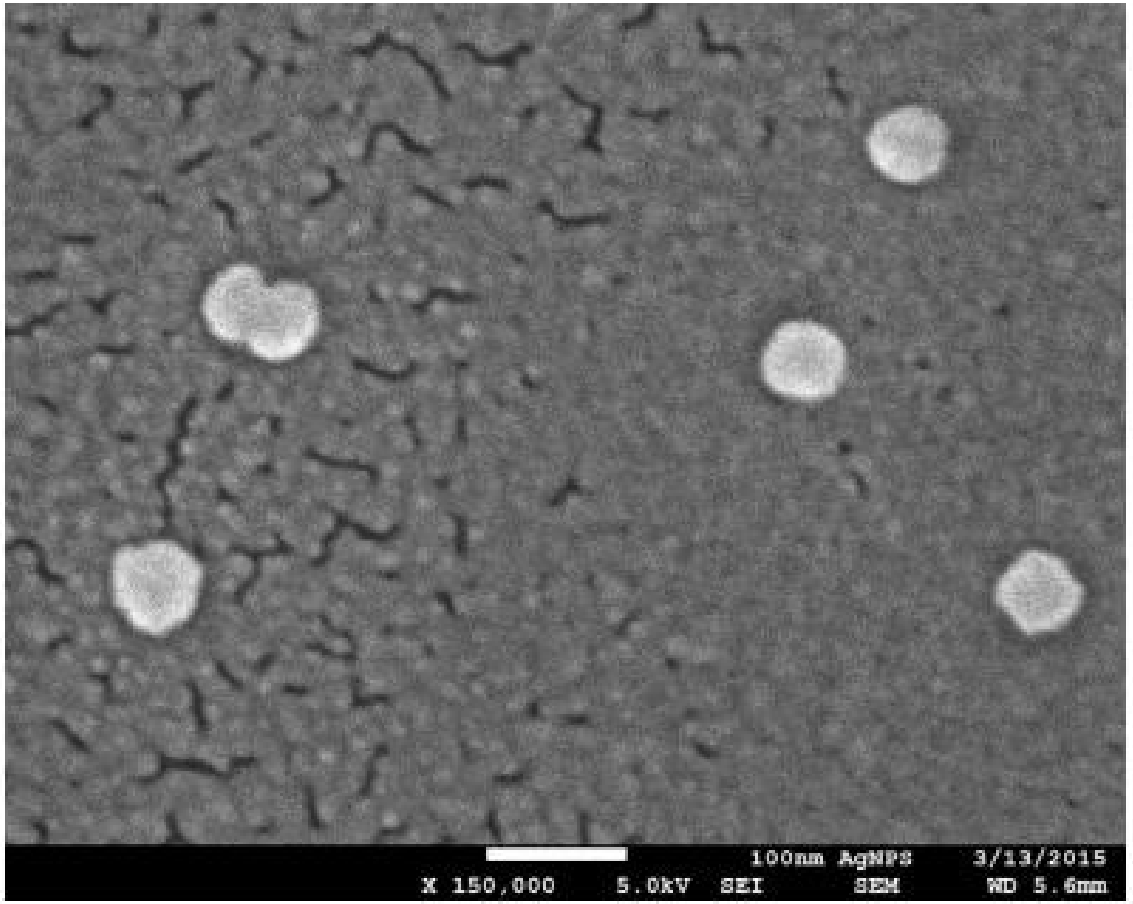


Fig. 2 Scanning electron microscope (SEM) image of tested silver nanoparticles. Scale (100 nm) is shown in the bottom of the picture

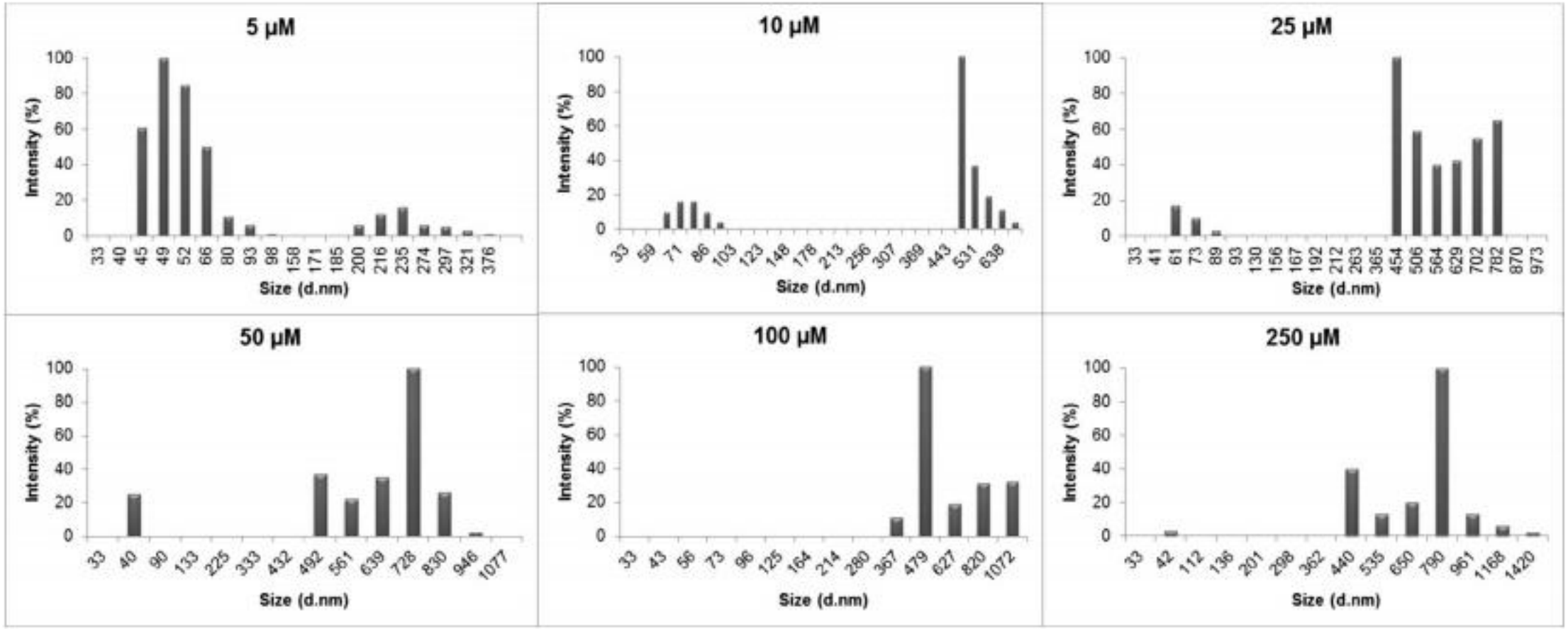


Fig. 3 Aggregate size distribution measured by DLS after mixing with the test media at different AgNP concentration. The original colloid (50-nm average size of particles) was maintained for 6 h in 75 % v/v medium 203 (prepared according to OECD 203 guideline). The values in the upper part of the figure represent the concentration of AgNPs

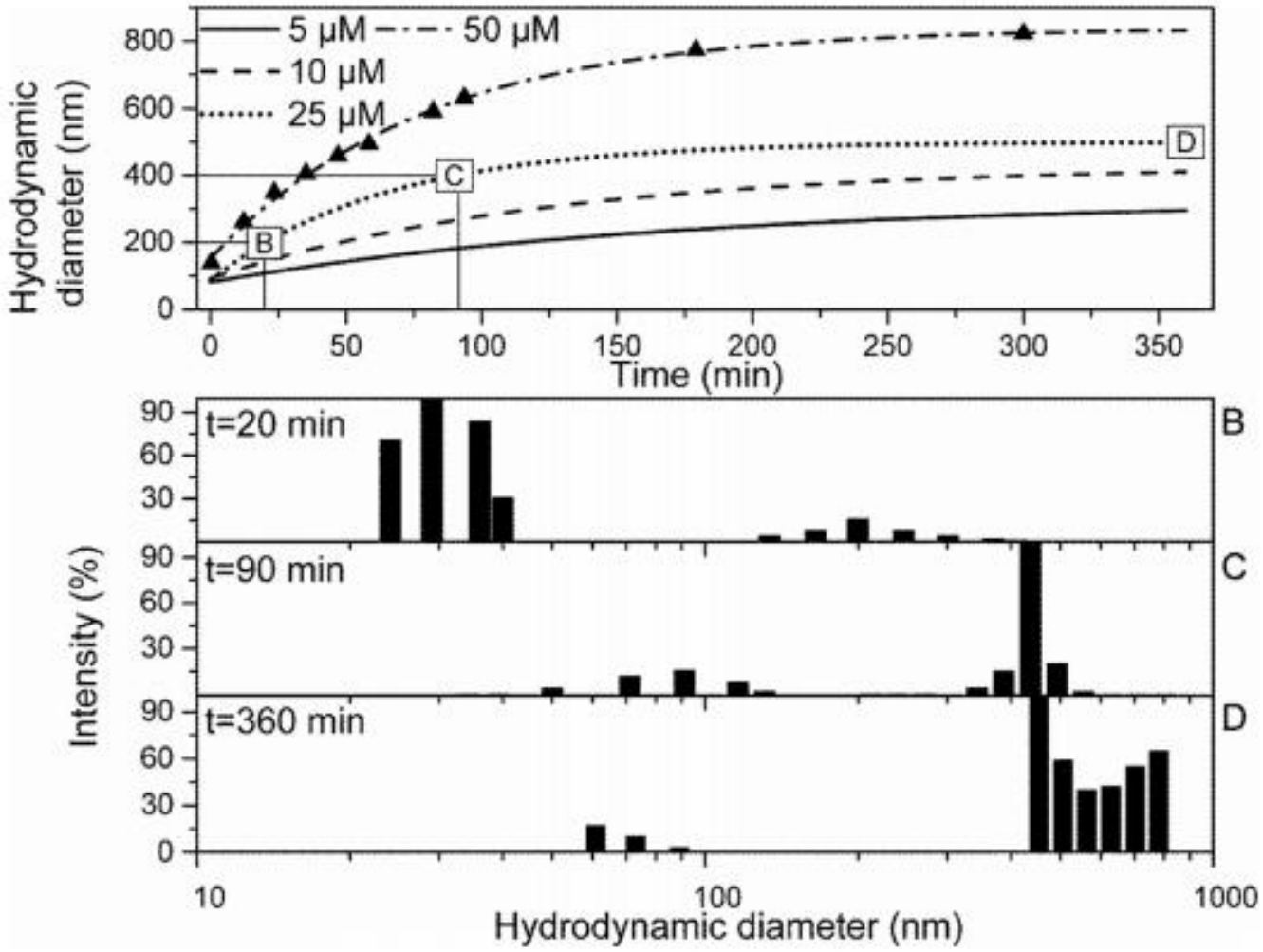


Fig. 4 Agglomeration behavior of AgNPs measured in time. The *upper panel* shows measured points in time for concentration ranges 5–50 μM. Measured points in time are presented like *triangles*. Kinetics of aggregate formation calculated according Eq. (1) at concentrations 5, 10, 25, and 50 μM are shown as *curves*. *Lower panel* shows actually measured particle size distribution of 25 μM AgNP colloid after 20 (*graph B*—corresponding also to point *B* in the upper panel), 90 (*C*) and 360 min (*D*) of the primary colloid dilution in 75 % medium 203 for better illustration of agglomerate size growing in time

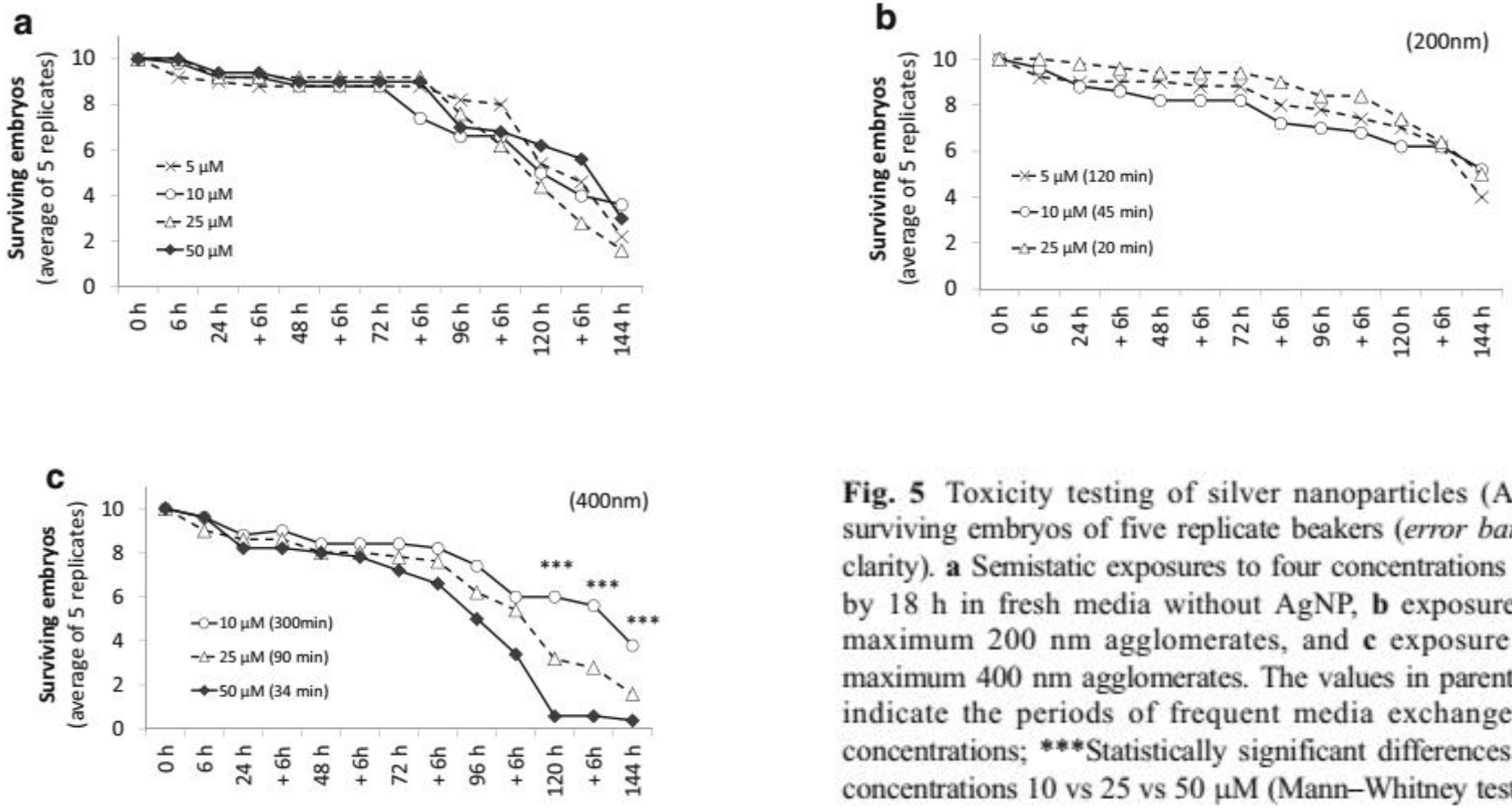


Fig. 5 Toxicity testing of silver nanoparticles (AgNPs): average surviving embryos of five replicate beakers (*error bars* not shown for clarity). **a** Semistatic exposures to four concentrations for 6 h followed by 18 h in fresh media without AgNP, **b** exposure controlling for maximum 200 nm agglomerates, and **c** exposure controlling for maximum 400 nm agglomerates. The values in parentheses in **b** and **c** indicate the periods of frequent media exchanges for the given concentrations; ***Statistically significant differences among all three concentrations 10 vs 25 vs 50 µM (Mann–Whitney test, $p<0.05$)

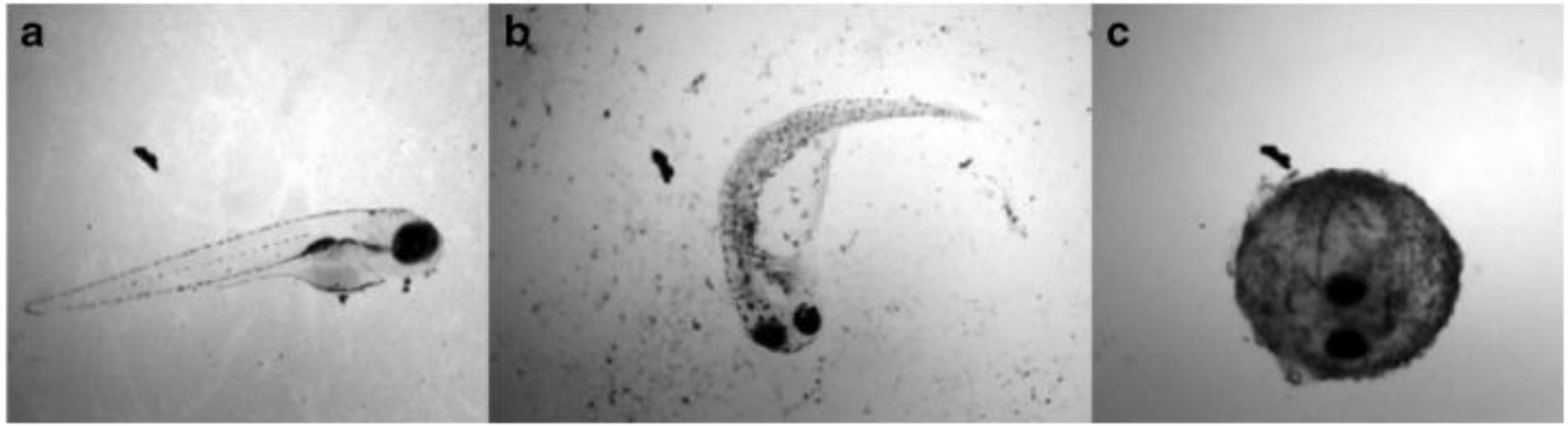


Fig. 6 Example of the influence of AgNPs on the development of carp embryos after 144 h of the experiments. **a** Control embryo, **b** embryo with apparent spine curvature and pericardial edema (exposure to media with AgNP concentration 5 μM) in the experiment controlling for 200 nm agglomerates), and **c** embryo with a chorion covered by AgNP agglomerates (exposure to 25 μM AgNP in the experiment controlling for 400 nm agglomerates)

Fate of silver nanoparticles in wastewater and immunotoxic effects on rainbow trout

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Aquatic Toxicology 174 (2016) 70–81

Table 1
Physical characteristics and concentration of total organic carbon (TOC) and dissolved organic carbon (DOC) in the control water utilized for experiments exposure test. The samples were non-exposed to silver.

Control water	TOC (mg/L)	DOC (mg/L)	pH	Conductivity (μS/cm)
Tap water	2.33	2.03	7.45	290
Wastewater	30.3	21.4	7.7	683
10% effluent, T = 0H	7.7	3.44	7.55	273
10% effluent, T = 96H	10.3	8.4	8.45	340

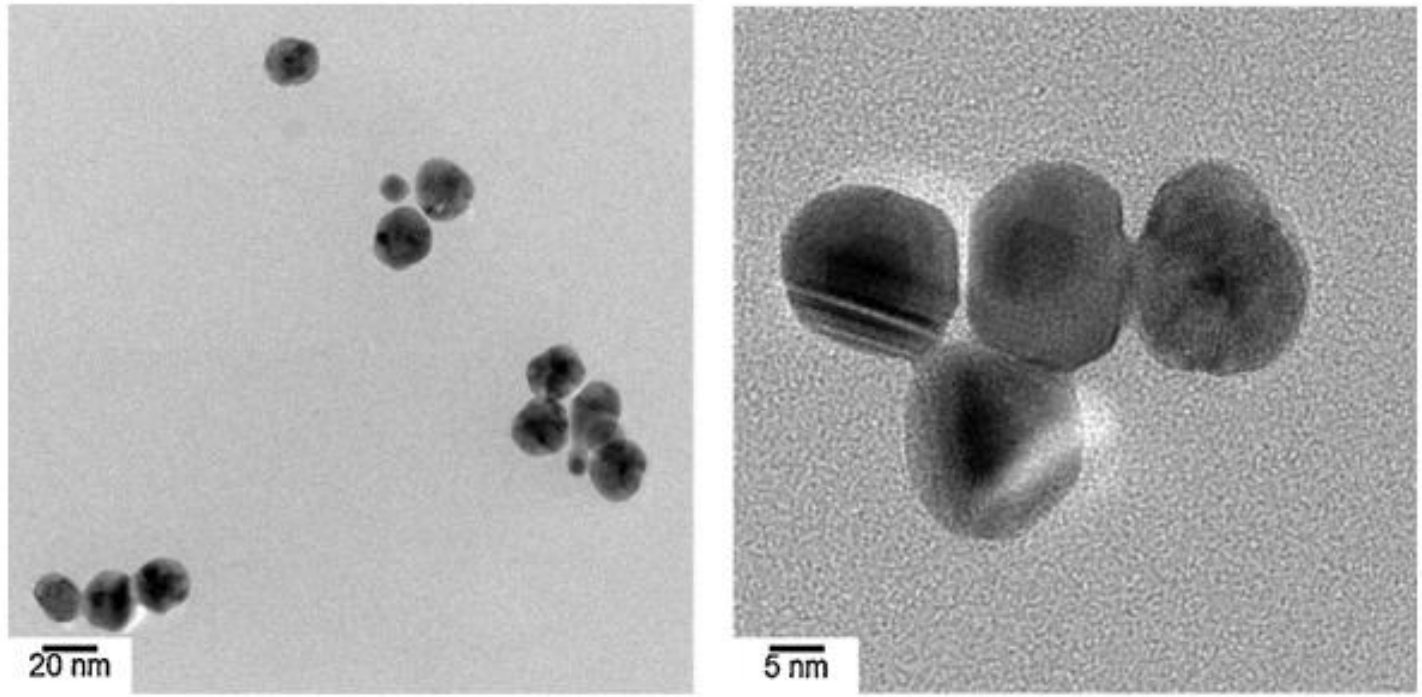

Fig. 1. Transmission electron microscope (TEM) of AgNPs in Ted Pella stock solution. All the nanoparticles were individually separated and present a spherical shape. The scale bars indicate 20 and 5 nm.

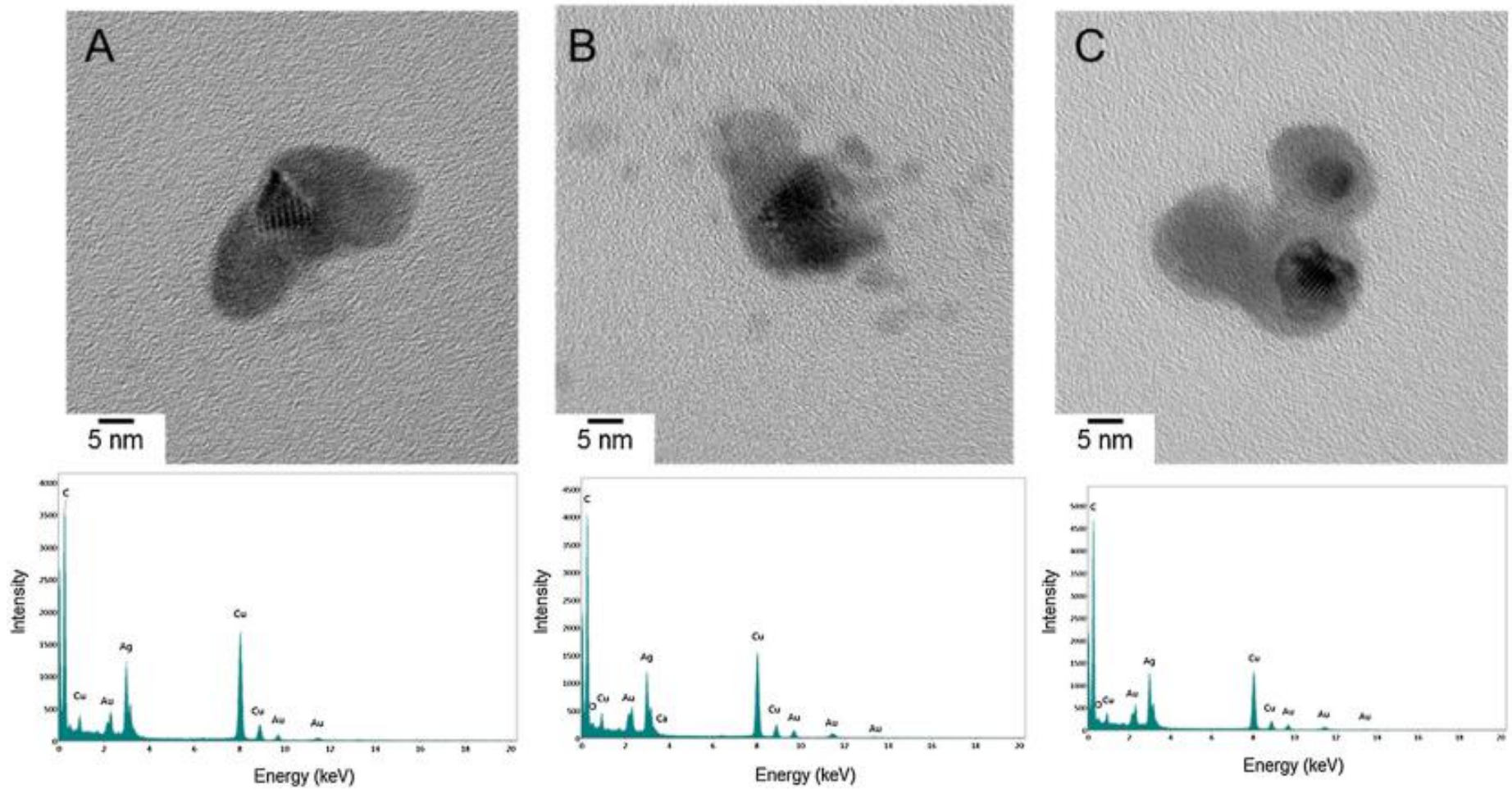

Fig. 2. Silver morphology in wastewater after 96 h exposure and elemental composition analysis. Ag nanoparticles were retrieved in small aggregates. The energy dispersive spectra (EDS) elemental analysis data are presented below for each images confirm that each image show silver nanoparticles. The scale bars indicate 5 nm.

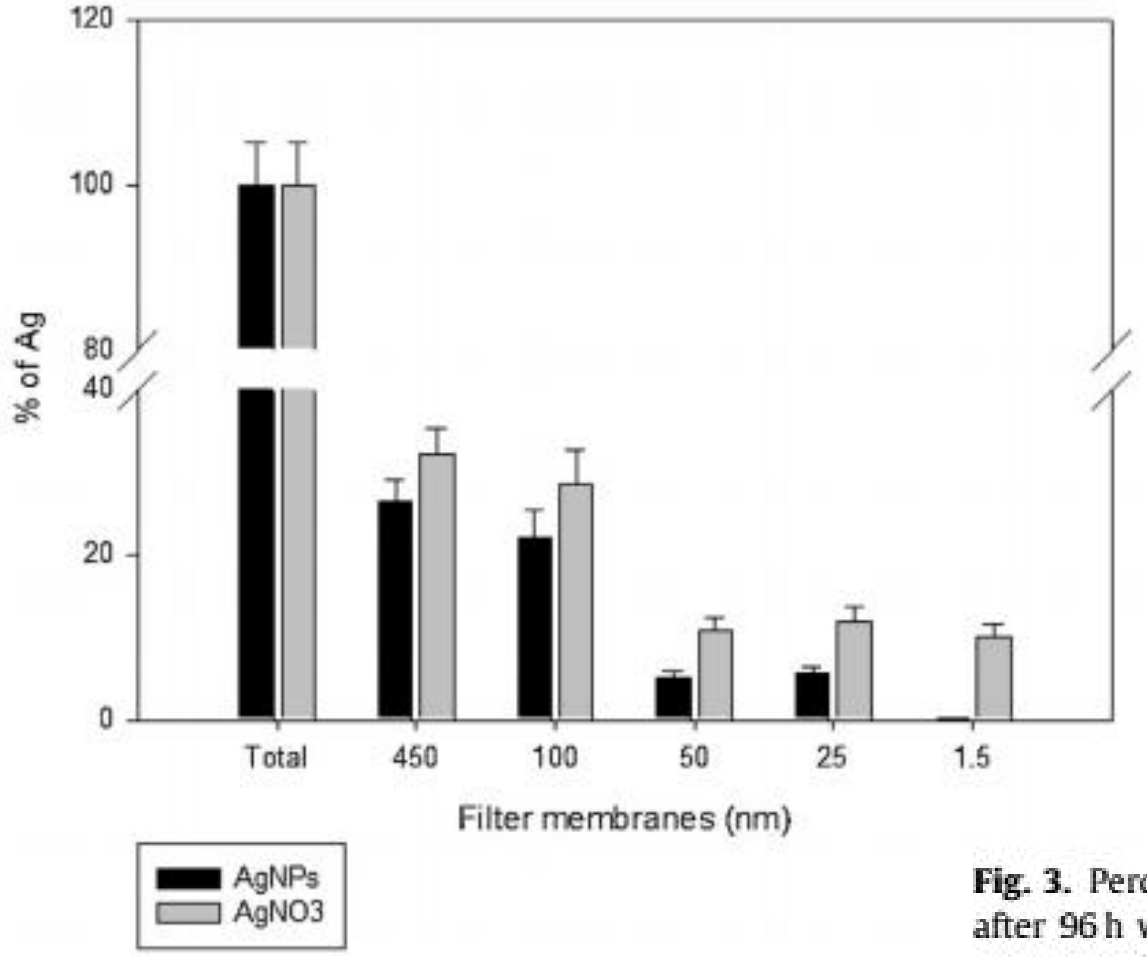

Fig. 3. Percentage of Ag in water after 96 h exposure. The total Ag concentration after 96 h were 25.7 μg/L and 3.75 μg/L for AgNPs and $AgNO_3$ respectively. Total corresponds to the non-filtered fraction, 450 nm, 100 nm, 50 nm, and 25 nm stands for filtered fractions; 1 kDa corresponds to the truly dissolved fraction (Ag^+).

Table 2
Mean size and zeta potential of AgNPs in stock solution and 10% effluent. These results were observed with a DLS after filtration through a membrane of 0.45 μm in order to eliminate large aggregates in the solution and measure the mean diameter size of the particles.

Samples	Mean diameter size (nm)	Zeta potential (mV)
Stock solution	19.2 ± 0.3	−58.02 ± 4.21
10% effluent	11.7 ± 2.1	0.00 ± 0.00

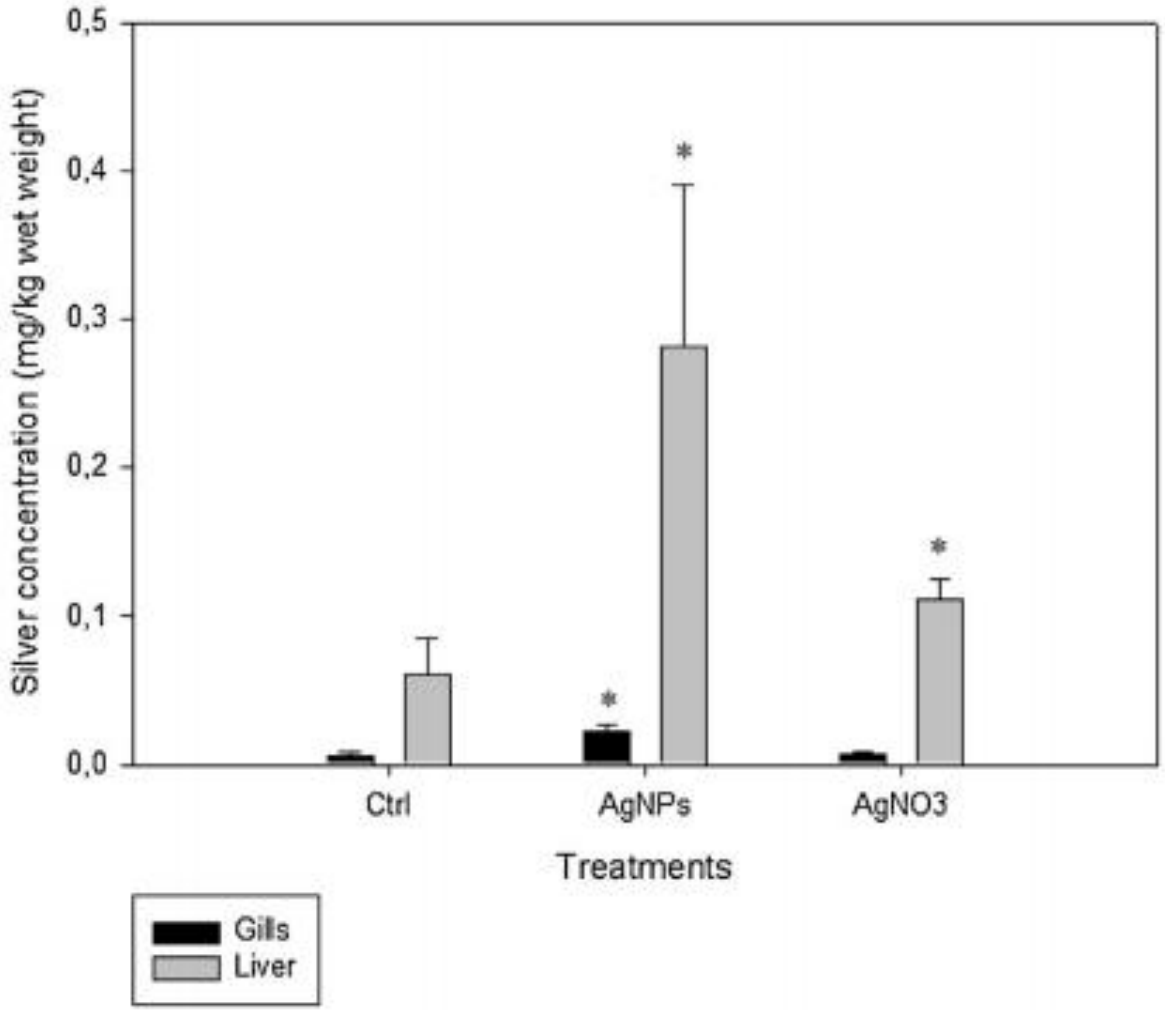

Fig. 4. Ag concentrations (mg/kg wet weight) in gills and livers of rainbow trout exposed 96 h to AgNPs and $AgNO_3$. Fish were initially exposed to 40 μg/L of AgNPs and 4 μg/L of $AgNO_3$. Error bars correspond to standard error. Stars indicate significant differences between silver treatments and control ($p < 0.05$).

Table 3
Pearson correlations calculated between 9 biomarkers (COX = cyclooxygenase, GST = glutathione S-transferase, SOD = superoxide dismutase, DNA = DNA strand breaks, lab Zn = labile zinc, LPO = lipid peroxidation, MT = metallothioneins, Phago activity = immunoactivity, Phago efficiency = immunoefficiency) and Ag bioaccumulation data (Bio liver = bioaccumulation in liver and Bio gills = bioaccumulation in gills) from all the data of AgNPs and $AgNO_3$.

	COX liver	GST liver	SOD liver	DNA liver	Lab Zn liver	LPO liver	Bio liver	MT liver	GST gills	SOD gills	DNA gills	Lab Zn gills	LPO gills	Bio gills	Phago activity	Phago efficiency
COX liver	1.00															
GST liver	0.36	1.00														
SOD liver	0.07	0.28	1.00													
DNA liver	−0.35	−0.17	−0.10	1.00												
Lab Zn liver	−0.41*	0.34	0.14	0.02	1.00											
LPO liver	0.12	0.28	−0.11	−0.46*	0.12	1.00										
Bio liver	0.55*	−0.07	0.09	0.01	−0.35	−0.25	1.00									
MT liver	0.41*	0.81*	0.26	−0.20	0.25	0.18	0.03	1.00								
GST gills	0.44*	0.16	−0.05	0.33	−0.09	−0.46*	0.54*	0.01	1.00							
SOD gills	−0.14	−0.17	0.28	0.25	0.11	−0.51*	0.55*	−0.15	0.46*	1.00						
DNA gills	0.32	0.42*	−0.10	0.04	0.32	−0.19	0.32	0.28	0.77*	0.34	1.00					
Lab Zn gills	0.41*	−0.01	0.19	−0.11	−0.23	−0.13	0.47*	0.05	0.29	0.32	0.02	1.00				
LPO gills	0.30	−0.15	−0.11	−0.26	−0.53*	0.16	−0.14	−0.25	−0.07	−0.48*	−0.20	0.14	1.00			
Bio gills	0.31	−0.20	0.14	−0.08	−0.36	−0.16	0.86*	−0.05	0.18	0.52*	0.00	0.40*	−0.19	1.00		
Phago activity	−0.43*	−0.28	−0.19	0.26	0.31	−0.26	0.15	−0.28	−0.02	0.30	0.08	−0.23	−0.52*	0.23	1.00	
Phago efficiency	−0.44*	−0.17	−0.06	0.39*	0.36	−0.33	−0.05	−0.30	0.08	0.25	0.10	−0.21	−0.42*	−0.06	0.89*	1.00

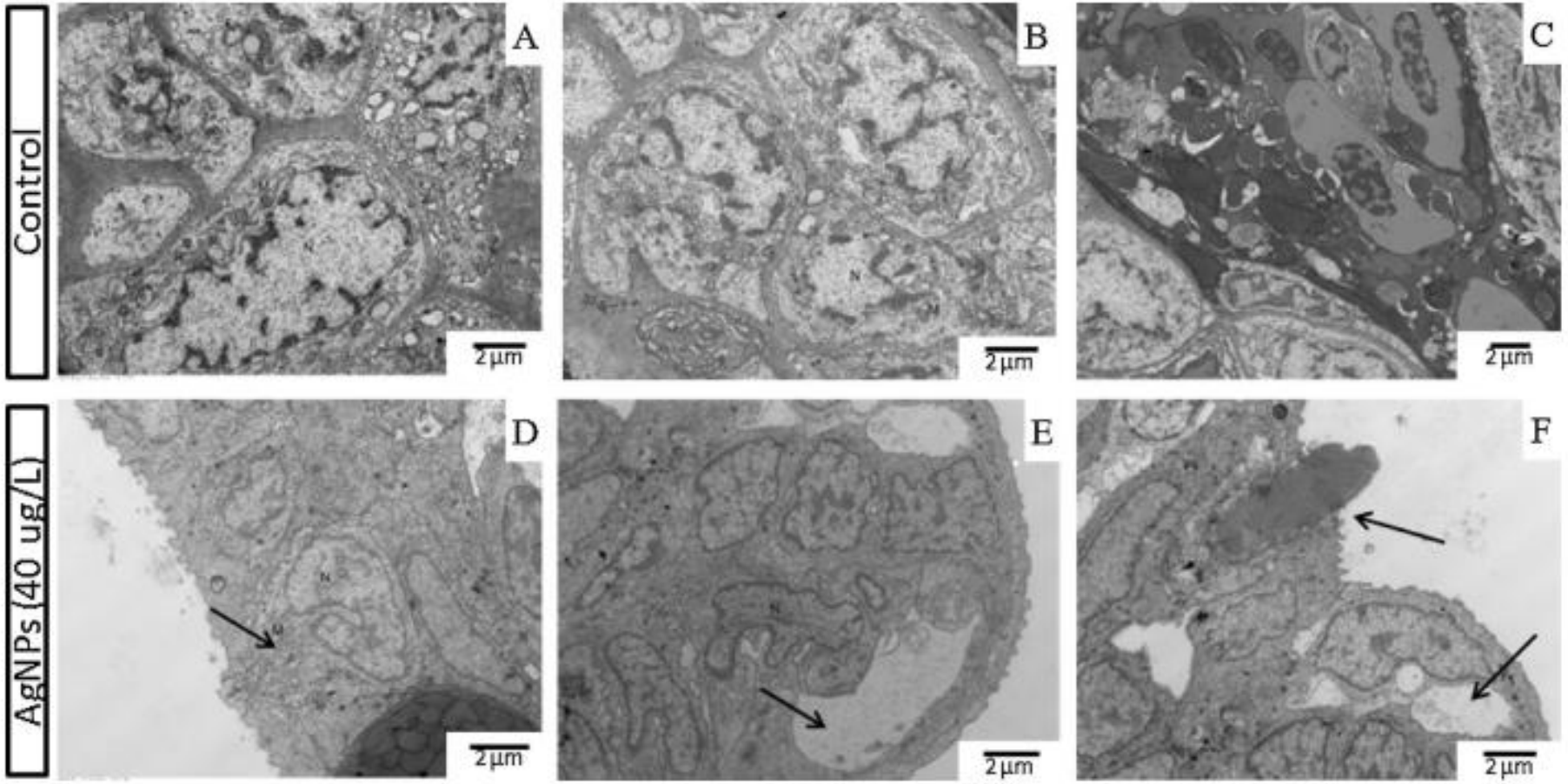

Fig. 5. TEM imagery of gills of non-exposed (A–C) and exposed fish (D–F) to AgNPs for 96 h. M = mitochondria, N = nucleus. Arrows indicated morphological gills modifications. No significant accumulation of AgNPs was observed in gills, but structural changes were observed, such as: mitochondria number increase (D), nucleus invagination (E) and compaction of the reticulum (E) and vacuoles (E and F). All the pictures were made with ×2900 magnification and 120 KeV. The scale bars indicate 2 µm.

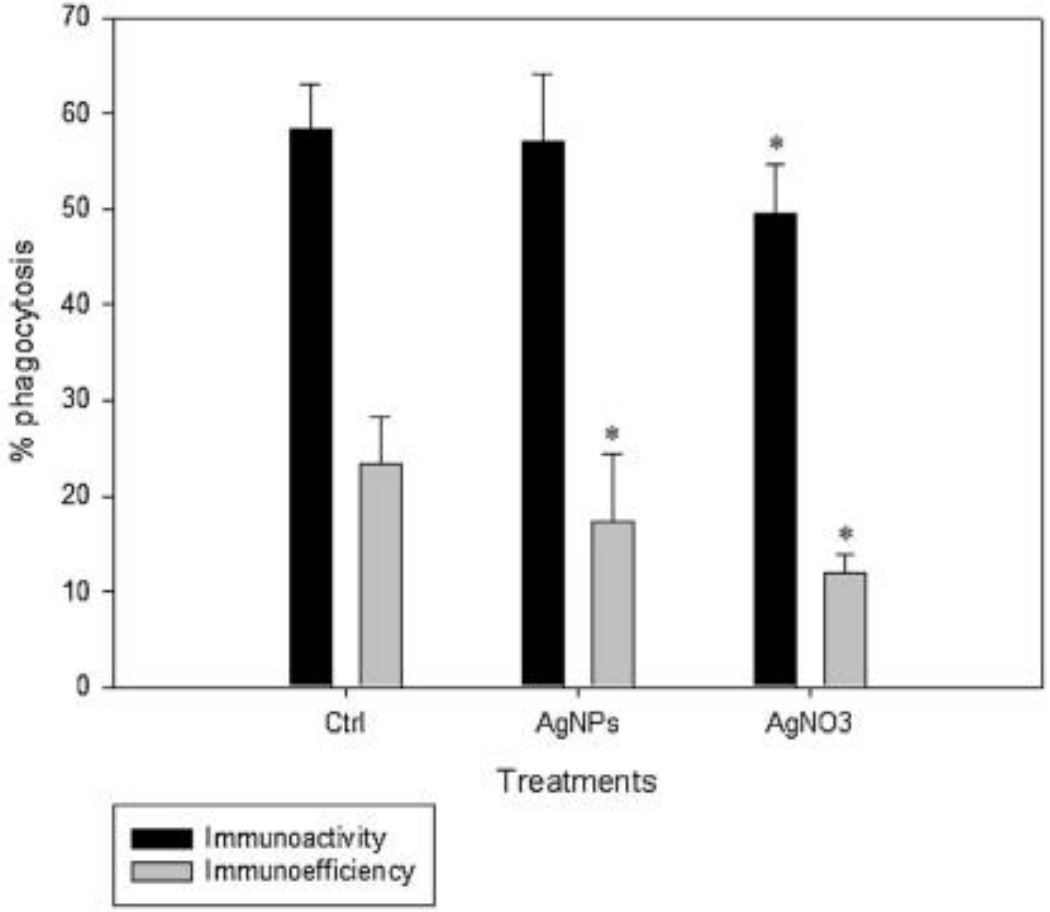

Fig. 6. Immunoactivity (phagocytosis of 1 bead and more) and immunoefficiency (phagocytosis of 3 beads and more) of rainbow trout exposed to AgNPs and $AgNO_3$ for 96 h. Error bars correspond to standard error. Stars indicate significant differences between silver treatments and control ($p < 0.05$).

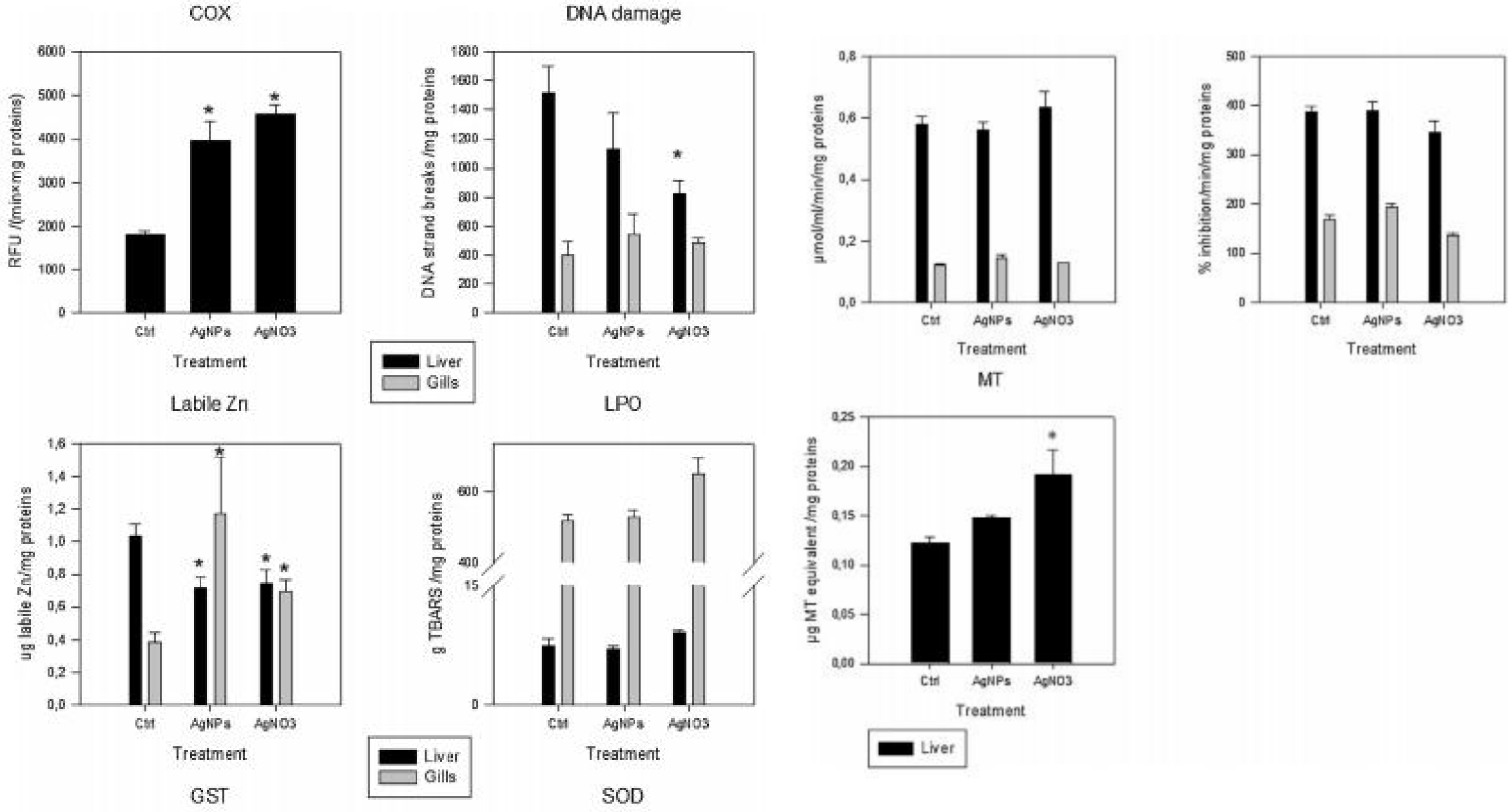

Fig. 7. Biomarkers in gills and liver of fish exposed to AgNPs and $AgNO_3$ for 96 h. COX = cyclooxygenase activity, DNA damage, Labile Zn = labile linc, LPO = lipid peroxidation, GST = glutathione S-transferase activity, SOD = superoxide dismutase, and MT = metallothionein level. Error bars correspond to standard error, stars indicate significant differences between silver treatments and control ($p < 0.05$).

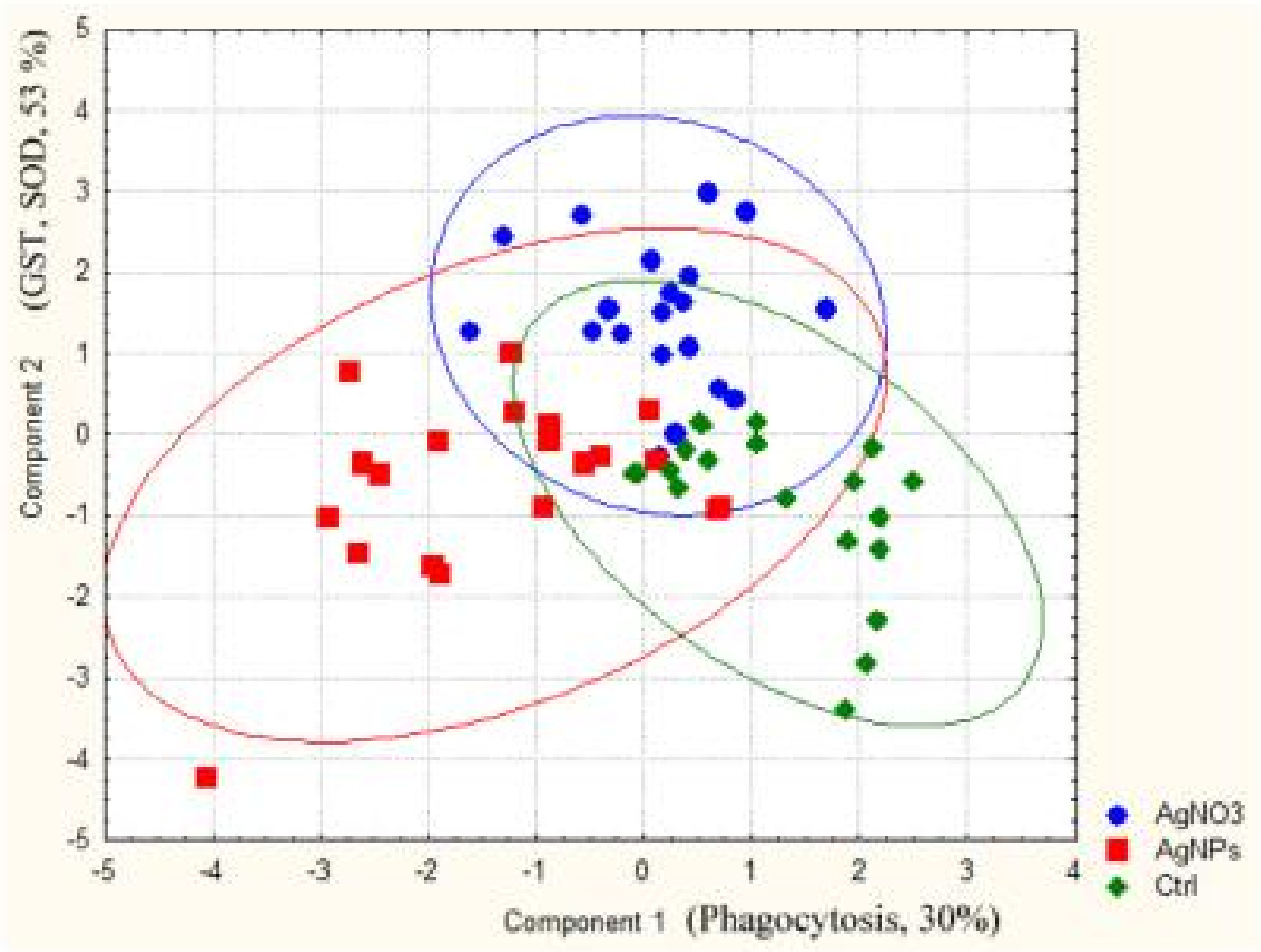

Fig. 8. Discriminant analysis between all the results and treatments. No association between treatments was observed. Phagocytic activity and efficiency were the main biomarkers that discriminated the biochemical response of exposed from non-exposed fish and explained 30% of the data variance (component 1). Glutathione S-transferase (GST) and superoxide dismutase (SOD) were the main biomarkers that discriminated the $AgNO_3$ from AgNPs treatments and explain a cumulated percentage of 53% of variance (component 2). Ellipses correspond to the confidence interval around all the data for all the treatment. Confidence intervals are fixed at 95%. According to the figure, there is some degree of similarity of Ag and AgNPs. AgNPs differs from $AgNO_3$ from the *x* axis i.e., phagocytosis.

Oxidative stress and immunotoxicity induced by graphene oxide in zebrafish

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Aquatic Toxicology 174 (2016) 54–60

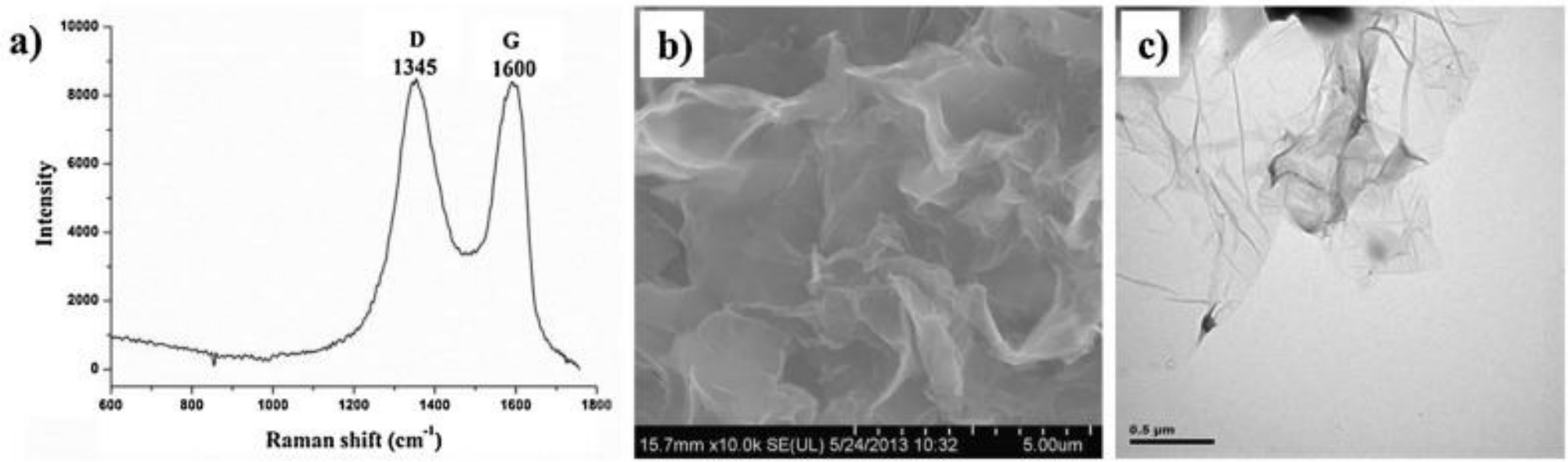


Fig. 1. Characterization of GO: Raman spectra (a), SEM image (b), and TEM images (c).

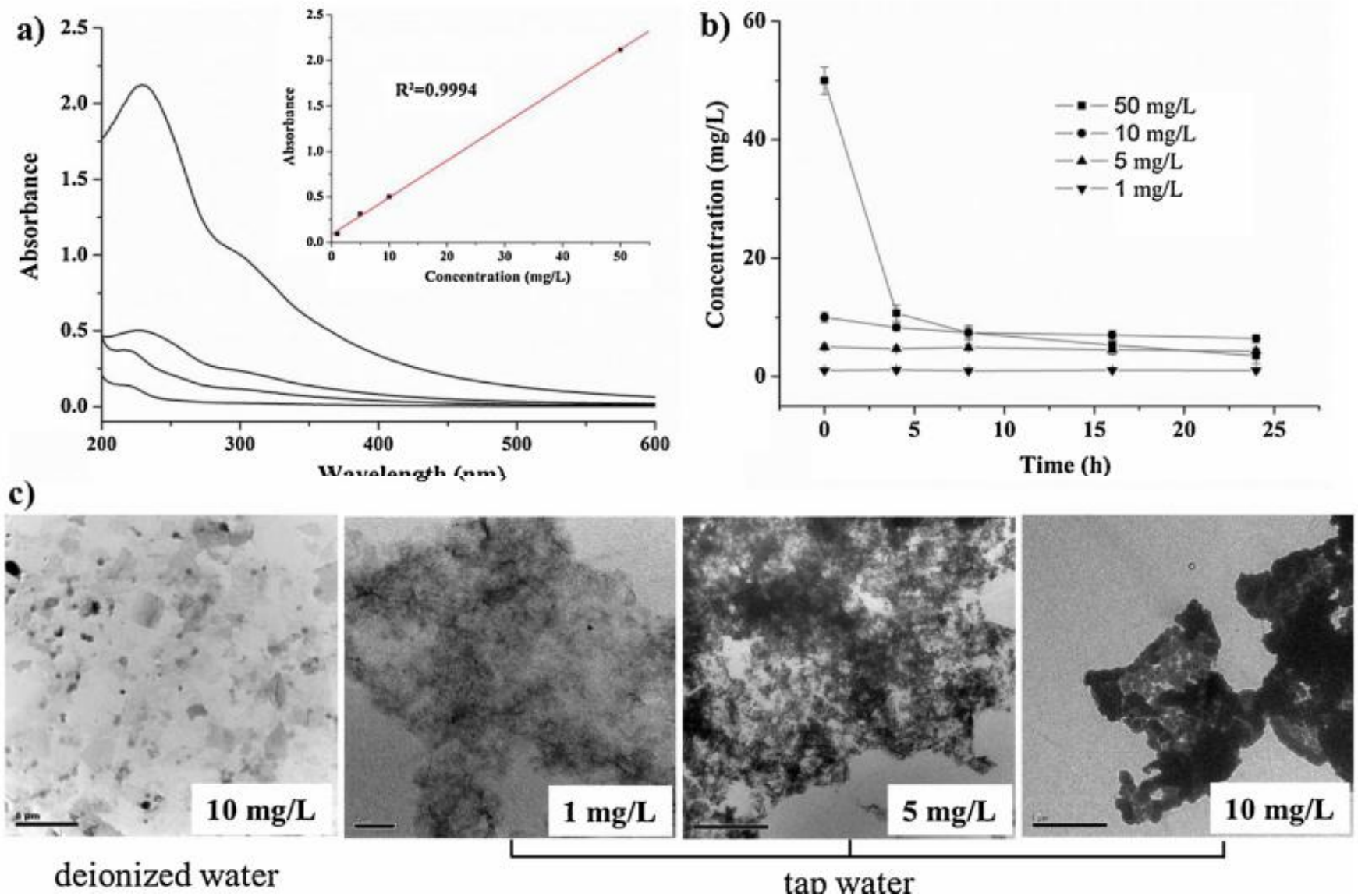
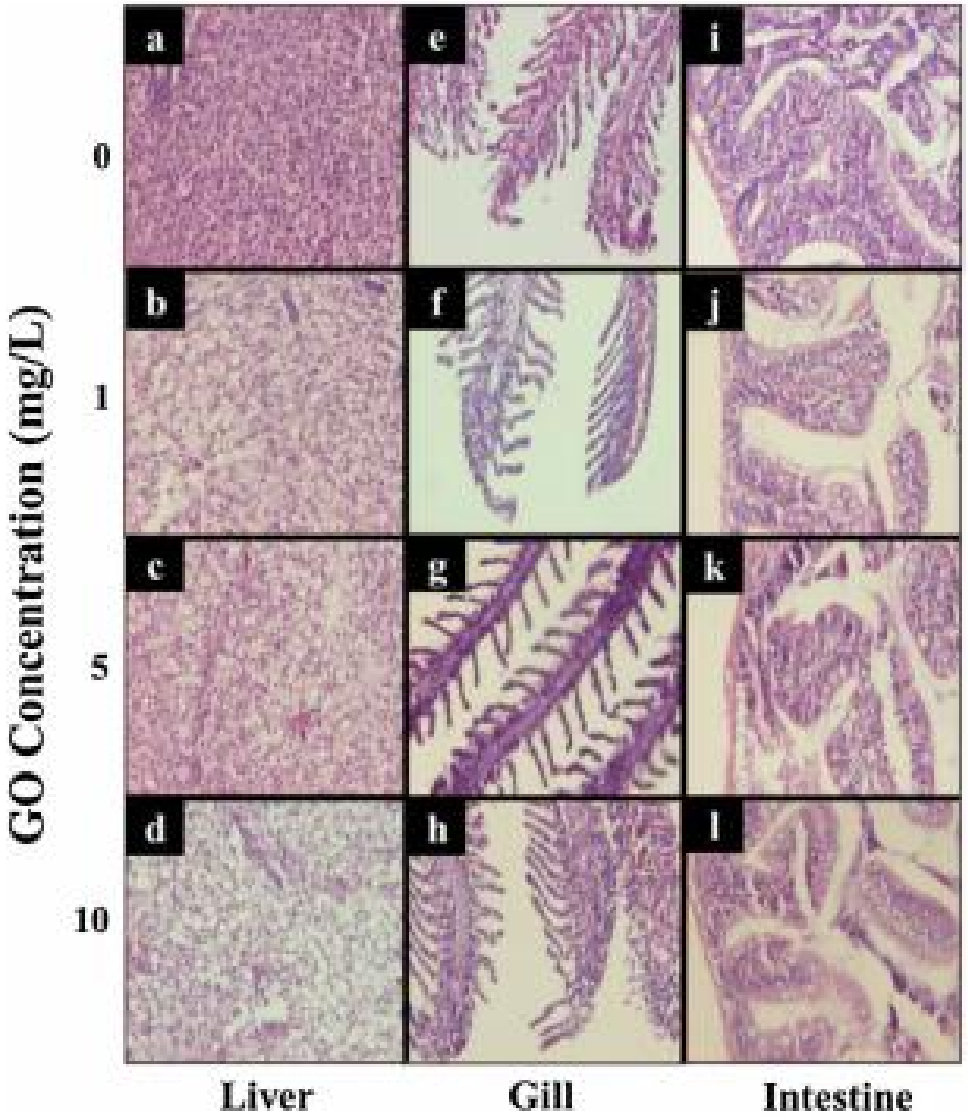


Fig. 2. (a) UV–vis spectra of GO aqueous dispersions at 1, 5, 10 and 50 mg/L (bottom to top) and the linear relationship between absorbance and concentration (insert figure). (b) The residual concentrations of GO in water during exposure. (c) TEM images of GO in deionized water and tap water. All suspensions were laid at room temperature for 24 h, and were diluted to 1 mg/L for TEM image.



	GO (mg/L)	Va	LA	Di
Liver	0	–	–	–
	1	+	–	–
	5	++	+	+
	10	+++	++	++
Gill	0	–	–	–
	1	–	–	–
	5	–	–	–
	10	–	–	–
Intestine	0	–	–	–
	1	+	–	+
	5	++	+	++
	10	+++	++	++

Va: vacuolation
LA: loose arrangement of cells
Di: disintegration of cell boundaries

Fig. 3. Light micrographs of liver, gill, and intestinal tissue samples from control and GO treated zebrafish. The degree of organ damage including vacuolation, loose arrangement of cells, and disintegration of cell boundaries was expressed by the number of "+".

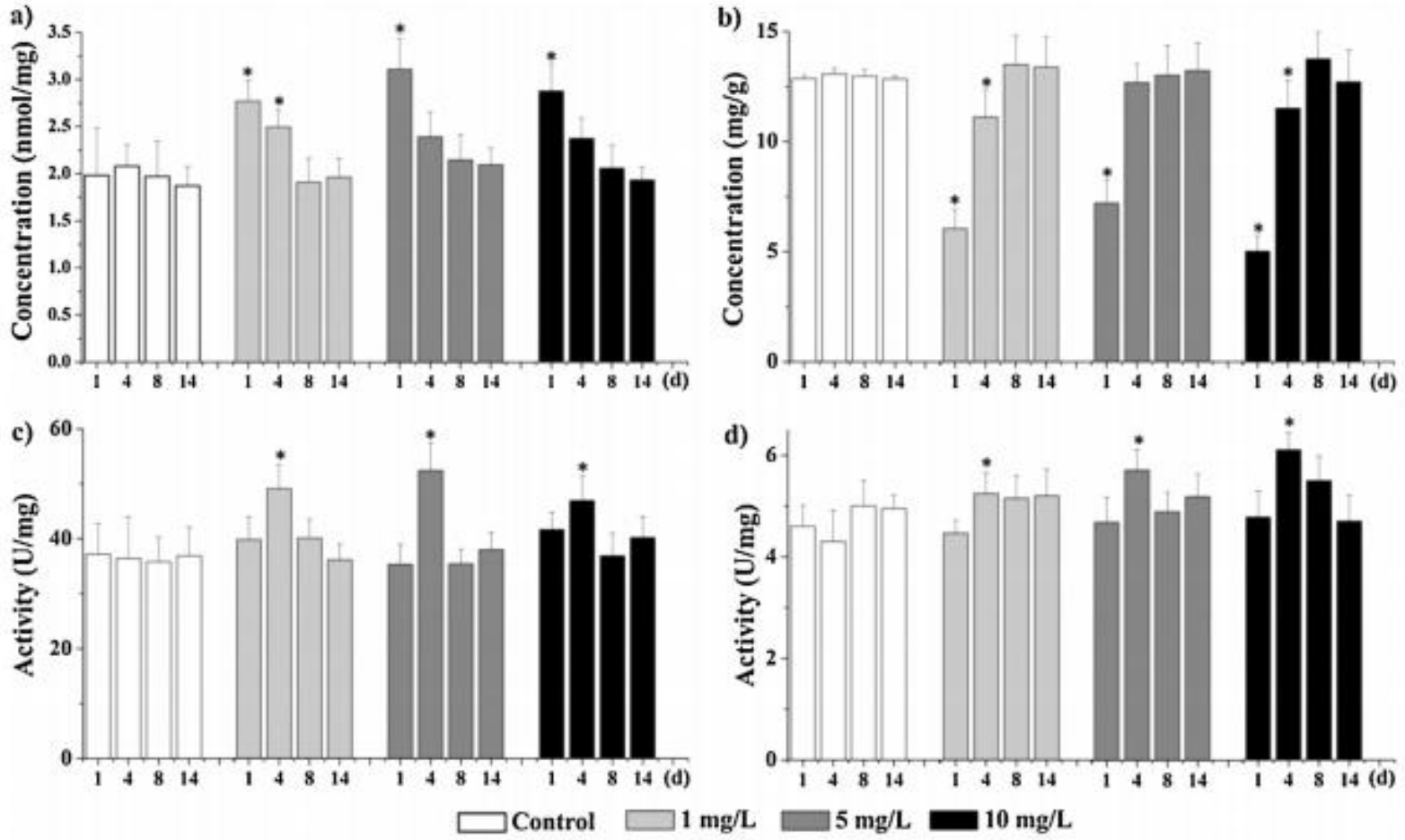


Fig. 4. Changes in concentrations of MDA (a) and GSH (b) and activities of SOD (c) and CAT (d) in liver tissue from GO treated zebrafish. Values are means ± SD ($n = 4$). Statistically significant differences from the corresponding exposure day control values are indicated by *, $p < 0.05$.

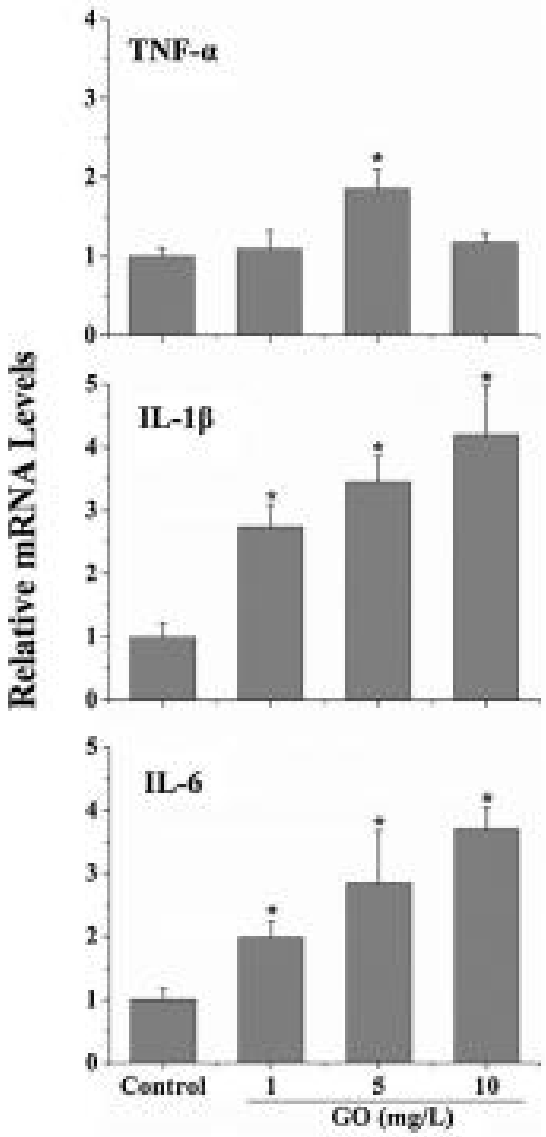


Fig. 5. mRNA expression levels of TNF-α, IL-1β and IL-6 in spleens from zebrafish after 14 days exposure to GO (1, 5 and 10 mg/L). Values are means ± SD ($n = 4$). Statistically significant differences from the control group are indicated by *, $p < 0.05$.

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