

## Electronic Supplementary File

Influence of humic acid and UV-irradiation on iron-based nanoparticles toxicity in *Girardia tigrina*.

Natasha Yadav<sup>a,b</sup>, Anurag Nath<sup>a,b</sup>, Pushplata Prasad Singh<sup>a,b</sup>, Himadri B. Bohidar<sup>a</sup>, Damien L. Callahan<sup>c</sup>, Antoine M. Dujon<sup>b,d</sup>, Luis O.B. Afonso<sup>b</sup>, and Aaron G. Schultz<sup>b\*</sup>

<sup>a</sup>*National Centre of Excellence for Advanced Research in Agricultural Nanotechnology, TERI - Deakin Nanobiotechnology Centre, Sustainable Agriculture Division, The Energy and Resources Institute (TERI), DS Block, India Habitat Centre, Lodhi Road, New Delhi, 110003, India*

<sup>b</sup>*School of Life and Environmental Sciences, Deakin University, Geelong, Victoria, 3217, Australia.*

<sup>c</sup>*School of Life and Environmental Sciences, Deakin University, Burwood, Victoria, 3125, Australia.*

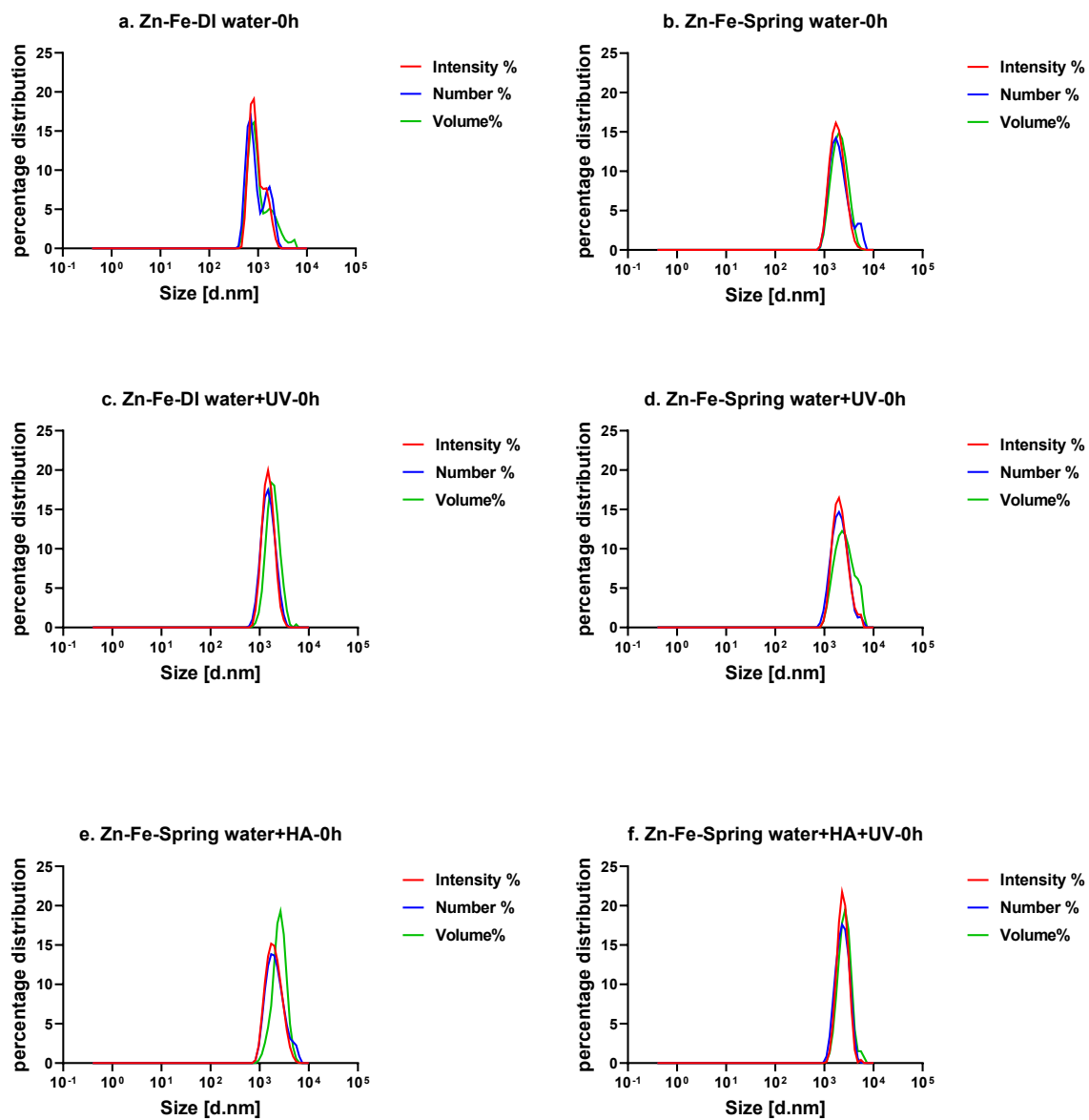
<sup>d</sup>*CREEC/(CREES), MIVEGEC, Unité Mixte de Recherches, IRD 224–CNRS 5290–Université de Montpellier, Montpellier, France*

\* **Corresponding author:** aaron.schultz@deakin.edu.au

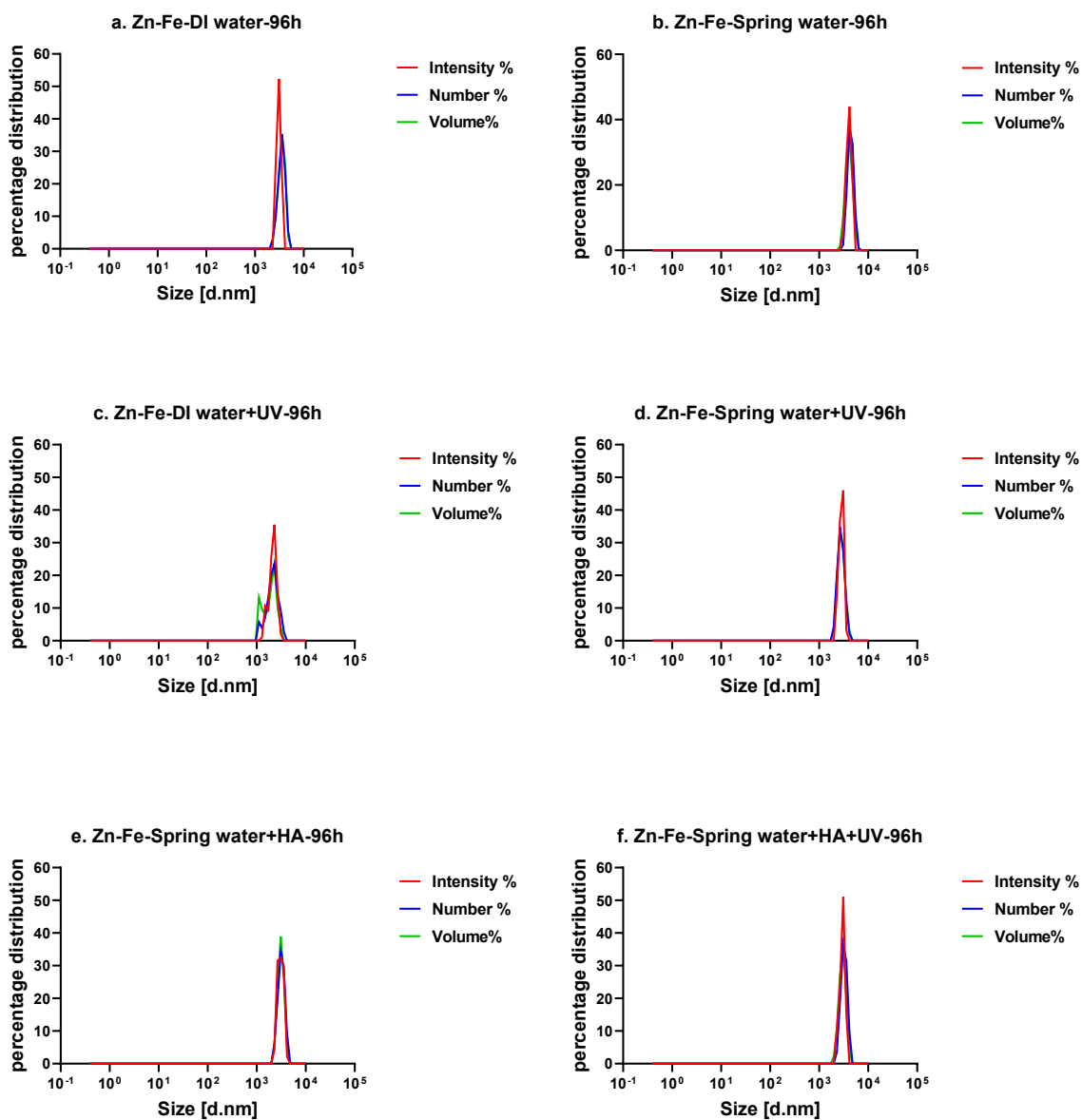
**TABLE S1** Physical characteristics of pristine NPs suspended in DI water and used in this study.

<b>Sample ID</b>	<b>Synthesis Method</b>	<b><math>\lambda_{max}</math> (nm)</b>	<b>Hydrodynamic Size (nm)</b>	<b>Zeta potential (mV)</b>	<b>PDI</b>	<b>TEM size (nm)</b>	<b>Shape</b>
1.Nano Fe-Zn	Biogenic	400-500	1704±94	-19± 1	0.8	>20	Quasi-spherical
2.BS_IO_MNP	Biogenic	309	288±30	-19± 1	0.2	15±5	Spherical
3.Sig_IO_MNP	Chemical	330	623 ±30	-16± 1	0.9	104± 15	Spherical

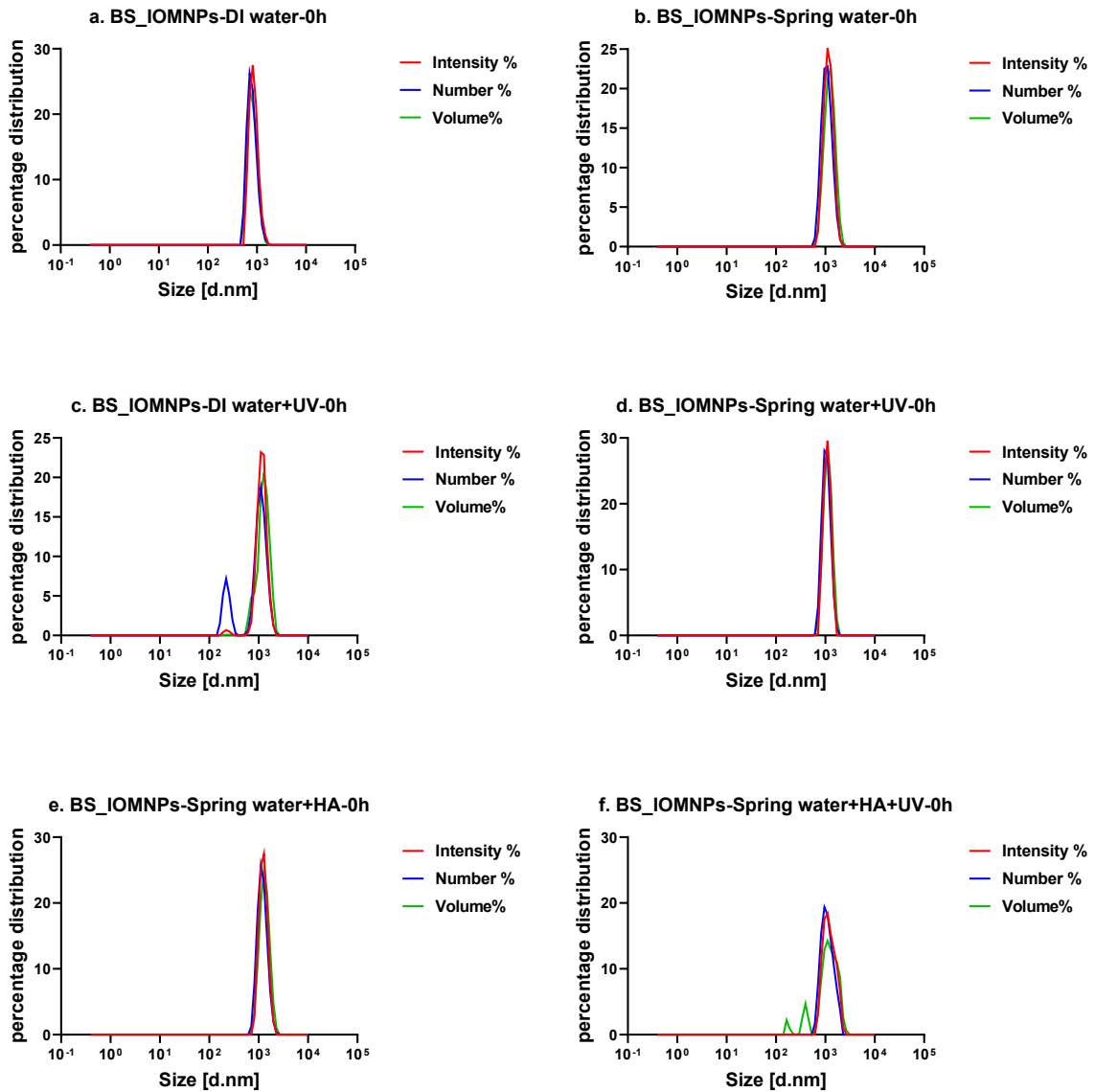
Nano Fe-Zn: iron zinc composite nanoparticles; BS\_IOMNPs: biologically synthesised iron oxide nanoparticles; Sig\_IOMNPs: chemically synthesised iron oxide nanoparticles.



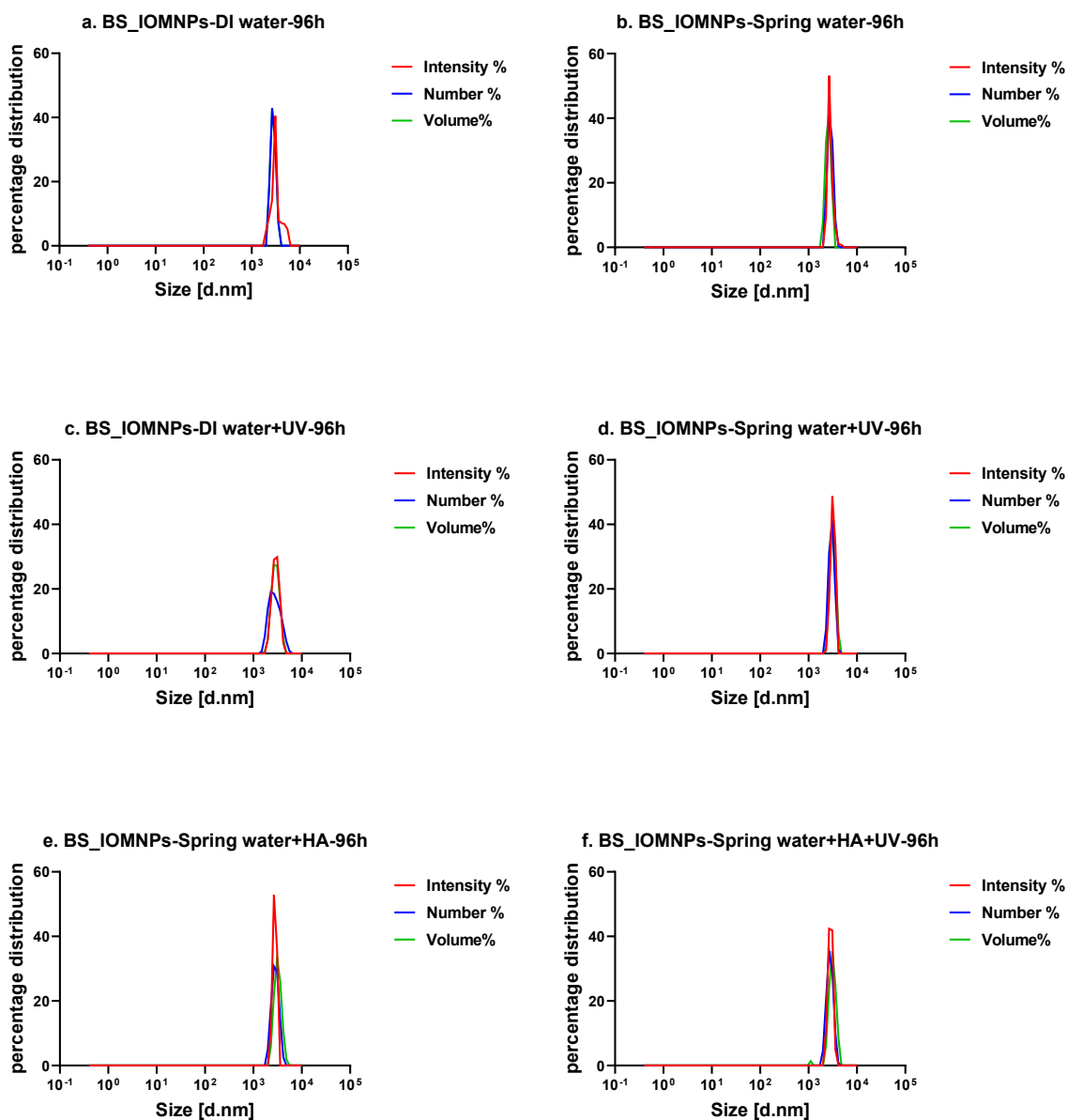
**Fig. S1** Particle size histograms for 0th-time point observations w.r.t size against intensity (red line graph), number (blue line graph) and volume (green line graph) for nano Zn-Fe in different test media for highest test concentration  $200 \mu\text{g.mL}^{-1}$  (a-f).



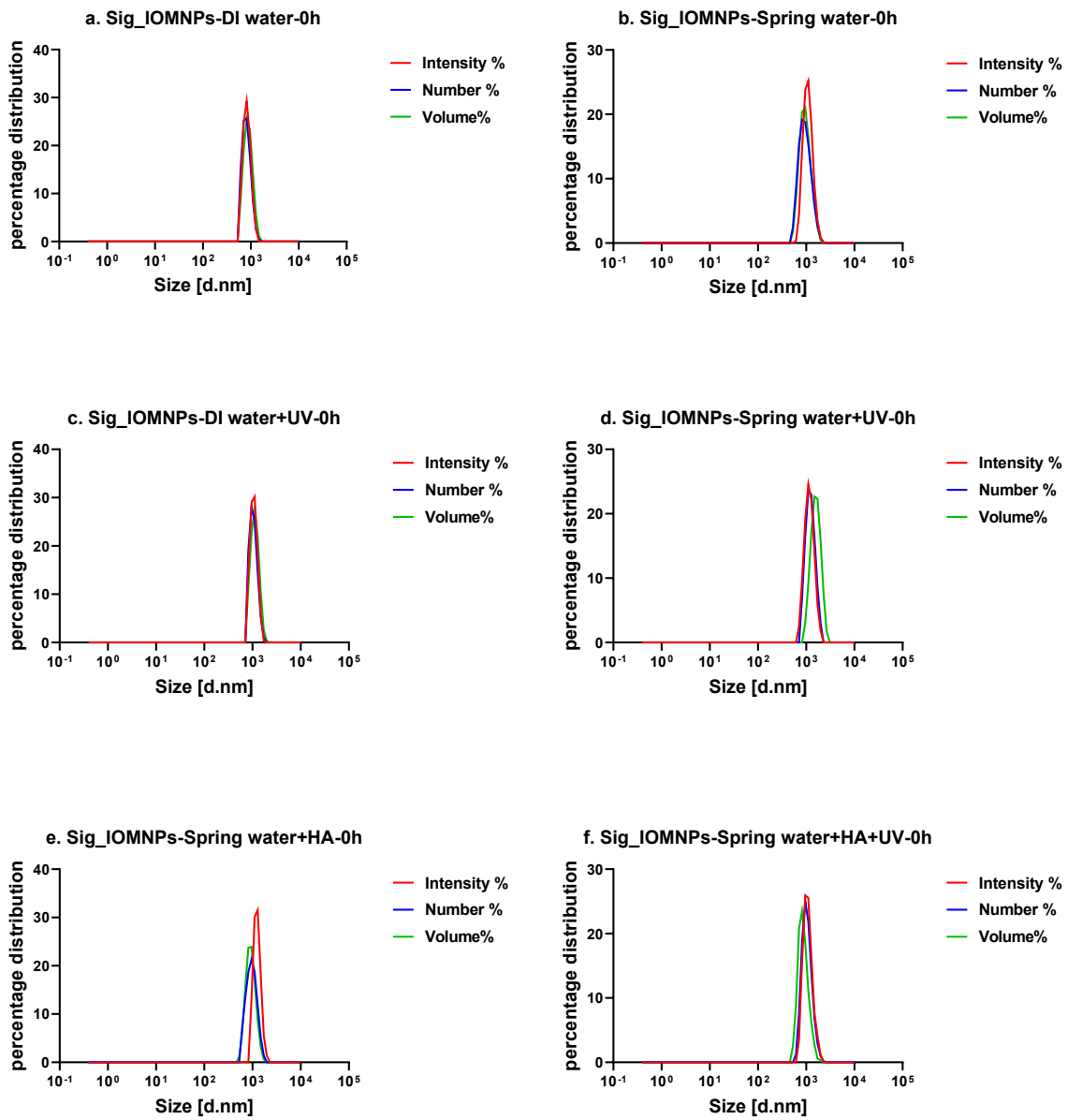
**Fig. S2** Particle size histograms for 96h-time point observations w.r.t size against intensity (red line graph), number (blue line graph) and volume (green line graph) for nano Zn-Fe in different test media for highest test concentration  $200 \mu\text{g.mL}^{-1}$  (a-f).



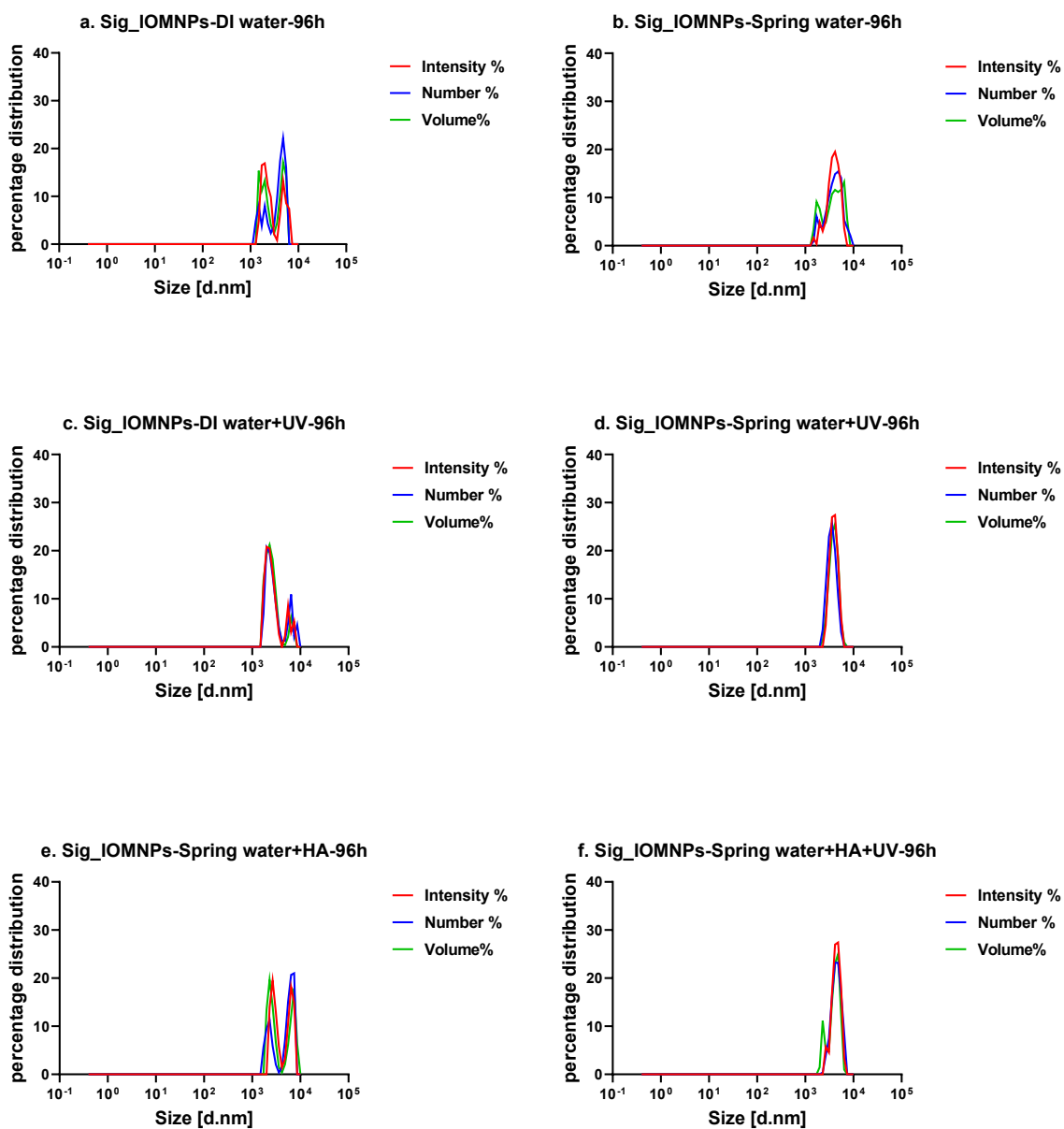
**Fig. S3** Particle size histograms for 0th-time point observations w.r.t size against intensity (red line graph), number (blue line graph) and volume (green line graph) for BS\_IOMNPs in different test media for highest test concentration 200  $\mu\text{g.mL}^{-1}$  (a-f).



**Fig. S4** Particle size histograms for 96h-time point observations w.r.t size against intensity (red line graph), number (blue line graph) and volume (green line graph) for BS\_IOMNPs in different test media for highest test concentration  $200 \mu\text{g.mL}^{-1}$  (a-f).

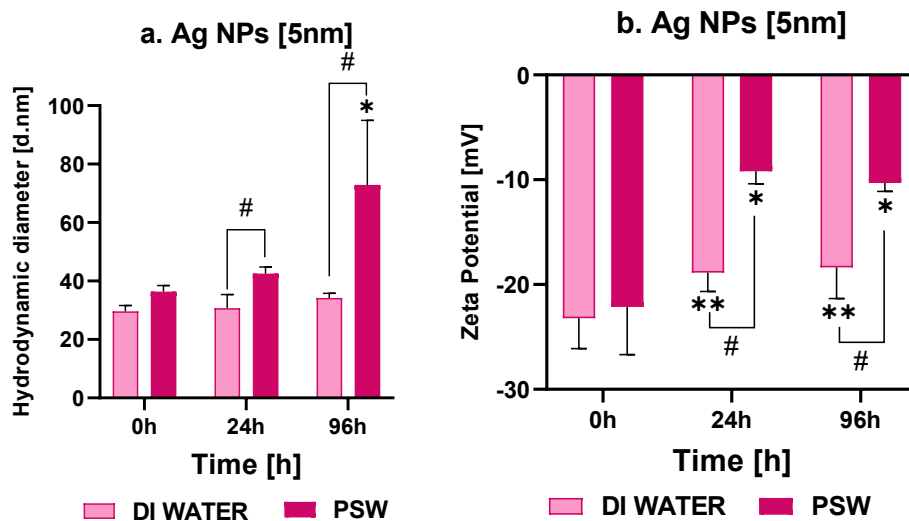


**Fig. S5** Particle size histograms for 0h-time point observations w.r.t size against intensity (red line graph), number (blue line graph) and volume (green line graph) for Sig\_IOMNPs in different test media for highest test concentration  $200 \mu\text{g.mL}^{-1}$  (a-f).

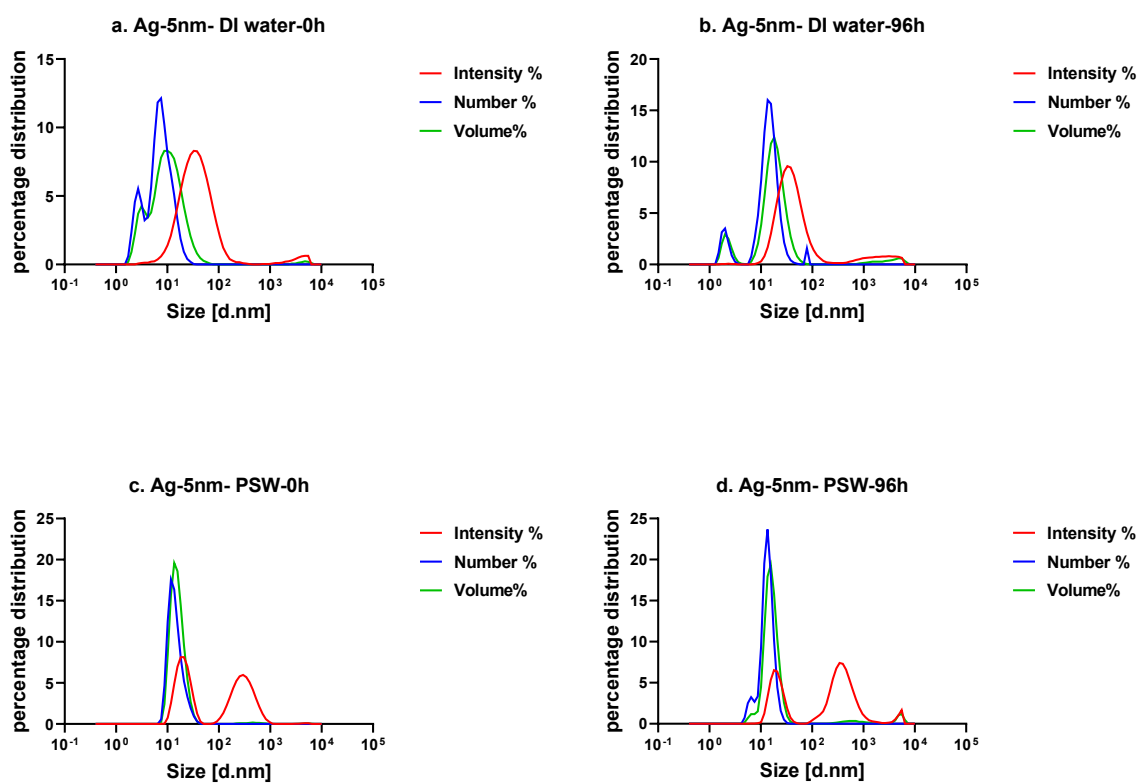


**Fig. S6** Particle size histograms for 96h-time point observations w.r.t size against intensity (red line graph), number (blue line graph) and volume (green line graph) for Sig\_IOMNPs in different test media for highest test concentration 200  $\mu\text{g.mL}^{-1}$  (a-f).

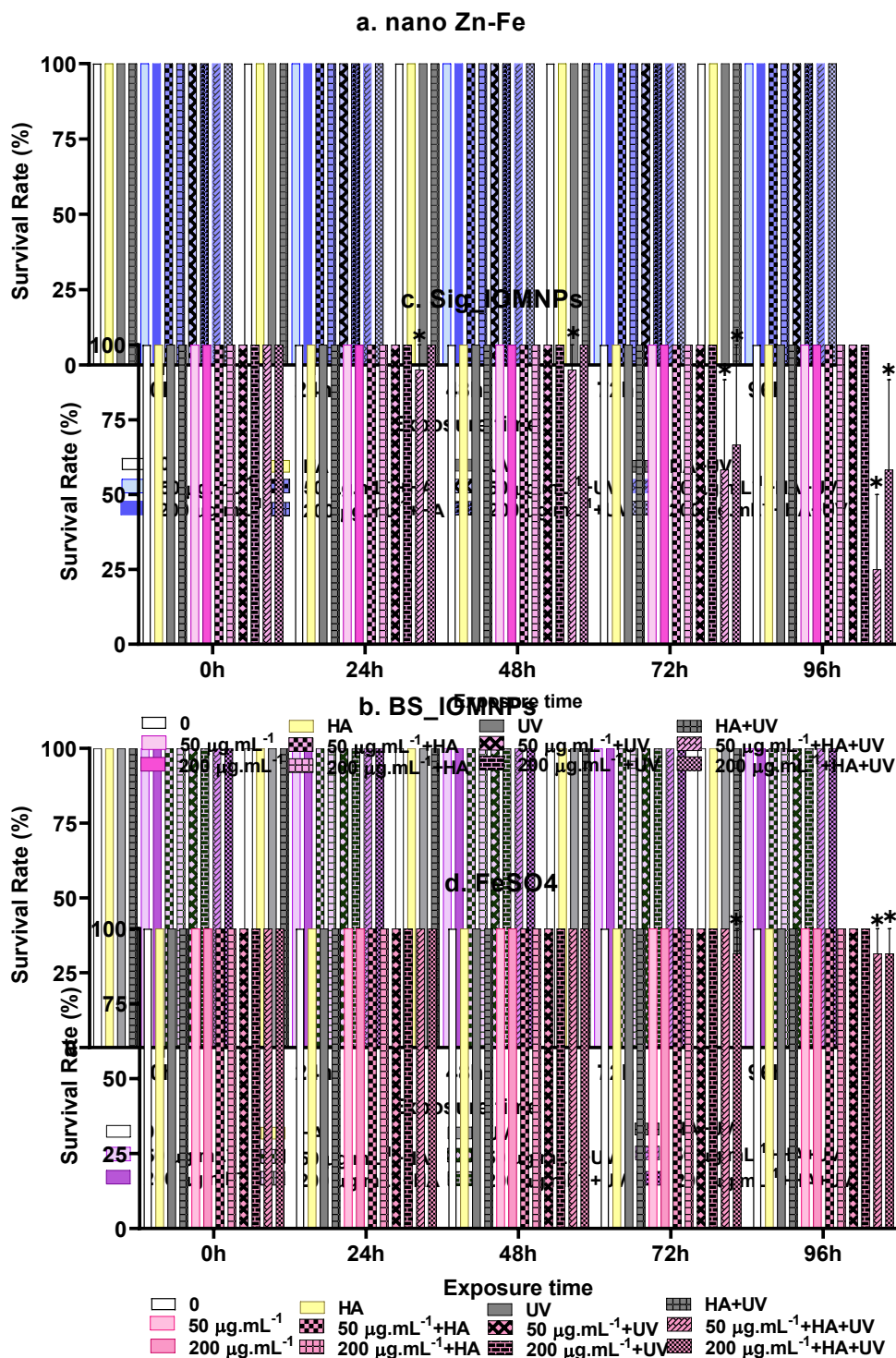




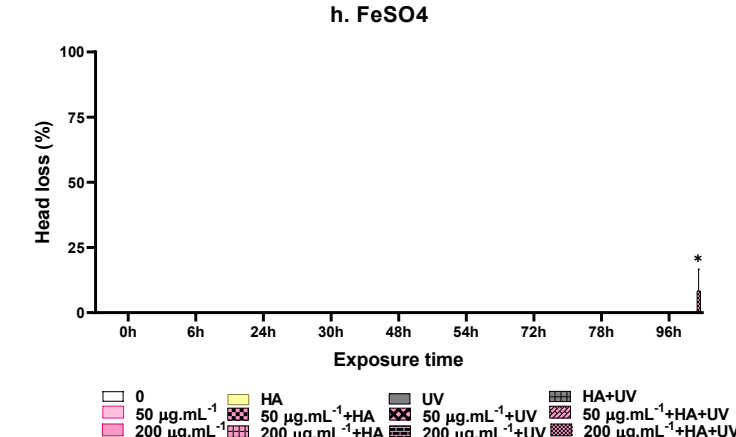
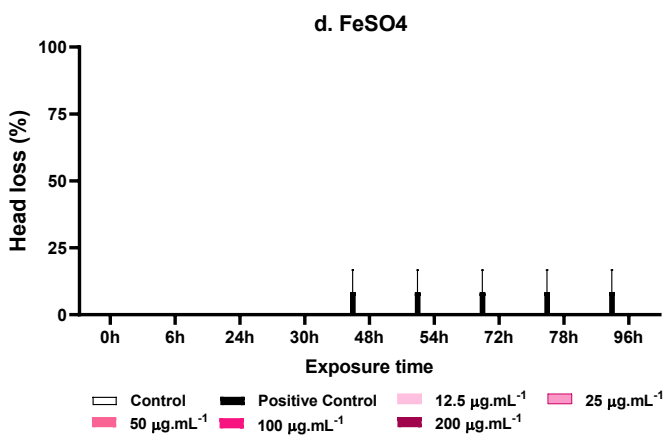
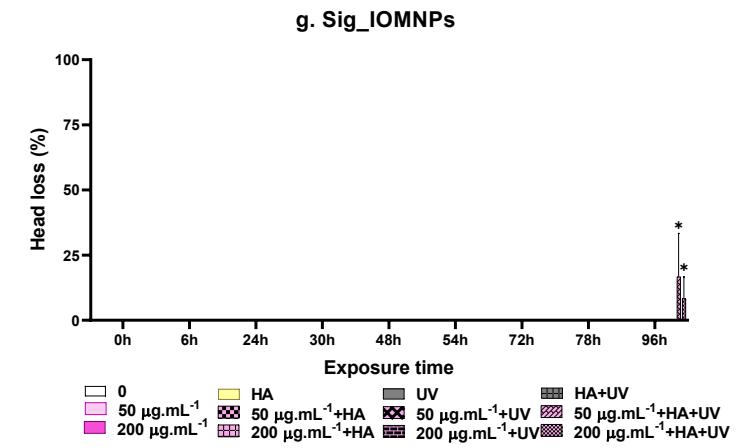
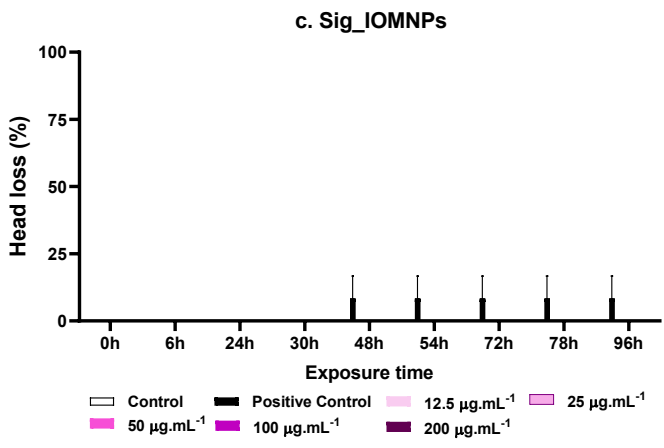
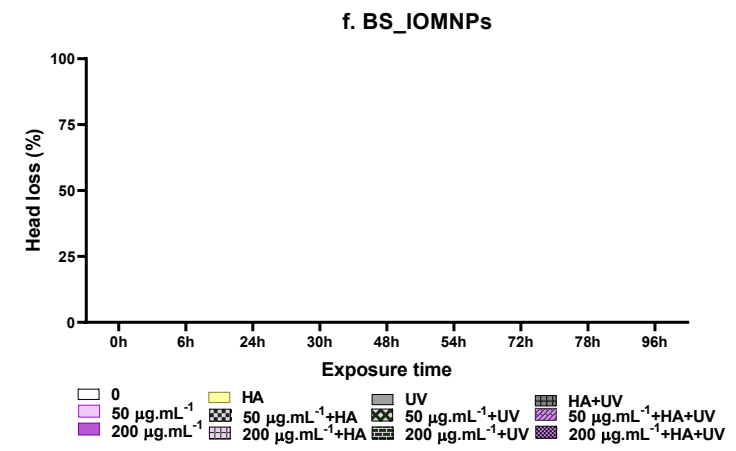
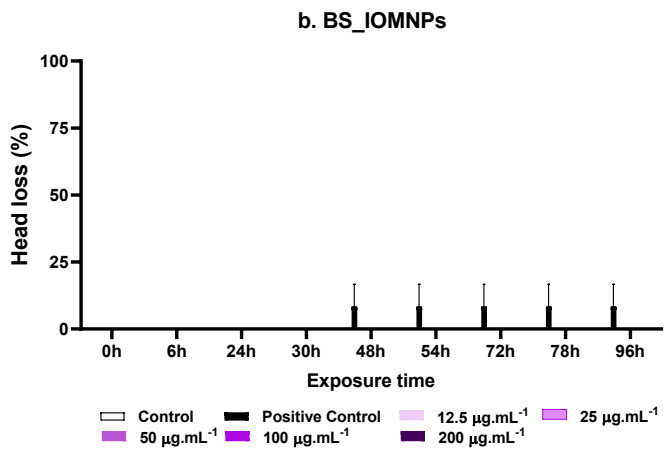
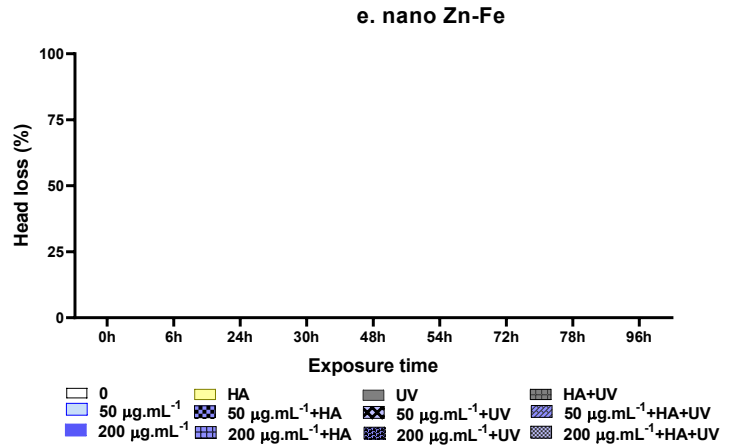
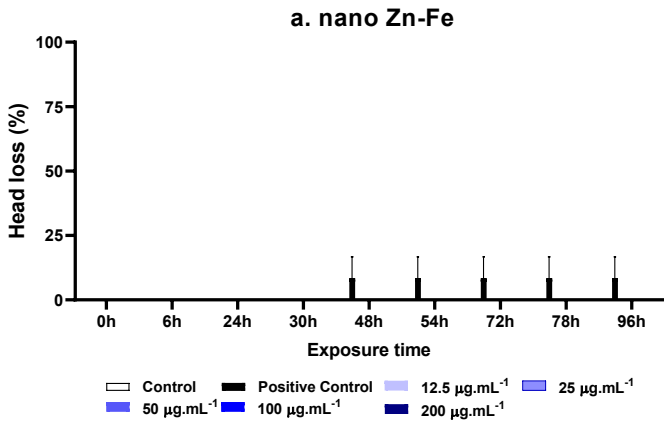
**Fig. S7** a. Hydrodynamic diameter and b. zeta potential of Ag NPs in DI water and PGM, Values are presented as mean  $\pm$  SD. [Here, the asterisk (\*) and the double asterisk (\*\*) indicates statistical significance against time 0 for PGM and for DI water conditions, respectively, and the single hash (#) indicates statistical significance between DI water and PGM for each time point, when P-value  $<0.05$ ,  $n = 3$ ].



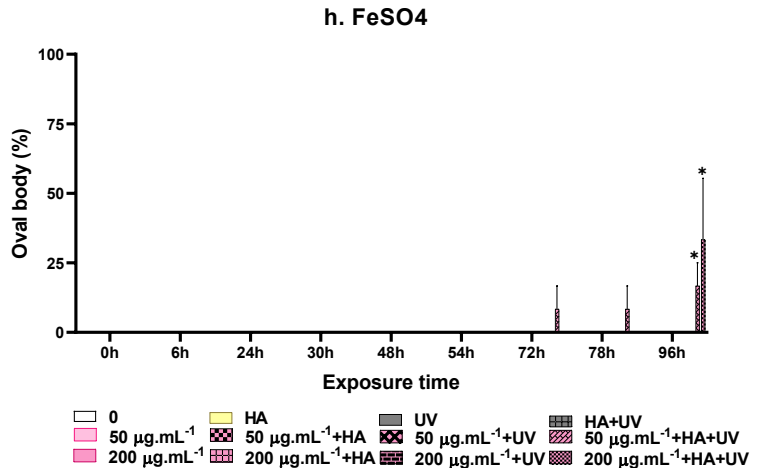
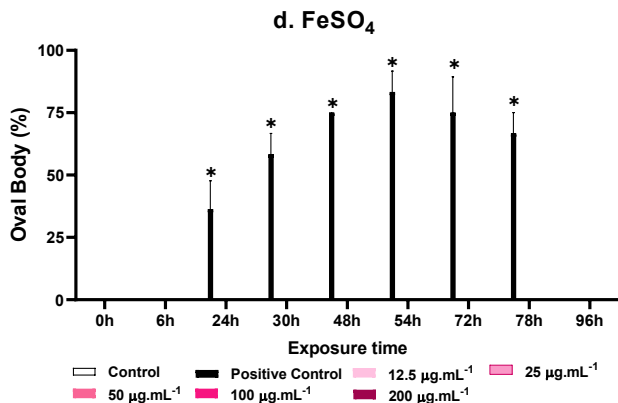
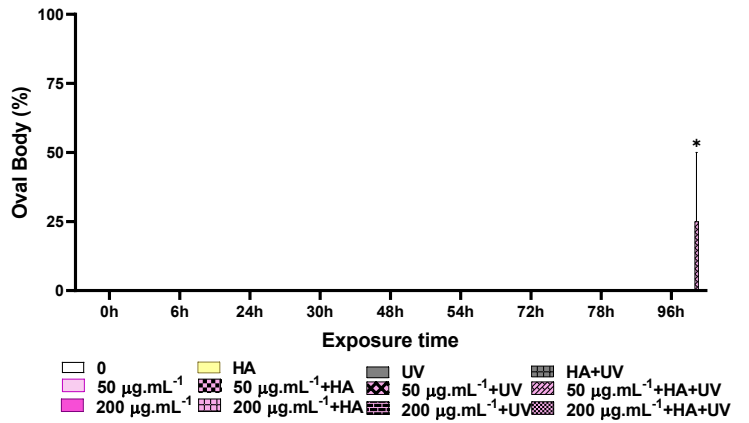
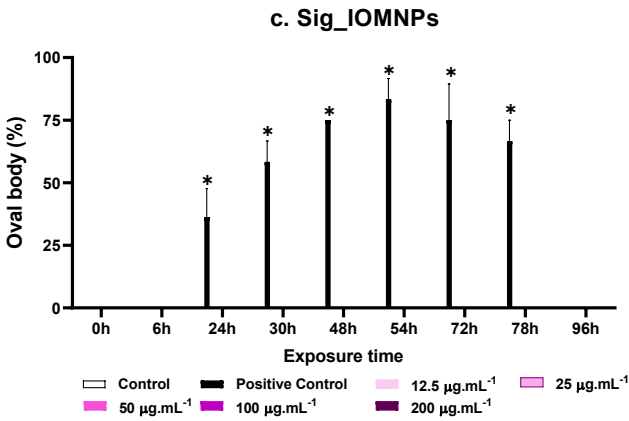
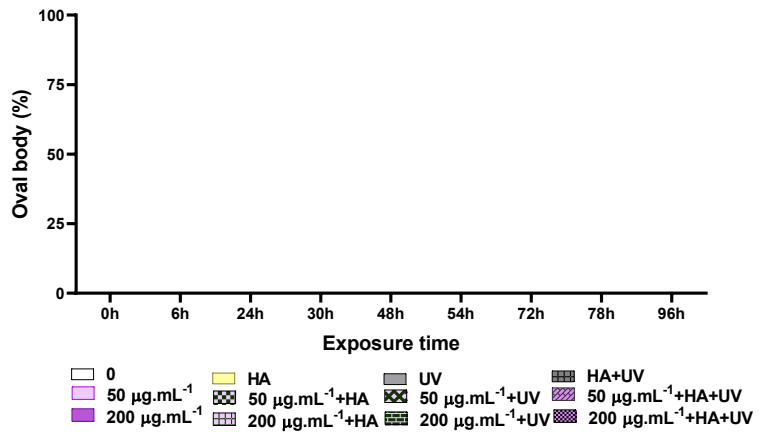
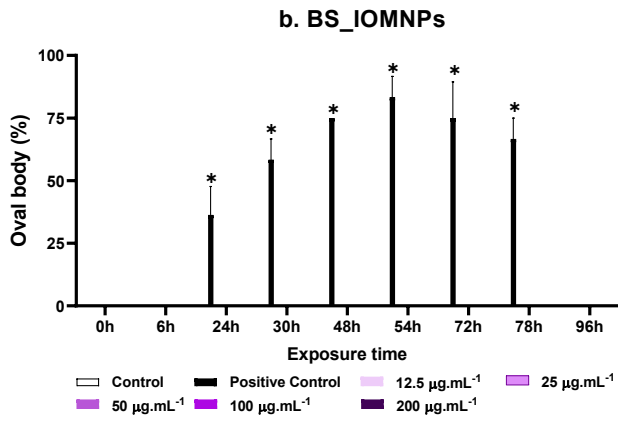
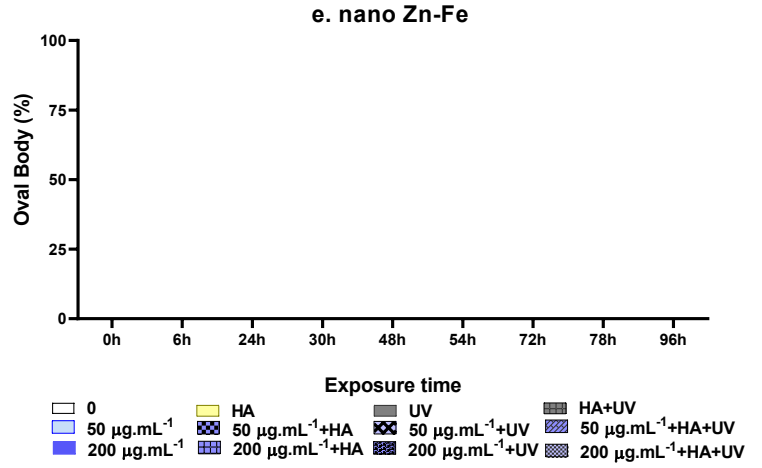
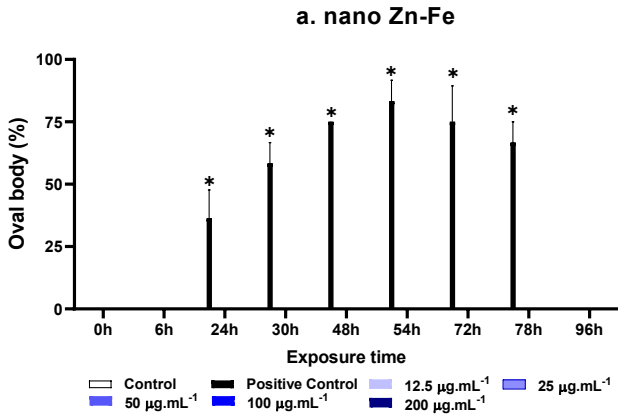
**Fig. S8** Particle size histograms for 96h-time point observations w.r.t size against intensity (red line graph), number (blue line graph) and volume (green line graph) for Ag NPs in DI water (a-b) and PGM (c-d) for highest test concentration 200  $\mu\text{g.mL}^{-1}$ .



**Fig. S9** Effects of a. Nano Zn-Fe, b. BS\_IOMNPs, c. Sig\_IOMNPs and d. Bulk FeSO<sub>4</sub>, on survival of planaria at test concentrations (50, and 200  $\mu\text{g}\cdot\text{mL}^{-1}$ ) in presence of abiotic factors [HA (20  $\mu\text{g}\cdot\text{mL}^{-1}$  and UV]. Values are presented as mean  $\pm$  S.E.M. [Here, the asterisk (\*) indicates statistical significance between control and the treatment for each time point, when P-value  $<0.05$ ,  $n = 3$ ].



**Fig. S10** Effects of a. Nano Zn-Fe, b. BS\_IOMNPs, c. Sig\_IOMNPs and d. Bulk FeSO<sub>4</sub>, at increasing concentrations (0, 12.5, 25, 50, 100, and 200 µg.mL<sup>-1</sup>) and at test concentrations (50, and 200 µg.mL<sup>-1</sup>) in presence of abiotic factors [HA (20 µg.mL<sup>-1</sup> and UV] on head loss of planaria. Values are presented as mean ± S.E.M. [Here, the asterisk (\*) indicates statistical significance between control and the treatment for each time point, when P-value <0.05, *n* = 3].

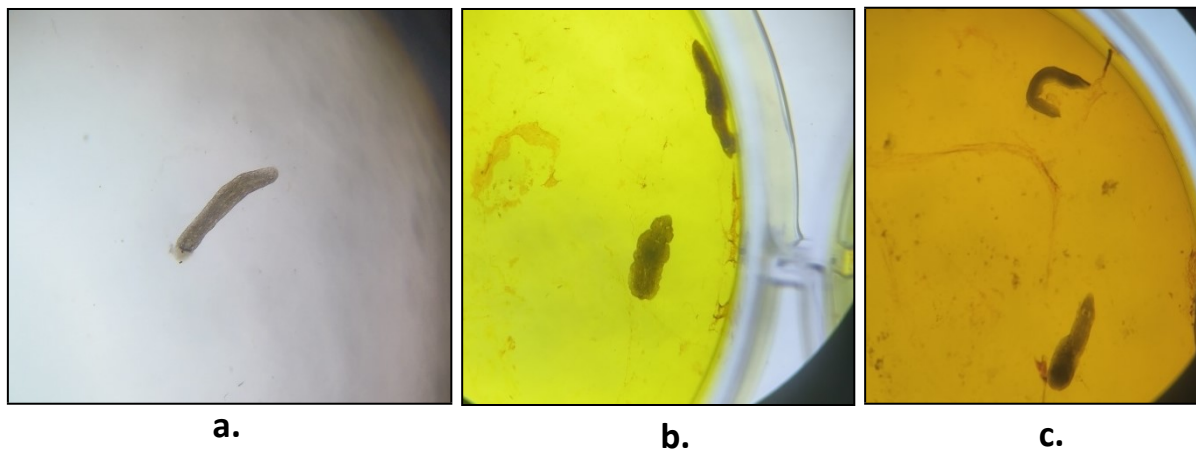


**Fig. S11** Effects of a. Nano Zn-Fe, b. BS\_IOMNPs, c. Sig\_IOMNPs and d. Bulk FeSO<sub>4</sub>, at increasing concentrations (0, 12.5, 25, 50, 100, and 200  $\mu\text{g.mL}^{-1}$ ) and at test concentrations (50, and 200  $\mu\text{g.mL}^{-1}$ ) in presence of abiotic factors [HA (20  $\mu\text{g.mL}^{-1}$ ) and UV] on oval body of planaria. Values are presented as mean  $\pm$  S.E.M. [Here, the asterisk (\*) indicates statistical significance between control and the treatment for each time point, when P-value  $<0.05$ ,  $n = 3$ ].





**Fig. S12** Effects of a. Nano Zn-Fe, b. BS\_IOMNPs, c. Sig\_IOMNPs and d. Bulk FeSO<sub>4</sub>, at increasing concentrations (0, 12.5, 25, 50, 100, and 200  $\mu\text{g.mL}^{-1}$ ) and at selected test concentrations (50, and 200  $\mu\text{g.mL}^{-1}$ ) in presence of abiotic factors [HA (20  $\mu\text{g.mL}^{-1}$  and UV] on auricles of planaria. Values are presented as mean  $\pm$  S.E.M. [Here, the asterisk (\*) indicates statistical significance between control and the treatment for each time point, when P-value  $<0.05$ ,  $n = 3$ ].



**Fig. S13** Images representing morphological alternation in planarian exposed to Ag NPs, namely (a). Head loss, (b). Shortening of auricles, and (c). Oval body.