The Nano-Bio Interactions of Rare-Earth Doped BaF_2 Nanophosphors Shape the Developmental Processes of Zebrafish

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MATERIALS AND METHOD

Chemicals: Barium acetate (Himedia, 99%), Ammonium fluoride (Himedia, 97%) Europium nitrate hexahydrate (Alfa Aesar 99.9%), Cerium nitrate (Alfa Aesar 99.99%), Terbium nitrate (Alfa Aesar 99.90%), 1-methylimidazolium (Alfa Aesar, 99%), acetone (Loba chemie, 99%), Acetylacetate (Loba chemie, 99.5%), Ethanol (Merck, ACS grade), Deionised water (CDH), Methanol (Loba chemie, 99%).

Synthesis of [C_2mim][Br] IL: This IL is synthesized by modifying a previously reported procedure, under an inert environment (Ar gas).[1] In a typical synthesis, 10 ml of 0.126 mole of 1-methyl imidazole was taken into a three-necked round bottom (RB) flask on a cold water bath. Thereafter, 12.40 ml of 0.166 mole C_2H_5Br was drop-wise added to the RB flask and then the reaction mixture was allowed to reflux for 3-4 hours and obtained white solid crystal was crushed and washed for 2-3 times with ethyl acetate. The obtained product was dried under

vacuum for 24 hours.

Characterization Techniques of materials: PXRD was carried out on a D8 Advance BRUKER, equipped with Cu Kα (1.54060 Å) as the incident radiation. The crystallite size was calculated using Scherer equation $D = K\lambda/\beta\cos\theta$, where K = 0.9, D represents crystallite size (Å), λ is the wavelength of Cu Kα radiation and β is the corrected half-width of the diffraction peak. Atomistic level growth of nanoparticle was confirmed using FEI TALOS 200S instrument at a working voltage of 200 kV. The high-resolution TEM/HRTEM is used to map the shape, size, and lattice structure of the nanocrystals dispersed on a carbon-coated copper grid from acetone solution. Morphological characterization was also carried out by SEM using a NOVA NANO SEM-450, FEI. Photoluminescence emission and excitation spectra were measured using a HORIBA JOBIN YVON made Fluoromax-4 spectrofluorometer. Hydrodynamic diameter, size distribution (polydispersity index or PI) and Zeta Potential of pure BaF_2 and $BaF_2:Ce^{3+}/Tb^{3+}$ nanoparticles were determined using NanoPlus-3 (Version 5.01, Micromeritics Instrument Corporation, Particulate Systems, Norcross, GA, USA). Nanoparticles at suitable concentrations (range 10 – 100 mg/L) were dispersed in 10 mM NaCl solution by sonication in a water bath for 20 minutes. All the measurements were carried out at an equilibrium temperature of 25 °C. Smoluchowski formula for the approximation of zeta potentials from electrophoretic mobility is selected in the present case.

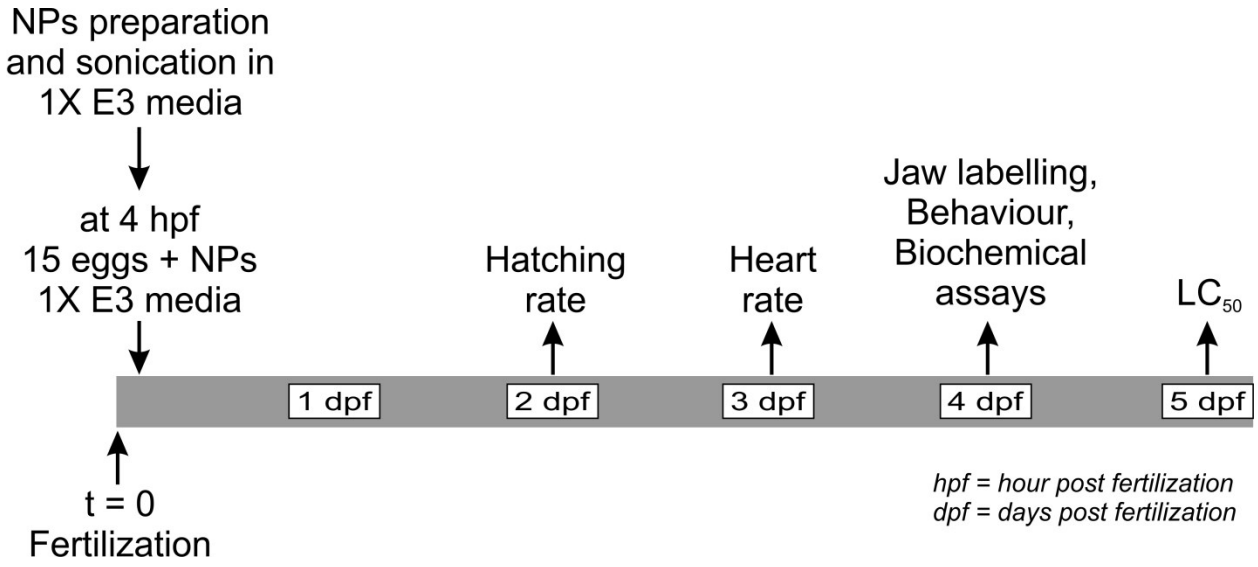


Figure SI 1. The layout of the experimental design used in the present study. All nanoparticles (NPs) were prepared in the 1X E3 aqueous media by sonication. All treatment (control and NPs) were started on zebrafish embryo of 4 hpf stage until the indicated time. We have used 15 eggs per treatment at the start of every experiment per treatment group. At the indicated time eight larvae (except for hatching rate and LC_{50}) were randomly selected and different endpoints were measured e.g. at 2 dpf (hatching rate), at 3 dpf (heart rate), at 4 dpf (jaw labeling, behavior and other biochemical assays) and at 5 dpf stage LC_{50} was determined.

Lattice strain

Wiliamson and Hall Equation: Williamson and Hall method is used for determining the lattice strain of the as-prepared nanoparticles:[2]

$$\frac{\beta cos\theta}{\lambda} = \frac{1}{D} + \eta\frac{sin\theta}{\lambda} \qquad (1)$$

Where, the graph is plotted between βcosθ/λ vs sinθ/λ. From the slope of the graph and intercept, lattice strain (η) crystallite size (D) can be quantitatively determined respectively. The negative and positive magnitudes of the slope of the graph indicate the compressive and tensile strains respectively.

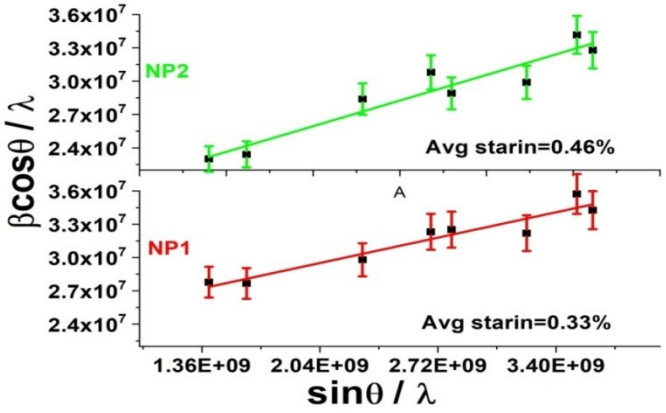


Figure SI 2: Lattice strain graph of as-prepared NP1 (un-doped BaF_2) and NP2 (1 % Ce^{3+}/Tb^{3+} doped BaF_2) nanoparticles.

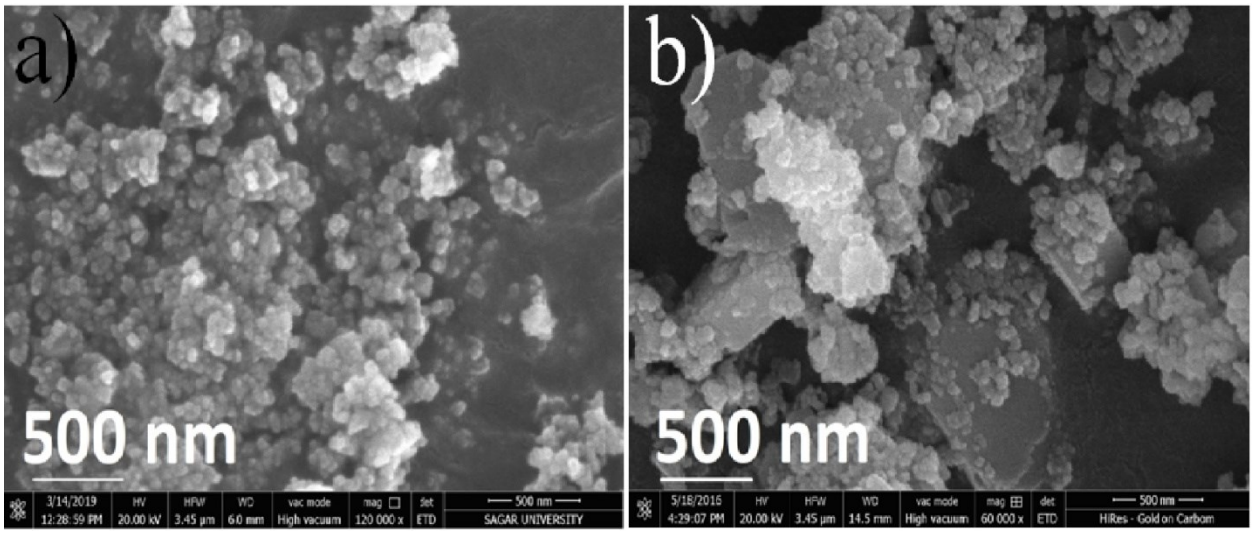


Figure SI 3. High magnification FESEM images of as-prepared nanoparticles: a) NP1 (un-doped BaF_2) and b) NP2 (1 % Ce^{3+}/ 1% Tb^{3+} doped BaF_2).

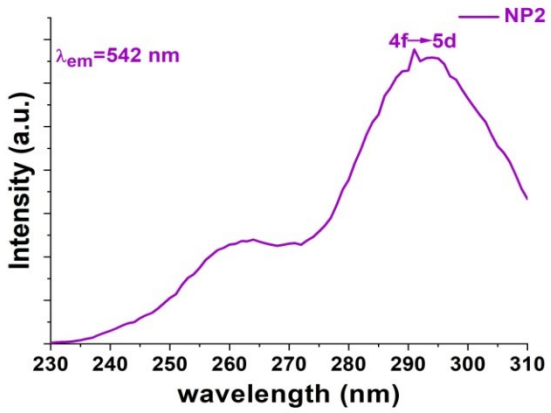


Figure SI 4. The excitation spectrum of as-prepared NP2 (1 % Ce^{3+}/ 1% Tb^{3+} doped BaF_2) nanoparticles measured using λ_{em} = 542 nm.

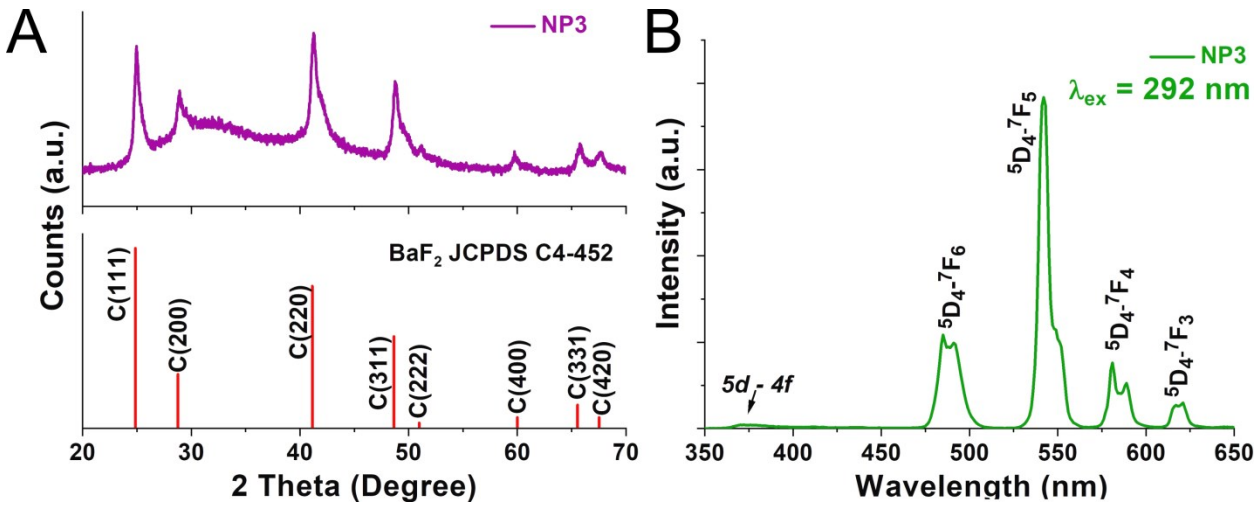
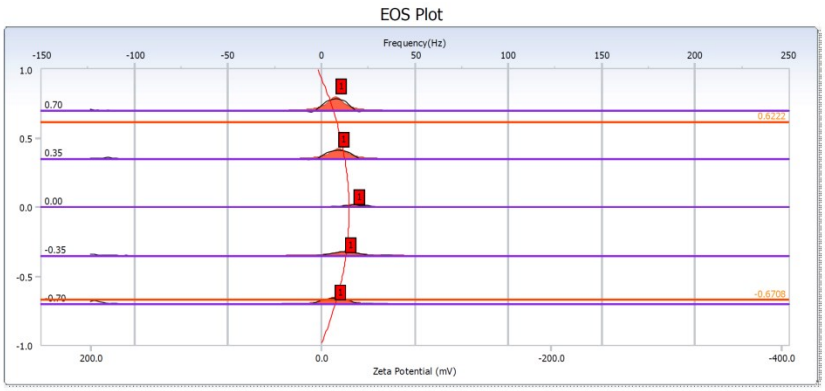
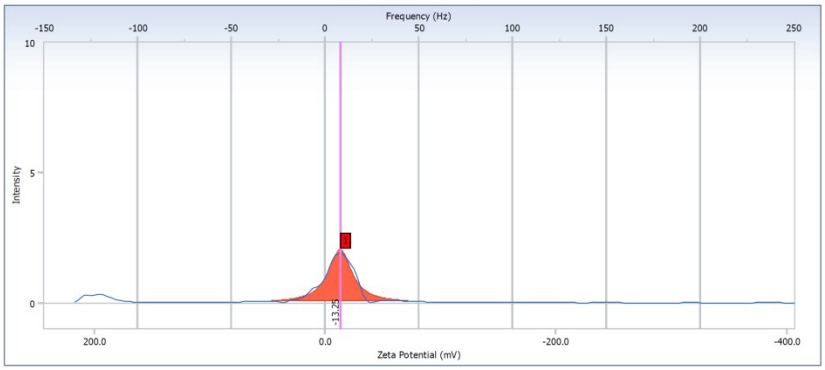


Figure SI 5. A) PXRD patterns of NP3 (5 % Ce^{3+}/Tb^{3+} doped BaF_2 nanoparticles) synthesized via ionic liquid. B) Emission spectra of NP3 (λ_{exct} = 292 nm).

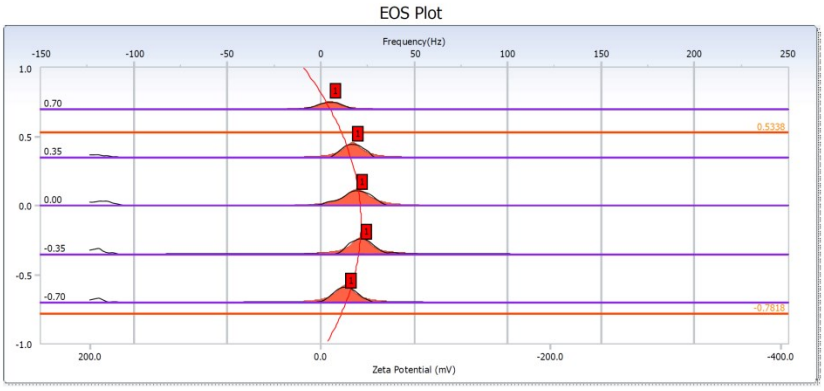
A


EOS Plot
Frequency(Hz)
-150 -100 -50 0 50 100 150 200 250
1.0
0.5
0.0
-0.5
-1.0
0.70
0.35
0.00
-0.35
-0.70
0.6222
-0.6708
200.0 0.0 -200.0 -400.0
Zeta Potential (mV)

B


Mobility Distribution
Frequency (Hz)
-150 -100 -50 0 50 100 150 200 250
10
5
0
Intensity
-13.25
200.0 0.0 -200.0 -400.0
Zeta Potential (mV)

C


EOS Plot
Frequency(Hz)
-150 -100 -50 0 50 100 150 200 250
1.0
0.5
0.0
-0.5
-1.0
0.70
0.35
0.00
-0.35
-0.70
0.5338
-0.7818
200.0 0.0 -200.0 -400.0
Zeta Potential (mV)

D

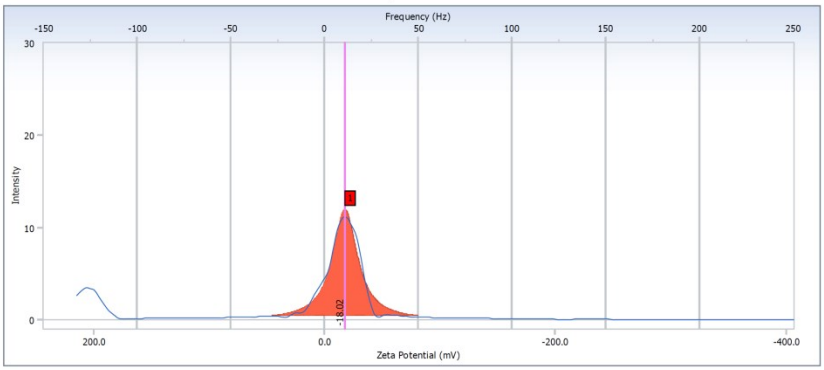

Mobility Distribution
Frequency (Hz)
-150 -100 -50 0 50 100 150 200 250
30
20
10
0
Intensity
-15.02
200.0 0.0 -200.0 -400.0
Zeta Potential (mV)

Figure SI 6. EOS flow plot and mobility distribution of A, B) NP1 (BaF_2) and C, D) NP2 (1 % Ce^{3+}/Tb^{3+} doped BaF_2) nanoparticles. Using flow cell type the zeta potential and other parameters of NP1 and NP2 was found to be under following conditions. For NP1: -13.25 mV (zeta potential), -1.035e-004 cm^2/V s (mobility), 0.9092 mS/cm (conductivity), -16.43 V/cm (avg electric field) and -0.75 mA (avg current). For NP2: -18.02 mV (zeta potential), -1.411e-004 cm^2/V s (mobility), 1.1781 mS/cm (conductivity), -16.40 V/cm (avg electric field) and -0.97 mA (avg current).

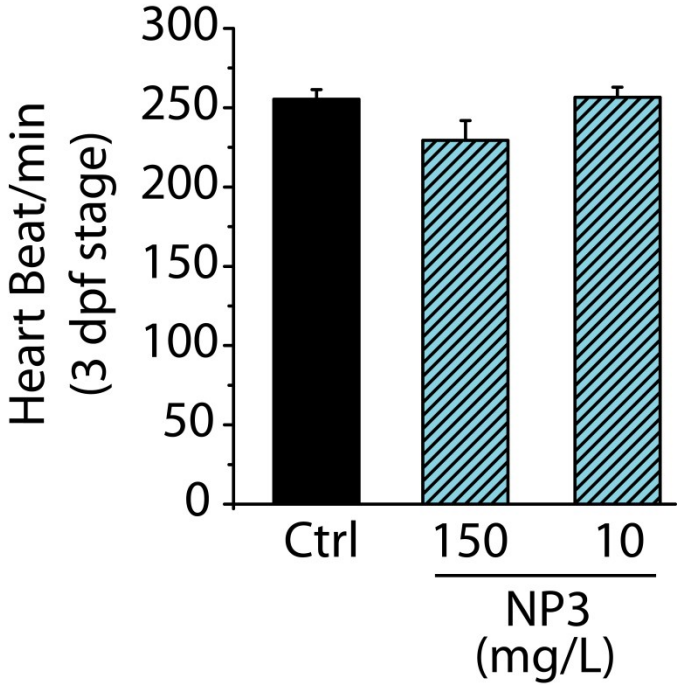


Figure SI 7. Impact of NP3 (5 % Ce^{3+}/ 5% Tb^{3+} doped BaF_2) on the heart rate. The data represents mean ± SEM of 8 – 16 zebrafish larvae of 3 dpf stage. We have seen no significant difference in heart rate between control and NP3 treated (at both concentrations) groups.

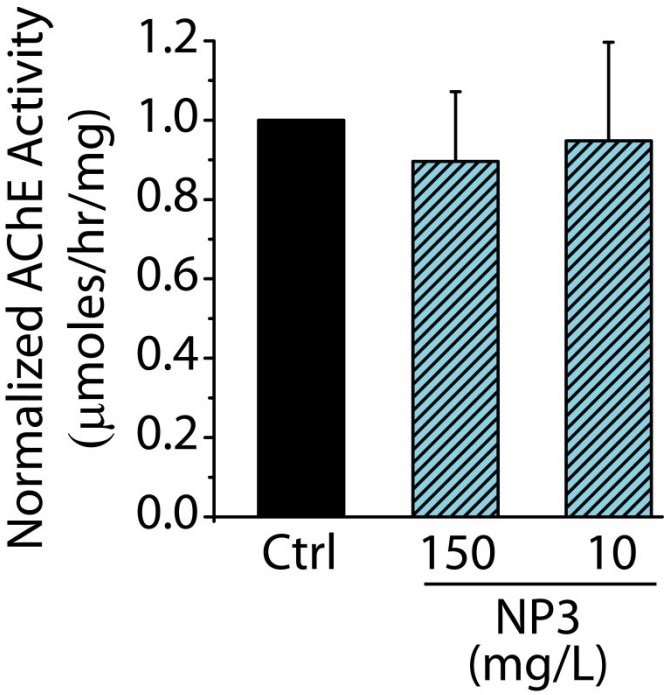


Figure SI 8. Impact of NP3 (5 % Ce^{3+}/Tb^{3+} doped BaF_2) on the acetylcholinesterase (AChE) enzyme activity. The data represents mean ± SEM of two experiments in which a pool of 8 larvae per experiment per treatment group. Like NP1 and NP2 effect on AChE enzyme activity, we have also seen no significant difference in the AChE enzyme activity between control and NP3 treated (at both concentrations) groups.

Table SI 1. Phase, lattice strain and crystallite size of the as-prepared 1% RE doped BaF_2 nanoparticles under similar reaction conditions (IL= $[C_2mim]Br$), (Y=Yes and N=No).

Sample code	**Sample name**	**IL (Y/N)**	**As-prepared /calcined**	**Cell parameter**		**Phase**	**Crystallite size(nm) (±2)**	**Average lattice strain(%)**	**Lattice strain**
				A (A^o)	Volume				
NP1	BaF_2	Yes	As-prepared	6.201(19)	238.55(14)	Cubic	16.00	0.336	Tensile
NP2	BaF_2:Ce^{3+}/ Tb^{3+}	Yes	As-prepared	6.2039(17)	238.78(11)	Cubic	17.60	0.462	Tensile

Table SI 2: Electrochemical and physical characterization of as-prepared nanoparticles.

Nanoparticles	Structural dimensions (Average) (a x b) (nm) *	Zeta potential (mV)	Polydispersity Index (PdI)	Hydrodynamic diameter (D) (nm)
NP1 (un doped BaF_2)	20 nm & 19 nm	-13.25	0.487	D (10%) = 154.30 nm. D (50%) = 367.00 nm
NP2 (1 % Ce^{3+}/Tb^{3+} doped BaF_2)	22 nm & 17 nm	-18.02	0.652	D (10%) = 202.10 nm. D (50%) = 458.40 nm

* Our nanoparticles are cuboidal shaped which is evident from TEM images. Thereby we have measured the edge length of the nanoparticle which is evident from TEM images.

Supplementary table SI 3: Percent morphological abnormalities in the developing zebrafish larvae.

Morphological endpoint	Treatment	% Mean ± SEM	N (out of number of larvae)#
Tail bend	Control	3.12 ± 3.12	32
	NP1 (150 mg/L)	62.5 ± 12.5	32
	NP1 (10 mg/L)	12.5 ± 8.84	32
	NP2 (150 mg/L)	56.25 ± 10.83	32
	NP2 (10 mg/L)	9.38 ± 5.98	32

#This data is from 4 dpf larvae.

Morphological endpoint	Treatment	% Mean ± SEM	N (number of larvae)†
Pericardial edema	Control	10.42 ± 3.84	48
	NP1 (150 mg/L)	23.21 ± 7.43	46
	NP1 (10 mg/L)	5.79 ± 3.87	49
	NP2 (150 mg/L)	18.15 ± 11.41	43
	NP2 (10 mg/L)	8.33 ± 4.17	48

†This data is from 3 dpf larvae.

Morphological endpoint	Treatment	% Mean ± SEM	N (number of larvae) †
Yolksac/tube edema	Control	12.5 ± 6.45	48
	NP1 (150 mg/L)	68.85 ± 10.48	45
	NP1 (10 mg/L)	3.94 ± 2.5	49
	NP2 (150 mg/L)	60.81 ± 14.15	43
	NP2 (10 mg/L)	20.83 ± 10.54	48

†This data is from 3 dpf larvae.

Supplementary table SI 4: Percent frequency distribution of zebrafish larvae in different concentric zones during open-field behaviour for different treatment groups.

Treatment group	Zones	% Mean ± SEM	N (out of number of larvae)
Control	Zone-1	0	32
	Zone-2	21.88 ± 5.98	
	Zone-3	78.13 ± 5.98	
NP1 (150 mg/L)	Zone-1	21.88 ± 7.86	32

	Zone-2	43.75 ± 6.25	
	Zone-3	34.38 ± 13.86	
NP1 (10 mg/L)	Zone-1	12.5 ± 5.1	32
	Zone-2	31.25 ± 8.07	
	Zone-3	56.25 ± 6.25	
NP2 (150 mg/L)	Zone-1	25 ± 11.41	32
	Zone-2	46.88 ± 7.86	
	Zone-3	28.13 ± 7.86	
NP2 (10 mg/L)	Zone-1	9.38 ± 5.98	32
	Zone-2	21.88 ± 5.98	
	Zone-3	68.75 ± 8.07	

REFERENCES

1. E. R. Parnham, A. M. Z. Slawin, and R. E.Morris, J. Solid State Chem. 2007, **180 (1),** 49–53.

2 R. K. Sharma, A. V. Mudring and P. Ghosh, *J. Lumin.*, 2017, **189**, 44–63.