Electronic Supplementary Material (ESI) for Environmental Science: Nano.

Supplementary Information for

Toxicity of 12 metal-based nanoparticles to algae, bacteria and a protozoa

Villem Aruoja*[1], Suman Pokhrel[2], Mariliis Sihtmäe[1], Monika Mortimer[1], Lutz Mädler[2], Anne Kahru[1]

[1] Laboratory of Environmental Toxicology, National Institute of Chemical Physics and. Biophysics, Akadeemia tee 23, Tallinn 12618, Estonia

[2] Foundation Institute of Materials Science IWT, University of Bremen, Bremen, Germany

Villem Aruoja Tel./Fax: +372 6398368, E-mail: villem.aruoja@kbfi.ee

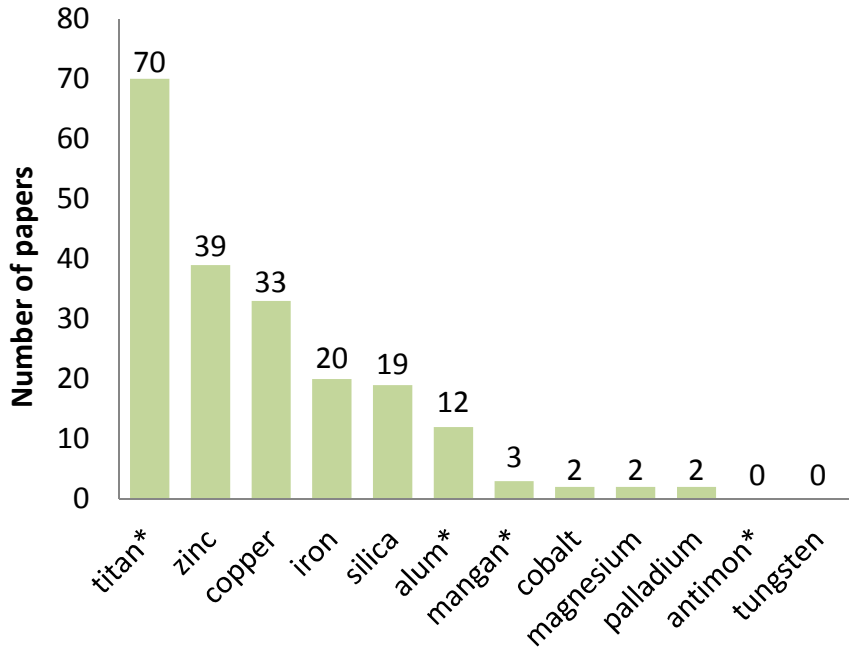


Figure S1 Number of toxicology papers on algae for different metal-containing nanoparticles in the Web of Science™ as of Jan. 21, 2015; TOPIC: (toxic* AND alga* AND nano*AND keywords shown in the figure), Total of 369 papers were retrieved, 202 concerned substances in this paper and were plotted in the graph.

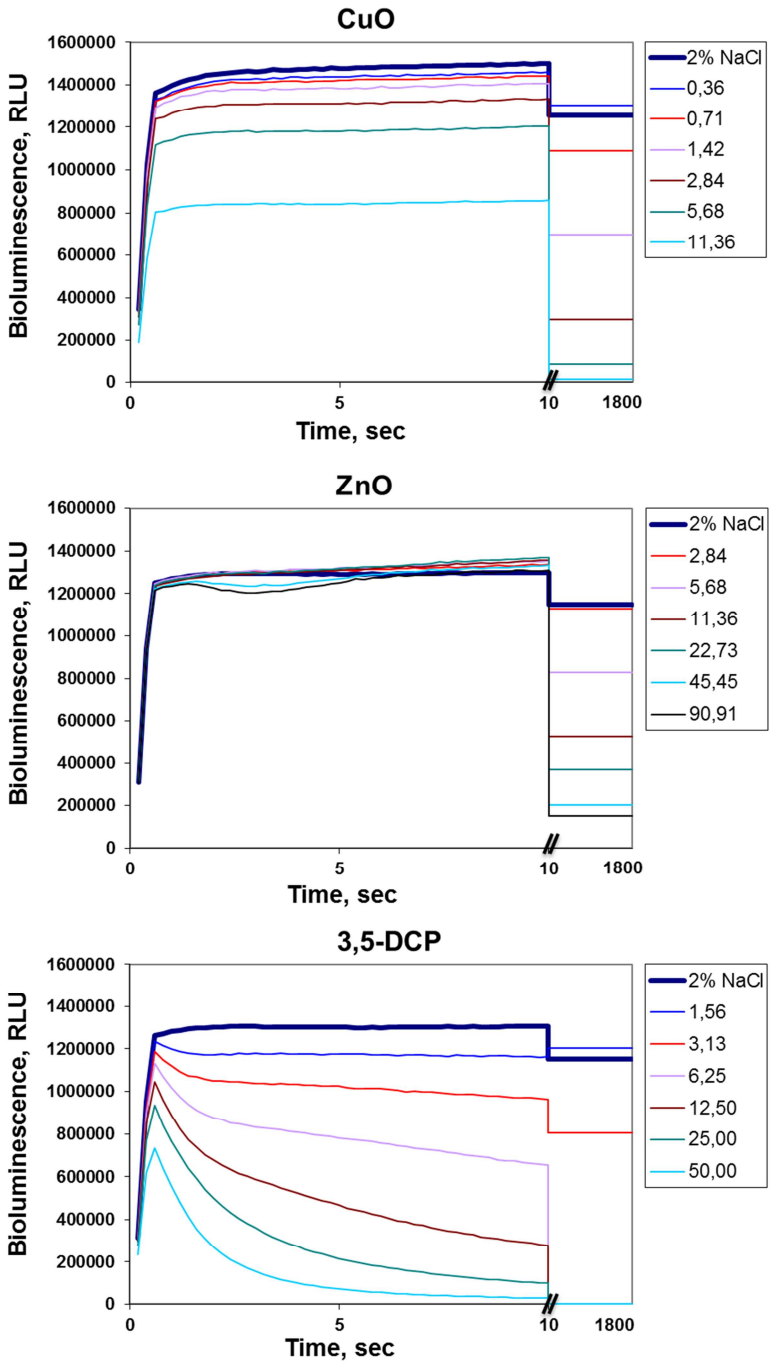


Figure S2 Kinetics of luminescence of *Vibrio fischeri* exposed to different concentrations (0,36-91 mg/l) of CuO and ZnO NPs suspensions measured in microplate format of Flash Assay. 2% NaCl served as a negative control and 3,5-dichlorophenol (3,5-DCP) as a positive control. Concentrations of metal based NPs are nominal. RLU – relative light units.

Table S1 Composition of the algal test medium (OECD 201).

No	Component	Concentration, mg/L
1	NH_4Cl	15
2	$MgCl_2*6H_2O$	12
3	$CaCl_2*2H2O$	18
4	$MgSO_4*7H_2O$	15
5	KH_2PO_4	1.6
6	$NaHCO_3$	50
7	$Na_2EDTA*2H_20$	0.1
8	$FeCl_3*6H_2O$	0.08
9	H_3BO_3	0.185
10	$MnCl_2*4H_2O$	0.415
11	$ZnCl_2$	0.003
12	$CoCl_2*6H_2O$	0.0015
13	$Na_2MoO_4*2H_2O$	0.007
14	$CuCl_2*2H_2O$	0.00001

Table S2 Specific surface area, primary particle sizes (d_{BET}, d_{XRD} and d_{TEM}) of the nanoparticle library prepared for the experiments. See also Figure S2.

Sample	Specific surface	BET size (d_{BET})	Crystallite size (d_{XRD}) nm	TEM size (d_{TEM})
ZnO	53	20.4	19.2	15-20
CuO	72	13.1	12.2	9-15
Al_2O_3	134	11.4	9.9	-
SiO_2	289	7.8	-	-
TiO_2	123	12.2	*A-14.5, R = 8.5	9-15
Sb_2O_3	56	20.5	-	10-15
Mn_3O_4	81	15.2	11.6	-
Fe_3O_4	120	9.7	*M = 8.2, H = 9.4	10-15
MgO	123	13.6	-	10-15
Co_3O_4	85	11.5	10.5	9-15
WO_3	79	10.6	-	-
Pd	33	15.1	15.1	-

*The formation of the double phase present in the nanoparticle were also analyzed using Rietveld fittings where A and R, denote anatase and rutile TiO_2 and M and H denote magnetite (Fe_3O_4) and hematite (Fe_2O_3) nanoparticles.

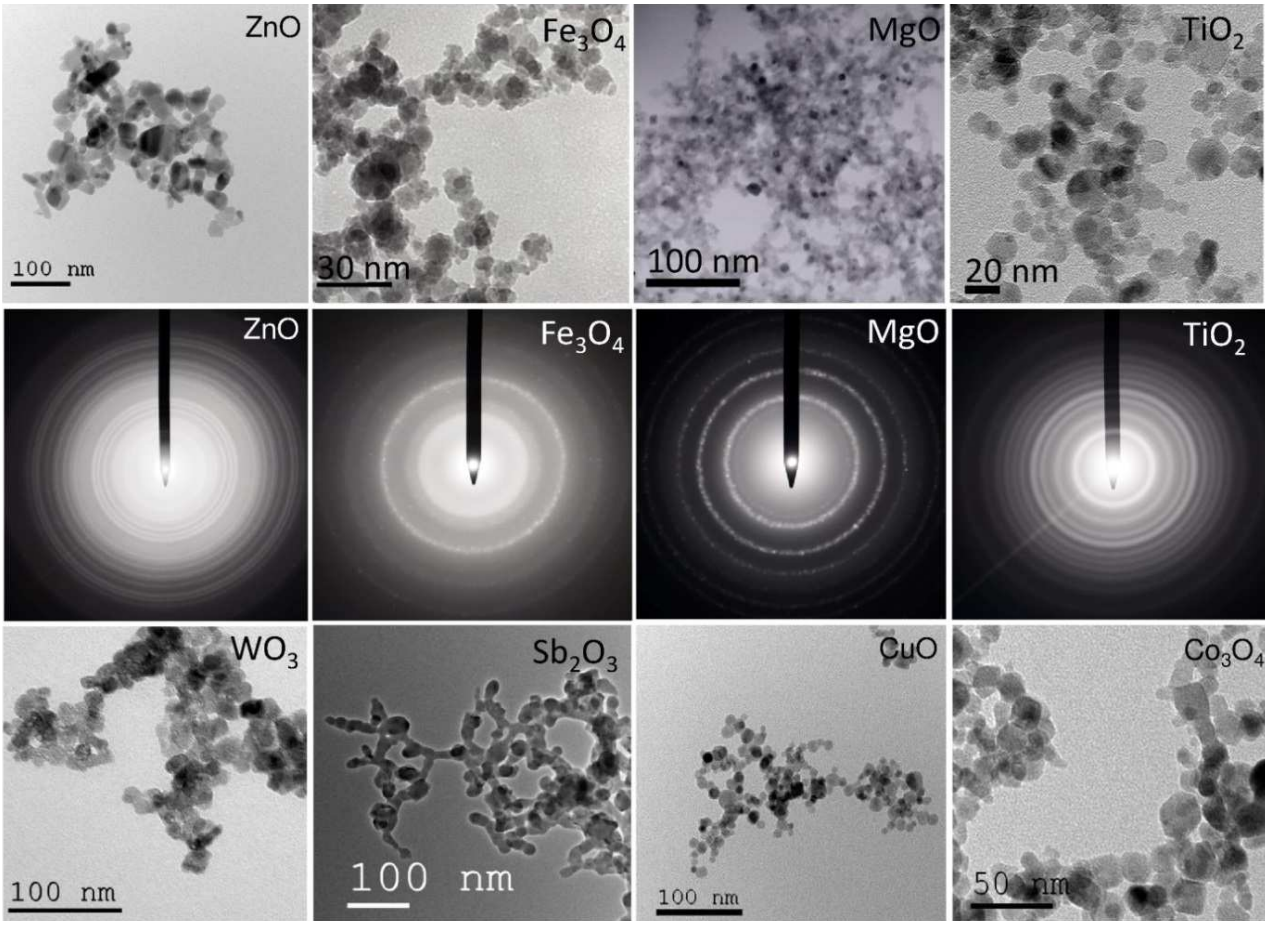


Figure S3 The FSP synthesized particles are ultrafine, spherical and highly crystalline. Upper panel: low resolution TEM images of ZnO, Fe_3O_4, MgO and TiO_2; middle panel: selected area diffraction patterns of ZnO, Fe3O4, MgO and TiO2; lower panel: low resolution TEM images of WO_3, Sb_2O_3, CuO, and Co_3O_4.

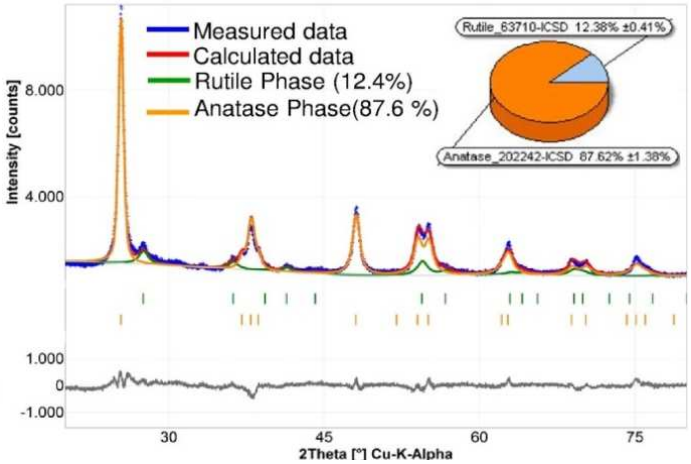


Figure S4 Rietveld refinement of the XRD patterns of TiO_2, quantitative anatase and rutile content.

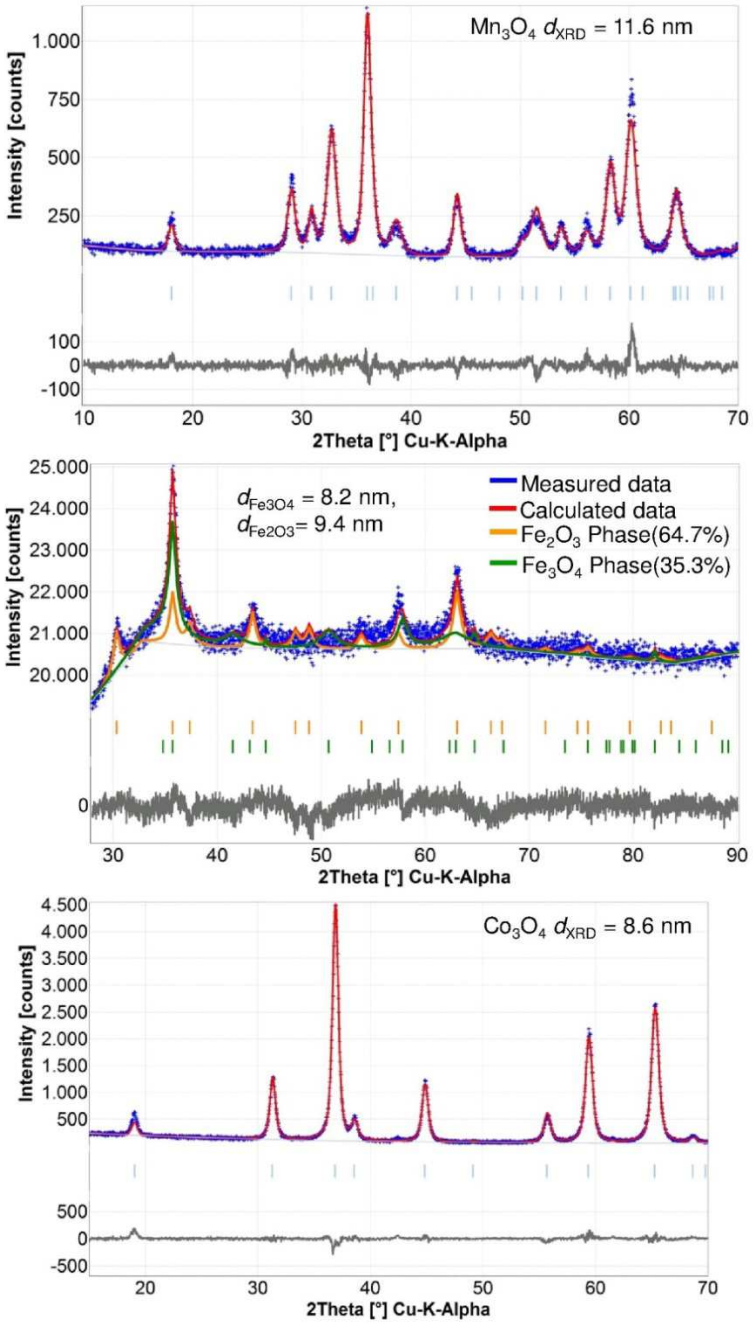
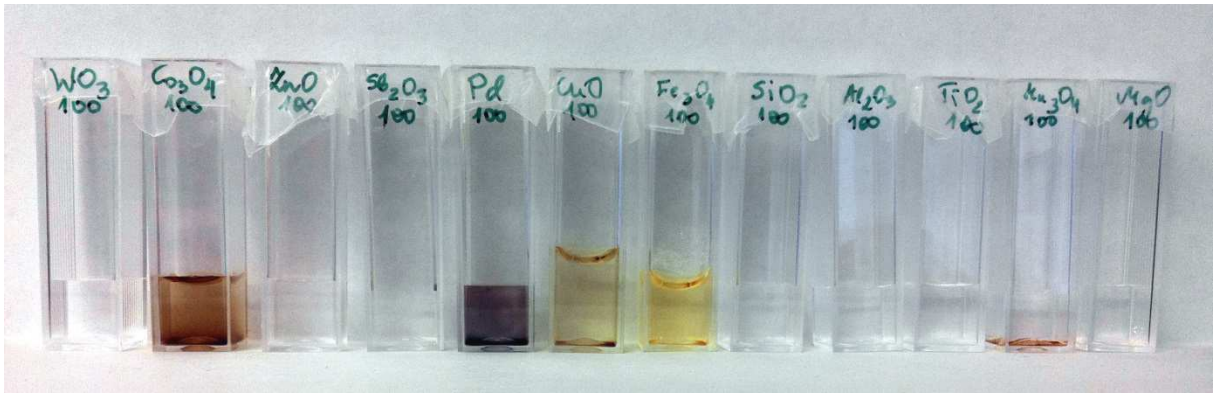


Figure S5 XRD patterns and structure refinement of Mn_3O_4, Fe_3O_4 and Co_3O_4 spinel mixed oxides

A



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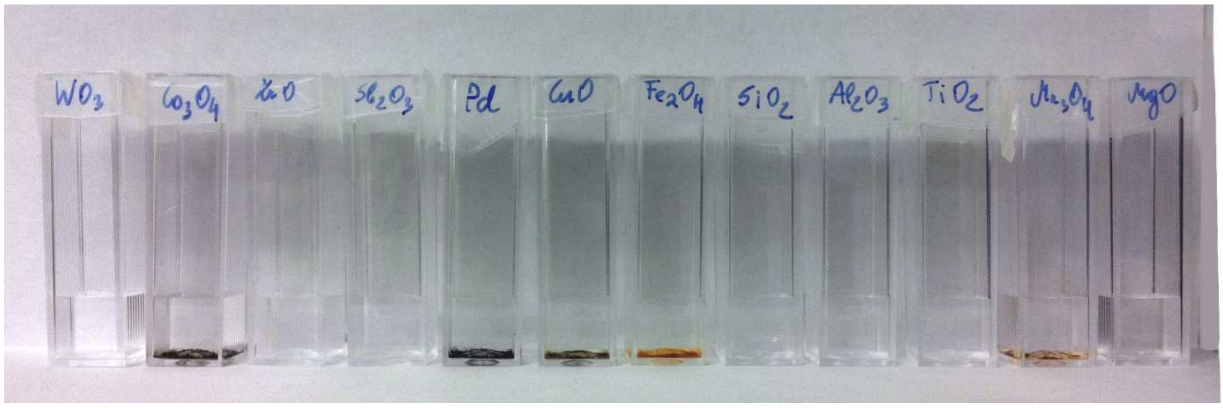


Figure S6. The stock suspensions (200 mg/l) of metal containing NPs were vortexed and sonicated for 4 minutes (40W, Branson probe sonicator, USA), diluted 1:1 with DI water or 200% algal medium and stored for 7 days (A: DI water) or for 1 day (B: algal medium).

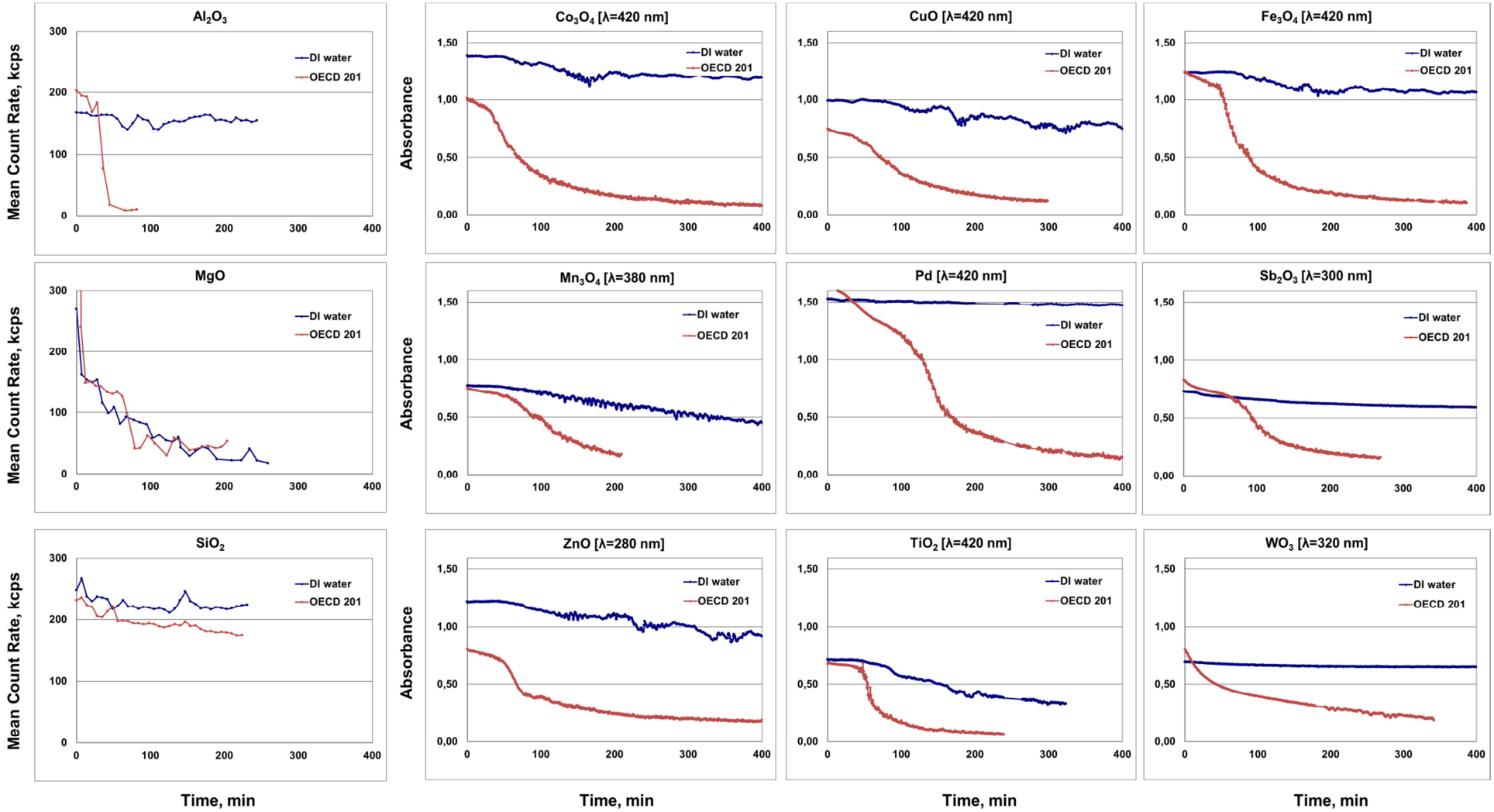


Figure S7. Stability of metal containing nanoparticle suspensions. The stock suspensions (200 mg/l) of metal containing NPs were vortexed and sonicated for 4 minutes (40W, Branson probe sonicator, USA) then further diluted 1:1 with DI water (blue lines) or 200% algal medium (purple lines)and analyzed immediately with UV-Vis spectrophotometry (in case of Co_3O_4, CuO, Fe_3O_4, Mn_3O_4, Sb_2O_3, ZnO, TiO_2, WO_3, and metallic Pd) using 1cm path quartz cuvette (Multiskan Spectrum, Thermo Electron Corp., Finland) or light scattering/count rate (in case of Al_2O_3, MgO, SiO_2) (Malvern Zetasizer Nano-ZS, Malvern Instruments, UK).

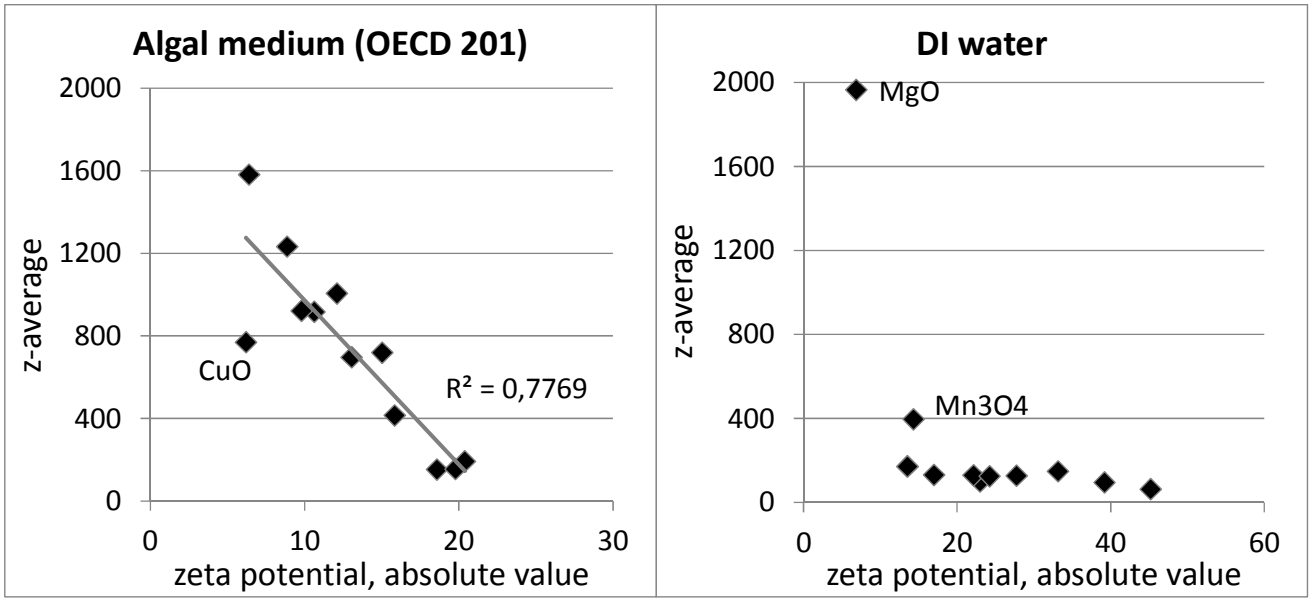


Figure S8 Relationship between particle hydrodynamic size (z-average) and absolute zeta potential in the algal growth medium and deionized water.

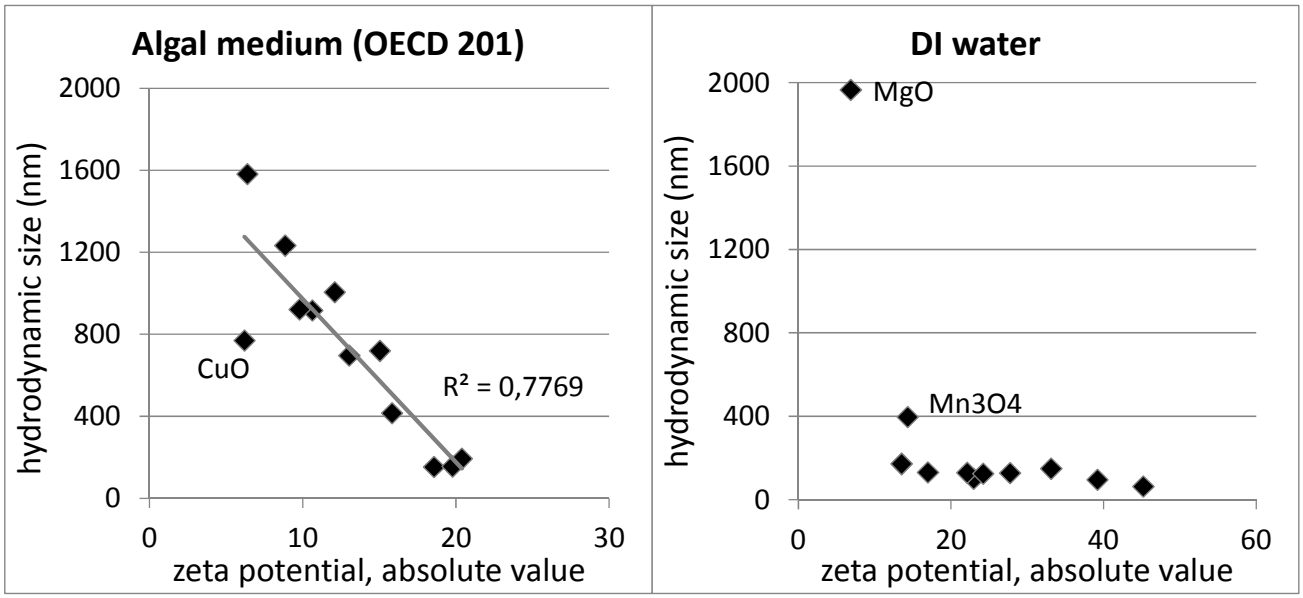


Figure S9 Relationship between sedimentation % and absolute zeta potential at 200 minutes after suspension in the algal growth medium and deionized water.

Table S3. Toxicities of nanoparticle (NP) suspensions and the respective soluble salts to algae *Pseudokirchneriella subcapitata*, protozoa *Tetrahymena thermophila* and bacteria *Vibrio fischeri*.

	NP suspensions						**Soluble salts**					
	P. subcapitata		*T. thermophila*		*V. fischeri*			*P. subcapitata*		*T. thermophila*		*V. fischeri*	
NP	72h EC_{50}[a]	95% confidence limits	24h EC_{50}[a]	95% confidence limits	30min EC_{50}[a]	95% confidence limits	Metal salt	72h EC_{50}[b]	95% confidence limits	24h EC_{50}[b]	95% confidence limits	30min EC_{50}[b]	95% confidence limits
ZnO	0.10	0.03	1.84	1.04	11.52	1.41	$ZnSO_4x7H_2O$	0.02	0.01	7.00	0.90	2.68	0.09
Pd	0.41	0.26	>100	-	55.42	4.60	$PdCl_2$	0.03	0.01	NT	-	0.23[c]	0.02
CuO	0.43	0.06	2.00	1.14	1.78	0.10	$CuSO_4$	0.02	0.01	0.70	0.43	0.42	0.01
Co_3O_4	1.11	0.17	>100	-	>100	-	$CoCl_2x6H_2O$	0.10	0.01	NT	-	NT	-
TiO_2	1.26	0.23	52.62	12.97	>100	-	$TiOSO_4xH_2SO_4xH$	NT	-	4.00[d]	0.62	NT	-
Mn_3O_4	1.34	0.26	>100	-	>100	-	$MnCl_2x4H_2O$	14.32	1.72	NT	-	NT	-
Fe_3O_4	1.93	0.69	26.03	5.71	>100	-	$FeSO_4x7H_2O$	23.14	10.22	7.25	3.23	NT	-
Al_2O_3	30.80	14.13	>100	-	>100	-	$AlCl_3x4H_2O$	50.98	20.84	NT	-	NT	-
SiO_2	34.58	1.26	>100	-	>100	-	Na_2SiO_3	NT	-	NT	-	NT	-
WO_3	57.80	7.24	>100	-	87.07	12.88	$Na_2WO_4x2H_2O$	>100	-	>100	-	>100	-
Sb_2O_3	>100	-	>100	-	73.74	4.85	$SbCl_3$	NT	-	13.26[e]	7.62	2.03[f]	0.97
MgO	>100	-	>100	-	>100	-	MgCl2	NT	-	>100	-	>100	-

NT – not tested;

[a] The presented toxicity values are based on nominal exposure concentrations, mg compound/l.

[b] The presented toxicity values are based on nominal exposure concentrations, mg metal/l.

[c] Acidic – pH of the $PdCl_2$ solution at 125 and 1.9 mg metal/l in the test was 4.2 and 6.2, respectively.

[d] Acidic – pH of the $TiOSO_4xH_2SO_4xH$ solution at 100 mg metal/l was <4.5.

[e] Acidic – pH of the $SbCl_3$ solution at 100 mg metal/l in the test was <4.5.

[f] Acidic – pH of the $SbCl_3$ solution at 125 and 1.9 mg metal/l in the test was 2.9 and 6.0, respectively.

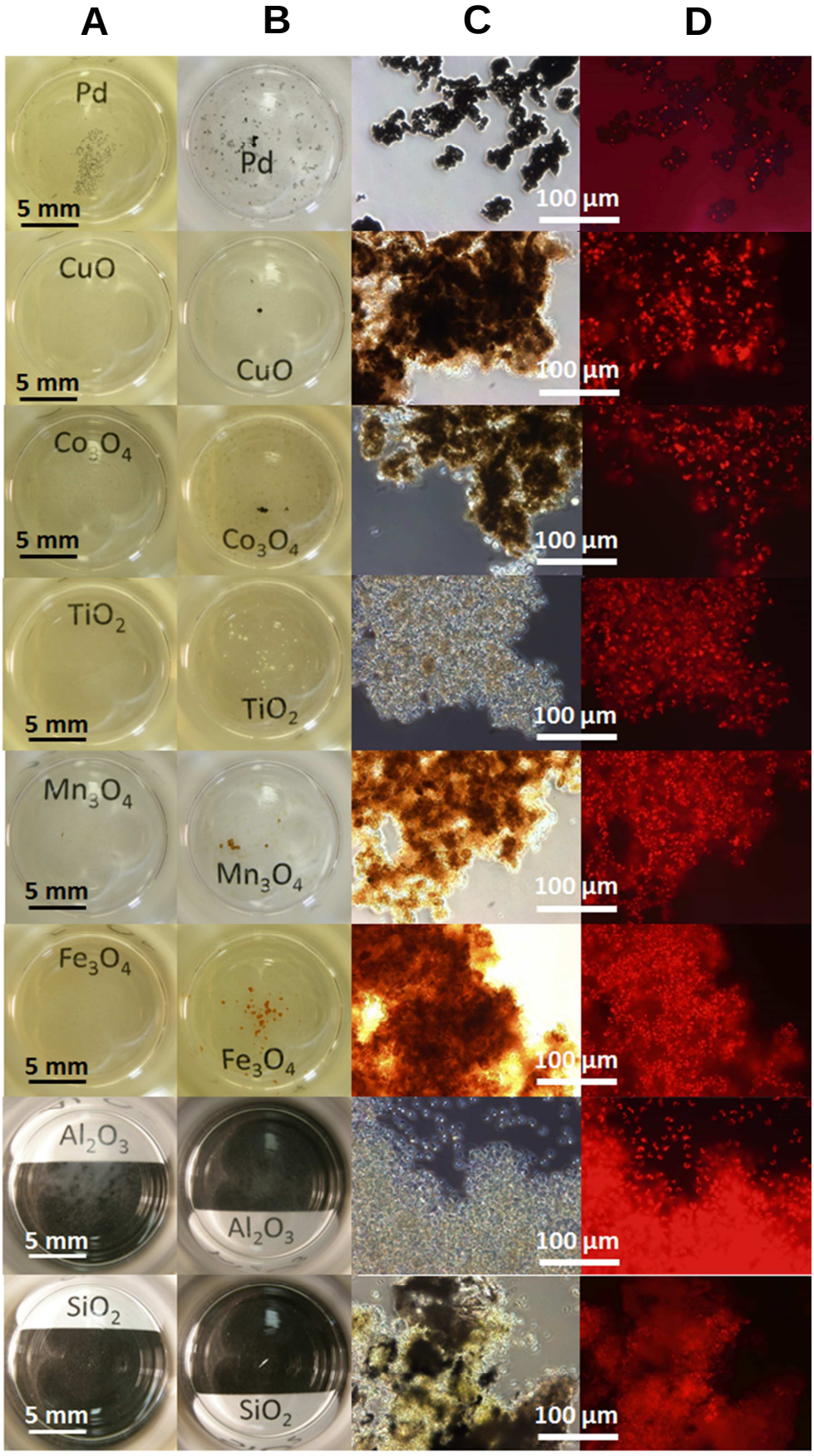


Figure S10. Nanoparticle agglomerates entrap algae. Nanoparticles in the algal medium without algal cells (A) and agglomerates that entrapped algal cells visible with a naked eye (B), in phase contrast microscope (C) and fluorescence microscope (D).

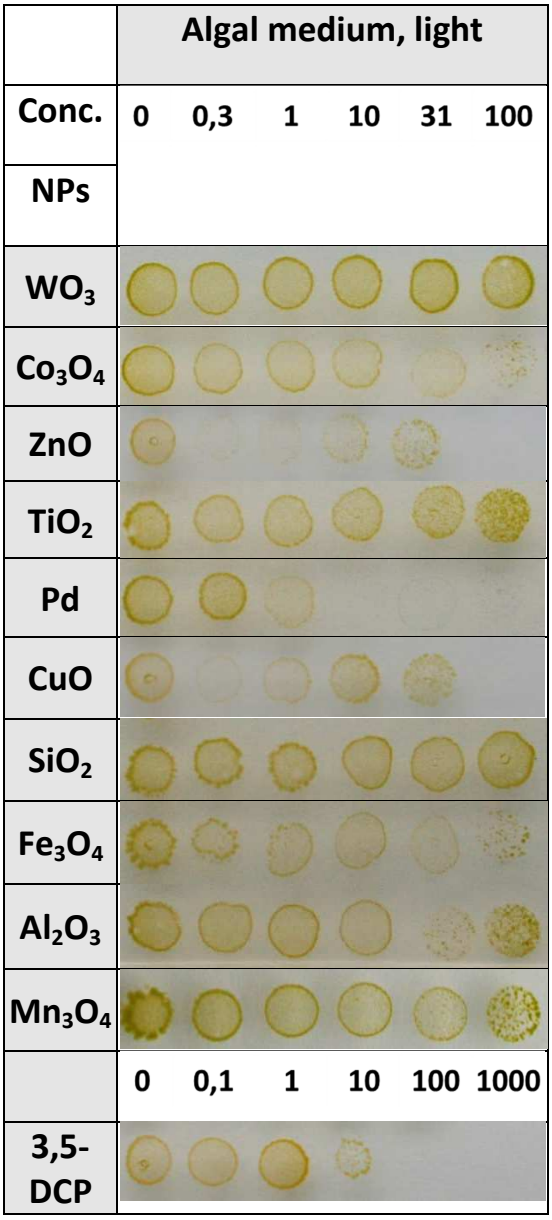


Figure S11 Colony-forming ability of *Pseudokirchneriella subcapitata* after 24 h exposure to nanoparticles (NPs) in algal medium (OECD 201) under illumination at 25◦C. After the 24 h exposure, 5 µl of cells was transferred onto toxicant-free agarized algal growth medium. The growth of the alage was evaluated visually after incubation of the agar plates for 7-10 days at 25 °C under illumination. The concentrations of the NPs are given as mg compound/l.

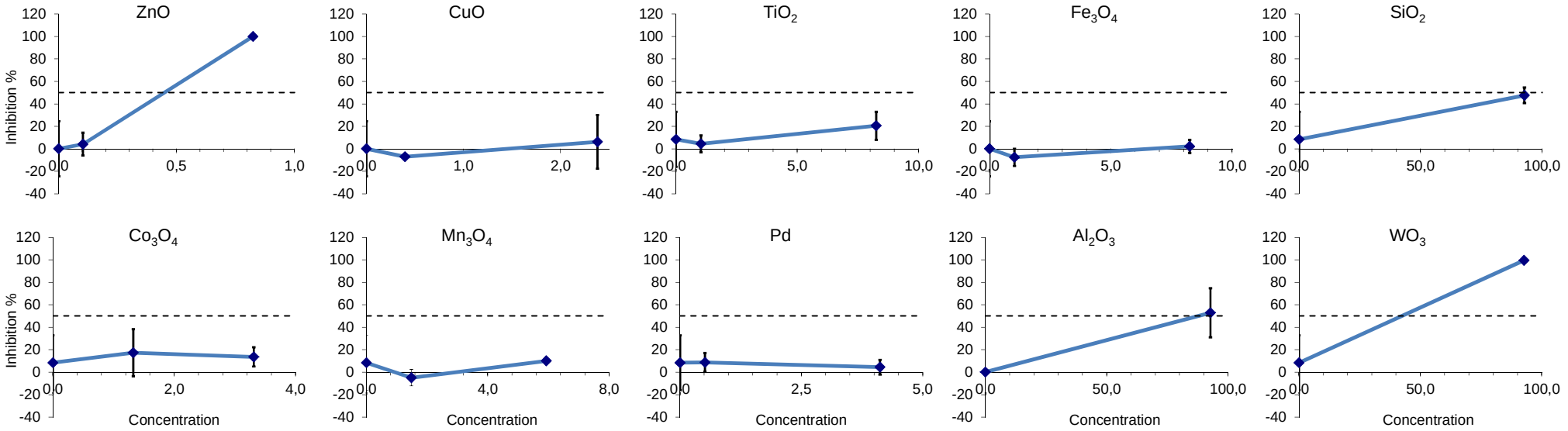


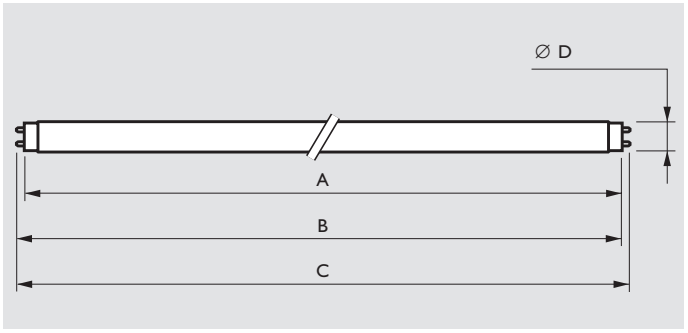
Figure S12 Growth inhibtion of *Pseudokirchneriella subcapitata* by supernatants of nanoparticle suspensions

Special fluorescent lamps

Aquarelle

'TL'D Ø 26 mm

'TL' Miniature



Dimensions in mm

The well-defined light spectrum of this low-pressure mercury vapour fluorescent lamp is optimally tuned to reveal the true beauty of fish and plant life in freshwater aquaria.
The Aquarelle light output closely matches the natural light wavelength which is required for successful photosynthesis and chlorophyll synthesis.
An additional benefit of Aquarelle is the particularly high energy in the blue region providing a well-balanced spectrum which stimulates oxygen production, promotes thriving aquarium plant and fish life and gives a good colour rendering.

According to personal preference, Aquarelle can be combined with Philips 'TL'D/80 New Generation or 'TL'D/90 de Luxe colours to create different visual impressions without impairing the biological qualities of the Aquarelle light output.
Lamps are to be operated on AC mains on switch-start or HF control gear.

Applications

- Freshwater aquaria.

Type	A max.	B min.	B max.	C max.	D max.
Cap/base G5					
'TL' 8W /89	288.3	293.0	295.4	302.5	16.0
Cap/base G13					
'TL'D 14W /89	361.2	365.9	368.3	375.4	28.0
'TL'D 15W /89	437.4	442.1	444.5	451.6	28.0
'TL'D 18W /89	589.8	594.5	596.9	604.0	28.0
'TL'D 25W /89	740.0	744.7	747.1	754.2	28.0
'TL'D 30W /89	894.6	899.3	901.7	908.8	28.0
'TL'D 36W /89	1199.4	1204.1	1206.5	1213.6	28.0
'TL'D 38W /89	1047.0	1051.7	1054.1	1061.2	28.0
'TL'D 58W /89	1500.0	1504.7	1507.1	1514.2	28.0

Type	Cap/ base	Lamp voltage V	Lamp current A	Colour rendering index	Correlated colour temp. K	Lumen output lm	Useful life h	Nett weight g	Ordering number	EOC
/89										
'TL' 8W /89	G5	56	0.15	70	10000	340	8000	29	9280 010 08900	627810
'TL'D 14W /89	G13	45	0.38	70	10000	600	8000	66	9280 243 08900	642530
'TL'D 15W /89	G13	51	0.34	70	10000	750	8000	76	9280 248 08900	620217
'TL'D 18W /89	G13	59	0.36	70	10000	1020	8000	100	9280 480 08900	620248
'TL'D 25W /89	G13	82	0.38	70	10000	1440	8000	85	9280 244 08900	640321
'TL'D 30W /89	G13	98	0.36	70	10000	1820	8000	145	9280 254 08900	620279
'TL'D 36W /89	G13	103	0.44	70	10000	2450	8000	186	9280 485 08900	620309
'TL'D 38W /89	G13	104	0.43	70	10000	2380	8000	162	9280 457 08900	628718
'TL'D 58W /89	G13	111	0.67	70	10000	3800	8000	233	9280 490 08900	620330

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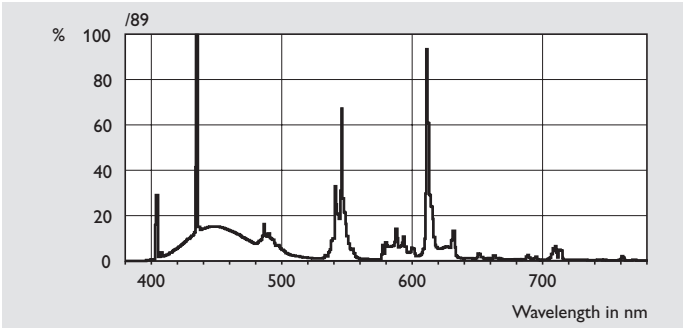

Spectral power distribution

PHILIPS