

# **Supplementary data**

## ***Corbicula fluminea* gene expression modulated by CeO<sub>2</sub> nanomaterials and salinity**

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Gene Name	Accession Number (GenBank)	Forward (5'-3')	Reverse (5'-3')	Amplicon length (bp)	Concentration used for qPCR (nM)	GO biological process	Reference
Beta Actine	EF446608.1	CCTTTACCACAAACAGCCGA	AGCATAGCCTCAGGGCA	191	300	Cell morphogenesis - mitotic cytokinesis - ...	Arini et col. 2014
Catalase	EF446609	CCTTCAGTGCCTAGTCTGG	CCTGTTCTCTGACCGTGG	196	200	Response to oxidative stress	Arini et col. 2014
Cu-Zn Superoxide dismutase	KF218347.1	GAATGTTGTGGCTGATGAGG	GGATCGTTCTCCTGTCAATG	76	250	Response to oxidative stress	Unpublished probes
GABARAP	KF218343	AAAGCCCGTGTAGGAGACTTG	AAGAGATCCCATACTCGCACT	150	250	Autophagosome assembly	Chen et col, 2013
Se-GPx	EF547366.1	TATGCCCTGTTCCCGTGTTC	CTGCCATCTGGACCAATCAG	148	200	Response to oxidative stress	Chen et col. 2015
GST-Pi	AN AY885667.1	CTGGGCGACCGAATACAAATC	GATGGCATTGGACTGGGCTA	90	250	Response to reactive oxygen species	Chen et col. 2015
HSP70	KC979064	CGCCGACGCTGATTACCTTA	AAACGGTTGATAGGACGCAAG	84	200	Cellular response to stress, heat, protein refolding...	Chen et col, 2013
Metallothionein (isoform 1)	EF185126	CGGCTATCTCCCGCGA	AGCTTTACCAGAACCAAACAGT	210	200	Cellular metal ion homeostasis, cellular response to oxidative stress	Arini et col. 2014
Mn Superoxide dismutase	EF446611	CTGTAGGTAGTAAGCGTGCTCC	GGATCATTGACGAGATGAAGGC	200	200	Response to oxidative stress	Arini et col. 2014
MRP1	KJ027659	GTGGTCGGGTCTGTTGGTA	AGGGTGGCGTTCTGTATCC	139	200	Drug transmembrane transport, xenobiotic transport	Chen et col. 2015
PGP / MDR	KJ001772	ATCCTGGTTGATGGCACTGA	AGGTTCCGGCTCACAAATACC	80	200	Xenobiotic transport	Chen et col. 2015
Ribosomal protein S3	AJ517687	CAGTGTGAGTCCTGAGATACAAG	AAACGGTTGATAGGACGCAAG	154	250	Translation (Used as housekeeping gene)	Navarro et col., 2001
Thioredoxin reductase	KF275126	AATGCGGTGGCTTCCTACA	TCTGCTGGTCAAATCCTCGC	89	200	Cell redox homeostasie	Chen et col. 2015

Table S1: Primer sequences used. Cited references: Navarro A, Faria M, Barata C, Piña B. 2011. Transcriptional response of stress genes to metal exposure in zebra mussel larvae and adults. Environ Pollut. 159, 100-7. ; Arini A, Daffe G, Gonzalez P, Feurtet-Mazel A, Baudrimont M. 2014. Detoxification and recovery capacities of Corbicula fluminea after an industrial metal contamination (Cd and Zn): a one-year depuration experiment. Environ Pollut. 192, 74-82. ; Chen H, Zha J, Liang X, Bu J, Wang M, Wang Z. 2013. Sequencing and de novo assembly of the Asian clam (Corbicula fluminea) transcriptome using the Illumina GAIIx method. PLoS One. 2013 8:e79516. ; Chen H, Zha J, Yuan L, Wang Z. 2015. Effects of fluoxetine on behavior, antioxidant enzyme systems, and multixenobiotic resistance in the Asian clam Corbicula fluminea. Chemosphere. 119, 856-62.

Pairwise comparison	Gills				Digestive gland			
	Control	NM-212	Envirox™	Combusted Envirox™	Control	NM-212	Envirox™	Combusted Envirox™
Gills	Control	0.62						
	NM-212	0.17	0.17					
	Envirox™	0.36	0.24	0.70				
Digestive gland	Control	< <b>0.05</b>	NT	NT	NT			
	NM-212	NT	< <b>0.05</b>	NT	NT	0.62		
	Envirox™	NT	NT	< <b>0.05</b>	NT	0.36	0.52	
	Combusted	NT	NT	NT	< <b>0.05</b>	0.70	0.91	0.36
	Envirox™							

Table S2: Pairwise comparison (Hotelling T<sup>2</sup> test) made on PLS-DA performed with whole data collected in organisms exposed 28 days to each contamination conditions in two organs (gills and digestive gland). Bold letters represent significant differences between groups. Non-tested conditions are shown as “NT”.

Day	Salinity	Condition	Beta-actin	Catalase	MRP1	MT	PGP	GST-Pi	GABARAP	CuZnSOD
7 days	1,5 PSU	Control	0.0 ± 0.4 NS	0.0 ± 0.7 NS	0.0 ± 0.3 ab	0.0 ± 0.5 NS	0.0 ± 0.7 NS	0.0 ± 0.4 NS	0.0 ± 0.5 NS	0.0 ± 0.4 a
		NM-212	-0.5 ± 0.4 NS	-0.4 ± 0.6 NS	0.2 ± 0.5 ab	-0.4 ± 0.5 NS	-0.2 ± 0.3 NS	-0.1 ± 0.4 NS	-0.1 ± 0.5 NS	0.2 ± 0.4 ab
		Envirox™	-0.4 ± 0.3 NS	-1.1 ± 0.5 NS	-0.5 ± 0.4 a	0.0 ± 0.3 NS	-0.7 ± 0.4 NS	-0.2 ± 0.3 NS	-0.7 ± 0.4 NS	-0.1 ± 0.3 a
	15 PSU	Combusted Envirox™	-0.6 ± 0.3 NS	-1.1 ± 0.6 NS	-0.1 ± 0.3 ab	0.3 ± 0.5 NS	-0.3 ± 0.3 NS	-0.5 ± 0.3 NS	-0.8 ± 0.2 NS	-0.2 ± 0.3 a
		Control	-1.0 ± 0.9 NS	-0.3 ± 0.5 NS	-0.4 ± 0.6 a	-0.1 ± 0.6 NS	-0.1 ± 0.6 NS	0.1 ± 0.5 NS	0.3 ± 0.7 NS	0.8 ± 0.6 b
		NM-212	-1.0 ± 0.8 NS	-0.4 ± 0.6 NS	-0.3 ± 0.4 ab	0.5 ± 0.4 NS	0.0 ± 0.4 NS	-0.1 ± 0.4 NS	-0.5 ± 0.5 NS	0.4 ± 0.4 ab
14 days	1,5 PSU	Envirox™	-0.6 ± 0.2 NS	-0.4 ± 0.5 NS	0.4 ± 0.3 b	0.2 ± 0.6 NS	0.2 ± 0.4 NS	-0.3 ± 0.2 NS	-0.3 ± 0.4 NS	0.4 ± 0.2 ab
		Combusted Envirox™	-0.5 ± 0.4 NS	-0.7 ± 0.7 NS	-0.3 ± 0.3 ab	-0.1 ± 0.4 NS	-0.6 ± 0.6 NS	-0.1 ± 0.3 NS	-0.6 ± 0.4 NS	0.4 ± 0.2 ab
		Control	0.00 ± 0.33 NS	0.00 ± 0.39 NS	0.00 ± 0.45 NS	0.00 ± 0.34 a	0.00 ± 0.64 NS	0.00 ± 0.28 bc	0.00 ± 0.29 NS	0.00 ± 0.32 NS
	15 PSU	NM-212	-0.23 ± 0.33 NS	-0.57 ± 0.43 NS	-0.46 ± 0.45 NS	0.45 ± 0.33 ab	-0.44 ± 0.44 NS	-0.16 ± 0.31 ab	0.39 ± 0.40 NS	-0.18 ± 0.43 NS
		Envirox™	0.27 ± 0.48 NS	0.29 ± 0.51 NS	-0.23 ± 0.45 NS	1.05 ± 0.40 b	-0.22 ± 0.47 NS	0.55 ± 0.42 c	0.49 ± 0.61 NS	0.05 ± 0.48 NS
		Combusted Envirox™	0.16 ± 0.42 NS	0.13 ± 0.50 NS	-0.21 ± 0.54 NS	0.82 ± 0.47 b	-0.47 ± 0.47 NS	0.20 ± 0.32 bc	0.38 ± 0.43 NS	-0.12 ± 0.41 NS
		Control	-0.26 ± 0.67 NS	0.11 ± 0.65 NS	-0.12 ± 0.50 NS	0.60 ± 0.56 ab	0.01 ± 0.51 NS	-0.61 ± 0.64 a	0.25 ± 0.57 NS	0.12 ± 0.64 NS
		NM-212	-0.02 ± 0.28 NS	0.05 ± 0.50 NS	-0.47 ± 0.36 NS	0.45 ± 0.49 ab	-0.17 ± 0.58 NS	-0.30 ± 0.41 ab	0.50 ± 0.67 NS	0.30 ± 0.37 NS
		Envirox™	0.11 ± 0.35 NS	0.50 ± 0.31 NS	0.04 ± 0.35 NS	0.73 ± 0.30 b	0.07 ± 0.42 NS	0.18 ± 0.20 bc	0.74 ± 0.45 NS	0.48 ± 0.14 NS
		Combusted Envirox™	0.28 ± 0.27 NS	0.17 ± 0.26 NS	0.23 ± 0.52 NS	0.64 ± 0.36 ab	0.33 ± 0.42 NS	-0.22 ± 0.34 ab	0.62 ± 0.29 NS	0.43 ± 0.34 NS

Table S3: Relative gene expression (expressed in log 2) of 8 genes (Beta-actin, catalase, MRP1, MT, PGP, GST-Pi, GABARAP & CuZnSOD) after mRNA extraction from gills of *C. fluminea* exposed in mesocosm during 7 and 14 days. Control 1.5 psu was chosen as reference for relative expression. Groups that do not share the same letter are significantly different. NS stands for no significant differences.

Day	Salinity	Condition	Beta-actin	Catalase	MRP1	MT	PGP	GST-Pi	GABARAP	CuZnSOD
21 days	1,5 PSU	Control	0.0 ± 0.3 NS	0.0 ± 0.2 NS	0.0 ± 0.4 NS	0.0 ± 0.3 NS	0.0 ± 0.4 NS	0.0 ± 0.3 NS	0.0 ± 0.3 <sup>a</sup>	0.0 ± 0.23 NS
		NM-212	-0.7 ± 0.8 NS	-0.7 ± 0.5 NS	-0.2 ± 0.3 NS	0.3 ± 0.2 NS	-0.3 ± 0.4 NS	0.0 ± 0.4 NS	0.1 ± 0.3 <sup>a</sup>	-0.2 ± 0.21 NS
		Envirox™	-0.1 ± 0.4 NS	-0.3 ± 0.4 NS	0.0 ± 0.4 NS	0.6 ± 0.4 NS	0.1 ± 0.6 NS	0.2 ± 0.4 NS	0.0 ± 0.4 <sup>a</sup>	0.1 ± 0.23 NS
	15 PSU	Combusted Envirox™	-0.1 ± 0.4 NS	0.2 ± 0.4 NS	-0.6 ± 0.3 NS	0.3 ± 0.2 NS	-0.1 ± 0.2 NS	-0.3 ± 0.3 NS	-0.1 ± 0.4 <sup>a</sup>	0.1 ± 0.23 NS
		Control	-0.5 ± 0.4 NS	-0.3 ± 0.3 NS	-0.3 ± 0.4 NS	0.1 ± 0.2 NS	-0.4 ± 0.4 NS	-0.2 ± 0.3 NS	-0.4 ± 0.3 <sup>ab</sup>	0.3 ± 0.20 NS
		NM-212	-0.3 ± 0.4 NS	-0.4 ± 0.4 NS	-0.9 ± 0.4 NS	0.5 ± 0.6 NS	-0.8 ± 0.5 NS	-0.1 ± 0.3 NS	-0.2 ± 0.4 <sup>ab</sup>	0.2 ± 0.19 NS
28 days	1,5 PSU	Envirox™	-0.6 ± 0.5 NS	-0.6 ± 0.3 NS	-0.8 ± 0.4 NS	0.2 ± 0.3 NS	-0.6 ± 0.4 NS	-0.3 ± 0.4 NS	-0.3 ± 0.5 <sup>ab</sup>	0.2 ± 0.42 NS
		Combusted Envirox™	-0.4 ± 0.7 NS	-0.6 ± 0.4 NS	-0.4 ± 0.6 NS	-0.2 ± 0.4 NS	-0.4 ± 0.5 NS	0.2 ± 0.4 NS	-1.0 ± 0.4 <sup>b</sup>	-0.3 ± 0.33 NS
		Control	0.0 ± 0.6 NS	0.0 ± 0.4 NS	0.0 ± 0.4 NS	0.0 ± 0.4 NS	0.0 ± 0.5 NS	0.0 ± 0.2 NS	0.0 ± 0.2 NS	0.0 ± 0.5 NS
	15 PSU	NM-212	0.1 ± 0.6 NS	0.8 ± 0.5 NS	-0.2 ± 0.4 NS	0.4 ± 0.4 NS	0.7 ± 0.6 NS	0.6 ± 0.4 NS	-0.1 ± 0.5 NS	0.5 ± 0.4 NS
		Envirox™	-0.8 ± 0.6 NS	-0.6 ± 0.5 NS	-0.5 ± 0.7 NS	-0.3 ± 0.7 NS	0.1 ± 0.5 NS	-0.4 ± 0.5 NS	-0.5 ± 0.5 NS	-0.3 ± 0.5 NS
		Combusted Envirox™	-0.7 ± 0.6 NS	-0.7 ± 0.6 NS	-0.4 ± 0.5 NS	-0.9 ± 0.6 NS	-0.4 ± 0.6 NS	-0.3 ± 0.5 NS	-0.7 ± 0.5 NS	-0.1 ± 0.5 NS
		Control	-0.4 ± 0.6 NS	0.3 ± 0.7 NS	0.1 ± 0.6 NS	-0.3 ± 0.6 NS	0.3 ± 0.5 NS	0.0 ± 0.5 NS	0.3 ± 0.5 NS	0.6 ± 0.5 NS
		NM-212	-1.1 ± 1.2 NS	0.4 ± 0.6 NS	0.1 ± 0.6 NS	0.5 ± 0.8 NS	0.7 ± 0.7 NS	0.0 ± 0.5 NS	0.3 ± 0.5 NS	0.5 ± 0.5 NS
		Envirox™	-0.3 ± 0.3 NS	0.4 ± 0.8 NS	0.2 ± 0.5 NS	0.2 ± 0.5 NS	0.3 ± 0.4 NS	-0.1 ± 0.4 NS	0.1 ± 0.4 NS	0.6 ± 0.3 NS
		Combusted Envirox™	-0.4 ± 0.4 NS	0.1 ± 0.4 NS	-0.2 ± 0.6 NS	0.7 ± 0.5 NS	0.4 ± 0.7 NS	0.1 ± 0.4 NS	0.1 ± 0.4 NS	0.8 ± 0.4 NS

Table S4: Relative gene expression (expressed in log 2) of 8 genes (Beta-actin, catalase, MRP1, MT, PGP, GST-Pi, GABARAP & CuZnSOD) after mRNA extraction from gills of *C. fluminea* exposed in mesocosm during 21 and 28 days. Control 1.5 psu was chosen as reference for relative expression. Groups that do not share the same letter are significantly different. NS stands for no significant differences.

Day		1.5 psu			15 psu		
		Control	Envirox™	Combusted Envirox™	Control	Envirox™	Combusted Envirox™
<b>Day 7</b>	Envirox™	< <b>0.05</b>					
1.5 psu	Combusted Envirox™	< <b>0.05</b>	< <b>0.05</b>				
	NM-212	0.11	< <b>0.05</b>	< <b>0.05</b>			
	Envirox™				< <b>0.05</b>		
15 psu	Combusted Envirox™				0.06	0.31	
	NM-212				0.17	0.06	0.31
<b>Day 14</b>	Envirox™	< <b>0.05</b>					
1.5 psu	Combusted Envirox™	< <b>0.05</b>	< <b>0.05</b>				
	NM-212	< <b>0.05</b>	< <b>0.05</b>	< <b>0.05</b>			
	Envirox™				< <b>0.05</b>		
15 psu	Combusted Envirox™				0.05	0.06	
	NM-212				0.06	0.06	< <b>0.05</b>
<b>Day 21</b>	Envirox™	< <b>0.05</b>					
1.5 psu	Combusted Envirox™	< <b>0.05</b>	< <b>0.05</b>				
	NM-212	< <b>0.05</b>	0.15	< <b>0.05</b>			
	Envirox™				0.92		
15 psu	Combusted Envirox™				< <b>0.05</b>	< <b>0.05</b>	
	NM-212				0.26	0.26	< <b>0.05</b>
<b>Day 28</b>	Envirox™	< <b>0.05</b>					
1.5 psu	Combusted Envirox™	0.22	0.11				
	NM-212	< <b>0.05</b>	0.11	< <b>0.05</b>			
	Envirox™				0.57		
15 psu	Combusted Envirox™				0.80	0.80	
	NM-212				0.22	0.21	0.22

Table S5: Pairwise comparisons (Hotelling T<sup>2</sup> test) made on PLS-DA performed at 7, 14, 21 and 28 days of exposure to each contamination. Data were treated time per time and salinity was treated separately for each time step. Bold letters represent significant differences between groups.