

Supplementary Materials for

Genotoxic effects on *Daphnia magna* fed with aquatic green algae exposed to silver nanoclusters

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Table S1. The composition of SE culture medium.

No.	Ingredient	Concentration of stock solution	Usage
1	NaNO ₃	25g/100mL of ultrapure water	1mL/L
2	K ₂ HPO ₄	7.5g/100mL of ultrapure water	1mL/L
3	MgSO ₄ ·7H ₂ O	7.5g/100mL of ultrapure water	1mL/L
4	CaCl ₂ ·2H ₂ O	2.5g/100mL of ultrapure water	1mL/L
5	KH ₂ PO ₄	17.5g/100mL of ultrapure water	1mL/L
6	NaCl	2.5g/100mL of ultrapure water	1mL/L
7	FeCl ₃ ·6H ₂ O	0.5g/100mL of ultrapure water	1mL/L
8	EDTA-Fe	① 4.1 mL of concentrated hydrochloric acid was diluted with 50 mL of ultrapure water (solution one); ② 0.9306g of EDTA-Na ₂ was dissolved into 50 mL of ultrapure water (solution two); ③ 0.901g of FeCl ₃ ·6H ₂ O was dissolved into 10 mL of the solution one (solution three); ④ 10 mL of solution two was added into solution three (solution four); ⑤ Solution four was then diluted to 1L of ultrapure water (stock solution).	1mL/L
9	H ₃ BO ₃ MnCl ₂ ·4H ₂ O ZnSO ₄ ·7H ₂ O Na ₂ MoO ₄ ·2H ₂ O CuSO ₄ ·5H ₂ O Co(NO ₃) ₂ ·6H ₂ O	2.86g/100mL of ultrapure water 1.86g/100mL of ultrapure water 0.22g/100mL of ultrapure water 0.39g/100mL of ultrapure water 0.08g/100mL of ultrapure water 0.05g/100mL of ultrapure water	1mL/L
10		① 200g of garden soil with fertilizer was dissolved into 1 L of ultrapure water; ② The mixture was heated in boiling water for 3h, cooled down, and was deposited for 24h; ③ The second step was done in triplicate, and the mixture was then filtered to obtain the supernatant; ④ The supernatant was sterilized in a SANYO autoclave (121 °C, 30 min) and stored at 4 °C before use.	40mL/L

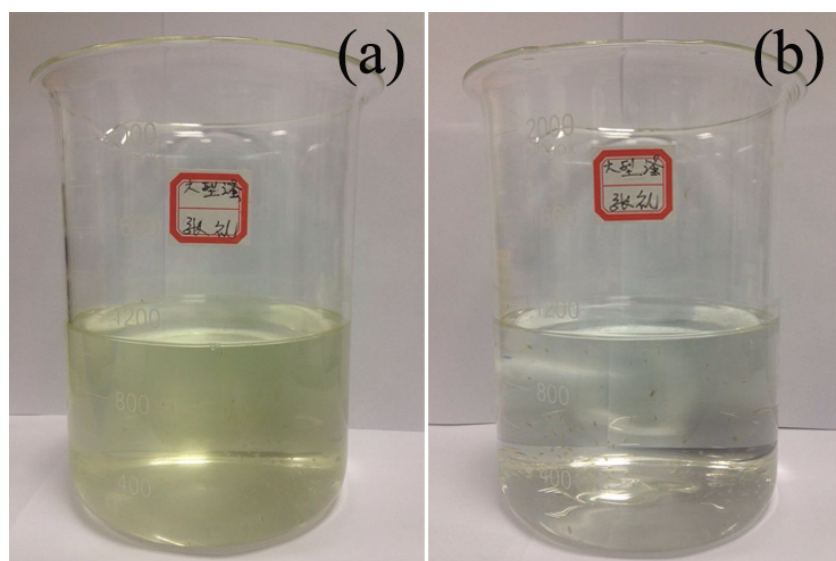


Figure S1. The toxicity test of *Daphnia magna* through the diet of *Scenedesmus obliquus* treated with silver nanoparticles, and two photos representing the beginning (a) and end (b) of toxicity test.

Table S2. Total RNA was checked using a NanoDrop 2000 UV-vis Spectrophotometer. Samples A, B, C and D represent the control (*Daphnia magna* from the diet of green algae only), *Daphnia magna* from the diet of green algae treated with 135 $\mu\text{g/L}$ r-AgNCs (without 0.5 mM of L-cysteine), *Daphnia magna* from the diet of green algae treated with 135 $\mu\text{g/L}$ r-AgNCs (with 0.5 mM of L-cysteine) and *Daphnia magna* from the diet of green algae treated with 10 $\mu\text{g/L}$ silver ions, respectively.

Swimming Lane (No.)	Samples	Concentration (ng/ μL)	260/280	260/230	Volume (μL)	Total RNA (μg)
1	Sample A	247.31	2.38	0.88	40.00	9.89
2	Sample B	271.21	2.38	0.93	40.00	10.85
3	Sample C	384.49	2.27	1.30	40.00	15.38
4	Sample D	277.78	2.34	1.14	40.00	11.11

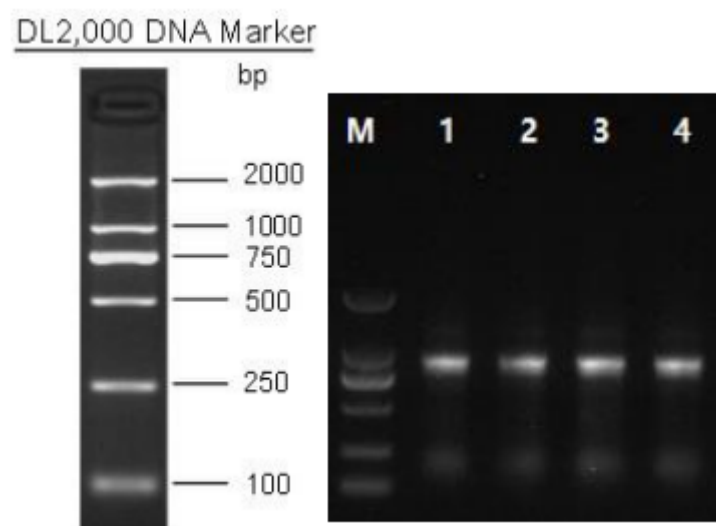


Figure S2. Total RNA was checked by agarose gel electrophoresis. Swimming lane numbers (1~4) represent the control (*Daphnia magna* from the diet of green algae only), *Daphnia magna* from the diet of green algae treated with 135 $\mu\text{g/L}$ r-AgNCs (without 0.5 mM of L-cysteine), *Daphnia magna* from the diet of green algae treated with 135 $\mu\text{g/L}$ r-AgNCs (with 0.5 mM of L-cysteine) and *Daphnia magna* from the diet of green algae treated with 10 $\mu\text{g/L}$ silver ions, respectively. Each treatment was done in biological quadruplicates.

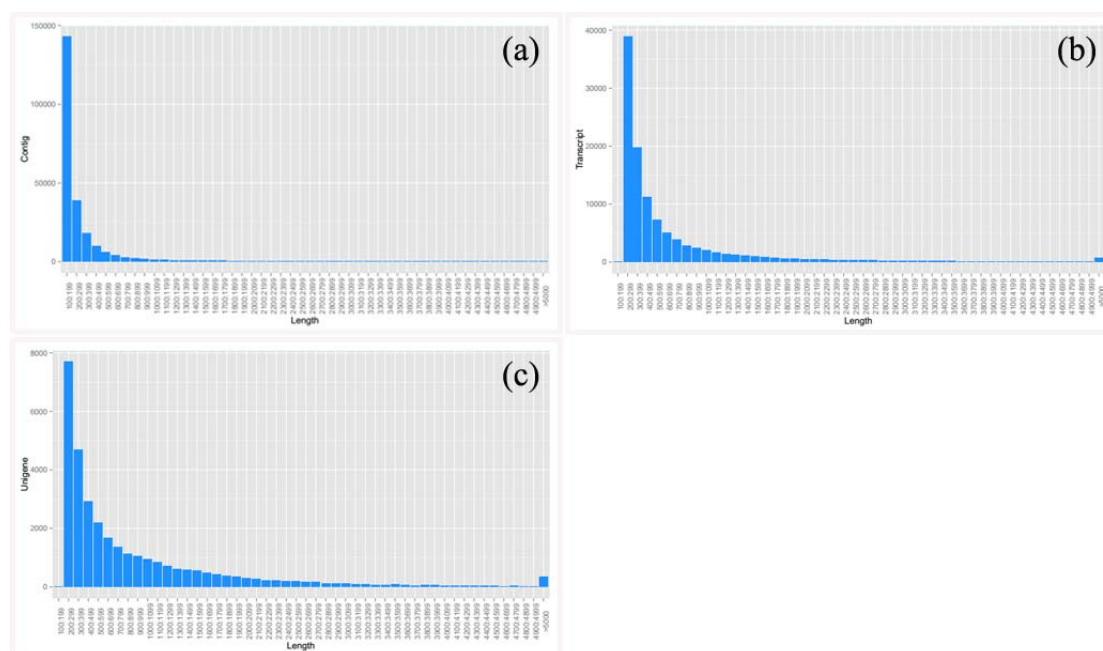


Figure S3. Histogram of length distribution of contiges (a), transcripts (b) and unigenes (c), with the number of contiges, transcripts and unigenes on the y-axis and the length distribution on the x-axis.

Table S3. Illumina de novo assembly statistics of transcriptomic profiles of *Daphnia magna* from green algae diet. (1) Samples A, B, C and D represent the control (*Daphnia magna* from the diet of green algae only), *Daphnia magna* from the diet of green algae treated with 135 µg/L r-AgNCs (without 0.5 mM of L-cysteine), *Daphnia magna* from the diet of green algae treated with 135 µg/L r-AgNCs (with 0.5 mM of L-cysteine) and *Daphnia magna* from the diet of green algae treated with 10 µg/L silver ions, respectively. (2) Q20: proportion of nucleotides with a quality value > 20 in raw sequencing reads. Q30: proportion of nucleotides with a quality value > 30 in raw sequencing reads. N50: unigene length-weighted median. GC %: percentage of G and C bases.

Raw Sequencing Reads							
Samples	Reads	Raw Reads	Raw Data (bp)	Q20 (%)	Q30 (%)	N%	
A	Paired	34,015,174	5,102,276,100	98.26	95.93	0.000264	
B	Paired	45,970,242	6,895,536,300	98.29	95.9	0.000211	
C	Paired	34,935,830	5,240,374,500	98.3	95.97	0.000251	
D	Paired	45,869,432	6,880,414,800	98.36	96.05	0.000186	
High Quality Reads							
Samples	Reads	Clean Reads No.	Clean Data (bp)	Clean Reads %	Clean Data %		
A	Paired	33,908,648	5,008,006,022	99.68	98.15		
B	Paired	45,829,500	6,731,987,734	99.69	97.62		
C	Paired	34,803,358	5,106,294,418	99.62	97.44		
D	Paired	45,735,154	6,728,898,660	99.7	97.79		
Sequence Assembly Statistic							
	Total Length (bp)	Sequence No.	Max. Length (bp)	Ave. Length (bp)	N50 (bp)	> N50 Reads No.	GC %
Contig	221,287,727	3,596,325	23,735	62	48	859,954	50.4
Transcript	72,901,724	108,954	23,712	669	1,009	17,277	48.7
Unigene	29,549,817	32,009	23,712	923	1,517	5,454	49.6

Table S4. Annotation of unigenes of *Daphnia magna* transcriptome.

Database	Numbers of annotated unigenes	Percentage of annotated unigenes
NR	32,009	100 %
GO	22,532	70.39 %
KEGG	5,761	18 %
eggNOG	29,260	91.41 %
Swissprot	26,943	84.17 %
In all database	4,841	15.12 %

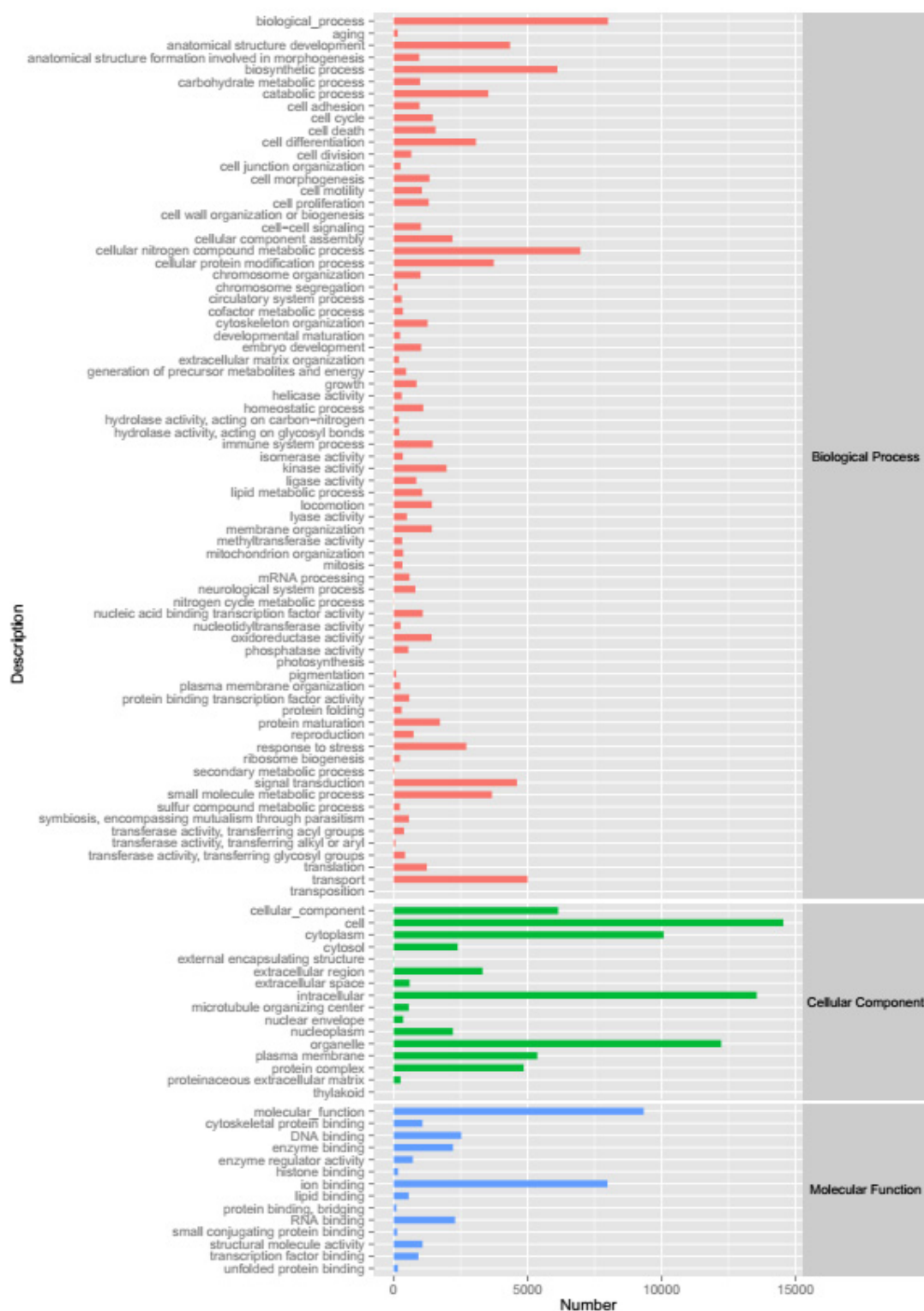


Figure S4. The Gene Ontology (GO) annotations of unigenes. The bars represent the number of unigenes (x-axis) in different GO terms (y-axis).

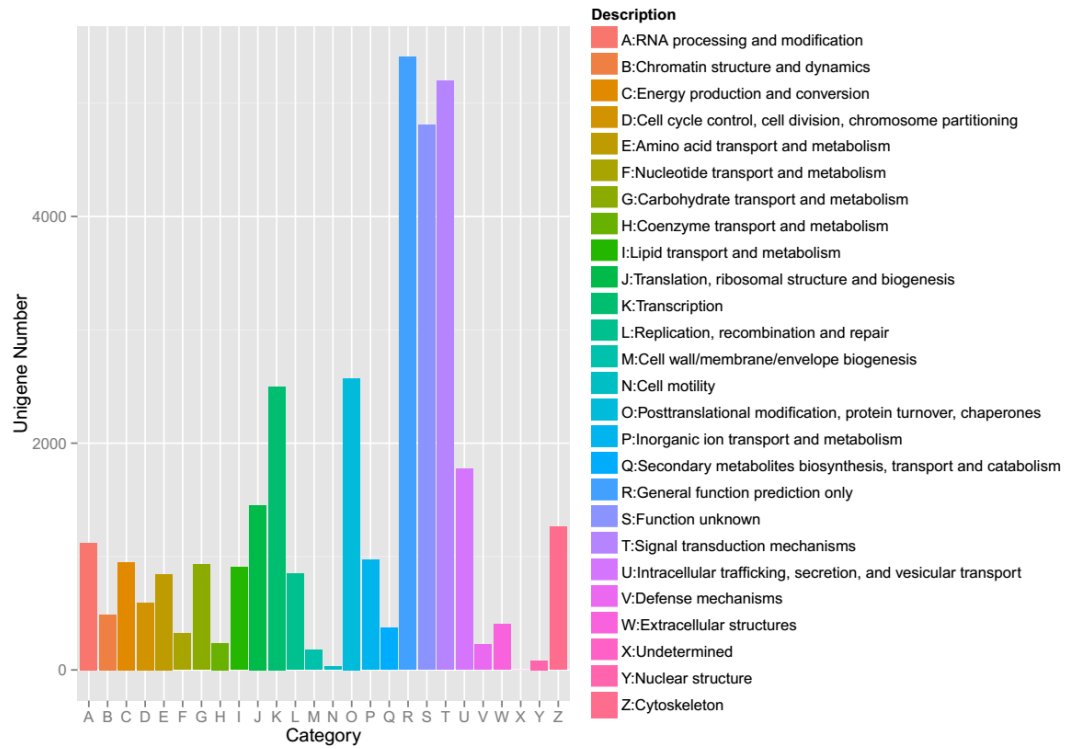


Figure S5. The Evolutionary genealogy of genes: Non-supervised Orthologous Groups (eggNOG) annotations of unigenes. The bars represent the number of unigenes (y-axis) in different eggNOG categories (x-axis).

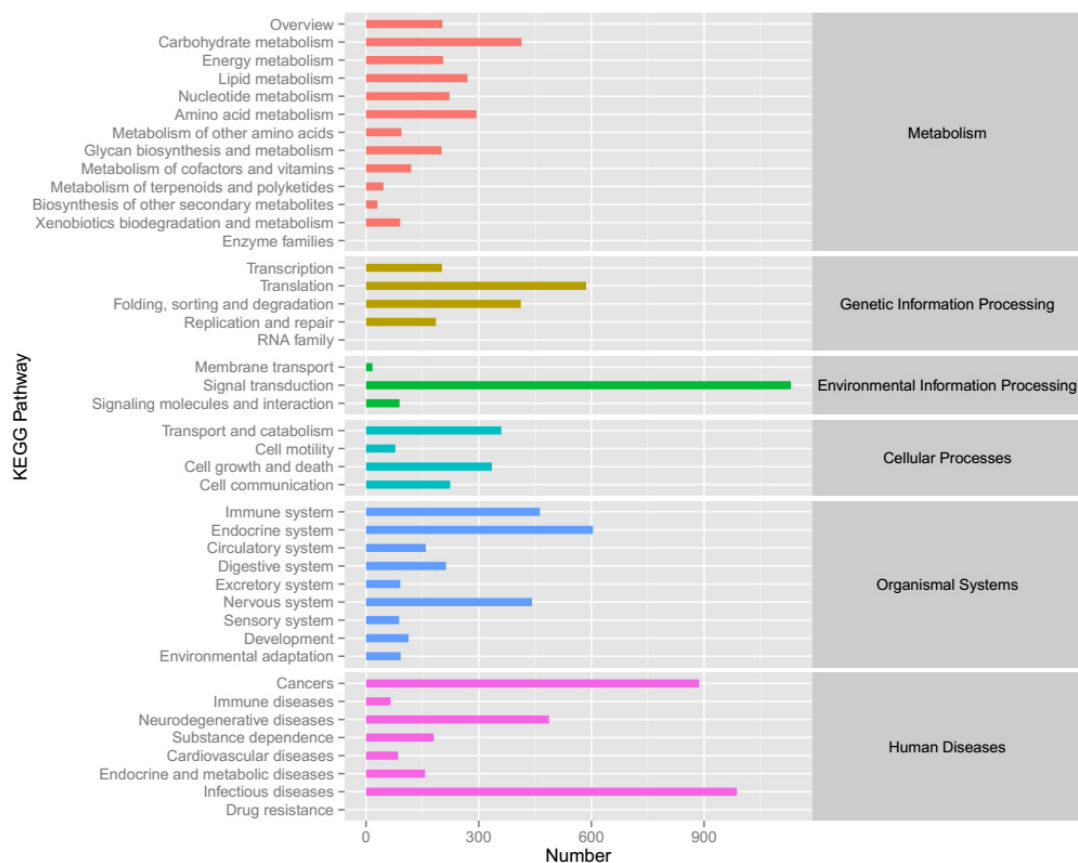


Figure S6. The Kyoto Encyclopedia of Genes and Genomes (KEGG) annotations of unigenes. The bars represent the number of unigenes (x-axis) in different KEGG metabolic pathways (y-axis).

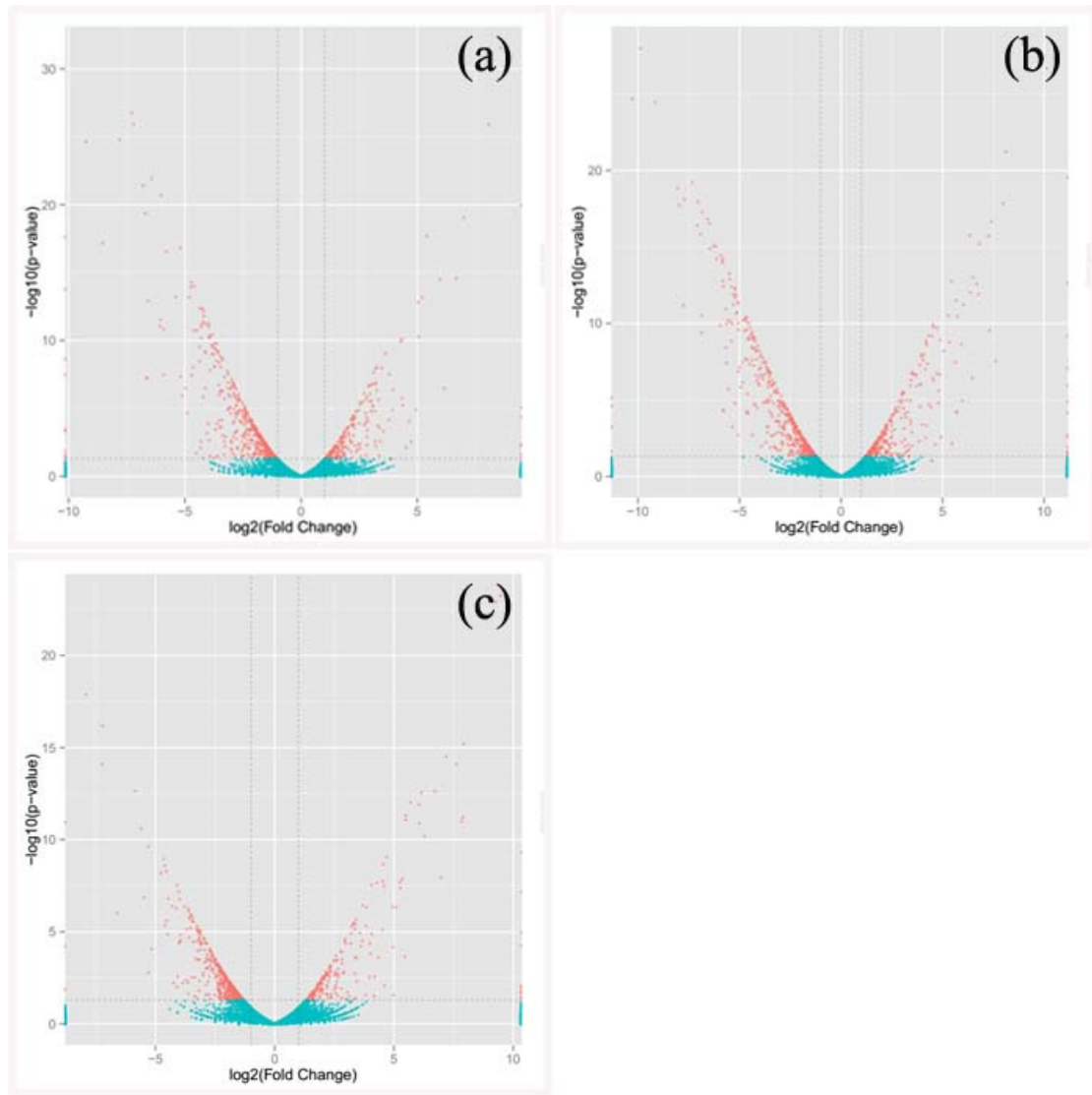


Figure S7. “Volcano plot” of $\log_2(\text{fold change})$ versus $-\log_{10}(p\text{-value})$ of differentially expressed genes derived from *Daphnia magna* fed a diet of green algae treated with 135 $\mu\text{g/L}$ r-AgNCs (without 0.5 mM of L-cysteine) (a), 135 $\mu\text{g/L}$ r-AgNCs (with 0.5 mM of L-cysteine) (b) and 10 $\mu\text{g/L}$ silver ions (c), compared to the control (*Daphnia magna* from the diet of green algae only), respectively. Red spots and blue spots represent differentially expressed genes ($|\text{fold change}| > 2$ and $p\text{-value} < 0.05$) and no difference in gene expression, respectively.

Table S5. List of differentially expressed genes for the digestive system and lipid metabolism of *Daphnia magna* after being fed a diet of algae treated with silver for 48h. Shades of red, green and blue indicate up- and down-regulation of target genes from each silver treatment compared with the controls and no differential expression of genes (Unigenes, background genes), respectively.

Digestive system of <i>Daphnia magna</i>					
Pancreatic secretion					
Gene ID	Gene Symbol	Ag NCs	Ag NCs + L-cysteine	Ag ions	Gene Description
c75537_g1_i1	PLA2G, SPLA2				secretory phospholipase A2
c22033_g1_i2	PLA2G, SPLA2				secretory phospholipase A2
c36635_g1_i1	CLCA4				calcium-activated chloride channel regulator 4
c27143_g2_i1	ATP1A				sodium/potassium-transporting ATPase subunit alpha
c35469_g1_i2	ADCY3				adenylate cyclase 3
Bile secretion					
c26535_g1_i1	HMGCR				hydroxymethylglutaryl-CoA reductase (NADPH)

c27143_g2_i1	ATP1A				sodium/potassium-transporting ATPase subunit alpha
c35469_g1_i2	ADCY3				adenylate cyclase 3
Protein digestion and absorption					
c28996_g1_i1	COL1A5				collagen, type I/II/III/V/XI/XXIV/XXVII, alpha
c36721_g2_i4	COL9A				collagen, type IX, alpha
c27143_g2_i1	ATP1A				sodium/potassium-transporting ATPase subunit alpha
c30005_g1_i1	SLC16A10				MFS transporter, MCP family, solute carrier family 16 (monocarboxylic acid transporters), member 10
c33004_g1_i1	SLC8A, NCX				solute carrier family 8 (sodium/calcium exchanger)
Fat digestion and absorption					
c22033_g1_i2	PLA2G, SPLA2				secretory phospholipase A2
c75537_g1_i1	PLA2G, SPLA2				secretory phospholipase A2
Lipid metabolism of <i>Daphnia magna</i>					

Fatty acid elongation					
Gene ID	Gene Symbol	Ag NCs	Ag NCs + L-cysteine	Ag ions	Gene Description
c34043_g1_i2	HSD17B12, KAR, IFA38				17beta-estradiol 17-dehydrogenase / very-long-chain 3-oxoacyl-CoA reductase
c27461_g1_i1	ELOVL4				elongation of very long chain fatty acids protein 4
c14600_g1_i2	ELOVL4				elongation of very long chain fatty acids protein 4
Cutin, suberine and wax biosynthesis					
c41268_g1_i1	FAR				fatty acyl-CoA reductase
c52253_g1_i1	FAR				fatty acyl-CoA reductase
c8335_g1_i1	FAR				fatty acyl-CoA reductase
Steroid biosynthesis					
c30643_g1_i1	LIPA				lysosomal acid lipase/cholesteryl ester hydrolase
c35983_g1_i1	LIPA				lysosomal acid lipase/cholesteryl ester hydrolase
Steroid hormone biosynthesis					
c34043_g1_i2	HSD17B12, KAR, IFA38				17beta-estradiol 17-dehydrogenase / very-long-chain 3-oxoacyl-CoA reductase
c73788_g1_i1	UGT				glucuronosyltransferase

c35122_g1_i1	UGT				glucuronosyltransferase
Glycerophospholipid metabolism					
c41909_g1_i1	ACHE				acetylcholinesterase
c30655_g1_i4	DGK, dgkA				diacylglycerol kinase (ATP dependent)
c75537_g1_i1	PLA2G, SPLA2				secretory phospholipase A2
c22033_g1_i2	PLA2G, SPLA2				secretory phospholipase A2
c26159_g1_i1	ACHE				acetylcholinesterase
Ether lipid metabolism					
c75537_g1_i1	PLA2G, SPLA2				secretory phospholipase A2
c22033_g1_i2	PLA2G, SPLA2				secretory phospholipase A2
Arachidonic acid metabolism					
c75537_g1_i1	PLA2G, SPLA2				secretory phospholipase A2

c22033_g1_i2	PLA2G, SPLA2				secretory phospholipase A2
Linoleic acid metabolism					
c75537_g1_i1	PLA2G, SPLA2				secretory phospholipase A2
c22033_g1_i2	PLA2G, SPLA2				secretory phospholipase A2
alpha-Linolenic acid metabolism					
c22033_g1_i2	PLA2G, SPLA2				secretory phospholipase A2
c75537_g1_i1	PLA2G, SPLA2				secretory phospholipase A2