Supplementary data for

Toxicity of mercuric chloride, when combined with ionic and nanoparticulate silver on *Artemia salina*: Growth, fatty acid composition, oxidative stress, and lipid peroxidation

Nahid Ravantab[a], Zahra Ghasemi[a, b, *], Seyed Ali Johari [c], Richard D. Handy[d]

[a] Department of Fisheries, Faculty of Marine Science and Technology, University of Hormozgan, Bandar Abbas, Iran
[b] Nanoscience, Nanotechnology and Advanced Materials Research Centre, University of Hormozgan, Bandar Abbas, Iran
[c] Department of Fisheries, Faculty of Natural Resources, University of Kurdistan, Sanandaj, Kurdistan, Iran
[d] School of Biological and Marine Sciences, University of Plymouth, UK.

***Corresponding author. E-mail:** z.ghasemi@hormozgan.ac.ir, zghasemi446@yahoo.com

S1: Fig. *S1* and *S2* show the X-ray diffraction (XRD) and scanning electron microscopy (SEM) of silver nanoparticles (AgNPs**)** respectively.

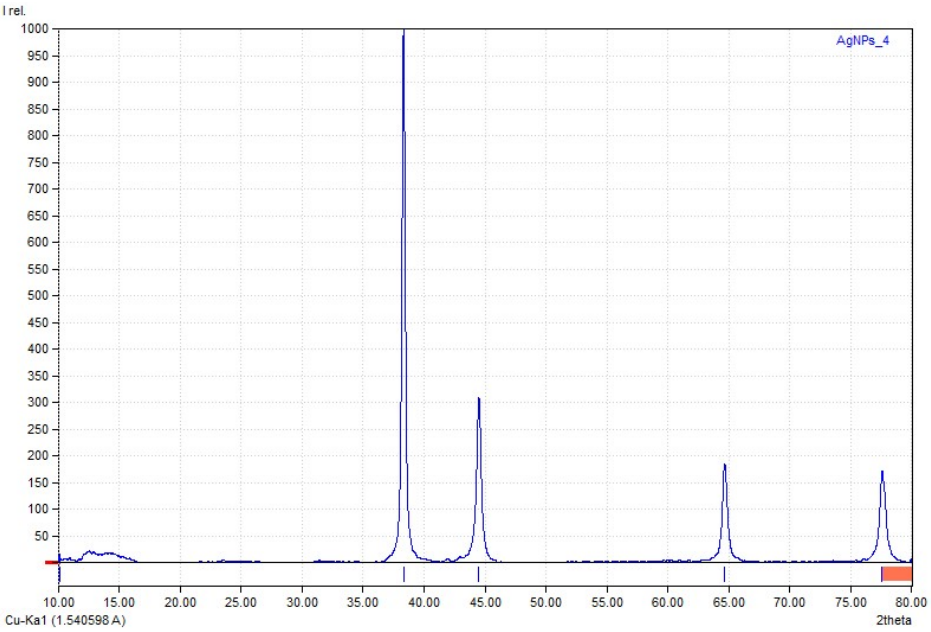


Fig. *S1.* XRD spectra of AgNPs

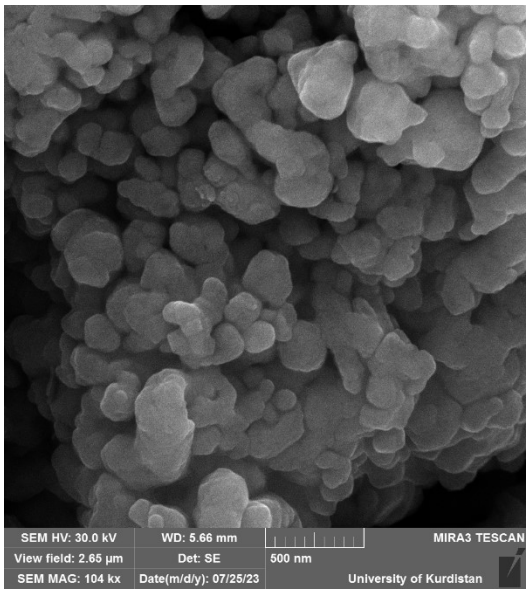


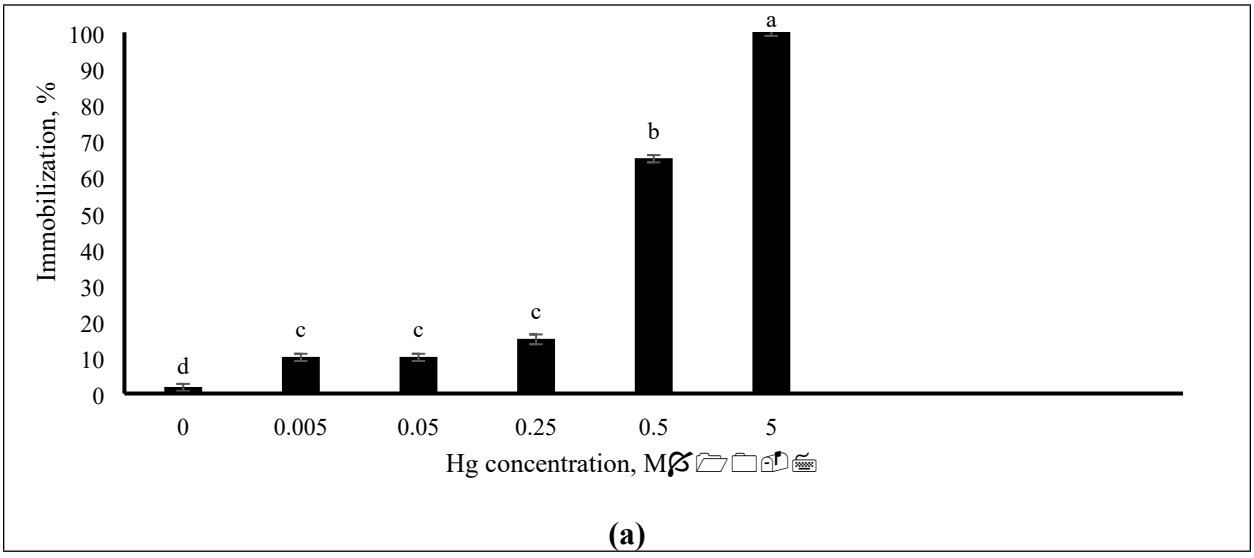
Fig. *S2.* SEM image of AgNPs

S2: SOD activity was quantified using the protocol explained by Kono (1978). Reagents including 50 mM Na_2CO_3 (pH 10), 96 mM NBT, 0.6% Triton X-100, and 20 mM hydroxylamine hydrochloride were added to 70 µL of the obtained supernatant. The mixtures

were incubated under visible illumination (fixed wavelength) at 37 °C for 20 min. The absorbance values were recorded using a UV-vis spectrophotometer (Cecil Instruments, CE 2501, UK) at 560 nm.

CAT activity was estimated based on the method explained by Yilancioglu et al. (2014). An amount of 100 µL of the obtained supernatant was added to 2 mL of 10.8 mM H_2O_2, and 100 µL of 50 mM potassium phosphate buffer (pH 7.0). Using a UV–vis spectrophotometer, the absorbance value of the mixture was measured at 240 nm.

The MDA content was determined via the 2-thiobarbituric acid (TBA) according to the methodology described by Vyncke (1970). A volume of 50 µL of the supernatant was moved into a screw-capped tube and 200 µL of 20 mM TBA was added. The procedure was then followed by vigorous agitation of the mixture in a vortex and placing it in a boiling water bath for 60 min. After cooling, the MDA-TBA complex was measured by a spectrophotometer at 530 nm.



(a)

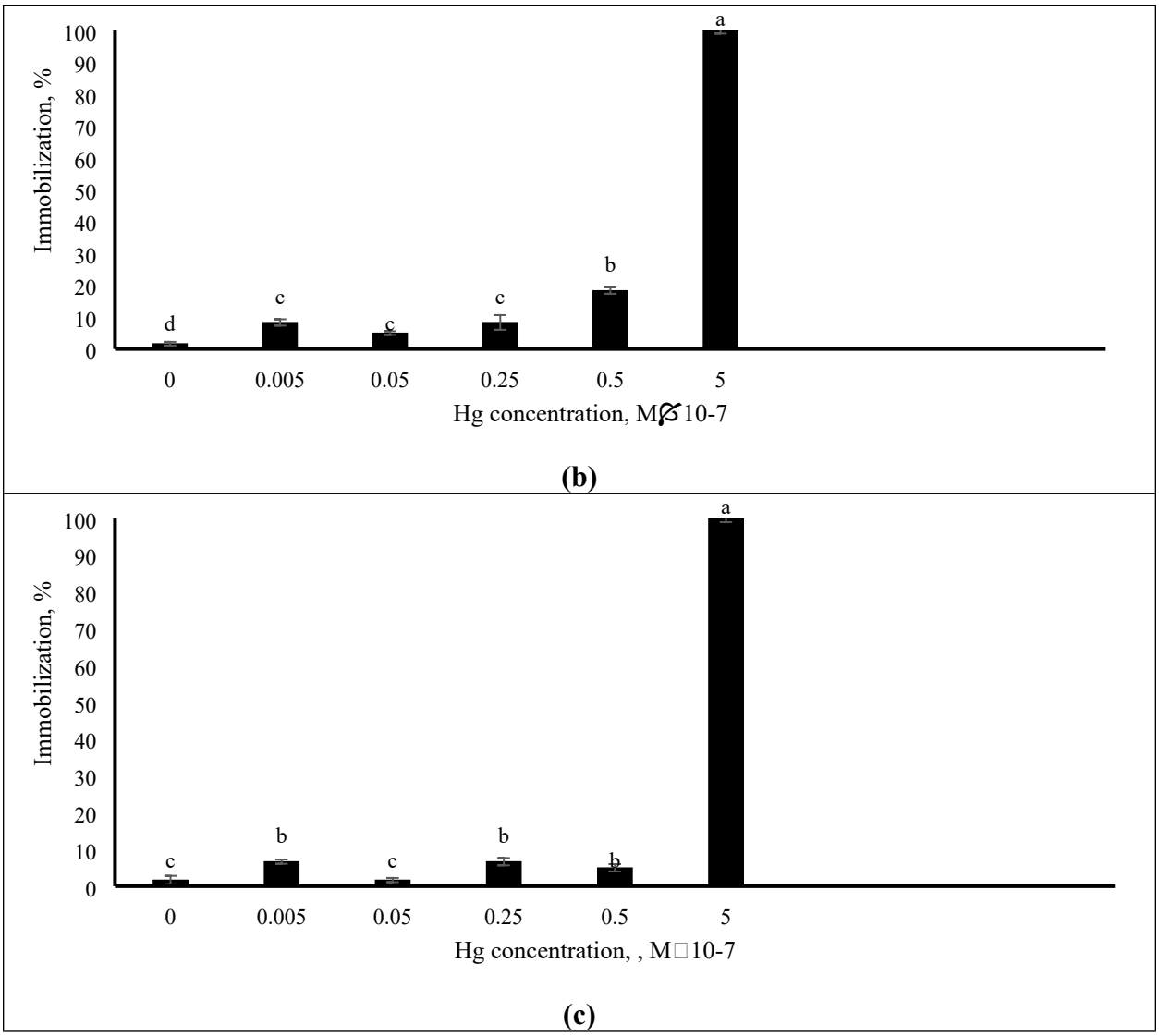


Fig. *S3*. Comparison of the immobilization percentage of *A. salina* nauplii exposed to different concentrations of Hg^{2+} ions in (a) Hg (Hg as $HgCl_2$ alone), (b) Hg+Ag (Hg in the presence of a constant concentration of 1×10^{-4} M Ag as silver nitrate) and (c) Hg+AgNPs (Hg in the presence of a constant concentration of 1×10^{-4} M Ag NPs) after 48 h. The bars with different letters are significantly different (mean ± SD, ANOVA, $P < 0.05$, n = 20).

S3: The FA composition of *A. salina* nauplii signified alterations against Hg, Hg-AgNPs, and Hg-Ag, as shown in Fig. *S4*, *S5* and *S6*. In response to 2.5×10^{-8} Hg^{+} at Hg (Fig. *S4*), SFAs including myristic acid (C14:0), palmitic acid (C16:0), and ∑SFA increased significantly but the increase of stearic acid (C18:0) was not significant. MUFAs including vaccenic acid (C18: 1-n7), oleic acid (C18:1-n9), and ∑MUFAs decreased while palmitoleic acid (C16:1-n7) and erucic

acid (C22: 1-n9) increased, and myristoleic acid (C14:1-n5) showed no significant change. All PUFAs, including linoleic acid (C18: 2-n6), α-linolenic acid (C18: 3-n3), eicosapentaenoic acid (C20: 5-n3), and ∑PUFAs showed a decrease response except arachidonic acid (C20: 4-n6). C20: 5-n3 showed an undetectable level (i.e., below the detection limit) at Hg.

Similarly, at Hg-Ag with 2.5ᔓ10^{-8} of Hg and 1ᔓ10^{-4} M of Ag^{+} ions (Fig. *S5*), the FA composition of *A. salina* nauplii changed. C14:0, C16:0 and ∑SFA raised significantly though C18:0 decreased significantly. MUFAs including C16:1-n7, C18:1-n9, and ∑MUFAs decreased while C14:1-n5, C22: 1-n9 increased, and C18: 1-n7 showed no significant change. ∑PUFAs, C18: 2-n6, and C18: 3-n3 showed a decrease response while C20: 4-n6 increased and C20: 5-n3 showed no significant change.

At Hg-AgNPs with 2.5ᔓ10^{-8} of Hg and 1ᔓ10^{-4} M AgNPs, respectively (Fig. *S6*), the FA composition of *A. salina* nauplii changed less than that at Hg and Hg-Ag treatments. SFAs, consisting C14:0, C16:0, C18:0, and ∑SFA signified no significant alterations compared with the control. MUFAs, including C14:1-n5, C16:1-n7, C22: 1-n9, and ∑MUFAs exhibited an increasing response while C18: 1-n7 decreased and C18:1-n9 showed no significant change. ∑PUFAs, C18: 2-n6, and C18: 3-n3 showed a decrease response while C20: 4-n6 and C20: 5-n3 showed no significant change.

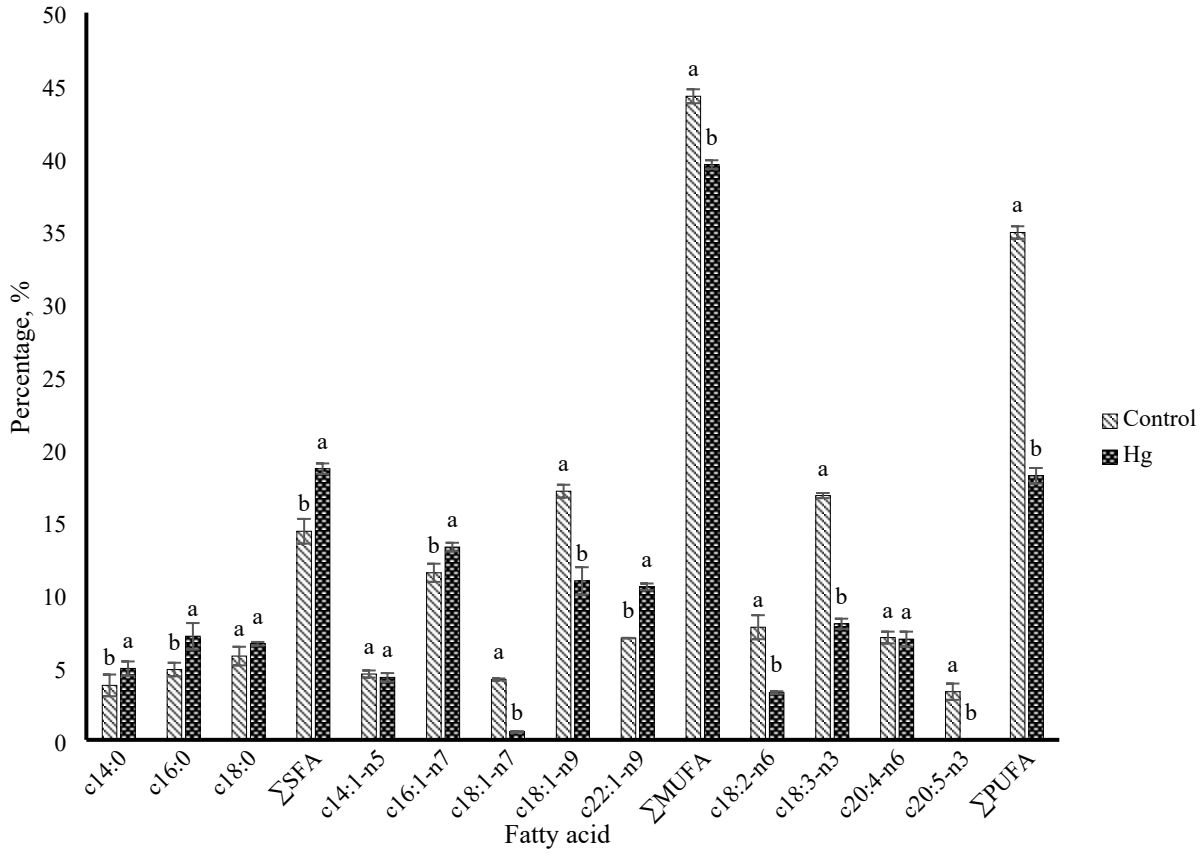


Fig. *S4*. Fatty acid profile of *A. salina* nauplii after 48 h exposure to 2.5ϕ10^{-8} of Hg^{+} ions at Hg treatment and control. The bars with different letters are significantly different (mean ± SD, ANOVA, $P < 0.05$).

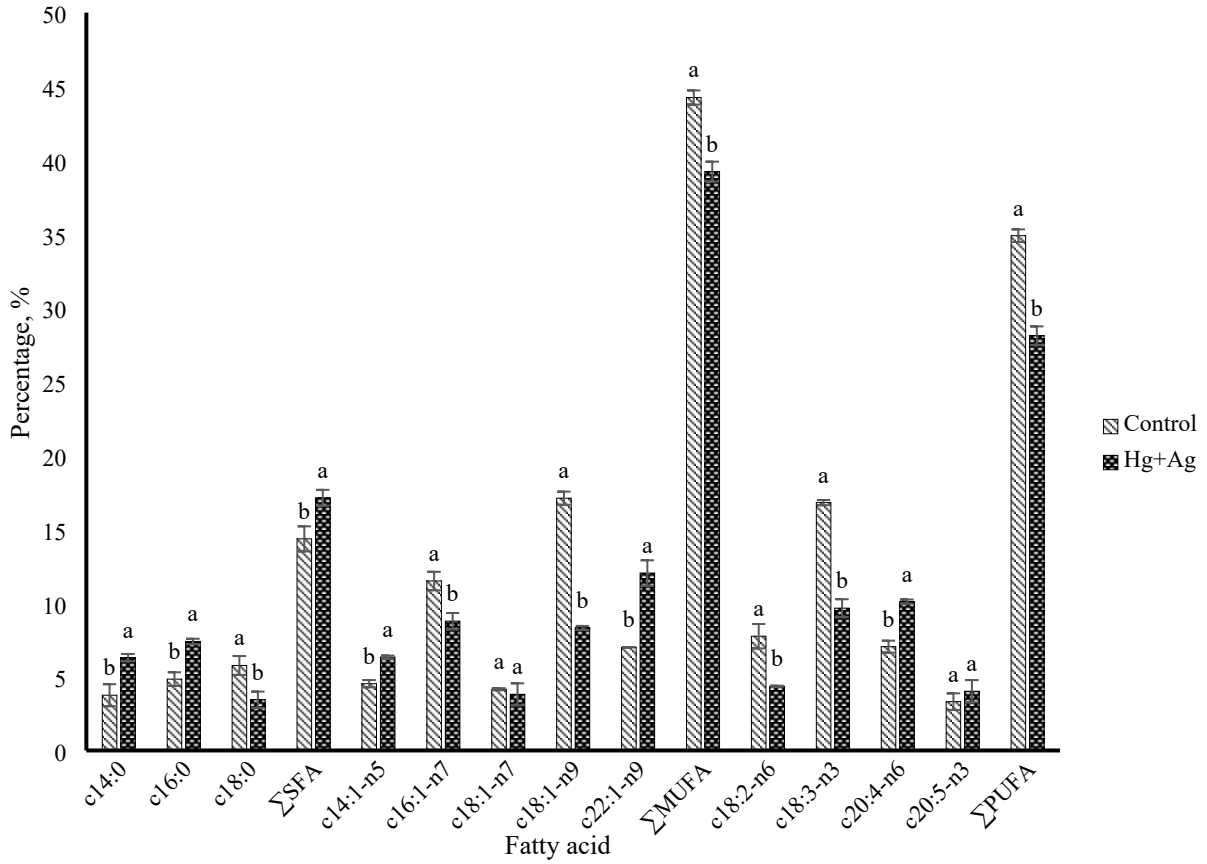


Fig. *S5*. Fatty acid profile of *A. salina* nauplii after 48 h exposure to 2.5ᔓ10^{-8} of Hg^{+} ions and 1ᔓ10^{-4} M of Ag^{+} ions at Hg-Ag treatment and control. The bars with different letters are significantly different (mean ± SD, ANOVA, $P < 0.05$).

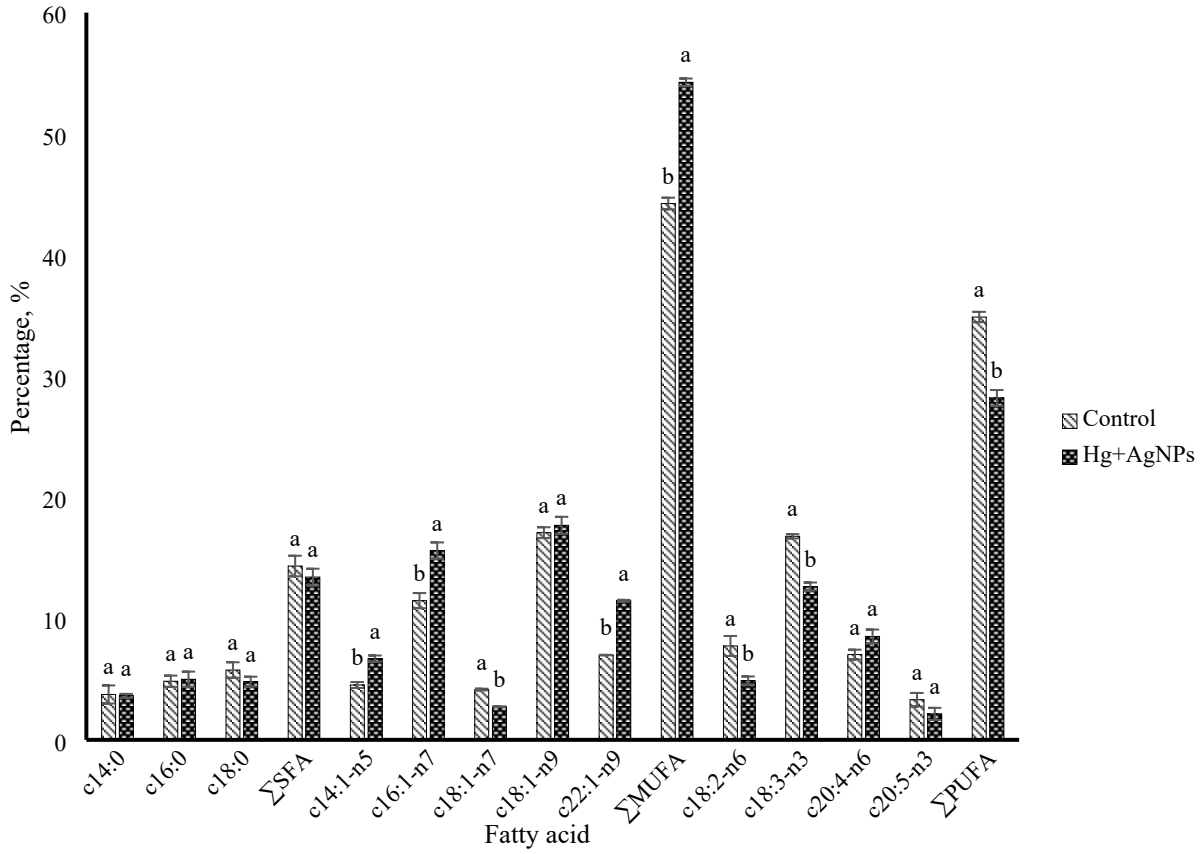


Fig. *S6*. Fatty acid profile of *A. salina* nauplii after 48 h exposure to 2.5×10^{-8} of Hg^{+} ions and 1×10^{-4} M of AgNPs at Hg-AgNPs treatment and control. The bars with different letters are significantly different (mean ± SD, ANOVA, $P < 0.05$).

References

Kono, Y. (1978) Generation of superoxide radical during autoxidation of hydroxylamine and an assay for superoxide dismutase. Archives of Biochemistry and Biophysics 186(1), 189-195.
Vyncke, W. (1970) Direct Determination of the Thiobarbituric Acid Value in Trichloracetic Acid Extracts of Fish as a Measure of Oxidative Rancidity. 72(12), 1084-1087.
Yilancioglu, K., Cokol, M., Pastirmaci, I., Erman, B. and Cetiner, S. (2014) Oxidative Stress Is a Mediator for Increased Lipid Accumulation in a Newly Isolated Dunaliella salina Strain. PLOS ONE 9(3), e91957.