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Co-exposure effects of mercury chloride ($HgCl_2$) and silver nanoparticles (Ag-NPs) on goldfish *(Carassius auratus)*: Histopathological changes, oxidative stress response, and bioaccumulation

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ABSTRACT

The toxicity effect of nanoparticles when exposed to other environmental pollutants is not completely known. Therefore, the aim of this study was to evaluate the effect of coexisting mercury chloride ($HgCl_2$) and silver nanoparticles (Ag-NPs) on the oxidative stress response, behavioral pattern, and histopathological changes of goldfish. In this study, goldfish (*Carassius auratus*) with mean length of 5 ± 0.4 cm and mean weight of 3 ± 0.3 g were assigned randomly to four experimental groups (15 fish in each group); exposed to a non-lethal concentration of Ag-NPs, a non-lethal concentration of $HgCl_2$, a mixture of Ag-NPs and $HgCl_2$, and a control group. In the end of experiment, activities of antioxidant enzymes (SOD, CAT, GPx, TAC, and MDA), bioaccumulation of Hg, and histopathological alterations in gill and intestine tissues were studied. Intensity of histopathological anomalies in goldfish tissues when $HgCl_2$ and AgNPs mixed group was higher than the anomalies observed in $HgCl_2$ or Ag-NPs nonlethal concentration. Aneurism, hyperplasia, fusion of lamellae in gills and degeneration, increase in the number of goblet cells and swelling of goblet cells in intestines were observed as notable damages in combined group. Moreover, co-exposure of $HgCl_2$ and Ag-NPs significantly curtailed the length of secondary lamellae ($p < 0.001$) but increased the diameter of primary and secondary lamellae ($p < 0.001$). We observed significant differences in the mean activities of GPx, CAT, and concentrations of TAC and MDA in gills between experimental groups ($p < 0.05$). No significant differences between groups in the mean activity of these parameters (except for MDA) were found in intestines. Compare to $HgCl_2$ and control groups, Hg bioaccumulation was significantly higher under $HgCl_2$ and AgNPs mixed group ($p < 0.05$). We conclude that in the sub-acute term, simultaneous presence of silver nanoparticles and mercury in the aquatic environment can induce a synergistic effect on goldfish tissues.

Keywords: Sub-acute toxicity; Mercury chloride; Silver nanoparticles; Goldfish disease

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1. Introduction

Heavy metal pollution of aquatic ecosystems has been a worldwide concern over the past decades, mainly due to their toxicity, low biodegradability, bioaccumulation through the food-chain, and the potential for human exposure [1–3]. Therefore, studying the bioaccumulation potential of various pollutants are very important aspect of toxicological studies. Aquatic environments are threatened by various pollutants, but mercury (Hg) and nanoparticles (NPs) are the most common ones these days in aquaculture [4–6]. It appears that metal contaminations and nano-toxicity studies are crucial for environmental protection to build a proper policy, manage water resources and aquatic health. Mercury (Hg) is well known for its bioavailability and high toxicity at low concentrations [6,7]. Its toxicity depends greatly on the form of Hg compounds and its oxidation state. Both organic and inorganic forms are cumulatively toxic and impose considerable risk to aquatic biota [8]. Hg causes pathologic effects such as histopathological damages, oxidative stress, immunotoxicity, and Na^+-K^+ ATPase inhibition in different tissues of fish [9,10].

Silver nanoparticles (Ag-NPs) are widely used in various industrial and commercial applications, such as consumer products, medical device coatings, healthcare related products, optical sensors, cosmetics, food industry, orthopedics, drug delivery, textiles, keyboards, wound dressings, and biomedical devices [11–13]. AgNPs may discharge to environment by means of manufacturing, incorporation into goods, and goods recycling or waste [14]. Recent studies have reported the release of AgNPs through biocidal plastics, textiles, paints and other home products [15–17]. Various studies have evaluated on toxic effects of Hg in fish under controlled conditions [9,18,19] and effects of Ag-NPs on aquatic organisms [20,21]. During the last decade, vast majority of those studies have dealt with the effect of their separate effects only. Simultaneous exposure of two pollutants can behave differently when animal are co-exposed with other pollutants (additive, synergistic, or antagonistic impacts). Thus, in this study we aim to evaluate the interaction effects of mercury chloride on ecosystems when joint with silver nanoparticles. This issue has been recently outlined by several independent researches. Kim et al. [22] reported that Ag-NPs reduced the uptake of As and Cu but increased the uptake and toxicity of Cd in *Daphnia magna*. We specifically aim to evaluate the joint-presence of mercury chloride ($HgCl_2$) and silver nanoparticles (Ag-NPs) on the oxidative stress response, Hg bioaccumulation, and histopathological alterations in goldfish (*Carassius auratus*) under controlled conditions.

2. Materials and methods

2.1. Mercury chloride ($HgCl_2$) and silver nanoparticles (AgNPs)

Mercury chloride ($HgCl_2$) was purchased from Azmiran Co. (Iran) and a stock solution of Hg (1000 mg/L) was prepared by dissolving $HgCl_2$ (Merck) in deionized water. This study was conducted using a colloidal silver nanoparticles solution (Ag-NPs, Nanocid®) which was kindly provided by Nano Nasb Pars Co. (Tehran, Iran). TEM micro graph and size distribution of Ag-NPs are presented in Fig. 1. For more information about this product, interested readers are advised to refer to the study of Johari et al. [14]. The particle zeta potential, the particle size distribution, and the average silver hydrodynamic diameter were 53.33 ± 7.8 mV, 54.1%, and 54.8 nm respectively.

2.2. Test organism and experimental conditions

The goldfish (*Carassius auratus*) were procured from local aquaculture shop in Sanandaj city in 2017. The mean length and weight of fish were 5 ± 0.4 cm and 3 ± 0.3 g respectively. After transferring fish to laboratory, they were placed in a 100 L aquarium for one month to adapt to the laboratory circumstances. Aquarium was supplied with tap water and continuously aerated under 12 h-light/12 h-dark. The water characteristics of aquarium were measured by standard methods for the examination of water; hardness 5°dGH, pH 7.5 ± 0.23, temperature 23.5 ± 3°C, conductivity 610 ± 15 µS/cm, and dissolved oxygen content (DO) 6.7 ± 0.3mg L^{-1}. For sub-acute experiment, one sub lethal Hg concentration (20 µg L^{-1}) was chosen based on environmental relevant concentrations [10,23]. For Ag-NPs, solution of 1 mg/L was chosen as sub lethal concentration [24]. The fish were exposed to sub lethal concentration of Ag-NPs, sub lethal concentration of $HgCl_2$, and a mixture of Ag-NPs and $HgCl_2$ (1 mg L^{-1} + 20 µg L^{-1}) for 14 consecutive days. For comparison purposes, a control group without any exposure was also prepared. Changes in behavioral pattern of fish were observed regularly [25]. After the exposure period, the gill and intestine tissues of goldfish were dissected for histopathological studies, oxidative stress protocol, and evaluation of Hg bioaccumulation. The fish were quarantined for 2 weeks in laboratory and examined for any signs of diseases. Eventually only healthy fish were used. Ethical considerations and animal rights were considered and study was approved by Ethics Committee of the Payame Noor University.

2.3. Histopathological study

Flaps of gill and intestine were placed in Bouin solution immediately after dissection. One hour later, samples were dehydrated and inserted into paraffin wax. Using a microtome, a 5 µm slice was derived and stained with haematoxylin and eosin [26,27]. The diameter and length of secondary gill lamellas as well as diameter of gill filaments were measured using Axio Vision (Release 4.8.2). Severity of histopathological alterations in gills and intestines were evaluated as: none (–), mild (+), moderate (++), and severe (+++).

2.4. Oxidative stress response

Glutathione peroxidase (GPx) and superoxide dismutase (SOD) activities were determined colorimetrically (enzymatically), using an ELISA microplate reader (Tecan Co., Grodingen, Austria), MDA assay kit (Cayman Chemical Co., Ann Arbor, MI, USA), TAS kits (Randox, Crumlin, UK), GPx assay kit (Cayman Chemical Co., Ann Arbor, MI, USA), CAT kits (ZellBio, Germany) and SOD assay kit (Cayman Chemical Co., Ann Arbor, MI, USA). All assays were

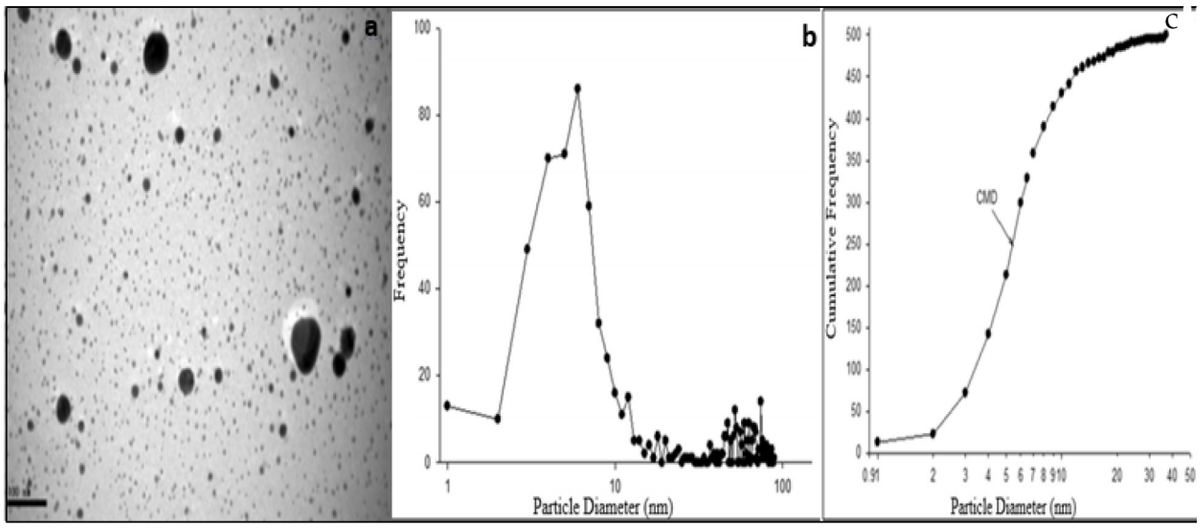

Fig. 1. A. TEM micrograph (a) and size distribution of silver nanoparticles based on number frequency (b) and cumulative frequency (c)

carried out according to the manufacturer's instructions without modifications

2.4.1. Glutathione peroxidase (GPx) activity

Glutathione peroxidase activity was measured following the method of Flohé and Günzler [28]. GPx catalyzes the oxidation of glutathione (GSH) by cumene hydro peroxide. In the presence of glutathione reductase (GR) and NADPH, the oxidized glutathione (GSSG) is immediately converted to the reduced form with concomitant oxidation of NADPH to NADP+. The decrease in absorbance at 340 nm for 5 min (25°C) was measured. The tissue homogenate (50 µL) was incubated with 25 m*M* potassium phosphate, 0.5 m*M* EDTA, pH 7.4, 0.5 mM $NaNO_3$, 0.3 mM NADPH, 0.64 U GSH-Rd and 1 m*M* GSH. The reaction was started with 0.1 mM Cumene Hydro peroxide. Values were corrected for non enzymatic oxidation of GSH and NADPH by hydrogen. The results were expressed in unit of GSH-Px activity using a molar extinction coefficient of GPX U/L of sample = 8412 × ΔA 340 nm/min (ΔA = deference Blank with sample). The unit defined as either U/mg or U/g protein.

2.4.2. Super oxide dismutase (SOD) activity

Super oxide dismutase (SOD) activity was measured following the method of McCord and Fridovich [29]. Briefly, tissues homogenate (50 µL) was incubated for 5 min at 25°C with 20 m*M* potassium phosphate, 1 m*M* EDTA of pH 7.8, 0.25 m*M* xanthine, and 0.17 m*M* cytochrome C. The reaction was initiated by adding xanthine oxidase (0.16 U) and assayed following the reduction of cytochrome c at 550 nm for 5 min (25°C) in the presence or absence of xanthine oxidase and SOD. The results were expressed as units of activity (U/mg protein). One unit of the activity was defined as the amount of SOD that inhibited the rate of cytochrome c reduction by 50%.

2.4.3. Concentrations of TAC

For TAC, ABTS (2, 2'-azino-di-[3-ethylbenzthiazoline sulphonate]) was incubated with a peroxidase (metmyoglobin) and H_2O_2 to produce the radical cation $ABTS^+$. It was measured at 600 nm with relatively stable blue-green color. Antioxidants within the sample caused to some extent the suppression of this colorful reaction which was proportional to their concentrations. For evaluation, serum (20 µL) was incubated at 37°C with 1 mL tissue chromogen (6.1 m*M*/L) and ABTS 610 µ mol/L. The absorbance was measured at 600 nm. The result was incorporated with 250 µmol/L Hydrogen peroxide (25% µmol/L), and after 3 s aberrance detected in 600 nm. The reduced amount of $ABTS^+$ resulted in antioxidants which was compared with standards [30]. The results were expressed as units of activity (mmol/L).

2.4.4. Concentrations of MDA

The MDA in tissue was determined following the method of Vila et al. [31] with minor modifications as described in Frankic et al. [32]. A Waters Alliance HPLC (Waters, Milford, MA) equipped with a Waters 474 scanning fluorescence detector was used to determine MDA-TBA adducts. The mobile phase consisted of 50 mmol/L of KH_2PO_4 buffer (pH 6.8) and CH_3OH in a gradient mode. A 10–L aliquot was injected on to a reversed-phase C18 HPLC chromatographic column (HyperClone 5u ODS (C18) 120A, 4.6 × 150 mm; Phenomenex Inc., Torrance, CA]. The flow rate of the mobile phase was 1 mL/min, and column temperature was set at 30°C. The chromatographic data were evaluated by the Millenium 32 Chromatography Manager program (Waters).

2.5. Mercury bioaccumulation

At the end of the experiment period, the uptake of Hg was measured in gill and intestine tissues (two fish pooled

to take two sample tissues of gill and intestine of 0.5 g). Samples were digested in a solution of nitric acid (HNO_3; 65%) and perchloric acid ($HClO_4$; 70%) mixture. Samples were accurately weighed and separated into 50 mL Erlenmeyer flasks and 5 mL of nitric acid (65%) was added to each sample. Samples were left overnight to get slowly digested. After one day, 2.5 mg of nitric acid was added. Digestion was performed on the bain-marie (water bath) at 100°C until the solutions were cleared. The digested samples were diluted with 25 mL deionized water [33]. Finally, the Hg concentrations in gill and intestine tissues were measured using atomic absorption spectrophotometer model of Phoenix 986 graphite furnace (Biotech engineering management Co. Ltd. UK).

2.6. Statistical analysis

The data was analyzed with SPSS statistical software (version 16; SPSS, Chicago, IL). The values are reported as mean ± SD. One-way ANOVA was used to assess the mean differences between the studied groups by Turkey's Honest. Significance level in all the analyses was set as 0.05.

3. Results and discussion

3.1. Histopathological anomalies

The goldfish in all experimental groups showed activities such as rapid swimming, hyperactivity, convulsions, and loss of balance. These activities were seen more often in mixed group (Table 1). Histopathological alterations in the gill of fish were also studied (Figs. 2 and 3). Only minor alterations were observed under control group but damages were more prevalent in fish at $HgCl_2$ and Ag-NP groups. Aneurism, hyperplasia, oedema, curvature, fusion of lamellae, and increased mucous secretion in gills were notable. Alterations observed in intestine were degeneration, vacuolation, increase in the number of goblet cells, swelling of goblet cells, increase in the number of lymphocyte, integration of villi, and expansion at villi structure. Furthermore, the co-exposure of $HgCl_2$ and Ag-NPs significantly reduced the length of secondary lamellae ($p < 0.001$) but increased the diameter of primary lamellae and diameter of secondary lamellae ($p < 0.001$) (Table 2). The semiquantitative evaluation of lesions showed abundant anomalies such as fusion of lamellae, hyperplasia, epithelium shortening, and aneurism for fish treated with $HgCl_2$ and Ag-NP (Table 3). Moreover, for these fish, anomalies such as degeneration, increase in the number of goblet cells, swelling of goblet cells, and increase in the number of lymphocyte were markedly high (Table 4).

Histopathological studies are good biomarkers for monitoring alterations under variety of environmental stress. These studies can assess the health of an organism better than any another biochemical assessment tool [34–36]. The behavioral pattern and damages we found in gills and intestines of fish in Ag-NPs and $HgCl_2$ mixed group were in agreement with other studies [26,27,35–38]. In response to environmental pollutants such as Ag-NPs and $HgCl_2$ epithelial hyperplasia may occur in gills [36,39]. Hyperplasia leads the proliferation of adjacent lamellae cells to exchange gas between the environment and gills in order to build a barrier for accumulation of Ag-NPs and $HgCl_2$ [40,41]. As the level of severity of tissue injuries increases, fusion of lamellae occurs. This is known as the defense mechanism of gills to guard against environmental pollutants. Gill shrinkages the total respiratory area to uptake less oxygen for total metabolic activities [37,42–44].

Table 1
Impact of Ag-NPs and $HgCl_2$ on the behavior of goldfish studied

Parameter	Control	$HgCl_2$	Ag-NPs	$HgCl_2$ + Ag-NPs
Hyperactivity	–	+	+	++
Loss of balance	–	+	–	++
Rate of swimming	–	+	+	++
Rate of opercular activity	–	+	+	+
Convulsions	–	+	–	+

Severity of the symptoms: (–) none, (+) mild, (++) moderate, (+++) strong

The branchial aneurism was one of the major injuries we observed in fish treated with Ag-NPs-$HgCl_2$ mixed. This injury has been also reported in Al-Bairuty et al. [45] and Rajkumar et al. [46] studies. Branchial aneurisms occurs after dilatation of lamellar blood vessels and collapsing of pillar cells and vascular integrity. With the increase in the level of severity of aneurysm, there is a risk of rupture, severe haemorrhage, and other complications or death [47].

3.2. Oxidative stress responses

In the presence of Ag-NPs, it turns out that $HgCl_2$ increases the activity of SOD, CAT, and GPx. The elevated levels of TAC and MDA was also expected in gill. However, we found a lower activity of SOD and GPx, but higher levels of CAT, TAC and MDA (Table 5). Moreover, under Ag-NPs-$HgCl_2$ mixed group, we found, i) low GPx activity but high CAT, MDA, and TAC activities in both gill and intestine tissues; ii) SOD activity high in gill but low in intestine. The results also showed the increasing activity of antioxidant enzymes. In other studies, the alteration activity of antioxidant enzymes and expression of P2X7R in fish when exposed to mercury have been found [23,48]. Moreover, Monteiro et al. [49] reports alteration in the oxidative stress biomarkers such as SOD, CAT, and GPx for neotropical fish (*Hoplias malabaricus)* in contact with sub-lethal inorganic Hg. Exposure to Hg can interfere with physiological and biochemical activities via the oxidative stress. This stress is generated when the production of reactive oxygen species (ROS) increases and antioxidant is decreased to modify ROS to the less reactive intermediate. Oxidative stress can cause DNA damages, protein oxidations, nitric oxide formations and peroxidation of cell constituents, especially lipid peroxidation [50–52]. SOD, CAT, and GPx are involved in the recycling of GSH and generation of NADPH. The GSH and other thiols depletion will render susceptible cells to an oxidative damage when the antioxidant enzymes activity is elevated [53,54]. Glutathione, when adhere to Hg, prevents

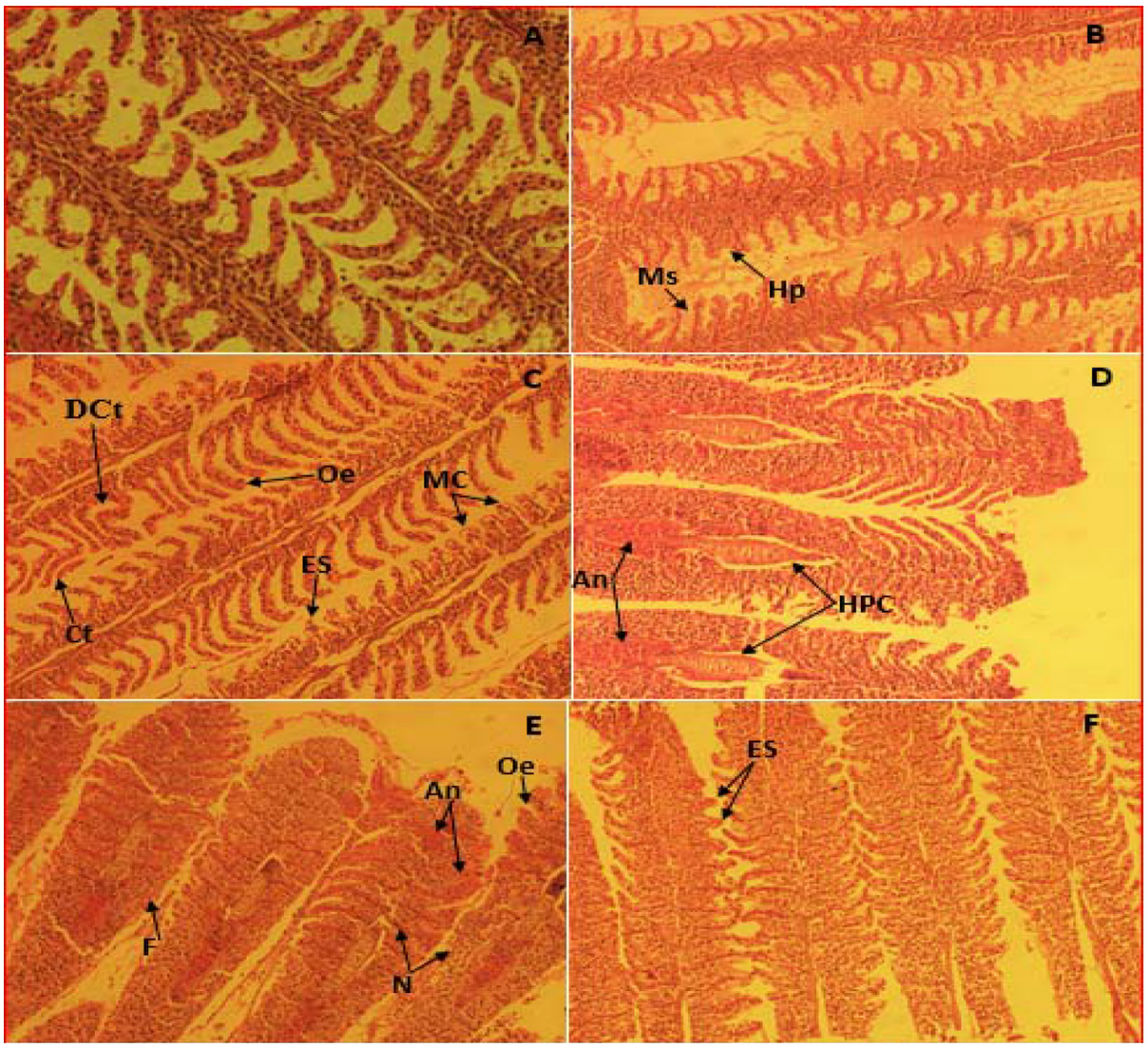

Fig. 2. Gill morphology in goldfish in sub-acute period (14 days; 400× magnification). The gills of control fish (A; 600× magnification) indicated only some small histopathological alterations, whilst all treatments (Ag-NPs: (B), Hg (C), and Hg + Ag-NPs (D to F)) showed injuries that include aneurism (An), dilated and clubbed tips (DCt), hyperplasia (Hp), oedema (Oe), curvature (Ct), fusion of lamellae (F), hypertrophy and proliferation in the erythrocytes of cartilaginous core (HPC), epithelium shortening (ES), dilated marginal channel (MC), increased mucous secretion (Ms), necrosis (N).

Hg of binding to cellular proteins. Both GSH and cellular antioxidant enzymes play an important role in tissues injuries caused by $HgCl_2$ [48,55].

3.3. Bioaccumulation of Hg

Bioaccumulation of contaminants in fish can be used to trigger the toxicity of environment. For fish treated with $HgCl_2$-Ag-NPs mixed, we found high concentration of Hg in both gill and intestine tissues (Table 6). Accumulation of Hg was higher in intestine. Hg uptake by gill depends on water chemistry so that increasing the dissolved organic carbon (DOC) decreases the uptake but increasing chloride concentration helps further absorption of Hg [56,57]. Significant fractions of ingested Hg may be localized in intestinal mucosa and posterior intestines. After 30 d of exposing rainbow trout to $HgCl_2$, Boudou and Ribeyre [58] reported that 36% of relative Hg burden were accumulated in posterior intestine of rainbow trout. Some studies suggest that the water soluble Ag-NPs (supported on Al_2O_3) are efficient in removal of Hg^{2+} from aqueous environments [59–61]. Although this may encourage the use of Ag-NPs in wastewater treatment, its toxicity effects on Hg ions are still unknown. Eventually, more studies are needed to evaluation the toxicity effects of co-exposure nanoparticles in the presence of other pollutants to understand the potential risks of these materials in aquatic ecosystems.

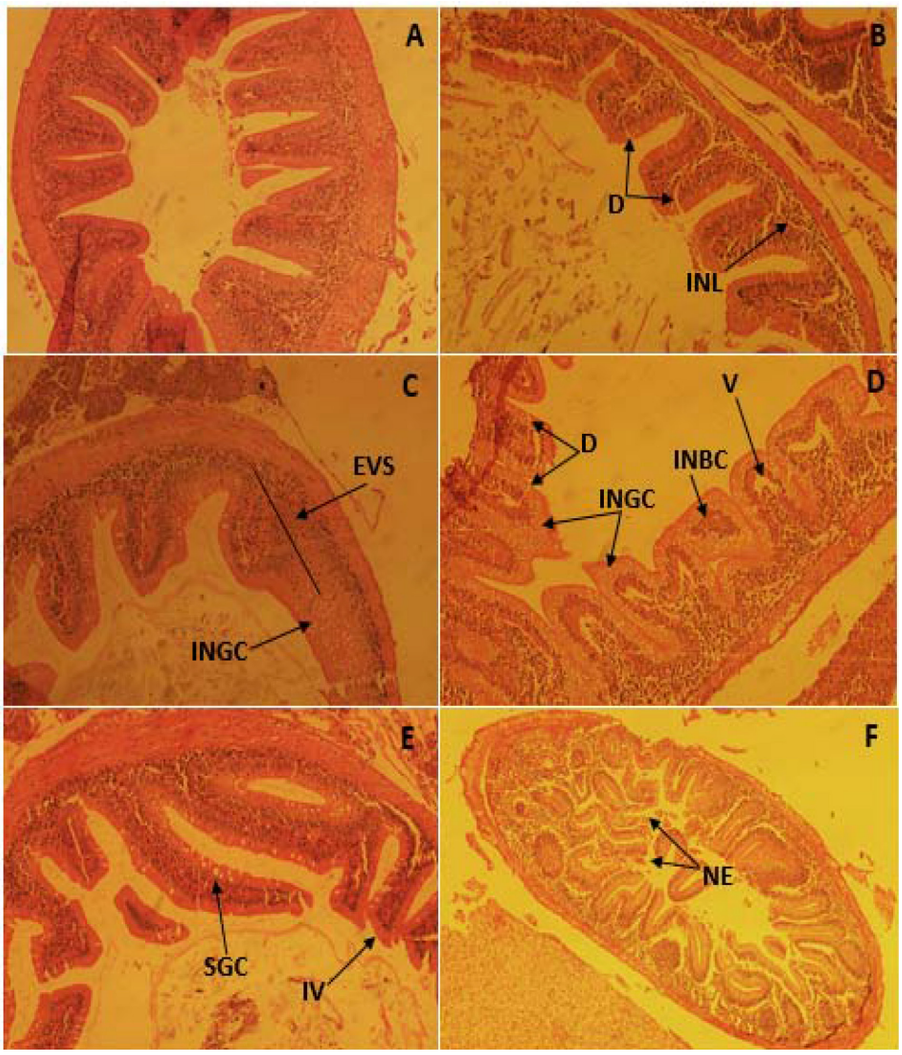

Fig. 3. Intestine morphology in goldfish in sub-acute period (14 days; A and F: 200× magnification; B, C, D, and E: 400× magnification). The intestine of control fish (A) indicated only some small histopathological alterations, whilst all treatments (Ag-NPs: (B), Hg (C), and Hg + Ag-NPs (D to F)) revealed injuries that include degeneration (D), vacuolation (V), necrosis and erosion (NE), increase in the number of goblet cells (INGC), swelling of goblet cells (SGC), increase in the number of blood cells (INBC), increase in the number of lymphocyte (INL), integration of villi (IV), expansion at villi structure (EVS).

Table 2
Quantitative parameters of goldfish's gills (µm (Mean ± SD)) following exposure to different concentrations of Hg and Ag-NPs and their mixture

Groups	Diameter of secondary lamellae	Diameter of primary lamellae	Length of secondary lamellae
Control	23.7 ± 2.6[a]	5.2 ± 0.9[a]	35.7 ± 2.2 [a]
Hg	35 ± 0.8[b]	9 ± 0.8[b]	28 ± 0.8[b]
Ag-NPs	33 ± 1.8[b]	8 ± 1.1[b]	31 ± 1.4[b]
Hg + Ag-NPs	41.7 ± 2.7[c]	13.2 ± 2.7[c]	17.5 ± 1.2[c]
p-value	<0.001	0.001	<0.001

*In each column, the numbers with different letters differ significantly ($p < 0.05$).

Table 3
Semi quantitative evaluation of lesions recorded in gills of goldfish studied

Damages Groups	*Ct	Oe	Hp	Ms	F	DCt	ES	An	N
Control	**+	+	+	+	−	+	−	−	−
Hg	+	++	++	+	−+	++	+	++	+
Ag-NPs	+	+	++	++	−	+	+	+	−
Hg + Ag–NPs	++	++	+++	++	−++	++	++	++	++

* curvature (Ct), oedema (Oe), hyperplasia (Hp), dilated marginal channel (MC), fusion of lamellae (F), dilated and clubbed tips (DCt), epithelium shortening (ES), aneurism (An), necrosis (N).
**None (−), mild (+), moderate (++) and severe (+++).

Table 4
Semi quantitative evaluation of lesions recorded in intestines of goldfish studied

Damages Groups	*D	NE	INGC	SGC	INL	V	IV	EVS	INBC
Control	**–	–	–	–	–	–	+	+	–
Hg	+	+	++	++	−+	+	+	+	+
Ag-NPs	–	–	+	+	–	+	+	+	+
Hg + Ag–NPs	++	++	+++	+++	−++	++	+	++	++

*Degeneration (D), vacuolation (V), necrosis and erosion (NE), increase in the number of goblet cells (INGC), swelling of goblet cells (SGC), increase in the number of lymphocyte (INL), increase in the number of blood cells (INBC), integration of villi (IV), and expansion at villi structure (EVS).
**None (–), mild (+), moderate (++) and severe (+++).

Table 5
Activities of SOD, GPx, and CAT, and concentrations of TAC, MDA, and protein carbonyl in different tissues of goldfish following the co-exposure to AgNPs and $HgCl_2$ treatments

Parameters						
Tissues	MDA	TAC	CAT	GPx	SOD	Protein
Gills						
Control	6.94 ± 1.3	0.64 ± 0.1a*	0.08 ± 0.02a	0.18 ± 0.03a	11.1 ± 1.7a	63 ± 18.1
Hg	7.22 ± 1.4	0.76 ± 0.1a	0.12 ± 0.01a	0.19 ± 0.02a	12.4 ± 0.7a	63 ± 4.5
AgNPs	7.51 ± 0.8	1.32 ± 0.23b	0.14 ± 0.04a	0.26 ± 0.03b	14.4 ± 0.3b	55.3 ± 7.3
Hg + AgNPs	7.05 ± 1.1	0.27 ± 0.09c	0.18 ± 0.01b	0.27 ± 0.09b	14.8 ± 0.7b	49 ± 2.6
F value	0.12	19.36	5.29	6.63	9.68	1.33
P value	0.942	0.0005	0.026	0.014	0.005	0.354
Intestine						
Control	36.1 ± 8.1	10.1 ± 1.4	0.84 ± 0.29	0.18 ± 0.03	0.48 ± 0.08a	19.3 ± 5.5
Hg	38.1 ± 8.6	10.8 ± 2.3	0.96 ± 0.19	0.25 ± 0.12	0.52 ± 0.08a	16.3 ± 3.1
AgNPs	29.8 ± 9.8	6.6 ± 3.5	0.67 ± 20	0.20 ± 0.10	0.34 ± 0.06a	23.3 ± 5.5
Hg + AgNPs	31.1 ± 6.5	6.9 ± 2.7	1.16 ± 0.26	0.49 ± 0.26	0.86 ± 0.29b	14 ± 3.6
F value	0.83	1.92	2.13	2.56	5.50	2.34
P value	0.515	0.204	0.174	0.128	0.024	0.098

*In each column, the numbers with different letters differ significantly ($p < 0.05$).

Table 6
Hg concentrations (μg/g) in the tissues of goldfish following the co-exposure to AgNPs and $HgCl_2$ treatments

Groups	Gill	Intestine
0.06 ± 0.004	0.04 ± 0.008	Control
0.20 ± 0.01	0.11 ± 0.005	Hg
0.34 ± 0.01	0.20 ± 0.02	Hg + AgNPs
0.001	0.01	p value

p value for one-way ANOVA; Significant at $p < 0.05$

4. Conclusion

In summary, in this study we found that the severity level of histopathological anomalies was high when both Ag-NPs and $HgCl_2$ are present in environment. Aneurism, hyperplasia, and fusion were notable on gill. Degeneration, necrosis, and erosion increasing the number and swelling of goblet cells were common on intestine. This study showed that Hg alone or in combination with other contaminants poses different modes of toxicity. Furthermore, accumulation of Hg was increased in both gill and intestine when Ag-NPs presented in this study.

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References

[1] W. Tang, B. Shan, H. Zhang, W. Zhang, Y. Zhao, Y. Ding, N. Rong, X. Zhu, Heavy metal contamination in the surface sediments of representative limnetic ecosystems in eastern China, Sci. Rep., 4 (2014) 7152–7161.
[2] B. Mansouri, R. Baramaki, M. Ebrahimpour, Acute toxicity bioassay of mercury and silver on *Capoeta fusca*, Toxicol. Ind. Health., 28 (2012) 393–398.

[3] E. Hoshyari, A. Pourkhabbaz, B. Mansouri, Contaminations of metal in tissues of Siberian gull (*Larus heuglini*): gender, age, and tissue differences, Bull. Environ. Contamin. Toxicol., 89 (2012) 102–106.
[4] P. Swain, S.K. Nayak, A. Sasmal, T. Behera, S.K. Barik, S.K. Swain, S.S. Mishra, A.K. Sen, J.K. Das, P. Jayasankar, Antimicrobial activity of metal based nanoparticles against microbes associated with diseases in aquaculture, World. J. Microbiol. Biotechnol., 30 (2014) 2491–2502.
[5] L. Peng, F. Xinbin, Y. Qiongzhi, G, Xuefei, X. Jialin, W. Minghung, C. Peter, W. Sheng-Chun, The effects of aquaculture on mercury distribution, changing speciation, and bioaccumulation in a reservoir ecosystem, Environ. Sci. Pollut. Res., 24 (2017) 25923–25932.
[6] L. Peng, G. Xuefei, Y. Qiongzhi, Z. Jin, C. Yucheng, Z. Chan, W. Ming-Hung, W. Sheng-Chun, Role of mariculture in the loading and speciation of mercury at the coast of the East China, Sea. Environ. Pollut., 218 (2016) 1037–1044.
[7] M. Ebrahimpour, M. Mosavisefat, R. Mohabbati, Acute toxicity bioassay of mercuric chloride: An alien fish from a river, Toxicol. Environ. Chem., 92 (2010) 169–173.
[8] L.R. Skubal, N.K. Meshkov, Reduction and removal of mercury from water using arginine-modified TiO_2, J. Photochem. Photobiol. A: Chem., 148 (2002) 211–14.
[9] Q.F. Zhang, Y.W. Li, Z.H. Liu, Q.L. Chen, Exposure to mercuric chloride induces developmental damage, oxidative stress and immunotoxicity in zebra fish embryos-larvae, Aquat. Toxicol., 181 (2016) 76–85.
[10] Q.F. Zhang, Y.W. Li, Z.H. Liu, Q.L. Chen, Reproductive toxicity of inorganic mercury exposure in adult zebra fish: Histological damage, oxidative stress, and alterations of sex hormone and gene expression in the hypothalamic-pituitary-gonadal axis, Aquat. Toxicol., 177 (2016) 417–424.
[11] S. Chernousova, M. Epple, Silver as antibacterial agent: Ion, nanoparticle, and metal, Angew. Chem. Int. Ed., 52 (2013) 1636–1653.
[12] C.Y. Li, Y.J. Zhang, M. Wang, Y. Zhang, G. Chen, L. Li, D. Wu, Q. Wang, In vivo real-time visualization of tissue blood flow and angio genesis using Ag_2S quantum dots in the NIR-II window, Biomaterials, 35 (2014) 393–400.
[13] I. Sondi, B. Salopek-Sondi, Silver nanoparticles as antimicrobial agent: A case study on E. coli as a model for Gram-negative bacteria, J. Colloid. Interface. Sci., 275 (2004) 177–182.
[14] H.S. Jiang, L. Yin, N.N. Ren, L. Xian, S. Zhao, W.L.B. Gontero, The effects of chronic silver nanoparticles on aquatic system in microcosms, Environ. Pollut., 223 (2017) 395–402.
[15] C. Lorenz, L. Windler, N. von Goetz, R.P. Lehmann, M. Schuppler, K. Hungerbuhler, M. Heuberger, B. Nowack, Characterization of silver release from commercially available functional (nano) textiles, Chemosphere, 89 (2012) 817–824.
[16] T. Kunniger, A.C. Gerecke, A. Ulrich, A. Huch, R. Vonbank, M. Heeb, A. Wichser, R. Haag, P. Kunz, M. Faller, Release and environmental impact of silver nanoparticles and conventional organic biocides from coated wooden facades, Environ. Pollut., 184 (2014) 464–471.
[17] D.K. Tripathi, A. Tripathi, S. Singh, Y. Singh, K. Vishwakarma, G. Yadav, S. Sharma, V.K. Singh, R.K. Mishra, R.G. Upadhyay, N.K. Dubey, Y. Lee, D.K. Chauhan, Uptake, accumulation and toxicity of silver nanoparticle in autotrophic plants, and heterotrophic microbes: a concentric review, Front. Microbiol., 8 (2017) 1–16.
[18] J.H. Kim, J.S. Lee, J.C. Kan, Effect of inorganic mercury on hematological and antioxidant parameters on olive flounder *Paralichthys olivaceus*, Fish. Aquat. Sci., 15 (2012) 215–220.
[19] T. Cappello, P. Pereira, M. Maisano, A. Mauceri, M. Pacheco, S. Fasulo, Advances in understanding the mechanisms of mercury toxicity in wild golden grey mullet (Liza aurata) by 1H NMR-based metabolomics, Environ. Pollut., 219 (2016) 139–148.
[20] G. Laban, L.F. Nies, R.F. Turco, J.W. Bickham, M.S. Sepulveda, The effects of silver nanoparticles on fathead minnow (*Pimephales promelas*) embryos, Ecotoxicol., 19 (2010) 185–195.
[21] L. Murray, M.D. Rennie, E.C. Enders, K. Pleskach, J.D. Martin, Effect of nanosilver on cortisol release and morphometrics in rainbow trout (*Oncorhynchus mykiss*), Environ. Toxicol. Chem., 36 (2015) 1606–1613.
[22] I. Kim, B.T. Lee, H.A. Kim, K.W. Kim, S.D. Kim, Y.S. Hwang, Citrate coated silver nanoparticles change heavy metal toxicities and bio accumulation of *Daphnia magna*, Chemosphere, 143 (2016) 99–105.
[23] F.F. Cruz, C.E. Leite, T.C. Pereira, M.R. Bogo, C.D. Bonan, A.M. Battastini, M.M. Campos, F.B. Morrone, Assessment of mercury chloride-induced toxicity and the relevance of P2X7 receptor activation in zebra fish larvae, Comp. Biochem. Physiol. Part C, 158 (2013) 159–164.
[24] S. Abarghoei, A. Hedayati, R. Ghorbani, H.K. Miandareh, T. Bagheri, Histopathological effects of waterborne silver nanoparticles and silver salt on the gills and liver of gold fish Carassius auratus, Int. J. Environ. Sci. Technol., 13 (2016) 1753–1760.
[25] A. Kumar, B. Sharma, R.S. Pandey, Preliminary evaluation of the acute toxicity of cypermethrin and k-Cyhalothrin to Channa punctatus, Bull. Environ. Contamin. Toxicol., 79 (2007) 613–616.
[26] B. Mansouri, A, Maleki, S.A. Johari, N, Reshahmanish, Effects of cobalt oxide nanoparticles and cobalt ions on gill histopathology of zebra fish (*Danio rerio*), AACL Bioflux., 8 (2015) 438–444.
[27] B. Mansouri, A. Maleki, S.A. Johari, B. Shahmoradi, E. Mohammadi, S. Shahsavari, B. Davari, Histopathological effects of copper oxide nanoparticles on the gill and intestine of common carp (*Cyprinus carpio*) in the presence of titanium dioxide nanoparticles, Chem. Ecol., 4 (2017) 295–308.
[28] L. Flohé, W.A. Günzler, Assay of glutathione peroxidase, Methods. Enzymol., 105 (1984) 115–121.
[29] J.M. McCord, J. Fridovich, Super oxide dismutase: an enzymatic function for erythrocuprein (hemocuprein), J. Biol. Chem., 244 (1969) 6049–6055.
[30] P. Prieto, M. Pineda, M. Aguilar, Spectrophotometric quantitation of antioxidant capacity through the formation of phosphomolybdenum complex, specific application to the determination of vitamin E, Ann. Biochem., 26 (1999) 337–341.
[31] B.Z.W. Vila, J.R.R. Marquardt, A.A. Frohlich, Effect of T-2 toxin on in vivo lipid peroxidation and vitamin E status in mice, Food Chem. Toxicol., 40 (2002) 479–486.
[32] T. Frankic, T. Pajk, V. Rezar, A. Levart, J. Salobir, The role of dietary nucleotides in reduction of DNA damage induced by T-2 toxin and deoxynivalenol in chicken leukocytes, Food. Chem. Toxicol., 44 (2006) 1838–1844.
[33] F. Majnoni, B. Mansouri, M.R. Rezaei, A.H. Hamidian, Contaminations of metals in tissues of common crap, *Cyprinus carpio* and Silver crap, *Hypophthalmichthys molitrix* from Zarivar wetland, western Iran, Arch. Polish. Fish, 21 (2013) 11–18.
[34] B.K. Greenfield, S.J. Teh, J.R.M. Ross, J. Hunt, G.H. Zhang, J.A. Davis, G. Ichikawa, D. Crane, S.S.O. Hung, D.F. Deng, F.C. Teh, P.G. Green, Contaminant concentrations and histopathological effects in Sacramento splittail (*Pogonichthys macrolepidotus*), Arch. Environ. Contamin. Toxicol., 55 (2008) 270–281.
[35] V. Poleksic, M. Lenhardt, I. Jaric, D. Djordjevic, Z. Gacic, G. Cvijanovic, B. Raskovic, Liver, gills, and skin histopathology and heavy metal content of the Danube sterlet (*Acipenser ruthenus Linnaeus, 1758*), Environ. Toxicol. Chem., 29 (2010) 515–521.
[36] Y. Wu, Q. Zhou, Silver nanoparticles cause oxidative damage and histological changes in medaka (*Oryzias latipes*) after 14 days of exposure, Environ. Toxicol. Chem., 32 (2013) 165–173.
[37] W. Jiraungkoorskul, S. Sshaphong, N. kangwanrangsan, M. Kim, Histopathological study: the effect of ascorbic acid on cadmium exposure in fish (*Puntius altus*), J. Fish. Aquat. Sci., 1 (2006) 191–199.
[38] R. Macirella, E. Brunelli, Morpho functional alterations in zebra fish (*Danio rerio*) gills after exposure to mercury chloride, Int. J. Mol. Sci., 18 (2017) 824–842.
[39] C.A. de Oliveira Ribeiro, L. Belger, E. Pelletier, C. Rouleau, Histopathological evidence of inorganic mercury and methyl mercury toxicity in the arctic charr (*Salvelinus alpinus*), Environ. Res., 90 (2002) 217–222.

[40] R. Fracário, N.F. Verani, E.L.G. Espíndola, O Rocha, O Rigolin-Sá, C.A. Andrade, Alterations on growth and gill morphology of *Danio rerio* (pisces, ciprinidae) exposed to the toxic sediments, Brazil, Arc. Biol. Technol., 46 (2003) 685–695.
[41] T. Wang, X. Long, Z. Liu, Y. Cheng, S. Yan, A Comparison effect of copper nanoparticles versus copper sulphate on Juvenile *Epinephelus coioides*: growth parameters, digestive enzymes, body composition, and histology as biomarkers, Int. J. Genom., 2015 (2015) 1–10.
[42] E.F. Pane, A. Haque, C.M. Wood, Mechanistic analysis of acute, Niinduced respiratory toxicity in the rainbow trout (*Oncorhynchus mykiss*): an exclusively branchial phenomenon, Aquat. Toxicol., 69 (2004) 11–24.
[43] S. Pereira, L.A. Pinto, R. Cortes, A. Fontanhas-Fernandes, A. M. Coimbra, S.M. Monteiro, Gill histopathological and oxidative stress evaluation in native fish captured in Portuguese northwestern rivers, Ecotoxicol Environ. Safe, 90 (2013)157–166.
[44] D.M.S. Santos, M.R.S. Melo, D.C.S. Mendes, I.K.B.S. Rocha, J.P.L. Silva, S.M. Cantanhêde, P.C. Meletti, Histological changes in gills of two fish species as indicators of water quality in Jansen Lagoon (São Luís, Maranhão State, Brazil), Int. J. Environ. Res. Public. Health, 11 (2014) 12927–12937.
[45] G.A. Al-Bairuty, B.J. Shaw, R.D. Handy, T.B. Henry, Histopathological effects of waterborne copper nanoparticles and copper sulphate on the organs of rainbow trout (*Oncorhynchus mykiss*), Aquat. Toxicol., 126 (2013) 104–115.
[46] K.S. Rajkumar, N. Kanipandian, R. Thirumurugan, Toxicity assessment on haemotology, biochemical and histopathological alterations of silver nanoparticles-exposed freshwater fish *Labeo rohita*, Appl. Nanosci., 6 (2016) 19–29.
[47] T. Ostaszewska, M. Chojnacki, M. Kamaszewski, E. Sawosz-Chwalibóg, Histopathological effects of silver and copper nanoparticles on the epidermis, gills, and liver of Siberian sturgeon, Environ. Sci. Pollut. Res., 23 (2016) 1621–1633.
[48] G. Sener, A.O. Sehirli, G. Ayanoglu-Dülger, Melatonin protects against mercury (II)-induced oxidative tissue damage in rats, Pharmacol. Toxicol., 93 (2003) 290–296.
[49] D.A. Monteiro, F.T. Rantin, A.L. Kalinin, Dietary intake of inorganic mercury: bioaccumulation and oxidative stress parameters in the neotropical fish Hoplias malabaricus, Ecotoxicol., 22 (2013) 446–456.
[50] X.N. Verlecar, K.B. Jena, G.B. Chainy, Biochemical markers of oxidative stress in *Perna viridis* exposed to mercury and temperature, Chem. Biol. Interact., 167 (2007) 219–226.
[51] N.J. Miller, C.A. Rice-Evans, Factors influencing the antioxidant activity determined by the ABTS radical cation assay, Free. Radic. Res., 26 (1997) 195–199.
[52] E.O. Oruç, N. Uner, Marker enzyme assessment in the liver of cyprinus carpio (L) exposed to 2,4-D and azinphosmethyl, J. Biochem. Mol. Toxicol., 16 (2002) 182–188.
[53] R. Thirumavalavan, Effect of mercury on lipid peroxidation and antioxidants in gill tissue of fresh water fish, *labeo rohita*, Ijrsr, 5 (2010) 122–124.
[54] A. Margarat, G. Jagadeesan, S. Sethupathy, Comparative effect of penicillamine and taurine on mercury poisoned mice, Mus musculus, Pollut. Res., 20 (2001) 1–4.
[55] J.L. Franco, H.C. Braga, A.K.C. Nunes, C.M. Ribas, A.P. Silva, Lactational exposure to inorganic mercury: evidence of neurotoxic effects, Neurobehav. Toxicol. Teratol., 29 (2007) 360–367.
[56] P.C. Pickhardt, M. Stepanova, N.S. Fisher, Contrasting uptake routes and tissue distributions of inorganic and methylmercury in mosquito fish (*Gambusia affinis*) and redear sunfish (*Lepomis microlophus*), Environ. Toxicol. Chem., 25 (2006) 2132–2142.
[57] R. Wang, W.X. Wang, Importance of speciation in understanding mercury bioaccumulation in tilapia controlled by salinity and dissolved organic matter, Environ. Sci. Technol., 44 (2010) 7964–7969.
[58] A. Boudou, F. Ribeyre, Experimental study of trophic contamination of *Salmo gairdneri* by two mercury compounds—$HgCl_2$ and CH_3HgCl—Analysis at the organism and organ levels, Water. Air. Soil. Pollut., 26 (1985) 137–148.
[59] E. Sumesh, M.S. Bootharaju, A.T. Pradeep, A practical silver nanoparticle based adsorbent for the removal of Hg^{2+} from water, J. Hazard. Mater., 189 (2011) 450–457.
[60] K.V. Katok, R.L. Whitby, T. Fukuda, T. Maekawa, I. Bezverkhyy, S.V. Mikhalovsky, Hyperstoichiometric interaction between silver and mercury at the nanoscale, Angew. Chem. Int. Ed. Engl., 51 (2012) 2632–2635.
[61] T. Yordanova, P. Vasileva, I. Karadjova, D. Nihtianova, Submicron silica spheres decorated with silver nanoparticles as a new effective sorbent for inorganic mercury in surface waters, Analyst., 139 (2014) 1532–1540.