



Research Article
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# Evaluation of toxicity and lethal concentration (LC<sub>50</sub>) of silver and selenium nanoparticle in different life stages of the fish *Tenualosa ilish* (Hamilton 1822)



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#### Abstract

The anadromous *Tenualosa ilish* is the most economically important fish in the world and consequently in Iran. The national fish of Iranian contributes about 12% of the total fish production and about 1% of GDP. About 4,500,000 people are directly involved with the catching for livelihood; around four to five million people are indirectly involved with the trade. The present study aimed to estimate  $96hLC_{50}$  of silver and selenium on  $LC_{50}$  of nanoparticle of silver and selenium in different life stages of the fish *T. ilish* in the environment experimental. The  $LC_{50}$  for silver in four stages of fish *T. ilish* were larvae (0.23ppm), fry (0.45ppm), juvenile (0.90ppm) and fingerling (1.45ppm), respectively. There was significance difference between  $LC_{50}$  levels in four stages of fish species (P<0.05) and the  $LC_{50}$  for selenium in four stages of fish *T. ilish* were larvae (0.89ppm), fry (0.95ppm), juvenile (1.12ppm) and fingerling (1.65ppm), respectively. There was significance difference between  $LC_{50}$  levels in four stages of fish species (P<0.05). Variability in acute toxicity even in a single species and single toxicant depending on the size, age and condition of the test species along with experimental factors. Finally, in order of the toxicity of heavy metal in different stage of fish were larvae > fry > juvenile > fingerling, respectively. In conclusion, the toxicity tended to elevate with decreasing fish size.

Keywords: Nanotechnology; Heavy metal; Nanoparticle; LC50; Tenualosa ilish

## Introduction

The aquatic environment makes up the major part of our environment and resources. Therefore, its safety is directly related to human health. The excessive contamination of aquatic ecosystems has evoked major environmental and health concerns worldwide because the aquatic environment is the ultimate recipient of pollutants produced by natural and anthropogenic sources [1].

Heavy metals are defined as metallic elements that have a relatively high density compared to water. With the assumption that heaviness and toxicity are inter-related, heavy metals also include metalloids, such as silver and selenium that are able to induce toxicity at low level of exposure [2]. In recent years, there has been an increasing ecological and global public health concern associated with environmental contamination by these metals. Also, human exposure has risen dramatically as a result of an exponential increase of their use in several industrial, agricultural, domestic and technological applications. Reported

sources of heavy metals in the environment include geogenic, industrial, agricultural, pharmaceutical, domestic effluents, and atmospheric sources. Environmental pollution is very prominent in point source areas such as mining, foundries and smelters, and other metal-based industrial operations [1,3].

Heavy metals become toxic when they are not metabolized by the body and accumulate in the soft tissues [4]. Heavy metals may enter the human body via food, water, air, or absorption through the skin in agriculture, manufacturing, pharmaceutical, industrial, or residential settings. Industrial exposure is common in adults. Ingestion is the most common route in children [5]. Children may develop toxic levels from normal hand-to-mouth activity (ie, coming in contact with contaminated soil or eating objects that are not food such as dirt or paint chips). Less common routes of exposure include a radiological procedure, inappropriate dosing or monitoring during intravenous (parenteral) nutrition, a broken thermometer, or a suicide or homicide attempt [6].

Silver nanoparticles (AgNPs) are widely used worldwide as spectrally selective coatings for solar energy absorption, chemical catalysts and especially for antimicrobial sterilisation [6]. After their discharge, AgNPs will most likely enter the ecosystems and may produce a physiological response in many animals, possibly altering their fitness, and ultimately changing their densities or community populations. Open access literature regarding the toxicity of nanoparticles is still emerging, and gaps still exist in our knowledge of this area. The mechanism of toxicity of silver NPs in fish has not been determined. An enhanced toxicity of nanoparticulate silver, compared to silver ions (Ag\*), may result from their shape and/or size, the release of silver ions (silver ions are well known to be toxic to aquatic organisms) or a combination of both [7-9].

Yeo and Kang examined the rate of photosynthesis in zebrafish embryogenesis exposed to silver NPs or silver ions in both the presence and the absence of cysteine (which binds free silver ions) and showed that silver NPs were more toxic than silver ions, based on the concentration of ions present, requiring a higher concentration of cysteine to eliminate the toxicity. Wu et al. [10] studied the effects of silver nanoparticles on the development and histopathology of Japanese medaka (*Oryzias latipes*) and after observing many changes, suggested further research on toxicity of silver nanoparticle in fish species. Yeo & Kang confirmed fatal effects of nanometer sized silver materials on zebrafish embryogenesis. Also, Solatni et al. [11] reported the toxic effect of nanosilver particles on hatchability of rainbow trout (*Oncorhynchus mykiss*) eggs and the survival of the produced larvae.

Ishikawa et al. [12] studied the effects of selenium nanoparticles on the histopathology of Goldfish (*Carassius auratus*). They suggested further research on toxicity of selenium nanoparticle in fish species. Karasu et al. [13] studied the effects of selenium nanoparticles on the histopathology on zebrafish. Also, Kamunde et al. [2] reported the toxic effect of nanoselenium particles on hatchability of zebrafish.

The LD<sub>50</sub> is the dose of a given chemical product (pesticide,...) which causes the mortality of 50% of a group of test animals in a specified period. It is commonly used in bioassays assessments to measure the acute toxicity of a chemical active ingredient. The lower the LD<sub>50</sub> value the more toxic the chemical [13]. These calculations are based on two important assumptions. The first assumption is that the exposure time associated with the specified LC<sub>50</sub> is sufficient to allow almost complete chemical equilibration between the fish and the water. The second assumption is that the specified  $LC_{50}$ , the minimum  $LC_{50}$  that kills the fish during the associated exposure interval [14]. Fortunately, most reliable  $LC_{50}$  satisfy these two assumptions [15]. The 96h  $LC_{50}$  tests are conducted to measure the susceptibility and survival potential of organisms to particular toxic substances such as oils pollution. Higher LC<sub>50</sub> values are less toxic because greater concentrations are required to produce 50% mortality in organisms [7].

*Tenualosa ilisha* is a species of fish in the herring family (Clupeidae), and a popular food fish in South Asia. It is also the national fish of Iran. The national fish of Iranian contributes about 12% of the total fish production and about 1% of GDP [1]. About 4,500,000 people are directly involved with the catching for livelihood; around four to five million people are indirectly involved with the trade. The average annual landing of this species in 2006 was 4989.83t and constituted about 15% of Khouzestan Province total commercial fish landing. During 2008, 4645t of *T. ilisha* was landed in the Khouzestan Province (Northwest of Persian Gulf). The present study aims to investigate the  $LC_{50}$  of selenium and silver in four life stages of, *Tenualosa ilish*. This fish species was chosen for its economic important, easily transported and maintained in the laboratory.

#### **Materials & Methods**

The fish used in the present experiments (Anadromous fish, *Tenualosa ilish*) were reared in the outdoor pond of NIOF fish farm. Four Different life stages were picked after 5, 15, 30, 45 and 90 days (larvae, fry, juvenile and fingerlings, respectively.) from the hatching. Fish stages and they were transported to the laboratory in containers containing water from the same pond. They have been acclimated for a day in a stock tank. Table 1 show length and weight in different stage fish species *T. ilish*.

Table 1: Length and weight in different stage fish species Tenualosa ilish

Stage	Length (cm)	Weight (gm)
Larvae	1.8- 2.5	0.05-0.5
Fry	2.5-5.5	0.5-1.00
Juvenile	5.5-9.5	12.5
Fingerling	10.0-13.5	5.0-10.3

Stock solutions of the heavy metals selenium and silver were prepared by using pure grad of  ${\rm CuSO_4.5H_2O}$  and  ${\rm HgCl_2}$ . During the experiments, test solutions of selenium and silver were freshly prepared from the stock solutions. Test solutions of Se ranged from 0.5 to 5ppm, while Hg varied from 0.05 to 2ppm for all 4 life stages [12,14].

All samples were acclimated for 1 week in 15 aerated fiberglass tanks at 25  $^{\circ}$ C under a constant 12:12h light:dark photoperiod. Acclimatized fish were fed daily with a formulated feed. Dead fish were immediately removed with special plastic forceps to avoid possible deterioration of the water quality.

Silver tested concentrations were 0, 0.1, 0.5, 1.00 and 1.5ppm of silver, and selenium tested concentrations were 0, 0.1, 0.2, 0.3 and 0.5ppm of selenium. Groups of 21 fish were exposed for 96h in fiberglass tank. Test medium was not renewed during the assay and no food was provided to the animals. Values of mortalities were measured at time 0, 24, 48, 72 and 96h.

Acute toxicity tests were carried out in order to calculate the 96h  $LC_{50}$  for metals, based on the study conducted by Hotos

and Vlahos. Mortality was recorded after 24, 48, 72 and 96h and  $LC_{50}$  values and its confidence limits (95% CLs) were calculated. Percentages of fish mortality were calculated for each metal concentration at 24, 48, 72 and 96h of exposure.

Also,  $LC_{50}$  values were calculated from the data obtained in acute toxicity bioassays, using Finney's method of 'probit analysis' and with SPSS computer statistical software. In Finney's method, the  $LC_{50}$  value is derived by fitting a regression equation arithmetically and also by graphical interpolation by taking logarithms of the test chemical concentration on the x-axis and the probit value of percentage mortality on the y-axis.

The 95% CLs of the  $LC_{50}$  values obtained by Finney's method was calculated with the formula of Mohapatra and Rengarajan. Probit transformation adjusts mortality data to an assumed

normal population distribution that results in a straight line. Probit transformation is derived from the normal equivalent deviate approach developed by Tort who proposed measuring the probability of responses (i.e. proportion dying) on a transformed scale based in terms of percentage of population or the SDs from the mean of the normal curve.

#### Results

All controls resulted in low mortalities, fewer than 5%, which indicated the acceptability of the experiments. The mortality of roach for silver and selenium was examined during the exposure times at 24, 48, 72 and 96h in Tables 2 & 3, respectively. With increasing concentration, fish exposed during the period of 24–96h had significantly increased number of dead individual. There were significant differences in number of dead fish between the duration of 24 and 96h in each exposure.

Table 2: Cumulative mortality of fish during acute exposure to selenium (n = 20).

Chama	C	No. of mortality			
Stage	Concentration (ppm)	24h	48h	72h	96h
Larvae	Control	0	0	0	0
	0.1	0	0	0	0
	0.2	0	0	0	0
	0.3	19	19	20	20
	0.5	21	22	22	24
	Control	0	0	0	0
	0.1	0	0	0	0
Fry	0.2	0	0	0	0
	0.3	0	0	0	0
	0.5	28	28	29	30
	Control	0			
	0.1	0			
Juvenile	0.2	0			
	0.3	0	0	0	0
	0.5	32	33	33	34
	Control	0	0	0	0
Fingerling	0.1	0	0	0	0
	0.2	0	0	0	0
	0.3	0	0	0	0
	0.4	34	34	36	37

Table 3: Cumulative mortality of fish during acute exposure to silver (n = 20).

Stage	Concentration (ppm)	No. of mortality			
		24h	48h	72h	96h
Larvae	Control	0	0	0	0
	0.1	0	0	0	0
	0.5	0	0	0	0
	1	0	0	0	0
	1.5	8	15	15	16

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Fry	Control	0	0	0	0
	0.1	0	0	0	0
	0.5	0	0	0	0
	1	0	0	0	0
	1.5	18	18	19	20
	Control	0			
	0.1	0			
Juvenile	0.5	0			
	1	0	0	0	0
	1.5	20	21	21	22
Fingerling	Control	0	0	0	0
	0.1	0	0	0	0
	0.5	0	0	0	0
	1	0	0	0	0
	1.5	23	23	25	26

The  $LC_{50}$  for silver in four stages of fish T. ilish were larvae (0.23), fry (0.45), juvenile (0.90) and fingerling (1.45), respectively. There was significance difference between  $LC_{50}$  levels in four stages of fish species (P<0.05). The highest of Toxicity of silver were detected fingerling stage of fish species. Considering the silver bioassay, the lowest concentration causing 100% of fish mortality was 1.00mg/l at 96h, while the highest concentration causing no fish mortality was 1.5mg/l at 96h.

The  $LC_{50}$  for selenium in four stages of fish T. ilish were larvae (0.89), fry (0.95), juvenile (1.12) and fingerling (1.65), respectively. There was significance difference between  $LC_{50}$  levels in four stages of fish species (P<0.05). The highest of Toxicity of silver were detected fingerling stage of fish species. Considering the selenium bioassay, the lowest concentration causing 100% of fish mortality was 0.3mg/l at 96h, while the highest concentration causing no fish mortality was 0.5mg/l at 96h.

## Discussion

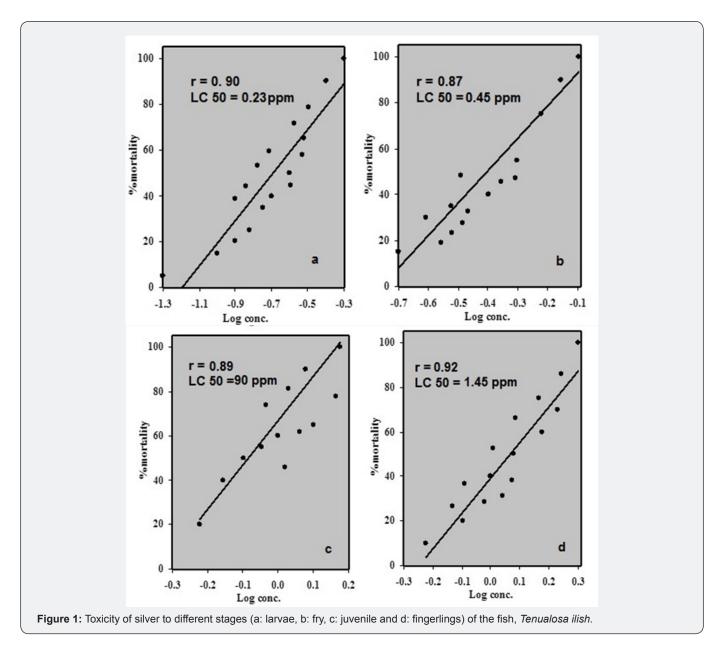
Variability in acute toxicity even in a single species and single toxicant depending on the size, age and condition of the test species along with experimental factors. The differences in acute toxicity may be due to changes in water quality and test species. It is evident from the results that the heavy metal concentration has a direct effect on the  $LC_{50}$  values of the respective fish.  $LC_{50}$  values indicated that silver is more toxic than others.  $LC_{50s}$  obtained in the present study were compared with corresponding values that have been published in the literature for other species of fish.

The degree of studied fish to lower concentrations of selenium may be attributed to the altered physiological response of every species to the specific metal and the level of solubility of metals. The fish exposed to selenium can compensate for the

pollutant. If it cannot successfully compensate for contaminant effects, an altered physiological stage may be reached in which the fish species continues to function and, in extreme cases, the acclimation response may be exhausted with a subsequent effect on fitness.

The susceptibility of fish species to a particular heavy metal is a very important factor for  $LC_{50}$  levels. The fish that is highly susceptible to the toxicity of one metal may be less or even nonsusceptible to the toxicity of another metal at the same level of that metal in the ecosystem. Also, the metal which is highly toxic to a fish species at low concentration may be less or even nontoxic to other species at the same or even higher concentration. Because of the lack of available data on the effects of selenium on the respective  $LC_{50}$  values of all studied species, the results of the present study have not been compared with those of other studies and discussed accordingly. However, some justifications have been provided following various studies.

Silver is highly toxic to aquatic organisms and interacts with numerous inorganic and organic compounds which affect its bioavailability and toxicity to aquatic biota. Its toxicity depends on environmental factors that change through time and space (e.g. temperature and water quality) and on the affected organism's species, age, size, and reproductive condition [16]. Figure 1 shows the toxicity patterns of silver to fish, *T. ilish*, at different life stages (larva, fry, juvenile and fingerling). Estimation of the median lethal concentrations of silver showed that the 96h LC5 $_{\rm 0s}$  were 0.1, 0.5, 1.00 and 1.5ppm, respectively. It can be noticed that the LC5 $_{\rm 0s}$  in the 1st 3 length categories were similar and the 4th category was the highest one which means that the tolerance of fish increases at the 4th length category than the others, also indicated that the 1st stages were more venerable than the older stages.



Few studies examined the relation between fish size and the silver toxicity for finfish. Howarth and Sprague reported that silver toxicity to rainbow trout, *Oncorhynchus mykiss*, decreased with fish growth, and the toxicity for 0.71g fish was three times higher than that for 10g in freshwater. Hedayati et al. reported that silver toxicity to *Rutilus rutilus* decreased with fish growth, and the toxicity for 2gr fish was four times higher than that for 10g in freshwater. They reported that  $LC_{50}$  for silver in *Rutilus rutilus* was 0.36ppm of silver. Also, Hedayati et al. reported that the toxicity for 3gr fish was three times higher than that for 9g in fish *Acanthopagrus latus*. They reported that  $LC_{50}$  for silver in *Rutilus rutilus* was 0.45ppm of silver.

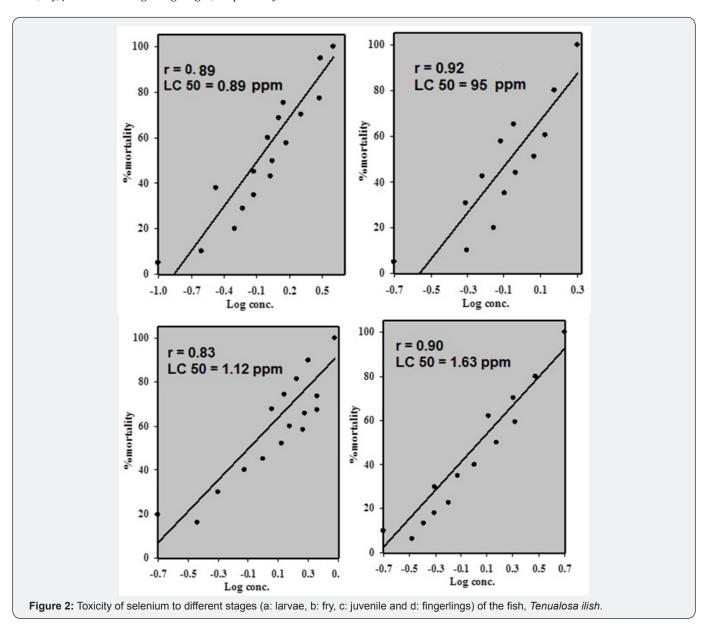
There were numerous studies examining the silver toxicity to fresh and marine fish, 96h  $LC_{s_0}$  reported a range between 2 to

5ppm and 0.1 to 15ppm, respectively [17]. Straus [18] recorded a range of (0.23 to 28.39ppm) within a total alkalinity of 11-215mg/l  $CaCO_3$ , and stated that the Se sulfate can be extremely toxic to fish in water of low alkalinity. In the same manner the mean total alkalinity in the present study condition was 218mg/l  $CaCO_3$ . As well as, more studies recorded that Cu is less toxic in hard water than in soft water [16,18]. For fresh water fish, Oliva et al. [19] reported 0.35ppm silver as 96h  $LC_{50}$  for juvenile singales, 0.25 for chequered rainbow, 0.14 for black striped rainbow and 0.021ppm for fly specked hardhead. This indicates that the present studied species was more tolerance to silver toxicity than other species studied.

Silver is classified as one of the most toxic metals, which are introduced into the natural environment by human interferences.

Inorganic silver is the most common form of the metal released in the environment by industries, presenting a stronger acute effect on fish tissues than that of its organic form [20]. Figure 2 shows the toxicity patterns of selenium to the different studied stages of the fish T ilish. 96h Hg LC $_{50}$  were 0.1, 0.2, 0.3 and 0.5ppm for larva, fry, juvenile and fingerling stages, respectively. It was found

that the toxicity of selenium was significantly decreased (r=0.98,  $p\le0.05$ ) with increasing the fish life stage (Figure 3). Unlike the present study, some studies on other aquatic fish species indicated that selenium toxicity did not change significantly with varying size [21].



Hedayati et al. reported that selenium toxicity to *Rutilus rutilus* decreased with fish growth, and the toxicity for 2gr fish was three times higher than that for 10g in freshwater. They reported that  $LC_{50}$  for selenium in *Rutilus rutilus* was 0.87ppm. Also, Hedayati et al. reported that the toxicity for 5gr fish was five times higher than that for 20g in fish *Acanthopagrus latus*. They reported that  $LC_{50}$  for silver in *Rutilus rutilus* was 1.37ppm.

From the previous studies, Ishikawa et al. [12] recorded 0.45ppm 96h  $LC_{50}$  for *Oreochromis leucostictus* fingerlings which was compare to the present study (Table 4). In the

context, Ramamurthi et al. recorded Hg LC $_{50}$  0.87ppm for *Tilapia mossambicus*. As well as, many studies recorded a range of 0.045–0.21ppm selenium LC $_{50}$  for different fish species which was more or less lower than the present study [22]. It was indicated that the present examined species was more tolerant than the previous studied species. For both of the present studied metals (selenium and silver), it can be noticed that the toxicity tended to elevate with decreasing fish size, this mean that the earlier stages were more sensitive than the older one. Grosell et al. [23] stated that the size was an effective factor for acute toxicity in fresh water

organisms. It has been mentioned that small fish or younger organisms were more susceptible to metal poisoning than the larger or more mature fish. Thongra-ar et al. [21] and Furuta et

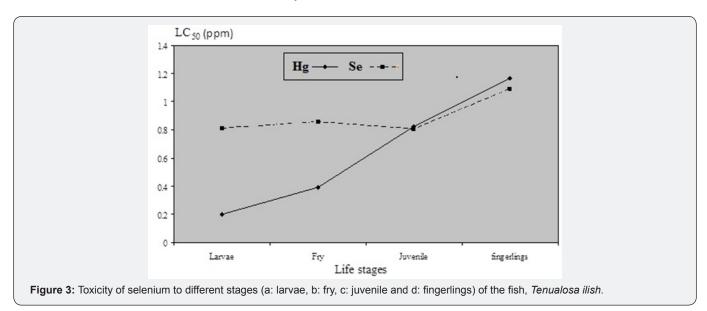
al. [6] found that the seabass larvae were more sensitive to Hg toxicity than the juvenile stages.

Table 4: The comparison of LC<sub>50</sub> of silver and selenium in Tenualosa ilish in this study with different studies.

Consider a	L	n 6		
Species	Selenium	Silver	References	
Tenualosa ilish	1.65	1.45	This study	
Oreochromis leucostictus	0.81	0.2	Ramamurthi et al. (1982)	
Tenualosa ilish	1.45	0.73	Verma et al. (1985)	
Oreochromis leucostictus	0.85	0.4	Chen (2000)	
Liza abu	0.8	0.82	Shyong and Chen (2000)	
Oreochromis leucostictus	1.09	1.17	Gooley (2000)	
Tilapia mossambicus	1.1	0.79	Grosell et al. (2000)	
Oreochromis leucostictus	1.4	0.22	Thongra-ar et al. (2003)	
Tenualosa ilish	0.87	0.45	Straus (2003)	
Carcharhinus dussumieri	0.76	1.04	Furuta et al. (2007)	
Chanos chanos	0.34	0.32	Hedayati et al et al.( 2011)	
Liza abu	0.23	0.67	Hedayati et al et al. (2012)	
Euryglossa orientalis	1.78	1.71	Hosseini et al. (2015)	

By comparing the toxicity of selenium and silver in the present study (Figure 3), it can be stated that the silver is more toxic than selenium during the  $1^{\text{st}}$  2 life stages, where they recorded the same toxicity during the last 2 life stages with slight increase of Se toxicity at the last stage. Gooley and Shyong and Chen found that selenium was more toxic than silver to *Acrosscheilus paradoxus* 

and  $Zacco\ barbata$ , respectively. Hedayati et al. reported that silver toxicity to  $Rutilus\ rutilus\$ was more toxic than other metal and  $LC_{50}$  of silver was higher than selenium, cobalt and silver. Also, Verep et al. and Vieira et al. reported that silver toxicity to  $pomatoschistus\$ microps and  $Rainbow\$ trouts was higher than other heavy metal.



The results of toxicity test of silver and selenium in current study were compared to those reported for fish in the Persian Gulf and different regions of the world (Table 4). In general, the  $LC_{50}$  of silver and selenium in our study were higher than those in *Oreochromis leucostictus* by Ramamurthi et al. *Tenualosa ilish* 

by Verma et al. *Oreochromis leucostictus* by Chen and *Tilapia mossambicus* in study of Grosell et al. [23]. The  $LC_{50}$  of silver and selenium in *Tenualosa ilish* in this study was higher than all studies that present in Table 4, expect in *Euryglossa orientalis* in study by Hosseini et al.

#### Conclusion

However, in the current study, the LC<sub>50</sub> values vary from each species and the accumulation of heavy metals in the body of fish depends upon several factors, it is evident from the present study that concentrations of selenium and physiological response affect the  $LC_{50}$  values of fish. It may be due to the increased resistance of carp to the selenium through acclimatization. During acclimatization, some proteins are released in the body of fish and detoxify the metal ions. This may cause higher levels of heavy metals being required to cause effects, resulting in higher LC<sub>50</sub> amounts. The selection of heavy metals may be an important tool for the assessment of the effects of pollutants in aquatic ecosystems; both metals used in our experiment demonstrated their potential for use in bioassays. In conclusion, comparing the sensitivity of these metals to common reference toxicants, we suggest using Roach for toxicity determinations as a suitable model of ecotoxicological studies. Clearly, there is a need to conduct further studies with specific contaminants on this species to assess its suitability for detecting toxicity, as well as experiments involving a complex mixture of pollutants, in order to determine aquatic ecosystems monitoring program.

#### Acknowledgment

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