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Full Length Research Paper

Evaluation of LC50 of Nanoparticle of Mercury and Selenium in Different life Stages of the Fish *Tenualosa ilish* (Hamilton 1822) in the Environment Experimental

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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 96 hLC_{50s} of mercury and selenium on LC_{50} of nanoparticle of mercury and selenium in different life stages of the fish *T. ilish* in the environment experimental. The LC_{50} for mercury in four stages of fish *T. ilish* were larvae (0.23 ppm), fry (0.45 ppm), juvenile (0.90 ppm) and fingerling (1.45 ppm), 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 (0.89 ppm), fry (0.95 ppm), juvenile (1.12 ppm) and fingerling (1.65 ppm), 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: Nanoparticle, LC_{50}, Heavy metal, Toxicity, *Tenualosa ilish*, Environment Experimental

INTRODUCTION

Selenium nanoparticles, nanodots or nanopowder are typically 10 - 45 nanometers (nm) with specific surface area (SSA) in the 30 - 50 m^2/g range and also available in with an average particle size of 75 - 100 nm range with a specific surface area of approximately 2 - 10 m 2 /g. Nano Selenium Particles are also available in passivated and Ultra high purity and high purity and coated and dispersed forms (Hedayati et al. 2012). They are also available as a

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nanofluid through the AE Nanofluid production group. Nanofluids are generally defined as suspended nanoparticles in solution either using surfactant or surface charge technology. Nanofluid dispersion and coating selection technical guidance is also available. Other nanostructures include nanorods, nanowhiskers, nanohorns, nanopyramids and other nanocomposites (Bury et al. 2002).

Mercury is considered to be one of the most notorious metal pollutants present in food, water, air and soil, but the process of eliminating it is limited. Mercury toxicity impacts proteins in the body, where elements and element groups (compounds) make chains in specific formations, with just the right molecular bends, turns, and pockets to enable cells to function. Important elements in the protein chains include sulfur (S) and selenium (Se). When mercury (Hg) binds to sulfur or selenium, it can disrupt the protein chain to the point of impairing cell function and damaging cells, affecting organs, muscles and neurological functions (Hosseini et al. 2015).

Heavy metals such as mercury are formed on the earth's crust and made into solutions with ground water through certain natural processing and pH changes occurring in the soil (Beaumont et al. 2000). There are traditional methods that are used to extract mercury from the natural water sources and industrial waste water, such as chemical precipitation, amalgamation, reverse osmosis, membrane filtration and photochemical method. However, these methods are expensive, time consuming, and inefficient, hence the need for a nanofiltration technology that overcomes all of these issues. Nanofiltration technology is very efficient in removal of mercury species due to its characteristics of having high surface area-to-volume and the fact that it's easily chemically functionalized (Basha and Rani 2003). Additionally, Brownian motion of nanomaterials allows them to scan large volume of solvent in short times (Braunbeck and Lammer 2006). There are many copolymer nanoparticles (NPs) that can be used as scavengers to eliminate mercury species via redox reactions such as selenium NPs, manganese dioxide nanowhiskers and mercury NP-based materials. Among these adsorbents, citrate-capped gold NP-based materials have been used intensively to capture mercury species from nature water (Hedayati et al. 2012).

The LD_{50} 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_{50} value the more toxic the chemical (Grosell et al. 2002). These calculations are based on two important assumptions. The first assumption is that the exposure time associated with the specified LC_{50} 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. Fortunately, most reliable LC_{50} satisfy these two assumptions (Ishikawa et al. 2007). The 96-h LC_{50} tests are conducted to measure the susceptibility and survival potential of organisms to particular toxic substances such as oils pollution. Higher LC_{50} values are less toxic because greater concentrations are required to produce 50% mortality in organisms (Ferrer et al. 2007; Furuta et al. 2007).

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. 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 4989.83 t and constituted about 15 % of Khouzestan Province total commercial fish landing. During 2008, 4645 t of *T. ilisha* was landed in the Khouzestan Province (Northwest of Persian Gulf) (Roomiani and Jamili, 2011). The present study aims to investigate the LC_{50} of selenium and mercury in four life stages of, *Tenualosa ilish*. This fish species was chosen for its economic important, easily transported and maintained in the laboratory.

MATERIALS AND 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 *Tenualosa ilish*.

Stock solutions of the heavy metals selenium and mercury were prepared by using pure grad of $CuSO_4.5H_2O$ and $HgCl_2$ (Ishikawa et al. 2007; Ezemonye et al. 2008). During the experiments, test solutions of selenium and mercury were freshly prepared from the stock solutions. Test solutions of Se ranged from 0.5 to 5 ppm, while Hg varied from 0.05 to 2 ppm for all 4 life stages (Kamunde et al. 2002; Ishikawa et al. 2007).

All samples were acclimated for 1 week in 15 aerated fiberglass tanks at 25_C under a constant 12:12 h 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 (Karasu et al. 2005).

Mercury tested concentrations were 0, 0.1, 0.5, 1.00 and 1.5 ppm of mercury and selenium tested concentrations were 0, 0.1, 0.2, 0.3 and 0.5 ppm of selenium. Groups of 21 fish were exposed for 96 h in fiberglass tank. Test medium was not renewed during the assay and no food

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	1.-2.5
Fingerling	10.0-13.5	5.0-10.3

was provided to the animals. Values of mortalities were measured at time 0, 24, 48, 72 and 96 h (Hedayati et al., 2011).

Acute toxicity tests were carried out in order to calculate the 96-h LC_{50} for metals, based on the study conducted by Hedayati et al (2012). Mortality was recorded after 24, 48, 72 and 96 h 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 96 h of exposure.

Also, LC_{50} values were calculated from the data obtained in acute toxicity bioassays, using Finney's (1971) method of 'probit analysis' and with SPSS computer statistical software. In Finney's method, the LC50 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 (McGeer et al. 2000).

The 95% CLs of the LC_{50} values obtained by Finney's method was calculated with the formula of Karasu Benli et al. (2009). 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 (Oliva et al. 2009).

RESULTS

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

The LC 50 for mercury 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 mercury were detected fingerling stage of fish species. Considering the mercury bioassay, the lowest concentration causing 100% of fish mortality was 1.00 mg/l at 96 h, while the highest concentration causing no fish mortality was 1.5 mg/l at 96 h.

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 mercury were detected fingerling stage of fish species. Considering the selenium bioassay, the lowest concentration causing 100% of fish mortality was 0.3 mg/l at 96 h, while the highest concentration causing no fish mortality was 0.5 mg/l at 96 h.

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 (Hedayati et al. 2011). It is evident from the results that the heavy metal concentration has a direct effect on the LC_{50} values of the respective fish.

Table 2 Cumulative mortality of Roach during acute exposure to selenium (*n* = 20)

Stage	Concentration (ppm)	No. of mortality			
		24 h	48 h	72 h	96 h
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
Fry	Control	0	0	0	0
	0.1	0	0	0	0
	0.2	0	0	0	0
	0.3	0	0	0	0
	0.5	28	28	29	30
Juvenile	Control	0			
	0.1	0			
	0.2	0			
	0.3	0	0	0	0
	0.5	32	33	33	34
Fingerling	Control	0	0	0	0
	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 Roach during acute exposure to mercury (*n* = 20)

Stage	Concentration (ppm)	No. of mortality			
		24 h	48 h	72 h	96 h
Larvae	Control	0	0	0	0
	0.1	0	0	0	0
	0.5	0	0	0	0
	1.00	0	0	0	0
	1.5	8	15	15	16
Fry	Control	0	0	0	0
	0.1	0	0	0	0
	0.5	0	0	0	0
	1.00	0	0	0	0
	1.5	18	18	19	20

Table 3: Continue

Juvenile	Control	0			
	0.1	0			
	0.5	0			
	1.00	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
	1.00	0	0	0	0
	1.5	23	23	25	26

LC_{50} values indicated that mercury 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 (Shyong et al. 2000).

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 (Straus 2003; Hedayati et al., 2011).

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 (Sunderland et al. 2000; Hedayati et al., 2010). 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.

Mercury 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 (Woody, 2007). Figure (1) shows the toxicity patterns of mercury to fish, *T. ilish*, at different life stages (larva, fry, juvenile and fingerling). Estimation of the median lethal concentrations of mercury showed that the 96h $LC5_{0s}$ were 0.1, 0.5, 1.00 and 1.5 ppm, respectively. It can be noticed that the $LC5_{0s}$ 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 mercury toxicity for finfish. Sunderland (2000) reported that mercury toxicity to rainbow trout, *Oncorhynchus mykiss*, decreased with fish growth, and the toxicity for 0.71 g fish was three times higher than that for 10 g in freshwater. Hedayati et al. (2011) reported that mercury toxicity to *Rutilus rutilus* decreased with fish growth, and the toxicity for 2 gr fish was four times higher than that for 10 g in freshwater. They reported that LC_{50} for mercury in *Rutilus rutilus* was 0.36 ppm of mercury. Also, Hedayati et al. (2012) reported that the toxicity for 3 gr fish was three times higher than that for 9 g in fish *Acanthopagrus latus*. They reported that LC_{50} for mercury in *Rutilus rutilus* was 0.45 ppm of mercury.

There were numerous studies examining the mercury toxicity to fresh and marine fish, 96h LC_{50} reported a range between 2 to 5 ppm and 0.1 to 15 ppm, respectively (Thongra-ar et al. 2003; IPC INCHEM, 1998). Straus (2003) recorded a range of (0.23 to 28.39 ppm) within a total alkalinity of 11- 215mg/l selenium, and stated that the selenium 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 218 mg/l selenium. As well as, more studies recorded that selenium is less toxic in hard water than in soft water (Straus 2003; Woody 2007; Hosseini et al. 2013). For fresh water fish, Oliva et al. (2009) reported 0.35 ppm mercury as 96h LC_{50} for juvenile singales, 0.25 for chequered rainbow, 0.14 for black striped rainbow and 0.021 ppm for fly specked hardhead. This indicates that the present studied species was more tolerance to mercury toxicity than other species studied.

Mercury is classified as one of the most toxic metals, which are introduced into the natural environment by

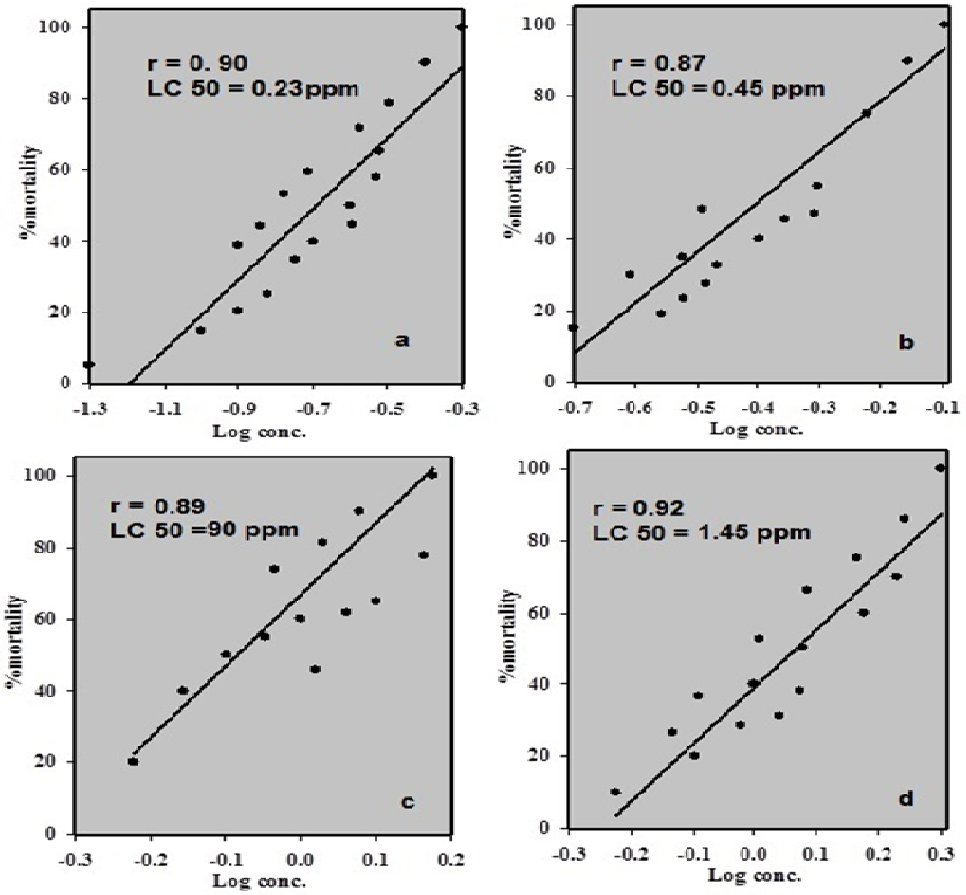

Figure. 1 Toxicity of mercury to different stages (a: larvae, b: fry, c: juvenile and d: fingerlings) of the fish, *T. ilish*

human interferences (Hosseini et al. 2013). Inorganic mercury 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 (Sunderland and Chmura 2000). Figure (2) shows the toxicity patterns of selenium to the different studied stages of the fish *T. ilish*. 96h Hg LC50 were 0.1, 0.2, 0.3 and 0.5 ppm for larva, fry, juvenile and fingerling stages, respectively. It was found that the toxicity of selenium was significantly decreased ($r=0.98$, $p \leq 0.05$) 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 (Thongra-ar et al. 2003; Hedayati et al. 2012).

Hedayati et al. (2011) reported that selenium toxicity to *Rutilus rutilus* decreased with fish growth, and the toxicity for 2 gr fish was three times higher than that for 10 g in freshwater. They reported that LC_{50} for selenium in *Rutilus rutilus* was 0.87 ppm. Also, Hedayati et al. (2012) reported that the toxicity for 5 gr fish was five times higher than that for 20 g in fish *Acanthopagrus latus*. They reported that LC_{50} for mercury in *Rutilus rutilus* was 1.37 ppm.

From the previous studies, Ishikawa et al. (2007) recorded 0.45 ppm 96h LC_{50} for *Oreochromis leucostictus* fingerlings which was compare to the present study (Table 4). In the context, Ramamurthi et al. (1982) recorded Hg LC_{50} 0.87 ppm for *Tilapia mossambicus*. As well as, many studies recorded a range of 0.045 – 0.21 ppm selenium LC_{50} for different fish species which was more or less lower than the present study (Valavanidis and Vlachogianni 2010). 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 mercury), 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. (2002) stated that the size was an effective factor for acute toxicity in fresh water organisms. It has been mentioned that small

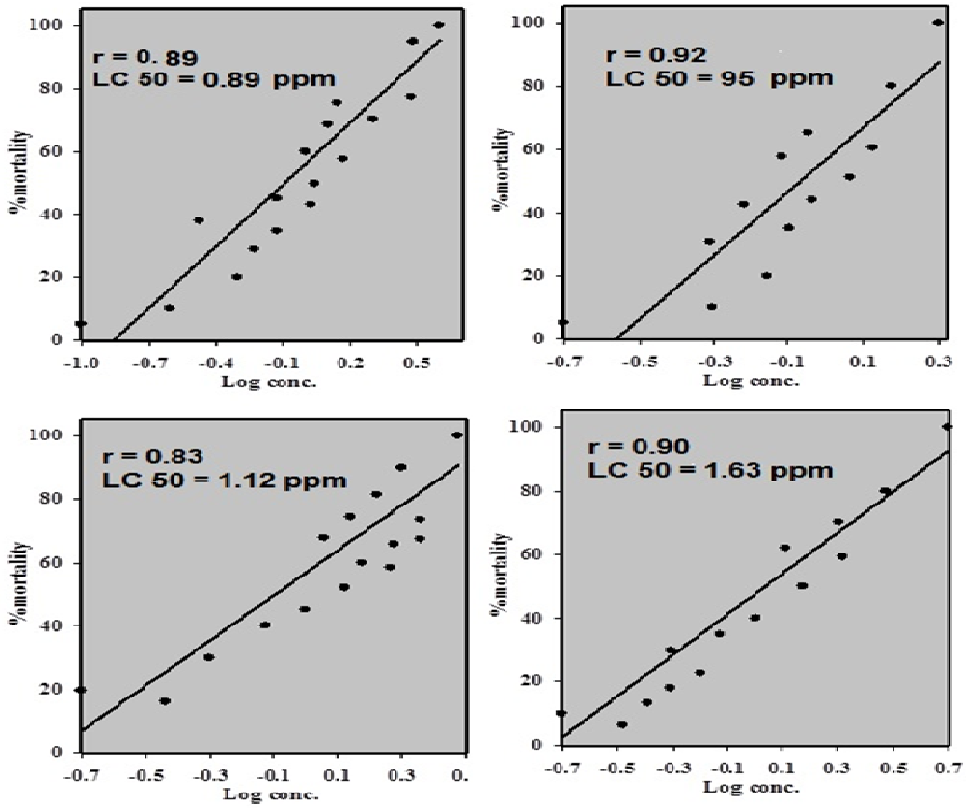

Figure 2: Toxicity of selenium to different stages (a: larvae, b: fry, c: juvenile and d: fingerlings) of the fish, *T. ilish*

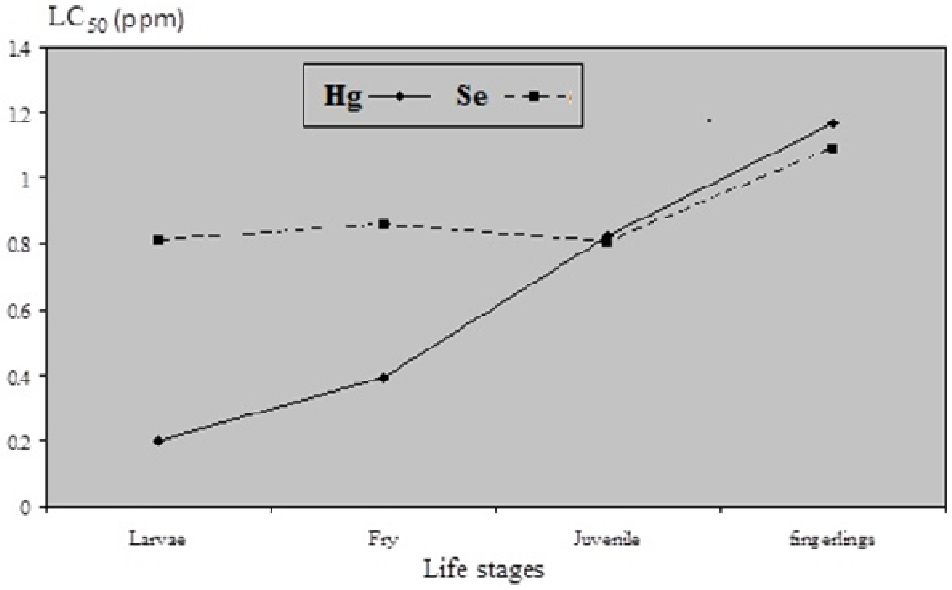

Figure 3: Toxicity pattern of selenium and mercury to different stages (larvae, fry, juvenile and fingerlings) of the fish, *T. ilish*

Table 4 The comparison of LC_{50} of mercury and selenium in *Tenualosa ilish* in this study with different studies

Species	LC_{50}		References
	Selenium	Mercury	
Tenualosa ilish	1.65	1.45	This study
Oreochromis leucostictus	0.81	0.20	Ramamurthi et al. (1982)
Tenualosa ilish	1.45	0.73	Verma et al. (1985)
Oreochromis leucostictus	0.85	0.40	Shyong and Chen (2000)
Liza abu	0.80	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)

fish or younger organisms were more susceptible to metal poisoning than the larger or more mature fish (Headayati et al. 2012). Thongra-ar et al. (2003) and Furuta et al. (2007) found that the seabass larvae were more sensitive to Hg toxicity than the juvenile stages.

By comparing the toxicity of selenium and mercury in the present study (Figure. 3), it can be stated that the mercury is more toxic than selenium during the 1st 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 (2000) and Headayati et al. (2012) found that selenium was more toxic than mercury to *Acrosscheilus paradoxus* and *Zacco barbata*, respectively. Hedayati et al. (2011 and 2012) reported that mercury toxicity to *Rutilus rutilus* was more toxic than other metal and LC_{50} of mercury was higher than selenium, cobalt and silver. Also, Verep et al. (2007) and Vieira et al. (2009) reported that mercury toxicity to *pomatoschistus microps* and *rainbow trouts* was higher than other heavy metal.

The results of toxicity test of mercury 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 mercury and selenium in our study were higher than those in *Oreochromis leucostictus by* Sunderland and Chmura (2000), *Tenualosa ilish* by Oliva et al. (2000), *Oreochromis leucostictus* by Shyong and Chen (2000) and *Tilapia mossambicus* in study of Grosell et al. (2000). The LC_{50} of mercury 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. (2015).

CONCLUSION

However, in the current study, the LC50 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_{50} amounts (Deb and Fukushima, 1999). 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.

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