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Original Article

Acute toxicity bioassay of the mercury chloride and copper Sulphate in *Rutilus caspicus* and *Rutilus kutum*

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Abstract: The purpose of the present study was to determine the acute toxicity (LC_{50}) of $HgCl_2$ and $CuSO_4$ in Caspian roach (*Rutilus caspicus*) and $CuSO_4$ in Caspian kutum (*Rutilus kutum*). The Caspian roach LC_{50} values for $HgCl_2$ at 24, 48, 72, and 96-hrs of exposure, were 0.64, 0.61, 0.42, and 0.28 mg L^{-1}, respectively, and for $CuSO_4$ were 11.55, 5.08, 2.49, and 1.47 mg L^{-1}, respectively. The Caspian roach LC_{50} values for $CuSO_4$ at 24, 48, 72, and 96-hrs of exposure, were 5.31, 4.17, 3.20, and 2.25 mg L^{-1}, respectively. The results of this study showed that the toxicity of $HgCl_2$ is higher than that of $CuSO_4$ for the studied species. The mortality decreased with time, and most of the deaths were occurred during the first 24 hrs.

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Introduction

Pollution pressures are associated with urbanization endangering the coastal ecosystems. The biota may be stressed by discharged specific point sources (e.g. sewage effluents and industrial wastes) and general non-point pollution (e.g. harbor activities, storm drainage and agricultural drainage). The aquatic ecosystems that receive pollutants are often important fishery and recreational areas (Pirbeigi et al., 2016; Nasrolah Pourmoghadam et al., 2015). It is therefore essential that techniques are established to monitor the pollutants that pose a danger to the aquatic biota and humans (Gopalakrishnan et al., 2008).

Metals are an important group of the water pollutants that disturb the integrity of biochemical and physiological mechanisms in aquatic organisms, such as fishes. Among metals, copper and mercury are of special concern since they are considerably toxic to aquatic animals at ecologically relevant concentrations (Zhang et al., 2005; Allen et al., 1994). Copper is a trace element that plays a fundamental role in the biochemistry of organisms, including aquatic organisms that can take it up directly from water (Grosell et al., 2003; Erickson et al., 1996). However, it can become toxic at high concentrations (Alquezar et al., 2008). Among metals, mercury is of special concern since it is considerably toxic to aquatic animals at ecologically relevant concentrations (Vieira et al., 2009; Oliveira et al., 2002). Mercury is considered one of the most dangerous metals in the aquatic environment (Goyer et al., 1995; Ribeiro et al., 1996), mainly its organic forms that can be biomagnified in trophic chains representing an increased risk for top predators (MacDougal et al., 1996), including humans consuming contaminated fishes. Chronic exposure and accumulation of these chemicals by aquatic biota can result in tissues that produce adverse effects not only in the exposed organisms, but also in human beings (IARC., 1990; Karthikeyan et al., 2007). Therefore, it is essential to study the detrimental

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Table 1. Lethal concentration (LC_{50}) of mercury and copper estimated by EPA method on the Caspian Roach (*Rutilus caspicus*).

Metal	LC_{50} values (mg L^{-1})			
	24 hours	48 hours	72 hours	96 hours
Copper				
LC_{10}	0.69	0.56	0.48	0.43
LC_{50}	5.31	4.17	3.20	2.25
LC_{90}	40.07	30.11	21.04	11.36

effects of such hazardous pollutants so as to formulate the strategies for safeguarding aquatic organisms.

Caspian roach (*Rutilus caspicus*) and Caspian kutum (*Rutilus kutum*) are found in the southern part of the Caspian Sea, particularly in the coastal waters of Iran, Turkmenistan and Azerbaijan. These species are economically important fishes (Coad, 2015). Hence, this study aimed to investigate the toxic effects of the mercury chloride and copper sulphate on the Caspian roach and Caspian kutum by determination of 96-hour LC_{50} values

Materials and Methods

The specimens of Caspian roach (n=120) and Caspian kutum (n=120), with mean weight of 3±0.6 g were obtained from Sijval restocking center (Bandar Turkaman, north of Iran) during August to September 2009. The fishes were transported in polythene bags to the fisheries laboratory of the Gorgan University of Agricultural Sciences and Natural Resources. Then, they were maintained in 20 L pre-cleaned glass aquaria filled with dechlorinated tap water with water temperature of 26±1°C in order to adaptation to the laboratory conditions. Thereafter, sets of 10 specimens (in triplicate) were randomly introduced to the aquarium systems. The exposure time to Hg (as $HgCl_2$) and Cu (as $CuSO_4$) were 96 hours, without any feed. Control group was designed with three replicates. No mortality was observed during the experimental period in control groups as well. In addition, four different concentrations of the mercury (as $HgCl_2$) in geometric decreasing amounts of 0.02, 0.03, 0.04, and 0.05 mgL^{-1} were used. Also, four different concentrations of copper (as $CuSO_4$) in geometric decreasing amounts of 1, 4, 7, and 10 mg L^{-1} in three-replicates were designed. For determination of the mortality limits of the mercury and copper as well as survival rate, the treatments and replications were considered based on OECD (OECD, 1988).

Stock solutions (1000 mg L^{-1}) were prepared by dissolving analytical-grade of the mercury (as $HgCl_2$, Merck) and copper (as $CuSO_4$; Merck) in distilled water. Preliminary tests were carried out to estimate the minimum lethal and maximum non-lethal concentrations of the mercury (as $HgCl_2$) and copper (as $CuSO_4$). Dissolved Oxygen (DO) (mg L^{-1}), temperature (°C), total hardness (mg L^{-1}) and pH were recorded in each aquarium during experiment. The recorded water parameters were as: temperature=26±1°C, pH=7.7±0.2; total hardness=205 mg L^{-1} as $CaCO_3$, and DO=7 mg L^{-1}. Water quality of the experimental tank was determined according to standard procedures. Lethal concentration for 50% (LC_{50}) values were calculated using the EPA computer probit analysis program (Version 1.5) (Vieira, 2009; Das et al., 2005; Pyle et al., 2002).

Statistical analyses were performed using SPSS software (ver. 16.0, SPSS Co., Chicago, IL, USA). All the data were tested for normality using Kolmogorov-Smirnov test.

Results

The LC_{50} value for $HgCl_2$ and $CuSO_4$ in the Caspian roach and Caspian kutum at 24, 48, 72, and 96-hrs of exposure period were presented in Tables 1 and 2. The LC_{50} value in Caspian roach for $HgCl_2$ at 24, 48, 72 and 96-hrs of exposure period were 0.64, 0.61,

Table 2. Lethal concentration (LC_{50}) of copper estimated by EPA method on the Caspian kutum (*Rutilus kutum*).

Metal	LC_{50} values (mg L^{-1})			
	96 hours	72 hours	48 hours	24 hours
Mercury				
LC_{10}	0.15	0.17	0.25	0.31
LC_{50}	0.28	0.42	0.61	0.64
LC_{90}	0.51	1.01	1.31	1.47
Copper				
LC_{10}	0.27	0.48	0.76	2.23
LC_{50}	1.47	2.49	5.08	11.55
LC_{90}	7.85	12.73	33.22	58.88

0.42, and 0.28 mg L^{-1}, respectively; while for $CuSO_4$, the LC_{50} values at 24, 48, 72 and 96-hrs were 11.55, 5.08, 2.49, and 1.47 mg L^{-1}, respectively (Table 1).

Discussion

The 96-hrs LC_{50} values of fish is species and metal dependent. In this study, the 96-hrs LC_{50} for $HgCl_2$ was determined to be 0.28 mg L^{-1}. In other studies the acute toxicity thresholds for inorganic mercury (typically as $HgCl_2$) in freshwater organisms vary from approximately 0.005-0.230 mg L^{-1} in crustaceans, to 0.06-0.8 mg/L in fish (Ramamoorthy and Baddaloo, 1995). The 96-hrs LC_{50} values for freshwater fish ranges 0.033-0.4 mg L^{-1}, while the LC_{50} is generally higher for marine fish (Boening, 2000). The 96-hrs LC_{50} values of $HgCl_2$ on *Capoeta fusca* was 0.154 mg L^{-1} (Mansouri and Baramaki, 2011), on *Gambusia holbrooki* was 0.36 mg L^{-1} (Ebrahimpour et al., 2010a, b; Pourkhabbaz et al., 2011), and on *Acanthoparus latus* was 0.648 mg L^{-1} (Hedayati and Safahieh., 2010). In addition, the 96-hrs LC_{50} values of $HgCl_2$ on *Heteropneustes fossilis*, *Oncorhynchus mykiss*, *Roccus saxatilis*, and *Salvelinus fontinalis* were found to be 0.35, 0.22, 0.09, and 0.075 mg L^{-1}, respectively (Pandey et al., 2005).

The acute toxicity of the copper sulphate decreased with increasing exposure time in both species. Copper sulphate was significantly (96-hrs LC_{50} values) more toxic on *R. caspicus* than *R. kutum*. The observed differences in the acting copper might be species dependent and their susceptibility rates to the test chemical, which resulted in their subsequent toxicity values. Copper salts combine with proteins present in the mucus of the fish's mouth, gills, and skin, preventing respiration causing death (Richey and Roseboom 1987; Peres et al., 1991).

As conclusion, the mercury chloride was more toxic than copper sulphate on the Caspian Roach. In general, the toxicity of $HgCl_2$ was higher than that of $CuSO_4$ in both studied species.

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چکیده فارسی

زیست‌سنجی سمیت حاد کلرید جیوه و سولفات مس برروی کلمه ترکمنی *Rutilus caspicus* و ماهی سفید، *Rutilus kutum*

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چکیده:

هدف از مطالعه حاضر تعیین سمیت حاد (LC_{50}) کلرید جیوه و سولفات مس در ماهی کلمه ترکمنی (*Rutilus caspicus*) و ماهی سفید (*Rutilus kutum*) بود. میزان LC_{50} ماهی کلمه ترکمنی برای کلرید جیوه در ۲۴، ۴۸، ۷۲، و ۹۶ ساعت مواجهه به‌ترتیب ۰/۶۴، ۰/۶۱، ۰/۴۲، و ۰/۲۸ میلی‌گرم بودند و برای سولفات مس نیز به ترتیب ۱۱/۵۵، ۵/۰۸، ۲/۴۹ و ۱/۴۷ میلی‌گرم در لیتر بدست آمد. همچنین مقادیر LC_{50} ماهی سفید برای سولفات مس در ۲۴، ۴۸، ۷۲، و ۹۶ ساعت مواجهه به‌ترتیب ۵/۳۱، ۴/۱۷، ۳/۲۰ و ۲/۲۵ میلی‌گرم در لیتر بود. نتایج حاصل از این مطالعه نشان داد که سمیت کلرید جیوه بالاتر از سولفات مس برای گونه‌های مورد مطالعه بوده است. میزان مرگ‌و میر با روند زمان کاهش یافت، و بالاترین میزان مرگ و میر در طول ۲۴ ساعت اول رخ داد.

کلمات کلیدی: سمیت، LC_{50}، زیست سنجی استاتیک، ماهی.