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Effect of Water Salinity on the Acute Toxicity of Mercuric Chloride on Rainbow Trout (*Oncorhynchus mykiss*, Walbaum 1792)

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Abstract: The acute toxicity of mercuric chloride ($HgCl_2$), one of most toxic pollutants for aquatic ecosystems, in rainbow trout (*Oncorhynchus mykiss*) was investigated in the present study at three different salinities (<0.005‰, 7.5‰ and 17‰). The actual concentration ranged of $HgCl_2$ was ranged from 0.10 to 8.00 mg L^{-1}. The acute toxicity tests were performed by 96-h static tests in different water salinity conditions. The lethal concentrations of mercuric chloride for trout, at the end of the acute toxicity tests were evaluated by Finney's Probit Analysis. Concentrations of $HgCl_2$ that killed 50% of the rainbow trout within 96 hours (96 h LC_{50}) were estimated as 0.808, 0.602, and 0.583 mg L^{-1}, for 0, 7.5, and 17 ppt (parts per thousand) of salinity, respectively. The sensitivity of trout to mercuric chloride was increased with increasing concentration of salinity.

Keywords: Water pollution, aquatic toxicology, fish, heavy metal, mercury.

Gökkuşağı Alabalıklarında (*Oncorhynchus mykiss*, Walbaum 1792) Civa Klorür ($HgCl_2$) Akut Toksisitesine Tuzluluğun Etkisi

Özet: Bu çalışmada sucul ekosistem için en toksik kirleticilerden biri olan civa-II-klorür ($HgCl_2$)'ün 3 farklı tuzlulukta Gökkuşağı alabalığı (*Oncorhynchus mykiss*) üzerindeki akut toksisitesi incelenmiştir. Deneme için uygulanan $HgCl_2$ konsantrasyonu 0.10 ile 8.00 mg L^{-1} arasında değişim göstermiştir. Civa-II-klorür'ün alabalıklarla ilgili olarak letal konsantrasyonları, akut toksisite testleri sonucunda Finney'in Probit Analiz'i ile değerlendirildi. Farklı tuz konsantrasyonları (<0.005‰, 7.5‰ and 17‰) için 96 saat içinde Gökkuşağı alabalıklarının %50'sini öldüren (96 h LC_{50}) $HgCl_2$ konsantrasyonları sırasıyla 0.808, 0.602, ve 0.583 mg L^{-1} olarak bulundu. Gökkuşağı alabalığının $HgCl_2$ akut toksik duyarlılığının tuz konsantrasyonu artışı ile paralel olarak arttığı gözlenmiştir.

Anahtar kelimeler: Su kirliliği, akuatik toksikoloji, balık, ağır metal, civa

1. Introduction

Water pollution, which is caused by the heavy metals, is one of the most serious problems for aquatic environments and its biota. There are more than 40 metals and metal alloys in the industry

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today (Güley and Vural, 1987). Mercury (Hg) has great importance among harmful heavy metals (Sorensen, 1991). Mercuric chloride has been used in agriculture as a fungicide, in medicine as a topical antiseptic and disinfectant. Mercury-II- chloride is commonly used both for industrial, scientific and agricultural purposes around the world as well as in Turkey (Başer et al., 2003; Svobodova et al., 2003).

Mercury is present mainly as inorganic mercury compounds or methyl mercury (CH_3Hg^+) in aquatic media (Driscoll et al., 1994; Van et al., 1994). There are two different ways for biological accumulation of mercury in aquatic organisms. These are the result of direct (by the metal present in the water) or tropic exposures (by the metal in food) (Boudou and Ribeyre, 1983). Mercury is deposited predominantly accumulated in gills of fish and also a small extent in liver, muscles and mucus (Handy and Penrice, 1993). Mercury mainly attaches sulfhidryl proteins in fish's various tissues (Olson et al., 1978). In this way, mercury may cause death or retardation of growth of fish (Wiener and Spry 1996; Snarski and Olson, 1982; Klaverkamp et al., 1983; Perry et al., 1988).

Many research have been conducted to determine the toxicity of heavy metals in trouts, especially for mercury substances (Terzi and Verep, 2011; Gül et al., 2008; Verep et al., 2007; Beşli, 2006; Pardey et al., 2005). A little study has been conducted to determine the acute toxicity of $HgCl_2$ on seabass species at different salinities (Tongra-ar et al., 2003) but no study for rainbow trout.

This study was concerned with the determination of the acute LC_{50} value of mercury-II-chloride upon *Oncorhynchus mykiss* individuals at three different salinities using acute bioassay tests. Rainbow trout was selected for bioassays because it can easily be obtained from local trout farms throughout the year, and its growth is excellent under laboratory conditions (Bernstein and Montgomery 2008; Molony, 2001; U.S.D.A, 2000).

2. Materials and Methods

Experimental Setup

Rainbow trouts (*Oncorhynchus mykiss*) were selected for the bioassay test study. The trouts were taken from a local trout breeding farm near laboratory and then transferred to the laboratory in Faculty of Fisheries, Rize University. The trouts were put in tanks filled with fresh water conditioned for 10 days to acclimate to the laboratory conditions prior to experiments. Throughout the acclimation period and subsequent periods of mercuric chloride exposure, fish were held under a photoperiod of 12-h light and dark cycle. During acclimation, fish were fed with commercial trout pellets twice a day at 5% of their body weight. The feeding was terminated 2 days before the bio-experiments and not fed during experiments and care was taken to keep the mortality rate below 5% during this period (APHA, AWWA and WPCF, 1971). No fish died during this period. After acclimatization, the fish were placed into the maintenance tanks. Each tank was stocked with 10 fish. Fish's average weight was 27.40±1.00 g and average fork length was 13.20±0.20 cm. A Dikomsan DS-30 brand weighing machine, which has 1 mg precision, was used weighing to fish. A Calipper brand digital caliper, which has ±1 mm precision, was used for the measurement of fish's total length. Although the fiberglass tanks (Armaplast brand, 1.10 x 1.10 x 0.42 m) had 500 L capacity, test tanks were failed with 250 L of conditioned tap water. The total fish number must be added by autors in this section

Water quality

Some characteristics of water in these tanks were showed Table 1. YSI-85 brand oxygen-meter was used to determine of these concentrations. Resun Aco-008 brand air pump was used to keep the oxygen level at the desired level. The temperature was regulated at 10±1°C by using heaters. Such as whether there is no statistically significant difference between doses for average temperatures (F=0.12; P=0.98), there was no statistical difference between the salinity concentrations, too (F=0.17; P=0.84). Dolphin Marine Salt used to provide salinity. Amount of salinity was measured before and after the participation with YSI-85 brand oxygen-meter, too.

Table 1: Physicochemical water quality of test water

Parameters	Value
Temperature (°C)	10 ± 1
Dissolved oxygen (mg L^{-1})	7.8 – 8. 2
pH	7.3 – 7.5
Conductivity (µS cm^{-1})	158 – 182
Total hardness (as mg$CaCO_3$/L)	61 – 65
Salinity (‰)	Freshwater,7.5, 17.0

The toxic compound used in the bio-experiments was mercury-II- chloride ($HgCl_2$). Mercury-II-chloride was prepared to give the stock material by diluting in pure water. A Precisa brand electronic scale, which has 1 mg precision, was used to weigh the toxic substance. The dosing solutions were prepared from this stock. The dosing concentrations were 0.00, 0.28, 0.56, 0.84, 1.12, 1.40, 2.00, 4.00 and 8.00 mg L^{-1}. The effect of the compound was determined in a period of 96 hours (Henle, 1981). Behavioral changes were recorded in detail at 1 – 6 h and every 12 h during test period. Mortality was controlled and the dead fish were immediately removed from the tank during this period. The numbers of dead and surviving fish in each tank were recorded at the end of 96 hours. Care was taken to keep the mortality rate of the control group below 10% (APHA, AWWA and WPCF, 1971). The bioassay system was as described in standardized methods (APHA, AWWA and WEF, 1998) and national regulation (Turkish Official Gazette, 1991). LC_{50} and 95% confidence limits were calculated by a computer program (US EPA, 1999; Finney, 1971). Following the preliminary experiments, all determinations were repeated three times.

3. Results

The calculated 96-h acute LC_{50} value (95% confidence limits) of mercury-II- chloride, using a static bioassay system to fry rainbow trout (*Oncorhynchus mykiss*) were 0.808, 0.602, and 0.583 mg L^{-1} for <0.005 (freshwater), 0.75, 17 ppt salinities, respectively. Results are in Table 2. Probit Analysis was used for this (Finney, 1971). The results show that mercury-II- chloride is very toxic to the fish in the doses of higher than 1 mgL^{-1} $HgCl_2$ (Table 2, Figure 1).

Table 2: Acute 96-h toxicity of mercury-II-chloride on *Oncorhynchus mykiss*

	SALINITY CONCENTRATION (‰)					
POINT (LC)	**<0.005 (freshwater)**		**7.5 (Saltwater 1)**		**17 (Saltwater 2)**	
	Concentration (mg L^{-1})	**95% Confidence limits**	**Concentration (mg L^{-1})**	**95% Confidence limits**	**Concentration (mg L^{-1})**	**95% Confidence limits**
1.00	0.487	0.264-0.604	0.223	0.124-0.304	0.247	0.095-0.355
5.00	0.565	0.354-0.672	0.298	0.190-0.381	0.318	0.152-0.425
10.00	0.611	0.414-0.714	0.348	0.238-0.432	0.363	0.195-0.470
15.00	0.645	0.459-0.744	0.387	0.276-0.470	0.398	0.230-0.504
50.00	0.808	0.682-0.922	0.602	0.503-0.694	0.583	0.442-0.706
85.00	1.012	0.891-1.301	0.938	0.808-1.164	0.856	0.707-1.188
90.00	1.068	0.934-1.435	1.042	0.887-1.341	0.937	0.768-1.383
95.00	1.155	0.996-1.668	1.216	1.012-1.665	1.071	0.859-1.750
99.00	1.340	1.113-2.231	1.627	1.280-2.525	1.378	1.042-2.769

Fish Behavior

The behavioral changes of trouts were compared among control group and each exposed to various doses of $HgCl_2$. The control group showed normal behavior during the test period, and mortality was 0%. The changes in behavioral response started between 1 - 3 hours after dosing. The lowest concentration (0.28 mg L^{-1}) had similar behavior with the control group. From the dose of 0.56 mg L^{-1}, the fish started to show behavioral disorders such as excessive mucus secretion. From 0.84 mg L^{-1} onwards, there were a loss of equilibrium, rapid gill movement, swimming at the water surface and gulping for air. At the concentrations of 1.12 mg L^{-1}, behavioral abnormalities of the fish reached maximum, such as, increased difficulty in respiration, besides the accumulation of air transport, accumulation in fish together, and motionless laying down on the tank bottom. At the same time, there was 100% mortality at 1.40, 2.00, 4.00, and 8.00 mg L^{-1} concentrations within the first 30 min after dosing (Figure 1).

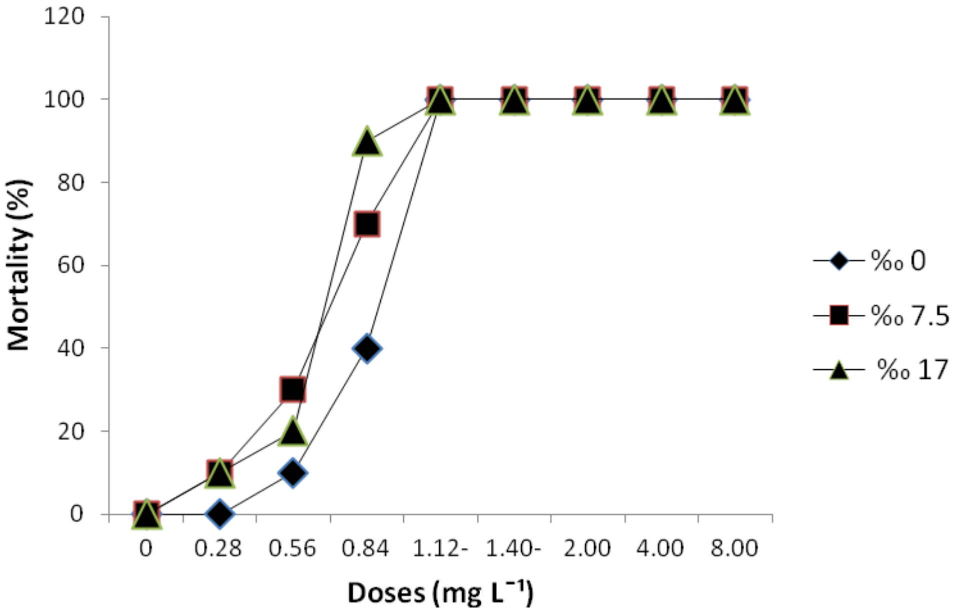

Figure 1: Mortality values of rainbow trout due to mercury-II-chloride ($HgCl_2$) toxicity at the three different salinities (<0.005‰, 7.5‰ and 17‰)

4. Discussion

The behavioral changes in fish are one of the most important indicators of contamination in aquatic environments (Richmonds and Dutta, 1992). The 96 h LC_{50} value of mercury-II- chloride in fry trout was 0.808 mg L^{-1} in the present study. In aquatic toxicology, if LC_{50} (mg L^{-1}) concentration is smaller than 1 mg L^{-1}, the chemical is highly toxic and if between 1 – 10 mg L^{-1}, then it is considered to be a moderately toxic (Louis et al., 1996). Therefore, we report mercury-II- chloride to be highly toxic to fry rainbow trout and increasing in salt concentration increases the toxic effect.
Duncan and Klaverkamp (1983) determined the LC_{50} value of $HgCl_2$ upon *Catostomus commersoni* as 0.687 mg L^{-1}. The 96 h LC_{50} values of inorganic mercury was reported as 0.002 mg L^{-1} (30 days) for crayfish (*Oronectes limosus*), 0.005 mg L^{-1} for cladoceran (*Daphia magna*), 0.010 mg L^{-1} for scud (*Gammarus pseudolimnaeus*), 0.200 mg L^{-1} for juvenile rainbow trout, 0.440 mg L^{-1} for *Notopterus notopterus*, 0.007 mg L^{-1} for leopard frog (*Rana pipiens*), 0.107 mg L^{-1} for marbled salamander (*Ambystoma opacum*), 0.006 mg L^{-1} for blue mussel (*Mythilus edulis*), 0.330 mg L^{-1} for adult slipper limpet (*Crepidula fornicata*), 0.089 mg L^{-1} for bay scallop (*Argopecten irradiance*), 0.004 mg L^{-1} for juvenile mysid shrimp (*Mysidopsis bohia*), 0.015 mg L^{-1} for adult copepod

(*Acartia tonsa*), 0.015 mg L^{-1} for prawn (*Penaeus indicus*), 0.014 mg L^{-1} for polychaete larva (*Capitella capitata*), 0.098 mg L^{-1} for haddock (*Melanogrammus aeglifinus*) (Eisler 1987). According to FAO/UNEP (1991), the 96h LC_{50} values for $HgCl_2$ are 0.35, 0.22, 0.09, 0.08, and 2.00 mg L^{-1} for catfish, rainbow trout, striped bass, brook trout, and mummichog, respectively (FAO/UNEP, 1991). The LC_{50} value of mercury-II- chloride in other aquatic organisms was reported as 0.037, 0.160, and 0.903 mg L^{-1} for fathead minnow, bluegill sunfish (size: 0.6 g), and rainbow trout, respectively (URL-1).

Finally, Wobeser (1975) found the 48-hour LC_{50} of mercury on rainbow trout (*Oncorhynchus mykiss*) at 10 °C as 280 µg L^{-1}. Matida et al. (1971) also investigated the toxic effect of $HgCl_2$ upon rainbow trout and found the 48-hr LC_{50} value as 0.21 mg L^{-1}. Leblond and Hontela (1999) determined the same value as 199 µm. According to present study, trouts (*Oncorhynchus mykiss*) are less sensitive than the before-mentioned species, but increasing in salt concentration increases the toxic effect. Toxicity of metals is lower than in saline water compared to freshwater in most cases. But some authors suggested that the increase of metal toxicity in saline water may be linked to incomplete hypoosmoregulatory ability of the fish (Witeska and Jezierska, 2003). Tongra-ar et al. (2003) have been conducted to determine the acute toxicity of mercury on seabass species at different salinities. In this study, after 7-d exposure to various Hg concentrations and at four salinities (2, 10, 20 and 30 psu), salinity did not have any significant effects on the toxicity of Hg on survival and growth of seabass larvae. One possible explanation is that Hg preferentially forms very strong complexes with sulfhydryl groups (-SH) in proteins rather than with chloride. But no study for rainbow trout. Mercury was found to be more highly toxic to larval invertebrates than larval fish. The seabass larvae were more sensitive to Hg toxicity than the juvenile stage of seabass, milkfish *Chanos chanos* and cresent grunter *Therapon jarbua*. This is because early life stages of aquatic organisms are generally more sensitive to metal toxicity than older stage or adults. Also in this study declared that increasing salinity from freshwater to 7.5 and 17 ppt increases the acute toxicity of mercuric chloride on trout. For freshwater in this study, LC_{50} 96-h of mercuric chloride is 0.808 mgL^{-1} for trout, but 0.602 and 0.583 mgL-1 for saltwater (7.5 and 17 ppt, respectively).

Mercuric chloride has slightly toxicity to fish and other aquatic organisms. Unfortunately, because of lack of tight controls and waste waters that produced during the processes of industrial plants and other sources in many countries, there may be unexpected potential risks to the environment. Especially, depending on the increased water temperature in summer increases the solubility of mercury and salt. Thus its accumulation in fish increases, too. Fish is the major source of food from the aquatic media. Because the toxic metals accumulate though the food chain, the metal pollution in fish poses a threat to human health. It is necessary to determine the lethal effect limits of the metals accumulated in fish.

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