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RESEARCH PAPER

Environmental risk assessments of $CuCl_2$ and $AgSo_4$ toxicity in *Gambusia holbrooki* based on ISO 31000

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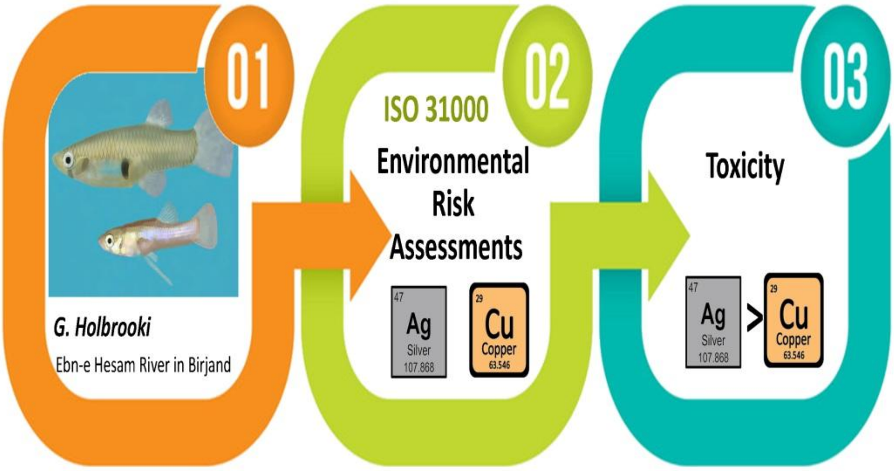
Highlights

- Heavy metal pollution and its effect on biota are one of the human concerns nowadays.
- Risk assessment provides a basis for decisions about the most appropriate approach to be used to treat the risks.
- Gambusia fish is widespread in both freshwater and estuaries environment of temperate regions and considered as a suitable model in Ecotoxicology.
- *G. holbrooki* is sensitive to Ag and Cu and were more susceptible to Ag than Cu.
- Environmental risk assessments based on ISO 31000 is a useful tool for all areas of risk.

Graphical Abstract



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Abstract

Heavy metal pollution and its effect on biota are one of the human concerns nowadays. The amount of heavy metal in a medium, reaches more than a specific limit, it becomes toxic for those animals that live in the environment. The aim of this study was to assessments environmental risk based on ISO 31000 in water and the toxicity of heavy metal in fish (*Gambusia holbrooki*) through conduction of a static bioassay. Environmental risk management provides a basis for decisions about the most appropriate approach to be used to treat the risks. According to our results, Concentrations for Ag were 0.0016, 0.0035, 0.00625, 0.0125, and 0.025 mg/kg, respectively. Concentrations for Cu were 0.16, 0.35, 0.625, 1.25, and 2.5 mg/kg, respectively. Results showed that heavy metal toxicity (Ag and Cu) had a significant effect on fish. No Observable Effect (NOEL) for Ag and Cu were 0.0016, and 0.16 mg/kg, respectively, and No Observable Adverse Effect (NOAEL) of them were 0.025 and 2.5 mg/kg, respectively. Therefore, Ag toxicity was significantly very higher than Cu toxicity for fish. Environmental risk assessments based on ISO 31000 is a useful tool for all areas of risk and permits the identification of how and where it may be possible to improve controls.

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1. Introduction

Establish national standards to standardize risk management and actions have begun in the world, and some organizations, such as the International standard organization (ISO), were pioneers; finally, ISO 31000 in November 2009 published officially (Kimbrough and Componation, 2009). Some researchers expected this standard used by organizations and institutes in all of the world, soon (Kiyani et al., 2013). Risk assessment provides decision-makers and responsible parties with an improved understanding of risks that could affect the achievement of objectives, and the adequacy and effectiveness of controls already in place. This provides a basis for decisions about the most appropriate approach to be used to treat the risks. The output of risk assessment is an input to the decision-making processes of the organization. Risk assessment is the overall process of risk identification, risk analysis and risk evaluation (Fig. 1). How it is used depends not only on the context of the risk management process but also on the methods and techniques used to assess the risk. Risk assessment may require a multidisciplinary approach since risks may cover a wide range of causes and consequences (Amjadian et al., 2021; Farokhian et al., 2021).

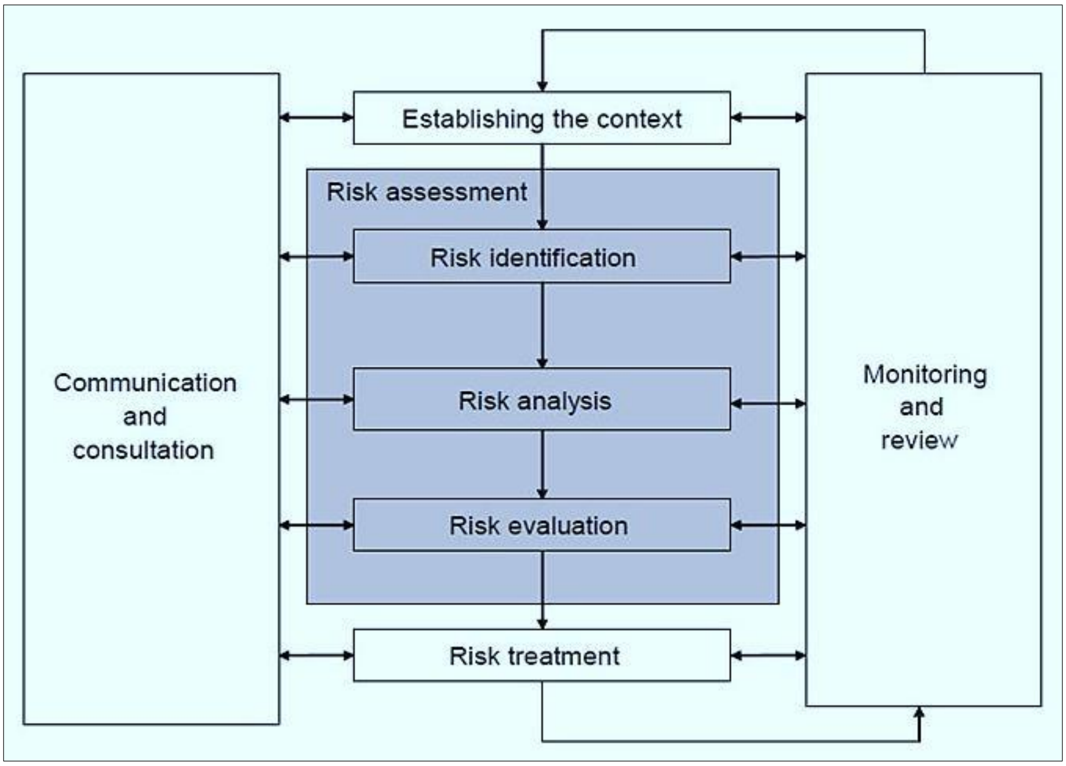

Fig. 1. Contribution of risk assessment to the risk management process (Kiyani et al., 2013).

Environmental risk assessments (Toxicity assessment) are used here to cover the process followed in assessing risks to plants, animals and humans as a result of exposure to a range of environmental hazards. Risk management refers to decision-making steps, including risk evaluation and risk treatment. The method involves analyzing the hazard or source of harm, and how it affects the target population and the pathways by which the danger can reach a susceptible target population. This information is then combined to estimate the likely extent and nature of harm (IEC/FDIS 31010, 2009E). This process is used in several stages to assess the dangers of plants, animals, and humans as a result of exposure to hazards such as chemicals, microorganisms, or other species.

1.2. Problem formulation

This includes setting the scope of the assessment by defining the range of target populations and hazard types of interest.

1.3. Hazard identification

This involves identifying all possible sources of harm to the target population from hazards within the scope of the study. Hazard identification relies typically on expert knowledge and a review of literature.

1.4. Hazard analysis

This involves understanding the nature of the hazard and how it interacts with the target. For example, in considering human exposure to chemical effects, the hazard might include acute and chronic toxicity, the potential to damage DNA, or cause cancer or birth defects. For each hazardous effect, the magnitude of the impact (the response) is compared to the amount of hazard to which the target is exposed (the dose), and, wherever possible, the mechanism by which the effect is produced is determined. The levels at which there is No Observable Effect (NOEL) and No Observable Adverse Effect (NOAEL) are noted. These are sometimes used as criteria for the acceptability of the risk (Fig. 2).

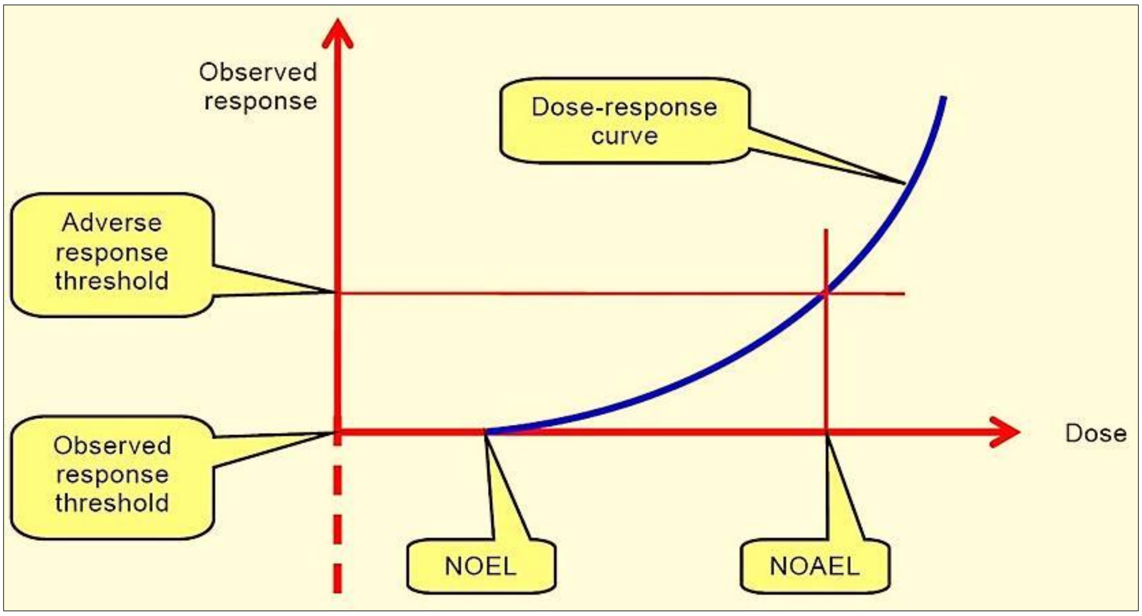

Fig. 2. Dose-response curve.

For chemical exposure, test results derive dose-response curves such as that shown schematically in Fig. 2. These are usually derived from tests on animals or from experimental systems such as cultured tissues or cells.

1.5. Exposure analysis

This step examines how a hazardous substance or its residues might reach a susceptible target population and in what amount. It often involves a pathway analysis that considers the different routes the hazard might take, the barriers which might prevent it from reaching the target and the factors that might influence the level of exposure.

1.6. Risk characterization

In this step, the information from the hazard analysis and the exposure analysis are brought together to estimate the probabilities of particular consequences when effects from all pathways are combined. Where there are large numbers of hazards or pathways, an initial screening may be carried out, and the detailed hazard and exposure analysis and risk characterization carried out on the higher risk scenarios.

Heavy metals are widely used in various industries and considered as common water pollutants. When the amount of heavy metal in a medium, reaches more than a specific limit, it becomes toxic for those animals that live in the environment. Low concentrations of some heavy metals are essential for aquatic animals; however, at high concentration levels, they accumulate in different organs, damage tissues and interfere with normal growth and proliferation (Alkarkhi et al., 2009). That is why knowledge regarding the toxicity of heavy metals to aquatic organisms is of paramount importance (Biodegradability, 1992). Silver (Ag) and Copper (Cu) are often present in industrial wastewaters, are hazardous to the aquatic ecosystem, and pose possible human health risk effects. Besides their toxic and harmful effects on aquatic organisms, heavy metals accumulate throughout the food chain and may affect human health (Martins et al., 2004) and ecological. All aquatic organisms are directly or indirectly affected by the physical characteristics of their environment, especially the chemical composition of the water (Gillis et al., 2008; Yi et al., 2011). Studies have also shown that environmental factors play an important role in modifying the toxicity of metals (Vedamanikam and Shazilli, 2008). The effects of metals on aquatic organisms have been the subject of numerous investigations (Martins et al., 2004; Kim et al., 2001). In the other study, indicated histopathological changes induced by mercury, adversely affected the proper functioning of the organs in these fish (Hedayati and Khsoravi Katuli, 2016). In the other study, was showed that *Clarias Gariepinus* (Cat Fish) accumulated Zn and Cu in its tissues and morality responses depend on concentrations and period of exposure (Chidiebere, 2019).

The process is used to assess risks to plants, animals and humans as a result of exposure to hazards such as chemicals, micro-organisms or other species. Other researchers did risk assessment for advent heavy metal which existed in Acid sludge in recycling factory of motor oil to underground water and presentation suitable cover in the landfill of Industrial Park (Rathore and Khangarot, 2003). In Iran, during 1922–1930, Gambusia spp. were imported from Italy and introduced to Ghazian marshes on the Caspian littoral for the biological control of malaria (Ebrahimpour et al., 2010 a). In 1966, these fish were introduced to other parts of the country. Due to the importance of Gambusia fish in controlling malaria in local freshwater, it was introduced to many countries and now is considered one of the major freshwater fish species all around the world (Ebrahimpour et al., 2010 a). this species has a variety of habitats (Leyse et al., 2005), and it is widespread in both freshwater and estuaries environment of temperate regions and because of high fecundity and representative of secondary consumers in aquatic ecosystems, considered as a suitable model in Ecotoxicology (Annabi et al., 2009). Ebn-e-Hesam is a seasonal river in Birjand (in eastern Iran). Seasonal rivers are the major water resources of this city-Lack of rainfall and droughts prevents the creation of permanent rivers that are used for drinking and agricultural purposes. It is characterized by agricultural activity and thought to be affected, mainly, by run off contain fertilizers and pesticides leading to significant risks of river water pollution. In this research, a short acute toxicity assay (lethal concentration) was designed to assess the toxicity effect of Ag and Cu on fish *Gambusia holbrooki*. The main objective of this study was to assessments environmental risk based on ISO 31000 in water and a case study: toxicity of heavy metal in fish (*Gambusia Holbrooki)* through conduction a static bioassay (Dural et al., 2006).

2. Materials and Methods

G. Holbrooki were collected from the Ebn-e-Hesam River in Birjand (Fig. 3) and transported to the laboratory in polythene bags filled with river water. The fish were acclimated to the laboratory conditions eight days before the experiments. The fish density in aquariums was kept about 1 g/L to make the adaptation more convenient. The fish were divided into three groups and placed in separate glass aquaria filled with dechlorinated tap water (temperature 20 °C) and supplemented with sodium chloride (6 g/L). Ten fish were used for each group, and no food was provided during the tests. The duration of exposure was 96 h. Temperature and pH were monitored at 0, 24, 48, 72, and 96 h for test validation purposes. Aquariums held 50 liters of water and were fitted with artificial aeration to maintain oxygen levels (±SD) at an appropriate level (6.8±0.2). The experiment was repeated thrice, and the number of dead fish was recorded at 24, 48, 72, and 96 h. The fish were exposed to Ag (as $AgSO_4$ – (ready-to-use'' solutions)) and Cu (as $CuCl_2$) in the aquarium systems. The exposure time of fish to Ag and Cu was 96 hours. The concentration ranges of tested heavy metals were determined by providing serially diluted solutions (Zeidali et al., 2021).

Fig. 3. Gambusia fish and site of its sampling in Ebn-e-Hesam River (in eastern Iran).

Preliminary tests were carried out to estimate the minimum nonlethal and maximum lethal concentrations of Ag and Cu. Stock solutions (1000 mg/kg) were prepared by dissolving analytical-grade of Ag and Cu (Merck Chemicals, Darmstadt, Germany) in distilled water. Concentrations for Ag were 0.0016, 0.0035, 0.00625, 0.0125, and 0.025 mg/kg, respectively. Concentrations for Cu were 0.16, 0.35, 0.625, 1.25, and 2.5 mg/kg, respectively. Control was used for the tests with three replicates of each treatment. No mortality was observed over the experiment in controls. The hardness of the water used over the experiment was the same as river water (>350 mg/L $CaCO_3$). Physicochemical parameters of the water of the experimental tanks were determined using standard protocols EPA (2010). Total hardness, magnesium, nitrate, and ammonia (mg/kg) were determined prior to start the experiments by photometer, Plain test 8000. Mortalities were recorded at 24, 48, 72, and 96 hours of fish exposure to heavy metals and the dead fish were removed regularly from the test solutions. Lethal Concentration (LC) values were calculated through the data collected in the acute toxicity bioassays using the Environmental Protection Agency (EPA) method "Probit analysis program (version 1.5)" (Ferrer et al., 2010).

3. Results and Discussion

The average wet weight and length(±SD) of the fish were 0.39 (±0.08) g and 4.8 ±1.3 centimeters, respectively (Table 1).

Table 1. Fish species ecological characteristics.

Scientific name	English name	Feeding habit	Number of samples in each experiment	Length (cm)	Weight (g)
Gambusia holbrooki	Eastern mosquitofish	Carnivore	10	4.8 ± 1.3	0.39 ±0.08

Table 2 shows Physio-Chemical Characteristic, variables maintained in the river and aquaria for the toxicity testing of the fish species. Measured physio-chemical parameters after adding metals did not show any significant difference.

Table 2. Physicochemical parameters of test and river water.

Type of water	Total hardness (mg/kg)	Ca^{2+}	Mg^{+2}	NO_2	NH_4	Cl_2	DO*	T°C	pH
River water	1200±50	176±7	173±5	0.05±0.01	0.79±0.1	0.1±0.02	6.7±0.2	12.9±0.2	7.82±0.1
Test water	350±10	52±4	45±3	0.07±0.001	.01±0.02	0.2±0.06	6.8±0.2	13.6±0.3	7.75±0.2

The relationship between the different exposure concentrations and the mortality response rate of *G. holbrooki* showed in Tables 3 and 4 and Figs. 4 and 5.

Table 3. The relationship between the copper concentration and the mortality rate of (*G. holbrooki*) for the 96 hour exposure.

Cu concentration (mg/L)	No. of the test animals	Period of exposer			
		24 hours	48 hours	72 hours	96 hours
2.5	10	9	-	1	-
1.25	10	2	1	1	-
0.62	10	3	6	1	-
0.31	10	-	1	-	-
0.16	10	-	-	-	-

Table 4. The relationship between the $AgSO_4$ concentration and the mortality rate of (*G. holbrooki*) for the 96 hour exposure.

Ag concentration (mg/L)	No. of the test animals	Period of exposer			
		24 hours	48 hours	72 hours	96 hours
0.0016	10	-	-	-	-
0.0035	10	-	-	1	-
0.00625	10	2	5	1	-
0.0125	10	6	3	-	-
0.025	10	9	1	-	-

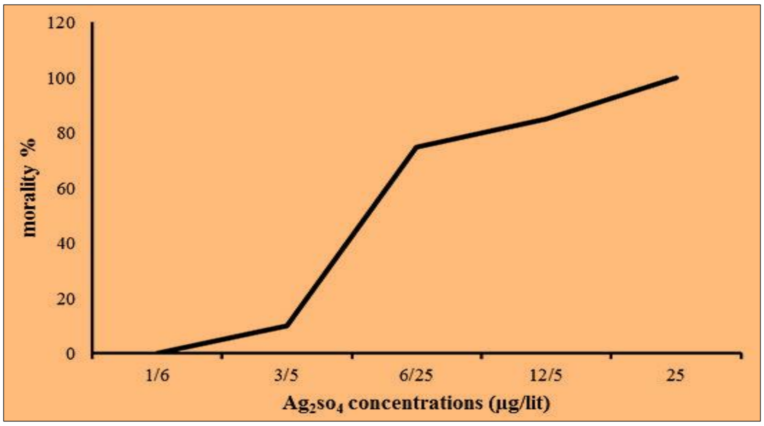

Fig. 4. Mortality (%) of G. holbrooki after 96 hours exposure to different concentrations of $AgSO_4$ (mg/L).

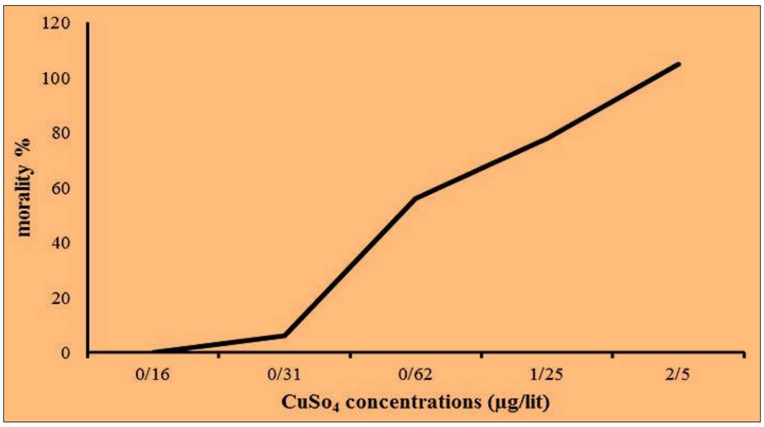

Fig. 5. Mortality (%) of G. holbrooki after 96 hours of exposure to different concentrations of $Cucl_2$ (mg/L).

Mouths moving rapidly are an indication that the toxic effect of the heavy metals caused the depletion of the oxygen content of the medium. Swimming activity and frequent surfacing reduced drastically, color change from black to pale with mucus covering the body were observed. The mucus covering, the entire body of the test organisms might have resulted from response to the toxic effect of the heavy metals. The strength of risk assessment provides a very detailed understanding of the nature of the problem and the factors which increase risk. The result of the experiment showed mortality rate has a direct relationship whit the concentration of heavy metal (Ag and Cu); in another word, increase the concentration of heavy metal caused an increase in mortality rate in fish (Figs. 1 and 2). Most mortality occurred at 24 hours of exposure; this issue indicated acute effect heavy metal due to accumulation of metal concentration in the body. Also, in the other study, observed that *G. holbrooki* when exposed to acid mine drainage, showed increases in mortality during the first 12 h of exposure (Gerhard et al., 2005). At 96 hours of exposure, no mortality was observed at 0.0016 mg/kg Ag and 0.16 mg/kg Cu. Increase concentration from 0.35 to 0.625 mg/kg for Cu increase of mortality rate to 40%, while increase concentration from 0.0035 to 0.00625 mg/kg for Ag increase of mortality rate to 60%.

Therefore, the result is increased concentration from 0.35 to 0.625 mg/kg for Cu and increase concentration from 0.0035 to 0.00625 mg/kg for Ag as so as critical range, the most sensitivity observed in this range of concentration. The least sensitivity was increased concentration from 0.0016 to 0.0035 mg/kg for Ag, and the least sensitivity was increased concentration from 0.16 to 0.35 mg/kg for Cu. So, according to this range should do the possible use in the environment with creation limit degradation. What is more important in assessing the chemical toxicity of a compound are a threshold concentration and the concentration gradient curve. According to the result of the experiment, no observable effect (NOEL) for Ag was 0.0016 mg/kg and no observable adverse effect (NOAEL) was 0.025 mg/kg. In comparison, no observable effect (NOEL) for Cu was 0.16 mg/kg, and no observable adverse effect (NOAEL) was 2.5 mg/kg (Fig. 6).

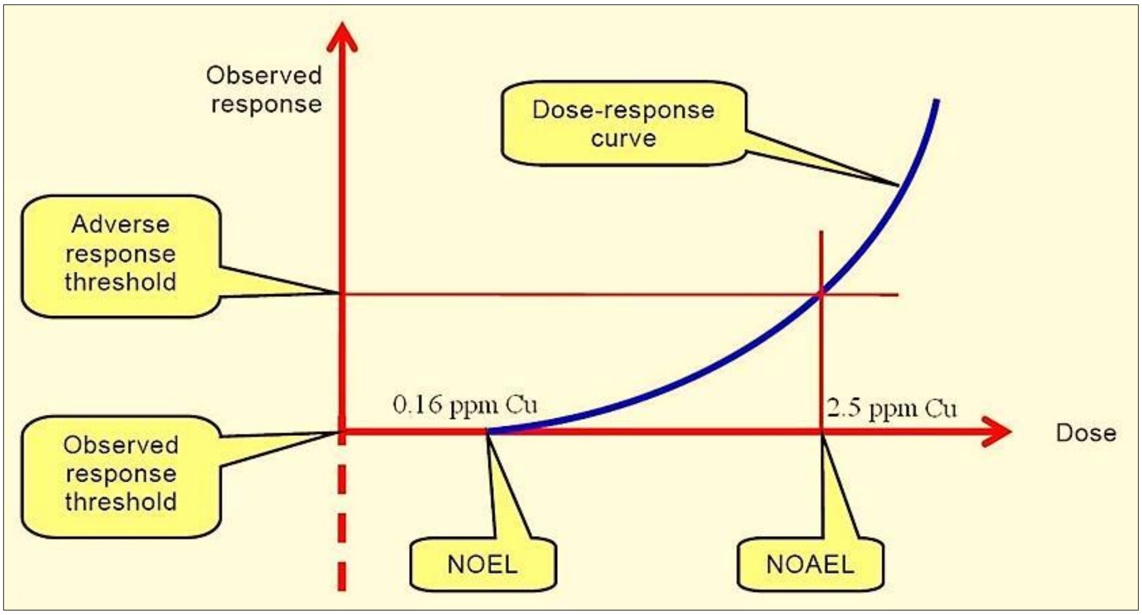

Fig. 6. Dose-response curve Ag and Cu (unit data is mg/kg).

The acute toxicity of Ag and Cu to the *Gambusia holbrooki* was evaluated by static bioassays for calculating the LC50. Comparing the effects of the two metals on fish showed Cu was less toxic; in other words, toxicity of Ag was 100 times than toxicity of Cu: Ag>>Cu (Tables 3 and 4).

The toxicity rate is affected by the kind and the concentration of heavy metals, species under the test (Ebrahimpour et al., 2010 b) and the degree of water hardness (Kiyani et al., 2013). In the other study, was observed that an increase in water hardness reduces the toxicity of Cu and Zn to *G. holbrooki* at 96 hours of exposure, and *G. holbrooki* is more sensitive to Cu and Zn toxicity in soft water, and Cu in soft water is more toxic to the fish (Pourkhabbaz et al., 2011). Water hardness and pH levels, could affect the acute toxicity of $HgCl_2$ on the *G. holbrooki* (Ebrahimpour et al., 2010 b; Gerhard et al., 2005). A suitable strategy for avoiding of environmental pollution by heavy metal is prevention; for example, recycling of heavy metal created from industrial and other pollution sources, prevent pollution of water and soil and reduce the cost of disposal to 10 times, too. A large sum of money can be saved if material recovery processes are opted by sludge generators. It also helps promote greener technology and enables sustainable development.

4. Conclusion

The amount of heavy metal in a medium, reaches more than a specific limit, it becomes toxic for those animals that live in the environment. Low concentrations of some heavy metals are essential for aquatic animals; however, at high concentration levels, they accumulate in different organs, damage tissues and interfere with normal growth and proliferation. This research indicated that *G. holbrooki* is sensitive to Ag and Cu and was more susceptible to Ag than Cu. However, in polluted areas, it is recommended to expand the aquaculture activities in the water resources that their hardness is higher than the average water hardness in the environment. The toxicity effect of other heavy metals on more diverse aquatic animals should be investigated in future research in the sensitive environment particularly. Environmental risk assessments based on ISO 31000 are a useful tool for all areas of risk and permits the identification of how and where it may be possible to improve controls that need appropriate data, which is often not available or has a high level of uncertainty associated with it. For example, dose-response curves derived from exposing animals to high levels of a hazard should be extrapolated to estimate the effects of shallow levels of the contaminants on humans, and there are multiple models by which this is achieved. Where the target is the environment rather than humans and the hazard is not chemical, data which is directly relevant to the particular conditions of the study may be limited.

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