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The effect of copper and cadmium on oxygen consumption of the juvenile common carp, *Cyprinus carpio* (L.)

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Abstract - This study was carried out on juvenile *Cyprinus carpio* (L.) to investigate the effects of sub-lethal concentrations of copper and cadmium (0.1, 0.25, 0.4 ppm) on the survival rate, oxygen consumption and histopathological changes in the gills of exposed fish. The results showed a decrease in survival rate with increasing concentration of each metal. Copper has the most toxic effect compared with cadmium, the survival rate has decreased from 50% to 10% for copper and from 70% to 20% for cadmium with increasing concentration for 15 days. The half lethal time (LT50) was (14 and >15) days for copper and cadmium, respectively. Oxygen consumption rate decreased with increasing concentration and there was a negative correlation between oxygen consumption and metal concentration. The exposure to each metal caused histopathological changes in the gill and resulted in separation of epithelial secondary gill lamellae, hyperplasia, fusion of secondary lamellae and necrosis.

Keywords: Pollution, heavy metals, oxygen consumption, histopathological changes, *Cyprinus carpio*.

Introduction

The contamination of fresh waters with a wide range of pollutants has become a matter of concern over the last few decades (Vindodhini and Narayanan, 2008). Increased human activities especially with rapid development of agriculture and industry has resulted in a considerable increase in levels of pollutant such as heavy metals which is the main anthropogenic pollution causing serious and long lasting damage to all living organisms (Sastry and Sukla, 1993; Murugan *et al*., 2008).

Some toxic metals like copper, which is also essential for cellular metabolism, has become extremely toxic for aquatic animals as its concentration increases in water (Carvaho and Fernandes, 2006). However, cadmium which is not an essential element for life, is toxic in low concentrations for all forms of life in the environment (Eisler, 1985), and is an important challenge to toxicologists and ecological transportation (Aardt and Booysen, 2004).

Fish have the ability to accumulate heavy metals in their tissue to higher level than the toxic concentration in their environment by absorption along the gill surface and gut, and their respiratory system differs from all other systems because the gills are the main target of pollutants and damage to gills has immediate impact on the rest of the fish body (Al-Yacoob *et al*., 1994), and human can be at a great risk through contamination of the food chain (Costa and Hartz, 2009).

Common carp was selected as it is one of the important economic species and easily adapted to laboratory conditions.

Many researchers have reported the harmful effects of copper and cadmium on aquatic life (Able and Papoutsoglou *et al.,* 1994: Olaifa *et al.*, 2004; Muthukumarvel *et al.,* 2007).

The aim of this study is to investigate the effect of essential copper (Cu) and toxic cadmium (Cd) on the survival rate and oxygen consumption rate after metal exposure for 15 days on juvenile of common carp *Cyprinus carpio*.

Materials and Methods

Specimens of common carp *C. carpio* of 10.3 ± 0.7g weight and 9.6 ± 1.2cm length were collected from Garmet Ali river. The fish starved for 24 hr before use and the active juvenile divided into seven groups; three of which were exposed to 0.1, 0.25 and 0.4 ppm of copper and three were exposed to similar concentrations of cadmium (six fish in each group), and the last group of fish (six in number) were not exposed to neither copper nor cadmium and served as control group.

An aqueous stock solution of 1.000 liter of copper and cadmium prepared by dissolving 3.9294 and 6.8466 gm of $CuSO_4$. $5H_2O$ and $CdSO_4$ $8H_2O$ in a litre of distilled water. Three concentrations of each metal were made these are 0.1, 0.25 and 0.4 ppm. The survival rate was recorded for 15 days.

Four juvenile carp fish were taken, each with a weight 4 ± 1.6 gm, and kept singly in one liter conical flask containing dechlorinated tap water and closed firmly. Aeration was made by passing plastic tube through the plastic stopper. Each flask was covered with opaque plastic cover to reduce stress.

The acclimation to this enclosed environment was continued for 24hr. The experiment began by stopping aeration and adding one volume of either of the copper and the cadmium to one of the flasks in the concentrations of 0.1, 0.25 and 0.4 ppm, while the 4^{th} flask was kept as control.

Oxygen consumption in each flask was determined by using DO meter (OSI 325-A-SET) at intervals of 30, 60, 90, 120 and 180 minutes. Oxygen consumption rates were calculated as $mgO_2/g/h$ (Sumich, 1996).

Gill specimens from the control and experimental animals were taken from juveniles exposed to 0.4 ppm of each metal (copper and cadmium) for 6 days (LT50 for the highest concentration), fixed in Bouin's solution and dehydrated in graded series of alcohol, cleared in xylene and embedded in paraffin wax. Sectioning was carried out using rotating microtome to 5-7µ, and stained with hematoxylin-eosin method (Humason, 1979).

Results

The results showed a decrease in the LT_{50} from 14 days at 0.1 ppm to 6 days at 0.4 ppm for the copper. Similarly a decrease in the survival rate was observed with increasing concentration from 50% at 0.1 ppm down to 10% in 0.4 ppm after 15 days. Cadmium, likewise, showed similar results, LT_{50} decreased from >15 days at 0.1 ppm to 8 days at 0.4 ppm and the survival rate has decreased from 70% at 0.1 ppm to 20% at 0.4 ppm (Table 1).

Table 1. Survival rate % and half lethal time (LT_{50}) of exposed *C. carpio* to three levels of copper and cadmium for 15 days

	Concentration of copper (ppm)			Concentration of cadmium (ppm)		
	0.1	0.25	0.4	0.1	0.25	0.4
LT50 (days)	14	11	6	>15	13	8
Survival rate (%)	50%	35%	10%	70%	40%	20%

There was a decrease in the oxygen consumption rate of *C. carpio* with increasing concentration of each metal copper and cadmium for 24 hr of exposure, ranging from 0.243 at 0.1 ppm to 0.137 $mgO_2/g/h$ at 0.4 ppm for the copper, and from 0.381 at 0.1ppm to 0.323 $mgO_2/g/h$ at 0.4 ppm for cadmium, compared with the control 0.454 $mgO_2/g/h$ (Figure 1). The differences are statistically not significant ($P > 0.05$) with the concentration used in experiment ($r = -0.846$ for the copper and - 8.98 for the cadmium).

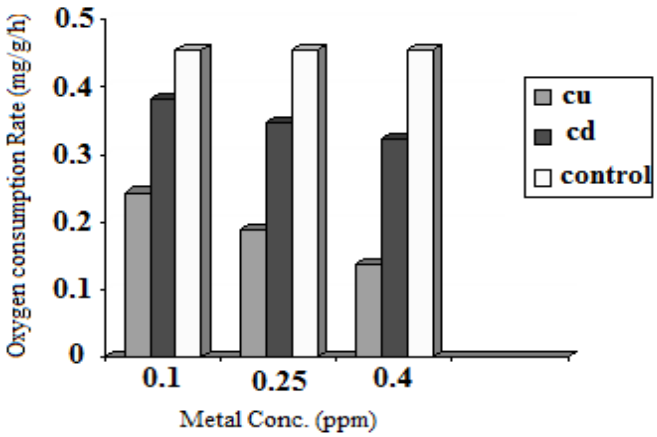

Figure 1. Oxygen consumption rate in C. carpio after 24 h of exposure to different levels of copper and cadmium.

Fish gill arches carry two rows of filaments known as primary lamellae or gill filaments, on the upper and lower surface of each primary lamellae there is a row of secondary lamellae covered by a thin layer of epithelial cells (Plate 1).

The gills of *C. carpio* were exposed to 0.4 ppm of each metal (copper and cadmium) for 6 days. The results indicated the separation of the epithelium of the secondary lamellae, hyperplasia, fusion of secondary lamellae and necrosis (Plate 2-6).

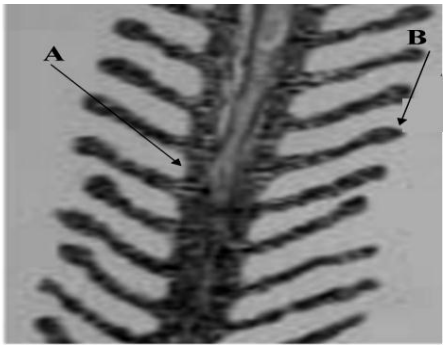

Plate 1. Longitudinal section in the gill of normal *C. carpio* shows the primary lamellae (A), and secondary lamellae (B) (200 X).

Plate 2. Longitudinal section in the gills of *C. carpio* exposed to 0.4 ppm of copper for 6 days shows fusion of secondary lamellae (A) and hyperplasia (B) (400 X).

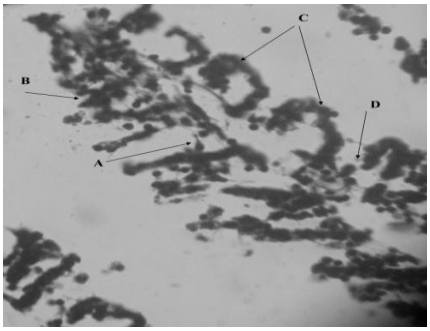

Plate 3. Longitudinal section in the gills of *C. carpio* exposed to 0.4 ppm of copper for 6 days shows separation of epithelial gill lamellae (A), hyperplasia (B), fusion of secondary lamellae (C) and necrosis (D) (400 X).

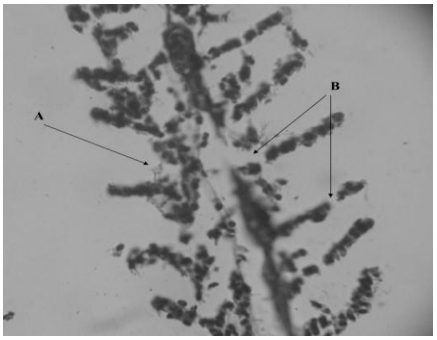

Plate 4. Longitudinal section in the gills of *C. carpio* exposed to 0.4 ppm of copper for 6 days, demonstrates separation of epithelial gill lamellae (A) and necrosis (B) (400 X).

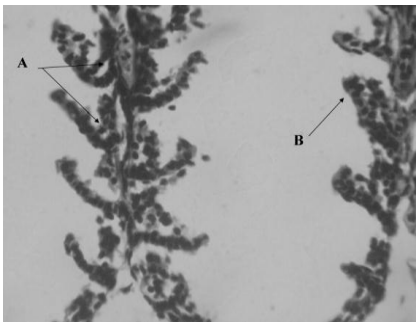

Plate 5. Longitudinal section in the gills of *C. carpio* exposed to 0.4 ppm of cadmium for 6 days illustrates separation of epithelial gill lamellae (A) and hyperplasia (B) (400 X).

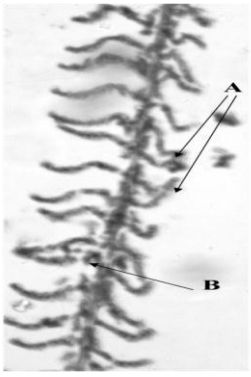

Plate 6. Longitudinal section in the gills of *C. Carpio* exposed to 0.4 ppm of cadmium for 6 days, shows fusion of secondary gill lamellae and necrosis (B) (200 X).

Discussion

The negative effect of heavy metals on fish is related to the disturbance in their biochemical and physiological processes (Villela *et al*., 1999). In the present study the survival rate was decreased from 50% to 10% for the copper and from 70% to 20% for the cadmium after exposing *C. carpio* to sub-lethal concentrations of each of both metals for 15 days. Similar results were reported by other studies, Abdullah and Ahmed (1998) on *C. carpio*, Hassan (2005) on *Carassius carassius* and Vutukuru (2005) when exposed *Labeo rohita* to different concentrations of chromium.

The increase of death with increasing concentration and increasing of the duration of exposure could be because of the accumulation of metals in different tissues of body especially in the gills which are important sites for the entry of metals, therefore causing lesions and gill damage and failure of metabolic activities (Bols *et al*., 2001; James *et al*., 2003). So it is possible that the cumulative action of copper and cadmium at various metabolic sites is responsible for the death of the fish (Basa and Rani, 2003).

The main reason of death in fish exposed to heavy metals is the hypoxia because the metals act on the gill function and structure causing damage of the gill epithelia, disturbances in osmo-regulation process, decrease of oxygen consumption and then death (Albaster and Lioyd, 1982; Peuranen *et al*., 1994; Hassan, 2005). The gills are very susceptible to water-born metals and often show various metal induced lesion. This leads not only to osmotic imbalance but may also caused an impairment of the respiratory system function of the fish which differs according to the type of metal and site of action (Jezierska and Sarnowski, 2002; Dobreva *et al*., 2008).

The present study also reported on a decrease in the oxygen consumption during exposure to either metal. Copper seems to have more toxic effect than cadmium, as it has caused the highest mortality and reduction in the oxygen consumption. Goss and Wood (1988) suggested that heavy metals act on gill function resulting in a decrease in oxygen consumption rate because of ion regulatory and acid-base disturbance. The same result was reported for the common carp by DeBoeck *et al*., (1995) when they exposed fish to sub-lethal concentration of copper, and Jezierska and Sarnowski (2002) when they exposed *C. carpio* larvae to mercury, copper and cadmium, and reported that short-term copper exposure resulted in a strong decline of oxygen consumption by the larvae of *C. carpio* compared with cadmium. In addition, a decrease in oxygen consumption rate was reported by Dobreva *et al*. (2008) after exposing crussian carp *Carassius gibellio* to growing increase in the concentration of zinc for 96 hr, and Vutukuru (2005) when exposed major Indian carp *Labeo rohita* to chromium for 96 hr and suggested that there is an alteration in cellular components as a cause of depression in the respiratory activity in fish exposed to metallic stress.

Reduction of oxygen consumption rate in fish exposed to heavy metals indicate the onset of hypoxia under metallic stress (James, 1990), because metals accumulate in gill epithelium and induce lesions like necrosis, thickening and separation of respiratory epithelium (Peuranen *et al*., 1994; Hassan, 2005), also it may resulted in an increase of diffusion distance between the water and blood which makes oxygen absorption difficult

(Dalzell and MacFurtan, 1994; Aardt and Booysen, 2004). In addition, metals may impair the respiratory surface function by reducing the respiratory surface area through the atrophy and fusion of secondary lamellae, as well as the internal action of metal which enhances the action of respiratory inhibiting factors. (Muthukumarvel *et al*., 2007; Shereena and Logswamy, 2008).

The present histopathological study showed that the exposure to each copper and cadmium has caused a damage in the gill of juvenile *C. carpio* because fish gills are always in contact with water and they have a very thin epithelial layer of a few microns separating the interior of the fish from the external environment, that makes the gills are important target for pollutants and strongly affected by environmental contaminants (Gross *et al.,* 1987). Researchers divide the fish gill lesions into two groups, the direct effect of irritants and the defense responses of fish. The direct effects of metals were necrosis and rupture of the gill epithelium, because the exposure to heavy metals leads to the formation of insoluble protein compound which are toxic to the gill. (Albaster and Lioyd, 1982).

The results showed separation of epithelial gill lamellae, hyperplasia and lamellae fusion. These changes in the gill epithelial layer may be explained as a kind of protection against pollutants, because separation of the epithelial gill lamellae increases the distance through which the metals has to traveled to reach the blood stream, where hyperplasia results in fusion of secondary lamellae which could be protective for a larger vulnerable gills surface area (Leino *et al*., 1987; Pandey *et al*., 1997; Wangsongsak, 2003). A similar result was obtained by Muhvich (1995) when he exposed gold fish *Carassius auratus* to sub-lethal concentration of copper sulphate for 96 hr, the results showed hyperplasia in the top of the filament, and Pandey *et al.* (1997) when they exposed estuarine mullet *Liza parsia* to sub-lethal concentration of lead nitrate 0.5 ppm for 15 days, the results indicated a separation of the epithelial lining cells from the basement membrane of the secondary lamellae, hyperplasia and complete fusion of secondary lamellae. Wangsongsak (2003) and Hassan (2005) reported that the exposure of *Puntius gonionotus* and *Carassius carassius* - to sub-lethal concentrations of cadmium resulted in the separation of the epithelial secondary lamellae, fusion of secondary lamellae and necrosis.

In conclusion the exposure of the freshwater *C. carpio* to sub-lethal concentrations of copper and cadmium tends to increase mortality with increasing exposure time and concentration, decrease the level of oxygen consumption rate, histopathological changes in gill epithelia. In addition the strongest effect of copper on juvenile *C. carpio* resulted from copper uptake by the gills which induced epithelial lesions and gill disorders more than cadmium.

References

Aardt, W.V. and Booysen, J. 2004. Water hardness and the effects of Cd on oxygen consumption in *Tilopia sparrmanii*. Waster S.A., 30 (1):57-64.

Abdullah, A.A.M. and Ahmed, S.M. 1998. Effect of copper on ionic regulation and blood parameter on common carp *Cyprinus carpio* (L.) juvenile, Basr. J. Agri. Sci., 11 (1): 39-44.

Abdu-Alkareem, G. 1998. The effect of PH values and three metals on the survival rate and blood of common carp *Cyrinus carpio* (L.) juvenile. MSc. Thesis, college of Agriculture Basrah University, 73pp.

Able, P.D. and Papoutsoglou, S.E. 1986. Lethal toxicity of cadmium to *Cyprinus carpio* and *Tilapia aurea*. Bull. Environ. Contam. Toxicol., 37: 382-386.

Albaster, J.S. and Lioyd, R. 1982. Water Quality criteria for fresh water fish, FAO, Butter worths London, Boston, 297p.

Al-Yakoob, S., Bou-oluyan, A.H. and Bahlool, M. 1994. Trace metals in gills of fish from the Arabian Gulf. Bull. Environ. Contam. Toxicol., 53(5): 718-752.

Basa, S. and Rani, U.A. 2003. Cadmium induced a toxidant defense mechanism in fresh water teleosts *Dreochromis mossambicus* (Tilapia). Eco. Toxicol. Environ. Saf., 56(2):218-221.

Bols, N.C., Brubacher, J.L., Ganassin, R.C. and Lee, L.E. 2001. Ecotoxicology and innate immunity in fish. Dev. Comp. Immunol., 25(8): 853-873.

Carvalho, C.S. and Fernandes, M.N. 2006. Effect of temperature on copper toxicity and hematological responses in the neotropical fish *prochilodus scrofa* at low and high pH. Aqua. Cult., 251: 109-117.

Costa, S.C and Hartz, S.M. 2009. Evaluation of trace metals (cadmium, chromium, copper and zinc) in tissues of a commercially important fish (*Leporinus obtusidens*) from Guaiba lake, Southern Brazil. Braz. Arch. Biol. Technol., 52(1): 241-250.

Dalzell, D.J. and MacFurtane, N.A. 1994. The toxicity of iron to brown trout and effects on the gill, a comparison of two grades of iron sulfate. J. Fish. Biol., 55: 301-315.

DeBoeck, G., Desmet, H. and Blust, R. 1995. The effect of sub lethal levels of copper on oxygen consumption and ammonia excretion in the common carp *Cyprinus carpio* (L.). Aquat. Toxicol., 32: 127-141.

Dobreva, V., Tsekov, A. and Velcheva, I. 2008. Study of the effect of zinc on gill functions of the carassian carp *carassius gibelio* (Boch). Bulg. J. Agri. Sci., 14(2): 182-185.

Eisler, R. 1985. Cadmium hazards to fish, wild life and invertebrates: Asynptic review. V.S. Fish and Wild life services. Biological report, 85-112.

Goss, G.G. and Wood, C.M. 1988. The effect of acid and acid aluminium exposure in circulating plasma cortisol levels and other blood parameters in the rainbow trout *Salmo gairdenri*: J. Fish. Biol., 24: 299-309.

Gross, E.C., Richards, J.H., Haskot, R.H., Menache, M.G., Graham, J.A. and Dauterman, W.C. 1987. A comparative study of the effects of cadmium chloride and cadmium oxide: pulmonary response. J. Toxicol. Environ. Health, 12: 219-32.

Hassan, B.K. 2005. The effect of sub lethal concentration of cadmium on gill and liver of carassian carp *Carassius carassius* (L.). MSc. Thesis, college of Agriculture, Basrah University, 53pp.

Humasan, G.L. 1979. Animal tissue techniques. W.H. Freeman and company. U.S.A., 661pp.

James, R. 1990. Individual and combined effects of heavy metals on behavior and respiratory responses of *Oreochromis mossambicus*. Indian J. Fish, 37: 139-143.

James, R., Sampath, K. and Edward, D.S. 2003. Copper toxicity on growth and reproductive potential in an oruamental fish *Xiphophorus heller*. Asian Fishi. Sci., 16: 317-326.

Jezierska, B. and Sarnowski, P. 2002. The effect of mercury, copper and cadmium during single and combined exposure on oxygen consumption of *Oncorhynchus mykiss* (WAL.) and *Cuprinus carpio* (L.) larvae. Arch. Pol. Fish., 10(1): 15-22.

Leino, R.L., Wilknson, P. and Anderson, J.G. 1987. Histopathological changes in the gills of pearl dace *Semotilus margarita* and fathead minnows *Pimephalus promleas* from expirmently acidified Candian lakes. Can. J. Fish. Aquat. Sci., 44: 126-134.

Muhvich, A.G., Jones, R.T., Kane, A.S., Anderson, R.S. and Reimscheussel, R. 1995. Effects of chronic copper exposure on the macrophage chemiluminescent response and gill histology in gold fish *Carassius auratus* (L.). Fish and shell fish Immun., 5: 251-264.

Murugan, S.S., Karuppasamy, R., Poongodi, K. and Puvaneswari, S. 2008. Bioaccumulation pattern of zinc in fresh water fish *Channa punctatus* (Bloch) after chronic exposure. Turk. J. Fish. Aqua. Sci., 8: 55-59.

Muthukumarvel, K., Kumarasamy, P., Amsath, A. and Paulraj, M.G. 2007. Toxic effect of cadmium on the electrophoretic protein patterns of gill and muscle of *Oreochromis mssambicus*. J. Chemi., 4(2): 284-286.

Olaifa, F.G., Olaifa, A.K. and Onwude, T.E. 2004. Lethal and sub lethal effects of copper to the African catfish *Clarias gariepnus*. Afr. J. Biomed. Res., 7: 65-70.

Pandey, A.K., George, K.C. and Mohamed, M. 1997. Histopathological alterations in the gill and kidney of an esturine mullet, *Liza parsia* (Hamilton-Buchanan), caused by sub lethal exposure to lead (pb). Indian. J. Fish, 44(2): 171-180.

Pelgrom, S.M., Lamers, L.P., Garristen, J.A., Lock, R.A., Balm, P.H. and Wendelaar, W.S. 1994. Inter action between copper and cadmium during single and combined exposure in juvenile Tilapia *Oreochromis mossambicus*. Influence of feeding condition on whole body metal accumulation and the effect of the metal on tissue water and ion content. Aquat. Toxicol., 30: 117-135.

Peuranen, S., Vuroinen, P.J., Vuroinen, M. and Hollender, A. 1994. The effect of iron, humic acid and low pH on the gills and physiology of brown trout *Salmo trutta*. Ann. Zool. Fennici., 31: 389-396.

Sastry, K.V. and Sukla, V. 1993. Effect of cadmium on the rate of oxygen uptake by the fish *Channa punctatus*. J. Environ., 5(4):295-298.

Shereena, K.M. and Logaswamy, S. 2008. Impact of some heavy metals on oxygen consumption by the fish *Tilapia mossambicus*. Curr. Bio., 2(3): 300-307.

Sumich, J.L., Dudley, G.H. and Miller, R. 1996. Laboratory and field investigation in marine life WCB McGrow Hill. U.S.A. 203p.

Viella, S., Ingrossi, L., Lionetto, M., Schettino, T., Zonno, V. and .Stroelli, C. 1999. Effect of cadmium and zinc on the Na/H exchanger on the brush

border membrane vesicles isolated from eel kidney tankular cells. Aquat. Toxicol., 48: 25-36.
Vinodhini, R. and Narayanan, M. 2008. Bioaccumulation of heavy metals inorgans of fresh water fish *Cyprinius carpio*. Int. J. Environ. Sci. Tech., 5(2):179-182.
Vutukuru, S.S. 2005. Acute effects of hexavalent chromium on survival, oxygen consumption, hematological parameters and some biochemical profiles of the Indian major carp, *Labeo rohita*. Inter. J. Environ. Res. Public Health, 2(3): 456-462.
Wangsongsak, A. 2003. Toxicity of cadmium on histology of gill, liver and kidney in *Puntius gonionotus* (Bleeker). Thesis, Mahidol University. India. 92pp.

تأثير عنصري النحاس والكادميوم على استهلاك الأوكسجين في صغار أسماك الكارب الاعتيادي *Cyprinus carpio (L.)*

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المستخلص - أجريت الدراسة الحالية على يافعات اسماك الكارب الشائع *Cyprinus carpio* لمعرفة تأثير التعرض للتراكيز تحت المميتة لكل من عنصري النحاس والكادميوم (0.10، 0.25، 0.40 جزء بالمليون) على نسب البقاء ومعدل استهلاك الأوكسجين فضلاً عن التغيرات النسيجية المرضية للأسماك المعرضة. أظهرت النتائج إنخفاض نسب البقاء مع ارتفاع التراكيز المستخدمة لكلا العنصرين، وكان النحاس هو العنصر الأكثر سمية اذ تناقصت نسب البقاء من 50% الى 10% للنحاس و % 70الى 20% للكادميوم مع ارتفاع التراكيز المستخدمة ولمدة 15 يوماً، وقد كانت قيم نصف الزمن المميت LT50 (14، >15) يوم للنحاس والكادميوم على التوالي. كما بينت النتائج حدوث تناقصٍ في معدل استهلاك الأوكسجين مع ارتفاع التراكيز المستخدمة ولكلا العنصرين. كذلك سبب التعرض لكلا العنصرين حدوث تغيرات نسيجية مرضية في غلاصم الأسماك المعرضة إذ سُجلت حالات انفصال الطبقة الطلائية للصفائح الغلصمية الثانوية واندماج في الصفائح الغلصمية الثانوية فضلاً عن حالات النخر المصاحبة للتعرض.