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Review

Toxic, physiological, histomorphological, growth performance and antiparasitic effects of copper sulphate in fish aquaculture

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ABSTRACT

This paper provides the current state of knowledge available from the literature regarding the use of copper sulphate ($CuSO_4$) in culture of freshwater and marine fish as related to toxicity, growth performance, physiology, immunity, histomorphology and antiparasitic treatment. From this review, I have assessed and discussed all of these factors, as well as the potential strategies available for use in fish farming. Acute toxicity (96h-LD_{50}) to $CuSO_4$ varies widely among fish species (0.001–730 mg/L) depending on various water quality factors, and many fish species are sensitive to concentrations near those required for controlling and treating parasite infections. Acute exposure to $CuSO_4$ may lead to mortality while sublethal exposure in different organism causes changes in feeding and swimming behaviour, growth performance, histomorphology of gills, liver, kidney, and spleen, hematology, blood biochemistry, the antioxidant defense system, and oxygen consumption. After exposure to copper sulphate, copper ions often accumulate in the gills, liver, kidney and spleen, and in the gills provokes changes in mucus and chloride cells, hyperplasia and/or hypertrophy of primary and/or secondary lamellae, edema of the gill epithelium, and lamellar fusion. Long and short-term exposure to copper sulphate may negatively affect the body growth of fish exposed, and control and treat ectoparasite infections that are discussed here. Copper sulphate may be a chemotherapeutic for controlling and treating ectoparasites in farmed fish because of its effectiveness and low cost.

1. Introduction

The global demand for fresh and nutritious fish products has stimulated the growth of aquaculture production and consequently, the production scale of farmed fish has increased and will continue to increase with a growing world population. Freshwater and marine fish aquaculture are sources of food, nutrition, income, and livelihood for communities around the world (Luis et al., 2019; Carraschi and Cruz, 2019; Malheiros et al., 2020). However, this growth has led to problems with parasitic diseases. Such diseases have caused approximately 40% of losses of aquaculture production at a cost of over US$ 100 billion (Carraschi and Cruz, 2019). Advances in management of diseases may facilitate economic sustainability, improve livelihoods for producers, and eventually contribute to the growth of fish aquaculture on the local, regional and national levels. Copper sulphate ($CuSO_4$) has been used as a therapeutic to reduce infections caused by parasites in fish aquaculture (Paperna, 1984; Ling et al., 1993; Schlenk et al., 1998; Tavares-Dias et al., 2011; Virgula et al., 2017; Owatari et al., 2020). Various concentrations of $CuSO_4$ have been recommended for therapeutic purposes, which will be discussed in addition to other issues related to the use of this chemotherapeutic agent.

Copper sulphate can vary in copper ion concentrations depending on their commercial formulation, of which pentahydrate is the commonly used formulation in freshwater and marine aquaculture systems. This chemical agent acts as an algicide and fungicide and is used globally in agriculture and aquaculture. Dosages necessary for the control of algae were first described by Moore and Kellerman in 1905 (Hanson and Stefan, 1984). Copper sulphate has historically been used in many countries as a chemotherapeutic to control and treat diseases in freshwater and marine aquarium and aquaculture fish, and to eliminate snails from aquaculture ponds (Smith, 1940; Hanson and Stefan, 1984; Straus and Tucker, 1993; Karan et al., 1998; Schlenk et al., 1998; Ezeonyejiaku et al., 2011; Nekoubin et al., 2012; Jegede, 2013; Silva et al., 2014; Lasiené et al., 2016; Calomeni et al., 2018; Ghasemzadeh and Bahrekazemi, 2019). This chemical product has also been used to control unwanted fish that are predators and competitors of commercially produced fish (Smith, 1940).

Copper ions are present in natural aquatic environments at

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concentrations of less than 5 μg/L (Karan et al., 1998; Takasusuki et al., 2004; Hernandez et al., 2011; Jegede, 2013; Lasiené et al., 2016; Pourkhabbaz et al., 2016; Suchitra et al., 2017). Copper sulphate dissolves in water and splits into copper and sulphate ions. Ionic copper plays an important role in cellular metabolism by comprising part of the active sites of many proteins (Waiwood and Beamish, 1978; Hernandez et al., 2011; Monteiro et al., 2012; Ewa et al., 2018; Mitrašinović-Brulić and Suljević, 2019; Afaghi and Zare, 2020), but excess copper produces free radicals that are toxic for cells and becomes toxic to organisms with elevated concentrations in the environment.

Copper sulphate generally forms insoluble compounds with other elements rather than breaking down in the environment. Copper disappears from the water column rapidly after application and accumulates in the bottom sediments. In addition, dissolved oxygen in the water may become depleted, which occurs when large amount of algae or aquatic weeds die due to treatments with $CuSO_4$. The toxicity of $CuSO_4$ to fish is influenced by the chemistry properties of the water, and the concentrations of inorganic or organic material in water (Chakoumakos et al., 1979; Cardeilhac and Whitaker, 1988; Reardon and Harrell, 1990; Straus and Tucker, 1993; Gollon & Griffin, 1998; Mazon and Fernandes, 1999; Adhikari, 2003; Straus, 2006; Straus et al., 2009; Silva et al., 2014; Calomeni et al., 2018). Given that ionic copper is toxic, this chemotherapeutic agent can affect the aquatic fauna, including fish species (Smith, 1940; Lasiené et al., 2016).

The application of copper sulphate to freshwater and marine aquaculture has been investigated globally. Such studies are relevant since adequate knowledge regarding this chemotherapeutic agent may optimize its use for controlling and treating ectoparasitic infections in fish. However, the use of $CuSO_4$ in aquaculture to control ectoparasites has not been adequetly addressed. The present paper reviews the use of $CuSO_4$ in aquaculture focusing on its potential role as a therapeutic against fish ectoparasites. Emphasis was placed on reporting and discussing the use of $CuSO_4$ in therapeutic baths and its toxic effects in hematopoietic tissue structures and physiology of the fish during exposure. This review was performed in an exhaustive manner by searching databases (SciELO, ISI, Scopus, Science Direct, BioOne, Gale, Highwire Press, SpringerLink, Zoological Records, Periodicos Capes, CAB Abstracts databases and Google Scholar). Several database searches were conducted using the keywords “copper sulphate and fish”. Various papers were selected for this review upon demonstrating that they divulged information regarding exposure of freshwater and marine fish to copper sulphate. Articles with unsubstantiated information about the use of copper as $CuSO_4$ were excluded in this review. Here, I report and discuss the results that matched my criteria for this paper.

2. Acute toxicity of copper sulphate in freshwater and marine fish species

The lethal dose (LD_{50}) is known as the biological index in which 50% mortality occurs in a population exposed one time to a substance. The 96h-LD_{50} trials are conducted to measure the potential susceptibility and mortality of a species to certain toxic substances. Higher 96h-LD_{50} values indicate that the tested substance is less toxic because greater concentrations are required to produce 50% mortality of exposed fish. The toxicity tests are carried out based on universal guidelines (Domitrovic, 1997). Hence, the acute toxic effect of $CuSO_4$ is determined by a statistically significant decrease in the survival rate of fish exposed to this chemical product relative to the survival of the control fish. However, Basirun et al. (2019) highlighted that LD_{50} data are statistically generated and are not a biological constant. Therefore, acute toxicity bioassays are conventional tools that are extensively used to assess the toxicity of physiologically active therapeutic agents and the potential of these products to contaminate commercially and ecologically important species (Al-Tamimi et al., 2015). However, the safe concentration to farmed fish species should be known before $CuSO_4$ can be used as a therapeutant. This is especially important since the recommended concentrations and treatment durations for $CuSO_4$ are near the lethal concentration for many fish species. The initial use of $CuSO_4$ as a therapeutic agent in fish culture is unknown. However, Cardeilhac and Whitaker (1988) hypothesized that the use of copper began when aquarium hobbyists kept pennies or copper screens in tanks to control parasites. To our knowledge, the first study reporting lethal concentration of $CuSO_4$ to fish was conducted using *Carassius auratus* in 1863 by Penny and Adams. Since the 19th century, this chemotherapeutant has been used in freshwater fish culture to control parasites (Birdsong and Avault, 1971).

Tolerance to $CuSO_4$ varies among fish species. Some fish species are extremely sensitive to $CuSO_4$ and mortality is observed at extremely low concentrations, whereas other fish species are highly tolerant (Table 1). As such, 96h-LD_{50} values vary by fish species, the commercial $CuSO_4$ formulation used, and other factors related to fish and the environment. For *Oncorhynchus mykiss*, the 48h-LD_{50} was 0.75 mg L^{-1} (Brown and Dalton, 1970). For *Siganus rivulatus* juveniles, the 72h-LD_{50} was >3.0 mg L^{-1} (Nasser et al., 2017), while for *Ctenopharyngodon idella* juveniles, was 2.01 mg L^{-1} (Nekoubin et al., 2012). In contrast, the 72h-LD_{50} was 40.6 mg L^{-1} for *Oreochromis niloticus* (Mohamed et al., 2008).

Another factor that may affect survival of fish exposed to $CuSO_4$ includes acclimation period, i.e., exposing fish to increasing concentrations of ionic copper over the course of several days until the target concentration is obtained (Taylor et al., 2000; Pourkhabbaz et al., 2016). For some species, younger fish are more resistant to $CuSO_4$ toxicity than older fish, whereas the opposite occurs for other fish species (Table 1). For example, studies by Sellin et al. (2005) reported that *Pimephales promelas* larvae acclimate to $CuSO_4$ exposure more quickly than juvenile and adult fish and had better survival. Karan et al. (1998) found that 6 month old *Cyprinus carpio* were more tolerant to $CuSO_4$ than 30 day old fish.

Several studies have demonstrated that the acute toxicity of $CuSO_4$ decreases with increased exposure time (Priya et al., 1999; Ramesh, 2001, Park and Heo, 2008, Balambigai and Aruna, 2011, Thangam et al., 2014, Al-Tamimi et al., 2015, Delahaut et al., 2020). For example, the 96h-LD_{50} of $CuSO_4$ for *Oreochromis niloticus* is 12.85 mg L^{-1} (Mutlu et al., 2015), and sublethal exposure to 2.0 mg L^{-1} for 10 days caused a mortality rate of only 5% (Nouh and Selim, 2013). However, not all species adhere to the decrease in acute toxicity concentrations of $CuSO_4$ with increased exposure. Hoseini and Nodeh (2012) reported the 24 and 48h-LC_{50} were both 0.42 mg L^{-1} for *Rutilus rutilus caspicus* fry.

Water chemical characteristics also influence the toxicity of $CuSO_4$ in exposed fish (Smith and Heath, 1979; Chakoumakos et al., 1979; Straus, 2006; Furuta et al., 2008); Ezeonyejiaku et al., 2011; El-Moselhy et al., 2011; Nouh and Selim, 2013; Thangam et al., 2014; Closson and Paul, 2014; Mashifane and Moyo, 2014; Silva et al., 2014; Hassan and Tabarraei, 2015; Jagadeshwarlu et al., 2015; Delahaut et al., 2020). Toxicity of $CuSO_4$ tends to increase with a smaller fish age when comparing larvae, fry, fingerlings, and juveniles of *O. niloticus* (El-Moselhy et al., 2011). Perkins et al. (1997) reported mortality of *Ictalurus punctatus* adults (male and female) after exposure for 11 weeks to 0.35 and 0.46 mg L^{-1} $CuSO_4$.

The concentration of free copper ions in water is important to understanding the toxicity of $CuSO_4$. The concentration of copper ions decreases as the alkalinity of water increases and as a consequence, therapeutic concentrations of $CuSO_4$ used in high alkalinity water become lethal to the fish when the same treatment is carried out in low alkalinity water (Wurts and Perschbacher, 1994; Perschbacher and Wurts, 1999; Nouh and Selim, 2013). For *Oreochromis aureus*, the 96h-LD_{50} of $CuSO_4$ was 43.06, 6.61, 0.69 and 0.18 mg L^{-1} in waters with total alkalinity of 225, 112, 57 and 16 mg L^{-1} $CaCO_3$, respectively (Straus, 2003).

Concentrations of copper ions in aquaculture ponds increases immediately following $CuSO_4$ treatment, then rapidly decreases. Hence, no contamination of effluents in culture ponds occurs with applications of $CuSO_4$ due to the instability of copper ions in the water column after

Table 1
Median lethal concentrations (96-h LD_{50}) of copper sulphate for different fish species in aquaculture.

Fish species - Life phase	96h LD_{50} (mg L^{-1})	References
Catla catla (FW) - Larva	1.03	Kumari et al. (2018)
Oreochromis niloticus (FW) - Larva	0.74	Lyly et al. (2015)
Danio rerio (FW) - Larva	0.88	Hernandez et al. (2011)
Oreochromis niloticus (FW) - Larva	0.81	El-Moselhy et al. (2011)
Pimephales promelas (FW) - Larva	0.005–0.17	Welsh et al. (1996)
Pimephales promelas (FW) - Larva	0.002–0.18	Welsh et al. (1993)
Pimephales promelas (FW) - Fry	0.69	Closson and Paul (2014)
Salvelinus fontinalis (FW) - Fry	0.25	Closson and Paul (2014)
Salvelinus fontinalis (FW) - Fry	0.48	Closson and Paul (2014)
Cyprinus carpio (FW) - Fry	0.12	Quarashi et al. (2017)
Cyprinus carpio (FW) - Larva	0.50	Jezierska and Sarnowski, 2001
Cyprinus carpio (FW) - Fry	0.64	Karan et al. (1998)
Paralichthys olivaceus (MW) - Fry	8.70–12.20	Furuta et al. (2008)
Pagrus major (MW) - Fry	2.00–5.20	Furuta et al. (2008)
Oncorhynchus mykiss (FW) - Fry	0.09	Taylor et al. (2000)
Oncorhynchus mykiss (FW) - Fry	0.10	Flammarion et al. (1996)
Oncorhynchus mykiss (FW) - Fry	0.15	Taylor et al. (2000)
Morone saxatilis (FW) - Fry	0.62	Wellborn Jr (1969)
Lepomis macrochirus (FW) - Fry	2.66	Johnson et al. (2008)
Oreochromis niloticus (FW) - Fry	0.85	El-Moselhy et al. (2011)
Rutilus caspicus (MW) - Fry	0.42	Hoseini and Nodeh (2012)
Rutilus caspicus (MW) - Fry	0.23	Farhangi et al. (2014)
Pimephales promelas (FW) - Fry	0.17	Calomeni et al. (2018)
Pimephales promelas (FW) - Fry	0.23	Johnson et al. (2008)
Peprilus triacanthus (MW) - Fingerling	0.50	Jiraungkoorskul et al. (2007)
Rutilus rutilus (FW) - Fingerling	0.62	Jahanbakhshi et al. (2012)
Rhabdosargus sarba (MW) - Fingerling	1.03	Wong et al. (1999)
Rhabdosargus sarba (MW) - Subadult	1.24	Wong et al. (1999)
Rutilus caspicus (MW) - Fingerling	0.57	Hoseini et al. (2016a)
Rutilus caspicus (MW) - Fingerling	2.25	Pourkhabbaz et al. (2016)
Rutilus frisii kutum (MW) - Fingerling	4.02	Azarin et al. (2012)
Channa marulius (FW) - Fingerling	0.32	Khangarot (1981a)
Channa punctatus (FW) - Fingerling	3.60	Singh et al. (2008)
Channa punctatus (FW) - Fingerling	3.60	Singh et al. (2012)
Channa punctatus (FW) - Fingerling	11.78	Adhikari (2003)
Ictalurus punctatus (FW) - Fingerling	0.18	Christensen et al. (1972)
Ictalurus punctatus (FW) - Fingerling	0.71	Moore (2005)
Ictalurus furctatus (FW) - Fingerling	0.69	Moore (2005)
Ictalurus punctatus (FW) - Fingerling	6.89	Straus (2006)
Ictalurus punctatus (FW) - Fingerling	0.30	Goodwin and Straus (2006)
	2.610–3.76	Smith and Heath (1979)
Ictalurus punctatus (FW) - Fingerling	0.09	McKim and Benoit (1971)
Salvelinus fontinalis (FW) - Fingerling	0.11	McKim and Benoit (1971)
Aequidens portalegrensis (FW) - Fingerling	2.94	Domitrovic (1997)
Morone chrysops x *M. saxatilis* (FW) - Fingerling	3.35	Straus (2006)
Pimephales notatus (FW) - Fingerling	0.23	Horning and Neiheisel (1979)
Pimephales notatus (FW) - Fingerling	0.31	Richards and Beitinger (1995)
Pimephales notatus (FW) - Fingerling	0.45	Richards and Beitinger (1995)
Pimephales notatus (FW) - Fingerling	0.51	Richards and Beitinger (1995)
Pimephales promelas (FW) - Fingerling	1.60	Brungs et al. (1976)
Pimephales promelas (FW) - Fingerling	21.00	Brungs et al. (1976)
Carussius auratus (FW) - Fingerling	0.29	Ling et al. (1993)
Chelon parsia (FW) - Fingerling	85.6	Mohapatra and Rengarahan (1996); Mohapatra and Rengarahan (1997)
Heteropneustes fossilis (FW) - Fingerling	730.0	Razzaq et al. (2011a)
Heteropneustes fossilis (FW) - NI	10.49	Rajbanshi and Gupta (1988)
Heteropneustes fossilis (FW) - Fingerling	2.40	James and Sampath (1995)
Heterobranchus biborsalis (FW) - Fingerling	0.39	Jegede (2013)
Rasbora sumatrana (FW) - Fingerling	0.006	Shuhaimi-Othman et al. (2015)
Oncorhynchus mykiss (FW) - Fingerling	0.02–0.10	Miller and Mackay (1980)
Oncorhynchus mykiss (FW) - Fingerling	0.09	Taylor et al. (2000)
Oncorhynchus mykiss (FW) - Fingerling	0.11–0.92	Smith and Heath (1979)
Oncorhynchus mykiss (FW) - Fingerling	0.09	Gündoğdu (2008)
Oncorhynchus mykiss (FW) - Fingerling	0.19	Taylor et al. (2000)
Oncorhynchus mykiss (FW) - Fingerling	0.33	Dixon and Sprague (1981a)
Oncorhynchus mykiss (FW) - Fingerling	0.44	Hassan and Tabarraei (2015)
Oncorhynchus mykiss (FW) - Fingerling	1.05	Bulut et al. (2014)
Oncorhynchus mykiss (FW) - Fingerling	0.25–0.60	Lett et al. (1976)
Oncorhynchus clarkii (FW) - Fingerling	0.01–0.37	Chakoumakos et al. (1979)
Oncorhynchus clarkii (FW) - Fingerling	0.08–0.51	Chakoumakos et al. (1979)
Poecilia reticulata (FW) - Fingerling	1.23	Khangarot (1981b)
Poecilia reticulata (FW) - Fingerling	0.038	Shuhaimi-Othman et al. (2015)
Mugil cephalus - (MW) Fingerling	0.03	Ramesh et al. (2017)
Mugil cephalus - (MW) Fingerling	39.68	Erfanifar et al. (2018)
Hypophthalmicthys molitrix (FW) - Fingerling	0.98	Jahanbakhshi et al. (2012)
Cyprinus carpio (FW) - Fingerling	0.12	Thangam et al. (2014)
Cyprinus carpio (FW) - Fingerling	2.65	Al-Tamimi et al. (2015)
	5.45	Karan et al. (1998)

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Table 1 (*continued*)

Fish species - Life phase	96h LD_{50} (mg L^{-1})	References
Cyprinus carpio (FW) - Fingerling		
Oreochromis aureus (FW) - Fingerling	0.18	Straus (2003)
Oreochromis aureus (FW) - Fingerling	0.69	Straus (2003)
Oreochromis aureus (FW) - Fingerling	6.61	Straus (2003)
Oreochromis aureus (FW) - Fingerling	41.06	Straus (2003)
Oreochromis mossambicus (FW) - Fingerling	0.50	Mashifane and Moyo (2014)
Oreochromis niloticus (FW) - Fingerling	1.09	El-Moselhy et al. (2011)
Oreochromis niloticus (FW) - Fingerling	1.27	Monteiro et al. (2012)
Oreochromis niloticus (FW) - Fingerling	2.49	Osuala and Bawa-Allah (2013)
Oreochromis niloticus (FW) - Fingerling	4.30	El-Bouhy et al. (2016)
Oreochromis niloticus (FW) - Fingerling	10.01	Osuala and Bawa-Allah (2013)
Oreochromis niloticus (FW) - Fingerling	12.85	Mutlu et al. (2015)
Oreochromis niloticus (FW) - Fingerling	16.78	Osuala and Bawa-Allah (2013)
Oreochromis niloticus (FW) - Fingerling	27.78	Osuala and Bawa-Allah (2013)
Oreochromis niloticus (FW) - ND	30.00	Ullah et al. (2016)
Oreochromis niloticus (FW) - Fingerling	31.20	Alkobaby and El-Wahed (2017)
Oreochromis mossambicus (FW) - Fingerling	0.87	Mashifane and Moyo (2014)
Oreochromis mossambicus (FW) - Fingerling	1.21	Monteiro et al. (2009)
Oreochromis mossambicus (FW) - NI	4.27	James et al. (2000)
Oreochromis mossambicus (FW) - NI	4.27	James and Sampath (2003)
Oreochromis mossambicus (FW) - Fingerling	12.00	Jagadeshwarlu and Sunitha (2018)
Oreochromis mossambicus (FW) - Fingerling	20.00	Basirun et al. (2019)
Oreochromis mossambicus (FW) - Fingerling	47.00	Jagadeshwarlu et al. (2015)
Esomus danricus (FW) - Fingerling	5.50	Vutukuru et al. (2005)
Labeo rohita (FW) - Fingerling	0.56	Adhikari (2003)
Labeo rohita (FW) - Fingerling	3.15	Latif et al. (2013)
Notopterus notopterus (FW) - Fingerling	25.00	Ravikiran and Kulkarni (2015)
Notopterus notopterus (FW) - Fingerling	30.00	Barad and Kulkarni (2010)
Capoeta umbla (FW) - Fingerling	1.48	Kirici et al. (2019)
Rutilus kutum (FW) - Fingerling	1.47	Pourkhabbaz et al. (2016)
Catla catla (FW) – Fingerling	3.50	Senthamilselvan et al. (2010)
Peprilus triacanthus (MW) - Fingerling	0.50	Jiraungkoorskul et al. (2007)
Clarias batrachus (FW) - Fingerling	690.0	Razzaq et al. (2011a)
Clarias gariepinus (FW) - Fingerling	59.36	Chidiebere (2019)
Clarias gariepinus (FW) - Fingerling	40.86	Wani et al. (2013)
Clarias gariepinus (FW) - Fingerling	40.86	Wani et al. (2018)
Clarias gariepinus (FW) - Fingerling	59.36	Chidiebere (2019)
Piaractus mesopotamicus (FW) - Fingerling	10.36	Silva et al. (2014)

Table 1 (*continued*)

Fish species - Life phase	96h LD_{50} (mg L^{-1})	References
Cyprinus carpio (FW) - Fingerling	0.12	Suryawanshi et al. (2017)
Cyprinus carpio (FW) - Fingerling	0.12	Tembhre and Kumar (1995)
Cyprinus carpio (FW) - Fingerling	0.75	Kondera et al. (2014)
Carassius gibelio (FW) - Fingerling	0.50	Velcheva et al. (2013)
Lepomis macrochirus (FW) - *Fingerling*	2.31–3.80	Smith and Heath (1979)
Lepomis macrochirus (FW) - Fingerling	0.74	Trama (1954)
Notemigonus crysoleucus (FW) - Fingerling	0.31–0.41	Smith and Heath (1979)
Prochilodus lineatus (FW) - Juvenile	0.001	Takasusuki et al. (2004)
Salvelinus fontinalis (FW) - Juvenile	0.11	McKim and Benoit (1971)
Salvelinus fontinalis (FW) - Juvenile	0.09	McKim and Benoit (1971)
Prochilodus lineatus (FW) - Juvenile	0.03	Mazon and Fernandes (1999)
Prochilodus lineatus (FW) - Juvenile	0.01	Carvalho and Fernandes (2006)
Prochilodus lineatus (FW) - Juvenile	0.09	Carvalho and Fernandes (2006)
Danio rerio (FW) - Juvenile	0.07	Campagna et al. (2008)
Leporinus macrocephalus (FW) - Juvenile	0.09	Nunes et al. (2010)
Oncorhynchus tshawytscha (FW) - Juvenile	0.03	Finlayson and Verrue (1982)
Carassius auratus (FW) - Juvenile	0.17	Muhvich et al. (1995)
Carassius auratus (FW) - Juvenile	1.51–2.92	Smith and Heath (1979)
Carassius auratus (FW) - ND	0.50	Oliveira et al. (2018)
Carassius auratus (FW) - ND	3.02	Jahanbakhshi et al. (2012)
Prochilodus lineatus (FW) - Juvenile	0.20	Takasusuki et al. (2004)
Perca fluviatilis (FW) - Juvenile	0.30	Collvin (1984)
Carassius auratus (FW) - Juvenile	0.30	James et al. (2008)
Xiphophorus helleri (FW) - Juvenile	0.36	James et al. (2008)
Oreochromis niloticus (FW) - Juvenile	0.80	El-Moselhy et al. (2011)
Hypophthalmichthys molitrix (FW) - Juvenile	0.98	Hedayati and Ghaffari (2013)
Ctenopharyngodon idella (FW) - Juvenile	1.72	Nekoubin et al. (2012)
Centropomus parallelus (MW) - Juvenile	1.88	Oliveira et al. (2014)
Petenia kraussii (FW) - Juvenile	2.84	Lemus and Chung (1999)
Labeo rohita (FW) - Juvenile	3.15	Latif et al. (2014)
Morone saxatilis (FW) - Juvenile	3.57	Reardon and Harrell (1990)
Pomatoschistus microsps (MW) - Juvenile	0.57	Vieira et al. (2009)
Petenia kraussii (FW) - Juvenile	4.85	Lemus and Chung (1999)
Channa punctatus (FW) - Juvenile	11.78	Adhikari (2003)
Colossoma macropomum (FW) - Juvenile	15.50	Tavares-Dias et al. (2011)
Rita rita (FW) - ND	34.00	Suchitra et al. (2017)
Rita rita (FW) - ND	34.00	Tomar et al. (2015)
Lates calcarifer (MW) - Juvenile	68.32	Paruruckumani et al. (2015a)
Rutilus frisii kutum (MW) - Juvenile	2.31	Gharedaashi et al. (2013)
Trachinotus carolinus (MW) - Juvenile	1.40	Birdsong and Avault (1971)
	1.50	Birdsong and Avault (1971)

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Table 1 (*continued*)

Fish species - Life phase	96h LD_{50} (mg L^{-1})	References
Trachinotus carolinus (MW) - Juvenile		
Trachinotus carolinus (MW) - Juvenile	2.00	Birdsong and Avault (1971)
Xiphophorus helleri (FW) - Adult	0.36	James et al. (2003)
Rutilus rutilus (FW) - Adult	0.50	Paris-Palacios and Biagiantirisbourg (2006)
Danio rerio (FW) - Adult	0.63	Paris-Palacios and Biagiantirisbourg (2006)
Clarias gariepinus (FW) - Adult	70.13	Ezeonyejiaku et al. (2011)
Clarias batrachus (FW) - Adult	0.40	Kumar et al. (2015)
Cnesterodon decemmaculatus (FW) - Adult	0.16	Villar et al. (2000)
Oreochromis niloticus (FW) - Adult	58.84	Ezeonyejiaku et al. (2011)
Oreochromis mossambicus (FW) - Adult	6.50	De Vera and Pocsidio (1998)
Oreochromis mossambicus (FW) - Adult	20.00	Jafri and Shaikh (1998)
Pimephales promelas (FW) - Adult	1.60–21.00	Brungs et al. (1976)
Poecilia reticulata (FW) - Adult	0.05	Moosavi and Shamushaki (2015)
Paracheirodon axelrodi (FW) -Adult	1.65	Dias et al. (2018)
Rasbora daniconius (FW) - Adult	0.44	Mohate and Jagtap (2018)
Oncorhynchus mykiss (FW) - Adult	0.65	Bagdonas and Vosylienė (2006)
Heteropneustes fossilis (FW) - Adult	219.81	Shukla et al. (2017)
Heteropneustes fossilis (FW) - Adult	4.50	Dutta et al. (2016)
Capoeta fusca (FW) -ND	6.85	Zarei et al. (2013)
Phallocerus caudimaculatus (FW) -ND	0.05	Silva et al. (2014)
Hyphessobrycon eques FW) -ND	0.16	Silva et al. (2014)
Danio rerio (FW) - ND	0.13	Silva et al. (2014)
Danio rerio (FW) - ND	0.09	Oliveira-Filho et al. (2004)
Anabas testudineus (FW) - ND	1.74	Kumar and Nandan (2014)
Ameiurus nebulosus (FW) - ND	0.19	Brungs et al. (1973)

ND: Not determined, FW: freshwater fish, MW: marine water fish

application (McNevin and Boyd, 2004). The concentration of CuSO4 used should be based on total alkalinity of water because the $CuSO_4$ precipitates rapidly as copper carbonate with an alkalinity above 250 mg L^{-1} (Owatari et al., 2020).

Low water hardness increases the mortality of fish exposed to $CuSO_4$ because high calcium concentrations block or minimize the effects of copper ions at the sites action (Mazon and Fernandes, 1999; Adhikari, 2003). For *O. mykiss*, the toxicity of $CuSO_4$ decreased with the increase of water hardness (Miller and Mackay, 1980). Conversely, *Cyprinus carpio* exposed to 3.0 mg L^{-1} of copper sulphate in water without calcium, exposure to $CaCO_3$ increased mortality (Ghasemzadeh and Bahrekazemi, 2019). Adhikari (2003) reported that *Labeo rohita* and *Chana punctatus* showed an increase in tolerance to $CuSO_4$ with increases in water hardness. Similar results were reported for *Oncorhynchus clarkii* exposed to $CuSO_4$ in water with high alkalinity and hardness (Chakoumakos et al., 1979). For *I. punctatus* juveniles, Perschbacher and Wurts (1999) determined that the 48h-LC_{50} of $CuSO_4$ was 1.25 mg L^{-1} in water with hardness and total alkalinity of 20 mg/L $CaCO_3$. Based on these results, the recommended concentration of $CuSO_4$ as a therapeutant in fish culture should be based on total alkalinity and calculated as 1 mg L^{-1} $CuSO_4$ per 100 mg L^{-1} total alkalinity (Straus, 2006).

Acute toxicity (LC_{50-96h}) varied widely among the fish species depending on the chemical and physical characteristics of water (Table 1). Therefore, the concentrations of copper sulphate that are toxic for fish in culture ponds depend particularly on hardness and alkalinity in water. A safe and effective concentration for treatment using copper sulphate should be determined before treatment because the ionic copper concentrations in water are altered very rapidly after application.

Nearly all physiological and biochemistry processes may be affected by the water temperature since fish are poikilotherms. Temperatures above or below the tolerance of fish are considered to be harmful and may cause mortality of fish (Cairns et al., 1975; Smith and Heath, 1979). Cairns et al. (1975) noted that the rise of temperature can increase the toxicity of heavy metals, including copper, but the extent of these effects are negligible for long-term exposure (e.g. >48 h), whereas short-term acute exposure was shown to be more prone to temperature modulation.

Trials of 24h-LD_{50} for $CuSO_4$ in fingerlings of *Notemigonus crysoleucus, Lepomis macrochirus, Carassius auratus* and *I. punctatus* maintained at 5, 15 or 30 °C, and *O. mykiss* at 5, 12 and 18 °C for 21 days toxicity differences between these species. *Oncorhynchus mykiss*, *C. auratus* and *I. punctatus* had the greatest sensitivity at higher temperatures, whereas the opposite was observed in *L. macrochirus*. In addition, *I. punctatus* had greatest sensitivity at 15 °C rather than 30 °C (Smith and Heath, 1979). Lemus and Chung (1999) showed that an increase of temperature (22–30 °C) reduced the tolerance to $CuSO_4$ in *Petenia kraussii*. In contrast, exposure of *I. punctatus* to 20 mg/L $CuSO_4$ in water temperatures of 21–27 °C increased its tolerance (Perschbacher, 2005).

The salinity of water can influence the water hardness. Exposure of *Morone saxatilis* to variations in salinity (5–15 mg/L) increased the 96 h-LD_{50} of $CuSO_4$ from 2.68 to 7.88 mg L^{-1} due to the effects on water hardness (Reardon and Harrell, 1990). In *Trachinotus carolinus* exposed to $CuSO_4$, the 96h-LD_{50} increased with increasing salinity from 10 to 30 mg/L (Birdsong and Avault, 1971). For *O. niloticus*, the toxicity of copper sulphate increased with a rise or reduction in salinity, with a 96 h-LD_{50} of 27.78 mg L^{-1} $CuSO_4$ and at a salinity of 12 mg L^{-1}, and 2.49 mg L^{-1} and 10.01 mg/L in freshwater and in 2 mg L^{-1} salinity, respectively, while a salinity of 18 mg L^{-1} was 16.78 mg L^{-1} $CuSO_4$ (Osuala and Bawa-Allah, 2013). These studies suggest that salinity influences the toxicity of copper sulphate for *O. niloticus*, which is a euryhaline fish species. Reardon and Harrell (1990) reported that copper ions are highly toxic to marine fish in aquaculture systems. Ionic copper does not readily precipitate as copper carbonate at neutral pH in seawater because of the large concentration of chloride ions in the water. Ionic chloride is strongly attracted to copper ions and forms a copper-chloride complex that is negatively charged, soluble, and stable. The copper-chloride complex is more stable than the hydrated copper ion and less biologically available. This form of copper is also less available for the formation of other salts (Cardeilhac and Whitaker, 1988). Therefore, as saltwater has a higher concentration of ions than freshwater, $CuSO_4$ chemistry in marine aquaculture systems is more complicated to management than in freshwater systems because salts can affect the final concentration of copper ions in seawater.

An immediate and often rapid loss of copper ions occurs when $CuSO_4$ is added to a marine system containing commonly used filtrants (calcareous, activated carbon, silica gravel, or cured coral). No such loss was observed when the copper compounds were mixed with artificial seawater. The loss of copper ions to such a wide variety of materials suggests a relatively non-specific adsorption process for copper ions in marine environments, i.e., copper attaches to almost any available surface. No change in the ionic copper concentration occurred in the presence of coral or activated carbon when chelated or complex formulations of $CuSO_4$ were used, and only a slight decrease took place in aquariums with other filtrants (Keith, 1981). All these studies provide data regarding $CuSO_4$ toxicity in various environmental conditions and therefore contribute to the knowledge of the seawater quality adequate for the use of this chemotherapeutic agent.

For *P. promelas*, $CuSO_4$ dissipated with a half-life of 1.5 h in a static

water system. The 96h-LD_{50} with a half-life of 4 and 8 h nonstatic water system were between 2 and 3 times greater than the 96h-LD_{50} for the static water system. Correlations between tissue copper concentrations and percent survival based on static toxicity trials were predictive of *P. promelas* survival for exposures in toxicity trials (Calomeni et al., 2018). However, in fish culture ponds, the algae serve as ligands, rapidly sequestering the copper and rendering it unavailable to target species (Johnson et al., 2008).

The acclimation of fish to lethal $CuSO_4$ concentrations in the environment may increase tolerance to this chemotherapeutic agent. Tolerance by *O. mykiss* fingerlings increased to 60, 106 and 90% following exposure to 0.29, 0.40 and 0.59 mg L^{-1} $CuSO_4$ over 3 weeks, respectively (Dixon and Sprague, 1981b).

3. Physiological and behavioral changes due to exposure to copper sulphate

Changes in fish behavior after exposure to $CuSO_4$ are due to endogenous and exogenous processes, and they facilitate the understanding of the health and survival of fish populations exposed to this chemotherapeutic. In general, behavioral changes are related to alterations in physiological and histomorphological functions of fish exposed to $CuSO_4$. Some of the behavioral changes reported for fish species exposed to lethal or sublethal concentrations of $CuSO_4$ include: fins become hard and stretched following high excitability; fish jumping out of the tank, erratic swimming, discoloration and/or dark skin, hyperventilation, irregular operculum beat frequencies, respiratory difficulty, irregular tail beat frequencies, loss of reflex and loss of balance. Hence, after this stressful period, fish remain suspended in vertical position with the mouth near the water surface and the tail pointing downward, hitting against the walls of tanks before sinking and becoming motionless and eventually dieing (Ezeonyejiaku et al., 2011; Nekoubin et al., 2012; Jegede, 2013; Nouh and Selim, 2013; Al-Bairuty et al., 2013; Farhangi et al., 2014; Al-Tamimi et al., 2015; Ullah et al., 2016; El-Bouhy et al., 2016; Suryawanshi et al., 2017; Alkobaby and El-Wahed, 2017; Erfanifar et al., 2018; Basirun et al., 2019; Owatari et al., 2020).

In *Heteropneustes fossilis*, exposure to 0.24 to 0.72 mg L^{-1} $CuSO_4$ decreased the rate of oxygen consumption (James and Sampath, 1995). De Boeck et al. (1995) reported that *C. carpio* fingerlings exposed to 0.22, 0.34 or 0.84 μmol L^{-1} of $CuSO_4$ showed decreased oxygen consumption immediately after exposure to the two latter concentrations of $CuSO_4$, whereas nitrogen excretion remained stable. However, after 7 days of continuous exposure to 0.34 μmol L^{-1} $CuSO_4$, oxygen consumption increased to pre $CuSO_4$ exposure rates whereas ammonia excretion remained the same throughout the trial. Copper sulphate strongly influence oxygen consumption by larvae of *C. carpio* and *O. mykiss* exposed at 0.2 mg L^{-1} of this chemical (Jezierska and Sarnowski, 2001).

The copper ions can have a significant effect on swimming performance in exposed fish. The presence of copper causes oxygen consumption to decrease and the energy expenditure for a given swimming speed to increase. Copper induces an increased metabolic rate at a given swimming speed that can be due to stress in exposed fish (Waiwood and Beamish, 1978). Behavior is a sequence of actions, operating through the central and peripheral nervous systems and the effects of biochemical and physiological processes essential to fish. These are the results of adaptation to changes in the environment after exposure to copper sulphate, allowing the fish to adjust to its internal and external conditions. Therefore, since behavioral characteristics are indicators of lethal and sublethal contamination by $CuSO_4$, these should be included in evaluations of toxicity with this chemotherapeutant agent.

The mechanism of toxicity to $CuSO_4$ is not well understood in fish. Toxicity occurs when concentrations of copper ions exceed physiological thresholds and interrupt physiological functions in fish. Copper accumulates in gills and interferes in osmoregulation by decreasing branchial Na^+ and K^+ adenosine triphosphate activity. Such accumulation of copper causes severe damage to fish gills and affects energy metabolism, which compromise the swimming performance and equilibrium of fish (De Boeck et al., 1997; Monteiro et al., 2005; Kim et al., 2018). Given that swimming is central to many aspects of fish biology, a decreased performance may have implications for interspecific and intraspecific interactions, reducing the fitness of individuals. These behavior changes when exposed to $CuSO_4$ appear to be caused by the neurotoxic effects and by the irritation to the perception system of the fish. Therefore, strategies are needed to reduce toxicity of $CuSO_4$ in fish culture systems. Moreover, swimming performance of fish could be used as an indicator for measuring toxic effects of $CuSO_4$, which include the impairing of transport or exchange of respiratory gases, as well as alterations in energy transformations, or inhibition from activity of the nervous or muscular systems.

Nanotechnology is a science that is growing rapidly and has contributed to solving various problems in industries, including aquaculture (Abdel-Khalek et al., 2015; Luis et al., 2019; Malheiros et al., 2020). In aquaculture, nanotechnology may reduce economic losses caused by high mortality rates of farmed fish (Malheiros et al., 2020). Nanotechnology has produced nanoparticulates of metals, including copper nanoparticles used for $CuSO_4$. Nanoparticles form dispersions or emulsions rather than aqueous solutions in water. Hence, there are concerns that the toxicity of metal nanoparticles may be different from the traditional dissolved forms of the same metal (Griffitt et al., 2007; Shaw et al., 2012; Wang et al., 2015; Wang et al., 2016; Hedayati et al., 2016). Nanoparticles are sets of several atoms from a specific material. Important features of nanoparticles include small size, wider surface, and specific optical features and their surface coverage, which increases their activity when they enter into the body. The release of copper ions may contribute to the bioactivity of copper nanoparticles (Griffitt et al., 2007; Shaw et al., 2012; Al-Bairuty et al., 2016; Hedayati et al., 2016; Braz-Mota et al., 2018).

Recent studies have reported low toxicity of copper nanoparticles when compared with copper or $CuSO_4$ for different fish species (Griffitt et al., 2007; Shaw et al., 2012; Wang et al., 2015; Wang et al., 2016; Hoseini et al., 2016b; Al-Bairuty et al., 2016; Hedayati et al., 2016; Braz-Mota et al., 2018). In contrast, copper nanoparticles were shown to have a similar toxic effect as $CuSO_4$ to *O. mykiss*, *Epinephelus coioides* and *C. carpio* (Shaw et al., 2012; Al-Bairuty et al., 2013; Wang et al., 2014; Mazandarani and Hoseini, 2017). Furthermore, $CuSO_4$ nanoparticles were shown to cause injury to the intestine, liver and brain, whereas $CuSO_4$ affects more the gills and muscle of fish. Both nanoparticles of copper and $CuSO_4$ led to severe anemia in exposed fish (Al-Bairuty et al., 2013; Mazandarani and Hoseini, 2017). Hence, $CuSO_4$ and $CuSO_4$ nanoparticles have different effects in fish since they are absorbed via different routes, generating different effects with different magnitudes in animals. Perhaps, copper ions of $CuSO_4$ are absorbed in gills of the fish, which is the major site for absorption of these ions, whereas copper nanoparticles are absorbed via ingestion/gut (Hedayati et al., 2016). Therefore, these results indicate that $CuSO_4$ nanoparticles may be used as a therapeutic in fish, but more research is needed.

4. Histomorphological alterations on gills and hematopoietic organs of freshwater and marine fish exposed to copper sulphate

Histopathology has been considered as an excellent tool to evaluate the effects of heavy metals in fish tissues, such as copper, thus tissues have been widely used as biomarkers in evaluation of the health of fish exposed to contaminants in both laboratory and field studies (Balamurugan et al., 2012; Latif et al., 2013; Nouh and Selim, 2013; Paruruckumani et al., 2015a, 2015b; Basirun et al., 2019). Copper sulphate damages several organs and systems, including the gills, liver, kidney, spleen, and immune system of exposed fish. The gills of fish are directly in contact with water and are the first organ to respond to exposure to acute and sublethal concentrations of $CuSO_4$ (Table 2).

Exposure of *Cyprinus carpio* to 0.02–10.78 μM L^{-1} $CuSO_4$ caused

Table 2
Histopathological effects of exposure to copper sulphate on gills of different fish species.

Fish species	Concentration (mg L^{-1})	Exposure	Tissue alterations	References
Carassius auratus	0.10	Sublethal	Distention of gill plates, minor vacuolation and necrosis of gill tissue. The mucus cells show hypertrophy, while blood capillaries got shrinked due to decreased supply of blood	Sultan and Khan (1983)
Carassius auratus	1.00	Lethal	Necrosis in gill filaments, desquamation on some of filaments caused gradual separation of epithelial cells from pilaster cells and consequent disintegration, and effects were similar both on lamellar epithelium as well as filament epithelium. The crumpling of lamellae y due to the loss of supporting property of pilaster cells.	Sultan and Khan (1983)
Carassius auratus	0.03–0.17	Sublethal	Hyperplasia with light to severe degree, which was dose dependent	Muhvich et al. (1995)
Carassius gibelio	0.05–0.10	Sublethal	Gill epithelium degeneration, edema in the filamentary epithelium, vasodilatation, lamellar aneurysm, proliferation of filamentary epithelium and lamellar fusion	Velcheva et al. (2013)
Oncorhynchus mykiss	0.002–0.02	Sublethal	Increase in number of mucous and chloride cells in epithelium, necrosis in chloride cells, hypertrophy cellular and proliferation of epithelial cells	Nowak and Duda (1999)
Oncorhynchus mykiss	0.10	Sublethal	Areas of hyperplasia at the base of the secondary lamellae, edema of the gill epithelium, lamellar fusion, clubbed tips, the occasional aneurysm in the secondary lamellae, and swollen mucocytes	Al-Bairuty et al. (2013)
Oncorhynchus mykiss	0.13	Sublethal	Lesions diffuse, accumulation of cellular debris in the epithelium of lamellae and interlamellar regions, enlarged lamellar epithelial cells and lamellar fusion. Cellular debris consisted of either small, strongly basophilic granules or large pale acidophilic masses entrapped within the respiratory epithelium. Ultrastructurally, this cellular debris corresponded to cells undergoing coagulation necrosis or, more commonly, to membrane-bound fragments of cells containing densely packed organelles. These fragments were either intracellular (auto- and heterophagosomes) or extracellular. The latter were identified as apoptotic bodies. Phagosomes could be found within epithelial cells, neutrophils, or macrophages, although only a few leukocytes could be identified clearly. Many apoptotic bodies and some phagosomes contained morphologically recognizable organelles such as mitochondria, nuclear fragments in the form of very electron-dense masses, or portions of endoplasmic reticulum. The presence of nuclear fragments in some intraepithelial phagosomes identified the latter as heterophagosomes and enlarged lamellar epithelial cells with a pale cytoplasm and a large pale nucleus.	Daoustg et al. (1984)
Piaractus mesopotamicus	0.50–1.00	Sublethal	Discrete to moderate hyperplasia with increase of caliciform cells and mucus production, congestion, telangiectasia, interstitial hemorrhage, and mononuclear leukocytes infiltration	Tavares-Dias et al. (2002)
Cyprinus carpio	0.50–4.00	Sublethal	Lifting, hyperplasia and curling of secondary lamellae, focal hyperplasia; hypertrophy, proliferation and dislocation of chloride and mucous cells, telangiectasis and hyperemia	Karan et al. (1998)
Cyprinus carpio	0.16–0.53	Sublethal	Hypertrophy and hyperplasia of chloride cells, severe necrotic changes of secondary lamellar, the sharp fusion of secondary lamellar and clubbing of secondary lamellar	Afaghi and Zare (2020)
Cyprinus carpio	0.25–4.00	Sublethal	Lifting of gill epithelium, hyperplasia of basis of secondary lamellae, focal hyperplasia. Chloride cells changes as hypertrophy, proliferation, and dislocation. Mucous cell changes as hypertrophy, proliferation, and dislocation. Curling of secondary lamellae, lack of mucous cells, curling of secondary lamellae, telangiectasis and hyperemia	Karan et al. (1998)
Poecilia reticulata	0.12	Sublethal	No significant lesions occurred in the gills, exceptmild curling at the tips of gill lamellae	Park and Heo (2008)
Poecilia reticulata	1.17	Lethal	Severe hyperplasia and exfoliation of epithelial cells.	Park and Heo (2008)
Danio rerio	0.0008–0.02	Sublethal	Proliferation and second-stage alterations as rupture of lamellae and aneurisms, and fusion of the walls of the blood vessels in the secondary lamellae	Campagna et al. (2008)
Heteropneustes fossilis	3.00	Sublethal	Rupture of epithelial lining in some areas of primary lamellae, mucus deposition on the gill lamellae, and distortion and rough surface of secondary lamellae in some areas with epithelial detachment of the primary lamellae. Shrinkage and distortion in the tip of gill lamellae with degeneration of microridges, deposition of worn out tissue along with excessive mucus accumulation on the surface of the lamella and rupturing and uplifting of gill epithelium along with the release of RBCs on the gill surfaces. Distortion with breakage in some areas of secondary lamellae and deposition of mucus on the surface of the filament and secondary lamellae, and abnormal structure of secondary lamellae and secondary lamellae. Gill filament exhibiting rough surface with breakage and loss of alignment in some areas of secondary lamellae	Guite et al. (2015)
Clarias batrachus	0.50	Lethal	Separation of epithelium of secondary lamellae, hyperplasia, fusion of secondary lamellae and necrosis	Kumar and Ram (2015)
Clarias batrachus	0.25–0.40	Subletal	Mucus cell hyperplasia was generally more pronounced towards the proximal end of the filament and hyperplasia of epithelial cells resulted in the fusion of many lamellae. Mucus cells were become enlarged and some lamellae appeared thickened and retracted while some were reduced and subepithelial space developed. Buldging of taste bud gill rackers, formation of interlamellar space, fusion of secondary lamellae, breakage of lamellar blood capillaries, swollen tip, telangiectatic secondary lamellae and clotting of blood were observed.	Kumar et al. (2015)
Oreochromis niloticus	0.04–0.40	Lethal	Presence of edema, epithelial lifting and changes in filament epithelial thickness, changes in filament epithelium thickness, lamellar fusion, vasodilatation and aneurisms, and proliferation of the lamellar epithelium, necrosis, and adjacent lamellar fusion. Edemas and aneurisms were correlated with acute exposure periods and lamellar fusion with chronic exposure.	Monteiro et al. (2008)
Oreochromis niloticus	2.50	Subletal		

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Table 2 (*continued*)

Fish species	Concentration (mg L^{-1})	Exposure	Tissue alterations	References
			Edema, lifting of lamellar epithelia and an intense vasodilatation of the lamellar vascular axis, and less frequent, lamellar fusion caused by the filamentar epithelium proliferation and some lamellar aneurisms	Figueiredo-Fernandes et al. (2007)
Oreochromis niloticus	0.60–1.30	Subletal	Basal region of gill lamellae presenting edema, stretching of pillar cells, and the disappearance of pericytes, whereas in the apical region pillar cells remained intact, and pericytes and pavement cells were activated. Filament epithelium, pavement cells showed structural signs of high functional activity, while mitochondrion-rich and mucous cells were degenerated. In the deep filament region, there were edema, loss of neuroepithelial cells, proliferation of undifferentiated cells, and transformation of leukocyte-like cells into macrophages	Monteiro et al. (2012)
Oreochromis niloticus	2.00	Subletal	Gills showed telangiectasia and focal hyperplasia in the secondary lamellae.	Nouh and Selim (2013)
Oreochromis niloticus	10.00–35.00	Lethal and subletal	Complete fusion of several secondary lamellae, lifting of the lamellar epithelium and edema in the filamental epithelium, presence "curling" and clubbed tips of secondary lamella and increase in arithmetic thickness of gill lamellae epithelium	Alkobaby and El-Wahed (2017)
Solea senegalensis	0.10	Sublethal	Lifting and swelling of the lamellar epithelium, and lamellar fusion, hyperplasia, rupture of capillaries, and, consequently, release of erythrocytes. Size of the pavement cells of the secondary lamellae increased (hypertrophy). Changes in the pavement cells, with large nuclei and clear disperse heterochromatin. In the cytoplasm, a few rough and smooth endoplasmic reticulum dispersed, and with many free ribosomes presents, and the Golgi complex had disappeared. The mitochondria had an irregular shape and antiparallel cristae, and 5% of the chloride cells were altered, and in these cells most of the organelles were absent. Number of both chloride and goblet cells increased in the primary lamellae of contaminated specimens, and pavement and chloride cells were not altered in this region.	Arellano et al. (1999)
Ctenopharynogodon idella	2.50–5.00	Sublethal	Accentuated lifting of the lamellar epithelium, edema in the filamentary epithelium, an intense lamellar vasodilatation and exuding of erythrocytes from capillary of lamellar. Exuding of erythrocytes was due to lifting and necrosis of lamellar epithelium, and lamellar fusion in numerous areas because of the filamentary epithelium proliferation. Few aneurisms were observed at gill lamellae and necrotic cells and macrophages often occurred in the filament and the lamellar epithelium	Atabati et al. (2015)
Pseudopleuronectes americanus	0.56–3.20	Sublethal	Eithelial layer of lamellar cells was reduced in size, but there was no detachment from the basement membrane. Increase of presence of chloride cells and mucus cells, and in some instances the epithelial layer was made up entirely of chloride cells. The epithelial layer was separated from the remaining parts of the lamella. In some instances, it appeared that the lifting off of the epithelium was really a greatly expanded chloride cell, since the typical eosinophilic content of chloride cells was contained in the bubbles caused by epithelial detachment. Lamellae fuse in some instances, whereas others became swollen with red blood cells, presumably due to the pillar cells losing their supporting properties. Epithelial cells often became so disintegrated that there was little observable cellular detail. In some gills, there was complete cell destruction with some fusion of adjacent lamellae, in dependency of concentration of copper sulphate. Apart from red blood cells, only chloride cells were still recognizable. The remains of the epithelial layer became completely detached from the more central portion of each lamella	Baker (1969)
Pseudopleuronectes americanus	0.18	Sublethal	The most obvious change was in the relationship of mucus cells to chloride cells; very few mucus cells were found, whereas chloride cells were common note the extensive smooth endoplasmic reticulum and the large number of mitochondria. The granules that were present within the mitochondria seemed similar to those just outside the cell. Occasionally, occurred large clumps of dense particulate material adhering to the outer cell membrane, and numerous myelin like bodies within the cell. Large vacuoles were found within the cytoplasm of epithelial cells; and these vacuoles were either empty or filled with a fibrillar material. Near the surface of the epithelial cell were numerous vesicles, and these vesicles were bounded by a double membrane and some contained smaller vesicles. The apical homogeneous layer was found to be reduced in thickness. The epithelial cells also contained bodies that appeared to be autophagosomes, since the vesicular content was partially formed of rough endoplasmic reticulum. Other cellular vesicles were uniformly bounded by double membranes and they were either empty or contained a homogeneous moderately dense material.	Baker (1969)
Oreochromis mossambicus	2.50–20.00	Sublethal and lethal	Abnormalities of the nucleus shape, swollen cells, lipid droplet deposition, and increase in vacuolation according to the degree of damage associated with copper sulphate exposure concentration. Ultrastructure showed a severe deterioration on the secondary lamella, with communal anomalies as hyperplasia, desquamation, curling, degeneration, and formation of an aneurysm on the secondary lamella. Increased in the concentration of copper sulphate resulted in more than 90% deterioration, which included disarrangement and detachment of the secondary lamella (gill racker) and primary lamella. The consequence of damage was beginning at the secondary lamella prior to the primary lamella, and there was fusion of the secondary lamella. On the other hand, it indicates the destruction of a primary lamella, also known as distorted epithelial tissue at the secondary lamella.	Basirun et al. (2019)
Rutilus rutilus caspicus	0.10–0.40	Lethal	Hyperplasia, edema, hyperemia, hemorrhage and expansion of secondary lamellae	Farhangi et al. (2014)
Heterobranchus bidorsalis	0.20–0.50	Sublethal	Slight vacuolation, epithelium proliferation of mucous cells and hyperplasia, fusion of secondary lamella and gill filaments. Lifting of lamellar epithelium and edema, and Degeneration of the gill architecture with erosion of the gill filament and rakers, and severe edema.	Jegede (2013)

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Table 2 (*continued*)

Fish species	Concentration (mg L^{-1})	Exposure	Tissue alterations	References
Poronotus triacanthus	0.02–0.25	Sublethal	Mucosal cells of the lamellar epithelium appeared swollen and exhibited an increased thickness and microridges were irregularly formed and absent from many cells. The intercellular spaces between the mucosal and serosal epithelial layers increased markedly, filament epithelium exhibited an increased thickness and the microridges were absent. The lamellar epithelium generally contained higher densities of myelinoid bodies characterized by their onionoid whorls of membranous material, and the intercellular spaces had increased in both size and number. The filament epithelium had been presented necrotic cells showing cytoplasmic disorganization and containing electron dense granular material of undefined morphology.	Jiraungkoorskul et al. (2007)
Prochilodus lineatus	0.02–0.03		Intense proliferation of pavement cells and hypertrophy of both pavement and chloride cells, and epithelium height increased, usually consisting of hypertrophied cells layer in height and evidenced a dose dependent. Cell proliferation resulted in incomplete fusion of several lamellae and in complete lamellar fusion. Detachment of lamellar epithelium and necrosis were common and increased with increasing copper, and cell changes in the filament and lamellar epithelium, several histopathology in the vascular system were identified. Erythrocytes congestion was common in the marginal channel (telangiectasis) and erythrocytes congestion throughout the entire lamella (aneurysm), and rupture of the lamellar epithelium and the pillar cell system indicating hemorrhage foci	Mazon et al. (2001b)
Prochilodus lineatus	0.02–0.03		Hyperplasia of the gill filament and lamellar epithelia and decrease of interlamellar space and epithelial lifting of the lamellae. Incomplete fusion of several lamellae and complete fusion of several lamellae. Rupture of the lamellar epithelium and hypertrophy and hyperplasia of pavement cells. Hypertrophy and hyperplasia of chloride cells and cell degeneration	Mazon et al. (2002)
Lates calcarifer	6.83–13.7		Secondary gill lamellae exhibited hypertrophy and hyperplasia of the epithelial cells, the pavement cell appeared irregular with a considerable loss of microridges, and initial part of the protruding lamellae showing edema of the interstitial tissue and irregular capillary shapes, whereas vasodilatation was mostly confined to the lamellar basal region and was associated with stretched pavement cells and large hydropic vacuoles. Vasodilatation in many areas of the secondary lamellae with breakdown of the pillar cell system appeared by degenerative and necrotic changes of the pillar cells. Occasionally, occur proliferation of chloride cells and mucous cells in the secondary lamella, and chloride cells appeared with dilated vesicles within the cytoplasm, and chloride cells due to complete fusion of secondary lamellae and damaged mitochondria, while the mucous cells were completely filled with electron-dense mucous containing vacuoles and no other organelles could be visible in this cell. Enlarged filament intercellular spaces contained macrophage like cells, leucocyte-like cells, and macrophages with large digestive vacuoles that frequently showed autolysis. Numerous macrophages or apoptotic bodies and the external cover of pavement cells also exhibited some modifications. These cells rounded up and partially detached, resulting in coalescence and rupture of blood vessels and hypertrophic pavement cells with irregular shape, long cytoplasmic processes and without microridges were observed. Congestion of blood vessels by erythrocytes in the presence of different leucocytes has been observed. Dilation of the blood vessel walls allows hemorrhage. Due to marked interstitial edema, large epithelial cell spaces were formed and this progressively leads to lifting of the epithelium up to the tip of the lamellae. The enlarged filament intercellular spaces contained undifferentiated cells, leucocytes, hemorrhagic residues and macrophages with large digestive vacuoles which frequently showed autolysis. Swelling of the lamellar epithelium was seen frequently in surfaces leading to wrinkled and non-homogenous surfaces and extensive aneurism with some ruptures	Paruruckumani et al. (2015b)
Clarias batrachius	500	Sublethal	Mucosal cells severely swollen, microridges were absent, and lamellar epithelium usually contained higher densities of myelenoid bodies and intercellular spaces increased. Contraction of primary and secondary lamellae occurred, and tips of lamellae showed dissolution of tissues and vacuoles formation. Primary and secondary lamellae had dissolution and had vacuole formation.	Razzaq et al. (2011b)
Heteropneustes fossilis	8.17	Lethal	Fusion of secondary lamellae, rupture of various cellular components and deposition of excessive mucus and blood on the gill surface. The epithelial surface of treated gill thus lost the organized structures like microvilli, arborizing ridges and channels.	Rajbanshi and Gupta (1988)
Heteropneustes fossilis	600	Sublethal	Mucosal cells severely swollen, microridges were absent, and lamellar epithelium usually contained higher densities of myelenoid bodies and intercellular spaces increased. Contraction of primary and secondary lamellae occurred, and tips of lamellae showed dissolution of tissues and vacuoles formation. Primary and secondary lamellae had dissolution and had vacuole formation.	Razzaq et al. (2011b)
Catla catla	0.35	Sublethal	Vacuolation, fusion, and degeneration of gill lamellae and separation of basement membrane, with separation of basement membrane, necrosis, vacuolation, hyperplasia and degeneration of primary gill lamella	Senthamilselvan et al. (2010)
Clarias gariepinus	2.00	Sublethal	Lifting of lamellar epithelium and edema in the filamentary epithelium, lamellar disorganization, swollen and fusion of secondary gill lamellae tips	Wani et al. (2011)
Clarias gariepinus	5.00	Sublethal	Proliferation of filamentary epithelium as a resulted in fusion of 3–4 secondary gill lamellae, hypertrophy and hyperplasia of lamellar epithelium. Complete fusion of secondary gill lamellae, lamellar due to aneurysm (telangiectasia) and hemorrhage rupture of lamellar epithelium	Wani et al. (2011)
Synechogobius hasta	0.15	Sublethal	Intense lifting of lamellar epithelium and fusion with epithelium vascular congestion or lamellar aneurysms, and hyperplasia of primary lamellar epithelium	Song et al. (2013)

telangiectasis, hyperemia, edema of primary and secondary epithelium, hypertrophy, hyperplasia of the gill epithelium and hyperplasia of goblet cells, necrosis, and infiltration of leukocytes (Delahaut et al., 2020). Therefore, gills are the organ most affected after exposure to acute and sublethal concentrations of $CuSO_4$ and lose the ability to regulate ion concentrations in fish. Such histopathological changes increase the water-blood diffusion distance and consequently decreases the absorption of toxicants. Mucous cell proliferation increases mucus production, which also helps to prevent absorption of toxic compounds by the gills. However, these changes in the gills affect the gas exchange and reduce the O_2 uptake for metabolism and detoxification processes (Shiogiri et al., 2012).

Copper sulphate affects the respiratory system of the fish since ionic copper accumulates in gills of exposed fish, leading to interference in the cardiovascular and nervous systems. Fish gills are exposed to the environment, have a large surface area, and perform numerous functions such as respiration, osmoregulation, excretion of nitrogenous waste products, and acid-base balance. Hence, the gills are the first organ affected by exposure to $CuSO_4$ in water. The large gill surface area of fish favor copper uptake from the water, but the accumulation of ionic copper in the gill tissue can be lower than in the liver, spleen, and kidney. The uptake of this heavy metal is lower when absorbed through the body surface. Ionic copper is transported to the liver via the bloodstream, metabolized and then excreted through the bile. Increased concentrations of ionic copper in the water lead to the production of metal-binding proteins such as metallothioneins, which are stored in the hepatocytes. Excess of metals such as copper bind to the a-globulin in the liver, producing ceruloplasmin and are excreted through the kidney. However, when exposed to extremely high concentrations of copper and the capacity of the liver to remove copper is exceeded, toxic copper ions can be transported through the bloodstream to other organs (Mazon and Fernandes, 1999).

Ionic copper also suppresses immune system functions and can affect the lateral line of fish. In addition to general signs of distress during toxicity, the fish may display a darkening of the skin, various behavioral changes, problems with balance, and increased mortality. The copper ions of $CuSO_4$ enter through fish gills and accumulate in the blood by forming complexes with blood cells and plasma proteins, and subsequently accumulating in the kidney and liver (Brungs et al., 1973; Dixon and Sprague, 1981b; Ling et al., 1993; Pilgaard et al., 1994;Mazon and Fernandes, 1999; Ay et al., 1999; McGeer et al., 2000a; Chen et al., 2013; Paruruckumani et al., 2015b; Sevcikova et al., 2016; Ewa et al., 2018; Shokr, 2020). The accumulation of copper ions in gills, liver and kidney may be rapid. Accumulation occurs primarily in the liver and the response to copper suggests an active regulation in tissues of fish (Mazon and Fernandes, 1999; Ay et al., 1999; McGeer et al., 2000a). Hence, accumulation of copper in the gills, kidney, liver, and spleen tissues of fish leads to significant morphological alterations (Tables 2–5), which may cause changes in the hemostasis in fish. In fish exposed to $CuSO_4$, the copper residues fall rapidly after transferring the fish to clean water, and the mechanism responsible for acclimation to copper ions are inducted by the hepatoprotein synthesis that led to its production to mitigate the severe exposure to this metal (Dixon and Sprague, 1981b).

Fish gills are morphologically and physiologically complex in that they perform several functions such as gas exchange, ion exchange, acid base balance, nitrogenous waste excretion, and other metabolic transformations. Fish live in intimate contact with the water through their gills, of which the surface comprises over half of the body surface area and its delicate gill epithelium separates the internal environment from the external environment (Kumar et al., 2015; Basirun et al., 2019; Afaghi and Zare, 2020). *Centropomus parallelus* exposed to the sublethal concentrations of $CuSO_4$ of 0.47 and 0.94 mg L^{-1} showed an accumulation of copper in gills after 96 h, but no differences were shown between exposure concentrations (Oliveira et al., 2014). Gills can store a large amount of copper ions because fish have an extensive surface area and minimal diffusion distance between dissolved oxygen and blood capillaries for efficient gas exchange. This respiratory organ is equipped with a defense mechanism acting against environmental irritants, i.e, the mucus cells. The mucus cells react instantaneously to the chemical products and secrete copious mucus to form a thick protective layer over the entire exposed surface, which remain stuck to the mucus. The mucus layer creates a microenvironment that acts as an ion trap, concentrating trace elements in the water. Thus, the histomorphological response of the gills of fish exposed to copper sulphate is often manifested by alterations in mucus cells, chloride cells, hyperplasia and/or hypertrophy of primary and/or secondary lamellae, epithelium edema and lamellar fusion (Table 2).

Exposure to elevated concentrations of $CuSO_4$, and the subsequent disturbance in iono- and osmoregulatory processes typically results in morphological changes in fish gills. Due to the high plasticity of gill tissue, iono-and osmoregulatory mechanisms can induce tissue damage and remodeling. Secondary lamellae of the gills serve as the main site for gas exchange and ion transport in fish, and morphological adaptations either facilitate oxygen uptake or serve as a mechanism for increasing the blood-water barrier. If the presence of the irritant is persistent, different histopathological alterations can occur that reduce the respiratory surface and impair respiration and physiology of the gills (Delahaut et al., 2020).

In vitro incubation of gill filament cells of *Oreochromis mossambicus* at 50 and 100 μM of $CuSO_4$ caused an approximate 5- and 16-fold increase of necrosis, respectively, in addition to chloride cell necrosis. A 12 h incubation with 0.28 μM cortisol prior to exposure to 100 μM of $CuSO_4$ reduced necrosis by nearly 75%. The apparent protection provided by cortisol against copper toxicity may be blocked by the glucocorticoid receptor blocker RU 486. Incubation with 0.83 μM cortisol induced apoptosis to the same extent as that of camptothecin, which is a topoisomerase inhibitor. Therefore, $CuSO_4$ causes necrosis of chloride cells, while incubation with cortisol protects against copper ion toxicity at lower concentrations and induces apoptosis at higher concentrations, which is typical for severely stressed fish (Bury et al., 1998).

Oreochromis mossambicus exposure to $CuSO_4$ led to inhibitory effects on cholinesterase activity in gills depending on the concentration of exposure. Exposure to 20 mg/L caused 99.9% inhibition of cholinesterase activity. Acetylcholinesterase has an important physiological role in the degradation of acetylcholine and its inhibition can affect locomotion and equilibrium of exposed fish, indicating neurotoxicity. Cholinesterase is an adequate biomarker for the detection of heavy metals as an inhibition response to a wide range of inhibitors accompanied by an increase in fish mortality and the detection of lower levels of $CuSO_4$ contamination (Boareto et al., 2018; Basirun et al., 2019). Fish also show antioxidant enzymes such as superoxide dismutase, catalase, glutathione S-transferase, which can be used as biomarkers of contamination by $CuSO_4$, and carbonic anhydrase is important in physiological responses when fish are exposed to metals such as $CuSO_4$. The stress caused by this chemical agent could suppress enzymatic activities of the antioxidant defense, causing oxidative damage in exposed fish exposed (Boareto et al., 2018; Kirici et al., 2017). Hepatic superoxide dismutase and glutathione S-transferase activities in *Rhamdia quelen* increased after acute exposure to 0.20 mg L^{-1} $CuSO_4$, whereas catalase showed no differences between treatments. Hepatic superoxide dismutase activities increased in *O. niloticus* after exposure to $CuSO_4$, and the glutathione S-transferase decreased, while the catalase activity showed no significant differences between treatments. An increase in lipid peroxidation was observed at the lowest copper concentration for the *O. niloticus* (0.002 mg L^{-1}). Both *R. quelen* and *O. niloticus* showed carbonic anhydrase inhibition with an increased concentration of $CuSO_4$ (Boareto et al., 2018). In *Capoeta umbla*, exposure to 0.74 mg L^{-1} of $CuSO_4$ for 12–96 h, there was an increase in concentrations of malondialdehyde, superoxide dismutase and catalase in gill, kidney and liver, and a decrease in levels of glutathione reductase, glutathione peroxidase and glucose 6-phosphate dehydrogenase (Kirici et al., 2017).

Danio rerio larvae exposed to lethal concentrations of $CuSO_4$ showed

Table 3
Histopathological effects of exposure to copper sulphate on liver of different fish species.

Fish species	Concentration (mg L^{-1})	Exposure	Tissue alterations	References
Oncorhynchus mykiss	0.50	Sublethal	Exhibited cytoplasmic disruption, few degenerations extensive, pyknotic nuclei apparent and liver architecture lost, intensive cellular vacuolation, hemorrhage and complete loss of liver structure.	Williams and Wootten (1981)
Oncorhynchus mykiss	0.10	Sublethal	Cells with pyknotic nuclei or cytoplasmic vacuoles indicative of the early stages of necrosis in a few cells, small foci of hepatitis-like cell injury; increase in number of melanomacrophage, the occasional separation of the endothelium from the walls of blood vessels and changes in the sinusoid space	Al-Bairuty et al. (2013)
Oncorhynchus mykiss	0.60	Sublethal	Hepatocytes has dark, non-homogenous regions and congestion vein, with degenerations and sinusoidal dilatations, and increasing number of Kupffer cells, vascular degenerations, and congestion in vessel	Atamanalp et al. (2008)
Oreochromis niloticus	0.50–2.50	Subletal	Vacuolation and necrosis, and number of hepatocytes nucleus decreased with the increase of copper sulphate concentration.	Figueiredo-Fernandes et al. (2007)
Oreochromis niloticus	2.00	Subletal	Edema, hyperplasia in the bile ducts and focal aggregations of melanomacrophages, and hepatocytes revealed mild vesiculation of smooth endoplasmic reticulum rough endoplasmic with mitochondrial swelling and increase in the lipid droplets. Lysosomal activities were evident with marked swelling and vesiculation of reticulum rough endoplasmic and loss of some ribosomes. Mitochondrial swelling was evident in addition to condensation of nuclear heterochromatin, and marked decrease in glycogen together with increase lipid droplets, vacuolation and cavitation of cytoplasm were evident	Nouh and Selim (2013)
Oreochromis niloticus	25.00–40.00	Lethal and subletal	Hepatocytes polygonal cells with a homogenous cytoplasm, and a large central spherical heavily stained nucleus, also the pancreatic area with its pancreatic acini normal along the portal vessels within the liver. Cytoplasmic rarefaction and an increase of cytoplasmic vacuolation, the number of hepatocytes nucleus was decreased and nuclear pyknosis. Partial atrophy in the pancreatic tissue and deterioration of its acini and the acinar arrangement	Alkobaby and El-Wahed (2017)
Danio rerio	0.01–0.14	Subletal	Hepatocytes showed an increase of nuclear and nucleolar size and almost all the hepatocytes very basophilic and electron dense with an intranucleolar structure forming cordon or reticule, and this alteration evoked honeycomb organization. Pars granulosa and nucleoplasm showed clearly lower copper concentrations than reticular structure demonstrating that the copper deposit was very well localized in honeycomb like structure	Paris-Palacios and Biagiantirisbourg (2006)
Danio rerio	0.04–0.14	Sublethal	Parenchyma near hepatic veins were completely lysed, areas devoid of cell were more extensive and numerous and lysed areas covered about 50% of the total liver surface of fish. Lysing hepatocytes contained nuclei at various stages of pyknosis, disrupted plasma membranes, cytoplasmic membrane residues, few lysosomes, residual bodies, and swollen mitochondria with electron-dense matrix exhibiting typical cristae. Concentric arrangements of hepatocytes, scant in control livers, increased in number and size, and in these structures' vascularization (veins, sinusoids) was highly reduced, almost absent. Nearly all the mitochondria were affected but only slightly (increase in length, few disrupted cristae, slightly increased electron-density of the matrix). The mitochondria were of appreciable morphological diversity; regular spherical and oval forms were by far the most common. Mitochondrial bodies, relatively few in number were easily perceptible as amorphous grains of rather high electron-density. Typically, with cyprinidea, bile canaliculi were intercellular, mostly located between two hepatocytes, and also intracellular, invaginating the hepatocyte towards the nucleus area	Paris-Palacios et al. (2000)
Solea senegalensis	0.10	Sublethal	Liver demonstrated an increase in fat vacuolation; sinusoids and venules were filled with red blood cells and hepatocellular necrosis was observed occasionally in these contaminated specimens. Number of lipid droplets had increased in the hepatocytes. Approximately 10% of the lipid droplets present in the hepatocytes exhibited round inclusions. The endothelial lining of the sinusoids and the microvilli of the hepatocytes were often disrupted, and the endothelia, were presents membranous inclusions near the sinusoids	Arellano et al. (1999)
Pseudopleuronectes americanus	1.00–3.20	Sublethal	Nucleated red blood cells filled the sinusoids that separated the columns of hepatic cells and presence of fat in the cells around the central vein	Baker (1969)
Rutilus rutilus caspicus	0.10–0.40	Lethal	Hyperemia, hemorrhage, inflammatory cells infiltration and hepatocytes necrosis	Farhangi et al. (2014)
Carassius auratus	0.10	Sublethal	Some of the hepatocytes become completely and some partially vacuolated. The pyknotic nuclei migrate towards the periphery of cell due to vacuolation of precipitated cytoplasm. Sinusoids were more prone to disintegration. The spaces of blood sinusoids do not fill the entire cavity suggesting decreased blood supply	Sultan and Khan (1983)
Carassius auratus	1.00	Lethal	The hepatocytes become partially vacuolized due to precipitation of cytoplasm condensed in a granular form. The nuclei of hepatic cells become swollen and appear to be pyknotic, and sinusoids also undergo degeneration	Sultan and Khan (1983)
Carassius gibelio	0.10–2.00	Sublethal	Degeneration in the lamellae and disorder of blood circulation in the lamellae as lamellar aneurysms. Fusion, sticking together of two and more contiguous filaments, and hyperplasia of lamellae, whit increase in the number of erythrocytes in lamellae	Georgieva et al. (2010)
Heterobranchus bidorsalis	0.30–0.50	Sublethal	Vacuolation of the hepatocytes, and congestion in blood sinusoids, distorted liver cells, severe vacuolation of the hepatocytes and fibrosis in the hepatic parenchyma. Coagulative necrosis in the liver parenchyma and round cell infiltration	Jegede (2013)
Poronotus triacanthus	0.02–0.25	Sublethal	Hepatocytes exhibiting many large electron dense lipid droplets and nuclear outline irregular, mitochondrial swelling with loss of the matrix and cristae and	Jiraungkoorskul et al. (2007)

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Table 3 (*continued*)

Fish species	Concentration (mg L^{-1})	Exposure	Tissue alterations	References
			condensation of the nuclear chromatin. The ribosomes were detached from the surface of the rough endoplasmic reticulum. There was a random distribution of ribosomes throughout the cytoplasm. Mitochondria increased and some showed swelling, and a large number of vacuoles and lysosomes were present	
Channa punctatus	0.050.010	Sublethal	Marked proliferation of the smooth endoplasmic reticulum of. hepatocytes with a complete degeneration of the rough endoplasmic reticulum, loss of ribosomes from the surface of the rough endoplasmic reticulum, a random distribution of ribosomes throughout the cytoplasm, and an increased number and size of smooth endoplasmic reticulum cisternae after. The lysosomal matrix frequently displays a crystalline structure of considerable size and mitochondrial swelling with loss of the matrix and cristae. Loss of internal and external membranes of mitochondria and electron dense particles were visible in the nuclei. The nuclear size was reduced, and chromatin material clumped within the nucleus and at many places occurred rupture of the nuclear envelope, which allowed the continuity of nucleoplasm with the cytoplasm. A large number of vacuoles and lysosomes having dense bodies and lysosomal matrix frequently with crystalline structures of various sizes, sometimes resulting in deformation of the organelles. The autophagic vacuoles and myelinated bodies with heterogenous contents were also observed and prominent changes in nuclei of fish hepatocytes. Nuclei of necrotic cells showed marked clumping of chromatin with an aggregation of intrachromatin material, some of the nuclei had lost their envelope entirely and rupture of nuclear membranes was seen. The size and shape of the nucleus showed drastic changes and clusters of dense granules probably representing the dense chromatin are present in the nucleoplasm. These aggregates are generally located in the center of the nucleus. More dilation and vesiculation were observed in the rough endoplasmic reticulum and aggregation of smooth and rough endoplasmic reticula was recorded and the number of mitochondria drastically decreased. The outer and inner membranes of mitochondria ruptured, and the number of cristae was drastically decreased. The number of Golgi complexes increased, and in some cells, bilobed nuclei with dilated nuclear membranes were observed	Khangarot (1992)
Labeo rohita	3.15	Lethal	Liver exhibited accumulation of fat within the hepatocytes displacing and compressing the nucleus karyorrhexis whereby its chromatin is distributed irregularly throughout the cytoplasm), karyopyknosis, nuclear vacuolization, cytoplasmic degeneration or collapse leading to increase in size i.e. ballooning degeneration. Congestion of blood vessel with blood, and exhibition karyorrhexis and karyopycknosis, congestion in blood vessel, and nuclear vacuolization	Latif et al. (2013)
Lates calcarifer	6.83–13.7	Sublethal	Mitochondria, and hepatocytes had the smooth endoplasmic reticulum was highly developed and were swelling, disappearance of cristae, vacuolization, formation of myelinoid-bodies, and the hepatocytes showed massive swollen mitochondria with a loss of cristae and condensed mitochondria. Degranulation and fragmentation of rough endoplasmic reticulum, dilatation and vesiculation of the reticulum cisternae; some hepatocyte nuclei exhibited chromatin clumping. Flattened stack-like cisternae modified to numerous vesicles due to fragmentation. Hydropic swelling of hepatocytes with nuclear pyknosis and chromatin condensation was observed. With reference to storage vesicles, there appeared to be an increase in the lipid droplets (lipidosis, steatosis), within many hepatocytes. The nuclei also showed alterations with dilation of the nuclear envelope and an accumulation of heterochromatin. A slight accumulation of dark minute granules in some hepatocytes. Hepatocytes showed diffuse degenerative vacuolation (cellular edema or acute cell swelling) and cytoplasm rarefaction. In some instances, mylenoid bodies, and nuclei were affected by exposure, showing dilatation of nuclear envelope, rarefaction of karyoplasm and lipid inclusions and complete damage of mitochondria.	Paruruckumani et al. (2015b)
Catla catla	0.10–0.30	Sublethal	Cytolysis, vacuolization in perinuclear space and pyknotic nuclei, dilatation of sinusoids and fibrosis within sinusoids and hemorrhage within sinusoids. Hemorrhage in central lobular vein, blebbing of cytoplasm, dilated sinusoid, and focal necrosis	Patel and Bahadur (2011)
Catla catla	0.35	Sublethal	Mild vacuolation, pyknotic nucleus, and degeneration of hepatocytes and thrombosis in central vein. Hypertrophy of hepatocytes and dilation of central vein and hyperplasia of hepatocytes	Senthamilselvan et al. (2010)
Synechogobius hasta	0.15	Sublethal	Slight hyalinization, hepatic parenchyma with intense vacuolation, pyknotic nuclei and hyalinization, and slight cellular swelling	Song et al. (2013)
Clarias batrachius	500	Sublethal	Hepatocytes withered and were separated by vacuoles formed due to dissolution of some cells, and nuclear hypertrophy and vacuoles appear. Hepatic tissues rupture and disintegration and vacuole formation indicating tendency towards fibrosis	Razzaq et al. (2011b)
Heteropneustes fossilis	600	Sublethal	Hepatocytes withered and were separated by vacuoles formed due to dissolution of some cells. Nuclear hypertrophy and vacuoles appear, and hepatic tissues rupture and disintegration and vacuole formation.	Razzaq et al. (2011b)
Mollienesia sp.	1 mg/L	Lethal	Hepatocytes become swollen, due to condensed cytoplasm and portal vein undergoes partial blood coagulation. The pancreatic acinar cells lying vicinity of veins become more affected than hepatocytes. The cordal arrangement seems to be crumpled due to swollen and stretched hepatocytes, and nuclei of hepatocytes become large	Sultan and Khan (1981)
Mollienesia sp.	0.10	Sublethal	Vacuolization of hepatocytes with serious effects resulting total disruption of hepatic cords. Some of the hepatocytes become completely or partially vacuolated.	Sultan and Khan (1981)

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Table 3 (*continued*)

Fish species	Concentration (mg L^{-1})	Exposure	Tissue alterations	References
			This leads to forced shifting of nucleus towards periphery and may, therefore, be due to precipitation of cytoplasm. Nucleus get shrunk, and sinusoids also undergo disintegration. The hepatocytes in the vicinity of sinusoids are liable to more toxic hazards than the central ones	
Clarias gariepinus	2.00	Sublethal	Degeneration and hypertrophy of hepatocytes, blood congestion in vessels, and atrophy of hepatocytes. Cytoplasm appeared reticulated and mostly occupied by large vacuoles, hemorrhage, and hemolysis due to rupture of blood vessels. Hepatocytes were damaged around the blood vessels	Wani et al. (2010)
Clarias gariepinus	5.00	Sublethal	Hepatocytic nuclei were irregular in shape and become pyknotic and shifting of the nuclei towards the periphery of the hepatocytes was seen (eccentric nuclei). Nuclear degeneration in few hepatocytes, and necrosis of hepatic tissue and enlargement of bile passages. Cell membrane of the hepatocytes was ruptured and showing syncytial appearance, and extensive cytoplasmic vacuolation and focal necrotic areas	Wani et al. (2010)
Cyprinus carpio	0.04–0.07	Sublethal	Marked dystrophic lesions in hepatocytes, and hydropic-to-vacuolar degeneration of hepatocytes, the dilatation of capillaries, mild hyperemia, and cholestasis	Sevcikova et al. (2016)

Table 4
Histopathological effects of exposure to copper sulphate on kidney of different fish species.

Fish species	Concentration (mg L^{-1})	Exposure	Tissue alterations	References
Oncorhynchus mykiss	0.002–0.02	Sublethal	Increase in number of vacuoles in hepatocytes and "feathery" appearance of hepatocytes	Nowak and Duda (1999)
Oncorhynchus mykiss	0.10	Sublethal	Occasional degeneration of renal tubules, a few necrotic cells in the hematopoietic tissue, minor elevation in the number of melanomacrophage deposits throughout the kidney, as well as some enlargement in Bowman's space	Al-Bairuty et al. (2013)
Cyprinus carpio	0.16–0.53	Sublethal	Cell swelling in the tubules and glomerular, bleeding and infiltration of inflammatory cells in the interstitial tissue, degraded tubular tissue and hyaline cysts formation	Afaghi and Zare (2020)
Pseudopleuronectes americanus	1.00–3.20	Sublethal	Presence of hemopoietic tissue necrotic and very much reduced in volume and the tubule cells themselves were vacuolated and reduced in size. The apical portion of the tubule cells seem to disintegrate, and the lumen of the tubules contained much dense material	Baker (1969)
Rutilus rutilus caspicus	0.10–0.40	Lethal	Expansion of Bowman's capsule, hemorrhage, hyperemia, and degeneration in tubules	Farhangi et al. (2014)
Heterobranchus bidorsalis	0.30–0.50	Sublethal	Necrosis of proximal tubule and glomerular shrinkage, degeneration of interstitial tissue and renal tubules, and enlargement of globular lumen and damage of proximal tubules, vacuolization andlesions.	Jegede (2013)
Poronotus triacanthus	0.02–0.25	Sublethal	Apical vacuoles increased in diameter, some mitochondria were contracted and showed a considerable variability in size and shape. The nucleus displayed irregular outlines with condensed heterochromatin and there were dilations of rough endoplasmic reticulum cisternae. In addition, lysosomes were increased in number and no longer restricted to the apical cell portions. There was an increase in the size of mitochondria and proliferation of atypical mitochondrial profile	Jiraungkoorskul et al. (2007)
Labeo rohita	3.15	Lethal	Congestion of blood vessel, and tubular necrosis of glomerulus, and liquefactive necrosis of first and second proximal segments and irregularity in interstitial hematopoietic tissue due to tubular necrosis, and extravasation of blood from blood vessels, coagulative necrosis of first and second proximal segments, irregular blood congestion, and tubular necrosis	Latif et al. (2013)
Oreochromis niloticus	2.00	Subletal	Vacuolation in the renal epithelium with focal depletion of this hematopoietic tissue, and mitochondrial degeneration in the tubular epithelium with partial to complete loss of the matrix. Cristae dilatation and vesiculation of rough endoplasmic reticulum cisternae that changed into circular arrays and numerous fat globules in the cytoplasm with loss of peroxisomes, ribosome, and glycogen	Nouh and Selim (2013)
Poecilia reticulata	1.17	Sublethal	Obstruction of the internal cavities of renal tubules with necrotized renal epithelial cells sloughed from the basement membrane.	Park and Heo (2008)
Clarias batrachius	500	Sublethal	Nucleus displayed irregular nuclear membrane and condensed heterochromatin and granular heterochromatin dilated into cisternae. The lysosome increased number and scattered in cells, and increase in the mitochondrial size and its proliferation, and necrosis with dissolution of tubular kidney cells and Bowman's capsule. Hypertrophy of nucleus and appearance of vacuoles with dissolution and destruction of tissues.	Razzaq et al. (2011b)
Heteropneustes fossilis	600	Sublethal	Nucleus displayed irregular nuclear membrane and condensed heterochromatin and granular heterochromatin dilated into cisternae. The lysosome increased number and scattered in cells, and increase in the mitochondrial size and its proliferation, and necrosis with dissolution of tubular kidney cells and Bowman's capsule. Hypertrophy of nucleus and appearance of vacuoles with dissolution and destruction of tissues.	Razzaq et al. (2011b)

body necrosis, absence of heartbeat or failure to move with mechanical stimulation (Hernandez et al., 2011). Studies on free amino acids in the liver of *Channa punctatus* exposed to 1 mg L^{-1} $CuSO_4$ for 84 days showed that cystine and tryptophan disappeared, and few new amino acids appeared. These changes in the free amino acids may be due to the toxic effects of $CuSO_4$). In *C. punctatus*, exposure to 0.36 mg L^{-1} $CuSO_4$ increased alanine amino transferase and aspirate amino transferase concentrations in gill, liver and kidney, and a decrease in catalase activity due to damages in these tissues (Singh et al., 2012). In *Pomatoschistus micros* exposed to 0.02–0.40 mg L^{-1} $CuSO_4$ there was a decrease in acetylcholinesterase, 7-ethoxyresorufin-O-deethylase activity in gills and liver, and an increase in lactate dehydrogenase, glutathione S-transferases, catalase, superoxide dismutase, lipid peroxidation, glutathione reductase and glutathione peroxidase activity (Vieira et al.,

Table 5
Histopathological effects of exposure to copper sulphate on spleen of different fish species.

Fish species	Concentration (mg L^{-1})	Exposure	Tissue alterations	References
Oncorhynchus mykiss	0.002–0.02	Sublethal	Splenic depletion	Nowak and Duda (1999)
Cyprinus carpio	0.006–0.01	Sublethal	Splenic cells with vacuolization, necrosis and the nucleus become less prominent and outer capsule was ruptured	Khan (2016)
Carassius gibelio	0.10–2.00	Sublethal	Thickening of spleen capsule and no clumping of macrophages filled with hemosiderin, which can be due to the intensive usage of the hemoglobin secreted by the destroyed erythrocytes for formation of new erythrocytes. In the higher concentrations, besides the thickening of the capsule, a strong congestion of the red pulp, as well as narrowing of the sinuses was found	Georgieva et al. (2010)
Poecilia reticulata	1.17	Sublethal	There were no significant abnormal lesions in the tissues of spleen	Park and Heo (2008)
Synechogobius hasta	0.15	Sublethal	Splenic parenchyma with increased lymphoid cells and macrophages accumulations	Song et al. (2013)

2009).

Fish acclimated to soft water over 21 days were exposed to 0.01 mg L^{-1} $CuSO_4$ and 0.01 mg L^{-1} $CuSO_4$ plus 3.3 mM of sodium ions or 3.3 mM of calcium ions plus 0.01 mg L^{-1} $CuSO_4$. Although there was an effective reduction in ionic copper concentrations in all tissues, sodium in the presence of this metal did not decrease the degree of oxidative damages, particularly in the gills. Conversely, the presence of calcium with $CuSO_4$ decreased the accumulation of ionic copper in the gills, but not in the liver, and there was no reduction in oxidative damages. Transcriptional analyses of these genes mainly showed a down regulation of transcripts with the $CuSO_4$ only treatment, whereas treatment with calcium plus $CuSO_4$ restored some of the genes to concentrations similar to the control. However, the sodium plus $CuSO_4$ treatment had a strong opposing effect when compared to exposure to only the $CuSO_4$. Therefore, $CuSO_4$, under these environmental conditions, has complex effects on gene expression patterns (Craig et al., 2010).

The gills of *D. rerio* larvae showed green fluorescent protein (EGFP) expression after exposure to 100 μM of $CuSO_4$ from as early as 24 h post-fertilization. Results suggested that the most sensitive organs to stress induced by copper ions in water were the central nervous system and the liver, even though the most affected in terms of cell death were the gills and kidney pronephros. It is probable that the observed cell death had been elicited by the induction of reactive oxidative species (ROS), which may have caused death of the larvae exposed to $CuSO_4$ (Hernandez et al., 2011). Mohamed et al. (2008) reported that gill cells of *O. niloticus* exposed to $CuSO_4$ showed low mitotic activity. Also, positive inductions of macro-DNA damage as represented by different types of aberrations such as chromatid deletions, chromatid breaks, gaps, fragments, stickiness, translocations, ring chromosomes and centromeric attenuation were observed, of which chromatid deletion, stickiness and fragments were more frequent than other chromosomal aberrations.

The liver, spleen, and kidney are pivotal organs of the body that maintain homeostasis. The liver is the center of metabolism and detoxification, and the kidneys are involved in elimination of wastes from the body and selective reabsorption. The liver plays a central role in the metabolism of toxicants (Jegede, 2013; Latif et al., 2013). The fish spleen is an erythro- and leukopoietic organ involved in the synthesis of new erythrocytes and lymphocytes, and this hematopoietic tissue is the only organ in fish to trap antigens (Balamurugan et al., 2012; Taheri et al., 2016). The few studies available have shown that the spleen undergoes changes in its hematopoietic structure/function due to toxic effects of $CuSO_4$ (Table 5). Hence, these organs show morphological alterations as responses to toxicity caused by $CuSO_4$ (Tables 2–5).

Liver of *O. mykiss* after 24 h of exposure to 0.50 mg L^{-1} $CuSO_4$ presented signs of general improvement in structure, little vacuolation and the cytoplasm was more homogenous. After 72 h, sinusoids were still dilated but blood appeared normal. After 96 h, the liver structure resembled those of the control fish (Williams and Wootten, 1981). Na^+-K^+- ATPase activity decreased in liver of *C. carpio* after exposure to the lethal concentration of 8.0 mg L^{-1} $CuSO_4$ (Balambigai and Aruna, 2011). In contrast, in *Poecila reticulata* exposed to the sublethal concentration of $CuSO_4$ (0.12 mg L^{-1}), no significant lesions in liver, kidney or spleen were observed, as well as in the liver or spleen of fish exposed to the lethal concentration of 1.17 mg L^{-1} (Park and Heo, 2008).

Sublethal concentrations of $CuSO_4$ can also be toxic for fish with reduced metabolic capacity, which can be explained by the accumulation of ionic copper on the gill surface and the impairing of the O_2 diffusion capacity (Gündoğdu, 2008), and the involvement of other vital organs in fish (Tables 3–5). Given that the liver is the main organ for storing essential metals, the accumulation of copper ions in this tissue is related to the concentration of ions in the environment and the duration of exposure. Therefore, the ionic copper content in fish liver can be used as an indicator of $CuSO_4$ toxicity. However, many of the tissue changes that occur at exposure to sublethal concentrations of $CuSO_4$ can be reversible after a recovery period (Karan et al., 1998).

Toxicity of $CuSO_4$ to early developmental stages of fish has also been documented. Embryos and larvae are more sensitive to $CuSO_4$ than juvenile and adult fish (Witeska et al., 2014). In studies carried out with *C. carpio* larvae, the embryonic development in water at 0.2 mg L^{-1} $CuSO_4$ until day 20 post-hatch showed body malformations, which impaired larval locomotion and adversely affected feeding efficiency, thus reducing survival. Only the larvae that were able to take up exogenous food survived. Hence, the exposure to $CuSO_4$ affected the larvae survival (Ługowska and Witeska, 2004). Effects of $CuSO_4$ on the survival of embryos, time of hatching, size, and quality of newly hatched larvae of *Leuciscus idus* exposed to 0.10 mg L^{-1} has been evaluated. Results showed that exposure of embryos to $CuSO_4$ delayed hatching, reduced survival and increased frequency of body malformations and mortality in newly hatched larvae. Exposure during the larval period reduced survival, growth, and delayed development as indicated by yolk utilization, beginning of active feeding and swim bladder inflation. However, exposure of embryos to copper reduced toxicity in larvae with continuous exposure to ionic copper when compared to fish that were previously unexposed (Witeska et al., 2014). Lasiené et al. (2016) investigated the influence of $CuSO_4$ on the development of embryos of *Poecilia reticulata* exposed to 0.5, 1.0 and 1.5 mg L^{-1} $CuSO_4$ for 24 h and after 15 days. No visible lesion was observed in the embryos of fish

exposed to 0.5 mg L^{-1} of $CuSO_4$, whereas fish exposed to 1.0 mg L^{-1}, the embryos showed visible abnormalities from blastodisc to middle-eyed stages of development. Exposure to 1.5 mg L^{-1} $CuSO_4$ caused the death of fish and their embryos within 24 h. Therefore, these results indicate a compromise in larval development, which could interfere in the production of fish in aquaculture.

Understanding the differences in the effects of $CuSO_4$ concentrations on tissues and biological mechanisms may provide essential information to manage the use of this chemotherapeutic in aquaculture. Although studies on different species often involve the repetition of the same experimental methodologies, I attempt to demonstrate the advantage of such comparative studies for a thorough understanding of $CuSO_4$ bioaccumulation and its potential harm to tissues.

5. Physiological and immune alterations caused by copper sulphate in freshwater and marine fish

Copper is a trace metal that is essential for fish growth and metabolism because it is part of many enzymes and glycoproteins (e.g. superoxide dismutase, cytochrome-c oxidase, tyrosinase, monoamine oxidase, lysyl oxidase and phenylalanine hydroxylase), which are important for nervous system functions and necessary for hemoglobin synthesis (Waiwood and Beamish, 1978; Kamunde and Wood, 2004; Craig et al., 2010; Hernandez et al., 2011; Monteiro et al., 2012; Wani and Sikdar-Bar, 2013; Sevcikova et al., 2016; Ewa et al., 2018; Mitrašinović-Brulić and Suljević, 2019; Afaghi and Zare, 2020). However, excess or deficiency of ionic copper disrupts healthy metabolic functions by promoting the production of free radicals that are toxic for cells and organisms (Hernandez et al., 2011; Sevcikova et al., 2016; Padrilah et al., 2018).

Copper ions of $CuSO_4$ accumulate in the blood by forming complexes with blood cells and plasma proteins, and subsequently travel through the bloodstream and accumulate in the liver and kidney. The bioaccumulation of ionic copper in fish tissues mainly depends on the duration of exposure and the dose. Hence, high concentrations of copper sulphate cause damages in hematopoietic tissues of fish (Mazon and Fernandes, 1999; Tavares-Dias et al., 2011; Kondera et al., 2014; Ewa et al., 2018) and may lead to alterations in several physiological processes in exposed fish. It has been accepted that heavy metal uptake through the gills and the body surface is transported to the liver via the bloodstream, metabolized and then excreted through the bile (Mazon and Fernandes, 1999; Kim et al., 2018).

The analysis of blood of fish exposed to $CuSO_4$ is relevant because this parameter is a sensitive indicator of fish health. Blood variables are important in determining the influence of pathophysiological conditions on homeostasis of fish exposed to $CuSO_4$, because physiology and biochemistry information about the health status of fish exposed may be obtained (Ramesh, 2001; Tavares-Dias et al., 2002; Tavares-Dias et al., 2011; Quarashi et al., 2017). Assessments of physiology and biochemistry variables are therefore important in the studies on changes in blood and immune parameters because of exposure to $CuSO_4$ for different aquaculture species submitted to different concentrations of this chemotherapeutic agent.

The concentration of ionic copper in fish tissues may decrease during recovery (Jiraungkoorskul et al., 2007; Ewa et al., 2018). In some cases, copper levels in the kidney may remain high after a short exposure period, leading to short and long-term morphological changes in tissues (Ewa et al., 2018). Other studies have shown hematological and plasma ion recovery was faster than the morphological restoration of gill tissues after exposure to $CuSO_4$. The recovery processes appear to be adaptive responses of fish exposed to ionic copper, suggesting other possible compensatory responses that allow fish to quickly recover their baseline blood parameters and plasma ion concentrations (Cerqueira and Fernandes, 2002). Therefore, this indicates a high exposure to $CuSO_4$ may not contribute to quicker recovery of homeostasis with long-term exposure.

Increased levels of ionic copper in water lead to the production of metal-binding proteins such as metallothionein, which are stored in the hepatocytes bound to copper. Excess metals bind to alpha-globulin in the liver, producing ceruloplasmin that becomes excreted through the kidney. In fish exposed to $CuSO_4$, copper ion homeostasis occurs at the cellular level and in tissues. Generally, copper homeostasis entails regulated uptake, distribution, and excretion, and occurs by coordinated interactions of several organ systems. In the liver, ionic copper is incorporated into various proteins for biological function, detoxification, and storage. Protein bound copper ions, primarily caeruloplasmin-bound copper, enters the secondary phase of transport to the rest of the body. With expossure to $CuSO_4$, the uptake of ionic copper is balanced by excretory losses via bile, and gills, and other losses via the kidney (Fig. 1) and other organs. Several oxidation and hydrolytic reactions in addition to reduction reactions of the Phase 1 reactions are catalyzed by microsomal cytochrome P450 enzymes. The P450 are membrane-bound enzymes and therefore, metabolized substances must be somewhat lipophilic. In Phase 2 reactions, glucuronidation is often involved and is a system with a relatively high capacity. Conjugation Glutathione is particularly important in interrupting highly reactive intermediates (metabolites) formed by P450 in Phase 1 reactions. Conjugation with various amino acids (e.g. glutamic, glycine, etc) can occur, and acetylation may alter solubility depending on the polarity of the compound. Given that one of vital functions of the liver is to eliminate exogenous chemicals and endogenous intermediates, hepatocytes contain high levels of phase I enzymes, which have the capacity to generate reactive electrophilic metabolites. Hepatocytes also have a wide variety of phase II enzymes, which enhance the hydrophilicity by adding polar groups to lipophilic compounds and target these conjugates to certain carriers in the canalicular or plasma membrane for excretion. Generally, phase II reactions yield stable, nonreactive metabolites (Parkinson and Ogilvie, 2008).

When exposure to high $CuSO_4$ concentrations occurs and the capacity of the liver to remove copper is exceeded, toxic copper ions may be transported through the bloodstream to other organs (Mazon and

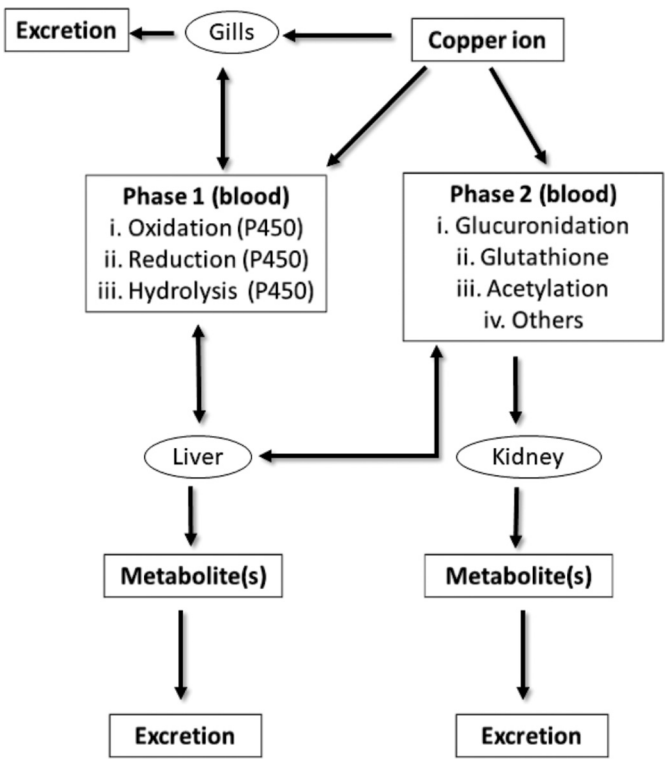

Fig. 1. Schematic diagram of copper ion metabolism in fish after exposure to copper sulphate. Adapted of Kamunde and Wood (2004).

Fernandes, 1999; Kim et al., 2018). Fish have been used in lethal and sublethal toxicity testing and biomonitoring with various chemicals, including $CuSO_4$. Fish may have a range of tolerance to this chemotherapeutic that depends on environmental conditions. Furthermore, fish have the ability to synthesize metallothionein. Metallothionein is a cysteine the participe in regulation of synthesis that involves altered gene expression and that binds to copper. This protein is inducible in liver, kidney, and intestine by glucocorticoids, as well as by acute exposure to copper ions of $CuSO_4$ (Shuhaimi-Othman et al., 2015; Kim et al., 2018).

In fish, stress may be induced by therapeutic treatments with $CuSO_4$ and depends on the concentration used and time of exposure. Stress is a general and non-specific response to any factor that disturbs homeostasis of exposed fish. Neural and hormonal control of stress response involves activation of the sympathetic neural system, and the release of hormones such as epinephrine and cortisol from the kidney (Barton and Iwama, 1991; Wendelaar Bonga, 1997; Urbinati et al., 2020). In low stress conditions caused by exposure to $CuSO_4$, the fish homeostasis balance is usually restored. Severe or prolonged stress may deplete fish coping mechanisms, resulting in physiological disturbances such as alterations in blood composition and immune mechanisms. Since exposure to $CuSO_4$ may cause damages on the gill epithelium and hematopoietic tissues such as in the kidney, spleen and/or liver of fish (Table 2–5), this accumulation of copper ions in tissues of fish may lead to alterations of physiological processes.

The increasing or decreasing of blood leukocytes and erythrocytes number, hematocrit and hemoglobin after acute or sublethal exposure of $CuSO_4$ may be attributed to generalized stress response rather than to a specific cytotoxic action of copper ions may led to increase in plasma cortisol and glucose concentrations in fish exposed, causing significant effect on erythropoiesis and leukopoiesis. Blood erythrocytopenia and leukocytopenia may be caused either by the inhibition of erythropoiesis and leucopoiesis or by the destruction of cells due to damages in hematopoietic tissues, particularly in the kidney and spleen of fish exposed to $CuSO_4$. Exposure to 5 mg L^{-1} $CuSO_4$ resulted in a decrease in protein content in the liver and gills of *C. punctatus*. Total protein is an important constituent of cells and tissues that play vital role in the physiology of living organisms (Bhure et al., 2011). Metallothionein content in the liver of *Cyprinus carpio* increased due to damages in this tissue (Tóth et al., 1996).

Immunosuppression induced by $CuSO_4$ exposure may be expressed through the decline of blood leukocytes in fish. The immune system is extremely sensitive to homeostatic adjustments via endocrine regulation and is influenced by the biochemical profile of the fish nervous system. Blood and biochemical parameters have been used to assess the health status of fish exposed to $CuSO_4$, and several studies have shown physiological and immune changes in fish caused by this chemical agent used as a therapeutic in aquaculture (Table 6). Thus, exposure to $CuSO_4$ may alter immune functions of the fish, resulting in immunosuppression, uncontrolled cell proliferation and/or alterations of the defense mechanism of the fish in response to exposure to $CuSO_4$. In *Carassius auratus*, exposure to 0.01 mg L^{-1} $CuSO_4$ increased the phagocytic response and 0.17 mg L^{-1} inhibited phagocytic response (Muhvich et al., 1995). In *Hemigrammus* sp., exposure to 0.30 mg L^{-1} $CuSO_4$ caused a decrease in monocytes, but had no influence on neutrophils, lymphocytes, and eosinophils in the blood (Paixão et al., 2013). In contrast, exposure of *O. mykiss* to 0.002–0.02 mg L^{-1} $CuSO_4$ had no effects on the proportions of blood lymphocytes, monocytes, and neutrophils (Nowak and Duda, 1999).

In *Ictalurus punctatus* exposed to 1.70 mg L^{-1} $CuSO_4$ for 78 h, no changes were shown for the hematocrit, plasma lactate dehydrogenase, alanine aminotransferase and aspartate aminotransferase concentrations (Griffin et al., 1999). Exposure to 0.30 mg L^{-1} $CuSO_4$ showed no influence on serum levels of metallothionein, alkaline phosphatase and aspartate aminotransferase in *C. carpio* (Al-Taee and Al-Hamdani, 2015). *Oncorhynchus mykiss* exposed to sublethal concentration of

Table 6
Hematological and biochemical effects of acute and subletal exposure to copper sulphate for different fish species after.

Fish species	Concentration (mg L^{-1})	Alterations	References
Salvelinus fontinalis	0.04–0.07	Increase in total erythrocytes number, hematocrit, hemoglobin, and increase in plasma chloride, protein, glutamic oxalacetic transaminerse and osmolarity levels	McKim et al. (1970)
Heteropneustes fossilis	0.24–0.72	Decrease in total erythrocytes number and hemoglobin	James and Sampath (1995)
Heteropneustes fossilis	0.25	Decrease in total erythrocytes number and total serum protein, and increase in total leukocytes number, hemoglobin, serum urea, cholesterol, glucose glutamic pyruvic transaminase, glutamic oxalacetic transaminase, alkaline phosphatase, sodium, and potassium levels	Singh and Reddy (1990)
Heteropneustes fossilis	4.50	Decrease in total erythrocytes number and hemoglobin, and increase in total leukocytes number	Dutta et al. (2016)
Ictalurus punctatus	1.70	Increase in plasma cortisol levels	Schlenk et al. (1999)
Ictalurus punctatus	1.70	Increase in plasma cortisol, glucose, and lactate levels, and decrease in plasma chloride levels	Griffin et al. (1999)
Piaractus mesopotamicus	0.50	Decrease in mean corpuscular hemoglobin concentration (MCHC), total erythrocytes and leukocytes number, and neutrophils percentage, and increase in mean corpuscular volume (MCV).	Tavares-Dias et al. (2002)
Piaractus mesopotamicus	1.00	Decrease in mean corpuscular hemoglobin concentration (MCHC), plasma glucose, total erythrocytes, and leukocytes number, and increase in mean corpuscular volume (MCV).	Tavares-Dias et al. (2002)
Leporinus macrocephalus	0.02–0.04	Increase plasma glucose, percentual of monocytes and decrease in thrombocytes percentual	Nunes et al. (2010)
Prochilodus lineatus	0.01–0.20	Increase the plasm glucose and lactate levels	Takasusuki et al. (2004)
	1.75–5.437		

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Table 6 (*continued*)

Fish species	Concentration (mg L^{-1})	Alterations	References
Colossoma macropomum		Decrease in plasma total protein, plasma sodium, total erythrocytes, and neutrophils number, and increase in mean corpuscular volume (MCV)	Tavares-Dias et al. (2011)
Colossoma macropomum	8.75	Decrease in total erythrocytes, leukocytes, lymphocytes, and PAS-positive granular leukocytes number, and increase in mean corpuscular volume (MCV)	Tavares-Dias et al. (2011)
Oncorhynchus mykiss	0.005	Decrease in plasma calcium and sodium levels	Reid and McDonald (1988)
Oncorhynchus mykiss	0.20	Plasma glucose, aspartate aminotransferase, alanine aminotransferase and acetylcholinesterase	Nemcsok and Hughes (1988)
Oncorhynchus mykiss	2.00	Plasma glucose, aspartate aminotransferase and alanine aminotransferase, and decrease in acetylcholinesterase	Nemcsok and Hughes, 1988
Oncorhynchus mykiss	0.50	Decrease in hematocrit, hemoglobin, plasma glutamate oxaloacetic transaminase lactate dehydrogenase and hydroxybutyric dehydrogenase activity, and increase in plasma glucose and plasma glutamate pyruvate transaminase	Williams and Wootten (1981)
Oncorhynchus mykiss	0.20	Increase in plasma glucose, aspartate aminotransferase, alanine aminotransferase and lactate dehydrogenase, and decrease in hematocrit	Hughes and Nemcsók (1988)
Oncorhynchus mykiss	0.30	Decrease in total leukocytes and lymphocytes number	Dick and Dixon (1985)
Oncorhynchus mykiss	0.12	Decrease in total erythrocytes, leukocytes, and lymphocytes number, and increase in total thrombocytes number	Dick and Dixon (1985)
Oncorhynchus mykiss	0.50	Decrease in hematocrit and hemoglobin, plasma sodium, chloride, potassium, and calcium, and increase in plasma lactate and magnesium levels	Pilgaard et al. (1994)
Oncorhynchus mykiss	0.002–0.02	Increase in plasma cortisol, and decrease in phagocytic activity of macrophages and circulating antibody, plasma sodium and potassium levels	Nowak and Duda (1999)
Oncorhynchus mykiss	0.01	Increase in total erythrocytes and leukocytes number, monocytes and neutrophils percentage, hemoglobin, and corpuscular hemoglobin concentration (MCHC), and decrease in mean corpuscular volume (MCV), lymphocytes percentage	Mitrašinović-Brulić and Suljević (2019)
Oncorhynchus mykiss	0.55	Decrease in plasma sodium and chloride levels, and increase in plasma potassium levels	Priya et al. (1999)
Oncorhynchus mykiss	0.05	Decrease in plasma sodium, chloride, and potassium levels	Priya et al. (1999)
Cyprinus carpio	1.00–4.00	Increase in serum alkaline phosphatase, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels	Karan et al. (1998)
Cyprinus carpio	0.02	Decrease in total erythrocytes and leukocytes number, and hemoglobin	Thangam et al. (2014)
Cyprinus carpio	0.16–0.53	Decrease in hemoglobin, hematocrit, total erythrocytes, and leukocytes number	Afaghi and Zare (2020)
Cyprinus carpio	0.30	Increase in serum alanine aminotransferase levels	Al-Taee and Al-Hamdani (2015)
Cyprinus carpio	0.50–1.20	Increase in total erythrocytes and leukocytes number and increase in mean corpuscular hemoglobin concentration (MCHC), decrease, or increase in hemoglobin and hematocrit	Al-Tamimi et al. (2015)
Cyprinus carpio	1.50–3.00	Decrease in hemoglobin, hematocrit, total erythrocytes, and leukocytes number	Ghasemzadeh and Bahrekazemi (2019)
Cyprinus carpio	10.00	Increase in serum L-alanine:2-oxoglutarate aminotransferase, laspartate:2-oxoglutarate aminotransferase, L-lactate: NAD oxidoreductase and glucose levels	Tóth et al. (1996)
Cyprinus carpio	1.00–4.00	Increase in serum alanine	Karan et al. (1998)

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Table 6 (*continued*)

Fish species	Concentration (mg L^{-1})	Alterations	References
		aminotransferase, aspartate aminotransferase and alkaline phosphatase levels	
Cyprinus carpio	0.55	Increase in total erythrocytes and leukocytes number, hemoglobin	Ramesh (2001)
Cyprinus carpio	0.05	Decrease in total erythrocytes and hemoglobin, and increase in leukocytes number	Ramesh (2001)
Cyprinus carpio	2.00	Decrease in total erythrocytes, hematocrit, hemoglobin and lymphocytes percentage, plasma total protein, glucose, and Aspartate aminotransferase, and increase in total leukocytes number and neutrophils percentage	Mottahari et al. (2013)
Cyprinus carpio	0.25	Decrease in total erythrocytes and leukocytes number, hemoglobin and mean corpuscular hemoglobin concentration (MCHC), and mean corpuscular hemoglobin concentration (MCHC), triglycerides and cholesterol	Mazandarani and Hoseini (2017)
Cyprinus carpio	2.00	Decrease in serum total protein and albumin, and increase in serum globulin, total bilirubin, and uric acid	Mutlu et al. (2016)
Cyprinus carpio	0.02	Decrease in total erythrocytes and leukocytes number, and hemoglobin,	Quarashi et al. (2017)
Cyprinus carpio	0.07	Increase in total erythrocytes, lymphocytes, monocytes and neutrophils number, hemoglobin, hematocrit, plasma cells, plasma ceruloplasmin activity, ferric-reducing ability of plasma, plasm glucose, ammonia, albumin, alanine aminotransferase, cholesterol, lactate dehydrogenase and calcium	Sevcikova et al. (2016)
Clarias gariepinus	4.00–8.00	Decrease in total erythrocytes number, hemoglobin, and hematocrit, and increase in mean corpuscular hemoglobin concentration (MCHC)	Wani and Sikdar-Bar (2013)
Notopterus notopterus	20.00–30.00	Increase plasma cholesterol and hemoglobin, and decrease in plasma glucose, total protein, urea, and creatine	Barad and Kulkarni (2010)
Pagrus major	0.03–0.04	Increase in plasma glucose, cortisol and metallothionein levels, plasma corticotrophin-releasing hormone and corticotrophin-adrenocorticotropic hormone levels, and decrease in in plasma Na^+/K^+-ATPase levels	Kim et al. (2018)
Oncorhynchus mykiss	0.25	Decrease in total leukocytes number	Bagdonas and Vosylienė (2006)
Anabas testudineus	0.11	Decrease in total erythrocytes number, hemoglobin, hematocrit, and oxygen carrying capacity, and increase in glutamate pyruvate transaminase and glutamate oxalate transaminase and lactate dehydrogenase levels	Kumar and Nandan (2014)
Anabas testudineus	0.34	Decrease in total erythrocytes number, hemoglobin, hematocrit, and oxygen carrying capacity, and increase in glutamate pyruvate transaminase, glutamate oxalate transaminase and lactate dehydrogenase levels	Kumar and Nandan (2014)
Rutilus frisii kutum	0.004–0.40	Decrease in hematocrit, mean corpuscular volume (MCV), and increase in mean corpuscular hemoglobin concentration (MCHC) and total leukocytes number	Azarin et al. (2012)
Rutilus rutilus caspicus	0.02–0.06	Increase in serum cortisol, glucose, and alanine aminotransferase (ALT) levels, and decrease in serum sodium, total protein, albumin, globulin, and albumin: globulin ratio	Hoseini et al. (2016a)
Prochilodus lineatus	0.09–0.10	Increase in total erythrocytes number, hematocrit, hemoglobin, and decrease in mean corpuscular volume (MCV) and mean corpuscular hemoglobin	Carvalho and Fernandes (2006)

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Table 6 (*continued*)

Fish species	Concentration (mg L^{-1})	Alterations	References
		concentration (MCHC)	
Prochilodus lineatus	0.01	Increase in hematocrit, total erythrocytes number, and mean corpuscular volume (MCV), and decrease in hemoglobin and mean corpuscular hemoglobin concentration (MCHC)	Carvalho and Fernandes (2006)
Prochilodus lineatus	0.03	Increase in hematocrit, hemoglobin, total erythrocytes, mean corpuscular hemoglobin concentration (MCHC) and plasma potassium levels, and decrease in mean corpuscular volume (MCV) and plasma sodium and chloride levels	Cerqueira and Fernandes (2002)
Ameiurus nebulosus	0.05–0.11	Increase in hematocrit and glucose level	Christensen et al. (1972)
Ameiurus nebulosus	0.05–0.10	Increase in hematocrit, hemoglobin, plasma total protein and glucose level, and decrease in plasma chloride levels	Christensen et al. (1972)
Oreochromis mossambicus	0.10–0.20	Increase in hematocrit and hemoglobin	Cyriac et al. (1989)
Oreochromis mossambicus	3.00–12.00	Increase in plasma glucose levels	Jagadeshwarlu and Sunitha (2018)
Oreochromis niloticus	0.04–0.40	Decrease in gill Na^+/K^+-ATPase activity, plasma sodium, chloride, plasma osmolality, and increase in plasma protein, glucose, and cortisol levels	Monteiro et al. (2005)
Oreochromis niloticus	2.00	Decrease in total erythrocytes and leukocytes, hemoglobin and hematocrit, plasma total protein, albumin, and increase in plasma glucose, aspartate aminotransferase, alanine aminotransferase, blood urea nitrogen and creatinine	Nouh and Selim (2013)
Oreochromis niloticus	0.10	Decrease in serum globulin, uric acid, urea, triglyceride and total cholesterol levels, phagocytic index, phagocytic activity, and increase in serum albumin: globulin ratio, catalase, aspartate aminotransferase (AST), alanine aminotransferase (ALT) levels	El-Keredy et al. (2017)
Oreochromis niloticus	0.02–0.07	Increase in total erythrocytes number, hemoglobin, hematocrit, corpuscular hemoglobin concentration (MCHC), plasma glucose, cortisol, lactate dehydrogenase, aspartate aminotransferase, alanine aminotransferase, calcium and lactate, and decrease in plasma total protein, and total leukocytes number and mean corpuscular volume (MCV)	Shokr (2020)
Oreochromis niloticus	0.21	Decrease in total erythrocytes, thrombocytes and leukocytes number, hemoglobin, hematocrit, number and mean corpuscular volume (MCV), corpuscular hemoglobin concentration (MCHC), perceptual of lymphocytes, monocytes, neutrophils, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels	El-Bouhy et al. (2016)
Oreochromis niloticus	0.43	Decrease in total erythrocytes, thrombocytes and leukocytes number, hemoglobin, hematocrit, number and mean corpuscular volume (MCV), corpuscular hemoglobin concentration (MCHC), perceptual of lymphocytes, monocytes, neutrophils, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels	El-Bouhy et al. (2016)
Carassius gibelio	2.00	Decrease in total erythrocytes and leukocytes number, and increase in hematocrit	Ewa et al. (2018)
Carassius gibelio	0.10–2.00	Decrease in hematocrit, corpuscular hemoglobin concentration (MCHC) and mean	Georgieva et al. (2010)

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Table 6 (*continued*)

Fish species	Concentration (mg L^{-1})	Alterations	References
		corpuscular volume (MCV)	
Hypophthalmichthys molitrix	0.09–0.49	Decrease hematocrit, hemoglobin, mean corpuscular volume (MCV) and total erythrocytes and total lymphocytes number, and increase in mean corpuscular hemoglobin concentration (MCHC) and total leukocytes and neutrophils number, plasma cortisol and glucose levels	Hedayati and Ghaffari (2013)
Labeo rohita	3.15	Decrease hematocrit, hemoglobin, total erythrocytes and leukocytes number, serum total protein, albumin, potassium, calcium, magnesium and ammonia, and increase in mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration (MCHC) and oxygen saturation, and increase in serum triglycerides, total cholesterol, gamma glutamyltransferase, uric acid and bilirubin and PCO_2	Latif et al. (2014)
Prochilodus lineatus	0.02–0.03	Increase in total erythrocytes number, hematocrit, hemoglobin, lymphocytes, and plasma potassium, and decrease in neutrophils, plasma sodium and chloride	Mazon et al. (2002)
Channa punctatus	0.36	Decrease in total erythrocytes number, hematocrit, hemoglobin, and mean corpuscular hemoglobin concentration (MCHC), monocytes, neutrophils, and basophils percentage, and increase in mean corpuscular volume (MCV), erythrocyte sedimentation rate, clotting time, lymphocytes, and eosinophils percentage	Singh et al. (2008)
Puntius sophore	0.40–0.80	Decrease in total erythrocytes number, hematocrit, hemoglobin, and increase in mean corpuscular volume (MCV),	Gupta et al. (2013)

$CuSO_4$ (0.06 and 0.25 mg L^{-1}) showed no alterations in hemoglobin, hematocrit, and total erythrocytes number, as well as in total leukocytes in fish exposed to 0.06 mg L^{-1} (Bagdonas and Vosylienė, 2006). *Clarias batrachus* exposed to sublethal concentration of $CuSO_4$ (0.5 mg L^{-1}) showed no alterations in hemoglobin and total erythrocytes, but poikilocytosis and anisocytosis in these cells occurred (Kumar et al., 2017).

Clarias batrachus exposed to sublethal concentration of $CuSO_4$ (1.5 mg L^{-1}) showed decreases in the carbohydrate levels in the brain, kidney, muscles, and liver (Siddiquie et al., 2009). In *Oreochromis mossambicus*, sublethal exposure to $CuSO_4$ (3.00–12.00 mg L^{-1}) for 40 days caused decreases in glycogen and lipid levels in muscle and liver (Jagadeshwarlu and Sunitha, 2018). *Carassius gibelio* exposed to 0.10 or 0.25 mg L^{-1} $CuSO_4$ presented a decrease in lactate dehydrogenase activity in the gills (Teodorescu et al., 2012). In *Carassius auratus*, exposure to 0.10 to 1.50 mg L^{-1} $CuSO_4$ increased the lipid peroxidation in liver and decreased protein content, superoxide dismutase, glutathione peroxidase, glutathione reductase, glutathione-s-transferase and catalase activities, and glutathione content (Trivedi et al., 2012).

The uptake of ionic copper occurs via two distinctive mechanisms in the gills: i) a transmembrane protein (copper transporter 1), which is unaffected by external copper concentrations; and ii) the apical Na+-uptake pathways located at branchial epithelial cells, which is influence by the external concentration of copper. In the latter case, intracellular sodium levels can decrease as a direct consequence of competition at the uptake site. In addition, once copper ions enter epithelial cells, they inhibit the activity of the membrane bound Na^+/K^+-ATPase (Reid and McDonald, 1988; Nowak and Duda, 1999; Ay et al., 1999; Kamunde and Wood, 2004; Monteiro et al., 2005; Al-Bairuty et al., 2016; Kim et al., 2018). Copper ions have also been known to induce oxidative stress, olfactory impairment, and increase plasma ammonia and acid-base imbalance (Reid and McDonald, 1988; Sevcikova et al., 2016). Exposure to the sublethal concentrations of copper sulphate for 24 and 96 h induced DNA damages in blood erythrocytes. Despite acute exposure to sublethal concentrations inducing the accumulation of ionic copper and DNA damages in fish, recovery is shown after 240 h in seawater without the addition of copper sulphate (Oliveira et al., 2014).

The antioxidant defense system consists of antioxidant enzymes such as superoxide dismutase, catalase, glutathione peroxidase, glutathione S-transferase, glutathione reductase, vitamin A, vitamin C, and vitamin E. Hence, the levels of antioxidant enzymes can be used as indicators of the antioxidant activity in fish and may serve as biomarkers of oxidative stress. In the liver and gills of *O. mykiss*, the exposure to 5 μg L^{-1} of $CuSO_4$ decreased the glutathione, glutathione peroxidase and catalase concentrations, and increased the malondialdehyde concentration (Yonar et al., 2016). Therefore, these results demonstrate that $CuSO_4$ has potential to induce oxidative stress in exposed fish.

6. Growth performance alterations of freshwater and marine fish exposed to copper sulphate

Increasing fish production greatly depends on adequate water quality, as well as feeding, handling, and the absence of stress. The control of parasites for increasing the fish growth rates is one most important issue in all phases of aquaculture prodution. Despite copper being a trace metal necessary for growth, the low or excess levels in fish may be harmful to their health and interfere in growth. Exposure to $CuSO_4$ may be toxic to fish and interfere in production and productivity (Table 7). The toxicity of $CuSO_4$ leads to physiological and behavior disturbances that generally affects food consumption or energy production in exposed fish.

Studies on the prophylaxis with copper sulphate (4.0–10.0 mg L^{-1}) in the hatchery of *C. gariepinus* suggest that continuous exposure is better for increasing hatch rate, growth and survival of eggs and fry (Ataguba et al., 2013). *Pimephales notatus* had smaller and higher mortality rates, for 30 or 60 days, after exposure to 0.12 mg L^{-1} $CuSO_4$ than those exposed to 0.02–0.04 mg L^{-1} and the controls. Fish exposed to 0.07 mg

Table 7
Body growth performance effects for different freshwater and marine fish species after exposure to copper sulphate.

Fish species	Concentration (mg L^{-1})	Alterations	References
Salvelinus fontinalis	0.003–0.03	Decrease in survival and growth	McKim and Benoit (1971)
Pimephales promelas	0.004–0.03	Decrease in growth	Eaton (1973)
Lepomis macrochirus	0.16	Decrease in survival and retarded growth	Benoit (1975)
Oncorhynchus mykiss	0.002–0.21	Decrease in growth rates	Waiwood and Beamish (1978)
Oncorhynchus mykiss	0.07–0.22	Decrease in food intake, appetite, and growth rate	Lett et al. (1976)
Oncorhynchus mykiss	0.09–2.70	Increase in length and weigh	Dixon and Sprague (1981a)
Oncorhynchus mykiss	0.06	Increase in length and specific growth rates	Dixon and Sprague (1981a)
Oncorhynchus mykiss	0.07	Increase on food consumption and weigh	McGeer et al. (2000b)
Oncorhynchus mykiss	0.002–0.02	Decrease in survival, weight gain and protein in muscles	Nowak and Duda (1999)
Perca fluviatilis	0.14–0.19	Decrease in growth rates	Collvin (1984)
Rhabdosargus sarba	0.15–0.45	Decrease in growth rates	Wong et al. (1999)
Ictalurus punctatus	0.46	Increase in length, and decrease in weight and condition factor	Perkins et al. (1997)
Xiphophorus helleri	0.04–0.12	Decrease in feeding rate, conversion efficiency and gonadosomatic index, and increase conversion rate	James et al. (2003)
Xiphophorus helleri	0.04–0.16	Decrease in and specific growth rate and gonadosomatic index	James et al. (2008)
Carassius auratus	0.02–0.10	Decrease in and specific growth rate and gonadosomatic index	James et al. (2008)
Ictalurus punctatus	1.5	Decrease in specific growth rate and increase in feed conversion ratio	Rábago-Castro et al. (2006)
Cyprinus carpio	0.2	Decrease in length and growth	Sarnowski and Witeska (2008)
Cyprinus carpio	1.5	Decrease in weight gain, length, condition factor and increase in mortality and feed conversion ratio	Ghasemzadeh and Bahrekazemi (2019)
Cyprinus carpio	3.0	Decrease in weight gain, length, condition factor and increase in mortality and feed conversion ratio	Ghasemzadeh and Bahrekazemi (2019)
Cyprinus carpio	0.07	Decrease in total length and weight and condition factor, and increase in hepatosomatic index	Sevcikova et al. (2016)
Oreochromis niloticus	0.15–0.50	Decrease in final weight, weight gain, specific growth rate, protein efficiency ratio, net protein retention and condition factor, and increase in hepatosomatic index in feed conversion ratio	Ali et al. (2003)
Oreochromis niloticus	0.10	Decrease in final weight, weight gain, relative growth rate and protein efficiency ratio, and increase in feed conversion ratio	El-Keredy et al. (2017)
Oreochromis niloticus	0.02–0.07	Decrease in final weight, weight gain, and hepatosomatic index	Shokr (2020)
Clarias gariepinus	2.0–10.0	Increase in survival, and decrease in mean final weight, mean weight gain and specific growth rate	Ataguba et al. (2013)
Rutilus frisii kutum	0.11	Decrease in weight	Gharedaashi et al. (2013)
Rutilus frisii kutum	0.23	Decrease in final weight, specific growth rate and survival rate, and increase in feed conversion ratio	Gharedaashi et al. (2013)
Rutilus caspicus	0.06	Decrease in weight gain, specific growth rate, food intake and survival of fish	Hoseini et al. (2016a)
Poecilia reticulata	0.004	Decrease in gonadosomatic index, surviving rate and offspring production	Moosavi and Shamushaki (2015)
Poecilia reticulata	0.01–0.02	Decrease in final weight, specific growth rate, relative fecundity, gonadosomatic index, surviving rate and offspring production, and increase in feed conversion ratio	Moosavi and Shamushaki (2015)
Danio rerio	0.02	Decrease in final weight and survival	Campagna et al. (2008)
Poecilia reticulata	0.03	Decrease in final length and weight, relative fecundity, gonadosomatic index, surviving rate and offspring production, and increase in feed conversion ratio	Moosavi and Shamushaki (2015)
Mystus viattatus	1.20–1.91	Decrease in feeding, metabolic and growth rates, conversion efficiency and absorption efficiency	Subathra and Karuppasamy (2007)
Synechogobius hasta	0.06–0.12	Increase in body weight, and decrease in specific growth rate, survival, hepatosomatic index and viscerosomatic index	Chen et al. (2013)
Mystus viattatus	0.47–0.75	Decrease in feeding, metabolic and growth rates, conversion efficiency and absorption efficiency	Subathra and Karuppasamy (2007)
Synechogobius hasta	0.08–0.15	Decrease in survival rate and weight gain, and increase in viscerosomatic index and hepatosomatic index	Song et al. (2013)

L^{-1} $CuSO_4$ also had smaller growth than those exposed to 0.02 mg L^{-1} and the controls (Horning and Neiheisel, 1979). Basirun et al. (2019) reported that exposure to high concentrations of $CuSO_4$ negatively affected the food intake in *O. mossambicus*. In contrast, long term exposure with 0.02–0.03 mg L^{-1} $CuSO_4$ had no impact on the growth rates of *Prochilodus lineatus* (Mazon and Fernandes, 1999). *Oncorhynchus mykiss* exposed to 0.03 mg L^{-1} $CuSO_4$ showed no changes in body weight (Dixon and Sprague, 1981a), and *O. niloticus* exposed to 0.1 mg L^{-1} of $CuSO_4$ showed no change in feed intake (El-Keredy et al., 2017),

suggesting that the growth of this fish was unaffected by exposure.

Ictalurus punctatus exposed to 1.5 mg L^{-1} of $CuSO_4$ showed no changes in the length, weight and condition factor (Rábago-Castro et al., 2006). In *Rutilus frisii kutum* exposed to 0.11 mg L^{-1} $CuSO_4$, the final body weight, specific growth rate, feed conversion ratio, condition factor and survival rate were unaffected (Gharedaashi et al., 2013). For *Poecilia reticulata* adults, exposure to a low concentration of $CuSO_4$ (0.004 mg L^{-1}) had no influence on the final length and weight, specific growth rate and feed conversion (Moosavi and Shamushaki, 2015). *Oreochromis niloticus* also exposed to a low concentration of $CuSO_4$ (1.5 mg L^{-1}) for 35–95 days showed no changes in growth rate (Mutlu et al., 2015). In *Pimephales promelas*, exposure to 0.1–2.0 μM L^{-1} $CuSO_4$ caused a decrease in body weight (Erickson et al., 1996). *Oncorhynchus mykiss* fry acclimated for 30 days to 20.0 and 60.0 mg L^{-1} $CuSO_4$ or 1.0 and 2.0 mg L^{-1} showed no changes in the specific growth rate (Taylor et al., 2000). Similarly, exposure of *I. punctatus* females to sublethal concentrations of $CuSO_4$ (0.22–0.46 mg L^{-1}) for 11 weeks showed no changes in length, weight, and body condition (Perkins et al., 1997). On the other hand, in *Hypophthalmichthys molitrix* larvae exposed to 0.15–0.30 mg L^{-1} $CuSO_4$, a decrease in the survival rate of fish was shown to be proportional to increases of the concentration of this chemical agent (El-Fiky, 2001). Exposure to $CuSO_4$ may change the feeding rate of fish, and also lead to negative effects on location and reach of food. Swimming behavior and avoidance of adverse conditions have direct effect on the fish appetite as both the activities influence fish survival.

7. Antiparasitic efficacy of copper sulphate in treated freshwater and marine fish species

In the aquaculture and aquarium industry, the occurrence of parasitic diseases causes high economic losses in production and has increased with the expansion of large-scale productions (Thoney and Hargis Jr, 1991; Tavares-Dias & Martins, 2017; Malheiros et al., 2020). Hence, concerns regarding the impacts of disease in fish farming has increased, becuase aquaculture and aquarium industry may suffer serious economic problems with inadequate management. This increase in parasitic diseases is mainly due to a high fish stocking density, handling stress and inadequate water quality, all factors that facilitate the occurrence of infections and epizootics particularly by ectoparasites as protozoans and metazoans, which often cause losses in production of fish farming (Tavares-Dias & Martins, 2017; Malheiros et al., 2020). These problems have led to many studies aimed at identifying suitable chemotherapeutics, for example $CuSO_4$ (Table 8), which has a long history of use in both aquaculture and aquarium industries due to its effectiveness and low cost. However, $CuSO_4$ was initially used to control bacterial diseases.

Thus, discussion is required to optimize treatment concentrations with $CuSO_4$ and to establish the most effective protocols to control the ectoparasites, while ensuring minimal impacts on fish performance in aquiculture and aquarium activities. Therapeutic strategies with copper sulphate for the control of ectoparasites should also provide environmentally friendly alternatives. To reduce the toxicity risk and maximize the therapeutic efficiency of $CuSO_4$, it is necessary to know the concentrations of copper sulphate that are effective for treatment of each ectoparasite disease for different species of fish. In addition, an improvement in treated fish welfare as well as in immune-related to overall health can help prevent diseases or help fish recover of parasitic infections

Various ectoparasites are problematic in freshwater and marine fish aquaculture, and therapeutics such as $CuSO_4$ have been effective in controlling protozoans and monogenean species (Table 8). Monogenea is a group of parasitic worms commonly found in fish that feed on mucus and epithelial cells of the skin and gills, and sometimes on blood. Monogeneans are usually well accommodated to fish hosts and have less effects, but they frequently cause severe epizootics in cultured and aquarium fish. In such cases, the short and direct life cycle of

Table 8
Management strategies of therapeutic baths with copper sulphate to control and treatment of ectoparasite species in different freshwater and marine fish.

Parasite species	Concentration (mg L^{-1})	Exposure	Results	References
Trichodina sp.	3.00	24 h	High efficacy	Diggles (2000)
Trichodina sp.	0.50	2 min	No efficacy	Fish and Burrows (1940)
Trichodina sp.	0.43	30 days	High efficacy	El-Bouhy et al. (2016)
Ichthyophthirius multifiliis	0.02–0.25	7 days	High efficacy	Straus (1993)
Ichthyophthirius multifiliis	0.40–2.00	5–10 days	High efficacy	Schlenk et al. (1998)
Ichthyophthirius multifiliis	2.20–4.40	7 days	High efficacy	Straus (2008)
Ichthyophthirius multifiliis	0.63	192 h	High efficacy	Carneiro et al. (2005)
Ichthyophthirius multifiliis	0.05– 0.12	*In vitro* (4 h)	High efficacy	Straus et al. (2009)
Ichthyophthirius multifiliis and *Myxobolus colossomatis*	1.75–8.75	48 h	No efficacy	Tavares-Dias et al. (2011)
Ichthyophthirius multifiliis	2.10	10 days	No efficacy	Farmer et al. (2013b)
Ichthyophthirius multifiliis	0.29	14 days	High efficacy	Ling et al. (1993)
Ichthyophthirius multifiliis	0.25	7–21 days	No efficacy	Ling et al. (1993)
Ichthyophthirius multifiliis	1.00–1.50	21 days	No efficacy	Tieman and Goodwin (2001)
Amyloodinium ocellatum	0.75	14 days	High efficacy	Paperna (1984)
Amyloodinium ocellatum	0.75	6 days	High efficacy	Aiello and D'Alba (1986)
Amyloodinium ocellatum	1.50	7 days	High efficacy	Abreu et al. (2007)
Amyloodinium ocellatum	1.0–3.0	1 h	High efficacy	Virgula et al. (2017)
Amyloodinium ocellatum	1.0–3.0	24 h	High efficacy	Virgula et al. (2017)
Amyloodinium ocellatum	0.30	2 h, for 14 days	Low efficacy	Bessat and Fadel (2018)
Amyloodinium sp.	0.20	10 day	High efficacy	Owatari et al. (2020)
Tetrahymena thermophila	1.00 and 5.00	100 h	High efficacy	Schlenk and Moore (1994)
Tetrahymena thermophila	7.00–10.00	100 h	No efficacy	Schlenk and Moore (1994)
Ichthyobodo necator				
Ichthyobodo necator	2.10	4–10 days	High efficacy	Farmer et al. (2013a)
Ichthyobodo necator	2.10	4–10 days	High efficacy	Farmer et al. (2014)
Ichthyobodo necator	2.00	5 days	High efficacy	Mitchell et al. (2008)
Cryptocaryon irritans	0.50–0.70	10 days	Low efficacy	Rigos et al. (2001)
Anacanthorus penilabiatus	0.50	1 day	High efficacy	Tavares-Dias et al. (2002)
Anacanthorus penilabiatus	0.50–1.00	8 day	No efficacy	Tavares-Dias et al. (2002)
Anacanthorus spathulatus	1.75–8.75	48 h	High efficacy	Tavares-Dias et al. (2011)
Onchocleidus mimus	2.10	10 days	High efficacy	Farmer et al. (2013b)
Cichlidogyrus sp.	0.43	30 days	High efficacy	El-Bouhy et al. (2016)
Monogenea gen. sp.	0.30	3 days	High efficacy	Paixão et al. (2013)

(*continued on next page*)

Table 8 (*continued*)

Parasite species	Concentration (mg L^{-1})	Exposure	Results	References
Caecognathia coralliophila	5.00–20.00	24 h	No efficacy	Thing et al. (2016)
Caecognathia coralliophila	40.00	24 h	High efficacy	Thing et al. (2016)

monogeneans enables them to reach epizootic levels quickly when hosts and parasites are confined closely together (Thoney, 1990; Thoney and Hargis Jr, 1991; Poynton et al., 1997; Tavares-Dias et al., 2002; Tavares-Dias et al., 2011; Farmer et al., 2013b; Paixão et al., 2013; Malheiros et al., 2020). Monogenean species that feed on the blood may transmit other disease (Thoney and Hargis Jr, 1991). Copper sulphate may control monogenean adults in freshwater fish and may also affect the free swimming oncomiracidium of monogeneans more than the adult stages in seawater (Thoney, 1991). However, 0.25 mg L^{-1} of $CuSO_4$ in seawater was reported to have little effect on the oncomiracidia and eggs of *Benedeniella posterocolpa* in skin of the shark *Rhinoptera bonasus*. In *in vitro* trials with adult *Microcotyle hiatulae* exposed to 0.24 mg L^{-1} $CuSO_4$, no effects were shown on the monogeneas (Thoney, 1990). In contrast, embryos contained in eggs of *M. hiatulae* incubated at 0.24 mg L^{-1} $CuSO_4$ died prior to hatching (Thoney and Hargis Jr, 1991). The monogenean *Neodermophthirius harkemai* was also eliminated in the shark *Negaprion brevirostris* after treatment with 0.25 mg L^{-1} $CuSO_4$ over 85 days (Poynton et al., 1997).

In Sea World of Florida, Thoney and Hargis Jr, 1991 observed that cownose and dasyatid rays are slightly more sensitive to $CuSO_4$ than many teleost fish species even though most elasmobranch fish are more sensitive. Furthermore, infestations of the monogeneans *Neobenedenia melleni* in marine teleosts in an aquarium were treated with 0.15–0.18 mg L^{-1} of copper sulphate for 14 days, and intensity of these ectoparasites in the fishes decreased to zero within 5 days, which is when the concentration of copper ions in the water reached 0.14 mg L^{-1}. *In vitro* studies using 1.60 mg L^{-1} $CuSO_4$ caused higher mortality of *Euryhaliotrema chrysotaeniae*, *Euryhaliotrema spirotubiforum*, *Haliotrema longitubocirrus*, *Haliotrema patellacirrus*, *Haliotrema anguiformis* and *Diplectanum fusiforme* when compared to 0.40 or 0.80 mg L^{-1}. However, when *Lutjanus kasmira* was submitted to a therapeutic bath with 0.80 mg L^{-1} $CuSO_4$ for 24 h, no antiparasitic efficacy was observed (Vignon et al., 2009), suggesting that short baths and in low doses are ineffective against monogeneans.

Some species of freshwater and marine protozoans are ectoparasites that invade the skin and gills of fish. When fingerlings are raised at high stocking densities, these parasites can cause mortality of an entire stock unless the fish are treated with an antiprotozoal agent and the disease cycle is interrupted. Copper sulphate has shown efficacy at eliminating protozoan species when used at low concentrations and with long-term baths (Table 8). In *I. punctatus*, treatments with 1.00 or 1.50 mg/L $CuSO_4$ for 24 or 48 h reduced the spread of *I. multifiliis* infection to healthy fish, but the severe infections were not controlled (Tieman and Goodwin, 2001). Similar findings were reported in other studies (Straus, 1993; Schlenk et al., 1998), which found that the invasion of healthy fish by *I. multifiliis* was prevented by $CuSO_4$.

In *Paralichthys orbignyanus*, treatment with 1.50 mg/L copper sulphate for 7 days caused the detachment of the *Amyloodinium ocellutum* trophonts and generated a high number of tomonts at the bottom of the tank. However, this same $CuSO_4$ concentration was ineffective to kill the tomont stages of *A. ocellutum* (Abreu et al., 2007). Hecht and Endemann (1998) suggested that marine *A. ocellatum* and the freshwater *Piscinoodinium pillulare* can be controlled with long term baths with 0.75 μg L^{-1} $CuSO_4$. *In vitro* studies showed efficacy of 100 mg/L copper sulphate against *Uronema marinun* of *Paralichthys olivaceus* (Jee et al., 2002). In contrast, exposure to 3.00 mg L^{-1} $CuSO_4$ for 6 h was moderately effective against *Trichodina* sp. in *Colistium nudipinnis* (Diggles, 2000).

In vitro exposures of *A. ocellatum* at 0.50–10.0 mg/L $CuSO_4$ was ineffective to inhibit the proliferation of the tomonts. However, at sporulation, with the cleavage of the tomont theca, all concentrations were lethal to sporulation of tomits and formation of dinospores. With exposure at 0.50 mg L^{-1}, few defective non-motile dinospores emerged, and at 0.10 mg L^{-1} sporulation progress was unaffected and motile dinospores were produced. Thus, exposure to all $CuSO_4$ concentrations, if interrupted during the sporulation it was ineffective to prevent the dividing tomites from completing its sporulation and producing viable dinospores. Furthermore, exposure with at least 1.00 mg L^{-1} for up to 12–24 h reduced the reproduction of the tomonts. However, dinospores were still produced in large numbers but were of low vitality, possibly due to the difficulty in completely eliminating the $CuSO_4$ residues after the interruption of the treatment (Paperna, 1984). Goodwin and Straus (2006) determined for *I. multifiliis* the $LD_{50–240min}$ of $CuSO_4$ to vary from 0.02 to 0.09 mg L^{-1}, depending on alkalinity. Xu et al. (2016) studied the *in vitro* LC_{50} for *Ichthyophthirius multifiliis*, *Tetrahymena thermophila*, *Tetrahymena pyriformis* and *Tetrahymena* sp., which varied from 0.15 to 0.28 mg L^{-1}

In the production of freshwater and marine ornamental fish, short and long-term baths may be used for controlling ectoparasites at concentrations varying from 0.05 to 1.0 mg L^{-1} of $CuSO_4$. Short-term baths of 30 min may be used with higher concentrations. Long-term baths for up to 7 days may be used and performed in holding tanks until obtaining a final concentration of less than 1.0 mg L^{-1} of copper ions. In contrast, for fish reared in marine and freshwater aquariums, the concentration should be lower because copper sulphate needs to be added to obtain a final concentration of 0.15–0.25 mg L^{-1} copper ions. *Cryptocaryon irritans* is a ciliated protozoan that causes marine white spot disease in fish and may be controlled using of 0.15–0.25 mg L^{-1} $CuSO_4$ in long-term baths for 3–5 days (Yanong, 2017). Nevertheless, to reduce the risk of toxicity to fish and maximize the chemotherapeutic efficiency, additional studies on the therapeutic doses of $CuSO_4$ are necessary to treat diseases in different fish species with specific water conditions.

8. Conclusions and perspectives

Copper sulphate is a popular chemotherapeutic to control parasites because of its effectiveness and low cost. However, it may be toxic fish and sensitivity varies between species. This chemotherapeutic is toxic to many fish species when using doses near the therapeutic concentration. Many studies in freshwater fish exposed to copper sulphate have been carried out, but few studies have been conducted with marine fish species, which have different osmotic and osmoregulatory strategies. Freshwater fish take up major ions actively through the gills from the environment, while marine fish actively excrete excess ions through the gills. Marine fish consume seawater to replenish water lost through osmosis to the environment, which increases the uptake of copper ions in the water. Therefore, studies in marine fish species need to be carried out given the lack of information regarding their tolerance to treatment with $CuSO_4$.

Laboratory data analyzed here provides estimates for exposures in the field and require extrapolation for each scenario due to the toxic action of copper ions. In practical situations in fish farming, producers must consider environmental conditions to treat a parasitic infestation with $CuSO_4$.Although the margins of safety are determined from laboratory bioassays, where environmental conditions are controlled, the risks of toxicity to $CuSO_4$ must be reduced or mitigated by the effective use of this chemotherapeutic and adequate manipulation in the field. Since $CuSO_4$ has a low margin of safety and its lethal toxicity for many fish species remains unknown, tests should be carried out on a small subsample of fish before application in cultivation ponds. Future research should be aimed at identifying the range of water quality parameters and effective concentrations of this compound to treat ectoparasitic infections without risk to farmed fish. In general, traditional laboratory toxicity tests focus on the response of a fish species

continuously exposed to a series of $CuSO_4$ concentrations with a fixed duration, but little attention has been given to the effects of the varying durations. Furthermore, data related to toxicity in these traditional methods should be investigated to verify if the duration was overestimated or underestimated for the $CuSO_4$ concentration to show the therapeutic effects in the field. Another problem related to the use of $CuSO_4$ in cultivation ponds is the mechanism of toxic action and predicting how the copper ions might interact in the environment. Since the copper sulphate may exist in multiple forms that are influenced by water quality characteristics, particularly temperature, pH, alkalinity, hardness and salinity, the efficacy of this chemotherapeutic in aquatic systems is highly dependent on its bioavailability.

I found that the use of $CuSO_4$ to control and treat ectoparasitic infections in fish farming has been successful in eliminating protozoans and monogeneans, but efficacy may vary when applied to treat other parasite species. Toxicity of $CuSO_4$ in fish is determined by the release of bioavailable ionic copper in the environment, which affects their homeostasis and immune system. Innate immunity plays a key role in the defense against disadvantageous factors to farmed fish, which also include the ectoparasites. Alkalinity and hardness are the main modifiers of $CuSO_4$ toxicity since they influence the availability of ionic copper. In low alkalinity, caution should be taken when applying copper sulphate to avoid high concentrations of ionic copper and/or copper hydroxide complexes because a high concentration of these toxic forms may disrupt homeostasis in fish. Therefore, careful measures of water quality parameters in cultivation tanks must be taken in order to ensure fish survival when using this therapeutic.

I am unable to suggest definitively that applications of $CuSO_4$ in freshwater aquaculture, fish mariculture and aquarium systems must be stopped under any circumstances, despite having negative impacts on the environment or presenting potential risks to food security. However, there appears to be no valid reasons for prohibiting the use of copper in aquaculture when heeding certain basic precautions. However, applications in fish farming should be made only when strictly necessary. Chemotherapeutic doses of copper should not exceed 0.01 mg/L of total alkalinity concentrations, and this should not be made when there is heavy rainfall, which could possibly cause overflow of cultivation ponds. Copper sulphate concentrations in the cultivation ponds should be checked daily to verify that therapeutic concentrations are maintained and to avoid toxic levels. Further studies are needed to include cultivation in net-cages, evaluation of alternative antifouling agents for cages and possible benthic effects of copper accumulation in areas near net-cages. The increasing resistance to chemotherapeutics and growing awareness for environmental protection are promoting research for alternative treatments. Since the use of nanoparticles is a promising strategy in the therapeutic drugs field, and $CuSO_4$ nanoparticles possess enhanced or even unique physicochemical properties and show lower toxicity when compared to non-structured copper sulphate, but more tests are needed for nanostructures in laboratory and field as an alternative chemotherapeutic for fish aquaculture. Given the known toxicity of copper sulphate when dissolved in water, it should be investigated whether copper-containing nanoparticles present a similar or different environmental hazard.

Declaration of competing interest

None

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