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Copper and zinc generated by the Aquascape IonGen pond clarifier system can be detrimental to koi (*Cyprinus carpio*) health

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ABSTRACT

Background Copper is frequently used as an algicide, and copper ion generators such as the Aquascape IonGen claim to be safe for use in systems containing fish. In 2012, a die-off of koi (*Cyprinus carpio*) in a pond in Raleigh, North Carolina, occurred after the IonGen was added to the system.

Methods Physical and postmortem examinations suggested that heavy metal toxicity was the likely cause of morbidity and mortality. This was supported by a heavy metal screening of the owners' pond. Additional experiments were performed to determine if the IonGen produced toxic levels of copper and zinc.

Results The tank containing the IonGen had higher concentrations of copper and zinc, and copper levels exceeded those associated with toxicity in both hard and soft water.

Conclusion The results of this study indicate that ion generators might not be safe for fish, and copper should only be used as an algicide if concentrations are closely monitored.

INTRODUCTION

Controlling algae growth is a common concern for freshwater ponds and aquaculture, and copper (Cu) has been shown to be an effective algicide under certain conditions.[1 2] Cu is also used as a treatment for ichthyophthiriosis,[3 4] although the therapeutic range is narrow and toxicity can occur at concentrations over 0.25 mg/l, depending on the species and water conditions.[5–9] The Aquascape IonGen claims to reduce algae through the release of Cu and zinc (Zn) ions.[10] While this product claims to be safe for systems containing fish, its safety has not been empirically tested.

Clinical case

In a backyard pond in Raleigh, North Carolina, two koi fish (*Cyprinus carpio*) out of an original population of 23 fish died. One of those fish died in winter of 2011 and the other in June 2012. The latter fish was frozen and submitted for postmortem examination at the North Carolina State University College of Veterinary Medicine. Other than being underweight no gross abnormalities were found. Around that same time, a tancho kohaku koi began to lose its white colour and brilliance and continued to do so for a period of about six months, and three other koi in the pond showed signs of lethargy and cachexia. Three fish were found dead on 13 October 2012. One was preserved in formalin at the owners' home, another was sent to Rollins Diagnostic Laboratory in Raleigh, North Carolina, for postmortem examination, and the third was too autolysed for pathological study.

The koi fish lived in an outdoor, 49,400–57,000-litre, 2.13-m deep, rubber-lined pond with a life support system containing two 400W UV filters, advanced biological filtration, two pumps and a Cu ion generator (IonGen, Aquascape, St Charles, Illinois, USA) (figure 1). The life support system was installed in March 2012 to control algae and parasites, although no parasite problem had been diagnosed in the fish. The pond also contained two bottom drains, a protein skimmer and a waterfall. The water was turned over every 60–90 minutes, and no supplemental heat or lighting was provided. The owners provided commercially available food (Microbe-Lift Legacy Koi and Goldfish Food, Cape Coral, Florida, USA).

On 14 October 2012 the owners of the koi reported that water quality values for temperature, pH, ammonia, nitrite and nitrate were normal, but Cu was frequently as high as 0.2 mg/l. The owners reported that Cu pipes were not being used, but that the Cu ion generator had been added, as described above. The owners turned off the ion generator two days before the three dead fish were

Figure 1 Life support system for backyard pond. Two 400W UV filters are shown in the red rectangle. IonGen copper ion generator is shown in the yellow circle. The life support system also contained two pumps and advanced biological filtration.

found on 13 October and a 30 per cent water change was performed. The veterinarian tested the Cu levels in the pond using a dip stick (SenSafe Copper (John's), Industrial Test Systems, Rock Hill, South Carolina, USA) and found that it was 0.05–0.1 mg/l Cu, compared with a well water control at 0 mg/l Cu (figure 2A,B).

Full physical examinations were performed on two of the remaining fish in the pond that were showing signs of lethargy and cachexia on 14 October. Both fish were anaesthetised in 200 mg/l tricaine mesylate (MS-222, Tricaine-S, Western Chemical, Vadodara, Gujarat, India) in 10 litres of water. The first fish (figure 3A,B) was in poor body condition and had a mild abrasion left of the dorsal fin, which was likely a result of being trapped in the skimmer when it was found. No parasites or pathogens were observed on gill and skin biopsies, but the gills had excessive mucus (figure 3C,D). The second fish (figure 3E) was also in poor body condition but not as severe as the first fish, and the gill and skin biopsies were normal. Both fish took an unusually long time to recover from anaesthesia, and they were returned to the pond on recovery. The primary differential diagnosis after the physical examination was Cu toxicity based on the skin and gill biopsies, the absence of ectoparasites, and the fact that the standard water quality parameters were normal. It was recommended to leave the Cu ion generator disconnected, add activated carbon to the system and continue performing 30 per cent water changes with 1.5 g/l salt (sodium chloride).

Figure 2 Copper test results from (A) the koi fish pond and (B) a well water control.

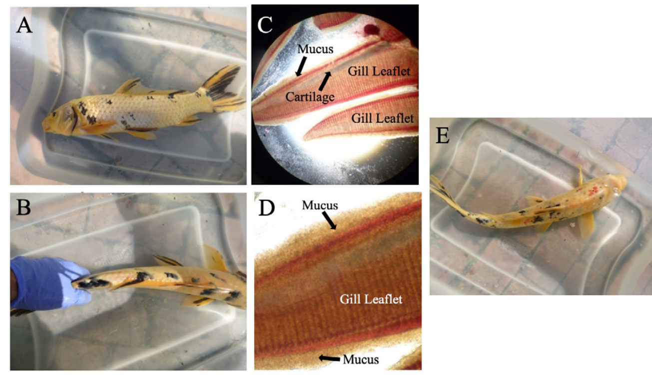

Figure 3 Photographs from the physical examination of two koi fish. (A) Anaesthetised koi fish in right lateral recumbency. (B) Dorsal view of koi fish in (A). (C) Gill biopsy of koi fish. (D) Higher magnification of gill biopsy showing excess mucus. (E) A second anaesthetised koi fish, dorsal view.

Water quality samples and liver tissue from one of the deceased fish were sent to the Pennsylvania Animal Diagnostic Laboratory in Kennett Square, Pennsylvania, for analysis. Results from the heavy metal screening of the water samples can be found in table 1. Briefly, Cu was found to be elevated at a concentration of 0.123 mg/l, compared with a typical range in freshwater of 0.001–0.02 mg/l. Zn was also elevated in the liver based on the toxicology results (table 2), with a concentration of 2950 mg/l, but Cu was at a concentration of 19.6 mg/l.

Based on these results, Cu toxicity was identified as the most likely cause of morbidity and mortality. The owners continued to keep the Cu ion generator disconnected in their pond, and the fish resumed normal activity levels and no further mortalities were observed.

MATERIALS AND METHODS

To determine whether the IonGen Cu ion generator used in the backyard pond could generate toxic levels of

Table 1 Heavy metal concentrations in water samples from a backyard koi pond in Raleigh, North Carolina

Metal	Concentration (mg/l)
Calcium	18.3
Copper	0.123
Iron	<0.05
Magnesium	5.61
Phosphorus	<0.05
Potassium	2.74
Selenium	<0.05
Sodium	975
Zinc	<0.05

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Table 2 Heavy metal concentrations in the liver of a deceased koi fish

Metal	Concentration (mg/l, dry weight)
Calcium	637
Cobalt	0.126
Copper	19.6
Iron	2320
Magnesium	1040
Manganese	3.04
Molybdenum	0.593
Selenium	4.30
Zinc	2950

heavy metals, an experiment was carried out to measure the output of Cu, Zn and silver (Ag) by the IonGen over time. One 1130-litre tank was fitted with an IonGen and another 1130-litre tank without the IonGen (control). All other parameters between the tanks were identical. Water samples were collected from both tanks before the addition of the IonGen, and then weekly for 12 weeks. The samples were sent to the Pennsylvania Animal Diagnostic Laboratory for analysis.

The water samples were analysed for Cu, Zn and Ag using NexION 300D inductively coupled plasma mass spectrometry (Perkin Elmer, Shelton, Connecticut, USA) by the Pennsylvania Animal Diagnostic Laboratory System New Bolton Center Toxicology Laboratory, University of Pennsylvania, School of Veterinary Medicine (Kennett Square, Pennsylvania). The analytical standards were purchased from SCP Science (Champlain, New York, USA), and trace metal grade nitric acid was purchased from Fisher Scientific (Pittsburgh, Pennsylvania, USA). All dilutions were done using in-house deionised water (≥18 megaohm cm) obtained from a Millipore water purification system.

Three-millilitre portions of the water samples were placed in Teflon perfluoroalkoxy vials (Savillex, Minnetonka, Minnesota, USA) and digested overnight at 70°C with 1 ml of concentrated nitric acid. The digested samples were cooled to room temperature and diluted with deionised water to a final volume of 10.0 ml after addition of four internal standards with a final concentration of 20 parts per billion (^{74}Germanium, ^{115}Indium, ^{89}Yttrium, ^{159}Terbium). Ag was determined by standard mode, whereas kinetic *energy discrimination* mode using helium as the collision gas was used to analyse Cu and Zn. To ensure proper instrument operation and accuracy of the results, standard curves were generated for all three elements of interest. All final concentrations were reported in parts per million (mg/l) on a wet weight basis. Method detection limits for Cu, Ag and Zn were established at 0.005 mg/l, 0.001 mg/l and 0.1 mg/l, respectively, in the water samples.

Eight of the samples were analysed in duplicate with the values within 20 per cent of each other for all three elements. Water standard reference material (1643e) with certified mineral values from the National Institute of Standards and Technology (Gaithersburg, Maryland, USA) was analysed with each batch of samples.

The concentrations of Cu, Zn and Ag between the two tanks were analysed over time using repeated-measures analysis of variance in RStudio (V.1.2.1335)[11] using the built-in packages as well as rcompanion[12] and ggplot2.[13] For samples where the metal concentration was less than the minimum detectable threshold, the value was set at the minimum detectable threshold for statistical analysis. For example, if the Cu concentration was reported as less than 0.005 mg/l, the value for the statistical test was set at 0.005 mg/l.

RESULTS

The concentration of Cu was 0.011 mg/l in the control tank and 0.009 mg/l in the treatment tank before the addition of the IonGen to the treatment tank. During the 12-week treatment period, the concentration of Cu was significantly higher in the IonGen tank than in the control tank (F_1=120.83, $P<0.001$), reaching concentrations as high as 16.7 mg/l, and differed significantly across weeks in the IonGen tank (F_1=14.96, $P<0.001$) (figure 4A). The Cu concentration in the control tank ranged between less than 0.005 and 0.013 mg/l. Zn concentrations were also higher in the IonGen tank compared with the control tank (F_1=134.82, $P<0.001$), but there was not a significant fluctuation in Zn concentrations in either tank across weeks (F_1=0.75, P=0.40) (figure 4B). Overall, the Zn concentration in the control tank did not get above <0.1 mg/l, while it ranged between 0.207 mg/l and 0.369 mg/l in the IonGen tank. Ag was not detectable in the control tank and did not rise above <0.001 mg/l in the IonGen tank, so statistical analysis was not conducted to compare Ag concentrations.

DISCUSSION

The results of this case study and experiment suggest than an ion generator such as the IonGen can yield toxic levels of Cu in freshwater ponds, and that chronic exposure to Cu at these levels can lead to poor health and mortality in koi. Koi fish from the backyard pond in this case began showing signs of lethargy and cachexia after the life support system was refurbished in March 2012, including the addition of the IonGen. Water quality parameters were normal with the exception of elevated Cu and Zn, and there was no evidence of infection, parasites or nutritional deficiencies in the fish. The liver toxicity screen revealed elevated Zn, but Cu concentrations were comparable with those typically found in common carp.[14] The history and diagnostic results indicate that metal toxicity is the most likely cause of disease in the koi in this case.

While Cu is commonly used as a therapeutic agent, it has a very narrow therapeutic index and must be kept between 0.15 and 0.20 mg/l.[4] The toxicity of Cu in aquatic systems is highly variable and depends on the physical and chemical characteristics of the water.[5 9] Cu is more toxic in soft water than in hard water because hard water contains more ions (eg, calcium and magnesium) that can compete with Cu ions for binding sites on organisms, such as on the gills of fish.[15 16] Increasing the hardness of water has been shown to decrease both acute toxicity and chronic toxicity of Cu, likely by decreasing the bioavailability and accumulation of Cu in tissues.[5 9] However, if the concentration of Cu in the water is high enough, toxicity can still occur in hard water. Cu, like other heavy metals, typically accumulates in the gills, kidney and liver, and cause osmoregulatory disturbances.[4] Cu exposure has been shown to increase the metabolic demands of common carp, leading to weight loss despite normal to increased food consumption.[14] Zn toxicity is similar to Cu toxicity, as it also decreases with increased water hardness. Zn and Cu toxicities are also considered to be additive in many conditions,[17–19] so increased concentrations of both can reduce the toxic threshold of each metal alone. While chronic exposures to Cu and Zn in aquatic systems are generally associated with Cu pipes and coins,[17] respectively, an ion generator such as the IonGen can also be a source of high concentrations of these heavy metals.

The concentration of Cu in the water sample from the backyard pond analysed in the heavy metal screening was 0.123 mg/l, which would be considered a sublethal toxic level in a freshwater system with soft water.[5 9] It should be noted that this value was the amount of Cu present after disconnecting the IonGen for three days and performing a 30 per cent water change, and the owners reported measuring Cu at 0.2 mg/l during regular water quality checks. Unfortunately, information about the hardness of the water in the pond is not available. The experiment showed that the IonGen released concentrations of Cu that would be consistently toxic in both hard and soft water. The authors did not directly measure the hardness of the water used in the experiments, but water from the city of Raleigh, North Carolina, is typically between 18 and 30 parts per million calcium carbonate and is considered soft water.[20 21] While the experimental set-up only included one experimental tank and one control tank, the high levels of Cu produced by the IonGen in the experimental tank cannot be ignored. Furthermore, the concentration of Cu released by the IonGen was not stable or predictable over time, so pond owners using the IonGen as an algicide would not be able to predict how much Cu was being released into their system. The complicated behaviour of Cu ions in relation to environmental conditions adds to the uncertainty of how much Cu produced by the IonGen is bioactively available in a pond. While the IonGen tank had higher levels of Zn than the control tank, the levels did not approach toxicity (above 1.5 mg/l in soft water)[9] and is therefore unlikely to be contributing to any additive toxicity in this scenario.[18 19] Interestingly, the liver sample analysed for heavy metal concentrations contained high Zn concentrations but relatively low Cu concentrations. One shortcoming of the analysis of this sample was that only one fish was used, so it might not be representative of the typical Cu concentrations of other fish in the pond.

In conclusion, Cu ion generators such as the IonGen can produce Cu concentrations that have detrimental effects on the health of koi. Although both Cu and Zn toxicities are dependent on water hardness and other geochemical parameters, the experiment suggests that the IonGen has the capacity to produce Cu at levels that are toxic regardless of water hardness. Cu ion generators should not be used in freshwater ponds that contain live plants and animals due to the risk of

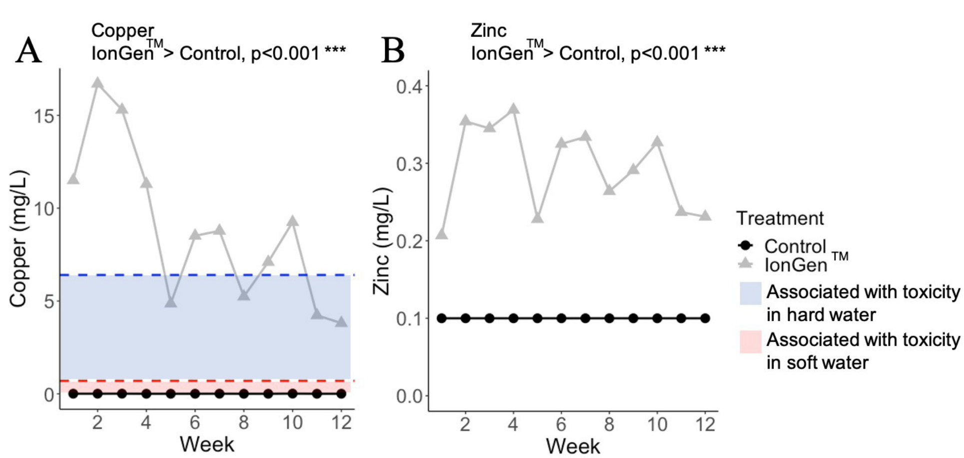

Figure 4 Copper and zinc concentrations in a tank containing the IonGen ion generator and a control tank without the IonGen. (A) Copper concentrations were significantly higher in the IonGen tank (grey triangles with grey line) and fluctuated significantly across weeks compared with the control tank (black circles with black line). The region highlighted in red indicates the concentration of copper associated with toxicity to freshwater fishes in soft water (0.03–0.70 mg/l copper), and the region highlighted in blue indicates the region of copper associated with toxicity in hard water (0.60–6.40 mg/l copper). (B) Zinc concentrations were significantly higher in the IonGen tank than in the control tank but did not fluctuate significantly across weeks in either tank. Zinc did not reach toxic levels in either tank Asterisks (***) indicate statistical significance of p<0.001.

chronic and unpredictable Cu exposure, and veterinarians should consider Cu ion generators as potential sources for Cu toxicity in freshwater fish, especially in ponds with soft water.

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Competing interests None declared.

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