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ACUTE TOXICITY OF THE SYNERGISM OF SURFACTANT (LAS) AND COPPER ON THE FRESHWATER FISH, *PUNTIUS GONIONOTUS*, BLEEKER.

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Abstract

Acute toxicities of linear alkylbenzene sulphonate (LAS) and copper on the freshwater fish, Puntius gonionotus *Bleeker, were different, with the 96-h* LC_{50} *values as 5.88 (5.68-6.08) mg/l MBAS and 91.96 (79.45-106.45)* µ *g/l, respectively. The toxicity of copper varied considerably according to concentration and exposure time, but for LAS, it depended on concentration and initial exposure time. The mixtures of LAS and copper were synergistic with each other in all toxicity ratios tested. The 96-h* LC_{50} *values for 1:1, 1:2 and 2:1 toxicity ratios were 0.53 (0.35-0.80), 0.36 (0.24-0.55) and 0.49 (0.32-0.77) toxic units, respectively, which exhibited more-than-additive effects. The acute toxicity of each toxicity ratio did not differ significantly, but the mode of action of each was influenced by the proportion of the LAS and copper in the mixed solution.*

Introduction

Water pollution is an important problem, which becomes more widespread and serious, resulting from wastes owing to the development of industry and the expansion of population. These waste waters frequently carry certain toxic substances, which can interact with each other and exhibit synergistic effects on aquatic organisms.

Two of the most ubiquitously distributed substances are linear alkylbenzene sulphonate (LAS), which is the main component of synthetic detergent, and copper, which is a widely utilized heavy metal. LAS is an anionic surfactant which is most widely used for household products[1]. Although LAS is easily degraded and its toxicity reduced, it exhibits an acute toxic effect before it changes configuration. Besides, LAS can also create water pollution problems in natural water sources, such as foaming, taste and odour. Copper is widely used in many industrial processes and is a compoment of pesticides, in the form of copper sulphate ($CuSO_4.5H_2O$). Copper causes acute damage on aquatic organisms and the environment because it does not degrade, and so it can be accumulated in water sources, sediments, and aquatic organisms.

It has been emphasized from studies in the past that LAS and copper are synergistic to each other[2]. Nevertheless, information concerning the toxicity of the mixture of toxicants is still limited. Therefore, the present study was designed to obtain the acute toxicity of the LAS-copper mixture on the freshwater fish, *Puntius gonionotus,* which is the most sensitive species to pollutants. It is hoped that information obtained from this study may be useful in predicting the long-term toxic effect of this mixture at low concentrations, and that it may also be useful as a guideline for the corresponding water quality standards set for the protection of aquatic lives.

Materials and Methods

The fingerling of *Puntius gonionotus*, with a mean weight of 0.21 g and a mean length of 2.0 cm, were held in aerated tap water for at least 7 days prior to conducting the assays. Water qualities of the tap water were as follows: Water temperature 28° ± 1°C, pH 7.5 ± 0.3, total alkalinity as $CaCO_3$ 100 ± 5 mg/l, total hardness as $CaCO_3$ 105 ± 3 mg/l (measured as the total concentration of bivalent cations using the Ethylene-diaminetetraacetic Acid or EDTA Method)[3], dissolved oxygen 7.5 ± 3 mg/l (measured by the azide modification of the Winkler Method), copper 0 mg/l (measured by Atomic Absorption Spectophotometer or AAS), and surfactant 0 mg/l.

The stock solution of linear alkylbenzene sulphonate (LAS) (active ingredient, 60%) was dissolved in distilled demineralized water, and the stock solution of copper was prepared in demineralized water from copper sulphate ($CuSO_4.5H_2O$). Concentrations of LAS and copper were determined using the Methylene Blue Active Substance Method (MBAS)[3] and AAS, respectively. The following concentrations were used : 5.070, 5.395, 5.785, 6.175 and 6.500 mg/l MBAS for LAS, and 75.0, 92.0, 112.0, 135.0 and 168.0 μg/l for copper. Their 96-h LC_{50} values, 95% confidence intervals and slopes of probit lines were determined by the method described by Finney[4]. The mixed concentrations of LAS and copper in the toxicity ratios of 1:1, 1:2 and 2:1 were calculated from the 96-h LC_{50} values obtained above using the formula described by Sprague and Ramsay[5]. Hence, the mixed concentrations used were 0.1, 0.3, 0.5, 0.7 and 0.9 toxic units, and their 96-h LC_{50} values, 95% confidence intervals and slopes of probit lines were determined using the same method as above.

Ten fingerlings from the acclimatization tanks were placed in each experimental container using a stratified randomization method[6]. Each experiment consisted of 5 concentrations and a control, and was replicated 3 times. During the bioassay test, continuous aeration was provided to maintain adequate dissolved oxygen level without excessive turbulence in the containers. Fish under experimentation and 2 days prior to exposure were not fed.

The test solutions were replenished 48 h after exposure by siphoning. Analyses for dissolved oxygen, pH, alkalinity, hardness, copper and surfactant were determined on each test initially, before replenishing, and at the end of the test period.

Results

The acute toxicity of LAS on *P. gonionotus* at 4 different exposure times is shown in Table 1 and Fig. 1. Mortality rates and toxicities increased with increasing concentrations and exposure times. The 96-h LC_{50} value was 5.88 mg/l MBAS. The LC_{50} values at various exposure times did not differ significantly. The fish began to die within 3 h at the concentration of 6.5 or 6.175 mg/l MBAS. After the 24-h exposure period, most of the survived fish regained their balance and recovered. The response of fish to LAS was indicated by the sharpness of the toxicity curve and the slope of probit, indicating that the toxicity of LAS on fish was acute.

The initial sign of distress was inflammation of the area around the gills. The fish showed a change in their behaviour shortly after they were exposed to the toxicant. Normal active behaviour ceased and general movement increased. They swam up and down actively. Their respiration rate also increased rapidly. Finally, they came to the surface where they gulped air regardless of dissolved oxygen concentration and became less active. Then, they lost balance and swam in an uncoordinated manner, sank to the bottom of the containers and died with raised gill covers and open mouths. Mucilaginous material was extruded from the gills.

The acute toxicity of copper on *P. gonionotus* at 4 different exposure times is shown in Table 2 and Fig. 2. Mortality rates and toxicities increased with increasing concentrations and exposure times. The 96-h LC_{50} value was 91.96 μg/l. The LC_{50} values at various exposure times did not differ significantly. The fish began to die within 6 h at the concentration of 168.0 μg/l. The fish exposed to the toxicant became alarmed and moved quickly without direction. The respiration rate was observed to be higher than that of the control fish. The fish began to lose their balance, flipped over, stopped swimming, sank to the bottom of the containers and died with their mouths open, fin spread and gills darkened with red colour.

The acute toxicities of the mixtures of LAS and copper on *P. gonionotus* at 4 different exposure times are shown in Table 3 and Fig. 3. Mortality rates and toxicities

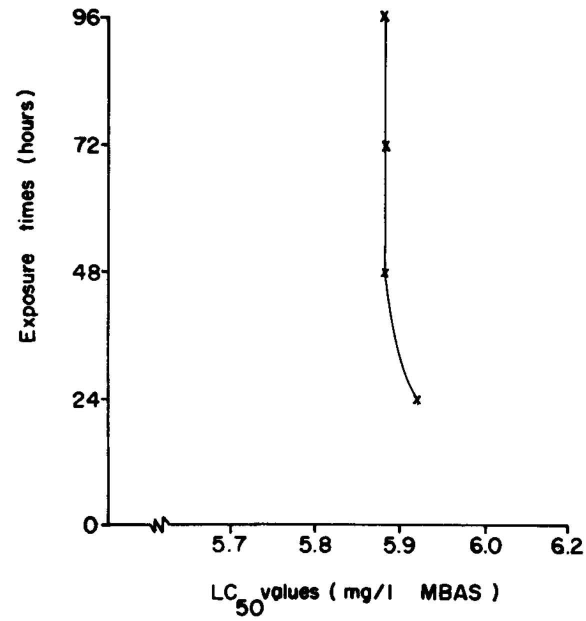

Figure 1. Toxicity curve of LAS on *P. gonionotus*

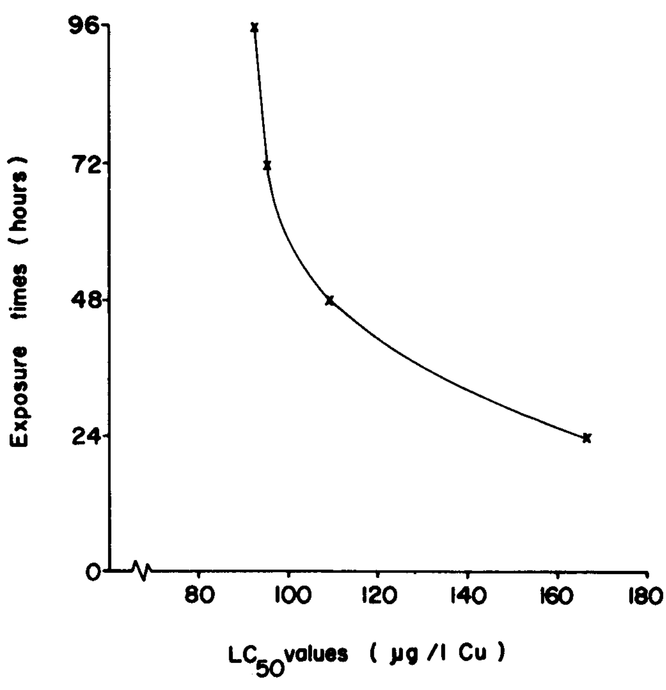

Figure 2. Toxicity curve of copper on *P. gonionotus*

TABLE 1 ACUTE TOXICITY OF LAS ON THE FINGERLING STAGE OF *P. GONIONOTUS* AT DIFFERENT EXPOSURE TIMES.

LAS concentrations (mg/l MBAS)	Mortality rates (%) of *P. gonionotus*			
	24 h	48 h	72 h	96 h
5.070	10.00	10.00	10.00	10.00
5.395	13.33	13.33	13.33	13.33
5.785	33.33	36.67	36.67	36.67
6.175	63.33	63.33	63.33	63.33
6.500	90.00	96.67	96.67	96.67
LC_{50} values	5.92	5.88	5.88	5.88
95% confidence limits	5.71-6.15	5.68-6.08	5.68-6.08	5.68-6.08
Slopes of probit lines	24.13	26.22	26.22	26.22

TABLE 2 ACUTE TOXICITY OF COPPER ON THE FINGERLING STAGE OF *P. GONIONOTUS* AT DIFFERENT EXPOSURE TIMES.

Cu concentrations (μg/l)	Mortality rates (%) of *P. gonionotus*			
	24 h	48 h	72 h	96 h
75.0	0	13.33	23.33	26.67
92.0	3.33	40.00	53.33	53.33
112.0	13.33	53.33	70.00	73.33
135.0	20.00	60.00	73.33	80.00
168.0	53.33	93.33	96.67	96.67
LC_{50} values	166.18	109.05	94.82	91.96
95% confidence limits	121.60-227.10	96.57-123.14	82.27-109.98	79.45-106.45
Slopes of probit lines	7.41	6.18	6.07	6.31

TABLE 3 ACUTE TOXICITIES OF THE MIXTURE OF LAS AND COPPER ON *P. GONIONOTUS* AT VARIOUS TOXICITY RATIOS.

Toxicity ratios	96-h LC_{50} (toxic units)	95% confidence intervals	Slopes of probit lines
1:1	0.53	0.35-0.80	2.02
2:1	0.49	0.32-0.77	1.76
1:2	0.36	0.26-0.51	1.98

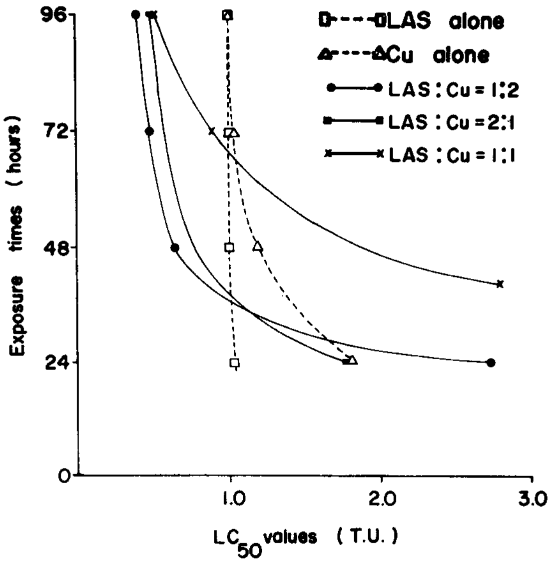

Figure 3. Toxicity curves to *P. gonionotus* of LAS alone, copper alone, and mixtures of various toxicity ratios.

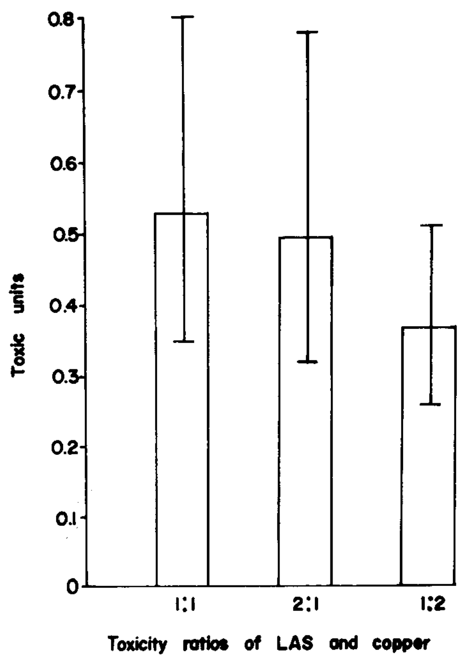

Figure 4. The 96-h LC_{50} values and 95% confidence limits of LAS-copper mixtures to *P. gonionotus*

increased with increasing concentrations and exposure times. The 96-h LC_{50}values were 0.53, 0.49 and 0.36 toxic units for 1:1, 2:1 and 1:2 toxicity ratios, respectively. At the 96-h exposure period, it was found that the LC_{50} values of the LAS-copper mixtures in each ratio did not differ significantly ($P > 0.05$, t-test : $t = 0.225$ for 1:1 and 1:2, $t = 0.258$ for 1:1 and 2:1, and $t = 0.605$ for 1:2 and 2:1 toxicity ratios). In addition, the results show that the 95% confidence intervals of the toxicity ratios overlapped each other (Fig. 4), that the slope of probit line of each toxicity ratio varied slightly, and that the mode of action of each toxicity ratio was different depending on the influence of the proportion of the components.

Discussion

This investigation has shown that LAS demonstrated a fairly narrow range of toxicity, indicating that the toxic property of LAS was manifested within the first 24 h of exposure[7]. Bardach *et al.*[8] reported that acute toxicity of surfactant was confined to the gill and epidermis of *Ictalurus natalis,* and Cairns and Scheier[9] found that LAS damaged the gill of sunfish. Surfactants are toxic by lowering the surface tension, thus causing an increase in cellular permeability and rendering a greater penetration of both the surfactants and other substances[10]. LAS also acts as solvent, thus causing cell membranes of the fish to be susceptible to damage, such as the lifting away of the gill epithelium from the underlying tissue and the invasion of lymphocytes and granulocytes into the subepithelial spaces. The surfactants, which were adsorbed on the surface of the fish gills increased the number of red blood cells causing multiple hematomas to develop on the gill tissue and the respiratory folds of the gills. These resulted in diminished oxygen uptake, which might be accompanied by a similar impairment of salt balance and of metabolism of urea[11]. Hassler *et al.*[12] and Shinichi[11] reported that the toxicity of surfactant might be due to the formation of a surfactant-protein complex in gills.

In the case of copper, the toxicity increased with increasing concentration and exposure time. Lloyd[13] and Skidmore and Tovelle[14] found that copper damaged the epithelial cell of the gill and lessened its efficiency. Seller *et al.*[15] reported that mucous secretion and limitation of gas exchange were the cause of less diffusion of oxygen into the blood of fish. These caused tissue hypoxaemia which was associated with a greater respiratory demand and an increase in ventilation rate[17]. Thus, there was a change in ventilatory volume and, subsequently, an increase in the rate at which copper passed over the gills. Cremlyn[18] found that copper inhibited acetylcholinesterase in the fish.

The toxicity curves in Figs.1 and 2 show that the slope of LAS curve was steeper than that of copper, indicating that the fish was more sensitive to LAS than to copper.

This investigation has also shown that the mixtures of LAS and copper were more toxic than any of the single toxic substances, and that the interaction exhibited more-than-additive effect (Table 3, Fig. 3). The mode of action of 1:1 and 1:2 toxicity ratios was similar to copper alone, and 2:1 ratio to LAS alone. The 96-h LC_{50} values indicated that the toxicity of 1:2 ratio was highest, followed by 2:1 and 1:1 ratios. Calamari and Marchetti[2] found that the mixture of LAS-copper exerted a more-than-additive effect. Bolle *et al.*[19] observed that the toxicity of alkylbenzene sulphonate (ABS) could be increased by simply raising the concentration of Ca^{2+}, Mg^{2+} or Ba^{2+}, and this could be applied to copper and LAS. Surfactant-metal-surfactant compounds could be formed in which the metal substitutes for Na in LAS and ABS molecules. The toxico-dynamic process of the mixture of LAS and copper probably involves enzymatic reactions as described by Solon *et al.*[20] for the mixture of LAS and pesticides. Copper in the mixture could bind to free sulhydryl groups of proteins and inhibit acetylcholinesterase, and could induce lesion at the lateral line epithelium and kidney[20]. The mixture of LAS and copper also caused histological damage similar to the acute heavy metal poisoning and LAS alone, such as damaging the gill epithelium, thickening of the lamellae which was caused by both the reduction of the surface tension by the presence of LAS and copper[20].

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บทคัดย่อ

ความเป็นพิษเฉียบพลันของสารลดแรงตึงผิว Linear alkylbenzene sulphonate (LAS) และทองแดง ต่อปลาตะเพียนขาวมีความแตกต่างกัน ค่า LC_{50} ที่ 96 ชม. ของ LAS มีค่า 5.88 (5.68-6.08) มก/ ล MBAS และของทองแดงมีค่า 91.96 (79.45-106.45) ไมโครกรัม/ ลิตร ตามลำดับ โดยที่ความเป็นพิษของทองแดงจะเพิ่มขึ้นเมื่อความเข้มข้นและเวลาเพิ่มขึ้น แต่สำหรับ LAS ความเป็นพิษจะเพิ่มขึ้นเมื่อความเข้มข้นเพิ่มขึ้นในช่วงเวลาเริ่มต้นของการทดลองเท่านั้น หลังจากนั้นก็จะเริ่มมีความต้านทานต่อ LAS สารผสมของ LAS และทองแดงนั้นจะเสริมพิษซึ่งกันและกันทุกอัตราส่วนความเป็นพิษ โดยที่ค่า LC_{50} ที่ 96 ชม. มีค่า 0.53 (0.35-0.80), 0.36 (0.24-0.55) และ 0.49 (0.32-0.77) toxic units สำหรับอัตราส่วนความเป็นพิษ 1:1, 1:2 และ 2:1 ตามลำดับ ซึ่งเป็นการเสริมพิษแบบ more-than-additive- และความเป็นพิษเฉียบพลันของแต่ละอัตราส่วนความเป็นพิษไม่แตกต่างกันอย่างมีนัยสำคัญ แต่การแสดงความเป็นพิษ- (mode of action) ของแต่ละอัตราส่วนความเป็นพิษต่อปลาจะขึ้นอยู่กับสัดส่วนของสาร LAS และทองแดงในสารผสมนั้น