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EFFECTS OF WATER HARDNESS AND TEMPERATURE ON THE TOXICITY OF DETERGENTS TO THE FRESHWATER FISH, *PUNTIUS GONIONOTUS*, BLEEKER.

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Abstract

The toxicity of detergents to fish varied considerably according to the hardness of the water in which the fish were treated. The 96-hr. LC_{50} values of soft detergent were, respectively, 13.6, 11.8 and 11.4 mg/l products in soft, moderate and hard waters, whereas the 96-hr. LC_{50} values of hard detergent were, respectively, 24.7, 13.6 and 10.5 mg/l products in soft, moderate and hard waters. The detergent was more toxic in hard water than in soft water. Increasing water temperature increased slightly the toxicity of detergent to fish. At 28° and 35° C, the 96-hr. LC_{50} values of soft detergent were, respectively, 11.8 and 11.5 mg/l products, and of hard detergent were, respectively, 13.6 and 12.1 mg/l products.

Introduction

It is now recognized that water pollution is an important problem which becomes more widespread and serious as population and industry expand, and that something must be done if aquatic life resources are to be saved and their productivity to be maintained.

Detergents are pollutants commonly found in natural waterways. Anionic detergents are the most studied group since they are the most widely used and represent the major source of detergent pollution. The most common anionic detergents are the alkyl aryl sulphonates such as alkyl benzene sulphonate (ABS) and alkyl sulphates. An ABS with unbranched hydrocarbon chain is usually referred to as linear alkylate sulphonate (LAS)[1]. Since 1965, most detergent manufacturers have used "soft" biodegradable LAS surfactant in their formulations.

Two components of detergents are of particular interest as water pollutants: a surface active agent and a phosphate builder. Surfactants appear to be the primary toxic component in the formulation[2], and LAS has been demonstrated to be two or four times more toxic than the old ABS compound[3-5]. The removal of LAS by biodegradation is accompanied by a reduction in toxicity without accumulation of toxic intermediates[6]. The phosphate builders are a significant source of phosphorus in streams and lakes and increase algal and weed growth. The switch to degradable detergents has had no appreciable effect on this aspect of the detergent problem[7].

In the evaluation by bioassay of the toxicity of specific materials to fish, there will be physical and chemical factors of the aquatic environment, as well as physiological mechanism of the living system, that need to be considered as influencing factors[8].

The toxicity of detergents has been variously reported to increase, decrease or be unaffected by altering the level of water hardness[1]. Sprague[9] stated that it is important to investigate effects of water temperature on the toxicity of detergents to determine lethal threshold concentrations, but information on the influence of the temperature is scarce.

The purpose of this study was to determine effects of water hardness and temperature on the toxicity of soft and hard detergents to the freshwater fish by means of short-term bioassay studies, and it is hoped that this study may help to evaluate the effects of these detergents changeover on the aquatic population in streams where detergents have been or are problem.

Materials and Methods

Either sex of the fingerling of *Puntius gonionotus* Bleeker, with a mean weight of 0.3 g and a mean length of 2.4 cm, were held in aerated local tap water. Water qualities of the tap water were as follows : total hardness as $CaCO_3$ 104 ± 8.0 mg/1, total alkalinity 110 ± 8.4 mg/l, dissolved oxygen 7.2 ± 0.3 mg/l (measured by oxygen meter, YSI model 54 A), pH 8.0 ± 0.6, water temperature 28°±1.5° C, and surfactant 0 mg/l.

Water hardness (total hardness equivalent to mg/l $CaCO_3$) was found by measuring the total concentration of bivalent cations using the Ethylene-diaminetetraacetic Acid Method (EDTA)[10]. Soft water was obtained by mixing 50 parts of distilled water and 50 parts of tap water, and hard water was obtained by adding 192.0 mg/l $NaHCO_3$, 120.0 mg/l $CaSO_4 2H_2O$, 120.0 mg/l $MgSO_4$ and 8.0 mg/l KCl to tap water[11]. These waters were aerated vigorously for about 2 days to bring them into equilibrium with the atmospheric gases before use[2]. The average hardness concentrations of dilution waters were 50, 110 and 260 mg/l $CaCO_3$.

Two water temperatures, 28° and 35° C, were selected and were maintained by means of a constant temperature waterbath in which the test vessels were immersed[12].

Batches of fish from holding tanks were acclimatized for at least 7 days in test water condition[13]. The increase or reduction in water hardness was achieved by gradual mixing and the increase of water temperature was made at the rate of 2° C per day.

The detergents used were household powder detergents of LAS (soft detergent) and ABS (hard detergent) types. The approximate surfactant levels of soft and hard detergents were 18% and 20%, as measured by Methylene Blue Active Substance Method[10], and the test solutions were replenished 48 hours after exposure by siphoning.

Five fingerlings from the acclimatization tank were placed in each experimental container using a stratified randomization method[14]. Each experiment consisted of 5 concentrations and a control, and was replicated 4 times. During the bioassay test, continuous aeration was provided to maintain adequate dissolved oxygen level without excessive turbulence in the container[15, 16]. Fish under experimentation and 2 days prior to exposure were not fed.

Analyses for dissolved oxygen, pH and alkalinity were determined on each test initially and at the end of the test period. The 96-hr. LC_{50}, its 95% confidence intervals and slopes of probit lines were determined for each experiment by the method described by Finney[17].

Results

The acute toxicity of detergents on *P. gonionotus* at 3 levels of water hardness is shown in Table 1, and the mortality rates of *P. gonionotus* exposed to various concentrations of detergents at 3 levels of water hardness for 96 hours are shown in Figs. 1 and 2. The 96-hr. LC_{50} values of soft detergent in soft, moderate and hard waters were 13.6, 11.8 and 11.4 mg/l product respectively, and of hard detergent in soft, moderate and hard waters were 24.7, 13.6 and 10.5 mg/l product respectively.

The acute toxicity of detergents on *P. gonionotus* at 2 different water temperatures is shown in Table 2, and the mortality rates of *P. gonionotus* exposed to various concentrations of detergents at 2 different water temperatures for 96 hours are shown in Figs. 3 and 4. The 96-hr. LC_{50} values of soft detergent to the fish were 11.8 and 11.5 mg/l product, and of hard detergent to the fish were 13.6 and 12.1 mg/l product at water temperatures of 28° and 35° C respectively.

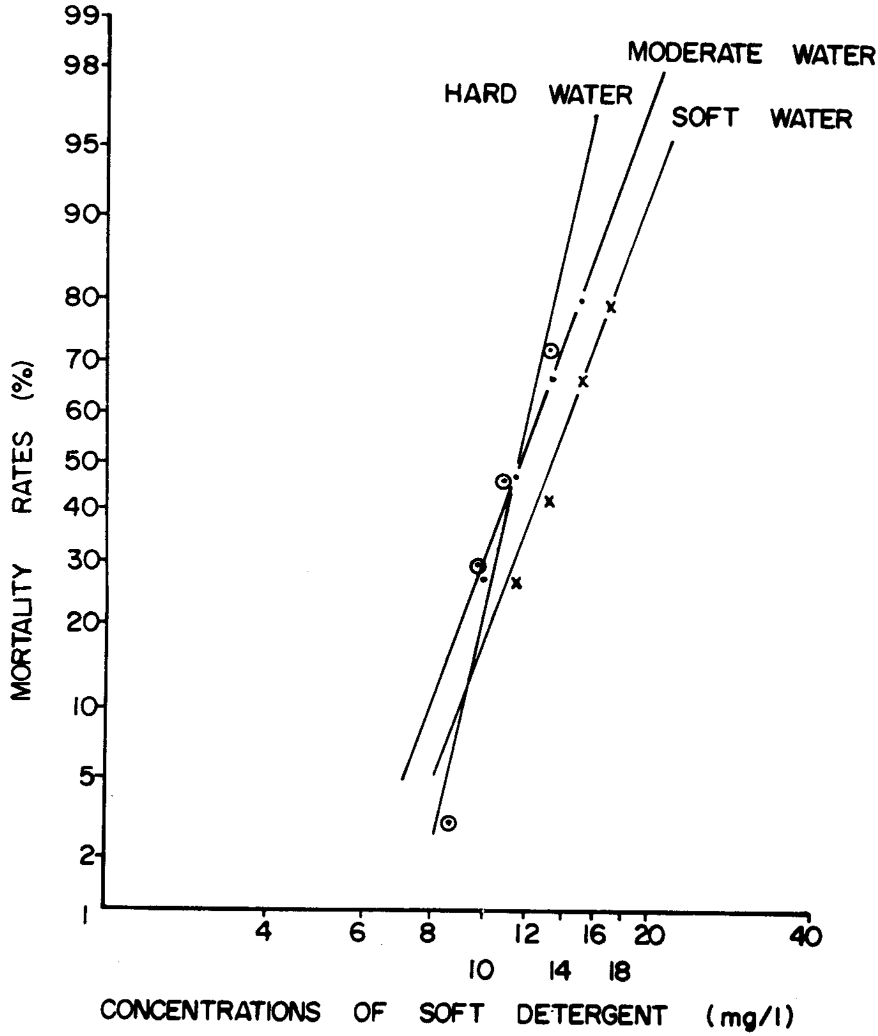

Figure 1. Mortality rates of *P. gonionotus* exposed to various concentrations of soft detergent at 3 levels of water hardness for 96 hours.

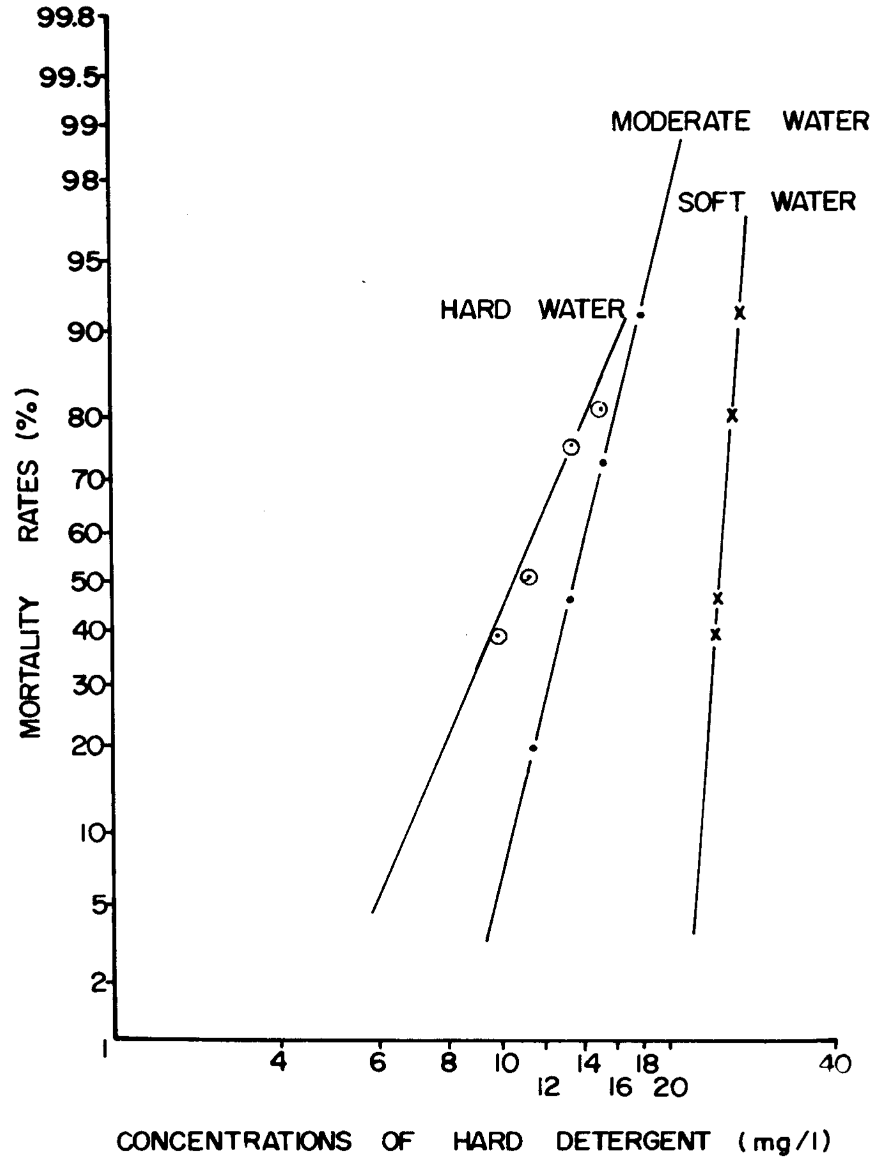

Figure 2. Mortality rates of *P. gonionotus* exposed to various concentrations of hard detergent at 3 levels of water hardness for 96 hours.

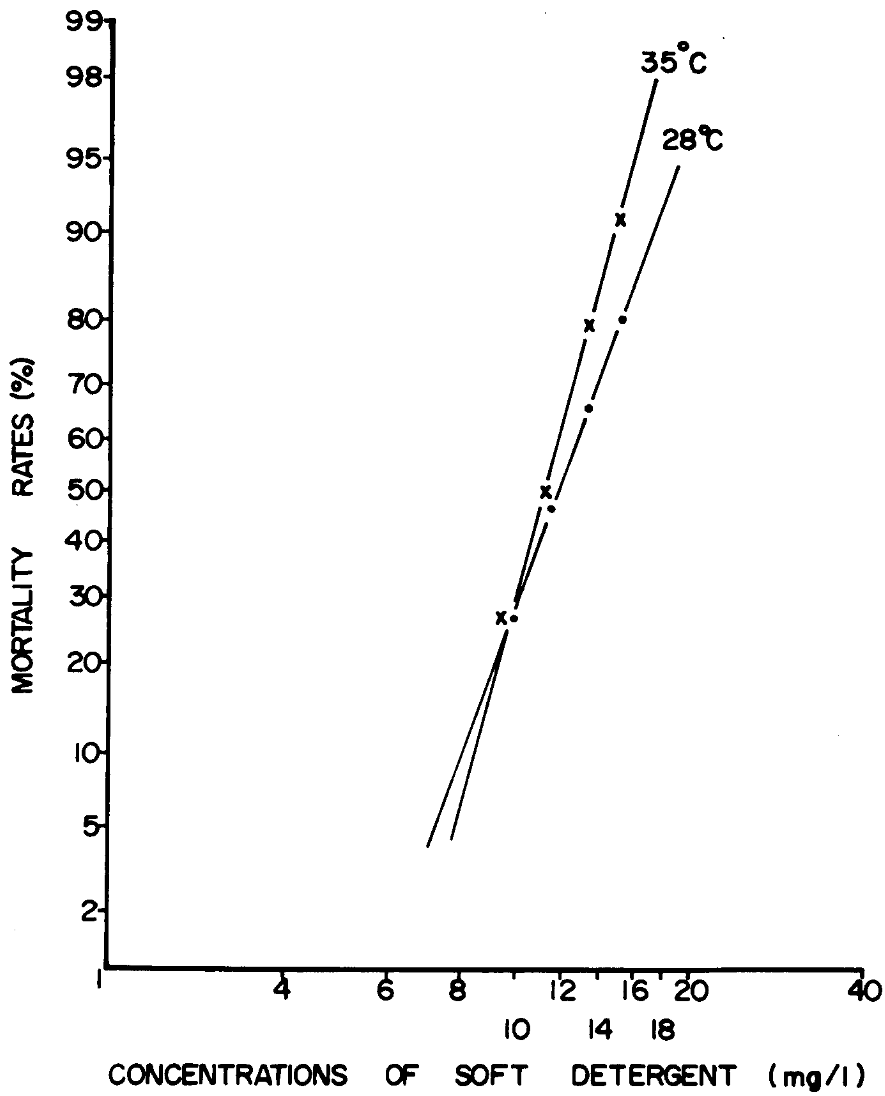

Figure 3. Mortality rates of *P. gonionotus* exposed to various concentrations of soft detergent at 2 different water temperatures for 96 hours.

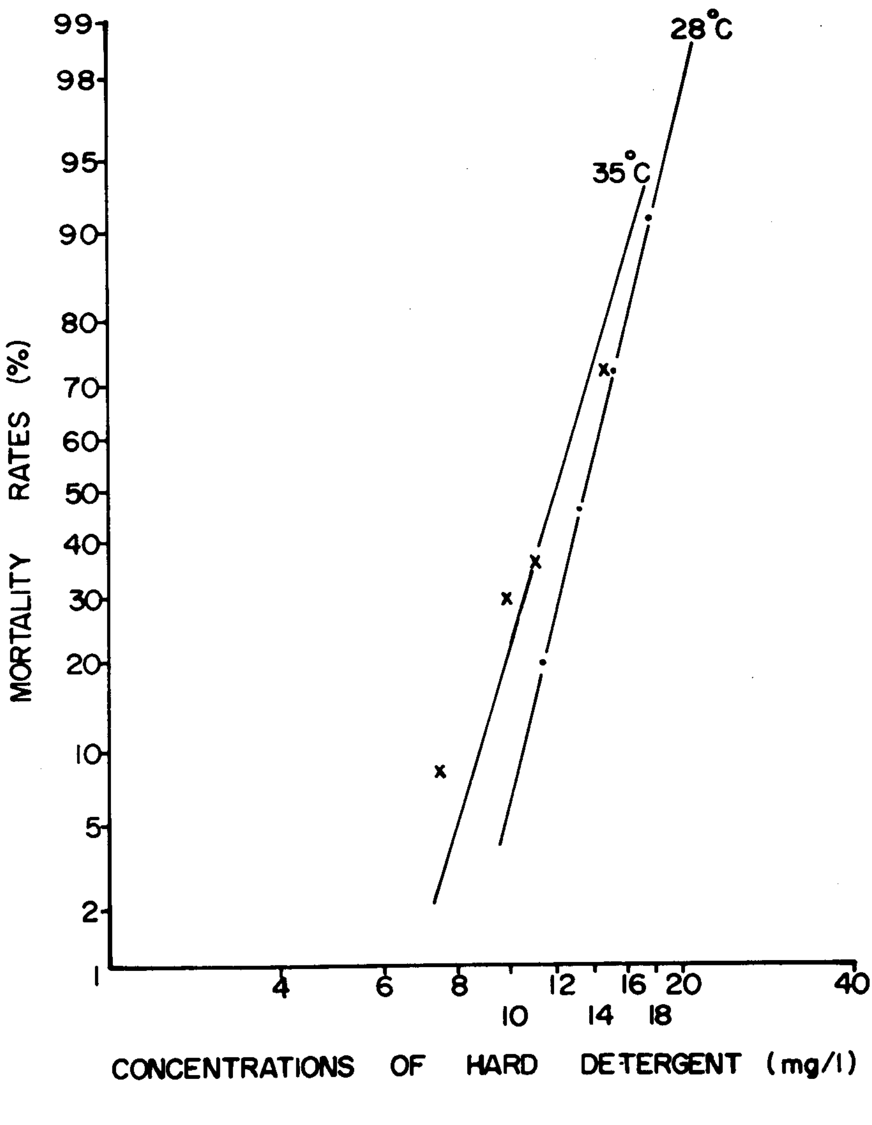

Figure 4. Mortality rates of *P. gonionotus* exposed to various concentrations of hard detergent at 2 different water temperatures for 96 hours.

TABLE 1. ACUTE TOXICITY OF DETERGENTS ON *P. GONIONOTUS* AT 3 LEVELS OF WATER HARDNESS.

Detergents	Water hardness levels	96-hr. LC_{50} (mg/l)	95% confidence intervals	Slopes of probit lines
Soft detergent	Soft	13.6	12.94 - 14.30	10.97
	Moderate	11.8	11.13 - 12.61	9.02
	Hard	11.4	10.76 - 12.11	10.55
Hard detergent	Soft	24.7	24.70 - 24.71	38.09
	Moderate	13.6	12.96 - 14.35	12.17
	Hard	10.5	9.10 - 12.02	5.89

TABLE 2. ACUTE TOXICITY OF DETERGENTS ON *P. GONIONOTUS* AT 2 DIFFERENT WATER TEMPERATURES.

Detergents	Water temperatures (° C)	96-hr. LC_{50} (mg/l)	95% confidence intervals	Slopes of probit lines
Soft detergent	28	11.8	11.13 - 12.61	9.02
	35	11.5	10.94 - 12.11	10.95
Hard detergent	28	13.6	12.96 - 14.35	12.17
	35	12.1	11.27 - 12.91	7.72

Discussion

This investigation has shown that the toxicity of detergents to fish was affected by water hardness, and was more pronounced in hard water than in soft water. Henderson et al[2]. found that alkyl benzene sulphonate (ABS) was more toxic to fathead minnow in hard water, sodium alkyl sulphate was more toxic in soft water, and a nonionic ethoxylate was unaffected. Hokanson and Smith[18] reported that linear alkylate sulphonate (LAS) was significantly less toxic to the bluegill in soft water compared to hard water. Tovell et al[13]. recently showed that the anionic detergent, sodium lauryl sulfate (SLS) was more acutely toxic to goldfish and rainbow trout in hard water than in soft water.

The present study shows that in soft water, soft detergent was significantly more toxic than hard detergent (Table 1). Fish in hard water absorbed more anionic detergent due to an acute change in the permeability of certain tissues (gill in particular), or to a change in the availability of detergent for absorption due to some interaction with other dissolved constituents (Ca^{2+} in particular)[13]. The presence of Ca^{2+} (or other divalent cations) did not affect the toxic response to detergent by some action on the diffusion membranes of the gill, instead the toxic response relies on an interaction between the ions in the solution and the detergent which in turn modifies the solubility and/or diffusion characteristic of the detergent itself.

Marchetti[19], treating *Salmo gairdneri* with anionic detergents, found a numerical increase of chloride cells, enlargement of single cells, and enhanced activity documented by the numerical increase of mitochondria and the swelling of the tubules of the smooth endoplasmic reticulum. The increase toxicity of ABS in the presence of Ca^{2+} (or other divalent cations) could be due to the formation of ABS-Ca-ABS by replacement of sodium from Na-ABS.

Bioassay studies of temperature influence in this investigation indicated that increasing water temperature slightly increased the toxicity of detergent. Marchetti[19] found that a significant change in threshold concentration occurred with increasing temperature. High temperature is usually assumed to cause a pollutant to be more toxic[9], and resistance to disease becomes lower[16]. The sensitivity of fish to toxic substances and disease increases at high temperature, basically due to decrease in oxygen concentration. More water must be passed over the gills and, therefore, more toxic material passes over the permeable membrane[20].

Some variation in effect of water hardness or temperature would be expected due to different species, type of detergent (particularly surfactant and builders). The immediate cause of death from acute detergent poisoning where extensive gill damage occurs is likely to be either asphyxiation or loss of osmotic or ionic stability. Abel and Skidmore[21] showed four main signs of damage in fish gills exposed to anionic detergent.

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บทคัดย่อ

ความเป็นพิษของผงซักฟอกต่อปลาในน้ำที่มีความกระด้างระดับต่าง ๆ แตกต่างกัน พบว่าค่า LC_{50} ที่ 96 ชั่วโมง ของผงซักฟอกชนิด soft detergent มีค่า 13.6, 11.8 และ 11.4 มก/ล ในน้ำอ่อน น้ำค่อนข้างกระด้างและน้ำกระด้าง ตามลำดับ ค่า LC_{50} ที่ 96 ชั่วโมง ของผงซักฟอกชนิด hard detergent มีค่า 24.7, 13.6 และ 10.5 มก/ล ในน้ำอ่อน น้ำค่อนข้างกระด้าง และน้ำกระด้าง ตามลำดับ ความเป็นพิษของผงซักฟอกในน้ำกระด้างมีพิษมากกว่าในน้ำอ่อน เมื่ออุณหภูมิเพิ่มขึ้นความเป็นพิษของผงซักฟอกที่มีต่อปลาก็จะเพิ่มขึ้นด้วย พบว่าที่อุณหภูมิ 28° และ 35°ซ ค่า LC_{50} ที่ 96 ชั่วโมง ของผงซักฟอกชนิด soft detergent มีค่า 11.8 และ 11.5 มก/ล ตามลำดับ และของผงซักฟอกชนิด hard detergent มีค่า 13.6 และ 12.1 มก/ล ตามลำดับ