Interactive Effect of Chromium Compounds and a Fungal Parasite on Carp Eggs[1,2]

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ABSTRACT

A fungal parasite (Saprolegniales) of carp eggs was exposed to hexavalent and trivalent chromium compounds at concentrations identical to those used in a study of the effects of the same compounds on carp egg hatchability. Comparison of data from both experiments revealed that increased egg mortality and increased fungal growth were coupled at low concentrations of the compounds. A mechanism for the interaction of a chemical stress and a biological stress on carp egg survival in natural systems is presented.

INTRODUCTION

Chromium compounds are important industrial chemicals and are used extensively as corrosion inhibitors in cooling towers operation. These compounds may enter the environment in cooling tower drift (TAYLOR et al. 1974), or they may be discharged directly into aquatic environments in chromium-treated cooling water. Considerable data exists on the acute and long-term toxicities of chromium compounds to aquatic organisms (McKEE and WOLF 1963; BECKER and THATCHER 1973). These reviews disclose that chromium toxicity and effects are dependent on several variables such as the organism, temperature, water quality and water hardness, and pH.

White Oak Lake (WOL), a holding pond for laboratory wastes on the ERDA Reservation at Oak Ridge, Tennessee, receives processed chromium-treated cooling water (as well as other chemical and sanitary waste discharges). Studies of WOL fish populations have disclosed a high incidence of gross physical abnormalities. The abnormalities have been correlated with environmental conditions such as temperature, oxygen concentration, water chemistry, and radiation during the ontogeny of the organisms (AUERBACH 1974).

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Biotic conditions such as hormone imbalance, genetic factors, and parasitism may also account for the abnormalities; however, their influence is difficult to evaluate.

TRABALKA (1974) incubated fertilized carp eggs in water containing hexavalent (sodium chromate) or trivalent (chromium acetate and chromium chloride) chromium at concentrations ranging from 0.10 ppm to 30.0 ppm. Eggs exposed to hexavalent chromium from 1.0-30.0 ppm demonstrated significantly greater hatch (99.5-100.0%) than controls (98.6%; $P \leq .05$). On the other hand, eggs exposed to trivalent chromium demonstrated a LD_{50} response at concentrations greater than 3.0 ppm (Fig. 1). Data on proportional hatch of eggs exposed to hexavalent chromium indicated an interaction effect between chromium concentrations and environmental conditions or biotic factors. Direct light microscope observation of chromium-exposed eggs suggested that the other factor was a fungal parasite. It was postulated that low concentrations of some chromium compounds stimulated fungal growth, which would be detrimental to carp eggs. This report tests the hypothesis and suggests a mechanism responsible for the interaction in natural environments.

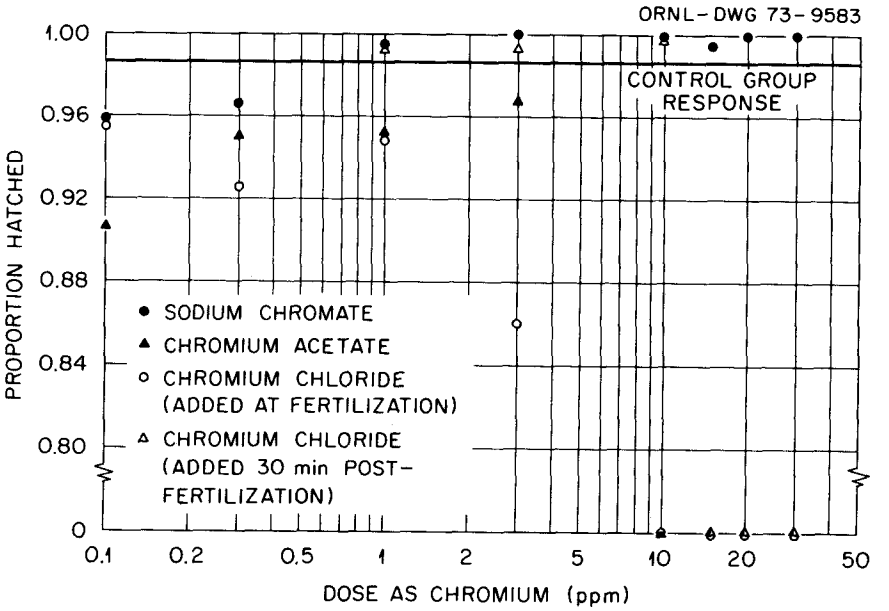

Fig. 1. Hatchability of carp eggs exposed to chromium during embryonic development.

MATERIALS AND METHODS

Light microscope observation of the fungal parasite, freshly collected from carp eggs, disclosed its natural association with

aquatic bacteria, algae, and protozoa (hereafter referred to as hyposphere microorganisms[1]). To simplify determination of the role of the fungus in carp egg survival, the fungus was isolated and maintained in axenic culture.[2] The fungal cultures provided inocula for the determination of hexavalent and trivalent chromium effect on fungal biomass or growth.

A modified liquid medium[3] was chosen to support the growth of the facultative parasite, in the presence of chromium compounds. Ten milliliters of either sodium chromate, chromium acetate, or chromium chloride (in .001 M sodium citrate stock solutions) were added to 90.0 ml of modified growth medium, in 250.0 ml flasks. Stock solutions were formulated to provide chromium concentrations of 0, 0.10, 0.30, 1.0, 3.0, 10.0, 15.0, 20.0, and 30.0 ppm. Each treatment was run in triplicate.

Uniform fungal inocula were obtained by homogenization of stock fungi grown in the liquid culture. One milliliter of the homogenate was introduced into each flask and cultures were agitated continuously for six days at 26 C. All procedures were carried out under aseptic conditions. On the sixth day, all growth medium and fungal biomass within each flask was passed on to a preweighed filter paper disc (Watman #40 Ashless) with suction. Discs, and the adherent fungal biomass were rinsed with distilled water and allowed to air dry for 48 hours. Filter paper discs, with adherent fungi, were then reweighed with fungal biomass as the parameter measured.

RESULTS

Data were analyzed with one-way analyses of variance to determine significant differences in fungal response to each chromium compound at each concentration. Identification of treatments significantly different ($P < .05$) from the control treatments was made using Duncan's Multiple Range Test.

Sodium chromate significantly inhibited fungal growth at all concentrations, except 0.30 and 0.10 ppm. At these concentrations fungal growth was stimulated; the stimulatory effect occurring at

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[1]Microorganisms inhabiting the area immediate to fungal hyphae; for senescent hyphae, the hyphal interior is included.

[2]Czapek-Dox Agar: Sucrose, 30.0 g; $NaNO_3$, 2.0 g; K_2HPO_4, 0.1 g; KCl, 0.5 g; $MgSO_4 \cdot 7H_2O$, 0.5 g; $FeSO_4$, trace; agar, 15.0 g; distilled water, 1.0 l.

[3]Czapek-Dox Liquid Medium: Sucrose, 30.0 g; $NaNO_3$, 2.0 g; K_2HPO4, 0.1 g; KCl, p.5 g; $MgSO_4 \cdot 7H_2O$, 0.5 g; $FeSO_4$, trace; spring water, 1.0 l.

0.10 ppm was significantly different from the control (Fig. 2a). Fungal growth was stimulated by the three lowest concentrations of chromium acetate. However, only at 0.10 ppm was growth significantly greater than the control. Growth was inhibited at 20.0 and 30.0 pp, chromium acetate, and significantly lower than the controls at 3.0, 10.0 and 15.0 ppm (Fig. 2b). Chromium chloride concentrations from 0.10 to 3.0 ppm caused significant stimulation of fungal biomass; concentrations of 10.0, 15.0, 20.0, and 30.0 ppm significantly inhibited growth when compared to controls (Fig. 2c).

DISCUSSION

Comparison of data on carp egg mortality (Fig. 1) and on fungal growth response indicate that increased egg mortality and increased fungal growth success were coupled at low concentrations of hexavalent and trivalent chromium. The interactive effect was most evident in the responses of carp eggs and fungi to hexavalent sodium chromate; where fungal growth was effectively limited (sodium chromate concentrations: 1.0-30.0 ppm) egg hatchability exceeded the control response (TABLE I). Data for trivalent chromium acetate effects demonstrated that with decreasing chromium acetate concentration there was a decrease in egg hatchability and a corresponding increase in fungal biomass. Results from chromium chloride treated carp eggs and fungi support the trends described.

Chemical toxicity tests, due to their design, infrequently demonstrate, or account for, the action of several stresses on test organisms. Clear demonstration of the interaction of a chemical stress and a biological stress is rare due to problems encountered in separate analysis of the effect of each stress in the interactive system. The observations presented here indicate a complex interaction between chromium compounds and a parasitic fungus on carp egg survival. Low levels of chromium compounds may have a direct stimulatory effect on fungal growth potential and the success of parasitemia. Studies of chromium toxicity to aquatic bacteria, algae, protozoa, and fungi have shown that fungi are the least sensitive to the action of chromium compounds (BUI et al. 1971; POON and BHAYANI 1971; SADO and AIBA 1971). Direct stimulation is an adequate explanation for fungi grown and tested in axenic culture; however, it inadequately accounts for occurrences in complexly interactive natural environments.

A suggested mechanism for the interaction involves the carp egg, the fungus and the hyposphere microorganisms associated with it, and chromium compounds. The fungal hyposphere provides a habitat rich in exudates capable of supporting numerous bacteria and microbial grazers (BIRKINSHAW 1965). These natural inhabitants of the hyposphere may also function as stresses limiting growth of the fungal parasite. Low concentrations of hexavalent and trivalent chromium limit the growth of the relatively chromium-sensitive hyposphere microorganisms, thereby removing a fungal

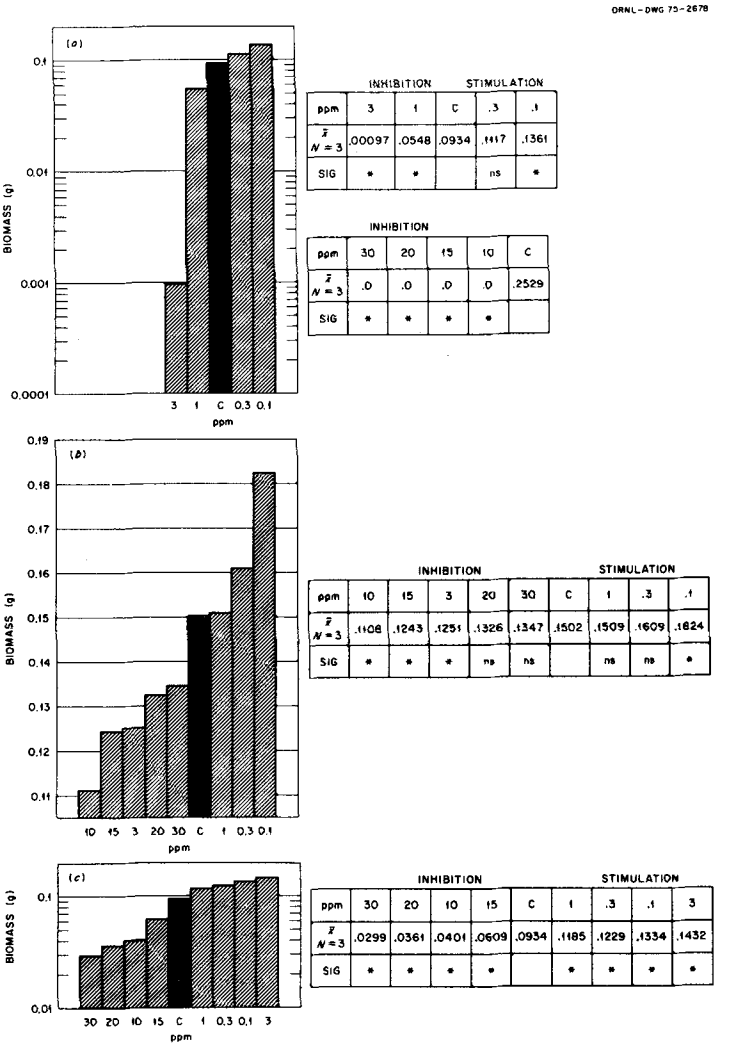

Fig. 2. Effect of sodium chromate (a), chromium acetate (b), and chromium chloride (c) on biomass (g dry wt) of a parasitic fungus. Progressively increasing inhibition is shown to the left of the control response; while, progressively increasing stimulation is shown to the right.

*denotes significance of $P \leq 0.05$.

growth inhibitor. Such removal may initiate lush fungal growth in the presence of appropriate substrates such as carp eggs. The high concentrations of chromium used in this study, especially of sodium chromate, limit growth of both fungus and hyposphere microorganisms; this effectively diminishes the potential for establishment of the fungal parasite on carp eggs.

TABLE I

Effect of sodium chromate, chromium acetate, and chromium chloride on carp egg hatchability and growth of fungus

	Sodium chromate (hexavalent)		Chromium acetate (trivalent)		Chromium chloride (trivalent)	
ppm	Carp egg	Fungus	Carp egg	Fungus	Carp egg	Fungus
0						
0.1	-	+	-	+	-	+
0.3	-	+	-	+	-	+
1	+	-	-	+	-	+
3	+	-	-	-	-	+
10	+	-	∞	-	∞	-
15	+	-	∞	-	∞	-
20	+	-	∞	-	∞	-
30	+	-	∞	-	∞	-

+Stimulation of hatchability (carp egg) or growth (fungus) as compared to control response.

-Inhibition of hatchability or growth as compared to control response.

∞Mortality.

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