Prediction of Incipient Lethal Levels of Copper to Juvenile Atlantic Salmon in the Presence of Humic Acid by Cupric Electrode

by
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Humic acid increases the incipient lethal level (ILL)* of copper by binding cupric ions and does not affect the ILL of zinc to juvenile Atlantic salmon (*Salmo salar*). Measurements with a cupric ion selective electrode in conjunction with a known ILL at a given concentration of humic acid can be used to predict ILL's of copper to juvenile Atlantic salmon at different concentrations of humic acid.

The chelation of cupric ions by fulvic acid was studied in detail (SCHNITZER 1971, GAMBLE et al. 1970). Humic acid also strongly adsorbs cupric ions (RIEMER and TOTH 1970), and an analytical procedure was described for the determination of different cupric complexes, including those with humic acid, in natural waters (STIFF 1971). Experimental evidence indicates that humic acid decreases the acute toxicity of copper and zinc mixtures to fish (GRANDE 1966, ANON. 1971, WILDISH et al. 1971, COOK and COTE 1972). It has been suggested that a cupric ion selective electrode could be used to measure the acute toxicity of copper to fish (MONTGOMERY and STIFF 1971).

This paper deals with the toxicity of copper and zinc to juvenile Atlantic salmon in the presence of humic acid, and with predictions of ILL's of copper from the potential of a cupric ion selective electrode.

EXPERIMENTAL

Bioassay conditions. Juvenile Atlantic salmon, size range 8.8-9.8 cm were kept in running tap water (500 mℓ/min) in fiberglass tanks (32 ℓ, 5 or 10 fish) at 3.8-4.8°C. Constant head Mariotte bottles were used for copper or zinc sulfate and humic acid solutions, delivered at a rate of 0.7 and 3.5 mℓ/min, respectively. Water samples were analysed for copper or zinc and humic acid 3-4 times a day. Hardness of tap water was 14 mg/ℓ, expressed as calcium carbonate.

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*ILL is defined as "that level of the environmental entity beyond which 50% of the population cannot live for an indefinite time" (SPRAGUE 1969).

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Humic acid, technical, Aldrich Chemical Company, was dissolved by suspending in tap water and adjusting pH = 10-10.5 with 5N sodium hydroxide. After 15 min, pH was adjusted to 6 with concentrated hydrochloric acid and the solution was filtered through glass wool and diluted further with tap water as required.

Fulvic acid, supplied by Dr. M. Schnitzer, Canada Department of Agriculture, was well soluble in tap water.

Amberlite XAD-1 resin was used to remove humic acid from water samples (RILEY and TAYLOR 1969). A portion of the sample (300-500 mℓ was adjusted to pH = 2 with 1N sulfuric acid and passed (3 mℓ/min) through an Amberlite XAD-1 column (1.9 x 40 cm). The effluent was neutralized (pH = 6.8-7.0) with 1N sodium hydroxide. An equivalent amount of sodium sulfate was added to the untreated portion of the original sample. The column was regenerated by 0.2N potassium hydroxide and distilled water.

Chemical analyses. A Perkin-Elmer Model 303 Atomic Absorption Spectrophotometer, operated under routine conditions, was used to determine copper and zinc. Samples containing humic acid were acidified to pH = 1.8-2.0 with concentrated sulfuric acid before the determination. Humic acid was determined before the acidification using either a Beckman DK-2A, or a Coleman Junior 6D Spectrophotometer at 250 and 400 nm, respectively. Low concentrations of humic acid (<2 mg/ℓ) were determined fluorimetrically on a Perkin-Elmer MPF-2A Spectrofluorimeter at 452 nm on excitation at 270 nm. In all 3 cases standard solutions of Aldrich humic acid were used for calibration. The activity of cupric ions was determined by an Orion Cupric Ion Electrode Model 94-29, Model 90-02 Double Junction Reference Electrode and a Model 801 Digital pH/mV Meter. Known amounts of a copper sulfate solution (copper concentration 40 μg/mℓ) were added to a water sample (100 mℓ), stirred magnetically in a 250-mℓ beaker, and the electrode potential was plotted against the concentration of copper.

RESULTS AND DISCUSSION

The ILL of copper increases with increasing concentration of humic acid (Fig. 1). The potential of the cupric ion selective electrode is a linear function of the total copper concentration in semilogarithmic coordinates (Fig. 2). The potential was unstable at low copper concentrations and was determined by extrapolation. ILL's in the presence of humic acid were predicted as indicated in Fig.2, assuming that they depend on the potential of the cupric ion selective electrode, and that ILL = 25 μg/ℓ in the absence

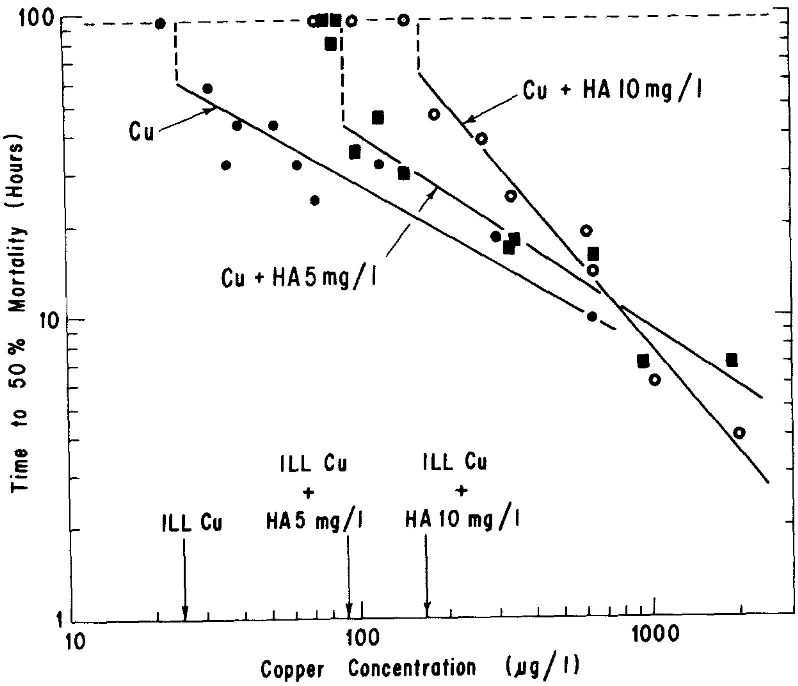

Fig. 1. Median lethal time of juvenile Atlantic salmon at different copper and humic acid (HA) concentrations. Mortality of less than 50% was observed at 96 h.

of humic acid (Fig. 1). The predicted ILL's (58 and 110 µg/ℓ at 5 and 10 mg/ℓ of humic acid, respectively) are lower than ILL's determined experimentally (90 and 165 µg/ℓ), but in view of the biological variability of ILL's and the long extrapolation of the potential of the cupric ion selective electrode the agreement is fairly good.

Fulvic acid is not readily available in amounts sufficient for a bioassay with fish and its effect on the ILL of copper may be predicted by the technique described above. The ILL's thus obtained are 110 and 240 µg/ℓ at 5 and 10 mg/ℓ of fulvic acid in tap water, respectively.

The ILL of copper to salmonid fishes increases with increasing hardness (LLOYD and HERBERT 1962). Cupric ion activity is a function of ionic strength and composition of the solution and it has been suggested that the effect of hardness on ILL is due to the formation of soluble cupric carbonate (STIFF 1971). In the present work, no attempt was made to evaluate the effect of ionic strength

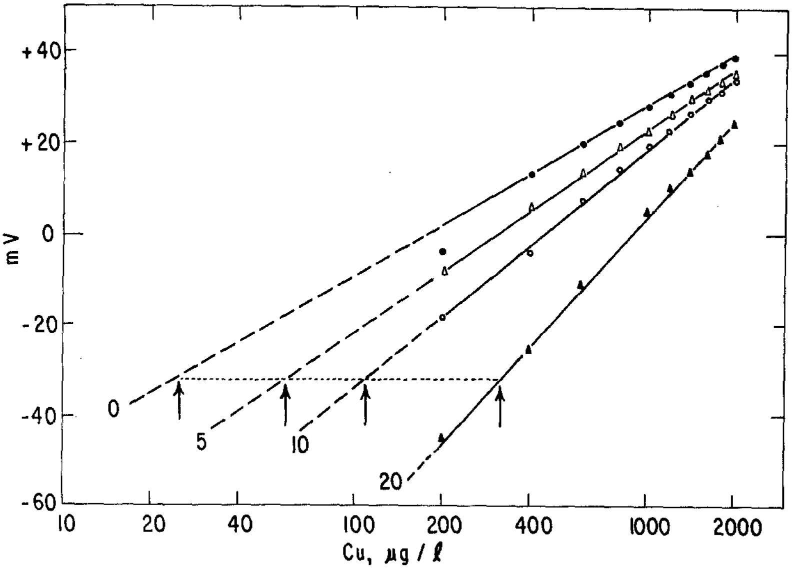

Fig. 2. Potential of cupric ion selective electrode at different copper and humic acid (0-20 mg/ℓ) concentrations. Arrows indicate the reference ILL (0) and the predicted (5-20) ILL's (see text). Tap water, total hardness 14 mg/ℓ.

and composition on the activity of cupric ions and, in experiments with tap water, tap water without added humic acid served as a blank. In the case of natural water such a blank is not available and, therefore, three artificial blanks were prepared:

(1) A solution of calcium sulfate in distilled water, with hardness equal to that of the sample. (Effluents from sulfide ore mining areas are limed and yield waters with calcium sulfate hardness).

(2) Humic acid was removed from a portion of the sample by the Amberlite XAD-1 resin. (The treatment did not remove humic acid below a residual concentration of 1 mg/ℓ, and did not change hardness expressed as calcium carbonate. Some loss of bicarbonate may occur due to the pH manipulation).

(3) Well water with hardness of 95-100 mg/ℓ and containing less than 1 mg/ℓ humic acid was diluted with distilled water to match the hardness of the sample.

The ILL's of copper in water with hardness different from that used in the bioassay (14 mg/ℓ) were calculated from the equations:

$$ILL = 2.02H + 7.87 \quad (0<H\leq 53)$$

$$ILL = 1.56H + 32.2 \quad (H>53)$$

derived from the data of LLOYD and HERBERT (1962) and were used as the reference ILL's (ILL in µg/ℓ, H = total hardness, mg/ℓ, as calcium carbonate).

The predicted ILL's of copper in the Northwest Miramichi River, New Brunswick, Canada (average hardness 19 mg/ℓ, average concentration of humic acid 16 mg/ℓ), were 265, 168, and 170 µg/ℓ, based on the calcium sulfate (1), Amberlite (2), and well water (3) blank, respectively. Copper-complexing ions are practically absent from solutions of calcium sulfate in distilled water. Consequently, for a given reference ILL the potential of the cupric ion selective electrode is higher in the calcium sulfate solution than in the other two blanks and so is the predicted ILL. The ILL's in the Northwest Miramichi, predicted on the basis of the Amberlite and well water blank, are in good agreement with values obtained in fish bioassays (COOK and COTE 1972).

The effects of calcium sulfate hardness on the predicted ILL's at different concentrations of humic acid are summarized in Table 1.

TABLE 1

The effect of calcium sulfate hardness on the predicted ILL's of copper at different concentrations of humic acid.

Humic acid concentration, mg/ℓ	5	10	20
Hardness as $CaCO_3$, mg/ℓ	Ratio of predicted to calculated ILL's		
15	1.82	2.37	6.60
30	1.81	2.57	4.05
90	1.65	1.77	3.20

As can be seen from Table 1, the ratio of predicted to calculated ILL's decreases with increasing hardness due to decreased binding of copper by humic acid at higher hardness. This effect has been suggested on the basis of fish bioassays (WILDISH et al. 1971, COOK and COTE 1972).

Humic acid at concentrations of 5 and 10 mg/ℓ has no effect on the ILL of zinc to juvenile Atlantic salmon, possibly due to lower stability of humic acid - zinc complexes. The zinc - fulvic acid complex is approximately 10 times less stable than the corresponding copper - fulvic acid complex (SCHNITZER 1971). In addition, the ILL of zinc (740 μg/ℓ at a hardness of 14 mg/ℓ) is much higher than the ILL of copper and the metal-binding capacity of humic acid may be saturated before the ILL is reached.

The presented data indicate that the cupric ion selective electrode is a useful tool for predicting ILL of copper to juvenile Atlantic salmon in the presence of humic acid. The technique may very likely be extended to other copper-complexing substances such as lignosulfonates, pulp mill effluents in general, and various organic compounds.

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REFERENCES

ANON., Department of the Environment, Water Pollution Research 1970, Her Majesty's Stationery Office, London (1971).

COOK, R.H. and R.P. COTE, The influence of humic acids on the toxicity of copper and zinc to juvenile Atlantic salmon as derived by the toxic unit concept. Environmental Protection Service Manuscript Report 72-5, Halifax (1972).

GAMBLE, D.S., M. SCHNITZER, and I. HOFMAN, Can. J. Chem. 48, 3197 (1970).

GRANDE, M., Advan. Water Pollut. Res., Proc. Int. Conf., 3rd, 1, 97 (1966).

LLOYD, R. and D.W.M. HERBERT, J. Inst. Public Health Eng. 61, 132 (1962).

MONTGOMERY, H.A.C. and M.J. STIFF, Differentiation of chemical states of toxic species, especially cyanide and copper, in water, IN: Identification and Measurement of Environmental Pollutants, B. Westley, Editor, 375, National Research Council of Canada, Ottawa, 1971.

RIEMER, D.N. and S.J. TOTH, J. Amer. Water Works Ass. 62, 195 (1970).

RILEY, J.P. and D. TAYLOR, Anal. Chim. Acta 46, 307 (1969).

SCHNITZER, M., Metal-organic matter interactions in soils and water, IN: Organic Compounds in Aquatic Environment, S.D. Faust and J.V. Hunter, Editors, 297, Marcel Dekker, Inc., New York, 1971.

SPRAGUE, J.B., Water Res. 3, 793 (1969).

STIFF, M.J., Water Res. 5, 171 (1971).

STIFF, M.J., Water Res. 5, 585 (1971).

WILDISH, D.J., W.G. CARSON, and W.V. CARSON, The effect of humic substances on copper and zinc toxicity to salmon, *S. salar* L., Fish. Res. Bd. Canada MS Rep. No. 1160, St. Andrews, 1971.