Asian Journal of Chemistry Vol. 13, No. 2 (2001), 439–443

Static Bioassay and Toxicity of Azo Dye Stuff to the Air Breathing Fish *Heteropneustes fossilis* and Its Possible Rectification

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This paper deals with the calculation of the toxicity of synthesized azo dye stuffs and its complexes with 3d transition metal ions on *H. fossilis* up to 30 days at a durational interval of 5, 10, 20 and 30 days. Sublethal, LD_{50} and LD doses were found out by taking different concentrations of azo dye and its complexes with 3d transition metals like Fe^{2+} and Co^{2+}. The extent of lethality was measured by behavioural changes all over the body on exposure to azo dye and its complex.

INTRODUCTION

Dyes have been used in a wide variety of areas, *viz.*, pisciculture, textile, paper, rubber, plastics, paints, drugs and cosmetic industries. But the use of certain synthetic dyes in food and medicine has been decreased because of their side effects[1] and health hazards to workers coming in contact with these dyes.

Dermatitis, hyperpigmentation, biochemical and haematological disturbances and also carcinogenic effects caused by dyes have been reported in aquatic organisms[2].

Dyes are released into aquatic environment from different sources, enter the food chain and may produce carcinogenic, mutagenic and teratogenic effects on human health[3, 4]. Hence, much importance is being given to the treatment of dying industry waste water, before its disposal to land or water. Along with the pollutants, dyes have also proved to be hazardous to aquatic organisms and alter the colour and quality of water. The effluents even reduce the rate of germination of seeds and growth of crop plants[5].

The textile industry is highly water intensive and hence it is one of the major contributors to water pollution. Textile bleaching, mercerization, dyeing, printing etc. have led to a slow but steady deterioration of aquatic ecosystems and render it unfit for the various aquatic organisms, particularly for fishes[6–9]. It has been seen that the dyeing operations contribute 10 to 20% of the COD load in aqua[10].

The present work is aimed at evaluating the toxicity of azo dyes on the

freshwater fish *H. fossilis* by static bioassay experiment[11, 12] with reference to its behavioural changes[13] and its possible rectification or waste minimization through compellation, effluent treatment and through advanced dyeing techniques such as lowering of liquors ratio during dyeing, optimizing dyeing processes and recipes, process of innovation and by substituting offending dyestuffs with more ecofriendly substituents[14] etc. for pollution control. This can be effectively implemented by source reduction instead of "end of pipe" solutions.

EXPERIMENTAL

2-Hydroxy-5-methoxy azo benzene-2'-carboxylic acid phenyl dye and its two complexes with Fe^{2+} and Co^{2+} were used which were highly coloured and having dyeing potentialities and biological activities. One hundred healthy fishes *H. fossilis* were collected from the local fish market. They were acclimated to laboratory conditions for 10 days before commencing the experiments. They were provided goat liver pieces as their diet along with proper aeration supplied by additional dissolved O_2 using a gas diffuser for their proper adaptation.

Acclimated fishes were used to determine the toxic level of the azo dye and its complexes with Fe^{2+} and Co^{2+} at different doses including LD_{50}, LD and sublethal concentration The experiment was carried out in 3 : 1 : 1 size of glass aquaria according to the method of Feeny (1964).

The preliminary experiments were conducted for complete survival and mortality for 30 days. Five replicas of each dose were tested and a parallel set of experiments with 10 fishes were also made.

RESULTS AND DISCUSSION

When experimental fishes were exposed to the azo dye and its complexes, behavioural changes in LD_{50} and subsequent higher doses were observed, such as erratic jumping movements. Hyper and hypo activity changes in opercular movements rate, irregular swimming activity of the body, floating upside down with abdomen directed upwards in between normal movements, convulsions, spiralling jerky movements, loss in equilibrium, vertical movement, spreading of excess mucus all over the surface of the body and ultimately death occurred. No death occurred in the controlled set of experiments.

Table-1 indicates the percentage mortality of experimental fishes on exposure to different concentrations of azo dye and its complexes for a period of 30 days. It was seen that 50% and 100% mortality of *H. fossilis* upto 30 days was caused by 2.4 and 3.2 ppm of 5-methoxy azo dye respectively and similarly 50% and 100% mortality was caused by 5.8 and 7.6 ppm of Fe^{2+} complex and 5.1 and 6.7 ppm of Co^{2+} complex respectively. The concentration at which in 30 days there was no mortality or changes in behaviour of test fish was defined as Non-lethal

concentration. The nonlethal concentration. for 5-methoxy azo dye is 0.8 ppm, for its Fe^{2+} complex 2.2 ppm and for its Co^{2+} complex 1.9 ppm. Graphical representation of effect of concentration of azo dye on toxicity is shown in Fig. 1.

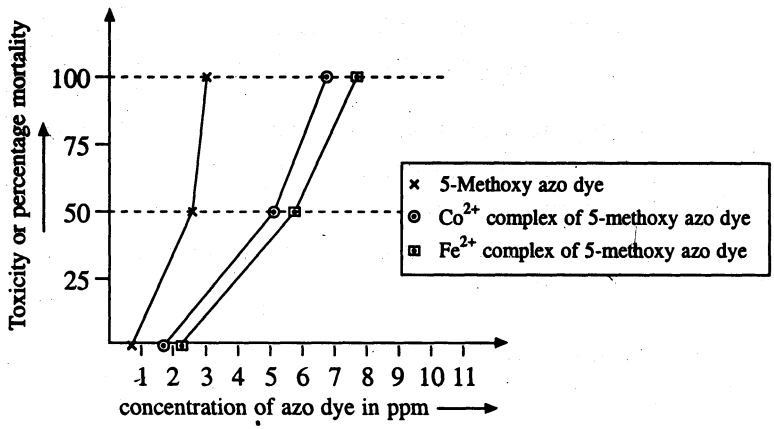


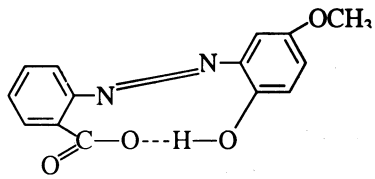
Fig. 1. Relation between concentration of azo dye and its complexes *vs.* toxicity

TABLE-1
% MORTALITY OF *H. fossilis* ON EXPOSURE TO DIFFERENT CONCENTRATION OF AZO DYE AND ITS COMPLEXES FOR A PERIOD OF 30 DAYS

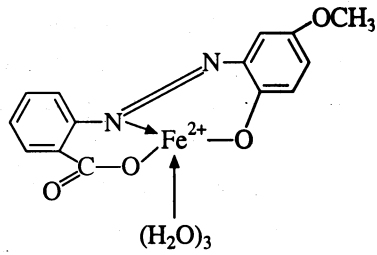
Azo dye and its complexs	Conc. of azo dye in ppm	Average mortality in 30 days (%)	Results
5-methoxy azo dye	0.8	0	Non-lethal
	2.4	50	LD_{50}/30 days
	3.2	100	LD_{100}/30 days
Fe^{2+} complex of 5-methoxy azo dye	2.2	0	Non lethal
	5.8	50	LD_{50}/30 days
	7.6	100	LD_{100}/30 days
Co^{2+} complex of 5-methoxy azo dye	1.9	0	Non lethal
	5.1	50	LD_{50}/30 days
	6.7	100	LD_{100}/30 days

The above observations were a result of behavioural changes in LD_{50} and subequent higher doses and ultimately death occurred as a result of exposure to azo dye and its Fe^{2+} and Co^{2+} complexes.

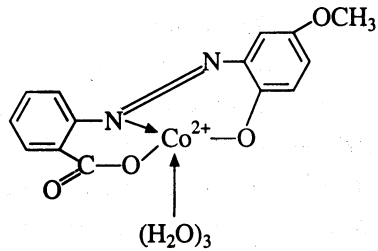
Increased opercular movement observed in the present experiment might be necessary to satisfy the requisite O_2 demand for survival. The observed mucous accumualations were probably due to toxic effects of azo dye, because respiratory epithelium might be the main target site of toxicity during the period of experiment. The mucous is supposed to be an adaptive response perhaps providing additional protection against the corrosive nature of compounds, and also excessive secretion of mucous by skin made the body more slippery for quick



5-Methoxy azo dye



Fe^{2+} complex of 5-methoxy azo dye



Co^{2+} complex of 5-methoxy azo dye

movement in test solution and avoid the absorption of toxicant by general body surface. No death resulted in controlled set of experiment.

The experimental fishes when exposed to different concentration of azo dye and its complexes for different periods of time showed considerable variations in the degree of intoxication at different rates.

Nonlethal, LD_{50} and LD_{100} concentration were found out on 30 days exposure and from observations the following results can be obtained:

1. The mortality % is dose dependent.
2. The toxicity in fishes increased with increased concentration of azo dye and its complexes.
3. Observations also revealed that azo dye was more toxic to fishes than its complexes because *H. fossilis* exhibited a higher level of tolerance to its complexes than the azo dye itself.

The results obtained show that some physiological factors affect mortality and that behavioural changes are directly related to complex biochemical and physiological responses underlying the animals and have often being used as a measure of stress syndrome in the organisms experiencing them.

Thus this experiment helps in determining the concentration of azo dye and

its complexes which can be less toxic for aquatic life and can minimize the extent of water pollution. Before disposal to the water, it can be treated up to the nonlethal or less toxic concentration and can help in maintaining the colour and quality of water.

Other advanced techniques are also used for minimization of waste which are:

1. M.L.R.—lowering of liquor ratios.
2. Optimizing dyeing processess and recipes.
3. Process of innovation.
4. By substituting offending dyestuffs with more ecofriendly substitutes etc.

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(*Received*: 24 August 2000; *Accepted:* 24 November 2000) AJC-2199