UNIVERSITI PUTRA MALAYSIA

BIOLOGICAL MARKERS IN RIVER CATFISH, *MYSTUS NEMURUS* (C&V) EXPOSED TO HYDROGEN SULPHIDE

MUHAMMAD TAFAZZAL HOQUE

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By

MUHAMMAD TAFAZZAL HOQUE

Dissertation Submitted in Fulfilment of the Requirements for the Degree of Doctor of Philosophy in the Faculty of Science and Environmental Studies Universiti Putra Malaysia

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This dissertation is dedicated

to my mother,

who inspired me to do this

and to my father,

who has taught me to aspire and persevere

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LIST OF ABBREVIATIONS

CDNB	1-chloro-2,4-dinitrobenzene
EDAX	Energy Dispersive Analysis X-ray
EDTA	Ethylenediamine tetra-acetic acid
GST	Glutathione *S*-transferase
Hb	Haemoglobin
HbO_2	Oxyhaemoglobin
KCN	Potassium cyanide
$K_3Fe(CN)_6$	Potassium Ferricyanide
LSI	Liver-somatic index
MS-222	3-aminobenzoic acid ethyl ester
SDS	Sodium dodecyl sulphate
SEM	Scanning Electron Microscope
SHb	Sulphaemoglobin
TEM	Transmission Electron Microscopy

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Chairperson : Assoc. Prof. Dr. Fatimah Md. Yusoff
Faculty : Science and Environmental Studies

Biochemical, histopathological, histochemical and bioenergetic parameters were studied to determine their suitability as biological markers for hydrogen sulphide toxicity detection using the river catfish *Mystus nemurus* (C&V) exposed to H_2S in laboratory experiments as well as those caught from the wild. In the laboratory, the toxic effects of H_2S to *M. nemurus* juveniles were determined by using a flow-through bioassay technique.

The 96-h LC_{50} value of unionized H_2S was 3.20 µg/L, and 0.003 µg/L unionized H_2S was recommended as the safety level for *M. nemurus* juveniles under tropical environmental condition. Sulphaemoglobin and thiosulphate concentrations significantly increased ($p<0.01$) with increasing hydrogen sulphide (H_2S) concentrations and exposure time. However, H_2S reduced the oxygen carrying capacity of haemoglobin by reducing oxyhaemoglobin. Glutathione *S*-transferase (GST) specific activities significantly increased ($p<0.01$) in fish exposed to H_2S higher than 30% of LC_{50}.

Gill lesions such as epithelial separation, club-shaped lamellae and interlamellar fusion were observed at different concentrations of H_2S. The evidence of neurotoxicity was elucidated by necrosis and damaged mitochondria in fish brain tissue. Sulphur accumulation in gills progressively increased with the increase of H_2S concentrations and exposure time.

The liver-somatic index (LSI) and growth rate significantly decreased ($p<0.05$) with increased concentrations of H_2S and exposure time. Fulton's condition factor failed to predict ($p>0.1$) stress effects in fish exposed less than six weeks to H_2S. However, RNA-DNA ratios showed high correlations with H_2S concentrations from the second ($r^2 = 0.83$; $p<0.01$) to sixth week ($r^2 = 0.98$;$p<0.01$) of exposure.

Thiosulphate and sulphaemoglobin showed positive correlations with H_2S concentrations ($r^2 = 0.79$; $p<0.01$ and $r^2 = 0.89$; $p<0.01$ respectively). Sulphur accumulation in gills was positively correlated with thiosulphate and sulphaemoglobin concentrations in blood ($r^2 = 0.74$; $p<0.01$), indicating that these compounds resulted from H_2S exposure. In addition, H_2S levels in water were directly correlated with GST activities and sulphaemoglobin concentrations. However, H_2S concentrations showed an inverse relationship with oxyhaemoglobin concentrations.

The field study supported the laboratory findings for two indicators; thiosulphate and sulphur accumulation, were potential biological markers for H_2S toxicity. Other markers such as Fulton's condition factor, liver-somatic index, growth rate, RNA-DNA ratio, histopathology and histochemistry did not reflect specific toxic effect, although they can be used to indicate the general health condition of fish exposed to H_2S. Among all the indicators, thiosulphate was found to be the simplest and fastest biological marker for detecting H_2S toxicity.

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Lesion insang seperti pemisahan epitelial, lamela berbentuk belantan dan penyatuan interlamela dapat dilihat pada beberapa kepekatan H_2S yang berbeza. Bukti ketoksikan saraf ditunjukkan oleh nekrosis dan kerosakan mitokondria di dalam tisu otak ikan. Pengumpulan sulfur di dalam insang meningkat secara berterusan dengan peningkatan kepekatan H_2S dan masa pendedahan.

Indeks somatik hati (LSI) dan kadar pertumbuhan menurun dengan bererti ($p<0.05$) dengan peningkatan kepekatan H_2S dan masa pendedahan. Faktor keadaan Fulton gagal ($p>0.01$) meramalkan kesan tekanan di dalam ikan yang terdedah kepada H_2S kurang dari enam minggu. Walaubagaimana pun, nisbah RNA-DNA menunjukkan pertalian yang tinggi dengan kepekatan H_2S pada minggu yang kedua ($r^2 = 0.83$; $p<0.01$) ke minggu yang keenam ($r^2 = 0.98$; $p<0.01$).

Tiosulfat dan sulfamoglobin menunjukkan pertalian yang positif dengan H_2S ($r^2 = 0.79$; $p<0.01$ dan $r^2 = 0.89$; $p<0.01$ masing-masing). Pengumpulan sulfur di dalam insang berkait secara positif dengan kepekatan tiosulfat dan sulfamoglobin di dalam darah ($r^2 = 0.74$; $p<0.01$), menunjukkan bahawa kompaun-kompaun ini terhasil daripada ketoksikan H_2S. Tambahan pula, paras H_2S di dalam air berkadar terus dengan aktiviti GST dan kepekatan sulfamoglobin. Namun begitu, kepekatan H_2S menunjukkan pertalian songsang dengan oksihemoglobin.

Kajian lapangan menyokong penemuan di dalam makmal bahawa dua penunjuk: tiosulfat dan pengumpulan sulfur adalah penanda biologi yang berpotensi bagi ketoksikan H_2S. Penanda biologi yang lain seperti faktor keadaan Fulton, indeks somatik hati, kadar pertumbuhan, nisbah RNA:DNA, histopatologi dan histokimia tidak mencerminkan kesan ketoksikan yang spesifik, walaupun kesemuanya boleh digunakan untuk menentukan tahap kesihatan am bagi ikan yang terdedah kepada H_2S. Di antara kesemua penanda-penanda yang dikaji, tiosulfat adalah penanda biologi yang paling mudah dan pantas untuk mengesan ketoksikan H_2S.

CHAPTER I

INTRODUCTION

Background of the Study

Hydrogen sulphide (H_2S) is a colourless gas, heavier than air and moderately water soluble (6g /L at 10^0C; Keith and Walters, 1985). It occurs in many natural situations where decomposition of organic matter in bottom deposits is a normal phenomenon. H_2S is also generated in sludge deposits from paper mills, leather tanning and finishing, rubber processing, rayon manufacture, dyeing, untreated sewage effluent, and other sources of organic debris. In Malaysia, six pulp and paper mills are discharging about 295,000 mt effluent per year which contribute a significant amount of H_2S (FAO, 1991).

Various explanations have been suggested for high concentrations of H_2S in waterbodies. The predominant hypothesis is anthropogenic eutrophication (Zaytsev, 1976, 1977; Nesterova, 1977; Tolmazin, 1977). H_2S availability in nature is limited by oxygen availability. Due to oxidation of sulphide in the water column, oxygen

availability is decreased which leads to hypoxia and ultimately to anoxic condition in the bottom layers of water (Bella et al., 1972). In prolonged hypoxia fish are synergistically exposed to oxygen deficiency and higher unionised H_2S concentration as pH decreases.

H_2S is a rapid and powerful systematic poison (Gleason et al., 1969) and its unionised form has been demonstrated to be toxic to fish (Smith and Oseid, 1972b; Broderius et al., 1977). Its toxicity is strongly influenced by pH together with temperature. H_2S occurs naturally at levels which affect the survival of fish and production in both freshwater (Smith and Oseid, 1974; Torrans and Clemens, 1982) and marine (Fenchel and Riedl, 1970; Breaten et al., 1983; Liefrig, 1985; Jorgensen, 1984; Bagarinao, 1991a, 1995) ecosystems. The lethality of H_2S is comparatively higher in freshwater than that in the marine environment since the former maintain a higher unionised stage of H_2S (Millero, 1986) due to low pH and lack of buffering capacity (Poole et al., 1978).

Various approaches, which are mainly acute and chronic toxicity tests have been used to evaluate or predict the effects of environmental stress on fish. Although these approaches are frequently adopted, they have little ecological realism (Cairns, 1981; National Research Council, 1981). So, a new approach through selecting other stress-related parameters that are biologically and ecologically relevant and has maximum predictive capabilities was suggested.