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BIOACCUMULATION OF COPPER AND ITS EFFECT ON HUMORAL IMMUNE RESPONSE IN GOLDFISH CARASSIUS AURATUS LINNAEUS

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By

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TO

MY

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LIST OF ABBREVIATIONS

APDC = Ammonium Pyrrolidine Dithiocarbamate

APHA = American Public Health Association

AWWA = American Water Works Association

DDTC = Diethyl Ammonium Diethyldithiocarbamate

DO = Dissolved Oxygen

EIFAC = European Inland Fisheries Advisory Commission

EPA = Environmental Protection Agency of U.S.A

FAO = Food and Agriculture Organisation of U.N

h = Hours

huRBC = Human Red Blood Cell

IPNV = Infectious Pancreatic Necrosis Virus

MATC = Maximum Allowable Toxicant Concentration

MIBK = Methyl Isobutyl Ketone

mon. = Months

PBS = Phosphate Buffer Saline

S.D = Sample Standard Deviation

S.E = Sample Standard Error

SRBC = Sheep Red Blood Cells

Pre-IM = Pre-Immunised

Post-IM = Post-Immunised

V/V = Volume by Volume

WPCF = Water Pollution Control Federation of U.S.A

Wt. = Weight

Abstract of thesis submitted to the Senate of Universiti Pertanian Malaysia in fulfillment of the requirements for the degree of Master of Science

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By

MD. ABUL HOSSAIN

December, 1990

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This study reports on the acute toxicity levels, bioaccumulation of copper and their effects on the humoral immune response of goldfish (Carassius auratus). This study also provides information on the levels of bioaccumulation of copper in organs such as kidney, liver and spleen, which plays a role on the humoral antibody production of goldfish under tropical conditions.

The acute toxicity levels of copper to goldfish (10.5 ± 1.43 g) for 12, 24, 48, 72, 96 and 120 hours were estimated as 0.44 ± 0.06, 0.36 ± 0.04, 0.20 ± 0.03,

0.19 ± 0.02, 0.15 ± 0.02 and 0.14 ± 0.02 ppm respectively under fresh water static conditions.

Goldfish (17 ± 0.2 g) were exposed to sublethal levels (10, 30 and 50 µg/l) of copper for a period of six and eight weeks to facilitate bioaccumulation. The group which was exposed to copper for eight weeks was injected with SRBC at the end of sixth and seventh weeks. This was done to activate the antibody production in test fish and to monitor the effect of copper on humoral antibody response. The bioaccumulation of copper in kidney, liver, spleen and muscle of goldfish was directly proportional to the exposure concentration. The highest bioaccumulation was observed in liver and was followed by spleen, kidney and muscle. Bioaccumulation of copper at 14.19-23.51 µg/g in kidney, 60.28-102.99 µg/g in liver, 15.09-26.30 µg/g in spleen and 2.66-4.11 µg/g in muscle (dry weight) was evident following six weeks exposure. Bioaccumulation of copper in organs of test fish exposed for eight weeks was as follows: kidney 20.39-28.84 µg/g, liver 82.01-152.77 µg/g, spleen 22.38-39.31 µg/g and muscle 3.39-5.29 µg/g (dry weight).

At the end of eighth week, serum anti-SRBC agglutinating antibody titres in goldfish, which were exposed to copper for eight weeks and subjected to immunisation at sixth and seventh weeks, were partially suppressed when compared to unexposed fish. The suppression of antibody titre was inversely proportional to the exposure concentration of copper. The fish unexposed and exposed to copper at 50 µg/l showed highest 1:256 (mode) and lowest 1:32 (mode) antibody titres respectively. The recorded pre-immunisation heterophilic (nonspecific) antibody titre was 1:4 (mode). Antibody titre of fish unexposed and exposed to copper at different sublethal levels were significantly different.

The weekly rate of bioaccumulation of copper in post-immunised fish was significantly higher when compared to the pre-immunised fish.

Abstrak tesis yang dikemukakan kepada Senat Universiti Pertanian Malaysia, sebagai memenuhi keperluan untuk mendapat Ijazah Master Sains.

BIOAKUMULASI KUPRUM DAN KESANNYA TERHADAP RANGSANGAN IMUN HUMORAL DALAM IKAN MAS CARASSIUS AURATUS LINNAEUS

Oleh

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Kajian ini melaporkan tahap toksisiti akut, bioakumulasi kuprum dan kesan terhadap pengeluaran antibodi humoral dalam ikan mas (C. auratus). Kajian ini juga menyatakan tahap bioakumulasi kuprum dalam organ seperti ginjal, hati dan limpa yang mana memainkan peranan dalam pengeluaran antibodi humoral ikan mas di dalam keadaan tropika.

Tahap toksisiti akut kuprum pada ikan mas (10.5 ± 1.43 g) untuk 12, 24, 48, 72, 96 dan 120 jam masing-masing dianggarkan pada 0.44 ± 0.06, 0.36 ± 0.04, 0.20 ± 0.03, 0.19 ± 0.02, 0.15 ± 0.02 dan 0.14 ± 0.02 bpj, di dalam keadaan air tawar stasis.

Ikan mas (17 ± 0.2 g) telah didedah kepada tahap subletal (10, 30 dan 50 µg/l) kuprum selama enam dan lapan minggu untuk memudahkan bioakumulasi. Kumpulan yang didedahkan kepada kuprum selama lapan minggu telah disuntik dengan SRBC pada akhir minggu ke enam dan ke tujuh. Ini adalah untuk mengaktifkan pengeluaran antibodi dalam ikan yang diuji dan untuk meneliti kesan kuprum terhadap gerak-balas antibodi humoral. Bioakumulasi kuprum dalam ginjal, hati dan limpa adalah berkadaran langsung dengan kepekatan pendedahan. Bioakumulasi tertinggi telah dilihat dalam hati, dituruti oleh limpa, ginjal dan otot. Bioakumulasi kuprum dalam ginjal ikan mas adalah 14.19-23.51 µg/g, hati 60.28-102.99 µg/g, limpa 15.09-26.30 µg/g dan otot 2.66-4.11 µg/g berat kering apabila didedahkan selama enam minggu. Bioakumulasi kuprum dalam ikan mas yang didedahkan selama lapan minggu adalah seperti berikut: ginjal 20.39-28.84 µg/g, hati 82.01-152.77 µg/g, limpa 22.38-39.31 µg/g dan otot 3.39-5.29 µg/g (berat kering).

Pada minggu ke lapan, titer serum anti-SRBC antibodi pengaglutinatan dalam ikan mas yang didedahkan pada kuprum selama lapan minggu dan diimunkan dalam minggu ke enam dan ke tujuh, didapati separa tertekan jika dibandingkan dengan ikan yang tidak terdedah. Penekanan titer antibodi adalah berkadar songsang dengan kepekatan pendedahan kuprum.

Ikan yang tidak didedah dan yang didedah kepada kuprum (50 µg/l) masing-masing menunjukkan titer antibodi tertinggi, 1:256 (mod) dan terendah 1:32 (mod). Titer antibodi heterofilik (bukan khusus) sebelum imunisasi dirakamkan pada 1:4 (mod). Titer antibodi ikan yang tidak didedah dan didedahkan kepada kuprum pada paras subletal yang berlainan, adalah berbeza keertian, di antara satu sama lain.

Kadar mingguan bioakumulasi kuprum dalam ikan pasca-imun adalah lebih tinggi keertian berbanding dengan ikan praimun.

CHAPTER I

GENERAL INTRODUCTION

Sources of Copper in Aquatic Ecosystem

Copper is one of the most commonly available, toxic and bioaccumulable heavy metals present in water. With the rapid industrialisation of the present world, and the indiscriminate discharge of copper oriented products/complexans, the metal is continuously increasing in both fresh and sea water ecosystems. The industrial sources of copper from widely used major industrial products are fertilizers, algicides, pesticides, therapeutic agents, bronze, wiring, storage batteries, electrical goods, etc.

Copper is also widely distributed in nature as rocks and earth crust. Copper is introduced into the aquatic ecosystem as a result of weathering of soils and rocks from volcanic eruption and also from a variety of human activities, including mining process. Contamination of copper from the industries occur when the metals

or other substances that contain copper contaminants are discharged into the aquatic ecosystem (Mance, 1987; Nriagu, 1979; EIFAC/FAO, 1976). The sources of copper rich water is usually from contaminated mine water or run-off from aged spoil heaps (Mance, 1987). Thus the ecosystem is being constantly polluted by unextracted copper compounds from the soil and from the abandoned industrial products containing copper (Nriagu, 1979).

Copper can exist in the form of at least 5000 complexans in water (EIFAC/FAO, 1976). Copper when present in combination with other heavy metals especially zinc, becomes more harmful to aquatic organisms. Copper is safe for normal biochemical life processes if its presence is below 5 µg/l in water. The atmospheric loading of copper into most terrestrial and aquatic ecosystem now exceeds the combined copper requirement of flora and fauna for their normal biochemical life processes (Kelly et al., 1975). In Kelang Estuary, Malaysia, the mean total and dissolved copper content was 10 µg/l and 4.3 µg/l respectively (Law and Singh, 1986).