BIOLOGICAL MONITORING 1998 ANNUAL REPORT FOR FLETCHER CHALLENGE PAPER ALBURY

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TABLE OF CONTENTS

PAGE

1.0 INTRODUCTION:

The 1998 biological monitoring program focuses on Fletcher Challenge Paper's 2100ML Lake Ettamogah used as an irrigation storage dam for tertiary treated wastewater. Lake Ettamogah provides habitat for water fowl including the protected musk duck (*Biziura lobata*) and blue-billed duck (*Oxyura australis*), is rich in microcrustaceans (copepods, daphnids and shrimp) and has been stocked with silver and golden perch. The assessment of long-term bioaccumulation is essential to address questions with respect to the potential impact on local wildlife.

2.0 AIMS

To undertake biological and chemical monitoring of FCP's wastewater discharged to the River Murray in accordance with New South Wales Environment Protection Agency Licence No.01272; Sections W9 (Long term bioaccumulation monitoring using fish) and W10 (Microbial oxidation of manganese on artificial substrates) for May 1997 to April 1998. The null hypothesis tested in all cases "that there is no difference between control water and wastewater treatments."

3.0 METHODS

3.1 Bioaccumulation Monitoring

Trials were conducted to determine the levels of bioaccumulation of metals from FCP's final outfall wastewater using a crustacean (yabby, *Cherax destructor*) and stocked fish species (golden perch (*Macquaria ambigua*) and silver perch (*Bidyanus bidyanus*)).

3.1.1 Yabby *(Cherax destructor)*

Bioaccumulation trials were undertaken using FCP's stored wastewater from the irrigation storage dam Lake Ettamogah.

Yabby trials were conducted in six preconditioned 1000L flow through plastic tanks fed with stored water pumped from Lake Ettamogah, or Murray River water. Three tanks were randomly assigned to each treatment, the control tanks fed with river water and the test tanks fed with FCP stored wastewater. All tanks contained short sections of PVC water pipe as habitat.

125 Male yabbies were purchased from a local commercial yabby farm - 20 animals were randomly allocated to each tank and 5 retained as an initial (pre-trial) sample for chemical analysis. Each tank contained a minimum/maximum thermometer. Physicochemical water quality (temperature, dissolved oxygen, conductivity, pH, hardness and alkalinity) was assessed in each tank fortnightly. 10 animals were measured (weight and carapace length) each month and 4-7 animals removed at the termination of the trial for chemical analysis. The animals retained for chemical analysis were frozen, freezedried and divided in half dorsoventrally. One half of each was then homogenised and acid digested in preparation for assay of metals (Al, As, Cd, Cu, Fe, Mn, Pb and Zn) by ICP at Australian Government analytical Laboratories (AGAL). The other half of each was separated into three major body parts; tail/claw (muscle), exoskeleton (shell), and other organs gills/gastrolith/hepatopancreas/gut (viscera), homogenised, acid digested, and assayed for manganese by AAS at MDFRC's chemistry Laboratory.

Growth and general metals data were analysed and reported in quarterly progress reports to FCP. A synopsis of these and the Mn assays to determine the site of Mn deposition in the animals are included in this annual report.

3.1.2 Stocked Fish from FCP Wastewater Holding Ponds

Growth, sexual maturity and tissue metals concentrations were assessed for 3 year old silver and golden perch population released into Lake Ettamogah as fingerlings. The surveys were conducted in summer using nets. The fish were anaesthetised and killed using benzocaine™ and assessed for size, age (using otoliths), and gonad development. 20g muscle/skin samples were removed from behind the head, frozen, freezedried and homogenised then acid digested in preparation for assay of metals (Al, As, Cd, Cu, Fe, Mn, Pb, and Zn) by ICP at Australian Government Analytical Laboratories (AGAL).

3.1.3 Stocked Fish v Hatchery and Wild Fish

It is intended that bioaccumulation of metals in 4 year old fish living in wastewater will be compared with similar fish from two other sources;
(1) grown in a commercial hatchery/farm (control)
(2) from the Murray/Murrumbidgee Rivers (control)
The surveys will be conducted in summer during the breeding season. Fish in Lake Ettamogah will be captured using nets or electrofishing. Angling Clubs and NSW Fisheries will be consulted for their cooperation in supplying data and flesh samples from fish caught in the rivers. Ten fish from each source will be anaesthetised and killed using benzocaine™ and assessed for size, age and sex. Flesh samples will be assayed as above. A report will be submitted to FCP by June 1999 for incorporation as an appendix to their annual report of compliance with EPA licence requirements and development consent conditions.

3.2 Manganese-oxidising Bacteria

3.2.1 Isolation and characterisation of Mn-oxidising bacteria

Mn oxidising bacteria were obtained from wastewater by serial dilution and inoculation onto WW media, as well as from surficial biofilms by scraping artificial substrates and yabbies and inoculating Pedomicrobium (PC) agar (Tyler and Marshall 1967c).

Wastewater (WW) medium was prepared by supplementing (1L) wastewater with NH_4Cl (0.1g), KH_2PO_4 (0.2g), $MnSO_4$ $4H_2O$ (0.02g) and agar (15g). All plates were incubated at 27°C and kept under observation for the presence of colonies which turned brownish/black as Mn oxide formed. Colonies testing positive for manganese oxide with Leucocrystal Violet (LV) indicator (Spratt *et al*. 1994, ASTM 1990) were subcultured onto fresh media until a pure culture was obtained. Subsequent liquid culture was carried out as described by Green and Madgwick (1988). Fresh colonies were tested for catalase and oxidase activity with 3% pharmaceutical H_2O_2 and Pyo-test™ strips (for detection of cytochrome oxidase) respectively. Standard Plate Count agar (BBL, Cockeysville, USA) was used to test growth in the absence of manganese.

3.2.2 Mn^{2+} oxidation studies on wastewater

Mn-oxidation in the wastewater was determined by measuring the rate of loss of added Mn^{2+} from both unfiltered wastewater and wastewater which had passed through a "Spin-Klin™" filtration plant (nominal cut-off 115 μm). 40 mL of filtered wastewater, unfiltered wastewater or Milli-Q water were placed into two series of 50 mL screw-cap polyethylene centrifuge tubes. 1000 μg Mn^{2+} L^{-1} (as Mn_2SO_4) was added to one series and none to the other. Microbial activity in half of the samples was inhibited by the addition of 0.25 mL of 1.75 M NaN_3. The tubes were placed on an orbital shaking table and maintained at 20 ± 1 °C. Aliquots were periodically removed from the tubes and the concentration of Mn^{2+} was determined by the tetra(*p*-carboxyphenyl) porphyrin method of Ishii *et al*. (1982) as modified by Johnson *et al*. (1995).

3.3 Lake Ettamogah Biological Survey

A survey of the biota of Lake Ettamogah was conducted using light traps, sweep nets, and artificial substrates to supplement existing survey data (Klomp and Costello 1997, Merritt unpublished). An inventory of micro-invertebrates, macro-invertebrates, amphibians, fish and birds, along with habitat notes was compiled. This inventory was submitted to FCP in a form appropriate for an educational resource for visiting students.

3.4 Reporting

Quarterly reports containing all test results and observations including physico-chemical data were submitted to FCP. This Annual Report containing a summary of results from the monitoring program was submitted to FCP for incorporation as an appendix to their annual report to fulfil their requirements for Condition W16 of Licence No.01272 issued by the NSW Environment Protection Authority.

4.0 RESULTS AND DISCUSSION

4.1 Bioaccumulation Monitoring

4.1.1 Yabby *(C. destructor)*

A two month *C. destructor* trial commenced in November 1997. Those grown in wastewater were significantly (P = 0) smaller than the controls. Metals assayed (aluminium, arsenic, cadmium, copper, iron, lead, manganese and zinc) are presented in Figure 1. The mean and standard deviation (n = 4 to 7) for each are plotted against treatment (initial control, river water control and wastewater treatment). Animals exposed to wastewater accumulated aluminium, iron and manganese. Those in river water accumulated cadmium, copper, lead and zinc; and lost aluminium, iron and manganese.

Manganese concentration in major body parts of *C. destructor* (Figure 2) identifies the shell as the primary location of Mn accumulation and to a lesser extent the viscera in animals exposed to wastewater. This confirms the preliminary investigation documented in our 1997 annual report and supports the theory of Mn being associated with biofilms adhering to the surface of the carapace or being ingested by benthic grazing.

4.1.2 Stocked Fish from FCP's Lake Ettamogah Wastewater Storage Dam

17 silver perch and 4 golden perch were netted from Lake Ettamogah in February. All of which were estimated to be three years old. Growth rates and condition factors were greater than those obtained by NSW Fisheries in surveys of silver perch from impoundments and rivers (Mallen-Cooper *et al*. 1995, Paul Brown pers. comm.). The

elevated year round water temperatures and abundance of invertebrates and lack of competition are all contributing factors.

Most of the captured fish were sexually mature and ripe to spawn, but no fish larvae or fry were found with trawls or trapping at night, so it likely that either the cues to spawn are not present in the Lake, or fertilisation / larvae survival is unsuccessful due to the salinity of the water (Brett Ingram pers. comm.).

Muscle metals concentrations were low (<4mg/kg), with iron, zinc and aluminium the most abundant (9-20mg/kg). These results are documented in the second quarter progress report to FCP.

4.1.3 Stocked Fish v Hatchery and Wild Fish

Fishing is scheduled for December 1998 or January 1999. The comparison of metals in muscle of fish from the three sources will then be undertaken and included in a report to FCP by 30 June 1999.

4.2 Manganese-oxidising Bacteria

4.2.1 Isolation and Characterisation of Mn-oxidising Bacteria

Isolation on agar in the laboratory of two manganese-oxidising bacteria from an artificial substrate and a yabby carapace was successful. Pure cultures were obtained with one isolate each from the artificial and yabby surfaces selected for further study. Strain ANM1 (*Sphingomonas* sp. B. Patel pers. comm.) obtained from an artificial substrate was a budding gram negative curved rod. After 2 weeks incubation colonies on PC medium were less than 1 mm, black and irregular, whereas the colonies on medium without manganese were 0.5-2.0 mm, yellow and circular with an entire margin. Strain ANM2 (*Leptothrix*-like bacterium G. Rees pers. comm.) isolated from the carapace of the yabby was filamentous and slow growing. The colonies took upwards of 3-4 weeks before they were clearly visible on PC agar.

4.2.2 Mn^{2+} Oxidation studies on Wastewater

Both native and added Mn^{2+} was oxidised in wastewater (Figs. 3 & 4). The shape of the oxidation curve is consistent with a biotically mediated process rather than an abiotic process. A lag phase can be observed where little oxidation occurs in about the first 4 days of incubation. However, much of the Mn is oxidised in the next 4 days of incubation. The rate of oxidation was significantly reduced when microbial activity was inhibited by the addition of NaN_3 (Fig 3). The rate of Mn^{2+} oxidation was also severely reduced in wastewater filtered to a nominal 115μm (to remove particulates) by the filtration plant (Fig. 4) (King *et al*. in press).

Indications for FCP are that the holding ponds whilst aerobic are active in reducing the concentration of manganese in wastewater and further supports the theory that Mn accumulation in yabbies is microbially mediated. Also it appears that by removing suspended particles, the irrigation system's filtration plant, reduces the potential for fouling of the reticulation infrastructure.

4.3 Lake Ettamogah Biological Survey

The inventory ".....Fauna of FCP's Lake Ettamogah Forest Wastewater Re-Use Scheme and Environs" included: 163 species of vertebrates; 126 Birds, 11 Mammals (including 4 marsupials), 12 Reptiles, 10 Amphibians, 5 fish; and 24 species of Invertebrates

5.0 COMMUNICATION

The revised monitoring program entitled "Biological Monitoring Program Proposal for Australian Newsprint Mills Albury for the 1998/99 Financial Year" was submitted to NSW Fisheries and EPA NSW in April 1998 and approved. The program incorporated modifications to the program following the Annual Review on 25 November 1997.

The paper : King, H.M., Baldwin, D.S., Rees, G.N. and McDonald, S. (in press) Apparent bioaccumulation of Mn by the freshwater crayfish *Cherax destructor* - the role of Mn-oxidising bacteria; was presented at the Annual Congress of the Australian Sociey for Limnology in Brisbane, 3-6 July 1998.

Resource material for visiting school groups was prepared in consultation with FCP communication staff. The 9 page handout "The Fauna of FCP's Lake Ettamogah Forest Wastewater Re-Use Scheme and Environs" was submitted to FCP along with the second quarter progress report in September 1998.

5.0 REFERENCES

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Tyler, P.A. and Marshall K.C. (1967c). Form and function of manganese oxidising bacteria. *Archiv fur mikrobiologie*, 56, 344-353.

7.0 FIGURES

Figure 1: Concentration of metals in whole yabbies prior to the commencement of the 1998 bioaccumulation trial (initial) and those exposed to river water (control) and FCP wastewater (mean & standard deviation).

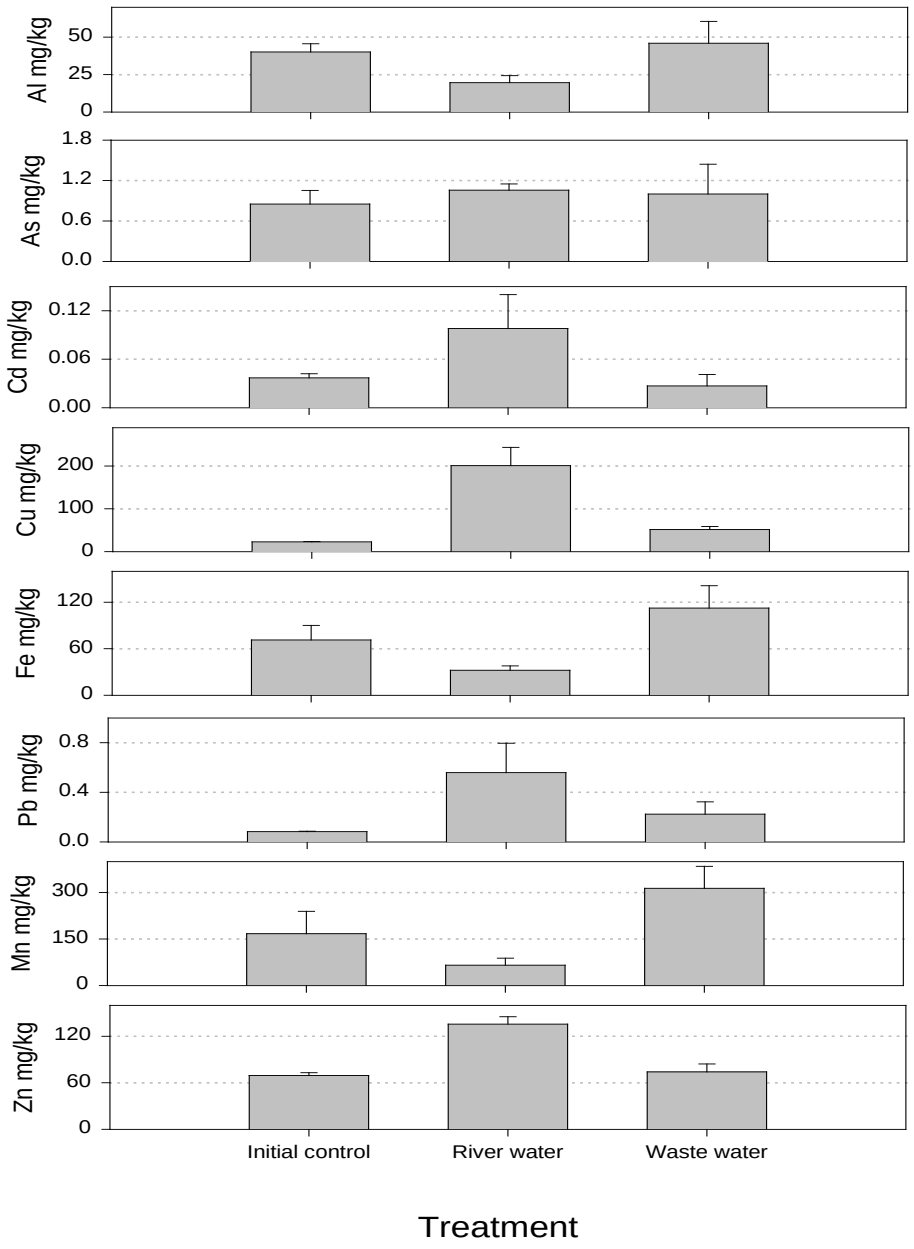


Figure 2: Concentration of Mn in major body parts of yabbies exposed to river water (control) and wastewater in the 1998 bioaccumulation trial.

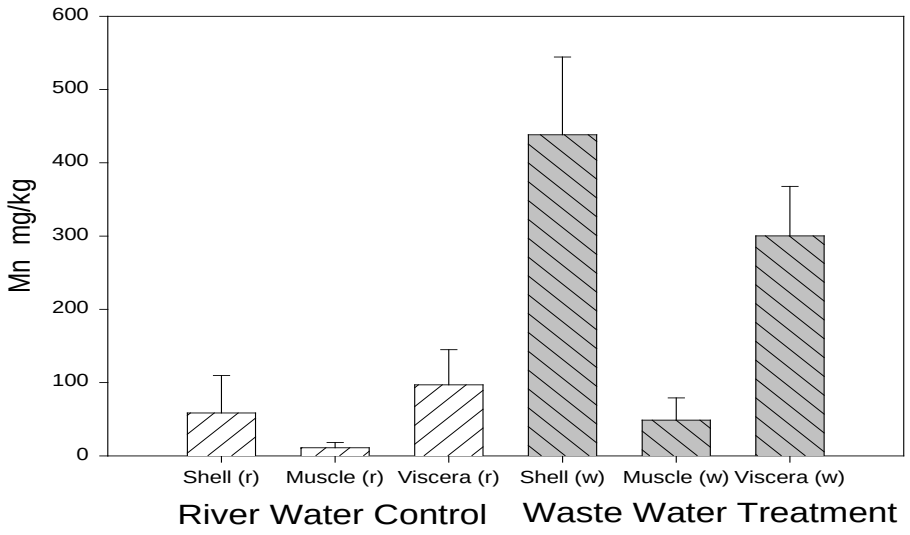


Figure 3: Oxidation of Mn^{2+} in unsterilised (closed circles) and sterilised (open circles) storage dam wastewater, to which; (A) no Mn^{2+} and (B) 1000 μg/L Mn^{2+} was added.

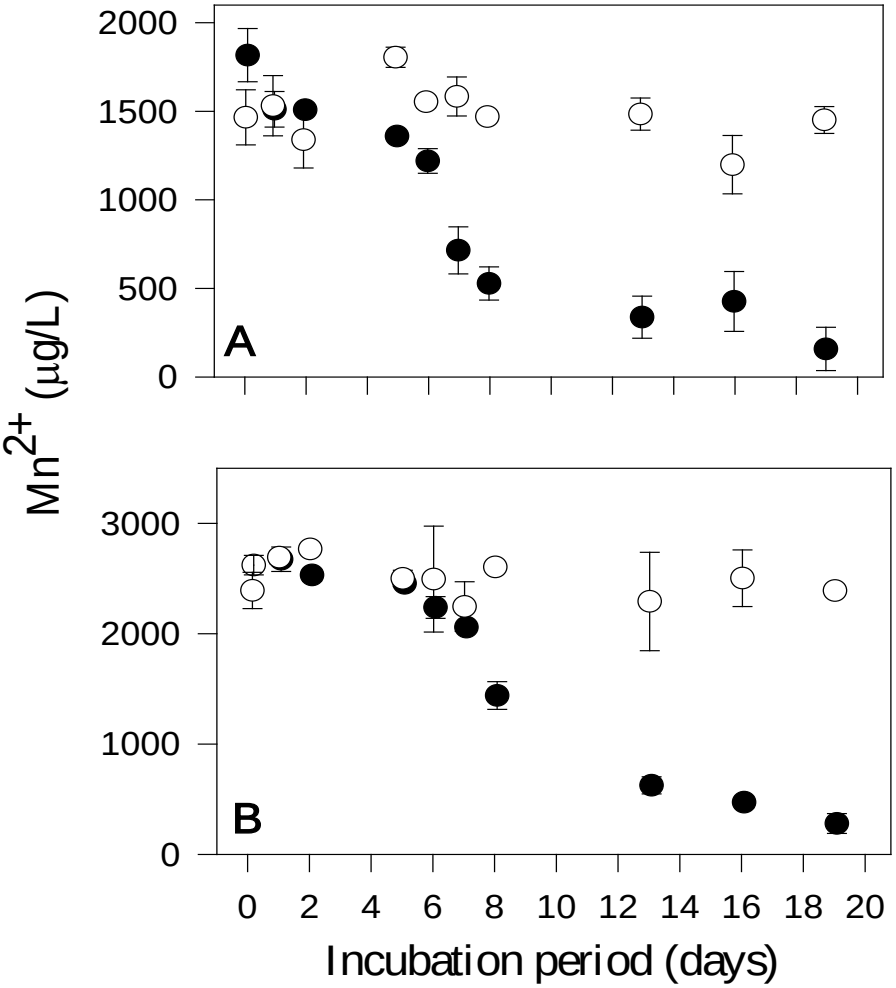
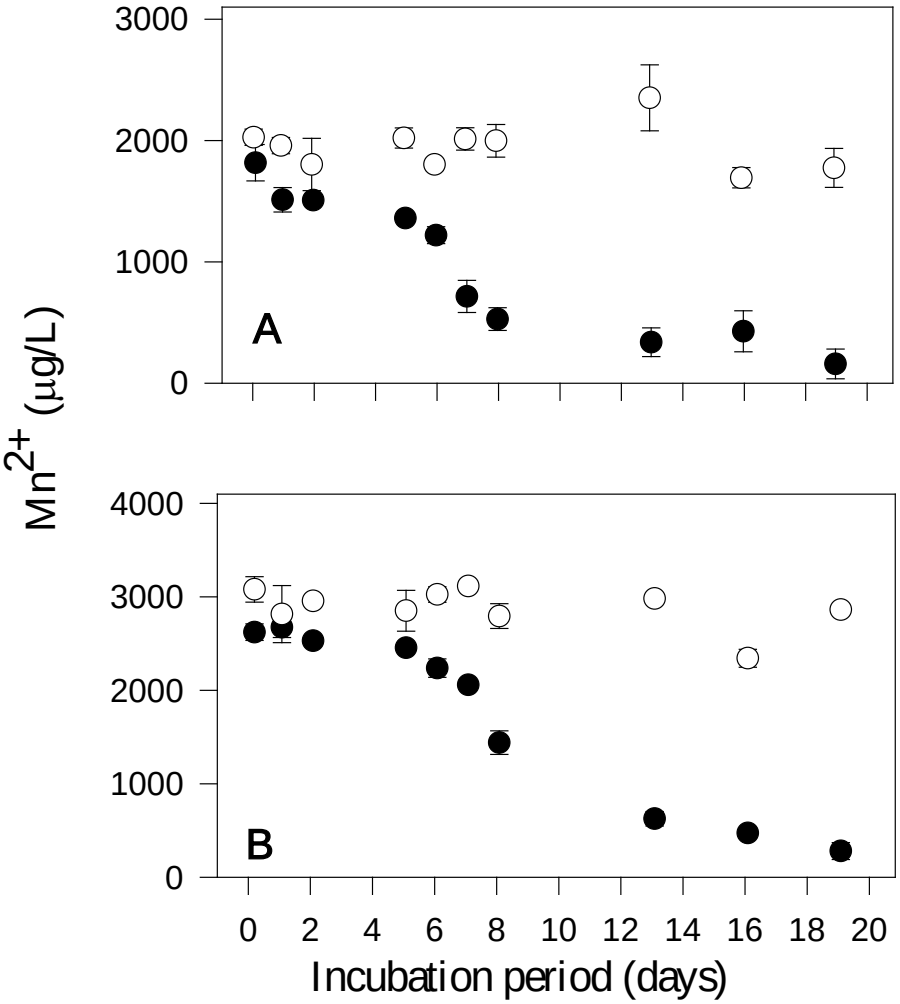


Figure 4: Oxidation of Mn^{2+} in unfiltered (closed circles) and filtered (open circles) storage dam wastewater, to which; (A) no Mn^{2+} and (B) 1000 μg/L Mn^{2+} was added.



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29 June, 2011

Our Ref: YH/6/21/1 and YH/6/21/3

Mr Jeff Lassman
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Dear Jeff,

1998 ANNUAL REPORT OF BIOLOGICAL MONITORING

Please find enclosed an unbound copy of the 1998 Annual Report of Biological Monitoring for Fletcher Challenge Paper, undertaken by The Murray-Darling Freshwater Research Centre. This Annual Report complies with the Licence Condition of bioaccumulation monitoring.

Please do not hesitate to contact me on 60582355 for any additional information.

Yours sincerely

Helen King

Scientific Officer

Enc. 1998 Annual Report.