

**1994 ANNUAL REPORT OF
CHEMICAL AND BIOLOGICAL
MONITORING FOR
AUSTRALIAN NEWSPRINT MILLS Ltd
ALBURY**

by

**The Murray-Darling Freshwater
Research Centre**

AUSTRALIAN NEWSPRINT MILLS
CHEMICAL AND BIOLOGICAL MONITORING
1994

1.0 INTRODUCTION

The Murray-Darling Freshwater Research Centre was contracted to undertake environmental chemical and biological monitoring of ANM's wastewater and its impact on the River Murray at Albury in accordance with specifications in the SPCC Licence No. 01272 sections W10 and W11 and NSW Department of Planning's 'Instrument of Consent' dated 26 June 1991, Condition 4 'Bioassay Testing and Environmental Monitoring Programmes'.

2.0 METHODS

2.1 ECOTOXICOLOGICAL AND BIO-ACCUMULATION MONITORING [W10]

2.1.1 Bioassay

Bioassay test methods are based on the standard methods defined by APHA (1989), OECD (1981), USEPA (1989) and USEPA (1991).

Laboratory bioassays were conducted on river water below ANM's point of discharge and on treated wastewater from three sources on site at ANM; the point of discharge from the water treatment plant into the four day holding pond (pond inlet), the point of exit from the four day holding pond (pond) and the final discharge, which includes wastewater from the holding pond as well as cooling water and treated sanitary wastewater (pond outlet). Control/dilutant water was obtained from the River Murray upstream of local point source discharges. All water samples were sieved to 90 micrometres to remove micro crustaceans.

Chironomus tepperi and *Daphnia carinata* acute (96 hour) toxicity tests, were run concurrently each month during 1994, using wastewater at concentrations ranging from 0.1% to 100%. *Daphnia carinata* chronic (21 day) toxicity tests, have been conducted every two months this year, in wastewater concentrations of 1% to 100%. Primary untreated effluent and potassium dichromate (30 - 80 ug/L) were used as reference toxicants.

2.1.2 Bioaccumulation

Bioaccumulation trials on Yabbies (*Cherax destructor*) were conducted using three 8 m³ concrete tanks on site at ANM. Tank 1 (the control) fed only by river water and Tanks 2 and 3 (test tanks) fed by a mixture of 50% river water and 50% pond outlet wastewater. 100 animals were added to each tank, approximately twenty of these were weighed and measured each month, and a smaller subsample removed every three months for whole body chemical assay. The whole samples were freeze dried then acid digested at The Murray-Darling Freshwater Research Centre's chemistry laboratory.

Bioaccumulation trials on Silver Perch (*Bidyanus bidyanus*) were conducted using six 90L flow through tanks housed in the laboratory. Three tanks fed by river water

and three fed by a mixture of 50% river water and 50% wastewater. Sampling was conducted as for *C.destructor* .

The samples were sent to Amdel Laboratories, Thebarton, SA, where three subsamples from each were assayed for metals. Elements assayed for were: aluminium, chromium, barium, iron, zinc, copper, cobalt, nickel, manganese, phosphorus, magnesium, arsenic, cadmium, lanthanum, molybdenum, silver, lead and yttrium.

2.2 RIVER ENVIRONMENT MONITORING SURVEYS [W11]

2.2.1 Water

Sample Collection and Handling

Grab samples were taken at three locations on the river on a monthly basis. Until 21 February 1994 site 1 samples were taken at a point approximately 1 km upstream of the ANM outfall (locally known as Grey's farm). Site 1 samples collected after this date were taken from Mungabareena Reserve (approximately 3 Km upstream of the outfall. The change in sampling location was necessary because of severe bank erosion at the grey's farm site. Site 2 samples were taken at a point approximately 200 m downstream of ANM's outfall (adjacent to the railway bridge). Site 3 samples were taken at a point approximately 1 km downstream of ANM's outfall (adjacent to Union Bridge).

5 samples were taken at each location (for analysis of physical parameters, phosphorus, forms of nitrogen, metals and mercury respectively). All samples were collected and preserved in accordance with Australian Standards AS2031.1 and AS2051 - all preservatives were "ANALAR" grade or better and, clean polyethylene gloves were worn at all times. Sampling blanks were handled and analysed in a similar manner to the samples.

Analysis of water samples.

All metal analyses (except for mercury analyses) were performed by :-
EML (Chem) Pty Ltd
425 -427 Canterbury Road
Surrey Hills Vic 3127.

The parameters determined were extractable:-

Aluminium, Cadmium, Cobalt, Chromium, Copper, Iron, Manganese, Lead and Zinc.

Analyses for total Mercury were performed by :-
Australian Newsprint Mills Ltd
Boyer Tas 7140.

Physical and nutrient analyses were performed at the Murray-Darling Freshwater Centre (MDFRC). Turbidity, Colour, Specific Conductance, Total Dissolved Solids, Ammonia, Nitrate, Nitrite, Organic Nitrogen, Total Phosphorus were determined according to the methods outlined in the MDFRC Chemistry laboratory's methods manual.

2.2.2 Sediment.

Sample Collection and Handling.

A series of forty sediment samples were taken on the 9th of June 1994. Sediment samples were collected from two deposition zones approximately equidistant (ca 500 m upstream and ca 500 m down stream) of ANM mills outfall. Samples were collected at 10 metre intervals along the 60 cm depth contour (approximately 2 metres from, and parallel to, the river bank). A total of 20 samples were taken above and 20 samples taken below the outfall.

Approximately the top 5 - 10 cm of sediment was directly scooped into 500 mL wide mouthed polyethylene bottles which had previously been acid washed (5% HCl) and repeatedly rinsed with Milli-Q water. Sampling was such that every effort was made to completely fill the sampling bottle with sediment. The bottle was sealed while under water to minimise the loss of fine material.

The samples were immediately returned to the laboratory and air dried. The air dried samples were sieved (2 mm) - the fraction retained by the sieve was weighed and then discarded, the fraction passing through the sieve was weighed and then thoroughly mixed. All subsequent analysis were performed only on the sieved fraction (Grimshaw 1989).

Particle Size Analysis.

Approximate particle fractionation was carried out on all samples. Fractionation was by the method described by Grimshaw (1989). Essentially, that portion of the sample which was retained by a 2 mm sieve was considered gravel. The portion of the sample that passed through a 2 mm sieve was considered a mixture of silt, clay and sand.

The percentage of silt + clay in this fraction was determined by the 4 minute 48 second hydrometer method described by Grimshaw (1989). The sand content was estimated by difference. No distinction between silt and clay content, or fine sand and coarse sand content was attempted.

Analysis of Acid Extractable Metals.

The fraction of acid extractable metals in the samples was determined by a modification of the method of Anon (1989). 5 g of sediment was accurately weighed into 50 mL polyethylene centrifuge tubes (which had previously been washed with 5% HCl and extensively rinsed with MILLI - Q water). 25 mL of 0.1 M "ARISTAR" grade HCl was subsequently added to the sediment. The tubes were then capped and placed on a Ratek orbital shaking table for one hour. The samples were allowed to settle overnight and, subsequently filtered through acid washed Whatman GF/C filters. The filtrate was placed in 100 mL polyethylene bottles (which had previously been washed with 5% HNO₃ and repeatedly rinsed with MILLI-Q water) and dispatched to :-

Amdel Laboratories,
Brown St, Therbaton

South Australia,

for analysis by Inductively Couple Plasma Atomic Emission Spectroscopy (ICP-AES). The elements assayed for were aluminium, arsenic, barium, boron, cadmium, calcium, chromium, cobalt, copper, iron, lanthanum, lead, magnesium, manganese, molybdenum, nickel, silica, silver, strontium, tin, yttrium and zinc. An extraction blank and a standard reference material (Buffalo River sediment - SRM 2707) were processed in exactly the same manner as the samples.

Analysis for Total Mercury.

Approximately 10 g of air dried sample was placed in clean polyethylene bags and dispatched to :-

Australian Newsprint Mills Ltd
Boyer Tas 7140.

for digestion and subsequent analysis by Cold Vapour Generation Atomic Absorption Spectroscopy.

Analysis for Total Nitrogen.

Total nitrogen was determined by a modification of the technique of Hosmoi and Sudo (1986). Approximately 0.25 g of sediment was accurately weighed into acid washed 50 mL centrifuge tubes. 10 mL of an alkaline persulfate digestion medium (0.9 % NaOH, 4.0 % K₂S₂O₄) was added to each tube. The tubes were sealed and subsequently heated in an autoclave for one hour. The solution was analysed for nitrate by an automated version of the cadmium reduction method (Clesceri *et al* 1989). All analyses were done at least in duplicate.

Analysis for Exchangeable Phosphorus.

Exchangeable phosphorus was determined by a modification of the method of Anon (1982). About 5 g of sediment was accurately weighed into 50 mL acid washed centrifuge tubes. The sediment was extracted into 25 mL of a 0.5 M sodium bicarbonate solution (pH adjusted to 8.5 with NaOH). The level of soluble reactive phosphate in the extractant was determined by an automated version of the ascorbic acid method (Clesceri *et al* 1989).

2.2.3 Biota

Macroinvertebrates

Macroinvertebrate samples were collected using artificial substrates from six paired sites on the River Murray (Figure 1) according to the method set out in Bennison *et al.* 1989. Monthly sampling continued in 1994. Samples were sorted into taxonomic groups, counted and compared statistically by site using cluster analysis in "Statistica".

Fish

Fish sampling was conducted bi-annually, under low flow and high flow conditions at the above sites, using 10 bait nets at each site, set for 12 hours and using a chemical light as an attractant.

3.0 RESULTS AND DISCUSSION

3.1 ECOTOXICOLOGICAL AND BIO-ACCUMULATION MONITORING [W10]

3.1.1 Bioassay

A summary of acute toxicity test results in which animals in the test solutions reacted differently to those in the control solutions is provided in Table 1. A summary of chronic toxicity test results in which the reproductive capacity of the animals was significantly different ($p < 0.05$, Students t-Test) when compared with the control is provided in Table 2. Significant adverse reactions occurred almost exclusively in 100% wastewater solutions. ANM wastewater was not toxic to *C.tepperi* in any of the tests conducted.

Fish Ventilation Monitoring

The fish ventilation monitor, which measures stress on fish by detecting changes in electrical potential between two passive electrodes caused by opercular movement, is operational on eight channels. Validation of the system was conducted using primary untreated effluent at the EC50 (mortality) concentration for *D.carinata*.

Bioaccumulation

The 1993 Yabby (*Cherax destructor*) bioaccumulation trial commenced in April 1993 and continued through to December 1993. The results of the metals assays are presented in Table 3 for the eight month trial. Arsenic, molybdenum, chromium, cadmium, lead, cobalt, silver, lanthanum, nickel, and yttrium were only recorded at levels close to the detection limit for all samples. Barium and Aluminium data were highly variable both within and between treatments. Zinc, iron, copper and phosphorus data showed no difference between treatments over time, however manganese increased significantly over time in the test animals exposed to 50% ANM wastewater.

The 1994 *C.destructor* trial was conducted from January to July 1994. The results of the metals assays are presented in Table 4 for the six month trial. Arsenic, molybdenum, chromium, cadmium, lead, cobalt, silver, lanthanum, nickel and yttrium were only recorded at levels close to the detection limit for all samples. Barium and aluminium data were highly variable both within and between treatments. Zinc, iron, copper, magnesium and phosphorus data showed no difference between treatments over time, however manganese increased over time in the test animals exposed to 50% ANM wastewater.

A new *C.destructor* trial commenced in July 1994

The 1994 Silver Perch (*Bidyanus bidyanus*) trial commenced in March and was terminated in May 1994. The results of the metals assays for the two month trial are presented in Table 5. Arsenic, molybdenum, chromium, cadmium, lead, cobalt, silver, lanthanum, nickel and yttrium were only recorded at levels close to the detection limit for all samples. Barium, aluminium, manganese, zinc, iron, copper, magnesium and phosphorus data were highly variable both within and between treatments.

There were no differences in growth between treatments for any of the trials (reported in quarterly reports)

3.2 RIVER ENVIRONMENT MONITORING SURVEYS [W11]

3.2.1 Water

A summary of the water quality data is presented in Figure 2. The figure shows the variation of metals (manganese, iron, aluminium, and zinc), nutrients (total phosphorus, ammonia, organic nitrogen and oxides of nitrogen), and, physical parameters (turbidity, conductivity, colour and total dissolved solids) between the three sites over time. (Site 1 samples are represented by open circles, site 2 samples are represented by closed circles and site 3 samples are represented by open triangles; lines are included for only for clarity and no interpolation between data points is intended.) The data for 1993 has also been included for purpose of comparison. The figure does not include those analytes not detected in any of the samples or, those whose levels remained very close to their detection limit. Cadmium (0.001 mg/L), cobalt (0.006 mg/L), chromium (0.02 mg/L), lead (0.03 mg/L) and mercury (0.0002 mg/L) were not detected in any of the samples (detection limits in brackets). Copper (detection limit of 0.004 mg/L) was detected on five occasions - site 1 on the 22/11/93 (0.11 mg/L), sites 1 and 3 on the 24/1/94 (0.007 mg/L at both sites), site 2 on the 21/3/94 (0.005 mg/L), and site 3 on the 22/8/94 (0.005 mg/L).

Generally, most of the data show little (if any) variation between sites although, there may be significant variation over time (seasonal effects). From the figure it is evident that iron, manganese, total phosphorus, turbidity, conductivity, colour, total dissolved solids, and oxides of nitrogen (NO_x) vary little between sites. The levels of ammonia and organic nitrogen, seem to be more variable between sites - possibly reflecting variable inputs of organic matter to the river from what is essentially a rural riverine environment. Relatively high levels of zinc were detected at site 1 on the 22/11/9, at site 2 on the 19/9/94 and at site 3 on the 22/8/94. The cause of these spikes is unknown.

3.2.2. Sediments

Arsenic, cobalt, molybdenum and silver were not detected in any of the sediment samples either upstream or downstream of the outfall (detection limit 0.05 mg/kg). Tin, cadmium and boron were not detected in any of the upstream samples; tin was detected in one downstream sample (0.1 mg/kg), boron was found in six downstream samples (range 0.05 - 0.4 mg/kg, average 0.15 mg/kg) and cadmium was found in twelve downstream samples (range 0.05 - 0.15 mg/kg, average 0.1 mg/kg). Chromium was present in one upstream sample (0.05 mg/kg) and six downstream samples (range 0.05-0.35 mg/kg, average 0.15 mg/kg). Mercury was present in fourteen of the upstream samples (range 2 - 3 µg/kg, average 2 µg/kg) and eleven downstream samples (range 2 - 6 µg/kg, average 4 µg/kg).

The results for the sediment analyses for total persulphate nitrogen, exchangeable phosphorus and acid extractable aluminium, barium, calcium, copper, iron, lanthanum, lead, magnesium, manganese, nickel, silica, strontium, yttrium and zinc are summarised in Figure 3. For each analyte a box plot showing the distribution above and below the outfall is presented. The solid horizontal lines of the box plot represent the 10th, 25th, 50th, 75th and, 90th percentiles of the data - the box itself represents the 25th to 75th percentile. All data outside the 10th and 90th percentiles are shown as open circles on the plots. The mean of the data is represented by a dotted line. From the figure it is clear that almost all of the downstream samples have analyte concentrations greater than those of the upstream sites. For all of these analytes, with the exception of nickel and strontium, there is a statistically significant difference ($p < 0.05$; Student's t-test) between the mean concentration in the upstream and downstream samples

Sediment characteristics.

The crude sediment characteristics (% gravel, % sand, and % silt and clay,) of the upstream and down stream sample sites are summarised in Figure 3. As in last years survey, the sediments predominantly consisted of sand and gravel with little silt or clay. The level of silt and clay was lower than the level found in the 1992 survey and similar to that found in the 1993 survey.

Chemical characteristics of the sediment.

A summary of the chemical characteristics of the sediment samples taken upstream and down stream of ANM's outfall are also presented in Figure 3. An extensive series of elements were determined. These included total persulfate nitrogen, exchangeable phosphorus and acid extractable aluminium, arsenic, barium, boron, cadmium, calcium, chromium, cobalt, copper, iron, lanthanum, lead, magnesium, manganese, molybdenum, nickel, silica, silver, strontium, tin, yttrium and zinc

From the figure it can be seen that the down stream sediments tend to have a greater distribution in the concentration of analytes than the upstream sites and, the mean concentration for the down stream sites tends to be significantly higher than for the upstream sites.

Figure 4 shows the distribution of iron along the upstream and downstream transects. From the figure it can be seen that the upstream transect has an essentially uniform distribution of iron. This is not the case for the downstream transect. The early stations (closer to the outfall) appear to have a similar range of iron concentrations as that of the upstream transect. However, after approximately 90 metres along the transect there is a significant rise in the iron concentration. Most of the other analytes studied show a similar distribution pattern. Unlike last years survey (where the presence of a snag along the transect confounded the data analysis) there is no obvious difference in the physical composition of the sediment (for example more silt and clay or higher gross organic matter content) to account for the observed distribution.

It should be noted that the railway bridge immediately upstream of the start of the lower transect was being painted at the time of sampling. One possible hypothesis to account for the distribution of analytes along the transect is that the rise in concentration represents the beginning of a depositional zone (either water or airborne) for material derived from the bridge. Given the available data it is not possible to test this hypothesis.

3.2.3 Biota

Macroinvertebrates

The 1993 monthly macroinvertebrate data are analysed seasonally in the average linkage dendrogram using Euclidean Distance metric (Figure 5) which clusters similar samples by comparing taxonomic data. The dendrogram shows no biological differences between sites with limited grouping according to sampling dates. The samples for 1994 are currently being identified and analysed.

Fish

Fish surveys of the River Murray in 1994 yielded no data. After discussion at the ANM Monitoring Program Annual Review, the sampling method has been changed from bait nets to cylindrical bait traps for 1995 and will continue to comprise intensive sampling (10 traps) at the six sites bi-annually, under low flow and at high flow conditions.

4.0 REFERENCES

- Anon, 1982. "Chemical Analysis of Polluted Soils" Victorian Environment Protection Authority Publication 139.
- APHA 1989. "Standard Methods for the Examination of Water and Wastewater" 17th Edition, Clesceri, L.S., Greenburg, A.E. and Trussel, R.R. (Eds), American Public Health Association Washington.
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- Hosmoi, M. and Sudo, R. 1986. Simultaneous determination of total nitrogen and total phosphorus in freshwater samples using persulfate digestion. *Intern. J. Environmental Studies* 27, 267-275.
- OECD 1981. "OECD Guidelines for Testing of Chemicals" (Adopted 12 May 1981).
- USEPA 1989. "Short-term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms" (2nd Edition) EPA-600/4-89/001.
- USEPA 1991. "Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms" (4th Edition) EPA-600/4-90/027.

Figure 2: A summary of the water quality data for 1993 and 1994. The figure shows the variation of metals (manganese, iron, aluminium, and zinc), nutrients (total phosphorus, ammonia, organic nitrogen and oxides of nitrogen), and, physical parameters (turbidity, conductivity, colour and total dissolved solids) between the three sites over time. Site 1 samples are represented by open circles, site 2 samples are represented by closed circles and site 3 samples are represented by open triangles; lines are included for only for clarity and no interpolation between data points is intended.

Figure 3: Box plots for the distribution of total persulphate nitrogen, exchangeable phosphorus and acid extractable aluminium, barium, calcium, copper, iron, lanthanum, lead, magnesium, manganese, nickel, silica, strontium, yttrium and zinc in the sediment from transects above and below ANM's outfall.

Figure 4: Distribution of Iron along the upstream and downstream transects.

Figure 5: Seasonal analysis of the 1993 monthly macroinvertebrate data in the River Murray from paired sites - sites 1 and 2 downstream, sites 3 and 4 mixing zone and sites 5 and 6 (control) upstream from ANM wastewater discharge.

Table 1: 1994 summary of significant results for acute toxicity bioassays where :
 PI = ANM pond inlet; P = ANM pond; PO = ANM pond outlet (final outfall); Murray
 = River below ANM outfall.

TEST	WATER	MONTH
<i>D.carinata</i> 96h	PI 100% (48h)	March
<i>D.carinata</i> 96h	PI 100% (72h)	April
<i>D.carinata</i> 96h	Murray (24h)	May
<i>D.carinata</i> 96h	PO 100% (48h) PI 100% (72h) P 100% (96h) P 10% (96h)	July
<i>D.carinata</i> 96h	PI 100% (72h) P 100% (72h) Murray (72h) PO 100% (96h)	August

NOTE: No significant results from *C.tepperi* tests.

Table 2: 1994 summary of significant results for *Daphnia carinata* chronic toxicity bioassays where : PI = ANM pond inlet; P = ANM pond; PO = ANM pond outlet (final outfall); Murray = River below ANM outfall; < = Inhibitory effect on reproduction potential and > = stimulatory effect on reproductive potential.

TEST	WATER	MONTH
<i>D.carinata</i> 21day	PI 100 % (<) P 100% (<) P 10% (>)	January
<i>D.carinata</i> 21day	PO 100% (<) PI 1% (>) PI 0.1% (>) P 10% (>) PO 1% (>)	March/April
<i>D.carinata</i> 21day	P 100% (>) P 10% (>) PO 10% (>) Murray (>)	May
<i>D.carinata</i> 21day	P 100% (>) P 10% (>) PO 100% (>)	July
<i>D.carinata</i> 21day	PI 100% (<) PO 100% (<)	August/September

Table 3: Yabby eight month bioaccumulation trial 1993 - metals assay data; where CON1 = control tank 1, TEST2 = 50% ANM wastewater tank 2 and TEST3 = 50% ANM wastewater tank 3.

Table 4: Yabby six month bioaccumulation trial 1994 - metals assay data; where CON1 = control tank 1, TEST2 = 50% ANM wastewater tank 2 and TEST3 = 50% ANM wastewater tank 3.

Table 5: Silver perch two month bioaccumulation trial 1994 - metals assay data; where CON2, CON5 and CON6 = control tanks 2, 5 and 6 respectively and TEST1, TEST3 and TEST4 = 50% ANM wastewater tanks 1,3 and 4 respectively.

16 December 1994

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

Mr Ralph Coghill
Technical Services Manager
Australian Newsprint Mills Limited
Private Bag
LAVINGTON NSW 2641

Dear Ralph

1994 ANNUAL REPORT - BIOLOGICAL AND CHEMICAL MONITORING

Please find enclosed an unbound copy of the 1994 Annual Report outlining the monitoring undertaken by The Murray-Darling Freshwater Research Centre for Australian Newsprint Mills Limited. This Annual Report complies with Licence Condition W16 on the ecotoxicological and bio-accumulation monitoring and the river environment monitoring surveys.

Please do not hesitate to contact me on 43 1002 for any additional information.

Wishing you and your staff a Merry Xmas and a Happy New Year.

Yours sincerely

H M KING

Scientific Officer

Enc. 1994 Annual Report.

Disk copy of text and Figure captions.

TOXICITY TEST PROTOCOLS

APHA 1989. Standard Methods for the Examination of Water and Wastewater, 17th Edition, Clesceri, L.S., Greenburg, A.E. and Trussel, R.R. (Eds), *American Public Health Association*, Washington, USA.

ASTM 1990. Annual Book of Standards. Section 11 (Water and Environmental Technology), Volume 11.04 (Pesticides; Resource Recovery; Hazardous Substances and Oil Spill Responses; Waste Disposal; Biological Effects). *American Society for Testing and Materials*, Philadelphia, USA.

OECD 1981. OECD Guidelines for Testing of Chemicals. *Organisation for Economic Co-operation and Development*, Paris, France. (Adopted 12 May 1981).

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MACROINVERTEBRATE SAMPLING METHOD

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