Technical Memorandum 11

In Situ Experiments to Determine the Uptake of Copper by the Aquatic Macrophyte *Najas tenuifolia* R.Br.

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Hart, B.T., Jones, M.J. and Breen, P. (1984). *In situ* experiments to determine the uptake of copper by the aquatic macrophyte *Najas tenuifolia* R.Br. Supervising Scientist for the Alligator Rivers Region, Technical Memorandum 11.

In situ experiments, in which ionic copper was added to an enclosed area of the aquatic macrophyte *Najas tenuifolia* R.Br., showed that this plant can rapidly (in around six hours) take up considerable amounts of the added copper. Epiphytes (and their associated microfauna) present on this macrophyte took up the added copper even more rapidly, but also seemed to release the copper equally fast. At the end of the three day experiment 30% to 60% of the added copper was associated with the macrophytes and 15% to 20% was still in the water column. The remainder of the copper was associated with the 'epiphyte component', the sediments and probably also the walls of the plastic enclosure.

1 INTRODUCTION

There are two major uranium deposits, Ranger and Jabiluka, in the Magela Creek catchment in the Alligator Rivers Region of northern Australia. The Ranger deposit is currently being mined but government approval has not been granted to permit mining of the Jabiluka deposit. Before approval of the Ranger development, an extensive public inquiry was held into the environmental implications of uranium mining and ore-processing in the Alligator Rivers Region (Fox et al. 1977). This inquiry identified the release of toxic concentrations of metals from the mine sites as one of the main potential causes of adverse impacts from the operations. The report suggested that the billabongs and the flood plain would be likely sites for the deposition of any released metals.

The Magela Creek catchment (area 1565 km^2) (Fig. 1), has a monsoonal climate with a very distinct Wet Season between December and May, and a Dry Season for the remainder of the year. In its upper reaches the creek is confined to a sandy, 'braided' channel and starts to flow soon after the first substantial rains. To the north (i.e. downstream) of Mudginberri, the creek runs through a broad flood plain which, in most years, is inundated between January and May. The flood plain (area around 230 km^2) sustains a very large biomass of macrophytes (Williams 1979; Pancontinental 1981).

Aquatic plants are known to take up (and release) heavy metals present in water at trace concentrations (Hutchinson 1975; Stuckey 1975; Welsh and Denny 1976; Wells et al. 1980; Schierup and Larsen 1981; Larsen and Schierup 1981). In the case of floating macrophytes, uptake must occur directly from the water column. With rooted macrophytes, such as *Nymphoides*, *Najas* and *Phragmites*, uptake may also occur from the sediments via the root system. Depending upon both the metal and the macrophyte species, heavy metals may be accumulated in different parts of the plants. For example, Schierup and Larsen (1981) found that copper, cadmium and lead accumulated mainly in the roots of *Phragmites* growing in sewage-polluted lakes in Denmark. Zinc accumulated in both the roots and the aerial parts of the plants. It has also been reported that some macrophytes can excrete heavy metals, presumably in the form of organic complexes. Once macrophytes die some of the associated heavy metal is released back to the water column and the rest is incorporated into the sediments.

In view of the large biomass of macrophytes known to exist on the Magela Creek flood plain during each Wet Season, it is important to know the role they play in the cycling of any metals that may be added to the system. In this report we discuss the results of an *in situ* experiment, run in Gulungul Billabong in March 1981, which aimed to provide information on the ability of one macrophyte, *Najas tenuifolia* R.Br., to take up copper.

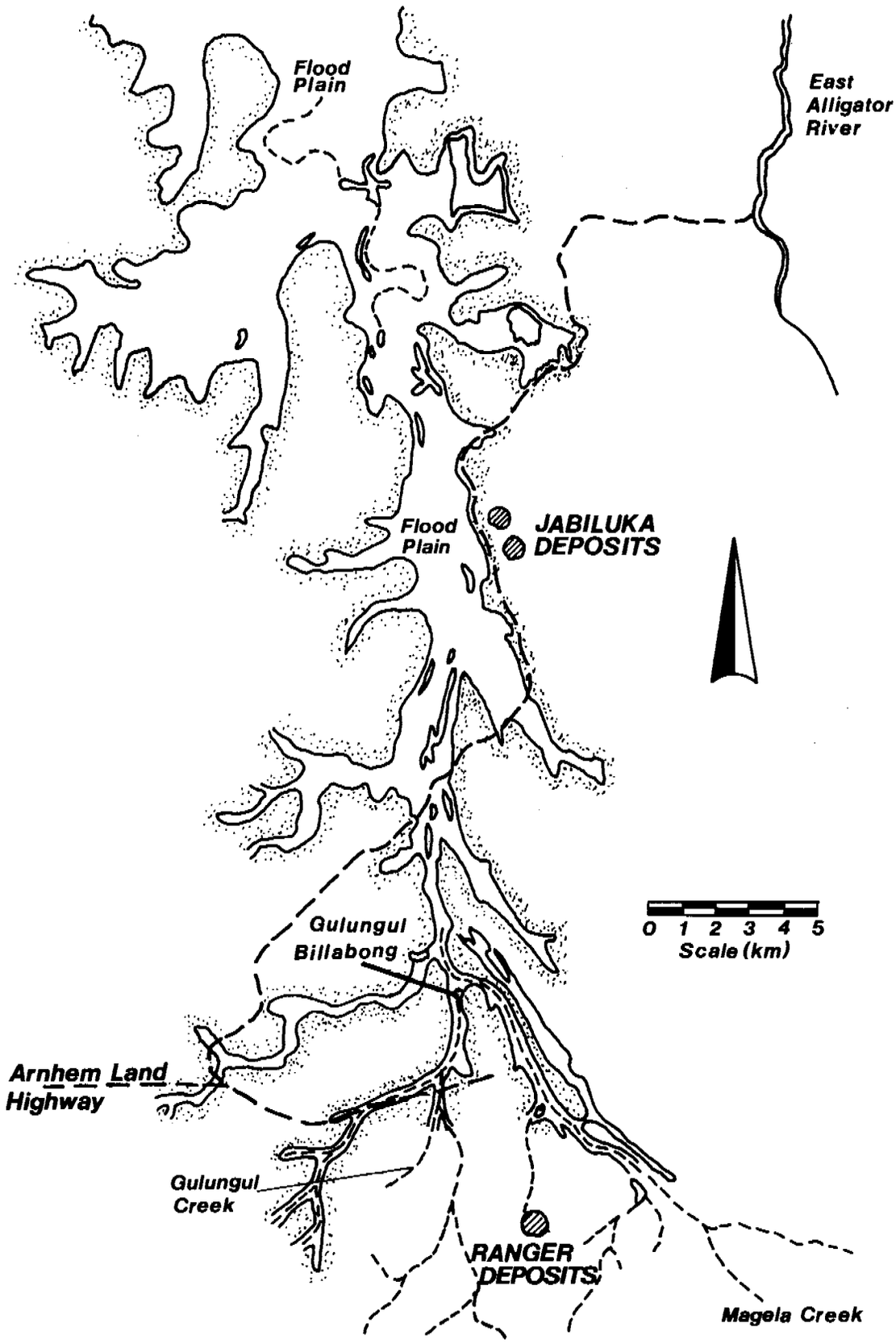

FIGURE 1 MAGELA CREEK SYSTEM.

2 METHODS

2.1 Setting up the Enclosure

An area of macrophytes in Gulungul Billabong (Fig. 1) was isolated by means of a rectangular, open-ended enclosure consisting of a metal frame supporting sides made of clear plastic. The frame measured 1 m x 1 m x 1.5 m. The plastic walls (1.3 m high) were attached to the metal frame by Selley's 'Kwik-Grip' adhesive. The enclosure was positioned in the billabong on a slight slope and maximum and minimum depths were measured. The depth of water in the enclosure was around 85 cm and varied as shown in Table 1.

TABLE 1 DEPTH OF WATER IN THE ENCLOSURE.

	Depth (cm)			
Date	19.3.81		20.3.81	21.3.81
Time	0900 h	1905 h	0800 h	0850 h
maximum	90	89	88	85
minimum	83	82	81	79

The enclosure was placed in position at 1650 h on 18 March 1981, and as the sediments and epiphytes were disturbed by this, the structure was allowed to settle overnight. The site chosen was approximately 10 m from the water's edge and was in the vicinity of a depth gauge board. The area had a dense growth of *Najas tenuifolia* with a large amount of associated epiphytic growths.

Copper was added, in the form of $Cu(NO_3)_2$ dissolved in 5 L of water (Milli-Q, acidified with 2 mL of concentrated Suprapur HNO_3), within 2 hours of the solution being prepared. To ensure even distribution of the added copper, a length of clean PVC pipe, through which a number of holes had been drilled, was placed in the enclosed water and approximately 500 mL of the solution poured into it. This was repeated at different positions within the enclosure until the complete amount had been added (0856 h on 19 March 1981). The amount of copper added (8.1 mg) was sufficient to raise the concentration of copper in the enclosure to around 11 µg/L.

2.2 Sampling Program

Water samples were taken from both inside and outside the enclosure and dissolved oxygen, pH, alkalinity and temperature were measured (Table 2).

Water samples were taken (by submerging acid-washed, 500 mL polypropylene bottles) at a depth of either 10 cm ('surface sample') or approximately 50 cm ('bottom sample') below the water surface. Each water sample was divided into two parts; one was acidified with the equivalent of 5 mL of Suprapur HNO_3 per 1 L of water, and the other part was filtered through an acid-washed 0.4 µm Nuclepore membrane filter and then acidified with HNO_3.

Both samples were then analysed for copper, the former giving the total concentration and the latter giving the concentration of filterable (soluble) copper. Both filtered and unfiltered samples were stored in acid-washed polypropylene bottles until analysis, at the Water Studies Centre laboratory in Melbourne, could be carried out. Unfortunately several of the bottles containing filtered samples were damaged and the samples contaminated before analysis could be carried out.

Samples of epiphytes were removed from the host plant by suction; a Pasteur pipette connected via plastic tubing to a hand-operated Nalgene vacuum pump was used. The sample containing the epiphytes and approximately 100 mL of water was transferred from the 1 L receiving flask to a 100 mL acid-washed polypropylene bottle. The epiphytes were collected by filtering the sample through a weighed, acid-washed 0.4 µm Nuclepore filter which was then dried at 105°C. Reweighing of the filter gave the dry weight of epiphytes collected.

Macrophyte samples were taken from near the surface by cutting off a portion with secateurs and shaking off any loosely associated epiphytes. The samples were stored in acid-washed 100 mL polypropylene bottles.

Sediment samples were taken with a 10 cm diameter perspex tube. Due to the dense plant growth and associated root systems it was very difficult to obtain a sample at a depth greater than 3 cm. All samples were stored in plastic bags in a freezer until analysis could be carried out.

2.3 Analytical Methods

Alkalinity and concentration of dissolved oxygen were determined using APHA standard methods (Sections 403 and 422B respectively, APHA 1975). Copper was determined using either atomic absorption spectroscopy with electrothermal furnace (Varian Techtron CRA 90) or anodic stripping voltammetry (ASV) (PAR 174 with hanging mercury drop electrode). In each case calibration was achieved by the technique of standard additions. Before analysis by ASV, water samples were digested by gently boiling the acidified samples for 10 minutes. Epiphyte and macrophyte samples were digested using hydrogen peroxide and nitric acid as outlined in Hart and Thomas (1984).

TABLE 2 DISSOLVED OXYGEN, pH, TEMPERATURE AND ALKALINITY.

Date	Time	Outside Enclosure				Inside Enclosure			
		surface		bottom		surface		bottom	
	(h)	DO[a]	°C	DO	°C	DO	°C	DO	°C
19.3.81	1100	8.4		7.7		6.1		5.9	
	1900	8.7		7.1		8.9		8.7	
20.3.81	1100[b]		31.5		30.0		32.2		30.0
	1800	8.9		8.2		9.8		9.8	
21.3.81	1850		33.6		31.7		34.0		31.7

[a] Dissolved oxygen (mg/L).
[b] 1100 h: outside enclosure - alkalinity 4.8 mg/L, pH 8.06
inside enclosure - alkalinity 4.8 mg/L, pH 8.08

3 RESULTS AND DISCUSSION

The concentrations of copper found in water (surface and bottom), epiphyte and macrophyte samples are given in Table 3 and shown also in Figure 2.

Some effort was taken to ensure that the added copper was evenly distributed in the enclosure. The similarity in the concentrations of the filterable copper in surface and bottom waters (5.9 µg/L and 4.9 µg/L respectively) sampled just after the copper solution was added, suggests that this operation was successful.

The concentration of total copper in the surface water increased to 18 µg/L immediately after addition of the copper solution, then decreased rapidly in the next 4 hours, and, apart from a small increase after approximately 10 hours, continued to decrease over the course of the experiment to about 3.6 µg/L (Fig. 2). From the amount of copper added to the enclosure, it can be calculated that the copper concentration before any uptake should have been about 11 µg/L. The higher-than-expected concentration of total copper in the surface water could have been due to the presence of epiphytes, containing some newly sorbed copper, that were dislodged when the copper solution was added.

Due to problems outlined in Section 2, there are fewer data for the concentrations of filterable copper in the water samples. However, the data that are available show a trend similar to that of the total copper, but without the marked variations. The concentration of filterable copper

TABLE 3 CONCENTRATIONS OF COPPER IN THE SAMPLES.
Asterisks denote samples contaminated during transport.

Location	Date	Time	Water (µg/L)				Epiphyte	Macrophyte
		(h)	total		filterable		(µg/g dry wt)	
			S[a]	B[b]	S	B	S	S
Outside	18.3	1655	0.84	-	-	-	-	-
Enclosure	19.3	0846	3.0	-	2.6	-	2.4	-
	20.3	1100	2.0	2.4	0.49	0.64	11	8.5
Inside	18.3	1056	2.5	-	-		-	-
Enclosure	19.3[c]	0850	1.9	3.7	1.6	2.3	4.4	14
		0900	18	5.4	5.9	4.9	12	26
		1120	8.3	4.4	4.8	*	33	26
		1305	4.7	4.2	*	*	15	31
		1500	5.8	6.7	*	5.0	13	42
		1700	5.5	6.7	*	4.5	12	40
		1900	8.9	4.3	3.9	*	15	33
	20.3	0805	3.5	3.3	2.1	*	19	50
		1800	3.5	2.5	*	*	16	34
	21.3	1840	3.8	4.0	1.5	2.4	14	41

[a]Surface; [b]Bottom; [c]Copper solution was added at 0856 h on 19.3.81.

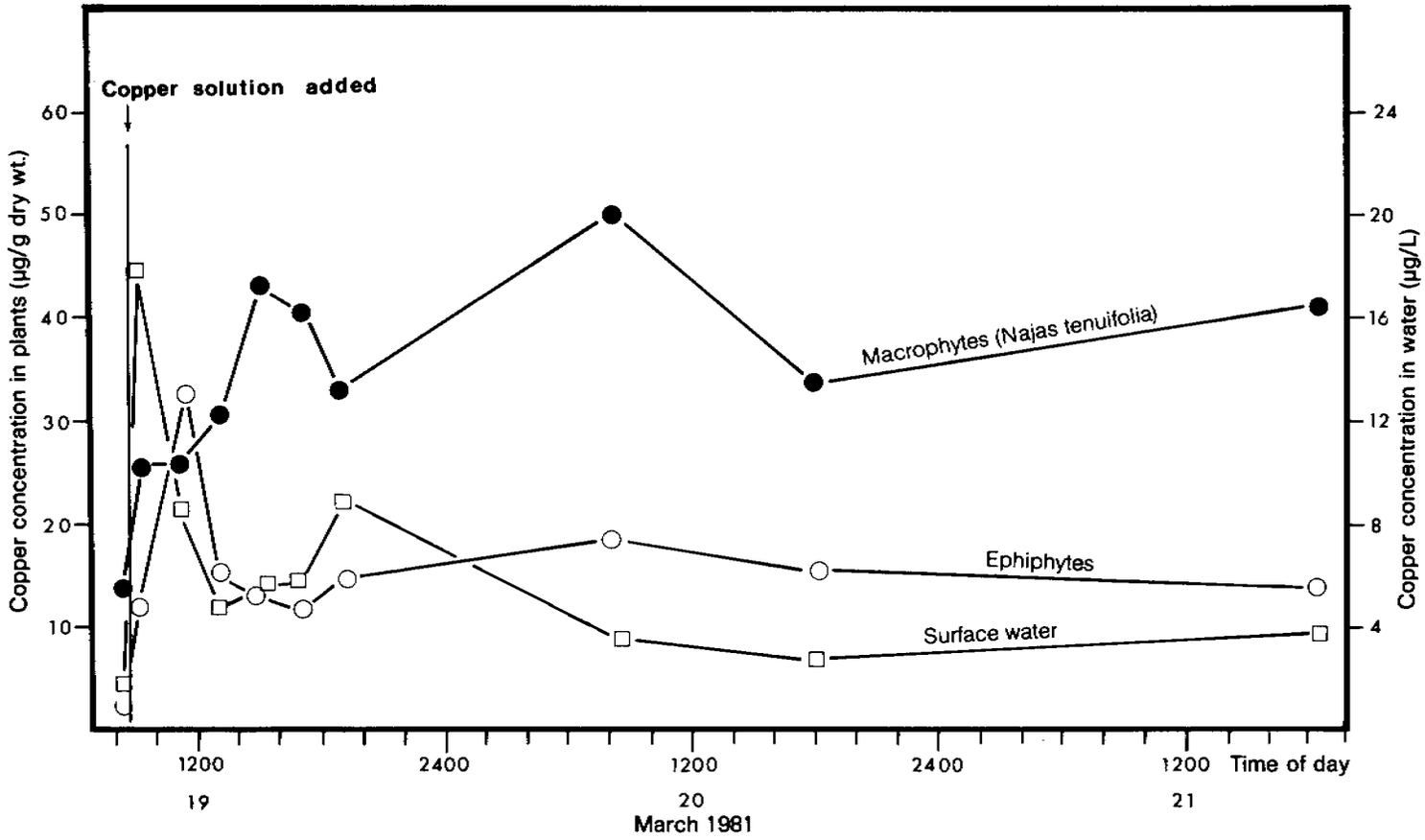

FIGURE 2 CONCENTRATIONS OF COPPER IN SAMPLES TAKEN FROM THE ENCLOSURE.

initially increased to 5.9 µg/L and then gradually decreased over the course of the experiment to 1.5 µg/L.

There was a very rapid increase in the concentration of copper in the epiphytes; but they lost most of this copper almost as rapidly (Fig. 2). Uptake and release of copper took place in around four hours. Uptake was probably due to adsorption at the cell surface, a process that occurs rapidly with phytoplankton (Davies 1978). For example, Bates et al. (1981) found that *Chlamydomonas variabilis* and *Scenedesmus subspicatus* adsorbed most zinc from the water within the first few minutes of incubation. It is more difficult to explain the subsequent rapid release of the adsorbed copper. There was insufficient time for epiphytes killed by the initial addition of copper to become detached from the macrophytes, sink to the bottom of the enclosure, and be replaced by other epiphytes containing a lower concentration of copper. A more likely mechanism would be that the added copper rapidly killed the epiphytes, which then released most of their copper to the water without the dead cells becoming detached.

Before the copper solution was added, *Najas tenuifolia* contained around 8.5 µg/g (dry wt) of copper. Three samples of this macrophyte, taken from Gulungul Billabong in May 1980, had copper concentrations of 9.2, 12 and 13 µg/g. These values are similar to, but slightly higher than, those found in ten samples taken from the flood plain (3.5-8.5 µg/g (dry wt); Pancontinental 1981).

The data presented in Figure 2 show that *Najas* is able to take up added copper rapidly. In around 6 hours the concentration in these plants increased from less than 10 µg/g to around 40 µg/g, on a dry weight basis, and stayed around this level until the end of the experiment some 52 hours later.

To assess the relative importance of the plants (epiphytes and macrophytes) in removing added copper from the water, the final distribution of copper amongst the various components in the enclosure was calculated. This calculation is very tentative, since the total biomass of the epiphytes and macrophytes was not known. The total biomass of macrophytes was estimated from data obtained for this billabong during the previous Wet Season when *Najas* was collected from three 0.25 m^2 quadrats. The plants were initially dried with absorbent paper and weighed to obtain the 'wet' weight, they were then dried to constant weight at 105°C and re-weighed to obtain the 'dry' weight.

Although sediment samples were taken, they were not analysed, since simple calculations indicated that even if all of the added copper had been taken up by the sediment, the very small increase in the concentration of copper would not have been detectable analytically.

The copper 'budget' calculation, shown in detail in Table 4, indicated that 30% to 60% of the copper was associated with *Najas*, 15% to 20% was left in the water column, and the rest was presumably associated with epiphytes, sediments and the walls of the enclosure.

TABLE 4 MASS-BALANCE CALCULATION FOR THE FINAL DISTRIBUTION OF COPPER.

Weight of added copper	=	8.1 mg
Volume of enclosed water	=	0.82 m^3
In water:		
Copper concentration:		
outside enclosure	=	1.9 µg/L[a]
inside enclosure	=	3.6 µg/L[b]
Difference in concentration	=	1.7 µg/L
Mass of added copper present in water	=	1.4 mg
Percentage of added copper present in water	=	17%
In macrophytes:		
Mass of macrophytes (estimated)	=	1000-2000 g/m^2 (wet wt)
	=	75-150 g/m^2 (dry wt)
Concentration of copper in macrophytes	=	40[c]-8.5
	=	32 µg/g dry wt
Mass of copper in macrophytes	=	0.032 x 75 to 0.032 x 150
	=	2.4-4.8 mg
Percentage of added copper present in macrophytes	=	29% to 58%

[a]values used: 0.84, 3.0, 2.0; mean 1.9 µg/L; SD 1.1 µg/L.
[b]values used: 3.5, 3.5, 3.8; mean 3.6 µg/L; SD 0.2 µg/L.
[c]values used: 42, 40, 33, 50, 34, 41; mean 40 µg/g
SD 6 µg/g.

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