PHYCOMICROBIAL ECOLOGY OF ACID MINE DRAINAGE IN THE PIEDMONT OF VIRGINIA[1]

by

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Abstract. Acid mine drainage encompasses 18 km^2 of Louisa County, Virginia. Heavy metal laden acidic leachate flows from abandoned mines along the Piedmont's Gold-Pyrite Belt. The oxidation of pyrite, sphalerite, chalcopyrite and other sulfide minerals that are disseminated throughout the mine tailings release H_2SO_4, Fe, Cu, Zn, Ni, Cd, As, Pb and other heavy metals into the Contrary Creek watershed and beyond, into Lake Anna. Downstream of these abandoned pyrite mines, high levels of acidity and heavy metals have made this a severely stressed environment incapable of supporting a healthy creek ecosystem. In an effort to assess *in-situ*, bioaccumulatory remediation of acid mine drainage by phyco-microbial mats, surveys have been conducted for 11 months in Contrary Creek; several extremophiles that are tolerant of acid mine systems have been found. Twelve to thirteen genera of algae and a few cocci and bacilli have been identified in surface waters. Predominant genera include Ulothrix, Pinnularia and Oscillatoria. Preliminary results demonstrate that the phycomicrobial communities found in this acid mine system maintain density and species diversity independent of pH and heavy metal fluctuations. These extremophiles also demonstrate high potential for heavy metal sorption. Phyco-microbial mats bioaccumulate 60-70% more heavy metals than concentrations found in surface waters and the creek. To date, remediatory attempts to restore Contrary Creek have not been successful. Our results suggest that the extremophile ecology found in this system will facilitate the remediation process of other, similar acid mine affected ecosystems.

Additional Key words: bioremediation, acid mine drainage, phycomicrobial mats

Introduction

Industrial pyrite mining for the production of sulfuric acid was begun in the late nineteenth century within Contrary Creek's watershed (Figure 1) in Virginia. This area was well known for its pyrite lenses which were reportedly as much as fifty feet in thickness and several hundreds of feet in length, occurring as part of the Chopawamsic Formation (Sweet, 1976). The resulting exposure of bedrock and disseminated sulfide minerals to precipitation and weathering are largely responsible for the release of toxic metals well in excess of EPA limits; over 1000 ppm sulfate, 17,000 ppm iron, 108 ppm copper, 200 ppm lead, 80 ppm arsenic have been measured (Krishnaswamy, 1997). In addition, in accordance with previous work (Kleinmann et al., 1995, 1979 and 1978; Nordstrom, 1977; Mills et al., 1987; Robbins et al., 1996) Thiobacillus ferrooxidans, a sulfide oxidizing bacterium, is believed to be responsible for the production of acidity and water pH values of less than 3.3 on average per year.

The effects of acid mine drainage (AMD) from Contrary Creek on Lake Anna are variable both geochemically and biologically (Mills et al., 1987; Herlihy et al., 1988; McIntire et al., 1988; Bruckner et al., 1989; Bell et al., 1990; Anderson, 1995, 1996). Lake Anna has a nominal volume of 4.45 x 10^{11} liters which, for the time being, appears adequate for diluting Contrary Creek runoff (Leech, 1973; Simmons, 1971; Miorin et al., 1974). The average pH of Lake Anna ranges between 6-7.5 (Krishnaswamy, 1997; Dagenhart, 1980). There is, however, much concern over the biological effects of AMD adversely impacting Lake Anna due to the lake's recreational activities, especially fishing. Over the last three decades, several fish kills have been reported in Lake Anna (Mount, 1966; Odum et al., 1978; Dagenhart, 1980). Although, not all the fish kills were located in proximity to the mouth of Contrary Creek, a high percentage did occur subsequent to large storms that increased the output of Contrary Creek. Exposure to toxic heavy metals does not correlate to immediate death of biologic organisms, but rather, upon reaching critical residence time within the body (Fergusson, 1990).

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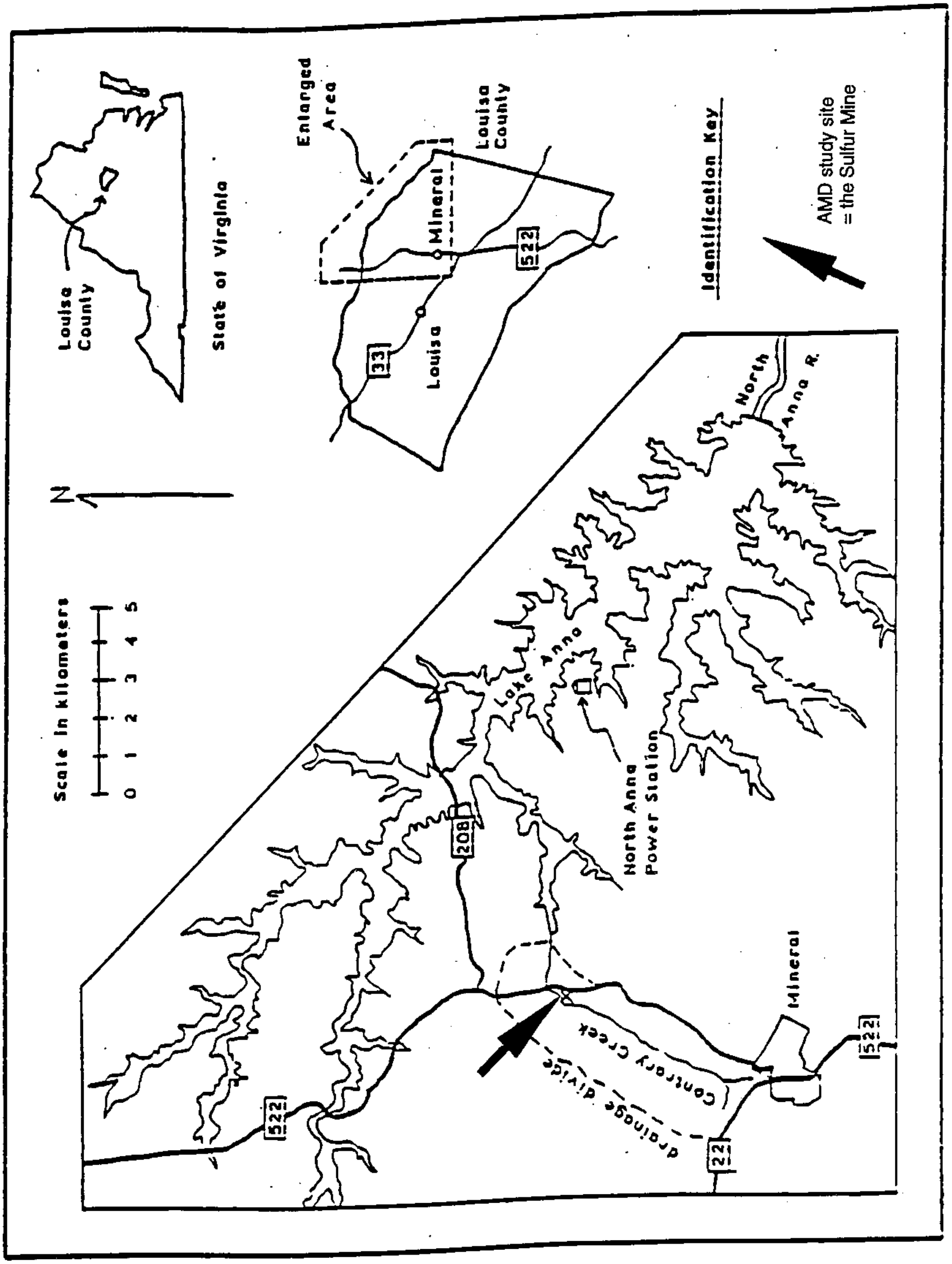

Figure 1. Location of Contrary Creek in Louisa County, Virginia. Black arrow shows location of study site at abandoned Sulfur mine (Modified from Dagenhart, 1980).

Furthermore, the density and diversity of macrobenthic organisms and microbial populations in proximity to Contrary Creek's mouth, in Lake Anna, appear diminished (Ayers, 1975; Odum et al., 1978). Water quality sampling proved that even small tributary streams carrying mine storm runoff, when inundated with toxic metal concentrations, can severely degrade much larger river systems (Dagenhart, 1980). Both Contrary Creek and its mouth, draining into Lake Anna, reflect poor water quality and mobile, toxic sediments (Krishnaswamy, 1997; Dagenhart, 1980).

Among the cocci and bacilli of the AMD system found at Contrary Creek, two species were identified Thiobacillus ferrooxidans and Leptothrix discophora (Nordstrom, 1977; Robbins et al., 1995; Krishnaswamy, 1997). T. ferrooxidans and L. discophora are found in two locations in the Contrary Creek system: in water samples collected from the creek and in surface waters, in association with algal blooms.

Previous work dealing with bacteria-metal interactions including metal uptake, metal sorption and absorption, bacterial adaptations and mechanisms that facillitate metal concentration and bacteria-metal and clay interactions is extensive (Carrol, 1958; Barghoon and Tylor, 1965; Razzaghe-Karimi and Robert, 1975; Pickering, 1979; Beveridge, 1980, 1981, 1984, 1985, 1989, 1995; Schultze-Lam et al., 1985; Munier-Lamy et al., 1987; Geesey and Jang, 1989; Mayers and Beveridge, 1989; Ferris et al., 1989; Walker et al., 1989; Urrutia et al., 1993). The effects of acid/ low pH inhibition on bacteria-metal binding are poorly documented.

Although microbes are always found in water samples obtained from Contrary Creek, the algae of this AMD system appear restricted to surface runoff that are metal and acid enriched and form as water seeps out of mine tailings along the banks of the creek. Significant phycological density decreases occur in response to thermal stress rather than in response to pH and/or heavy metal concentration flux. Predominant algal species include Ulothrix sp., Eunotia sp., Euglena mutabolis, Cylindrocapsa sp., Navicula sp. and Pinnularia sp. The presence of cyanobacteria species such as Chroococcus turgidus and Phormidium sp. was confirmed via microbiological techniques of aseptic pure culture isolation, identification and classification.

A relatively large body of work is available about algal-metal interactions in non-acid systems (Crist et al., 1981, 1988, 1992; Darnall et al., 1986(a), 1986(b); Greene et al., 1986; Watkins et al., 1987; Majidi et al., 1988; Gardea-Torresday et al., 1988, 1990; Mahan et al., 1989; Kubiak and Wang, 1989; Harris et al., 1990). This study, unlike previous work, not only provides evidence that algae can bind metals in acid systems, but that algae and microbes, together, bind metals *in-situ*, in AMD systems. Lastly, this study indicates that the potential for combined phyco-microbial metal binding capacity may be greater than that of individual algal and microbial metal capture, especially in AMD systems. Preliminary data indicate potential for *in-situ* phyco-microbial bioremediation of AMD systems such as Contrary Creek. Details of processes and statistical analysis of success rates of combined phyco-microbial metal capture in acid mine systems require further monitoring and research.

Methodology

Two surface water point sources along the length of Contrary Creek were sampled to obtain geochemical data. The two point sources emanate from the Sulfur mine, are 50 feet apart and in reference to highway 522 are labeled 1 and 2. Creek water samples and phyco-microbial mat samples were obtained along transects leading from point sources 1 and 2 and that spanned the width of the creek. The creek water samples collected along the transects are labeled T1 and T2. The phyco-microbial mat samples collected from point sources 1 and 2 are labeled PM1 and PM2, respectively. pH and temperature measurements were also made at sites 1, 2, T1 and T2. pH was measured monthly, alternating between wet (during rain) and dry (during sunny) conditions.

In order to decontaminate sample containers the following laboratory procedures were employed prior to field sampling: 99 ml capacity glass bottles were 'acid washed' with 10 M concentrations of HNO_3 and HCl acids, consecutively. The acids were then rinsed 10 times with double distilled, biofiltered, deionized water. Finally, the cleaned glass bottles were capped and placed within an acid washed plastic Ziploc bag.

Mixed algal and bacterial (phyco-microbial) samples were lifted out of surface waters at both point sources and held for a few seconds to allow for excess surface water to drip off the mat. The biological samples were placed in a Ziploc bag.

Within 24 hours after sample collection all water samples were filtered using a .45 micron biofilter. The phyco-microbial samples were examined and all chironomids and polychaetes were removed. Approximately 90% of the phyco-microbial samples were freeze dried. All samples were stored at 4°C until shipped to Colorado State University for geochemical processing via ICP.

Ten percent of all phyco-microbial samples were studied under the light microscope at 630X magnification and under the SEM. Permanent slides were made for samples from each collection site. Standard microbiological, aseptic pure culture isolation, identification and classification of algal species was carried out at The George Washington University.

Geochemical analysis encompasses September, 1995. All pH, temperature measurements and microbiological techniques, algal and microbial identification and classification were analyzed over an 11 month study regime.

Results and Conclusions

In-situ bioremediatory processes require microorganisms and/or plants for accumulation of environmentally hazardous pollutants. Preliminary geochemical data indicate that phyco-microbial mats found at Contrary Creek bioaccumulate heavy metals, sulfate and iron in concentrations that are several orders of magnitude greater than concentrations found in the creek and the surface water point sources (Table 1). The fact the these phyco-microbial mats are located *in-situ*, in the point sources is promising with regard to maximizing the mats' bioaccumulatory potential because the surface water point sources appear to contribute most of the heavy metal load found in this system.

Total algal generic diversity and density of each algal genus undergo seasonal density and diversity shifts (Table 2) which are independent of pH flux, but dependent on temperature (Table 3). Chlorophycean generic diversity decreased from about 11, where pH ranged from 3.5 to 2.8 and temperature ranged from 12-6°C in September 1995, to 8-9, where pH ranged from 3 to 2.8 and temperature ranged from 6-2°C in April 1996. Chlorophycean generic diversity then increased to 12 and 15 genera in August 1996, in point sources 1 and 2 respectively, in response to temperature flux (Table 3). The pH and temperature values for the creek are given in Tables 4 and 5, respectively.

In contrast to diversity decreases in Chlorophycean genera induced by thermal stress, diatom generic diversity increased from about 4 genera in September 1995 to about 13 genera in December 1995 as temperatures declined. Diatom generic diversity increased to over 10 genera in May 1996. Cyanobacteria, in both point sources 1 and 2, make their first appearance in October 1995. Lowest, cumulative algal genera diversity occurs between February 1996 and March 1996.

Conclusions

Specific trends exist between associations of algal generic diversity/density and a particular temperature-pH range (Table 3). Despite the harsh environmental conditions of acid mine drainage, the phyco-microbial community not only survives seasonally, but also demonstrates its usefulness as a metals sink and as a temperature-pH-algal genera specific monitoring technique. The results of this study support the potential for bioremediation as technology for the future in terms of sustainable and cost-effective, environmental management, hazard prevention and pollution prediction.

Table 1. Geochemical data for point sources 1 and 2, creek water samples T1 and T2 and phyco-microbial mats PM1 and PM2.

Samples	pH	SO4	Cl	NO3	Al	Fe	Mn	Cu	Zn	Ni	Cd	As	Pb
1	2.8	1460.4	9.9	0.1	56.5	114.71	10.5	2.32	9.31	0.07	0.065	0.001	0.28
2	2.8	1393.6	7.8	0.1	57.8	114.22	11.82	2.77	9.57	0.11	0.072	0.001	0.3
T1	3.1	258.9	0	0.1	4.5	4.2	2.3	0.66	3.73	0.02	0.006	0.001	0.05
T2	3.3	242.8	0	0.1	3.1	2.64	2.08	0.57	3.18	0.01	0.005	0.001	0.05
PM1	3.2	13970		0.1		717757	48.3	186	94	7.82	12.7	73.47	146
PM2	3.5	18440				94350	82.3	129	124	13.4	16.6	20.71	170

Table 2. Phycological Profile September 1995-September 1996. 1 and 2 represent surface water point source sites 1 and 2.

1	2	1	2
Sep-95	**Sep-95**	**Dec-95**	**Dec-95**
Chlorella	Chroomonas	Spirogyra ?	Acanthes
Rhizochrysis limneticus	Eunotia	Eunotia	Geminella
Chroococcus limneticus	Euglena	Phacus	Eunotia
Desmidium	Stichococcus	Geminella	Docidium
Euglena	Cyclotella	Trachelomonas	Pinnularia
Fragilaria	Pleurogaster	Tabellaria	Gomphonema
Eunotia	Closteriopsis	Euglena	Cylindrocapsa
Oedogonium	Bacillaria	Acanthes	Diatoma
Ulothrix	Cylindrocapsa geminella	Ulothrix	Opephora
Pinnularia	Zygnemopsis desmidiodes	Oscillatoria plantonica	Meridion
Oct-95	Odeogonium	Chrococcus	Nitzschia
Chroococcus	Desmidium	Pinnularia	Hormediopsis ellipsoideum
Lepocincilis	**Oct-95**	**Jan-96**	**Jan-96**
Trachelomonas	Tabellaria	Euglena	Geminella
Phacus	Melosira	Eunotia	Melosira
Euglena mutabolis	Eunotia	Oscillatoria	Gomphonema
Stipitococcus	Nitzschia	**Feb-96**	Gyrosigma
Synedra ulna	Chroococcus turgidus	Euglena	Cyclotella
Nitzschia	Achanthes	Oscillatoria	Navicula
Cyclotella	Trachelomonas	**Mar-96**	Mallomonas
Pleurogaster	Cylindrocapsa geminella	Euglena	Chroococcus
Desmidium	Odeogonium	Oscillatoria	**Feb-96**
Oedogonius	Leponcincilis	Eunotia	Geminella
Ulothrix	**Nov-95**	Oscillatoria plantonica	Melosira
Nov-95	Geminella	**Apr-96**	Gomphonema
Cylindrocapsa geminella	Eunotia	Eunotia	Gyrosigma
Eunotia	Gomphonema	Tabellaria	Navicula
Cyclotella	Diatoma	Acanthes	Mallomonas
Mallomonas	Synedra	Pinnularia	Chroococcus
Chroococcus	Cyclotella	Eunotia	**Mar-96**
Gomphonema	Navicula	Gomphonema	Geminella
Trachlemonas	Pinnularia	Synedra	Melosira
Achanthes	Melosira	Cyclotella	Gomphonema
Synedra	Closterium		Gyrosigma
Melosira	Coconeis		Navicula
Leponcincilis	Lepocincilis		Mallomonas
Coconeis			Chroococcus
Navicula			Synedra
			Pinnularia

Table 2. Phycological Profile September 1995-September 1996 (continuation).

1	**2**	**2**
May-96	**Apr-96**	**Sep-96**
Eunotia	Coconeis	Coconeis
Tabellaria	Eunotia	Zygnemopsis desmidiodes
Acanthes	Tabellaria	Chroomonas
Pinnularia	Acanthes	Odeogonium
Eunotia	Pinnularia	Desmidium
Gomphonema	Euglena	Cyclotella
Synedra	Oscillatoria	Euglena
Cyclotella	Oscillatoria plantonica	Stichococcus
Euglena	Chroococcus	Eunotia
Oscillatoria plantonica	**May-96**	Pleurogaster
Aug-96	Coconeis	Closteriopsis
Oedogonium	Melosira	Navicula
Euglena	Tabellaria	
Chlorella	Gomphonema	
Chroococcus limneticus	Gyrosigma	
Eunotia	Pinnularia	
Desmidium	Navicula	
Desmidium	Mallomonas	
Rhizochrysis limnetica	Chroococcus	
Ulothrix	Synedra	
Stichococcus	Pinnularia	
Chroomonas	Oscillatoria	
Netrium ?	Eunotia	
Sep-96	**Aug-96**	
Chlorella	Chroomonas	
Chroococcus limneticus	Eunotia	
Chroomonas	Euglena	
Eunotia	Stichococcus	
Euglena	Cyclotella	
Desmidium	Navicula	
Rhizochrysis limnetica	Pleurogaster	
Ulothrix	Closteriopsis	
Stichococcus	Bacillaria	
Oedogonium	Pinnularia	
Pinnularia	Cylindrocapsa geminella	
Synedra ulna	Coconeis	
	Zygnemopsis desmidiodes	
	Odeogonium	
	Desmidium	

Table 3. Phycological genera, pH and Temperature (°F) in Surface water point sources.
1 and 2 represent point source sites.

	1 # Genera	1 pH	1 Temp.	2 # Genera	2 pH	2 Temp.
9/95 (Dry)	10	3.5	32	12	2.8	25
10/95 (Wet)	13	2.5	27	10	2.6	21
11/95 (Dry)	13	3	22	12	3.4	18
12/95 (Wet)	12	2.8	17	12	2.8	12
1/96 (Dry)	3	3.5	6	8	3	10
2/96 (Wet)	2	3	10	7	2.9	12
3/96 (Dry)	4	3.5	8	9	3.2	9.5
4/96 (Wet)	8	3	2	10	2.8	6
5/96 (Dry)	10	3.5	12	13	3.6	10
8/96 (Wet)	12	2.3	36	15	2.5	25
9/96 (Dry)	12	3.4	30	13	4	21

Table 4. pH of point source sites 1 and 2 and creek water along transects T1 and T2.

Months/yrs.	1	2	T1	T2
09/95 (Dry)	3.5	2.8	3.1	3.3
10/95 (Wet)	2.5	2.6	3.5	3.4
11/95 (Dry)	3	3.4	3.3	3.2
12/95 (Wet)	2.8	2.8	4.2	4.5
01/96 (Dry)	3.5	3	3.3	3.2
02/96 (Wet)	3	2.9	4.1	4
03/96 (Dry)	3.5	3.2	3.8	3.5
04/96 (Wet)	3	2.8	4	3.8
05/96 (Dry)	3.5	3.6	3.5	3.3
08/96 (Wet)	2.3	2.5	3.9	3.8
09/96 (Dry)	3.4	4	3.4	3.3

Table 5. Contrary Creek Temperatures in °F.
T1 and T2 represent two sites along the transects at which temperature was measured.

Months/yrs.	T1	T2
09/95 (Dry)	20	20
10/95 (Wet)	20	20
11/95 (Dry)	19	19
12/95 (Wet)	17.5	17.5
01/96 (Dry)	10	10
02/96 (Wet)	11	11
03/96 (Dry)	9	9
04/96 (Wet)	6	6

Months/yrs.	T1	T2
05/96 (Dry)	14.2	14.2
08/96 (Wet)	19.3	19.3
09/96 (Dry)	20	20

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