

Evaluating the factors limiting algal biomass in acidic pit lakes of the Collie lake district, Western Australia

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Abstract Acidic pit lakes often have elevated metal concentrations, very low nutrient concentrations and very low algal primary productivity. A microcosm experiment was performed to identify the main limiting factor(s) for algal biomass. Nutrients (N, P and C) were added in all possible combinations to pit lake water in the presence or absence of pit lake sediment. Microcosms without sediment showed higher chlorophyll *a* concentrations compared to the treatments with sediment. Microcosms where nitrogen and phosphorus were added showed highest chlorophyll *a* concentrations. Results suggest that algal biomass in pit lakes may be primarily limited by low nitrogen and phosphorus concentrations.

Key Words Acidic pit lakes, algae, primary production, nutrients, chlorophyll *a*.

Introduction

Relative to natural aquatic systems, acidic pit lakes have a water chemistry mainly dominated by iron and sulfur and greater depth:area ratios, reduced bankside stability and very limited bankside vegetation (Castro and Moore 2000; McCullough 2008). All these factors reduce the pit lake's water quality and biodiversity and slow the establishment of a functioning ecosystem. Poor water quality in acidic pit lakes may limit realisation of environmental beneficial end uses which might be otherwise possible (McCullough *et al.* 2009) and may pose an environmental hazard to groundwater and nearby aquatic and terrestrial ecosystems (McCullough and Lund 2006).

Acidic pit lakes are often oligotrophic to ultraligotrophic due to low concentrations of nutrients such as carbon and phosphorus (Nixdorf *et al.* 2001; Lessmann *et al.* 2003). Nutrients are required to sustain algal primary productivity which is fundamental for the ecological structural and functional components of the pit lake ecosystem. However, in acidic pit lakes phosphorus unavailability often strongly limits algal primary productivity. Although phosphorus is highly soluble in low pH pit lakes phosphorus may co-precipitate with elevated concentrations of iron and aluminium limiting bioavailability (Lessmann *et al.* 2003; Lund and McCullough 2009). Acidic pit lakes are often deficient in nitrate/nitrite concentrations as nitrogen typically remains as ammonia due to limited nitrification at low pH (Nixdorf *et al.* 2001). Availability of organic carbon (for mixotrophs) is also often limited.

Although nutrient availability in acidic pit lakes appears to be limiting primary production, it is not clear whether the acidity, water toxicity or nutrient limitation; or a combination of all; are the impediments (Woelfl *et al.* 2000; Neil *et al.*

2009). Additionally, sediment in lakes can alter nutrient availability by adsorptive processes (Kleeberg and Grüneberg 2005; Simmons 2010) and may therefore influence the net outcome of any nutrient addition treatment especially in shallow acidic pit lakes. The wide range of pit lake depths and areas means that pit lakes will differ in volume: sediment area ratios. Determining the effect of sediment on the nutrient dynamics in acidic pit lakes and algal primary productivity would therefore provide information on the important relationship between lake depth and nutrient availability. The present microcosm study is designed to evaluate the effects of nutrient amendments individually or in combination, presence or absence of sediment, and effect of chemical neutralisation on enhancing biomass of naturally present algae. The major aim of this study was to determine the limiting factor(s) for algal primary productivity in an acidic pit lake rather than tracking the water quality improvement.

Methods

Study site description

In Western Australia (WA), coal mining is a significant industry and is based in Collie (Kumar *et al.* 2009). The Collie coal region, in the south-west of WA, accounts for the whole of coal production for the state. All coal is extracted by open cut mining. Coal mining in the Collie region has resulted in 13 pit lakes that range from <0.1–1.0 km² in surface area, <10–70 m in depth, 5–50 years in age, in pH from 2.4–5.5 and vastly differ in the extent of rehabilitation (Lund and McCullough 2008; McCullough *et al.* 2010). Acidic pit lakes in Collie are typically not light limited (McCullough *et al.* 2010) with euphotic depths extending past their maximum depths, however they are oligotrophic and algal biomass is negligible. This makes the acidic

pit lakes in Collie an excellent site to investigate strategies for enhancing algal biomass production. Lake Kepwari (33.36°S, 116.15°E) is the largest of the Collie pit lakes. Lake Kepwari was rapidly filled by a diversion of first-flush saline water from the south branch of the Collie River from 1999 to 2005 (Salmon *et al.* 2008) and the volume is now $\approx 0.025 \text{ km}^3$, with a maximum depth of 65 m and surface area of 1.03 km^2 . Although neutral river water initially raised the lake water pH, the lake pH is now low (≈ 4 , author's unpublished data) and appears to be still decreasing.

Water and sediment sampling

Water samples were collected from the epilimnion of Lake Kepwari in several 20 L clean carboys for the microcosm experiment in September 2010. Sediment samples were collected in acrylic cores by scuba divers. Water and sediment samples were returned to the laboratory on the same day and allowed to equilibrate to room temperature (22 °C). The microcosm experiment was commenced within 24 h of pit lake water and sediment collection.

Microcosm experiment

Two sets of microcosms in 2.5 L plastic jars were prepared; one set had pit lake sediment added to a depth of 20 mm and the other set contained only acidic pit lake water. All the microcosms were amended with nutrients both individually for each nutrient and in all combinations. Nutrients (C as glucose for organic carbon 10 mg/L, N as nitrate 1.5 mg/L and P as phosphate 0.09 mg/L) were added stoichiometrically following the Redfield ratio (C:N:P, 106:16:1) (Redfield and Ketchum 1963). Two different controls were used throughout the experiment. In both the controls, no nutrients were added, however, acidic mine water was neutralised in one control using 0.1 N NaOH to facilitate the increase of dissolved inorganic carbon concentration through development of a bicarbonate buffering system at circum-neutral pH (Wetzel and Likens 2000). All the treatments and controls were carried out in triplicate, with randomised placement at a temperature of 22 °C and maintained under artificial lighting (18,000 K) with a light:dark cycle (12 h:12 h).

Analysis

A vertical profile of physico-chemical data was collected in September 2010 in Lake Kepwari. A Datasonde 4a (Hydrolab, USA) was used to measure pH, oxidation reduction potential (ORP, platinum reference electrode, mV), electrical conductivity (EC, mS/cm) and dissolved oxygen (DO, mg/L) at 2 m intervals top to bottom. Water samples from the epilimnion and hypolimnion were also collected using a Kemmerer bottle for nutrients, anions and

metals analysis. Acidity was measured in the laboratory on an auto-titrator (Metrohm, Switzerland) using 0.1 N NaOH as titrant.

Physico-chemical measurements from the microcosm experiments were taken on Day 1 and finally at the end of the experiment on Day 35. A multi-parameter probe (Hydrolab Datasonde 4a) was used for the physico-chemical measurements, recording temperature, pH, EC, DO and ORP. At the end of the experiment water samples from the microcosms were filtered using $0.45 \mu\text{m}$ glass fibre (GFC) filter paper for chlorophyll *a* analysis following the N,N-Dimethylformamide (DMF) method (Speziale *et al.* 1984). Before filtering the water samples, the microcosm walls were scrubbed with a fine brush to remove algal biomass. A similar procedure was followed for the microcosms with sediment where benthic algae on the top of the sediment layers were scraped in to 50 mL polypropylene centrifuge tubes. Chlorophyll *a* concentrations were used to assess the algal biomass as influenced by the different nutrient treatments.

Results and Discussion

There were few differences between pH, ORP, temperature and EC throughout the lake water column indicating that the lake was well mixed (Fig 1). Although September in south-western Australia is early spring time, lake temperatures were still low enough to permit mixing of the lake water column. Lake Kepwari nutrients, metals and acidity concentrations are shown in Table 1. Although high EC in Lake Kepwari is typical of many Australian pit lakes (Kumar *et al.* 2009), the high EC is largely due to the saline first-flush from the Collie River that was used to rapid-fill the pit void (Salmon *et al.* 2008). Lake Kepwari is mainly an aluminium buffered system with acidity inputs from aluminium cycling and groundwater iron inputs (Salmon *et al.* 2008). Uncharacteristic of most acid mine drainage influenced pit lakes, Collie pit lakes have only low acidity, low sulfate and metal concentrations (Lund *et al.* 2006). Such uncharacteristic water quality is mainly due to the low sulfur concentrations in the coal combined with very low buffering from surrounding geologies (Sappal *et al.* 2000).

pH increased in all the treatment and control microcosms irrespective of the sediment's presence or absence (Fig 2 a, b). However, microcosms without sediment showed slightly higher pH than those containing sediment. Microcosms with sediment generally showed a ≈ 0.6 pH unit increase from the initial values regardless of the nutrient treatment. Alternatively, microcosms without sediment showed a mixed pH increase depending on the nutrient treatment applied. For instance, microcosms amended with C, N & P showed a maxi-

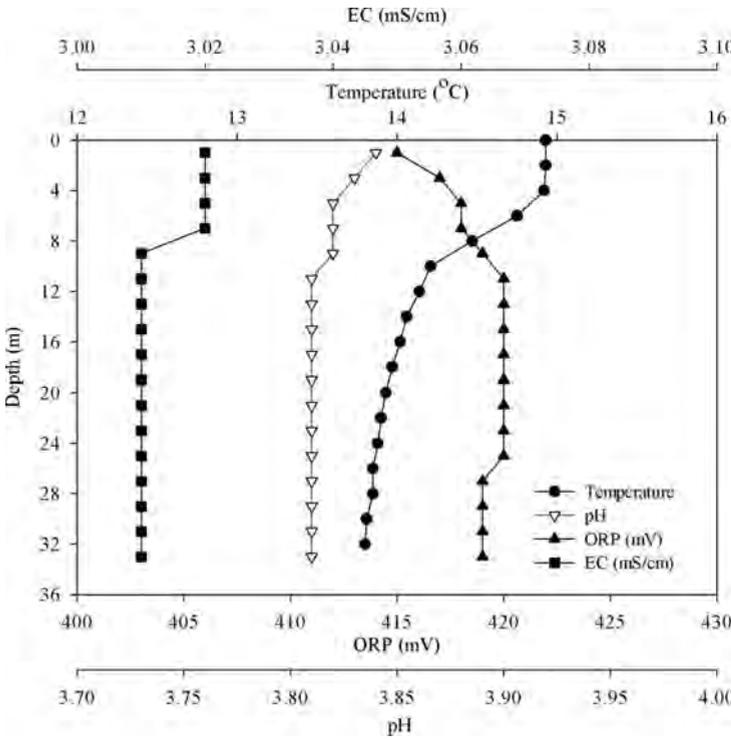


Figure 1 Vertical profiles of Lake Kepwari for pH, ORP, temperature and EC (Data from September 2010).

Parameter	Epilimnion	Hypolimnion
Total Nitrogen	0.9	0.8
NO _x -N	0.5	0.5
NH ₄ -N	0.04	0.04
PO ₄	<0.002	<0.002
Chloride	850	780
Sulfate	210	180
DOC	1.7	3.5
Total Carbon	1.8	3.7
Al	1.8	1.8
Ca	37	37
Co	0.1	0.1
Cr	0.01	0.02
Fe	0.5	0.6
K	6.6	6.5
Mg	115	120
Mn	0.4	0.4
Na	470	480
Ni	0.1	0.2
S	63	64
Zn	0.8	0.8
Acidity mM (K _B 8.2)	0.3	0.3

Table 1 Nutrients, anions, metals/metalloids and acidity concentrations in water samples from the epilimnion and hypolimnion of Lake Kepwari (Data from September 2010). All the values are in mg/L except for acidity.

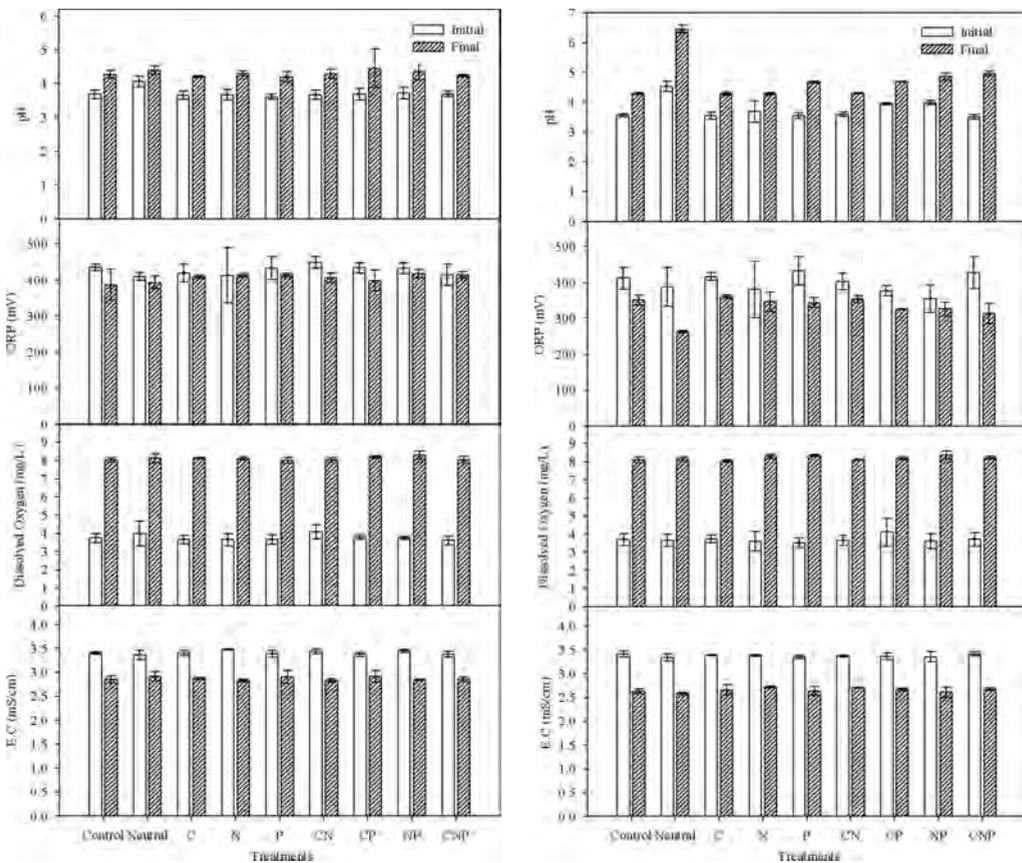


Figure 2 Mean ($n = 3$, \pm standard deviation) changes in (a & b) pH, (c & d) ORP, (e & f) dissolved oxygen and (g & h) E.C. in microcosms with sediment and without sediment following nutrient addition treatments.

mum increase of 1.5 pH units, with final pH 5.0 from an initial pH of 3.5, whilst microcosms amended with P alone showed a 1.1 pH unit increase. The slightly higher pH increase observed in microcosms without sediment than with sediment could be because sediment had served as a sink, adsorbing some of the added nutrients. Similar results have been reported for loss of amended P from the water column to sediments during a mesocosm study due to binding of P with dissolved Al (Neil *et al.* 2009).

ORP in the acidic pit lake water was around 400 mV, indicative of highly oxidic conditions favourable for acidity-generating processes of biogeochemical iron and sulfur oxidation. ORP declined slightly in all the microcosms (Fig 2 c, d) following the nutrient treatments, with ORP reduction slightly higher in microcosms without sediment. DO almost doubled in all nutrient treatments and controls (Fig 2 e, f). However, there were only slight differences between the increases recorded in microcosms with and without sedi-

ment. At the end of the experiment, EC had also declined slightly in all the microcosms (Fig 2 g, h); it was slightly greater in microcosms without sediment than with. Electrical conductivity decreases correlated well with the pH increases recorded, most likely due to solute precipitation at elevated pH (Kumar *et al.* 2011).

The changes in physio-chemical parameters in the control were similar to all the treatments, illustrating that the algal growth had not obviously resulted in water quality improvements. The water in the controls was isolated (compared to the lake) from incoming sources of acidity. This coupled with some algal growth and potential binding of solutes to the microcosm container probably explain the changes seen. Over a longer treatment time, the nutrient treatments may have improved the overall water quality, as enhancing algal primary productivity in acidic pit lakes is known to improve the water quality with time (Davison *et al.* 1995; Dessouki *et al.* 2005).

Nutrient amendments failed to increase algae

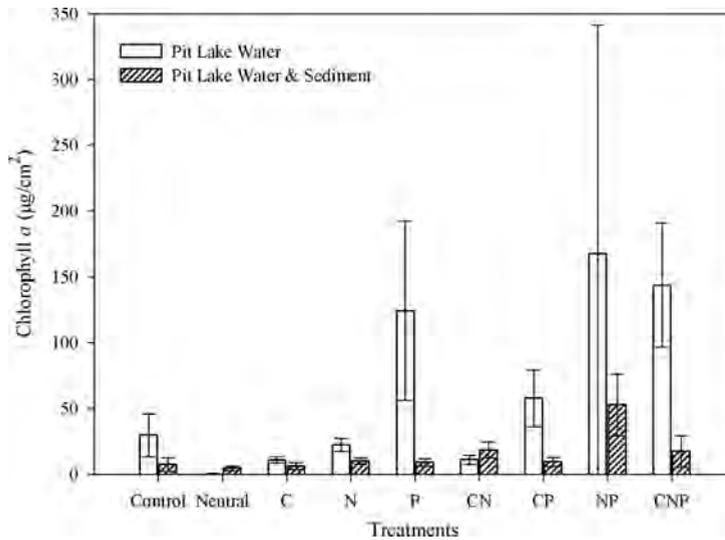


Figure 3 Mean ($n=3$, \pm standard deviation) increase in chlorophyll a concentration with different nutrient treatment in microcosms with and without sediment. Data for chlorophyll a presented is the total concentration in the microcosms.

in the water column with the majority of algal biomass occurring on microcosm sides and bottom; irrespective of sediment presence. Controls with and without sediment showed little algal biomass (Fig 3). Microcosms which were chemically neutralised failed to demonstrate increased algal biomass indicating that nutrients were also required in addition to a neutral pH. Carbon and nitrogen treatments, with or without sediment, did not significantly increase the algal biomass either individually, or when added in combination. However, P alone was able to increase algal biomass as indicated by the high chlorophyll *a* concentrations in microcosms without sediment but P in microcosms with sediment was not able to enhance algal biomass. This may have implications for shallow acidic pit lakes where the high sediment surface area: water volume relationship may result in greater nutrient removal and consequently lower algal biomass able to be achieved.

The most likely reason for reduced algal biomass in microcosms with sediment is that P added was lost from the water column by adsorption onto sediment through co-precipitation with Al and Fe as oxyhydroxides (Kleeberg and Grüneberg 2005; Nixdorf *et al.* 2005). Similar insignificant increases in algal biomass were also recorded in microcosms where P was added with C. However, when P was combined with N, the treatment seemed to be highly favourable for algal growth, as indicated by the higher chlorophyll *a* concentrations. The treatments, where all the nutrients were added (C, N, P) showed slightly lower chlorophyll *a* concentrations than with N & P combination. Fyson *et al.* (2006) have also reported similar results of algal biomass improvement in acidic pit lake water following

amendments with phosphate and acetate.

Conclusions

The present study showed that simple nutrient additions, especially P, were able to increase algal biomass in acidic pit lake water. The results also showed that sediment can decrease nutrient availability to algae by acting as a nutrient sink. As a result, where nutrient additions are considered as a strategy to improve algal biomass, shallow pit lakes may require greater and more frequent nutrient additions. Deep pit lakes therefore appear to be more appropriate for nutrient amendments to enhance algal biomass. This study was also able to highlight that nutrient limitation, rather than metal toxicity and/or water pH, are the primary limitations to algal biomass in the moderately acidic Collie pit lakes.

Acknowledgements

The authors thank the financial support provided by the Australian Coal Association Research Program (ACARP) through a research grant (C19018). We would also like to thank our industry partner Wesfarmers Premier Coal for their support and mine site access, particularly Dr. Digby Short. Thanks also to Mr. Clay Millar at the School of Natural Sciences, Edith Cowan University, Australia for technical assistance.

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