

# OPTIMISING ABATTOIR WASTEWATER IRRIGATED SOILS AS A SINK AND SOURCE OF PHOSPHORUS FOR PLANT BIOMASS PRODUCTION, AS AFFECTED BY FLYASH APPLICATION

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## Abstract:

Long-term discharge of abattoir wastewater can increase phosphorus (P) in soil, which may lead to loss of P and other nutrients under saturated conditions. Although some soils can retain P, the capacity of P sorption in most soils to adsorb and release P is low because of pH, since P availability to most crops requires a soil pH range of 6 to 7.5. Therefore, specific soil amendments based on pH of the native soil, such as addition of lime for acidic soils is required. Since lime application to soil involves high costs, an alternative alkaline material such as flyash (FA) is being used to improve the soil pH.

In this experiment, the biomass production of selected grass species such as napier grass (*Penisetum purpureum*), wallaby grass (*Themeda triandra*) and silky-blue grass (*Dicanthium sericeum*) were determined in abattoir wastewater irrigated soil (pH-5.25), both in presence and absence of FA. The plants were grown in plastic pots using tap water under controlled environment in a greenhouse and harvested after 60 days. Before the commencement of plant growth experiment, the abattoir wastewater irrigated soil was incubated (21 days under 80% water holding capacity) separately in the presence and absence of FA (15 % wt/wt), for each plant species. The entire experiment was conducted in triplicate.

The incubated soil was measured for changes in pH and Olsen P; the biomass was recorded from the plant growth experiment and compared between the crops in presence and absence of FA. The initial soil incubation showed that soil pH increased from 5.25 to 6.87 and the Olsen P improved from 29.12 to 94.66 mg P/kg soil. The plant growth was higher in FA amended soil compared to unamended soils. In FA amended soil, napier grass (6.14 g/pot) showed high biomass production followed by wallaby grass (4.64 g/pot) and silky-blue grass (4.59 g/pot). Hence, P in abattoir wastewater irrigated soils can be managed effectively using appropriate alkaline amendments for better soil productivity and higher biomass production.

## Introduction

Some water-intensive farm industries including animal slaughter houses generate high amounts of nutrients and contaminants, thereby contaminating our soil and water environment. In Australia and many other developed countries, meat consumption is high and hence the number of abattoirs (red meat processing) is on the rise over the last few decades (Stark et al. 2012). Abattoir wastewater contains blood, urine, other body fluids, animal faeces and contaminated storm water, which are the main sources of phosphorus (P). Effluent ponds in piggeries have been initiated in Australia since 1960s and land application of the piggery wastewater has been popular until the environmental regulations in the early 1990s

(Cusack 1995). Nowadays, land application of piggery wastewater is being carried out where discharging huge volume of untreated wastewater into land will impact on the fertility and productivity of the land, causing serious soil issues such as salinity, alkalinity and sodicity (Phillips 2002).

The enormous P resource (both organic and inorganic P) in abattoir wastewater has been a potential source of P in contemporary agriculture where the mode of P application has evolved in terms of volume, timing and additional amendments for enhanced utilisation of P. Being an essential nutrient for plant growth, adequate supply of P is necessary to optimise plant growth, especially in the early stages of development, which has both economic and environmental implications (Grant et al. 2001). The P applied from inorganic sources such as single superphosphate (SSP) and diammonium phosphate (DAP), and organic sources such as poultry manure (PM) and biosolids have different pathways for P release and also the rate of P release differs significantly between P sources (Headley and McLaughlin 2005; Turner et al. 2003). In the case of organic P sources, the P gets mineralised slowly and consequently the rate of supply of plant available P is slow compared to inorganic P application (Kwabiah et al. 2003). Hence, all applied P are not available to plants immediately, mainly because of either adsorption to soil particles or loss due to leaching/runoff or organic binding of P. An increase in pH increases the phosphatase activity, thereby increasing the mineralization of organic P (Fuentes et al. 2006; Lai et al. 1999).

Hence, there is a need for specific amendments which could mineralise organic P forms, immobilise the orthophosphate P forms and facilitate the release of bound P when plants require. Industrial alkaline by-products (pH>10) such as flyash (FA) and redmud (RM) can be effective in decreasing the soil P loss to the environment, thereby enhancing P utilisation in plants (Summers et al. 2001; Stout et al. 2000). These amendments are not only cost effective, but also a menace to the environment if not utilised. The increase in soil pH is one of the most significant characteristics of these coal combustion products (CCPs) for P transformation, thus enhancing the immobilisation of inorganic P and mineralisation of organic P (Seshadri et al. 2013). The use of CCPs on improving the bioavailability of P has been increasingly explored by many researchers (Bhattacharya and Chattopadhyay 2002; Pathan et al. 2002; Urvashi et al. 2007). Pathan et al. (2002) observed increase in bioavailable P from 18.5 to 43 mg kg<sup>-1</sup> soil in FA amended soil under laboratory conditions. Another alkaline industrial by-product from the alumina producing industry has also proved to be effective in minimising P loss and enhancing the utilisation of P by plants (Snars et al. 2004). In this paper, we report the effect of FA and RM on P mobility, and availability to Napier grass and selected native grass species grown in abattoir wastewater irrigated soil. Special emphasis was laid on the release of phosphatase by FA and RM amended soils and the relationships between Olsen P and relevant soil parameters such as pH and phosphatase activity have been discussed.

## **Materials and Methods**

### **Characterisation of soils and industrial by-products**

The influence of industrial by-products on P bioavailability in abattoir effluent irrigated (IS) and non-irrigated (NIS) samples were studied using the soils (sandy loam) collected from the treatment sites at Primos Port Wakefield abattoir, South Australia. The soil amendment used in the experiment was FA (Port Augusta Power Stations, South Australia, Australia) and RM (obtained from Rio Tinto Alcan Yarwun alumina refinery, Gladstone, Queensland, Australia). The P treatments used were a laboratory grade potassium dihydrogen phosphate (PP) and a commercial poultry manure (PM). The characterisation of the soil and the industrial by-

products were conducted as per standard procedures, which includes acid-digestion of samples for total elemental analyses, Olsen P for measuring bioavailable P and pH and electrical conductivity (EC) for all the samples involved (Rayment and Higginson 1992). The pH and EC for all the samples were determined by end-over-end equilibration of soils with water at a ratio of 1:5 for an hour and measured the solution with a pH/conductivity meter. For the selected elemental analysis, the samples were digested using aqua-regia (1:3 – HNO<sub>3</sub>:HCl) and the concentration of metals in the extract was determined by ICP-OES.

### **Incubation and Plant growth experiments**

The IS and NIS samples (200 g each) were incubated separately in plastic bags with FA and RM at the rates of 0 and 15 % (wt/wt soil) for 21 days at field capacity. The NIS samples were also treated with 200 mg kg<sup>-1</sup> (wt/wt soil) P using PP and PM, to compare the effects of various sources of P (inorganic and organic). The incubated samples were allowed to dry and 1 g samples were analysed for pH, EC and Olsen P. The dried samples (only the IS samples) were segregated into two parts – one for continuing incubation and the other (100 g) for plant growth experiment. The soil used for the incubation study was IS (control, FA and RM amended). The soil was incubated for five weeks and the selected parameters (pH, Olsen P and phosphatase activity) were used in the plant growth experiment to determine its P uptake ability in IS, amended with industrial by-products.

The grass saplings were grown on the incubated samples (100 g each) and were maintained with optimal moisture content at the greenhouse facility. The method devised by Stanford and Dement (1957) was used in this experiment to determine the effect of FA and RM on wastewater derived P towards soil retention and plant nutrition. Plastic pots (600 mL capacity; 10 cm diameter) with bottoms removed were nested in similar containers which had intact bottoms and were filled with 500 g sand. Three samplings of Napier grass were planted in each pot and Hoagland solution was supplied every day. Thirty days after planting, the pots containing saplings along with sand medium were transferred to another set of pots that contained 100 g of the incubated soil samples. A nylon mesh (pore size – 0.5 mm) was placed between the sand medium and the soil in order to separate the soil sample for analysis after the plant growth experiment. Rhizon samplers (Rhizosphere Research Products, Wageningen, Netherlands) were placed horizontally at 1 cm from the bottom of the pot. Pore water samples were collected using the Rhizon samplers at regular intervals after the transfer of saplings to the wastewater irrigated soils, and analysed for pH, and solution P. At the end of five weeks, the plants were harvested and the dry weights were recorded.

### **Soil and Pore water analyses**

The incubated soil samples were analysed for pH, EC, Olsen extractable P and phosphatase activity before and after commencing the experiment. The phosphatase activity of the soil samples was determined by measuring the release of para-nitrophenol from para-nitrophenyl phosphate (PNP). The incubated IS samples were exposed to a modified universal buffer (MUB) at pH 11 for the assay of alkaline phosphatase activity, as described by Tabatabai and Bremner (1969) and 1 mL PNP (0.025 M) was added to 1 g soil samples, followed by the addition of 0.2 mL toluene. The mixture was incubation at 37 °C for 90 minutes and centrifuged at 4000 rpm for 5 min. The p-nitrophenol formed was determined by spectrophotometer (Agilent 8453 UV-visible spectroscopy (Germany) system) at 440 nm.

The pore water samples were collected using rhizon samplers at 7, 14, 21, 28 and 35 days after the transfer of pots to the treated soil. Prior to pore water collection, the moisture content of the soil was maintained at 80 % of WHC and 60 mL of deionised water was added

for pore water collection and the pH was measured immediately. After pH measurement, the samples were analysed for soluble P content using phosphomolybdate method (Murphy and Riley 1962).

### Statistical analysis

All measurements, including pH, EC, Olsen P ( $\text{mg kg}^{-1}$ ), phosphatase activity ( $\mu\text{mol g}^{-1}$  PNP), and elemental composition ( $\text{mg kg}^{-1}$ ) were calculated for three replicates of each treatment. All the calculations and standard deviations of the replicates were determined using SPSS (version 17) (SPSS Chicago, Illinois). Variability in the data was expressed as standard deviation and a  $p < 0.05$  was considered to be statistically significant. The relationships for soil pH vs. Olsen P, phosphatase activity vs. Olsen P and pore water pH vs. soluble P were established by regression analysis in Grapher™ software (Golden software, version 9, USA). The acceptance limit was set to 95 % significance level.

### Results and discussion

#### Properties of the materials used

The pH of the IS and NIS were acidic (5.79) and alkaline (7.12), respectively. The abattoir wastewater irrigation may have lowered the soil pH and also added salts as reflected in the EC values (Table 1). The high amount of total P in IS showed P accumulation in these soils due to discharge of huge volume of nutrient-rich wastewater over the years. The FA used in this study also contained high P content compared to RM (Table 1). Olsen P values for IS and FA were around 35% of total P whereas NIS and RM showed lower Olsen P.

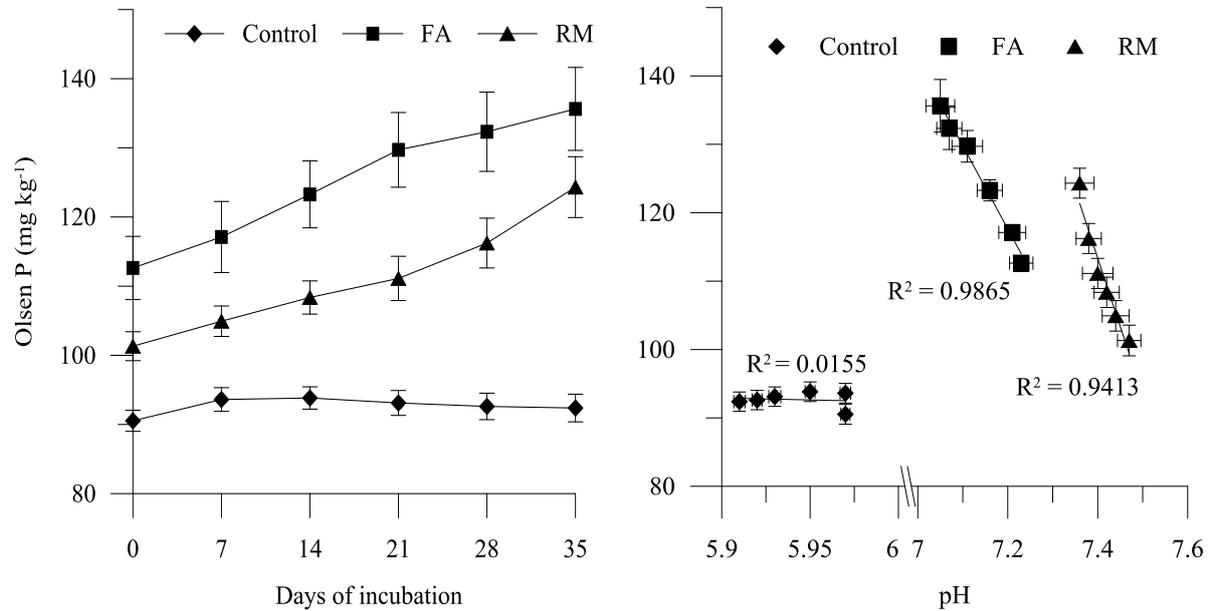
**Table 1** Characteristics of soils and industrial amendments (values are means  $\pm$  SD;  $n = 3$ , the mean difference is significant at  $p < 0.05$ )

Materials	pH	EC ( $\mu\text{S cm}^{-1}$ )	Total P ( $\text{mg kg}^{-1}$ )	Olsen P ( $\text{mg kg}^{-1}$ )	Major P sorptive elements ( $\text{mg kg}^{-1}$ )		
					Ca	Fe	Al
IS	5.79 $\pm 0.07$	1026 $\pm 23.12$	267.76 $\pm 8.23$	29.12 $\pm 4.56$	2436.54 $\pm 34.57$	10576.14 $\pm 62.85$	10741.82 $\pm 61.76$
NIS	7.12 $\pm 0.11$	634 $\pm 14.76$	76.82 $\pm 2.13$	13.42 $\pm 0.37$	1323.41 $\pm 25.84$	7631.42 $\pm 54.77$	7812.11 $\pm 53.68$
FA	10.53 $\pm 0.09$	1579 $\pm 27.28$	1154.31 $\pm 4.67$	51.33 $\pm 2.64$	18035.11 $\pm 51.62$	12130.11 $\pm 85.97$	25170.14 $\pm 50.21$
RM	11.37 $\pm 0.17$	5283 $\pm 36.77$	35.72 $\pm 1.32$	1.23 $\pm 0.09$	14321.31 $\pm 42.89$	15211.12 $\pm 91.62$	28630.12 $\pm 71.23$

#### Soil incubation experiment

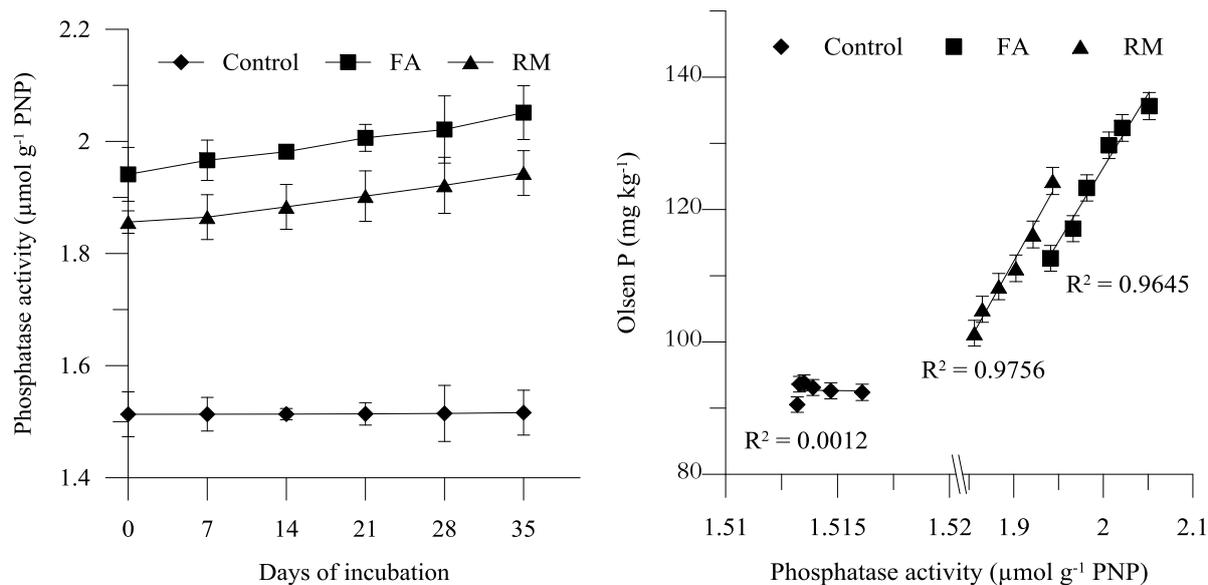
The soil used for the incubation study was IS (control, FA and RM amended). The soil was incubated for five weeks and the selected parameters (pH, Olsen P and phosphatase activity) were measured for five consecutive weeks. The Olsen P increased on the application of alkaline industrial amendments (FA and RM) and with increasing incubation time, where RM showed the highest increase (18.51 %) at the end of 35 days of incubation, followed by FA (16.96 %) and control (2.01 %). In the case of FA, there was a steady increase in Olsen P from 112.63 to 135.63  $\text{mg P kg}^{-1}$  soil and for RM, Olsen P increased from 101.32 to 124.32  $\text{mg P kg}^{-1}$  soil (Figure 1a). Pathan et al. (2002) observed an increase in bioavailable P from 18.5 to 43  $\text{mg kg}^{-1}$  soil in FA amended sandy soil under laboratory conditions. Summers et al.

(2001) and Snars et al. (2004) observed increases in bioavailable P in RM amended soils in the presence of plants. One of the major influencing factors for P availability is pH (Lindsay et al. 1989; McDowell 2005; Seshadri et al. 2013). A regression analysis between pH and Olsen P showed a negative correlation for both FA and RM, where Olsen P increased with decrease in pH (Figure 1b). In the case of control soil, there was no significant correlation ( $R^2 = 0.0155$ ,  $p > 0.05$ ).



**Fig 1** Effect of alkaline industrial amendments (FA and RM) on a. Olsen P in the incubated soils through five weeks and b. Relationship between soil pH and Olsen P through five weeks of soil incubation. Error bars represent the standard deviation between replicates.

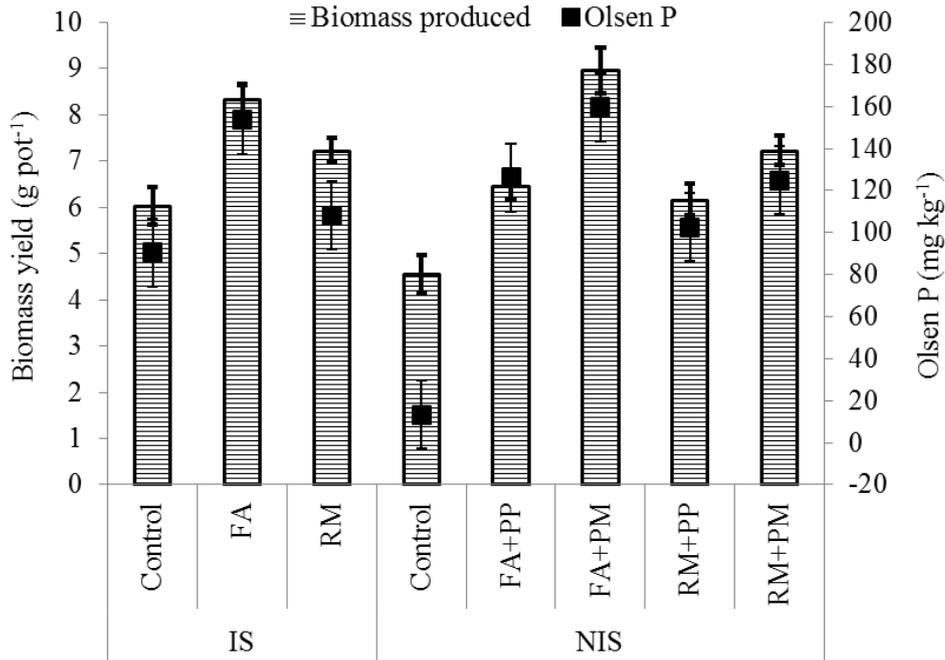
The phosphatase activity of soil in the presence of FA and RM over the five week incubation period showed increasing trends (Figure 2a). There was no significant increase in phosphatase activity for the control soil ( $R^2 = 0.0012$ ) over 35 days of incubation, whereas FA and RM showed increased phosphatase activity at 5.37 and 4.51 %, respectively. Both the amendments showed more than 90 % increase in phosphatase activity compared to the unamended soil. Lai et al. (1999) observed initial increase in phosphatase activity for FA and sludge amended soil samples. But after 30 days, they found decreased phosphatase activity, which they attributed to substrate depletion or accumulation of toxic substances, such as heavy metals. In this experiment, oxalic acid production also deteriorated as the incubation period increased, which can also be attributed to the accumulation of toxic substances. McCarty et al. (1994) observed a decrease in phosphatase activity as pH of the FA amended soil increased. Trasar-Cepeda and Carballas (1991) observed that an increase in pH reduced phosphatase activity when an acid soil was limed and also showed that mineralisation of P increased at pH above 6.5. In this incubation experiment, there was a significant positive correlation between soil phosphatase activity and Olsen P in FA ( $R^2=0.9645$ ) and RM ( $R^2=0.9756$ ) amended soils, whereas no significant relationship was noticed for the control soil ( $R^2=0.0012$ ,  $p > 0.05$ ) (Figure 2b). Therefore, for the soil incubation experiment, the increase in Olsen P for FA and RM amended samples was mainly due to phosphatase activity.



**Fig 2** Effect of alkaline industrial amendments (FA and RM) on a. Phosphatase activity in the incubated soils through five weeks and b. Relationship between soil phosphatase activity and Olsen P, through five weeks of soil incubation. Error bars represent the standard deviation between replicates.

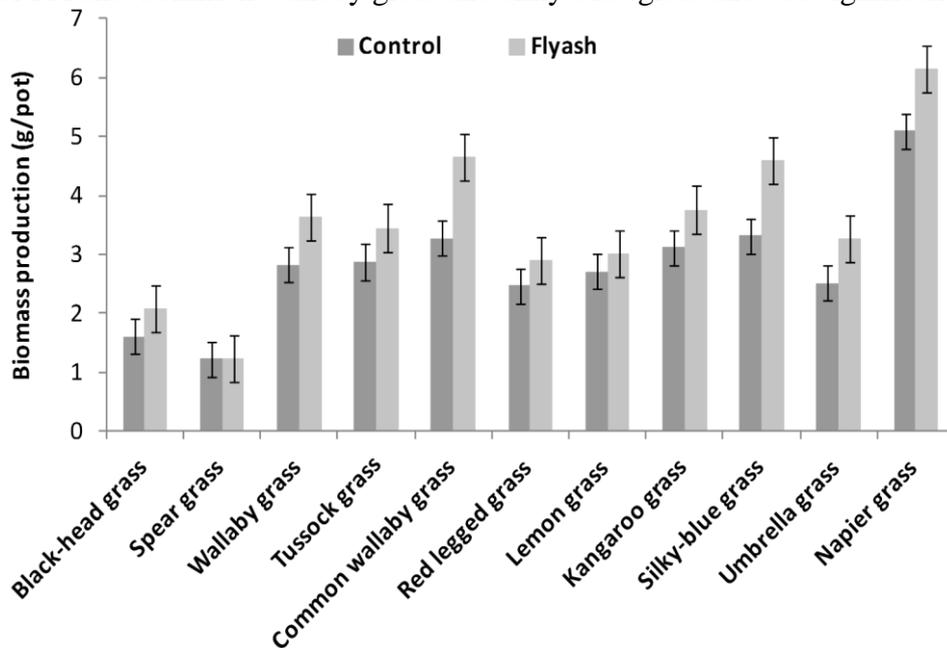
### Plant growth experiment

The biomass yields of the Napier grass plants and other grass species grown on the amended soils have been determined using their dry weights. The biomass of the Napier grass plants increased in the presence of amendments (FA and RM) for all the samples. The highest yields were recorded for FA amended samples, followed by RM. This can be attributed to the high Olsen P content of FA and pH increase as a result of FA and RM addition from 5.79 to slightly above 7 (Table 1; Pathan et al. 2002). In the case of IS, both FA and RM increased Olsen P in soil, with FA being the most effective amendment. The Olsen P value of FA amended IS was high (153.61 mg/kg) compared to control and RM amended IS (Figure 3). For NIS samples, there was a wide variation between P sources in FA amended soils. The PM treated soils produced higher biomass than PP treatment, which was also reflected in their Olsen P values. This can be due to the increase in phosphatase activity enhanced by FA and RM leading to mineralisation (Dou et al. 2003; Lai et al. 1999; Makoi et al. 2010).



**Fig 3** Effect of various treatments to abattoir wastewater irrigated and non-irrigated soils on biomass yield of Napier grass and their corresponding soil Olsen P. Error bars represent the standard deviation between replicates.

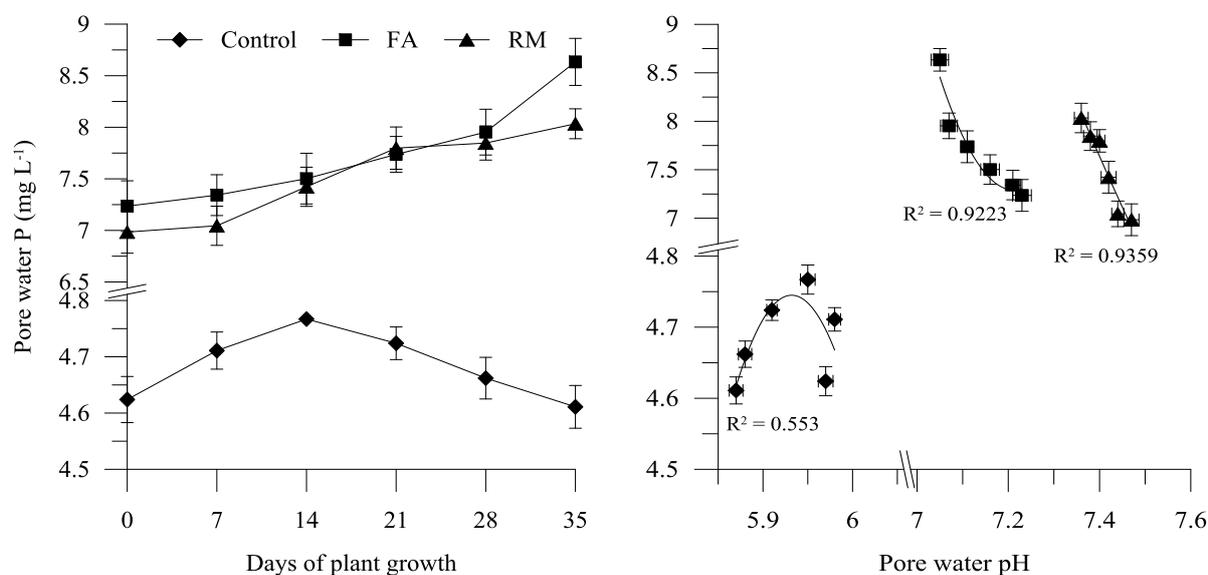
The overall results with other native grass species showed that although FA enhanced Olsen P in most of the plants, only two grass species were closer to the biomass yields of napier grass (Figure 4). Some of the grass species showed increased biomass production in the presence of FA and Common wallaby grass and silky-blue grass showed significant response.



**Figure 4** Effect of FA on biomass production of selected native grass species and napier grass. Error bars represent the standard deviation between replicates.

## Pore water analyses

The pore water samples are representative of the characteristics of the rhizosphere soil. The pH decreased (data not shown) with increasing days of plant growth which may be due to the  $H^+$  release from the roots (Dakora and Phillips 2002). The soluble P contents in the pore water of FA and RM amended soils showed increasing trends through the five week plant growth period (Figure 5a). A relationship between the pore water pH and pore water soluble P concentration showed significant negative correlation for FA ( $R^2 = 0.9223$ ) and RM ( $R^2 = 0.9359$ ) amended samples (Figure 5b). This can be attributed to the pH optimisation by OA exudated by Napier grass roots in response to their P demand. Shen et al. (2013) demonstrated that organic acids (including oxalic acid) exudated by Napier grass can transform P from sorbed forms to bioavailable forms as extracted by sodium bicarbonate solution.



**Fig 5** Effect of the industrial amendments (FA and RM) on (a) pore water P (mobilised P) through five weeks of plant growth and (b) Relationship between pore water pH and P. Error bars represent the standard deviation between replicates.

## Conclusions

This study showed that FA and RM can be effective amendments in mobilising inorganic and organic P components in soil, thereby increasing the biomass yield of Napier grass. Among the amendments, FA showed the highest increase in Olsen P and phosphatase activity, thereby showing maximum plant growth. The highest increase in Olsen P for PM treated NIS soils showed the ability of the amendments (FA and RM) on mobilising organic P better than inorganic P (PP). Phosphatase activity can be attributed to the mineralisation of P from organic P sources. The use of other plant species including native grass species can give us better ideas on the metabolic pathways involved in P mobilisation at the rhizosphere level.

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