The agronomic effectiveness of a fish waste based P fertiliser – spatial variability of Olsen P and pasture responses.

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Abstract
Phosphorus is one of the essential nutrients for pastures, particularly in P deficient soils such as those commonly found in Australia. A new phosphorus fertiliser, derived from activated (inoculated) rock phosphate composted with waste from the seafood industry and selected fungi and bacteria, has been proposed for agricultural use in Australia. Such a product has the dual advantages of supplying required nutrients to agricultural crops as well as utilising waste materials that are otherwise costly to dispose of. A two-year field trial was established on a low-marginal Olsen-P (< 18 mg/kg) soil at Tatura (northern Victoria) to examine the effectiveness of this product in comparison with a traditional P fertiliser (single superphosphate) on irrigated pasture. A spatial variability study of Olsen P levels was conducted. Soil Olsen P, plant dry matter and P uptake over the 2003-04 irrigation season were measured. The spatial variability study enabled us to select experimental plots with uniform Olsen P levels. No significant dry matter response to P fertiliser was observed for the pasture cuts during the first irrigation season. Olsen P levels at the end of the irrigation season were significantly lower, on average, for the fish based P fertiliser treatments than for superphosphate treatments.

Key words
Waste reuse, spatial analysis, P variability, irrigated pastures.

Introduction
Disposal of fish waste is a problem of increasing concern to Australia's seafood industry. Thousands of tonnes of fish waste are produced by processors and retailers each year in Australia (Gavine *et al* 2001). When fish are processed, the bulk (~60% by weight) of the product is discarded, often at a cost to the processor and ending up in landfill. As a result, the seafood industry in Australia is currently developing a fish waste based P fertiliser from fish waste, reactive phosphate rock (RPR), and selected fungi and bacteria.

Australian soils are generally deficient in phosphorus (P) in their native state. They require regular application of P fertilisers to satisfy the crop and pasture requirements for P. Irrigated pastures in northern Victoria support an important part of Australia's dairy industry. The application of P fertiliser is generally necessary to maintain pasture productivity on soils supporting the dairy industry.

A field trial was established on a low-marginal Olsen-P (< 18 mg/kg, Peverill *et al* 1991) soil at Tatura (northern Victoria) to examine the effectiveness of the fish waste P fertiliser (a new product) in comparison with traditional P fertiliser (superphosphate) on irrigated pasture. In this paper we evaluate (i) the spatial variability of soil Olsen P levels in relation to selection of experimental plots, and (ii) the first irrigation season pasture responses to fertiliser treatments.

Spatial variability of Olsen P in relation to plot selection
In order to compare the performance of P fertiliser products, we ideally needed an experimental site where the Olsen P levels are low (< 12 mg/kg, Peverill *et al* 1991) and fairly uniform across the site. A site (242 m x 70 m) on N5 block at DPI Tatura, northern Victoria, was chosen on the basis of previous fertiliser history. Preliminary soil testing was carried out (June 2002) in a traditional manner to assess the levels of Olsen P. A number of soil samples (0-10 cm) were collected at random locations across the chosen site, bulked together and the single bulked sample was used to estimate the Olsen P level for the whole site. At the DPI Tatura site, this method revealed an Olsen P level of 14 mg/kg, which was regarded as low-marginal (Peverill *et al* 1991) and therefore likely to respond to the application of P fertiliser in irrigated pastures. However, this method was crude because it does not take account of

possible spatial variability in Olsen P levels across the site in Olsen P levels, which could lead to bias in statistical comparisons among fertiliser treatments.

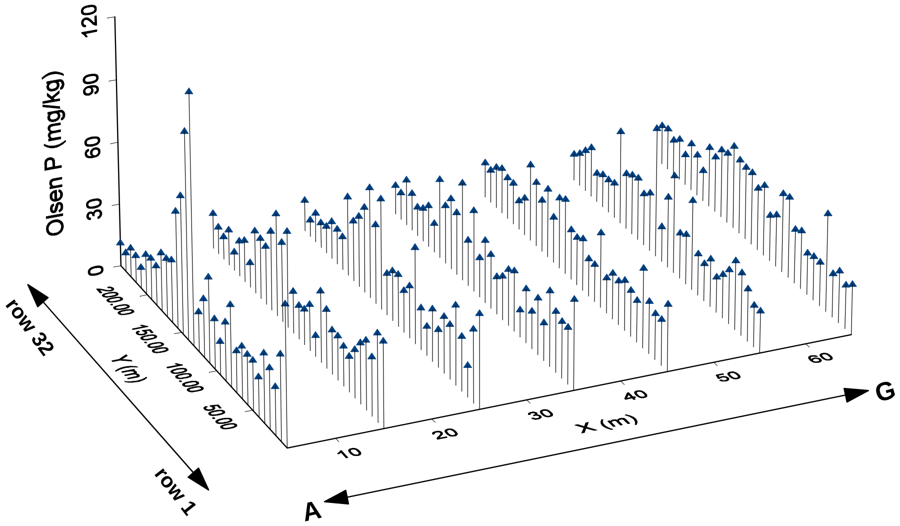

Figure 1. The spatial distribution of surface (0-10 cm) Olsen P values on the proposed pasture trial site.

The spatial variability in Olsen P levels across the site was examined in greater detail by sampling on a spatial grid. A two-dimensional regular grid with 7 columns (A-G in Figure 1) and 32 rows (Y- axis in Figure 1) was established on the site. The distance between columns was 10 m and the distance between rows was 7.62 m. At each of the 224 (7x32) spatial locations on the grid, four soil samples (0-10 cm) were collected, combined, and analysed for Olsen P levels. The raw data was plotted as a three-dimensional scatter plot in Figure 1.

Exploratory data analysis

By artificially assigning columns (A-G) and rows (1-32) to the sampling grid at the field site, we can describe the Olsen P data statistically. Olsen P levels on the site ranged from a minimum value of 8 mg/kg to a maximum of 120 mg/kg, with an average value of 26.45 mg/kg (s.e.m=0.88 mg/kg). In Tables 1 and 2 means and standard error of means are presented for the 7 columns and 32 rows, respectively. We can see, from Table 1, that mean Olsen P levels are higher but more variable in column A compared to other columns. This column corresponds to the bottom end of existing irrigation bays, where nutrients may have accumulated under previous flood irrigated conditions. In Table 2, Olsen P levels were particularly high (at or above 35 mg/kg) in rows 1, 5, 12, 18-21 and 24. A couple of these rows are likely to correspond to former fence lines where soil P is elevated in zones of stock camping. They are consistently lower between rows 25 and 32 where they do not exceed 19 mg/kg. No historical records exist for this site, but it is possible that rows 25-32 may have not been fertilized due to their proximity to major irrigation supply and drainage channels.

Table 1. Sampling statistics along columns at the proposed trial site for topsoil Olsen P concentrations (mg/kg).

Column	A	B	C	D	E	F	G
Mean	32.56	24.84	26.50	25.09	24.19	25.69	26.88
s.e.m	4.39	1.92	2.17	1.59	1.20	1.92	1.43

Table 2. Sampling statistics along rows at the proposed trial site for topsoil Olsen P concentrations (mg/kg).

Row	Mean	s.e.m	Row	Mean	s.e.m
1	38.86	5.93	17	22.00	2.31
2	29.71	3.60	18	55.86	11.31
3	23.57	1.63	19	42.14	9.73
4	27.57	1.48	20	43.14	5.89
5	36.29	2.16	21	37.43	3.45
6	21.86	1.22	22	30.43	1.29
7	22.71	1.36	23	28.43	0.53
8	20.00	1.09	24	35.00	2.35
9	24.14	0.99	25	15.86	0.91
10	19.14	1.72	26	18.14	0.94
11	27.43	3.41	27	18.43	0.95
12	36.00	2.68	28	13.57	1.36
13	19.86	2.11	29	18.00	1.68
14	20.86	0.86	30	16.86	1.52
15	30.71	3.96	31	13.57	1.59
16	26.43	2.25	32	15.14	1.03

Box-and-Whisker plots showing the median, interquartile range (middle 50% of values) and minimum and maximum values for the 7 columns (Figure 2a) and 32 rows (Figure 2b) display the distribution of Olsen P levels at the proposed trial site.

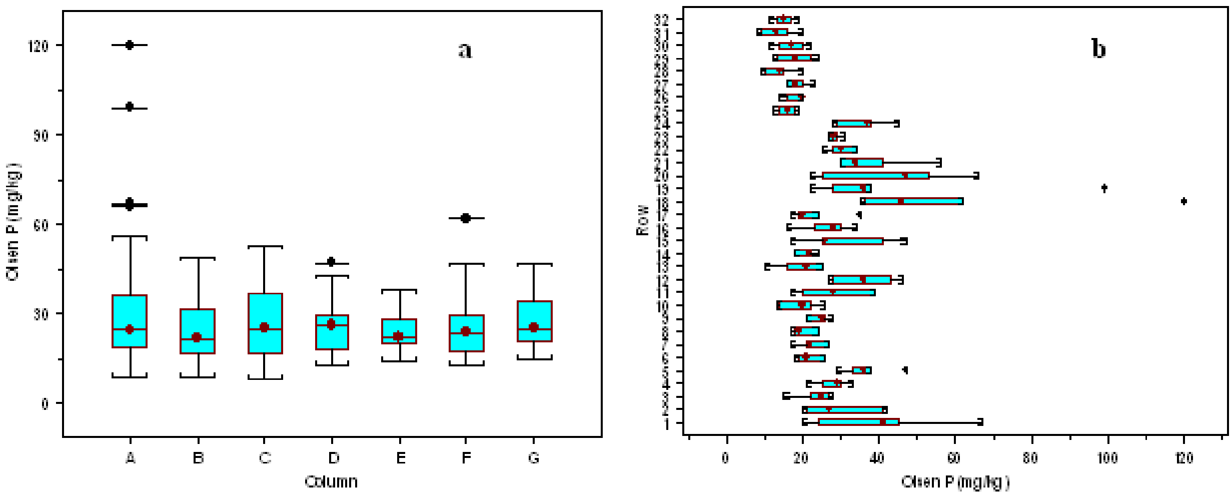

Figure 2. The distribution of values for the median, interquartile range, minimum and maximum for topsoil Olsen P measured along (a) columns and (b) rows of the proposed trial site.

It can be seen, from Figure 2a, that there are a number of large outlying values for column A, which has caused the larger mean recorded in Table 1. The medians for the 7 columns are fairly uniform. In Figure 2b, it is clear that the Olsen P levels are smaller and less variable in rows 25 to 32, and therefore more suitable for our trial purposes.

Spatial analysis of Olsen P data

In Figure 3, an omnidirectional variogram is shown for the Olsen P data, omitting two outlying observations (Olsen P levels of 120 and 99 mg/kg in column A). The variance (semi-variance) can be seen to increase until a distance of approximately 80 m after which it levels off and starts to decline. However, the decline should be treated with caution, as variograms become less stable at greater distances as the number of counts (point to point comparisons) decreases. It should be noted that we did not examine for spatial anisotropy because this is only recommended when there are greater than 300 data points.

Fitting a model to the observed variogram

A spherical model was also fitted to the variogram for surface Olsen P values (Figure 3). As a first approximation, this model explains 94.4% of the variation in the observed variogram, with sill=114.4, range=98.6 and nugget=34.7.

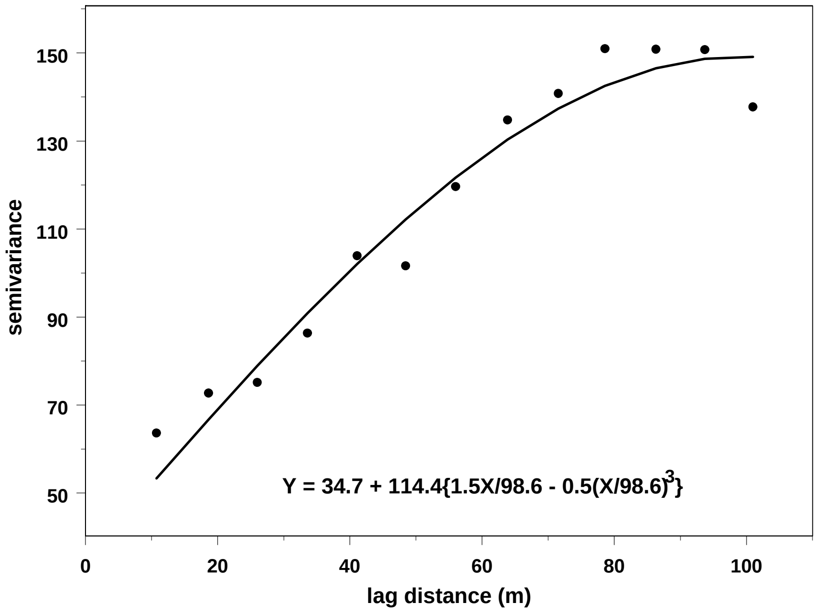

Figure 3. Experimental variogram and fitted spherical model for surface Olsen P at proposed trial site.

Mapping the data

The spherical variogram model was used to aid in the prediction of Olsen P at other locations on the site. This process, known as kriging, allowed us to obtain a grid of predicted values that can be mapped. Basically, these kriged predictions are weighted averages of the observed Olsen P values, with the weight being based upon the fitted variogram. Interpolation was performed on a grid of points 1 m apart, giving a total of 241×65=15, 906 predicted Olsen P values. These predicted values were plotted as a contour plot in Figure 4. It can clearly be seen from the contour plot that Olsen P levels vary considerably but they are at their lowest and most uniform in the top half of the plot, above 190 m, corresponding to rows 25 to 32.

Pasture fertiliser experiment

Based on the spatial variability analysis of topsoil P distribution, the fertiliser experiment was relocated in that part of the proposed trial site where Olsen P levels were at their lowest and most uniform. This new site corresponded to rows 25 to 32 of the original site. The experiment was laid out as a 2 x 3 factorial experiment with added control in four randomised blocks (blocks B, C, E and F in Table 3). The two factors used represented two types of fertilisers (biophosphate, B and superphosphate, S) and three rates of application (1/2 the recommended P rate, the recommended P rate and twice the recommended P rate). The recommended P rate at this site was 30 kg P/ha (G.N. Ward, pers. comm.). The total number of plots (9 m x 6 m) in the experiment is 28 (7 treatments x 4 replicates). Previous P fertiliser experiments at DPI Tatura had revealed coefficients of variation (CV) for dry matter production of <5%, indicating that four replicates were necessary to show treatment differences of 13% (assuming type 1 and 2 errors of 5 and 10%). The layout for the trial (Table 3) was deliberately chosen to further minimise soil Olsen P variation by judicious placement of lane-ways and buffer zones, and by preferential randomisation of the treatments within the selected blocks.

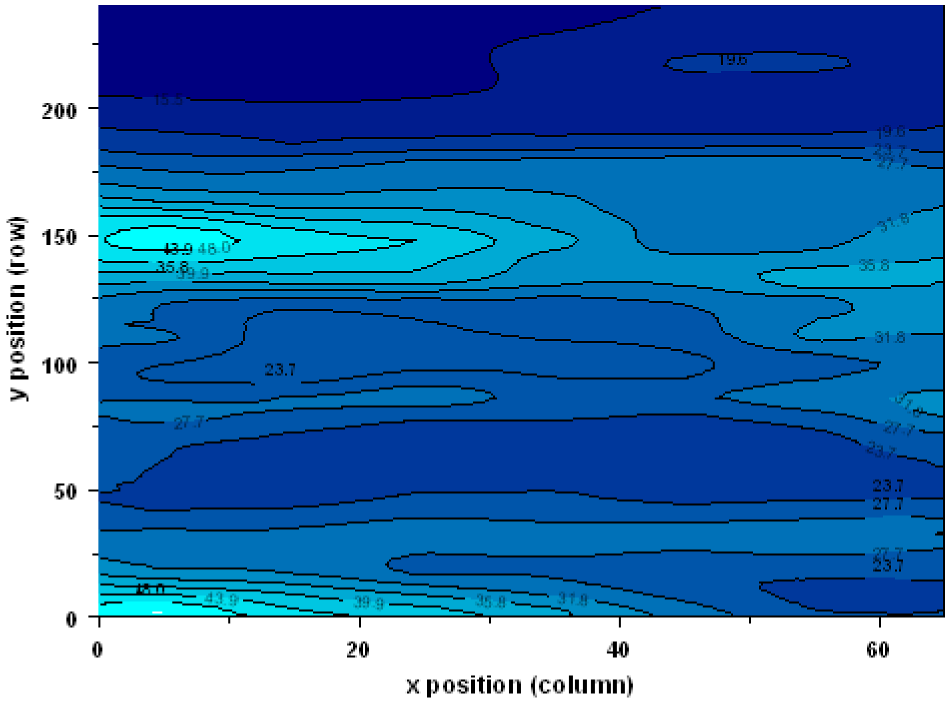

Figure 4. Predicted contour plot for surface soil Olsen P values at the proposed trial site.

Table 3. Layout of the P-fertiliser experimental site according to the rows and columns of an earlier proposed site.

Column/ Block	**Row**						
	25	**26**	**27**	**28**	**30**	**31**	**32**
G				Buffer zone			
F	B[1] 0.5X	Control	S[2] 2.0X	S 0.5X	B 1.0X	S 1.0X	B 2.0X
E	Control	B 1.0X	S 1.0X	B 2.0X	S 2.0X	S 0.5X	B 0.5X
D				Lane way			
C	B 2.0X	B 0.5X	B 1.0X	S 1.0X	Control	S 2.0X	S 0.5X
B	S 1.0X	S 0.5X	B 0.5X	B 1.0X	B 2.0X	Control	S 2.0X
A				Buffer zone			

[1]B=Biophosphate, [2]S=Superphosphate; 0.5X, 1.0X, 2.0X correspond to different ratios of the recommended annual application rate (1.0X) of P fertiliser for pasture soils.

First irrigation season (2003-04) data

Olsen P levels were assessed for the 28 plots in July/Aug 2003. White clover- ryegrass pasture was sown at 15 kg/ha on 23 Sep 2003 and a pre-treatment pasture cut was taken 104 days after sowing (5/01/04) to measure dry matter yield. The fertiliser treatments (Table 4) were applied on 6 Jan 2004 and further pasture cuts (numbered 1 to 5) were respectively taken on days 129 (30/01/04), 154 (24/02/04), 184 (25/03/04), 206 (16/04/04) and 247 (27/05/04) after sowing to assess pasture yields. The yields were expressed as kg dry weight/harvested strip (7.72 m^2)/plot. At cuts 2, 3 and 4, herbage P analysis was undertaken. P uptake was calculated from dry matter yields and herbage P concentrations. The significance of data was assessed using analysis of variance (ANOVA).

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Table 4. Analytical components of fertilizers used in the experiment (w/w).

Analyte	Superphosphate	RPR-Fish Waste based P fertiliser
Total P	7.52%	9.6%
P as water soluble	6.84%	<0.5%
P as citrate soluble	0.51%	1.6%
P as citrate insoluble	0.17%	7.6%
Total K	6.02%	8%
Total S	11.87%	6.4%
Total Ca	16.24%	25.6%
Total N	0	0.1%
Organic matter (loss on ignition at 550^0C)	-	8%
Total Carbon (Leco)	-	1.5%

Pre-treatment Olsen P levels and pasture dry matter

Olsen P levels prior to application of treatments ranged from 8 mg/kg to 18 mg/kg, with a mean level of 12.61 mg/kg (s.e.m=0.42 mg/kg) and there were no significant differences in mean levels among the plots allocated to the 7 treatments (Table 5). Similarly, pre-treatment pasture yields did not differ significantly among treatments (Table 5). Consequently, these data were not used as covariates in further analyses.

Table 5. Mean Olsen P for topsoil and pasture dry weight values for pre-treatment assessments.

Treatment	Soil Olsen P (mg/kg)	Pasture dry weight (kg)
Control	13.50	0.402
Biophosphate 0.5X	13.00	0.320
Biophosphate 1.0X	12.25	0.439
Biophosphate 2.0X	12.74	0.381
Superphosphate 0.5X	13.00	0.308
Superphosphate 1.0X	11.50	0.402
Superphosphate 2.0X	12.25	0.262
Residual mean square	5.73	0.065
sed[1]	1.65	0.180
lsd[2] (5%)	3.47	0.379
CV[3] (%)	18.5	71.0

[1]standard error of difference between two means, [2]Fisher's unprotected least significant difference, [3]coefficient of variation

Post-treatment pasture dry matter, P concentration and P uptake

There was no significant effect of fertiliser type or application rate on pasture dry matter weights at any of the five cuts or for the first irrigation season totals (Table 6). It should also be noted that there was a large block effect at cuts 2 and 3, with mean weights high in block F for both cuts (attributed to weeds) and low in block B for cut 2.

Table 6. Mean pasture dry matter weights (kg) for 5 cuts and total dry matter for the season.

Treatment	Cut 1	Cut 2	Cut 3	Cut 4	Cut 5	Season total
Control	0.486	0.713	0.911	0.514	0.317	2.94
Biophosphate 0.5X	0.433	0.604	0.818	0.506	0.356	2.72
Biophosphate 1.0X	0.452	0.667	0.895	0.487	0.354	2.86
Biophosphate 2.0X	0.402	0.552	0.625	0.524	0.359	2.46
Superphosphate 0.5X	0.461	0.616	0.726	0.488	0.380	2.67
Superphosphate 1.0X	0.434	0.646	0.833	0.612	0.455	2.98
Superphosphate 2.0X	0.527	0.715	0.990	0.521	0.484	3.24
Residual mean square	0.014	0.155	0.054	0.028	0.014	0.24
sed	0.085	0.088	0.165	0.117	0.082	0.35
lsd (5%)	0.179	0.185	0.346	0.246	0.173	0.73
CV (%)	26.3	19.3	28.1	31.8	30.1	17.4

Table 7. Mean herbage P (%) of pasture for cuts 2, 3 and 4.

Treatment	Cut 2	Cut 3	Cut 4
Control	0.393	0.365	0.365
Biophosphate 0.5X	0.405	0.398	0.380
Biophosphate 1.0X	0.408	0.360	0.383
Biophosphate 2.0X	0.400	0.390	0.383
Superphosphate 0.5 X	0.440	0.360	0.405
Superphosphate 1.0X	0.413	0.378	0.413
Superphosphate 2.0X	0.480	0.445	0.450
Residual mean square	0.001	0.002	0.001
sed	0.023	0.028	0.017
lsd (5%)	0.048	0.058	0.037
CV (%)	7.8	10.2	6.2

For the total P measured in herbage (Table 7) there was no significant effect of fertiliser type or application rate on measured responses until the fourth cut. At this latter harvest, herbage from treated plots (0.40 %P) had significantly ($p<0.05$) higher values than controls (0.37 %P). Superphosphate treatments (0.42 %P) also had significantly ($p<0.01$) higher herbage P than biophosphate treatments (0.38 %P).

Application of P at twice the recommended rate resulted in increased P uptake for the superphospate fertiliser when compared to biophosphate fertiliser, when using an unrestricted F-test ($p>0.05$, Table 8).

Table 8. Mean P uptake by pasture (g P/harvested strip) for cuts 2, 3 and 4.

Treatment	Cut 2	Cut 3	Cut 4	Total
Control	1.90	3.41	1.87	7.18
Biophosphate 0.5X	1.75	3.19	1.90	6.84
Biophosphate 1.0X	1.85	3.28	1.88	7.01
Biophosphate 2.0X	1.59	2.43	2.01	6.03
Superphosphate 0.5 X	2.04	2.60	1.97	6.61
Superphosphate 1.0X	1.81	3.16	2.53	7.50
Superphosphate 2.0X	2.57	4.31	2.27	9.16
Residual mean square	0.36	0.91	0.39	2.49
sed	0.42	0.68	0.44	1.12
lsd (5%)	0.89	1.42	0.92	2.34
CV (%)	31.1	29.9	30.0	21.9

Olsen P levels at end of the 2003-04 irrigation season

Soil samples (0-10 cm) were collected from each of the 28 plots at the end of the 2003-04 irrigation season and assessed for their level of Olsen P (Table 9). On average, significantly higher Olsen P levels were observed for treated than for control plots ($p<0.01$, control=8.0 mg/kg, treated=12.7 mg/kg).

In addition mean Olsen P levels were significantly higher, on average, for superphosphate than for biophosphate ($p<0.001$, S=16.3 mg/kg, B=9.2 mg/kg). The rate of application had a significant effect on the level of Olsen P remaining in the soil ($p<0.001$, 0.5X=10.5 mg/kg, 1.0X=11.1 mg/kg, 2.0X=16.5 mg/kg). There was evidence of a significant interaction between type and rate of fertiliser ($p<0.001$), with little difference between the three biophosphate rates but with superphosphate having a much larger mean Olsen P value for the highest rate 2.0X.

It should also be noted that Olsen P levels in the control treatment dropped from 13.5 mg/kg (marginal) to 8 mg/kg (low) over the course of the first irrigation season.

Table 9. Mean Olsen P of topsoil at the end of the 2003-04 irrigation season.

Treatment	End of season Olsen P (mg/kg)
Control	8.00
Biophosphate 0.5X	9.75
Biophosphate 1.0X	9.00
Biophosphate 2.0X	8.75
Superphosphate 0.5X	11.25
Superphosphate 1.0X	13.25

Superphosphate 2.0X	24.25
Residual mean square	5.14
sed	1.60
lsd (5%)	3.37
CV (%)	18.8

Conclusion

For assessing the agronomic effectiveness of a fish waste based P fertiliser, a spatial variability study enabled us to select experimental plots with uniform Olsen P levels. No significant dry matter response to P fertiliser application was observed for the pasture cuts during the first irrigation season. This is a likely response during early pasture establishment, especially with weed invasion of one experimental block during a period of wet weather. One irrigation season was required to achieve segregation of soil P treatments within plots initially selected for highly uniform Olsen P levels. Consequently it is expected that the experimental site will only show a dry matter response to P fertiliser in the second and subsequent irrigation seasons.

If the biophosphate treatments record similar yields to the superphosphate treatments over the remaining two irrigation seasons while exhibiting lower Olsen P levels (as occurred in the first irrigation season), there would be advantages of using this fish-based P fertiliser in intensive irrigated dairy industry. The likelihood of higher P losses and eutrophication of natural water bodies through surface run-off and leaching is higher from soils with higher Olsen P level. Consequently biophosphate P fertiliser may have an important role in environmental management of P losses from soil as well as limiting the disposal of fish waste to landfill for the seafood industry.

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