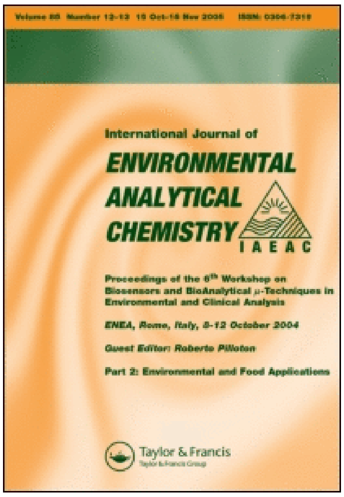

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INORGANIC MONOPHOSPHATE DETERMINATION IN OVERLAND FLOW FROM IRRIGATED GRAZING SYSTEMS

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This paper presents a novel technique for measuring soluble inorganic monophosphate in water based on high performance liquid chromatography and flow injection detection (IC-FI). The IC-FI technology was applied to irrigation water from the Macalister Research Farm in South Eastern Australia. Overland flow was measured exiting two irrigation bays. Inorganic monophosphate constituted over 97% of the DRP component and between 86–93% of the total phosphorus. Similar results were obtained from a channel that drained the excess irrigation water from the farm. This work supports the findings of others that show DRP contributes a large portion of the P in runoff from grazing systems. Additionally, it confirms this fraction is dominated by immediately bioavailable inorganic monophosphate, something that previously could only be inferred due to the limitations of the chemistry applied.

Keywords: Monophosphate; dissolved reactive phosphorus; irrigation water; ion chromatography; flow injection analysis; orthophosphate

INTRODUCTION

Phosphorus (P) is an essential nutrient in agricultural systems and is present in different forms. Transfer to aquatic systems of immediately bioavailable forms of P, such as inorganic monophosphate, increases the risk of algal blooms compared with refractory P compounds such as inositol phosphates. Inositol phosphates, as well as other complex organic phosphates, require hydrolysis by cyanobacteria to more available P forms, such as inorganic monophosphate, prior to uptake across the cell membrane [1].

Historically, P speciation has been based on physical separation, such as filtration through specified pore size filters [2, 3], or analysis with molybdate reagent

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pre- and post-digestion. These analytically defined groups contain numerous P compounds and are reviewed elsewhere [4]. Identifying specific P compounds in runoff water is a necessary prerequisite for investigating the processes by which P is mobilised and the likely effect on receiving waters.

P transferred from pasture based grazing systems to water is dominated by dissolved forms [5], particularly dissolved reactive phosphorus DRP [6, 7]. DRP is most commonly measured colorimetrically using the method described by Murphy and Riley [8]. Colour formation relies on the presence of inorganic monophosphate, implying that DRP and inorganic monophosphate are similar. However, the acidic conditions of the Murphy-Riley test may cause hydrolysis of acid-labile phosphorus compounds. Additionally, complexes formed between molybdate and contaminants found in some waters such as silica, germanium and arsenic are analytically indistinguishable from the mono-P complex [9]. Consequently, DRP may overestimate the true inorganic monophosphate concentration in water [10, 11].

This paper describes a novel technique for measuring the *true* inorganic monophosphate concentration in water based on high performance liquid chromatography and flow injection detection. Separation of inorganic monophosphate by ion chromatography eliminates interferences due to silica, arsenic and germanium ions, while hydrolysis of acid-labile phosphorus compounds is avoided because inorganic monophosphate is subject to acidic conditions only after it has been resolved from the sample proper. This technology was applied to water samples (overland flow) from an irrigated dairy farm in South Eastern Australia to measure the soluble inorganic monophosphate component transported from irrigated pastures in this system.

EXPERIMENTAL

Fieldsite description and irrigation management

The study area was located on the Macalister Research Farm in the Macalister Irrigation District, South Eastern Australia (37° 58' S, 146° 59' E). Samples were recovered from two bays, designated the Cypress and Fescue bays, and were 1.08 ha (360 m × 30 m) and 1.35 ha (270 m × 50 m), respectively. These bays are part of a 92 ha farm of which 66 ha have been developed for flood irrigation. Both bays had previously been laser graded (1:420), and the dominant pasture species were perennial ryegrass *(Lolium sp.)* and white clover (*Trifolium sp.*) on the Cypress Bay and tall fescue (*Festuca sp.*) on the Fescue bay. The farm is topdressed annually with 3 tonnes of phosphorus, 6 tonnes of potassium and

3 tonnes of sulphur (in half yearly dressings) and up to 20 tonnes of nitrogen (at monthly, or two monthly intervals) [12]. However, there had not been an application of P fertiliser for approximately 16 weeks prior to sampling.

Soil on the Cypress bay was classified as a red brown clay loam, while the Fescue bay contained a grey clay loam [13]. The range in Olsen P [14] values for the farm is 25–35 mg/kg. In addition to sampling the two bays, water was also recovered from the drainage channel servicing the Cypress and six other adjacent bays, encompassing an area of approximately 7.56 ha.

The Macalister Research Farm has an annual water allocation of 270 ML surface water and 97 ML groundwater. However, in a typical year about 480 ML of irrigation water is used (6 ML per hectare) [12]. Sampling occurred during the fourth irrigation for the 1998/99 irrigation season, and the water budget for each of the bays for the fourth irrigation is shown in Table I.

TABLE I Water balance for sampling locations

Sampling location	*Water on bay(s) (L)*	*Water off bay(s) (L)*	*Water loss (%)*
Cypress Bay	584 200	40 000	6.8
Fescue Bay	597 400	16 800	2.8
Drain	3 418 100[a]	518 000[b]	15.2

a. This hypothetical value has been estimated by multiplying the water on the Cypress Bay by seven. It indicates the maximum water volume that could be drained through this point

b. This is the actual water volume measured through the flume in the drain

Sample collection and pretreatment

The volume of irrigation water entering the Cypress bay was measured using a rectangular weir and a pair of Model 392 dataloggers (Dataflow, Queensland). Excess water leaving this bay was channeled through a 150 mm RBC flume [15] and samples collected in the flume using an ISCO storm-monitoring system comprising a Model 3700 automatic sampler, Model 4230 bubbler flowmeter, and Model 674 rain gauge (ISCO, Nebraska). Water was sampled across the entire flow with a self-elevating strainer. Water sampling was flow integrated to maximise sampling frequency at higher flow rates, when greater P loads were expected.

The Fescue bay was fitted with dual 200 mm flumes and four Dataflow 392 dataloggers to monitor the volume of water entering the bay. Excess water leaving the bay was channeled through a 150 mm RBC flume and sampled using an ISCO 3700 autosampler triggered by an ISCO liquid level actuator. Samples were automatically collected hourly thereafter. The water level was calculated using a pair of Dataflow 392 dataloggers.

TABLE II Phosphate concentrations and estimated load measurements in overland flow from the Cypress and Fescue bays, and in the flume draining the Cypress bays

Sampling location	*Monophosphate*		*Dissolved Reactive P*		*Total P*	
	Mean [mono-P] (mg P/L)	*Load P[a] (g/ha)*	*Mean [DRP] (mg P/L)*	*Load P (g/ha)*	*Mean [TP] (mg P/L)*	*Load P (g/ha)*
Cypress Bay	6.9 ± 0.2[b] [0.22 mM]	253	7.1 ± 0.2 [0.23 mM]	259	7.5 ± 0.1 [0.24 mM]	278 (265–291)[c]
Fescue Bay	5.3 ± 0.1 [0.17 mM]	148	5.4 ± 0.1 [0.17 mM]	151	6.2 ± 0.1 [0.20 mM]	77 (70–84)
Drain	3.5 ± 0.2 [0.1 mM]	230	3.5 ± 0.2 [0.11 mM]	232	3.9 ± 0.2 [0.12 mM]	267 (218–306)

a. Load calculated as mean P concentration × volume of water through flume divided by drainage area
b. Standard errors shown
c. Range values calculated based on minimum and maximum P concentrations through flume

Water from the drain was channeled through a 200 mm RBC flume in combination with a 600 mm rectangular weir, and samples were taken from the flume using the ISCO storm monitoring equipment described above for the Cypress bay.

Samples were filtered (<0.45μm) immediately on collection from the autosampler (cleared within 6 hours) and analysis for inorganic monophosphate and DRP commenced within two hours after filtration. Total P (TP) analysis was conducted within 5 days, with samples stored at –20 °C during this time.

Sample analysis

Water samples were collected and analysed for inorganic monophosphate, dissolved reactive phosphorus (DRP) and total phosphorus (TP). Inorganic monophosphate analysis was conducted using an on-line ion chromatographic system coupled to a flow injection detection system (IC-FI) described below.

Reagents were of analytical grade unless otherwise indicated. A standard phosphate stock solution (100 mg P/L) was prepared in deionised water from oven dried KH_2PO_4 (BDH, Victoria). This solution was stored for up to 3 months at <4 °C. Working standard solutions were prepared daily.

Ion chromatographic system

A Model 305 HPLC (Gilson, France) pump was used for the isocratic separation of inorganic monophosphate. Sample solution was injected using a Rheodyne 7125 injection valve into an Alltech (Australia) Wescan Anion/R separation column (250 mm × 4.6 mm i.d.) packed with a poly(styrene-divinylbenzene) trimethylammonium anion exchanger and eluted at a flow rate of 1.0 mL/min. The column effluent was diverted into the post-column reaction system using 0.3 mm i.d. teflon tubing, and was detected at 690 nm as phosphomolybdenum blue by spectrophotometry using a Cecil (United Kingdom) detector with a flow through cell (light path = 10 mm, volume = 75 μL). A schematic of the system is shown in Figure 1.

The mobile phase solution comprised 0.05 M KCl (BDH, Dorset) and 1.32×10^{-3} M Na_4EDTA (Sigma, St Louis). The ammonium molybdate reagent consisted of 8.90×10^{-3} M ammonium molybdate (BDH, Dorset) in 0.61 M H_2SO_4 (AJAX, Sydney), and the reducing agent consisted of 1.05×10^{-3} M $SnCl_2$ (BDH, Dorset) and 1.54×10^{-2} M hydrazine sulfate (BDH, Dorset) in 0.52 M H_2SO_4. All solutions were made weekly.

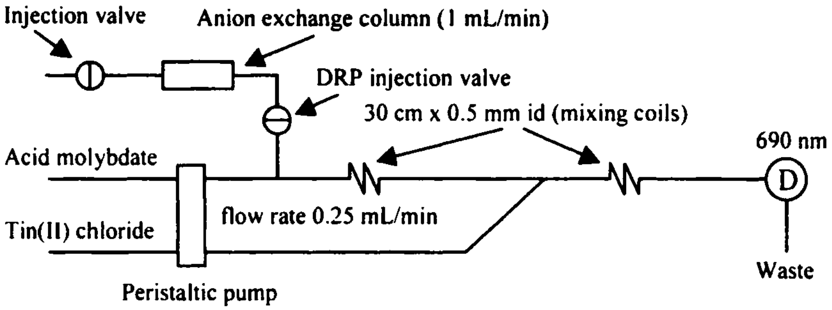

FIGURE 1 Schematic of the IC-FI system

Flow injection analysis of dissolved reactive phosphorus (DRP)

The flow injection determination of DRP was conducted using the same manifold as described in Figure 1 with two minor modifications. A low-pressure injection valve was introduced after the analytical column, and the salt carrier was replaced with deionised water to remove potential interference caused by refractive index differences between sample and carrier. Sample solution (50 µL) was injected post-column where molybdate reactive phosphorus was detected as phosphomolybdenum blue by spectrophotometry.

Total phosphorus analysis

Samples were analysed for TP using an alkaline persulphate autoclave digestion procedure adapted from La Chat method number 30–115–01–1-B [16] and analysed on a La Chat Quickchem 8000 (Milwaukee) flow injection system.

RESULTS AND DISCUSSION

Ion chromatography flow injection determination of orthophosphate

The IC-FI technique used in this study has been adapted from a more complex condensed phosphate separation published elsewhere [17]. The major modification to the original technique was the removal of the on-line digestion system. The modified IC-FI procedure improves on previous ion chromatographic systems used for inorganic monophosphate determinations by employing a rapid on-line flow injection detection system [18–20]. This sensitive and selective post-column detection system is superior to that traditionally used in conductivity detection because it overcomes the problem of background noise caused by the high ionic strength of the mobile phase.

Inorganic monophosphate determinations were conducted in <5 minutes. The detection limit for the IC-FI system was 20 µg P/L, based on the technique of Miller and Miller [21], and the linear working range was easily adjusted from 0.02 – 25 mg P/L by altering the size of the injection loop. Lower detection limits are achievable with better quality detectors [22]. For this work, an injection loop of 100 µL was used to provide a working range of 0.25 – 6 mg P/L. The regression equation was $[P] = (1.01 \times 10^{-5} \times \text{peak height}) + 0.01$ ($R^2 = 0.999$, $n = 5$). The technique was validated using Setpoint Laboratory Reference Standard No. 2184 (Graham B Jackson Pty. Ltd., Melbourne), with monophosphate recoveries ranging between 99–108%.

Comparison of monophosphate with DRP

Water budgets for the Cypress bay, Fescue bay and the Drain monitoring sites are shown in Table I. These values were used to calculate loads of inorganic monophosphate, DRP and TP in overland flow (and the drainage water) from the Macalister Research Farm based on the mean P concentrations in Table II. Inorganic monophosphate constituted greater than 97% of the DRP component from each of the monitoring locations (Table III).

TABLE III Relative abundance of P species in overland flow

Sampling location	*Mono-P vs DRP[a] (%)*	*Mono-P vs TP (%)*	*DRP vs TP (%)*
Cypress Bay	97.7 ± 0.6[b]	93.0 ± 1.3	95.2 ± 1.4
Fescue Bay	98.0 ± 0.5	86.0 ± 1.0	87.8 ± 1.4
Drain	99.1 ± 0.5	88.8 ± 1.3	89.6 ± 1.7

a. Based on estimated P loads from Table II

b. Standard errors shown

The DRP and inorganic monophosphate values from the Macalister Research Farm were highly correlated ($R^2 = 0.996$) with no statistical difference between inorganic monophosphate and DRP ($p = 0.79$) (Figure 2). This contrasts with numerous studies reporting often large differences between inorganic monophosphate and DRP concentrations in water [10, 11, 23–25]. Tarapchak [10] and Stainton [24] each cite up to two orders of magnitude difference between inorganic monophosphate and DRP in lake water samples. However, reported differences usually occur at low P concentrations (i.e. <0.1 mg P/L) and in natural systems [3, 10, 11, 22–24, 26]. Edwards and Withers [27] have cited a very limited data set that highlights differences determined between inorganic monophosphate and DRP measurements at concentrations of less than 0.02 μg P/L, suggesting that the values detected by ion chromatography probably reflect a more realistic P concentration present in *true* solution.

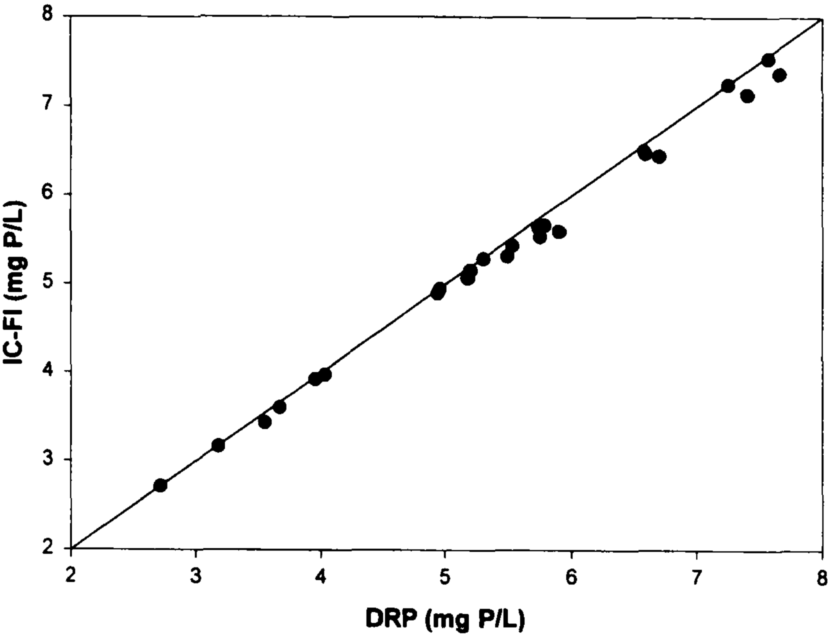

FIGURE 2 Comparison of DRP and monophosphate measured in overland flow from the Macalister Research farm (1:1 line shown)

There is little available data on the proportion of inorganic monophosphate in the DRP component of waters containing parts per million concentrations of P. However, it is reasonable to expect that in most cases a large portion of the DRP component in high P systems would contain a high inorganic monophosphate fraction, as these systems are usually the result of contamination of inorganic P sources (e.g. sewage/fertiliser). One exception to this could be the interstitial water in the soil. Soil solutions in farming systems have been reported to contain high concentrations of P [28, 29]. However they have also been reported to contain significant quantities of organic phosphates, particularly down the soil profile (>70 %) [30].

P species in overland flow from the Macalister Research Farm

The presence of a large inorganic monophosphate concentration in overland flow from the Macalister Research Farm is environmentally significant since inorganic monophosphate is considered to be the most bioavailable form of P [31]. Inorganic monophosphate contributed more than 97% of the DRP component, but also constituted between 86 – 93% of the TP. This data supports the findings of Nash and Murdoch [6] and others [32, 33] that report high percentages of dissolved reactive P transferred from grazing systems.

There is approximately 1–3 % of the DRP component in the overland flow that is not present as inorganic monophosphate in solution (Table III). This fraction could include easily hydrolysable organic P compounds that are converted to inoganic monophosphate during DRP analysis due to the acidic detection chemistry [9]. White and Payne [34] have reported the presence of a high-molecular weight reactive phosphate that could also account for differences between DRP and inorganic monophosphate. However, there is a growing body of evidence suggesting that a significant portion of the difference between inorganic monophosphate and DRP may be attributed to associations between inorganic monophosphate and colloidal material in the <0.45 μm range, including evidence to support both molybdate reactive and unreactive colloidal P species [2, 24, 25, 35]. Haygarth et al. [2] investigated different size filtration fractions for molybdate reactive phosphorus and suggested that P may have the potential to be physically mobile (by attaching to colloids and particles), but remain chemically immobile. We suggest that inorganic monophosphate attached to colloids would not co-elute with *free* inorganic monophosphate in the ion chromatographic system due to the charge differences of such P-clay associations, and therefore would not be detected as inorganic monophosphate in the IC-FI system.

The TP component in the overland flow contained between 88–95% DRP suggesting that 5–12% of the P load was in a particulate and/or unreactive form. This fraction could include the organic P component in the runoff, although it could also include some inorganic molybdate reactive P that is >0.45 μm. The IC-FI technique is limited in its capacity to characterise particulate (>0.45 μm) P fractions. It could perhaps be used after an appropriate extraction procedure to elucidate P species, although it is difficult to determine the impact of extraction procedures on the original P equilibrium.

P concentrations in overland flow in Australian systems are often higher than similar agricultural systems in Europe and USA [4, 30, 31, 36]. In dairy production systems in Australia the DRP component accounts for the majority of the total P, with lesser amounts of soluble organic and particulate P [6, 32]. Haygarth *et al.* [4] has found much lower TP concentrations on similar agricultural systems in the

UK. Perhaps the most striking difference between values reported by Haygarth and those found in this study was that after having runoff occur immediately after application of triple super their "highest" P levels in runoff were 1.773 mg P/L. This is lower than some baseline values measured in Australia [6], and 100 times less than values measured when runoff coincided with fertiliser application [Nash, *unpublished data*].

Haygarth et al. [4] also reported varying proportions of soluble organic, particulate and reactive P, with DRP not always accounting for the majority. However, the concentrations of soluble organic and particulate P found overseas may not necessarily be much different than those found in this study. This is because the organic and particulate P fractions attributed only 5–12 % of the TP, which in concentration terms equates to between 0.2–0.8 mg P/L. We currently do not know enough about P transfer processes to understand why Australian soils transfer significantly more phosphorus, compared with similar agricultural systems from overseas. However, it does appear from our work that the additional P lost from the Macalister Research Farm was in the most highly bioavailable form of inorganic monophosphate.

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