



ATMOSPHERIC **D**EPOSITION AND **I**MPACT OF
POLLUTANTS, **K**EY ELEMENTS AND **N**UTRIENTS ON THE
OPEN MEDITERRANEAN **S**EA
ADIOS

Deliverable N°9
Development of sampling and analytical methodology
for the determination of ^{32}P and ^{33}P in rain water and sea-water

Dissemination level: PU
20 December 2001

ADIOS
Atmospheric Deposition and Impact of pollutants, key elements and nutrients
on the Open Mediterranean Sea

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Development of sampling and analytical methodology
for the determination of ^{32}P and ^{33}P in rain water and sea-water

by
J. M. Pates and M. W. Bescoby

Department of Environmental Science,
I.E.N.S.,
Lancaster University,
Lancaster, LA1 4YQ.

1. INTRODUCTION

Phosphorus is a key nutrient, both in the Mediterranean and marine ecosystems in general. Furthermore, P is the limiting nutrient in the Mediterranean (Thingstad and Rassoulzadegan, 1995). Critical to understanding the role of P in ecosystems is the rate at which it is cycled between available and non-available forms. To date, our view of P cycling in the oceans has been derived principally from stable P concentrations and *in vitro* experiments. However, simple measurement of P concentrations is not sufficient to gain detailed insight into cycling rates, and *in vitro* experiments are subject to perturbation of the system under study.

Phosphorus-32 and -33 (half-lives = 14.3 and 25.3 days, respectively) are predominantly produced by the interaction of cosmic rays with atmospheric argon (Lal, 1999), and are subsequently removed from the atmosphere principally by wet depositional processes (Waser and Bacon, 1995; Benitez-Nelson and Buesseler, 1999a). In aquatic systems, these radionuclides rapidly become incorporated into biogeochemical cycles: they are analogous to anthropogenic stable P in being bio-available. Over time the $^{33}\text{P}:^{32}\text{P}$ ratio changes as a function of their different half-lives, and, because the input term can be constrained, kinetic data such as turnover rates can be determined. Determination of natural radioactive isotopes of P has advantages over other types of tracer study, most importantly: (i) the system is not perturbed by the addition of tracers (especially important for P-limited ecosystems, such as the Mediterranean); and (ii) the system can be studied without resort to enclosures or bottle experiments. One study, utilising this alternative approach, produced evidence that very low concentrations of bio-available P can be rapidly turned over, calling into question traditional views of P-limitation (Benitez-Nelson and Buesseler, 1999b).

Until recently, the complexity of ^{32}P and ^{33}P determinations has prevented their widespread use as an oceanographic tracer (Lal and Lee, 1988; Waser *et al.*, 1996; Benitez-Nelson and Buesseler, 1999b). Indeed, work in the Mediterranean has been limited to a single study of phosphate turnover in three coastal regions (Testa *et al.*, 1997). However, this technique offers a unique method of examining the uptake and release of P from atmospheric inputs into the surface ocean, and is the most powerful tool we have to address the turnover and fate of P in the upper water column.

Concentrations of these radionuclides are very low in seawater, typically 1000 times lower than in rainwater, which has average concentrations from 0.003 to 0.1 Bq l⁻¹ (Benitez-Nelson and Buesseler, 1998). This necessitates the preconcentration from 1000 l of seawater and low level counting techniques. Developments in liquid scintillation spectrometry (LSS) in the late 1990s have allowed recent advances in the determination of ^{32}P and ^{33}P in natural systems (Benitez-Nelson and Buesseler, 1998), which we aim to exploit here. This report concerns the establishment and testing of this new method at Lancaster University, for the determination of ^{32}P and ^{33}P in dissolved inorganic phosphorus (DIP), total dissolved phosphorus (TDP) and particulate phosphorus in marine and rainwater samples.

2. INSTRUMENTATION

^{32}P and ^{33}P decay by β emission with maximum energies (E_{max}) occurring at 1.71 and 0.249 MeV, respectively. Given the low concentrations of these radionuclides in environmental materials, it is essential to utilise a counting technique with the lowest achievable background. Until recently, most work has been carried out using gas-proportional counting (GPC) (Lal *et al.*, 1988; Waser *et al.*, 1996). However, GPC requires that the sample be dried to a uniform and reproducible thin film. Variations in the thickness of this film lead to variable counting efficiency (self-absorption), and hence potential errors.

The alternative is to use ultra-low-level liquid scintillation spectrometry (LSS). The instrument used here is the Packard Tri-Carb 3170, which includes a quasi-active bismuth germanate (BGO) guard to reduce background to levels compatible with the detection of environmental concentrations of ^{32}P and ^{33}P . LSS has the advantage over GPC of measuring samples in an aqueous matrix, avoiding problems associated with self-absorption. A second advantage of this instrumentation is the facility to view spectra and select regions of interest (ROIs), particularly as two β emitters of differing energies are being determined (Fig. 1).

Optimisation of counting conditions

In LSS, the optimum instrumental parameters for a particular radionuclide are generally found using the E^2/B

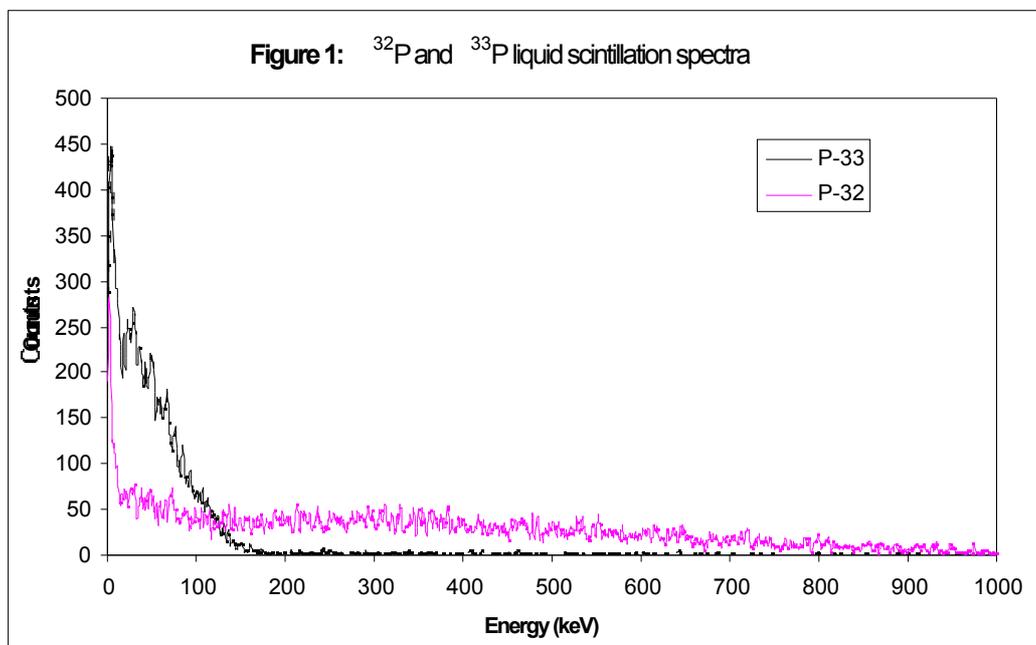


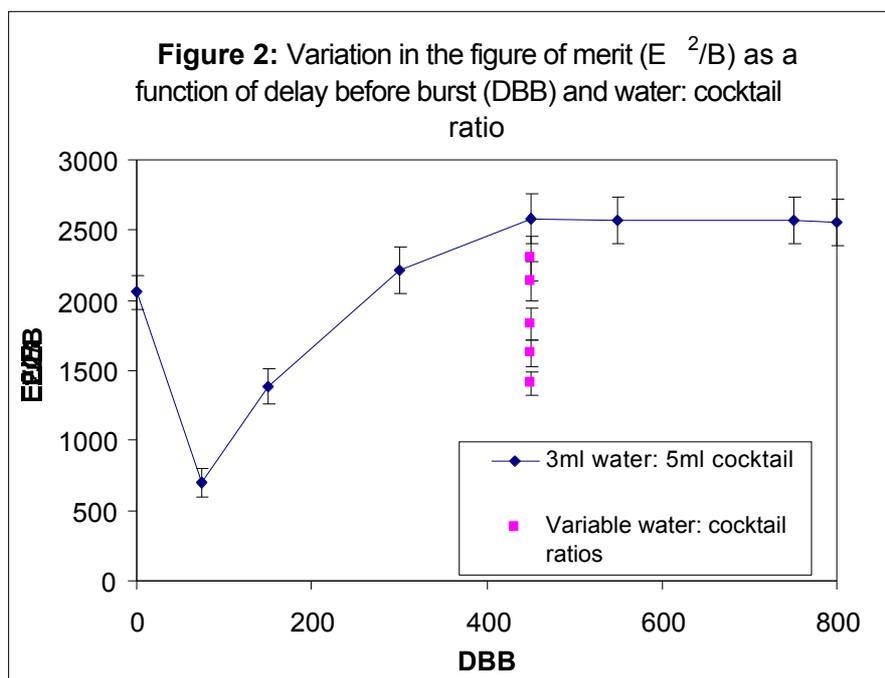
figure of merit, where E is the counting efficiency and B is the background. The lowest detection limits will be achieved when the efficiency is maximised and the background is minimised. In this instance, the parameters needing optimisation are the background reduction function and the water:cocktail ratio in the sample.

The Packard Tri-Carb 3170 uses pulse shape analysis to reduce the background during counting. Decay events detected in the sample result in electronic pulses, with a characteristic length according to event type. Background events, external to the instrument, result in longer pulses than those originating in the vial. The background reduction circuitry discriminates between true events and background events on this basis; pulses longer than a pre-set time (the delay before burst, DBB) are rejected. The BGO guard aids this process by causing external background events to produce longer pulses than normal. The default DBB is 75 ns, which has been optimised for low-energy β emitters. If this default is employed for higher energy β emitters, such as ^{32}P , not only would the background be reduced, but counting efficiency would be seriously affected too, as ^{32}P events are also rejected.

A further factor influencing the counting efficiency is the degree of quenching experienced by the sample (see below). A simple method of reducing the degree of quenching, and hence improve the counting efficiency, is to reduce the sample:cocktail ratio. However, the more cocktail there is in a vial, the higher the background will be. Therefore, this effect was also investigated to determine the optimum conditions.

Method: A pair of vials were prepared, each comprising 3 ml water and 5 ml Ultima Gold LLT™ scintillation cocktail in a 20 ml low-potassium glass vial. The first was the background, and the second was spiked with a known activity of ^{32}P tracer. The vials were counted for 200 minutes each at a range of DBB settings (from 75 to 800, the maximum) and the E^2/B figure of merit calculated for each setting. The efficiency was determined as in equation (1) below. In each case, the ^{32}P count rate was decay corrected to the time the experiment started (Fig. 2).

Once the optimum DBB had been determined, the experiment was repeated using a range of water:cocktail ratios, namely 3:7, 3:9, 3:12 and 3:15. The E^2/B figure of merit was determined in the same way for each of these combinations (Fig. 2).



The counting conditions selected on the basis of this work were a DBB of 450 and a water:cocktail ratio of 3:5.

Quench correction

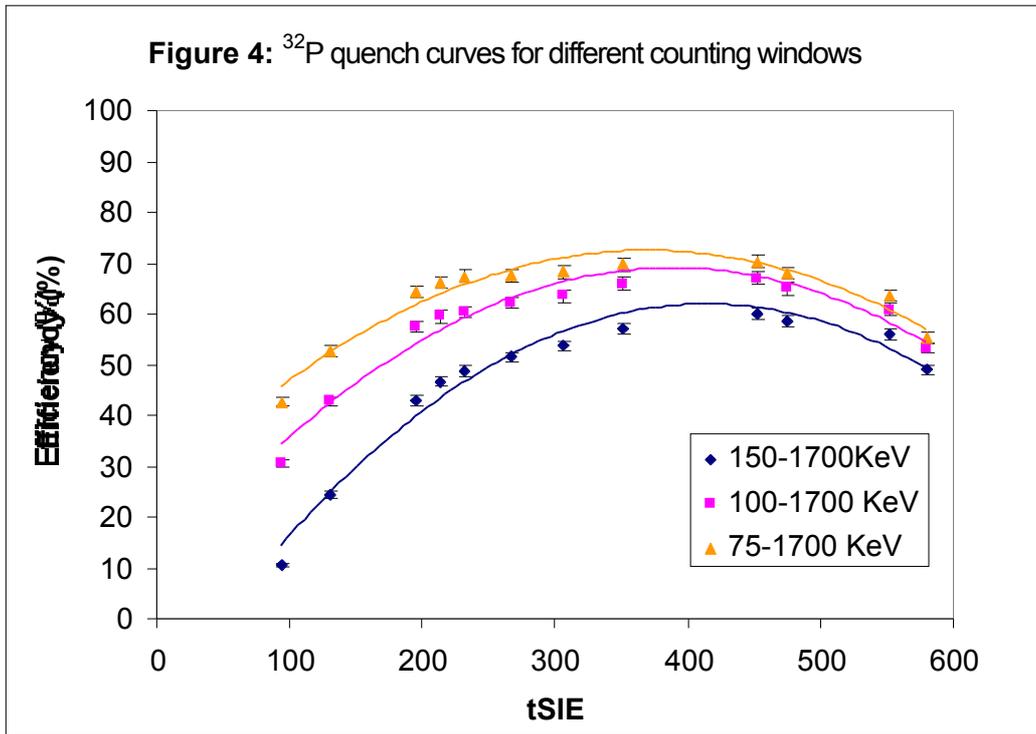
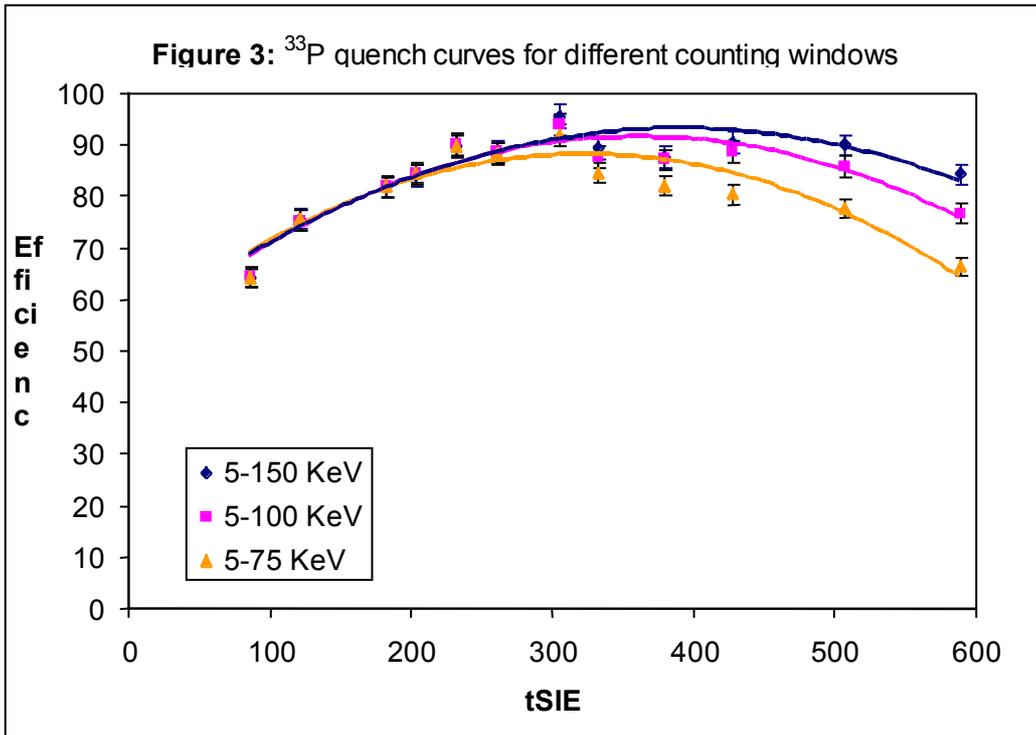
In LSS, ionising radiation in a sample passes through a fluorescent “cocktail” resulting in the production of light photons. Both the sample matrix and any impurities present in that sample matrix result in a reduction in the light produced, and, hence, the counting efficiency. A secondary effect is that the spectrum is shifted towards lower energies. Together these effects are called chemical quenching. The degree of quenching is determined through the use of the “transformed Index of the External Standard” or tSIE, where 0 indicates a fully quenched sample and 1000 is an unquenched sample. It is necessary to determine the impact of variations in the chemical quench on the counting efficiency, which can be achieved through the use of quench curves.

Method: A series of samples was prepared containing increasing amounts of a standard quenching agent, carbon tetrachloride (CCl_4), resulting in tSIEs ranging from ~ 85 to 600. Each sample consisted of 3 ml water and 5 ml Ultima Gold LLT™ in a 20 ml low-potassium glass vial. A known activity of ^{32}P or ^{33}P was added to half the samples, and the remainder were used to determine the background at each quench condition. All samples were counted for 100 minutes each.

Given that the spectra for ^{32}P and ^{33}P overlap (Fig. 1), and that chemical quenching causes spectra to shift to lower energies, it was necessary to use a series of regions of interest, or counting windows, to minimise interference between the two nuclides. Each counting window corresponds to a given tSIE range. The counting efficiency (E) was simply defined as:

$$E = \frac{\text{cpm}_S - \text{cpm}_B}{\text{dpm}_S} \quad (1)$$

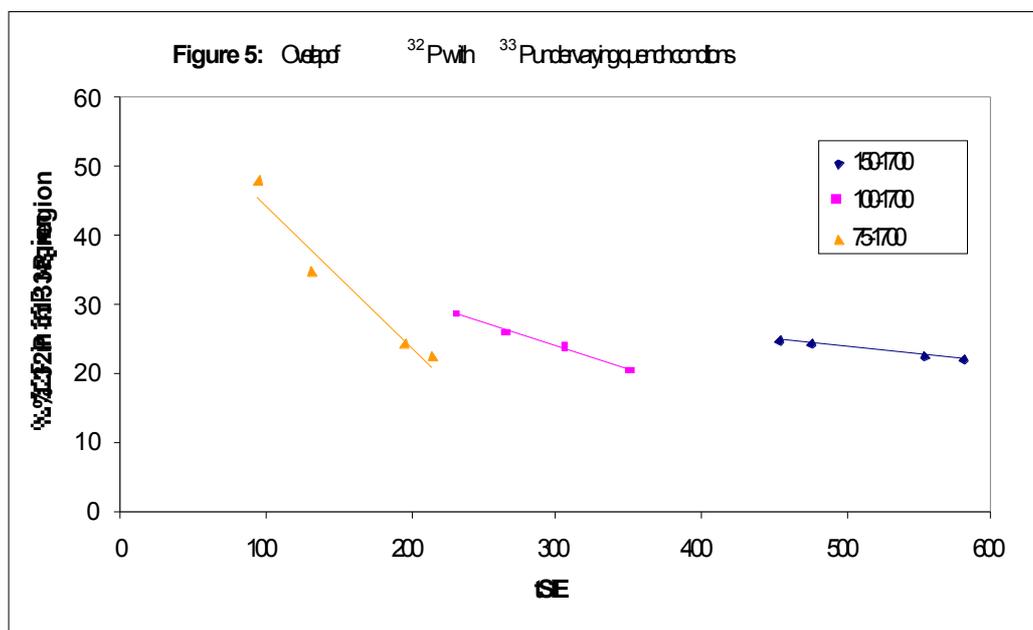
where cpm_S is the sample count rate in the appropriate window in counts per minute, cpm_B is the background count rate, and dpm is the known activity in the vial in disintegrations per minute (Figs. 3 and 4).



Natural samples contain both ³²P and ³³P. To account for the overlapping spectra, an overlap factor (F) was determined for each tSIE, where:

$$F = \frac{\text{cpm}_{33} - \text{cpm}_{B,33}}{\text{cpm}_{32} - \text{cpm}_{B,32}} \tag{2}$$

where "33" indicates the count rate in the ^{33}P region of interest in the ^{32}P spectrum, "32" is the count rate in the ^{32}P region of interest in the ^{32}P spectrum and "B" signifies the background in the respective windows (Fig. 5).



Detection limits

(Currie, 1968) defines the lowest detectable count rate (LLD) as

$$\text{LLD} = \frac{2.71 + 4.65\sqrt{\mu \times t}}{t} \quad (3)$$

where μ is the background in counts and t is the count time. Given typical background and efficiency data for marine samples, minimum detectable activities (MDAs) of 0.02 Bq can be readily achieved for both phosphorus isotopes. These MDAs can be lowered still further by use of long count times, but are conducive to the determination of naturally-occurring phosphorus isotopes in marine systems.

3. SAMPLING AND PRE-CONCENTRATION

Given the analytical constraints outlined above, it is necessary to sample several litres rainwater, and hundreds of litres of seawater to successfully determine natural ^{32}P and ^{33}P . There are therefore, two steps in the analytical process: (i) to pre-concentrate P from 10 – 1000 litres water; and (ii) to purify the phosphorus contained in that concentrate, ensuring complete separation from other radionuclides that may also be concentrated from the sample. The pre-concentration step will be discussed in association with the sampling procedures, given that they are carried out in tandem.

Rainwater

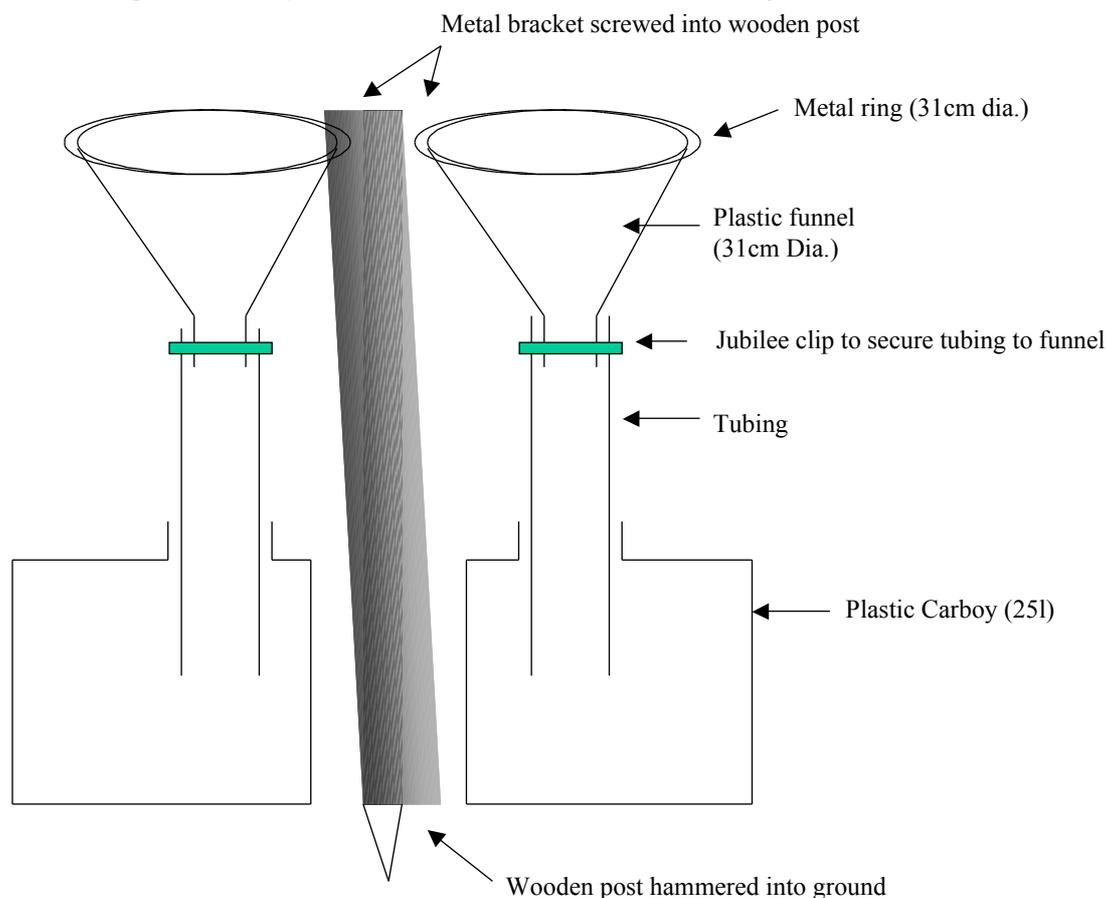
It is necessary to collect rainwater over the period that marine samples are collected, in order to evaluate the input flux of ^{32}P and ^{33}P from the atmosphere.

Rainwater collector design

A number of designs were considered for a system to collect rainwater samples from both Crete and Corsica. Initially, a single funnel design was constructed and tested at a site in Lancaster and a site in the Lake District, England. Although these worked well, it was felt that a larger collection area would be needed, due to the rainfall patterns in Corsica. Therefore, a system using four 31 cm diameter funnels was constructed (Fig. 6). This system allows the collection of up to 100 l of rainwater in 4 separate 25 l volume carboys. The collectors were set up in Crete and Corsica in October 2001 with rainwater collection beginning

in November 2001. Samples are collected biweekly and pumped through Fe-impregnated fibres (see Marine samples below) in situ before being sent to Lancaster for further analysis.

Figure 6: A diagrammatic representation of the rainwater collection system utilised in Corsica and Crete.



Marine samples

In seawater, dissolved P can be divided into two distinct fractions, namely DIP and dissolved organic phosphorus (DOP). DIP is defined as the fraction that forms a molybdenum blue complex in the presence of ascorbic acid (Koroleff *et al.*, 1983). The measurement of DOP is complicated by the multitude of chemical compounds present. Hence DOP is not measured directly, but inferred from the TDP minus DIP.

The aim of this study is to determine ^{32}P and ^{33}P activities in marine particulates and the two dissolved phases – DIP and DOP. In order to achieve this, the particulate P is first removed by filtration through 0.2 μm cellulose nitrate membrane filters. Subsequently, TDP is removed by pumping the water through $\text{Fe}(\text{OH})_3$ impregnated polypropylene fibres. DIP is removed by complexation with molybdenum blue (MB) and then pumping the resultant suspension through Na-form fibres.

Polypropylene fibres

Polypropylene felt (25 μm pore size) was used as a support for the chemical agents used to remove TDP and DIP from seawater. For removal of DIP, the fibres were treated with NaOH (6.25 M) at $\sim 100^\circ\text{C}$ for 20 min which converts the fibre into a cation-exchange type resin (Krishnaswami *et al.*, 1972). The Na-form fibres are not thought to adsorb DOP, because the surface charge of DOP will be neutralised at pH 1-2 as encountered in MB complexes (Lee *et al.*, 1992).

For TDP removal, the fibres were first treated with NaOH , as above, but then impregnated with FeCl_3 at $\sim 100^\circ\text{C}$ for 20 min. The fibres are then transferred to a NH_4OH (3 M) bath for >12 hours. It has been shown that it is possible to load 25 to 30 % $\text{Fe}(\text{OH})_3$ onto the fibres in this way (Benitez-Nelson and Buessler, 1998). The $\text{Fe}(\text{OH})_3$ is speculated to be in the form of goethite ($\alpha\text{-FeOOH}$), whose hydroxyl ligand facilitates instantaneous complexation (Honeyman and Santschi, 1989).

Total Dissolved Phosphorus

1000 l of seawater must be processed for TDP at each station and depth. The water was pumped onto deck and into two 500 l plastic containers using a 1/2 horsepower bronze gear-pump through 5 cm diameter reinforced tubing. Samples of 500 l could be collected in a matter of minutes. Sub-samples (50 ml) were collected in acid-cleaned plastic bottles for stable P analysis. The water was then filtered into another 500 l plastic container, through 0.2 μm cellulose nitrate membrane filters (Whatman, 147mm diameter) to separate the particulate phase. The filters were stored in clean zip-lock plastic bags for analysis in the laboratory. Following removal of the particulate phase, the water was pumped through a plastic tube (2 l volume) packed with $\text{Fe}(\text{OH})_3$ impregnated polypropylene fibres to remove TDP. A further 500 l seawater was filtered to remove particulates as above and then pumped through the same packed tube. The fibres were removed from the tube and stored in a clean zip-lock plastic bag for analysis in the laboratory, according to the procedure outlined below.

Dissolved Inorganic Phosphorus

A similar volume of water must also be processed for DIP at each station and depth. The samples were processed as for TDP. Following filtration, a DIP-molybdenum blue complex was formed by addition of mixed reagent (sulphuric acid, ammonium molybdate, potassium tartrate) and ascorbic acid. The sample was mixed and left for 30 min to allow the formation of a molybdate blue complex. The sample was then pumped through a plastic tube (2 l volume) containing Na-form polypropylene fibres. The filters were then removed from the tube and stored in a clean zip-lock plastic bag for analysis in the laboratory.

Testing $\text{Fe}(\text{OH})_3$ and Na – form fibres

Prior to a field campaign, the efficacy of the Fe-impregnated and Na-form fibres for P removal was tested using locally collected seawater. Two duplicate 20 l samples were collected, and the stable phosphorus concentration determined. One was pumped through a 300 cm^3 plastic tube packed with $\text{Fe}(\text{OH})_3$ impregnated fibres. The second was pumped through a plastic tube packed with Na-form fibres of a similar size. Samples of the water were taken after various volumes had been processed to check for breakthrough of phosphorus (Table 1).

Substantially larger volumes of Fe-impregnated / Na-form fibres are employed in field sampling. Therefore, this level of performance was felt to be adequate. Additional checks for P breakthrough are carried out on the effluent water during field sampling.

Table 1: Breakthrough of phosphorus from both $\text{Fe}(\text{OH})_3$ and Na-form fibres.

Sample	Volume Processed (l)	Initial P conc. (ppb)	Final P conc. (ppb)	% breakthrough
1	5	9429	23.81	0.25
1	10	9429	250.0	2.65
1	15	9429	642.9	6.82
2	5	11286	71.43	0.63
2	10	11286	107.1	0.95
2	15	11286	750.0	6.65

Problems encountered

1. Pump priming.

Deployment of the tubing to a depth of 100 m proved troublesome, as the pump needs to be primed. After attempting to fill the tubing whilst it was in situ, 30 m of tubing was removed and the remaining tubing laid out on deck and filled with sea water from a tap on board before being released overboard.

Proposed solution: A one-way valve, with a weight attached, will be fitted to the end of the tubing to aid in filling the tubing with water before commencing pumping.

2. Separation of particulate phase.

The main problem was the time taken to filter such large volumes of water through relatively small pore size filters. The speed of filtration is restricted by the pressure on the cellulose nitrate filters, which will split if the pressure is too high. It took approximately 10 hours to filter 1000 l through the membrane filters and a further 8 hours to pump the water through the polypropylene fibres. These times were achieved by changing the 0.2 μm filters more regularly than necessary in order to increase the flow rate.

Such long processing times require the ship to stay at each sample site for at least 36 hours per depth, or to return to the sample site on numerous occasions in order to sample different depths.

Proposed solution: The two separation steps could be combined into one for the TDP measurement. However for the DIP measurement an additional reaction needs to be carried out after the particulate phase is removed. The use of more and larger filter rigs (four, 300 mm diameter filters) will speed up the initial filtration process. These will be connected to the gear pump to remove the need for pumping into storage tanks prior to filtration.

4. CHEMICAL PURIFICATION

The result of the pre-concentration / sampling procedures are P samples in the form of either acrylic fibres or membrane filters. The filters/fibres were ashed in a muffle furnace (500°C for 4 hours), to reduce the volume and convert the phosphorus to inorganic forms. The samples are then processed following the procedure outlined in Figure 7.

Several stages in the chemical purification procedure were checked and optimised. In all cases, these experiments were conducted using stable P (in the form of NaPO_4) as a tracer. In all cases, stable P determinations were carried out using a standard method (Mackereth *et al.*, 1978).

DIPE extraction

Previous researchers (Benitez-Nelson and Buesseler, 1998) had found that most of the phosphorus lost during the chemical purification procedure occurs during the di-isopropyl ether (DIPE) extractions, due to some of the phosphorus becoming partitioned in the ether phase with the iron. Therefore, experiments were carried out to see how much phosphorus is lost during the DIPE extraction in our laboratory, to check that recoveries were not being hampered by this step.

Resin rinsing and preconditioning

The first batches of rainwater samples processed resulted in a coloured sample at the final stage. Sample coloration results in a reduction in the counting efficiency, but is difficult to quantify.

The most likely source of the problem was organic material being eluted from the AG1x8 column along with the sample. This can be overcome by pre-conditioning the column with a suitable volume of the elution agents. However, it is necessary to minimise this volume, so that the time taken for the column does not become excessive. Initially, the column was rinsed with 50 ml MilliQ water and pre-conditioned with 50 ml 9M HCl. Therefore, the volumes of rinsing water and acid were increased step-wise, until a colourless samples resulted (Table 3). It was found that 100 ml MilliQ and 100 ml 9M HCl were sufficient to ensure a clear sample.

Table 3: Investigation into the effect of changing the volumes of water and acid used to pre-condition the AG1X-8 column on final sample colour.

MilliQ (ml)	9M HCl (ml)	Sample colour
50	50	coloured
75	50	Slight colour
100	50	Slight colour
50	75	Slight colour
75	75	Slight colour
100	75	Very slight colour
50	100	Slight colour
75	100	Very slight colour
100	100	Colourless

5. FIELD SAMPLING

The field sampling and pre-concentration protocols were tested during the cruise ADIOS E-2 in October 2001. Samples were collected from station C at 5m depth for TDP and DIP, and 60m depth for TDP. Particulate fractions were additionally collected at both depths.

Samples were collected and processed as described above. They were counted for ^{32}P and ^{33}P on the Packard Tri-Carb 3170 for 100 minutes, using the low level count mode and a DBB of 450 ns, immediately on completion of the separation. Additional counts will be made at least 3 times over the 3 months following sampling. The data are regressed, using the respective half-lives of the two P isotopes, to the time of sample collection.

The regions of interest for ^{32}P and ^{33}P for each sample are chosen on the basis of the tSIE value (see above). The efficiency is then calculated from the corresponding quench curves and hence the activity determined calculated. Overlap between the two spectra is then corrected for using the procedure outlined above. Chemical recoveries are determined using the stable P data, and the results corrected for losses.

Preliminary results are presented in Table 4 below. Several of the determinations are below the detection limit. However, the samples have not been fully counted at this point. Additional experience and practise with the methodology will also act to improve both recoveries and speed up the analytical process, and hence reduce the LLDs still further.

Preliminary rainwater results are presented in Table 5. These samples were collected locally to test the methodology. In this instance, all the data are above the detection limit. The ^{33}P : ^{32}P ratio varies about a mean of 0.91.

Table 4: Preliminary results from the ADIOS-E2 cruise, October 2001. Activities are quoted in Bq 1000 litre⁻¹

Sample depth (m)	TDP		DIP		PP	
	^{32}P Activity	^{33}P Activity	^{32}P Activity	^{33}P Activity	^{32}P Activity	^{33}P Activity
5	0.116	0.032	< LLD	< LLD	< LLD	< LLD
60	< LLD		--	--	0.187	< LLD

Table 5: Preliminary results from locally collected rainwater sampling

Date of collection	^{32}P activity (Bq l^{-1})	^{33}P activity (Bq l^{-1})	$^{33}\text{P}:^{32}\text{P}$ ratio
16/05/01	0.013	0.012	0.98
30/05/01	0.0057	0.0032	0.55
18/06/01	0.0027	0.0036	1.3
14/08/01	0.0021	0.0017	0.80

References:

- Benitez-Nelson, C. R. and K. O. Buesseler (1998). "Measurement of cosmogenic ^{32}P and ^{33}P activities in rainwater and seawater." *Analytical Chemistry* **70**: 64-72.
- Benitez-Nelson, C. R. and K. O. Buesseler (1999a). "P-32, P-33, Be-7 and Pb-210: atmospheric fluxes and utility in tracing stratosphere / troposphere exchange." *Journal of Geophysical Research - Atmospheres* **104**(D9): 11745-11754.
- Benitez-Nelson, C. R. and K. O. Buesseler (1999b). "Variability of inorganic and organic phosphorus turnover rates in the coastal ocean." *Nature* **398**: 502-505.
- Currie, L. A. (1968). "Limits for qualitative detection and quantitative determination. Application to radiochemistry." *Analytical Chemistry* **40**: 586-593.
- Honeyman, B. D. and P. H. Santschi (1989). "A Brownian-pumping model for oceanic trace metal scavenging: Evidence from Th isotopes." *Journal of Marine Research* **47**: 951-992.
- Koroleff, F., K. Grasshoff, M. Ehrhardt and K. Kremling (1983). *Methods of Sea water Analysis*, Verlag Chemie.
- Krishnaswami, S., D. Lal, B. L. K. Somayajulu, F. S. Dixon, S. A. Stonecipher and H. Craig (1972). "Silicon, radium, thorium, and lead in seawater: In-situ extraction by synthetic fibre." *Earth and Planetary Science Letters* **16**: 84-90.
- Lal, D. (1999). "An overview of 5 decades of studies of cosmic ray produced nuclides in oceans." *Science of the Total Environment* **237/238**: 3-13.
- Lal, D., Y. Chung, T. Platt and T. Lee (1988). "Twin cosmogenic radiotracer studies of phosphorus recycling and chemical fluxes in the upper ocean." *Limnology and Oceanography* **33**: 1559-1567.
- Lal, D. and T. Lee (1988). "Cosmogenic ^{32}P and ^{33}P used as tracers to study phosphorus recycling in the upper ocean." *Nature* **333**: 752-754.
- Lee, T., E. Barg and D. Lal (1992). "Techniques for extraction of dissolved inorganic and organic phosphorus from large volumes of seawater." *Analytica Chimica Acta* **260**: 113-121.
- Mackereth, F. J. H., J. Heron and J. F. Talling (1978). *Water Analysis*, Freshwater Biological Association.
- Testa, C., D. Desideri, F. Guerra, M. A. Meli and C. Roselli (1997). "The radioanalytical determination of cosmogenic ^{32}P in marine samples for the study of the phosphate ion turnover in the Mediterranean Sea." *Radiochimica Acta* **78**: 159-161.
- Thingstad, T. F. and F. Rassoulzadegan (1995). "Nutrient limitations, microbial food webs, and 'biological C-pumps': suggested interactions in a P-limited Mediterranean." *Marine Ecology Progress Series* **117**: 299-306.
- Waser, N. A. D. and M. P. Bacon (1995). "Wet deposition fluxes of cosmogenic ^{32}P and ^{33}P and variations in the $^{33}\text{P}/^{32}\text{P}$ ratios at Bermuda." *Earth and Planetary Science Letters* **133**: 71-80.
- Waser, N. A. D., M. P. Bacon and A. F. Michaels (1996). "Natural activities of ^{32}P and ^{33}P and the $^{33}\text{P}/^{32}\text{P}$ ratio in suspended particulate matter and plankton in the Sargasso Sea." *Deep-Sea Research II* **43**: 421-436.