Water chemistry and algal phosphatase activity in zinc-contaminated streams

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by

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ABSTRACT

A study was carried out on algal phosphatase activity and water chemistry in zinc-contaminated streams in the Northern Pennine Orefield.

Fourteen field sites were studied; They ranged from low contaminated to high-zinc sites (0.1 mg l^{-1} to 19.4 mg l^{-1} with 7 of the 14 above 1 mg l^{-1} Zn). Phosphate concentrations were low in the majority of sites (2.2 µg l^{-1} to 22.8 µg l^{-1} with 13 of the 14 sites below 7.7 µg l^{-1} TFP and 1.8 µg l^{-1} to 8.5 µg l^{-1} with 13 of the 14 sites below 4.9 µg l^{-1} FRP).

Phosphatase activity was tested over a broad pH range (3.0 - 11.0) using at least two different buffers at each pH value. Phosphatase activity of samples tested showed different responses to pH. Most of the samples exhibited enzyme activity in the alkaline range. High phosphatase activity was observed in samples with Mougeotia and Stigeoclonium populations.

Possible relationships between phosphatase activity and selected environmental variables were examined. Phosphatase activity was significantly correlated with Zn (+ve), TFP (-ve) and FRP (-ve) in the water.

The role of phosphatases in these high-zinc environments is discussed.

ABBREVIATIONS

^{o}C	degrees Celsius
g	gramme
mg	milligramme
µg	micrograme
dry wt	dry weight
l	litre
ml	millilitre
µl	microlitre
m	metre
cm	centimetre
µm	micrometre
nm	nanometre
h	hour
min	minute
M	molar
mM	millimolar
µM	micromolar
µmol	micromole
P	phosphorus
P_i	inorganic phosphorus
FRP	filtrable reactive phosphorus
TFP	total filtrable phosphorus
PMEase	phosphomonoesterase
PDEase	phosphodiesterase
APA	alkaline phosphatase activity
bis-ρNPP	bis (ρ-nitrophenyl) phosphate
AMeP	2-amino-2-methyl-1-propanol
CAPS	3-(cyclohexylamino)-1-propanesulphonic acid
DMG	3, 3-dimethylglutaric acid
EDTA	ethylenediamine tetra-acetic acid (disodium salt)
EPPS	N-(2-hydroxyethyl) piperazine-N′-3-propanesulphonic acid
HEPES	N-2-hydroxymethylpiperazine-N′-2-ethanesulphonic acid
TES	N-tris(hydroxymethyl) methyl-2-aminoethane sulphonic acid
n	number of samples
p	probability
sem	standard error of mean

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CHAPTER 1
INTRODUCTION

1.1 General introduction

Measurements of environmental variables in aquatic systems are often used to assess biological needs and therefore it is logical to use biological systems in the assessment procedure. Much more work has been carried out using species presence or absence to give a broad indication of the water quality. Morphological and physiological characteristics of organisms are often related to environmental factors and in most cases, changes in these characteristics can reflect changes in the environmental conditions where these organisms grow.

1.2 Zinc in the aquatic environment

Zinc is a fairly abundant metal, representing 0.004 % of the earth's crust and is twenty-fifth in the order of abundance. According to Bowen (1966) the average concentration in the soil is 50 mg kg^{-1} and 10 $\mu g\ l^{-1}$ in freshwater.

The patterns and processes of zinc entry into freshwater are reviewed by Weatherley et al. (1980). Elevated level of zinc in freshwater can be expected in base-metal mining regions (derived from both groundwater drainage of mineral deposits and drainage of overburden of zinc or other ore bodies), in industrial regions and urban regions. In addition, acid mine drainages associated with coal mines may carry elevated levels of zinc, due to the increased mobilizing influence of acid waters.

The chemistry of zinc in freshwater is reviewed by Hem (1972) and Florence (1980). pH is particularly important in controlling the solubility of zinc in aqueous environments. Zinc may be precipitated at higher pH values in the following way:

$$Zn^{-2} + H_2O \rightleftharpoons ZnOH^+ + H^+ \rightleftharpoons Zn(OH)_2\downarrow + H^+$$

Based on thermodynamic calculations, Hem (1972), however, showed that the solubility of zinc carbonate ($ZnCO_3$) and zinc silicate ($Zn_2\ SiO_4$) were both less than that of zinc hydroxide and in the majority of freshwater, these form the major control of zinc solubility. Other chemical forms of zinc may also occur in the water, particularly organic complexes (e.g. Zn-humate, Zn-citrate) and as ions adsorbed into colloids (e.g. Zn^{2+}-humic acid) or inorganic colloids (Zn^{2+}-clay minerals). All complex forms of zinc in

natural waters occur more readily at higher pH values and above approximately pH 7 zinc complexes are likely to be formed; below pH 6 zinc is likely to exist as the simple divalent ion or as the hydrated ion (Hem, 1972; Florence, 1980).

Many authors state the average level of zinc in unpolluted freshwater can suitably approximate to 10 $\mu g\ l^{-1}$. Levels of zinc tend to be elevated in thermal or acid streams excepting these, levels of zinc above 0.1 $mg\ l^{-1}$ in streams are indicative of human activity and those above 1 $mg\ l^{-1}$ are usually associated with past or present mining activities. Say (1977) reported many streams containing >1 $mg\ l^{-1}$ zinc, in his survey of streams draining the Northern Pennine Orefield; the maximum was 22.3 $mg\ l^{-1}$ zinc. The maximum level reported in the literature is probably that for a site draining a smelter tip in Southern France (Say & Whitton, 1982), where a small seepage was found to contain 3840 $mg\ l^{-1}$ zinc.

1.3 Algal populations in high-zinc environments

Many algae populations have the ability to adapt in high-zinc environments. It has been shown that algae species present in flowing waters with high zinc levels were represented by populations genetically adapted for tolerance (Say et al., 1977). Filamentous green algal populations such as Stigeoclonium tenue and Mougeotia spp. are widespread and often abundant in waters with and without zinc pollution. At sites with mean zinc levels in water of about 0.2 $mg\ l^{-1}$ and above, populations of S. tenue showed increased tolerance to the metal in comparison with populations from sites with lower zinc levels. Experiments with Mougeotia spp. have indicated that populations of these species taken from high-zinc sites have acquired genetic tolerance (B.A.Whitton, unpublished). It thus seems probable that most filamentous green algae growing at sites with high zinc levels will prove to be genetically tolerant populations of species which normally do not show such tolerance.

Zinc is an important micro-nutrient for growth and metabolism of algae and plays a vital role in maintaining the integrity of ribosomes, although in most cases these requirements are fulfilled by low environmental levels. For instance stocks of Stigeoclonium tenue could be maintained in a medium with less than 0.002 mg Zn l^{-1} (Harding and Whitton, 1976). No zinc-tolerant population of either S. tenue or Klebsormidium rivulare have been found with an increased requirement for zinc.

1.4 Phosphorus

1.41 Occurrence of phosphorus

Phosphorus (P) is the eleventh most abundant element in nature, its concentration is estimated as 0.1% by weight in the lithosphere, and is thus classed as a trace element. 80 % of the P reserves are contained in phosphorite deposits in ocean sediments and 15 % in igneous and metamorphic rocks.

1.42 Phosphorus compounds

1.421 Phosphorus fractions

Phosphorus generally occurs in the oxidized form, either as phosphates or organic P compounds. Phosphate can be divided into:

1. Orthophosphates
2. Polyphosphates (chain phosphates)
3. Metaphosphates (ring phosphates)
4. Ultraphosphates (branched ring phosphates)

Orthophosphates are generated from the weathering of rocks or from biological metabolism or degradation. Polyphosphates and metaphosphates are produced by biological activity. Orthophosphates and polyphosphates are frequently introduced into waters by man. Analytically defined P fractions are categorized as:

Tot P = Total phosphorus

↓

PP = Particulate phosphorus > 0.45 μm

TFP = Total filtrable phosphorus < 0.45 μm

FRP = Filtrable reactive phosphorus

Particulate P includes:

1. Phosphorus in organisms as (**a**) relatively stable nucleic acids DNA, RNA, and phosphoproteins which are not involved in rapid cycling of phosphorus, (**b**) low-molecular-weight esters of enzymes, vitamins, etc., and (**c**) nucleotide phosphate, such as adenosine diphosphate (ADP) and adenosine-triphosphate

(ATP).

2. Mineral phases of rock and soil, such as hydroxyapatite, in which phosphorus is adsorbed into inorganic complexes such as clays, carbonates, and ferric hydroxides.

3. Phosphorus adsorbed into dead particulate organic matter or in microorganic aggregations.

In contrast to particulate P, TFP is composed of:

(1) Orthophosphate (PO_4^{-3}), (2) Polyphosphates, (often originates from synthetic detergents), (3) Organic colloids or phosphorus combined wuth adsorptive colloids, (4) low-molecular-weight phosphate esters. The term filtrable reactive phosphorus is more appropriate than orthohposphate for the phosphorus fraction reacting to give the colour with the molybdenum blue technique.

1.422 Organic phosphorus compounds in aquatic systems

The major share of the phosphorus resources of the earth's surface waters is in organic form. The principal pools or storage sites of organic phosphorus in the hydrosphere are:

1. The organic compounds of living and dead particulate suspended matter (seston),
2. A variety of filtrable organic compounds usually termed "dissolved",
3. The organic compounds of rooted and encrusting plants of the bottom,
4. The phosphorus of free-swimming animals,
5. Phosphorus present in bottom sediments.

Particulate organic P in aquatic ecosystems dominates total organic P, and is comprehensively reviewed by Broberg and Persson (1988). Although particulate organic P is the major constiuent of organic P forms it is not readily available. Dissolved organic P (DOP) can be regarded as a more important fraction as it is readily available to the biota and is rapidly turned over. It is not know whether DOP is primarily released by active cell metabolism or by cell death and decay.

Some pools of DOP do not undergo rapid hydrolysis by phosphatase, and these compounds may constitute a major part of the DOP pool. One such pool of DOP compounds are the nucleotides or polynucleotides. Broberg and Persson (1988) reported that up to 4.2% of the total P in bogs was attributed to nucleic acid. Hino (1989) found that 65% of DOP in lake waters was composed of compounds between 300 to 10000 daltons. Addition of phosphodiesterases (1.522) to high molecular weight DOP compounds did not release any measurable P_i, although a combination of phosphodiesterases

and phosphomonoesterases increased the amount of P_i released by 30% when compared to release of P_i by PMEase alone.

A major component of colloidal P in aquatic systems are the inositol phosphates or phytates, which are hydrolyzed by the group of phosphatases known as phytases. The phytates are esters of inositol and phosphoric acid. Phytates exist in many forms as there are many different isomers of inositol and each isomer exists with one to six esterified phosphate groups per molecule. Phytates can comprise up to 35% of the colloidal P, which is mainly derived from microbial storage and structural compounds.

1.43 Biologicaly available phosphorus

The forms of P most commonly utilized by biological systems are HPO_4^{2-} and $H_2PO_4^-$, the ionic forms of phosphorus which predominate according to pH. Many organisms have two uptake systems for phosphate, one being "diffusive" and the other "rapid". The rapid uptake system occurs when the internal phosphorus concentration is low. Both these uptake systems require energy but Whitton (1967) described colonies of Nostoc which appeared to passively take up P when the external concentration was very high.

Many different substrates can be utilized as P sources by algae which are capable of alkaline phosphatase activity (Livingstone et al., 1983; Al-Mousawi, 1984). In natural waters both high and low molecular weight organic phosphorus fractions have been identified and at least a part of each fraction has been shown to be available to some algae. Livingstone et al. (1983) found that Calothrix parietina D550 could utilize six different substrates as sources of phosphorus. Al-Mousawi (1984) found 10 substances could be utilized by nine strains of blue-green algae isolated from an Iraqi rice field. Most of the compounds used by algae are soluble but 84% of the particulate P was available to algae in the R. Amazon (Grobbelaar, 1983).

Broberg (1985) stated that the availability of different phosphorus compounds to algae is dependent on the algae enzyme pool, the phosphorus status of the alga, the orthophosphate uptake rate, the nature of the P-compounds and environmental conditions.

1.44 Algal requirements for phosphorus

Compounds containing phosphorus play major role in nearly all phases of metabolism, particularly in the energy transformation of phosphorylation reactions during photosynthesis. Phosphorus is required in the synthesis of nucleotides, phospholipids, sugar phosphates, and other phosphorylated intermediate compounds. Further, phosphate is bonded, usually as an ester, in a number of low-molecular-weight enzymes and vitamins essential to algal metabolism.

1.5 Phosphatase

1.51 Introduction

Phosphatase are enzymes which catalyze the hydrolysis of both esters and anhydrides of phosphoric acid and under some circumstances certain phosphatase act as transferases by catalyzing the transfer of phosphate from one substrate to another. They are often divided into acid and alkaline phosphatase according to their pH optima.

1.52 Classification of phosphatase

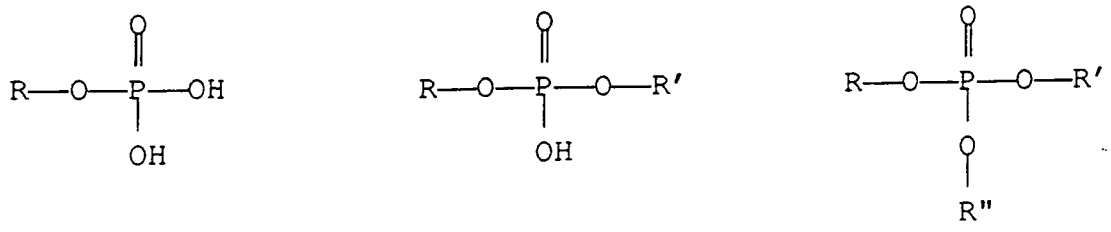
The commission on Enzymes of the International Union of Biochemistry has classified all of these enzymes into five major groups:

1. Phosphoric monoester hydrolases E.C 3.1.3 (phosphomonoesterases)
2. Phosphoric diester hydrolases E.C 3.1.4 (phosphodiesterases)
3. Triphosphoric monoester hydrolases E.C 3.1.5
4. Hydrolases splitting anhydride bonds in phosphoryl-containing anhydrides E.C 3.6.1
5. Hydrolases splitting P-N bonds E.C 3.9 (phosphoamidases)

1.521 Phosphomonoesterases

The phosphomonoesterases catalyze the hydrolysis of monoesters of othophosphoric acid. Most often the term "phosphatase" is used synonymously with phosphomonoesterases, abbreviated to PEMase. Similar but functionally different enzymes are the phosphodiesterases, abbreviated to PDEase, which include the nucleases. General formulae for phosphate esters are in Fig. 1.1.

Fig. 1.1 Formulae for phosphate esters



PHOSPHOMONOESTER PHOSPHODIESTER PHOSPHOTRIESTER

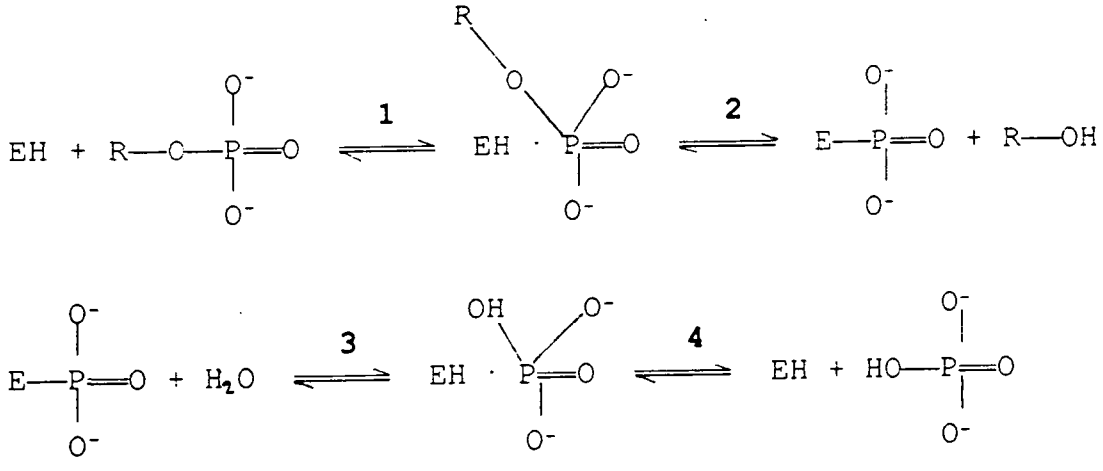
R represents the organic part of the phosphate esters

The most common catalytic breakdown studied is the breakdown of phosphomonoesters by PMEase. The reaction mechanism (Fig. 1.2) is divided into four steps (McComb et al., 1979):

1. Non-covalent binding of the substrate to the enzyme (EH).
2. Alcohol release from the complex and P_i become covalently bound to the enzyme forming a phosphoryl-enzyme compound.
3. Conversion of the phosphoryl-enzyme compound, through uptake of water, to a non-covalent complex.
4. Release of P_i and regeneration of free enzyme.

Any of the steps 2-3 can be rate-limiting for the overall reaction (McComb et al., 1979).

Fig. 1.2 Reaction scheme for the enzyme catalyzed hydrolysis of phosphate esters as described by McComb et al., (1979).



PMEase activity will primarily depend on the type and concentration of substrate and enzyme. Other factors which affect PMEase activity are temperature, ionic stength, pH and metal ions (McComb et al., 1979). Alkaline PMEases have been characterized as metallo-enzymes with an essential metal ion, which has been reported to be zinc in many cases (Spiro, 1973; McComb et al., 1979 and Torriani-Gorrini et al., 1987).

1.522 Phosphodiesterases

These enzymes catalyse the hydrolysis of a phosphoric diester to yield a phosphoric monoester and an alcohol. PDEases are able to hydrolyze a wide range of nucleotides. PDEases are distinguished into two categories on their ability to hydrolysze 3' and 5' nucleotides. PDEase I hydrolyzes nucleic acids to nucleoside 5'-phosphate and pDEase II hydrolyzes nucleic acids to nucleoside 3'-phosphates (Kelly et al., 1975). PDEases are typically alkaline and inhibited by EDTA. The activity of EDTA treated PDEases is completely restored by zinc and partially by calcium and magnesium (Ito et al., 1987), which suggests that PDEases are also zinc-metallo enzymes like PEMases. PDEases in all cases are inhibited by P_i and in most cases PDEases are inhibited by ascorbic acid.

1.53 Bacterial phosphatase activity

Many studies have been made on phosphatase in bacteria. Much of what is known about the function and physiology of phosphatases is derived from studies on E.coli (McComb et al., 1979) and phosphatase activity has been shown in bacterial strains isolated from lake water. More specific studies on aquatic bacterial phosphatase have been made with marine species. Marine bacteria, in general, appear to have phosphatases that are located in the periplasmac space (Thompson & Macleod, 1974a, b).

1.54 Algal phosphatase activity

Phosphatase activity has been found in all major groups and numerous species of algae. Synthesis of phosphatases with external function has been frequently demonstrated in cultured algae (Kuenzler, 1965; Healey, 1973; Wynne, 1981). Phosphatases are located on the cell surface or in cell membranes and the release of extacellular phosphatase in cultures is frequently reported (Healey, 1973; Aaronson & Patni, 1976).

Phosphatase activities in aquatic environments have often been attributed to algae (Petterson, 1980). The major amount of work on activity in aquatic environments has concentrated on extracellular phosphatases (Jansson et al., 1988), which can make up a substantial amount of the activity in lake waters. Extracellular phosphatases are generally defined as those which pass through 0.45 μm membrane filtrs. The characteristics of extracellular phosphatses do not differ from "cell-bound" phosphatases (Flynn et al., 1986). It is not clear how, or to what extent, active secretion of phosphatases takes place and whether it is more beneficial to release or to localize them on the external cell surface (Jansson et al., 1988).

1.55 Characteristics of algal phosphatases

1.551 Acid and alkaline

Phosphatases have maximum hydrolyzing capacity at different pH values and hence the common division into acid and alkaline phosphatases. Acid phosphatases generally have the highest activity between pH 4 - 6 and alkaline between pH 8 - 11 . Both acid and alkaline phosphatases have been found as extracellular and cell-bound enzymes in algae (Siuda, 1984). The alkaline phosphatases with extracellular function are the most studied type in aquatic ecosystems.

In many aspects acid and alkaline phosphatases share essential characteristics. Both types have a broad specificity against different substrates, i.e. their activity is restricted only to the P-O bond on the phosphomonoesters. However, alkaline phosphatases differ in that they require cations for activity and are inhibited by chelators such as EDTA, whereas acid phosphatases have no cationic requirement and spcifically inhibited by fluoride.

Acid and alkaline phosphatases show essential difference concerning their location in the cell and mode of synthesis. Acid phosphatases are intracellular (cellular) whereas alkaline are in contact with the surrounding medium i.e. bound to the cell membrane, wall or sheath (Wynne, 1977; Siuda, 1984). In contrast to alkaline the synthesis of acid phosphatase is generally not inhibited by P_i (Wynne, 1977). It is possible that acid phosphatases are constitutive enzymes produced for internal P-metabolism, whilst alkaline phosphatases have external functions and a synthesis which is induced or repressed depending upon the P status of the alga (Jansson et al., 1988).

1.552 Temperature dependence

Algal phosphatases have Q_{10} values between 1.5 and 3 and temperature optima between 30 - 60°C, which is usually above the temperature of the original environment. Huber and Kidby (1984) showed that temperature optima of algal cultures and field populations were between 25 - 50°C.

1.553 Effect of ions

The ionic requirements for algal phosphatases vary considerably. The activity of phosphatases in many algae can be enhanced by the addition of Ca^{2+} (Healey, 1973). The effect of Mg^{2+} on phosphatase activity is less clear. Zinc may slightly inhibit activity but has been reported as an activator in a multicellular marine alga (Walther & Fries, 1976). There are also reports of cobalt, iodine, manganese and potassium being stimulatory and cobalt and maganese being inhibitory (Walther & Fries, 1976)

1.554 Stability

Extrtacellular phosphatases are functional for long periods under axenic conditions. Alkaline phosphatases when incubated with chloroform-saturated water, decreased in activity by 20% over 10 days (Berman, 1970). Jansson et al., (1984) found that extracellular acid phosphatases remained active after 20 days and 10% of the original activity remained after 69 days. However, the mechanism for the inactivation or break down of phosphatases in situ remains unclear.

1.555 Control of synthesis

The synthesis of enzymes is influenced most by the substrate supply or reaction products. Inducible phosphatases are those where synthesis starts in the presence of suitable substrate, and constitutive are enzymes produced independently of an activator, i.e. they are more or less constantly synthezed in the cell. Induction, where phosphatase activity is enhaced by the addition of a substrate, seems uncommon or rarely investigated.

Algal acid phosphatases seem to be mostly constitutive while alkaline phosphatases are inducible. Acid phosphatases are located internally, whilst alkaline have external functions (1.551). Therefore, alkaline phosphatases supply algae with P_i from outside the cell and are regulated by the internal P_i pool (Fitzgerald & Nelson, 1966; Wynne, 1977). When the internal P_i pool is filled, synthesis of alkaline

phosphatases is stopped, and when the pool is depleted to a particular level, alkaline phosphatase synthesis is induced.

1.556 Alkaline phosphatase activity as a phosphorus-deficiency indicator

Studies on P-limited marine and freshwater algae have shown that the production of alkaline phosphatases stopped when the algae were P-sufficient (Kuenzler & Perras, 1965; Fitzgerald & Nelson, 1966). These two papers were the basis for the use of algal alkaline phosphatase as biological indicators of the P-status of the environment. Healey (1982) confirmed that many algal species respond sufficiently similarly to phosphorus deficiency to permit measurments of phosphatase activity on natural populations.

The induction of the alkaline phosphatase under P-limmited conditions seems to be a characteristic phenomenon which allows this enzyme assay to be used as a measure of P-limited growth. Studies have revealed that there is an inverse relationship between alkaline phosphatase activity and phosphorus in aquatic ecosystems (Wynne, 1977; Pettersson, 1980, 1985; Franko, 1984; Chrost et al., 1984). In each case low alkaline phosphatase activity was associated with high concentrations of P. High alkaline activity was only detected when the P concentration was low.

Induction of alkaline phosphatase activity is often associated with low total cellular P concentration, low polyphosphate concentration and high polyphosphate synthetase activity (Healey, 1982). Therefore, alkaline phosphatase activity, total cellular P, polyphosphate concentration and polyphosphate synthetase activity are in themselves P-deficiency indicators. However, they are not independent of each other since repression-induction of alkaline phosphatase activity is controlled by cellular P fractions, probably polyphosphate (Cembella et al., 1984a).

The usefulness of phosphatase as indicator of P-deficiency has been argued. Cembella et al.(1984a) concluded that "the current practice of using assays of alkaline phosphatase as bio-indicators of the nutritional status of the environment is probably reckless and fraught with undesirable complications". They agreed that phosphatases from zooplankton, bacteria, degenerating cells, external input of phosphatases, dissolved and constitutive phosphatases could all decrease the significance of the assay as an indicator of P-deficiency. Increased phosphatase activity can also be induced by factors other than P-deficiency. Wilkins (1972) showed that alkaline phosphatase activity in E. coli was induced by a deficiency in pyrimidines and guanine and not a lowering of the internal P_i pool. Variations in

phosphatase activity during the day have also been demonstrated (Reichardt, 1971; Chrost et al., 1984). This stresses the problem that even the time of sampling is a factor influencing phosphatase activity.

Aims

The overall aim of this study was to investigate the presence of phosphatase of algal populations in high zinc streams.

1. The initial aim was to study the distribution of phosphatase activity of algal populations growing in these high zinc environments.

2. Second aim was to examine the relationship between phosphatase activity and chemical variables and particularly with N : P ratio and phosphates in the water.

3. A further aim was to examine if phosphatase activity in these high-zinc streams is associated directly or indirectly with the high zinc concentrations.

4. The fourth aim was to examine the hypothesis that high phosphatase activity in these streams may be associated with high ratio of organic to inorganic phosphate.

CHAPTER 2
MATERIALS AND METHODS

2.1 Computing and statistical treatments

The text for this dissertation was processed with Microsoft word 5.0 running on an IBM PS/2 model 30. Alkaline phosphatase data conversion carried out with Quattro Pro Spreadsheets.

For phosphatase activity and environmental variables Spearman's rank correlation was employed. The test with histograms showed that the above data do not follow the normal curve by locating the center and indicating the spread around the center (Pisani, 1987).

2.2 Chemicals

2.21 Media

Reagents used in the preparation of media were of Analar grade, obtained from British Drug House Ltd (BDH), Poole, Dorset.

2.22 Substrates used for assaying phosphatase activity

Table 2.1 Substrates used for assaying phosphatase activity

Reagents	Supplier
(ρ-nitrophenyl phosphate disodium) (ρNPP)	Sigma Chemical Co., USA
bis (ρ-nitrophenyl) phosphate Sodium salt (bis-ρNPP)	Sigma Chemical Co., USA

2.3 Common procedures

2.31 Absorption

2.311 Shimadzu spectrophotometer

All large volume i.e. more than 300 µl colorimetric analysis were carried out using a Shimadzu Digital Double-Beam Spectrophotometer (model UV-150-2). Glass cuvettes with a path length of 4 cm were used for all readings between the visible and infra-red range of the spectrum.

2.312 MCC Plate Reader

The MCC plate Reader was used for a large percentage of colorimetric analysis on alkaline phosphatase activity (APA). Assays using ρNPP and bis-ρNPP as substrates, Absorbance program 1 and Filter Code 1 (405nm) were used.

2.32 Flame atomic absorption spectrophotometry

Samples for Zn, Ca and Mg measurement, were analysed with a Perkin-Elmer 5000 Atomic Absorption Spectrophotometer and Automatic Burner Control Unit using an air-C2H2 flame (gas box settings =28:35) and deuterium-arc lamp background correction. The sensitivity of the spectrophotometer was enhanced by replacing the flow spoiler with an impact bead, whilst concentrations of Zn below 0.2 mg l^{-1} were detected on a chart recorder with expansion factors of 1 to 12. The machine was calibrated with standards made from 1000 mg l^{-1} Zn solutions (BDH "spectrosol").

2.33 Assay for alkaline phosphatase activity

2.331 Preparation of algal material for analysis of alkaline phosphatase assays

Algae were washed in stream water and transferred into flasks with assay media. They were centrifuged in 50 ml MSE non-sealable polyethylene centrifuge tubes, in a SS-34 8 x 100 ml angle head rotor, using a Sorvall RC-5B refrigerated superspeed centrifuge at 8000 x g for 15 min. The algal pellet was washed in MQ water and recentrifuged. After the second centrifugation, the supernatant was poured

off and the pellet was resuspended in 25 ml assay medium. The algal material was homogenized by passing it through a graded series of syringe needles. The algae were sonicated in a MSE soniprep 150 at an amplitude of 26 μm for 1-3 min. During sonication the algal homogenate was cooled with an ice jacket.

2.332 Assay procedure for alkaline phosphatase activity using the MCC plate Reader

The assay was carried out in microwell plates with a total reaction volume of 330 μl. A multichannel pipette was used to fill all eight wells in a column at a time. The pH range used was 5.0 to 11.0 and at each pH unit the sample had 8 replicates i.e.each column corresponded to each pH unit. 100 μl of standard buffer and 50 μl of algal material were pipetted into the microwells. 30 μl of 4.95 M NaOH was pipetted into 4 wells at each column before substrate was added. These wells were the control of the same material at each column. The microwell plates were transferred to the growth room at 25°C where 150 μl of substrate solution was pipetted to all assay and control wells. The addition of the substrate was the start of the assay which was incubated for 60 minutes At the end of the incubation, 30 μl of 4.95 M NaOH was added to assay wells to terminate the activity and fully develop the yellow ρNP colour. The absorbance of all assay and control wells was read on the Titertek plate reader.

2.333 Measurement of phosphatase activity

The absorbance values obtained at each pH unit for each sample were converted into concentration of ρNPP hydrolysed (μmol ml^{-1}), using a series of standard solutions of ρNP standard solution (Appendix 3). The algal concentration used in the assay well was calculated from the dry weight of each sample (μg ml^{-1}). The enzyme activity(μmol mg $d.wt^{-1}$) was obtained by dividing the ρNP concentration by the algal concentration and multiplying by 1000. All the calculations were carried out by a Quattro Pro Spreadsheet.

2.334 Effect of pH on phosphatase activity

The buffers were 50 mM (final concentration), which was chosen as a suitable concentration for buffering physiological media. At each pH unit duplicate buffers and another third at the range of 7.0 to 10.3 pH were used to compensate for any inhibition of phosphatase activity by the buffers used (Table 2.2).

Table 2.2 Buffers used to investigate effect of pH on phosphatase activity, (final assay buffer conc.= 50 mM)

pH	buffer	buffering range	pKa at 25 ^{o} C
3.0	DMG-NaOH	3.2 - 7.6	3.66, 6.20
3.0	glycine-HCL	2.2 - 3.6	2.35, 9.60
4.0	DMG-NaOH	3.2 - 7.6	3.66, 6.20
4.0	succinic-acid-NaOH	3.8 - 6.0	4.18, 5.60
5.0	DMG-NaOH	3.2 - 7.6	3.66, 6.20
5.0	succinic-acid-NaOH	3.8 - 6.0	4.18, 5.60
6.0	DMG-NaOH	3.2 - 7.6	3.66, 6.20
6.0	succinic-acid-NaOH	3.8 - 6.0	4.18, 5.60
7.0	DMG-NaOH	3.2 - 7.6	3.66, 6.20
7.0	HEPES-NaOH	6.8 - 8.2	7.50
7.0	EPPS-NaOH	7.3-8.7	8.0
8.0	TES-NaOH	6.8 - 8.2	7.50
8.0	HEPES-NaOH	6.8 - 8.2	7.50
8.0	EPPS-NaOH	7.3 - 8.7	8.0
9.0	AMeP-NaOH	9.0 - 10.5	9.69
9.0	glycine-NaOH	8.6 - 10.6	2.35, 9.60
9.0	EPPS-NaOH	7.3 - 8.7	8.0
10.0	AMeP-NaOH	9.0 - 10.5	9.69
10.0	glycine-NaOH	8.6 - 10.6	2.35, 9.60
10.0	CAPS-NaOH	9.8 - 11.1	10.40
10.3	AMeP-NaOH	9.0 - 10.5	9.69
10.3	glycine-NaOH	8.6 - 10.6	2.35, 9.60
10.3	CAPS-NaOH	9.8 - 11.1	10.40
11.0	CAPS-NaOH	9.8 - 11.1	10.40
11.0	N_2CO_3-$NaHCO_3$	9.2-10.8	10.33

2.4 Standard culture techniques

2.41 Cleaning of apparatus

All glassware and plastic apparatus were cleaned by soaking in 2% Decon 90, a phosphate free detergent (Decon Laboratories Ltd, England) for 20 minutes. The apparatus were rinsed six times in distilled water. All volumetric glassware was dried at room temperature. Plastics were dried at 40°C and other glassware at 100°C.

2.42 Media

2.421 Chu 10-F

This medium, a modification of the No 10 formula of Chu (1942) was used for growth of algal field material. The medium was prepared according to a recipe using concentrated stock solutions (Sinclair, 1977) (Table 2.3). HEPES was used to adjust the pH to 7.5. The elemental composition of the standard media is presented in Table 2.4.

2.422 Assay medium

The concentration of mineral salts and elements of assay medium is presented in Tables 2.3 and 2.4, respectively.

Table 2.3 Concentration of mineral salts in Chu 10-F and in assay medium

Salt	Chu10-F		assay medium	
	$\mu g\ l^{-1}$	μM	$\mu g\ l^{-1}$	μM
KH_2PO_4	4.39	32.0	-	-
$MgSO_4\ 7H_2O$	25.0	101.4	25.0	101.4
$Ca(NO_3)_2\ 4H_2O$	40.0	169.4	-	-
$NaNO_3$	55.64	654.0	-	-
$NaHCO_3$	15.80	188.6	15.80	188.6
Na_2SiO_3	10.87	12.8	-	-
$FeCl_3\ 6H_2O$	1.94	7.2	0.97	3.6
Na EDTA	2.67	7.2	1.33	3.6
H_3BO_3	0.715	11.50	2.860	46
$MnCl_2\ 4H_2O$	0.453	2.30	1.810	9.10
$ZnSO_4\ 7H_2O$	0.055	0.20	0.222	0.77
$Na_2MoO_4\ 2H_2O$	0.007	0.03	0.390	1.61
$CuSO_4\ 5H_2O$	0.019	0.08	0.079	0.31
$CoSO_4\ 7H_2O$	0.010	0.04	-	-
$Co(NO)_3\ 6H_2O$	-	-	0.049	0.16
$NiSO_4\ 7H_2O$	-	-	0.048	0.16
NaOH	* c.60	1500.0	-	-
HEPES	600.0	2517.0	-	-
KCl	-	-	4.27	57.3
$CaCl_2\ 2H_2O$	-	-	35.87	278

* NaOH used to adjust pH

Table 2.4 The elemental concentration in Chu 10-F and in assay medium

Element	Chu 10-F		assay medium	
	$\mu g\ l^{-1}$	μM	$\mu g\ l^{-1}$	μM
Cl	0.92	25	22.72	641
N	14	9921	0.002	0.14
Ca	6.7	169	11.13	278
Na	55	2391	4.4	190
S	0.009	0.028	0.039	1.2
Mg	2.5	100	2.5	100
K	1.25	32	2.23	57
Si	1.43	51	-	-
P	1.0	32	-	-
B	0.125	11.5	0.46	46
Fe	0.4	7.2	0.2	3.6
Mn	0.12	2.3	0.50	9.1
Zn	0.01	0.019	0.05	0.77
Cu	0.005	0.079	0.02	0.31
Co	0.002	0.043	0.009	0.16
Ni	-	-	0.01	0.16
Mo	0.002	0.028	0.15	1.6

2.5 Sample collection and preparation

2.51 On-site

An number of physical and chemical factors were measured at the time of sampling. Conductivity and temperature were measured using a WTW (model FC910. pH was measured using a WTW (model pH91) pH meter with temperature display.

2.52 Algal samples

Samples of algae were collected using forceps; they were placed in polyethylene together with stream water and were kept on ice for transport back to the laboratory. After arrival at the laboratory, the material was kept in the fridge until the following day when the assaying procedure was carried out.

2.53 Water samples

Water was collected in acid-washed, iodized polypropylene bottles. Each bottle was rinsed three times and filled, expelling all air. Sample bottles were returned to the laboratory in a box filled with ice. The water for phosphate analysis was immediately passed through a GF/C filter. MQ-water was passed through the GF/C filter as a control sample for the phosphate analysis. After filtration, water for phosphate and nitrite analysis was stored at -20^{o}C until analysis could be carried out.

2.6 Chemical analysis

2.61 Nitrite

Nitrite was determined by the method of N-1-naphthylethylenediamine dihydrochloride (Stainton et al.,1977).

2.62 Nitrate

Nitrate was reduced to nitrite by a cadmium-copper couple and analysed as for nitrite (Stainton et al., 1977).

2.63 Ammonia

Ammonia was determined by the indophenol blue method (Stainton et al., 1977).

2.64 Phosphate

2.641 A simplified phosphorus analysis technique

Phosphorus analysis was carried out by a simplified phosphorus analysis technique by Eisenreich et al., 1975. The method results in a 70 % decrease in analysis time for multiple samples and about a 30 % increase in sensitivity. The method can be directly applied to water samples with P levels of 2 to 1100 µg $P\ l^{-1}$.

2.642 Phosphate fractions measured

The terminology of the various fractions measured corresponds approximately to that in American Public Health Association (APHA) (1980). In this study, Filtrable Reactive Phosphorus (FRP) and Total Filtrable Phosphorus (TFP) were determined. FRP is that which is detectable by colorimetry without further treatment. TFP is that which is detectable by colorimetry after digestion with acid sulphate, which hydrolyses most organic and any condensed phosphates. The difference between FRP and TFP gives filtrable organic phosphorus and filtrable acid hydrolysable phosphate in APHA terminology. For simplicity, the difference is termed simply "filtrable organic"-P.

2.643 Experimental

(i) Stock solutions and Reagents

Four stock solutions were prepared for the phosphate analysis. From these solutions, three reagents were prepared daily for use (Table 2.5).

Table 2.5 Stock solutions and reagents used for phosphate analysis

Stock solutions	Reagents
A H_2SO_4-Antimony	Digestion Reagent ($K_2S_2O_8$, C)
B Molybdate	Mixed Reagent I (A, B, ascorbic acid)
C Digestion acid (concentrated H_2SO_4)	Mixed Reagent II (A,B,C, ascorbic acid)
D Phosphate standard solution	

(ii) Procedure

1. Determination of FRP

From the phosphate standard solution, a calibration curve was prepared at the range of 10 $\mu g\ l^{-1}$ to 100 $\mu g\ l^{-1}$. 10 replicates of each sample were prepared and an addition of P was carried out at each replicate. The addition in the first replicate was 10 µg and in the tenth was 100 µg with a difference 10 µg between each replicate (Appendix 1, 2). All samples were prepared in a 125-ml Erlenmyer flasks of 25 ml volume. Two control samples also were carried out through the procedure. 15 min were allowed for the colour development after the addition of reagent II and the solution absorbance was measured at 882 nm in 4 cm cell against MQ-water.

2. Determination of TFP

For TFP, the calibration curve and samples were prepared in the same way as for FRP, except that a digestion reagent was added to the samples which were then autoclaved for 30 min at 15 psi. After this, reagent I was added and the absorbance was measured as above.

2.7 Algae tested for phosphatase activity

All algal samples tested for phosphatase activity consisted of filamentous green algae (Tables 5.1, 5.2). They were examined under a type 109 Nikon Fluophot microscope, fitted with a Nikon micrometer eyepiece. Some samples had mixed algal species. Estimates of abundance for species within each sample were made on slides under the microscope. Although the estimates were subjective, they indicated the relative abundance. This abundance was roughly estimated as a percentage. The samples were also examined for organisms other than algae. In this study, therefore, each algal sample consisted either of a single population or a mixture of populations.

The algal material was tested for phosphatase activity after 2 days of the collection date. For practical reasons it was impossible to test them in shorter time after collection (2.331) Care was taken by preserving them in the refrigerator in order to minimize changes in the material.

CHAPTER 3

3 FIELD SITES AND SAMPLING PROGRAMME

3.1 Indroduction

This chapter presents some background information on the sites, which were sampled for the study of phosphatase activity of algae present in these high Zn level streams sites. The sampling programme of this study is also presented here.

3.2 General background to areas of study

The study area is situated in the Pennines, which constitute the dominant feature in the physiography of the North England. The Northern Pennine Orefield covers an approximate 3885 km^2 in the counties of Cumbria, Northumberland, Durham and West Yorkshire. It is divided into two complementary halves by the Stainmore Gap. The area of study is situated in the northerly half. This area is drained by the three principal rivers of North-eastern England, the Tyne, Wear and Tees. The waters of of these catchments have been the subject of a wide range of studies (Horne, 1977) and are easily accessible from the University of Durham. In the present study, sites in the Tyne and Wear River systems were visited (Table 3.1, Fig. 3.1).

Anthropogenic influence comes mainly from agriculture and mining activities in these river systems studied. Mineral veins are reported to have been mined since pre-Roman times (Raistrick & Jennings, 1965), but most of the mining activity took place in the nineteenth century. Many mine drainage water empty into these river systems, most being from disused mines (Harding & Whitton, 1976), polluting many of the upland sreams with heavy metals, heavy metals are also introduced by seepage from heaps of mine tailings (Say & Whitton, 1981) (Fig. 3.2).

The upland areas are mainly covered by peat and heather moors, with pasture giving way to crops inthe lower reaches (Whitton & Crisp, 1984). In general the upland areas have higher rainfall, more persistent cloud cover and lower temperature than the lowlands areas. The banks of many of the upland tributaries have little or no tree cover.

3.3 Sites of study

1. Caplecleugh Low Level

This site is a small stream draining from an adit of the Caplecleugh Mine in the village of Nenthead, Cumbria. Caplecleugh mine was previously a major source of lead and zinc concentrates in the Northern Pennine Orefield (Dunham 1948). The stream drains into the R. Nent which itself drains into the R. South Tyne (Fig. 3.2).

2. Rampgill Level

This site is a smal, zinc-rich stream that flows into the Nent from a tunnel, in the village of Nenthead. The stream is exposed for about one metre of its path and then flows uderground until its mouth, which was the point of sampling.

3. "Shield Hilltop"

This site is a zinc-rich, low flow small stream draining from an adit, near to the village Garrigill. The stream flows for 15 m before drainig into another sream which is tributary to Brown Gill.

4. Gillgill Burn

Gillgill Burn is a Zn-rich small upland stream near to the village of Nenthead. It originates as a spring from old waste tailing tip, receives drainage and percolations from surrounding tips.

5. Mine at Newberryside

This site is a relatively low polluted stream near to the town Alston and is tributary to R.South Tyne.

6. Kilhope Burn

This site is an acid, small stream in a moorland, upland sheep grazing area. The water draining from the fells is acid and organic-rich.

7. R. West Allen

This site is a low polluted stream in upland sheep grazing pasture area. It drains from top of fells and moorland.

8, 9. R. East Allen

These two sites are the R. East Allen (8) and the spring stream (9) beside to river in the Sipton Shield area (Allendale common). R. East Allen drains from upper fells and receives tributaries from meadow pastures. Site 9 is a spring calcareous, slow flow stream with relatively high zinc concentration.

10. Sites in the village Nenthead (11, 13, 14)

These sites come from an area of old mine buildings including an old dressing floor, a smelting mill and a large number of tailings heaps right up the valley towards the upper Nent. Sites 10 and 14 are small, slow flow streams draining from adits, with relatively high levels of Zn. Site 13 is a small spring stream with high Zn concentration. Site 11 is a small stream with a low Zn concentration.

12. "Brown Gill tributary"

Brown Gill tributary" is a Zn-rich acid stream, direct run-off from spoil heaps of old mine workings, in a sheep-grazing area near to Garrigill.

3.4 Sampling programme

Samples were collected during June, July and August. The sampling was carried out in two surveys (A & B). In survey A, three sites were chosen for intensive study: Caplecleugh Low Level, Rampgill Level and "Shield Hilltop" (3.3). Water and algal samples were collected from each site on five occasions. Each time, phosphatase activity and environmental variables were measured. In survey B, 14 sites were sampled once on 29/08/90 (Table 3.1). Phosphatase activity of algal organisms as well as the environmental variables for each site were measured. In this survey, particular attention was paid to phosphate analysis as well as the influence of buffering on phosphatase activity.

Table 3.1 Sites visited for the study of phosphatase activity of algal samples

No	Site	Grid reference	Stream * reach	Environment
1	Caplecleugh Low Level	NY782435	0097-01	Zn-rich mine drainage
2	Rampgill Level	NY782435	0096-01	Zn-rich mine drainage
3	"Shield Hilltop"	NY752424		Zn-rich mine drainage
4	Gillgill Burn	NY795440	0093-01	Zn-rich upland stream
5	Mine at Newberryside	NY728448		Low polluted calcareous stream
6	Killhope Burn	NY807434		Acid small upland stream
7	R.West Allen	NY802449	0085-05	Low polluted upland stream
8	R.East Allen	NY847497		Low polluted calcareous river
9	R.East Allen	NY847497		Zn-rich spring small stream
10	Village Nenthead	NY787428		Mine drainage (slow flow)
11	Village Nenthead	NY784434		Small stream tributury to R. Nent
12	"Brown Gill tributary"	NY764423	0108-08	Zn-rich acid stream
13	Village Nenthead	NY785433		Zn-rich spring small stream
14	Village Nenthead	NY786420		Mine drainage (slow flow)

* Stream reach is a code number for recognised sites which are listed in Durham database of river environmental data.

Fig. 3.1 Location of 14 sampling sites in the Northern Pennine Orefield

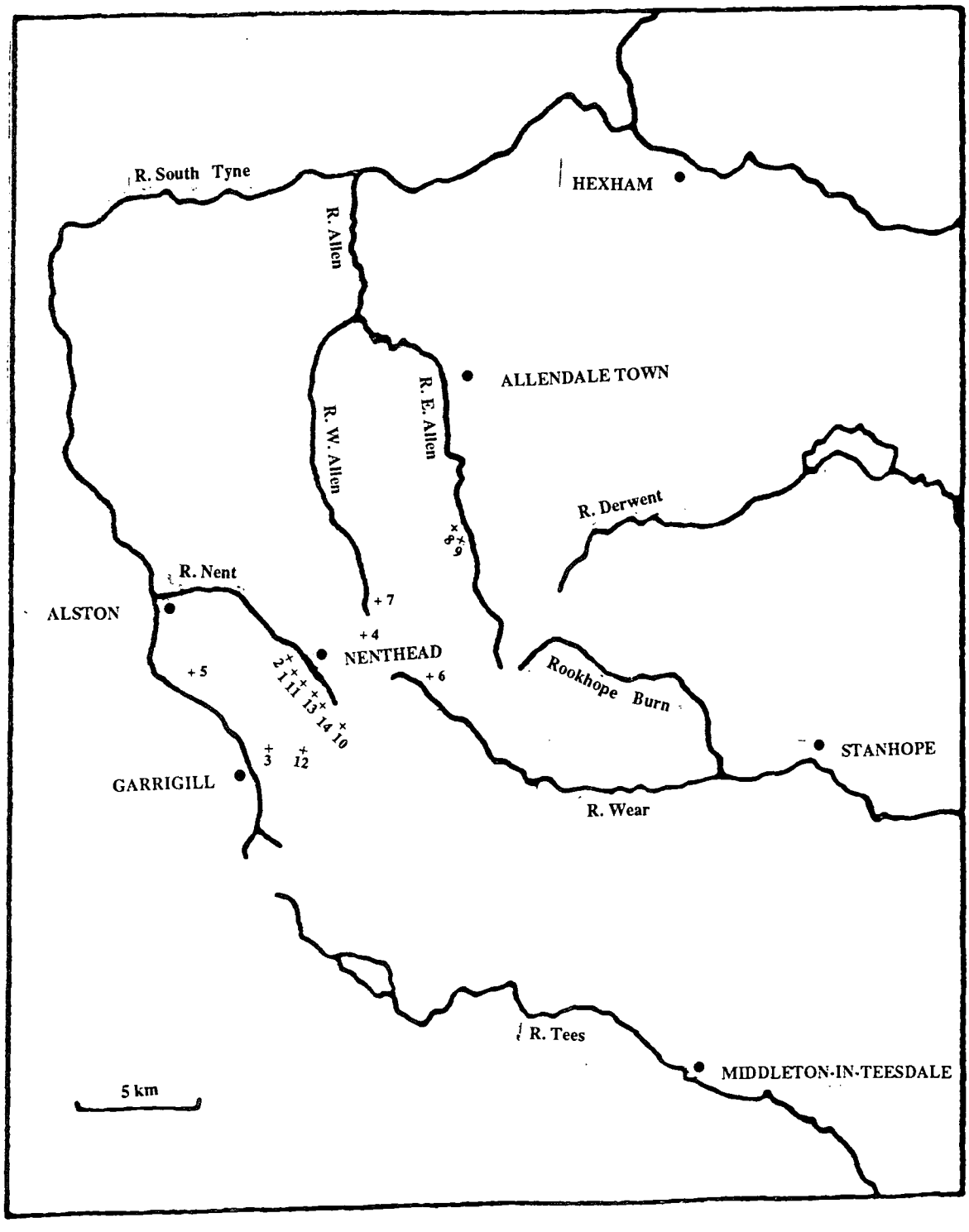
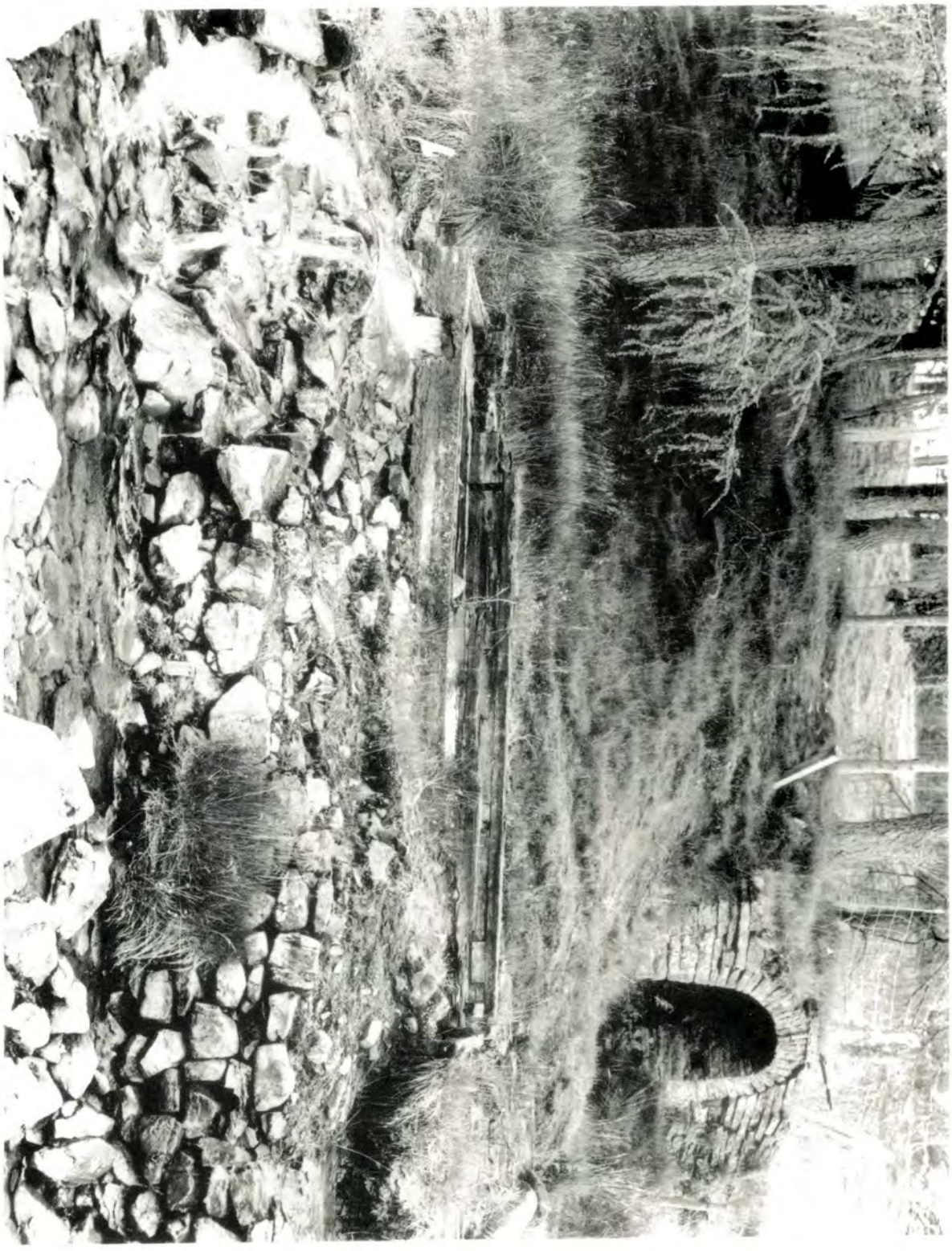


Fig. 3.2 View of Caplecleugh Low Level from which mine water is discharged. The mine water is directed into the river by the remnants of a water leat channel (The picture used was taken in summer 1981 by Dr B.A. Whitton)



CHAPTER 4

PHYSICAL AND CHEMICAL VARIABLES

4.1 Introduction

In order to provide environmental information for the study of phosphatase activity of algal samples from the field sites, selected variables were measured. These provided information about the environment where the algal populations had been growing so that relationships between phosphatase activity and envirnmental variables could be examined. Measurements were carried out in two surveys (A, B) (3.4). Results of survey A water analysis are given in Table 4.1. Phosphate measurement in this survey was incomplete and results therefore are not included in Table 4.1. Results for survey B water analysis are given in Table 4.2. Major emphasis was placed to phosphate analysis by employing the procedure of phosphate additions to the samples in order to minimise errors and to measure more accurately low concentrations. In this survey, water samples were designated from 1 to 14 corresponding to the sites from where they were collected (Table 3.1).

4.2 Mg, Ca and Zn concentration

Survey A. The three streams sampled in this survey are typically highly buffered, have a pH greater than 7.0, contain high concentrations of dissolved zinc and high concentrations of dissolved calcium. The feature of the water in these streams is the constancy of its chemistry. The concentrations of Mg, Ca and Zn were approximately at the same level on the five occasions of sampling (Table 4.1).

Survey B. The water chemistries of the 14 sites reflect the different origins of the water in these streams. pH values ranged from 3.9 to 8.7 and most sites had a pH greater than 7.0. Calcium concentrations ranged from 2 mg l^{-1} to 98.6 mg l^{-1}. Zinc concentration ranged from 0.1 mg l^{-1} to 19.4 mg l^{-1} with 7 of the 14 sites above 1 mg l^{-1} Zn (Table 4.2, Fig. 4.1).

4.3 Nitrate, nitrate and ammonia concentrations

Survey A. Nitrate-N concentrations were relatively high with small variations between the five occasions in each site. Nitrite-N and ammonia-N concentrations were below the detection limits of the analytical technique (Detection limit < 5 $\mu g\ l^{-1}$ N) (Table 4.1).

Survey B. Nitrate-N concentrations were relatively high in the majority of the sites. Nitrite-N concentrations were below 5 $\mu g\ l^{-1}$ in all sites and ammonia-N also concentrations were below 5 $\mu g\ l^{-1}$ in the majority of the sites (Table 4.2, Fig. 4.2).

4.4 Phosphate

Survey A. Only FRP concentrations were determined. All the values were below the detection limit on all occasions (Detection limit < 5 $\mu g\ l^{-1}$ P).

Survey B. For FRP and TFP determination in this survey, the procedure of adding known concentrations of P to each sample was employed. Each sample was measured eleven times, one as single and ten with additions, so that any contamination or interference of P with other elements in the sample was able to be detected. FRP and TFP were determined by comparing the absorbance value of each sample with the calibration curve using standards of known concentrations of P (Fig. 4.3, Appendix 1, 2). In the majority of the samples, the concentration of P in the replicates of each single sample was close to concentration of the single sample after substracting the added P values. However, there were some exceptions and particularly for TFP determination where the concentration of P in the replicates with the highest added P values was not close to the concentration of the single sample after substracting the added values (e.g. samples 1, 4). The above indicate that contamination or interference was negligible in most of the samples. The values of the single samples are taken into account for FRP and TFP determination.

TFP exceeded FRP for 9 of the 14 samples. In two samples the concentration of TFP was approximately the same as of FRP (sample 4, 12), but in three samples the concentration of TFP was far lower of FRP (samples 3, 10, 14). For practical purposes the concentration of TFP is assumed to be equal to FRP in the last three samples (Table 4.2, Fig. 4.2).

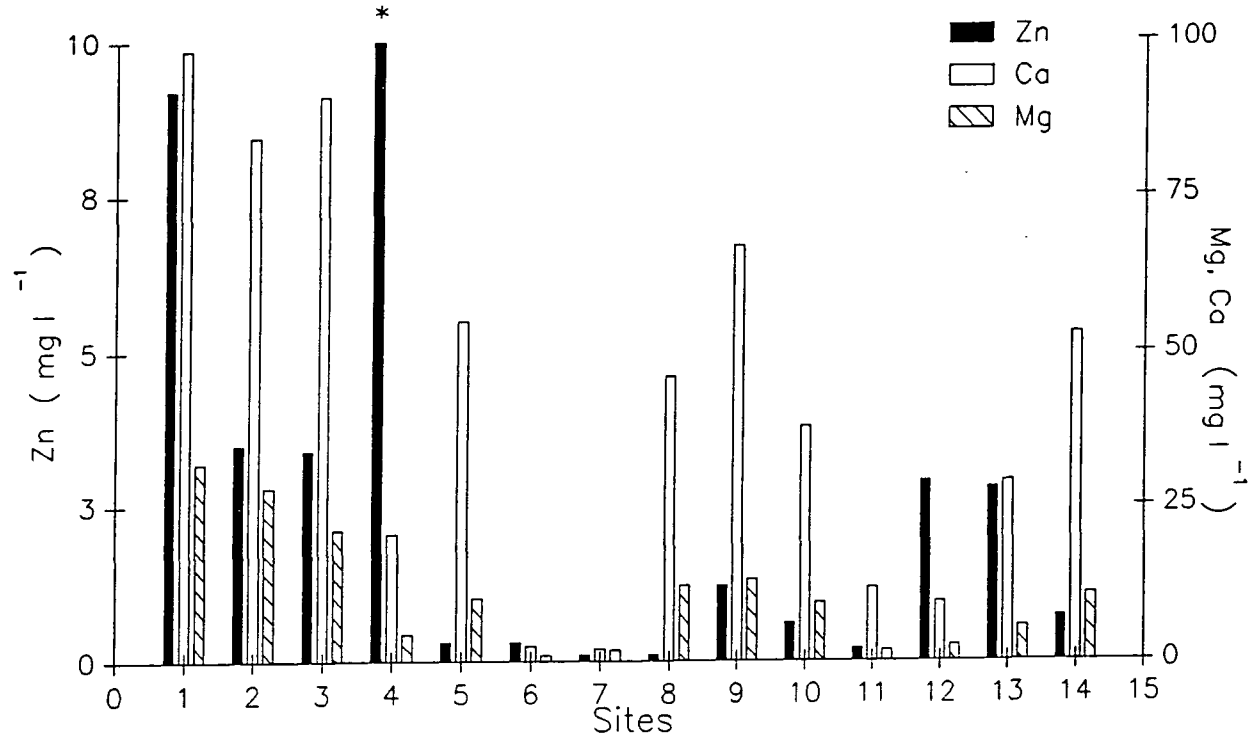
Table 4.1 Physical and chemical variables for three sites sampled on five occasions (survey A)

Site No	date	Temperature ^{o}C	Conductivity $\mu S\ cm^{-1}$	pH	Mg $mg\ l^{-1}$	Ca $mg\ l^{-1}$	Zn $mg\ l^{-1}$	NO_3-N $\mu g\ l^{-1}$
1	28/06/90	9.6	754	7.9	23.6	102.7	7.8	95
	05/07/90	10.2	723	8.1	27.2	102.1	8.2	112
	12/07/90	9.9	695	7.8	26.3	96.2	8.4	87
	19/07/90	12.8	725	7.8	22.2	97.1	7.7	115
	02/08/90	11.9	743	7.7	25.2	104.5	8.7	91
2	28/06/90	10.6	633	8.0	19.6	75.7	2.6	115
	05/07/90	9.5	532	8.1	20	77	2.8	133
	12/07/90	9.4	586	8.0	23.3	81.8	2.9	85
	19/07/90	10.7	606	7.9	22.2	80	2.8	96
	02/08/90	11.5	653	7.8	25.2	87.5	3.5	92
3	28/06/90	9.6	553	8.0	13.8	81.9	3.3	182
	05/07/90	9.2	467	8.0	13.6	74	3.0	172
	12/07/90	9.0	509	8.0	13.8	84.8	3.5	190
	19/07/90	10.9	507	7.9	13.1	80.1	3.3	235
	02/08/90	11.5	538	7.8	14.6	84.5	3.6	222

Table 4.2 Physical and chemical variables for 14 sites sampled on 29/08/90 (survey B)

Sample Site	Temperature oC	Conductivity $\mu S\ cm^{-1}$	pH	Mg $mg\ l^{-1}$	Ca $mg\ l^{-1}$	Zn $mg\ l^{-1}$	NO_3-N $\mu g\ l^{-1}$	NH_4-N $\mu g\ l^{-1}$	TFP $\mu g\ l^{-1}$	FRP $\mu g\ l^{-1}$
1	11.5	742	7.7	32	98.6	9.2	81	13	7.7	2.2
2	11.5	642	7.8	28	84.5	3.5	87	0	4.6	1.8
3	11.5	538	7.8	21	91	3.4	167	0	2.2	2.2
4	16.8	285	5.7	4.2	20.4	19.4	5	16	3.6	3.6
5	16.0	358	8.4	10	55	0.3	77	< 5	7.2	4.9
6	14.0	87	3.9	0.9	2.4	0.3	199	< 5	22.8	8.5
7	15.3	57	5.4	1.7	2	0.1	133	< 5	6.7	2.7
8	17.3	358	8.7	12	46	0.1	17	< 5	4.1	3.6
9	17.0	467	7.3	13	67	1.2	65	0	5.1	3.1
10	13.0	312	7.9	9.3	38	0.6	70	12	3.1	3.1
11	14.5	96	7.4	1.6	11.6	0.2	88	12	5.1	4.0
12	14.2	145	5.2	2.5	9.4	2.9	37	5	4.5	4.5
13	15.4	243	7.2	5.4	29	2.8	126	< 5	3.1	1.8
14	11.2	520	8.2	10.6	53	0.7	72	< 5	4	4

Fig. 4.1 Mg, Ca and Zn concentrations of 14 sites sampled in survey B



* Zn conc. in site 4 is higher (see Table 4.2)–

Fig. 4.2 Nitrate, TFP and FRP concentrations of 14 sites sampled in survey B

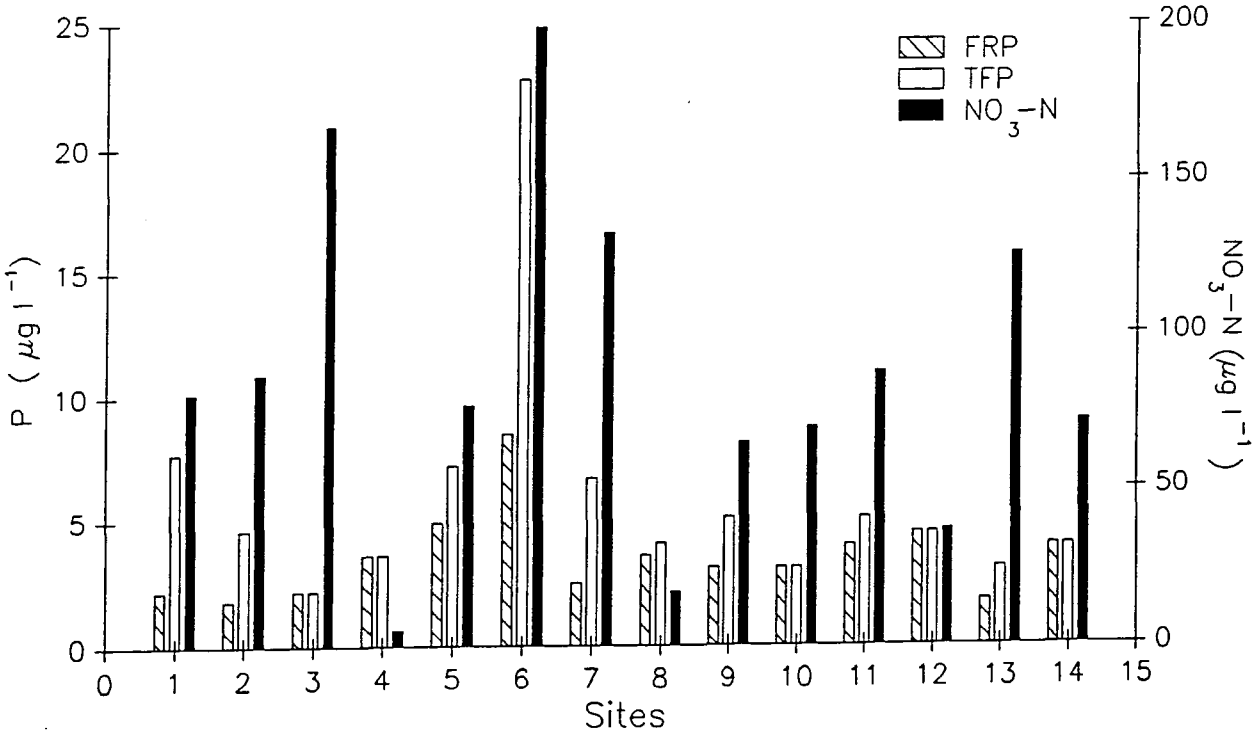


Fig. 4.3 Calibration curve and phosphate additions procedure employed for the determination of FRP and TFP of 14 samples from 14 sites in survey B

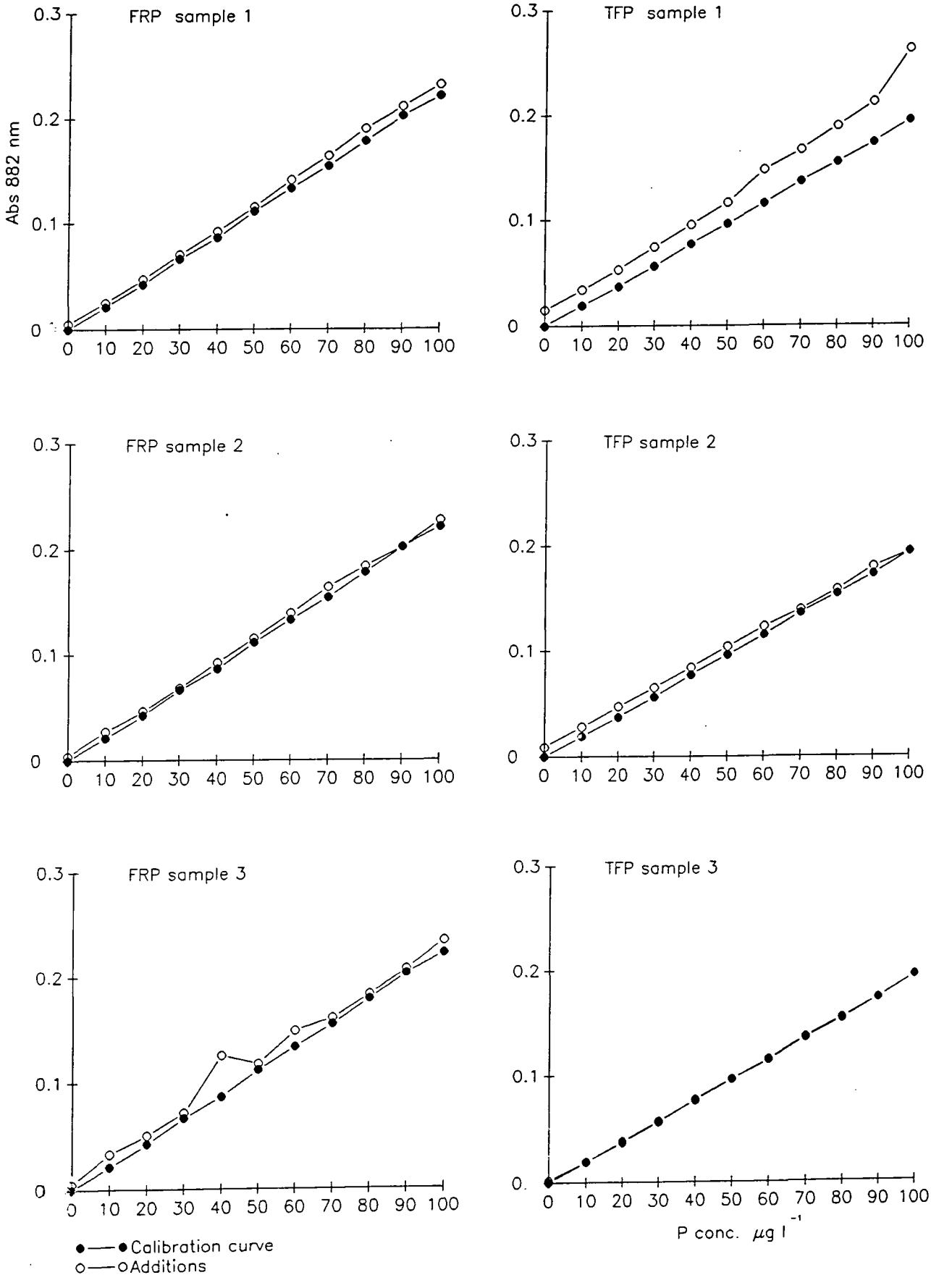


Fig. 4.3 continued

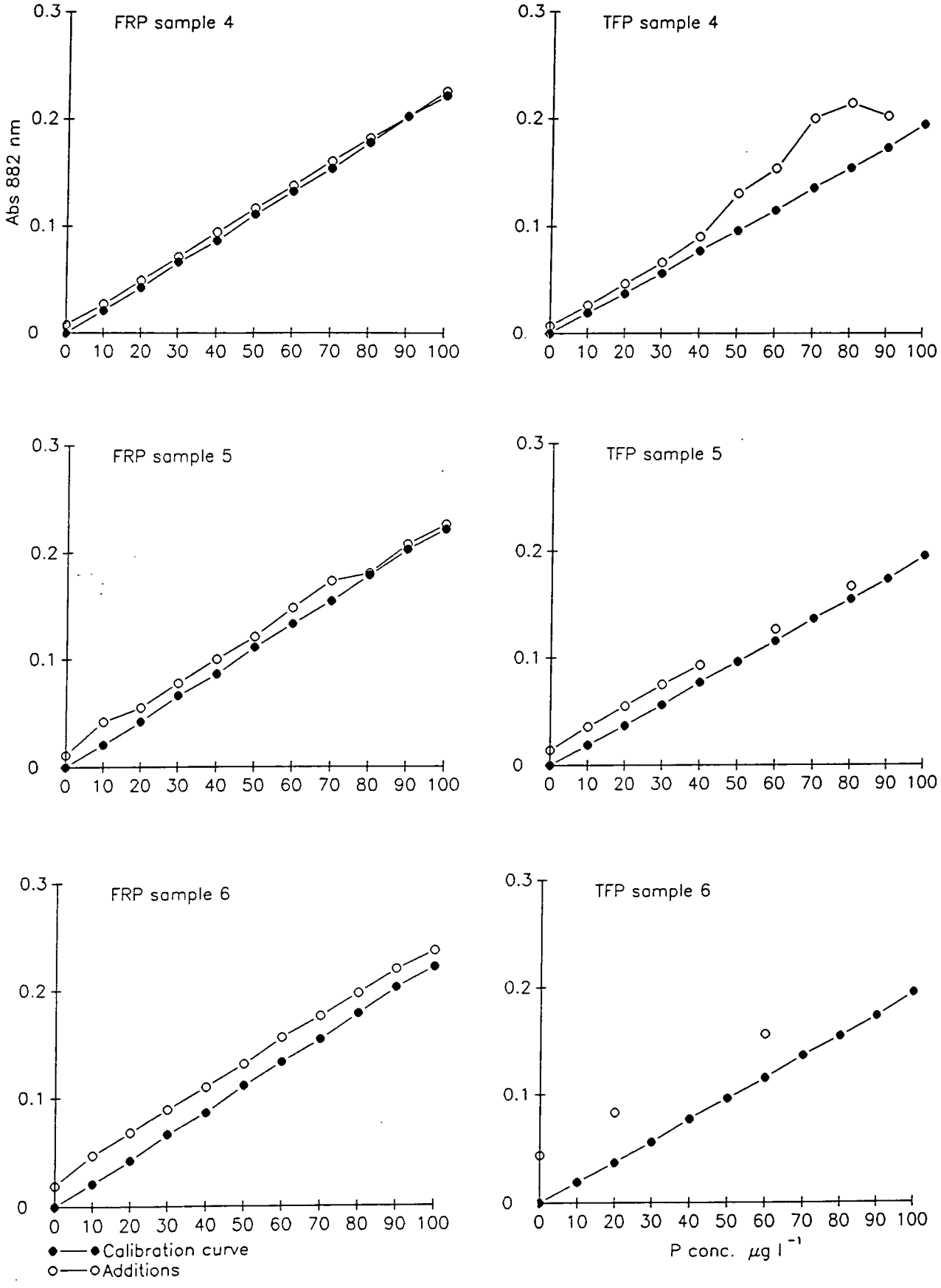


Fig. 4.3 continued

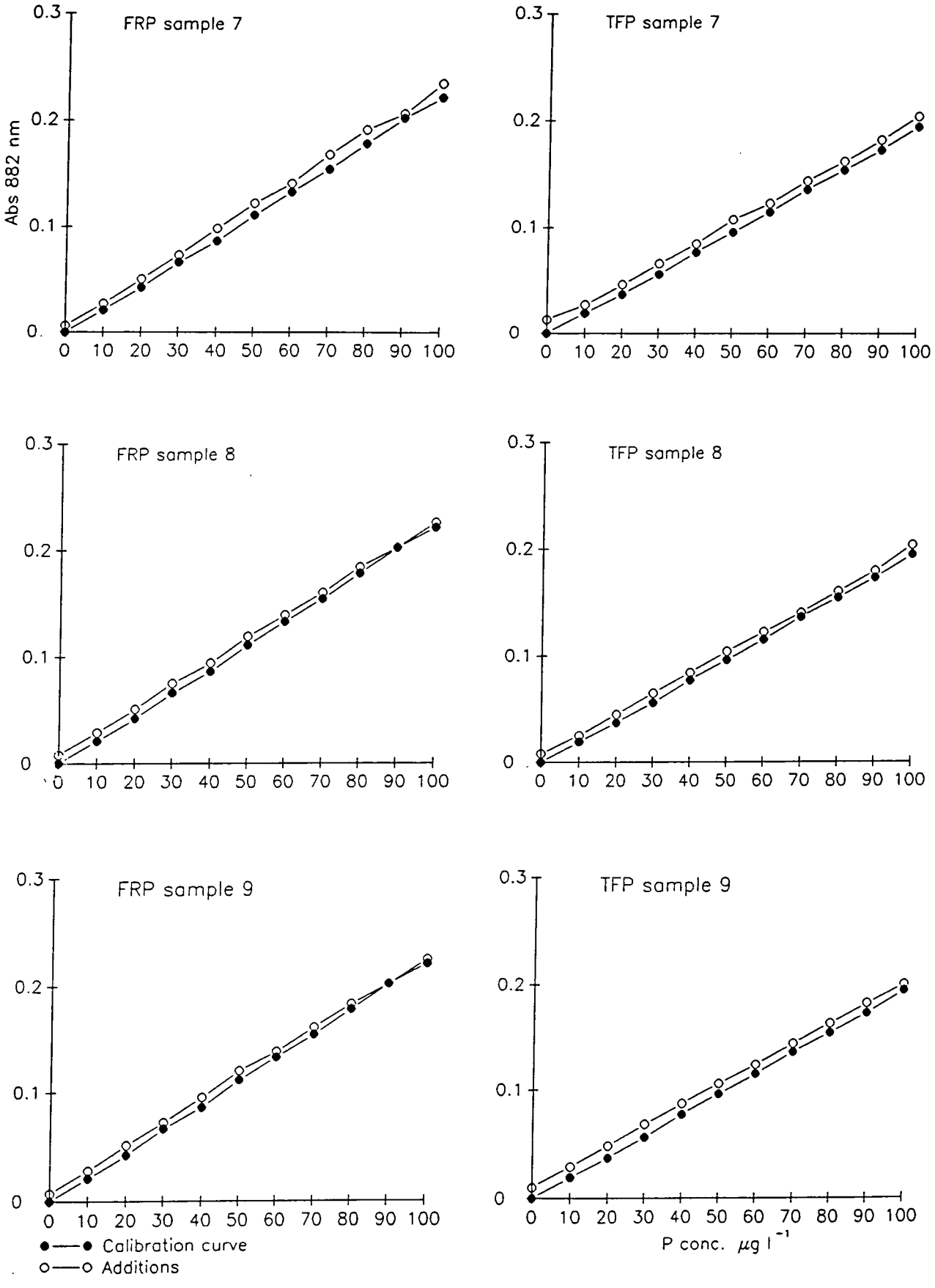


Fig. 4.3 continued

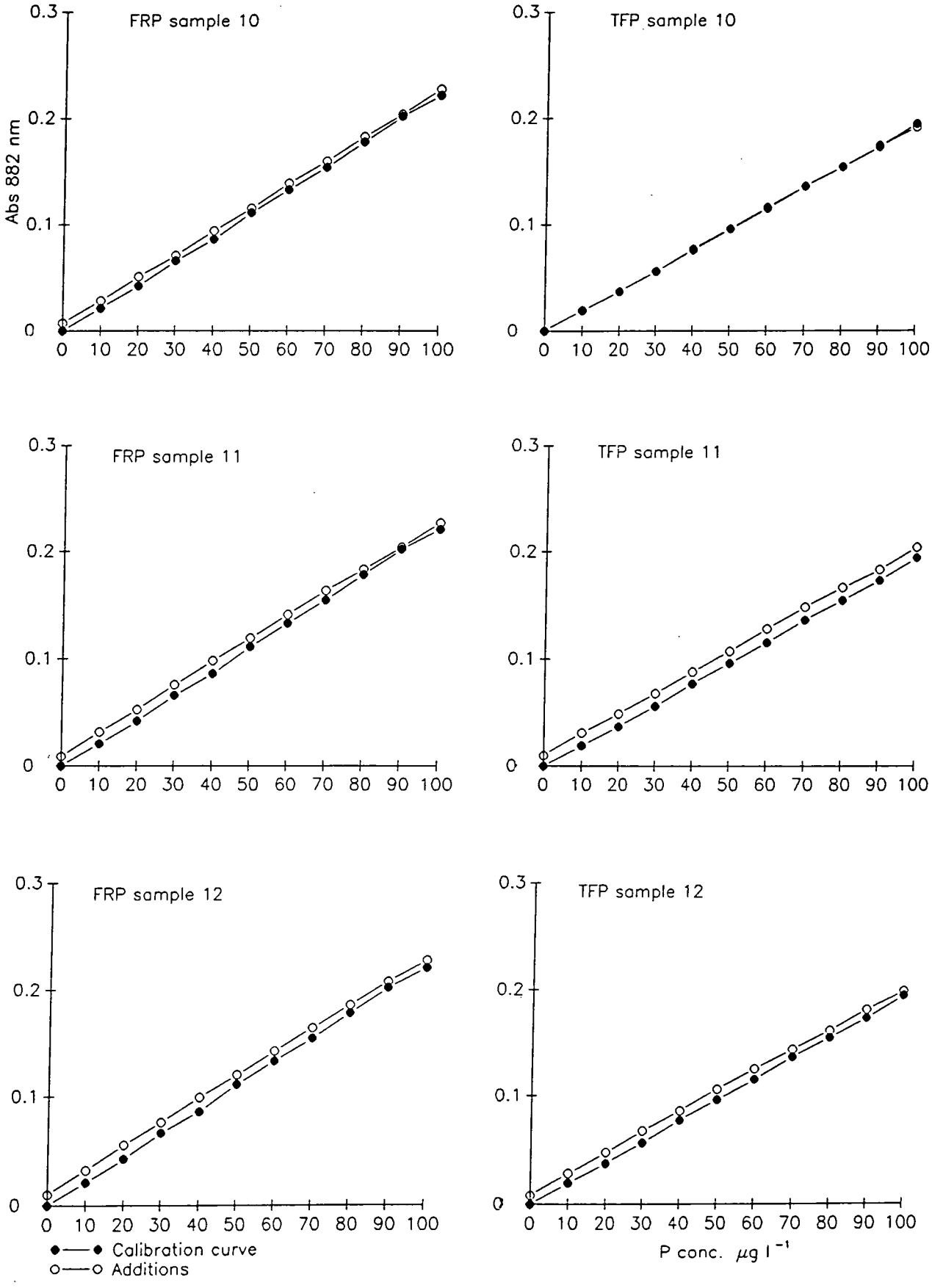
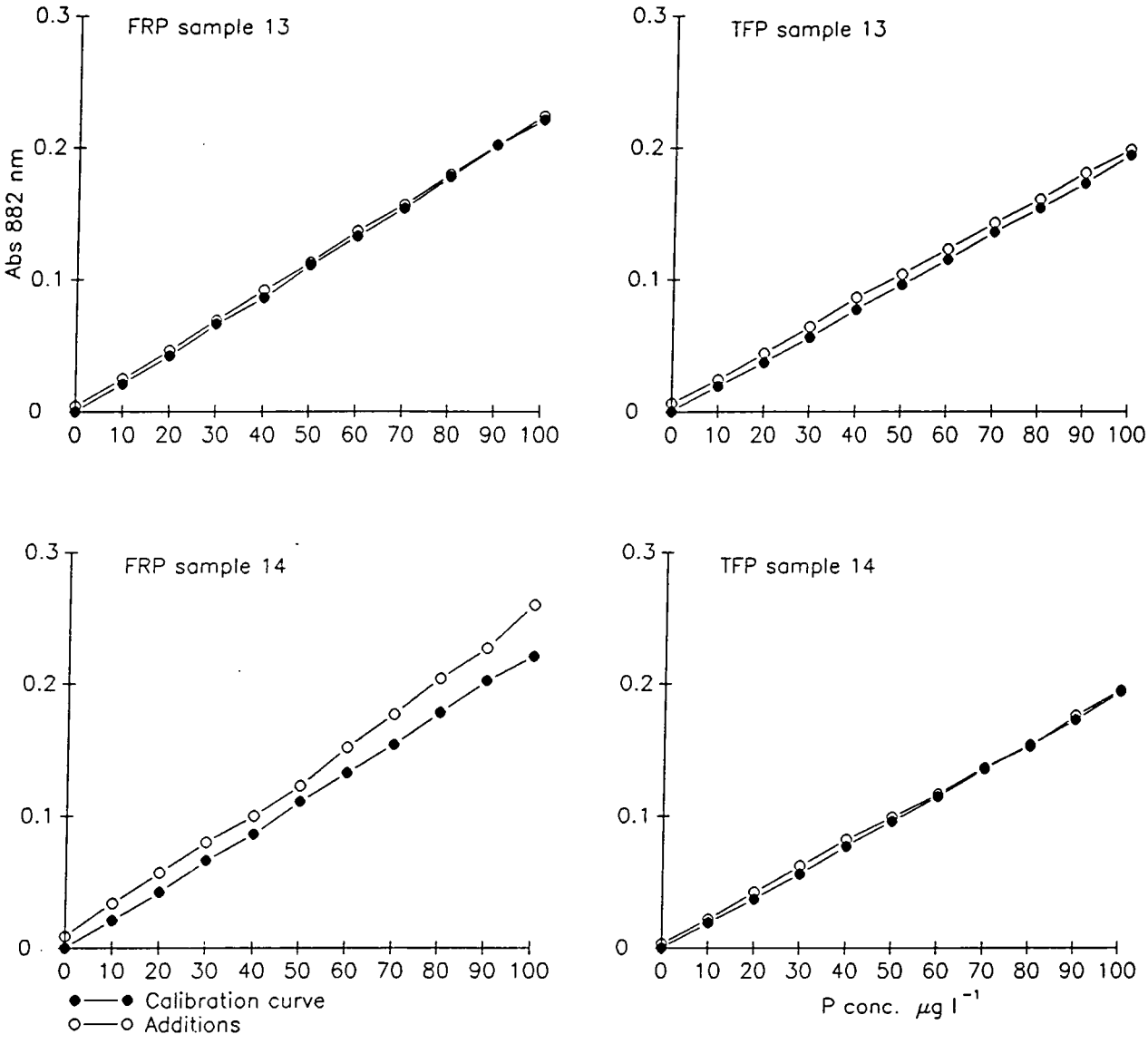


Fig. 4.3 continued



CHAPTER 5

PHOSPHATASE ACTIVITY

5.1 Introduction

The presence of phosphatase activity and its relation to water chemistry in the high-zinc streams was studied in two surveys (A, B) (3.4). In the first, the response of phosphatase activity to pH throughout the five occasions was examined (1.551). The presence of mixed populations in the samples was also examined. In all occasions the samples were tested for phosphomonoesterase (PMEase) activity and in one occasion apart from PMEase they were tested for phosphodiesterase (PDEase) activity (1.522).

In the second survey, the response of phosphatase activity to pH in these sites with the different water chemistries was examined(1.551, Table 4.2). The relationship between phosphatase acitvity and a number of environmental variables was examined. Particular attention was paid to buffering system used. In both surveys phosphatase activity was tested over a broad pH (3.0 - 11.0) using two different buffers at each pH unit. In survey B, a third buffer was used in the range from 7.0 to 10.3 (2.4). Samples in this survey were tested only for PMEase activity (1.52). The algal samples were designated from 1 to 14 corresponding to the sites from where they were collected (Tables 3.1, 5.2).

5.2 Effect of pH on phosphatase activity

5.21 Influence of the buffering system used on phosphatase activity

Survey A. Phosphatase activity of algal samples tested in survey A is expressed with buffers which led to the higher enzyme activity (Figs 5.1, 5.2, 5.3).

Servey B. The different buffers used at each pH unit showed variable values of phosphatase activity in each sample as well as different influences on phosphatase activity between the samples (Table 5.3). Because of these variations the phosphatase activity of each sample is presented in two ways with respect to the buffers (Fig. 5.5):

(a) Buffer which led to the higher enzyme activity

(b) Common group of buffers for all the samples

In Fig. 5.5 each sample is presented twice in both ways: a and b. Examples of the influence by buffers on

phosphatase activity are given below (Table 5.3 and Figure 5.5):

1. Succinic acid buffer permitted enzyme activity in the majority of samples at pH 4.0.

2. Glycine-NaOH permitted higher activity at pH 9.0 and 10.0 than the other two buffers (sample 1, 2, 3 & 13).

3. EPPS and CAPS permitted much higher activity at pH range from 7.0 to 10.3 than the other buffers at the same range (sample 7).

4. Glycine-NaOH permitted higher activity than AMeP-NaOH at pH 9.0 in sample 1, 2 and 3, whereas it permitted lower enzyme activity than AMeP-NaOH at the same pH in sample 4, 5 and 11.

Fig. 5.1 shows however, that the majority of samples exhibited similar pH profiles in both ways except the case of the succinic acid interference at pH 4.0 and the sample 7. In all samples the phosphatase activity that is expressed with the buffer which led to higher activity will be taken into account without considering the case of succinic acid and sample 7.

5.22 The pH profiles of phosphatase activity of algal samples tested

Survey A. The algal populations present in the samples collected from three sites were Mougeotia spp., Ulothrix spp. and Stigeoclonium (Table 5.1). Samples from sites 1 and 2 always had mixed populations. In order to examine the contribution of each algal population to phosphatase activity of the samples, one population was separated from each field sample collected on 19/07 and allowed to grow in the laboratory for 7 days. It was then tested for phosphatase activity.

Mougeotia spp. Two Mougeotia populations 3.5 μm and ≤ 8 μm were present in the samples from site 1. Phosphatase activity of the samples showed the same pH profile on all occasions except the sample collected on 02/08/90. The samples showed activity in the alkaline range with peak at pH 10.0 and in a broad range with peak at pH 4.0. From sample collected on 19/07/90, Mougeotia ≤ 8 μm was separated for growth in the laboratory. It showed low enzyme activity (Fig. 5.1).

Ulothrix spp. These were present with Stigeoclonium in the samples from site 2. The pH profiles of the samples varied with the occassions. Samples collected on 28/06, 19/07 and 02/08 showed high enzyme activity in the alkaline range with pH maximum at 9.0 and 10.0, whereas samples on 05/07 and 12/07 showed lower activity in a broad range with small peak at pH 9.0. From sample collected on 19/07, Stigeoclonium was separated for growth in the laboratory. It showed high enzyme activity in the

alkaline range with peak at pH 9.0 (Fig. 5.2).

Stigeoclonium. This was present in the samples from site 3. All samples had similar pH profile and showed high enzyme activity with peak at pH 10.0. The separated Stigeoclonium from sample collected on 19/07, after growing in the laboratory, showed high enzyme activity with peak at pH 10.0 and 10.3 (Fig. 5.3). Stigeoclonium in samples from both sites 2 and 3 had hairs.

Survey B. The algal populations present in the samples from 14 sites were Stigeoclonium, Mougeotia spp., Ulothrix spp., Microspora, Zygnema and Spirogyra (Table 5.2)

Stigeoclonium. This was abundant in sample 2 and 3. In both samples, Stigeoclonium had hairs and both showed high enzyme activity. The pH optima of samples 2 and 3 was at pH 9.0 and 10.0 respectively. The pH of the water from the site where the two samples were collected was near 7.8 (Fig. 5.5).

Mougeotia spp. Three Mougeotia populations were present in the samples: 3.5 μm, ≤ 8 μm and > 8 μm. Mougeotia 3.5 μm was abundant in sample 1 which showed acid phosphatase activity with pH optima at 3.0. This sample was collected from site with pH near to 7.7. Mougeotia ≤ 8 μm which was abundant in sample 9, 10 and 13 which showed enzyme activity in the alkaline range. These samples were collected from sites with pH near to 7.5. Mougeotia > 8 μm was abundant in sample 4 which showed maximum activity in the range from 5.0 to 8.0 with a peak at pH 6.0. This sample was collected from site with pH near to 5.7 (Fig. 5.5).

Microspora. This was present in samples 6 and 7 which showed different pH profile in the two samples. The sample 6 did not show activity whereas the sample 7 showed activity in the acid range with a peak at pH 6.0. They were collected from sites with pH near to 4.0 and 5.5, respectively (Fig. 5.5).

Ulothrix spp. Two Ulothrix populations were present in the samples: 7.5 μm and 10 μm. Ulothrix 7.5 μm was present in samples 5 and 14 which showed enzyme activity in the alkaline range. The sample 14 showed low activity with two peaks at pH 8.0 and 10.3, whereas the sample 5 showed higher activity with a peak at pH 10.3. Both were collected from sites with pH near to 8.2. Ulothrix 10 μm was present in sample 12 which showed activity in a broad range with the highest peak at pH 5.0. It was collected from site with pH near to 5.2 (Fig. 5.5).

Zygnema. This was present in sample 8 which showed low activity with a peak at pH 8.0 (Fig. 5.5).

Spirogyra. This was abundant in sample 11 which showed activity in the alkaline range with a peak

at pH 9.0 (Fig. 5.5).

5.23 Algal samples tested for PDEase activity (survey A)

Algal samples collected on 19/07 were also tested for PDEase. The sample from site 1 did not show PDEase activity, whereas the samples from sites 2 and 3 showed activity in the alkaline range with two peaks at pH 7.0 and 9.0 (Fig. 5.4).

Table 5.1 Algal samples collected from 3 sites on five occasions (survey A)

Sites	Algal samples				
	28/06/90	05/07/90	12/07/90	19/07/90	02/08/90
1	Mougeotia	Mougeotia	Mougeotia	Mougeotia	Mougeotia
	a 3.5 μm	a 3.5 μm	a 3.5 μm	a 3.5 μm	a 3.5 μm
	b ≤ 8 μm	b ≤ 8 μm	b ≤ 8 μm	b ≤ 8 μm	b ≤ 8 μm
2	Stigeoclonium	Stigeoclonium	Stigeoclonium	Stigeoclonium	Stigeoclonium
	Ulothrix spp.	Ulothrix spp.	Ulothrix spp.	Ulothrix spp.	Ulothrix spp.
3	Stigeoclonium	Stigeoclonium	Stigeoclonium	Stigeoclonium	Stigeoclonium

Table 5.2 Algal samples collected from 14 sites for phosphatase activity (survey B)

Sites	Alga	cell width	percentage	other organisms
1	Mougeotia	≤ 8 μm	10%	
		3.5 μm	90%	
2	Stigeoclonium		85%	
	Ulothrix	≤ 8 μm	10%	
	Mougeotia	≤ 8 μm	5%	
3	Stigeoclonium			
4	Mougeotia	> 8 μm	90%	
		≤ 8 μm	10%	
5	Ulothrix	7.5 μm	95%	obvious bacteria
	Mougeotia	≤ 8 μm		
6	Microspora	≤ 8 μm		
7	Microspora	≤ 8 μm		
8	Zygnema	> 16 ≤ 32 μm		
9	Spirogyra	> 16 ≤ 32 μm		diatoms
	Mougeotia	≤ 8 μm	90%	
10	Spirogyra	> 16 ≤ 32 mm		
	Mougeotia	≤ 8 μm	90%	
11	Spirogyra	> 16 ≤ 32 μm		
12	Ulothrix	10 μm		
13	Mougeotia	≤ 8 μm		narrow Plectonema
14	Ulothrix	7.5 μm		

Table 5.3 Phosphatase activity obtained using different buffers at each pH unit (survey B). Detection limit < **0.03** (µmol ρNP mg d. wt^{-1} h^{-1})

		Phosphatase activity (µmol ρNP mg d. wt^{-1} h^{-1}) of samples tested (1-14)													
pH	Buffers	1	2	3	4	5	6	7	8	9	10	11	12	13	14
3	DMG-NaOH	0.076	< 0.03	0.101	< 0.03	< 0.03	< 0.03	< 0.03	< 0.03	< 0.03	< 0.03	< 0.03	0.046	< 0.03	< 0.03
	glycine-HCl	0.151	< 0.03	< 0.03	< 0.03	< 0.03	< 0.03	< 0.03	< 0.03	< 0.03	< 0.03	< 0.03	0.033	< 0.03	< 0.03
4	DMG-NaOH	0.056	< 0.03	0.206	< 0.03	< 0.03	< 0.03	0.055	< 0.03	< 0.03	< 0.03	< 0.03	0.038	< 0.03	< 0.03
	suc.acid-NaOH	0.116	0.042	0.165	0.037	0.05	0.077	< 0.03	0.053	0.038	0.062	0.053	0.085	0.078	0.051
5	DMG-NaOH	0.07	0.034	0.247	0.076	< 0.03	< 0.03	0.063	< 0.03	< 0.03	< 0.03	< 0.03	0.095	< 0.03	< 0.03
	suc.acid-NaOH	0.078	0.072	0.16	0.081	< 0.03	< 0.03	< 0.03	< 0.03	< 0.03	< 0.03	< 0.03	0.086	< 0.03	< 0.03
6	DMG-NaOH	0.038	0.065	0.136	0.176	< 0.03	< 0.03	0.075	< 0.03	< 0.03	< 0.03	< 0.03	0.097	< 0.03	< 0.03
	suc.ac-NaOH	0.037	0.042	0.148	0.149	< 0.03	< 0.03	0.053	< 0.03	< 0.03	< 0.03	< 0.03	0.103	< 0.03	< 0.03
7	DMG-NaOH	0.031	0.092	0.172	0.163	< 0.03	< 0.03	0.048	< 0.03	< 0.03	< 0.03	< 0.03	0.044	< 0.03	0.032
	HEPES-NaOH	0.044	0.105	0.127	0.08	< 0.03	< 0.03	< 0.03	< 0.03	< 0.03	< 0.03	0.037	0.062	< 0.03	< 0.03
	EPPS-NaOH	< 0.03	0.123	0.076	0.098	< 0.03	< 0.03	0.095	< 0.03	< 0.03	< 0.03	< 0.03	< 0.03	< 0.03	
8	TES-NaOH	0.03	0.19	0.255	0.124	0.072	< 0.03	0.032	0.03	< 0.03	0.047	0.065	0.079	< 0.03	0.052
	HEPES-NaOH	< 0.03	0.161	0.173	< 0.03	< 0.03	< 0.03	< 0.03	< 0.03	< 0.03	< 0.03	0.051	0.036	0.04	< 0.03
	EPPS-NaOH	< 0.03	0.167	0.169	< 0.03	0.04	< 0.03	0.077	< 0.03	< 0.03	< 0.03	< 0.03	< 0.03	0.074	
9	AMeP-NaOH	< 0.03	0.269	0.431	0.047	0.08	< 0.03	< 0.03	< 0.03	< 0.03	< 0.03	0.08	0.05	0.065	< 0.03
	glyc.-NaOH	0.05	0.354	0.506	< 0.03	0.043	< 0.03	< 0.03	< 0.03	< 0.03	< 0.03	0.061	0.045	0.144	< 0.03
	EPPS-NaOH	< 0.03	0.198	0.28	< 0.03	0.051	< 0.03	0.083	< 0.03	0.033	< 0.03	0.034	< 0.03	0.071	
10	AMeP-NaOH	< 0.03	0.147	0.059	0.035	0.077	< 0.03	< 0.03	< 0.03	< 0.03	< 0.03	0.046	0.043	< 0.03	< 0.03
	glyc.-NaOH	0.04	0.194	0.813	< 0.03	0.056	< 0.03	< 0.03	< 0.03	< 0.03	0.039	0.083	< 0.03	0.255	< 0.03
	CAPS-NaOH	< 0.03	0.13	0.071	< 0.03	0.044	< 0.03	0.107	< 0.03	0.035	< 0.03	< 0.03	< 0.03	0.12	
10.3	AMeP-NaOH	< 0.03	0.129	0.076	0.03	0.105	< 0.03	< 0.03	< 0.03	< 0.03	0.084	0.04	0.079	< 0.03	0.037
	glyc.-NaOH	< 0.03	0.067	0.572	< 0.03	0.06	< 0.03	< 0.03	< 0.03	< 0.03	< 0.03	0.059	< 0.03	0.039	< 0.03
	CAPS-NaOH	< 0.03	0.139	< 0.03	0.034	0.058	< 0.03	0.106	0.044	< 0.03	< 0.03	< 0.03	< 0.03	0.041	
11	CAPS-NaOH	< 0.03	0.054	0.059	0.044	0.075	< 0.03	< 0.03	< 0.03	< 0.03	0.057	0.037	0.073	< 0.03	< 0.03
	Na_2CO_3-$NaHCO_3$	< 0.03	0.054	< 0.03	0.033	0.039	< 0.03	< 0.03	< 0.03	< 0.03	< 0.03	0.056	0.042	< 0.03	< 0.03

Fig. 5.1 PMEase activity of samples collected from site 1 on five occasions in survey A (Table 5.1)

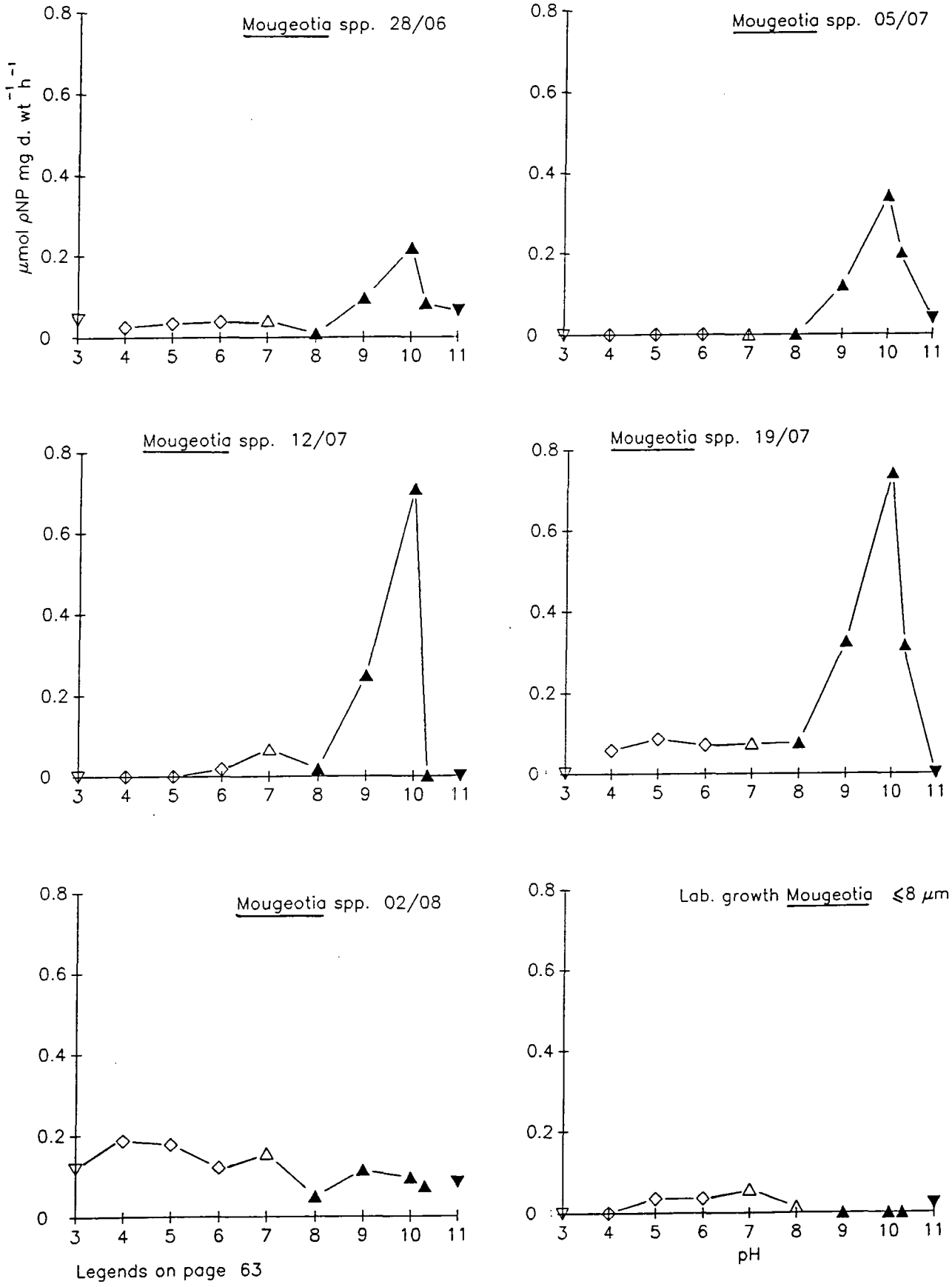


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Fig. 5.2 PMEase activity of samples collected from site 2 on five occasions in survey A (Table 5.1)

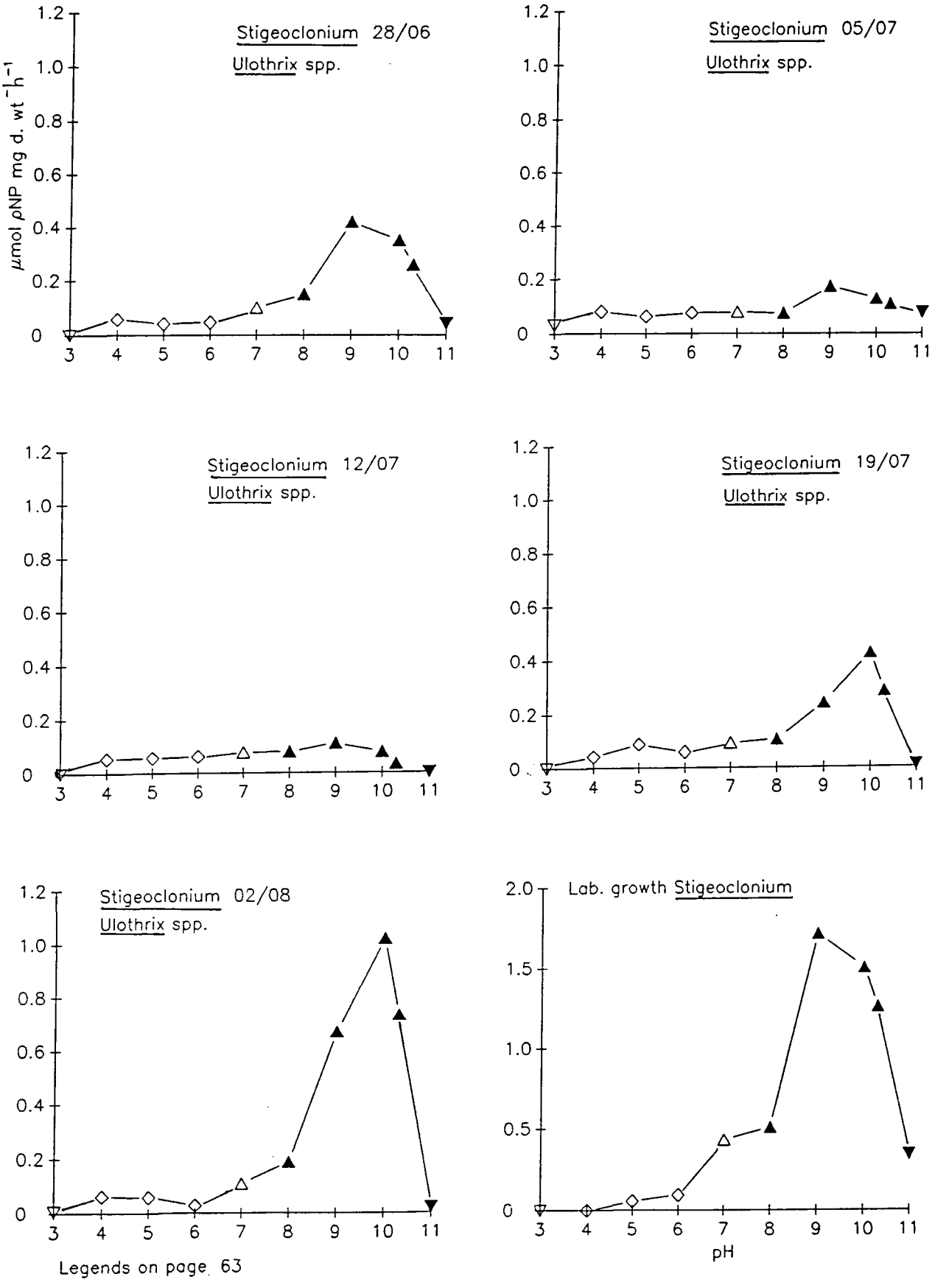

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Fig. 5.3 PMEase activity of samples collected from site 3 on five occasions in survey A (Table 5.1)

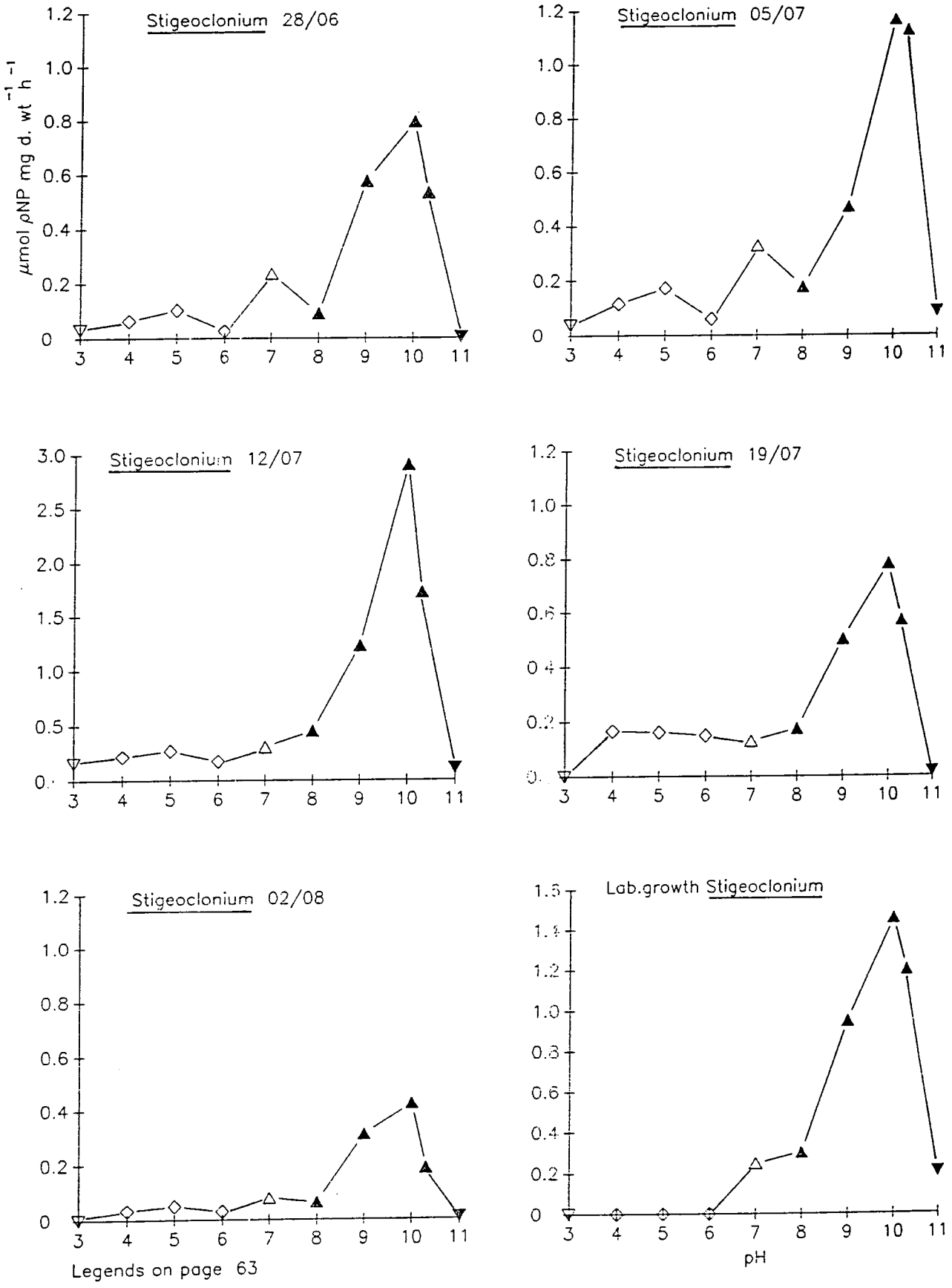

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Fig. 5.4 PDEase activity of samples collectet from 3 sites on 19/07/90 in survey A (Table 5.1)

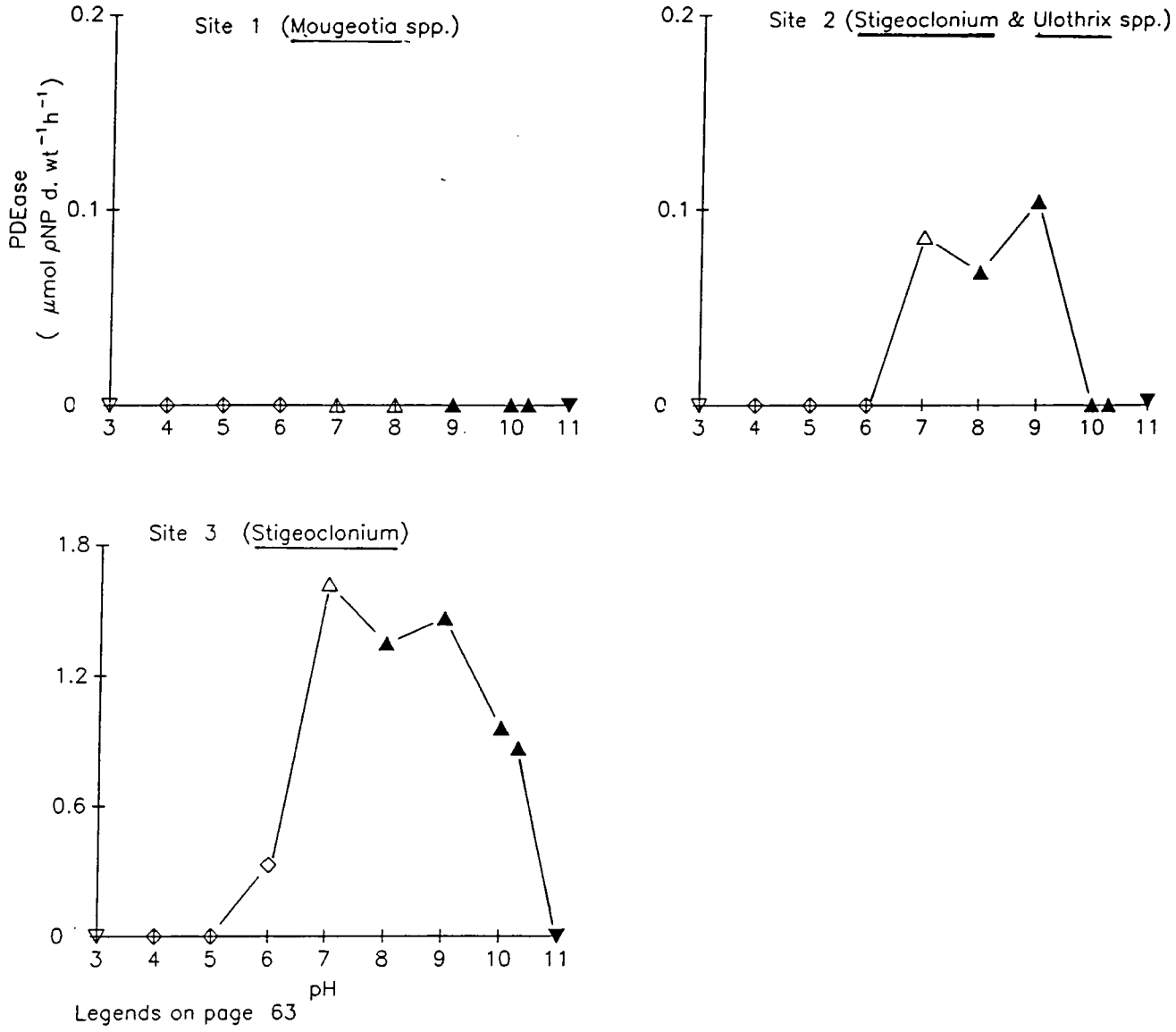

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Fig. 5.5 Effect of pH on phosphatase activity of algal samples collected from 14 sites in survey B (Table 5.2). Each sample is presented twice in two ways: a, b (5.21)

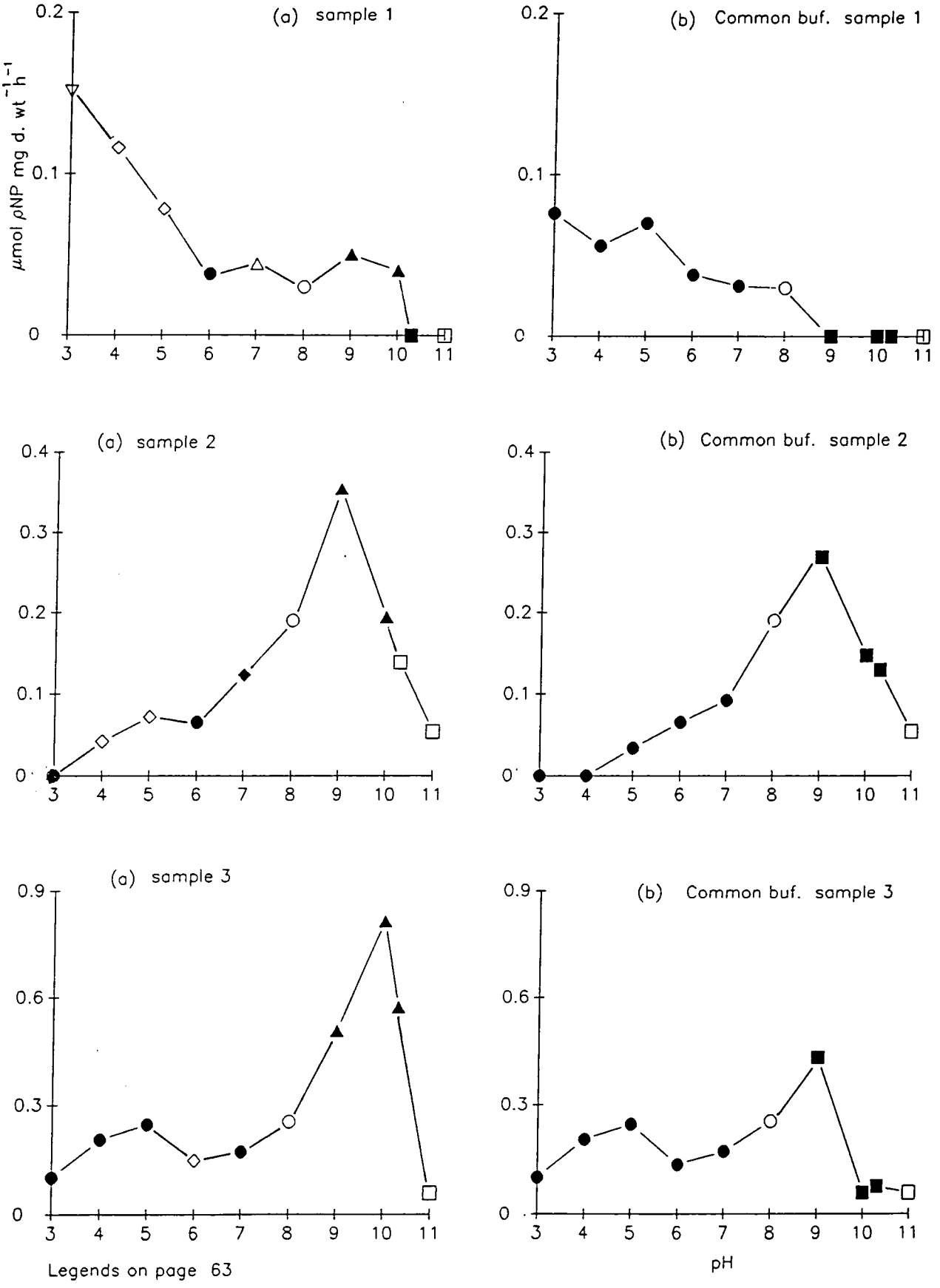

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Fig. 5.5 continued

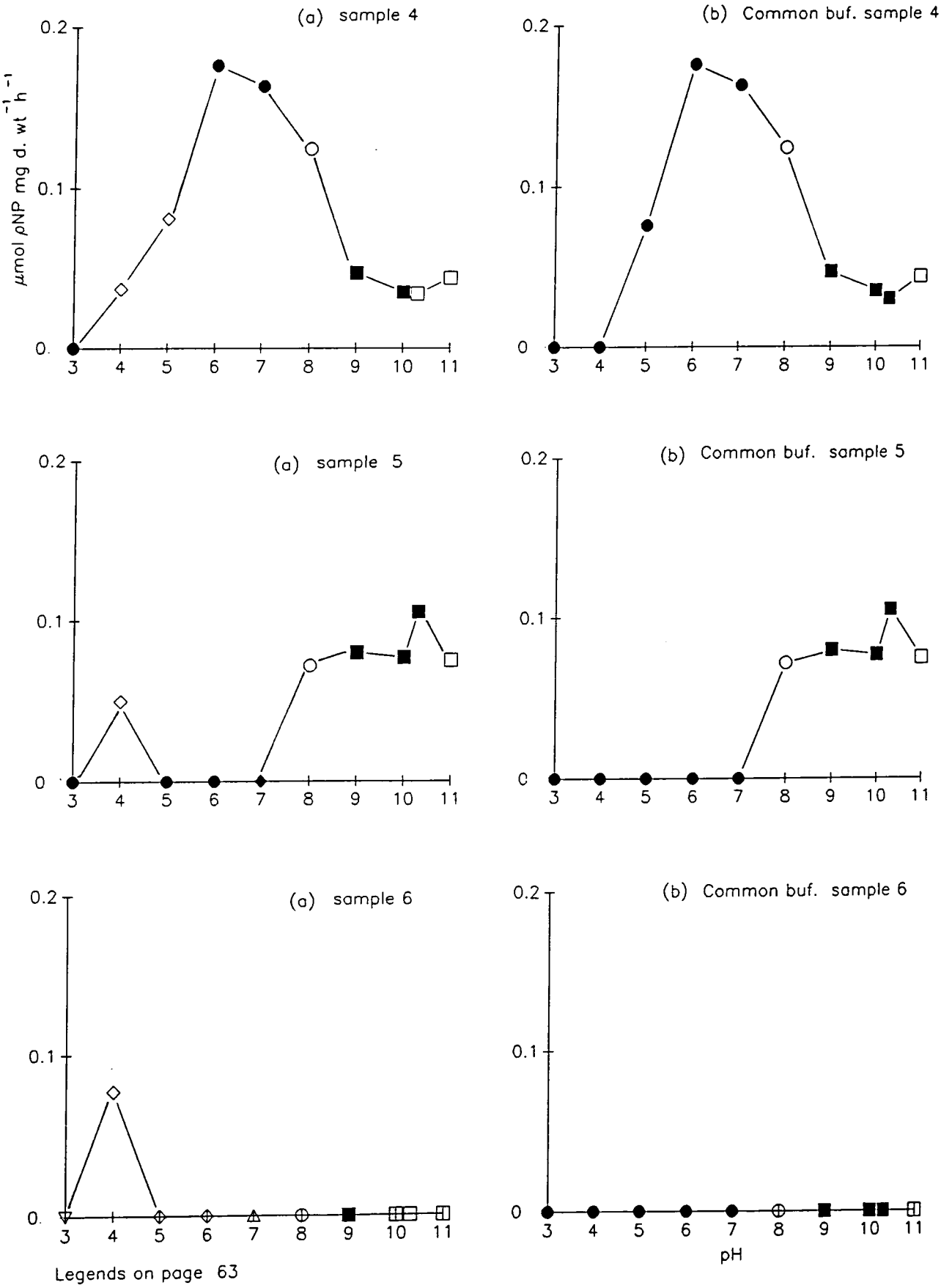

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Fig. 5.5 continued

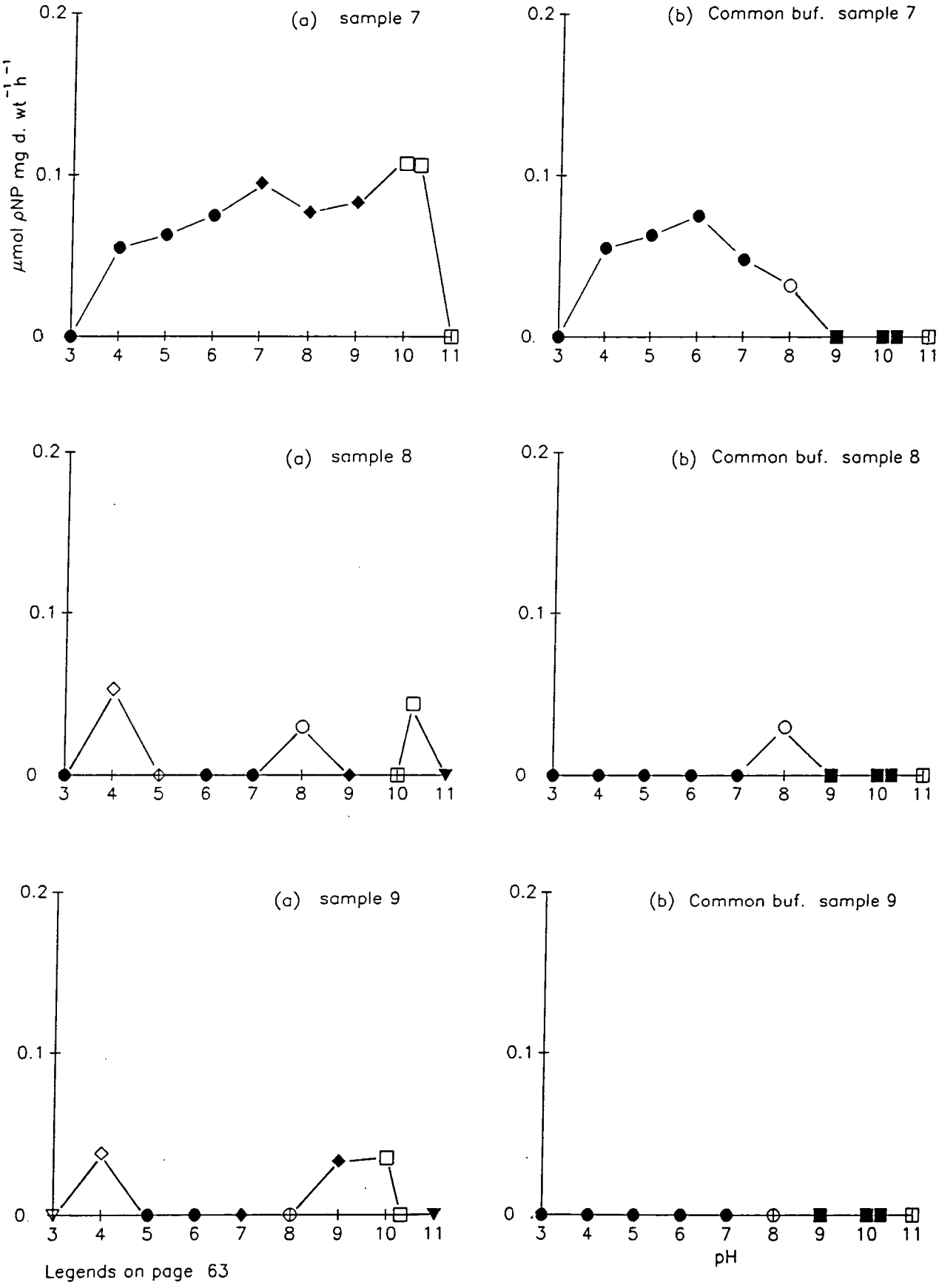

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Fig. 5.5 continued

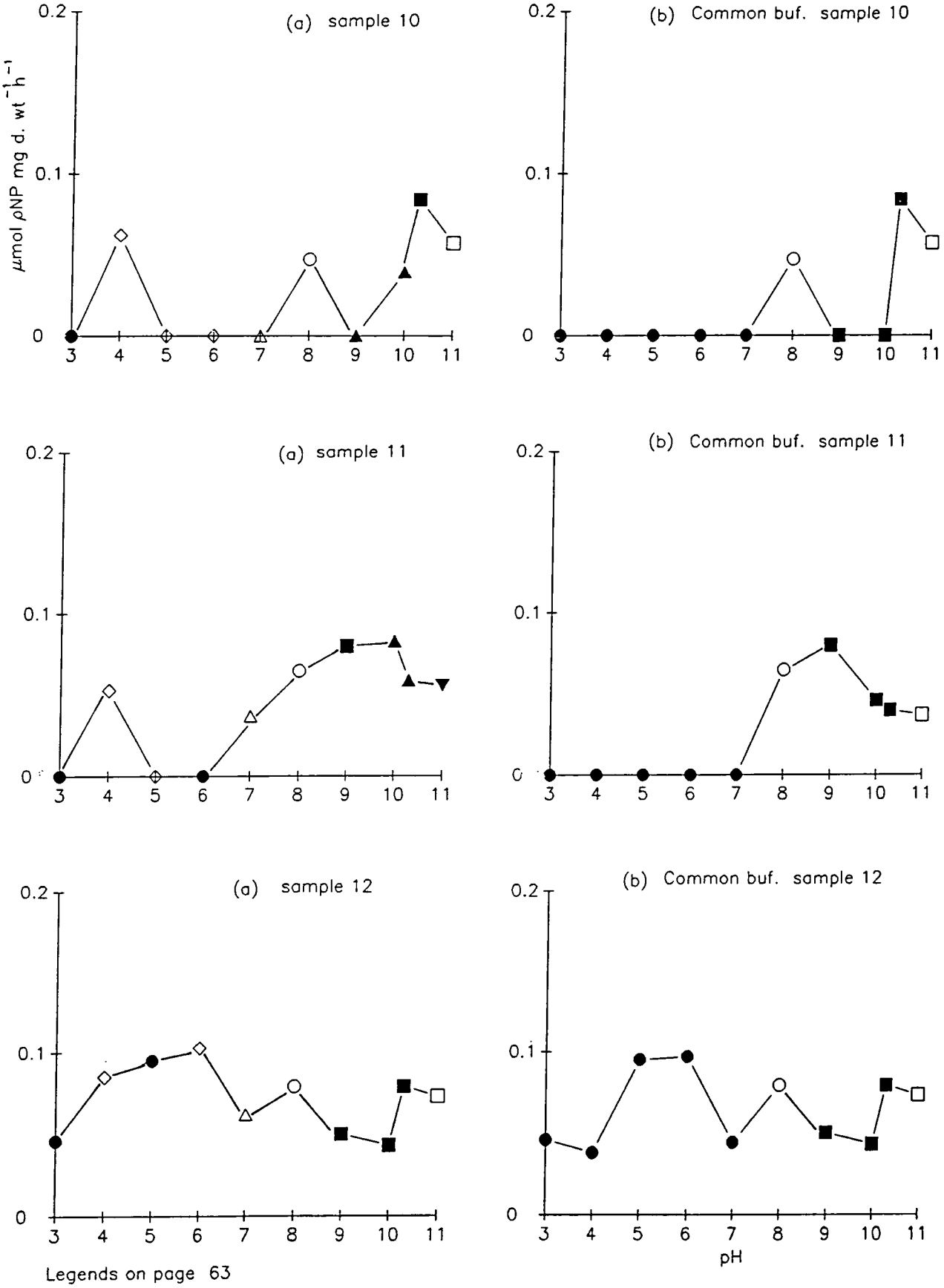

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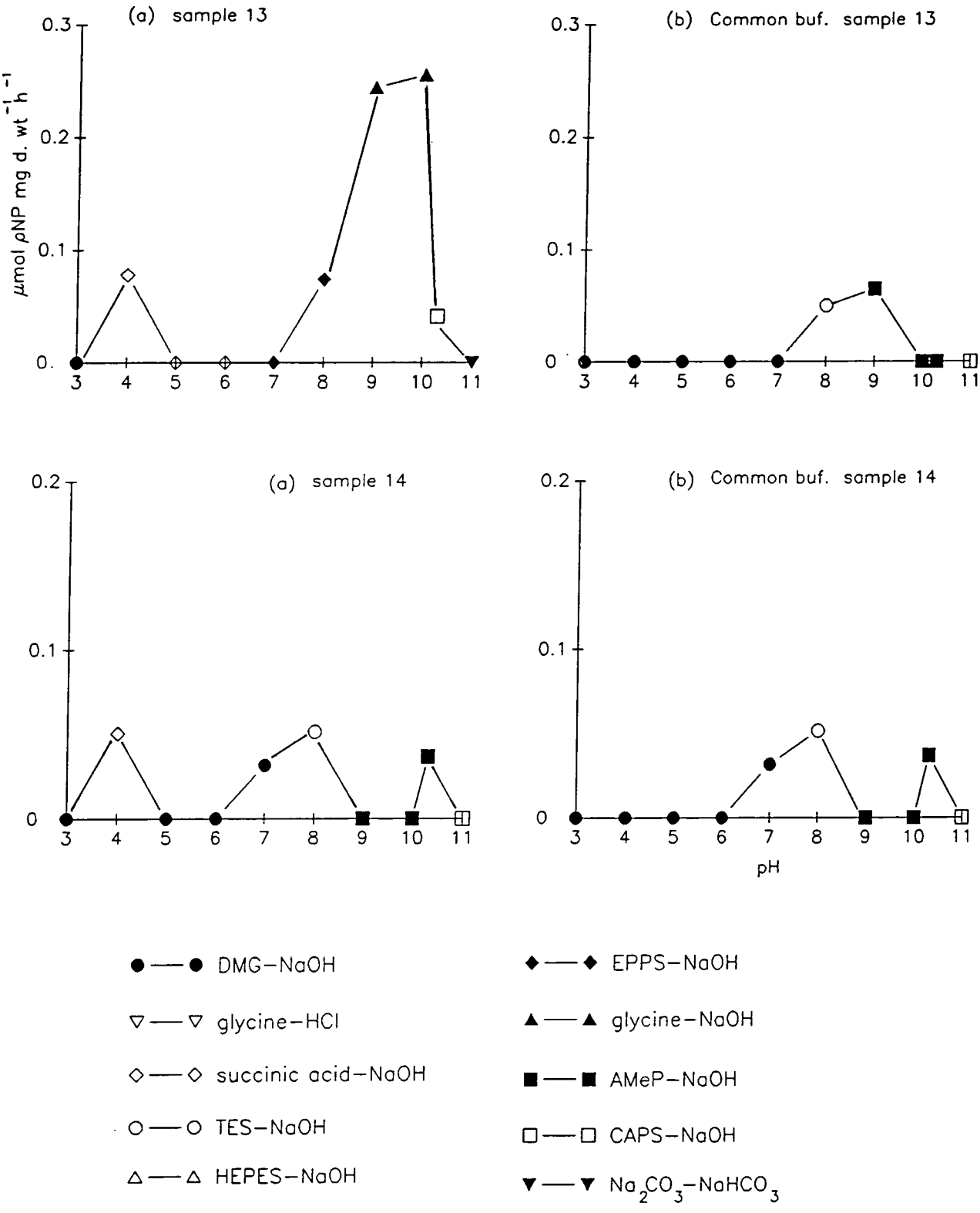
Fig. 5.5 continued



5.3 Phosphatase activity in relation to environmental variables (survey B)

The relationship between phosphatase activity of 14 samples tested in survey B and a number of environmental variables from 14 sites was examined in two ways:

(i) Phosphatase activity obtained at optimum pH for each sample was related to selected environmental variables: Ca, Zn, TFP, FRP and N : P ratio in the water (Tables 5.4, 5.6).

(ii) Phosphatase activity obtained at different pH values in the range from 7.0 to 10.3 was related to the above environmental variables (Tables 5.5, 5.6). This approach was intended to examine if there was any pH at which phosphatase activity was correlated to above variables.

In the first case, phosphatase activity was positively and negatively correlated with Zn and FRP, respectively (Table 5.6). In the second, phosphatase activity was positively correlated with Zn at ph 7.0 and negatively correlated with TFP at pH 8.0. The relationship also of phosphatase activity at optimum pH with FRP and N : P ratio is presented in Figures 5.6 and 5.7, respectively. The scattergram in Fig. 5.6 reflects the slight correlation found between phosphatase activity and FRP. In Fig. 5.7, although no significant correlation was found between phosphatase activity and N : P ratio, there are some sites where phosphatse activity seems to be related with N : P ratio. For example, sites 2, 3 and 9, 10, 13 show such relationships (5.22).

Table 5.4 Phosphatase activity (μmol ρNP mg d.wt^{-1} h^{-1}) obtained at optimum pH of the 14 samples tested (survey B). Detection limit < **0.03** (μmol ρNP mg d. wt^{-1} h^{-1})

Sample	optimum pH	Phosphatase activity
1	3.0	0.151
2	9.0	0.355
3	10.0	0.813
4	6.0	0.176
5	10.3	0.105
6	-	< 0.03
7	6.0	0.075
8	10.3	0.044
9	10.0	0.035
10	10.3	0.084
11	10.0	0.083
12	6.0	0.103
13	10.0	0.255
14	8.0	0.052

Table 5.5 Phosphatase activity obtained at different pH units in the alkalime range of the 14 samples tested (survey B). Detection limit < **0.03** (μmol ρNP mg d. wt^{-1} h^{-1})

Sample	Phosphatase activity (μmol ρNP mg d.wt^{-1} h^{-1})				
No	pH 7.0	pH 8.0	pH 9.0	pH 10.0	pH 10.3
1	0.044	0.030	0.050	0.040	< 0.030
2	0.123	0.190	0.354	0.194	0.139
3	0.172	0.255	0.506	0.813	0.572
4	0.163	0.124	0.047	0.035	0.034
5	< 0.030	0.072	0.080	0.077	0.105
6	< 0.030	< 0.030	< 0.030	< 0.030	< 0.030
7	0.048	0.032	< 0.030	< 0.030	< 0.030
8	< 0.030	0.030	< 0.030	< 0.030	0.044
9	< 0.030	< 0.030	0.033	0.035	< 0.030
10	< 0.030	0.047	< 0.030	0.039	0.084
11	0.037	0.065	0.080	0.083	0.059
12	0.062	0.079	0.050	0.043	0.079
13	< 0.030	0.074	0.244	0.255	0.041
14	0.032	0.052	< 0.030	< 0.030	0.037

Table 5.6 Spearman's rank correlation of phosphatase activity of 14 samples tested (survey B) with selected environmental variables: Ca, Zn, TFP, FRP, and N : P ratio in the water. (* = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$)

Environmental variable	Phosphatase activity (n=14) opt.pH	pH 7.0	pH 8.0	pH 9.0	pH 10.0	pH 10.3
Ca	0.410	0.098	0.140	0.389	0.384	0.304
Zn	0.685 **	0.572 *	0.516	0.505	0.440	0.106
TFP	-0.434	-0.218	-0.568 *	-0.122	-0.319	-0.482
FRP	-0.580 *	-0.294	-0.260	-0.348	-0.431	-0.091
N : P	0.450	0.221	0.200	0.429	0.507	0.120

Fig. 5.6 Scattergram showing relationship between phosphatase activity at optimum pH and FRP for 14 algal samples (Table 5.2) (n=14) from 14 sites (Table 3.1) in survey B

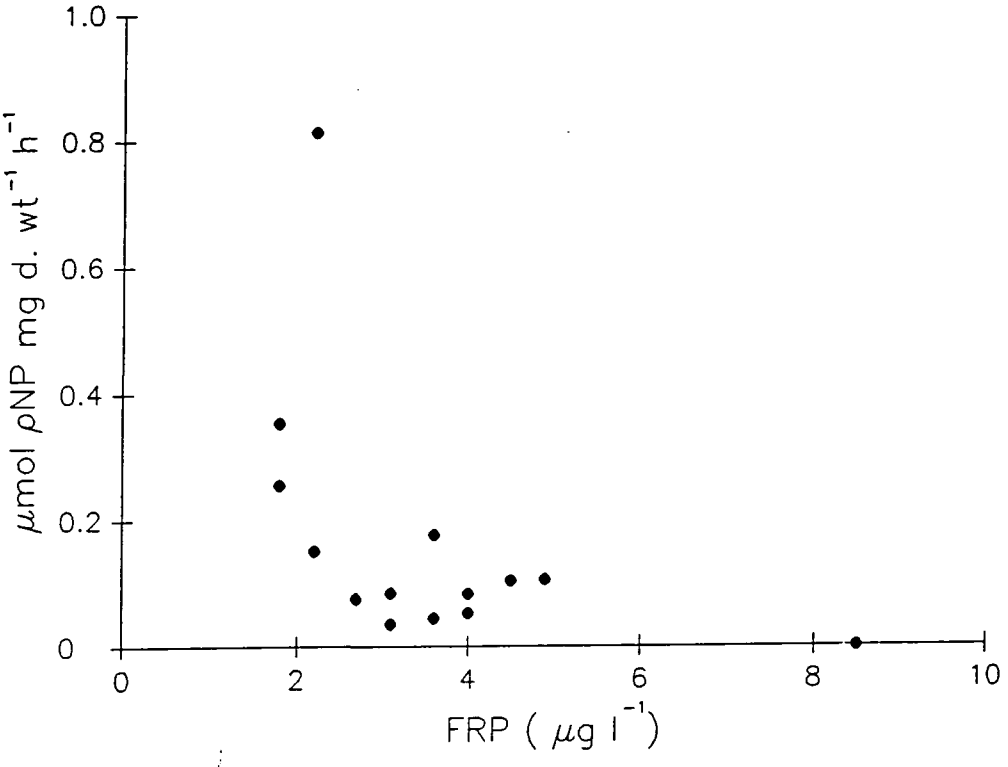
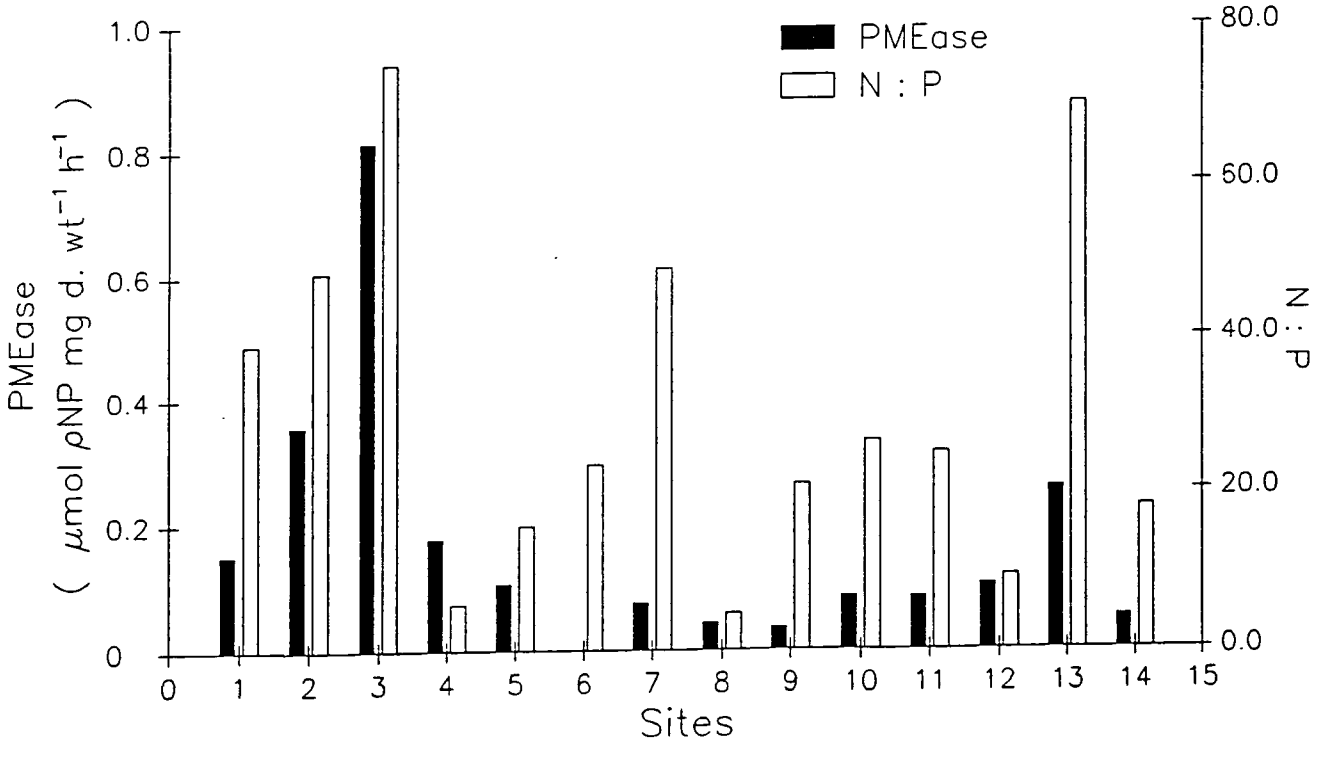


Fig. 5.7 Phosphatase activity at optimum pH and N : P ratio in the water for 14 algal samples from 14 sites in survey B



CHAPTER 6

DISCUSSION

6.1 Phosphatase activity

6.11 Effects of buffers used on phosphatase activity

The activity and stability of the enzyme can be influenced by the buffering system that is used. The buffers used in the present study, did influence the enzyme activity. Glycine and AMeP for example had a different influence on the enzyme activity. The enzyme lost activity in AMeP buffer in some samples, whereas the glycine buffer permitted activity and vice versa (5.21). Studies on the orthophosphate-repressible alkaline phosphatase in Neurospora.crassa, showed that this enzyme rapidly lost activity in an alkaline glycine buffer, and that this loss of activity was retarded by certain metals ions such as Ca^{2+}, Zn^{2+} and Mn^{2+} (Kadner & Joseph, 1969). It is probable that this influence of buffers on the enzyme activity is associated with such metals ions. The fact that the samples had mixed populations may also be associated with the different behaviour of buffers in different samples (5.21).

6.12 Phosphatase activity of algal samples tested

Phosphatase activity was detectable in all samples tested on five occasions (survey A) as well as in 13 of the 14 samples tested in survey B. Most of the samples showed activity in the alkaline range. The highest activity observed in Stigeoclonium and Mougeotia populations. It is assumed in the present study that bacterial phosphatase activity is negligible in the algal samples tested.

Mixed populations were present in the samples. It is expected that the enzyme activity obtained in samples with mixed populations is associated with the populations present in abundance. This abundance was estimated roughly as a percentage after examining each sample under the microscope. It is likely however, that the less abundant population in each sample may contribute to enzyme activity. As discussed below, phosphatase activity observed in samples collected in survey A may be associated with the mixture of populations.

Samples from site 1 showed phosphatase activity in the alkaline range on the four occasions but low acid activity on the fifth (Fig. 5.1). In the first two occasions the activity was lower than in the other two.

The two different populations of Mougeotia in this site are suspected for these variations in the enzyme activity. It is likely that the activity in the alkaline range is associated with the Mougeotia ≤ 8 μm, whereas the activity in the acid range with the narrow Mougeotia 3.5 μm The separated Mougeotia ≤ 8 μm for laboratory growth showed very low activity which is very difficult to be compared with the other samples. The fact that acid activity observed in sample 1 in survey B (Fig. 5.5) with Mougeotia 3.5 μm in abundance, suggests that the narrow Mougeotia is associated with the acid activity. Samples from site 2 showed activity in the alkaline range on all occasions (Fig. 5.2). A mixture of Stigeoclonium and Ulothrix spp. populations was present in these samples (5.3). It is likely that the low enzyme activity observed in samples collected on 05/07 and 12/07 is associated with the abundance of the two populations in the samples. The high activity of the separated Stigeoclonium suggests that Stigeoclonium was in abundance in samples with higher activity. Based on this interpretation, the low activity observed in the two samples is due to the presence of Ulothrix spp. as the abundant population. However, despite the indication of the separated sample, it is difficult to evaluate the contribution of Ulothrix spp. in the enzyme activity of each sample. Samples collected from site 3 with Stigeoclonium present as single population, showed high activity in the alkaline range (Fig. 5.3). The enzyme activity varied with the occasions. Both Stigeoclonium from sites 2 and 3 had hairs.

In survey B, Mougeotia spp. and Ulothrix spp. were the most widespread in the samples (Table 5.2). The samples showed differences in pH profiles and in phosphatase activity (5.22, Fig. 5.5). Nine samples had pH optima in the alkaline range, 3 samples in the neutral and 1 sample in the acid range. The highest enzyme activity was observed in samples 2, 3 which had Stigeoclonium populations in abundance. In both samples Stigeoclonium had hairs.

The response of phosphatase activity to pH varied with the samples and the sites from where they were collected. In survey A, all samples except one (sample from site 1) showed similar pH profiles but different phosphatase activity on the occusions of sampling. It is probable that the above differences may be associated to both phosphate concentrations in the water and mixed populations in the samples. It is difficult in this case to attribute these differences to one of the above reasons since phosphate analysis was incomplete and therefore no relationships can be made between phosphatase activity and phosphates. In survey B, there were differences in pH profiles and phosphatase activity in the 14 samples. Samples which had the same algal population in abundance and were collected from sites with similar pH, had

similar pH profiles but exhibited different phosphatase activity. For example, samples 2, 3; 9, 10, 13; 5, 14 showed different activity in the alkaline range. It seems reasonable to conclude here that the above differences in the samples collected in survey B are associated with both: primarily, with the different water chemistries in the sites and secondary, with the mixed and different populations in the samples. The former fits with the correlations found betwwen phosphatase activity and a number of environmental variables (6.2) The later is confirmed by the examination of mixed populations in survey A.

Phosphatase activity is widespread in these high-zinc streams. The results indicate that algal populations growing in this environment synthesize phosphatases as a response to environmental conditions. Differences in phosphatases activity in the samples tested is more likely to be associated with environmental conditions in the sites and the different populations in the samples.

6.2 Phosphatase activity in relation to environmental chemistry

Environmental data were obtained at the same time as the algal samples, so relationships might be obscured if the organisms grew in waters subject to marked physical and chemical changes; overland flow streams can encounter very great differences in flow and hence also the concentrations of many chemicals variables. However all the samples were taken after periods of relatively constant stream flow, so the values for environmental variables are probably a reasonable indication of the environment in which the algae had been growing.

The relationship between phosphatase activity and selected environmental variables showed that phosphatase activity at optimum pH and pH 8.0 was negatively correlated with FRP and TFP, respectively (5.3, Tables 5.4, 5.5 and 5.6). No correlation found with N : P ratio in the water. The N : P ratio is widely used in freshwater systems as an indicator of nutrient limitation. Chiaudani and Vighi (1974) concluded that phosphorus was always limiting when the ratio inorganic N to inorganic P was higher than 10, using Selenastrum as test organism. The N : P ratio in the water of the sites sampled, was well above 10 in 12 of the 14 sites. This indicates that most of the sites were phosphorus limiting. The different and mixed populations of algae present in the samples may be associated with the fact that no correlation was found between the phosphatase activity and N : P ratio in the water. This is because different algae species may respond differently to P-deficiency. For example in Fig. 5.7, the phosphatase activity of the two Stigeoclonium populations (sites 2, 3) as well as the Mougeotia ≤ 8 μm populations (sites 9, 10 & 13)

seems to be related to N : P ratio. The slight correlation found between phosphatase activity and FRP may be due to the different algal populations present in the samples. As Fitzgerald and Nelson (1966) pointed out, there are differences in alkaline phosphatase production between algae species. It is likely also that the small amount of data may be responsible for these relationships.

The present results suggest that the algal populations tested for phosphatase activity had been growing under P-limited conditions. This fits with the negative correlation found between phosphatase activity and FRP as well as with the high N : P ratio in the water. For example, the presence of hairs found in the two Stigeoclonium populations is indication that these organisms had been growing under P-limited conditions. Gibson and Whitton (1987) showed that presence of hairs in Stigeoclonium populations was strongly negatively correlated with environmental P and positively with phosphatase activity.

Algal populations growing in these high-zinc and P-limlited streams exhibit phosphatase activity in order to meet the phosphorus requirement for their growth. Alkaline phosphatase synthesis in these streams results in the regeneration of orthophosphates and their production is determined mainly by the concentrations of orthophosphates at each time (1.556). It is reasonable to conclude that in sites with relatively high P concentrations, phosphatase activity is low. It seems ecologically probable that species able to produce large amounts of phosphatases would have an advantage when orthophosphate is in short supply.

The present data do indicate that alkaline phosphatase activity in thsese streams is related to the availability of orthophosphates. However more data would be required to show such relationships between phosphatases and environmental variables.

6.3 Phosphatase activity in relation to zinc concentrations

The sites visited in this study varied from low to high-zinc streams. Algal populations tested are widespread and often abundant in these waters. Zinc is an important micro-nutrient for growth and metabolism of algae (1.3). Alkaline phosphatases have been characterized as a metallo-enzymes with an essential metal ion, which has been reported to be zinc in many cases (Spiro, 1973; McComb et al., 1979). The metal is essential for catalytic activity and possibly also for maintenance of native enzyme structure. The zinc requirement for enzymatic activity was demonstrated by the inhibition of the enzyme with metal

binding agents in accord with the order of the stability constants of the zinc complexes. For example, EDTA inactivates the enzyme by removing zinc from the enzyme, but complete activity is restored by adding zinc. Workers have showed that Zn as well as Co and Cd induce binding of phosphate to alkaline phosphatase (Applebury et al., 1970). Studies on metal effect on phosphatase activity showed that zinc was inhibitory at high concetrations (Grainger et al., 1989). Few details are available on the kinetics of Zn inhibition, which is surprising in view of the absolute requirement for this ion. In the present study, phosphatase activity of the 14 samples tested (survey B) at optimum pH and pH 7.0 was positively correlated with zinc concentrations in the water (5.3, Tables 5.4, 5.5 and 5.6).

It is possible also that high zinc concentration can have an indirect effect on phosphatase activity. Phosphate is extremely reactive and interacts with many cations (e.g. Fe, Ca, Zn) to form relatively insoluble compounds that precipitate out of the water. It is probable therefore, that availability of inorganic-P in these high-zinc streams may be influenced by the high zinc concentrations. It thus seems likely that the low concentrations of inorganic-P recorded in these streams may be associated with the high zinc concentrations. Under these conditions phosphatase activity is associated with the low inorganic-P concentrations. The significant correlation found between phosphatase activity at pH optimum and Zn as well as FRP, suggests that zinc concentrations may play an important role in phosphatase activity in these high-zinc environments. Further studies would be required to demonstrate the way of zinc influence on phosphatase activity.

6.4 Organic phosphorus and phosphatase activity

"Filtrable organic" P concentrations were low and in the majority of the samples were lower than FRP. The level of phosphate in these sites is associated with the geology of the areas of sites as well as the rate of precipitation. The streams visited are waters draining from fells, pastures and moorland (overland or ground water flow). Phosphate concentration in these streams and particularly in peat drainage streams are influenced by climatic conditions. For example, temperature and precipitation can result in releasing P from peat which moves through the drainage system to the streams. Although most of the sites visited were not peat drainage, it seems probable that the low organic phosphate concentrations recorded in this study, are related with the low precipitation occured during summer (mainly May-June). It is expected that the concentrations of organic phosphorus in the majority of these

streams is much higher during the spring. Livingstone and Whitton (1984) in their study on streams in Upper Teesdale recorded very high concentrations of "filtrable organic" phosphates in the spring, whereas both organic and reactive phosphate were near or below detection limits for the other months.

Under P-deficiency conditions, phosphatase are produced in order to release orthophosphate from organic P substrates. Such substrates can be either as dissolved or particulate form (1.422). Although the exact chemical nature of "filtrable organic" P in the present study is uncertain, it seems probable that most of it is truly associated with organic molecules. DOP consists of sensitive and insensitive compounds for alkaline phosphatases. Hino (1989) showed that some of the DOP in natural water is colloidal and amorphous P compounds unavailable form to the organisms. Studies on lake water have shown that phosphomonoesters exist in very low concentrations and thus are a minor part of the soluble organic P (Berman, 1970). Phosphomonoesters is the most readily hydrolised form of dissolved organic P. Limnological phosphatase studies have demonstrated in situ hydrolysis of phosphomonoesters. Heath and Cooke (1975) observed that the high concentrations of phosphatase in East Twin Lake during parts of the year (60 μg esterified P l^{-1}) were almost entirely depleted in less than a week simultaneously with an increase in phosphatase activity.

Far fewer studies have been made on phosphatases and organic P compounds in streams waters. These aquatic environments are characterized by very low PO_4-P and somewhat higher dissolved organic P concentrations. It is probable that under these conditions, algal populations growing in these streams utilize these organic P substrates by producing phosphatases in order to meet their P requirements since the available inorganic P is not enough. The ability to use these organic P substrates would impose a real advantage to an organism at times of phosphate limitation.

In the present study, it seems unfruitful to try to find any relation between phosphatase activity and "filtrable organic" P. Firstly, phosphatase activity was relatively low in the majority of the samples, secondly, FRP was not very low and thirdly, "filtrable organic" P was low and even lower than FRP in the majority of the samples. It is probable, that phosphatase activity of algal populations tested is associated with the regeneration of phosphate within the organisms living in these P-limited environments.

SUMMARY

1. A study was carried out to examine the algal phosphatase activity in high-zinc streams in the Northern Pennine Orefield. These zinc-rich waters originate from both surface and ground sources. The most important sources of high zinc concentrations were shown to be continued discharge of mine waters from adits and drainage from exposed heaps of tailings. Algal populations are abundant in these streams. Mougeotia (spp). was the most widespread genus in the samples, but not always present.

2. The sampling was carried out in two surveys (A, B). Details of these two surveys are given which were designed to provide information on the chemistry of the streams studied and to examine the presence of phosphatase activity and its relation to water chemistry in these high-zinc streams. In survey A, water and algal samples were collected from 3 sites on five occasions. This study was intended to examine: (i) the response of phosphatase activity to pH throughout the five occasions of sampling, (ii) the presence of mixed populations in the samples and (iii) phosphodiesterase (PDEase) activity apart from phosphomonoesterase (PMEase). In survey B, water and algal samples were collected from 14 sites once. This study was intended to examine: (i) the response of phosphatase activity to pH in these sites with the different water chemistries, (ii) the relationship between phosphatase activity and a number of environmental variables, and (iii) the influence of buffering system on phosphatase activity. The samples in this survey were tested only for PMEase activity. In both surveys phosphatase activity was tested over a broad pH range (3.0 - 11.0) using at least two different buffers at each pH value.

3. Survey A: The three streams sampled were zinc-rich mine drainage streams. They contained high concentration of zinc (2.6 - 8.7 mg l^{-1}) and high concentration of calcium (74 - 104.5 mg l^{-1}). Nitrate-N concentrations were relatively high (85 - 222 µg l^{-1}). Nitrite-N and ammonia concentrations were below the detection limit (Detection limit < 5 µg l^{-1} N). Phosphate analysis in this survey was incomplete.

The algal populations present in the samples from these 3 sites were Mougeotia spp., Stigeoclonium and Ulothrix spp.. Samples from sites 1 and 2 always had mixed populations. All samples except one

showed similar pH profiles but differences in phosphatase activity throughout the occasions of sampling. For the examination of mixed populations, one algal population was separated from each sample in one occasion and was allowed to grow in the laboratory, and then was tested for phosphatase activity. The results showed that these mixtures were likely to be associated with the above differences in phosphatase activity. Algal samples in one occusion were also tested for PDEase activity. Two of the 3 samples exhibited PDEase activity.

4. Survey B: Zinc concentrations in these streams ranged from 0.1 mg l^{-1} to 19.4 mg l^{-1} with 7 of the 14 streams above 1 mg l^{-1}. Calcium concentrations ranged from 2 mg l^{-1} to 98.6 mg l^{-1}. Nitrate-N concentrations ranged from 5 µg l^{-1} to 199 µg l^{-1}. Nitrite-N concentrations were below the detection in all sites and ammonia-N concentrations were also below the detection limit in the majority of samples (Detection limit < 5 µg l^{-1} N). Phosphate concentration were low in the majority of the samples. TFP concentrations ranged from 2.2 µg l^{-1} to 22.8 µg l^{-1} with 13 of the 14 samples below 7.7 µg l^{-1} TFP. FRP concentrations ranged from 1.8 µg l^{-1} to 8.5 µg l^{-1} with 13 of the 14 samples below 4.9 µg l^{-1} FRP.

The algal populations present in the samples from the 14 sites were Microspora, Mougeotia spp., Spirogyra, Stigeoclonium, Ulothrix spp. and Zygnema. Some samples did have mixed populations. Thirteen of the 14 samples exhibited phosphatase activity. The samples showed differences in pH profiles and in phosphatase activity. Most of the samples exhibited enzyme activity in the alkaline range.

Possible relationships between phosphatase activity and environmental variables were examined. Phosphatase activity was significantly correlated with Zn, TFP and FRP in the water. In particular, phosphatase activity at optimum pH for each sample was positively correlated with Zn and negatively with FRP. Phosphatase activity at pH 7.0 for each sample was positively correlated with Zn and at pH 8.0 was negatively correlated with TFP.

The examination of the influence of buffering on phosphatase activity involved the use of a third buffer in the range from pH 7.0 to 10.3. Some buffers appeared to have marked influence on phosphatase activity. In both surveys values obtained with buffers which led to higher activity were taken into account.

5. Phosphatase activity is widespread in these high-zinc streams. High phosphatase activity was observed in samples with Stigeoclonium and Mougeotia populations. Most of the samples showed phosphatase activity in the alkaline range. Differences in phosphatase activity in the samples tested are more likely to be associated with the different water chemistries at the sites and the different populations in the samples. The results suggest that these algal populations tested had been growing under P-limited conditions.

6. The possibility that algal populations in these high-zinc streams can utilize organic phosphates is discussed.

Appendix 1: Phosphate calibration sheet. The determination of **FRP** by employing the phosphate additions procedure. Ten known P concentrations were added to each sample

Standards :

P conc. [µg/l]	Abs 882 nm [units]
100	0.221
90	0.202
80	0.178
70	0.154
60	0.133
50	0.111
40	0.086
30	0.066
20	0.042
10	0.021
0	0

Regression Output:

Constant		0
Std Err of Y Est		0.001505
R Squared		0.999588
No. of Observations		11
Degrees of Freedom		10
X Coefficient(s)	0.002216	
Std Err of Coef.	7.67E-06	

Sample No

1

Added P	Abs 882nm	P conc.
100	0.232	104.6931
90	0.211	95.21661
80	0.19	85.74007
70	0.164	74.00722
60	0.141	63.62816
50	0.115	51.89531
40	0.092	41.51625
30	0.07	31.58845
20	0.047	21.20939
10	0.025	11.28159
0	0.005	2.256318

2

Abs 882nm	P conc.
0.227	102.4368
0.202	91.15523
0.184	83.03249
0.164	74.00722
0.139	62.72563
0.115	51.89531
0.092	41.51625
0.068	30.68592
0.046	20.75812
0.027	12.18412
0.004	1.805054

3

Abs 882nm	P conc.
0.233	105.1444
0.206	92.96029
0.182	82.12996
0.16	72.20217
0.148	66.787
0.117	52.79783
0.125	56.40794
0.071	32.03971
0.05	22.56318
0.033	14.8917
0.005	2.256318

4

Added P	Abs 882nm	P conc.
100	0.225	101.5343
90	0.202	91.15523
80	0.182	82.12996
70	0.161	72.65343
60	0.138	62.27437
50	0.117	52.79783
40	0.094	42.41877
30	0.071	32.03971
20	0.049	22.11191
10	0.027	12.18412
0	0.008	3.610108

5

Abs 882nm	P conc.
0.225	101.5343
0.207	93.41155
0.18	81.22744
0.173	78.06859
0.148	66.787
0.121	54.60289
0.1	45.12635
0.078	35.19856
0.055	24.81949
0.042	18.95307
0.011	4.963899

6

Abs 882nm	P conc.
0.236	106.4982
0.219	98.82671
0.197	88.89892
0.176	79.42238
0.156	70.39711
0.131	59.11552
0.11	49.63899
0.089	40.16245
0.068	30.68592
0.047	21.20939
0.019	8.574007

Appendix 1 continued

Samples No

7

Added P	Abs 882nm	P conc.
100	0.234	105.5957
90	0.206	92.96029
80	0.191	86.19134
70	0.168	75.81227
60	0.141	63.62816
50	0.122	55.05415
40	0.098	44.22383
30	0.073	32.94224
20	0.05	22.56318
10	0.027	12.18412
0	0.006	2.707581

8

Abs 882nm	P conc.
0.225	101.5343
0.202	91.15523
0.184	83.03249
0.16	72.20217
0.139	62.72563
0.119	53.70036
0.094	42.41877
0.075	33.84477
0.051	23.01444
0.029	13.08664
0.008	3.610108

9

Abs 882nm	P conc.
0.225	101.5343
0.202	91.15523
0.183	82.58123
0.161	72.65343
0.138	62.27437
0.12	54.15162
0.095	42.87004
0.072	32.49097
0.051	23.01444
0.028	12.63538
0.007	3.158845

10

Added P	Abs 882nm	P conc.
100	0.227	102.4368
90	0.204	92.05776
80	0.183	82.58123
70	0.16	72.20217
60	0.139	62.72563
50	0.115	51.89531
40	0.094	42.41877
30	0.071	32.03971
20	0.051	23.01444
10	0.028	12.63538
0	0.007	3.158845

11

Abs 882nm	P conc.
0.227	102.4368
0.204	92.05776
0.183	82.58123
0.163	73.55596
0.141	63.62816
0.119	53.70036
0.098	44.22383
0.076	34.29603
0.053	23.91697
0.032	14.44043
0.009	4.061372

12

Abs 882nm	P conc.
0.228	102.8881
0.208	93.86282
0.186	83.93502
0.164	74.00722
0.142	64.07942
0.12	54.15162
0.099	44.67509
0.076	34.29603
0.055	24.81949
0.032	14.44043
0.01	4.512635

13

Added P	Abs 882nm	P conc.
100	0.224	101.083
90	0.202	91.15523
80	0.18	81.22744
70	0.157	70.84838
60	0.137	61.8231
50	0.113	50.99278
40	0.092	41.51625
30	0.069	31.13718
20	0.046	20.75812
10	0.025	11.28159
0	0.004	1.805054

14

Abs 882nm	P conc.
0.26	117.3285
0.227	102.4368
0.204	92.05776
0.177	79.87365
0.152	68.59206
0.123	55.50542
0.1	45.12635
0.08	36.10108
0.057	25.72202
0.034	15.34296
0.009	4.061372

Appendix 2: Phosphate calibration sheet. Determination of **TFP** by employing the phosphate additions procedure. Ten known P concentrations were added to each sample

Standards :

P conc. [µg/l]	Abs 882 nm [units]
100	0.194
90	0.173
80	0.154
70	0.136
60	0.115
50	0.096
40	0.077
30	0.056
20	0.037
10	0.019
0	0

Regression Output:

Constant	0
Std Err of Y Est	0.000968
R Squared	0.999774
No. of Observations	11
Degrees of Freedom	10
X Coefficient(s)	0.001927
Std Err of Coef.	4.93E-06

Sample No

1

Added P	Abs 882nm	P conc.
100	0.262	135.9434
90	0.212	110
80	0.189	98.06604
70	0.166	86.13208
60	0.147	76.27358
50	0.116	60.18868
40	0.095	49.29245
30	0.074	38.39623
20	0.053	27.5
10	0.034	17.64151
0	0.015	7.783019

2

Abs 882nm	P conc.
0.194	100.6604
0.18	93.39623
0.158	81.98113
0.139	72.12264
0.123	63.82075
0.104	53.96226
0.084	43.58491
0.065	33.72642
0.047	24.38679
0.028	14.5283
0.009	4.669811

3

Abs 882nm	P conc.
0.194	100.6604
0.173	89.76415
0.153	79.38679
0.135	70.04717
0.114	59.15094
0.096	49.81132
0.076	39.43396
0.057	29.57547
0.038	19.71698
0.019	9.858491
0.002	1.037736

4

Added P	Abs 882nm	P conc.
100	0.202	104.8113
90	0.214	111.0377
80	0.2	103.7736
70	0.174	90.28302
60	0.15	77.83019
50	0.131	67.9717
40	0.09	46.69811
30	0.066	34.24528
20	0.046	23.86792
10	0.026	13.49057
0	0.007	3.632075

5

Abs 882nm	P conc.
0	0
0	0
0.166	86.13208
0	0
0.126	65.37736
0	0
0.093	48.25472
0.075	38.91509
0.055	28.53774
0.036	18.67925
0.014	7.264151

6

Abs 882nm	P conc.
0	0
0	0
0	0
0	0
0.156	80.9434
0	0
0	0
0	0
0.083	43.06604
0	0
0.044	22.83019

Appendix 2 continued

Sample No

7

Added P	Abs 882nm	P conc.
100	0.204	105.8491
90	0.182	94.43396
80	0.162	84.0566
70	0.144	74.71698
60	0.123	63.82075
50	0.108	56.03774
40	0.085	44.10377
30	0.066	34.24528
20	0.046	23.86792
10	0.027	14.00943
0	0.013	6.745283

8

Abs 882nm	P conc.
0.203	105.3302
0.179	92.87736
0.16	83.01887
0.14	72.64151
0.122	63.30189
0.104	53.96226
0.084	43.58491
0.065	33.72642
0.045	23.34906
0.025	12.9717
0.008	4.150943

9

Abs 882nm	P conc.
0.2	103.7736
0.182	94.43396
0.163	84.57547
0.144	74.71698
0.124	64.33962
0.106	55
0.087	45.14151
0.068	35.28302
0.048	24.90566
0.029	15.04717
0.01	5.188679

10

Added P	Abs 882nm	P conc.
100	0.191	99.10377
90	0.174	90.28302
80	0	0
70	0.136	70.56604
60	0.116	60.18868
50	0.096	49.81132
40	0.076	39.43396
30	0.056	29.0566
20	0.037	19.19811
10	0.019	9.858491
0	0	0

11

Abs 882nm	P conc.
0.204	105.8491
0.183	94.95283
0.166	86.13208
0.148	76.79245
0.128	66.41509
0.107	55.51887
0.088	45.66038
0.068	35.28302
0.049	25.42453
0.031	16.08491
0.01	5.188679

12

Abs 882nm	P conc.
0.198	102.7358
0.181	93.91509
0.161	83.53774
0.143	74.19811
0.125	64.85849
0.106	55
0.086	44.62264
0.067	34.76415
0.047	24.38679
0.028	14.5283
0.008	4.150943

13

Added P	Abs 882nm	P conc.
100	0.198	102.7358
90	0.181	93.91509
80	0.161	83.53774
70	0.143	74.19811
60	0.123	63.82075
50	0.104	53.96226
40	0.086	44.62264
30	0.064	33.20755
20	0.044	22.83019
10	0.024	12.45283
0	0.006	3.113208

14

Abs 882nm	P conc.
0.195	101.1792
0.176	91.32075
0.153	79.38679
0.137	71.08491
0.117	60.70755
0.099	51.36792
0.082	42.54717
0.062	32.16981
0.042	21.79245
0.022	11.41509
0.003	1.556604

Appendix 3 : APA calibration curve of ρNP concentration versus absorbance at 405 nm, with eight well-replicates per concentration

ρNP concentration (mM)	absorbance at 405 nm ± sem
0.050	0.739 ± 0.0030
0.040	0.598 ± 0.0025
0.030	0.460 ± 0.0026
0.020	0.300 ± 0.0015
0.010	0.138 ± 0.0018
0.005	0.077 ± 0.0013

Regression line : $Y = 14.193151\ X - 0.0004$ $r^2 = 0.9990$

REFERENCES

Aaronson, S. & Patni, N.J. (1976). The role of surface and extracellular phosphatase in the phosphorus requirement of Ochromonas. Limnol. Oceanogr. **21:** 838-845.

Al-Mousawi, A.H. (1984). Biological studies on algae in rice-field soil from the Iraqi marshes. Ph.D. Thesis, University of Durham, England.

American Public Health Association (1980). Standard methods for examination of water and wastwater. American Public Health Association, Washington, D.C.

Applebury, M., Johnson, B. & Coleman, E. (1970). Phosphate binding to alkaline phosphatase activity. J. Biol. Chem. **245:** 4968-4976.

Berman, T. (1970). Alkaline phosphatases and phosphorus availability in Lake Kinneret. Limnol. Oceanogr. **15:** 663-674.

Bowen, H.J.M. (1966). Trace Elements in Biochemistry. New York, Academic Press.

Broberg, B. (1985). Biological availability of different fractions of dissolved phosphorus in two natural waters: algal assay experiments. Verh. Int. Verein. Limnol. **22:** 2898-2903.

Broberg, O. & Persson, G. (1988). Particulate and dissolved phosphorus forms in freshwater: composition and analysis. Hydrobiologia **170:** 61-90.

Cembella, A.D., Antia, N.J. & Harrison, P.J. (1984a). The utilization of inorganic and organic phosphorus compounds as nutrients by eukaryotic microalgae. A multi-disciplinary perspective. Part I. Crit. Rev. Microbiol. **10:** 317-391.

Chiaudani, G. & Vighi, M. (1974). The N : P ratio and test with Selenastrum to predict eutrophication in lakes. Water Res. **8:** 1063-1069.

Chrost, R.J., Siuda, W. & Halemejko, G. (1984). Longterm studies on alkaline phosphatase activity (APA) in a lake with fish-aquaculture in relation to lake eutrophication and phosphorus cycle. Arch. Hydrobiol. Suppl. **70:** 1-32.

Chu, S.P. (1942). The influence of the mineral composition of the media on the growth of planktonic algae. I. Methods and culture media. J. Ecol. **30:** 284-325

Dunham, K.C. (1948). Geology of the Northern Pennine Orefield. I. Tyne to stainmore 357 pp. Mem. Geol. Surv. U.K., H.M.S.O., London.

Eisenreich, S.J., Bannerman, R.T. & Armstrong, D.E. (1975). A simplified phosphorus analysis technique. Environ. Lett. **9:** 45-53.

Fitzgerald, G.P. & Nelson, T.C. (1966). Extractive and enzymatic analysis for limiting or surplus phosphorus in algae. J. Phycol. **2:** 32-37.

Florence, M.T. (1980). Speciation of zinc in natural waters. pp. 199-227. In: Nriagu, J.O. (eds) Zinc in the environment. Part I. Ecological cycling. John Wiley and Sons, Chichester, New York, Brisbaan and Toronto.

Flynn, K.J., Opik, H. & Syrrett, P.J. (1986). Localization of alkaline phosphatase and 5'-nucleotidase activities of the diatom Phaeodactylum tricornutum. J. gen. Microbiol. **132:** 289-298.

Franco, D.A. (1984). Phytoplankton metabolism and cyclic nucleotides. II. Nucleotide-induced perturbations of alkaline phosphatase activity. Arch. Hydrobiol. **100:** 409-421.

Gibson, M.T. & Whitton, B.A. (1987). Hairs, phosphatase activity and environmental chemistry in Stigeoclonium, Chaetophora and Draparnaldia (Chaetophorales). Br. Phycol. J. **22:** 11-22.

Grainger, S.L.J., Peat, A., Tiwari, D.N. & Whitton, B.A. (1989). Phosphomonoesterase activity of the cyanobacterium (blue-green alga) Calothrix parietina. Microbios **59:** 7-17

Grobbelaar, J.U. (1983). Availability to algae of N and P adsorbed on suspended solids in turbid waters of the Amazon River. Arch. Hydrobiol. **96:** 302-316.

Harding, J.P.C. & Whitton, B.A. (1976). "Resistance to zinc of Stigeoclonium tenue in the field and laboratory". Br. phycol. J. **11:** 417-426.

Healey, F.P. (1973). Characteristics of phosphorus deficiency in Anabaena. J. Phycol. **9:** 383-394.

Healey, F.P. (1982). Phosphate. In: The Biology of Cyanobacteria, N. G. Carr and B. A. Whitton (eds), pp. 105-124. Blackwell, Oxford, England.

Heath, R.T. & Cook, G.D. (1975). The significance of alkaline phosphatase in eutrophic lake. Verh. Int. Ver. Limnol. **19:** 959-965.

Hem, J.D. (1972). Chemistry and occurence of cadmium and zinc in surface water and groundwater. Wat. Resour. Res. **8:** 661-679.

Hino, S. (1989). Characterization of orthophosphate release from dissolved organic phosphorus by gel filtration and several hydrolytic enzymes. Hydrobiologia **174:** 49-55.

Horne, J.E.M. (1977). A Bibliography of the Rivers North Tyne, Wear, Tees and Swale. 54 pp. Freshwater Biological Association Occasional Publication No. 3.

Huber, F.P. & Kidby, D.K. (1984). An examination of the factors involved in determining phosphatase activities in estuarine water. 1: Analytical procedures. Hydrobiologia **111:** 3-11.

Ito, K., Yamamoto, T. & Minimiura, N. (1987). Phosphodiesterase I in human urine: Purification and characterization of the enzyme. J. Biochem. **102:** 359-367.

Jansson, M., Olsson, H. & Pettersson, K. (1988). Phosphatases; origins, characteristics and function in lakes. Hydrobiologia **170:** 157-175.

Kadner, R.J. & Nyc, J.F. (1969). A repressible alkaline phosphatase in Neurospora crassa. J. Biol. Chem. **244:** 5125-5130.

Kelly, S.J., Dardinger, D.E. & Butler, L.E. (1975). Hydrolysis of phosphate esters catalyzed by 5′-nucleotide phosphodiesterase. Biochemistry **14:** 4983-4988.

Kuenzler, E.J. (1965). Glucose-6-phosphate utilization by marine algae. J. phycol. **1:** 156-164.

Kuenzler, E.J. & Perras, J.P. (1965). Phosphatases of marine algae. Biol. Bull. Woods Hole. **128:** 271-284.

Livingstone, D., Khoja, T.M. & Whitton, B.A. (1983). Influence of phosphorus on physiology of a hair-forming blue-green alga (Calothrix parietina) from an upland stream. Phycologia **22:** 345-350.

Livingstone, D. & Whitton, B.A. (1984). Water chemistry and phosphatase activity of the blue-green alga Rivularia in upper Teesdale streams. J. Ecol. **72:** 405-421.

McComb, R.B., Bowers, G.N. & Posen, S. (1979). Alkaline phosphatases, 986 pp. Plenum Press, NY., USA.

Pettersson, K. (1980). Alkaline phosphatase activity and algal surplus phosphorus as phosphorus-deficiency indicators in Lake Erken. Arch. Hydrobiol. **89:** 54-87.

Pettersson, K. (1985). The availability of phosphorus and the species composition of the spring phytoplankton in Lake Erken. Int. Revue ges. Hydrobiol. **70:** 1226-1230.

Pisani, R., Freedman, D. & Purves, R. (1987). Statistics eds w.w Norton & Company.

Raistrick, A. & Jennings, B. (1965). A History of Lead Mining in the Pennine. 347 pp. Longmans, London.

Reichardt, W. (1971). Catalytic mobilization of phosphate in lake water and by Cyanophyta. Hydrobiologia **38:** 377-394.

Say, P.J. (1977). Microbial ecology of high zinc level sreams. 295 pp. Ph.D. Thesis, University of Durham, England.

Say, P.J., Diaz, B.M. & Whitton, B.A. (1977). Influence of zinc on lotic plants. I: Tolerance of Hormidium species to zinc. Freshwater Biol. **7:** 357-376.

Say, P.J. & Whitton, B.A. (1981). Chemistry and plant ecology of zinc-rich streams in the Northern Pennines. pp. 55-63. In: Say, P.J. & Whitton, B.A. (eds). Heavy Metals in Northern England: Environmental and biological aspects. University of Durham, Dept. of Botany.

Say, P.J. & Whitton, B.A. (1982). Chimie et ecologie de la vegetation de cours d' eau en France a fortes teneurs en zinc. I. Massif Central. Ann. Limnol. **18:** 3-18.

Sinclair, C. (1977). Physiological and morphological studies on tapering blue-green algae. Ph.D. Thesis, University of Durham, England.

Siuda, W. (1984). Phosphatases and their role in organic phosphorus transformation in natural waters. A review. Pol. Arch. Hydrobiol. **31:** 207-233.

Spiro, T.G. (1973). Phosphate transfer and its activation by metal ions; alkaline phosphatase. In G. L. Eichhorn (eds), Inorganic Biochemistry Vol. I. Elsevier, Amsderdam: 549-581.

Stainton, M.P., Capel, M.J. & Armstrong, F.A.J. (1977). The Chemical Analysis of Freshwater, 2nd edn. Fish. Mar. Serv. Misc. Spec. Publs **25:** 166 pp.

Thompson, L.M.M. & MacLeod, R.A. (1974a). Factors affecting the activity and stability of alkaline phosphatase in a marine pseudomonad. J. Bact. **117:** 813-818.

Thompson, L.M.M. & MacLeod, R.A. (1974b). Biochemical localization of alkaline phosphatases in the cell wall of a marine pseudomonad. J. Bact. **117:** 819-825.

Torriani-Gorini, A., Rothman, F.G., Silver, S., Wright, A. and Yagil, E. (eds) (1987). Phosphate metabolism and cellular regulation in microorganisms, 316 pp. ASM, Washington D.C., USA.

Walther, K. & Fries, L. (1976). Extracellular alkaline phosphatases in multicellular marine algae and their utilization of glycerophosphate. Physiol. Pl. **36:** 118-122.

Weatherley, A.H., Lake, P.S. & Rogers, S.C. (1980). Zinc pollution and the ecology of the freshwater environment. pp. 337-418. In: Nriagu, J. O. (eds) Zinc in the environment. Part I. Ecological cycling. John Wiley & Sons, Chichester, New York, Brisbane & Toronto.

Whitton, B.A. (1967). Phosphate accumulation by colonies of Nostoc. Pl. Cell Physiol., Tokyo **8:** 293-296.

Whitton, B.A. & Crisp, D.T. (1984). Tees pp. 145-178. In Whitton, B.A. (eds) Ecology of European rivers. Blackwell Scientific publications, Oxford.

Wilkins, A.S. (1972). Physiological factors in the regulation of alkaline phosphatase synthesis in Escherichia coli. J. Bact. **110:** 616-623.

Wynne, D. (1977). Alternations in activity of phosphatases during the Peridinium bloom in Lake Kinneret. Physiol. Pl. **40:** 219-224.

Wynne, D. (1981). The role of phosphatases in the metabolism of Peridinium cinctum from Lake Kinneret. Hydrobiologia **83:** 93-99.