

Copyright is owned by the Author of the thesis. Permission is given for a copy to be downloaded by an individual for the purpose of research and private study only. The thesis may not be reproduced elsewhere without the permission of the Author.

ASSESSMENT OF THE BIOLOGICAL  
AVAILABILITY OF  
PARTICULATE-PHASE PHOSPHORUS

A thesis presented in partial fulfilment of  
the requirements for the degree of  
Doctor of Philosophy in Soil Science  
at Massey University

Michael James Hedley

1978

## ABSTRACT

A bioassay procedure for particulate-phase phosphorus (P), using Anabaena subcylindrica was developed and evaluated. Measurements of chlorophyll concentration and whole cell alkaline phosphatase activity were established as reliable indices of biomass and algal P status, respectively. Algal P content was found to be dependent on external P availability and was directly related to biomass, only when P availability was constant. The availability of P to Anabaena was controlled by culturing Anabaena in systems containing P sorbed on hydrous ferric oxide gel, saturated to varying proportions of the sorption maximum. By manipulating the amounts of P and gel, algae of similar P status to those grown in soil systems were produced.

The combined bioassay-chemical fractionation procedure developed was used to chemically characterize the amounts and forms of biologically-available particulate inorganic P (IP) and organic P (OP) in potential surface runoff fractions from a wide range of soils. The simultaneous fractionation of Anabaena of similar P status to those in the bioassay systems, enabled a correction to be made for the algal-P contribution to extractable soil + algal P. In this way, the depletion of particulate IP and OP could be monitored. Algal growth depleted P from the 0.1M NaOH-soil-P fraction only; in several bioassays, 0.1M NaOH-soil-OP constituted the larger part of the P depleted. For most of the materials studied, except allophanic material, 0.1M NaOH-soil-P was depleted by 70 to 100% during the growth of Anabaena. Extractability in 0.1M NaOH suggests that biologically-available IP is present as surface-sorbed IP. A similar origin is probable for particulate-phase OP.

The amounts of particulate P extracted by persulphate digestion, a

commonly-used extraction procedure, were greater than those of biologically-available particulate P. Conversely, the amounts of isotopically-exchangeable P underestimated those of biologically-available particulate IP, as determined by the developed procedure.

Algal-soil contact was an important factor influencing the depletion of soil P. Soluble, algal-extracellular products, acting in isolation from the algae, had little influence on particulate IP desorption. Also, the simple desorption of particulate IP was unable to account for the release of large amounts of P to the algae. The initial solution P concentration maintained by a particulate P source material considerably influenced the amount of algal growth and the extent to which particulate P was subsequently depleted.

Biologically-available OP in two soil materials was extracted with 0.1M NaOH and the extracts were separated into humic and fulvic material, which were fractionated by agar gel and Sephadex gel chromatography, respectively. Most of the OP in the humic extract was present as high molecular weight organic matter-Fe-P complexes. In the fulvic extract, both high and low molecular weight organic matter-Fe-P complexes were identified. Inositol polyphosphates, both free and complexed, were identified by ion-exchange chromatography in the fulvic material.

A major objective of the present study was the development of a chemical test for estimating the amount of biologically-available P in stream sediment-source materials. Except for samples containing allophanic material, the extraction of a water sample with 0.1M NaOH is proposed as a rapid and simple test for estimating the maximum amount of biologically-available P present in the sample.

ACKNOWLEDGEMENTS

I am extremely grateful to:

Professor J. Keith Syers for supervision, encouragement, translation of the Norfolk dialect into English, and close friendship during my studies.

All past and present people in the T.V.L. lab., for making it a pleasure to arrive for work, and to the assistance given from time to time by Lance, Ann, and Ken.

Drs. Andy Sharpley, John Ryden, John McLaughlin, and Neil McGregor for stimulating discussions.

Dianne for the transcription from Norfolk into English.

The Ministry of Agriculture and Fisheries and the Department of Scientific and Industrial Research for providing research funds from which my Research Assistantship was derived.

All past and present members of 37 Ranfurly St., and to the occupants of 2 Duna Place, 180 Victoria Ave., and 44 Campbell St., for making my stay in New Zealand extremely enjoyable.

Lastly, but most importantly, to my family at home.

## TABLE OF CONTENTS

	Page
ABSTRACT . . . . .	i
ACKNOWLEDGEMENTS . . . . .	iii
TABLE OF CONTENTS . . . . .	iv
LIST OF FIGURES . . . . .	x
LIST OF TABLES . . . . .	xv
SECTION 1	
INTRODUCTION . . . . .	1
SECTION 2	
LITERATURE REVIEW . . . . .	3
2.1 Eutrophication . . . . .	3
2.1.1 Phosphorus : a key nutrient? . . . . .	3
2.1.2 Importance of particulate-phase phosphorus . . . . .	6
2.1.3 Transport of particulate-phase phosphorus in waters. . . . .	9
2.2 Particulate-Phase Phosphorus . . . . .	10
2.2.1 Origin and forms . . . . .	10
2.2.1.1 Inorganic . . . . .	10
2.2.1.2 Organic . . . . .	12
2.2.2 Chemical characterization . . . . .	15
2.2.2.1 Inorganic . . . . .	15
2.2.2.2 Organic . . . . .	17
2.2.3 Assessment of biological availability . . . . .	19
2.2.3.1 Inorganic . . . . .	19
2.2.3.1.1 Sorption and desorption . . . . .	20
2.2.3.1.2 Isotopic exchangeability . . . . .	21
2.2.3.1.3 Bioassay procedures . . . . .	22
2.2.3.2 Organic . . . . .	26

	Page
2.3 Bioassays as Analytical Tools . . . . .	28
2.3.1 Algal bioassays. . . . .	28
2.4 Conclusions . . . . .	33

### SECTION 3

GENERAL METHODOLOGY . . . . .	35
3.1 Algal Culture Procedures . . . . .	35
3.1.1 Bioassay organism . . . . .	35
3.1.2 Media . . . . .	35
3.1.3 Preparation of inocula . . . . .	36
3.1.4 Bioassay technique . . . . .	36
3.1.5 Alkaline phosphatase activity . . . . .	37
3.1.6 Development and evaluation of biomass measurements . .	37
3.1.6.1 Organic carbon and dry weight . . . . .	38
3.1.6.2 Chlorophyll concentration, organic carbon, and dry weight . . . . .	39
3.1.6.3 Adopted method of biomass measurement . . . . .	47
3.2 Phosphorus Determination . . . . .	49
3.2.1 Definition of terms . . . . .	49
3.2.2 Phosphorus fractionation procedure . . . . .	50
3.2.2.1 Solution-P . . . . .	50
3.2.2.2 Sodium hydroxide-extractable-P . . . . .	50
3.2.2.3 Citrate-dithionite-bicarboante extractable-P (CDB-P) . . . . .	51
3.2.2.4 Acid-extractable-P (1M HCl-P) . . . . .	51

### SECTION 4

#### EFFECT OF PHOSPHORUS AVAILABILITY ON EXTRACTABLE

ALGAL CELL PHOSPHORUS . . . . .	52
4.1 Introduction . . . . .	52
4.2 Materials and Methods . . . . .	54
4.2.1 Preparation of Fe gel . . . . .	54
4.2.2 Soil materials . . . . .	55
4.2.3 Experimental procedure . . . . .	55

4.3	Results and Discussion . . . . .	57
4.3.1	Solution P concentrations . . . . .	57
4.3.2	Biomass as measured by chlorophyll concentration . . . . .	57
4.3.3	Algal P status . . . . .	61
4.4	General Discussion . . . . .	64

## SECTION 5

## CHARACTERIZATION OF BIOLOGICALLY AVAILABLE

	PARTICULATE-PHASE PHOSPHORUS . . . . .	67
5.1	Introduction . . . . .	67
5.2	Materials and Methods . . . . .	68
5.2.1	Soils . . . . .	68
5.2.2	Analytical methods . . . . .	68
5.2.3	Experimental procedures . . . . .	68
5.3	Results and Discussion . . . . .	69
5.3.1	Combined bioassay-phosphorus fractionation procedure . . . . .	69
5.3.1.1	Algal-growth characteristics . . . . .	69
5.3.1.2	Algal-phosphorus fractions . . . . .	75
5.3.1.3	Soil-phosphorus fractions . . . . .	76
5.3.1.4	Interpretations . . . . .	77
5.3.2	Modification of phosphorus fractionation procedure . . . . .	78
5.3.2.1	0.1M NaOH-extractable soil phosphorus . . . . .	79
5.3.2.2	Interpretations . . . . .	79
5.3.3	Evaluation of the modified bioassay- phosphorus fractionation procedure . . . . .	81
5.3.3.1	General aspects of the bioassay . . . . .	82
5.3.3.2	Extractable-algal phosphorus . . . . .	86
5.3.3.3	Extractable-soil phosphorus . . . . .	89
5.3.3.4	General discussion . . . . .	97

## SECTION 6

ASSESSMENT OF BIOLOGICALLY-AVAILABLE PARTICULATE PHASE P	
IN POTENTIAL SURFACE RUNOFF MATERIALS . . . . .	102
6.1 Introduction . . . . .	102
6.2 Materials and Methods . . . . .	105
6.2.1 Soils . . . . .	105
6.2.2 Experimental procedure . . . . .	105
6.3 Results and Discussion . . . . .	108
6.3.1 Shaken bioassays of soil material . . . . .	108
6.3.1.1 Bioassay of the <30- $\mu$ m material from Waimakariri, Atawhai, and Egmont soils . . . . .	108
6.3.1.2 Bioassay of the <30- $\mu$ m material from Manawatu and Waiotu soils, and Manawatu River sediment . . . . .	121
6.3.1.3 Bioassay of stream-sediment source materials and the <30- $\mu$ m material from earthworm casts . . . . .	128
6.3.1.4 Correlation between the amounts of biologically-available P and 0.1M NaOH- extractable P in a range of soil materials. . . . .	137
6.3.2 Non-shaken bioassay of <30- $\mu$ material from all source materials . . . . .	141
6.4 General Discussion . . . . .	152

## SECTION 7

COMPARISON OF ALTERNATIVE METHODS FOR THE DETERMINATION	
OF BIOLOGICALLY-AVAILABLE PARTICULATE P . . . . .	
7.1 Introduction . . . . .	160
7.2 Materials and Methods . . . . .	161
7.2.1 Ammonium persulphate digestion . . . . .	162
7.2.2 Isotopic exchangeability . . . . .	162
7.3 Results and Discussion . . . . .	163
7.3.1 Particulate P extracted by persulphate digestion . . . . .	163
7.3.2 Exchangeable particulate phase P . . . . .	165
7.4 General Discussion . . . . .	173

## SECTION 8

## FACTORS INFLUENCING THE AVAILABILITY OF

PARTICULATE-PHASE PHOSPHORUS . . . . .	177
8.1 Introduction . . . . .	177
8.2 Materials and Methods . . . . .	178
8.2.1 Desorption studies . . . . .	178
8.2.2 Algal soil contact study . . . . .	179
8.2.3 Solution P concentration studies . . . . .	181
8.2.4 Sterilization of Tokomaru soil material . . . . .	182
8.3 Results and Discussion . . . . .	182
8.3.1 Effect of algal-extracellular products on the desorption of soil phosphorus . . . . .	182
8.3.2 Importance of algal cell-soil contact to soil phosphorus availability . . . . .	186
8.3.3 Effect of solution phosphorus concentration on the availability of particulate phase phosphorus to algae . . . . .	190
8.3.4 Algal growth on sterile soil . . . . .	195
8.4 General Discussion . . . . .	198

## SECTION 9

## CHARACTERIZATION OF BIOLOGICALLY-AVAILABLE

ORGANIC PHOSPHORUS IN TWO SOILS . . . . .	202
9.1 Introduction . . . . .	202
9.1.1 Aims of study . . . . .	202
9.1.2 Techniques . . . . .	203
9.2 Materials and Methods . . . . .	206
9.2.1 Soil extracts . . . . .	206
9.2.2 Gel chromatography . . . . .	206
9.2.2.1 Fractionation of humic material . . . . .	207
9.2.2.2 Fractionation of fulvic material . . . . .	208
9.2.3 Determination of inositol polyphosphates . . . . .	208

	Page
9.3 Results and Discussion . . . . .	210
9.3.1 Fractionation of humic material . . . . .	210
9.3.2 Fractionation of the fulvic extract from Tokomaru soil . . . . .	212
9.3.2.1 Using Sephadex G-25 - 80 . . . . .	212
9.3.2.2 Using Sephadex G-50 - 80 . . . . .	214
9.3.2.3 Identification of inositol polyphosphates by ion-exchange chromatography . . . . .	216
9.3.3 Fractionation of the fulvic extract from Manawatu soil . . . . .	220
9.3.3.1 Using Sephadex G-50 - 80 . . . . .	221
9.3.3.2 Identification of inositol polyphosphates by ion-exchange chromatography . . . . .	224
9.3.3.3 The association of iron with the low molecular weight organic phosphate fractions obtained using Sephadex gel chromatography. . . . .	228
9.4 General Discussion . . . . .	231
SUMMARY AND CONCLUSIONS . . . . .	241
BIBLIOGRAPHY . . . . .	245
APPENDIX . . . . .	274

## LIST OF FIGURES

	Page
FIGURE 3.1 Effect of time of heating the oxidising mixture at 100°C on the determined carbon content of <u>Anabaena</u> (4 mg, A) and of <30-µm soil material (40 mg, B) . . . . .	40
3.2 Relationship between chlorophyll and carbon concentrations during the exponential growth of <u>Anabaena</u> . . . . .	43
3.3 Chlorophyll concentration (a), dry weight of algae (b), and alkaline phosphatase activity (APA) (c) during the growth of <u>Anabaena</u> in ASM-1, N- media at varying P concentrations . . . . .	44
3.4 Variation in the chlorophyll concentration-algae dry weight relationship during the growth of <u>Anabaena</u> in ASM-1, N- media at varying P concentrations . . . . .	46
3.5 Relationship between maximum chlorophyll concentration or maximum dry weight of <u>Anabaena</u> and the available P concentration of the growth media . . . . .	48
4.1 Amount of algal 0.1M NaOH-TP over and above that in the inoculum (a), amount of 0.1M NaOH-algal-TP per unit biomass (b), alkaline phosphatase activity (APA) (c), and chlorophyll concentration (d) during the growth of <u>Anabaena</u> in Fe gel systems of increasing P content . . . . .	58
4.2 Amount of algal 0.1M NaOH-TP over and above that in the inoculum (a), the amount of 0.1M NaOH-algal-TP per unit biomass (b), alkaline phosphatase activity (APA) (c), and chlorophyll concentration (d) during the growth of <u>Anabaena</u> in solutions of increasing P content . . . . .	59
4.3 Chlorophyll concentration (a) and alkaline phosphatase activity (APA) during the growth of <u>Anabaena</u> on the <30-µm material from Manawatu (A) and Waiotu (B) soils, and on Manawatu River sediment (C) . . . . .	62
5.1 Phosphorus fractionation procedure . . . . .	70

FIGURE 5.2	Chlorophyll concentration (a) and alkaline phosphatase activity (APA) (b) during the growth of <u>Anabaena</u> on Tokomaru soil (<30 $\mu\text{m}$ ) (S) and on Fe gels containing increasing amounts of sorbed P . . . . .	71
5.3	Amounts of TP, OP, and IP extracted from Tokomaru soil (<30 $\mu\text{m}$ ) by 0.1M NaOH as a function of time at a soil solution ratio of 1:750 . . . . .	80
5.4	Chlorophyll concentration (a) and alkaline phosphatase activity (APA) (b) during the growth of <u>Anabaena</u> on the <30 $\mu\text{m}$ material from Tokomaru (A) and Okaihau (B) soils. . . . .	83
5.5	Chlorophyll concentration (a) and alkaline phosphatase activity (APA) (b) during the growth of <u>Anabaena</u> on Fe gels containing increasing amounts of sorbed P. . . . .	84
5.6	Kjeldahl nitrogen concentration in the culture systems during the growth of <u>Anabaena</u> on the <30- $\mu\text{m}$ material from Tokomaru (A) and Okaihau (B) soils, and in Fe gel system 4 (Fig. 5.5a) (C). . . . .	85
6.1	Chlorophyll concentration during the growth of <u>Anabaena</u> on the <30- $\mu\text{m}$ material from Egmont (A, $0.33\text{g l}^{-1}$ ), Waimakariri (B, $1\text{g l}^{-1}$ ) and Atawhai (C, $1\text{g l}^{-1}$ ) soils . . . . .	109
6.2	Alkaline phosphatase activity (APA) during the growth of <u>Anabaena</u> on the <30- $\mu\text{m}$ material from Egmont (A, $0.33\text{g l}^{-1}$ ), Waimakariri (B, $1\text{g l}^{-1}$ ) and Atawhai (C, $1\text{g l}^{-1}$ ) soils . . . . .	111
6.3	Chlorophyll concentration during the growth of <u>Anabaena</u> on the <30- $\mu\text{m}$ material from Manawatu (A) and Waiotu (B) soils, and Manawatu River sediment (C). . . . .	122
6.4	Alkaline phosphatase activity (APA) during the growth of <u>Anabaena</u> on the <30- $\mu\text{m}$ material from Manawatu (A) and Waiotu (B) soils, and Manawatu River sediment (C) . . . . .	124
6.5	Chlorophyll concentration during the growth of <u>Anabaena</u> on the <30- $\mu\text{m}$ material from earthworm casts (A), surface-runoff material (B), and <30- $\mu\text{m}$ stream-bank material (C). . . . .	129

FIGURE 6.6	Alkaline phosphatase activity (APA) during the growth of <i>Anabaena</i> on the <30- $\mu\text{m}$ material from earthworm casts (A), surface-runoff material (B), and <30- $\mu\text{m}$ material from stream-bank material (C) . . . . .	130
6.7	Relationship between maximum chlorophyll concentration produced during the growth of <i>Anabaena</i> on a range of soil materials (<30 $\mu\text{m}$ ) and the amount of extractable TP in each system (given by the sum of solution-TP and 0.1M NaOH-soil-TP before algal growth). . . . .	140
6.8	Chlorophyll concentration ( $\blacktriangle$ ), amounts of 0.1M NaOH-IP ( $\bullet\text{--}\bullet$ ) and -TP ( $\bullet\text{---}\bullet$ ), and alkaline phosphatase activity (APA) ( $\ominus$ ) during the growth of <i>Anabaena</i> in the non-shaken bioassay of Tokomaru soil (<30 $\mu\text{m}$ ) . . . . .	142
6.9	Chlorophyll concentration ( $\blacktriangle$ ), amounts of 0.1M NaOH-IP ( $\bullet\text{--}\bullet$ ) and -TP ( $\bullet\text{---}\bullet$ ), and alkaline phosphatase activity (APA) ( $\ominus$ ), during the growth of <i>Anabaena</i> in the non-shaken bioassay of Okaihau soil (< 30 $\mu\text{m}$ ) . . . . .	143
6.10	Chlorophyll concentration ( $\blacktriangle$ ), amounts of 0.1M NaOH-IP ( $\bullet\text{--}\bullet$ ) and -TP ( $\bullet\text{---}\bullet$ ), and alkaline phosphatase activity (APA) ( $\ominus$ ) during the growth of <i>Anabaena</i> in the non-shaken bioassay of Waiotu soil (<30 $\mu\text{m}$ ) . . . . .	144
6.11	Chlorophyll concentration ( $\blacktriangle$ ), amounts of 0.1M NaOH-IP ( $\bullet\text{--}\bullet$ ) and -TP ( $\bullet\text{---}\bullet$ ), and alkaline phosphatase activity (APA) ( $\ominus$ ), during the growth of <i>Anabaena</i> in the non-shaken bioassay of Waimakariri soil (< 30 $\mu\text{m}$ ). . . . .	145
6.12	Chlorophyll concentration ( $\blacktriangle$ ), amounts of 0.1M NaOH-IP ( $\bullet\text{--}\bullet$ ) and -TP ( $\bullet\text{---}\bullet$ ), and alkaline phosphatase activity (APA) ( $\ominus$ ) during the growth of <i>Anabaena</i> in the non-shaken bioassay of Egmont soil (<30 $\mu\text{m}$ , 0.33g l <sup>-1</sup> ) . . . . .	146
6.13	Chlorophyll concentration ( $\blacktriangle$ ), amounts of 0.1M NaOH-IP ( $\bullet\text{--}\bullet$ ) and -TP ( $\bullet\text{---}\bullet$ ), and alkaline phosphatase activity (APA) ( $\ominus$ ) during the growth of <i>Anabaena</i> in the non-shaken bioassay of Manawatu soil (<30 $\mu\text{m}$ ) . . . . .	147
6.14	Chlorophyll concentration ( $\blacktriangle$ ), amounts of 0.1M NaOH-IP ( $\bullet\text{--}\bullet$ ) and -TP ( $\bullet\text{---}\bullet$ ), and alkaline phosphatase activity (APA) ( $\ominus$ ) during the growth of <i>Anabaena</i> in the non-shaken bioassay of Manawatu River sediment . . . . .	148

FIGURE 6.15	Chlorophyll concentration ( $\blacktriangle$ ), amounts of 0.1M NaOH-IP ( $\bullet\text{---}\bullet$ ) and -TP ( $\bullet\text{---}\bullet$ ), and alkaline phosphatase activity ( $\ominus$ ) during the growth of <i>Anabaena</i> in the bioassay of Atawhai soil (<30 $\mu\text{m}$ ) . . . . .	149
7.1	Relationship between amounts of 0.1M NaOH-IP and exchangeable P (30 min) for the <30- $\mu\text{m}$ material from Tokomaru (A), Waimakariri (B), Manawatu (D), Atawhai (E), Waiotu (F) and Okaihau (G) soils, and from earthworm casts (C) . . . . .	169
8.1	Design of diffusion cell . . . . .	180
8.2	Chlorophyll concentration and alkaline phosphatase activity (APA) during the growth of <i>Anabaena</i> on earthworm-cast material ( $\Delta$ ), earthworm-cast material enclosed in a permeable membrane ( $\square$ ), and in a P-control system ( $\bullet$ ). . . . .	189
8.3	Chlorophyll concentration during the growth of <i>Anabaena</i> on Tokomaru soil (<30 $\mu\text{m}$ ) to which P had been added. . . . .	191
8.4	Chlorophyll concentration, concentration of alkaline phosphatase activity+ ( $\mu\text{ moles pNP hr}^{-1}\text{ ml}^{-1}$ ), and P availability (reciprocal of alkaline phosphatase activity) during the growth of <i>Anabaena</i> on earthworm-cast material (<30 $\mu\text{m}$ ) from which varying amounts of water-extractable P had been removed . . . . .	194
8.5	Chlorophyll concentration during the growth of <i>Anabaena</i> on sterilized and non-sterilized Tokomaru soil (< 30 $\mu\text{m}$ ) . . . . .	196
9.1	Fractionation of the 0.1M NaOH humic extract from Tokomaru soil (<30 $\mu\text{m}$ ) on 12% agar gel (<250- $\mu\text{m}$ bead size) using carbonate-bicarbonate buffer as the eluant . . . . .	211
9.2	Fractionation of the 0.1M NaOH fulvic extract from Tokomaru soil (< 30 $\mu\text{m}$ ) on Sephadex G-25 using 0.1M NaOH as the eluant . . . . .	213
9.3	Fractionation of the material (A), excluded from and partially included by Sephadex G-25 on Sephadex G-50 using 0.1M NaOH as the eluant . . . . .	215
9.4	Fractionation of the 0.1M NaOH fulvic extract from Tokomaru soil (<30 $\mu\text{m}$ ) on Sephadex G-50 using 0.1M NaOH as the eluant . . . . .	217

FIGURE 9.5	Fractionation of inositol hexaphosphate hydrolyzate on ion-exchange resin (100 - 200 mesh, chloride form) using gradient elution with HCl (—) . . . . .	218
9.6	Fractionation of inositol polyphosphates, prepared from the 0.1M NaOH fulvic extract of Tokomaru soil (<30 $\mu$ m) by the method of Baker (1977), on ion-exchange resin (100 - 200 mesh, chloride form) using gradient elution with HCl . . . . .	220
9.7	Fractionation of inositol polyphosphates, prepared from Sephadex G-50 fractions 7 - 22 by the method of Moyer and Thomas (1970), on ion-exchange resin (100 - 200 mesh, chloride form) using gradient elution with HCl . . . . .	222
9.8	Fractionation of inositol polyphosphates, prepared from Sephadex G-50 fractions 23 - 37 by the method of Moyer and Thomas (1970), on ion-exchange resin (100 - 200 mesh, chloride form) using gradient elution with HCl . . . . .	223
9.9	Fractionation of the 0.1M NaOH fulvic extract from Manawatu soil (< 30 $\mu$ m) on Sephadex G-50 using 0.1M NaOH as the eluant . . . . .	225
9.10	Fractionation of inositol polyphosphates, prepared from Sephadex G-50 fractions 8 - 17 by the method of Moyer and Thomas (1970), on ion-exchange resin (100 - 200 mesh, chloride form) using gradient elution with HCl . . . . .	226
9.11	Fractionation of inositol polyphosphates prepared from Sephadex G-50 fractions 18 - 28 by the method of Moyer and Thomas (1970), on ion-exchange resin (100 - 200 mesh, chloride form) using gradient elution with HCl . . . . .	227
9.12	Fractionation of inositol polyphosphates prepared from Sephadex G-50 fractions 29 - 35 by the method of Moyer and Thomas (1970) using gradient elution with HCl . . . . .	229
9.13	Fractionation of the 0.1M NaOH fulvic extract from Manawatu soil (< 30 $\mu$ m) on Sephadex G-50 using 0.1M NaOH as the eluant . . . . .	230
9.14	Fractionation of Sephadex G-50 fractions 27 - 32 on Sephadex G-10 using 0.1M NaOH as the eluant . . . . .	232

## LIST OF TABLES

	Page
TABLE 3.1	Effect of increasing algal mass on the amount of oxidizable algal carbon . . . . . 38
3.2	Effect of increasing algal mass and soil on extractable chlorophyll concentration . . . . . 41
4.1	Solution IP concentrations during the growth of <u>Anabaena</u> in Fe gel and soil systems and in P solutions . . . . . 56
5.1	Algal P fractions during the growth of <u>Anabaena</u> in Fe gel systems containing different amounts of sorbed P . . . . . 73
5.2	Algal and soil P fractions at various times during the bioassay of <30- $\mu$ m material from Tokomaru soil . . . . . 74
5.3	Algal P fractions calculated from the P fractionation data obtained from Fe gel systems . . . 87
5.4	Solution P concentrations for soil and soil + algal systems during the bioassay of Tokomaru and Okaihau soils (<30- $\mu$ m material), and for the algal control . . . . . 88
5.5	Amounts of 0.1M NaOH-extractable P for soil and soil + algal systems, during the bioassay of Tokomaru soil (<30- $\mu$ m material) and for the algal control . . . . . 90
5.6	Amounts of 0.1M NaOH-extractable P for soil and soil + algal systems during the bioassay of Okaihau soil (<30- $\mu$ m material) and for the algal control . . . . . 91
5.7	Amounts of 1M NaOH-extractable P for soil and soil + algal systems during the bioassay of Tokomaru soil (<30- $\mu$ m material) and for the algal control . . . . . 94
5.8	Amounts of 1M NaOH-extractable P for soil and soil + algal systems during the bioassay of Okaihau soil (<30- $\mu$ m material) and for the algal control . . . . . 95
5.9	Amounts of CDB- and HCl-extractable IP from soil and soil + algal systems during the bioassay of Tokomaru and Okaihau soils (<30- $\mu$ m material) . . . 98

TABLE 6.1	Soils and soil materials used in the study . . . . .	103
6.2	Amounts of P extracted from the <30- $\mu$ m material of several soils, Manawatu River sediment, earthworm casts, stream-bank material, and surface runoff material. Iron and aluminum contents are included for some soils . . . . .	106
6.3	Chlorophyll and solution IP concentrations and algal and soil P fractions at various times during the bioassay of <30- $\mu$ m material from Waimakariri soil. . . . .	113
a.	Chlorophyll and solution IP concentrations and data for 0.1M NaOH-extractable P . . . . .	113
b.	Data for 1M NaOH-TP and CDB- and HCl-IP . . . . .	114
6.4	Chlorophyll and solution IP concentrations and algal and soil P fractions at various times during the bioassay of <30- $\mu$ m material from Atawhai soil. . . . .	116
a.	Chlorophyll and solution IP concentrations and data for 0.1M NaOH-extractable P . . . . .	116
b.	Data for 1M NaOH-TP and CDB- and HCl-IP . . . . .	117
6.5	Chlorophyll and solution IP concentrations and algal and soil P fractions at various times during the bioassay of <30- $\mu$ m material from Egmont soil . . . . .	119
a.	Chlorophyll and solution IP concentrations and data for 0.1M NaOH-extractable P . . . . .	119
b.	Data for 1M NaOH-TP and CDB- and HCl-IP . . . . .	120
6.6	Chlorophyll and solution IP concentrations, and algal and soil P fractions at various times during the bioassay of <30- $\mu$ m material from Manawatu soil . . . . .	125
6.7	Chlorophyll and solution IP concentrations, and algal and soil P fractions at various times during the bioassay of <30- $\mu$ m material from Waiotu soil . . . . .	126
6.8	Chlorophyll and solution IP concentrations, and algal and soil P fractions at various times during the bioassay of Manawatu River sediment . . . . .	127

TABLE 6.9	Chlorophyll and solution IP concentrations, and algal and soil P fractions at various times during the bioassay of <30- $\mu$ m material from earthworm casts . . . . .	132
6.10	Chlorophyll and solution IP concentrations, and algal and soil P fractions at various times during the bioassay of surface-runoff material . . . . .	134
6.11	Chlorophyll and solution IP concentrations, and algal and soil P fractions at various times during the bioassay of the <30- $\mu$ m material from stream-bank material . . . . .	136
6.12	Maximum chlorophyll concentration and amount of 0.1M NaOH-extractable P <sup>1</sup> in a bioassay of <30- $\mu$ m material from a range of soils . . . . .	139
7.1	Amounts of P extracted by persulphate digestion, 0.1M NaOH, and reagents of the fractionation scheme (total extractable P) from <30- $\mu$ m material of four soils . . . . .	164
7.2	Amounts of 0.1M NaOH-extractable IP and of exchangeable P as a function time allowed for exchange in <30- $\mu$ m material of several soils . . . . .	166
7.3	Comparison between amount of measured solution IP and solution IP calculated by using the ratio of solution <sup>32</sup> P:soil <sup>32</sup> P at equilibrium, and the effect of exchange time on the recovery of soil <sup>32</sup> P by 0.1M NaOH . . . . .	168
8.1	Amounts of solution IP and 0.1M NaOH-extractable IP and TP, organic P increase, and pH for Manawatu and Okaihau soils (both <30 $\mu$ m) as influenced by the culture media (M), the algal culture filtrate (C) in the presence and absence of Fe gel . . . . .	183
8.2	Effect of algal culture media and algal culture filtrate on the release of IP from <30- $\mu$ m material from three soils at a soil:solution ratio of 1:500 . . . . .	187
8.3	Effect of sterilization on solution IP and 0.1M NaOH-extractable P in Tokomaru soil material . . . . .	197