'A study into the effect of phosphate concentrations on the growth of phytoplankton'

1. Introduction

Phosphorus is one of the basic building blocks of living matter and is present in every living creature. It is the eleventh-most abundant mineral in the earth's crust, and these deposits occur primarily as phosphate in the mineral apatite. Phosphorus is a component of DNA, and plants and animals must have it to live and grow. It is one of several essential plant nutrients required for photosynthesis and is found in all lakes and streams. It occurs in several different forms, including inorganic phosphate PO^{3-}_4, HPO and organic P.

It has already been found that varying concentrations of phosphorus affect the amount of phytoplankton found in water systems. The actual concentration of phosphate in phosphorus-limited waters is difficult to calculate because commonly used chemical and radiochemical techniques overestimate the concentration. Thus by carrying out this experiment I hope to find more exact figures as to the relationship between phosphorus/phosphate concentrations and the growth of phytoplankton over a period of time. This information could be useful to the managers of the Ardingly Reservoir as excess phosphorus could result in eutrophication, which would be extremely detrimental to the water-life.

Eutrophication is one of the most significant causes of water quality problems. Eutrophication occurs in lakes, rivers, ponds and coastal waters. High levels of organic matter, nitrogen and phosphorus in water can cause an algae bloom. These nutrients can come from urban sources like waste water treatment facilities and runoff from fertilized lawns. In the case of the Ardingly Reservoir they are more likely to arise from agricultural practices which can produce large amounts of nutrient runoff from fertilized croplands, animal feed and from septic tank discharges. At first, eutrophication results in an increase in dissolved oxygen as there is more photosynthesis going on from the algae blooms, this stimulates fish production. However, as the algae continue to reproduce, they cloud the water making it difficult for larger submerged vegetation to get enough light. When the plants and algae die they undergo bacterial decomposition, dissolved oxygen is thus removed from the water by the rapidly multiplying bacteria. Lowered oxygen levels and reduced vegetation make it difficult for other aquatic organisms, including fish, to survive.

Carrying out a project of the nature intended would give some useful information to the wardens at Ardingly reservoir in order to help them with the monitoring of the water quality through the species phytoplankton found in the reservoir. Algae are the main primary producers of most rivers and lakes in temperate regions so it is not surprising that routine biological monitoring of water bodies is carried out throughout Europe as a means of supplementing chemical monitoring. Many of the general principles and

examples of how this has been carried out throughout Europe have been well documented (e.g. Whitton, B.A. E. Rott, G. Friedrich, 1991) The monitoring of numbers of particular species of algae can help pinpoint what contaminants are in the water, as they will have either antagonistic or synergistic effects on the algae.

2. Hypothesis: I can hypothesise that with an increasing concentration of phosphate there will be an increasing growth rate of the phytoplankton. However at some point where phosphate is not the limiting factor any more this growth curve will level out.

3. Materials and Methods

3.1. For the collection of phytoplankton

A source of phytoplankton
53µm Phytoplankton net
5l bucket
Distilled water
50ml beaker

It was first necessary to collect the phytoplankton. Running the phytoplankton net several times through the water in the Ardingly reservoir was how I collected the required amount for my experiment. However it is also possible to purchase cultures of phytoplankton. Once a sufficient amount had been collected I transferred it from the filter into the 5l bucket in order to keep them healthy. Once I had returned to the lab I filtered the water again and then washed the contents out with distilled water in order to create a stronger concentration of the solution. This solution was labelled and covered with tinfoil in order to prevent contamination from other sources in the lab, and placed to the side.

3.2. For the creation of solutions of different phosphate concentration

Stock solution of phosphate
Distilled water
4x100cm^3 beakers
20cm^3 syringes

It was then necessary to create the different concentrations of phosphate to be used in the experiment. This was done by taking equal amounts of the stock solution, placing them in separate flasks and then diluting these solutions with varying amounts of distilled water. Thus solutions of 0, 1, 10 and 100 ppm were created.

3.3. For the creation of culture solutions in which to grow the phytoplankton

12 100cm³ Conical Flasks

Several dropping pipettes

Several 5cm³ syringes

The following nutrient solutions:

- Carbonate
- Sulphate and Trace Salts
- Nitrate
- Ammonium Chloride
- EDTA

A stock solution of phosphate

Cotton Wool

Tinfoil

The various other nutrient solutions were collected from the fridge and 1 cm³ of each one was placed in each of the 12 conical flasks. These flasks were then separated into 4 groups of three, each group was labelled with the concentration of phosphate to be placed therein, i.e. 0%, 10% etc. 1 cm³ of each respective phosphate solution was placed in the conical flasks. A tuft of cotton wool was placed in the neck of each flask which was then covered in tinfoil.

3.4. For the sterilisation of the solutions

An autoclave, or if not available a pressure cooker would be suitable

Autoclave tape

The autoclave was checked to make sure it had a suitable amount of water in after which the flasks were placed in the autoclave and, after placing a strip of autoclave tape on one of the flasks, it was sealed and set off. The autoclave used automatically runs through the process of heating up, maintaining the temperature and allowing time to cool down. If an autoclave machine is not available using a simple pressure cooker is also possible however it is essential to ensure that a temperature of 126°C is reached, thus it may be helpful to search for instructions as to how this should be done.

3.5. For inoculation, growth and collection of results

10cm³ syringes

A bank of fluorescent, (Supplying around 60-80$\mu Em^{-2}s^{-1}$) or if not available a suitably light area

Cuvettes

Colorimeter, (A suitable on would be the 'WPA CO75 Colorimeter'

A suitable light filter, (520)
Microscope with a minimum magnification 0f 10x

In order to achieve an even distribution of phytoplankton when inoculating the culture solutions it was necessary to agitate the stock solution before taking up the required amount of solution with a sterile syringe, 10cm³. This was then placed in the conical flask, a sample of 2cm³ was taken from the flask and the light absorption of it was measured with the colorimeter; the cotton wool was then replaced in the neck of the flask, which was in turn recovered with tinfoil. This process was repeated with each flask, ensuring that the stock solution of phytoplankton was agitated each time for a uniform distribution of cells. These were then placed under a bank of fluorescent lights and allowed to stand for 7 days.

After the solutions had been left for the seven days it was necessary to measure the amount by which the populations of phytoplankton had increased or decreased in each flask. This was done by measuring the turbidity of the solutions with a colorimeter. A cuvette was filled with distilled water, a reference cuvette, this was placed in the CO75 colorimeter and the 'R' (Reference) button was briefly pressed. This cuvette was then removed and another cuvette containing a solution from one of the conical flasks was then placed in the CO75. The 'T' (Test) button was then briefly pressed and the reading was recorded. This was repeated three times in order to ensure reliability. The reference cuvette was then replaced and the 'R' button pressed briefly. This was done to reset the colorimeter in order to avoid any discrepancies in the readings due to instrument drift. This process was repeated for each individual flask, using a new cuvette each time.

4. Preliminary Investigation

In order to enable me to conduct a better and more accurate investigation a preliminary experiment was carried out. The purpose was to be able to ascertain as to whether the concentrations of phosphate to be used would give a suitable range of results. In this experiment I created cultures similar to those to be used in the main experiment and inoculated them with a solution containing phytoplankton collected from the Ardingly Reservoir.

The cultures were left to incubate for a period of seven days, after which I could compare the differences in the growth of the phytoplankton. However as this was only a preliminary experiment I did not carry out a numerical comparison. It was possible to tell from simply looking at the solutions whether there were significant differences between the populations of phytoplankton in the different solutions. The results from this experiment were satisfactory and therefore it was decided to use the same concentrations of phosphate used in the preliminary experiment for the main investigation.

The preliminary experiment also helped me improve my technique as I had not had any previous experience in the culturing of phytoplankton. Thus it served as a practice run for the main investigation as well as a method of acquiring a better knowledge of the relationship between phosphate concentration and its ability to inhibit the growth of phytoplankton.

5. Results

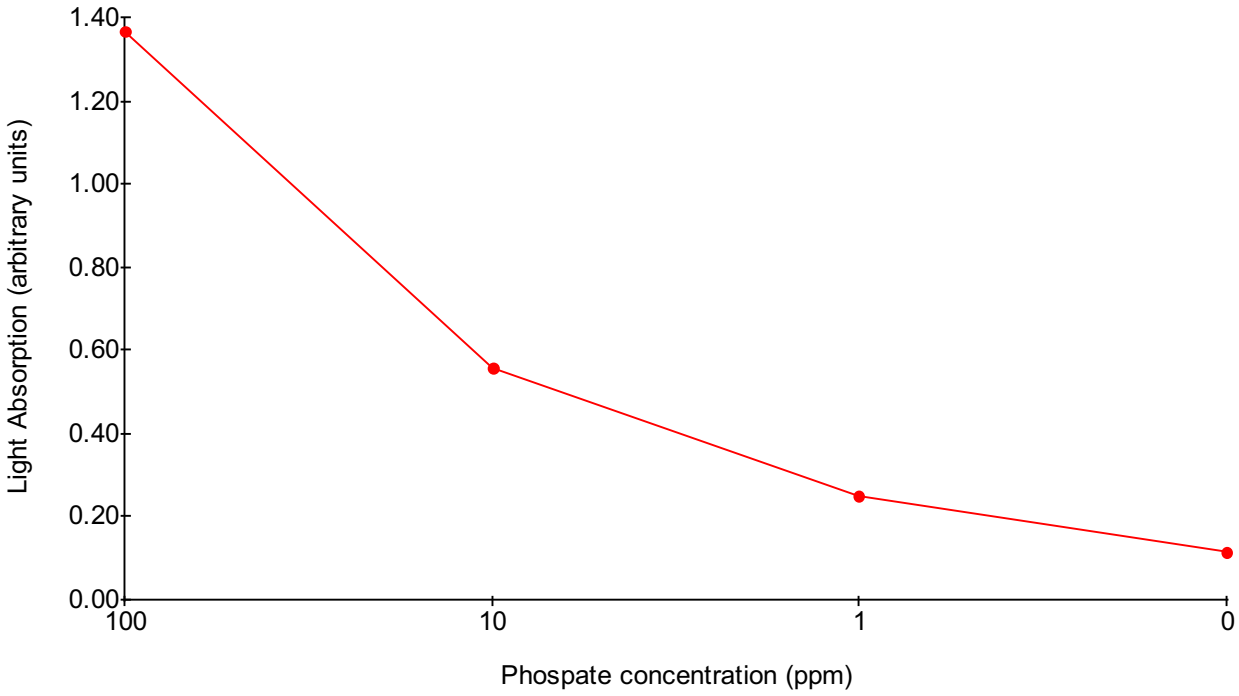
Before the flasks were left to incubate for the period of seven days, 2cm^3 of each solution was taken up and the turbidity, (light absorption) of each was measured. There was only a variation of 0.01 arbitrary units for all the results and so these were not placed in the table, the average light absorption was 0.10 arbitrary units.

It was found that the phytoplankton grown in a concentration of 100ppm of phosphate showed a dramatic increase in population size as compared to that of the phytoplankton in the culture containing no phosphate. It was also noted that although there was no phosphate in the 0ppm phosphate flasks there was still some light absorption.

5.1. A table to show how the concentration of phosphate affects the rate of growth of phytoplankton

Conc. of Phosphate	Light Absorption (arbitrary units)									
	1			2			3			Average
100	1.35	1.4	1.36	1.46	1.45	1.43	1.25	1.33	1.26	1.37
10	0.54	0.53	0.53	0.62	0.62	0.62	0.51	0.53	0.53	0.56
1	**0.12**	**0.1**	**0.09**	0.25	0.26	0.27	0.3	0.3	0.29	0.22
0	0.12	0.12	0.1	0.08	0.08	0.07	0.15	0.16	0.15	0.11

5.2. A graph to show how the concentration of phosphate affects the rate of growth of phytoplankton



6. Analysis

The rate at which phytoplankton takes up phosphate depends on their nutritional history; if phytoplankton are grown under conditions where they are saturated by nutrients the growth rate is equal to the uptake rate when both are expressed in units of time^{-1}. However if nutrient-starved cells are placed in an environment where there is an addition of the limiting nutrient their maximum uptake rate can be a lot higher than their maximum growth rate. This helps the cells to overcome their nutrient debt quickly.

One can see from these results that the phosphate concentration in the growth culture for the phytoplankton has a significant effect on the growth rate of the populations of phytoplankton. There are large differences between each concentration rate as well, this shows that phosphate has an essential role in the growth of micro algae. Phosphate is an essential nutrient for plants; it is required for photosynthesis, cell production and is contained in DNA and in cell walls, in the cell membrane. Thus the amount of phosphorus available in the environment in which the phytoplankton is growing will have a significant effect on the rate at which it can multiply.

There is a 99% reduction in the concentration of the phosphate solution from 100ppm to 1ppm. However there is only a fall of between 60% and 70% in the population size. This would indicate that although there is a significant relationship between the phosphate concentration and growth of the phytoplankton, it is not directly proportional.

7. Conclusion

From the results I have collected it is possible to make several conclusions. The first of which would be that there is a significant relationship between the concentration of phosphate present in a solution and the rate at which a community of phytoplankton will grow. The importance of phosphate to phytoplankton can be seen in graph 5.2, where one can clearly observe the rate at which the amount of growth decreases with the phosphate concentration.

As there was some light absorption in the flasks containing 0ppm of phosphate one can conclude that there was still suspended matter in these flasks. The average light absorption however, was half of that of the flasks containing 1ppm phosphate. This would indicate that although there was very little or no growth, a difference of 0.01 arbitrary units, (this could be discounted due to inaccuracy of instruments) the phytoplankton still remained living and thus we can conclude that although phosphate is a vital nutrient for growth of phytoplankton it is not vital for survival over a seven day period.

The results for the first flask of concentration 1ppm, (in bold) appear to be anomalous as they are quite a bit lower than the other 2 sets of results, in fact they are almost as low as those of a concentration of 0ppm. This may have been caused by several things, it could have simply been that when part of the solution for inoculating was taken up I had not agitated the stock solution enough and thus not as much phytoplankton would have been included, it could also have been that when the growth cultures were created my measurements of the stock solutions were not as accurate as others and so I may not have put as much phosphate, or other nutrients into the solution.

8. Evaluation

When conducting this experiment it was imperative to ensure that all the factors, except for the phosphate concentration were kept the same in each of the flasks. This included ensuring the original cultures had the same mineral solutions in them, ensuring that there was the same amount of fluid in each flask, light intensity was similar in all the flasks and that the temperature was kept as constant as possible.
When creating the cultures to be inoculated with the phytoplankton I ensured that I carried the process out in a methodical way, placing $1cm^3$ of a mineral salt into each flask and then moving on to the next one. This way I did not get confused as to which solutions I had placed into which flask. It also meant that there

was no contamination between the mineral solutions as I was only using one dropping pipette at a time. These were then labelled before I added the different concentrations of phosphate to them, this way I could be sure of the contents of each individual flask.
During the autoclave process the contents of the autoclave are heated to approximately 126ºC, at this temperature the contents of the flasks boil and thus it was important to place the cotton wool and tinfoil over them to ensure that there was little or no loss of fluids due to evaporation. The cotton wool and tinfoil also served as a barrier to other airborne organisms that could have contaminated the flasks during the growth period, causing anomalous results.
In order to ensure reliable results several repeats are necessary, thus for each individual concentration I used three separate flasks, this way it was possible for me to ascertain as to whether there were any anomalies among the results. Furthermore for each of these flasks I checked the results three times on the CO75, this again helped me to ensure that any anomalies were checked and could be disregarded.

There were certain areas of the experiment that could have been carried out in a more suitable method had there been better equipment. Although I managed to collect a suitable amount of phytoplankton from the Ardingly Reservoir I was not able to be very exact as to the numbers and species of phytoplankton that I had collected. This meant that there was an aspect of uncertainty in the experiment and thus could have had an effect on the end results. This could have been avoided had I been able to grow some phytoplankton from spore. This way I would have been surer about the populations and the species and thus I could have carried out some research into the particular species of phytoplankton which might have aided me in my final analysis and conclusion.

Although using a bank of fluorescent lights was better then simply relying on sunlight there were quite large temperature changes between day and night as the heating in the laboratory is turned off at night. Thus growth might have been reduced by quite a large amount at night. Therefore using an incubator might have helped acquire a better set of results. Several species of phytoplankton also benefit from a period of darkness, so it might have given more reliable results had I used a timer which would have turned the bank of lights off at certain times. It might have also been beneficial to have carried the experiment on for a longer period of time; this might have given more definite results as to the effect of the various phosphate concentrations on the phytoplankton. This is because, although after seven days there were differences between the populations in the different phosphate concentrations, a longer incubation period would have made these results more significant. Using a larger variance of phosphate concentrations as well as using more concentrations, i.e. 1; 10; 20; 30ppm might have produced a better set of results, enabling me to come to a more definite set of conclusions. If there had been more time it might have also helped to investigate the effect of phosphate concentration on different species of micro algae. Comparing these would give more reliable results as to the general behaviour of phytoplankton in different environments.

I feel that using the turbidity of the solutions as a method for comparing, was not really as accurate as would have been desired. Although it did give very definite results the turbidity of the solutions would not only have been affected by the phytoplankton in the solutions, the nutrients added could also have had an effect on the results thus adding some uncertainty to them. I also feel that only gathering results once at the end of the experiment was not a very good way of getting results. There are some other tests that could have helped to give more information on the growth rates of the micro algae; these include testing dissolved oxygen levels in the solutions, testing amounts of phosphate present in the solutions, and counting numbers of algae under a microscope. Although the former two would involve inserting probes into the solutions which might result in contamination the latter could be done using an inverted microscope which would not involve removing any of the solution from the flask. Although using the colorimeter gave suitable results, if a method of counting exact populations of phytoplankton within a certain volume of water had been used as well I could have attained more accurate results.

9. Bibliography

Books

Lobban, C. S., D.J. Chapman, B.P. Kremer. (1988) *Experimental phycology, A laboratory manual.* Cambridge: Cambridge University Press.

Indge, B. (2000) *The complete A-Z biology handbook* (2nd Edition). Kent: Hodder and Stoughton.

Whitton, B.A., E. Rott, G. Friedrich. (1991) *Use of algae for monitoring rivers*. Innsbruck: Dr. Eugen Rott.

WPA ltd. (1990) *Instruction manual CO75 colorimeter.*

Web sites

Author/s: Charles J. O'Kelly and Tim Littlejohn.

Latest modifications: 18 May 2003

http://megasun.bch.umontreal.ca/protists/protists.html

CD ROMs

Microsoft® Encarta® Reference Suite 2001

http://www.microsoft.com/encarta/

People

G. R. W. Hawley, B.Sc., Ph.D. (Dunelm)