

# **SPECTROPHOTOMETRIC DETERMINATION OF PHOSPHATE IN RIVER WATER (GROUP 3)**

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## **Aim**

Phosphorus is one of the most abundant elements on earth, though not found alone but in a combined form as phosphate. Phosphate is found in earth's crust, rivers and lakes. In these experiment known concentrations of phosphates in solutions was used to determine the concentration of water samples from a river, lake and an unknown water sample. This was determined assuming the colour intensity and absorbance of

each solution using a spectrophotometer, the experiment aims at familiarising each group with a typical spectrophotometer analysis.

The experiment also aims at comparing the river water value and set guidelines by the European Union. The error associated with the class values and spectrophotometer determination of values, was achieved using, rank correlation, regression and ANOVA one – way to check the mean values, standard deviation and 95% confidence intervals.

## **INTRODUCTION**

Nature has provided human being a source of natural occurring elements and compounds. Phosphorus is the eleventh most abundant element on earth and exists in four allotropic forms; red, white (or yellow), black (or violet). When pure phosphorus is colourless and transparent. Phosphorus is insoluble in water, although, elemental phosphorus is extremely reactive and will combine with oxygen ( $O_2$ ) when exposed to

air, the eleventh most abundant element is not found in air but rather mostly in earth's crust in a combined state as phosphates (or orthophosphate).

Rock phosphates are found in earth's crust at commercial deposits in China, South Africa, Morocco, United State of America, Finland and Russia. "The known reserves of current exploitable rock phosphate are estimated at about 40 billion tons"

([www.nhm.ac.uk/mineralogy/phos](http://www.nhm.ac.uk/mineralogy/phos)). There are two main types of phosphate rock deposit; igneous and sedimentary. Phosphate rocks are found combined with calcium (Ca), sodium (Na), Iron (Fe), aluminium (Al), magnesium (Mg) and many other elements.

Phosphate is a salt of phosphoric acid, a polyatomic ion or radical consisting of one phosphorus atom and four oxygen ( $\text{PO}_4^{-3}$ ). Phosphates are extremely important in plants and animal growth and metabolism. The importance and the role of phosphate are;

- The transfer of energy.
- Contribution of the generation of vitamins.
- The participation of metabolic process in soft tissues.
- Maintenance of appetite.
- Growth in plant.
- Bone development.

Phosphates are basically used for the following purposes;

#### *Agriculture*

Natural occurring phosphate in the soil provides a source of nutrient for plants, these process depends on the geochemical and biological rate in the soil, however, these is a slow process because of excess Nitrogen in the soil, this causes phosphate to be a growth-limiting nutrient. In other to improve and increase the growth of plants,

agricultural farmers add fertilizers to the surface or plant roots in their farms.

Phosphate is one of the major constituents of fertilizer.

#### *Detergent*

Detergents are added to water to remove dirt from contaminated surfaces, phosphates are major constituents of detergent.

#### *Food and animal feed supplement*

Phosphates are extremely important in animal growth and metabolism, bones are mainly composed of calcium phosphate ( $\text{Ca}(\text{PO}_4)_2$ ). Phosphates are used in the oxidation of glucose, that is, energy transfer and are used in the manufacture of animal feed supplements.

Phosphates are important in living organisms, however, continued over usage has created enormous problems to the environment. Man's quest to improve the growth of plants applied fertilizer and animal manure to plant roots, plants do not absorb all phosphates applied and the unused phosphates are washed into rivers and lakes. Detergents and water softeners contain polyphosphates and are discharged into sewage treatment plants. After due process, sewage is discharged into the rivers. Detergents contribute about 3 – 10% loads to surface waters ([www.ceep-phosphate.org](http://www.ceep-phosphate.org)). Deposition of rocks, minerals, agricultural runoff, sedimentation, erosion, stormwater runoff and atmospheric deposition all contribute to the level of phosphate in water. Although, algae for growth and proper maintenance of ecosystem need phosphates, excessive phosphate in water causes imbalance in the nutrient pattern. The cycling process is disturbed because there is increase in population of algae. Algae's now cover the surface of the water thereby preventing sunlight from reaching the lower part of the water. This reduces photosynthesis by plants in the water. The amount of oxygen ( $\text{O}_2$ ) drops drastically (low dissolved oxygen DO), fishes and other aquatic organisms are deprived of oxygen ( $\text{O}_2$ ) thereby shifting the ecosystem. The process described above is termed *eutrophication*; this is when there is excess nutrient in water thereby causing a shift in the ecosystem. Nitrogen and phosphorus are the main cause of eutrophication in water.

There are set guidelines by the European Union as to the expected quantities of phosphate in water, these measures are put in place to maintain a balance in the ecosystem. In some European countries, phosphate has been banned in some products, for instance, Switzerland has banned the use of phosphate in pesticides. Full-scale plants recovering phosphates from sewage for industrial recycling are currently

operative in Europe at Geesmerambacht Netherlands, Treviso Italy and Slough United Kingdom ([www.ceep-phosphates.org](http://www.ceep-phosphates.org)). Phosphorus concentrations in some EU rivers have fallen since the mid-1980s, particularly in the largest and most polluted rivers. The decline in phosphorus concentrations in major EU rivers is due to improved wastewater treatment and less phosphorus in household detergents. Since May 1999 all Member States have fully or partly transposed this Directive into their national laws and developed implementation plans. ([www.eea.eu.int](http://www.eea.eu.int)). Figure 1 below clearly shows the decline in phosphorus level in surface water.

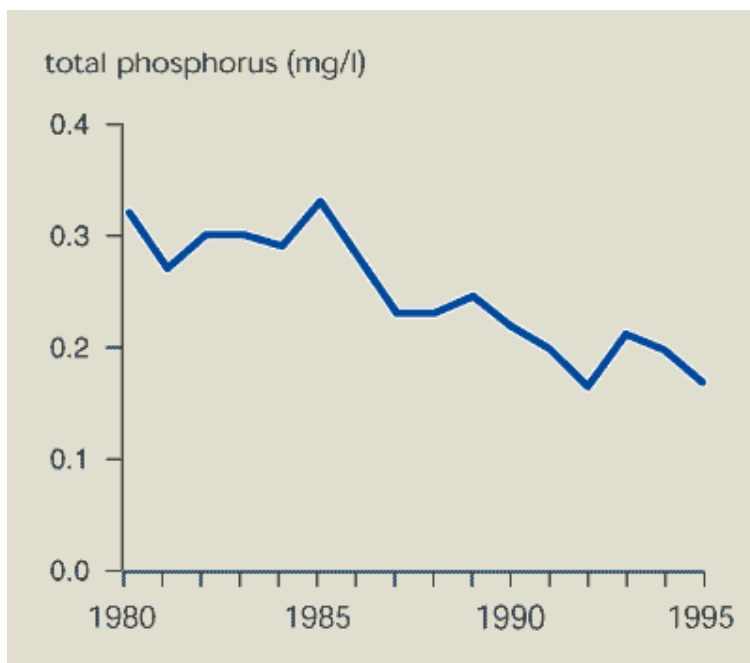


Figure (1) Source: EEA-ETC/IW based on country returns under EC Exchange of Information Decision (77/795/EEC)

The phosphorus concentration has fallen significantly over the past 15 years.

## **Spectrophotometer**

A spectrophotometer was used in the determination of absorbance of the sample provided. "Substances may be identified by its absorption spectrum" (Calder A.B). Spectrophotometric and colorimetric are a measure of light transmitted and absorbed over a given wavelength, the concentration of the absorbing solution is determined by the amount of light absorbed. The more concentrated a solution, the more light will be

absorbed by the solution. From figure (1), we can assume that there exist a linear relationship between absorbance and concentration.

Beer – Lambert’s law, can best describe the relationship between absorbance and concentration. The law relates the light intensity to the pathlength in an absorbing medium and the concentration of the solution. This is represented thus;

$$\text{Log}I_0/I = A = ecl \quad (1)$$

Where,

$I_0$  = Intensity of incident light

$I$  = Intensity of transmitted light

$A$  = Absorbance

$e$  = Molar absorptivity

$c$  = Concentration of solution (in these experiment mg/l)

$l$  = Length of path through the sample.

There are limiting factors associated with the use of spectrophotometer, this will be enumerated upon in the discussion. In this experiment the concentration of phosphate in river, lake and an unknown water sample will be determined using spectrophotometric methods. To determine concentration in each sample, a colour reagent was added to give a blue colour, the intensity of each colour depends on the amount of phosphate present. The spectrophotometer used for this analysis was **Pyu Unicam uv – vis SP 8390**

## Methodology

### *Sample preparation*

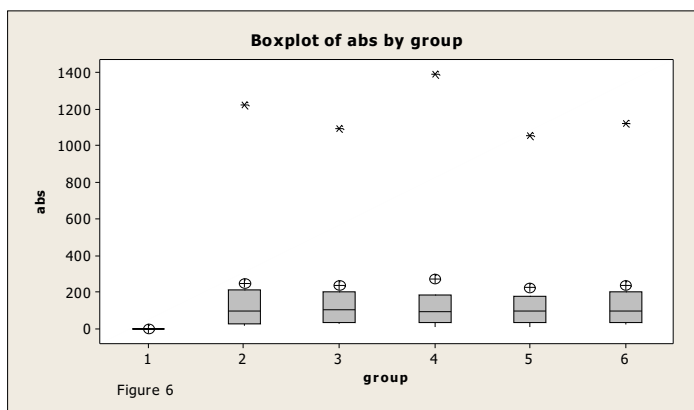
A series of standard solution was prepared from the stock solution, 5, 10, 20 and 40ml were pipette into a 100ml volumetric flask then made to the mark with distilled water. The prepared concentrations were 0.125mg/l, 0.250mg/l, 0.500mg/l and 1.000mg/l.

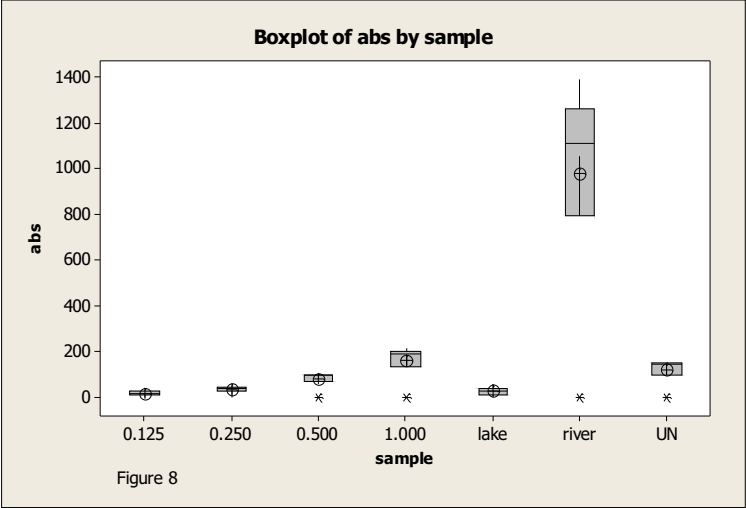
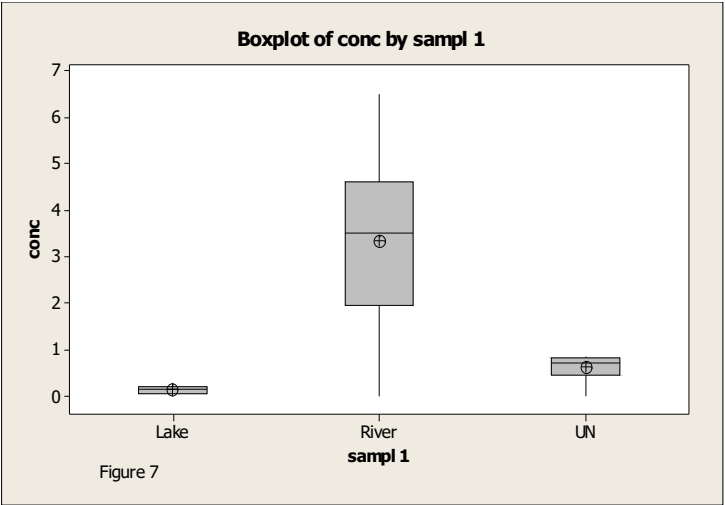
### *Colour development*

50ml of each standard solution including that of lake, river and unknown water sample were pipette into a conical flask and one drop of phenolphthalein indicator was added. 8ml of the combined reagent was added to each solution and was allowed to stand for 30minutes. This was to allow the blue colour develop, the blank sample was treated as above but turned slightly blue. Phosphate reacts with ammonium molybdate to form  $(\text{NH}_4)_3\text{PO}_4 \cdot 12\text{MoO}_4$ , the molybdate that is bound with the phosphate forms the blue colour.

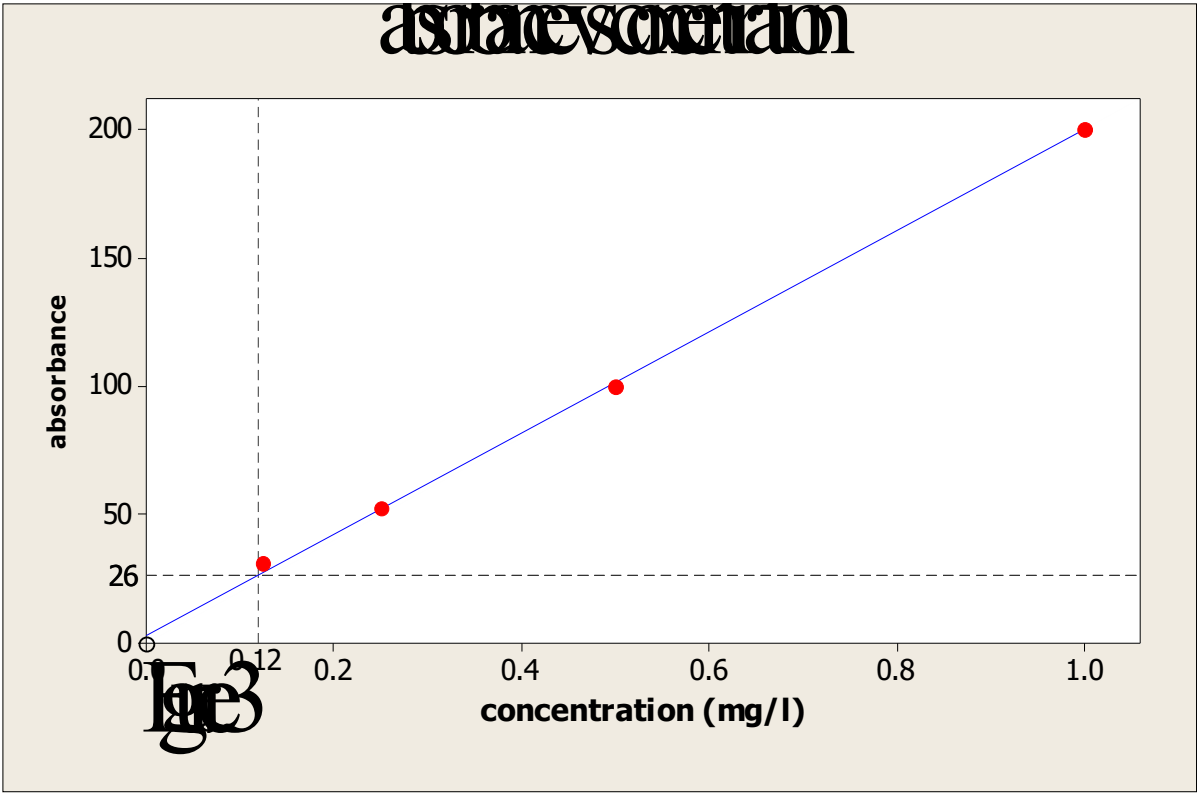
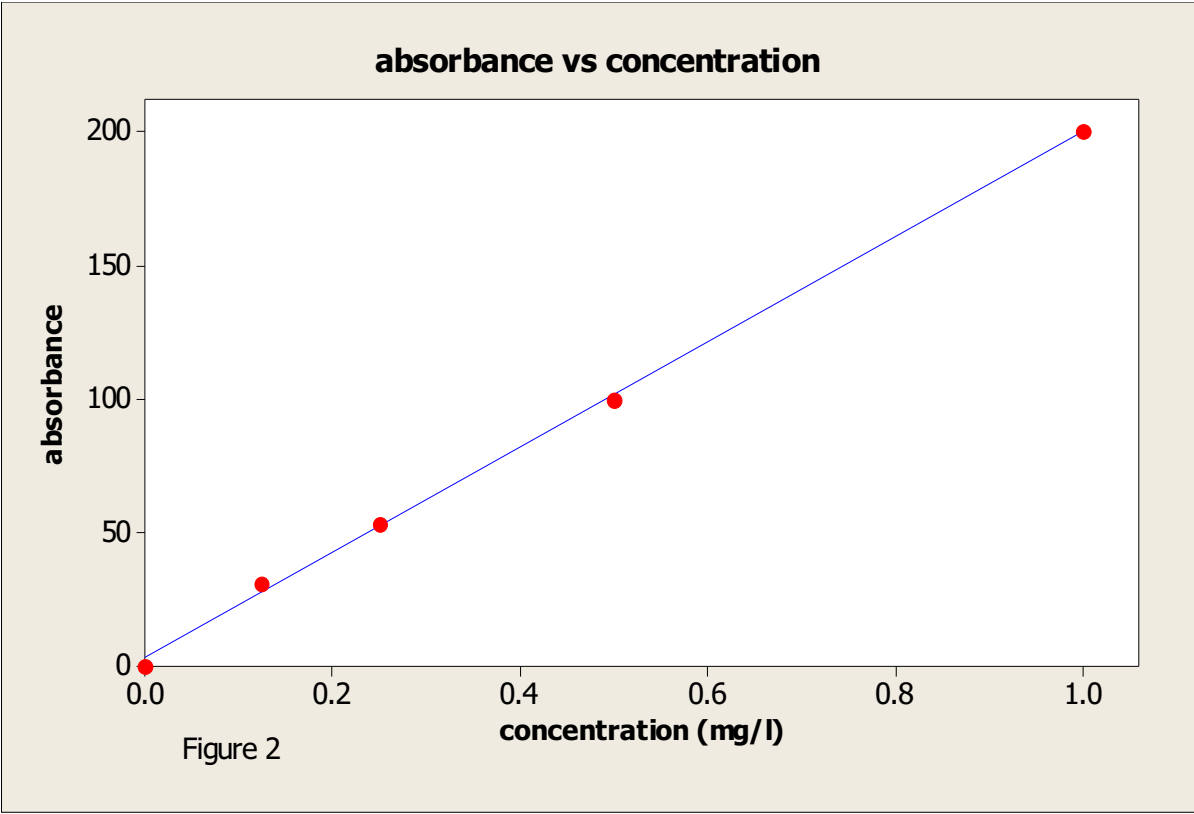
### *Spectrophotometer measurement*

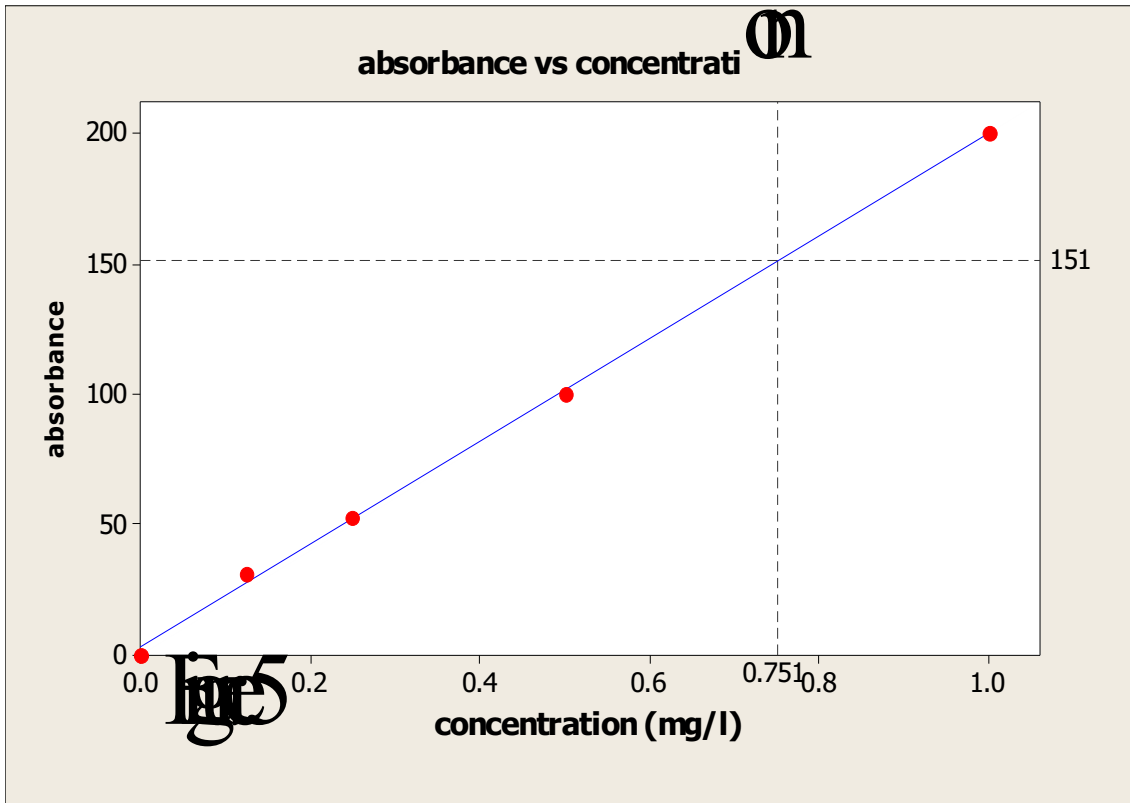
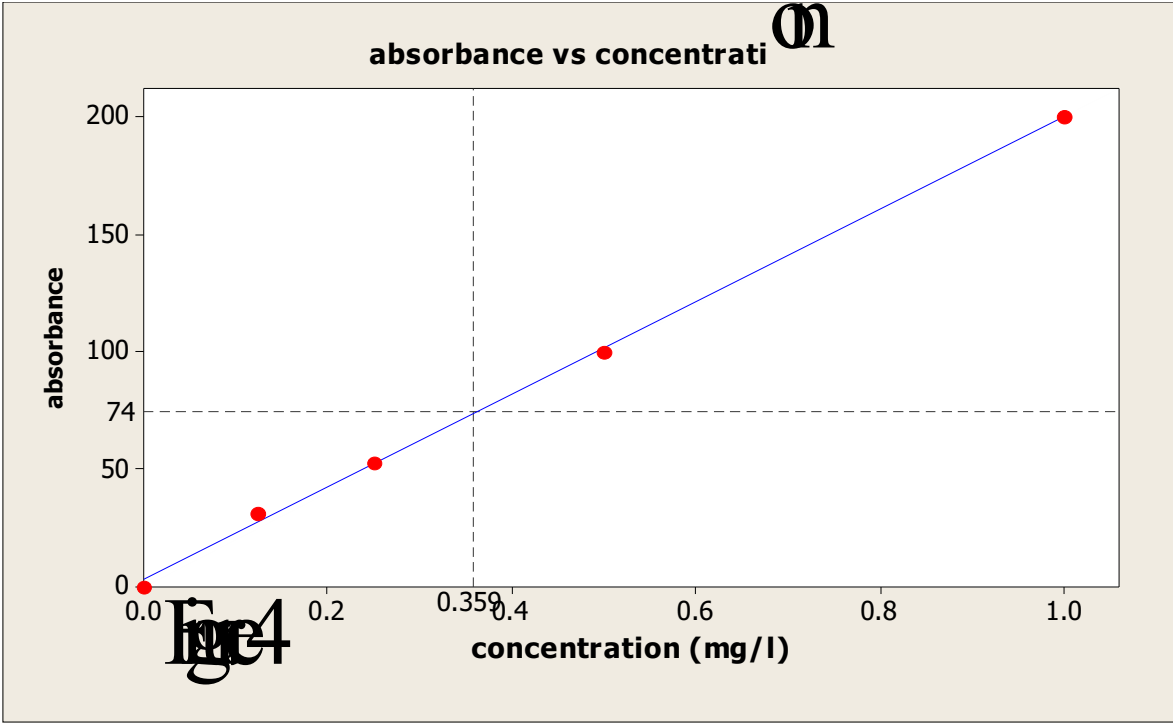
The samples were transferred into cuvettes and the absorbance readings were determined using a spectrophotometer (Pyu unicom uv- vis SP8390), the wavelength was set at 880nm. The values are shown in table (1) below.











# Results/Calculation

Spectrophotometric and Colorimetric method was used in determining the absorbance of the samples, various absorbance values were obtained from the spectrophotometer analysis, which are shown in table (1) below. The known concentration was plotted against the corresponding absorbance values figures (2,3,4 and 5). A 95% confidence interval was obtained for the accuracy of the spectrophotometer.

Groups	0.125 mg/l	0.250 mg/l	0.500 mg/l	1.000 mg/l	Lake A	River A	UN A	Lake mg/l	River mg/l	UN mg/l	River A (diluted)
1											
2	29	40	97	213	14	135	134	0.07	6.5	0.625	1228
3	<b>31</b>	<b>53</b>	<b>100</b>	<b>200</b>	<b>26</b>	<b>74</b>	<b>151</b>	<b>0.125</b>	<b>3.65</b>	<b>0.745</b>	<b>1096</b>
4	10	35	89	184	54	59	149	0.3	3.35	0.85	1387
5	12	34	98	177	34	74	151	0.19	4.0	0.82	1055
6	20	40	98	199	30	50	136	0.15	2.6	0.685	1123

Table (1) Spectrophotometric reading and final concentration values for lake, river and unknown water samples (class result)

Where;

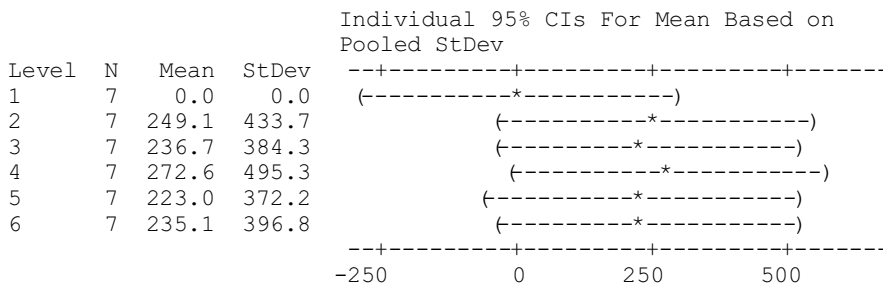
UN = Unknown

A = Absorbance

## One-way ANOVA: absorbance versus group

Source	DF	SS	MS	F	P
group	5	355235	71047	0.49	0.784
Error	36	5262417	146178		
Total	41	5617652			

S = 382.3    R-Sq = 6.32%    R-Sq(adj) = 0.00%

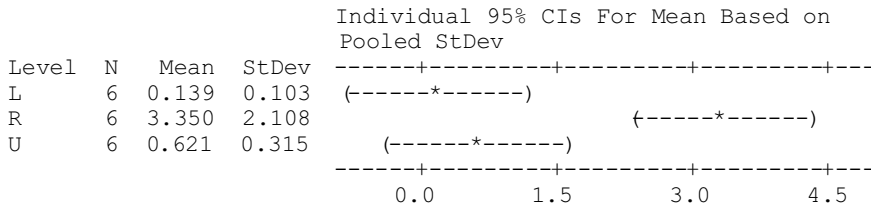


Pooled StDev = 382.3

## One-way ANOVA: concentration versus samples

Source	DF	SS	MS	F	P
sample	2	35.98	17.99	11.85	0.001
Error	15	22.77	1.52		
Total	17	58.75			

S = 1.232    R-Sq = 61.24%    R-Sq(adj) = 56.07%



Pooled StDev = 1.232

## Correlations: concentration, absorbance

Pearson correlation of concentration and absorbance = 1.000  
P-Value = **0.000**

## Regression Analysis: absorbance versus concentration

The regression equation is  
absorbance = 2.925 + 197.0 concentration

S = 2.77939    R-Sq = 99.9%    R-Sq(adj) = 99.9%

Analysis of Variance

Source	DF	SS	MS	F	P
Regression	1	24255.6	24255.6	3139.89	0.000
Error	3	23.2	7.7		
Total	4	24278.8			

## Calculation

The concentration values for lake, river and unknown water samples were determined from figures (3,4 and 5), however the values obtained by group 3 in class is different from the actual result obtained from the plot reason being that the final value from class result was estimated roughly without computer generation.

The lake absorbance value is 26;therefore, concentration from figure (3) is **0.12mg/l**.

River sample absorbance is 74 before dilution, the concentration from figure (4) is 0.359mg/l, however, sample was diluted by a factor of 10 because of over range during spectrophotometer reading. Therefore, the actual concentration of the river sample is **3.59mg/l**.

Unknown sample absorbance is 151; the concentration from figure (5) is **0.751mg/l**.

SAMPLE	Concentration (mg/l)
Lake	0.12
River	3.59
Unknown	0.751

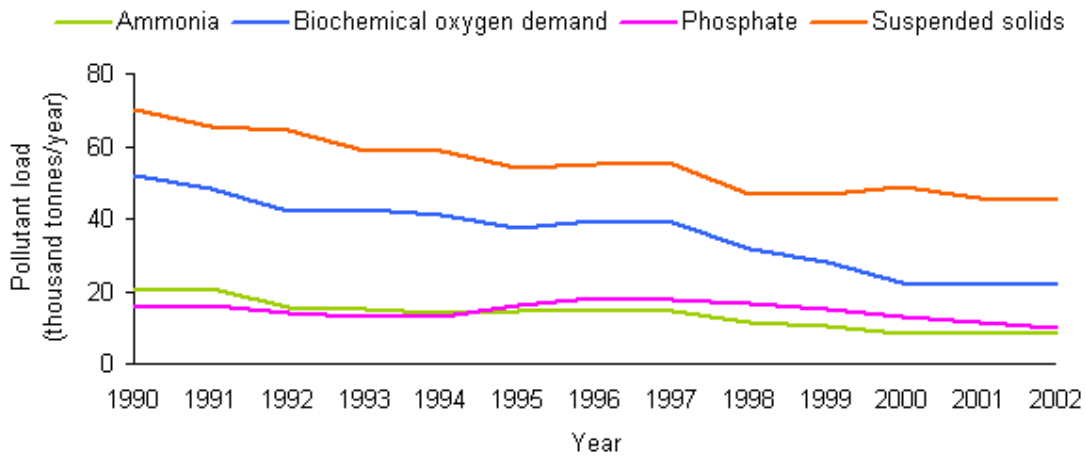
Table 2 concentration values of samples

Sewage treatment works pollution discharges in England and Wales, 1990 to 2002

Units: thousand tonnes/year

	Phosphate
1990	15.76
1991	16.28
1992	13.77
1993	12.73
1994	13.01
1995	16.30
1996	18.32
1997	17.67
1998	16.54
1999	15.02
2000	13.04
2001	11.33
2002	9.87

Source: Environment Agency



Source: Environment Agency

Figure 10

The value above shows how there has been a reduction in the phosphate discharge from sewage treatment plants in rivers in United Kingdom.

## Discussion

The spectrophotometric analysis was carried out using prepared standard solutions with known concentrations, different absorbance values were determined for each prepared sample including lake, river and an unknown water sample. However, before the analysis was carried out using spectrophotometer, it was observed visually, the effect of colour change on the samples. This showed clearly that there was a high phosphate concentration present in the river water as compared with the other samples, that is, lake and unknown. The concentrations of both samples lied within the colour range of the prepared standard solutions. From table (1), samples with high absorbance values were intensely coloured than the ones with lower absorbance values (using group 3 values).

The measured absorbance of the standardized solutions was plotted against the known concentrations to obtain a linear relationship, Pearson correlation of concentration and absorbance was determined and there is strong evidence to suggest that there exist a relationship between the concentration and absorbance. A further statistical analysis was carried out using regression, and there was very strong evidence that there is a relationship between absorbance and concentration, this therefore obeys Beer – Lambert's law. Furthermore, from the equation in figure (9), it can be deduced that an increase in increase in concentration there is also an increase in absorbance. Figure (2) shows clearly this relationship, the various concentration values of lake, river and unknown water samples were determined by extrapolating their absorbance values to the concentration values. This is illustrated in figures (3,4 and 5), the value of river water sample from the calculation above is higher than that of lake and unknown water samples. Figure (8), shows that this method can be used in the determination of phosphate present in water samples, the boxplot show that the degree of spread of lake and unknown water sample lie within the ranges of the standardized solutions. However, the river sample lies above the rest. Therefore this shows that the river sample has a high phosphate concentration.

### *Class result*

The problem with each group was limited, table (1) demonstrate that the result did not vary significantly from one group to another. However, from figure (7) and the statistical values obtained, it is evident that the mean phosphate concentrations for lake, river and unknown water samples are not equal. The mean phosphate level in the river water sample is 20 and 6 times greater than the lake and unknown water samples respectively. A 95% confidence interval level was determined and also the standard deviation, all showed clearly that the phosphate concentrations in the samples are not equal.

### *Spectrophotometer*

The use of Pyu Unicam UV – VIS can be attributed to its ease and speed of analysing samples, and also the absorbance data can be related to concentration. There are limiting factors that affect the reading from the spectrophotometer and these are;

- Lack of precision may arise from the many volumetric transfer of solution from one container to another.
- Stray light causes decrease in absorbance and reduces linearity range of instrument at high absorbance.
- Variation in lamp intensity and electronic output, this may lead to error in the value of the measurement.
- Noise from the uv – vis from the light source and electronic component.
- In absorbance spectrophotometer, at low absorbance that is low concentration, any change in  $I_0$  will give rise to a significant change in apparent absorbance. At high absorbance very little light is transmitted. (Cairns J. et al)

Standard deviation is the statistical method used in determining the accuracy of a spectrophotometer, from the statistical values obtained and from figure (6), there is evidence that the spectrophotometer reading was accurate. The standard deviation of all groups are almost equal, the spread from figure (6) shows that they are almost equal. There is also a 95% confidence interval that the equipment (spectrophotometer) reading was accurate, this can be attributed to the high p – value.



## **Conclusion/recommendation**

The phosphate level in lake, river and unknown water samples was successfully monitored using the Pyu Unicam UV – VIS. Concentrations in the lake and unknown samples did not exceed the mandatory or guideline levels in European legislation, however, the water sample level far exceeded the levels in the past 15 years, as shown in figure (1). Phosphate concentrations between 0.01 mg/l – 0.1 mg/l is enough to accelerate eutrophication (Peirce J.J et al. . . 1998). The recommended level acceptable on surface waters by European Union is between 0.22 – 0.3 mg/l (Directives 2000 / 60). With the statistical and empirical values obtained, the value of the river water sample far exceeded the set levels, therefore, the river water is rich in phosphate.

This experiment has familiarised each group on the working of the spectrophotometer and how it can be used to determine concentrations using the Beer – Lambert's relation. This experiment shows the relationship between absorbance and concentration, from the statistical data and figures (2 & 9) there exist a relationship between absorbance and concentration.

## **Recommendation**

- Further studies should be carried out on the river water sample so as to inform the appropriate authorities.
- Ion chromatography can be used instead of spectrophotometer because it does not depend on light absorption and colour intensity.
- The Pyu Unicam UV – VIS SP 8390 should be upgraded with a newer version.

## References

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[www.who.int](http://www.who.int)

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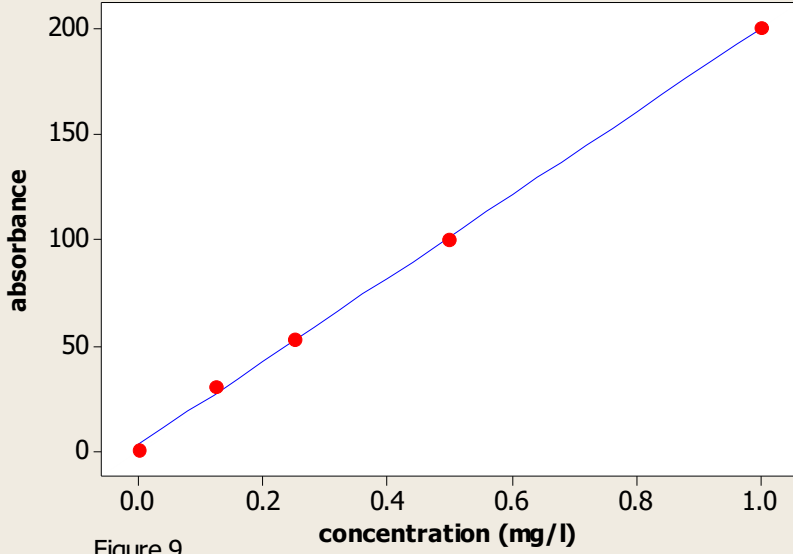
[www.eea.eu.int](http://www.eea.eu.int)

[www.environment-agency.gov.uk](http://www.environment-agency.gov.uk)

[www.nhm.ac.uk/mineralogy/phos](http://www.nhm.ac.uk/mineralogy/phos)

### Fitted Line Plot

$$\text{absorbance} = 2.925 + 197.0 \text{ concentration}$$



S	2.77939
R-Sq	99.9%
R-Sq(adj)	99.9%

Figure 9