

Detection and Determination of Phosphorus in Water Samples Using Optimized and Portable Technology

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Abstract: Detection as a method for determining the presence of a chemical or biological agent is a vital function within the domain of environmental monitoring and remediation. Nutrient pollutants (NPs), mainly nitrites, nitrates and phosphates that are applied commonly as fertilizers throughout American Midwest agricultural zones, have added to the problems of Eutrophication of streams and other bodies of water that has been established among many researchers [1-4]. In this present work, we used colorimetry and ion chromatographic methods for detection and determining the presence of orthophosphate from Grays Creek in Jefferson City, Missouri, and try to introduce a sensitive and less expensive method. The margin of error between the ion chromatographic (IC) instrument and the handheld optical sensor was found to be 10.35%. The absorbance was taken at 610nm, the concentration ranged from 0.3-13.93ppm and the electric conductivity (EC) ranged from 236-418 μ S/cm. Normality Test (Shapiro-Wilk) on the IC data revealed passed ($P= 0.787$) whereas the equal Variance Test (Brown-Forsythe) has shown failed ($P < 0.050$). Going by the Holm-Sidak method, the differences in the mean values among the treatment groups are greater than would be expected by chance; there is a very statistically significant difference ($P = <0.001$), while with handheld optical sensor on the hand, both the Normality Test (Kolmogorov-Smirnov and Equal Variance Test (Brown-Forsythe) Passed with P-values 0.155 and 0.713 respectively.

Keywords: Eutrophication, colorimetry, ion chromatography, fertilizer runoff, nutrient pollution, handheld optical sensor, detection

1.Introduction

Nitrogen and phosphorus are essential nutrients for all living organisms that are required to support the growth of plants, feed animals, and ultimately provide adequate nutrition for an expanding global population. Phosphorus is an essential element for all forms of life, particularly with regards to storage and transfer of energy through phosphorylation, which is a process of turning on many protein enzymes to play a vital role in a broad range of cellular processes. It is necessary for seed and root formation as well as a constituent of nucleic acids, cytoplasmic membranes, teeth and bones [5]. To maintain a healthy ecosystem, these environmental nutrients must be properly balanced. However, in several developed and rapidly developing regions of the world, excessive nutrients enrichment has caused significant water quality and health issues. Over the last 50 years, the use of nitrogen fertilizers on a global scale increased 9-fold, while phosphorus use has tripled. In the next 40 years, the global mineral fertilizer consumption for nitrogen and phosphorus is estimated to rise to about 40–50%, to feed a growing world [6]. For example, in the United States, 89% of total N inputs into the Mississippi River come from agricultural runoff and drainage [7]. Although there are still major areas of cropland in the Midwest of United States that are rated as having high N balances, resulting in soils highly susceptible to losses of N_2O to the atmosphere, the N input to output balances have become narrowed with slight increase in overall N fertilizer consumption since 1975 [8]. The impact of nutrient pollution in the U.S. has been identified as one of the most widespread, costly, and challenging environmental issues of the 21st century. Nutrient pollution causes excess algae growth (i.e., algal blooms) in bodies of water, which has a significant impact on the environment, human health, and the economy. Algal blooms consume a significant

amount of oxygen due to subsequent decomposition and thus deprive fish, shellfish, and other aquatic organisms of the oxygen needed to survive. In addition, algae can have a negative impact on human health by emitting toxins that can cause stomach aches, rashes, and more serious health issues. It is estimated that the U.S. tourism industry loses approximately \$1 billion annually because of algae-related decreases in fishing and recreational activities [9]. Nixon (1995) defined eutrophication as “an increase in the degree of supply of organic matter to an ecosystem,” most commonly caused by nutrient enrichment [10]. Sources of eutrophication in coastal marine environments are often anthropogenic in nature and include agriculture runoff, human sewage, urban waste, industrial effluent, and fossil fuel combustion [11]

2.Materials and Methods

2.1 Site Description

The sample collection site is Grays Creek, (Fig.1); the stream is geographically located between latitude 38.603090°N and longitude -92.2057396°W with an approximate elevation of 574 feet (175 meters) above sea level. Although the site is not listed by the Missouri Department of Natural Resources as being an impaired creek, the site was chosen due to its sampling access, proximity to the laboratory so that no sample preservation was required, and “Grays Creek empties into Missouri River which connects with Mississippi River and finally emptied into the Gulf of Mexico where there are dead zones due to these nutrient pollutants. In this present work, colorimetric EPA method 365.2 approved by EPA and APHA was used to determine the concentrations of phosphorus in water samples using a Handheld Optical Sensor and Ion Chromatography. Water

samples were collected from a flowing source in Grays Creek during summer 2016, fall 2016 and spring. The three sampling points were located using Trimble Geo 7X and the coordinates were post-processed with Geo Explorer Pathfinder Software to obtain more accurate positions. The map was produced using Arc Map 10.3 coupled with ArcSWAT.

Google Earth Pro was used for georeferencing. Sample collections were randomized within the three locations to prevent bias and with four replicates per sample. Samples were collected in thoroughly clean polyethylene (Nalgene) bottles by soaking them overnight with a de-ionized (DI) water and drying them. The collected samples were immediately brought back to the lab and analyzed.

2.2. Ion Chromatography (IC)

Ion Chromatography was used to determine the concentration of phosphate in the water samples utilizing a Thermo Scientific™ Dionex™ ICS-5000 + Reagent-Free HPLC System (Dionex™ Corp., Sunnyvale, CA). Since the ICS-5000+ system regenerates the eluent for reuse, variations in eluent preparation procedures were eliminated. Stable eluent concentration yields reproducible results, with little variability in peak retention times or areas. The system does not need to be recalibrated as it would if eluent were prepared manually. However, accuracy was routinely checked by the inclusion of quality assurance samples, calibration standards, blanks and duplicates in the analysis. The equipment is Thermo Scientific™ Dionex™ ICS-5000+ RFICTM system equipped with Thermo Scientific™ Dionex™ DP Dual Pump module, Thermo Scientific™ Dionex™ EG Eluent Generator module, Thermo Scientific™ Dionex™ DC Detector/Chromatography module, Thermo Scientific™ Dionex™ AS-DV Autosampler, Thermo Scientific™ Dionex™ EGC III KOH cartridge, Thermo Scientific™ Dionex™ CR-ATC Continuously Regenerated Anion Trap Column, utilizing Thermo Scientific™ Dionex™ Chromeleon™ 7 Chromatography Data System - Version 7.2

The calibration standards were prepared from Dionex Seven Anion Standard II (Thermo Scientific, Sunnyvale, CA) stock solutions at concentration levels; 0.2, 2, 6, 20, 100 and 200 ppm. Chromeleon™ 7 Chromatography Data System was used to develop calibration curves and calculate sample concentrations. Calibration standards were evaluated for linearity. Peaks were evaluated on their area. The minimum coefficient of determination accepted was 0.9990. The maximum acceptable relative standard deviation was 5.0%. During the analysis for the dissolved ortho-phosphate, the samples were filtered using 10 ml syringe coupled with 0.45µm filter from Fisher Scientific. Samples, calibration standards and blanks were dispensed into 5 mL PolyVials™ and capped with PolyVials™ filter caps (Thermo Scientific™, Sunnyvale, CA). As for the conditions maintained for the phosphate determination, the columns: Guard Column Dionex IonPac™ AG19, 4 × 50 mm, Separator Column Dionex IonPac™ AS19, 4 × 250 mm are maintained at 30 °C.

The eluent Source: Dionex™ EGC III KOH cartridge with Dionex™ CR-ATC column. A multi-step gradient was

regulated at 20mM KOH from 0-12 min, 20-55mM KOH from 12-22min and 55-20mM KOH from 22-25min. The Injection Volume was 25 µL with a Flow Rate: 1.0 mL/min. Detection was through suppressed conductivity, Thermo Scientific™ Dionex™ ASRS™ 300 4 mm. The suppressor current and the temperature for the detector were kept at 149 mA and 30 °C respectively.

2.3. Handheld Optical Sensor

The standards for the Handheld Optical Sensor were prepared from a crystalline certified ACS potassium phosphate monobasic. Other reagents for the test include the ammonium molybdate(VI), technical grade stannous chloride anhydrous etc. and the required concentrations were prepared using de-ionized water (refer appendix for reagents preparation). The DR900 Hach Unit with a wavelength ranges 420, 520, 560, and 610nm having accuracy of +/- 1.0nm was used for the detection of orthophosphate in the water samples. The instrument required AA Alkaline batteries as a source of power and has a USB mini interface. The lamp source was Light emitting diode (LED) and the detector was Silicon photodiode. The sample cells were 16mm, 1 cm/19mL, 1- inch (25mm) round. The photometric linearity and repeatability were both ±0.002Abs (0-1 Abs). The instrument was equipped with a data readout graphical display of 240 x 160 pixel.

2.4. Statistical analysis

Data analysis was performed using Systat Statistical Software (SigmaPlot; Version 13). The differences between the mean values were calculated using One-way Analysis of Variance (ANOVA) test. Measurements were replicated at least twelve times and the data results were represented by the mean ± Standard Error of the Means (SEM). The statistical significance of the difference between the mean values was determined by post comparison Holm-Sidak method with * *p* value of 0.05.

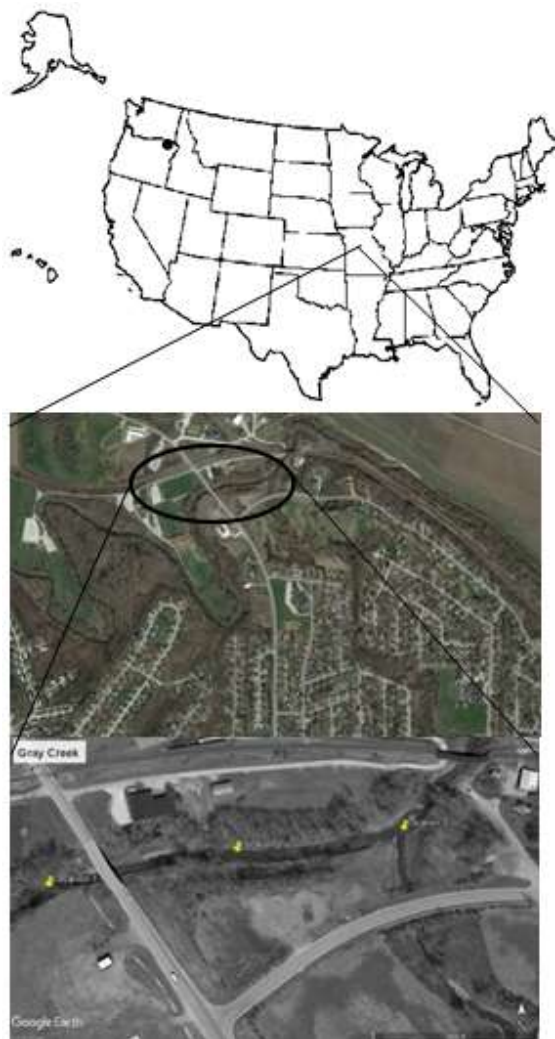


Figure 1: Location of Grays Creek and Sample collection points.

water contributed to the creek was from the surrounding areas which are some farmlands, fields and residential sites.

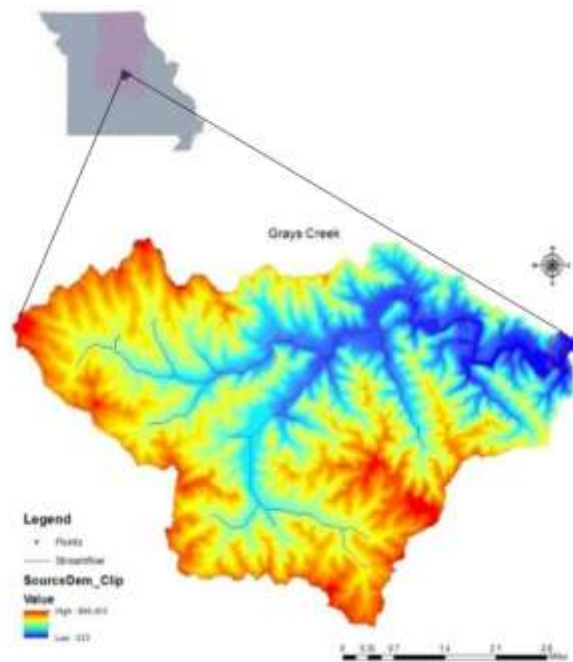


Figure 2: Drainage network and sample collection points in Grays Creek

3. Results

3.1 Delineation of Channel Networks in Grays Creek

The three little red dots (points) to the extreme righthand side of the map in the Figure 2 indicates the sample collection points; the bands as leveled are the red for the high elevation of 846.455 meters and blue as low elevation of 533 meters which indicated that the water is draining and finally empties into the Missouri River Location one was found to contain higher phosphorus concentration as compared to location two and three which contain flowing water. This may be due to the fact that location one contains a stagnant pool of water as compared to locations two and three which are running. The sample preparation procedure and running time for the Ion Chromatography (IC) is higher compared to that of the handheld optical Sensor with about 1hr 30 mins for equilibrating the instrument and 20 more minutes for the sequence run, whereas it only takes about 30-35 mins with the handheld optical device. The electric conductivity (EC) determined during summer happened to be higher but I did not consider the spatial and temporal distribution of the water, my main concern was the detection and determination the concentration of phosphate alone. The delineated drainage network in the creek indicated that most of the

3.2. Colorimetric Result for phosphate

The experiment was conducted using the specific materials in the materials and methods section. The serial dilutions were made as 1ppm, 3ppm, and 5ppm, by taking 10mL from the stock and diluting it to 100mL, and then 1mL, 3mL, and 5mL were pipetted and placed into labeled flasks# 1,2, and 3 and the concentration were verified using the ion chromatography instrument by comparing them with that of the instrument anion standards and the anion standards.

Table 1: Validation of the reagents prepared for the handheld optical sensor by means of ion chromatography.

Injection RetentionName	Retention Time	Amount
	min	ppm
IC PO40.2 ppm	16.77	0.102
IC PO42ppm	16.76	1.583
IC PO46ppm	16.76	5.337
HandHeld PO4 1ppm	16.87	0.761
HandHeld PO4 3ppm	16.85	3.034
HandHeld PO4 5 ppm	16.90	4.868

Table 1 indicates the validation of the prepared reagents to be used in the handheld optical device. The reagents prepared at various concentrations were run by ion chromatography and then their concentrations were compared with that of the standards in the ion chromatographic instrument.

3.3. Handheld optical sensor standard reagents validation using IC

Table 1 indicates the prepared standards to be used in the handheld optical sensor were validated using ion chromatographic instrument to ascertain accuracy. The Milli-Q is the DI water used as a rinse. The retention time

represent the time it takes for the component to elute out of the column and it was measured in minutes. The amount represents the concentrations which were recorded in ppm. The anion standards, labeled S0.1ppm – S3 ppm were prepared in such a way that their concentrations are doubled and the PO₄ are the phosphate (PO₄³⁻) standards 1ppm-5 ppm prepared to be use in the handheld optical device. An aliquot of 50 mL of sample solution was then taken in 125mL conical flask and aliquot 4 mL of ammonium molybdate was added slowly followed by the addition of 4-5 drops of stannous chloride with through mixing after each addition. Samples were left unshaken for 10 minutes for the blue color development. The chemistry behind it is that the ammonium molybdate produces $\text{PMo}_{12}\text{O}_{40}^{3-}$ anion which is then reduced by SnCl_2 to form a blue complex of β -keggin ion $\text{PMo}_{12}\text{O}_{40}^{7-}$ [12]. The amount of blue coloration produced is in direct proportion with the quantity of phosphate ions inside the solution. A vial with 10mL size was then filled with processed sample and then inserted into the instrument and the absorbance was measured and recorded. The absorbance was measured at 610nm using the handheld optical Sensor and the results were recorded. The plot of absorbance as a function of concentration was obtained and the R² – value was determined (Fig. 3)

Table 2: Indicates validation of the reagents prepared for the handheld optical Sensor by means of Ion Chromatography

Sample#	Concentration (ppm)	Absorbance(nm)
1	1	0.368
2	3	0.458
3	5	0.774

plot of absorbance versus concentration was done to obtain the calibration curve for the handheld instrument and then it was used to measure the concentration of the samples. The slope was found to be 0.0123 and the intercept along the y-axis was found to be 0.1679 according to the straight-line equation represented in the plotted area in Figure 3.

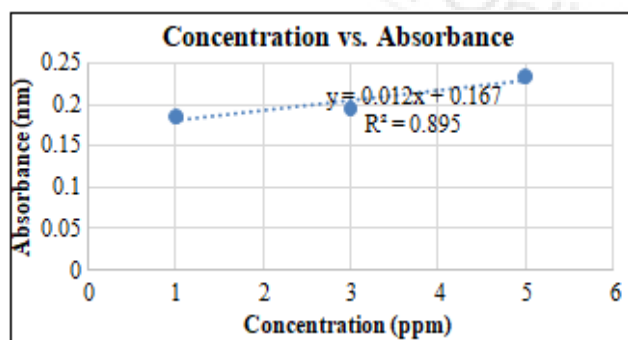


Figure 3: Plot of Absorbance versus Concentration

3.4. Ion Chromatographic Result for Phosphate

Table 3: Phosphate parameters

Injection Name	Retention Time	Amount
	min	ppm
Milli-Q	n.a.	n.a.
S0.1 ppm	17.051	0.1735
S 1 ppm	17.067	1.8610
S 3 ppm	16.958	5.7802
Location1	17.074	0.1725
Location1D	17.048	0.1503
Location2	17.071	0.0941
Location2D	17.054	0.1110
Location3	17.101	0.0901
Location3D	17.058	0.1004

Table 3 Phosphate retention time, amount in ppm.

Table 4: Measured concentrations, peak area and labeled concentration of Phosphate

Sample	Measured	Area	Labelled
	ppm	μS*min	ppm
Anion Mix - 0.1 ppm	0.174	0.017	0.2
Anion Mix - 1 ppm	1.861	0.180	2
Anion Mix - 3 ppm	5.780	0.560	6

Table 4 is basically used to validate the calibration curve obtained from the standards. The phosphate calibration curve (Fig. 4) was generated automatically and the data was then processed using the chameleon software to refine the data and eliminates unnecessary peaks that may alter the true coefficient of determination (R² value).

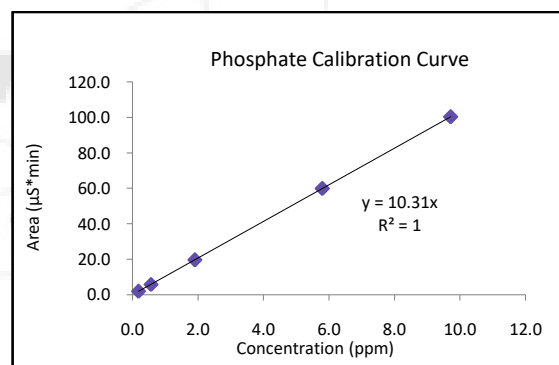


Figure 4: Phosphate Calibration Curve

After initial analysis, the samples were analyzed again after 30 days, a validation curve was constructed using Microsoft Excel to check the quality of the anionic standards used in the ion chromatographic instrument. This is based on the fact that the samples to be tested can be stored for about 28 days, so the validation of the standards is usually checked around 30 days to make sure it is fit to test for the concentrations.

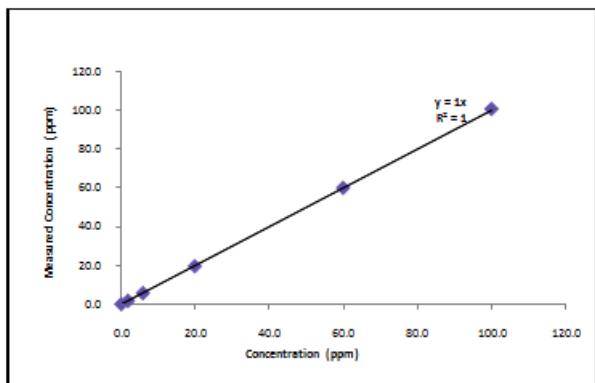
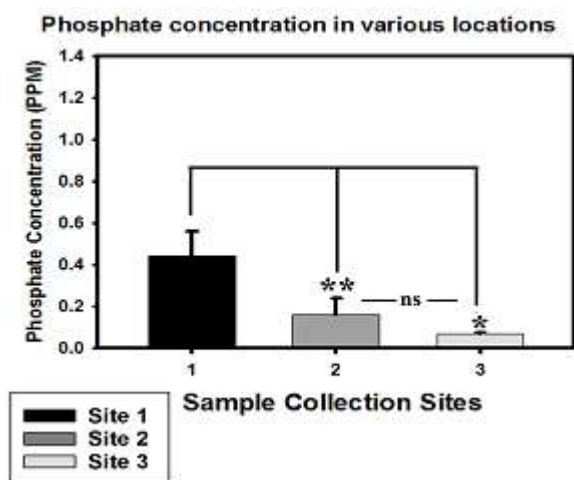


Figure 5: Phosphate Validation Curve

Keys: S = Anionic Standard, labeled = Known

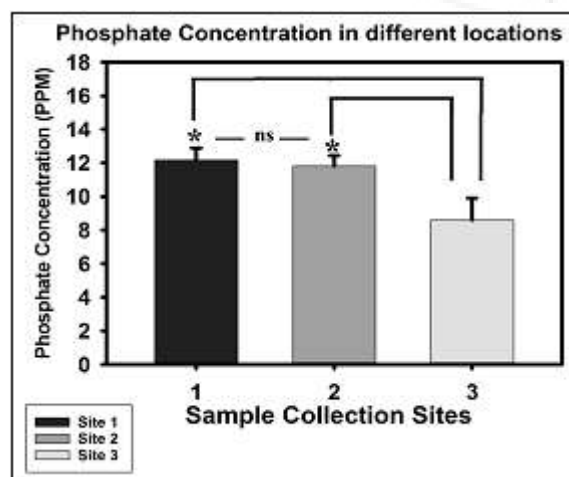
3.5. Ion Chromatography



*ns = Statistically not significant

Figure 6: Phosphate Concentration determined with Ion chromatography

3.6. Handheld Optical Sensor



*ns = Statistically not significant

Figure 7: Phosphate Concentration determined with Optical handheld sensor

4. Discussion

The absorbance was first taken at 429 nm looking at the fact that the blue region of the electromagnetic spectrum is around 400nm, but the result came up with a very low coefficient of determination of 0.113 and 0.2316 which is way low compared to what they should be which is around 0.999. The optimization of the procedure was continued by changing the wavelength to 610nm. The R^2 square values were raised to 0.60, 0.7128 and so on (Refer appendix Figure: 8 and 9) but still there wasn't consistency and then later, the experiment was run without adding phenolphthalein indicator and that was when the result started to make more sense. Although the handheld optical Sensor procedure took time before it was figured out and optimized, there is still need for some work to be done especially optimizing the handheld optical sensor itself. Comparably, it is still easier to use the handheld optical sensor than to use the ion chromatographic instrument which has a complex data processing issue during refining of the result and is time consuming and requiring an expertise. There is an improvement and tendency of upgrading the optical sensor to reach potentiality. The optical detection method provides a promising result of being sensitive in detection of statistically significant differences in phosphate levels in different geographic locations that confirms at least partially with their IC counterpart, less time consuming and cheaper as compared to buying reagents from the company as I have run a sample at a rate of 60 cents compared to the company's rate at \$1.14, while when considering the cost of running a sample in the ion chromatography, it cost \$5.50 (see cost run analysis in the appendix).

4.1. Comparison of the two Instruments

4.2. Detection-wise and comfortability

The detector used in the ion chromatography (IC) is a conductivity detector whereas in the handheld optical Sensor is a light emitting diode (LED). The IC can detect up to 100ppb (part per billion) whereas the handheld optical Sensor has a wavelength range of 420nm, 520nm, and 610nm and measures concentration only in part per million (ppm). The power source in the IC is alternating current whereas in the handheld optical sensor is 4 triple A size alkali cells. In term of portability, the handheld is far more portable and comfortable to use with less procedural processes compared to the IC which has a long sample analytical procedure and cannot be moved from one sample site to another.

4.3. Timing

Time wise, the running time for the IC from sample preparation where you will filter the sample, going into the chameleon window and set the parameters to bring it up to run sequence will take roughly about 1 hour and 30 minutes and it takes 22 minutes again to run the sequence, but with the handheld optical device, it takes not more than 35 minutes to run a sample. There is no issue of purging in the handheld optical sensor whereas with IC, whenever there is an air bubble in the system due to suction of air from the sample vial space, the system needs to be purged.

4.4. Cost and cost run analysis

The ion chromatography instrument cost \$60,000 whereas the handheld optical sensor can cost only about \$1341. (Refer to appendix Cost analysis).

5. Conclusion

In conclusion, there is every tendency that if this optical sensor is upgraded and the procedures were optimized and used to determine the concentrations of the remaining nutrient pollutants, it could be a promising sensor substitute for the costly and bulky ones that we do have in the laboratories.

References

- [1] Phosphorus in rivers--ecology and management. *Mainston CP, Parr W. Sci Total Environ. 2002 Jan 23; 282-283(1):25-47.*
- [2] Fang YY, Yang XE, Pu PM, Chang HQ, Ding XF. Water eutrophication in Li-Yang Reservoir and its ecological remediation countermeasures. *Journal of Soil and Water Conservation. 2004;18(6):183-186*
- [3] Tong CH, Yang XE, Pu PM. Degradation of aquatic ecosystem in the catchment of Mu-Ge Lake and its Remediation countermeasures. *Journal of Soil and Water Conservation. 2003;17(1):72-88.*
- [4] Palaeoecology, reference conditions and classification of ecological status: the EU Water Framework Directive in practice. *Andersen JH, Conley DJ, Hedal S. Mar Pollut Bull. 2004 Aug; 49(4):283-90.*
- [5] Havlin, J.L., J.D. Beaton, S.L. Tisdale, and W.L. Nelson. 1999. Soil fertility and fertilizers: An introduction to nutrient management. 6th ed. Prentice Hall, Upper Saddle River, NJ.
- [6] Brian M. De Borba, Richard F. Jack, Jeffrey S. Rohrer, Joan Wirt, Dongmei Wang., Simultaneous determination of total nitrogen and total phosphorus in environmental waters using alkaline persulfate digestion and ion chromatography. *Journal of Chromatography A, 1369 (2014) 131-137. doi.org/10.1016/j.chroma.2014.10.027*
- [7] EPA. Nutrient Pollution > The Effects: Economy [website]. Washington, DC: Office of Water, U.S. Environmental Protection Agency (updated 16 March 2014).
- [8] Millar N, Robertson GP, Grace PR, Gehl RJ, Hoben JP (2010) Nitrogen fertilizer management for nitrous oxide (N₂O) mitigation in intensive corn (Maize) production: an emissions reduction protocol for US Midwest agriculture. *Mitig Adapt Strateg Glob Change 15: 185-204*
- [9] EPA. Nutrient Pollution > The Problem [website]. Washington, DC: Office of Water, U.S. Environmental Protection Agency (updated 16 March 2014). Available: <http://www2.epa.gov/nutrientpollution/problem> [accessed 29 October 2014].
- [10] Nixon, S. W. (1995) Coastal marine eutrophication: a definition, social causes, and future concerns. *Ophelia, 41, 199-219*
- [11] Christian Jessen, Christian R. Voolstra, and Christian Wild., In situ effect of simulated overfishing and eutrophication on settlement of benthic coral reef

invertebrates in the Central Red Sea, 2014 Apr 8. DOI: 10.7717/peerj.339

- [12] A single solution method for the determination of soluble phosphate in sea water", Murphy J., Riley J.P., J. Mar. Biol. Assoc. UK, 1958, **37**, 9-14

Appendices

Cost Run Analysis of the experiment.

For the handheld instrument

Our Cost

The handheld optical sensor cost \$1341

1. Ammonium molybdate(IV)..... \$55.26 for 200g

Therefore, 25g will cost \$13.815

For 25g in 500mL of De-ionize water (DI water) will be enough to run 120 samples.

DI water from RICCA \$22.62 for 1 Liter

Therefore, 500mL will cost \$11.31

2. Stannous Chloride \$55.05 for 100g

Therefore, 2.5g will cost \$1.376

Glycerol ...\$162.02 for 500mL

Therefore, 100mL will cost \$32.404

3. Phenolphthalein Indicator ... \$ 33.76 for 100g

Therefore, 0.5g will cost \$0.1688

Ethanol \$61.47 for 500mL

Therefore, 100mL will cost \$12.294

Total cost to run 120 samples will be \$ 71.3678 ≈ \$72 (0.60 cents per sample run)

Company's Cost

- 1) Phosphate Standard Solution, 1mg/L as PO₄ (NIST), 500 mL ... \$24.85

- 2) Phosphate Standard Solution 500mg/L as PO₄ (NIST) PK/16 - 10mL Voluette ampules (16 Qty). \$59.19

- 3) Stannous Chloride Solution 100mL \$ 45.15

- 4) Ammonium Molybdate Reagent 100mL \$ 14.89 X 5 = 74.45

- 5) Glycerin (Glycerol) 500g..... \$ 48.95 / 5 = \$ 9.79

- 6) Phenolphthalein Indicator pillow (powder) pk/100 ... \$18.39. Therefore, 2.5g will cost 0.09

- 7) De-ionize water 4L \$27.39/4 will cost \$ 6.84

Total = \$136.32 (\$1.136 per sample run)

For Ion Chromatography Instrument's Cost

Ion Chromatography Thermo Scientific Cost \$60,000

Anion standard 200\$ (\$1 per injection) Anionic Cartridge

1200\$ for 6 months

Vial and Cap \$1

Syringe and filter \$950 per 1500

Injection of eluent \$2 for 6 months

Column and Suppressors \$2400 (\$200 a month per 50 samples)

(\$5.50 per sample run)

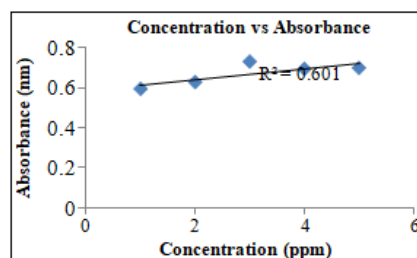


Figure 8: Low value of coefficient of determination

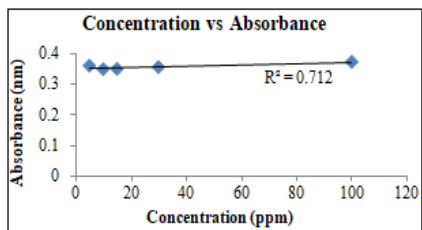


Figure 9: Low value of coefficient of determination

