

**DETERMINATION OF PHOSPHORUS SPECIES IN WATER BY  
CHROMATOGRAPHIC, COLORIMETRIC AND SPECTROMETRIC  
TECHNIQUES**

by

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## **DECLARATION BY CANDIDATE**

“I hereby declare that the dissertation/thesis submitted for the degree  
Magister Technologiae: Chemistry, at Tshwane University of Technology, is my own original work  
and quotes are indicated and acknowledged by means of a comprehensive list of references”.

TP Khumalo.

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## FOR

My mother, Nomfanekiso Thabanini KaMasoka, who, by her bravery and commitment, fought for her children's education.

In loving memory of my grandmother, KaMgqamela.

“Nhliziyo ungekhohlwe.”

To my son Kaelo “Robala ka khotso.”

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Finally to my caring husband, Sello, and my two sons, Sbonelo and Tshiamo.

“Love, faith, hope and, most of all, life in me, is your reason.”

## ABSTRACT

Excess nutrients in wastewater, mainly phosphorus can cause an increase in chemical requirements in water treatment, eutrophication, excessive growth of algae and other aquatic plants which can cause a deterioration in the aesthetic value of the receiving water body, destruction of the habitat and depletion of dissolved oxygen. This process in turn causes death of aquatic life because of the lowering of the oxygen level. In South Africa a special phosphate standard was introduced restricting the concentration of phosphorus in wastewater discharges to 1.0 mg P/l as orthophosphate according to Government Gazette, 1984 (quoted by De Haas and Wentzel, 2000:439). It is for these reasons that much attention has been given to phosphorus determination and removal in the sewage treatment process.

Different techniques are used in phosphorus determination. However, the choice and suitability of the best techniques raises concern. It is therefore the purpose of this dissertation to review some methods used in analysing phosphorus and identifying forms of phosphorus that are actually being measured. Four sensitive analytical methods were investigated for the analysis of phosphorus in water and wastewater, that is, Ion Chromatography (IC), Flow Injection Analysis (FIA), Inductively Coupled Plasma Optical Emission Spectrometry (ICPOES) and Inductively Coupled Plasma Mass Spectrometry (ICPMS). Sample preparation as a factor was examined by looking at FIA samples prepared using two methods.

The investigation showed that understanding sample type is essential when selecting a technique for phosphorous analysis in water and wastewater samples. Further, significance

cost savings can be achieved if the correct option is followed. For example, the colorimetric determination of phosphorus in a composite effluent sample shows equivalent phosphorus result to those determined by ICPOES. Therefore, at low concentration levels, a single method can be used for determination of phosphorus instead of using both techniques with equivalent results. Further, the study has determined that the FIA persulphate phosphorus concentrations are equivalent to the ICPOES phosphorus concentrations for both influent and effluent samples.

**Keywords:** phosphorus, eutrophication, influent, effluent, water quality, colorimetric, chromatography, spectrometry.

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## ABBREVIATIONS AND ACRONYMS

The chemistry notation and other abbreviations tabled below were used in this dissertation.

<b>ABBREVIATION</b>	<b>FULL NAME</b>
ATP	Adenosine triphosphate
DNA	Deoxyribonucleic acid
DWAF	Department of Water Affairs and Forestry
EBPR	Enhanced Biological Phosphorus Removal
FIA	Flow Injection Analyser
IC	Ion Chromatography
ICP	Inductive Coupled Plasma
ICPOES	Inductive Coupled Plasma Optical Emission Spectrometry
ICPMS	Inductive Coupled Plasma Mass Spectrometry
OP	Orthophosphate
RNA	Ribonucleic acid
SANAS	South African National Accreditation System
TP	Total phosphorus

## GLOSSARY

- Effluent - Wastewater or other liquid - partially or completely treated - flowing from a tank, treatment process or treatment plant.
- Eutrophication - The process of nutrient over-enrichment, usually phosphorus, leading to excessive growth of algae and other aquatic plants.
- Influent - Wastewater or other liquid partially treated, flowing into a tank treatment process or treatment plant.
- Limnology - Study of physical, chemical and biological characteristics of the fresh water of rivers and lakes.
- Pollution - Any interference with the use or reuse of water, or a failure to meet quality requirements.
- Sewage - The liquid conveying the wastes from domestic dwellings, including both the human excrete and the “grey water” influent resulting from dishes, clothes etc.
- Wastewater - The water discharged from factories and industrial premises that may or may not involve sewage.

## CHAPTER 1

### INTRODUCTION

#### 1.1. BACKGROUND: WHY PHOSPHORUS ANALYSIS IS IMPORTANT IN WATER AND WASTEWATER

Phosphorus is one of the major nutrients in water, as well as one of the essential elements required for growth of plants and animals. It is a constituent of nucleic acids, phosphor lipids and numerous phosphor-related compounds.

According to Twort, Ratnayaka and Brandt (2000:216), phosphates originate mainly from sewage effluents that contain phosphate-based synthetic detergents, industrial effluents and land run-off where inorganic fertilisers are used for farming. Schaum and Cornel (2005:57) pointed out that phosphorus is an essential nutrient in fertiliser and cannot be replaced.

Twort, Ratnayaka and Brandt (2000:201-300) mentioned that one of the effects of wastewater effluent discharge in water causeways and dams is eutrophication. Kennedy (1997:96) stated that in freshwater receiving waters, phosphorus has generally been considered the limiting nutrient in eutrophication.

Excess nutrients in wastewater, mainly phosphorus, cause a detrimental aesthetic effect, increase chemical requirements in water treatment and excessive growth of algae and other aquatic plants, which can cause destruction of the habitat and depletion of dissolved oxygen. This process in turn causes death of aquatic life because of the lowering of the oxygen level. It is for these reasons that much attention has been given to phosphorus removal in the sewage treatment process.

In freshwater, phosphorus pollution can lead to blooms of cyanobacteria, which are symptoms of eutrophication. This contributes to a wide range of water quality problems, including unpleasant odours and unpalatable taste in drinking water. When such water is processed, the high load of organic matter reacts with chlorine to form trihalomethanes.

The water-soluble compounds that are toxic to the nervous system and liver are released when cyanobacteria blooms die or are ingested. These toxic compounds can kill life stock and may impose a serious health risk to mankind. Therefore analysis of phosphorus is extremely essential to those concerned with water quality. According to Kennedy (1997:96), control of nutrients is becoming increasingly important, as more stringent discharge requirements are imposed. Erdinçler (2005:75) suggested that plans of future nutrients management should be based on phosphorus.

The increasing awareness of eutrophication effects has led to the introduction of legislation that controls the discharge of phosphorus in water. In South Africa a special phosphate standard was introduced to restrict the concentration of phosphorus in wastewater discharges to 1.0 mg P/l as orthophosphate, according to the Government Gazette, 1984 (quoted by De Haas and Wentzel, 2000:439). Wastewater treatment plants use orthophosphate and total phosphorus results to monitor or meet effluents standard.

Phosphorus is classified as dissolved,  $\text{PO}_4^{3-}$ , generally as orthophosphate, condensed phosphorus, particulate (organic) phosphorus and total phosphorus, which include polyphosphate. There is no chemical method for determining condensed phosphorus. “Condensed” phosphorus and “organic” phosphorus are calculated as small differences between orthophosphate and total phosphate and are consequently of limited validity according to Harwood and Hattingh (1973:291).

This study is concerned with the methods being used for orthophosphate, phosphorus and total phosphorus differentiation. In this investigation, four analytical techniques are proposed for phosphorus analysis in wastewater, namely ion chromatography (IC), flow injection analysis (FIA), inductive coupled plasma optical emission spectrometry (ICPOES) and inductively coupled plasma mass spectrometry (ICPMS). Furthermore, the study contributed to quality of test results assurance and Sebokeng Wastewater Works process control, optimisation and effluent monitoring.

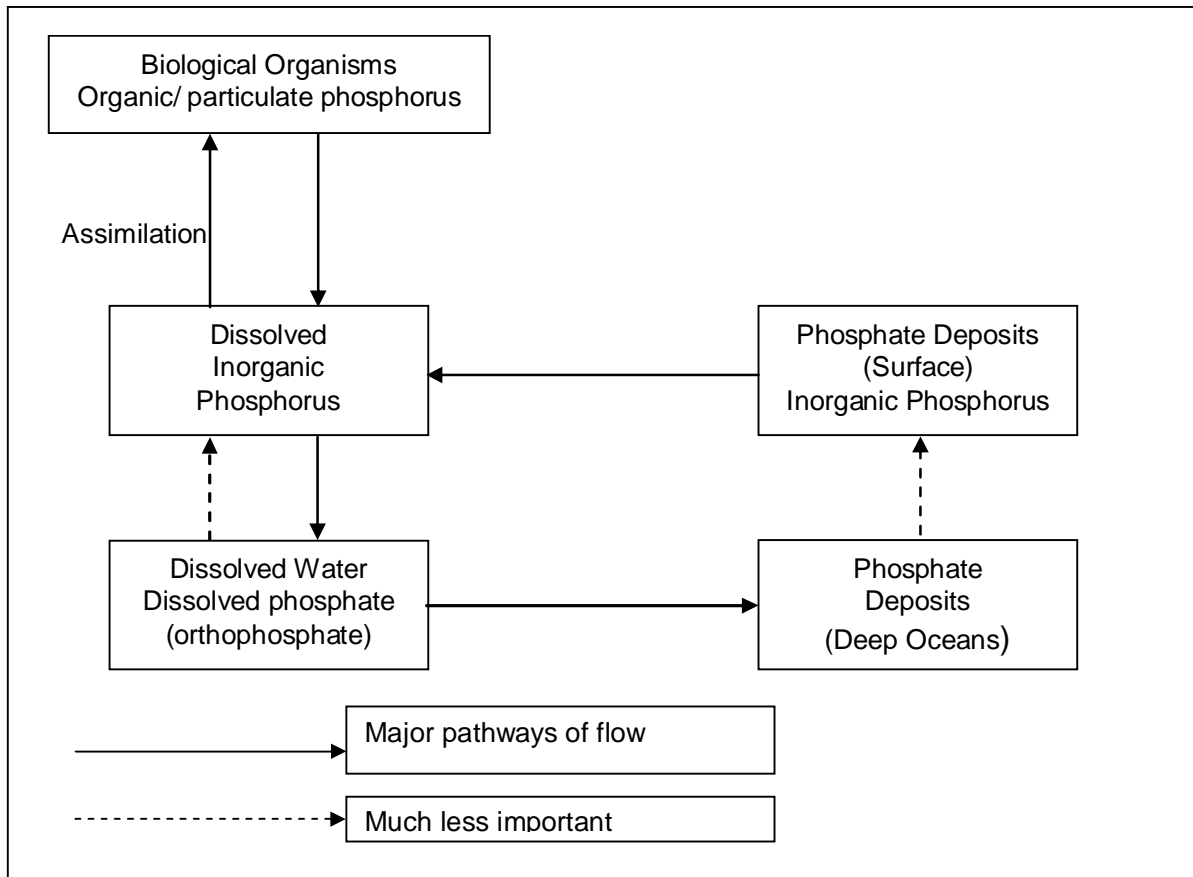
## **1.2. NATURAL FORMS OF PHOSPHORUS**

Phosphorus is required in all life processes as constituents of nucleic acids, phospholipids and numerous phosphor-related compounds. Schaum and Cornel (2005:57) described phosphorus as the key element for biochemical processes.

Natural phosphorus occurs in biological organisms. Henry and Heinke (1989:312) explained that plants and bacteria require phosphorus in the phosphate (dissolved) form, like orthophosphate, for its nutrition. It assimilates it directly, converting the  $\text{PO}_4^{3-}$  to the organic (insoluble) form in its protoplasm. The decay of these organisms dissolves and releases the phosphorus for reuse.

The phosphorus cycle in Figure 1.1 illustrates the three forms of particulate phosphorus (organic, dissolved  $[\text{PO}_4^{3-}]$  and inorganic [polyphosphates]), which would eventually hydrolyse in water to  $\text{PO}_4^{3-}$ . Particulate phosphorus is contained in the dead and living cells. The dissolved inorganic phosphorus in water is derived from this organic material by excretion and decomposition.





**Figure 1.1 - Phosphorus cycle (Environmental Science and Engineering, 1989:313)**

### 1.3. FORMS OF PHOSPHORUS

Phosphorus occurs in water and wastewater as orthophosphates, condensed phosphates (pyro-, meta-, and other polyphosphates) and organically bound phosphates.

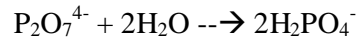
Phosphorus is found in the environment in various forms, as shown below.

- Monophosphates: Orthophosphoric acid,  $\text{H}_3\text{PO}_4$   
Sodium phosphate,  $\text{NaH}_2\text{PO}_4$   
Disodium phosphate,  $\text{Na}_2\text{HPO}_4$   
Diammonium phosphate,  $(\text{NH}_4)_2\text{HPO}_4$   
Trisodium phosphate,  $\text{Na}_3\text{PO}_4$
- Polyphosphates: Pyrophosphate,  $\text{P}_2\text{O}_7^{4-}$   
Triphosphate,  $\text{P}_3\text{O}_{10}^{4-}$

Trimetaphosphate,  $P_3O_9^{3-}$

Hexametaphosphate,  $(PO_3)_6^{3-}$

- Polyphosphates hydrolyse in aqueous solution to revert to the monophosphates:



- Organic phosphates: DNA, RNA, ATP and phospholipids

Total phosphorus in water is used as a measure of algal growth or concentration of chlorophyll.

The high concentration levels of phosphorus indicate water pollution. Therefore, those concerned with water quality are interested in the amount of ortho-, poly-, organic and total phosphorus present in waters.

#### **1.4. CURRENT SITUATION REGARDING THE ANALYSIS OF PHOSPHORUS IN WATER AND WASTEWATER**

Spivakov and Maryutina (1999:2161-2179) mentioned that phosphorous analysis is generally based on conversion of phosphorous forms of interest to dissolved orthophosphate, which is then determined by colorimetric or chromatographic techniques.

According to Spivakov and Maryutina (1999:2161-2179), most water laboratories use 0.45  $\mu\text{m}$  pore diameter membrane filters to separate dissolved from suspended forms of phosphorus. To determine total phosphorus, the persulphate digestion method is the most commonly used. Spivakov and Maryutina (1999:2161-2179) mentioned that automated analysers measure both orthophosphate and total phosphate.

Other laboratories determine orthophosphate by IC and total phosphate by colorimetric technique. However, there are few laboratories that analyse phosphorous by inductive coupled plasma according to Cai (2001:853) and Li *et al.* (2005).

Also, there are those laboratories that use all three techniques (colorimetric, chromatographic and spectrometry) for phosphorous determination. In South Africa, Wastewater Works monitor and manage their plant performance with different parameters, including phosphorus. Composite effluent and influent samples are analysed weekly for orthophosphate and total phosphorus to check whether effluent complies with the general standard set by DWAF, in order to improve and maintain effluent compliance.

Therefore, it was decided to commence an investigation to compare the form of phosphorous being measured by different techniques (flow injection analyser, FIA, ion chromatography, IC, inductive coupled plasma optical emission and mass spectrometry, ICPOES and ICPMS).

#### **1.5. APPLICATIONS OF PHOSPHORUS DATA IN THE ENVIRONMENT**

The phosphorus data is utilised as follows:

- to monitor compliance of the effluent discharge to receiving waters;
- for routine monitoring of pollution originated from wastewater treatment plants;
- for routine monitoring of pollution in water for environmental studies;
- to assess the potential biological productivity of surface waters; and
- to control phosphate dosages in water systems for corrosion prevention.

#### **1.6. MOTIVATION FOR THE CURRENT INVESTIGATION**

The operation of wastewater treatment plants is subject to national legislation. Water is a scarce commodity and it is important to protect this valuable resource. The National Water Act (1998) and Water Service Act (1997) are the mechanisms by which to protect water resources. It states regulation of waste standards that pertain to effluent discharge to a water source. According to Du Plessis (2004:17), the National Water Act includes strict regulations regarding the phosphate levels in water that are released by industry back into South Africa's river system. Du Plessis (2004:17)

mentioned that DWAF's director general initiated the formation of an institute by the end of 2003 to focus on preventative-based eutrophication management.

In South Africa wastewater (including sewage, industrial effluent and storm water infiltration) is cleaned and treated by wastewater treatment works to a standard specified by the Department of Water Affairs and Forestry before being discharged to a natural environment. According to the Department of Water Affairs and Forestry, the discharge limit for orthophosphate is 1 mgP/l for effluent in receiving waters.

As a result of the Water Service Act (1997), Rand Water interacts with wastewater works to ensure that effluent discharge conforms to legislation, in order to promote utilisation of sewage, effective wastewater treatment and to secure the preservation of the ecology and environment.

The Sebokeng Wastewater Works treats sewage emanating from Sebokeng (35%), Vanderbijlpark (35%) and Johannesburg (30%). Domestic sewage is treated through biological trickling filters and a biological nutrient removal (BNR) system with a capacity of 70 Ml/day, which discharges to the Rietspruit River, then to Vaal Barrage Catchments, which is one of Rand Water's sources to supply over 10 million people with drinking water after purification processes. This project was based on the interaction of Rand Water Bulk Sanitation (committed to wise resource use, waste minimisation and ongoing drive to conserve the environment) with Sebokeng Wastewater Works in process monitoring with the aim to improve health and to create an enabling environment for sustainable sanitation facilities.

Limnologists, wastewater specialists and customers raised concerns regarding the phosphorous analysis in water. The main concern was based on the techniques that were used for orthophosphate and total phosphorous determination for quality assurance of results. It was questionable whether

the inductive coupled plasma, mass spectrometry, ion chromatography or flow injection analyser gives orthophosphate or total phosphate. Harwood and Hattingh (1973:289) felt that although standard procedures are available for phosphorus determination, the methods chosen are not the best available phosphorus differentiation. It has therefore become a necessity to investigate and compare these instruments with regard to their capability of phosphorus determination.

The current four methods that are applied for the determination of phosphorus are Ion Chromatography (IC), Flow Injection Analyser (FIA), Inductive Coupled Plasma Optical Emission Spectrometry (ICPOES) and Inductive Coupled Plasma Mass Spectrometry (ICPMS). Instrumentation, sample preparation and techniques all differ. IC is a chromatographic technique and determines ions, while FIA is the colorimetric method, which determines  $\text{PO}_4^{3-}$  using ultra-violet (UV) absorption. ICPOES and ICPMS both determine total phosphorus and are based on emission. The ICPOES detects light from the plasma with an optical spectrophotometer while the detector in an ICP-MS is the mass spectrometer that separates ions according to their mass charge ratios. Rand Water laboratories are equipped and are able to apply all four techniques. Currently, orthophosphate is determined by FIA and “total phosphate” by ICPOES. The purpose of this study was to find which of these methods is the most cost-effective but still yield accurate and reliable results.

## **1.7. HYPOTHESIS OF THE CURRENT INVESTIGATION**

The most cost-effective, accurate and reliable method for the analysis of phosphorus in water can be determined.

## **1.8. GENERAL OBJECTIVES**

- To develop simple and reliable methods for orthophosphate, phosphorus and total phosphorus differentiation.
- To examine the phosphorous determined by spectrometry.
- To compare all techniques being used for phosphorous analysis.
- To determine phosphorus species in water using IC, FIA, ICPOES and ICPMS.

## **1.9. SPECIFIC OBJECTIVE**

- To select an accurate and reliable technique for the analysis of orthophosphate and total phosphate in water and wastewater samples based on comparative analytical data obtained from this study.

The phosphorus procedures and methods used are in Appendix A.

The next chapter will review the literature on various forms of phosphorous and the techniques and methods chosen for phosphorous determination.

## CHAPTER 2

### LITERATURE SURVEY

#### 2.1 ANALYTICAL VIEW OF THE TOPIC

The presence of phosphorus in wastewater is essential for the proper functioning of all biological wastewater treatment processes. Organisms use phosphorus during cell synthesis, maintenance and energy transport. However, excessive phosphorus can create harmful effects, including fish kills and making water unfit for potable use and recreation. According to Guo *et al.* (2005:160-167) it is well known that phosphate is an indicator of water quality and the most widely used methods for its analysis were automated colorimetry and IC conductivity detection. Most of the work done in developing molybdenum blue colorimetric methods dates from the 1970's.

Nesbitt (1973:649) points out that sewage works effluents contain nitrogen and phosphorus that are major nutrients to control biomass growth, and which are the limiting factors for eutrophication. Therefore, control of both phosphorus and nitrogen is necessary to prevent deterioration of water quality. According to Erdinler (2005:75), pertaining to nutrient management, phosphorus has been given priority over nitrogen by both environmental scientists and soil scientists in the last decade. Environmental engineers are trying to reduce phosphorus concentrations in the treatment plant effluents and soil scientists to establish favourable phosphorus levels in arable soils. It is for these reasons that much attention has been given lately to phosphorus removal in the sewage treatment process. Enhanced biological phosphorus removal (EBPR) is described by Erdinler (2005:75) as the recently developed technique for the removal of phosphorus in wastewater. This process is based on the biological uptake that phosphorus in excess of the normal metabolic requirements, as well as wastage of excess sludge, removes the phosphorus from the system. The wastewater works ensure that the effluent discharge conforms to the National Water Service Act (Act no. 108 of 1997), the National Water Act (Act no. 36 of 1998) and legislation and standards set by DWAF.

Phosphorus removal is monitored and assessed by determining orthophosphate and total phosphorus from both the influent and the effluent samples. An accurate, simple and reliable method is essential to water resource quality monitoring, effective wastewater treatment and to secure the preservation of the ecology and environment.

According to Kimerle and Rorie (1973:367), a need for accurate methods by which to determine phosphorus, is particularly relevant to water quality problems. Different techniques chosen and used in phosphorus determination are questionable regarding whether it is best suitable for analysing such forms of phosphorus. It is therefore the purpose of this paper to review some methods used in analysing phosphorus and identifying forms of phosphorus that are actually being measured.

## **2.2 DIFFERENT FORMS OF PHOSPHOROUS IN WATER AND WASTEWATER**

Nesbitt (1973:649) discussed forms of phosphorus in wastewater as organic phosphorus found in organic matter and protoplasm, soluble inorganic orthophosphate, such as those used in agriculture, wastewater treatment and soluble inorganic orthophosphate.

Phosphorus may be present in water samples in many different forms. According to Harwood and Hattingh (1973:290), chemical forms of phosphorus in a sample are classified as follows:

- orthophosphate as  $\text{PO}_4^{3-}$ ;
- condensed phosphorus;
- organic phosphorus, which is algal metabolites; and
- total phosphorus (the sum of 1, 2, and 3 above) as mg/l.

Henry and Heinke (1989:312) classified forms of phosphorus as



- particulate or organic phosphorus,
- dissolved phosphorus generally as orthophosphate, and
- inorganic (polyphosphates), which would eventually break down by hydrolysis in water to  $\text{PO}_4^{3-}$ .

Generally phosphorus can be classified as orthophosphate, organic phosphate and total phosphorus. The sum of all the phosphorus concentrations determined chemically, rather than additional, represents the total phosphorus content of the sample, according to standard methods of chemical analysis, as quoted by Harwood and Hattingh (1973:289).

According to Olsen, quoted by Kimerle and Rorie (1973:372), all forms of phosphorus – ortho-, poly- and residual - cannot be measured accurately because of being poorly separated. Although the standard methods by Greenberg *et al.* (1985:441) recommended the use of 0.45  $\mu\text{m}$  membrane filters to divide sample into soluble and particulate portions, Olsen, quoted by Kimerle and Rorie (1973:367-374), felt that membrane filtration resulted in a liquid and solid fraction with undefined portions of colloidal phosphorus. The use of ultra filtration or centrifugation techniques is recommended to achieve absolute separation of dissolved and colloidal fractions of phosphorus. He claimed that total phosphate is the only analysis that can be and have been satisfactorily performed ever since the determination of phosphorus in waters.

The sample handling and stability have also received much attention. Clesceri and Lee (quoted by Kimerle and Rorie 1973:372) have shown that condensed and organic phosphorus can undergo degradation; therefore the best way is to perform the analysis immediately after sampling.

Numerous methods have been developed for separation preconcentration and determination of phosphorus (Li *et al.*, 2005; Wilbur, 2001; Nhapi, 2004; Cartwright *et al.*, 2002; Tatro, 1997). These methods differ widely in the complexity of analytical procedures, detection, significance and interpretation of data. However, only a few of these techniques are used for phosphorus studies in water and wastewater. Orthophosphate, or reactive phosphorus, is the most frequently determined phosphorus analysis in water and wastewater without previous hydrolysis or oxidation. Table 2.1 includes lower oxidation state phosphorus compounds, which may be detected only in wastewater.

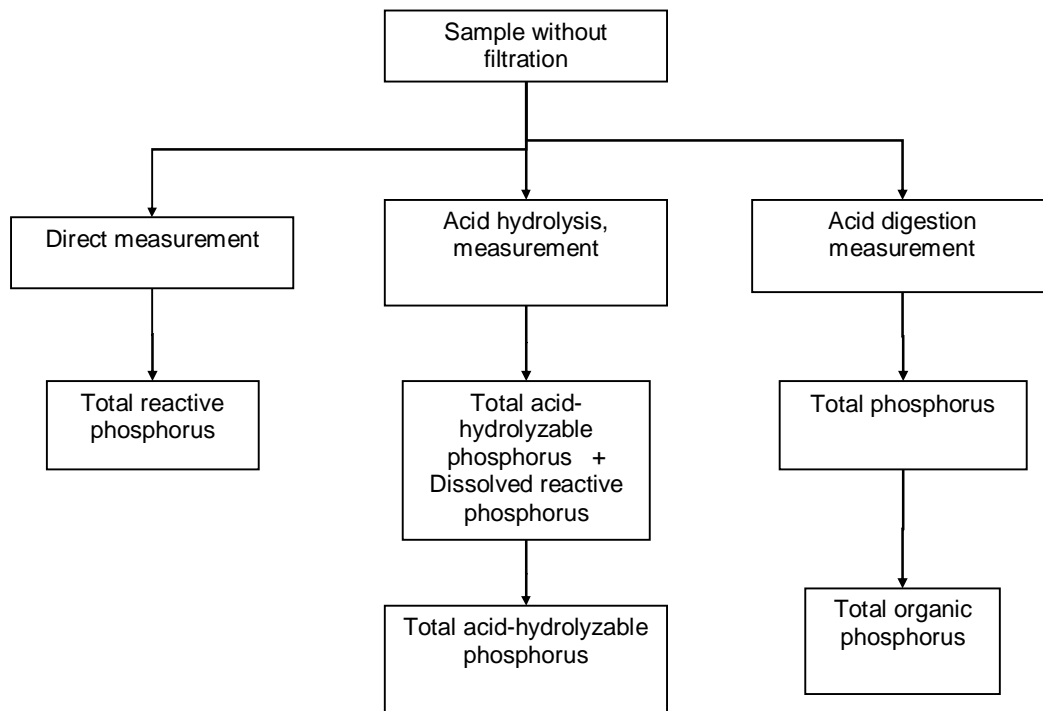
**Table 2.1 - Oxo acids of phosphorus (IUPAC, phosphorus speciation in water sediments, 1999)**

<b>Formula</b>	<b>Phosphorus species</b>
H <sub>3</sub> PO <sub>4</sub>	Orthophosphate
H <sub>4</sub> P <sub>2</sub> O <sub>7</sub>	Diphosphate
H <sub>5</sub> P <sub>3</sub> O <sub>10</sub>	Triphosphate
H <sub>6</sub> P <sub>4</sub> O <sub>13</sub>	Tetraphosphate
H <sub>3</sub> P <sub>3</sub> O <sub>9</sub>	Trimetaphosphate
H <sub>4</sub> P <sub>4</sub> O <sub>12</sub>	Tetrametaphosphate
H <sub>3</sub> PO <sub>2</sub>	Phosphinate
H <sub>3</sub> PO <sub>3</sub>	Phosponate
H <sub>4</sub> P <sub>2</sub> O <sub>6</sub>	Hypophosphate
H <sub>4</sub> P <sub>2</sub> O <sub>7</sub>	Diphosphonate
H <sub>4</sub> P <sub>2</sub> O <sub>6</sub>	Isohypophosphate

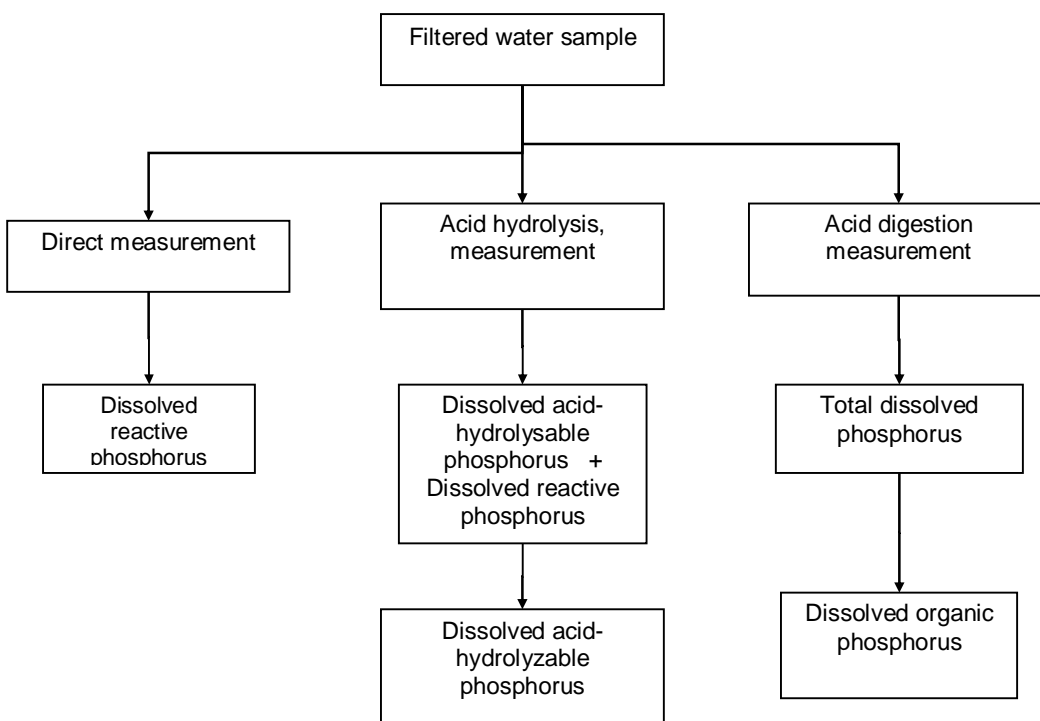
The most critical step in phosphorus analysis is the separation of the different forms of phosphorus and the conversion of phosphorus species into orthophosphate. In the standard methods Greenberg

*et al.* (1985:441) recommend the 0.45 µm membrane filtration process separation between dissolved phosphorus and total phosphorus.

Operational schemes for the analysis of several forms of phosphorus in water may be seen in Figures 2.1 and 2.2. The direct determination of phosphorus in a sample yields “total reactive phosphorus”. Acid hydrolysable phosphorus is achieved by acid hydrolysis at 100 °C, which converts different condensed phosphates into orthophosphates. Total phosphorus is obtained by digesting the sample with an acid (perchloric acid, nitric-sulphuric acid and persulphate sulphuric acid are recommended by Spivakov and Maryutina, 1999:2165) for the conversion of all organic matter present in a sample into orthophosphate.



**Figure 2.1 - Analytical scheme of phosphorus species determination in water without sample filtration (IUPAC, phosphorus speciation in water sediments, 1999)**



**Figure 2.2 - Analytical scheme for phosphorus species determination in water with sample filtration (IUPAC, phosphorus speciation in water sediments, 1999)**

Several rapid automatic methods have been developed for the separation and determination of phosphorus in water. Flow injection analysis, IC, high performance liquid chromatography and few other techniques were successfully used for phosphorus studies in water samples. Spivakov and Maryutina (1999:2164-2169) explained that these methods differ widely in the complexity of detection, procedures, significance of obtained data and possibilities of interpretation.

## **2.3 SELECTION OF METHODS**

### **2.3.1 Colorimetric technique**

The molybdenum blue method developed by Murphy and Riley (quoted by Harwood and Hattingh 1973:289-298) for determining orthophosphate is the most widely used method. Chamberlain and Shapiro (1973:355) maintain that the method is simple, sensitive and reproducible. Zenhai *et al.* (2004:263) claimed that a simple flow injection analyser coupled with fluorescence detector gives satisfactory results with good linearity for phosphorus determination in water samples. However,

the major problem is that, under acidic conditions, phosphorus in other forms might be converted to orthophosphate. This may result in overestimation of dissolved phosphorus or orthophosphate. Therefore Strickland and Parsons (quoted by Chamberlain and Shapiro, 1973:355) introduced the term “soluble reactive phosphorus”(defined as that phosphorus in a sample of Millipore filtrate that reacts in five minutes with acidified molybdate) to distinguish what was actually being measured from what was present in the water sample. They recommended the use of the Murphy-Riley method, as the analysis is rapid and easy to perform.

In the past, reductant, stannous chloride has been used as standard method of analysis. Burton and Riley (quoted by Harwood and Hattingh, 1973:289-298) discussed its major disadvantages as follows:

- the method is unsatisfactory at high concentration levels of phosphorus,
- colour is relatively unstable, so that accurate time intervals for reading the colour are necessary,
- there is an appreciable salt error,
- there are copper interferences appreciably above 50 µg/l, and
- the absorbance is temperature-dependent.

However, the use of ascorbic acid as reducing agent is claimed by Fogg and Wilkinson (1958:406) to increase colour stability and to reduce copper and arsenate interferences. Harwood and Hattingh (1969:289-298) have also stipulated that ascorbic acid has a very stable colour and no copper or arsenate interferences. Spivakov and Maryutina (1999:2166) recommended the extraction of molybdophosphoric acid into a benzene-isobutanol mixture or into another organic solvent to eliminate special interferences like silica, or to increase sensitivity.

Strickland and Parsons (quoted by Harwood and Hattingh, 1973:293) stated that ascorbic acid-antimony reagents have a major advantage over stannous chloride and other reductants. In addition, Harwood and Hattingh (1973:109) also pointed out that ascorbic acid gave constant readings.

Although Murphy and Riley (quoted by Harwood and Hattingh, 1973:293) considered the method to be free of interferences from hydrolysis of organic phosphorus, they admitted that very labile phosphorus compounds might lead to high orthophosphate results.

According to Snell and Snell (quoted by Harwood and Hattingh, 1973:291), however, orthophosphate is the only form of phosphorus that forms a complex with molybdate. Blomqvist and Sjosten (1997:1818) stated that the formation rate in water of phosphoantimonylmolbdenum complex declines with decreasing phosphate concentrations and decreasing reaction temperature. When determining low concentration of phosphate, slow formation rate of coloured complex is possible Blomqvist and Sjosten (1997:1818).

The vanadomolybdophosphoric acid, the stannous chloride and the ascorbic acid phosphorus speciation in water sediment techniques are the most often used colorimetric techniques. Table 2.2 shows the difference in quantifiabilities of the three techniques.

**Table 2.2 - Phosphorus concentration ranges for colorimetric methods (IUPAC, phosphorus speciation in water sediments, 1999)**

<b>Method</b>	<b>Concentration range (µg/l)</b>
Ascorbic acid	0.01 – 2.0
Stannous chloride	0.007 – 2.0
Vanadomolybdophosphoric acid	0.1 – 20

The three colorimetric techniques used in orthophosphate determination in water and wastewater have been tested in inter-laboratory studies, as shown in Table 2.3.

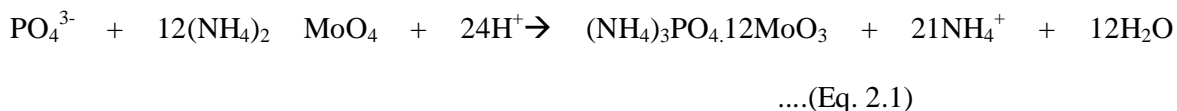
The vanadomolybdophosphoric acid method demonstrated a high relative standard deviation (75% for 0.1 µg/l). The stannous chloride has shown standard deviation of 26% for 0.1 µg/l. Therefore it is not advisable to use these two methods at lower concentrations. The ascorbic method has shown excellent results at both lower and higher concentrations (low relative standard deviation). At higher concentrations (7.0 µg/l), all three methods are acceptable.

Reactions involved in the colorimetric molybdate method.

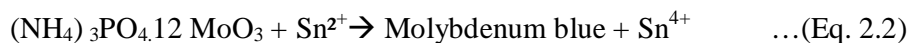
- Phosphorus occurring as orthophosphates



- Phosphate ion combines with ammonium molybdate and antimony potassium titrate under acidic conditions to form an antimony-phospho-molybdate complex. The reaction is carried out as:

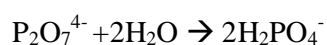


- Either ascorbic acid or stannous chloride is used as a reducing agent to increase colour stability. The coloured compound formed is referred to as molybdenum or heteropoly blue.

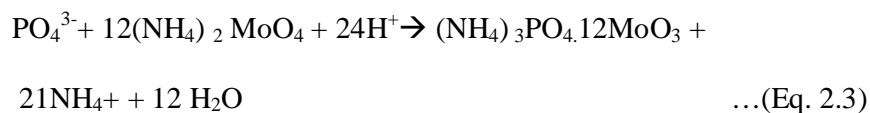


- Polyphosphates including organic compounds

When samples are acidified with sulphuric acid or persulphate, polyphosphates are converted to orthophosphates according to the following equation:



The digested sample is then analysed for orthophosphate.



**Table 2.3 – Inter-laboratory testing of spectrophotometric orthophosphate determination, (standard methods, 1985)**

Method	Orthophosphate (µg/l)	No. of laboratories	Relative standard deviation (%)
Vanadomolybdophosphoric acid	0.1	45	75
	0.6	43	20
	7.0	44	9
Stannous chloride	0.1	45	26
	0.6	44	14
	7.0	45	8
Ascorbic acid	0.1	3	9
	0.6	3	4
	7.0	3	5

The perchloric acid, nitric-sulphuric acid mineralisation and persulphate oxidation are three essentially different methods used for digestion of organic phosphorus and all other chemical forms of phosphorus present in a water sample and subsequent conversion to orthophosphate. Spivakov and Maryutina (1999:2165) recommended the perchloric acid as the most severe and



effective oxidising agent for organic-rich and inert carbon containing samples. The persulphate oxidation is most widely used and is gaining much appreciation.

The final digested sample can be performed for phosphorus analysis by a wide choice of analytical techniques. The colorimetric method is preferred for routine analysis.

### **2.3.2 Chromatographic technique**

The most important parameter in the ion chromatographic separation is the distribution ratio of a given ion between a resin phase and a solution phase. In IC, the detectors usually measure electrical conductivity, although UV detection can also be used.

This technique can be used to measure the concentration of several important inorganic anions such as fluoride, sulphate, phosphate and nitrate, all in one analysis. Although sample contamination is possible during sample filtration in auto-injectors, rinsing filters with 20 ml of deionised water prior to filtration of the sample has been shown to remove most inorganic contaminants.

Ion exchange chromatography can separate orthophosphate, linear polyphosphate, cyclic condensed phosphates and lower oxidation state anions of phosphorus in water and wastewater before determination.

According to Spivakov and Maryutina (1999:2169), comparison of results obtained using ICPOES and IC has shown that the IC method is reliable and applicable to phosphorus speciation studies in water samples. IC enables the determination of orthophosphate, diphosphate and triphosphate, along with chloride, nitrate and sulphate anions when indirect UV detection is used.

Although phosphate can be determined by IC, greater sensitivity is obtained by colorimetric methods that measure orthophosphate. Spivakov and Maryutina (1999:2169) reported a method for the separation and detection of condensed phosphate in wastewater using unsuppressed IC with post column FIA. Short analysis time (12 minutes for each sample) and low detection limits are the advantages of this technique. Ohashi (1973:303-311) claimed that ion chromatography gives better resolution for the separation of a series of linear phosphates than any other chromatographic techniques. According to Olsen (quoted by Kimerle and Rorie, 1973:367-378), the total phosphorus is the only form of phosphorus that can be determined accurately in samples that contain different forms of phosphorus in the dissolved, colloidal and particulate phases.

A greater need exists for better methods for characterising the forms of phosphorus in water. Many limnologically essential phosphorus fractions cannot be measured, as sufficient separation techniques are not available (Olsen, as quoted by Kimerle and Rorie, 1973:367-375). Although the chromatographic technique by Karl-Kroupa (quoted by Kimerle and Rorie, 1973:375) seems suited for the separation of chemical states of phosphorus at low concentration levels, it is not commonly used.

Ion chromatography, including other liquid chromatography techniques, is required for the speciation of individual phosphorous compounds, but few studies have referred to the analysis of real samples using this technique. Furthermore, comparison of the results obtained with measurements using IC and ICPOES has shown the method's reliability and capability in phosphorus determination.

### 2.3.3 Spectrometric techniques

Emission spectroscopy is a very sensitive method, applicable to all metals and non-metals, including phosphorus. Kaeleble (1973:345) stated that the lines 253,57 nm and 255,36 nm are the mostly used for phosphorus determination. According to Bradford and Cook (1997:6), other wavelengths may be substituted if the needed sensitivity can be assured and if the sample is treated with the same corrective techniques for spectral interferences. The selection of the best line for the element of interest in ICPOES requires experience.

One of the main advantages of emission spectroscopy is the ability to determine many elements simultaneously and quite rapidly. The ICPOES is most preferred among other techniques for phosphorus analysis, due to the following unique features, according to Cai (2001:853):

- multi-element analysis capability with high sample throughput,
- the possibility of element determinations in broad concentration range, including detection limit levels, and
- a less expensive approach for each analysis.

Cai (2001:853) also stated that sensitive phosphorus lines at 213.618 and 214.914 nm are hindered by spectral interferences.

A further spectrometric technique for broad-spectrum surveys of trace impurities is spark source mass spectrometry. There are few specific references to phosphorus determination by spark source mass spectrometry because of its primary use as a survey technique and the high cost of the equipment. According to Zeiner *et al.* (2007:593), the high operational cost is due to the amount of argon used and this is one of the disadvantages of ICP-MS.

Mass spectrometry offers advantages in precision, sensitivity and speed, while sacrificing versatility. An investigation shows that there are few water laboratories using ICPMS for phosphorus determination. Bradford and Cook (1997:1-7) discussed the disadvantages of ICPMS, including isobaric interferences produced by polyatomic species that arise from the plasma, gas and atmosphere. Although ICPMS is expected to analyse phosphorus, Bradford and Cook (1997:1-7) stated that it is not useful in the detection of non-metals. Non-metallic compounds, such as phosphorus, are less sensitive to conventional spectroscopic techniques such as AA, ICPAES and ICPMS, according to Li *et al.* (2005:263). Zeiner *et al.* (2007:593) stipulated that best results for sample analysis are usually achieved by combining different methods and concluded that coupling ion chromatography with ICPMS can be used for determination of metals.

A high background is reported on phosphorous determination when using ICPMS. Phosphorous sits at mass 31 (the only naturally occurring isotope), and this signal may be due to other plasma major species at mass number 31.

Examples of major species at mass 31

- Mass number 30 species are  $^{14}\text{N}^{16}\text{O}$ ,  $^{15}\text{N}^{15}\text{N}^+$ ,  $^{12}\text{C}^{18}\text{O}^+$
- Mass number 31 species are  $^{14}\text{N}^{16}\text{O}^1\text{H}^+$ ,  $^{15}\text{N}^{15}\text{N}^1\text{H}^+$ ,  $^{15}\text{N}^{16}\text{O}^+$ ,  $^{14}\text{N}^{17}\text{O}^+$ ,  $^{13}\text{C}^{18}\text{O}^+$ ,  $^{12}\text{C}^{18}\text{O}^1\text{H}^+$
- Mass number 32 species are  $^{16}\text{O}_2^+$ ,  $^{32}\text{S}^+$ ,  $^{14}\text{N}^{18}\text{O}^+$ ,  $^{15}\text{N}^{17}\text{O}^+$ ,  $^{14}\text{N}^{17}\text{O}^1\text{H}^+$ ,  $^{15}\text{N}^{16}\text{O}^1\text{H}^+$ ,  $^{14}\text{N}^{16}\text{O}^1\text{H}_2^+$ .

Phosphorus is also difficult to determine by ICPMS due to the polyatomic species interfering directly at mass 31 and indirectly at mass 32 from  $^{16}\text{O}_2$  and  $^{32}\text{S}$ . According to Cai *et al.* (2005:164), it is well-known that phosphorus, at trace level, is very difficult to determine using an ICPMS and

this is partially due to the reason that phosphorus has a high first ionisation potential (10.49 eV) and is only about 35% ionised in the argon plasma.

Wilbur (2001:1-5) also claimed that the major obstacle to accurate quantitative analysis of phosphorus is the possibility of interfering polyatomic species. He also concluded that because of its high first ionisation potential, relatively poor conversion of phosphorus atoms to phosphorus ions ( $P^+$ ) in the inductively coupled plasma. Kozono *et al.* (2002:542) utilized high resolution double-focussing sector ICPMS to distinguish P from polyatomic ions at nominal mass of  $m/z = 31$  and Becker *et al.* (2003:561), Yang *et al.* (2001:1302) and Koplík *et al.* (2002:261) determined phosphorus at  $m/z = 47$  based on the formation of  $PO^+$  in the argon plasma. According to Cartwright *et al.* (2002), using chromatographic techniques (high performance liquid chromatograph (HPLC) membrane desolvation system) prior to inductive coupled plasma mass spectrometry analysis reduces the amount of polyatomic interferences (see Table 2.4).

**Table 2.4 - Accurate masses of  $^{31}P$  and of the most abundant background ions at  $m/z$  31 (liquid chromatography coupled to inductively coupled plasma spectrometry for the determination of phosphorylation sites in tryptic digests of pesticides, 2002)**

Ion	Accurate mass $m/z$
$^{31}P^+$	30.9738
$^{15}N^{16}O^+$	30.9950
$^{14}N^{16}O^1H^+$	31.0058
$^{12}C^1H_3^{16}O^+$	31.0184

Spivakov and Maryutina (1999:2161-2176) recommended a combined method for the differential determination of phosphates and total phosphorus in wastewater. In this method, FIA is used for analysing orthophosphate and ICPOES for total phosphorus quantification.

### **2.3.4 Flow Injection Analysis**

FIA is the most common analysis of this type where the liquid sample is injected into a moving, non-segmented continuous stream of a suitable liquid. The first patent was filed in 1994 and the first publication appeared a year later (Hansen, 1995). The components of a simple system are a pump to propel the carrier stream, an injection valve to introduce the sample, a reactor to aid mixing of the two solutions and one or more detectors. In the case of colorimetric determination, the detector would be a single channel UV/Vis cell, set at a pre-determined wavelength. The detector could, however, be an ICPOES (Ortega *et al.*, 2003) or ICPMS (Vickers *et al.*, 1989).

## **2.4 CONCLUSIONS MADE ON THE RESULTS OF LITERATURE SURVEY**

Although phosphorus analysis, using segmented flow, has been reported for colorimetric techniques, the continuous flow (FIA) was chosen for this study. This was based on time taken for completion of analysis, including data calculation and printout. Although phosphorous determination by FIA requires preparation of reagents, the method is quick and easy to perform with satisfactory repeatability and sensitivity.

It was concluded that the most used reductants for colorimetric techniques are stannous chloride and ascorbic acid. The sensitivity of the two methods differs considerably, as shown in Table 2.3. The serious shortcomings of stannous chloride made ascorbic acid a method of choice for phosphorus determination by colorimetric technique.

In a routine laboratory, IC is recommended because of rapid automatic methods for separation and determination of phosphorus, including other anions such as fluoride, nitrite, nitrate, chloride and sulphate. Although IC has a serious drawback in analysis time (18 minutes for each sample), only eluent reagent is required to perform an analysis. Another advantage of IC is the ability to give

satisfactory results for orthophosphate and phosphorus when coupled with techniques such as ICPOES and ICPMS.

Phosphorus is a difficult element to be determined by ICPMS, due to the presence of isobaric and adjacent interfering species at mass 31.

In both ICPOES and ICPMS, phosphorus is determined at the same time as other elements of interest, thereby reducing sample volume, labour and analysis cost. ICPMS is expensive, therefore it is recommended for research laboratories.

According to this literature study, the species of phosphorus tabled below are determined by the different techniques.

**Table 2.5 - Different phosphorus species determined by the different techniques**

Techniques	P-species determined
FIA-colorimetric-molybdate	Orthophosphate
FIA-persulphate	Total phosphate
Ion chromatography	Orthophosphate
ICPOES	Total phosphate
ICPMS	Total phosphate

The next chapter will review different experimental procedures and methods used for determining phosphorus in water and wastewater.

## CHAPTER 3

### METHODOLOGY

#### 3.1 SAMPLING AND ANALYSIS

The Sebokeng Wastewater Works' influent and effluent samples were taken weekly from June 2002 to April 2004. All samples were collected in plastic containers washed with phosphate free detergent and stored at 4°C prior to analysis.

Samples for ICPOES and ICPMS were filtered through 0.45 µm pore size filter papers (Whatman, Clifton, NJ, USA) to remove insoluble particles from water sample prior to its injection into the instrumental system and prepared using 1% nitric acid (Merck, Midrand, Gauteng) to preserve phosphorus. The FIA and IC samples were only filtered with 0.45 µm filter papers. The total phosphate was determined by digesting samples with persulphate in a Kjeldahl system (Labotech, Gauteng, SA), followed by analysis using the phospho-molybdic photometric method.

The analysis was performed in a SABS ISO/IEC 17025 (1999) accredited laboratory, in accordance with Rand Water procedures (described in Appendix B) and standards methods (Eaton *et al.*, 1995). Operating manuals were followed for instruments used. The calibration, quality control (verification standard) and blank standards were used in all the instruments to achieve accuracy and quality of data and to monitor compliance for accuracy in determining phosphorus analysis.

#### 3.2 MATERIALS

All reagents used were of analytical grade and used without further purification and deionised water (18.2 MΩ cm) prepared by Milli-Q Plus system (Millipore, Bedford, USA) was used as water for preparing solutions. Dihydrogen dodecahydrate phosphate (Merck), with a certified purity of 99.9%, was used to prepare standard stock by dissolving it in water. For verification standard stock



preparation, a product of Riedel-de Haën was used. The working solutions of phosphate were freshly diluted from the stocks. According to the proposed methods, the reagents tabled below were used.

**Table 3.1 - Colorimetric reagents**

<b>REAGENTS</b>	<b>SUPPLIER</b>	<b>HAZARD</b>
Ammonium molybdate	Merck, Halfway House, Gauteng, SA	None
Antimony potassium nitrate	Anatech	Harmful
Ascorbic acid	Merck, Halfway House, Gauteng, SA	None
Sodium dodecyl sulphate	Merck, Halfway House, Gauteng, SA	Irritant
Sodium hydroxide	Merck, Halfway House, Gauteng, SA	Corrosive
Dihydrogen dodecahydrate Phosphate	Merck, Halfway House, Gauteng, SA and Riedel de Haën	Irritant
Sulphuric acid	Merck, Halfway House, Gauteng, SA	Corrosive
Helium gas	Air-Liquide	None
Mercuric oxide	Merck, Halfway House, Gauteng, SA	Very toxic
Potassium sulphate	Merck, Halfway House, Gauteng, SA	None

The mercury oxide used in the persulphate digestion method is kept in a poisonous cupboard, and the key for the cupboard is located in the safety room. A special plastic container labelled “toxic waste” is used for persulphate digestion waste. Toxic waste companies handle this waste for proper disposal, health and the safety of the environment.

**Table 3.2 - Ion chromatography reagents**

<b>REAGENT</b>	<b>SUPPLIER</b>	<b>HAZARD CLASSIFICATION</b>
Sodium carbonate	Merck, Halfway House, Gauteng, SA	Irritant
Sulphuric acid	Merck, Halfway House, Gauteng, SA	Corrosive
Dihydrogen dodecahydrate Phosphate	Merck, Halfway House, Gauteng, SA and Riedel de Haën,	Irritant

**Table 3.3 - Spectrometry reagents**

<b>REAGENT</b>	<b>SUPPLIER</b>	<b>HAZARD CLASSIFICATION</b>
Potassium dihydrogen Phosphate	Merck, Halfway House, Gauteng, SA	None
Dipotassium hydrogen Phosphate	Merck, Halfway House, Gauteng, SA	None
Orthophosphoric acid	Merck, Halfway House, Gauteng, SA	Corrosive
Nitric acid	Merck, Halfway House, Gauteng, SA	Corrosive
Argon gas	Air-Liquide, Artkin St, Germiston, Gauteng	Asphyxiate

### **3.3 EXPERIMENTAL PROCEDURES**

#### **3.3.1 Ion chromatography**

A Metrohm 761(Metrohm, Woodmead, Sandton, SA) compact chemically suppressed ion chromatograph with a conductivity detector was used in the determination of orthophosphate. Separation was performed on an ion exchange analytical column and a peak chromatography workstation was used for data collection. The chromatographic conditions tabled below were used.

**Table 3.4 - The optimised chromatographic conditions**

PARAMETER	VALUE
Eluent	1.7 mM NaHCO <sub>3</sub> +1.8 mM Na <sub>2</sub> CO <sub>3</sub> (Merck, Halfway House, Gauteng, SA)
Injection volume	20 µL
Flow rate	0.5 mL min <sup>-1</sup>
Separation Column	Dionex IonPac AS16, 4.0 mm × 250 mm (Sunnyvale, CA, USA)
Guard column	Dionex IonPac AG16, 4.0 mm × 50 mm (Sunnyvale, CA, USA)

According to the standard methods (Eaton *et al.*, 1995), although conventional colorimetric and electrometric methods are available for analyzing individual anions, only IC provides a single instrumental technique that may be used for rapid, sequential measurements of anions. IC is used in the assessment and control of receiving water quality, the treatment and supply of potable water and the measurement of operation and process efficiency in wastewater treatment. IC is also appropriate and applicable in the evaluation of environmental water quality. This method eliminates the use of hazardous reagents and distinguishes among halides and anions effectively.

In this present work, separated anions in the acid form were measured by conductivity detector and identified on the basis of retention time and compared to that of standards. The quantization was based on peak height. For quality assurance, calibration and verification standards were used.

### **3.3.2 Flow injection analysis**

Measurements were based on the formation of phospho-molybdic blue complex, which absorbs light at a wavelength of 880 nm. The ascorbic acid was used as a reducing agent, which is claimed, according to Harwood and Hattingh (1973:289-298), to increase colour stability and reduce silicate interferences from other ions, including silicate.

A FIA (Anatech, Slaone Park, Gauteng) was used and the method was based on the reactions that are specific for phosphate ions. The phosphate ions react with ammonium molybdate and antimony potassium nitrate, under acidic conditions, to form a phospho-molybdic complex, which was reduced with ascorbic acid to form a blue complex absorbing light at 880 nm. The absorbance was proportional to the concentration of orthophosphate in the sample.

To determine the influence of sample preparation of the results, FIA was applied to samples prepared in two different ways. All samples were first filtered and either injected directly into the system for the determination of orthophosphate, FIA (OP) or digested with persulphate for measuring total phosphate, FIA (TP).

A calibration and verification standard was used for quality assurance purposes.

### **3.3.3 Inductively coupled plasma optical emission spectrometry**

An ICPOES (Spectro Analytical Instruments, Freight Park, Gauteng) was used and emission measured at 177.495 nm, detected by a Spectro circular optical system charged coupled (CCD) detector (Spectro, Kleve, Germany). The operational conditions tabled below were used.

**Table 3.5 - Optimised ICPOES conditions**

<b>PARAMETER</b>	<b>VALUE</b>
RF forward power	1.5 kW
Plasma gas flow rate	15 l/min
Carrier gas flow rate	1.0 l/min
Auxiliary gas flow rate	0.8 l/min

In the inductively coupled plasma, 0.3 ml/min of the sample was transported into the instrument as a stream of liquid sample, which was converted into an aerosol through nebulisation. The aerosol was then transported to the plasma where it was desolvated, vaporised, atomised and ionised by the plasma.

The ICPOES was also used for the determination of a selection of elements (Al, B, Ca, Cd, Co, Cr, Cu, Fe, K, Mg, Mn, Mo, Na, Ni, Pb, S, Si, V and Zn) divided into two groups to avoid possible interferences, both chemical and physical. The particulars regarding wavelengths used for each element as well as optimal conditions is not relevant to this study. The three elements determined in one group were P, S and Si. This is important when considering the cost of each analysis.

Quality assurance was followed by preparing a calibration and verification standard for every 10 samples analysed.

### 3.3.4 Inductively coupled plasma mass spectrometry

A multi-element mode Micromass platform ICP (Micromass, UK) was used to carry out the measurements. A concentric Mynhardt nebuliser was used to develop the aerosol. An analytical program was established for calibration and analysis. Ions were resolved according to its atomic mass to charge ratio and detected using a diode-photo multiplier system. The operating conditions tabled below were used.

**Table 3.6 - Optimised ICPMS conditions**

<b>PARAMETER</b>	<b>VALUE</b>
ICPMS power	1.35 kW
Coolant argon flow	13.5 l/min
Nebuliser argon flow	0.75 l/min
Sample depth	6.0 mm
Torch	Standard quartz, 2.5 mm
Nebulizer	Quartz concentric, Meinhard
Spray chamber	Double-pass quartz, Scott type, 2 °C
Sampling and skimmer cones	Nickel
Isotope mass to charge ratio monitored	m/z = 31
Detector mode	Pulse

### 3.4 SELECTION OF METHOD FOR CALCULATION OF RESULTS

In order to draw differences between all four techniques used for the determination of the phosphorus concentration, statistical tests were performed, including the Student's t-test and the Mann-Whitney test. The t-test compares two means for significant difference. If the calculated t-value exceeds the tabulated value, then the means are significantly different. In order to make a decision, the calculated t-statistics must fall in the critical region with at least a 0.05 level of significance for a two-tailed test. The critical values in appendix F-9 of Johnson (1992:202-702) were calculated using the degrees of freedom (n-1) and critical alpha ( $\alpha$ ). If the calculated t-values exceed the tabulated value, then the means are significantly different.

The Mann-Whitney U-test, which is referred to as the rank sum test in Jongman *et al.* (1995:201-203), was used on results from FIA and ICPOES, FIA and FIA-TP, as well as ICPOES and FIA-TP. This test uses ranks instead of actual values in order to determine sample mean differences, which is particularly useful when dealing with non-normally distributed data, and the effect of outliers is eliminated. Furthermore, the Mann-Whitney test was important to ensure the determination of the reliability and robustness of the t-test results. The calculated z-value, at 95% confidence limit, should be between -1.96 and +1.96 for the results to have no significant difference.

Df = degrees of freedom or (n-1), where "n" is the number of analysis, df > or =36.

Critical t values for Mann-Whitney when  $\alpha = 0.05$  is -/+1.96.

Source: Appendix F-9 in Johnson (1992:202-702).

In conclusion, ANOVA was applied to ensure the reliability of both the t-test and the Mann-Whitney test. The next chapter will highlight the laboratory investigations that were carried out.

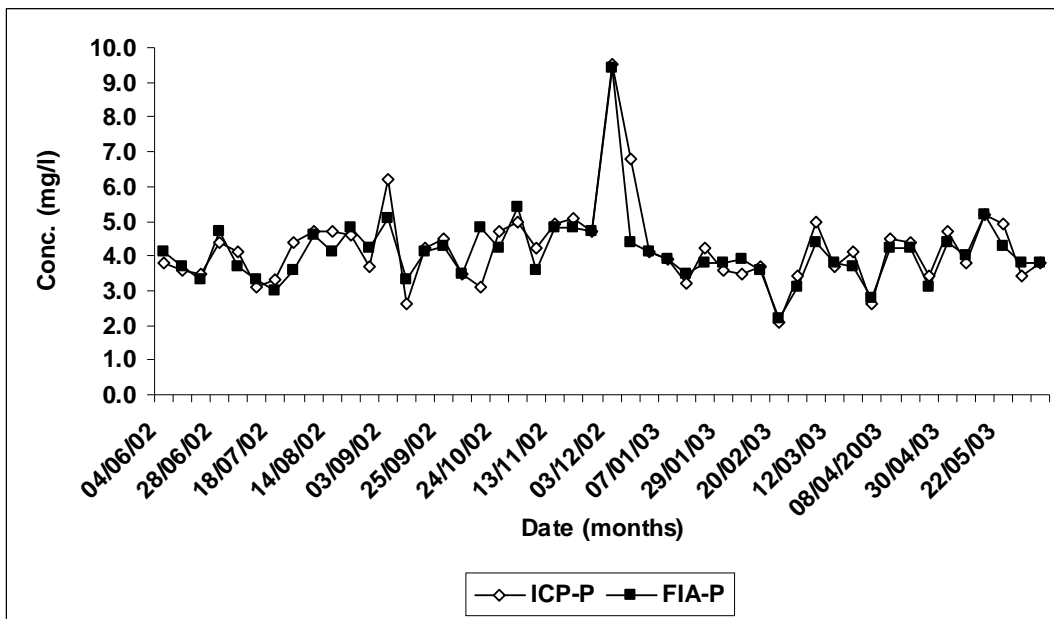


## CHAPTER 4

### RESULTS AND DISCUSSION

#### 4.1 RESULTS OF THE COMPARISON OF THE DIFFERENT TECHNIQUES

The phosphorus raw data obtained for a composite influent sample from June 2002 to June 2003 is shown in Table B-1, Appendix B.



**Figure 4-1 - Phosphorus composite influent concentrations taken using ICPOES (ICP-P) and FIA (FIA-P)**

Figure 4.1 is a graph that demonstrates the composite effluent phosphorus concentrations from Table B-1, Appendix B, and the statistical calculation of the results is shown in Table 4.1.

Statistic results in Table 4.1 show that calculated t-statistics are less than the critical t-value. Hence it can be concluded that there is no significant difference between the measurements of ICP and FIA for a composite influent sample over a 48-day period.

**Table 4.1 - Phosphorus composite influent statistic results, using ICPOES and FIA, using raw data from Table B-1, Appendix B**

	<i>ICPOES</i>	<i>FIA</i>
Mean (mg/l)	4.21	4.11
Variance	1.36	1.03
Observations	48	48
Pearson correlation	0.878	
Hypothesized mean difference	0	
Df	47	
t-stat	1.30	
P(T<=t) one-tail	0.101	
t-critical one-tail	1.68	
P(T<=t) two-tail	0.201	
t-critical two-tail	2.01	

**Table 4.2 - Phosphorus composite influent statistic results using ICPOES and FIA, using raw data from Table B-1, Appendix B**

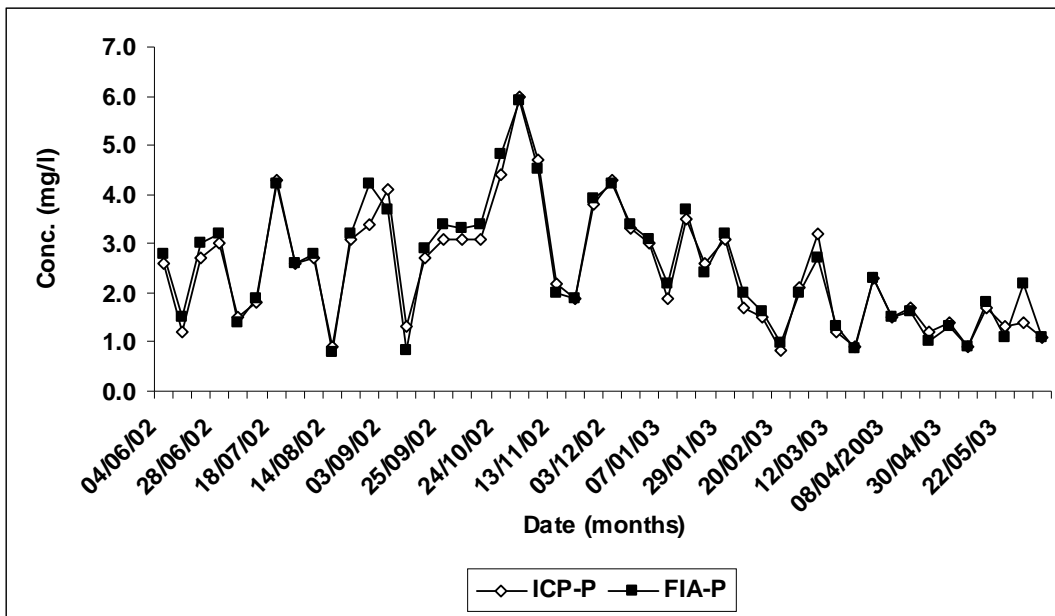
<i>Source of variation</i>	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F</i>	<i>P-value</i>	<i>F-crit</i>
Rows	105	47	2.23	14	2.47E-16	1.62
Columns	0.260	1	0.260	1.68	0.201	4.05
Error	7.29	47	0.155			
Total	112	95				

Table 4.2 shows that the F-statistics (1.68) is less than the F-critical (4.05) value; therefore there is no significant difference in the results between FIA and ICPOES. This supports the conclusion

drawn from the paired t-test in Table 4.1. The between-rows variation is significant because F-calculated (2.23) is larger than F-critical (1.62), indicating that the P-content of the different days varied significantly.

The phosphorus raw data obtained for a composite effluent sample from June 2002 to June 2003 is demonstrated in Table B-2, Appendix B.

Figure 4.2 - is a graph that demonstrates the composite effluent phosphorus concentrations from the raw data in Table B-2, Appendix B.



**Figure 4-2 - Phosphorus composite effluent concentrations taken using ICPOES and FIA**

The statistical calculation of results for a composite effluent, using the raw data phosphorus concentrations (ICPOES and FIA) from Table B-2, Appendix B is shown in Table 4.3.

**Table 4.3 - Phosphorus composite effluent statistic results, using ICPOES and FIA**

	<i>ICPOES</i>	<i>FIA</i>
Mean (mg/l)	2.45	2.51
Variance	1.40	1.47
Observations	48	48
Pearson correlation	0.977	
Hypothesized mean difference	0	
Df	47	
t-stat	1.58	
P(T<=t) one-tail	0.061	
t-critical one-tail	1.68	
P(T<=t) two-tail	0.122	
t-critical two-tail	2.01	

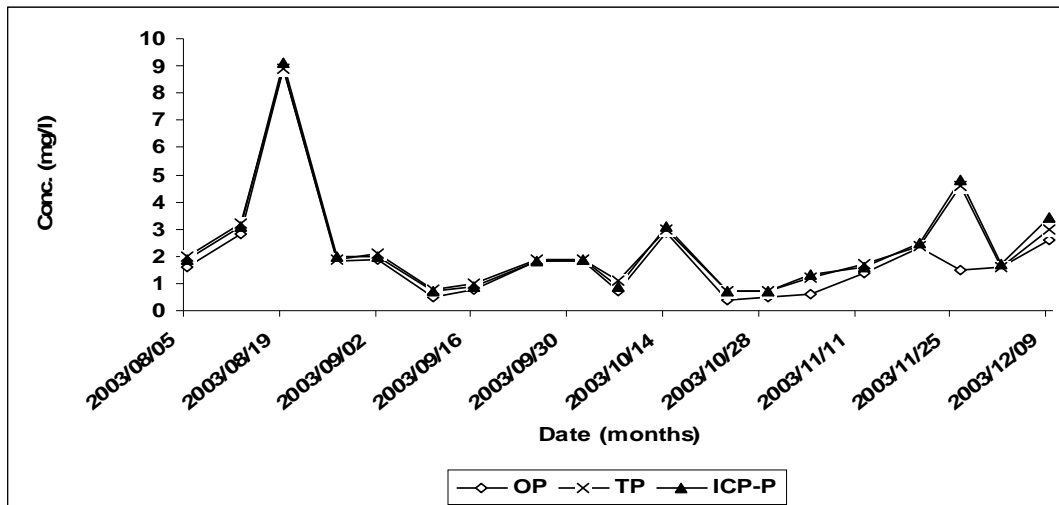
There is no significance difference between ICPOES and FIA phosphorus measurements for the composite effluent sample, since t-statistic in Table 4.3 is less than the critical t-value.

The measurements of the two techniques in Table B-2, Appendix B were also compared using two-factor ANOVA without replication. The results are depicted in Table 4.4.

**Table 4.4 - Phosphorus composite effluent statistic results, using ICPOES and FIA**

<i>Source of variation</i>	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F</i>	<i>P-value</i>	<i>F-crit</i>
Rows	104	47	2.23	14	2.47E-16	1.62
Columns	0.260	1	0.260	1.68	0.201	4.05
Error	7.29	47	0.155			
Total	112	95				

Since the F-calculated (1.68) is less than the F-critical value (4.05) (see Table 4.4), there is no significant difference in the results between ICPOES and FIA, which supported the results of the previous paired t-test. The between-rows variation proved to be significant, since F-calculated (14.0) was larger than F-critical (4.05), and therefore the P-concentrations in the effluent on different days differed significantly. The phosphorus raw data for a composite effluent taken using FIA, FIA-persulphate and ICPOES from August 2003 to December 2003 is shown in Table B-3, Appendix B. Figure 4.3 is a graph that illustrates the composite effluent phosphorus concentrations from the raw data in Table B-3, Appendix B.



**Figure 4-3 - Phosphorus composite effluent concentrations determined using FIA (OP), FIA-persulphate (TP) and ICPOES (ICP-P)**

The three sets of measurements were compared using two-factor ANOVA without replication.

The results are depicted in Table 4.5.

**Table 4.5 - Phosphorus composite effluent concentrations taken using FIA (OP), FIA-persulphate (TP) and ICPOES (ICP-P)**

<i>Source of variation</i>	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F</i>	<i>P-value</i>	<i>F-crit</i>
Rows	189	18	10	62	8.62E-22	1.89
Columns	1.98	2	0.992	5.89	0.006	3.26
Error	6.07	36	0.168			
Total	197	56				

Since the F-calculated (5.89) is greater than the critical t-value (3.26) (see Table 4.5), the null hypothesis is rejected. Therefore, there is a significant difference in the results for a composite effluent sample for FIA, FIA-TP and ICPOES. The ANOVA exercise was repeated on the data from table 3, Appendix B for the measurements of FIA-TP and ICPOES. The results are depicted in Table 4.6.

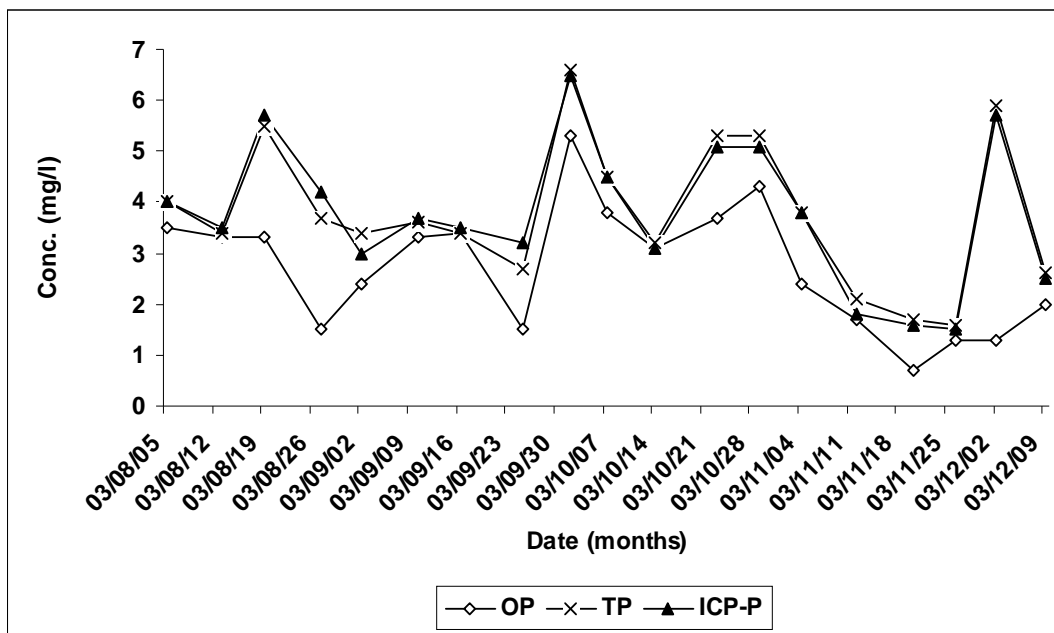
**Table 4.6 - Phosphorus composite effluent concentrations taken using raw data from Table B-3. FIA-persulphate (TP) and ICPOES (ICP-P) measurements were compared**

<i>Source of variation</i>	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F</i>	<i>P-value</i>	<i>F-crit</i>
Rows	132	18	7.38	653	1.1E-21	2.22
Columns	0.005	1	0.005	0.410	0.530	4.41
Error	0.204	18	0.011			
Total	133	37				

There is no significant difference between the measurements of TP and ICPOES phosphorus concentrations for composite effluent samples, because the calculated F-calculated (0.410) in Table 4.6 is less than the critical F-value (4.41). The between-rows variation was significant, since F-calculated (653) was greater than F-critical (2.22), indicating that the P-content on the different days differed significantly.

The phosphorus raw data for a composite influent taken using FIA, FIA-persulphate and ICPOES from August 2003 to December 2003 is shown in Table B-4, Appendix B.

Figure 4.4 is a graph that shows the composite influent phosphorus concentrations from the raw data in Table B-4, Appendix B, and the statistical calculation of results is demonstrated in Table 4.7.



**Figure 4-4 - Phosphorus composite influent concentrations taken using FIA (OP), FIA-persulphate (TP) and ICPOES**

**Table 4.7 - Phosphorus composite influent statistic results, using raw data from Table B-4, Appendix B for FIA and FIA-TP and ICPOES**

<i>Source of variation</i>	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F</i>	<i>P-value</i>	<i>F-crit</i>
Rows	83	18	4.64	11	6.37E-10	1.90
Columns	14	2	7.27	18	4.19E-06	3.26
Error	14	36	0.408			
Total	112	56				

Table 4.7 shows that the calculated t-test statistics for the between-columns variation exceeded the critical value. Therefore, for a composite influent sample, there was a significant difference in the results for FIA and FIA-TP and ICPOES.

Since there was a significant difference in results for FIA, FIA-TP and ICPOES, ANOVA was used to compare the measurements of FIA-TP and ICPOES (See Table 4.8).

**Table 4.8 - Phosphorus composite influent statistic results using raw data from Table 4.10 for FIA-TP and ICPOES**

<i>Source of variation</i>	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F</i>	<i>P-value</i>	<i>F-crit</i>
Rows	71	18	3.97	145	7.67E-16	2.22
Columns	0.002	1	0.002	0.086	0.772	4.41
Error	0.493	18	0.027			
Total	72	37				



Since the F-calculated for the between-columns variation was less than the F-critical value (Table 4.8), the null hypothesis is not rejected. Therefore there is no significant difference in the measurement for a composite influent sample for FIA-TP and ICPOES.

To conclude the significance between FIA and TP for a composite influent sample, the Mann-Whitney test was used.

The Mann-Whitney test demonstrated sufficient evidence, at 0.05 level, that there is a significant difference between FIA and TP phosphate concentrations (Table 4.9), which shows a high calculated t-test statistic value as compared to 1.96, the tabulated value.

**Table 4.9 - Phosphorus composite influent statistic results using raw data from Table 4.10 for FIA, actually FIA(OP), and FIA-persulphate (TP)**

		<b>Mann-Whitney U-test</b>								
		<b>By variable Var1</b>								
		<b>Marked tests are significant at p&lt;.05000</b>								
variables	<b>Rank sum</b>	<b>Rank sum</b>	<b>U</b>	<b>Z</b>	<b>p-level</b>	<b>Z</b>	<b>p-level</b>	<b>Valid N</b>	<b>Valid N</b>	<b>2*1sided</b>
	<b>FIA</b>	<b>FIA-TP</b>				<b>adjusted</b>		<b>FIA</b>	<b>TP</b>	<b>exact p</b>
		289	452	99	-2.38	0.017	-2.38	0.017	19	19

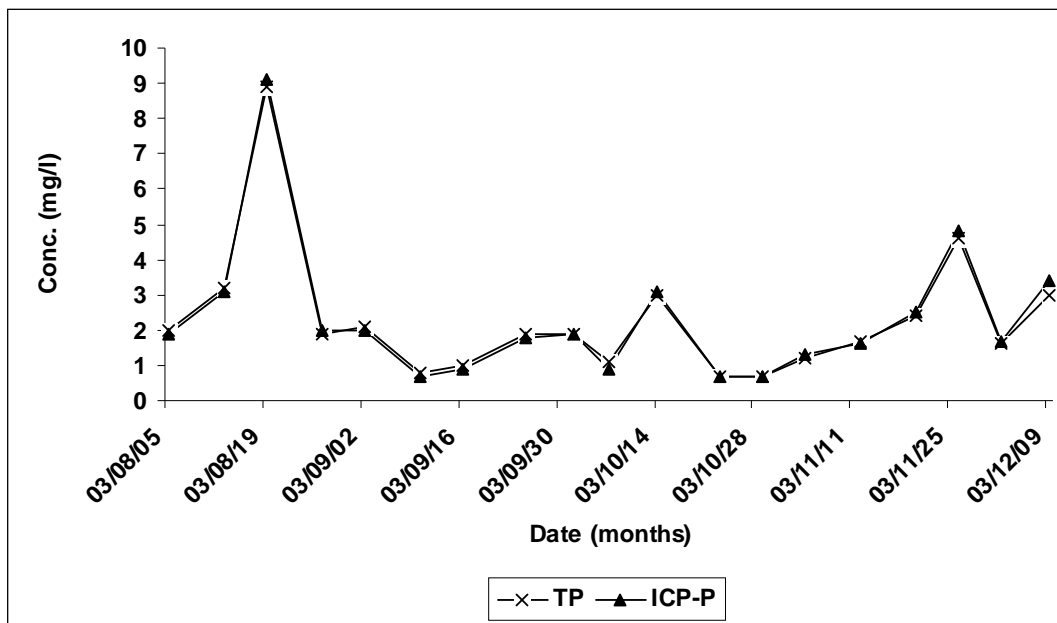
**Table 4.10 - Phosphorus composite influent statistic results using raw data from Table 4.10 for FIA and ICPOES**

variables	<b>Mann-Whitney U-test</b>									
	<b>By variable Var1</b>									
	<b>Marked tests are significant at p&lt;.05000</b>									
	<b>Rank sum</b>	<b>Rank sum</b>	<b>U</b>	<b>Z</b>	<b>p-level</b>	<b>Z</b>	<b>p-level</b>	<b>Valid N</b>	<b>Valid N</b>	<b>2*1sided</b>
<b>FIA</b>	<b>ICPOES</b>				<b>adjusted</b>		<b>FIA</b>	<b>ICP</b>	<b>exact p</b>	
292	448	102	-2.23	0.227	-2.28	0.022	19	19	1.00	

The Mann-Whitney test in Table 4.10 gives sufficient evidence that there is a significant difference between FIA and ICPOES results for a composite influent sample, since the calculated value of t-test statistics is higher than the critical t-value.

The phosphorus raw data for a composite effluent taken using FIA-persulphate and ICPOES from August 2003 to December 2003 is shown in Table B-5, Appendix B.

Figure 4.5 is a graph that illustrates the composite effluent phosphorus concentrations from the raw data in Table B-5, Appendix B, and Table 4.11 demonstrates the statistic calculation of results for FIA-persulphate and ICPOES using the paired t-test.



**Figure 4-5 - Phosphorus composite effluent concentrations taken using FIA-persulphate (TP) and ICPOES**

There is no significant difference between the FIA-TP and ICPOES phosphorus measurements for a composite effluent sample, because the calculated t-statistics in Table 4.11 does not exceed the critical t-value of 2.10. Both the paired t-test and two-factor ANOVA proved that the two techniques gave measurements that did not differ significantly for composite effluent.

The process was repeated for composite influent. The phosphorus raw data for a composite influent taken using FIA Persulphate and ICPOES from August 2003 to December 2003 is shown in Table B-6, Appendix B.

**Table 4.11 - The composite effluent statistical evaluation with the paired t-test on raw data from Table B-5, Appendix B for FIA-TP and ICPOES**

	<i>FIA-TP</i>	<i>ICPOES</i>
Mean (mg/l)	2.30	2.32
Variance	3.54	3.85
Observations	19	19
Pearson correlation	0.998	
Hypothesized mean difference	0	
Df	18	
t-stat	0.641	
P(T<=t) one-tail	0.265	
t-critical one-tail	1.73	
P(T<=t) two-tail	0.530	
t-critical two-tail	2.10	

Figure 4-6 is a graph that shows phosphorus composite influent concentrations from the raw data in Table B-6, Appendix B. The composite influent statistical calculation of results for TP and ICPOES is demonstrated in Table 4.12.

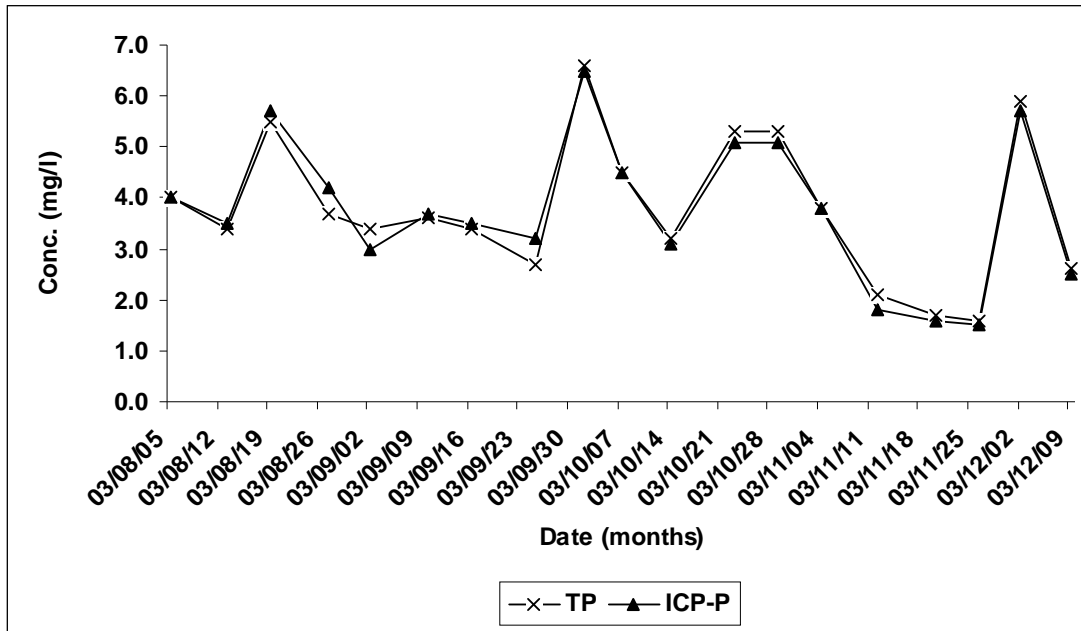


Figure 4-6 - Phosphorus composite influent concentrations taken using FIA-persulphate (TP) and ICPOES

**Table 4.12 - The composite influent statistic results, using raw data from Table B-6, Appendix B for FIA-persulphate and ICPOES**

	<i>FIA-TP</i>	<i>ICPOES</i>
Mean (mg/l)	3.81	3.79
Variance	1.99	2.00
Observations	19	19
Pearson correlation	0.986	
Hypothesized mean difference	0	
Df	18	
t-stat	0.294	
P(T<=t) one-tail	0.386	
t-critical one-tail	1.73	
P(T<=t) two-tail	0.772	
t-critical two-tail	2.10	

Table 4.12 indicates that there is no significant difference between the measurements of FIA-TP and ICPOES, since the calculated t-statistic is less than the critical t-value.

The phosphorus raw data for a composite effluent taken using FIA, IC, FIA-persulphate ICPOES and ICPMS from February 2004 to April 2004 is shown in Table B-7, Appendix B.

Figure 4-7 is a graph that demonstrates the composite effluent concentrations using raw data from Table B-7, Appendix B. The composite effluent statistical calculation of results for FIA, IC, FIA-TP, ICPOES and ICPMS is shown in Table 4.13.

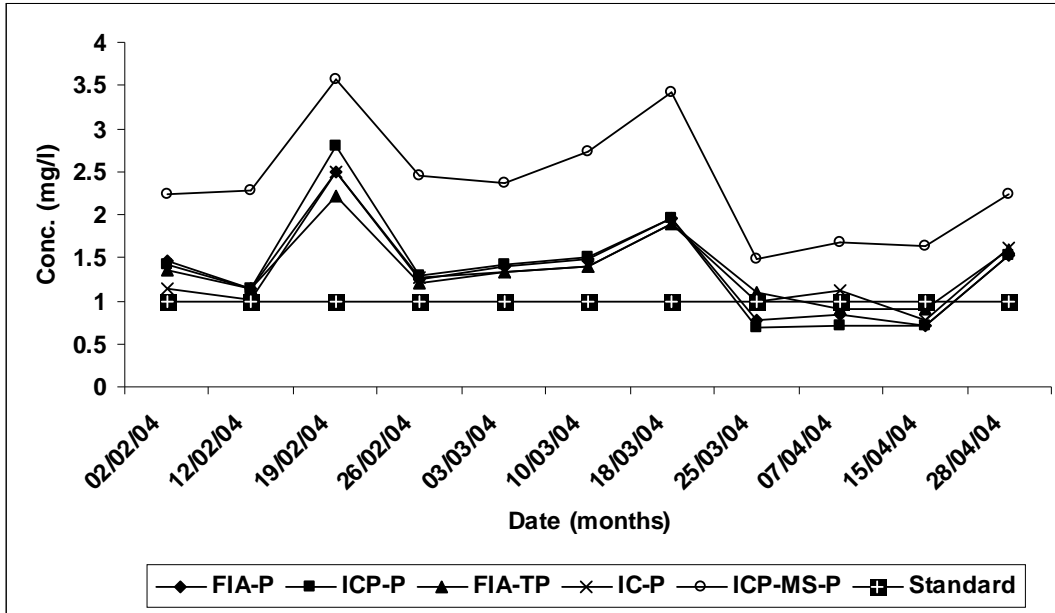


Figure 4-7 - Phosphorus composite effluent concentrations taken using FIA (FIA-OP), ICPOES, FIA-persulphate (FIA-TP), IC and ICPMS measured against 1.0 mg/l phosphorus standard

Table 4.13 - The composite effluent statistic results using raw data from Table 4.15 for FIA, IC, FIA-TP, ICPOES and ICPMS

<i>Source of variation</i>	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F</i>	<i>P-value</i>	<i>F-crit</i>
Rows	15	10	1.52	38	2.9E-17	2.08
Columns	8	4	2.04	51	4.01E-15	2.61
Error	1.61	40	0.04			
Total	25	54				

Since the F-calculated for the between-columns variation is greater than the F-critical value (Table 4.13), the null hypothesis is rejected. Therefore there is a significant difference in the results for a composite effluent sample using FIA, IC, FIA-TP, ICPOES and ICPMS.

By inspection it appears that the measurements of the ICPMS are not in line with the other four techniques. It was decided to repeat the ANOVA calculation without the ICPMS measurements.

Phosphorus composite effluent statistic results for FIA, IC, FIA-TP, ICPOES are shown in Table 4.14.

**Table 4.14 - Phosphorus composite effluent statistic results, using raw data from Table 7 for FIA, IC, FIA-TP, and ICPOES**

<i>Source of variation</i>	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F</i>	<i>P-value</i>	<i>F-crit</i>
Rows	10	10	1.02	62	2.92E-17	2.16
Columns	0	3	8.79E-05	0.005	0.999	2.92
Error	0.492	30	0.016			
Total	11	43				

The F-calculated for the between-columns variation is less than the F-critical value in Table 4.14; therefore there is no significance in the results for a composite effluent sample for FIA, IC, FIA-TP and ICPOES.

The phosphorus raw data for composite influent results obtained using FIA, IC, ICPOES, ICPMS and FIA- TP is shown in Table B-8, Appendix B.



Figure 4-8 is a graph that shows phosphorus composite influent concentrations using raw data in Table B-8, Appendix B. The phosphorus composite influent statistic calculation of results is demonstrated in Table 4.15.

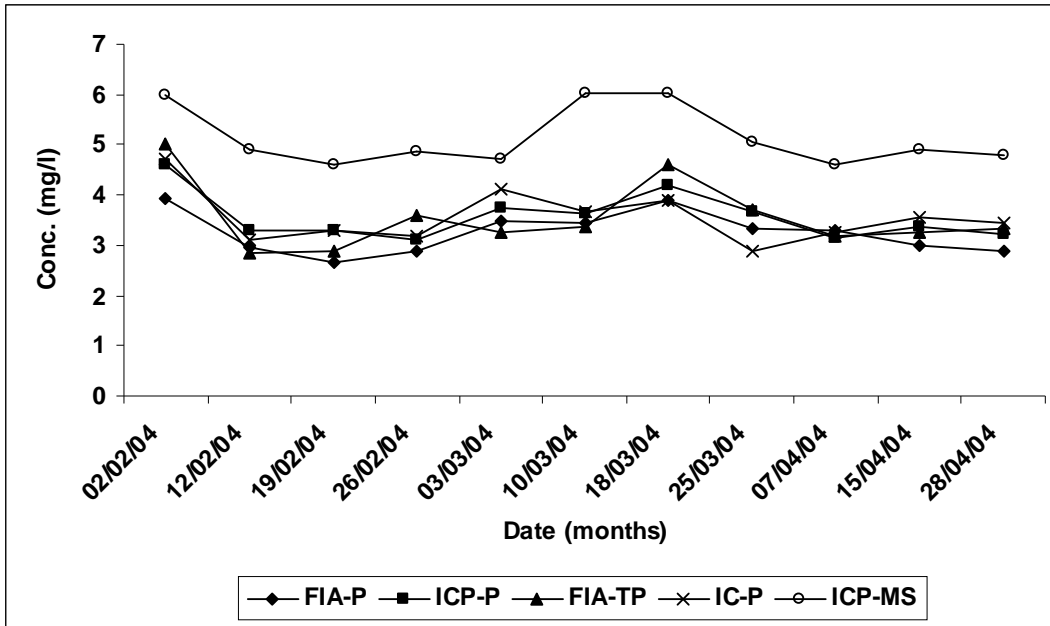


Figure 4-8 - Phosphorus composite influent concentrations using FIA, ICPOES, FIA-per sulphate (TP), IC and ICPMS

Table 4.15 - Phosphorus composite influent statistic results using raw data from Table B-8, Appendix B for FIA, IC, FIA-TP, ICPOES and ICPMS

<i>Source of variation</i>	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F</i>	<i>P-value</i>	<i>F-crit</i>
Rows	11	10	1.14	14	1.96E-10	2.08
Columns	25	4	6.23	79	1.77E-18	2.61
Error	3	40	0.08			
Total	39	54				

Since the F-calculated for the between-columns variation is greater than the F-critical value (Table 4.15), the null hypothesis is rejected. Therefore there is a significant difference in the results for a composite influent sample analysed by FIA, IC, FIA-TP, ICPOES and ICPMS.

Since the ICPMS measurements appeared to be out of line, the ANOVA calculations were carried out with regard to the other four techniques.

The phosphorus composite influent statistic calculation of results measured by FIA, IC, FIA-TP and ICPOES is demonstrated in Table 4.16.

**Table 4.16 - Phosphorus composite influent statistic results using raw data from Table B-8, Appendix B for FIA, IC, FIA-TP and ICPOES**

<i>Source of variation</i>	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F</i>	<i>P-value</i>	<i>F-crit</i>
Rows	9	10	0.909	13	2.3E-08	2.16
Columns	0.801	3	0.267	3.81	0.020	2.92
Error	2	30	0.070			
Total	12	43				

Since the F-calculated for the between-columns variation is greater than the F-critical value (Table 4.16), the null hypothesis is rejected. Therefore there is a significant difference in the results for a composite influent sample analysed by FIA, IC, FIA-TP and ICPOES.

By inspection it was decided to remove the data of the FIA and to do the ANOVA with the FIA-TP, IC and ICPOES.

The phosphorus composite influent statistical calculation of results measured by FIA, IC, FIA-TP and ICPOES is demonstrated in Table 4.17.

**Table 4.17 - Phosphorus composite influent statistic results using raw data from Table B-8, Appendix B for FIATP, IC and ICPOES**

<i>Source of variation</i>	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F</i>	<i>P-value</i>	<i>F-crit</i>
Rows	7	10	0.786	9	9.69E-06	2.35
Columns	0	2	0	0.019	0.980296	3.49
Error	1.60	20	0.079			
Total	9	32				

There is no significant difference in results for a composite influent sample analysed by IC, FIA-TP and ICPOES, since F-calculated for the between-columns variation is less than the F-critical value in Table 4.17.

## 4.2 CONCLUSIONS ON STATISTICS COMPARISONS

For a composite effluent sample, both t-test (Tables 3 and 4.11) and ANOVA (Table 4.4) show that there is no difference in results between FIA and ICPOES. Tables 4.1 and 4.2 demonstrated that there is no difference in results between FIA and ICPOES for a composite influent sample.

When looking at FIA, FIA-TP and ICPOES in Table 4.5, there is a difference in results among these three techniques for a composite effluent sample, but Tables 4.6 and 4.12 show that there is no significance in results between FIA-TP and ICPOES for a composite effluent sample.

Also, when looking at FIA, FIA-TP and ICPOES, it is clear that there is difference in results among these three techniques for a composite influent sample. Again, Table 4.8 shows no difference in results between FIA-TP and ICPOES.

Therefore, for both composite effluent and composite influent samples, there is no difference in results between FIA-TP and ICPOES, but a significant difference in results between FIA and FIA-TP and FIA and ICPOES (See Tables 4.9 and 4.10).

For both composite effluent and composite influent samples (see Tables 4.13 and 4.15 respectively), there is a significant difference among five techniques (FIA, FIA-TP, IC, ICPOES and ICPMS). For a composite effluent sample there is no difference between FIA and IC, but a significant difference between FIA and ICPMS and IC and ICPMS.

Since there is no difference in results between FIA-TP and ICPOES for both composite effluent and composite influent, it can be concluded that there is a difference in results between FIA-TP and ICPMS and ICPOES and ICPMS.

Results given in Tables 4.13 and 4.14 showed that there was no difference between FIA, IC, FIA-TP and ICPOES for an effluent. Since an effluent is at low concentration of phosphorus, this is expected, as an orthophosphate seems to be equivalent to total phosphate if phosphorus concentrations are at low levels. This proves that orthophosphate can be either less or equal to total phosphate.

A difference between FIA, IC, FIA-TP and ICPOES for an influent can be seen in Table 4.16. This is expected, since FIA and IC measure orthophosphate while FIA-TP and ICPOES give the total phosphate measurements.

In conclusion, that for both composite effluent and composite influent results, statistically there is a significant difference between the following techniques:

- FIA and FIA-TP;
- FIA and IC;
- FIA and ICPOES;
- FIA and ICPMS; and
- FIA-TP and ICPMS.

Tables 4.6 and 4.8 proved no difference in results for both composite effluent and composite influent for FIA-TP and ICPOES. This is confirmed in Tables 4.11 and 4.12.

**Table 4.18 phosphorus species determined from composite effluent and composite influent using FIA, FIA-TP and ICPOES**

SAMPLE	TECHNIQUES	RESULTS	COMPARISON
Composite influent	FIA and FIA- TP	Anova-F-calculated > F-critical value.	FIA phosphorus concentrations are less than FIA-TP phosphorus concentrations.
	FIA and ICPOES	1. t-calculated < t-critical = t-test. 2. F-calculated < F-critical value = Anova. 3. t- statistic is greater than the t-critical = Mann Whitney.	1 & 2 shows FIA phosphorus concentrations equivalent to ICPOES phosphorus concentrations, but Mann Whitney confirms that at 95% confidence level, FIA is not equivalent to ICPOES.
	FIATP and ICPOES	1. t-calculated > t-critical = t-test. 2. F-calculated > F-critical value = Anova. 3. t- statistic is greater than the t-critical = Mann Whitney.	Anova, Mann Whitney and t-test show sufficient evidence that FIA-TP phosphorus concentrations are equivalent to ICPOES phosphorus concentrations.

SAMPLE	TECHNIQUES	RESULTS	COMPARISON
	FIA & ICPOES	1. $t\text{-statistics} < t\text{-critical value}$ . 2. $F\text{-calculated} < F\text{-critical value} = \text{Anova}$ .	FIA phosphorus concentrations are equivalent to ICPOES phosphorus concentrations.
	FIA-TP and ICPOES	1. $t\text{-statistic} < t\text{-critical value}$ . 2. $F\text{-calculated} < F\text{-critical value} = \text{Anova}$ .	Both t-test and Anova shows that FIA-TP phosphorus concentrations are equivalent to ICPOES phosphorus concentrations.

### 4.3 CONCLUSIONS

The investigation has shown that sample type is essential when selecting a technique for phosphorous analysis in water and wastewater samples. This is based on the following reasons:

- For a composite effluent sample FIA and FIA-TP phosphorus concentrations are all equivalent in Figure 4.3, but for a composite influent sample FIA phosphorus concentrations are less than FIA-TP concentrations as can be seen in Figure 4.4.
- Also, with the composite effluent colorimetric FIA and ICPOES gives equivalent phosphorus concentrations (Figure 4.3), but FIA phosphorus concentrations are not equivalent to ICPOES phosphorus concentrations for a composite influent (Figure 4.4).
- In Figure 4.7 it can be seen that equivalent phosphorus concentrations were found for FIA, FIA-TP, IC and ICPOES, for all four techniques, for a composite effluent sample.
- Therefore, for a composite effluent sample any technique, FIA, IC, FIATP and ICPOES can be used to determine phosphorus concentration, since all four techniques yielded equivalent phosphorus concentrations.
- With the composite influent, FIA phosphorus concentrations are less than those found when using an ICPOES.
- Therefore, for a composite influent sample, it is essential to determine phosphorus concentrations using both colorimetric FIA and ICPOES techniques to differentiate between orthophosphate and total phosphate concentrations.

Significance cost savings may be achieved if the nature of the sample type is being known when selecting a technique for phosphorus analysis.



For example, both composite influent and composite effluent samples, the phosphorus concentrations obtained using FIA-TP were the same as those from ICPOES, but ICPOES detected practically all the elements required simultaneously, including phosphorus, with satisfactory results and great rapidity. The limited sample preparation involved with ICPOES has advantages in cost savings. Additional personnel, reagents for sample digestion and safety, health and hazardous environment of the FIA-TP technique contribute to high cost.

The study shows that further investigation is essential if accuracy, precise and sensitive results are to be obtained from ICPMS for phosphorus determination because for both composite effluent and composite influent samples ICPMS differed from all other techniques.

Based on the results of the study, the FIA and ICPOES technique were selected and validated for routine phosphorus analysis in water (Table 4.19 and Table 4.20).

**Table 4.19 – FIA and ICPOES figures of merit**

<b>PARAMETER</b>	<b>FIA</b>	<b>ICPOES</b>
Linear dynamic range (mg/l)	0-10	0-60
Estimated error at 95% confidence limit (%)	10	10
Lowest measurable concentration (mg/l)	0.05	0.04

The standard deviation (stdve) was used to determine both the repeatability and reproducibility of Flow Injection Analysis (FIA) and ICPOES techniques using quality control results as shown in Table 4.20.

**Table 4.20 - Quality control results for different techniques investigated**

<b>METHOD</b>	<b>QUALITY CONTROL MEASURED VALUE (mg/l)</b>	<b>QUALITY CONTROL ACTUAL VALUE (mg/l)</b>	<b>STANDARD DEVIATION (%)</b>
Colorimetric-FIA	0.998	1.0	0.01
ICPOES	29.4	30	0.02

The verification standard of 1.0 mg/l and 30 mg/l dihydrogen dodecahydrate phosphate was used in FIA and ICPOES respectively, after every nine to 10 samples during phosphorus analysis.

The next chapter will highlight the analysis cost implications carried out.

## CHAPTER 5

### ANALYSIS COST IMPLICATIONS

#### 5.1 COMPARING TECHNIQUES

To determine a detail comparison to make a final selection of a technique, the following criteria (Thomas, 1999) were used.

##### 5.1.1 Detection limit

Detection limits achievable for individual techniques represent a significant criterion for instrument selection. ICPMS would seem to have greater sensitivity and lower detection limits for most elements. However, high operational cost because of the volume of argon used and susceptibility to high salt concentrations present in digested samples which could result in measurements which may not be reliable, limits the ICPMS as the method of choice.

##### 5.1.2 Analytical working range

This can be viewed as the concentration range over which quantitative results can be obtained without recalibrating the instrument. The calibration function for both ICPOES and ICPMS is spread over a wider dynamic range when compared to FIA and IC.

##### 5.1.3 Sample throughput

The number of samples that can be determined per unit time is defined as sample throughput. Both ICPOES and ICPMS offer multi-element capabilities and high sample throughput when compared to FIA and IC.

##### 5.1.4 Purchasing cost

The multi-element instruments (ICPOES and ICPMS) are more expensive when compared to less complex FIA and IC.

### 5.1.5 Interferences

The study has shown that few analytical techniques are free from interferences. However, with the determination of phosphorus by ICPMS, most interference found were possibly due to isobaric effects, in particular those resulting from molecular ions  $^{15}\text{N}^{16}\text{O}^+$  and  $^{14}\text{N}^{16}\text{O}^1\text{H}^+$  that are formed in argon plasma.

### 5.1.6 Skills level in the methodology

This is based on the ease of use of the technique, operator skill level and whether application methods are readily available. Colorimetric-FIA is simple, very easy to use and IC applications are well documented. Although ICPOES is perhaps the most widely used multi-element technique it requires operator with a higher skill level and experience to achieve good quality data. ICPMS is relatively new and expensive when compared to FIA, IC and ICPOES and therefore routine applications for the technique are not as extensive as the other three techniques, especially for phosphorus determination.

### 5.1.7 Application

The ICPOES is faster than an ICPMS, which is a sequential technique and many laboratories routinely determining a number of elements in thousands of samples per day. Hence, ICPOES will be preferable than ICPMS if sample throughput is important. Time intensive sample preparation, when using FIA and IC, is a major disadvantage.

Table 5.1 shows how the techniques used differ in cost per sample for phosphorus analysis. The average cost was determined using the prices from three well-known water laboratories in South Africa.

**Table 5.1 - Average cost of phosphorus according to the technique selected, based on cost as in the year 2005**

<b>Technique</b>	<b>Cost for each sample (Rands)</b>
Phosphorus, ortho (colorimetric)	50.00
Phosphorus, ortho (chromatographic)	42.00
Phosphorus persulphate digestion	42.00
Phosphorus ICPOES	42.00
Phosphorus ICPMS	Not available

The investigation from the previous chapter shows that commercially available equipment varies widely in complexity, but a colorimeter is a standard method in practically every laboratory. Although the method depends on reagent preparation, this technique yields satisfactory results without requiring expensive equipment. Colorimetric methods save time because analyses are rapid, easy to perform and instruments are readily automated, including data output. The technique is amenable to the capabilities of the personnel in laboratories where phosphorus is routinely measured, as it is relatively easy technique.

The advantage of using ICPOES for phosphorus analysis is that it can detect practically all the elements required simultaneously, including phosphorus, with satisfactory results.

High speed and little sample preparation involved when an ICPOES is used, are other advantages with regard to cost-savings. Although both IC and colorimetric techniques involve reagent preparation, time taken per sample for anions, and phosphorus analysis in an IC seems to be a disadvantage, especially in the water and wastewater treatment works, where results are urgently needed for optimisation of the plant on a daily basis.

Additional personnel and reagents for sample digestion increases the costs associated with the persulphate method. The safety, health and hazardous environment issues of the method also contribute to high cost. As mentioned, clear waste disposal procedures must be in place when using the hazardous chemicals involved in the oxidative step for FIA (TP).

During this study it has been difficult to obtain the cost of phosphorus analysis using ICPMS, as little or no laboratories use ICPMS due to high purchase price. According to Dulude (2007), only academic and environmental laboratories use ICPMSs. Academic laboratories make use of the capability the ICPMS has to achieve ultra low detection limits and to distinguish between isotopes.

## **5.2 COST ANALYSIS**

For integrated cost analysis, the following were taken into consideration to determine operational cost of each technique used for phosphorus analysis. It must be remembered that only a comparative analysis can be achieved and then only when applying a pre-determined set of parameters. If, for example, an ICPOES is used to determine phosphorus in one sample then that analysis would take approximately 60 min. The same time would yield results for perhaps 40 elements, greatly reducing the cost of the phosphorus determination.

### 5.2.1 Direct cost

Direct cost was simply based on reagents or chemicals, consumables including gas used for phosphorus for the operating of an instrument. Time taken for sample preparation, preparation of working standards, data analysis, reporting time and capturing time were also taken into consideration.

### 5.2.2 Fixed cost

This cost was based on accommodation for an instrument, electricity charges and staff salaries.

### 5.2.3 Indirect cost

Indirect cost was determined by staff involvement that are not operators of the instruments, yet performing tasks enabling sample analysis to take place. This cost includes sampling personnel and laboratory assistants who assist in preparation of samples. Depreciation on instruments and services were also indicated as operational cost. Table 5.2 was used in determining the operational cost when selecting the technique for phosphorus analysis.

**Table 5.2 Integrated cost analysis**

<b>Technique</b>	<b>Sample throughput (min/sample)</b>	<b>Chemicals/reagent</b>	<b>consumables</b>	<b>Power</b>	<b>Operator</b>
IC	13.5	Eluent preparation	Vials and column		Skilled operator
FIA	1.0	Time-intensive reagent preparation	Vials and tubing		Unskilled operator
ICPOES	3.0	No reagents	Argon gas	1.5 kW	Skilled operator
ICPMS	8.0	No reagents	High argon gas	1.3 kW	Highly skilled operator

It is essential to reiterate that the ICPOES is faster (20 elements in 3 min) than the ICPMS with good detection limit for phosphorus analysis. ICPMS is a sequential technique, taking 8 min to measure 20 determinants in one sample. Although time intensive reagent preparation is required in FIA for phosphorus determination, analysis is simple, rapid and automated.

**Table 5.3 - Proposed cost implications**

<b>Technique</b>	<b>Sample throughput</b>	<b>Operational cost</b>	<b>Purchasing cost</b>
FIA	Moderate sample throughput	Low operational cost	R250,000; Anatech, Sloane Park, Gauteng, SA
IC	Low sample throughput	High operational cost	R220,000; Metrohm, Woodmead Sandton, SA
ICP-OES	High sample throughput	Low operational cost	R950,000; Spectro Analytical Instruments, Freight Park, Gauteng SA
ICPMS	moderate sample throughput	High operational cost	R1,700,000; Micromass, UK

The prices indicated above are applicable for the year 2006.

The next chapter will explain the conclusions and recommendations of this investigation.



## CHAPTER 6

### CONCLUSIONS AND RECOMMENDATIONS

#### 6.1 ACHIEVEMENT OF THE OBJECTIVES

This investigation was initiated with the aim of determining the best, most suitable technique for orthophosphate and total phosphate in water and wastewater. The results obtained from all four techniques for composite effluent samples are similar. The FIA phosphorus results for composite effluent are equivalent in all techniques (FIA, IC, ICPOES, and FIA-TP).

The colorimetric method has shown the lowest measurable concentration at 0.05 mg/l, with 100% quality control compliance at 95% confidence limit (Table 4.20).

The techniques used demonstrated that for the composite effluent sample, the FIA orthophosphate concentration was equivalent to that of the total phosphate with the lowest concentration determined 0.05 mg/l for both methods, and 100% and 99% compliance respectively. Hence, for the composite effluent, the orthophosphate technique is adequate.

The composite influent results obtained from FIA, IC and ICPOES indicate that the orthophosphate concentration will be either equal to or less than total phosphorus. For the composite influent, the total phosphorus would be suitable, but orthophosphate would be preferable for quality results assurance.

Both the composite influent and composite effluent results show that the results obtained from the ICPOES are for total phosphorus concentration. This is expected as the instrument determined phosphorus atom concentration and is independent of the molecule containing the atom. The ICPOES technique is preferable for total phosphorus due to linear dynamic range of 0-60 mg/l, with 99.8% compliance (Tables 4.19 and 4.20).

Sample type and stability are essential in the selection of techniques to be used for phosphorus analysis. Methods and problems associated with analysis of different forms of phosphorus in water and wastewater samples have been indicated. The colorimetric determination using molybdate remains a simple technique for orthophosphate analysis.

Although the persulphate method generally yielded satisfactory results for the total phosphorus, the ICPOES is quick, safe, accurate and cost-effective. There is no elaborate sample preparation required and no hazardous waste to dispose at the end of the analysis.

## **6.2 CONTRIBUTION OF THE STUDY**

Currently, orthophosphate is being analysed on colorimetric-FIA. Total phosphorus is being analysed simultaneously with other metals on an ICPOES. These methods were validated and are presently accredited.

Although this study was primarily undertaken for quality assurance of results, replacing the persulphate method with ICPOES resulted in significant cost saving. Also, the use of colorimetric-FIA instead of IC is quick, simple and satisfactory.

The reagents used for the persulphate method are unsafe (sulphuric acid is corrosive and mercury oxide very toxic); therefore substituting the persulphate method with the ICPOES technique has contributed to safety, health, hazardous circumstances and the environment.

### 6.3 SHORTCOMINGS OF THE CURRENT INVESTIGATIONS

Few specific references to phosphorus determination by emission source mass spectrometry could be found presumably because of the associated costs of ICPMS, Therefore, it will remain difficult to carry out inter-laboratory studies and investigations using an ICPMS as instrument of choice.

### 6.4 RECOMMENDATIONS

Based on the work done in this investigation, the analysis of different forms of phosphorus is recommended to be performed, as shown in Table 6.1.

**Table 6.1 - Proposed techniques for effluent and influent sample**

<b>Sample type</b>	<b>Technique</b>	<b>Forms of phosphorus</b>
Composite effluent	Colorimetric-FIA	Orthophosphate
Composite effluent	Chromatography-IC	Orthophosphate
Composite influent	Colorimetric-FIA	Both orthophosphate and Total
Composite influent	Spectrometry-ICPOES	Total phosphate

## 6.5 FURTHER RESEARCH

Realising that the orthophosphate can be measured accurately using colorimetric techniques, separation and quantification of each phosphorus component of the total phosphorus still poses a problem. Investigation is recommended into the use of rapid gravity sand filters to achieve the accurate separation of soluble and particulate phosphorous fractions.

Sample preservation is essential but this may include addition of preservatives, temperature control, sample storage and choice of proper containers which will depend on the analyte or element of interest. Further, sample preservation must ensure that the sample retains its physical and chemical characteristics so that results of the analysis represent the value of the element investigated.

Although, theoretically much has been mentioned with reference to challenges for determination of phosphorus by ICPMS due to isobaric interferences, little or no work has been done in terms of the influence of these interferences for phosphorus determination.

Therefore, investigations that require further study are as follows:

- separation of chemical states of phosphorus analysis by chromatographic technique prior to ICPMS phosphorus determination;
- effect of sample preservation for phosphorus analysis; and
- interferences of phosphorus analysis by ICPMS.

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# APPENDIX A

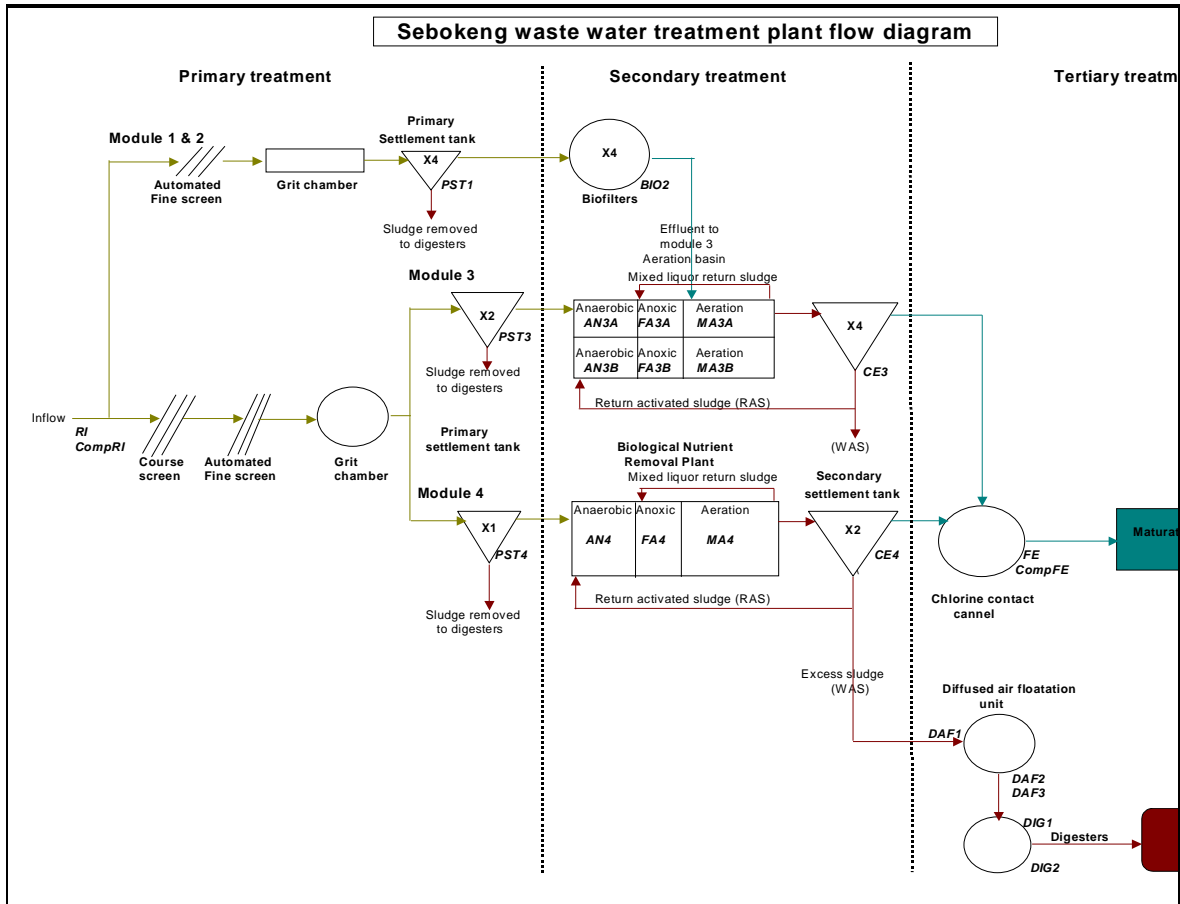
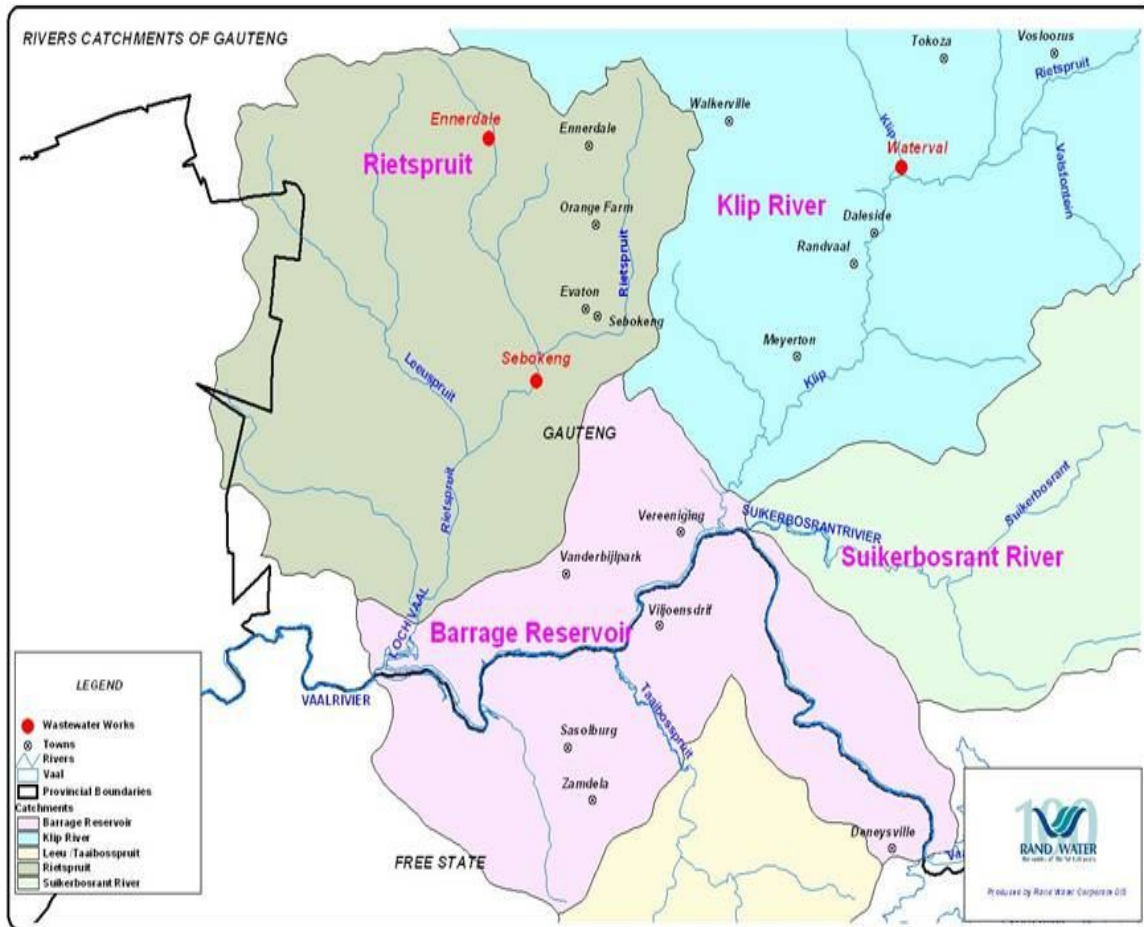


Figure A-1 - Process flow diagram of Sebokeng Wastewater Works



**Figure A - 2 - Study area - Sebokeng Waste Water Works**



**Figure A-3 - Settled sewage to the Bioreactor**



**Figure A-4 - The main purpose of the bioreactor is to remove nutrients (nitrate, nitrite, phosphate, ammonia) from wastewater**





**Figure A-5 - Rotating Disc Screens for the removal of coarse material from the flow stream**



**Figure A-6 - Secondary Settling Tank**



**Figure A-7 - Secondary Settling Tank from another view**



**Figure A-7 - Chlorine Contact Chamber, which is used for disinfection purposes to destroy pathogenic bacteria**



**Figure A-8 – Secondary settling tanks used to separate solid particles from water**





**Figure A-8 - Sludge fine screens before the digesters**



**Figure A-9 - Maturation pond provides a degree of polishing of the treated effluent discharged from the mechanical treatment process**

## APPENDIX B

**Table B-1 - The raw data for composite influent phosphorus concentrations, taken using ICPOES and FIA**

<b>Date</b>	<b>ICPOES (mg/l)</b>	<b>FIA-P (mg/l)</b>
04/06/02	3.8	4.1
12/06/02	3.6	3.7
20/06/02	3.5	3.3
28/06/02	4.4	4.7
02/07/02	4.1	3.7
10/07/02	3.1	3.3
18/07/02	3.3	3.0
26/07/02	4.4	3.6
06/08/02	4.7	4.6
14/08/02	4.7	4.1
22/08/02	4.6	4.8
30/08/02	3.7	4.2
03/09/02	6.2	5.1
11/09/02	2.6	3.3
19/09/02	4.2	4.1
25/09/02	4.5	4.3
08/10/02	3.5	3.5
16/10/02	3.1	4.8
24/10/02	4.7	4.2
30/10/02	5.0	5.4
05/11/02	4.2	3.6
13/11/02	4.9	4.8
21/11/02	5.1	4.8
27/11/02	4.7	4.7
03/12/02	9.5	9.4
11/12/02	6.8	4.4

<b>Date</b>	<b>ICPOES (mg/l)</b>	<b>FIA-P (mg/l)</b>
19/12/02	4.1	4.1
07/01/03	3.9	3.9
15/01/03	3.2	3.5
23/01/03	4.2	3.8
29/01/03	3.6	3.8
04/02/03	3.5	3.9
12/02/03	3.7	3.6
20/02/03	2.1	2.2
26/02/03	3.4	3.1
04/03/03	5.0	4.4
12/03/03	3.7	3.8
20/03/03	4.1	3.7
26/03/03	2.6	2.8
08/04/03	4.5	4.2
16/04/03	4.4	4.2
24/04/03	3.4	3.1
30/04/03	4.7	4.4
06/05/03	3.8	4.0
14/05/03	5.2	5.2
22/05/03	4.9	4.3
28/05/03	3.4	3.8
03/06/03	3.8	3.8

**Table B-2 - The raw data for a composite effluent phosphorus concentration, taken using ICPOES and FIA from June 2002 to June 2003**

<b>Date</b>	<b>ICPOES (mg/l)</b>	<b>FIA-P (mg/l)</b>
04/06/02	2.6	2.8
12/06/02	1.2	1.5
20/06/02	2.7	3.0
28/06/02	3.0	3.2
02/07/02	1.5	1.4
10/07/02	1.8	1.9
18/07/02	4.3	4.2
26/07/02	2.6	2.6
06/08/02	2.7	2.8
14/08/02	0.9	0.8
22/08/02	3.1	3.2
30/08/02	3.4	4.2
03/09/02	4.1	3.7
11/09/02	1.3	0.8
19/09/02	2.7	2.9
25/09/02	3.1	3.4
08/10/02	3.1	3.3
16/10/02	3.1	3.4
24/10/02	4.4	4.8
30/10/02	6.0	5.9
05/11/02	4.7	4.5
13/11/02	2.2	2.0
21/11/02	1.9	1.9
27/11/02	3.8	3.9



<b>Date</b>	<b>ICPOES (mg/l)</b>	<b>FIA-P (mg/l)</b>
03/12/02	4.3	4.2
11/12/02	3.3	3.4
19/12/02	3.0	3.1
07/01/03	1.9	2.2
15/01/03	3.5	3.7
23/01/03	2.6	2.4
29/01/03	3.1	3.2
04/02/03	1.7	2.0
12/02/03	1.5	1.6
20/02/03	0.8	1.0
26/02/03	2.1	2.0
04/03/03	3.2	2.7
12/03/03	1.2	1.3
20/03/03	0.9	0.9
26/03/03	2.3	2.3
08/04/03	1.5	1.5
16/04/03	1.7	1.6
24/04/03	1.2	1.0
30/04/03	1.4	1.3
06/05/03	0.9	0.9
14/05/03	1.7	1.8
22/05/03	1.3	1.1
28/05/03	1.4	2.2
03/06/03	1.1	1.1

**Table B-3 - The raw data for a composite effluent phosphorus concentration taken using FIA, FIA-persulphate (TP) and ICPOES (ICP-P) from August 2003 to December 2003**

<b>Date</b>	<b>FIA (mg/l)</b>	<b>TP (mg/l)</b>	<b>ICP-P (mg/l)</b>
5 Aug 03	1.6	2.0	1.9
13 Aug 03	2.8	3.2	3.1
19 Aug 03	8.9	8.9	9.1
27 Aug 03	1.8	1.9	2.0
02 Sep 03	1.9	2.1	2.0
10 Sep 03	0.5	0.8	0.7
16 Sep 03	0.8	1.0	0.9
25 Sep 03	1.8	1.9	1.8
02 Oct 03	1.8	1.9	1.9
07 Oct 03	0.7	1.1	0.9
14 Oct 03	2.9	3.0	3.1
23 Oct 03	0.4	0.7	0.7
29 Oct 03	0.5	0.7	0.7
04 Nov 03	0.6	1.2	1.3
12 Nov 03	1.4	1.7	1.6
20 Nov 03	2.3	2.4	2.5
26 Nov 03	1.5	4.6	4.8
02 Dec 03	1.6	1.6	1.7
09 Dec 03	2.6	3.0	3.42

**Table B-4 - The raw data for composite influent phosphorus concentrations taken using FIA (OP), FIA-persulphate (TP) and ICPOES (ICP-P) from August 2003 to December 2003**

<b>Date</b>	<b>FIA (mg/l)</b>	<b>TP (mg/l)</b>	<b>ICPOES (mg/l)</b>
05 Aug 03	3.5	4.0	4.0
13 Aug 03	3.3	3.4	3.5
19 Aug 03	3.3	5.5	5.7
27 Aug 03	1.5	3.7	4.2
02 Sep 03	2.4	3.4	3.0
10 Sep 03	3.3	3.6	3.7
16 Sep 03	3.4	3.4	3.5
25 Sep 03	1.5	2.7	3.2
02 Oct 03	5.3	6.6	6.5
07 Oct 03	3.8	4.5	4.5
14 Oct 03	3.1	3.2	3.1
23 Oct 03	3.7	5.3	5.1
29 Oct 03	4.3	5.3	5.1
04 Nov 03	2.4	3.8	3.8
12 Nov 03	1.7	2.1	1.8
20 Nov 03	0.7	1.7	1.6
26 Nov 03	1.3	1.6	1.5
02 Dec 03	1.3	5.9	5.7
09 Dec 03	2.0	2.6	2.5

**Table B-5 - The raw data for a composite effluent sample taken using FIA-persulphate (TP) and ICPOES (ICP-P) from August 2003 to December 2003**

<b>Date</b>	<b>TP (mg/l)</b>	<b>ICP-P (mg/l)</b>
05 Aug 03	2.0	1.9
13 Aug 03	3.2	3.1
19 Aug 03	8.9	9.1
27 Aug 03	1.9	2.0
02 Sep 03	2.1	2.0
10 Sep 03	0.8	0.7
16 Sep 03	1.0	0.9
25 Sep 03	1.9	1.8
02 Oct 03	1.9	1.9
07 Oct 03	1.1	0.9
14 Oct 03	3.0	3.1
23 Oct 03	0.7	0.7
29 Oct 03	0.7	0.7
04 Nov 03	1.2	1.3
12 Nov 03	1.7	1.6
20 Nov 03	2.4	2.5
26 Nov 03	4.6	4.8
02 Dec 03	1.6	1.7
09 Dec 03	3.0	3.42

**Table B-6 - Raw data for composite influent concentrations taken using FIA-persulphate (TP) and ICPOES (ICP-P) from August 2003 to December 2003**

<b>Date</b>	<b>TP (mg/l)</b>	<b>ICPOES (mg/l)</b>
05 Aug 03	4.0	4.0
13 Aug 03	3.4	3.5
19 Aug 03	5.5	5.7
27 Aug 03	3.7	4.2
02 Sep 03	3.4	3.0
10 Sep 03	3.6	3.7
16 Sep 03	3.4	3.5
25 Sep 03	2.7	3.2
02 Oct 03	6.6	6.5
07 Oct 03	4.5	4.5
14 Oct 03	3.2	3.1
23 Oct 03	5.3	5.1
29 Oct 03	5.3	5.1
04 Nov 03	3.8	3.8
12 Nov 03	2.1	1.8
20 Nov 03	1.7	1.6
26 Nov 03	1.6	1.5
02 Dec 03	5.9	5.7
09 Dec 03	2.6	2.5

**Table B-7 - Phosphorus raw data for composite effluent sample taken using FIA, IC, ICPOES, FIA-persulphate (TP) and ICPMS from February 2004 to April 2004**

<b>Date</b>	<b>FIA (mg/l)</b>	<b>ICPOES (mg/l)</b>	<b>FIA-TP (mg/l)</b>	<b>IC (mg/l)</b>	<b>ICPMS (mg/l)</b>
02 Feb 04	1.47	1.41	1.35	1.14	2.24
13 Feb 04	1.15	1.14	1.15	1.01	2.29
19 Feb 04	2.50	2.80	2.22	2.50	3.58
26 Feb 04	1.24	1.27	1.20	1.26	2.45
03 Mar 04	1.39	1.40	1.34	1.33	2.36
10 Mar 04	1.49	1.49	1.40	1.39	2.74
18 Mar 04	1.95	1.95	1.90	1.89	3.42
25 Mar 04	0.78	0.69	1.10	0.98	1.48
07 Apr 04	0.83	0.70	0.90	1.12	1.68
15 Apr 04	0.70	0.70	0.90	0.78	0.89
28 Apr 04	1.53	1.53	1.60	1.61	2.50

**Table B-8 - Phosphorus raw data for composite influent results using FIA, IC, ICPOES, ICPMS and FIA-TP taken from February 2004 to April 2004**

<b>INF</b>	<b>FIA (mg/l)</b>	<b>ICPOES (mg/l)</b>	<b>FIA-TP (mg/l)</b>	<b>IC (mg/l)</b>	<b>ICPMS (mg/l)</b>
02 Feb 04	3.92	4.59	5.02	4.70	5.99
13 Feb 04	2.97	3.31	2.86	3.09	4.92
19 Feb 04	2.64	3.29	2.90	3.28	4.59
26 Feb 04	2.87	3.00	3.61	3.18	4.86
03 Mar 04	3.47	3.74	3.25	4.10	4.72
10 Mar 04	3.43	3.62	3.36	3.65	6.02
18 Mar 04	3.89	4.21	4.60	3.88	6.03
25 Mar 04	3.35	3.65	3.70	2.90	5.05
07 Apr 04	3.28	3.16	3.18	3.24	4.60
15 Apr 04	2.99	3.38	3.27	3.57	4.90
28 Apr 04	2.90	3.22	3.33	3.44	4.80