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Phosphorus fractions in biosolids, biosolid-amended soils, runoffs and its impact on primary productivity in aquatic ecosystems

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PHOSPHORUS FRACTIONS IN BIOSOLIDS, BIOSOLID-AMENDED SOILS, RUNOFFS AND ITS IMPACT ON PRIMARY PRODUCTIVITY IN AQUATIC ECOSYSTEMS

by

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Bachelor of Science in Biology, University of Guyana, 2007

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Abstract

Phosphorus fractions in biosolids, biosolid-amended soils, runoffs and its impact on primary productivity in aquatic ecosystems

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The impact of land application of biosolids on soil phosphorus (P) and subsequent transfer to aquatic ecosystems were assessed. Boxed reference soils were amended with two biosolids at a rate of 8 dry t/ha. Biosolids and soil samples taken over four months were sequentially fractionated to determine various inorganic and organic P pools. Also, within three weeks of biosolids application, four storm events were simulated and surface runoff and leachate from the soils were collected and analyzed for different P forms. The runoffs and equivalent inorganic nutrient were added to different mesocosms that mimicked stratified lakes. Samples from the mesocosms were periodically collected and analyzed for various physical, chemical and biological parameters. The results indicated that biosolids significantly affect different P pools in soils. Also, P loading from biosolids was expected to drive the mesocosms to hypereutrophication, yet the response was moderately eutrophic, followed by decline in chlorophyll a.

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List of Abbreviations

BAP	Bioavailable phosphorus
BAPP	Bioavailable particulate phosphorus
CCME	Canadian Council of Ministers of the Environment
DRP	Dissolved reactive phosphorus
DO	Dissolved oxygen
ECO	Environmental Commissioner of Ontario
ELI	Environment Leverage Inc.
EU	European Union
EPA	Environmental Protection Act (Ontario)
IP	Inorganic Phosphorus
MOE	Ministry of Environment (Ontario)
MRP	Molybdate reactive phosphorus
N	Nitrogen
OMAFRA	Ontario Ministry of Agriculture, Foods and Rural Affairs
OP	Organic phosphorus
PP	Particulate phosphorus
TP	Total phosphorus
UNEP	United Nation Environment Program
USEPA	United States Environmental Protection Agency
WWTP	Wastewater treatment plant

1 Introduction

1.1 Overview

For thousands of years, human waste has been successfully land applied by the Chinese society. The development of this ecologically sound practice helped maintain their soil fertility by recycling various nutrients. Nutrients from farmlands that were providing food for city dwellers were being returned to the farmlands in the municipal wastes. Thus, it was the ideal tool of getting rid of human waste: it recycled the nutrients and it prevented pollution in cities (USEPA 1999).

It was a different picture in the west. It was just over 150 years ago that Western Europe and North America began the large-scale land application of municipal wastewater where waste were removed from outhouses and transported to farms. Previously, the untreated wastewater was discharged directly into streams, rivers and lakes resulting in the pollution of these receiving water bodies. Sometimes, the recourse was to simply move the discharge to a lower point in the river (USEPA 1999).

Sir Edwin Chadwick, an environment advocate, came up with the brilliant slogan "the rain to the river and the sewage to the soil" (USEPA 1999). This led to the creation of sewage farms and by 1875; there were around 50 such plants in England and many more in other major European cities. Building sewage farms quickly caught on in the United States where by the beginning of 1900s, there were over ten such farms scattered in various cities (USEPA 1999).

The term 'biosolids' first came into officially usage in 1991 by the Name Change Task Force of the Water Environment Federation (WEF) in order to differentiate between untreated sewage

sludge and treated sewage sludge that can be safely land applied. UNEP (2009) defines biosolids as 'nutrient-rich organic material separated during the wastewater treatment process that, after receiving additional treatment and passing rigorous quality requirements, is used as an agricultural or commercial fertilizer and soil-conditioning material.'

The United States and Canada currently produce about 10 million dry tonnes of biosolids/yr (Lystek 2011). Biosolids that are produced from the WWTP may be utilized in a number of ways that include land and mine reclamation, agricultural land fertilization, forest fertilization, erosion control, horticulture and slope stabilization. Also, biosolids are used indirectly as a source of energy via incineration, composts, soil amendment mixes and fabricated soils (USEPA 2005; UNEP 2009).

In Canada, biosolids have been applied to agricultural lands for the past 40 years. About 50% of all biosolids produced are recycled on lands that amount to less than 1% of Canada's landmass (CWWA 2010). In Ontario, 43% of biosolids produced are land applied, 47% incinerated, and 4% are sent to a landfill (Apedaile 2001). Prior to summer of 1996, much of Ontario's biosolids were sent to Michigan due to lack of landfill spaces and suitable farmlands in Ontario. However, after 1996 Michigan closed its borders to Ontario's biosolids due to odour complaints, the result was an increased urgency to land apply biosolids on farmlands as a disposal means (Environmental Commissioner of Ontario 2007).

Biosolids contain valuable nutrients, both macronutrients and micronutrients, and organic matter that are required for healthy plant growth (Sommers 1977; Shober *et al.* 2003; Atalay *et al.* 2007). The major nutrients provided by applied biosolids are nitrogen and phosphorus. Farmers benefit due to lower costs when compared to synthetic fertilizers.

There are major concerns with the land application of biosolids. Some of these concerns include heavy metal leaching and accumulation, pathogens, organic contaminants and excess nutrient application (especially phosphorus). In response to the public perception of and concern about biosolids land application, from 1999 to 2003, the U.S. Geological Survey investigated the effects of biosolids applications to the Metro Wastewater Reclamation District sites near Deer Trail, Colorado. Such concerns included the potential contamination of soil, crops, ground water and surface water by the nine regulated trace metals: arsenic, cadmium, copper, lead, mercury, molybdenum, nickel, selenium, and zinc. Other parameters were also investigated. After four years, the USGS found that the concentrations of the nine regulated trace elements in the biosolids-amended soil were relatively uniform. In addition, their concentration did not exceed the regulatory standards of the USEPA rule 403. Also, there was no significant increase in arsenic, cadmium, chromium, lead, mercury, nickel, and zinc concentrations in ground water. However, concentrations of nitrate, copper, molybdenum, and selenium did have a significant increase at one or more wells (Yager *et al.* 2004). Also, the Ministry of Environment of Ontario maintains that from biosolids land application, 'the potential for harm to the environment is low based on the current regulatory standards associated with the management of this material.' (ECO 2007).

Biosolids contain water soluble nutrients that could affect water quality and the growth and health of organisms dependent on the water. Nutrients from biosolids may be removed from the amended soils by a number of processes such as runoff from precipitation, erosion of soluble and particulate components, and leaching to ground water (USEPA 2000). These nutrients are rapidly transported via waterways to many vulnerable lakes where catastrophic events unfold over a short period of time.

Although the earth is over seventy percent water, more than 97.5% of it is found in the oceans. Glaciers and the polar ice caps trap 70% of the remaining 2.5% freshwater with less than 1% of found in lakes, rivers, reservoirs and in aquifers (WHO 2011). There are more than 3 million lakes in Canada. However, the Great Lakes are the largest system of fresh surface water on the planet and they contain approximately 18% of the world's fresh surface water (Environment Canada 2010). Therefore, protecting the delicate aquatic ecosystems and health of lakes and waterways from agricultural pollutants is a major concern of federal, provincial/territorial and municipal authorities. Land application of biosolids may represent a potential non-point pollution source thus having much environment relevance and missing concerns.

With respect to nutrient loss from biosolids, phosphorus is the major concern. This is due to two main reasons: P is limiting in most oligotrophic lakes (Correll 1998) and biosolids are applied based on the nitrogen agronomic needs of plants which always result in the over-application of phosphorus to the amended soils since biosolids have a lower N:P ratio than algal biomass. During runoff after heavy precipitations, sediments enriched with phosphorus (particulate and soluble) get eroded and transported to watercourses that are tributaries of major lake systems. Phosphorus is the element most commonly limiting to primary productivity in lakes, and therefore often controls the extent of eutrophication. The results from the Experimental Lakes research area in northwestern Ontario provide conclusive evidence of the role of P in eutrophication for these lakes (Schindler 1974).

Yet, not all the phosphorus that is transported in the waterways that empties into lakes and reservoirs is bioavailable or potentially bioavailable. This is due to the formation of different P compounds in the soil and that these compounds have varying degrees of lability. Some are mobile while the majority remain immobile. Therefore, the impacts of P on receiving aquatic

bodies can only be determined after a knowledge of phosphorus fractionation which will give indications as to what percentage of the phosphorus is bioavailable, potentially bioavailable and unavailable for biological uptake. Relevant to this thesis, phosphorus applied in biosolids in excess of agronomic need could be environmentally harmful to receiving waters contributing to eutrophication. Alternately, if the form of P does not permit its use, then loading of P to surface waters from biosolids could be environmentally benign.

This thesis sets out to determine what impact P from biosolids may have on eutrophication of receiving waters. A complete P fractionation procedure was carried out on two different biosolids (anaerobically digested and alkali stabilized), reference soil and amended soils over a four-month period to address the questions: Does phosphorus become transformed in soil with respect to its bioavailability? After a storm event, how much phosphorus is removed from the amended soils in surface runoff and tile drains? In what form is the phosphorus removed? On entering oligotrophic lakes, what is the effect on primary productivity? What forms of phosphorus are present in the lake water? The answers to these questions can definitely help us to better gauge the impacts or potential impacts of land application of biosolids. The objectives of this thesis were to:

1. Characterize the inorganic and organic P fractions in biosolids and in reference and biosolids amended soils over time.

2. Determine P levels in runoff from reference and biosolids amended soils under simulated rainfall. And,

3. Conduct mesocosm experiments to investigate the impact of runoffs from amended and reference soils and inorganic fertilizers (as comparator) on primary productivity.

1.2 The Phosphorus Cycle

Phosphorus (P) is one of the most important elements in the ecosystem and it participates in and limits many biogeochemical processes in the biosphere. P is needed in all living cells as a component of essentials molecules that take part in energy storage and transfer, reproduction, structure (Conley *et al.* 2009; Ingall *et al.* 2011), growth (Benitez-Nelson 2000), and nucleic acids (Correll 1998; Conley *et al.* 2009).

P plays a major role in fresh water and estuarine ecosystems by acting as the major limiting nutrient controlling eutrophication (Correll 1998). In some oceanic systems such as restricted (Krom *et al*. 1991) and shallow-marine communities (MacRae *et al.* 1994) as well as oligotrophic regions of the North Atlantic and North Pacific (Cotner *et al.* 1997), P does play a limiting nutrient role. Furthermore, at the transition between fresh and saline water, P in most cases is the limiting nutrient (Conley 2009).

P can also be a limiting nutrient for terrestrial biological productivity and thus limits net carbon uptake (Lajtha and Schlesinger 1998). In soils, P limitation is related to the age of regolith. P is not limiting on young soils but it can be co-limiting if enough time has not elapsed in order for the weathering of parent material to release the phosphorus into a bioavailable form (Vitousek 2004). However, as the soil ages, P becomes more fully weathered and leached out resulting in a deficiency in old soils. This is especially pronounced in soils in the tropics marked by warm and wet climate as compared to temperate soils (Tanner *et al.* 1998). In short, chronosequence studies have shown that P availability in soils increases at first and then decreases as the soils age (Wardle *et al.* 2004).

With an ever increasing demand for more food, fertilizer application is at its highest in human history. Fertilizers provide crops with the complete spectrum of macro- and micronutrients required for plant growth. These nutrients were removed from the soils due to over-cropping and have to be artificially replaced since replenishing through the weathering of bedrock takes millions of years. Biosolids and farm manures are also widely applied to soils in order to provide much needed nutrients to growing plants. Biosolids can alone provide the complete spectrum of nutrients needed by plants. However, there is a major drawback to biosolids application. Their lower N/P ratio often results in over application of P especially when they are applied based on the nitrogen (N) agronomic needs of crops (this application based on N is due to N-limitation of most younger temperate soils). Over time, P in soil becomes greater than the crops' agronomic need, soil P sorption capacities are exceeded (Liu *et al.* 2007), and runoff water can contain environmentally unacceptable levels of dissolved and particulate P (Maguire *et al.* 2005).

1.2.1 Occurrence of Phosphorus

Phosphorus is the tenth most abundant element in the earth's crust and it is found in around 300 naturally occurring minerals in which orthophosphate is present as a structural constituent (Slansky 1986).

The occurrence of P differs considerably compared with other major biogeochemically-cycled elements – N, S, C and O. Unlike the other elements, P does not have a major gaseous form in the natural environment. This limits the role of the atmosphere in P cycling. Although phosphine (PH_3) may be produced via the anaerobic enzymatic reduction of phosphate and escapes into the atmosphere (Glindermann *et al*. 1996), it quickly reverts to phosphate in an oxic environment. However, P is transported as particulate matter on dust particles and may be dissolved in rain

drops. Yet, its contribution to the P cycle is very insignificant. It should be noted that particulate P that is transported by the atmosphere can be a significant P source in some areas such as deep island lakes and the surface waters in the central gyres of oceans. These areas are marked by extremely low P pools and P input from other sources is either relatively non-existent or extremely slow (Delaney 1998; Jacobson *et al.* 2000).

Another major difference when compared to other cycles is the role of redox reactions in influencing the reactivity and distribution of P. Although there is a plethora of P-containing compounds, P mainly exists in the +5 oxidation state. P is found almost exclusively as the tetrahedral oxy-anion – phosphate – which is the most reactive and readily available form of P. Nearly all dissolved and particulate P forms are modifications of the phosphate ion (Jacobson *et al.* 2000).

Finally, there is only one major isotopic form of P, ^{31}P. In addition to ^{31}P, there are two naturally occurring isotopes - ^{32}P (half life: 14.3 days) and ^{33}P (half life: 25.3 days). Due to the relatively short half-lives of these isotopes, they only account for a small fraction of the P interactions in the environment. However, they are quite important in open oceans where their formation in the atmosphere as a spallation product of Ar by cosmic rays (Marquez and Costa 1955) and their subsequent deposition into the ocean by rainfall may be the most significant source of the much needed limiting nutrient (Benitez-Nelson and Buesseler 1999).

1.2.2 The Global Phosphorus Cycle

The global phosphorus cycle has four main components: 1) Tectonic uplift and exposure of phosphorus containing minerals to the elements of weathering; 2) Weathering and subsequent erosion of these parent rocks resulting in the formation of soils with soluble and particulate phosphorus; 3) Transport of the soluble and particulate components by water – rainfall and snowmelt – to streams and finally to lakes and oceans; and, 4) Sedimentation and subsequent lithification of deposited sediments into new rocks. The cycle then repeats itself with tectonic uplift (Ruttenberg 2003).

The main P reservoirs are sediments (crustal rocks and soil > 60 cm deep and marine sediments), soils (0-50 cm), organic and inorganic P, land and oceanic biota (zoomass, anthropomass, marine and terrestrial phytomass), surface and deep ocean, mineable phosphorus and the atmosphere (Ruttenberg 2003; Jasinski 2009; Smit *et al.* 2009). Guano is also a reservoir of P. Guano is the excrement of bats, seabirds and other vertebrae deposited thousands of years ago mainly in caves. Guano is extremely rich in phosphorus and nitrogen and is used as a commercial fertilizer (Wetzel 2001).

1.2.3 Weathering of Terrestrial Bedrocks

In rock, P is present mainly as mineral apatite, igneous fluorapatite (FAP) and sedimentary carbonate fluorapatite (CFA), which undergo weathering to release phosphate ions during paedogenesis (Froelich *et al.* 1982; Compton *et al.* 2000). Apatite reacts with carbon dioxide and releases phosphate ions according to the equation:

$$Ca_5(PO_4)_3OH + 4\ CO_2 + 3H_2O \rightarrow 5\ Ca^{2+} + 3\ HPO_4^{2-} + 4\ HCO_3^-$$

This weathering of apatite takes place as a result of many processes. Organic acids released by plant roots can dissolve apatite and release P which can then either be absorbed or incorporated into biomass or it can be leached (Jurinak *et al.* 1986). Dissolution may also result from the reduced pH environment created by the decomposition of DOM thus releasing P into pore spaces (Schlesinger 1997).

In active cycling of P, weathering of apatite and release of new phosphate plays a limited role in mature soils. Most of the actively cycled P in soil is found within organic matter and thus not directly accessible to plants. Plants and other soil biota have developed two main strategies to retrieve the much needed P supply. First, both plants and microbes secrete the enzyme phosphatase which hydrolyses organic P into orthophosphate (Tarafdar and Claasen 1998; Hayes *et al.* 2000; Kizilkaya *et al.* 2007). Second, many plants have developed symbiotic relationships with fungal mycorrhizae which interact with the root hairs. The mycorrhizae secrete phosphatase and other organic acids into the surrounding soil which then cleave the phosphodiester bonds in organic matter thereby releasing P which is then absorbed and channelled to the roots (Dodd *et al.* 1987). In exchange for the increased P supply to plants by the mycorrhizal fungi, plants give the fungi their food (reduced carbon compounds).

Phosphorus which is present in soil may be grouped into either of two categories: labile (bioavailable) and non-labile or refractory (not readily bioavailable). However, these forms change over time due to the extent of paedogenesis. Labile P includes P in soil pores (as dissolved P), P adsorbed onto soil particles such as clays and P that is found in some organic matter. Refractory P is any P that is within the crystal lattice structure of apatite minerals and also any P that was precipitated along with and/or absorbed onto iron and manganese oxyhydroxides (Filippelli 2002). In short, as time progresses, primary apatite decreases and less-

soluble secondary minerals and organic P compounds increase. P is now partitioned mainly as refractory P and organic P (Ruttenberg 2003).

1.2.4 Phosphorus Transfer in Freshwater Systems

Orthophosphate is the most important inorganic phosphorus species present in aquatic systems and it is the only form of P that can be directly assimilated into organic compounds by primary producers (Cembella *et al.* 1984). However, more than 90% of phosphorus in freshwater systems exists as organic phosphates and cellular compounds adsorbed onto inorganic and particulate organic matter (Froelich 1988; Jacobson *et al.* 2000). Various biogeochemical processes during riverine transport can alter the form of P. Thus, P which was once unavailable to producers may now be bioavailable to producers in the lower stages of the river (Ruttenberg 2003).

In freshwater systems, four general fractions of phosphorus have been identified. These phosphorus fractions are: (a) soluble phosphate, (b) acid-soluble seston (suspended) as calcium phosphate and ferric phosphate, (c) soluble and colloidal organic P, and (d) organic seston.

P buffering in rivers is a major factor which determines availability of P for uptake by producers. Suspended river sediments can maintain the bioavailable P to almost constant levels. Likewise, these sediments may act as a reservoir of potentially available P which may be released when changes in equilibrium and redox potential occur. Also, suspended sediments play a major role in sequestering excess P that may have been loaded into the aquatic ecosystems (Ruttenberg 2003). Sequestration may take place either by adsorption or chemical incorporated into the matrix of the sediments.

Lakes are often classified by their trophic levels which are, in turn, often related to their phosphorus content, again as phosphorus is commonly the biolimiting nutrient. Lakes with total phosphorus concentrations below 0.010 mg/L are classified as oligotrophic, phosphorus concentrations between 0.010 and 0.020 mg/L are indicative of mesotrophic lakes, and eutrophic lakes have phosphorus concentrations exceeding 0.020 mg/L (Table 1.1). Notably, there is overlap in P concentrations among trophic categories as 1) P may not always determine chlorophyll and 2) total P does not account for differences in form and may not always reflect available P.

Table 1.1 Phosphorus and chlorophyll a characteristics for oligotrophic, mesotrophic and eutrophic lakes. Adapted from LAKE CLASSIFICATION SYSTEMS – PART 1 by Niles R. Kevern, Darrell L. King and Robert Ring. The Michigan Riparian February 1996.

Measured Parameter	Oligotrophic	Mesotrophic	Eutrophic
Total Phosphorus (mgm^{-3}) Mean (range)	8 (3.0 - 17.7)	26.7 (10.9 - 95.6)	84.4 (16 - 386)
Chlorophyll a ($mg\ m^{-3}$) Mean (range)	1.7 (0.3 - 4.5)	4.7 (3 - 11)	14.3 (3 - 78)

1.2.5 Phosphorus Cycling in the Epilimnion

The epilimnion is the upper layer of a thermally stratified lake that rests on the deeper colder hypolimnion. As a result of exposure to the wind, there is mixing of gases and nutrients within the epilimnion and light penetration (although light may penetrate to the hypolimnion of some lakes and not fully penetrate the epilimnia of others). Thus the raw materials are readily available for cyanobacteria and algae to photosynthesize in the epilimnion. However, as organic matter sinks to the hypolimnion, nutrients within the organic matter are lost from the epilimnion.

Consequently, primary productivity is reduced in stratified lakes in summer months (Wetzel 2001).

The little available nutrients in the epilimnion are rapidly recycled. The microbial loop plays an important role in nutrient cycling in microbial food webs. The microbial loop is a model of the pathway of nutrient cycling within the microbial constituents of aquatic communities and is used to describe carbon flow through DOC-bacterium-protozoan food chains rather than the classical movement of organic carbon from phytoplankton-zooplankton-predator (Azam *et al.* 1983). Bacteria and viruses form the base of the microbial loop where they play a major role in converting dissolved organic matter to particulate form, and in acting as a valuable source of carbon in the microbial food web. The major sources of dissolved organic matter in aquatic systems include phytoplankton exudates as well as excretion by grazers such as protozoa (Azam *et al.* 1983; Wetzel 2001; Fenchel 2008; Withers 2008).

In addition, C, N or P availability do not always translate into abundant primary productivity. Martin and Fitwater (1988) first demonstrated iron limitation as a one of the reasons for such a phenomenon. Further studies have shown that heterotrophic bacteria are directly competing with cyanobacteria and algae for iron in the microbial iron wheel thus limiting primary productivity (Kirchman 1996).

1.2.6 Phosphorus and Lake Sediments

In natural waters, the exchange of phosphorus between bottom sediments and the overlying water contributes greatly to the cycling to phosphorus. Over geological time sediments act as a sink which will sequester phosphorus for millions of years. However, over more ecologically relevant time scales physical, chemical, and biological factors interact and determine whether

these sediments will become a sink or source for phosphorus. A number of factors can affect such outcome, including: (a) the nature of the sediments (mineral content), (b) the condition of the overlying water, and (c) the biota within the sediments (Wetzel 2001).

The sequestering of phosphorus by lake sediments is controlled by five mechanisms. (1) Uptake of phosphate via direct absorption by alga and to a lesser extent, macrophytes. (2) Sedimentation of organic particulate matter (autochthonous or allochthonous). (3) Remineralization of organic P in sediments, releasing phosphate to the water column or leading to (4) adsorption of phosphate with elements such as Fe (III) and Mn (IV), reversible under conditions of low pH and Eh. (5) Deposition of mineral particles with adsorbed P. A fraction of the phosphorus deposited with mineral particles, sedimented as organic matter, or sorbed to minerals in the sediments can ultimately be buried and sequestered in sediment. However, in the shorter term, the balance between flux from sediments caused by microbial activity (remineralization of organic P, creation of low pH or Eh conditions favoring desorption) and deposition of organic matter to sediments has important implications for ecosystem function. Internal loading of P from sediments can be an important driver of primary production in lakes during the summer and fall, when external nutrient loading is reduced (Jacobson *et al.* 2000; Wetzel 2001; Filippelli 2002; Amirbahman *et al.* 2003; Ruttenberg 2003).

1.3 Forms of Phosphorus

For all practical purposes, phosphorus can be divided into two broad categories: particulate and dissolved. Within each category, there are numerous subcategories. Alternatively, P forms can be broadly divided into organic and inorganic.

1.3.1 Particulate Phosphorus (PP)

Particulate phosphorus is referred to as the fraction of phosphorus that contains all material, inorganic and organic, particulate and colloidal, that can be captured on a filter paper (Carlson and Simpson 1996). PP includes: (1) P in organisms such as nucleic acids (DNA and RNA), phosphoproteins, low-molecular weight esters of different compounds such as enzymes and vitamins, energy storage molecules such as ATP, ADP and AMP that are used in the pathways of CO_2 fixation and respiration; (2) mineral phases in rocks and regolith in which the P is adsorbed to inorganic complexes such as clays and iron (III) hydroxides; and (3) P in association with dead particulate organic matter (Jacobson *et al.* 2000; Wetzel 2001; Ruttenberg 2003).

PP is very important as a source of available P in both aquatic and terrestrial ecosystems (Jacobson *et al.* 2008). P associated with suspended sediments may account for as much as half of the total phosphorus in some aquatic systems (Jacobson *et al.* 2008). Of the particulate P, about 40% exists in the organic phase while the remainder is most likely trapped with the lattice structure of various minerals or is sorbed onto clay and calcium carbonate surfaces where is it transported as a colloid in aquatic systems (Jacobson *et al.* 2008). Apatite is the most abundant orthophosphate containing mineral and represents more than 95% of all P-containing minerals. Apatite is the primary phosphorus sink in the earth's exosphere and exists in both igneous and sedimentary rocks. Apatite is not a single mineral; rather, it is a group of orthophosphate containing minerals with chemical formula ($Ca_{10}(PO_4)_6(X)_2$) that include hydroxyapatite, fluorapatite, chlorapatite and bromapatite. These forms of apatite are named for high concentrations of OH^-, F^-, Cl^- or Br^- ions, respectively in their hexagonal dipyramidal crystal system. In addition, the Ca^{2+} can be substituted for by various Group 1, Group 2 and transition elements (Jacobson *et al.* 2008).

1.3.2 Dissolved phosphorus

Dissolved Inorganic Forms of Phosphorus

Plants and microbes absorb inorganic phosphorus only in solution. The most reactive form of phosphorus is the orthophosphate species (PO_4^{3-}) (Karl and Yanagi 1997). Phosphate ions are fully dissociated from phosphoric acid according to the following stepwise reactions:

$$H_3PO_{4(s)} + H_2O_{(l)} \rightleftharpoons H_3O^+_{(aq)} + H_2PO_4^-{}_{(aq)} \qquad K_{a1} = 7.25\times10^{-3}$$

$$H_2PO_4^-{}_{(aq)} + H_2O_{(l)} \rightleftharpoons H_3O^+_{(aq)} + HPO_4^{2-}{}_{(aq)} \qquad K_{a2} = 6.31\times10^{-8}$$

$$HPO_4^{2-}{}_{(aq)} + H_2O_{(l)} \rightleftharpoons H_3O^+_{(aq)} + PO_4^{3-}{}_{(aq)} \qquad K_{a3} = 3.98\times10^{-13}$$

These reactions demonstrate the triprotic nature of phosphoric acid. As seen above and in Figure 1.1, each of these successive reactions have a different value for the dissociation constant due to the fact that it is energetically less favourable to lose another H^+ if one (or more) has already been lost and the ion becomes more negatively-charged. Thus, the chemical reactivity and the availability of the phosphate ion are highly dependent on the pH of the solution.

Polyphosphates are another important class of inorganic phosphate compounds. In these condensed compounds, two or more phosphate molecules are joined together by forming P-O-P bonds resulting in the formation of chains or cyclic compounds. However, these compounds only account for minute portions of the total P found in the environment.

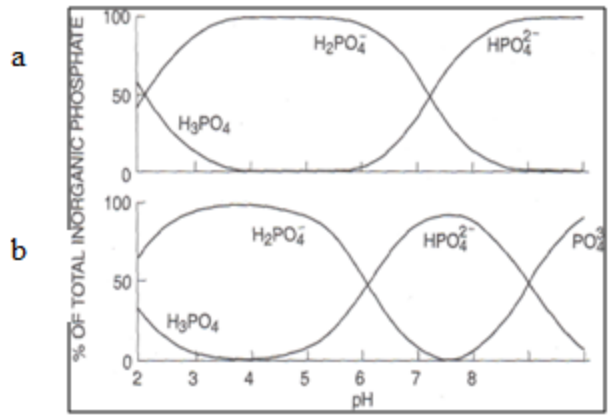


Figure 1.1 The dissolution of phosphoric acid is dependent on the pH of the water. At different pH, different species are present due to different extent of protonation. a. Distilled water. b. Seawater. Diagram adapted from Jacobson *et al.* 2008 p 361.

Organic Forms of Phosphorus

Many essential biomolecules are linked by the phosphodiester bond. Such compounds include nucleic acids, energy carrying molecules and even some vitamins and enzymes. Phosphorus is an important constituent of phospholipids – an essential component of cell membranes. Reactive P may also be sourced from dissolved organic compounds which may be readily available to organisms (Björkman and Karl 1994). Although the specific nature of most of these dissolved organic sources may be unknown, they are however dominated by the presence of the monophosphate esters and nucleotides (Karl and Yanagi 1997; Clark *et al.* 1998). This is especially true in the euphotic zones where the dissolved organic P may far exceed the dissolved inorganic P. Björkman and Karl (2003) have shown that microbial communities could derive up to 50% of their P from dissolved organic pools thus showing the importance of mineralization and transformation of one form to the other.

1.3.3 Phosphorus in Soil

The phosphorus contained in soil originates by both pedogenic and anthropogenic means; however, new P is introduced mainly as fertilizers, both mineral (Bolan *et al.* 2005) and organic residues (USEPA 1995).

In order to understand P supply, removal and transformation in soils, it is necessary to know about its chemical speciation. X-ray Absorption Near Edge Structure (XANES) is the most reliable tool that has been used to determine P mineralogy and transformation in different settings ranging from poultry litter to biosolids amended soils (Hesterberg *et al.* 1999; Maguire *et al.* 2000; Sato *et al.* 2005; Lombi *et al.* 2006; Shober *et al.* 2006; Brandes *et al.* 2007; Güngör *et al.* 2007; Turner *et al.* 2007; Ajiboye *et al.* 2008; Diaz *et al.* 2008; Kruse and Leinwebe, 2008; Seiter *et al.* 2008; Eveborn *et al.* 2009; Prietzel *et al.* 2010; Ingall *et al.* 2011).

Soil P exists in many different chemical forms that include both organic P and inorganic P. These forms widely differ in their partitioning, role and fate in soils (Shand and Smith 1997; Hansen *et al.* 2004). This is especially true for their bioavailability since different forms can be cycled at different rates, therefore supplying the needs of plants at different rates (Chen *et al.* 2003).

P minerals that are present in the soil may be grouped into: apatite minerals, non-apatite calcium phosphate minerals, aluminum phosphate minerals, iron and manganese phosphate minerals and others (Ingall *et al.* 2011).

Apatite minerals

Apatite is a common phosphate-bearing mineral that is found in all rock types - igneous, sedimentary and metamorphic (Chang *et al.* 1996). Apatite as fluorapatite (FAP) and sedimentary carbonate fluorapatite (CFA) is the most important exogenic P sink (Ruttenberg 1993; Compton *et al.* 2000). It represents the initial endogenic source of P in the form of crystalline rocks (Guidry and Mackenzie 2000). P in apatite is least available to plants since the phosphate is strongly sequestered away.

The general formula for apatite is $Ca_5(PO_4)_3(OH,F,Cl)$. However, there is much deviation from this formula due to the many conditions under which the precipitation of apatite occurs. In apatite, the hydroxyl, fluorine and chlorine atoms may replace each other. Calcium may even be partly substituted for by other metal cations such as manganese, strontium and rare-earth elements. In marine environment, bacteria play a major role resulting in the formation of authigenic apaptites. In such formations, the carbonate anion is often found substituting for the phosphate ion (Deer *et al.* 1992; Chang *et al.* 1996; Ingall *et al.* 2011).

Examples of apatitic minerals and their formulae are as follows (Ingall *et al.* 2011):

Apatite (poorly crystalline)	$Ca_5(PO_4)_3(OH,F)$
Carbonate apatite	$Ca_5(PO_4,CO_3)_3(OH,F)$
Carbonate fluorapatite	$Ca_5(PO_4,CO_3)_3(F)$
Carbonate hydroxylapatite fluorian	$Ca_5(PO_4,CO_3)_3(OH,F)$
Fluorapatite	$Ca_5(PO_4)_3F$
Hydroxylapatite chlorian	$Ca_5(PO_4)_3(OH,Cl)$

Non-apatite Calcium Phosphate Minerals

These minerals may represent pools of precursors to apatite minerals. Van Cappellen and Berner (1988) have demonstrated that monetite is a precursor phase for apatite formation in natural settings. Examples of non-apatite calcium phosphate minerals and their formulae are as follows (Ingall *et al.* 2011):

Anapaite	$Ca_2Fe(PO_4)_2.4H_2O$
Herderite	$CaBe(PO_4)F$
Messelite	$Ca_2(Mn,Fe^{2+})(PO_4)_2.2H_2O$
Monetite	$CaHPO_4$
Scholzite	$CaZn_2(PO_4)_2.2H_2O$
Whiteite	$(Ca,Fe,Mg)_2Al_2(PO_4)_4(OH)_2.8H_2O$

Aluminium phosphate minerals

This group of phosphate containing minerals has been the primary interest of many researchers owing to the fact that these minerals may influence P bioavailability (Hesterberg *et al.* 1999; Shober *et al.* 2006). Alum has been widely applied to farm animal litter and biosolids in order to limit the solubility of P (Smith *et al.* 2004; Warren *et al.* 2006; Moore *et al.* 2007). Moore *et al.* (1999) have shown that alum addition to poultry litter can reduce the solubility of P by as much as 99% by sequestering the soluble P into relatively insoluble aluminum phosphates. Examples of aluminum phosphate minerals and their formulae are as follows (Ingall *et al.* 2011):

Augelite	$Al_2(PO_4)(OH)_3$
Brazilianite	$NaAl_3(PO_4)_2(OH)_4$

Childrenite manganoan	$(Mn,Fe)Al(PO_4)(OH)_2.H_2O$
Eosphorite	$MnAl(PO_4)(OH)_2.H_2O$
Lazulite	$(Mg,Fe)Al_2(PO_4)_2(OH)_2$
Montebrasite	$(Li,Na)Al(PO_4)(OH,F)$
Variscite	$AlPO_4.2H_2O$
Wardite	$NaAl_3(PO_4)_2(OH)_4.2(H_2O)$
Wavellite	$Al_3(PO4)_2(OH,F)_3.5(H_2O)$

Iron and manganese phosphate minerals

Because of the similarity of charge and atomic radii, iron and manganese often substitute for each other at the same site in the lattice structure of a mineral (Ingall *et al.* 2011). Precipitates of iron and manganese that bind P can be formed under oxidizing conditions, and frequently dissolve or are modified when iron and manganese are reduced by changes in the redox potential in the sediments. This shifting from one redox state to the other is often observed in aquatic and soil ecosystems due to formation of anoxic and oxic zones (Ruttenberg 1993; McManus *et al.* 1997; Ingall *et al.* 2011). Reduction of these iron and manganese minerals often liberates phosphate, although some reduced iron and manganese minerals can continue to bind P. Oxidized iron and manganese phosphate minerals are usually formed from the weathering of parent rocks (Ingall *et al.* 2011) and represent a potential source of bioavailable P, once appropriate conditions favour release of P. Examples of iron and manganese phosphate minerals and their formulae are as follows (Ingall *et al.* 2011):

Phosphate minerals containing reduced iron and manganese [Fe(II), Mn(II)]:

Childrenite manganoan	$(Mn,Fe)Al(PO_4)(OH)_2$
Eosphorite	$MnAl(PO_4)(OH)_2.H_2O$
Hureaulite	$Mn_5(PO_3OH)_2(PO_4)_2.4H_2O$
Lazulite	$(Mg,Fe)Al_2(PO_4)_2(OH)_2$
Zwieselite	$(Fe,Mn)_2(PO_4)F$

Phosphate minerals containing oxidized iron and manganese [Fe(III), Mn(III)]:

Heterosite	$FePO_4$
Heterosite with Mn	$(Fe,Mn)(PO_4)$
Strengite	$FePO_4.2H_2O$ (orthorhombic)

1.4 Phosphorus Fractionation

Davidson *et al.* (1994) define chemical speciation as the process of identifying and quantifying different species, forms or phases present in a material. Species may be defined (i) functionally (for example those species that are bioavailable), (ii) operationally, according to the chemicals or procedures or reagents used in their extraction (for example water soluble P) and (iii) specifically, as particular compounds or oxidation states of an element (Davidson *et al.* 1994; Fuentes *et al.* 2003).

The fractionation procedure that is commonly used is an operational extraction that was first proposed by Hedley *et al.* (1982) and represents a variant of the Tessier procedure (1979). Tessier *et al.* (1979) used various chemical extractants to sequentially group particulate trace metals into five fractions: exchangeable, bound to carbonates, bound to Fe-Mn oxides, bound to organic matter, and residual. Hedley *et al.* (1982) used a sequential P extraction method to determine changes in the form and quantity of different inorganic and organic soil P fractions.

During the extraction procedure, progressively stronger chemical extractants are used to extract the more recalcitrant forms of P and inferences are drawn between the action of the sequential extractant and the potential availability of that P fraction that has been extracted (Linquist *et al*. 1997). The usefulness of quantifying these fractions lies in the fact that one can infer which fraction over time is acting as a sink or source in different soil types and it helps to develop better soil management practices (Verma *et al.* 2005).

Soluble P or readily available P: the solutions or materials that are widely used for extraction of this fraction include water (He *et al.* 2004), NH_4Cl (Akhtar *et al.* 2002), anion exchange resin or iron (III) oxide impregnated strips (Iyamuremye *et al.* 1996) and $NaHCO_3$ (Iyamuremye *et al.* 1996; Linquist *et al*. 1997).

Labile P: some inorganic and organic P are slightly adsorbed onto Fe and Al minerals and therefore easily extracted by NaOH (Iyamuremye *et al.* 1996; Linquist *et al*. 1997). However, NH_4F may be used initially to extract the Al-P pool from the Fe-P which is then extracted with NaOH (Zhang and Kovar 2008b).

Reductant (occluded) P: found within the matrices of various retaining aggregates and minerals is removed by CDB (sodium citrate-sodium dithionite-sodium bicarbonate) (Evans and Syers 1971).

Non-labile P: resistant inorganic P that is found in apatite or octocalcium phosphate. HCl is used to extract non-labile P (Iyamuremye *et al.* 1996; Linquist *et al*. 1997).

Residual fractions: this fraction is slowly exchangeable or non-exchangeable from the mineral surface and is extracted with strong digestion (Iyamuremye *et al.* 1996; Linquist *et al.* 1997).

In this research, the P fractionation scheme as outlined by Zhang and Kovar (2008b) has been used to operationally extract P from soil, biosolids and biosolids amended soils. This scheme operationally defines P species in both terrestrial and aquatic ecosystems. These forms are shown in Table 1.2 below:

Table 1.2 Terms for operational forms of P in runoff and tile water given in Kovar and Pierzynski (2009).

Form of Phosphorus	Abbreviation	Example of Extraction
Total Phosphorus *Total amount in dissolved and particulate phases*	TP	Digestion of sample (water, soil, biosolids) - Acid digestion - Kjeldahl method - Microwave acid digestion
Total Dissolved Phosphorus *Sum of dissolved inorganic and organic P*	TDP	Acid persulfate digestion of filtered sample
Orthophosphate	Ortho P	Ion chromatography
Bioavailable Phosphorus *Dissolved ortho P and a portion of particulate P that is algal available*	BAP	Extraction of unfiltered sample with -NaOH -C1- -saturated anion exchange resin -Ammonium fluoride

		-Iron-oxide filter paper strips
Molybdate Reactive Phosphorus *Dissolved ortho P and acid extractable particulate P (possibly algal available)*	MRP	Murphy and Riley colorimetric analysis of an unfiltered sample
Dissolved Reactive Orthophosphate *Immediately algal available and may include some <0.45µm readily labile organic and colloidal P*	DRP	Murphy and Riley colorimetric or ICP analysis of a filtered sample
Particulate Phosphorus *Inorganic and organic P associated with or bound to eroded sediment*	PP	By difference = [TP - TDP]
Dissolved Organic Phosphorus *Includes polyphosphates and hydrolyzable phosphates*	DOP	By difference = [TDP - DRP]

Biologically available P (BAP) is operationally defined as 'the amount of inorganic P, a P-deficient algal population can utilize over a period of 24h or longer' (Sonzogni *et al.* 1982). Furthermore, it is only the BAP that is utilized during primary productivity. BAP is the component of TP that is available for uptake by aquatic primary producers or by roots of macrophytes. It includes both dissolved P and bioavailable particulate P. Dissolved P includes both orthophosphate which is immediately available for algal uptake and some hydrolysable organic phosphates (Dils and Heathwaite 1998). More than 90% of dissolved inorganic P (MRP)

is bioavailable while less than 50% of dissolved organic P is bioavailable (Logan 1982); however Rigler (1968) has previously shown that the percentage of MRP which may actually be BAP varies from as much as 50-95%. Furthermore, DOP remains bio-unavailable unless hydrolyzed into orthophosphate. Therefore, in ecosystems, biological uptake closely relates to MRP rather than TDP. This being noted, some particulate P (PP) may also be transformed into BAP under various biological, chemical and physical reactions (Brostrom *et al.* 1988). This component is referred to as bioavailable particulate phosphorus (BAPP) and is of special environmental importance. In time, BAPP becomes dislodged from the particles and is then hydrolysed into orthophosphate which is readily available for algal uptake. BAPP release from PP is determined by various chemical processes such as adsorption–desorption, precipitation–dissolution and reduction–oxidation. While DRP is immediately available for uptake by algae and macrophytes, BAPP corresponds to a secondary and longer-term source of bioavailable phosphorus in lakes and receiving water bodies (Sharpley *et al.* 1993a). The percentage of BAPP in rivers and streams varies depending on the nature of land use. For agricultural streams, BAPP may be as high as 69% (Dorich *et al.* 1984). With so many variables contributing to the BAP pool, it is impossible to accurately correlate a particle source with BAP; thus, MRP, TDP and TP have to be measured in order to accurately determine the impact of each on the BAP pool (Dils and Heathwaite 1998).

There is also much variation in the method used to quantify BAP. Miller *et al.* (1978) showed that BAP can be estimated by algal assays that required up to 100-d incubations. However, other methods that used chemical extractants were developed so as to provide rapid results. Such extractants include NaOH (Butkus *et al.* 1998), NH_4F (Porcella *et al.* 1970) and citrate-dithionite-bicarbonate (Logan *et al.* 1979). Furthermore, there were differences in the values

obtained from the different extractants. The weaker extractants (NaOH and NH_4F) came to represent the BAP that could be utilized in the photic zones in lakes under aerobic conditions while the stronger extractant (CDB) came to represent the BAP present under anoxic conditions in the hypolimnion of stratified lakes (Sharpley 2008). The use of resins have also been developed to quantity available inorganic P in soil. This method normally uses chloride-saturated resin for up to 24 h (Amer *et al.* 1955; Huettl *et al.* 1979). Resins show higher correlations between plant responses and resin-extractable P when compared to results obtained from chemical extractants (Fixen and Grove 1990).

The time required to assess BAP with algal assays and resins and the ambiguity of results from chemical extraction (Bostrom *et al.* 1988) led to the development of the iron oxide impregnated filter paper strips to be used in aquatic systems as described by Sharpley (1993a). Previously, these strips were used to determine BAP in soils (van der Zee *et al.* 1987; Menon *et al.* 1988). In the iron oxide method, the iron on the strip reacts with P in the soil or solution resulting in the formation of insoluble iron phosphate. The iron phosphate on the paper can then be removed by reaction with dilute acids. Thus, the strips are acting as P sinks that mimic the removal of available P by algae (Sharpley 1993b). Sharpley (1993a and 1993b) showed that there was a strong correlation (r^2 = =0.90-0.95) between iron oxide extractable P and algal uptake. Sharpley *et al.* (1991) has also demonstrated that iron oxide method extracts most of the DIP fraction and some DOP along with labile PP.

1.4.1 Fate of P when applied to the soil

Phosphorus may be added to soil in an inorganic and/or organic form. Organic sources include biosolids, litter from farm animals and green manuring. When P is applied to the soil, it can be removed by biological uptake and chemical sequestering within the soil system. Biological uptake is mainly done via the roots of plants (Hook *et al.* 1973; Sopper and Kardos 1973). Within crops and trees, P is accumulated in all growing regions and will be removed when the crop is harvested (Conley and Tipton 1999).

Chemical sequestration is a three-step process that occurs simultaneously with biological uptake within the soil system. These processes are: adsorption, ion-exchange, and mineral precipitation (Aulenbach and Clesceri 2008). Adsorption takes place when the P is removed from the aqueous phase and becomes attached to the surface of soil particles (Abedin and Salaque 1998) via attractive forces. The smaller the particle, the greater the exposed surface area per unit volume and hence better adsorption capacity for free ions. Not only is the exposed area important to the adsorption capacity of a soil particle but also its charge. Thus, clays are better able to adsorb P onto their surfaces (Aulenbach and Clesceri 2008). Ige *et al.* (1995) stated that other soil physical and chemical properties influence P adsorption capacity. These include soil texture (Yuan and Lucas 1982), amount of organic matter (Daly *et al.* 2001) and iron and aluminum oxides (Toor *et al.* 1997), soil pH (Barrow 1984) and $CaCO_3$ content (Bertrand *et al.* 2003).

Ion-exchange is also responsible for the removal of P. When P comes in close proximity to the surface of the soil particle, phosphate with a -3 charge is preferentially exchanged for anions of lower charges such as chlorides and hydroxides which are then released into the soil solution. This results in P being held onto soil particles much more strongly than adsorptive forces

(Aulenbach and Clesceri 2008). Ca^{2+}, Al^{3+} and Fe^{3+} next play a major role in attracting and immobilizing any P that has been exchanged (Kardos 1973).

As a result of ion exchange, stable P precipitates with limited solubility may form in the soil. The most stable P compounds are formed by the interaction with calcium resulting in formation of different calcium minerals such as $Ca_3(PO_4)_2$ and $Ca_{10}(OH)_2(PO_4)_6$. However, the most common precipitate of P is amorphous aluminum phosphate, $AlPO_4$ (Galonian and Aulenbach 1973). Iron phosphate ($FePO_4$) is also stable at wide pH ranges but isn't under anaerobic conditions. Under such conditions, iron (III) is reduced to iron (II), releasing any locked P (Foster and Engelbrecht 1973).

The non-occluded P represents Fe- and Al-bound P, which is plant-available over extended periods of time, but is less available that soluble and loosely adsorbed P (Tiessen *et al.* 1984; Akhtar *et al.* 2002). It is suggested that initial P sorption takes place when P reacts with Fe hydroxides since the Fe-P bond is stronger than the Al-P bond. With the addition of amendments to soil, P increases and the Fe hydroxides become more saturated. At this point, Al hydroxides begin to sorb most of the available P rather than Fe hydroxides (Hartikainen 1989; Maguire *et al.* 2000). Furthermore, Fe hydroxides have a greater binding energy associated with P while Al hydroxides have a greater adsorption capacity (Parfitt 1989; Maguire *et al.* 2000). Together, the Al- and Fe-P contain a major potentially mobile P-pool in lake sediments (Bostrom *et al.* 1988; Rydin and Ottabong 1997) since they may be released under anoxic conditions during summer and winter in stratified lakes. These pools are important P sinks in agricultural soils that have been amended with P fertilizers. Simard *et al.* (1995) compared forest and agricultural soils that were amended with farm manure and showed that non-occluded P pools were important P sinks

in the A horizon of these soils. Zheng *et al.* (2002) further suggested that this occur though the formations of amorphous and crystalline A- and Fe-P compounds (Baley *et al.* 2008).

1.4.2 Nutrient Stoichiometry: The Redfield Ratio

The Redfield ratio or Redfield stoichiometry is the molecular ratio of carbon, nitrogen and phosphorus in phytoplankton. This molar ratio of C:N:P is 106:16:1 (Redfield 1962). The value of this ratio is somewhat constant throughout all oceanic bodies and thus indicating homogenous chemical compositions and the relatively stable nature of the oceans over the evolutionary history of phytoplankton. Estuaries, lakes and rivers do not have fixed ratios since their chemical composition are constantly changing due to a number of physiochemical, environmental and biological factors. For lakes, the seston C:P and N:P ratios are higher than the Redfield ratio of 16:1 for oceanic waters (Wetzel 2001; Cleaveland and Liptzin 2007). In addition to seston, the elemental ratios in the autochthonous phytoplankton and zooplankton communities can be a good indicator of nutrient availability and limitation. The availability or limitation of a nutrient is strongly related to the amount of nutrients being loaded to the aquatic body (Wetzel 2001). The C:N ratio has a twofold importance. First, it allows for the rate of the organic matter decay to a low C:N ratio of humus of approximately 8:1, and secondly the immediate availability of mineral nitrogen (NO_3^- and NH_4^+) to macrophytes. Conversely, a high C:N ratio to ecosystems would imply that the rate of decay and decomposition of organic matter can be delayed due to a lack of nitrogen (Brady 1999). Yet, not all the P or N that enter the receiving water body becomes incorporated into organic matter of living tissues. This depends totally upon the bioavailability of the nutrient and this is influenced by its form and whether it is bound loosely or strongly to soil particles and other particulate organic matter.

1.5 Biosolids

Biosolids are organic, stabilized material produced during the treatment of domestic sewage and septic sludge. Biosolids are treated to meet pollutant and pathogen requirements for land application and surface disposal. Biosolids are not sewage sludge. The term sewage sludge or sludge refers to the solids that have settled out from the liquid wastes during the wastewater treatment process. At this stage, this solid is not safe to handle since it is not pathogen free. On the other hand, biosolids are produced from the physico-chemical and or biological treatment of sludge (USEPA 1995a, b).

The main processes in the waste water treatment plant are: preliminary treatment, primary treatment, secondary treatment, advanced or tertiary treatment, disinfection and sludge treatment (Spinosa and Vesilind 2001).

A number of processes are employed to convert sludge to biosolids. These processes, in terms of the treatment effects and generated products, involve mainly five steps: clarification, stabilization, conditioning, thickening (concentration), dewatering, and drying. These stepwise processes use a combination of physical, chemical, and biological methods to eliminate water, organic matter, and pathogens from the solid residues (Spellman 1997; Clesceri *et al.* 2008).

Depending on the jurisdiction, land applied biosolids are classified differently. However, all of the classification systems include Class A and Class B biosolids. This differentiation is based on the level of pathogens present. Class A biosolids have no detectible levels of pathogens. In addition, class A biosolids satisfies strict vector attraction reduction conditions and low levels metals contents. These requirements are met through rigorous process in converting sewage to biosolids. In addition to digestion and alkaline stabilization, Class A biosolids go through "Processes to Further Reduce Pathogens" (PFRP) that include thermal drying, composting, pasteurization and testing the pathogen density limits (USEPA 2000).

Class B biosolids are treated similarly to Class A biosolids but the process is less rigorous and is referred to as a "Process to Significantly Reduce Pathogens" (PSRP) resulting in detectable levels of pathogens. Class B biosolids contain 2 x 10^6 fecal coliforms or less per gram of solid (Spinosa and Vesilind 2001). Digestion and limited alkaline stabilization technologies are only required for the production of Class B biosolids. However, Class B biosolids can be land applied with buffer requirements, public access and crop harvesting restrictions (USEPA 2000).

1.5.1 The Wastewater Treatment Plant: Stages in Sewage Treatment

Sewage treatment begins by separating the water phase from the solid phase via a preliminary treatment. This process reduces pathogens, odours and concentrates impurities into solid residues called sludge. Sludge at this stage is about 1-5% organic and inorganic solids suspended in water (Spinosa and Vesilind 2001).

There are three stages involved in all domestic sewage treatment: screening (or preliminary), primary and secondary, with some WWTPs operating with tertiary treatment to remove nutrients.

Screening treatment

The screening treatment removes or reduces coarse solids that are present either as floating or suspended solids. This is necessary to prevent damage to the treatment plant. This process is accomplished by forcing the sewage down a bar screen with about 15 mm between bars. These solids are not utilized in biosolid production and are rather disposed of by sending to incinerators or landfills. Leaving the bar screen, the wastewater flow is slowed down on entering a grit tank. This allows sand, gravel, and other heavy material that was small enough not to be caught by the bar screen to settle to the bottom. Like the debris collected by the bar screen, the debris from the

grit tank is also disposed of at a sanitary landfill (Spellman 1997; Spinosa and Vesilind 2001; Clesceri *et al.* 2008).

Primary treatment

The raw sewage that leaves the screening tank now enters the primary sedimentation tanks for a few hours where it undergoes additional treatment. The purpose of the primary treatment is mainly to reduce the amount of suspended solids present in the wastewater and the collection of grease and scum. Settling of the sewage is influenced by density, size, ability to flocculate, retention time and surface loading (Spellman 1997; Spinosa and Vesilind 2001; Amuda *et al.* 2008; Clesceri *et al.* 2008). During primary treatment, more than half of the suspended solids is removed and over one third of the biochemical oxygen demand is utilized (Amuda *et al.* 2008).

Secondary treatment

The products of the primary treatment then move on to secondary treatment. Wastewater at this stage still has a high biological oxygen demand (BOD) thus requiring secondary treatment either through biological filtration or sludge activation (Amuda *et al.* 2008). During its secondary treatment, growth of microbes is encouraged to consume most of the available nutrients (Spinosa and Vesilind 2001). The partially treated wastewater from the settling tank flows by gravity into an aeration tank. Here it is mixed with activated solids that contain aerobic microorganisms to break down the remaining organic matter. The aeration tank is equipped with inlets that bring air bubbles which provide mixing and oxygen, both of which are needed for the microorganisms to multiply. Bacteria, fungi, protozoa, and rotifers comprise majority of the biological mass of the activated sludge. While both heterotrophic and autotrophic bacteria are present in activated sludge, the former dominate the bacterial population. In addition, some metazoa, such as nematode worms, can be found at times in the activated sludge. However, the constant mixing in

the aeration tanks and sludge recirculation are deterrents to the growth of higher organisms (Spellman 1997; USEPA 2000; Spinosa and Vesilind 2001; Clesceri *et al.* 2008).

After the completion of this process, the effluent enters a clarifier. In the clarifier, the solids (mostly flocculated microorganisms and mineral precipitates) settle to the bottom. The bulk is removed and treated to form biosolids, while some is recirculated as activated sludge to replenish the population of microorganisms in the aeration tank to treat incoming wastewater (Spellman 1997; Clesceri *et al.* 2008).

1.5.2 Sludge Treatment: Formation of Biosolids

A series of steps are required to treat the sludge produced after secondary treatment into biosolids that can be land applied. The objectives of this stage of treatment include: (1) reducing the amounts of pathogens and vectors present within the biosolids, (2) dewatering them by at least 40% before being transported to a composting facility, and (3) producing a final product that is of suitable quality to enhance the composting process (Spellman 1997).

Sludge Stabilization

Stabilization is a physicochemical process applied to the sludge after clarification. The objectives of sludge stabilization are (1) to reduce or destroy pathogens, (2) minimize or eliminate the potential of odour generation, and (3) reduce the vector attraction potential of the biomaterials (Spellman 1997; Clesceri *et al.* 2008).

A number of factors are to be considered when selecting and designing the sludge stabilization method. These include the amount of the sludge to be processed, the complementary process, and the intended application of the treated sludge whether in landfill disposal or land applications for agricultural purposes. In the stabilization process, chemicals are added and the mixture is

heated resulting in reduction of volatile contents of the sludge and rendering the sludge unsuitable for the survival of microorganisms (Spellman 1997; Clesceri *et al.* 2008).

1 - Aerobic Stabilization

Aerobic stabilization is employed to treat organic sludge produced from various treatment operations. It is extensively applied in the treatment of waste-activated sludge (WAS), mixtures of primary and waste-activated sludge, waste sludge from operation plants, and waste sludge from activated-sludge plant without primary settling. As the available substrates become depleted in the sludge, the microbes undergo endogenous phase where approximately 80% of their cell tissue is oxidized aerobically. This results in the formation of carbon dioxide, water and ammonia. Consequently, the pH of the sludge decreases as the ammonia is further oxidized to nitrate (Spellman 1997; Clesceri *et al.* 2008). In the conventional system, aeration is achieved by leaving the sewage sludge is open tanks for an extended period of time. However, some plants now utilize a high-purity oxygen aerobic digestion process to replace air in the conventional system. Closed tanks are now used instead of open tanks and a more steady temperature is maintained when compared with conventional aerobic digestion. However, the major disadvantage of this newer technology lies in the high cost of supplying pure oxygen which is needed for efficient aerobic decomposition in the closed tanks (Tchobanoglous and Burton, 1995; Amuda *et al.* 2008).

2 - Anoxic-Aerobic Digestion

This is a pioneering process of digesting sludge intended to increase denitrification. The aerator functions intermittently by turning on and then off. When on, nitrifying organisms convert ammonium to nitrate, and when turned off, the nitrate is denitrified, reducing total N. The denitrification process prevents the acidification of sludge associated with nitrification and some

strictly anaerobic processes, thereby enhancing aerobic digestion when the system is on, nitrogen removal and pathogen destruction (Spellman 1997; Clesceri *et al.* 2008).

3 - Alkali Stabilization

Lime or any other suitable alkali can be added to raise pH and kill pathogens. The lowest US EPA requirement for lime stabilization is a pH of 12 for at least 2 hours. However, Class A biosolids requirement can be achieved only when the pH of the mixture is maintained at or above 12 for at least 72 hours and a temperature of 52^{o}C must be maintained for at least 12 hours during this period to facilitate killing of microbes. Alternatively, the temperature can be raised to 72 ^{o}C for at least half hour at pH 12 to achieve similar reductions in pathogen levels (Spellman 1997; Clesceri *et al.* 2008).

4 - Anaerobic Digestion

Anaerobic digestion is the oldest form of biological treatment that is currently being used. Anaerobic digestion is carried out in an airtight reactor and is responsible for the conversion of organic materials in the primary and secondary sludges to methane and carbon dioxide (Clesceri *et al.* 2008). Anaerobic digestion is accomplished through four major processes: hydrolysis, fermentation, acetogenesis and methanogenasis. During hydrolysis, large polymers in the sludge are broken down by hydrolytic enzymes into smaller compounds suitable for being utilized as a source in cellular respiration. Acidogenic fermentation then occurs resulting in acetate being the main end product. Volatile fatty acids are also produced along with carbon dioxide and hydrogen. Acetogenesis follows resulting in the breakdown of the products of hydrolysis and fermentation into intermediate compounds such as acetate and formaldehyde. Finally, methanogenesis converts these compounds to methane and water (Amuda *et al.* 2008; Clesceri *et al.* 2008).

Microorganisms, predominantly bacteria, are involved at each step of the reactions. A few examples include *Clostridium spp., Actinomycetes, Escherichia coli* (nonmethanogenic), *Methanobacterium, Methanococcus, and Methanosarcia* (methanogenic). Though they are all very specific in their activities, they complement one another in the two pathways that lead to formation of methane. End products of fermentation like hydrogen and acetate are converted to methane and carbon dioxide by the methanogens, while the acidogens produce the hydrogen (Amuda *et al.* 2008; Clesceri *et al.* 2008). Although the biological processes are the same, the physical set up of the chambers differ depending on which type of anaerobic digestion is carried out. Thus, the rate and method of mixing, odour control, flow versus batch, temperature, pH and chamber size all vary (Amuda *et al.* 2008).

Anaerobic digested biosolids have a higher N:P ratio than aerobic digested biosolids. This is because in aerobic digesters, nitrogen removal takes place in two sequential steps: oxidation of ammonium to nitrate (nitrification) during the secondary treatment and the additional reduction of the nitrate to nitrogen gas (denitrification) (Fux and Sieigrist 2004). Thus, additional N fertilizers may have to be used to supplement the N agronomic needs of croplands that have been amended with aerobic digested biosolids (OMAFRA 1998).

1.5.3 Phosphorus Management

The water treated by WWTPs is recycled into lakes and streams. Therefore, any pollution present in this water will impact aquatic ecosystems in lakes. P management is a major concern of WWTPs. Consequently, mechanisms that remove P from solution and cause it to precipitate are now widely used. The removal of P can occur either through microbial metabolism, converting available P into biomass, or by precipitation reactions. In the later, soluble P in the wastewater is converted into an insoluble form by a precipitating agent. The precipitate is removed physically

via filtration or settling out (Schönberger 1990; Choi *et al.* 2009), and is therefore often a major component form of P in biosolids. One such method is the addition of ferric chloride to precipitate the phosphate at low pH. A pH of around 2 is required for this reaction to take place since heavy metals do not precipitate below a pH of 3. In addition, higher pH results in the precipitation of ferric hydroxides and ferric oxides. The product in low pH, ferric phosphate is widely used as a commercial fertilizer to croplands (Kaschka and Weyrer 1999; Spinosa and Vesilind 2001). The precipitation reaction is as follows:

$Fe^{3+} + 3Cl^{-} + HPO_4^{2-} + 2H^{+} \rightarrow FePO_4 + 3HCl$

However, this reaction does not go to completion and furthermore, there are competing reactions for the Fe^{3+}. In practice, half a ton of ferric chloride is added during this stage to precipitate just 30.6 kg of phosphorus (Kaschka and Weyrer 1999).

Aluminum based alkalis can also be used to precipitate P. Sodium aluminate ($Na_2Al_2O_4$) reacts with the P to form insoluble aluminum phosphate. There are two main advantages to this process: hydroxide is produced as a by-product of this reaction which improves the biosolids index by containing less heavy metals and the acid capacity of the biosolids is raised instead of being lowered in the cases of iron salts (Kaschka and Weyrer 1999). Biosolids with low acid capacity due to lowered buffering capacity of water result in inhibition of denitrification (Kaschka and Weyrer 1999). The precipitation reaction is as follows:

$Na_2Al_2O_4 + 2\ PO_4^{2-} + 6H^{+} \rightarrow 2\ AlPO_4 + 2NaOH + 2H_2O$

Finally, slaked lime may also be used to precipitate P. This process entails the addition of an alkaline compound such as quicklime (CaO) (Spellman 1997; Clesceri *et al.* 2008). The Ca^{2+} combines with and precipitates the P by forming the thermodynamically stable and insoluble

calcium hydroxylapatite ($Ca_{10}(PO_4)_6(OH)_2$). This reaction is only possible at high pH which is achieved by the addition of the slaked lime (Kaschka and Weyrer 1999).

Guelph Biosolids

The city of Guelph WWTP produces its biosolids by the Lystek process that uses dewatered raw or digested sludges as its raw material. These biosolids are low viscosity liquid material even when solid concentration of 20-25% is present. The Lystek process utilizes a combination of heat, alkali (potassium hydroxide) and high shear mixing to breakdown biomass that is present within the biosolids. The liquefied product is further enhanced by anaerobic digestion which achieves two goals: at least a 40% increase in methane which is used as a biofuel and a reduction of more than 30% offsite disposal. Biosolids produced via the Lystek process exceeds the Class A definition for biosolids by the USEPA since it is pathogen free (Lystek 2011). Singh *et al.* (2007) has shown that there was no evidence of re-growth of harmful pathogens such as *Escherichia coli, Salmonella, Cryptosporidium* oocysts and *Giardia* cysts and fecal coliforms in Lystek produced biosolids even after storing the biosolids for nearly three years at room temperature.

Kitchener Biosolids

The Kitchener WWTP has a working capacity of 16200 m^3 and 11200 m^3 for its primary and secondary digestion. This plant utilizes a conventional secondary activated sludge process with chemical phosphorus removal and anaerobic sludge digestion. Anaerobic digesters further break down the sludge into biosolids over a two-week period. Like the Guelph treatment, ferric chloride is used to as an amendment to the primary effluent for phosphorus removal. Sodium hypochlorite is further added as a disinfectant with a minimum 30 minutes contact time. Kitchener biosolids are either used on agricultural lands or dewatered and landfilled (Region of

Waterloo 2010). However, as of present, Kitchener biosolids are only sent to landfills and not land applied (Frank Moffat, waste water treatment specialist with Waterloo Region, personal communication, August 2011).

1.5.4 Fate of Biosolids

Biosolids that are produced from the WWTP may be utilized in a number of ways. They are used directly in land and mine reclamation, agricultural land fertilization, forest fertilization, erosion control, horticulture and slope stabilization (USEPA 2005). Also, biosolids are used indirectly as a source of energy via incineration, composts, soil amendment mixes and fabricated soils (USEPA 2005). In Ontario, 43% of biosolids produced are land applied, 47% incinerated, and 4% are sent to a landfill (ELI 2006). In British Columbia, 90% of municipal biosolids are land applied; 70% is used for land reclamation, 25% for agriculture and the remaining 5% is retailed as compost (ELI 2006). In Quebec, 80% of biosolids are incinerated, 12% land filled and 8% was either land applied or composted (ELI 2006). Toronto generates about 195,000 tonnes of biosolids every year (City of Toronto 2008). Figure 1.2 shows how the biosolids produced in Toronto in 2008 were utilized:

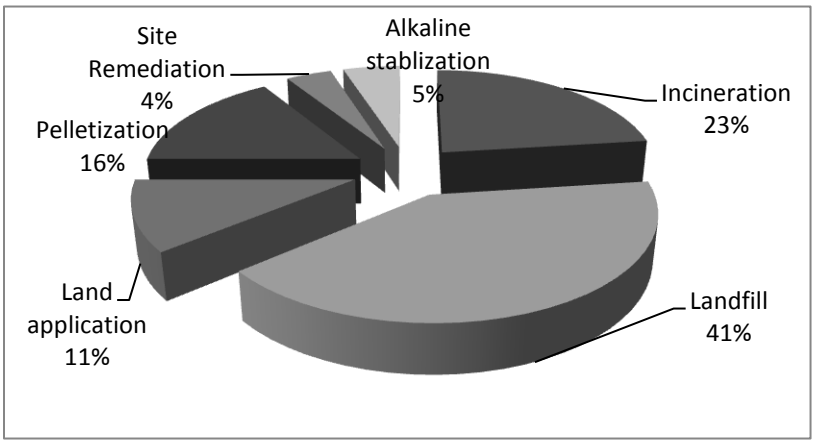


Figure 1.2 Utilization of Biosolids in City of Toronto in 2008.

Landfill Disposal

Landfill disposal of biosolids is the easiest and most common solution since it simply places all the waste into a single depositary. If properly managed, land filling reduces nutrient pollution and exposure to pathogens. However, there are risks associated with burying organic waste. Anaerobic decomposition results in the production of methane, a potent greenhouse gas. Also, if the containment chamber isn't properly lined, leaching of various chemicals and heavy metals may result in contaminated aquifers. At the same time, valuable plant nutrients and organic matter are lost (Evanylo 2009). In addition, valuable nutrients that include phosphorus are buried while environmentally devastating choices are made to extract P from phosphor deposits. Herring and Fantel (1993) have modeled known reserve bases and have shown that demand stabilization by 2025 will only leave 100 years before mines are exhausted. Furthermore, landfills should not be seen as long term solution but may be an unavoidable choice when wastes are contaminated (UNEP 2011).

Incineration

Incineration is sometimes used to get rid of biosolids and is a limited disposal option. It provides many benefits such as a reducing the biosolids volume, killing pathogens, and providing energy. Trace elements may also be concentrated up to a ten-fold from their original concentration. On the other hand, incineration releases carbon dioxide – another greenhouse gas – and it requires special systems to trap the fly ash thus increasing operational cost. Like landfill, the potential for recycling nutrients and organic matter on agricultural lands is absent (Evanylo 2009; UNEP 2011).

Land Application

For thousands of years, the Chinese have land applied their human waste onto surrounding farmlands. Currently, about 50% of biosolids produced in the USA are land applied (USEPA 2009). As of 2002, 40% of all biosolids produced were recycled on agricultural lands in the European Union (European Commission 2010). In Canada, biosolids have been applied to agricultural lands for the past 40 years. About 50% of all biosolids produced are recycled on lands that amount to less than 1% of Canada (CWWA 2010).

Land application of biosolids is the most cost effective means of disposal. Land application is most often carried out by liquid injection or surface spreading followed by incorporation into the upper horizon of the soil. Biosolids may also be applied onto rangeland and pastures in order to improve grazing. In addition, biosolids are now being used as soil amendments on marginal lands and reforested areas and in remediation schemes on mine sites (UNEP 2009).

Biosolids are rich in macronutrients (with the exception of K), micronutrients and organic matter might supply plants with all their nutrient needs (Atalay *et al.* 2007). In addition, biosolids enhance the soil physical properties (Khaleel *et al.* 1981). Soil structure, porosity, permeability, water retention capacity and drainage are improved which help to better retain various minerals and ions (Khaleel *et al.* 1981; Pentecost 2004). These properties are intrinsically linked to the rate of decomposition of soil amendment which is determined by a number of factors such as (i) chemical composition of the waste (for example, its C:N ratio), (ii) temperature, (iii) soil moisture, (iv) method of waste application (surface-applied or soil-incorporated), and (v) rate of application (Khaleel *et al.* 1981).

Soil microbial activity as well as enzymatic activity have been shown to increase due to a readily available source of food to decompose (Haynes *et al.* 2009). When biosolids are applied

to soils, there is an increase of soil microbial activity as well as enzymatic activity as the easily decomposable components become degraded (Haynes *et al.* 2009). However, a significant portion of carbon compounds present in the biosolids are resistant to microbial degradation and may persist in the soil on the order of centuries (Terry *et al.* 1979).

1.6 Biosolids Application and Management

1.6.1 Biosolids and the European Union

Since the implementation of the Urban Waste Water Treatment Directive 91/271/EEC in all Member States, there is an increase in the amount of biosolids being produced. This is the result of tertiary treatment of wastewater to reduce nutrient loading, creating additional material removed as chemical precipitates and flocculated microbial mass. In 1992, 5.5 million tonnes of dry matter were produced, while in 2005 almost 9 million tonnes were produced. Consequently, the Sewage Sludge Directive 86/278/EEC encouraged the application of biosolids on fields in such a way as to prevent any harm to the soil, vegetation, animals and humans. However, regulations are not as strict as it should be since it is possible to use untreated sludge if it is injected into the soil. The European Union has set maximum values of concentration for various heavy metals. Any biosolid that exceeds the maximum limit is banned from land application. As of 2002, 40% of all biosolids produced were recycled on agricultural lands; the rest went to landfills and incinerators (European Commission 2010).

1.6.2 Biosolids and the USA

In the USA, the Environmental Protection Agency regulates all biosolids land application. Since it is a federal mandate, all states follow the same legislations and guidelines. These regulations are found in the EPA Part 503 Biosolids Rule that took effect on March 22, 1993 (EPA 1994).

These regulations are set out to represent general standards, pollution limits, operational standards, management practices and record keeping and reporting frequencies. These standards are applied to all three means of disposal of biosolids: recycling and land use, incineration and burying in landfills (USEPA 1994).

USEPA classified biosolids into three groups: Class A, Class B and exceptional quality (EQ). The criteria used to classify the biosolids were based on pollutant concentrations (nine metals), pathogen criteria and process-control criteria that decreased vectors. EQ biosolids are those that meet the highest standard in each of the mentioned criterion and can be distributed without any further regulations under 40 CFR 503. Class A and Class B biosolids can be differentiated based on their pathogen content and control. Other biosolids will only be land applied under strict guidelines and further risk assessment (USEPA 1994).

1.6.3 Biosolids and Canada

In Canada, biosolids have been applied to agricultural lands for over 40 years. About 50% of all biosolids produced are recycled on lands that amount to less than 1% of Canada (CWWA 2010).

In Canada, federal regulations do not directly regulate and supervise the land application of biosolids. Few federal guidelines are provided in the *Canadian Environmental Protection Act*, 1999. In fact, there is no definition of biosolids in any federal acts or regulations (CCME 2010). The *Canadian Food Inspection Agency* (CFIA) regulates not only the sale but also the import of biosolids that are intended for use as a commercial fertilizer on lands. However, it is provinces and territories that directly manage all wastewater treatment and composting facilities along with the use and disposal of biosolids. Thus, land application of biosolids falls under the mandate of provincial/territorial acts and regulations that limit trace metals, pathogen and pathogen indicators, and organic contaminants present in the biosolids. Guidelines for Atlantic Canada are

contained in "*Atlantic Canada Standards and Guidelines Manual for the Collection, Treatment, and Disposal of Sanitary Sewage 2000*". Notably, land application of biosolids is not permitted in Newfoundland and Labrador. The Bureau Normalisation du Québec (BNQ) has developed various standards and guidelines for jurisdictions within the province (CCME 2010).

1.6.4 Biosolids and Ontario

In Ontario, the *Nutrient Management Act*, 2002 (Ontario Regulation 267/03) provides the necessary guidelines which the Ministry of Agriculture, Food and Rural Affairs (OMAFRA) implements. However, the Nutrient Management Act (2002) was preceded by the '*Guidelines for the Utilization of Biosolids and Other Waste, 1997*'. This document was intended to supplement Ontario Regulation 347 under the Environment Protection Act. In brief, this document strengthened earlier guidelines for land application of biosolids while seeking protection of the environment, consumer and animal health and food quality.

Provincial regulations have stipulated the maximum amount of biosolids that can be land applied in Ontario. The application rate may not exceed 8 tonnes of solids per hectare per five years or exceeding 135 kg of nitrogen/ha over a five-year period for crops. However, if metal concentrations in the biosolids are very low, the application rate may reach a maximum of 22 tonne/ha. However, in practice it is generally capped at 8 tonne/ha.

Biosolids may not be applied at all times or to every soil. A number of guidelines are in place in Ontario in order to limit potential runoff from biosolids from reaching waterways or being too close to residential areas. Some of these guidelines that are found in the Nutrient Management Act of 2002 (O. Reg. 338/09, s. 43) state that biosolids cannot be applied:

(a) During the restricted period (December 1 to March 31 of the following year) or at any other time when the soil is snow-covered (minimum depth of 5 cm of snow) or frozen (minimum 5 cm of soil).

(b) On land that is closer than 100 metres to a municipal well.

(c) Closer than 20 metres from the top of the nearest bank of the adjacent surface water.

(d) On fields that are next to waterways unless there is a vegetative buffer zone that lies in between the application site and the adjacent surface water.

(e) On lands that is subject to flooding at least once every five years or on lands where storm water collects.

(f) If the maximum sustained slope is more than 6%. In such a case, application of biosolids may be applied no less than 100 metres from the adjacent bank of the waterway.

1.6.5 Nutrients in Biosolids

The major nutrients provided by applied biosolids are nitrogen and phosphorus (Table 1.3). Nitrogen is present mainly as ammonium but it can also be present as organic nitrogen. Phosphorus is perhaps the most valuable nutrient present in biosolids. Unlike nitrogen, phosphorus ores are rapidly being depleted and within the next century, there will be no more reliable remaining P mines (Herring and Fantel 1993). Thus, P is recycled when biosolids are applied to enhance growth on forested areas or for that matter, any agricultural lands (Spinosa and Vesilind 2001).

Table 1.3 Biosolids organic matter and macronutrients (dry weight basis) Adapted from Sullivan *et al.* (2007).

Nutrient	Usual range %
Organic matter	45–70
Nitrogen (N)	3–8
Phosphorus (P)	1.5–3.5
Sulphur (S)	0.6–1.3
Calcium (Ca)	1–4
Magnesium (Mg)	0.4–0.8
Potassium (K)	0.1–0.6

In addition to macronutrients, biosolids contain significant quantities of trace elements (Table 1.4). Some of these trace elements, such as copper and molybdenum, are needed for healthy plant growth but may be toxic to humans, animals and plants if their concentration increases. Consequently, regulations are put in place to limit their concentrations in the soil (Evanylo 2009). Typical concentrations of these trace elements in biosolids are far below the maximum allowed concentrations.

Table 1.4 Metal concentrations in sewage biosolids (OMAFRA 2009).

Metal	Maximum concentration (mg/kg solids)	Typical Concentration (mg/kg solids)
Arsenic	170	4.3
Cadmium	34	3.4
Cobalt	340	6.5
Chromium	2800	80
Copper	1700	550
Mercury	11	1.4
Molybdenum	94	6.5
Nickel	420	12
Lead	1100	48
Selenium	34	2.7
Zinc	4200	506

Biosolids have an N:P ratio below Redfield, a priori suggesting that when applied to soil, N will be less available to plants than P, relative to biological demand (Table 1.5). Yet, biosolids are applied based on the N-agronomic needs of crops (Hue 1995; Smith 1996). The N content of biosolids are lower than the P content due to the anaerobic reactions that take place in the treatment of sewage sludge during its conversion into biosolids. Thus, excess P is being applied to soils. Eventually, the P buffering capacity of the soil becomes saturated and run-off waters may now contain environmentally unacceptable levels of both dissolved and particulate P from eroded particles (Maguire *et al.* 2005). These soils that contain enriched P can be an important source of nonpoint pollution that transfers P to surface and shallow ground water (Sharpley *et al.* 1994; Sims *et al.* 1998). Removal of P from agricultural lands is the leading cause of cultural eutrophication of lakes and streams (US EPA 1996; Daniel *et al.* 1998; Ulén *et al.* 2007). A number of factors, besides the actual process of the biosolids production, also influence solubility and hence the potential for P loss to water bodies. These factors include soil type (Penn and Sims 2002), method of biosolids application – surface versus injection (Deizman *et al.* 1989), nature of the cropping system – conventional versus no-tillage (White *et al.* 2010) and topography of the land (Dougherty *et al.* 2004).

Table 1.5 Typical biosolid characteristics for nitrogen and phosphorus in liquid versus dewatered anaerobically produced biosolids (OMAFRA 2002).

N and P in anaerobically produced biosolids	**Liquid sewage biosolids**	**Semi-solid (dewatered) sewage biosolids**
Total Solids (as % of total mass)	3.0%	26%
Total Nitrogen (as % of dry mass)	6.5%	4.0%
Fertilizer Equivalent Nitrogen (wet weight)	1.07kg/m^3	3.98 kg/m^3
Total Phosphorus (as % of dry mass)	3.6%	2.7%
Phosphate Fertilizer Equivalent (as P_2O_5) (wet weight)	1.0 kg/m^3	6.45 kg/m^3
Molar N:P	2.4	1.4

1.6.6 Phosphorus Form in Biosolids and other organic soil amendments

Shober *et al.* (2006) stated that knowledge of specific P forms would greatly assist researchers to better understand the potential for P loss from agricultural lands that have been amended with organic constituents such as biosolids. Knowledge of P speciation can only be derived from the use of advanced analytical techniques such as ^{31}P NMR. Both solid state ^{31}P NMR (Frossard *et al.* 1994) and solution ^{31}P NMR spectroscopy have been used to identify organic P species in different dairy manure (Hinedi *et al.* 1989) and in poultry litter (Maguire *et al.* 2004). However, Turner (2004) has shown that it is not possible to differentiate between different inorganic forms of P when using ^{31}P NMR. X-ray diffraction (XRD) may be used to identify inorganic forms of P; however, such forms have to be crystalline rather than amorphous (Ippolito *et al.* 2003). Ingel *et al.* (2010) highlighted that another technique, X-ray absorption near-edge structure (XANES) spectroscopy, has been employed successfully when studying P speciation in both soils (Beauchemin *et al.* 2003) and manures (Peak *et al.* 2002). Unlike XRD, XANES can be used to identify poorly crystalline and amorphous species (Shober *et al.* 2006).

Organic P in organic wastes may be as high as 50% and consists mainly of inositol phosphates, phospholipids, DNA, RNA and many sugar phosphates (Toor *et al.* 2006). In addition, there are many unidentified organic P compounds (Huang *et al.* 2008; Zhang and Kovar 2008b). Organic P can also show varying resistance to microbial degradation, thus impacting P lability and bioavailability (Chang *et al.* 1983). Microbial degradation and release of inorganic P from organic P compounds is solely dependent of the ability of phosphatases to hydrolyse the P substrate (Toor *et al.* 2006). Monoesters (sugar phosphates, mononucleotides) are more easily cleaved by phophatases than diesters (DNA, RNA, phospholipids) and polyesters. P is also bound to phytic acids, fulvic acids – these represent the more non-labile organic P pools in biosolids and organically amended soils (Zhang and Kovar 2008b).

In addition, P combines with iron, aluminum, calcium and other cations to form a range of inorganic compounds within the organic matrix and in soils (Zhang and Kovar 2009). Shober *et al.* (2006) has shown that P may be sorbed onto ferrihydrite, hydroxylapatite, β-tricalcium phosphate, oxyhydroxides and phytic acid. By a fractionation method, P in biosolids can be grouped as 'biologically bound' or physicochemically bound' (Choi *et al.* 2009). The biologically bound fraction is what is actually incorporated by and into living organisms. Physicochemically bound P is a result of van der Waals interactions between the adsorbate and the solid constituents or by covalent bonding with organic molecules (Choi *et al.* 2009).

Nutrient Loss from Biosolids

Nutrients from biosolids can be lost by a number of processes that include runoff from precipitation (rainfall and snowmelts), erosion of soluble and particulate components, and leaching to ground water of soluble nutrients and compounds (USEPA 2000).

Beyond the differences in biosolids stabilization methods, the quantity and quality of runoff is also strongly affected by application technique (applying biosolids as semisolids or applying to the soil surface or incorporating them into the soil), soil management, and application rate (Shigaki *et al.* 2007). Quite important also is the season of the year and the degree of water saturation of the soil at the time of application, and the physiochemical properties of the soil (Shigaki *et al.* 2007). Runoff will increase on saturated soil or if the soil particles are too compact and thus prevent infiltration.

Runoff is water flowing on the land surface after rainfall or snowmelt. It transports soil particles, dissolved nutrients and to a lesser extent, contaminants found in the soil. Runoff increases with increased precipitation since the surface soil becomes unable to absorb any more water. Ideally, when rain falls or snow melts, the water infiltrates the soil and percolates to the water table and

aquifers. This process repeatedly supplies groundwater for subsequent use. In addition, the soil acts a filter to remove particulates and other chemicals from reaching the aquifers. When the water is unable to infiltrate the soil due to impervious soils or heavy rainfall, runoff will occur and in the process, the runoff will erode the top soil and transport it into nearby streams and lakes (Wetzel 2001). Germane to this thesis, biosolids particulate matter applied to the soil can be cotransported with eroded soil.

Elliot *et al.* (2002) concluded that the leaching of phosphorus is quite small for most biosolids even when biosolids are applied to meet the nitrogen requirement of crops on sandy soils. This notwithstanding, Penn and Sims (2001) have shown that interactions between soil physiochemical properties and biosolids type affect the forms and mobility of phosphorus. They found that in the near term, wastewater treatment process affects extractable phosphorus concentrations in biosolids and, therefore, in biosolids-amended soils. Consequently, P concentrations in runoff from biosolids-amended soils will be related to treatment process in the short term. In the long term, as biosolids equilibrate with soils, the release of phosphorus to runoff will be affected more by soil properties than the actual biosolids physiochemical characteristics. Biosolids are produced by using lime and/or metal salts, and these processes affect the potential for biosolids phosphorus to cause runoff losses. Penn and Sims (2001) has shown that after biological nutrient removal biosolids, the available phosphorus concentrations in runoff were highest from no Fe + no lime biosolids, followed by Fe + lime biosolids, and then Fe + no lime biosolids. Thus, adding Fe to soils along with biosolids application may be beneficial in the long term, from the viewpoint of reducing phosphorus losses through runoff by increasing soil phosphorus sorption capacity.

Also, applying more biosolids to soils may increase the concentration of P in runoff. However, this may be mitigated by the effect of biosolids on increasing water holding capacity of the soil. This is generally true except for addition of slurries (liquid biosolids) in larger quantities, as slurries seal off the soil surface and prevent infiltration and thus increase runoff (Henry 2005).

Tile Drainage

Tiling is a common practice in Ontario. Subsurface permeable tiles drain excess water from the subsurface of agricultural soils. Tile drainage enhances optimal plant growth since it enhances drainage and air penetration into the soil. Most crops and soil organisms do not prefer waterlogged soils. Saturation of soils increases anaerobic processes such as denitrification, with a corresponding loss of nutrients (Lowell and Sands 2009). Unlike surface runoff which tends to be high in loss of nutrients and sediment, subsurface drainage in agricultural lands may reduce sediment loss by as much as 65% and phosphorus loss may be greatly reduced by as much as 45% (Zucker and Brown 1998). This is primarily due to well drained soils having less surface runoff and erosion by which phosphorus is chiefly lost (Lowell and Sands 2009; USEPA 2009). These tile drains empty into drainage ditches which constitute small streams in agricultural watersheds. Many of these small streams eventually join up and deliver their nutrients and sediments into rivers which make their way into larger rivers, lakes and coastal waters. While particulate P and sediments are greatly reduced in tile drainage, loss of soluble nutrients such as nitrate may actually increase in subsurface flow (Richards *et al.* 2008). Rabalais and Turner (2001) have shown that nitrogen loss from agricultural lands is the key nutrient responsible for the degradation of the healthy state of Gulf of Mexico and other marine ecosystems.

1.7 Fate of P in Receiving Waters

Role of Agriculture in Lake Eutrophication

Runoff from agricultural lands is a leading nonpoint source of pollution of streams and lakes. The runoff water carries nutrients in both dissolved and particulate forms which when enter waterways result in varying extents of eutrophication. Agriculture contributes to at least 50% of nutrient input into lakes and over 60% into rivers (USEPA 1996). Eutrophication is the process by which aquatic bodies become more eutrophic due to an increase in their nutrient supply (Edmondson 1995). Eutrophication is the most widespread water quality issue in the US and in many countries worldwide (Carpenter *et al.* 1996). However, Withers and Haygarth (2007) conclude that at present the precise role of agriculture in aquatic eutrophication remains poorly understood due to the complexity of the factors linking agriculture and nutrient enrichment of aquatic bodies. In this same light, the role of land application of biosolids to eutrophication is not clearly understood, as P loading and forms of P entering surface water following land application have not been well characterized, and the connection between this loading and eutrophication has not been thoroughly studied.

Surface runoff is the main form by which P is removed from watersheds. However, it is impossible to control surface runoff during storm events, snow melts and heavy rainfall. Runoff contains the highest amount of total P when rainfall erodes P enriched sediments that are found within the top 5cm of the upper horizon (Daniels *et al.* 1998). Sharpley *et al.* (1996) has shown that total P removed in runoff to surface waters is directly proportional to the total P of the soil. The relevance here, again, is P enriched particles derived from biosolids application could be eroded into surface water.

There are many harmful effects associated with eutrophication of aquatic bodies. Cooke *et al.* (1993) most succinctly states: 'Symptoms of eutrophication, such as algal blooms, low transparency, rapid loss of volume in reservoirs, noxious odours, tainted fish flesh, impaired potable water supplies, dissolved oxygen depletions, fish kills, and the development of nuisance or exotic animal populations can bring about economic losses in the forms of decreased property values, high cost treatments of raw drinking water, illness, depressed recreation industries, expenditures for management and restoration, and the need to build new reservoirs.'

In addition, some cyanobacteria produce toxins that are directly harmful to humans and various animal species and indirectly altering phytoplankton structure. Microcystins, for example, show great toxicity, widespread distribution and structural stability (Jiang *et al.* 2011). Fishes may even bioaccumulate these toxins. Palikova *et al.* (2011) has shown that microcystin concentration in tissues remained fairly constant after four weeks following exposure in tilapias. However, exposure to the microcystins had minimal impact on the feeding habit of the tilapias. Moore *et al.* (2009) has shown that the microcystin concentration found in the muscles of fish in the Jacarepaguá Basin, Rio de Janeiro, Brazil were far above the levels recommended for human consumption. Lotocka (2001) investigated the effect of *Microcystis aeruginosa* on the grazing intensity of *Daphnia magna*. The results clearly show that the cyanobacteria inhibited grazing intensity in *D. magna* and gut fullness never exceeded 58%. Ghadouani *et al.* (2003) have also shown that there was a decline in *Daphnia pulicaria* biomass when exposed to cyanobacterial blooms.

The potential for biosolids land application to contribute to eutrophication, then, has significant consequences for ecosystem function and the health of animals and humans relying on these ecosystems. This role of biosolids has not been demonstrated, and the purpose of this thesis is to

determine how biosolids land application, under worst-case scenario conditions – maximum allowable application rate, high frequency and intensity rainfall, maximum allowable slope, no buffer zone and no vegetation - could affect eutrophication of receiving waters. Should biosolids demonstrably contribute to eutrophication in a model system, then the potential for this to occur in natural systems must be weighed against potential benefits described previously in this Introduction.

1.8 Rationale for Thesis Research: Is biosolids application on agricultural lands a potential aquatic disaster?

With increasing population comes increasing waste. The province of Ontario is projecting that its population will increase by 34.4 per cent, or over 4.5 million, over the next 26 years, from an estimated 13.2 million persons on July 1, 2010 to 17.7 million on July 1, 2036. However, this is a median estimate. The high growth scenario is a 52.1% increase or 6.9 million new individuals (Ontario Ministry of Finance 2011). Additionally, tertiary treatment of wastewater is likely to become more rather than less common. Tertiary treatment increases volume of biosolids created in the wastewater treatment process, and coupled with increased population, an increase in biosolids requiring safe and environmentally responsible disposal will certainly increase.

At present, Ontario is under pressure to increase the land application of its biosolids since other alternatives are less eco-friendly and/or expensive. Biosolids application on croplands helps in the recycling of nutrients that were removed when crops were harvested and transferred through the agricultural food chain to humans. In addition to increased soil fertility, many other physical and chemical properties can be improved with biosolids amendment. Farmers also benefit, and by extension consumers, since they do not have to buy expensive inorganic fertilizers.

However, biosolids are applied based on the nitrogen agronomic needs of crops. Since biosolids have a lower N:P ratio, their application always results in the excess phosphorus being added to the soil. With repeated land application of biosolids, the soil's P-buffering capacity could become exceeded and phosphorus could become easily mobilized. In addition, P rich particles could be eroded from soils after heavy rainfall or snowmelts in surface run-off and tile drains. Eroded P rich sediments and soluble P compounds could then make their way to tributaries and eventually to lakes. Alternatively, land application may reduce erodability of soil through alterations of soil structure, reducing loading of particulate P to surface waters, even under worst-case scenarios.

While all the phosphorus supplied by synthetic fertilizers is in the form of orthophosphate which is completely bioavailable and the only form that can be assimilated by algae, phosphorus in biosolids occurs in many forms. Some of the phosphorus in biosolids and biosolids-amended soils are soluble and potentially bioavailable while other forms may remain unavailable in discrete insoluble minerals and organic compounds and thus have no impact on eutrophication even should land application of biosolids prove to increase loading of total P to surface waters. Determination of fractions of P lost from biosolids-amended soils, then, is an important component of this research to complement determination of phytoplankton response.

In this thesis, two different biosolids were applied onto reference soils at the rate of 8 dry tonne/ha. Rainfall events were simulated thrice after its application and the surface runoff and tile water were collected and analyzed for bioavailable phosphorus and total phosphorus. A combination of runoff and tile water was then fed into mesocosms that represented lakes. These mesocosms contained a mixture of algae, cyanobacteria and diatoms. A number of physical, biological and chemical parameters of the mesocosms were investigated to determine the impact

of nutrient enrichment on the community. Water samples from the mesocosms were collected and analyzed to determine changes in community function in response to nutrient enrichment. Furthermore, the biosolids and biosolids-amended soils were analyzed by a P fractionation procedure to determine P distribution in these matrices.

The specific hypotheses of the research were as follows:

1. Biosolids application will result in more nutrient loss from amended soils than from reference soil.

2. The forms and quantity of nutrients removed from biosolids-amended soils will correlate with biological and chemical parameters of the mesocosms.

3. The form and quantity of P removed from soil will differ between biosolids as a function of differences in biosolids stabilization methods and will affect the response of phytoplankton to P loading.

2 - Materials and Methods

In order to collect runoff water from soils with land applied biosolids, a series of wooden troughs containing various soil and biosolids treatments were set up in an indoor laboratory.

2.1 Experimental Setup

2.1.1 Setting up of Troughs

Wooden troughs to contain soil were constructed from 1.9 cm thick plywood and their dimensions were 1.0 m long, 0.35 cm wide and 0.40 cm deep. The inside of each box was lined with clear plastic (Film-Gard, 3.0 m x 2.0 m, clear polyethylene). Boxes were mounted on a ramp at a 9% slope. This slope represents the maximum slope allowed on lands where biosolids are applied (Ontario Ministry of Agriculture Food and Rural Affairs 2010). Nitex screen (mesh size = 1.0 mm) was placed on the top front end of each of the boxes so once the rain was simulated, the soil was retained instead of being eroded out of the boxes. The plastic overhung the front of the box to collect and funnel surface run-off to a single collecting jar. A weeping tile (1.15 m long x 0.15 m diameter) was installed at the bottom of each trough to drain water percolating through the soil. Elutriate collected from the weeping tile was to simulate tile drainage from agricultural fields. Replicates of three boxes were used for application of biosolids from Kitchener WWTP (anaerobic digestion), Guelph WWTP (anaerobic digestion followed by Lystek process) and the final three contained reference soil.

2.1.2 Gravel and Reference Soil

Gravel was added to the bottom 15 cm of all boxes in order to allow for easy percolation of water into the weeping tile, and to prevent tile pore blockage by soil particles. The soil that was added to the troughs was composed of a mixture that represents Environment Canada artificial

soil mix (Environment Canada 2005). This reference soil was composed of 70% silica sand, 20% kaolin clay and 10% peat moss by mass. An additional source of organic matter (< 1% w/w) was added to the soil in the form of regular garden soil. This was added to provide an inoculum of soil microorganisms. The constituents were thoroughly mixed in a cement mixer. Mixed soil was then added to a depth of 20 cm in all the troughs from above the gravel layer. De-chlorinated municipal tap water was added to bring total soil moisture to 80% as measured with a soil moisture probe (Fujan E-Inginst Electron Co). This moisture content was maintained throughout the length of the research time period by addition of three liters of deionized water once weekly.

2.1.3 Biosolids Application

Troughs were randomized by pulling assigned trough numbers from a black bag in order to determine which of the boxes would receive each treatment. The application rate for applying biosolids was the maximum allowed in Ontario which is 8 tonnes ha^{-1} (Ontario Ministry of Environment and Ontario Ministry of Agriculture Food and Rural Affairs 2004). This was calculated to be 0.288 kg of dry weight biosolids per trough. Dry weight content of each biosolids source was determined by drying a sample in an oven at 105^{o}C for 24 h. Guelph biosolids contained 3.09% solid content while Kitchener biosolids contained 1.47% solid content. Consequently, 9.32 kg (wet weight) Guelph biosolids were each applied to three troughs while 19.56 kg (wet weight) of Kitchener biosolids were applied to another three troughs. Immediately after the biosolids application, the biosolids were incorporated into the soil to a depth of 15 cm by the use of a hand shovel.

In order to investigate the impacts of nutrient runoffs water from troughs that were set up above, a series of mesocosms were set up in an indoor laboratory.

2.2 Mesocosms set up

A total of forty five aquatic mesocosms were setup using transparent plastic tubes (1.35 m height x 0.06 m diameter) held vertically in a wooden holder. Twenty seven mesocosms received runoff and tile water from the troughs (3 replicates corresponding to each of the troughs). An additional fifteen were fertilized with inorganic nitrogen and/or phosphorous containing fertilizers (potassium nitrate was used to provide nitrate, ammonium chloride for ammonia and potassium dihydrogenphosphate for phosphate) corresponding to the concentrations of nitrate, ammonia and bioavailable phosphorous found in the runoff and tile water, measured within hours of simulated rainfall events that generated runoff and tile drainage. These additional column sets were as shown in table 2.1.

Table 2.1 Inorganic treatments for additional mesocosms which were set up to compare the impact of inorganic fertilizer applications with that of biosolids.

High N	Correspond to the highest concentration of nitrate from runoff/tile water
High P	Corresponded to the highest concentration of bioavailable P from runoff/tile water
High N, High P	Corresponded to the highest concentration of nitrate and bioavailable P from runoff/tile water. Equivalent of Kitchener runoff/tile.
Low P	Corresponded to the lowest concentration of bioavailable P from runoff/tile water
Low N, Low P	Corresponded to the highest concentration of nitrate and bioavailable P from runoff/tile water. Equivalent of Guelph runoff/tile.

These control mesocosms were set up to simulate nutrient addition form agricultural lands that have been amended with synthetic fertilizers. The final three mesocosms had no addition of inorganic fertilizers or surface run-off/tile water from the soil troughs. These served as blanks to

determine changes in mesocosm properties over time in the absence of any fertilization. The positions of all the mesocosms were randomised under the light banks.

2.2.1 Preparation of Reference soil

The lake reference sediment was made up following the guidelines of OECD 207 (OECD 1984). However, peat moss concentration was reduced from 10% to 2% dry weight since the latter value corresponds more closely to the actual observed values of organic matter in natural sediments (Suedel and Rodgers 1993). The recommended cellulose source was also changed from *Urtica sp.* powder to finely ground Sugar Maple tree (*Acer saccharum*) (Table 2.2).

Table 2.2 Percentage dry constituents of the artificial sediment (OECD 1984).

Constituent	Characteristics	% of dry sediment
Peat	Sphagnum moss peat (particle size ≤ 0.5 mm), degree of decomposition: medium	2 ± 0.5
Quartz sand	Grain size: ≤ 2 mm	76
Kaolinite clay	Kaolinite content ≥ 30%	22 ± 1
Dried Maple Leaves	Powdered leaves of *Acer saccharum* with alpha-cellulose (1:1 ratio)	0.4 - 0.5%
Calcium carbonate	Pulverised and chemically pure	0.05 – 1
Deionised Water	Conductivity ≤ 10 µS/cm, in addition to dry sediment	30 – 50

The peat was air-dried under a fume hood and thereafter ground to a fine powder which was then sifted to remove large plant remains. Distilled water was added at a rate of 11.5 times the weighed peat powder in order to prepare a stirrable peat slurry whose pH was adjusted to 5.5 ± 0.5 by the addition of powdered calcium. The suspension was thereafter conditioned over a three day period by gentle stirring at room temperature. The pH was thereafter measured and readjusted to 6.0 ± 0.5 with the addition of more powdered calcium carbonate. This suspension

was then added to all the other dry constituents along with distilled water added and stirred to make up a homogeneous sediment mixture. The pH was again determined and thereafter adjusted to 6.5 with powdered calcium carbonate. Finally, an inoculum of actual lake sediment from a eutrophic pond was added into the artificial sediments (<0.5% w/w) in order to closely approximate the bacterial community of real lake sediments.

2.2.2 Preparing Mesocosms

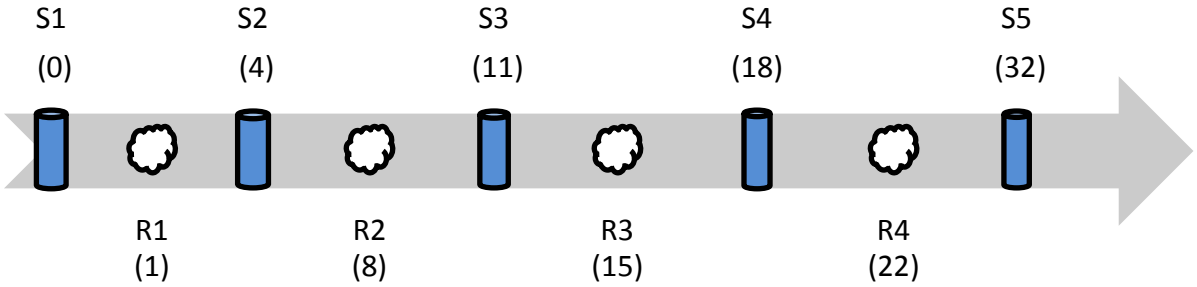
Sediments were added to the bottom 5 cm of each of the mesocosms which were then placed in placed in dark buckets (90 L, 38 cm tall storage bins) containing flowing cold water (10^{o}C ±2) to induce stratification and simulate an epilimnion and hypolimnion in the water column. Dark plastic was used to shade the bottom 1m of all the mesocosms so that the epilimnion would be a photic zone and the hypolimnion an aphotic zone. Six litres of de-chlorinated tap water were then added to each column. A light bank was set up above the mesocosms with twenty-one full-spectrum fluorescent lights (T8 VitaLux bulbs, 121.9 cm in length, MT-DTC) in order to provide a light intensity of 18000 1ux on the surface of the water. A timer was used to maintain light and dark cycles of 14 hr of light to 10 hr of dark throughout the experiment.

Eight days after introducing the dechlorinated water into the mesocosms, various photoautotrophs were added. These phytoplankton included (1) green algae: *Pseudokirchneriella subcapitata,* (2) diatoms*: Navicula pelliculosa and Synedra sp.* and, (3) cyanobacteria: *Microcystis aeruginosa, Nostoc sp., Anabaena sp., Oscillatoria sp.* and *Lynbyga sp.* All organisms were purchased from Ward's Scientific (St. Catharines, ON) and further sub-cultured at Ryerson University using protocols from Environment Canada (1992). On reaching a log

growth phase, 100 mL of each phytoplankton mixture was added to individual mesocosms. No further additions were made to the mesocosms.

Timeline for Events Pertaining to Mesocosms Sampling and Rain Events

A timeline for the events is shown below. This corresponds to July 24, 2010 (day 0) through August 25, 2010 (day 32).



( ): number in bracket is the day when event took place

R: rainfall simulation

S: sampling of mesocosms

Surface run-off and tile water that were generated from rainfall events were added to the mesocosms one day after the rain events.

2.3- Surface Run-off, Tile water and Nutrient Loading into Mesocosms

One week after the inoculation of the microphytes into the mesocosms, simulated rainfall was applied to the troughs using deionized water at a rate of 49.5 mm h^{-1} for 30 minutes. This rate represents an extreme storm event that occurs with a median frequency of once in 100 years in southern Ontario. Approximately 19 L of water were added to each of the troughs in this time. During the rainfall simulation, surface runoff and tile water were collected using 10 L pre-washed plastic bins. Surface runoff was collected from the front lower portion of the troughs while tile water was collected from the weeping tiles that protrude from the lower bottom end of the troughs. Collected runoff and tile samples were split into three aliquots: the first was immediately analysed for nitrate, ammonia and bioavailable P. The second aliquot was added to its corresponding mesocosm (10% v/v). Equivalent amounts of nitrate, ammonia and bioavailable P that were present in the run-off and tile water were then added to the inorganic nutrient control mesocosms, as mentioned above. The third aliquot was analyzed for various fractions of P, as described below. The above procedure was repeated on days 1, 8, 15 and 22.

2.3.1 Treatment and storage of surface run-off and tile water for P analysis

One third of the runoff and tile samples (described above) was further divided into two 250 mL aliquots. To reduce hydrolysis of some dissolved phosphates, one aliquot was immediately filtered through 0.22 µm membranes and thereafter refrigerated at 5^0C along with the second aliquot which was unfiltered. Aliquots were analysed for various phosphorus fractions within 48 h of sample collection, according to protocols established by Kovar and Pierzynski (2009). Molybdate reactive phosphorus (MRP) concentrations were analysed calorimetrically on samples that were filtered through 0.22 µm membranes, using the method of Murphy and Riley (1962).

The total phosphorus (TP) and total dissolved phosphorus (TDP) concentrations were determined using unfiltered and filtered samples, respectively. These samples were digested (acid–persulphate digestion) and P quantified using the colorimetric method of Murphy and Riley (1962). The Particulate Phosphorus (PP) fraction was determined from the difference between TP and TDP. Bioavailable Phosphorus (BAP) was determined by the iron oxide paper method (Myers *et al.* 1997).

Prior to the initial simulated rainfall on day 1, a sample of the reference soil was collected and air dried for P analysis. A sample from each trough was also collected on days 30, 60 and 120. All samples were air dried, crushed in a mortar and pestle, sieved (through 2 mm) and then analysed for P content as above.

2.3.2 Mesocosm Sampling and Analysis

On each sampling date, samples were collected from the upper and lower portions of the mesocosms. A weighted 2 m tygon tube was gently lowered into the mesocosms to approximately 15 cm from the air-water interface. This tube was attached to a 60 mL syringe which was used to collect the samples (3 syringes for each sampling depth). Samples were similarly collected from approximately 5 cm above the sediment surface. During sample collection, the tubing was lowered slowly to minimize mixing between the water in the top and lower layers within the columns.

Immediately after collecting, 45 mL of each sample was emptied into a 50 mL prewashed BD Falcon tube. Another 45 mL was filtered through a 0.22 µm filter (glass fibre filter) and collected into a 50 mL prewashed BD Falcon tube. Both tubes were immediately stored in a freezer at -20^{0}C for no more than 90 days until phosphorus analyses were performed. MRP, OP, TP, and TDP were analyzed in mesocosm samples as described above for runoff and tile drainage. The

filters from the epilimnion samples were immediately stored in black film canisters in a freezer at -20 ^{0}C for chlorophyll analysis. The remaining sample was used to measure pH, dissolved oxygen, and electrical conductivity.

2.3.2.1 Dissolved Oxygen Determination

Dissolved oxygen concentrations within the mesocosms were measured with a Clark-type oxygen microelectrode and picoammeter (Unisense A/S, Aarhus, Denmark). The microelectrode has a membrane diameter of 25 µm and precisely measures dissolved oxygen concentrations. The electrode does not require stirring due to exceedingly small oxygen consumption by the electrode. The electrode was calibrated using Millipore water at 100% oxygen saturation and 0% saturation by alternate bubbling of compressed air and nitrogen gas. According to the manufacturer the electrode has a 90% response time of < 1 second. Samples for dissolved oxygen were gently transferred to 20 mL glass scintillation vials, filling from the bottom using a short piece of tygon tubing, and allowing 1.5 volumes to overflow the scintillation vial. This was done to prevent reaeration of the sample during transfer. The tip of the electrode was fully immersed into each sample and readings were taken after 5 seconds.

2.3.2.2 Chlorophyll Extraction and Spectrophotometry (Trichromatic Method)

Ethanol (96%) was used to extract the chlorophyll from frozen filters. Under low light, the frozen filter was placed in a capped 25 mL tube and 10.0 mL of ethanol was added. The extraction tube was immediately closed and the sample was left in the dark at room temperature for 24 h (Jespersen and Christoffersen 1987; HELCOM 2011). The extraction tubes were shaken at approximately four equal intervals during this time. After extraction, the ethanol was poured into a centrifugation tube and it was centrifuged at 7000 rpm for 10 minutes to pellet particulate

residue from the filter. Concentrations of chlorophyll *a*, *b* and *c* were determined after the method of Jeffrey and Humphrey (1975), correcting for the use of ethanol rather than acetone (Jespersen and Christoffersen1987; HELCOM 2011). Results were then used to calculate concentrations of these phytopigments in mesocosm water based on volume of water filtered.

2.4 Analysis of Biosolids, Reference soil and Biosolids-amended soil

A complete P inorganic and organic fractionation procedure as outlined in figures 2.1 and 2.2 was carried on the two biosolids, reference soil and biosolids-amended soil. The procedure as outlined by Kovar and Pierzynski (2009) was used for the P fractionation into its inorganic and organic P operationally defined components.

2.4.1- Inorganic Phosphorus (IP) Fractionation

Step 1: DW-fraction (distilled water fraction)

0.5 g of soil (biosolids-amended or reference) or 0.1 g dried biosolids was placed into a 50 mL Naglene centrifugation tube and 25.0 ml Millipore water (18 MΩ) was added. The sample was shaken at 50 rpm for 30 minutes on a 3-D shaker and thereafter centrifuged at 15000 rpm for 15 minutes. The supernatant was then filtered through a 0.22 µm filter (glass fiber filter) and collected into a prewashed 50 mL Falcon tube. The sample was used to determine the DW-MRP (molybdate reactive phosphorus) calorimetrically using the ascorbic acid - molybdate method (Murphy and Riley 1962). The DW-MRP fraction represents the soluble inorganic phosphorus mainly as orthophosphate. The residual soil or biosolids from this step were kept for step 2.

Step 2: NH_4Cl fraction

Twenty-five mL of 1*M* NH_4Cl was added to the residue of step 1 and the suspension was then shaken for 30 minutes at 50 rpm and thereafter centrifuged at 15000 rpm for 15 minutes. The supernatant was then filtered through a 0.22 µm filter paper and collected into a prewashed 50 mL Falcon tube. The filtrate was then used to determine the loosely bound P. Determination of P in this fraction was as above.

The residual soil or biosolids from this step were kept for step 3.

Step 3: NH_4F fraction

Twenty-five mL of 0.5*M* NH_4F was added to the residue from step 2 and the suspension was then shaken for 1 h at 50 rpm and thereafter centrifuged at 15000 rpm for 15 minutes. The supernatant was then filtered through a 0.22 µm filter and collected into a prewashed 50 mL Falcon tube. The soil sample was then twice washed with 1.25 mL portions of saturated NaCl and centrifuged each time. The washings were combined with the filtrate and used to determine aluminum phosphates.

The residue from this step was kept for step 4.

Step 4: NaOH fraction

Twenty-five mL of 0.1*M* NaOH was added to the residue from step 3 and the suspension was then shaken for 17 h at 50 rpm and thereafter centrifuged at 15000 rpm for 15 minutes. The supernatant was then filtered through a 0.22 µm filter paper and collected into a prewashed 50 mL Falcon tube. The soil sample was then twice washed with 1.25 mL portions of saturated NaCl and

centrifuged each time. The washings were combined with the filtrate and used to determine iron phosphates.

The residue from this step was kept for step 5.

Step 5: CBD-fraction (citrate-bicarbonate-dithionate fraction)

Twenty mL of 0.3 M $Na_3C_6H_5O_7 \cdot 2H_2O$ and 5 mL of 1 M $NaHCO_3$ were added to the residue from step 4 and the suspension was then heated for 15 min in a water bath at 85°C. One gram of $Na_2S_2O_4$ (sodium dithionate) was then added and stirred rapidly to extract reductant-soluble P. The suspension was heated for another 15 min and then centrifuged at 15000 rpm for 15 minutes. The supernatant solution was decanted into a 50 mL volumetric flask. The soil sample was then twice washed with 12.5 mL portions of saturated NaCl and centrifuged each time and combined with the supernatant. The complete extract was exposed to air for 24 h to oxidize $Na_2S_2O_4$.

The residue from this step was kept for step 6.

Step 6: H_2SO_4 fraction

Twenty-five mL of 0.25 M H_2SO_4 was added to the residue from step 5 and the suspension was then shaken for 1 h at 50 rpm and thereafter centrifuged at 15000 rpm for 15 minutes. The supernatant was then filtered through a 0.22 µm filter paper and collected into a prewashed 50 mL Falcon tube. The soil sample was then twice washed with 1.25 mL portions of saturated NaCl and centrifuged each time. The washings were combined with the filtrate and used to determine apatite bound calcium phosphates.

P Analysis on each extract

An aliquot of 4.0 mL containing 2 to 40 μg P from each of extracts were added to separate 50-mL volumetric flasks. 10.0 mL deionized water and five drops of p-nitrophenol indicator were then added to the volumetric flasks containing NaOH and H_2SO_4 extracts and the pH was adjusted with 2.0 M HCl or 2.0 M NaOH until the indicator color just changes. (The indicator color changes from yellow to colorless for the NaOH extract while it changes from colorless to yellow for the H_2SO_4 extract). 7.5 mL 0.8 M H_3BO_3 was then added the volumetric flask containing NH_4F extract. Phosphorus concentrations in the various solutions were thereafter determined using the ascorbic acid - molybdate method (Murphy and Riley 1962). All absorbance readings were taken within 2 h of solution preparation. P standards that contained the same volume of extracting solution as in the extracts were used to prepare a P standard curve.

2.4.2 - Organic Phosphorus (OP) Fractionation

Step 1: Labile Organic P

Duplicates of 0.5 g (oven-dry weight basis) sieved soil samples or 0.2 g dried biosolids were weighed and carefully transferred into two 50 mL centrifuge tubes. To one tube, 50 mL of 0.5 *M* $NaHCO_3$ (pH 8.5) were added and the tube was placed horizontally on a mechanical shaker for 16 h. At the end of the extraction period, the sample was centrifuged at 15000 rpm for 15 min and the supernatant was filtered through 0.22 μm filter paper into a 25-mL volumetric flask. Millipore water was added to bring to volume.

Labile Pi was determined by transferring an aliquot containing 2 to 40 μg P into a 25-mL volumetric flask and thereafter adding five drops of *p*-nitrophenol indicator to the flask. The pH

of the solution was adjusted with 2 *M* HCl until the indicator color just changed from pale yellow to colorless. Approximately 20 mL of Millipore water was then added to the flask, followed by 8 mL of ascorbic acid-molybdate solution. After 20 minutes, P concentration was determined on a spectrophotometer at 880 nm.

Persulfate digestion was used to determine total labile P in the extract. 0.15g $K_2S_2O_8$ was mixed with 20 mL of the extract in an autoclavable flask. The solution was autoclaved for 1 h at 103.5 kPa and 120ºC. Neutralization and P determination were carried out using the same procedure as for Pi. The difference between total labile P following persulfate oxidation and labile Pi gives an estimate of labile Po. The residue from the labile P extraction was retained for the next step in the sequential extraction procedure.

Step 2: Moderately Labile Organic P

A two-step process was utilized in order to determine moderately labile Po. Twenty-five mL of 1 *M* HCl was added to the residue from the labile P extraction and the tube was placed on a mechanical shaker for 3 h. On completion, the sample was centrifuged at 15000 rpm for 15 min and the supernatant was filtered through 0.22 µm filter into a 25-mL volumetric flask. Millipore water was added to bring to volume. Total P and Pi in the extract was determined as outlined for labile organic P.

The residue from the HCl extraction was rinsed with Millipore water and thereafter shaken for 5 minutes then centrifuged at 15000 rpm for 15 minutes and the supernatant solution was then discarded. 25 mL of 0.5 *M* NaOH was then added to the residue and the sample was shaken for 3 h. At the end of the extraction time, the sample was again centrifuged at 15000 rpm for 15 min.

The supernatant contained both moderately labile OP (fulvic acid P) and nonlabile OP (humic acid P).

In order to separate the fulvic acid fraction from the humic acid fraction, 10 mL of the NaOH extract was measured and thereafter acidified to a pH of 1.0 to 1.5 with concentrated HCl in order to precipitate humic acids from the fulvic acids which is still soluble at this low pH. Total P in both the NaOH extract and the acidified sample was determined as previously mentioned. Humic acid P was determined by subtracting fulvic acid P from the total P measured in the NaOH extract. The residue from the moderately labile organic P extraction was retained for the next step in extraction procedure.

Step 3: Non-labile Organic P:

Highly-resistant non-labile Po was extracted by rinsing the residue from the NaOH extraction with Millipore water and thereafter shaking the sample for 5 minutes followed by centrifugation at 15000 rpm. The supernatant solution was discarded and the residue was then placed in a crucible and ashed at 550°C for 1 h. The ash was cooled and then dissolved by shaking in 25 mL of 1 *M* H_2SO_4 for 24 h. P were determined as previously described.

Calculations

The amount of P in each fraction was calculated with the following equation:

$$\text{P concentration in given fraction (mg kg}^{-1}) = \frac{[\text{Conc. of P (mg L}^{-1}) \times \text{Volume of extractant (L)}]}{\text{Mass of soil (kg)}}$$

All analyses were done in triplicates.

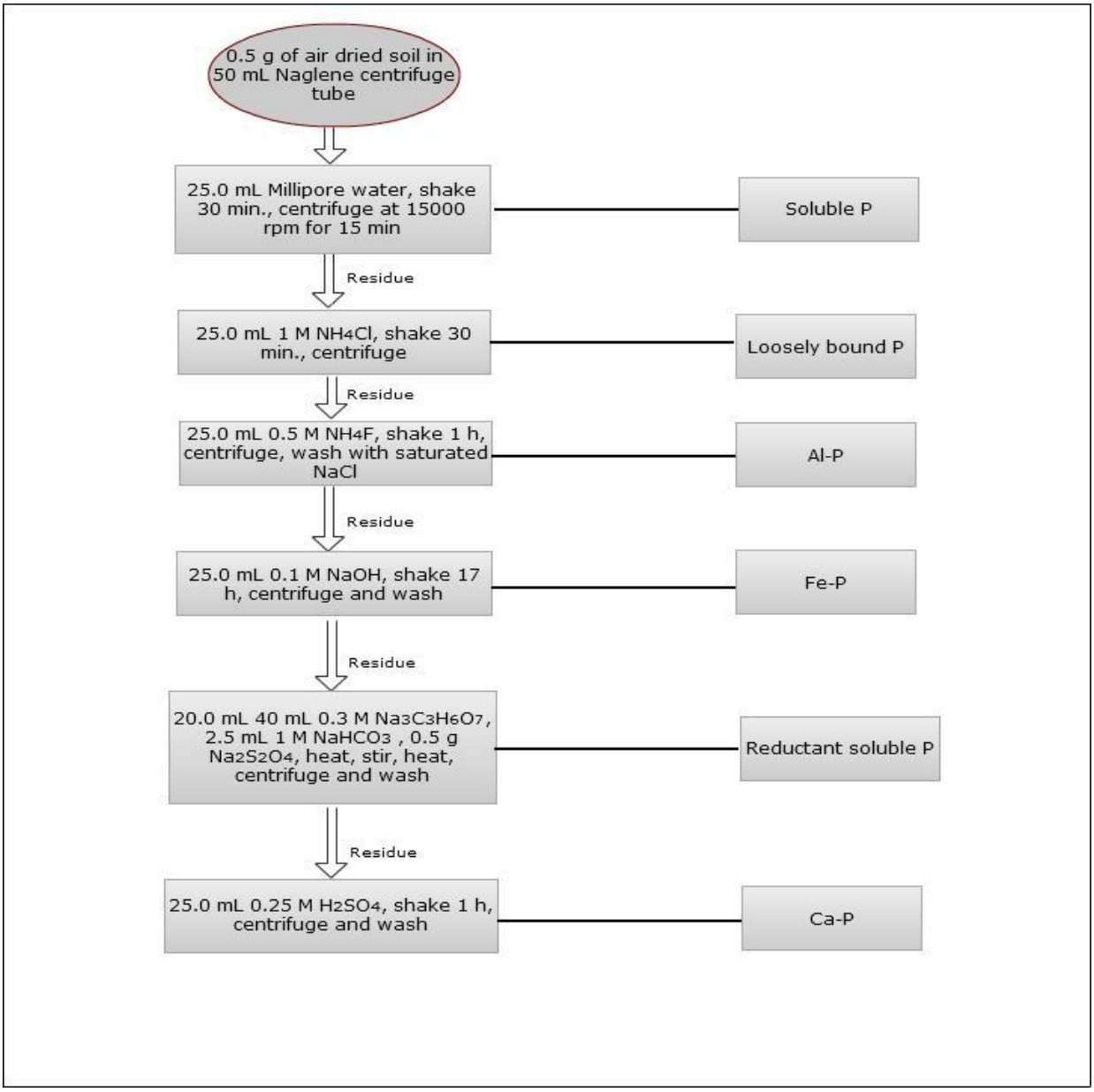


Figure 2.1 Sequential fractionation scheme for operationally defined IP. Modified from Zhang and Kovar (2008b).

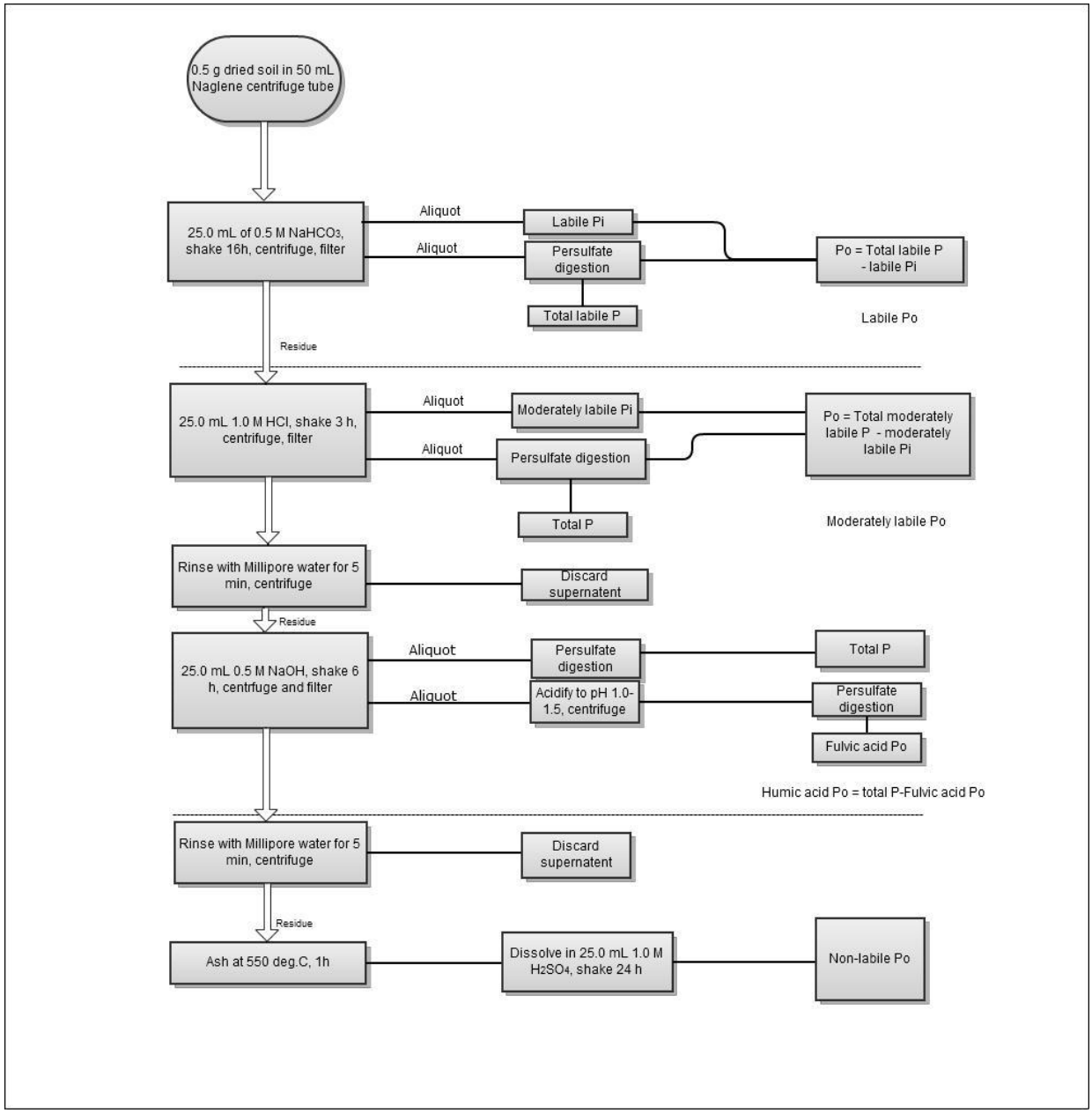


Figure 2.2 Sequential fractionation scheme for operationally defined OP. Modified from Zhang and Kovar (2008b).

2.4.3 Total P

Total P was determined by aqua regia digestion and semi-micro-Kjeldahl digestion.

Aqua regia digestion

0.2 g of finely ground soil (or 0.1 g dried and ground biosolids) was weighed and transferred into a 100 mL digestion flask. 10 mL aqua regia was added and the flask was then heated on a heating rack at 140°C for 4 h. After digestion, the supernatant was filtered into a 25 mL volumetric flask and its volume was brought up to 25 mL with Millipore water. An aliquot of 2 mL was then transferred to a second 25 mL flask and five drops of *p*-nitrophenol indicator were added. The pH of the solution was adjusted with 4 M NaOH and thereafter analysed for orthophosphate concentration as outlined by Murphy and Riley (1962).

Semi-micro-Kjeldahl digestion

0.2 g of finely ground soil (or 0.1 g dried and ground biosolids) was weighed and transferred into a 100 mL Kjeldahl digestion flask. 10 mL digestion reagent (appendix E) was carefully added to the Kjeldahl flask along with six glass beads (3- to 4-mm size) to prevent bumping during digestion. The heating unit on the micro-Kjeldahl digestion apparatus was set to its medium setting with a vacuum outlet connected to vent fumes from the flasks. The mixture was boiled until it became transparent and pale green and copious fumes were observed. Then, each heating unit was raised to its maximum setting and digestion was continued for an additional 30 minutes. After cooling, the digested sample was made up to a final volume of 30 mL by addition of rinse water (Millipore) from the micro-Kjeldahl distillation apparatus, and topped up with additional Millipore water. An aliquot of 2 mL was then transferred to a second 25 mL flask and five drops

of *p*-nitrophenol indicator were added. The pH of the solution was adjusted with 4 M NaOH and thereafter analyzed for orthophosphate concentration as outlined by Murphy and Riley (1962).

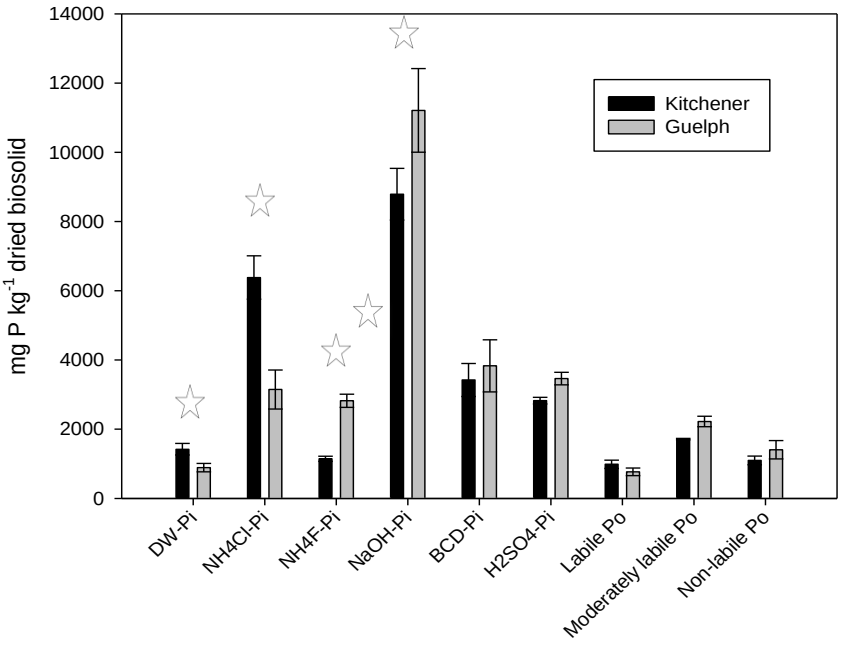
Statistical Analysis

Statistical analyses were performed using SYSTAT version 13 (Chicago, IL) software for PC computers. All response variable data were tested for normal distribution and when data failed to meet the assumption of normality in distribution, were log transformed. Differences within each P fraction in the reference and biosolids-amended soils, total phosphorus, chl analysis and dissolved oxygen were determined using repeat measures analysis of variance (rmANOVA). One-way ANOVA was used to determine statistical differences in P forms in runoff and tile leachate between treatments with post-hoc Tukey's HSD tests used to determine differences between individual treatments where a one-way ANOVA revealed a treatment effect. In all cases, statistical differences were accepted when probability was less than α of 0.05.

3 Results and Discussion

3.1 P in biosolids and soils

a)



b)

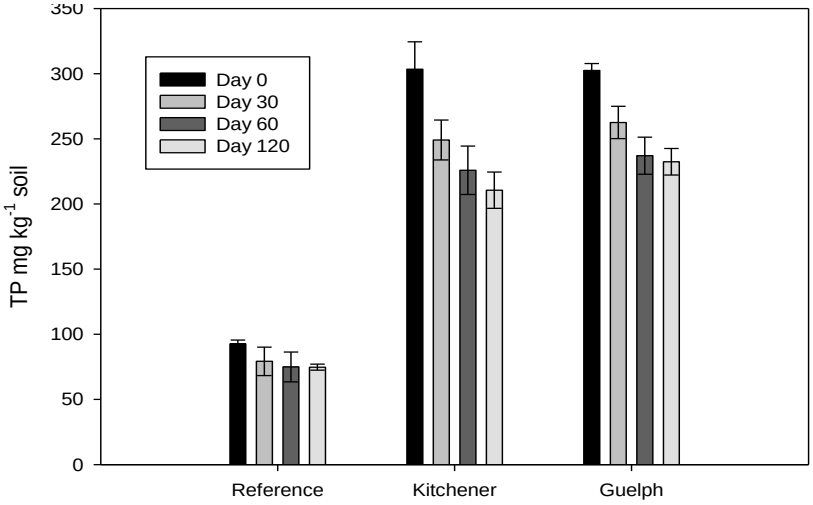


Figure 3.1 a) P fractions in biosolids, and b) Total Phosphorus in reference and biosolid-amended soils over 120 days. Values are mean ± standard error (n = 3). [☆represents significant difference]

The biosolids used in this study was representative of those produced through anaerobic digestion along with the addition of ferric chloride in the tertiary treatment process. However, sequential fractionation revealed a major difference in P pools between the two biosolids (figure 3.1a). Kitchener biosolids consistently contained more DW-IP, NH_4Cl-IP and labile OP when compared to Guelph biosolids. Furthermore, all these differences were significant ($p < 0.05$). Kitchener biosolids contained over 6000 mg kg^{-1} of loosely bound P which is over twice the amount contained in Guelph biosolids. This would indicate that Kitchener biosolids may pose a greater risk in the short term to receiving aquatic bodies resulting in more pronounced eutrophication. Labile OP was the smallest fraction in both biosolids. Although Kitchener biosolids had more labile OP, there was no significant difference in this pool ($p = 0.081$) when compared to Guelph biosolids and in addition, labile OP represented the smallest pool in both biosolids. There was also significant difference ($p < 0.05$) between the potentially available P pools in the two sources. Over 40% of P in both biosolids was retained in the NaOH-IP fraction since P in this fraction was retained mainly by Fe hydr(oxides). This is consistent with the fact that ferric chloride was added to both biosolids in order to reduce P lability. The NH_4F and NaOH fractions are potentially bioavailable and may become soluble under anoxic and reducing conditions such that frequently occur in the hypolimnion of stratified lakes. The unavailable P pools did not differ significantly ($p > 0.05$) between the two sources and represent on average, 28% of TP.

Figure 3.1b showed steady losses of total P from the upper 5 cm of reference and biosolid-amended soils during the 120 days of sampling. There was a significant difference in P between the biosolids amended soils and reference soil overall ($F_{2,6} = 814.2$; $p<0.001$). The temporal pattern of P loss also differed among soils (time x treatment: $F_{3,6} = 5.1$; $p = 0.003$). The rate of

loss was higher in biosolids amended soil compared with reference soil (Figure 3.1b). At the end of the 120 days, there was a decrease of 22% TP in reference soils compared to 31% in Kitchener and 22% in Guelph biosolid-amended soils. For all treatments, most of the P loss resulted from the four storm events simulated during the first thirty days of the experiment. During the remaining 90 days, simulated rainfall was light, maintaining soil moisture at 70% moisture content. The first thirty days saw 70% of total P loss in reference soils and 59 and 57% of the total P loss in Kitchener and Guelph biosolid-amended soils, respectively. These results highlight the important role of rainfall in removing P from surface soils at 0- to 5-cm depth due to its higher relevance to P loss in surface runoff (White *et al.* 2010). Runoff contains the highest amount of TP when rainfall erodes P enriched sediments that are found within the top 5cm of the upper horizon (Daniels *et al.* 1998). In addition to surface runoff, P was also removed from the upper layer of the soils to the lower layers via leaching. Percolating waters carried along both soluble and particulate P as it moved downwards into the lower horizons as observed in the high P levels in the tile water that was simultaneously collected with surface runoffs as seen in figure 3.2e. Since no surface runoff was generated during the latter 90 days, P was solely removed from the upper 5 cm of the soils by leaching to deeper layers. Thus, eluviation and illuviation are important processes that are responsible for the movement of nutrients from one horizon into deeper horizons within the regolith, especially on lands not receiving high levels of rainfall.

The addition of biosolids led to at least a threefold increase in the TP in soils during this study. However, less than 10% of the P applied to agricultural soil may be actually removed by plants during the first year of application (Stevenson and Cole 1999), the rest remaining in insoluble or fixed forms in the soil. Soils with high P levels are more prone to P loss via erosion and leaching.

Researchers have shown that soils with high P content consistently influence the DRP concentrations in runoff from these soils (Sharpley 1993; Daniel *et al.* 1994) which may accelerate eutrophication in P-limited receiving waters (Pote *et al.* 1996). This must be taken into consideration when developing P strategies to limit eutrophication but at the same time, maintain high crop yields (Pote *et al.* 1996). Since repeated biosolids application result in over application of P to soils, P accumulation and subsequent mobilization and removal during heavy rainfalls is a major challenge facing P management when soils are amended.

In order to reduce eutrophication potential of receiving water bodies, TP should be maintained below 25 mg m^{-3} in streams and 10 mg m^{-3} in lakes (Smith *et al.* 1999). The concentration of P and its forms in surface water frequently correlates with primary productivity and the trophic levels of rivers and lakes (Schindler 1974; Carpenter and Kitchell 1988; Marsden 1989, Correll 1998; Smith *et al.* 1999; Reynolds and Davies 2001). Huntsman (1948) added NPK fertilizers into an oligotrophic stream in Nova Scotia and found increases of filamentous algae and fish at downstream sites. Excessive nutrient input into receiving water bodies is a real problem affecting most North American streams and lakes. Agriculture contributes to at least 50% of nutrient input into lakes and over 60% into rivers (US EPA 1996). This study mimics worst case scenarios, as the soil had no crop over, had maximum allowed slope, four extreme storm events in a little over three weeks, and the runoff and leachate collected was directly added to water columns (i.e. no simulation of vegetated buffer strips).

3.2 Effect of biosolids on P fractions in soils

3.2.1 Inorganic P Fractions

Sequential chemical extraction can show changes in operationally defined P pools.

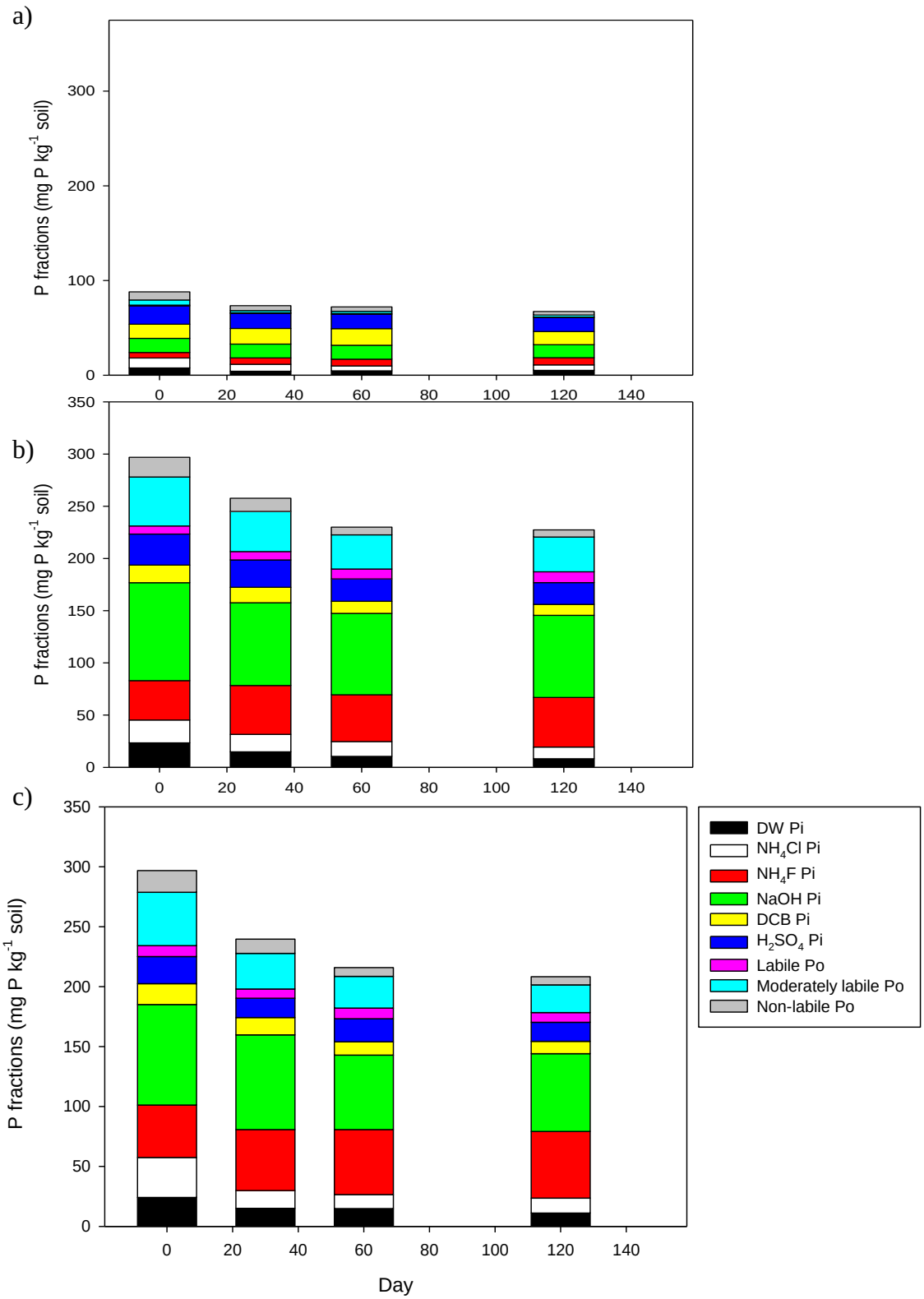


Figure 3.2 P fractions in biosolids amended and reference at different time points. a) Reference soils, b) Kitchener amended soils, and c) Guelph amended soils. Values are mean, n=3.

Changes in operationally defined P pools over the 120 days are shown in Figures 3 a-c.

With respect to bioavailability, the DW- and NH_4Cl-IP fractions are readily available for algal uptake since they are soluble and loosely adsorbed onto soil aggregates and detritus. One-way ANOVA showed that there was a significant difference in these fractions ($p<0.001$) for the different P sources – reference soils and soils amended with biosolids from Kitchener and Guelph. Furthermore, there was a significant difference in their temporal (time x treatment) DW-P distribution over the 120 days ($p<0.05$). A decrease of the DW-IP pool in amended soils over time agrees with the findings of Buehler *et al.* (2002) and Kashem *et al.* (2003) who both found that the DW-P pool was being transformed into $NaHCO_3$-P and NaOH-IP pools with increasing incubation time. It is possible that these fractions are being transformed into the potentially available fractions over time since these fractions saw small net increase.

These fractions consistently represented the smallest pool in all treatments throughout the 120 days of sampling. However, there was a slight decrease in this pool over time ranging from 3% for reference soils to 3 and 6% for Kitchener and Guelph amended soils respectively. These findings are in similar to those found by Cox *et al.* (1997), Maguire *et al.* (2000), Ippolito *et al.* (2007) and Baley *et al.* (2008) who observed decrease in the labile P and suggested that it was most probably retained into the Al-IP pool**.**

The potentially bioavailable NH_4F-IP and NaOH-IP fractions consist of P that is Al- and Fe-bound in soils. There was a significant difference in these P pools ($p < 0.001$) for the different treatments. However, there was no significant difference in their temporal (time x treatment) P distributions over the 120 days ($p>0.05$). P was being immobilized in this fraction by the P sorption capacity of the soils. Organic matter closely interacts with Al with organic matter

increasing the amorphous nature of soils and hence reactivity of Al as was mentioned by Maguire *et al.* (2000) in soils that were amended with biosolids. The Fe-bound P pool represents the largest P storage in amended soils. The average percentage of total P that is Fe-bound was 19% for reference, and 30% and 32% for soils amended with Kitchener and Guelph biosolids, respectively. Fe plays a major role in P sorption and it helps to restrict P mobility (Maguire *et al.* 2000; Akhtar *et al.* 2002). Furthermore, in this study, more than 50% of the soil P was found within the Al-P or Fe-P fractions in the amended soils. This is in conformity with the works of other researchers which showed that Al and Fe are the major soil components in responsible for retaining P in acidic and or near neutral soils (Williams *et al.* 1971; Maguire *et al.* 2000).

With the exception of reference soils, reductant soluble P slightly decreased for the biosolids amended soils over time. There was no difference in the CDB-IP pool ($F_{2,6} = 3.3$; $p = 0.106$) for the different P sources. Furthermore, there was no difference in the temporal (time x treatment) CDB-P distribution over the 120 days ($F_{3,6} = 1.3$; $p = 0.298$). The results show that with the exception of reference soils, reductant soluble P is not a significant P pool. The average percentage of total P as CDB was 21% for reference soils and 6 and 5% for soils amended with Kitchener and Guelph biosolids respectively. The reductant soluble or occluded P pool represents relatively stable pool in soils and it is found mainly in the inside of Fe oxides such as haematite and geotite (Chang and Jackson 1957; Maguire *et al.* 2000) and since it is fairly stable, it is not bioavailable on the short term to plants and algae (Akhtar *et al.* 2002).

H_2SO_4-IP which consists of Ca-bound P remained fairly unchanged for all treatments over time. There was a significant difference in the Ca-IP pool ($F_{2,\ 6} = 20.4$; $p = 0.002$) for the three different P sources; however, there was no difference in the temporal (time x treatment) Ca-IP distribution over the 120 days ($F_{3,6} = 3.2$; $p = 0.384$). The Ca-IP fraction is not a major sink in

biosolids amended soils. However, the Ca-IP pool represents the largest average P storage in unamended soils (22%). Ca is very effective at immobilizing and retaining P especially in calcareous soils where it reacts with P to form discrete minerals. However, in acidic soils, the role of Ca is limited due to the dissolution of Ca-IP minerals at lower pH. Its low solubility contributes to fairly slow soil reactions (Siddique and Robinson 2004) and may account for the slight change in the Ca-IP pool over time.

Total inorganic P was consistently higher in the biosolids amended soils when compared to the reference soils. On average, IP was 189.7 and 194.9 $mgkg^{-1}$ soil in the Kitchener and Guelph amended soils when compared to 65.9 mg kg^{-1} soil in reference soils. This represents at least a threefold increase in Pi in amended soils. Furthermore, Pi represented the greater part of the total P found in all treatments. Reference soils contain an average of 88% Pi while Kitchener and Guelph amended soils contained 79 and 77%, respectively.

3.2.2 Organic P fractionation of soils

As shown in figures 3 a-c, higher OP was found in amended soils when compared to reference soils. Total organic P was consistently higher in the biosolids amended soils when compared to the reference soils. On average, OP was 50.5 mg kg^{-1} and 58.1 mg kg^{-1} in the Kitchener and Guelph amended soils when compared to 18 mg kg^{-1} of reference soils. This represents at least a fivefold increase in OP in amended soils. This of course is expected since the reference soil did not receive any P amendment during the sampling period. Reference soils had on average 9% OP while Kitchener and Guelph amended soils contained 21 and 23%, respectively. With the exception of labile OP that slightly increased in amended soils, the remaining OP fractions consistently decreased over time. However, these changes were modest when compared to changes in some IP pools. This finding has been documented by many authors such as Antilen *et*

al. (2008) who showed that after 4 months, very little transformation had taken place in the organic fractions and also no significant amount of biosolids P were converted into plant accessible organic P. Baley *et al.* (2008) have shown that humic acid, fulvic acid and non-labile Po did not appear to play any major role in P transformations in soil. The main reason for the slow change within the different OP pools can be attributed to the slow rate of biological decomposition of the organic matter present in biosolids amended-soil over time. After biosolids application, inorganic P is likely to bond with the iron or aluminum present in soil (Chang *et al.* 1983); however, organic P is hydrolysed both in acid or basic soils (Hinedi *et al.* 1989). As confirmed by Kashem *et al.* (2003), although the change in fractions is small, there is a net conversion of OP pools into IP pools over time.

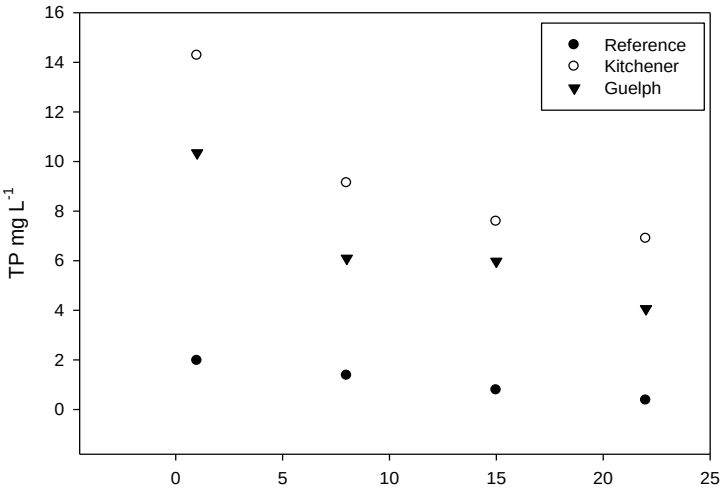
One way ANOVA showed that there was a significant difference ($p<0.001$) in the labile, moderately and non-labile OP pools in the three treatments. Labile-OP represents the smallest P pool and is therefore not a major P long-term source in any of the three treatments throughout the 120 days of sampling. The average percentage of total OP that is in the labile-OP pool is 1% for reference soils and 3% for both biosolids amended soils. Both amended soils did show a slight net gain of < 2% in the labile OP fraction. Antilen *et al.* (2008) and Baley *et al.* (2008) have shown that organic P species such as diesters, which are easily biodegradable in the soils, thus increase the labile OP pool over time. Soils that are amended with biosolids have a greater bacterial population than inorganic fertilizer-amended soils due to higher organic matter content in the top 5 cm of the soils (Baley *et al.* 2008) resulting in increased decomposition and release of OP.

Moderately labile-OP represents the largest organic pool in both amended soils and will therefore play an important role as a P long term sink or source in these soils. The average

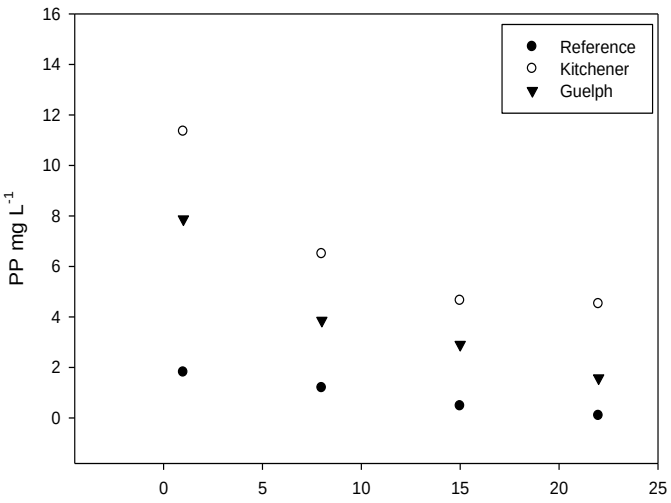
percentage of total OP that is in the moderately labile-OP is 4% for reference soils and 13 and 15% for soils amended with Kitchener and Guelph biosolids, respectively. P associated with fulvic acids accounted for over 50% of the total OP pool in the amended soils throughout the sampling period. All soils showed a net loss of <3% over the 120 days indicating slow mineralization of this pool over time. Humic acid P was not a significant pool in amended soils. This finding is in agreement with previous studies that examined OP transformations in biosolids amended soils. Since these fractions are highly resistant, they are relatively stable and thus act as sinks instead of sources (Sui *et al.* 1999; Baley *et al.* 2008).

3.3 Surface runoff and tile water P from soil troughs

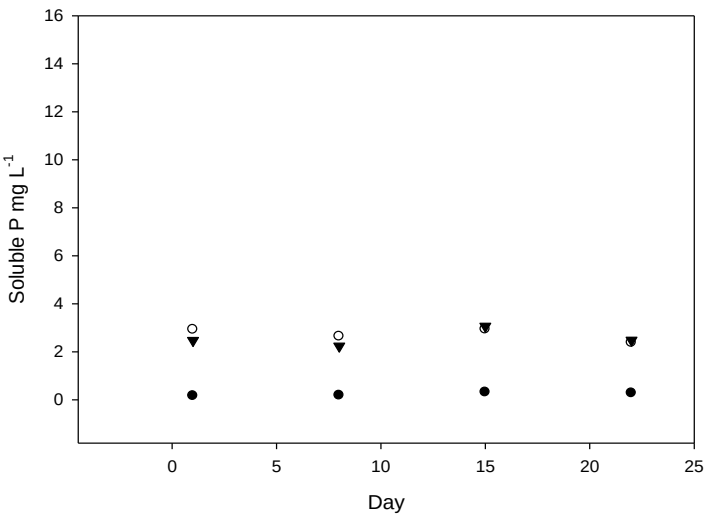
a)



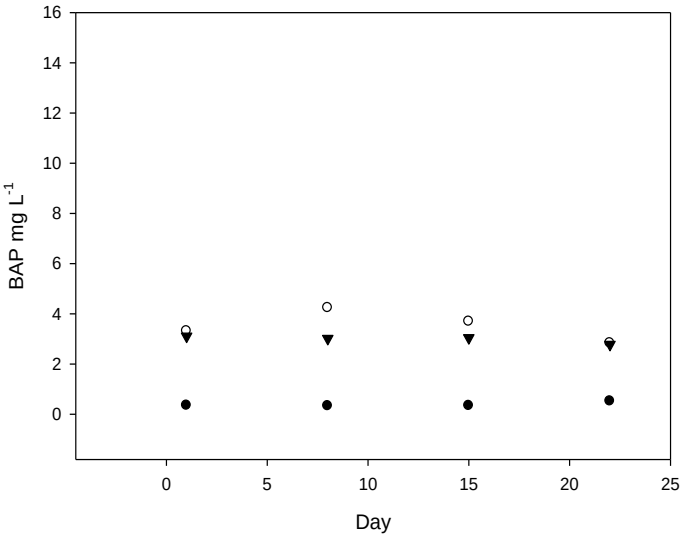
b)



c)



d)



e)

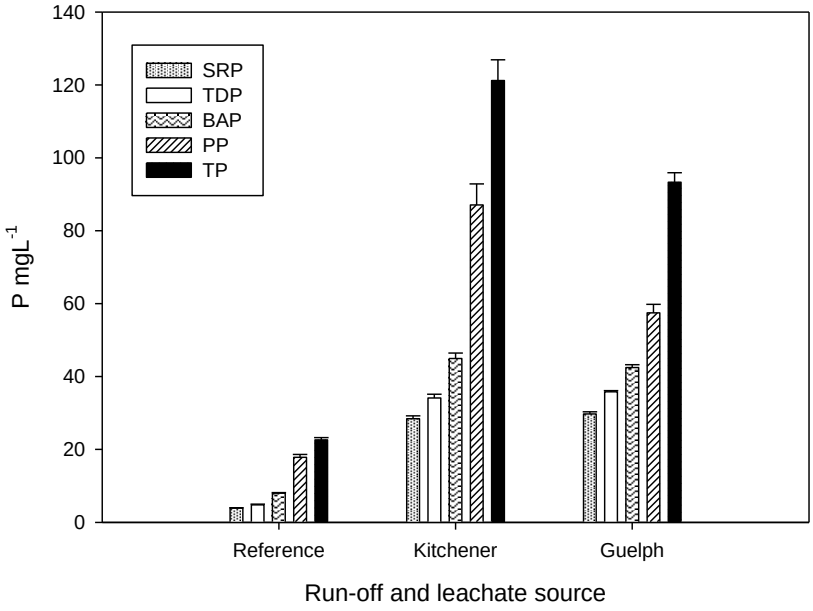


Figure 3.3 P loss from surface runoff and tile leachate in biosolids-amended and reference soils during simulated rainfall events. a) TP, b) PP, c) Soluble P, d) BAP, and e) comparison of P forms among treatments (average across all collection dates).

One-way ANOVA showed that TP in surface runoffs did differ significantly among treatments ($F_{2,6}$ = 1937, $p < 0.001$; Figure 3.3a). Post-hoc Tukey's HSD test determined that both TP concentrations from Guelph and Kitchener treatments differed from that of reference ($p < 0.001$ for each pair-wise comparison). Furthermore, the TP in runoff and tile leachate from Guelph also differed significantly from that of Kitchener ($p<0.001$). Average TP in runoff and tile leachate from Kitchener amended soils was 9.5 mgL^{-1} over the four rain events when compared to 6.6 mgL^{-1} from Guelph and a low 1.1 mgL^{-1} from reference soils. These TP values were relatively high compared to some similar studies. Application of biosolids in previous studies has resulted in an elevated TP loss relative to reference soil. This increase has ranged from to 2 mgL^{-1} to 10.38 mg L^{-1} (Mostaghimi *et al.* 1992; Sharply *et al.* 1992; Sharpley 1995; Cox and Hendricks

2000; Quinton *et al.* 2001; Withers *et al.* 2001; Andraski and Bundy 2003; Quilbe *et al.* 2005; Heathwaite *et al.* 2006; White *et al.* 2010) and was dependent on such variables as treatment process, application rate, tillage, chemical properties, rainfall and slope. Given these variations, such values obtained in this study may actually represent an upper limit of TP removed in unplanted soils that are amended with biosolids followed by frequent storm events.

On the basis of TP loss from soil to runoff which may enter surface waters, biosolids land application would seem to present a clear eutrophication risk. However, the use of TP as an indicator of eutrophication in water bodies is somewhat problematic. This is because TP consistently overestimates the amount of phosphorus that is bioavailable to phytoplankton and bacteria for uptake (Ellison and Brett 2006). Yet, when used with other management tools such as TDP and BAP, it can help managers better understand, plan and choose appropriate remediation measures if needed (Sharpley *et al.* 1992). In this experiment most of the TP in combined runoff and leachate was unavailable for algal uptake. Over 64% of the TP in the runoff from reference soils were in the unavailable fractions while 63 and 55% were unavailable in Kitchener and Guelph runoff, respectively.

Figure 3.3b shows the variation in the PP concentrations for each of the three treatments over the monitored period. Like TP, PP did differ significantly among treatments ($F_{2,6} = 738$, $p < 0.001$). Post-hoc Tukey's HSD test determined that both Guelph and Kitchener treatments differ from reference ($p < 0.001$ for each pair-wise comparison). Furthermore, PP in runoffs from Guelph also differed significantly from that of Kitchener ($p < 0.001$). PP represented the major form of P loss during the rain events ranging from an average of 72% from Kitchener amended soils, 62% in Guelph and not surprisingly 79% from reference soils. This finding is consistent with other studies that show higher percentages of PP in runoff from agricultural fields (Hooda *et al.*

1997; Miller and Hooda 2011) and also PP is the dominant form of P that is removed during storms (Ellison and Brett 2006). This study has shown a maximum of 13% of BAPP was actually contributed from PP (assuming that 100% TDP is bioavailable). This was probably due to the fact that this was a one-time biosolid application as opposed to repeated applications. Repeated applications risk exceeding the soil P sorption capacity since all available sites may be already occupied by phosphate and other competing anions (Penn and Sims 2002; Ippolito *et al.* 2007).

Figure 3.3c shows the variation in the TDP or soluble P concentrations for each of the three treatments over the monitored period. TDP is made of inorganic and organic components. TDP did differ significantly among treatments ($F_{2,6} = 6233$, $p < 0.001$). Post-hoc tests determined that both Guelph and Kitchener treatments differ from reference ($p < 0.001$ for each pair wise comparison) but not from each other ($p = 0.153$). TDP in runoffs averaged around a high of 2.9 mgL^{-1} from Kitchener soils and 2.3 mgL^{-1} from Guelph soils while TDP in runoffs from reference soils did not exceed 0.28 mgL^{-1}. All these values exceed the 0.01 mgL^{-1} limit recognized as stimulating eutrophication in receiving waters (Sharpley 1993). Also, the concentration of TDP in runoffs was highly correlated to TP ($r^2 = 0.8917$).

Organic P did differ significantly among treatments ($F_{2,6} = 116$, $p < 0.001$). Post-hoc tests determined that both Guelph and Kitchener treatments differ from reference ($p < 0.001$ for each pair-wise comparison) but not between the two amendments ($p = 0.848$). OP does contribute to the bioavailable pool after hydrolysis; however, less than 50% of dissolved organic P is bioavailable (Logan 1982). OP contributed no more than 2% of the TP in the runoffs from reference soils and 5% and 7% respectively in the runoffs from Kitchener and Guelph soils.

Figure 3.3d shows the variation in the BAP concentrations for each of the three treatments over the monitored period. BAP in runoffs and tile leachate did differ significantly among treatments ($F_{2,6}$ = 4469, $p < 0.001$). Post-hoc tests determined that both Guelph and Kitchener treatments differ from reference ($p < 0.001$ for each pair wise comparison) but not between the two amendments ($p = 0.074$). BAP accounted for an average 37% of TP in runoffs from Kitchener amended soils, 45% in Guelph and 35% in the reference soils. With the exception of an increase in the second rain event for Guelph runoffs, BAP concentrations decreased over time for all treatments. BAP measurement is perhaps the most important single parameter that will help in assessment of the impact of agricultural runoffs on the resultant biological productivity in receiving aquatic bodies (Sharpley 1992).

Land application of biosolids has dramatically increased over the past 25 years (Evanylo 2009). Release of P from biosolids-amended soils may result in an increase in non-point source pollution of surface and potentially of groundwater. This study has shown that runoff contains elevated P. However, not all this P is bioavailable and also this runoff will be further diluted by the volume of a river or receiving lake. In order to know how P in biosolids change with respect to its bioavailability and possible mobility, the fate of P and its different P pools needs to be investigated over time. In addition, sound management of applying biosolids on agricultural lands requires a comprehensive understanding of P loss and transformations in soils and in water bodies. This study showed that most of the P in amended soils was in an inorganic form and only a small quantity is bioavailable. Furthermore, this study showed that substantial amounts of P were removed via surface runoff and also from water passing through the soil as tile leachate. However, most was in the unreactive particulate pool which is unavailable for algal uptake. Over time, much of this P may be sorbed unto Al- and Fe-minerals and clay particles in soils resulting

in retention in soils that even have a modest amount of these minerals. This sorption may temporarily delay the P flux from amended soils to water bodies (Rydin and Ottabong 1997).

3.4 Impact of Runoffs on Receiving Waters (Mesocosm experiments)

Mesocosm treatments were set up to investigate the effects of organic and inorganic amendments when compared to runoff from unamended soils. The mesocosms were amended with runoffs from reference and Kitchener and Guelph-amended soils, and a combination of inorganic nutrients that represented the high and low N and P values that were measured from the runoffs of the biosolids applications. The intention of the inorganic amendments was to provide a similar loading of N and/or P experienced when biosolids run-off enters microcosms and have the total of this N or P in a fully bioavailable form. This should distinguish between the effects of total nutrient loading versus loading of bioavailable nutrients. One set of mesocosms was left without any nutrient addition and represented the blank.

3.4.1 Chlorophyll Analysis

a)

b)

c)

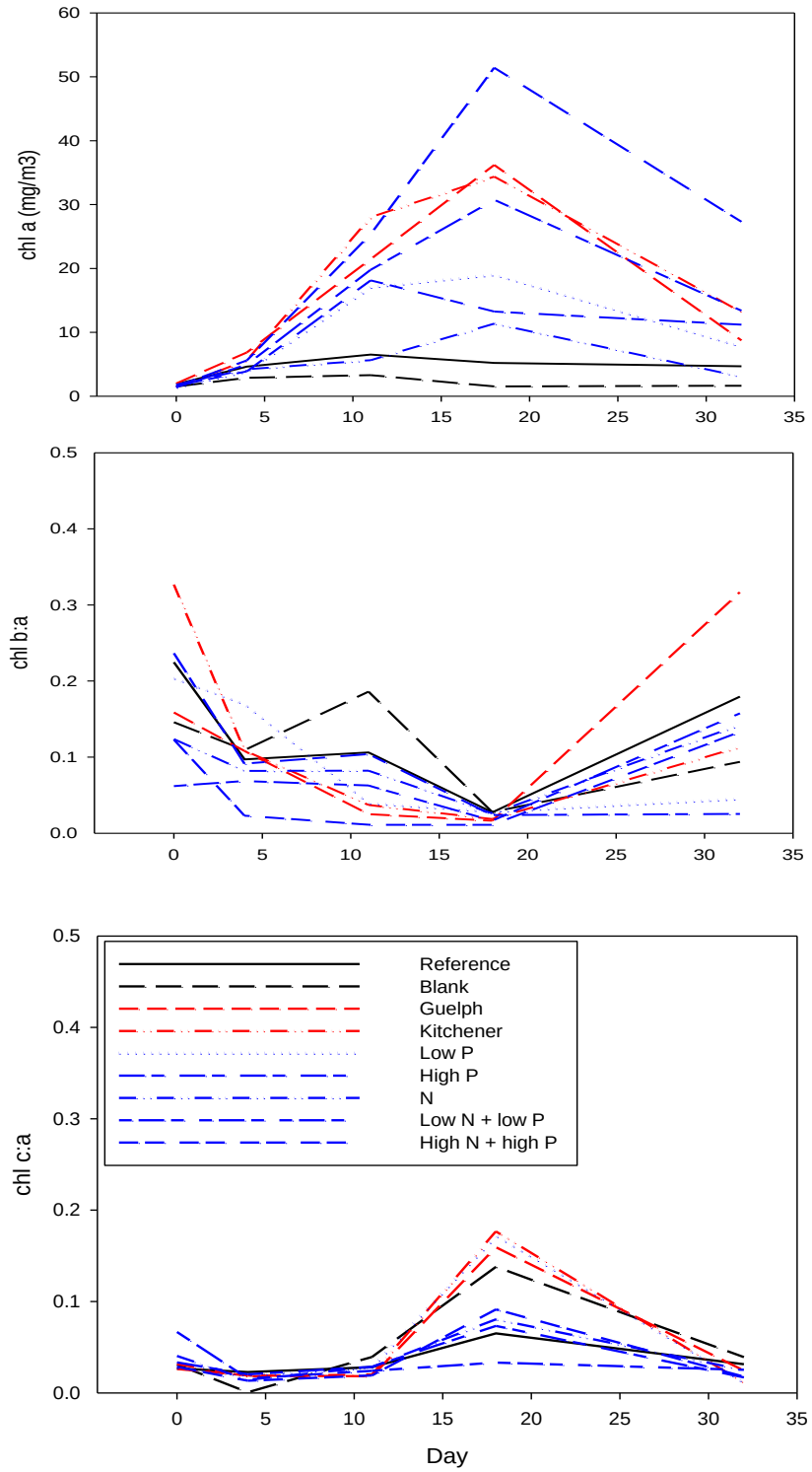


Figure 3.4 Changes in chlorophyll over time. a) Chl-a b) Chl-b:chl-a, and c) Chl-c:chl-a.

There was a statistical difference in chl-a ($F_{8,36} = 32.4$; $p < 0.001$) for the treatments and there was a difference in the temporal (time x treatment) chl-a distribution over the 32 days of sampling ($F_{4,32} = 8.8$; $p < 0.001$). Prior to the addition of nutrients into the mesocosms, chl-a averaged at 1.6 μgL^{-1}. The control mesocosms (reference soil run-off and blank) remained low in chl-a throughout the study (chl-a values consistent with oligotrophic systems). There is a strong relationship between the addition of nutrients and chl-a concentration in the mesocosms. As seen in figure 4a, there was a threefold increase in chl-a only four days after the addition of nutrients to the mesocosms. In all but the control and N only mesocosms, by day 11 chl-a levels were consistent with eutrophic (>9 μgL^{-1}) or hypereutrophic (>25 μgL^{-1}) conditions. The maximum chl-a concentrations for all fertilizer-amended mesocosms were consistently measured on day 18. The highest average recorded values of 51.6 μgL^{-1} chl-a were from a high N + high P inorganic fertilizer input (that is the inorganic loading analog of nutrients in Kitchener runoff). At this point in time, the Guelph and Kitchener mesocosms had also reached their maximum measured concentrations of 36.5 and 34.6 μgL^{-1} chl-a respectively. Low N + low P systems (that is the inorganic loading analog of nutrients in Guelph runoff) recorded their maximum values of 30.7 μgL^{-1} chl-a. Despite further additions of run-off or inorganic nutrients, chlorophyll a levels generally decreased toward the end of the experiment. By day 32, chl-a concentrations in Guelph mesocosms were consistent with mesotrophic conditions (8.6 μgL^{-1}) and Kitchener mesocosms were consistent with a low eutrophic state (13.2 μgL^{-1}). However, the N + high P mesocosms remained hypertrophic at day 32. Overall, as shown in figure 4a, the results indicate that chl-a increased from:

Control < N < reference < low P < high P < Guelph < Kitchener < N + low P < N + high P.

What is important about the chl-a data is the fact that a high nutrient input may not always translate into high primary productivity. For example, Jackson and Jeppesen (2007) recorded maximum chl-a of 26 μgL^{-1} while maximum TP was 937 μgL^{-1} for 30 shallow Canadian prairie lakes during the summers of 1998-2004. Similarly, Morgan *et al.* (2006) found that TP was as high as 2750 μgL^{-1} due to upstream sewage effluent at Salt Fork of the Vermillion river in 2004; yet, the sestonic chl-a was <3.5 μgL^{-1}. These authors suggested that sufficient light did not reach the periphyton on the stream bed. In this study, the inclusion of inorganic nutrient treatments demonstrated that the lower than anticipated response of algae to biosolids runoff was not due to light limitation, but was more likely due to the low availability of BAP to sustain growth. Any new BAP in the system had to have come from remineralisation of organic P from the microbial loop. Furthermore, SRP was below detection limits in all epilimnia in this study. This may in effect be the real limiting growth factor as suggested by Morris and Lewis (1988) and Stauffer (1992) and observed in this study.

There was no statistical difference in chl-b: chl-a ($F_{8,36} = 1.45$; $p = 0.21$) for the treatments. However, there was a difference in the temporal (time x treatment) chl-b:chl-a distribution over the 32 days of sampling ($F_{4,32} = 1.59$; $p = 0.035$) indicating a shift in relative abundance from green algae to cyanobacteria. In addition, biosolids treatments were moving toward dominance by cyanobacteria in the middle of the experiment, near the chlorophyll maximum. This is indicated by the high chl b:a ratio. Then late in the experiment, green algae become relatively more important, particularly in the Guelph mesocosms. Although Kitchener and Guelph mesocosms are not experiencing the same chlorophyll highs as their respective inorganic analogs, they are experiencing greater dominance by cyanobacteria in response to nutrient loading than is forced by the inorganic analogs. This observation has important ecosystem

implications as cyanobacteria are generally more a nuisance bloom than green algal blooms and also cyanobacteria are also less useful as a food source for zooplankton. In addition, no shift in relative abundance from green algae to diatoms was observed during this time since no significant difference ($F_{8,36} = 0.394$; $p = 0.916$) was observed between treatment or in the chl-c: chl-a temporal distribution over the 32 days ($F_{4,32} = 1.45$; $p = 0.21$). Yet, there was an observed reciprocal relationship between the chl-b:chl-a and the chl-c: chl-a. As seen in figures 4.2 and 4.3, on day 18 when chl-b: chl-a was at its lowest, chl-c: chl-a was at its highest.

3.4.2 Temperature, pH and DO

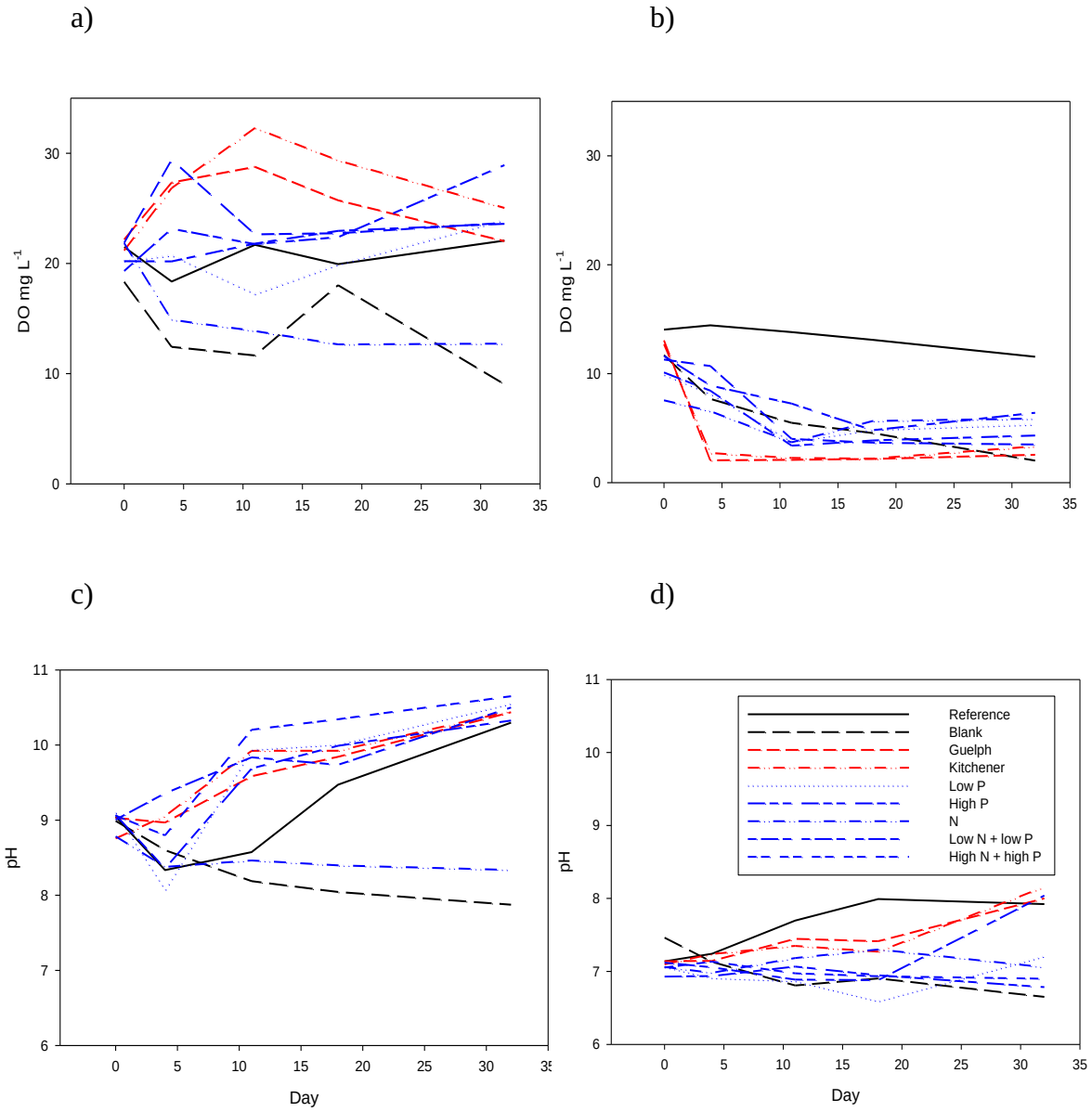


Figure 3.5 Dissolved oxygen concentration and pH for the different mesocosms over 32 days. a) DO in epilimnion, b) DO in hypolimnion, c) pH in epilimnion, and d) pH in hypolimnion (Data collected in collaboration with Denis Matiichine – MSc candidate, Env. Sc. Man., Ryerson, 2011.)

Thermal stratification was induced to represent stratification in temperate lakes during the summer months. Solar radiation heats up the upper portion of the lake resulting in an epilimnion with lower density water which remains on top of a hypolimnion of colder denser water. All mesocosms were stratified during the course of the experiment with little or no variation in the depth of the stratification (0.5 m from bottom). There was a distinct epilimnion and hypolimnion in all mesocosms with epilimnion temperatures of 22^{o}C (±2) and hypolimnetic temperatures of 10^{o}C (±2). The hypolimnion developed prior to the addition of runoffs.

There was a statistical difference in DO in the epilimnion versus the hypolimnion ($F_{8,36}$=14.5; $p<0.001$) between all treatments. In addition, there was a difference in the temporal (treatment x time) DO distribution over the 32 days of sampling ($F_{4,32}$=5.9; $p<0.001$). As shown in figure 5a, prior to the addition of nutrients into the mesocosms, DO averaged at 20.7 mgL^{-1}. DO remained high due to photosynthesis rather than diffusion as concentrations were well above 100% saturation (8.7 mgL^{-1} at 22^{o}C). DO in the control and N-amended mesocosms continued to decrease over time. For all eighteen the mesocosms that received biosolids amended runoffs, the highest DO values were observed on day 11. Average DO concentrations in the Kitchener mesocosms were at a high of 32.3 mgL^{-1} while Guelph were at 28.8 mgL^{-1}. However, in the mesocosms that received inorganic fertilizers the temporal patterns were different. DO in the low P (23.9 mgL^{-1}), high P (23.6 mgL^{-1}), and N + low P (28.9 mgL^{-1}) treatments continued to increase to a maximum on day 32, while DO concentration peaked at day 4 for the N + high P treatment (29.4 mgL^{-1}).

Interestingly, maximum DO concentrations were reached earlier than maximum chl-a concentrations. This would suggest that the algae were becoming less efficient at fixing carbon on a per biomass basis. Also, oxygen production was more strongly affected by a biosolids

addition than by inorganic nutrients addition. However, chl-a was more strongly affected by inorganic nutrients. This would imply that there is greater efficiency in carbon fixation in the mesocosms receiving biosolids runoff since they are producing more organic carbon per unit chlorophyll.

Hypoxia (<2 mg O_2 L^{-1}) is a widespread phenomenon in the hypolimnion of freshwater systems (Roberts *et al.* 2009). Figure 3.5b shows that hypolimnetic oxygen depletion occurred in all treatments. Oxygen concentrations in the hypolimnion crashed earliest (around day 4) in mesocosms with run-off from biosolids, suggesting a greater subsidy of organic carbon that was being remineralized in the hypolimnion. Inorganic nutrient addition treatments saw oxygen concentrations drop later, presumably as the newly produced algal biomass began to senesce and sink to the hypolimnion. Based on this, it would be predicted that hypolimnetic water would have the highest concentrations of inorganic P in biosolids-amended mesocosms and / or reach high concentrations of inorganic P sooner than inorganic nutrient-amended mesocosms. All other nutrient-amended hypolimnia developed hypoxic conditions around day 18 and remained as such throughout the study. The DO of the hypolimnion in mesocosms that received runoffs from reference soils remained saturated although it slightly decreased over time. This may have been caused by the low organic matter in the runoffs that were added and the slow deposition and subsequent decomposition of organic matter accumulated as a result of slower growth in these mesocosms. Based on chl-a data, these mesocosms were also only slightly mesotrophic and returned to being oligotrophic at the end of the study. In normal oligotrophic lakes, much oxygen is not consumed in the hypolimnion and it may actually be saturated although thermally stratified (Wetzel 2001).

Both large and small stratified lakes can be affected by hypoxia. Over the past two centuries, humans have altered the landscape around Lake Simcoe and P input in the lake has drastically increased due to anthropogenic sources resulting in eutrophication (Ontario Ministry of the Environment 2010). As of present, the oxygen concentration at the bottom of Lake Simcoe is too low to sustain the lakes coldwater fishes (Ontario Ministry of the Environment 2010). By volume, Lake Erie is the 11th largest freshwater lake in the world that experiences hypoxia every late summer which has been worsened by cultural eutrophication (Burns *et al.* 2005; Roberts *et al.* 2009). Roberts *et al.* (2009) examined the impacts of a hypoxic hypolimnion in Lake Erie on yellow perch foraging behaviour. They concluded that hypolimnetic hypoxia affect the spatial distributions of the perch which is a benthivorous and demersal species and in turn affect their foraging behaviour. Furthermore, Petrosky and Magnuson (1973) shown in laboratory experiments that hypoxia is lethal to bluegill after exposing them to oxygen concentrations ranging from 4.0 to 0.25 mgL^{-1} O_2. Similarly, Young *et al.* (2011) has mentioned that increase TP input from agriculture and sewage treatment plants has placed huge stress on the DO in Lake Simcoe, ON resulting in a hypoxic hypolimnion which is lethal to many cold water fish species such as lake trout, whitefish and lake herring (Evans *et al.* 1996). Kolar and Rahel (1993) investigated the tolerance of different invertebrate communities to hypoxia and predation and found that hypoxia alters benthic community composition mainly through direct mortality and also by increased vulnerability to predation when these organisms try to swim upwards into oxic environments.

Epilimnetic pH steadily increased in all mesocosms except the blank and N addition (figure 3.5 c). pH is controlled by the CO_2-HCO_3^--CO_2^{3-} buffering system. An increase of pH was due primarily to the high photosynthetic rate of the algae and cyanobacteria in the mesocosms. CO_2

was rapidly being utilized during photosynthesis and subsequent C fixation thus pushing the pH up. Due to insufficient nutrients in the blank and N addition mesocosms, photosynthesis was lower when compared to the other mesocosms thus resulting in an actual decrease in alkalinity in the epilimnion. As expected, pH dropped in the hypolimnion due to the deposition and subsequent microbial decomposition of the organic matter and the releasing of organic acids (figure 3.5d). However, after a consistent decrease in the pH of the hypolimnion for biosolids mesocosms, it increased from day 18 to 32. This was probably due to the denitrification of nitrates to nitrogen gas or the reduction of manganese and iron which will result in a net increase in pH in these systems (Dillon *et al.* 1997). Since a drop of pH is indicative of decomposition processes, one would expect a corresponding release of nutrients such as orthophosphates into the hypolimnion.

3.4.3 SRP and TP in the Mesocosms

a)

b)

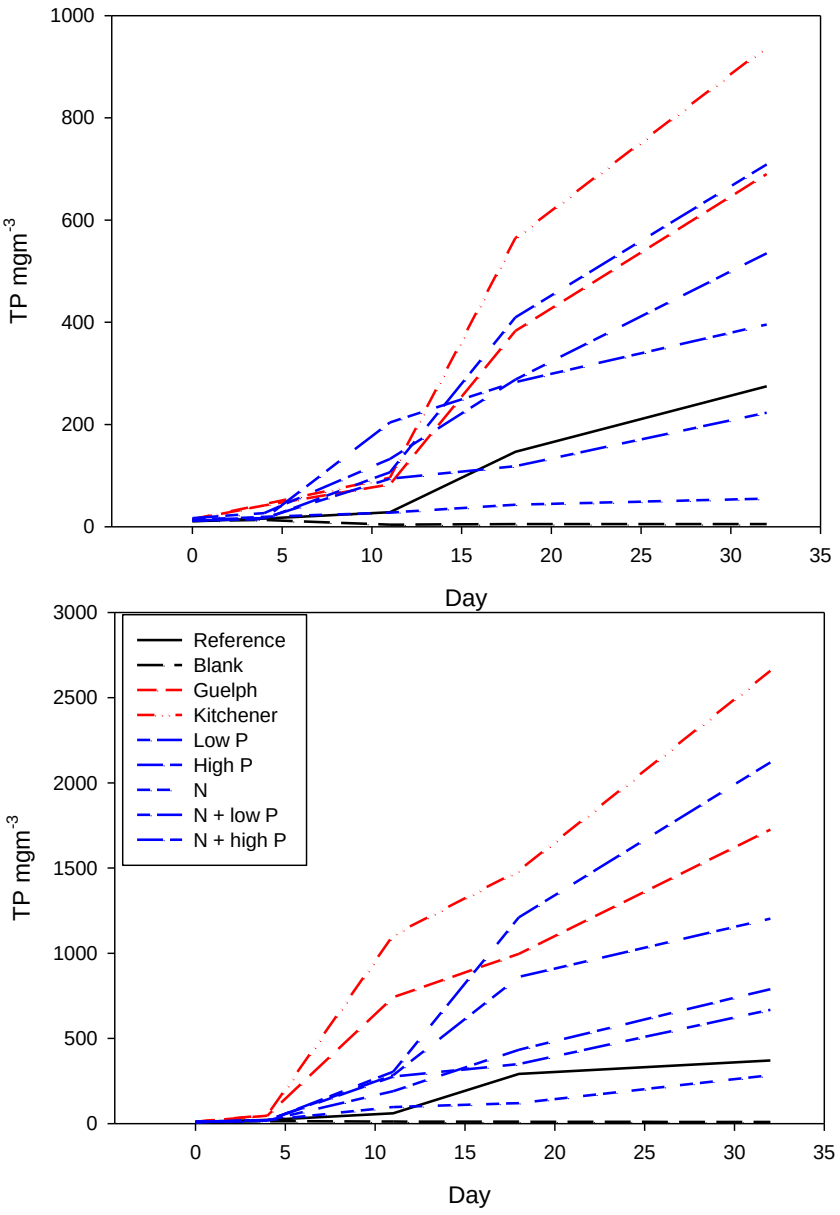


Figure 3.6 Total P in mesocosms over 32 days. a) TP in epilimnion, b) TP in hypolimnion.

No SRP was identified in the epilimnion of any of the mesocosms throughout the study. However, as predicted, SRP was found in the hypolimnion of the nutrient amended mesocosms almost two weeks after initial nutrient addition. Average SRP was highest in the Kitchener

mesocosms with 27 μgL^{-1} and followed closely by the high N + high P nutrient mesocosms with 24 μgL^{-1}. Guelph mesocosms averaged at 13 μgL^{-1}. The generation of SRP in the hypolimnion is a direct result of mineralization of organic matter that has accumulated from the addition of runoffs to the mesocosms and from the death and subsequent deposition of planktonic species in the epilimnion. Regenerating SRP is expected and consistent with other studies such as Özkundakci *et al.* (2011) who showed that in small eutrophic lakes with an anoxic hypolimnion, changes in P in the hypolimnion was affected by processes such as mineralisation, nutrient uptake, nitrification, adsorption/desorption and diffusion and these processes may influence as much as 48% of P hypolimnetic fluxes. Amirbahman *et al.* (2003) have shown that in eleven high-P lakes in Maine, USA, it is the reduction of ferric hydroxide in the sediments during summer stratification that directly control the hypolimnetic release of P. However, nitrate ions were present in these mesocosms and therefore would have inhibited P release via reduction of ferric hydroxide since it's a preferential electron acceptor when compared to Fe^{3+} (Wauer *et al.* 2005). Furthermore, any nitrate that enters a lake will directly increase the oxidizing capacity of the lake (Hemond and Lin 2010) and will therefore affect the mechanism of P release. Thus, most of P release may have actually originated from bacterial mineralization of organic matter especially since there was a corresponding increase of TOC in the hypolimnion (Denis Matiichine, MSc. En. Sci. Man., thesis results).

With the exception of the blank columns, TP consistently increased in the epilimnion and hypolimnion over the studied period. There was a statistical difference in TP in the epilimnion versus the hypolimnion ($F_{8,36} = 27.6$; $p < 0.001$) between all treatments. In addition, there was a difference in the temporal TP distribution over the 32 days of sampling ($F_{4,32} = 23.8$; $p < 0.001$). Average initial TP for all mesocosms was 11.4 μgL^{-1}. However, at day 32, TP values were

almost 1 mgL^{-1} in the epilimnion of the Kitchener mesocosms. Mesocosms receiving N + high P were next with 710 μgL^{-1} and these was followed by the Guelph mesocosms with 690 μgL^{-1}. The lowest TP (55 μgL^{-1}) values were recorded for the N-only mesocosms. On the basis of total P concentrations, these systems would be expected to function as hypereutrophic systems (>100 µg P L^{-1}) while a mesotrophic system has <30 μgL^{-1} TP. However, what is interesting is that despite the high levels of total P in these systems, those receiving biosolids did not function as hypereutrophic systems based on chlorophyll a content. Only those mesocosms receiving the equivalent amount of N and P in an inorganic form responded by functioning as hypereutrophic systems.

With the exception of the blank mesocosms which remained fairly steady, an increase of hypolimnetic TP occurred in all treatments. In addition, TP in the hypolimnion was significantly higher than TP in the epilimnion ($F_{1,8}$=8.4; $p<0.001$). At day 32, average TP value was 2650 μgL^{-1} in the hypolimnion of the Kitchener mesocosms. Mesocosms receiving N + high P were next with 2120 μgL^{-1} and these were followed by the Guelph mesocosms with 1725 μgL^{-1}TP. Although these values are environmentally significant and point to eutrophication potential, they are not at all unrealistic when compared to the TP and trophic state of many natural lakes, reservoirs and agricultural streams worldwide. Water quality monitoring of the Hartbeespoort Dam in South Africa showed that TP in the upper 5 m of the water column reached an all time high of 10530 μgL^{-1} in 1993 (van Ginkel and Silberbauer 2007). Morgan *et al.* (2006) investigated the relationship between nutrients, chl-a and DO in agricultural streams in Illinois and found that TP was as high as 2750 μgL^{-1} in Salt Fork of the Vermillion river in 2004 (average 630 μgL^{-1}). Maximum values of 30 shallow Canadian prairie lakes during the summers of 1998-2004 reached 937 μgL^{-1} TP (average 79 μgL^{-1}) while maximum TP for 222 Danish

lakes during the same time reached 930 μgL^{-1} TP (average 67 μgL^{-1}) (Jackson and Jeppesen 2007). Environment Canada (2010) stated that the concentrations of TP and TDP in rivers and the Great Lakes of Canada between 2004 to 2006 ranged between <0.5 and 1880 μgL^{-1} for TP while those for TDP varied from <0.5 to 1600 μgL^{-1}. In addition, the report showed that half of the sites that were sampled showed an increase in TP (8 out of 75 total sampled) resulting in a change in the trophic status of the water bodies. This being noted, although TP is routinely used as a trophic nutrient parameter, it may not really be. A more accurate P parameter should be BAP and not TP since growth actually depends on the available nutrient and not what is inaccessible.

3.5 Conclusion

Application of biosolids onto agricultural fields is highly regulated and is directly implemented and monitored by officials at all levels of government. Organic matter and a number of macro- and micronutrients are provided to the soil and crops through biosolids land application. However, its nutrient content is not in the ratio of plant needs. As of present, the biosolids application rate is determined by the N agronomic needs of crops which results in over application and build up of P over time since biosolids have a low N: P. In this study, the addition of biosolids led to at least a threefold increase in the TP in soils. Soils with high P levels are more prone to P loss via erosion and leaching. Also, the bioavailable and potentially available P pools in the soil were impacted most by biosolids application. During this study, the worst case scenario was mimicked as the soil had no crop over, had maximum allowed slope, four extreme storm events in a little over three weeks, and the runoff and leachate collected were directly added to water columns at a rate of 10% v/v. In addition, no grazers were added to mesocosms to modulate phytoplankton responses to nutrient loading.

The results from the study support the first hypothesis that biosolids will increase nutrient loss. it was shown that P content of soils significantly increased after biosolids application and also Runoffs from biosolilds-amended soils had significantly higher P than runoffs collected from the reference soil. While more TP was removed in the surface runoffs, BAP remained fairly consistent when compared to that in tile drains. P loss from heavy rainfall (and snowmelts) is a major challenge facing P management of biosolids-amended soils. Offsite migration to receiving aquatic bodies is a major concern since P is the limiting nutrient in most freshwater systems.

The results from the study support the first hypothesis that nutrient input from the surface run-off and tile water will increase algal blooms in the mesocosms. Nutrient input into aquatic systems has a dramatic effect on their trophic status. This study has shown that irrespective of the nutrient source – organic versus inorganic – excessive nutrients will stimulate primary productivity and subsequently alter the trophic state of the receiving body. Although the mesocosms receiving biosolids did go into eutrophic states during the study, at the end all systems returned to upper mesotrophic-lower eutrophic states. However, mesocosms receiving high inorganic nutrients that were the analog of Kitchener runoff remained eutrophic until the end.

The results also supported the second hypothesis that the amount of bioavailable P entering a mesocosm will highly correlate with biological parameters of the mesocosm. Chl-a increased with BAP and not with TP. This makes sense since not all the phosphorus which is present can be directly accessed by phytoplankton.

The third hypothesis was also found to be true. The impact of the runoff may vary depending on its source. Kitchener biosolids had more readily available P when compared to Guelph biosolids. This difference was translated into greater primary productivity in Kitchener mesocosms when

compared to Guelph mesocosms. Also, runoffs from biosolids-amended plots had higher organic carbon when compared to unamended soils. This not only affected the biological parameters but also physical parameters such as the dissolved oxygen levels and pH of the mesocosms. In this study, hypolimnetic hypoxia was observed as early as four days after the addition of runoffs from the biosolids-amended soils. This has far reaching consequences especially in stratified eutrophic lakes where oxygen is already low in the hypolimnion. By extension, fishes and other invertebrates that dwell in the hypolimnion will be seriously affected in anoxic conditions.

This study simulated summer stratification in a small lake. Consequently, the products of the decomposition of organic matter remained temporarily trapped in the hypolimnion of the mesocosms. During this time, there was a continuous build up of BAP (and other nutrients). However, in natural waters, during turn-over in spring, stratification ceases and the once trapped nutrients in the hypolimnion become freely available for algal uptake. Thus, the lakes that did not become hypereutrophic or remained eutrophic during the summer may after all become so in spring. This is a potential follow up study where the impact of lake turn-over on lake trophic status can be investigated.

Sustainable land application of biosolids will only occur when the criteria for land application are not based solely on the N agronomic needs of crops and pathogen levels. TP and BAP of the biosolids and the land P sorption capacity have to be included as relevant land application criteria. Such criteria can be used to formulate a more environmentally acceptable biosolids application index which will determine maximum sustainable land application rates. This approach may mitigate P loss from croplands and its subsequent transport to receiving aquatic bodies. This is the only way forward for sustainable land application. The alternative would be to continue mining commercial phosphor that utilizes intensive inputs of fossil fuels and result in

environmental degradation and habitat destruction at the mining site and of course when it is land applied, it ultimately ends up in rivers and lakes to do continuous harm.

In addition, this study has shown that TP levels in streams and lakes may not after all be truly reflective of their eutrophication potential. High TP values do not always translate into high primary productivity. TP is not readily accessible to algae. Consequently, P abatement strategies have to include BAP as an important criterion in assessing eutrophication potential. Furthermore, heavy emphasis has to be placed on P abatement in streams and lakes in order to reduce algal blooms. Control P and eutrophication can be greatly reduced. This being suggested, there are however, some inland aquatic bodies which are N limiting.

Lakes and their ecosystems have to be protected. For too long many have thought that the trophic status of lakes is not important. We need lakes for water, fishing and recreational activities. But how many really think about the harms we are doing to the actual lake ecosystem. When our needs are affected, only then do we become reactive. We have to shift from being reactive to being proactive. Proactiveness can only occur when all stakeholders understand their role in protecting, maintaining and restoring lakes to their former states. This underscores the huge role of educating farmers and the general public on the importance of having and maintaining healthy lakes.

Appendices

Appendix A: Determining Application Rates for Biosolids (Wet Mass)

Soil Surface area of each trough = 36 cm x 100 cm = 0.36 m^2

Application Rate = 8 dry ton per hectare = 8000 Dry kg / l x 10000 m^2

Let x represent the amount of dry mass biosolids to be land-applied to the trough:

8000 kg(Dry)/ 10000 m^2 = x kg(Dry) / 0.36 m^2

x = 0.288kg (Dry) of biosolids to be applied to each trough

1. Guelph Biosolids average dry weight was 3.09% of its wet weight (i.e. 30.9 g dry weight / kg wet weight biosolids)

   To provide 288 g dry weight, we need X g wet weight

   30.9 g dry weight: 1000 g wet weight = 288 g dry weight: X g wet weight

   X = 9320 g or 9.32 kg wet weight of Guelph biosolids were applied

2. Kitchener Biosolids average dry weight was 1.47% of its wet weight (i.e. 14.7 g dry weight / kg wet weight biosolids)

   To provide 288 g dry weight, we need X g wet weight

   14.7 g dry weight: 1000 g wet weight = 288 g dry weight: X g wet weight

   X = 19591.5 g or 19.56 kg wet weight of Kitchener biosolids were applied

Appendix B: Simulated Rainfall Quantity Calculations

"Multi-annual extreme storm event for South Ontario" = 49.5mm of rain

49.5mm = 4.95cm

Area of each trough = 3600 cm^2

Amount of water to be poured on the trough 4.95 cm x 36 cm x 100 cm = 17820 cm^3

Let X represent the amount of water per trough in Liters

1L has 1000 cm^3,

X = 17820 cm^3/1000 cm^3 L^{-1} = 17.82 L

Appendix C: Preparation of Growth Media and Culturing Phytoplanktons

Preparation of Working Stock Nutrient Solutions for *S. capricornutum* Liquid Growth Medium (Environment Canada, 1992)

Algae were grown at Ryerson using the protocols of Environment Canada (1992). The growth medium for the stock algal culture is made from five stock nutrient solutions and added to deionised water. The stock solutions were prepared in volumetric flasks using reagent grade chemicals (outlined in table 1) and deionised water. These stock solutions are ten times the final concentration of the algal growth medium. Stock solutions were then autoclaved and thereafter stored in the refrigerator.

To prepare the liquid growth medium for the stock algal cultures, 1 mL of each stock solution was aseptically transferred into a sterile Erlenmeyer flask containing about 50 mL deionised water. A volume-to-flask ratio of 20% for the growth medium was used to prevent growth inhibition due to carbon dioxide limitation. Two mL of the starter algal culture was aseptically transferred using a disposable sterile pipette into the 250 ml Erlenmeyer flask.

Parameters used for optimum growth are shown in table 2.

Table 1 Stock Nutrient Solution Compound Quantity per 500 mL of Deionised Water for algal culture medium.

Stock Solution	Compound	Amounts / 500 mL of Deionised Water	Quantity to add to make up 50 mL culture solution/mL
1	$NaNO_3$	12.75 g	1
2	$MgCl_2.6H_2O$	5.0 g	1
	$CaCl_2.2H_2O$	2.21 g	
	H_3BO_3	92.76 mg	
	$MnCl_2.4H_2O$	207.81 mg	
	$ZnCl_2$	1.64 mg[a]	
	$CoCl_2.6H_2O$	0.714 mg[b]	
	$CuCl_2.2H_2O$	0.006 mg[c]	
	$Na_2MoO_4.2H_2O$	3.63 mg[d]	
	$FeCl_3.6H_2O$	80.0 mg	
	$Na_2EDTA.2H_2O$	150.0 mg	
3	3 $MgSO_4.7H_2O$	7.35 g	1
4	4 K_2HPO_4	0.522 g	1
5	5 $NaHCO_3$	7.5 g	1

[a] Weigh out 164 mg of $ZnCl_2$ and dilute to 100 mL. Add 1 mL of this solution to Stock Nutrient Solution 2.

[b] Weigh out 71.4 mg of $CoCl_2.6H_2O$ and dilute to 100 mL. Add 1 mL of this solution to Stock Solution 2.

[c] Weigh out 60.0 mg of $CuCl_2.2H_2O$ and dilute to 1000 mL. Dilute 1 mL of this solution to 10 mL. Add 1 mL of this second solution to Stock Nutrient Solution 2.

[d] Weigh out 366 mg of $Na_2MoO_4.2H_2O$ and dilute to 100 mL. Add 1 mL of this solution to Stock Nutrient Solution 2.

Table 2 Parameters and ranges used to culture phytoplanktons

Parameters	Ranges
Temperature (°C)	24 ± 2
Light intensity (lux)	4000 (cool white fluorescent light)
Photoperiod (light: dark, hours)	16:8
pH	7.5
Shaking	100 rpm on a continuous shaker

Modified CHU 10 Medium

For culturing cyanobacteria and diatoms, the modified Chu-10 growth medium was used as outlined by Stein (1973).

The following stock solutions (table 3) were added to approximately 900 mL of deionised water and then the total volume was brought up to 1000 ml with deionised water. The pH was adjusted to 6.4 for diatoms or to 8.5 for cyanobacteria and the media was then autoclaved. The media was only inoculated after cooling to room temperature. Growth parameters are mentioned in table 2.

Table 3 Stock solutions for modified Chu-10 growth medium.

Stock Solution	g / 500 ml Deionised water	Quantity/ ml
$Ca(NO_3)_2 .4H_2O$	2.61 g	10
K_2HPO_4	0.5 g	10
$MgSO_4 . 7H_2O$	1.25 g	10
Na_2CO_3	1 g	10
Na_2SiO_3	1.25 g	10
Fe-citrate	0.15 g	10
citric acid	0.15 g	10
Trace metal	See table 4 below	1

Table 4 Trace metal stock solution for modified Chu-10.

Component	Primary Stock Solution / 1 L deionised water	Quantity
$FeCl_3 \cdot 6H_2O$	---	3.15 g
$Na_2EDTA \cdot 2H_2O$	---	4.36 g
$CuSO_4 \cdot 5H_2O$	9.8 g	1 mL
$Na_2MoO_4 \cdot 2H_2O$	6.3 g	1 mL
$ZnSO4 \cdot 7H_2O$	22.0 g	1 mL
$CoCl_2 \cdot 6H_2O$	10.0 g	1 mL
$MnCl_2 \cdot 4H_2O$	180.0 g	1 mL

Appendix D: Iron oxide paper

Preparation of Iron Oxide Paper and Testing for Bioavailable P

The iron oxide paper was prepared following the procedures of Myers et al. (1997) and Chardon et al. (1997) as outlined in Zhang and Kovar (2008b). Stiff 5.5 cm circles of Whatman no. 50 filter paper was used for making the FeO paper. The filter paper was submerged in acidified 0.65 *M* FeCl3 ·6H2O that contained 50 mL of concentrated HCl per litre of solution. The paper was left in the container overnight. On removal, the paper was air-dried on a rack and then immersed in 2.7 *M* NH4OH for 30 s and then drained for 15 s before completely rinsing in two buckets of deionised water. It is then placed in a third container and left for 1 h to permit dissipation of any remaining ammonia.

The paper is now ready for immediate use or dried for later use.

Appendix E: Kjeldahl Digestion reagent

Semi-micro-Kjeldahl Digestion reagent:

134 g K_2SO_4 and 7.3 g $CuSO_4$ were dissolved in about 800 mL water. Then, 134 mL concentrated H_2SO_4 was carefully added. When it has cooled to room temperature, the solution was diluted to 1 L with Millipore water and thoroughly mixed. Solution was kept at a temperature close to 20°C to prevent crystallization.

Appendix F: Total P in reference and biosolids amended soils

Kjeldahl and Aqua regia digestions

	Spectrophotometric readings at 880 nm				mg/kg P before dilution				Actual P present mg/kg soil			
Kjeldahl Digestion	Day 0	Day 30	Day 60	Day 120	Day 0	Day 30	Day 60	Day 120	Day 0	Day 30	Day 60	Day 120
Reference 1	0.200	0.190	0.181	0.151	0.374	0.357	0.342	0.288	94	89	86	72
Reference 2	0.190	0.141	0.130	0.160	0.357	0.271	0.251	0.305	89	68	63	76
Reference 3	0.203	0.170	0.161	0.160	0.380	0.323	0.307	0.304	95	81	77	76
Kitchener 1	0.242	0.225	0.190	0.181	0.450	0.419	0.357	0.342	281	262	223	214
Kitchener 2	0.280	0.217	0.210	0.165	0.517	0.406	0.393	0.312	323	254	246	195
Kitchener 3	0.265	0.198	0.177	0.189	0.490	0.371	0.334	0.356	306	232	209	223
Guelph 1	0.267	0.440	0.444	0.426	0.493	0.800	0.807	0.775	308	250	252	242
Guelph 2	0.258	0.464	0.393	0.389	0.478	0.842	0.716	0.710	299	263	224	222
Guelph 3	0.260	0.485	0.414	0.410	0.481	0.879	0.753	0.746	301	275	235	233
Aqua regia Digestion												
Reference 1	0.148	0.121	0.131	0.111	0.176	0.132	0.149	0.117	88	66	75	58
Reference 2	0.155	0.122	0.122	0.130	0.187	0.135	0.134	0.148	94	68	67	74
Reference 3	0.153	0.154	0.141	0.141	0.184	0.186	0.165	0.164	92	93	83	82
Kitchener 1	0.326	0.282	0.270	0.255	0.460	0.390	0.371	0.346	288	244	232	217
Kitchener 2	0.348	0.298	0.249	0.245	0.495	0.416	0.338	0.331	310	260	211	207
Kitchener 3	0.340	0.275	0.267	0.249	0.483	0.380	0.366	0.338	302	237	229	211
Guelph 1	0.332	0.548	0.244	0.237	0.470	0.816	0.329	0.318	294	255	206	199
Guelph 2	0.350	0.563	0.293	0.291	0.499	0.840	0.407	0.405	312	263	254	253
Guelph 3	0.343	0.573	0.284	0.275	0.487	0.855	0.393	0.379	304	267	246	237

Mean and *SD* of Total P in reference and biosolids amended soils

Treatment	Kjeldahl Digestion				Aqua regia Digestion			
	Day 0	Day 30	Day 60	Day 120	Day 0	Day 30	Day 60	Day 120
Reference	93	79	75	75	91	76	75	71
	3	*11*	*11*	*2*	*3*	*15*	*8*	*12*
Kitchener	304	249	226	211	300	247	224	212
	21	*15*	*19*	*14*	*11*	*12*	*11*	*5*
Guelph	303	263	237	232	303	262	235	230
	5	*12*	*14*	*10*	*9*	*6*	*26*	*28*

Appendix G: P fractions in biosolids

	Spectrophotometric readings at 880 nm						P before dilution (ppm)						Actual IP present mg P kg^{-1} soil					
IP fractions	DW	NH_4Cl	NH_4F	NaOH	BCD	H_2SO_4	DW	NH_4Cl	NH_4F	NaOH	BCD	H_2SO_4	DW	NH_4Cl	NH_4F	NaOH	BCD	H_2SO_4
Kitchener 1	0.108	0.3943	0.0817	0.2876	0.113	0.3419	0.156	0.630	0.113	0.453	0.165	0.543	1563	6299	1128	9068	3291	2716
Kitchener 2	0.102	0.3642	0.0877	0.2536	0.105	0.359	0.146	0.580	0.123	0.397	0.151	0.571	1463	5801	1227	7943	3026	2857
Kitchener 3	0.0883	0.4395	0.0788	0.2964	0.133	0.3644	0.124	0.705	0.108	0.468	0.198	0.580	1237	7047	1080	9359	3952	2902
Guelph 1	0.105	0.1857	0.3787	0.3496	0.148	0.2343	0.151	0.285	0.604	0.556	0.222	0.365	757	2848	3020	11119	4449	3652
Guelph 2	0.1336	0.1825	0.3331	0.3902	0.104	0.2124	0.199	0.280	0.529	0.623	0.150	0.329	993	2795	2643	12462	2993	3290
Guelph 3	0.1245	0.2431	0.3519	0.3173	0.136	0.2218	0.184	0.380	0.560	0.503	0.203	0.345	918	3798	2799	10050	4052	3445

Treatments	Spectrophotometric readings at 880 nm							Actual OP present mg P kg^{-1} soil					
OP fractions	$NaHCO_3$		HCl		NaOH		H_2SO_4	Labile	Moderately labile		Non-labile		
	Pi	P(T)	Pi	P(T)	P(acidified)	P (not acidified)	Ashing	$NaHCO_3$	HCl	Fulvic acid	Humic acid	Ashing	Total P_o
Kitchener 1	0.2688	0.4095	0.2574	0.3769	0.5913	0.1753	0.1547	1124	954	784	91	1137	4090
Kitchener 2	0.309	0.4215	0.4421	0.5527	0.5867	0.1769	0.1254	899	883	818	132	845	3577
Kitchener 3	0.319	0.4365	0.4478	0.5519	0.5981	0.1649	0.1359	938	831	870	139	949	3728
Guelph 1	0.2803	0.3917	0.5211	0.6018	0.6315	0.1937	0.1632	890	645	1412	194	1222	4363
Guelph 2	0.2953	0.3879	0.5151	0.6104	0.6284	0.1933	0.1843	740	761	1495	233	1433	4662
Guelph 3	0.3169	0.4012	0.4807	0.5915	0.6138	0.1904	0.1473	673	885	1468	71	1063	4161

Appendix H: P fractions in reference and biosolids amended soils

Inorganic fractions

	Spectrophotometric readings at 880 nm						P before dilution (ppm)						Actual IP present mg P kg^{-1} soil					
IP fractions	DW	NH_4Cl	NH_4F	NaOH	BCD	H_2SO_4	DW	NH_4Cl	NH_4F	NaOH	BCD	H_2SO_4	DW	NH_4Cl	NH_4F	NaOH	BCD	H_2SO_4
Day 0																		
Reference 1	0.1492	0.18	0.1214	0.1486	0.1253	0.2899	0.171	0.220	0.127	0.170	0.133	0.395	9	11	6	17	13	20
Reference 2	0.1208	0.1731	0.1121	0.1224	0.1306	0.2958	0.126	0.209	0.112	0.128	0.141	0.404	6	10	6	13	14	20
Reference 3	0.1457	0.1653	0.1112	0.1366	0.1509	0.2702	0.165	0.196	0.110	0.151	0.174	0.363	8	10	6	15	17	18
Kitchener 1	0.3579	0.5064	0.1375	0.2542	0.1613	0.1732	0.503	0.739	0.152	0.338	0.190	0.209	25	37	38	85	19	21
Kitchener 2	0.3117	0.4281	0.1635	0.2494	0.1441	0.1876	0.429	0.615	0.194	0.330	0.163	0.232	22	31	48	83	16	23
Kitchener 3	0.3655	0.4472	0.1543	0.2549	0.1467	0.1924	0.515	0.645	0.179	0.339	0.167	0.240	26	32	45	85	17	24
Guelph 1	0.4163	0.3166	0.1373	0.2786	0.1511	0.2343	0.596	0.437	0.152	0.377	0.174	0.306	30	22	38	94	17	31
Guelph 2	0.2846	0.3265	0.1239	0.2895	0.1366	0.2588	0.386	0.453	0.131	0.394	0.151	0.345	19	23	33	99	15	35
Guelph 3	0.3081	0.3051	0.1492	0.2641	0.1575	0.1926	0.424	0.419	0.171	0.354	0.184	0.240	21	21	43	88	18	24
Day 30																		
Reference 1	0.1014	0.1274	0.1326	0.1396	0.1475	0.2563	0.095	0.136	0.144	0.156	0.168	0.341	5	7	7	16	17	17
Reference 2	0.0947	0.1775	0.1248	0.1362	0.1436	0.241	0.084	0.216	0.132	0.150	0.162	0.317	4	11	7	15	16	16
Reference 3	0.0866	0.0978	0.1237	0.1238	0.1466	0.2278	0.071	0.089	0.130	0.130	0.167	0.296	4	4	7	13	17	15
Kitchener 1	0.2537	0.2124	0.165	0.3756	0.1286	0.2376	0.337	0.271	0.196	0.531	0.138	0.312	17	14	49	53	14	16
Kitchener 2	0.2217	0.2373	0.1842	0.2764	0.1465	0.2613	0.286	0.311	0.227	0.373	0.167	0.349	14	16	57	93	17	18
Kitchener 3	0.2185	0.2326	0.1605	0.2698	0.1213	0.2335	0.281	0.304	0.189	0.363	0.126	0.305	14	15	47	91	13	15
Guelph 1	0.2464	0.2631	0.145	0.5621	0.1337	0.3274	0.326	0.352	0.164	0.828	0.146	0.454	16	18	41	83	15	23
Guelph 2	0.2245	0.2714	0.1846	0.5519	0.1362	0.4804	0.291	0.365	0.227	0.812	0.150	0.698	15	18	57	81	15	35
Guelph 3	0.2158	0.2187	0.149	0.5051	0.1363	0.3042	0.277	0.281	0.170	0.737	0.150	0.418	14	14	43	74	15	21

Pi fractions in reference and biosolids amended soils (cont.)

	Spectrophotometric readings at 880 nm						P before dilution (ppm)						Actual IP present mg P kg^{-1} soil					
IP fractions	DW	NH_4Cl	NH_4F	NaOH	BCD	H_2SO_4	DW	NH_4Cl	NH_4F	NaOH	BCD	H_2SO_4	DW	NH_4Cl	NH_4F	NaOH	BCD	H_2SO_4
Day 60																		
Reference 1	0.1065	0.1514	0.1311	0.1354	0.1431	0.2515	0.103	0.174	0.142	0.149	0.161	0.334	5	9	7	15	16	17
Reference 2	0.1238	0.0632	0.1214	0.1242	0.1512	0.2439	0.130	0.034	0.127	0.131	0.174	0.322	7	2	6	13	17	16
Reference 3	0.0697	0.1074	0.1401	0.1421	0.1637	0.2007	0.044	0.104	0.156	0.160	0.194	0.253	2	5	8	16	19	13
Kitchener 1	0.2062	0.1689	0.1761	0.3399	0.1595	0.2992	0.262	0.202	0.214	0.474	0.187	0.410	13	10	53	59	12	21
Kitchener 2	0.2379	0.1324	0.1978	0.3412	0.1371	0.2675	0.312	0.144	0.248	0.476	0.152	0.359	16	14	62	60	10	18
Kitchener 3	0.2428	0.1738	0.1601	0.3821	0.1639	0.2814	0.320	0.210	0.188	0.542	0.194	0.381	16	11	47	68	12	19
Guelph 1	0.2237	0.1504	0.5738	0.5143	0.1604	0.3016	0.289	0.173	0.847	0.752	0.189	0.413	15	17	42	75	12	21
Guelph 2	0.1957	0.1997	0.6214	0.542	0.1338	0.3114	0.245	0.251	0.922	0.796	0.146	0.429	12	13	46	80	9	22
Guelph 3	0.1019	0.2013	0.6197	0.5391	0.1797	0.3207	0.096	0.254	0.920	0.791	0.219	0.444	5	13	46	79	14	22
Day 120																		
Reference 1	0.0408	0.0906	0.116	0.092	0.1516	0.2003	0.049	0.134	0.177	0.136	0.238	0.320	5	7	9	14	24	16
Reference 2	0.0546	0.0578	0.0827	0.0813	0.0529	0.1857	0.073	0.078	0.120	0.118	0.070	0.295	7	4	6	12	7	15
Reference 3	0.0313	0.0887	0.106	0.1049	0.0751	0.1706	0.033	0.131	0.160	0.158	0.108	0.270	3	7	8	16	11	14
Kitchener 1	0.1045	0.1927	0.1693	0.1931	0.0433	0.1755	0.157	0.307	0.268	0.308	0.054	0.278	8	15	67	62	8	14
Kitchener 2	0.1856	0.1024	0.1235	0.2191	0.0604	0.204	0.295	0.154	0.190	0.352	0.083	0.327	15	8	47	70	12	16
Kitchener 3	0.1392	0.1786	0.1352	0.1966	0.0529	0.215	0.216	0.283	0.210	0.314	0.070	0.345	11	14	52	63	11	17
Guelph 1	0.0883	0.1703	0.121	0.4763	0.0494	0.2662	0.130	0.269	0.186	0.789	0.064	0.432	7	14	46	79	10	22
Guelph 2	0.1086	0.1059	0.1377	0.4669	0.0531	0.2233	0.164	0.160	0.214	0.773	0.070	0.359	8	8	54	77	11	18
Guelph 3	0.1305	0.1511	0.1125	0.4818	0.0562	0.2819	0.202	0.237	0.171	0.799	0.075	0.459	10	12	43	80	11	23

Mean and *SD* of Inorganic P fractions in reference and biosolids amended soils

	Day 0							Day 30						
Treatment	DW	NH_4Cl	NH_4F	NaOH	BCD	H_2SO_4	Total	DW	NH_4Cl	NH_4F	NaOH	BCD	H_2SO_4	Total
Reference	8	10	6	15	15	19	73	4	7	7	15	17	16	65
	1	*1*	*0*	*2*	*2*	*1*	*3*	*1*	*3*	*0*	*1*	*0*	*1*	*5*
Kitchener	24	33	44	84	17	23	225	15	11	37	53	16	16	145
	2	*3*	*5*	*1*	*2*	*2*	*3*	*2*	*6*	*27*	*40*	*2*	*1*	*79*
Guelph	23	22	38	94	17	30	223	15	17	47	79	15	26	199
	6	*1*	*5*	*5*	*2*	*5*	*8*	*1*	*2*	*9*	*5*	*0*	*8*	*21*

	Day 60							Day 120						
Treatment	DW	NH_4Cl	NH_4F	NaOH	BCD	H_2SO_4	Total	DW	NH_4Cl	NH_4F	NaOH	BCD	H_2SO_4	Total
Reference	5	5	7	15	18	15	64	5	6	8	14	14	15	61
	2	*4*	*1*	*1*	*2*	*2*	*4*	*2*	*2*	*2*	*2*	*9*	*1*	*12*
Kitchener	15	12	54	62	11	19	173	11	12	56	65	10	16	170
	2	*2*	*8*	*5*	*1*	*1*	*6*	*4*	*4*	*10*	*5*	*2*	*2*	*3*
Guelph	11	14	45	78	12	21	181	11	12	49	71	11	18	171
	5	*3*	*2*	*2*	*2*	*1*	*2*	*2*	*3*	*5*	*1*	*1*	*3*	*2*

Organic fractions in reference and biosolids amended soils

Treatments	Spectrophotometric readings at 880 nm							Actual OP present mg P kg^{-1} soil					
OP fractions	$NaHCO_3$		HCl		NaOH		H_2SO_4	Labile	Moderately labile		Non-labile		
	Pi	P(T)	Pi	P(T)	P(acidified)	P(T: not acidified)	Ashing	$NaHCO_3$	HCl	Fulvic acid	Humic acid	Ashing	Total P_o
Day 0													
Reference 1	0.101	0.1104	0.002	0.005	0.0135	0.0169	0.038	1	1	2	0	4	7
Reference 2	0.097	0.1095	0.001	0.0024	0.0139	0.0187	0.041	1	0	2	0	4	8
Reference 3	0.093	0.0992	0.001	0.0039	0.0143	0.0156	0.042	1	0	2	0	5	8
Kitchener 1	0.125	0.2132	0.133	0.2416	0.2016	0.2549	0.027	8	9	18	5	3	43
Kitchener 2	0.118	0.2291	0.138	0.2318	0.2104	0.2547	0.029	10	8	19	4	3	44
Kitchener 3	0.1316	0.2414	0.137	0.2089	0.2004	0.2416	0.028	10	6	18	4	3	41
Guelph 1	0.11	0.2192	0.398	0.4736	0.3016	0.3624	0.025	10	7	27	5	3	51
Guelph 2	0.115	0.2243	0.394	0.4737	0.2901	0.3384	0.024	10	7	26	4	3	50
Guelph 3	0.113	0.2215	0.387	0.4711	0.2703	0.3109	0.026	9	7	24	4	3	48
Day 30													
Reference 1	0.0992	0.1076	0.0031	0.0036	0.0153	0.0168	0.027	1	0	2	0	3	6
Reference 2	0.096	0.1016	0.0016	0.0027	0.0143	0.0159	0.031	1	0	2	0	4	7
Reference 3	0.0995	0.1016	0.0015	0.0031	0.0149	0.0168	0.033	0	0	2	0	4	6
Kitchener 1	0.1246	0.2131	0.1314	0.1794	0.2019	0.2486	0.025	8	4	18	4	3	37
Kitchener 2	0.1216	0.2394	0.1356	0.1938	0.2216	0.2617	0.027	10	5	20	4	3	42
Kitchener 3	0.1381	0.2143	0.1364	0.1643	0.2106	0.2516	0.026	7	2	19	4	3	35
Guelph 1	0.1129	0.2214	0.3756	0.4532	0.3105	0.3316	0.054	9	7	28	2	6	51
Guelph 2	0.1098	0.2306	0.3826	0.4628	0.2956	0.3153	0.0479	11	7	27	2	5	51
Guelph 3	0.1149	0.2437	0.3892	0.4617	0.2847	0.3074	0.0392	11	6	26	2	4	49

Organic fractions in reference and biosolids amended soils (cont.)

Treatments	Spectrophotometric readings at 880 nm							Actual OP present mg P kg^{-1} soil					
OP fractions	$NaHCO_3$		HCl		NaOH		H_2SO_4	Labile	Moderately labile		Non-labile		
	Pi	P(T)	Pi	P(T)	P(acidified)	P(T: not acidified)	Ashing	$NaHCO_3$	HCl	Fulvic acid	Humic acid	Ashing	Total P_o
Day 60													
Reference 1	0.2393	0.2515	0.0866	0.0915	0.102	0.1125	0.1315	1	0	5	1	7	14
Reference 2	0.2321	0.2419	0.0876	0.0925	0.105	0.1184	0.1412	1	0	5	1	8	15
Reference 3	0.2495	0.2601	0.0876	0.0937	0.101	0.1094	0.1421	1	1	5	1	8	15
Kitchener 1	0.3092	0.4198	0.4316	0.5437	0.4932	0.5913	0.1658	9	9	36	8	10	72
Kitchener 2	0.309	0.4215	0.4421	0.5527	0.4843	0.5867	0.1653	9	9	35	8	10	71
Kitchener 3	0.319	0.4365	0.4478	0.5519	0.4892	0.5981	0.1626	9	8	36	9	10	72
Guelph 1	0.2803	0.3917	0.5211	0.6018	0.5431	0.6315	0.1865	9	6	40	7	12	74
Guelph 2	0.2953	0.3879	0.5151	0.6104	0.5348	0.6284	0.1869	7	8	40	8	12	74
Guelph 3	0.3169	0.4012	0.4807	0.5915	0.5219	0.6138	0.1835	7	9	38	7	11	73
Day 120													
Reference 1	0.089	0.093	0.002	0.005	0.012	0.016	0.044	0	0	2	0	5	8
Reference 2	0.091	0.099	0.001	0.008	0.014	0.019	0.045	1	1	2	0	5	9
Reference 3	0.087	0.095	0.001	0.004	0.011	0.017	0.046	1	0	2	1	5	8
Kitchener 1	0.195	0.2845	0.167	0.2874	0.2218	0.3154	0.031	8	10	20	8	4	50
Kitchener 2	0.205	0.2968	0.171	0.2689	0.2187	0.3118	0.032	8	9	20	8	4	48
Kitchener 3	0.194	0.2796	0.19	0.2983	0.2319	0.3314	0.03	7	9	21	9	4	50
Guelph 1	0.229	0.3124	0.312	0.4216	0.3418	0.4318	0.041	7	10	31	8	4	60
Guelph 2	0.252	0.3456	0.289	0.4109	0.3127	0.4087	0.043	8	11	28	8	5	60
Guelph 3	0.2109	0.3105	0.256	0.3729	0.2943	0.3914	0.041	9	10	26	8	4	58

Mean and *SD* of organic P fractions in reference and biosolids amended soils

	Day 0				Day 30			
Treatment	Labile	Moderately labile	Non-labile	Total	Labile	Moderately labile	Non-labile	Total
Reference	1	5	9	15	1	2	5	8
	0	*0*	*1*	*1*	*0*	*0*	*0*	*1*
Kitchener	9	45	18	72	8	30	12	49
	0	*1*	*0*	*0*	*0*	*1*	*0*	*1*
Guelph	8	47	19	74	8	38	13	59
	1	*0*	*0*	*1*	*1*	*2*	*0*	*1*

	Day 60				Day 120			
Treatment	Labile	Moderately labile	Non-labile	Total	Labile	Moderately labile	Non-labile	Total
Reference	1	2	5	8	1	2	4	6
	0	*0*	*0*	*0*	*0*	*0*	*0*	*0*
Kitchener	9	27	7	43	8	23	7	38
	1	*2*	*1*	*2*	*2*	*2*	*0*	*4*
Guelph	9	33	7	50	10	33	7	50
	0	*1*	*1*	*2*	*1*	*1*	*1*	*1*

Appendix I: P forms in surface runoff from reference and biosolids amended soils

Treatments	Spectrophotometric readings at 880 nm				P before dilution (ppm)				Actual P present mg L^{-1} surface runoff				
Rain 1													
	Pi	TDP	FeO-P	TP	Pi	TDP	FeO-P	T P	Pi	TDP	P(o)	FeO-P	T P
Reference 1	0.0359	0.0395	0.0452	0.1325	0.0670	0.0731	0.0828	0.1413	0.07	0.07	0.01	0.17	1.13
Reference 2	0.0377	0.0413	0.0497	0.1176	0.0701	0.0762	0.0904	0.1176	0.07	0.08	0.01	0.18	0.94
Reference 3	0.0432	0.0463	0.0528	0.1253	0.0794	0.0846	0.0957	0.1299	0.08	0.09	0.01	0.19	1.04
Kitchener 1	0.4532	0.4984	0.0872	0.3354	0.7750	0.8517	1.8487	0.4634	1.55	1.70	0.15	1.85	18.54
Kitchener 2	0.4984	0.5340	0.0919	0.2646	0.8517	0.9121	1.9444	0.3510	1.70	1.82	0.12	1.94	14.04
Kitchener 3	0.5185	0.5839	0.0905	0.3197	0.8858	0.9968	1.9158	0.4385	1.77	1.99	0.22	1.92	17.54
Guelph 1	0.3025	0.3725	0.0441	0.4215	0.5193	0.6381	0.9712	0.6001	1.30	1.60	0.30	1.94	15.00
Guelph 2	0.2781	0.3214	0.0421	0.4118	0.4779	0.5514	0.9304	0.5847	1.20	1.38	0.18	1.86	14.62
Guelph 3	0.2552	0.3060	0.0410	0.4429	0.4391	0.5253	0.9080	0.6341	1.10	1.31	0.22	1.82	15.85
Rain 2													
Reference 1	0.0265	0.0310	0.0437	0.1973	0.0511	0.0587	0.0803	0.2442	0.05	0.06	0.01	0.16	0.98
Reference 2	0.0215	0.0291	0.0415	0.1974	0.0426	0.0555	0.0765	0.2443	0.04	0.06	0.01	0.15	0.98
Reference 3	0.0287	0.0316	0.0486	0.1928	0.0548	0.0597	0.0886	0.2370	0.06	0.06	0.01	0.18	0.95
Kitchener 1	0.2142	0.2684	0.4709	0.2083	0.3695	0.4615	1.6101	0.2616	0.92	1.15	0.23	1.61	10.47
Kitchener 2	0.2014	0.2538	0.4452	0.2008	0.3478	0.4367	1.5228	0.2497	0.87	1.09	0.22	1.52	9.99
Kitchener 3	0.2237	0.2643	0.4925	0.1981	0.3856	0.4545	1.6834	0.2454	0.96	1.14	0.17	1.68	9.82
Guelph 1	0.2806	0.3214	0.4917	0.1618	0.4822	0.5514	1.6807	0.1878	0.96	1.10	0.14	1.68	7.51
Guelph 2	0.3302	0.3619	0.5034	0.1581	0.5663	0.6201	1.7204	0.1819	1.13	1.24	0.11	1.72	7.28
Guelph 3	0.2973	0.3394	0.5127	0.1531	0.5105	0.5819	1.7520	0.1740	1.02	1.16	0.14	1.75	6.96

P forms in surface runoff from reference and biosolids amended soils after simulated rainfall (cont.)

Treatments	Spectrophotometric readings at 880 nm				P before dilution (ppm)				Actual P present mg L^{-1} surface runoff				
Rain 3													
	Pi	TDP	FeO-P	TP	Pi	TDP	FeO-P	T P	Pi	TDP	P(o)	FeO-P	T P
Reference 1	0.0873	0.0986	0.1054	0.1721	0.1542	0.1734	0.1849	0.2042	0.15	0.17	0.02	0.19	0.82
Reference 2	0.0877	0.1012	0.1140	0.1605	0.1549	0.1778	0.1995	0.1857	0.16	0.18	0.02	0.20	0.74
Reference 3	0.0776	0.0891	0.0968	0.1624	0.1378	0.1573	0.1703	0.1888	0.14	0.16	0.02	0.17	0.76
Kitchener 1	0.2212	0.2509	0.3125	0.1786	0.4318	0.5363	1.0726	0.2145	1.08	1.34	0.26	2.15	8.58
Kitchener 2	0.2453	0.2897	0.3719	0.1853	0.4976	0.6371	1.2742	0.2251	1.24	1.59	0.35	2.55	9.01
Kitchener 3	0.2393	0.2841	0.3626	0.1867	0.4881	0.6213	1.2426	0.2273	1.22	1.55	0.33	2.49	9.09
Guelph 1	0.1845	0.2153	0.2346	0.1531	0.3191	0.3714	0.8083	0.1740	1.28	1.49	0.21	1.62	6.96
Guelph 2	0.1840	0.2273	0.2435	0.1518	0.3183	0.3918	0.8385	0.1719	1.27	1.57	0.29	1.68	6.88
Guelph 3	0.1813	0.2369	0.2617	0.1495	0.3137	0.4080	0.9002	0.1683	1.26	1.63	0.38	1.80	6.73
Rain 4													
Reference 1	0.0731	0.0841	0.0992	0.1634	0.1301	0.1488	0.3488	0.1903	0.13	0.15	0.02	0.35	0.19
Reference 2	0.0621	0.0927	0.1039	0.1527	0.1115	0.1634	0.3648	0.1734	0.11	0.16	0.05	0.37	0.17
Reference 3	0.0597	0.0993	0.1210	0.1735	0.1074	0.1746	0.4228	0.2064	0.11	0.18	0.07	0.42	0.21
Kitchener 1	0.2143	0.2943	0.2212	0.1736	0.3697	0.5054	0.7628	0.2065	0.74	1.01	0.27	1.53	8.26
Kitchener 2	0.2491	0.2738	0.2453	0.1627	0.4287	0.4706	0.8446	0.1892	0.86	0.94	0.08	1.69	7.57
Kitchener 3	0.1937	0.3253	0.2393	0.1801	0.3347	0.5580	0.8242	0.2169	0.67	1.12	0.45	1.65	8.67
Guelph 1	0.1627	0.2073	0.2200	0.1420	0.2822	0.3578	0.7587	0.1564	1.13	1.43	0.30	1.52	6.26
Guelph 2	0.1573	0.1937	0.2371	0.1530	0.2730	0.3347	0.8168	0.1738	1.09	1.34	0.25	1.63	6.95
Guelph 3	0.1791	0.2165	0.2407	0.1318	0.3100	0.3734	0.8290	0.1402	1.24	1.49	0.25	1.66	5.61

Mean and *SD* of P forms in surface runoff from reference and biosolids amended soils after simulated rainfall (mgP L^{-1})

Treatments	Rain 1					Rain 2				
	Pi	TDP	P(o)	FeO-P	T P	Pi	TDP	P(o)	FeO-P	T P
Reference	0.07	0.08	0.01	0.18	1.04	0.05	0.06	0.01	0.16	0.97
	0.01	*0.01*	*0.00*	*0.01*	*0.10*	*0.01*	*0.00*	*0.00*	*0.01*	*0.02*
Kitchener	1.68	1.84	0.17	1.90	16.71	0.92	1.13	0.21	1.61	10.09
	0.11	*0.15*	*0.05*	*0.05*	*2.36*	*0.05*	*0.03*	*0.03*	*0.08*	*0.34*
Guelph	1.20	1.43	0.23	1.87	15.16	1.04	1.17	0.13	1.72	7.25
	0.10	*0.15*	*0.06*	*0.06*	*0.63*	*0.09*	*0.07*	*0.02*	*0.04*	*0.28*

Treatments	Rain 3					Rain 4				
	Pi	TDP	P(o)	FeO-P	T P	Pi	TDP	P(o)	FeO-P	T P
Reference	0.15	0.17	0.02	0.19	0.77	0.12	0.16	0.05	0.38	0.19
	0.01	*0.01*	*0.00*	*0.02*	*0.04*	*0.01*	*0.01*	*0.03*	*0.04*	*0.02*
Kitchener	1.18	1.50	0.31	2.39	8.89	0.76	1.02	0.27	1.62	8.17
	0.09	*0.14*	*0.05*	*0.22*	*0.28*	*0.10*	*0.09*	*0.18*	*0.09*	*0.56*
Guelph	1.27	1.56	0.29	1.70	6.86	1.15	1.42	0.27	1.60	6.27
	0.01	*0.07*	*0.08*	*0.09*	*0.12*	*0.08*	*0.08*	*0.03*	*0.08*	*0.67*

Appendix J: P forms in tile leachate from reference and biosolids amended soils

Treatments	Spectrophotometric readings at 880 nm				P before dilution (ppm)				Actual P present mg L^{-1} surface runoff				
Rain 1													
	Pi	TDP	FeO-P	TP	Pi	TDP	FeO-P	T P	Pi	TDP	P(o)	FeO-P	T P
Reference 1	0.041	0.048	0.0468	0.124	0.076	0.088	0.086	0.128	0.08	0.09	0.01	0.17	1.03
Reference 2	0.0475	0.0488	0.0509	0.115	0.087	0.089	0.092	0.113	0.09	0.09	0.00	0.19	0.90
Reference 3	0.0359	0.0387	0.0395	0.111	0.067	0.072	0.073	0.107	0.07	0.07	0.01	0.15	0.86
Kitchener 1	0.2868	0.3165	0.0631	0.207	0.493	0.543	1.358	0.259	0.99	1.09	0.10	1.36	10.36
Kitchener 2	0.3162	0.3204	0.0659	0.230	0.543	0.550	1.415	0.296	1.09	1.10	0.01	1.42	11.83
Kitchener 3	0.2887	0.3129	0.0678	0.253	0.496	0.537	1.454	0.333	0.99	1.07	0.08	1.45	13.30
Guelph 1	0.0436	0.1261	0.0258	0.314	0.080	0.220	0.599	0.430	0.96	1.10	0.14	1.20	10.75
Guelph 2	0.0611	0.1172	0.0269	0.305	0.110	0.205	0.621	0.415	0.88	1.03	0.15	1.24	10.37
Guelph 3	0.064	0.1155	0.0274	0.321	0.115	0.202	0.631	0.440	0.92	1.01	0.09	1.26	11.01
Rain 2													
Reference 1	0.0208	0.0541	0.0461	0.165	0.041	0.098	0.084	0.193	0.08	0.10	0.02	0.17	1.16
Reference 2	0.0149	0.0702	0.0442	0.173	0.031	0.125	0.081	0.206	0.06	0.13	0.06	0.16	1.23
Reference 3	0.0376	0.083	0.0421	0.172	0.070	0.147	0.078	0.204	0.14	0.15	0.01	0.16	1.22
Kitchener 1	0.0914	0.1867	0.0614	0.297	0.161	0.323	1.323	0.403	1.29	1.61	0.33	2.65	8.06
Kitchener 2	0.0639	0.185	0.0625	0.316	0.115	0.320	1.346	0.432	0.92	1.60	0.68	2.69	8.64
Kitchener 3	0.0847	0.1519	0.0593	0.290	0.150	0.264	1.281	0.391	1.20	1.32	0.12	2.56	7.82
Guelph 1	0.0341	0.2236	0.0617	0.119	0.064	0.385	1.329	0.120	0.77	1.16	0.39	1.33	4.81
Guelph 2	0.0317	0.1936	0.05834	0.118	0.060	0.335	1.261	0.118	0.72	1.00	0.29	1.26	4.74
Guelph 3	0.0338	0.2013	0.0603	0.126	0.063	0.348	1.301	0.132	0.76	1.04	0.28	1.30	5.26

P forms in tile leachate from reference and biosolids amended soils after simulated rainfall (cont.)

Treatments	Spectrophotometric readings at 880 nm				P before dilution (ppm)				Actual P present mg L^{-1} surface runoff				
Rain 3													
	Pi	TDP	FeO-P	TP	Pi	TDP	FeO-P	T P	Pi	TDP	P(o)	FeO-P	T P
Reference 1	0.064	0.0763	0.0421	0.156	0.115	0.136	0.078	0.178	0.12	0.14	0.02	0.16	1.07
Reference 2	0.0618	0.0864	0.0399	0.183	0.111	0.153	0.074	0.221	0.11	0.15	0.04	0.15	1.33
Reference 3	0.0638	0.0769	0.0416	0.164	0.114	0.137	0.077	0.191	0.11	0.14	0.02	0.15	1.15
Kitchener 1	0.3685	0.3986	0.0593	0.226	0.631	0.682	0.640	0.290	1.26	1.37	0.10	1.28	7.25
Kitchener 2	0.3942	0.4315	0.0615	0.238	0.675	0.738	0.663	0.308	1.35	1.48	0.13	1.33	7.71
Kitchener 3	0.4115	0.4326	0.0601	0.258	0.704	0.740	0.648	0.341	1.41	1.48	0.07	1.30	8.53
Guelph 1	0.0888	0.1024	0.0613	0.125	0.157	0.180	0.661	0.130	1.25	1.44	0.19	1.32	5.20
Guelph 2	0.1021	0.1124	0.0645	0.120	0.179	0.197	0.693	0.121	1.44	1.57	0.14	1.39	4.83
Guelph 3	0.0864	0.1071	0.0619	0.126	0.153	0.188	0.667	0.131	1.22	1.50	0.28	1.33	5.24
Rain 4													
Reference 1	0.0531	0.0614	0.0391	0.136	0.096	0.110	0.072	0.146	0.10	0.11	0.01	0.15	0.88
Reference 2	0.0529	0.0764	0.0394	0.173	0.096	0.136	0.073	0.206	0.10	0.14	0.04	0.15	1.24
Reference 3	0.0315	0.0579	0.0338	0.153	0.060	0.104	0.063	0.173	0.06	0.10	0.05	0.13	1.04
Kitchener 1	0.3281	0.3937	0.0581	0.216	0.563	0.674	0.628	0.273	1.13	1.35	0.22	1.26	6.83
Kitchener 2	0.3461	0.4126	0.0532	0.204	0.593	0.706	0.578	0.255	1.19	1.41	0.23	1.16	6.37
Kitchener 3	0.3167	0.3816	0.0567	0.241	0.543	0.654	0.614	0.314	1.09	1.31	0.22	1.23	7.84
Guelph 1	0.0834	0.1023	0.0504	0.117	0.148	0.180	0.550	0.117	0.89	1.08	0.19	1.10	4.68
Guelph 2	0.0941	0.1009	0.0591	0.105	0.166	0.177	0.638	0.097	1.00	1.06	0.07	1.28	3.89
Guelph 3	0.0725	0.0991	0.0527	0.129	0.129	0.174	0.573	0.135	0.78	1.05	0.27	1.15	5.40

Mean and *SD* of P forms in tile leachate from reference and biosolids amended soils after simulated rainfall (mgP L^{-1})

Treatments	Rain 1					Rain 2				
	Pi	TDP	P(o)	FeO-P	T P	Pi	TDP	P(o)	FeO-P	T P
Reference	0.08	0.08	0.01	0.17	0.93	0.10	0.12	0.03	0.16	1.21
	0.01	*0.01*	*0.01*	*0.02*	*0.09*	*0.04*	*0.03*	*0.03*	*0.01*	*0.04*
Kitchener	1.02	1.09	0.07	1.41	3.55	1.14	1.51	0.38	1.32	4.90
	0.06	*0.01*	*0.05*	*0.05*	*0.44*	*0.20*	*0.17*	*0.29*	*0.03*	*0.25*
Guelph	0.92	1.05	0.13	1.23	5.14	0.75	1.07	0.32	1.30	4.94
	0.04	*0.05*	*0.03*	*0.03*	*0.15*	*0.03*	*0.08*	*0.06*	*0.03*	*0.29*

Treatments	Rain 3					Rain 4				
	Pi	TDP	P(o)	FeO-P	T P	Pi	TDP	P(o)	FeO-P	T P
Reference	0.11	0.14	0.03	0.15	1.18	0.08	0.12	0.03	0.14	1.05
	0.00	*0.01*	*0.01*	*0.00*	*0.13*	*0.02*	*0.02*	*0.02*	*0.01*	*0.18*
Kitchener	1.34	1.44	0.10	1.30	3.76	1.13	1.36	0.22	1.21	7.01
	0.07	*0.07*	*0.03*	*0.02*	*0.31*	*0.05*	*0.05*	*0.00*	*0.05*	*0.75*
Guelph	1.30	1.51	0.20	1.35	5.09	0.89	1.06	0.18	1.17	4.66
	0.12	*0.07*	*0.07*	*0.04*	*0.23*	*0.11*	*0.02*	*0.10*	*0.09*	*0.76*

Appendix K: Chlorophyll concentrations in epilimnion of mesocosms

Day 0

Treatment	Replica	Spectrophotometric readings at (nm)					Chl mg m^{-3}		
		630	647	664	665	750	a	b	c
Reference	1 A,B,C	0.0104	0.0117	0.0153	0.0096	0.0042	2.37	0.29	0.03
Reference	3 A,B,C	0.0126	0.0124	0.0127	0.0116	0.0043	1.67	0.29	0.05
Reference	8 A,B,C	0.0135	0.0135	0.0128	0.0118	0.0044	1.64	0.49	0.05
Blank	4 A,B,C	0.0123	0.0125	0.0127	0.0110	0.0043	1.67	0.41	0.05
Blank	6 A,B,C	0.0151	0.0139	0.0124	0.0120	0.0049	1.42	0.26	0.06
Blank	9 A,B,C	0.0109	0.0104	0.0116	0.0094	0.0046	1.43	0.02	0.04
Guelph	2 A,B,C	0.0148	0.0143	0.0141	0.0171	0.0044	1.90	0.35	0.06
Guelph	5 A,B,C	0.0135	0.0132	0.0146	0.0137	0.0041	2.13	0.21	0.05
Guelph	7 A,B,C	0.0119	0.0123	0.0138	0.0131	0.0044	1.93	0.31	0.04
Kitchener	A	0.0130	0.0133	0.0127	0.0116	0.0047	1.56	0.53	0.05
Kitchener	B	0.0123	0.0125	0.0130	0.0153	0.0048	1.64	0.36	0.04
Kitchener	C	0.0116	0.0114	0.0103	0.0074	0.0045	1.11	0.37	0.04
Low P	A	0.0128	0.0126	0.0131	0.0125	0.0034	1.94	0.32	0.05
Low P	B	0.0096	0.0102	0.0133	0.0066	0.0047	1.83	0.08	0.03
Low P	C	0.0114	0.0112	0.0097	0.0078	0.0038	1.11	0.45	0.04
High P	A	0.0117	0.0115	0.0106	0.0088	0.0041	1.25	0.38	0.04
High P	B	0.0132	0.0122	0.0118	0.0115	0.0038	1.56	0.16	0.05
High P	C	0.0118	0.0118	0.0113	0.0105	0.0049	1.25	0.37	0.04
N	A	0.0195	0.0162	0.0133	0.0131	0.0018	2.14	0.10	0.10
N	B	0.0156	0.0134	0.0120	0.0117	0.0029	1.73	0.05	0.07
N	C	0.0126	0.0126	0.0120	0.0118	0.0041	1.54	0.46	0.05
N + low P	A	0.0250	0.0191	0.0106	0.0142	0.0042	0.89	0.04	0.12
N + low P	B	0.0136	0.0127	0.0131	0.0124	0.0045	1.71	0.09	0.05
N + low P	C	0.0132	0.0122	0.0120	0.0118	0.0039	1.59	0.14	0.05
N + high P	A	0.0129	0.0123	0.0118	0.0115	0.0046	1.40	0.25	0.05
N + high P	B	0.0102	0.0101	0.0114	0.0149	0.0043	1.46	0.10	0.03
N + high P	C	0.0133	0.0128	0.0131	0.0129	0.0047	1.67	0.21	0.05

Chlorophyll concentrations in epilimnion of mesocosms – Day 4

Treatment	Replica	Spectrophotometric readings at (nm)					Chl mg m^{-3}		
		630	647	664	665	750	a	b	c
Reference	1 A,B,C	0.0233	0.0230	0.0275	0.0292	0.0029	5.03	0.34	0.12
Reference	3 A,B,C	0.0224	0.0222	0.0240	0.0318	0.0028	4.28	0.62	0.11
Reference	8 A,B,C	0.0183	0.0190	0.0245	0.0257	0.0026	4.57	0.35	0.09
Blank	4 A,B,C	0.0022	0.0071	0.0184	0.0019	0.0011	3.91	0.35	0.00
Blank	6 A,B,C	0.0018	0.0045	0.0119	0.0024	0.0013	2.41	0.06	0.00
Blank	9 A,B,C	0.0022	0.0058	0.0119	0.0018	0.0016	2.31	0.49	0.00
Guelph	2 A,B,C	0.0351	0.0337	0.0372	0.0640	0.0032	6.86	0.67	0.18
Guelph	5 A,B,C	0.0197	0.0238	0.0348	0.0401	0.0029	6.77	0.86	0.09
Guelph	7 A,B,C	0.0243	0.0268	0.0359	0.0483	0.0030	6.90	0.77	0.12
Kitchener	A	0.0245	0.0247	0.0295	0.0544	0.0032	5.42	0.51	0.12
Kitchener	B	0.0177	0.0200	0.0301	0.0274	0.0029	5.80	0.32	0.08
Kitchener	C	0.0174	0.0212	0.0299	0.0281	0.0033	5.65	0.87	0.08
Low P	A	0.0180	0.0201	0.0212	0.0263	0.0019	3.88	1.28	0.09
Low P	B	0.0159	0.0171	0.0239	0.0205	0.0025	4.51	0.25	0.07
Low P	C	0.0139	0.0151	0.0192	0.0190	0.0037	3.24	0.40	0.06
High P	A	0.0156	0.0151	0.0192	0.0203	0.0019	3.58	0.03	0.08
High P	B	0.0197	0.0209	0.0236	0.0247	0.0038	4.04	0.81	0.09
High P	C	0.0151	0.0161	0.0218	0.0208	0.0028	3.99	0.26	0.07
N	A	0.0157	0.0180	0.0238	0.0240	0.0038	4.21	0.64	0.07
N	B	0.0142	0.0159	0.0243	0.0198	0.0041	4.34	0.08	0.06
N	C	0.0143	0.0161	0.0229	0.0194	0.0038	4.06	0.31	0.06
N + low P	A	0.0151	0.0171	0.0226	0.0193	0.0026	4.19	0.60	0.07
N + low P	B	0.0178	0.0191	0.0284	0.0266	0.0037	5.27	0.04	0.08
N + low P	C	0.0171	0.0187	0.0271	0.0230	0.0019	5.33	0.29	0.08
N + high P	A	0.0169	0.0189	0.0284	0.0263	0.0028	5.46	0.24	0.08
N + high P	B	0.0163	0.0182	0.0287	0.0258	0.0019	5.73	0.11	0.08
N + high P	C	0.0158	0.0183	0.0301	0.0301	0.0043	5.59	0.03	0.06

Chlorophyll concentrations in epilimnion of mesocosms – Day 11

Treatment	Replica	Spectrophotometric readings at (nm)					Chl mg m^{-3}		
		630	647	664	665	750	a	b	c
Reference	1 A,B,C	0.0405	0.0395	0.0426	0.0319	0.0044	7.67	1.01	0.20
Reference	3 A,B,C	0.0442	0.0401	0.0404	0.0444	0.0043	7.13	0.52	0.23
Reference	8 A,B,C	0.0236	0.0233	0.0274	0.0242	0.0044	4.72	0.34	0.11
Blank	4 A,B,C	0.0032	0.0081	0.0194	0.0019	0.0021	3.91	0.35	0.00
Blank	6 A,B,C	0.0202	0.0211	0.0215	0.0190	0.0016	3.97	1.09	0.10
Blank	9 A,B,C	0.0358	0.0285	0.0172	0.0102	0.0046	2.00	0.38	0.18
Guelph	2 A,B,C	0.0453	0.0563	0.1022	0.0782	0.0044	21.25	0.33	0.22
Guelph	5 A,B,C	0.0725	0.0694	0.0845	0.0733	0.0041	16.50	0.53	0.39
Guelph	7 A,B,C	0.0983	0.0984	0.1321	0.1058	0.0044	26.62	0.70	0.53
Kitchener	A	0.0925	0.0898	0.1141	0.0564	0.0047	22.60	0.58	0.50
Kitchener	B	0.0838	0.1163	0.2336	0.1814	0.0048	50.17	1.03	0.42
Kitchener	C	0.0610	0.0564	0.0608	0.0591	0.0045	11.29	0.69	0.32
Low P	A	0.1059	0.0964	0.1086	0.1052	0.0031	21.31	0.44	0.59
Low P	B	0.0879	0.0827	0.0931	0.0819	0.0028	18.26	1.16	0.48
Low P	C	0.0799	0.0716	0.0762	0.0758	0.0021	14.80	0.49	0.44
High P	A	0.0771	0.0753	0.0758	0.0812	0.0039	14.26	2.75	0.42
High P	B	0.1028	0.0931	0.1024	0.0986	0.0026	20.06	0.57	0.57
High P	C	0.0863	0.0809	0.0867	0.0830	0.0031	16.75	1.51	0.47
N	A	0.0345	0.0284	0.0175	0.0106	0.0031	2.38	0.72	0.18
N	B	0.0241	0.0231	0.0250	0.0204	0.0028	4.47	0.46	0.12
N	C	0.0799	0.0716	0.0562	0.0758	0.0021	10.06	2.66	0.45
N + low P	A	0.0794	0.0734	0.0804	0.0773	0.0016	15.84	0.95	0.44
N + low P	B	0.0890	0.0911	0.1173	0.1062	0.0018	23.93	1.82	0.49
N + low P	C	0.0859	0.0823	0.0980	0.0920	0.0024	19.53	1.01	0.47
N + high P	A	0.0908	0.0887	0.1165	0.1050	0.0022	23.72	0.40	0.50
N + high P	B	0.0855	0.0852	0.1176	0.1030	0.0026	24.05	0.20	0.47
N + high P	C	0.0892	0.0928	0.1374	0.1202	0.0033	28.34	0.23	0.48

Chlorophyll concentrations in epilimnion of mesocosms – Day 18

Treatment	Replica	Spectrophotometric readings at (nm)					Chl mg m^{-3}		
		630	647	664	665	750	a	b	c
Reference	1 A,B,C	0.0298	0.0270	0.0282	0.0343	0.0044	4.73	0.18	0.14
Reference	3 A,B,C	0.0315	0.0310	0.0336	0.0393	0.0043	5.90	0.79	0.15
Reference	8 A,B,C	0.0267	0.0252	0.0289	0.0280	0.0044	4.98	0.15	0.13
Blank	4 A,B,C	0.0102	0.0099	0.0103	0.0085	0.0043	1.20	0.13	0.03
Blank	6 A,B,C	0.0124	0.0123	0.0127	0.0123	0.0038	1.78	0.32	0.05
Blank	9 A,B,C	0.0121	0.0117	0.0120	0.0138	0.0042	1.55	0.21	0.04
Guelph	2 A,B,C	0.1322	0.1539	0.1951	0.2338	0.0044	39.56	8.19	0.71
Guelph	5 A,B,C	0.1018	0.1177	0.1527	0.1376	0.0041	30.94	5.63	0.54
Guelph	7 A,B,C	0.0992	0.1127	0.1824	0.1603	0.0044	38.10	0.99	0.53
Kitchener	A	0.0896	0.1070	0.1873	0.1640	0.0047	39.45	0.60	0.47
Kitchener	B	0.1073	0.1172	0.1422	0.1268	0.0048	28.28	5.08	0.57
Kitchener	C	0.1418	0.1607	0.1786	0.2540	0.0045	35.35	10.25	0.77
Low P	A	0.0614	0.0596	0.0621	0.0512	0.0034	11.72	1.83	0.33
Low P	B	0.0744	0.0741	0.0765	0.0741	0.0047	14.32	2.85	0.39
Low P	C	0.0729	0.0704	0.0725	0.0833	0.0038	13.68	2.17	0.39
High P	A	0.0779	0.0793	0.0994	0.0977	0.0041	19.68	1.65	0.42
High P	B	0.0879	0.0817	0.0896	0.0879	0.0038	17.26	1.08	0.48
High P	C	0.0841	0.0836	0.1021	0.1010	0.0049	19.98	1.48	0.45
N	A	0.0479	0.0493	0.0594	0.0977	0.0038	11.42	1.37	0.25
N	B	0.0778	0.0687	0.0696	0.0879	0.0049	12.79	0.42	0.42
N	C	0.0423	0.0436	0.0521	0.1010	0.0041	9.85	1.24	0.22
N + low P	A	0.0819	0.0846	0.1122	0.1041	0.0042	22.50	1.42	0.44
N + low P	B	0.1020	0.1177	0.1928	0.1695	0.0045	40.36	1.23	0.54
N + low P	C	0.0898	0.0944	0.1424	0.1353	0.0039	29.35	0.17	0.48
N + high P	A	0.0982	0.1359	0.2022	0.1617	0.0046	42.04	8.87	0.51
N + high P	B	0.1202	0.1503	0.2719	0.2168	0.0043	58.00	1.52	0.63
N + high P	C	0.1072	0.1388	0.2545	0.2096	0.0047	54.25	2.01	0.56

Chlorophyll concentrations in epilimnion of mesocosms – Day 32

Treatment	Replica	Spectrophotometric readings at (nm)					Chl mg m^{-3}		
		630	647	664	665	750	a	b	c
Reference	1 A,B,C	0.0258	0.0232	0.0206	0.0161	0.0025	3.46	0.55	0.13
Reference	3 A,B,C	0.0241	0.0236	0.0219	0.0178	0.0043	3.40	0.93	0.11
Reference	8 A,B,C	0.0251	0.0272	0.0384	0.0262	0.0044	7.18	0.39	0.12
Blank	4 A,B,C	0.0133	0.0115	0.0099	0.0088	0.0021	1.47	0.13	0.06
Blank	6 A,B,C	0.0127	0.0116	0.0124	0.0113	0.0019	2.10	0.06	0.06
Blank	9 A,B,C	0.0126	0.0112	0.0095	0.0093	0.0023	1.35	0.22	0.06
Guelph	2 A,B,C	0.0281	0.0356	0.0431	0.0487	0.0037	8.16	2.65	0.13
Guelph	5 A,B,C	0.0222	0.0286	0.0399	0.0221	0.0041	7.59	1.60	0.10
Guelph	7 A,B,C	0.0727	0.0694	0.0590	0.0536	0.0044	10.40	3.25	0.39
Kitchener	A	0.0243	0.0281	0.0448	0.0298	0.0047	8.63	0.26	0.11
Kitchener	B	0.0330	0.0462	0.0894	0.0839	0.0048	18.54	0.73	0.15
Kitchener	C	0.0284	0.0432	0.0621	0.0536	0.0045	12.28	3.66	0.13
High P	A	0.0212	0.0277	0.0464	0.0401	0.0034	9.30	0.82	0.10
High P	B	0.0337	0.0398	0.0646	0.0421	0.0047	12.88	0.54	0.16
High P	C	0.0239	0.0301	0.0559	0.0324	0.0038	11.38	0.06	0.11
Low P	A	0.0223	0.0257	0.0422	0.0401	0.0041	8.22	0.11	0.10
Low P	B	0.0369	0.0362	0.0446	0.0421	0.0038	8.41	0.39	0.19
Low P	C	0.0239	0.0251	0.0356	0.0324	0.0049	6.50	0.11	0.11
N	A	0.0222	0.0237	0.0264	0.0401	0.0034	4.68	1.04	0.11
N	B	0.0212	0.0207	0.0215	0.0121	0.0047	3.36	0.52	0.09
N	C	0.0126	0.0112	0.0095	0.0324	0.0038	1.05	0.15	0.05
N + low P	A	0.0614	0.0566	0.0621	0.0512	0.0043	11.63	0.53	0.33
N + low P	B	0.0544	0.0674	0.0765	0.0741	0.0038	14.87	5.40	0.28
N + low P	C	0.0729	0.0674	0.0725	0.0833	0.0046	13.61	0.87	0.39
N + high P	A	0.1032	0.1161	0.1542	0.1352	0.0043	31.29	4.44	0.55
N + high P	B	0.0856	0.0921	0.1137	0.1102	0.0050	22.43	3.39	0.45
N + high P	C	0.0816	0.0946	0.1372	0.1439	0.0040	28.16	3.00	0.43

Mean and *SD* of chlorophyll in epilimnion of mesocosms

	Day 0			Day 4			Day 11			Day 18			Day 32		
Treatment	chl a	chl b	chl c	chl a	chl b	chl c	chl a	chl b	chl c	chl a	chl b	chl c	chl a	chl b	chl c
Reference	1.89	0.36	0.04	4.63	0.44	0.10	6.50	0.62	0.18	5.20	0.37	0.14	4.68	0.62	0.12
	0.63	*0.29*	*0.02*	*0.60*	*0.19*	*0.02*	*1.87*	*0.63*	*0.06*	*1.25*	*0.41*	*0.04*	*3.23*	*0.39*	*0.02*
Kitchener	1.99	0.29	0.05	6.84	0.77	0.13	21.46	0.52	0.38	36.20	4.94	0.59	8.71	2.50	0.21
	0.44	*0.19*	*0.02*	*1.55*	*0.62*	*0.05*	*4.85*	*0.30*	*0.15*	*8.21*	*5.30*	*0.21*	*3.32*	*1.97*	*0.14*
Guelph	1.43	0.42	0.04	5.62	0.57	0.09	28.02	0.77	0.41	34.36	5.31	0.60	13.15	1.55	0.13
	0.46	*0.17*	*0.01*	*1.69*	*0.38*	*0.03*	*18.08*	*0.40*	*0.13*	*15.37*	*6.43*	*0.30*	*6.37*	*2.78*	*0.04*
N(L)+P(L)	1.40	0.09	0.08	4.93	0.31	0.08	19.77	1.26	0.47	30.74	0.94	0.49	13.37	2.27	0.33
	0.44	*0.05*	*0.04*	*0.64*	*0.28*	*0.01*	*4.05*	*0.49*	*0.02*	*9.01*	*0.67*	*0.05*	*1.63*	*2.72*	*0.05*
N(H)	1.80	0.20	0.07	4.20	0.34	0.06	5.64	1.28	0.25	11.35	1.01	0.29	3.03	0.57	0.08
	0.31	*0.22*	*0.03*	*0.14*	*0.28*	*0.01*	*3.97*	*1.20*	*0.17*	*1.47*	*0.52*	*0.11*	*1.83*	*0.45*	*0.03*
P(H)	1.63	0.28	0.04	3.88	0.64	0.07	18.12	0.70	0.50	13.24	2.29	0.37	11.19	0.47	0.12
	0.45	*0.19*	*0.01*	*0.64*	*0.55*	*0.02*	*3.26*	*0.40*	*0.07*	*1.36*	*0.52*	*0.04*	*1.80*	*0.38*	*0.03*
N(H)+P(H)	1.51	0.18	0.04	5.59	0.13	0.07	25.37	0.28	0.48	51.43	4.13	0.57	27.29	3.61	0.48
	0.14	*0.08*	*0.01*	*0.14*	*0.11*	*0.01*	*2.58*	*0.11*	*0.02*	*8.35*	*4.11*	*0.06*	*4.49*	*0.74*	*0.06*
P(L)	1.35	0.31	0.05	3.87	0.37	0.08	17.02	1.61	0.49	18.97	1.40	0.45	7.71	0.20	0.13
	0.18	*0.12*	*0.01*	*0.25*	*0.40*	*0.01*	*2.91*	*1.09*	*0.08*	*1.49*	*0.29*	*0.03*	*1.05*	*0.16*	*0.05*
Blank	1.51	0.23	0.05	2.87	0.30	0.00	3.29	0.61	0.10	1.51	0.22	0.04	1.64	0.14	0.06
	0.14	*0.19*	*0.01*	*0.90*	*0.22*	*0.00*	*1.12*	*0.42*	*0.09*	*0.29*	*0.09*	*0.01*	*0.40*	*0.08*	*0.00*

Appendix L: Total P (mg m^3) in epilimnion and hypolimnion of mesocosms

Treatment	Replica	Epilimnion/Day					Hypolimnion/Day				
		0	4	11	18	32	0	4	11	18	32
Reference	1 A,B,C	11	16	42	183	286	10	20	54	264	346
Reference	3 A,B,C	10	13	22	196	266	11	21	61	297	382
Reference	8 A,B,C	12	19	22	61	273	11	23	66	315	383
Kitchener	4 A,B,C	16	44	93	558	927	11	48	1020	1064	2302
Kitchener	6 A,B,C	12	45	96	610	955	9	51	1113	1829	2880
Kitchener	9 A,B,C	15	43	98	527	935	11	50	1186	1560	2791
Guelph	2 A,B,C	15	40	83	388	716	11	43	235	765	1431
Guelph	5 A,B,C	13	44	84	378	713	10	48	1044	1112	1803
Guelph	7 A,B,C	15	40	81	384	641	12	48	945	1112	1942
N(L)+P(L)	A	16	19	149	297	477	11	23	196	427	729
N(L)+P(L)	B	18	38	115	266	545	12	4	164	408	782
N(L)+P(L)	C	16	23	134	301	582	9	28	212	465	855
N(H)	A	13	16	36	51	91	9	27	113	146	278
N(H)	B	17	27	30	33	48	13	30	93	90	291
N(H)	C	9	15	17	45	26	7	2	86	123	283
P(L)	A	11	13	91	121	240	7	15	269	378	711
P(L)	B	16	22	105	128	225	12	24	318	369	676
P(L)	C	15	16	85	106	204	11	20	239	301	617
N(H)+P(H)	A	8	14	112	444	753	7	16	326	1260	2301
N(H)+P(H)	B	11	13	103	380	692	7	14	304	1173	2105
N(H)+P(H)	C	16	18	105	404	682	11	20	283	1199	1954
P(H)	A	10	15	203	252	356	7	16	343	782	1104
P(H)	B	15	17	213	304	397	11	27	280	883	1197
P(H)	C	12	14	196	293	433	9	16	230	917	1307
Blank	A	11	12	4	6	7	7	13	12	11	9
Blank	B	10	13	3	4	5	7	14	6	10	9
Blank	C	12	14	5	5	4	9	16	15	11	7

Mean and *SD* of Total P (mg m^3) in epilimnion and hypolimnion of mesocosms

Treatment	Epilimnion/Day					Hypolimnion/Day				
	0	4	11	18	32	0	4	11	18	32
Reference	11	16	28	147	275	11	21	60	292	370
	1	*3*	*11*	*75*	*10*	*1*	*1*	*6*	*26*	*21*
Kitchener	14	44	96	565	939	11	49	1106	1485	2657
	2	*1*	*2*	*42*	*15*	*1*	*1*	*83*	*388*	*311*
Guelph	14	42	83	383	690	11	46	741	996	1725
	2	*2*	*1*	*5*	*43*	*1*	*3*	*441*	*200*	*264*
N(L)+P(L)	17	27	132	288	535	11	18	190	433	789
	1	*10*	*17*	*19*	*53*	*2*	*12*	*24*	*29*	*63*
N(H)	13	19	28	43	55	10	20	97	119	284
	4	*7*	*10*	*9*	*33*	*3*	*15*	*14*	*28*	*7*
P(L)	14	17	94	118	223	10	19	275	349	668
	3	*5*	*10*	*12*	*18*	*3*	*5*	*40*	*42*	*48*
N(H)+P(H)	12	15	107	410	709	8	17	304	1211	2120
	4	*3*	*5*	*32*	*38*	*2*	*3*	*22*	*45*	*174*
P(H)	12	15	204	283	395	9	20	284	861	1203
	2	*2*	*8*	*27*	*39*	*2*	*6*	*57*	*71*	*102*
Blank	11	13	4	5	5	8	14	11	11	8
	1	*1*	*1*	*1*	*2*	*1*	*2*	*4*	*0*	*1*

Appendix M: DRP (mg m^3) in epilimnion and hypolimnion of mesocosms

		Epilimnion/Day					Hypolimnion/Day				
Treatment	Replica	0	4	11	18	32	0	4	11	18	32
Reference	1 A,B,C	0	0	0	0	0	0	0	0	0	5
Reference	3 A,B,C	0	0	0	0	0	0	0	0	0	0
Reference	8 A,B,C	0	0	0	0	0	0	0	0	0	0
Kitchener	4 A,B,C	0	0	0	0	0	0	0	14	25	29
Kitchener	6 A,B,C	0	0	0	0	0	0	0	13	22	31
Kitchener	9 A,B,C	0	0	0	0	0	0	0	15	26	34
Guelph	2 A,B,C	0	0	0	0	0	0	0	0	0	10
Guelph	5 A,B,C	0	0	0	0	0	0	0	0	0	0
Guelph	7 A,B,C	0	0	0	0	0	0	0	0	0	7
N(L)+P(L)	A	0	0	0	0	0	0	0	19	60	77
N(L)+P(L)	B	0	0	0	0	0	0	0	19	56	64
N(L)+P(L)	C	0	0	0	0	0	0	0	19	59	71
N(H)	A	0	0	0	0	0	0	0	9	15	22
N(H)	B	0	0	0	0	0	0	0	10	12	17
N(H)	C	0	0	0	0	0	0	0	9	14	21
P(L)	A	0	0	0	0	0	0	0	17	50	63
P(L)	B	0	0	0	0	0	0	0	18	52	70
P(L)	C	0	0	0	0	0	0	0	17	49	61
N(H)+P(H)	A	0	0	0	0	0	0	0	11	26	54
N(H)+P(H)	B	0	0	0	0	0	0	0	12	24	38
N(H)+P(H)	C	0	0	0	0	0	0	0	13	20	46
P(H)	A	0	0	0	0	0	0	0	0	0	7
P(H)	B	0	0	0	0	0	0	0	0	0	0
P(H)	C	0	0	0	0	0	0	0	0	0	8
Blank	A	0	0	0	0	0	0	0	22	33	55
Blank	B	0	0	0	0	0	0	0	19	33	69
Blank	C	0	0	0	0	0	0	0	25	40	63

Mean and *SD* of DRP (mg m^3) in epilimnion and hypolimnion of mesocosms

Treatment	Epilimnion/Day					Hypolimnion/Day				
	0	4	11	18	32	0	4	11	18	32
Reference	0	0	0	0	0	0	0	0	0	2
	0	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*3*
Kitchener	0	0	0	0	0	0	0	14	25	31
	0	*0*	*0*	*0*	*0*	*0*	*0*	*1*	*2*	*2*
Guelph	0	0	0	0	0	0	0	0	0	5
	0	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*5*
N(L)+P(L)	0	0	0	0	0	0	0	19	58	71
	0	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*2*	*6*
N(H)	0	0	0	0	0	0	0	9	14	20
	0	*0*	*0*	*0*	*0*	*0*	*0*	*1*	*1*	*3*
P(L)	0	0	0	0	0	0	0	17	51	65
	0	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*2*	*4*
N(H)+P(H)	0	0	0	0	0	0	0	12	23	46
	0	*0*	*0*	*0*	*0*	*0*	*0*	*1*	*3*	*8*
P(H)	0	0	0	0	0	0	0	0	0	5
	0	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*5*
Blank	0	0	0	0	0	0	0	22	35	62
	0	*0*	*0*	*0*	*0*	*0*	*0*	*3*	*4*	*7*

Appendix N: Dissolved Oxygen (mg L^{-1}) in epilimnion and hypolimnion of mesocosms

		Epilimnion/Day					Hypolimnion/Day				
Treatment	Replica	0	4	11	18	32	0	4	11	18	32
Reference	1 A,B,C	20.0	16.7	23.8	18.1	22.7	6.7	9.9	11.0	9.5	8.5
Reference	3 A,B,C	21.6	17.8	21.1	20.7	21.7	12.6	12.8	10.3	8.8	4.2
Reference	8 A,B,C	22.9	20.6	20.2	21.0	21.9	22.9	20.6	20.2	21.0	21.9
Kitchener	4 A,B,C	24.2	30.2	34.0	28.9	25.4	12.0	3.0	1.8	2.2	2.8
Kitchener	6 A,B,C	20.4	25.9	31.5	27.4	25.1	13.4	3.1	2.9	2.3	5.3
Kitchener	9 A,B,C	18.8	24.4	31.4	31.8	24.5	12.8	2.0	2.1	2.1	1.9
Guelph	2 A,B,C	21.0	26.5	27.9	24.8	23.9	13.1	3.0	1.9	2.5	2.7
Guelph	5 A,B,C	20.2	21.8	28.7	24.8	19.9	13.8	1.6	2.4	2.3	2.1
Guelph	7 A,B,C	25.3	33.7	29.7	27.5	22.2	12.3	1.5	2.0	1.7	2.9
N(L)+P(L)	A	16.8	29.7	19.4	25.4	28.2	10.7	5.1	5.0	5.5	8.4
N(L)+P(L)	B	24.1	24.5	27.6	21.5	29.0	9.9	14.1	10.0	5.4	7.8
N(L)+P(L)	C	17.1	15.2	18.2	20.2	29.6	14.3	7.5	6.7	3.5	3.0
N(H)	A	20.4	13.8	12.3	11.8	12.1	9.7	10.7	5.4	5.3	3.9
N(H)	B	21.0	15.2	16.2	13.2	13.1	8.2	3.9	3.3	5.9	9.2
N(H)	C	24.2	15.7	13.1	13.0	13.0	4.7	5.1	2.3	5.8	4.6
P(L)	A	21.7	20.2	19.1	19.3	28.4	10.8	7.3	3.0	5.3	7.3
P(L)	B	19.4	22.6	15.7	20.0	18.1	10.0	8.4	4.6	4.9	7.0
P(L)	C	19.5	19.3	16.9	20.4	25.3	8.9	8.7	3.7	4.5	1.8
N(H)+P(H)	A	21.4	31.3	22.8	23.4	25.6	8.2	10.0	3.3	4.0	2.6
N(H)+P(H)	B	21.6	27.6	22.7	23.0	23.4	10.7	11.5	2.9	3.5	3.7
N(H)+P(H)	C	22.6	29.3	22.4	21.8	22.0	15.0	10.6	5.8	3.4	4.2
P(H)	A	20.0	16.7	23.8	18.1	22.7	6.7	9.9	11.0	9.5	8.5
P(H)	B	21.6	17.8	21.1	20.7	21.7	12.6	12.8	10.3	8.8	4.2
P(H)	C	22.9	20.6	20.2	21.0	21.9	22.9	20.6	20.2	21.0	21.9
Blank	A	24.2	30.2	34.0	28.9	25.4	12.0	3.0	1.8	2.2	2.8
Blank	B	20.4	25.9	31.5	27.4	25.1	13.4	3.1	2.9	2.3	5.3
Blank	C	18.8	24.4	31.4	31.8	24.5	12.8	2.0	2.1	2.1	1.9

Mean and *SD* of Dissolved Oxygen (mg L^{-1}) in epilimnion and hypolimnion of mesocosms

Treatment	Epilimnion/Day					Hypolimnion/Day				
	0	4	11	18	32	0	4	11	18	32
Reference	21.5	18.4	21.7	19.9	22.1	14.0	14.4	13.8	13.1	11.5
	4.2	*4.5*	*4.0*	*3.1*	*0.7*	*7.9*	*5.7*	*5.2*	*6.4*	*8.4*
Kitchener	22.2	27.3	28.8	25.7	22.0	13.1	2.0	2.1	2.2	2.5
	5.4	*8.3*	*4.2*	*2.3*	*2.1*	*2.6*	*1.2*	*0.8*	*0.6*	*0.9*
Guelph	21.2	26.8	32.3	29.3	25.0	12.7	2.7	2.3	2.2	3.3
	4.4	*5.3*	*4.0*	*2.7*	*1.4*	*3.0*	*0.8*	*1.0*	*0.4*	*2.3*
N(L)+P(L)	19.3	23.1	21.7	22.4	28.9	11.6	8.9	7.2	4.8	6.4
	4.1	*7.4*	*5.1*	*2.7*	*0.7*	*2.3*	*4.7*	*2.6*	*1.1*	*3.0*
N(H)	21.8	14.9	13.9	12.7	12.7	7.5	6.6	3.7	5.7	5.9
	2.0	*1.0*	*2.0*	*0.8*	*0.5*	*2.5*	*3.6*	*1.6*	*0.3*	*2.9*
P(L)	20.2	20.7	17.2	19.9	23.9	9.9	8.1	3.8	4.9	5.3
	1.3	*1.7*	*1.8*	*0.6*	*5.3*	*1.0*	*0.7*	*0.8*	*0.4*	*3.1*
N(H)+P(H)	21.9	29.4	22.6	22.7	23.7	11.3	10.7	4.0	3.7	3.5
	0.6	*1.9*	*0.2*	*0.8*	*1.8*	*3.4*	*0.7*	*1.6*	*0.3*	*0.8*
P(H)	20.2	20.2	21.8	23.0	23.6	10.1	8.4	3.4	3.9	4.3
	2.3	*1.8*	*4.6*	*0.4*	*1.6*	*2.4*	*3.6*	*1.7*	*0.3*	*2.5*
Blank	18.3	12.4	11.6	18.0	9.0	11.7	7.7	5.5	4.5	2.0
	1.8	*0.3*	*0.8*	*1.2*	*0.4*	*3.1*	*2.0*	*2.4*	*0.3*	*0.8*

Appendix O: pH in epilimnion and hypolimnion of mesocosms

Treatment	Replica	Epilimnion/Day					Hypolimnion/Day				
		0	4	11	18	32	0	4	11	18	32
Reference	1 A,B,C	9.03	7.80	9.18	9.33	10.33	7.46	6.96	7.98	8.23	8.59
Reference	3 A,B,C	9.06	8.79	7.56	9.51	10.26	6.93	7.49	7.56	7.69	7.40
Reference	8 A,B,C	9.05	8.41	8.99	9.57	10.30	7.02	7.28	7.56	8.05	7.78
Kitchener	4 A,B,C	8.64	9.29	9.91	9.55	10.44	7.03	7.18	7.39	7.26	7.78
Kitchener	6 A,B,C	8.84	9.12	9.94	9.72	10.41	7.27	7.38	7.39	7.33	8.36
Kitchener	9 A,B,C	8.79	8.75	9.92	10.50	10.51	6.99	7.15	7.27	7.21	8.33
Guelph	2 A,B,C	9.23	9.12	9.41	9.87	10.44	7.28	7.22	7.58	7.48	8.06
Guelph	5 A,B,C	8.78	8.46	9.54	9.84	10.37	7.06	6.88	7.26	7.21	7.81
Guelph	7 A,B,C	9.09	9.32	9.80	9.82	10.49	7.08	7.33	7.49	7.55	8.13
N(L)+P(L)	A	8.90	9.82	10.73	10.11	10.40	7.12	6.89	6.98	7.10	8.86
N(L)+P(L)	B	9.76	9.58	9.83	9.93	10.51	7.03	7.19	6.85	6.72	8.25
N(L)+P(L)	C	8.36	8.67	8.95	9.17	10.59	7.21	7.09	6.83	6.82	7.01
N(H)	A	8.56	8.23	8.15	8.16	7.95	7.19	6.94	6.90	7.02	6.90
N(H)	B	8.56	8.44	8.85	8.71	8.67	7.34	6.90	8.01	8.15	7.39
N(H)	C	9.23	8.46	8.39	8.32	8.39	6.65	7.10	6.64	6.73	6.88
P(L)	A	9.33	8.16	9.66	9.89	10.58	7.03	6.94	6.77	6.71	7.31
P(L)	B	9.02	7.99	10.12	10.19	10.58	7.01	6.87	7.16	6.53	7.39
P(L)	C	8.95	8.06	10.03	9.94	10.49	7.14	6.92	6.67	6.53	6.92
N(H)+P(H)	A	8.80	9.09	10.39	10.44	10.76	6.82	7.07	7.06	7.00	6.87
N(H)+P(H)	B	9.06	8.24	10.21	10.34	10.63	7.10	6.91	7.01	7.00	6.92
N(H)+P(H)	C	9.32	9.06	10.01	10.25	10.56	7.24	7.42	6.85	6.80	6.91
P(H)	A	8.85	8.01	9.56	9.73	10.49	6.88	6.73	6.56	6.60	6.54
P(H)	B	9.21	8.68	9.58	9.92	10.39	6.90	7.07	6.85	6.82	6.82
P(H)	C	9.15	8.38	9.92	10.32	10.10	7.01	7.01	7.79	7.42	6.99
Blank	A	8.89	8.81	7.97	8.02	8.30	7.43	7.11	6.98	6.95	6.58
Blank	B	9.01	8.36	8.09	7.92	7.49	6.82	7.00	6.77	6.92	6.58
Blank	C	9.07	8.63	8.50	8.19	7.83	8.14	7.28	6.67	6.84	6.79

Mean and *SD* of pH in epilimnion and hypolimnion of mesocosms

Treatment	Epilimnion/Day					Hypolimnion/Day				
	0	4	11	18	32	0	4	11	18	32
Reference	8.33	8.57	9.47	10.30	7.14	7.24	7.70	7.99	7.92	8.33
	0.27	*0.02*	*0.50*	*0.05*	*0.15*	*0.13*	*0.07*	*0.06*	*0.33*	*0.27*
Kitchener	9.05	9.92	9.92	10.45	7.10	7.24	7.35	7.27	8.16	9.05
	0.27	*0.02*	*0.50*	*0.05*	*0.15*	*0.13*	*0.07*	*0.06*	*0.33*	*0.27*
Guelph	8.97	9.58	9.84	10.44	7.14	7.14	7.45	7.41	8.00	8.97
	0.45	*0.20*	*0.03*	*0.06*	*0.12*	*0.24*	*0.16*	*0.18*	*0.17*	*0.45*
N(L)+P(L)	9.36	9.84	9.74	10.50	7.12	7.06	6.89	6.88	8.04	9.36
	0.61	*0.89*	*0.50*	*0.10*	*0.09*	*0.15*	*0.08*	*0.20*	*0.94*	*0.61*
N(H)	8.38	8.46	8.40	8.34	7.06	6.98	7.18	7.30	7.06	8.38
	0.13	*0.36*	*0.28*	*0.36*	*0.36*	*0.11*	*0.73*	*0.75*	*0.29*	*0.13*
P(L)	8.07	9.94	10.01	10.55	7.06	6.91	6.87	6.59	7.21	8.07
	0.09	*0.24*	*0.16*	*0.05*	*0.07*	*0.04*	*0.26*	*0.10*	*0.25*	*0.09*
N(H)+P(H)	8.80	10.20	10.34	10.65	7.05	7.13	6.97	6.93	6.90	8.80
	0.48	*0.19*	*0.10*	*0.10*	*0.21*	*0.26*	*0.11*	*0.12*	*0.03*	*0.48*
P(H)	8.36	9.69	9.99	10.33	6.93	6.94	7.07	6.95	6.78	8.36
	0.34	*0.20*	*0.30*	*0.20*	*0.07*	*0.18*	*0.64*	*0.42*	*0.23*	*0.34*
Blank	8.60	8.19	8.04	7.87	7.46	7.13	6.81	6.90	6.65	8.60
	0.23	*0.28*	*0.14*	*0.41*	*0.66*	*0.14*	*0.16*	*0.06*	*0.12*	*0.23*

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