VERTICAL PHOSPHORUS MIGRATION IN BIOSOLIDS-AMENDED SOILS: CONCENTRATIONS IN SOILS AND LEACHATES

by

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Abstract

Vertical phosphorus migration in biosolids-amended soils: concentrations in soils and leachates

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The impacts of biosolids land application on soil phosphorus and subsequent transfer to aquatic ecosystems in the condition of the minimal slope were assessed. Soil, representing typical “Non response” Ontario soil, was amended with anaerobically digested biosolids at a rate of 8 tonnes/ha. Over five months, soil samples from two different depths were sequentially fractionated to determine various inorganic and organic phosphorus pools in order to evaluate phosphorus vertical migration within a soil profile. Soil leachate was analyzed for soluble reactive phosphorus and added to the aquariums mimicking receiving surface waters. Water from aquariums was tested for the presence of eutrophication. The results indicated that biosolids application did not significantly affect phosphorus concentrations in soil and did not cause phosphorus vertical migration. The concentrations of soluble reactive phosphorus also were not affected by biosolids. No signs of eutrophication were observed in receiving waters.

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List of Abbreviations

AB	Alberta
ADP	Adenosine diphosphate
AMP	Adenosine monophosphate
ATP	Adenosine triphosphate
BC	British Columbia
BOD	Biochemical oxygen demand
CBP	Canadian Biosolids Partnership
CBP	Calcium-bound phosphorus
CCME	Canadian Council of Ministers of the Environment
CDD	Chlorinated dibenzo-*p*-dioxins
CDF	Chlorinated dibenzofurans
CFA	Carbonate fluorapatite
CFIA	Canadian Food Inspection Agency
CFU	Colony forming units
CWWA	Canadian Water and Wastewater Association
DOM	Dissolved organic matter
DNA	Deoxyribonucleic acid
EQ	Exceptional quality
EuLA	European Lime Association
FAP	Fluorapatite
FOG	Fats, oils, and grease
GMSC	Greater Moncton Sewerage Commission
LBP	Loosely-bound phosphorus
LP	Labile phosphorus
MB	Manitoba
MBP	Metal-bound phosphorus
MLP	Moderately-labile phosphorus
MPN	Most probable number
NASM	Non-agricultural source materials

NB	New Brunswick
NBMA	Northwest Biosolids Management Association
NDIR	Non-dispersive infrared gas analyzer
NLP	Non-labile phosphorus
NS	Nova Scotia
NWT	Northwest Territories
OMAFRA	Ontario Ministry of Agriculture, Food and Rural Affairs
ON	Ontario
OU	Odour unit
PEI	Prince Edward Island
QC	Quebec
RNA	Ribonucleic acids
SK	Saskatchewan
SRP	Soluble reactive phosphorus
TCDD	2,3,7,8-tetrachlorodibenzo-*p*-dioxin
TEF	Toxic equivalency factor
TEQ	Toxic equivalent
TOC	Total organic carbon
TP	Total phosphorus
UNEP	United Nations Environment Programme
US EPA	United States Environmental Protection Agency
WEF	Water Environment Federation
WSP	Water-soluble phosphorus

1. Introduction

Even though the term “biosolids”, as it is understood now, only appeared at the end of 20th century, the concept of human waste application to agricultural lands has been known for thousands of years. This practice has demonstrated an ability to improve soil fertility. However, along with the potential advantages of biosolids application, come several environmental concerns, including heavy metal leaching and accumulation, potential presence and potential spread of pathogens, presence of organic contaminants, and nutrient enrichment, especially in phosphorus, that might contribute to cultural eutrophication. The concerns regarding high phosphorus levels in biosolids, potential transfer of this phosphorus through the soil in leachate, and the impact of this leachate on receiving waters are addressed in this paper.

1.1. Biosolids

1.1.1. The History of Biosolids Land Application

The concept of human waste application to agricultural lands derives from thousands of years ago (US EPA, 1997a; WEF, 2004; Sanin *et al.*, 2011), when Chinese societies began applying sewage to surrounding farmlands. Such practice provided a closed nutrient cycle that helped to maintain soil fertility and provided a means of disposal for waste. Chinese farmers so highly valued the ability of sewage to increase land cropping that they competed with one another to obtain as much sewage as possible. Other early urban civilizations, such as Egypt and Indus Valley, at that time had not yet discovered the advantages of human waste application to agricultural lands and were focused on developing sewage

systems that helped cities to get rid of the produced waste. However, these systems often just created problems, such as heavy downstream water contamination (WEF, 2004).

The large-scale land application of municipal waste began only about 160 years ago when flush toilets and municipal sewage systems were first introduced in Western Europe and North America. Until then, the untreated wastewater was discharged directly into streams, rivers, and lakes. Such practice resulted in heavy pollution of receiving water bodies (US EPA, 1999). The stink of the rivers became unbearable. Water supplies located downstream from discharge points became the source of various water-borne diseases, such as cholera. In 1850, the first sewage farms, the predecessors of current wastewater treatment plants, were established in England to treat discharges from interceptors (WEF, 2004). The concept of sewage farms quickly spread in Europe and around the globe. By 1875, many similar farms already served many European cities. About a dozen sewage farms were established in North America by the end of the century. While sewage farms alleviated water bodies' pollution, sewage sludge produced on these farms created a different set of problems, including the disposal of sewage sludge.

To address the sludge disposal problem, it was put to beneficial uses, such as land application (WEF, 2004). New, efficient methods of sewage sludge treatment were introduced in the 1900s to minimize the contamination of food crops (CWWA, 2009). All sewage sludge intended for land application had to be stabilized in a digestion process. The stabilization of biosolids served to decompose the solids, to reduce odours, and to destroy most of the bacteria in the material (CWWA, 2009). To differentiate between untreated sewage sludge and treated sewage sludge that can be safely land applied, the term

"biosolids" came into usage in 1991 (CCME, 2010; CCME, 2012, US EPA 2012). Today, biosolids are defined as a "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" (UNEP, 2009).

Today, the use of biosolids in agriculture is a worldwide practice. In this research, Canadian biosolids application practices were addressed. In the early 2000s, annual generation of biosolids in Canada reached 555,000 tonnes (CBP, 2007), 17% of which were landfilled, 22% of which were incinerated, 52% of which were land applied, and 9% of which were put in other beneficial uses (GMSC, 2006).

1.1.2. Biosolids Production

1.1.2.1. Sludge Formation

As stated above, biosolids represent the sludge produced during the wastewater treatment process. As wastewaters differ greatly, so do wastewater treatment plants and the sludge produced at them. Overall, most treatment steps could be divided into screening, primary treatment, secondary treatment, and tertiary treatment (Sanin *et al.*, 2011).

Screening:

Screening is a preliminary treatment meant to remove coarse solids that are present in wastewater in order to prevent damage to the treatment plant. Removed solids are too bulky to be utilized in biosolids. Instead they are sent directly to a landfill or an incinerator. Sand, gravel, and other heavy material that settle down in a grit tank following the

screening system are also commonly disposed of at a landfill (Spellman 1997; Spinosa and Vesilind 2001; Clesceri *et al.* 2008). The screening step of a wastewater treatment plant does not contribute to the production of biosolids.

Primary Treatment:

Primary treatment is a wastewater treatment step that follows preliminary screening. The purpose of this step is to reduce the amount of suspended solids in wastewater. At this stage, wastewater is held in a settling tank or clarifier, where all heavier solids settle at the bottom and lighter solids float to the top (Sanin *et al.*, 2011). Usually, more than half of the suspended solids are removed and over one third of the biochemical oxygen demand is reduced during primary treatment (Amuda *et al.* 2008). Solids removed from the bottom of the clarifier and floating materials skimmed from the top are combined together to become a raw primary sludge. This sludge is often very watery and has a high concentration of pathogens (Sanin *et al.*, 2011).

Secondary Treatment:

Secondary treatment of the wastewater is designed to remove BOD and residual solids (Sanin *et al.*, 2011), which are usually removed by either biological filtration or sludge activation (Amuda *et al.* 2008). At this step the biomass is cultured in the aeration tank and suspended in the wastewater. Cultured microorganisms reduce the oxygen demand of water by consuming organic material producing CO_2 (Spinosa and Vesilind 2001; Sanin *et al.*, 2011). At the end, biomass is settled out in the final clarifier and part of this is returned to the aeration tank (Spellman 1997; Clesceri *et al.* 2008; Sanin *et al.*, 2011). The portion of

the biomass that is not returned to the head of the system is called waste activated sludge. It is usually mixed with the raw primary sludge for further formation of biosolids (Sanin *et al.*, 2011). A typical wastewater treatment system combining primary and secondary treatments is presented in Fig. 1.1.

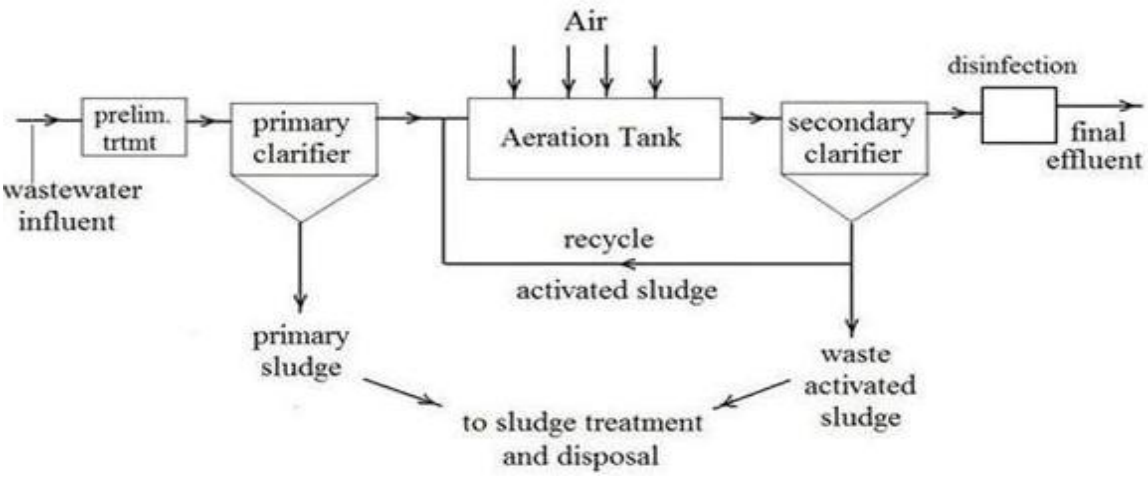


Fig. 1.1. Typical secondary wastewater treatment system (Bengtson, 2011).

Tertiary Treatment:

Tertiary treatment is the last step of a wastewater treatment process. It came into practice only several decades ago to address the issue of high nutrient levels in wastewaters. Nitrogen and phosphorus are removed during tertiary treatment by the use of physicochemical or biological methods (Sanin *et al.*, 2011). Tertiary treatment nutrient removal includes processes such as nitrification/denitrification, ammonia stripping, phosphorous precipitation, and overland flow. The sludge collected at the end is added to the mixture of the raw primary sludge and the waste activated sludge for further biosolids formation.

1.1.2.2. Biosolids Formation

Sludge collected from all three steps of wastewater treatment is stabilized by a digestion process. There are several different digestion processes applicable for sewage sludge stabilization. The type of process varies from one wastewater treatment plant to another.

Anaerobic Digestion:

One possible type of stabilization process is anaerobic digestion. This is the oldest and the most common type of biosolids formation used at wastewater treatment plants. Anaerobic digestion is a series of processes where microorganisms, in the absence of oxygen, break down biodegradable organic materials in sludge. Four main processes involved in anaerobic digestion are hydrolysis, fermentation, acetogenesis, and methanogenesis. Microorganisms, predominantly bacteria, are involved at each of the reactions. Undergoing all four steps of the digestion process, large polymers present in the sludge are finally converted to methane and water (Amuda *et al.* 2008; Clesceri *et al.* 2008).

Anaerobic digestion is performed in airtight reactors for an extended period of time. Time required for the process is dictated by the temperature regime. At 35^0C to 55^0C, anaerobic digestion typically requires approximately 15 days; at 20^0C, sludge digestion typically requires at least 60 days (US EPA, 1997a).

Anaerobic digestion results in about 40% reduction of volatile solids, significant reduction of pathogens, and reduction of objectionable odour (Sanin *et al.*, 2011). However, the resulting biosolids from the anaerobic digestion are still rich in ammonium and phosphorus.

Aerobic Digestion:

Another type of the stabilization process used at wastewater treatment plants is aerobic digestion. Overall this process can be described as of oxidizing and decomposing of the organic part of the sludge by microorganisms in the presence of oxygen. During this process, sludge is usually left in open or closed, but aerated, tanks for an extended period of time (i.e., several days). This results in the formation of CO_2, water and ammonia. Further oxidation of ammonia to nitrates decreases the pH of the sludge to the required levels for use in land application (Spellman, 1997; Clesceri *et al.,* 2008). Aerobic digested sludge is stabilized in terms of its oxygen demand and fraction of volatile solids (Sanin *et al.*, 2011).

Anoxic-Aerobic Digestion:

The combination of anaerobic and aerobic processes, called anoxic-aerobic digestion, could be used as an alternative to the two digestion processes described above. During this process, collected sludge is kept in an airtight tank where aerators are turned on and off intermittently. When oxygen accesses the system, microorganisms convert ammonium present in sludge into nitrate, which is then denitrified while aerators are turned off. Anoxic-aerobic digestion is efficient not only in pathogen removal, but also in reduction of nitrogen concentrations in resulting biosolids by enhancing nitrification-denitrification (Clesceri *et al.,* 2008).

High pH – High Temperature Digestion:

Finally, high pH – high temperature digestion could be applied for sludge stabilization. During this process, lime or any other suitable alkali is added to the sludge to raise its pH

and kill pathogens. To achieve the reduction of pathogens below detectable levels, sludge is held at pH 12 or above for at least 72 hours. For at least 12 out of these 72 hours, the temperature is maintained at 52^0C (US EPA, 1997a). High pH – high temperature digestion results in elimination of pathogens (i.e., with no risk of pathogen regrowth) and significant reduction of vector attraction and objectionable odour (EuLA, 2010). The resulting biosolids from the high pH – high temperature digestion are rich in organic carbon and nutrients.

As a result of the different types of digestion processes, as well as differences in the waste streams that generate the sludge, biosolids vary greatly in their chemical composition, pathogen load, nutrient content, and odour levels.

1.1.3. Biosolids Standards

Land applicable biosolids resulting from digestion processes could be classified based on the various quality criteria such as trace elements, heavy metals, pathogen reduction, and vector attraction and odour reduction. Classification systems vary broadly among Canadian provinces. Small territories, such as Nunavut and Yukon, adopted Canadian Food Inspection Agency's (CFIA) standards in their biosolids classification. In Saskatchewan (SK) and Manitoba (MB) there is only one category of biosolids; whereas two categories of biosolids (i.e., Class A and Class B) are recognized in British Columbia (BC), Nova Scotia (NS), and Northwest Territories (NWT). Three categories of biosolids (i.e., Exceptional quality (EQ), Class A and Class B) are recognized in Prince Edward Island (PEI) and New Brunswick (NB). In Quebec (QC) and Alberta (AB) biosolids are classified into entirely different categories and different naming conventions are adopted. There, biosolids can be

classified based on the levels of chemical contamination (i.e., Category C1 and C2), levels of pathogens (i.e., P1 and P2) and odour characteristics (i.e., O1, O2 and O3); consequently, biosolids are classified into one of the 12 possible classes (e.g., C1P1O1, the best quality product, or C1P2O3 etc). In Ontario (ON), biosolids are categorized into metal (i.e., CM1 and CM2), pathogen (i.e., CP1 and CP2) and odour (i.e., OC1, OC2 and OC3) categories. Accordingly, all biosolids in ON could be classified into one of the 12 possible classes (CM1CP1OC1 (the best quality product) or CM1CP2OC3, etc.) (CCME, 2010).

As biosolids are primarily managed under provincial legislation, standards and parameters to ensure the quality of biosolids are also often developed provincially. The requirements for various parameters utilized to establish quality criteria at the federal and provincial levels are outlined below.

1.1.3.1. Standards for Metals

Canadian Food Inspection Agency does not establish specific requirements for metal levels in biosolids or biosolids-amended soils, but most provinces have their own standards for maximum acceptable metal levels in biosolids products (CCME, 2010). Metal standards of several provinces are presented in Table 1.1.

For biosolids to be given the best product category, all metal concentrations of these biosolids should be within the limits of this category. If even one metal concentration exceeds the standards established for this category, biosolids cannot be given the best product category. For example, if biosolids produced in Ontario had 2 mg/kg Cd, 80 mg/kg

Cu, 100 mg/kg Pb, but 240 mg/kg Cr, it would be given the category CM2 instead of the category CM1.

Table 1.1. Standards for metals in biosolids in Canada (CCME, 2010).

Jurisdiction	Metal Concentration in Biosolids (mg/kg of dry mass)										
	Cd	Cr	Cu	Hg	Ni	Pb	Zn	As	Se	Mo	Co
SK	20	1060	760	5	180	500	1850	75	14	20	150
NS (Class A)	3	210	400	0.8	62	150	700	13	2	5	34
NS (Class B)	20	1060	760	5	180	500	1850	75	14	20	150
PEI (EQ)	39	1200	1500	17	420	300	2800	41	100	-	-
PEI (Classes A, B)	85	-	4300	57	420	840	7500	75	100	75	-
BC (Class A)	3	100	400	2	62	150	500	13	2	5	34
BC (Class B)	20	1060	2200	15	180	500	1850	75	14	20	150
QC (C1)	3	210	400	0.8	62	150	700	13	2	5	34
QC (C2)	10	1060	1000	4	180	300	1850	41	14	20	150
ON (CM1)	3	210	100	0.8	62	150	500	13	2	5	34
ON (CM2)	34	2800	1700	11	420	1100	4200	170	34	94	340

1.1.3.2. Standards for Pathogens

Not all Canadian provinces have standards for pathogen levels in biosolids. Instead, these provinces specify the treatment that should be used for biosolids formation. For example, MB requires all biosolids meant for land application to be obtained through anaerobic digestion. In Alberta, the three-level digestion process of sludge is specified. In other provinces, *Salmonella, E. coli*, and fecal coliforms are used as indicators of pathogen contamination of biosolids. According to the CFIA's standards for pathogens, *Salmonella* must be absent (non-detectable) and the level of fecal coliforms must not exceed 1000 Most Probable Number (MPN) per gram of the total solids (CCME, 2010).

In QC, based on the pathogen levels, all biosolids are divided into 2 categories, P1 and P2. Sewage biosolids that meet P1 standards must demonstrate an undetectable level of *Salmonella* in 10g wet weight of the sample. For P2 *E. coli* is used as an indicator. To meet P2 standards, the level of *E. coli* should be lower than $2x10^6$ colony forming units (CFU)/g of total solids' dry weight.

US EPA standards are used in NB for pathogen levels in biosolids. According to these standards, sewage biosolids must meet levels of *Salmonella* < 3 CFU or MPN/4g or 100 ml and levels of fecal coliforms < 3 MPN/g.

Ontario requires all biosolids to be treated in an approved process to reduce pathogens. Under the NASM (non-agricultural source materials) Plan, land applicable biosolids are sub-categorized into CP1 and CP2. Sewage biosolids that meet the CP1 standard must meet levels of *E. coli* ≤1,000 CFU/g dry weight, *Salmonella* < 3 CFU or MPN/4g or 100 ml, and *Viable Helminth ova* and total culturable enteric virus non detectable per 4g or 100 ml. The CP2 category does not have specific standards regulating *Salmonella* or total culturable enteric virus, but sewage biosolids categorized as CP2 have to meet the standard for *E. coli* < $2x10^6$ CFU/g of total solids dry weight standard (CCME, 2010).

In all other provinces, the *Salmonella* standard is < 3 MPN/ 4g, and the standard for fecal coliforms is < 1000 MPN/g of the total solids meant for the highest quality biosolids. For the lower quality products, the standard for fecal coliforms is below 2,000,000 MPN/g (CCME, 2010).

In order to be land applied, the biosolids must meet the highest category criterion (CCME, 2010).

1.1.3.3. Standards for Organic Chemical Contaminants

Dioxins and furans are used as indicators of organic chemical contamination of biosolids (CCME, 2010). The dioxins that might be present in biosolids include seven chlorinated dibenzo-*p*-dioxins (CDDs), 10 chlorinated dibenzofurans (CDFs), and 12 coplanar PCB congeners (National Research Council, 2002). Each of these compounds is assigned a toxic equivalency factor (TEF) that represents a potency to activate the aryl hydrocarbon receptor (AhR) relative to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). Multiplying the concentrations of each CDD, CDF, or dioxin-like PCB in biosolids by their TEFs and summing the products yields the toxic equivalents (TEQs) in that material (National Research Council, 2002).

According to CFIA, the maximum acceptable cumulative addition of organic chemicals to soil should not exceed 5.4 mg Toxic Equivalent (TEQ)/ha over 45 years. The standard for dioxins and furans are therefore calculated for each province individually regarding permitted biosolids application rate (CCME, 2010). As such, at a rate of 8 tonnes/ha, the level of dioxins and furans should not exceed 15 ng TEQ/kg of biosolids.

1.1.3.4. Standards for Odour

Defined standards for an odour level are not found in many provinces. Ontario, however, addresses potential odour issues associated with biosolids land application. Under NASM Plan, land applicable biosolids are sub-categorized into OC1, OC2, and OC3, based on the

detection threshold of the material. OC1 biosolids are the least odourous; OC3 are the most odourous (OMAFRA, 2012a). The corresponding ranges of odour detection thresholds for all three categories are shown in Table 1.2.

Table 1.2. Odour categories and their corresponding ranges of odour detection thresholds.

Odor Category	Odour Detection Threshold
OC1	Less than 500 odour units (OU) (OC1 <500 OU)
OC2	More than or equal to 500 odour units but less than 1,500 odour units (500 OU ≤ OC2 < 1,500 OU)
OC3	More than or equal to 1,500 odour units but less than 4,500 odour units (1,500 OU ≤ OC3 < 4,500 OU)

The odour unit (OU) is the number of unit volumes of odourless gas required to dilute one unit volume of odourous gas to reach the odour panel's detection threshold.

Given the complexity of the human sense of smell to odours, odour categories of biosolids are defined by a standardized method using dynamic olfactometry.

The odour category affects land application standards, such as separation distances to neighboring properties and methods of application. In general, greater setback distances and incorporation are required for more odourous materials (OMAFRA, 2012a).

1.1.3.5. Standards for Application Rates

In Canada, if the biosolids are treated and meet the federal fertilizers act regulatory requirements then they can be classified as a commercial fertilizer or soil amendment. Biosolids suppliers provide the directions for their biosolids use, including the frequency, timing and rate of application, as well as target crops on which the product is intended for use, which are required to be on the label. These directions should be created to minimize the potential environmental risks associated with the biosolids use.

In ON, in agreement with the NASM Plan, biosolids intended for land application must meet beneficial use criteria (i.e., demonstrate beneficial use for either organic matter content, nutrients, pH balance or irrigation) and comply with maximum application rates for nitrogen, phosphate, and depending on the treatment method and digester feedstock. Ontario also has numerous land application requirements that specify waiting periods for harvesting tree fruits and grapes, vegetables, hay and haylage, and sod as well as grazing horses, cattle, swine, sheep and goats (CCME, 2010). Some waiting periods and other requirements for land application of biosolids in Ontario are provided in Table 1.3.

Separation requirements for land application of biosolids in Canada are also established provincially. These requirements are designed to protect the surface and ground water quality, and human health. The separation requirements for land application of biosolids in ON are listed in Table 1.4.

Table 1.3. Waiting periods and other requirements for land application of biosolids as regulated under O.Reg 267/03 (CCME, 2010).

Application Rate	Regulation application of sewage biosolids cannot exceed 22tones/ha/5yrs dry weight based on regulated metals. Maximum application rates may also be restricted by other parameters such as metals, boron, sodium, fats and oils, and grease upon Director request). The most restrictive rate will govern.
Pasture	3 weeks for CM1 and CP1 and 2 months for CM2 and/or CP2 for horse, beef or dairy cattle 3 weeks for CM1 and CP1 and 6 months for CM2 and/or CP2 for swine, sheep or goats
Livestock feed	3 weeks for hay and haylage
Vegetables	3 weeks for CM1 and CP1 and 12 months for CM2 and/or CP2
Other Crops	3 weeks for CM1 and CP1 and 3 months for CM2 and/or CP2 for tree fruits & grapes 3 weeks for CM1 and CP1 and 15 months for CM2 and/or CP2 for small fruits 3 weeks for CM1 and CP1 and 12 months for CM2 and/or CP2 for tobacco
Commercial Sod	3 weeks for CM1 and CP1 and 12 months for CM2 and/or CP2

Table 1.4. Separation requirements for land application of biosolids as regulated under O.Reg 267/03 (CCME, 2010).

From	Distance (m)
Residential/ Dwellings	Residential (single dwelling): - OC1 – no application <25m - OC2 – no application <25m, 25-90m injection or spreading and incorporation within 6 hours, >90m no restriction - OC3 – no application <100m, 100-450m injection or if injection not possible spreading and incorporation within 6 hours, >450m injection and incorporation within 24 hours.
Institutional/ Commercial/Schools/ Parks and playgrounds	Residential areas, commercial, community or institutional uses: - OC1 - <50m no application - OC2 – no application <50m, 50-450m injection or spreading and incorporation within 6 hours, >450m no restriction -OC3 – no application <200m, 200-900m injection or spreading and incorporation within 6 hours, >900m injection or spreading and incorporation within 24 hours
Surface Water/ Ground Water/Wells	Surface Water: -CM1 and CP1 – if a 3m vegetable buffer is along surface water, in the next 10m biosolids must be injected, incorporated within 24 hours or applied to a living crop or on a field with at least 30% crop residue -20m if no vegetated buffer. - CM2 and/or CP2 – 20m Water Table: -CM1 and CP1 – no application <30cm - CM2 and/or CP2 – no application <30 cm, 30-90cm based on risk of groundwater contamination

From	Distance (m)
	Wells: CM1 and CP1- Municipal – 100 m, Drilled (6m water tight casing & ≥ 15m well depth) – 15m, other – 30m CM2 and/or CP2 – Municipal – 100m, Drilled (6m watertight casing & ≥ 15m well depth) – 15 m, other – 90m Depth to Bedrock: <30cm no application 30-100cm based on material quality and state (solid vs. liquid) >100cm no restriction based on bedrock
Bedrock	Depth to Bedrock: < 30cm – no application 30-100 cm – application based on material quality and state (solid or liquid) >100 cm – no restrictions for application
Other	Prohibition on land application anytime when the ground is frozen, snow covered and in winter (between Dec 1 and March 31st of the following year)

1.1.4. Fates of Biosolids

The greatest portion (52%) of biosolids produced at wastewater treatment plants in Canada is land applied (GMSC, 2006). Nevertheless, there are several other mechanisms of biosolids disposal, such as incineration, mine reclamation, incorporation into building materials, and landfill disposal (US EPA, 1993; Sanin *et al.*, 2011).

1.1.4.1. Incineration

Incineration of biosolids is attractive both for volume reduction and energy recovery (Roy *et al.,* 2011). Additionally, high-temperature processes ensure the elimination of pathogens that may be present in biosolids. Usually, biosolids incineration is accomplished in two steps. The first step is the drying process, where biosolids are dewatered to between 15 and 35% solids. The second step is the actual combustion of the volatile fraction of the solids that occurs at temperatures > 480^0C. As 65 to 75% solids are combustible, the volume of ash produced is significantly lower than that of the original biosolids (Sanin *et al.,* 2011). This ash then can be used in construction materials as a binding agent (Sanin *et al.,* 2011) or disposed of in landfill. If solids are dewatered to approximately 30% solids or higher and their heat value is sufficient, the combustion process can be self-sustaining, and supplemental fuel is not required (US EPA, 2003a).

There are several disadvantages of incineration as a method of biosolids disposal. The greatest disadvantage is the release of greenhouse and other gasses, such as CO2, NOx, and SOx. Moreover, trace elements may also be concentrated up to ten-fold from their original concentration, which complicates further handling of the combustion product. Finally, incineration eliminates any potential for recycling nutrients and organic matter (UNEP, 2009).

1.1.4.2. Mine Reclamation

Mining is a very important branch of Canadian industry. However, the mining process can severely damage soil structure at mining sites and around them. In the aftermath of this

damage, soils are often unable to support plant life due to the altered pH, lack of nutrients and organic matter, and other ecosystem changes (NBMA, 2004). To address this problem, nutrient-rich biosolids can be utilized at abandoned mining sites to replace lost topsoil. Such practice can improve soil fertility and stability, thus decreasing erosion and aiding in re-vegetation (NBMA, 2004).

There are several great advantages of biosolids use in mining reclamation. The first advantage is the ability to dispose great volume of biosolids at once. Secondly, this allows restoration of vegetation that would be very hard to restore otherwise (Stehouwer *et al.,* 2006). Additionally, as lower classes of biosolids can be applied at mining sites, mine reclamation is a way to use of lower class biosolids that cannot be applied to agricultural land. However, the research on nutrient and trace element leaching following mine reclamation with biosolids demonstrated that large applications of low-C/N biosolids could negatively impact an area's water quality (Stehouwer *et al.,* 2006). Even though nutrient losses could be minimized by appropriate application rates, blending with other residuals, and vegetative establishment, biosolids reclamation practices could not yet be called the safest method of biosolids disposal from the environmental perspective.

1.1.4.3. Landfill Disposal

Landfill disposal of biosolids is the easiest solution since it simply places all the waste into a monofill (i.e., a landfill that accepts only wastewater treatment plant biosolids), or in a co-disposal landfill (i.e., a landfill that combines biosolids with municipal solid waste). In some cases, landfilling of biosolids can also be the most economical management solution, as it requires little more than transportation. Nonetheless, due to the environmental risks

associated with burying of biosolids, the landfilling option is only considered when land application or other beneficial reuse is not possible. Among these risks is methane production as well as leaching of various chemicals and heavy metals (Evanylo, 2009). Additionally, landfilling of biosolids eliminates their reuse potential (US EPA, 2003b). Valuable plant nutrients and organic matter are lost (Evanylo, 2009). For these reasons, landfilling should not be seen as a long-term solution for biosolids disposal (UNEP, 2009).

1.1.5. Biosolids Land Application

Given the negative consequences of other biosolids disposal practices discussed above and driven by increased interest in beneficial re-use of waste residuals, land application has become the most common way to manage biosolids produced at wastewater treatment plants (US EPA, 1997a; GMSC, 2006). In many communities, it is now the most cost-effective and environmentally safe method (US EPA, 1997a). Land application of biosolids is possible on both forestlands and agricultural lands. Considering the area, biosolids could be spread or sprayed on the soil surface, or incorporated or injected into soil (US EPA, 1997a).

1.1.6. Benefits of Biosolids Land Application

Agricultural land application is a viable way to manage municipal biosolids. Biosolids are valued as a source of macro- and micronutrients, and organic matter necessary for healthy growth (Shober *et al.* 2003; Atalay *et al*., 2007). Two major nutrients provided to the crops by the biosolids are nitrogen (N) and phosphorus (P) (Pritchard *et al.,* 2010). Both nitrogen and phosphorus are important for plant growth and are used in a large amount.

Unfertilized soil, however, may be deficient in these major nutrients (Silva and Uchida, 2000). Land application of biosolids can also improve soil physical properties. It can increase the amount of pore space available for water and air entry and root growth (US EPA, 1997a). Moreover, if applied to sandy soils, biosolids increase water-holding capacity of the soil and provide chemical sites for nutrient exchange and absorption (US EPA, 1997a).

Finally, biosolids are valued for their relatively low cost compared to synthetic fertilizers (Vasileski, 2007). Both farmers and the general public may benefit from the cost savings resulting from biosolids land application (US EPA, 1997a).

1.1.7. Environmental Concerns Related to Biosolids Land Application

Even though municipalities, farmers, and the general public may benefit from the use of biosolids on agricultural lands, there are some significant environmental concerns related to this practice. Some of these concerns include potential for heavy metals leaching and accumulation. However, the US Geological Survey conducted in Colorado from 1999 to 2003 determined that concentrations of nine regulated trace elements in the biosolids-amended soil were relatively uniform, and their concentration did not exceed the regulatory standards (Yager *et al*., 2004). A study by Korbouwlesky *et al.* (2002), conducted in France, also established that neither total nor available heavy metal concentrations increased in soil after application of up to 90 tonnes/ha of biosolids. A later study of Gasco *et al.* (2005) found that the average metal concentrations and maximum metal concentrations in leachate were below the regulatory limits for irrigation water and that most of the metals, except Pb and Ni, were below regulatory limits for drinking water. Gove

et al. (2001) demonstrated that when biosolids are composted prior to application, they are unlikely to increase the risk of groundwater contamination with any heavy metals. Recently, some Canadian provinces have since significantly decreased permitted rates of Pb and Ni in biosolids destined for land application (CCME, 2010). Based on these more stringent limits, the likelihood of exceeding soil standards of these elements in future will be even lower.

Another environmental concerned related to biosolids land application is high levels of nutrients and their potential leaching to surface and groundwater (Oenema and Roest, 1998). In the past, nitrogen was the nutrient of concern due to its ability to leach into the ground water consequentially increasing the levels of nitrates in waters and accelerating the process of eutrophication (Sharpley *et al.,* 1987; Foy and Withers, 1995). Now, nitrogen application rate is regulated and biosolids are land applied according to the plants' nitrogen needs. Since biosolids have a low N:P ratio, their application rate based on restriction of nitrogen may still result in excess phosphorus being added to the soil. Such over-application has made phosphorus a nutrient of interest, as it provides the potential for phosphorus transport to near-by water bodies. Phosphorus has been recognized as an important nutrient in determining the function and productivity of freshwater bodies (Levine, *et al.,* 1997), but its overabundance can lead to eutrophication (Daniel *et al.,* 1998), which in turn can result in fish-kills (Glasgow and Burkholder, 2000), interdiction of shellfish aquaculture (Joint *et al*., 1997), loss or degradation of aquatic plants (McGlathery, 2001; Burkholder *et al.*, 1992), partial or complete elimination of recreational uses of the water (Smith *et al.*, 1999), and overall decrease in water quality.

Several studies (Elliot *et al.,* 2000; Elliot *et al.,* 2005; Alleoni *et al.,* 2008; Hanief 2011) have confirmed high phosphorus levels in runoff losses from soils amended with biosolids, while other studies (Ryden *et al.,* 1974; Foy and Withers, 1995; Correll, 1998; Pierzynski *et al.,* 2000; Manahan, 2001) demonstrated that phosphorus loss from agricultural land significantly contributes to surface water eutrophication. Nonetheless, these studies did not show direct connection between surface water eutrophication and biosolids land use. One might assume that if biosolids are the source of phosphorus loss and if phosphorus loss contributes to surface water eutrophication, then biosolids application may cause surface water eutrophication. However, this leap may be premature. The abilities of phosphorus to migrate from soil into water bodies and to affect aquatic systems are directly related to the forms of phosphorus present in soil (Heathwaite and Johnes, 1996). The forms of phosphorus and their proportions, however, vary among different types of fertilizers (Irshad *et al.,* 2008). To properly assess potential risk of biosolids land application to surface waters, it is crucial to fully understand their behaviour in soil. It is important to know which forms of phosphorus are present in biosolids and how these forms change within soil as biosolids weather, as this affects phosphorus transport towards water bodies. Even if the form in which phosphorus was initially applied is known, this is not necessarily the same form in which phosphorus enters water bodies (Heathwaite and Johnes, 1996).

1.2. The Phosphorus Cycle

Phosphorus is one of the most important elements in nature. It participates in many biogeochemical processes in the environment. Every living cell requires phosphorus as a

component of ATP, DNA and RNA, phospholipids, and many other biomolecules that take part in energy storage and transportation, reproduction, structure (Conley *et al.* 2009; Ingall *et al.* 2011), and growth (Benitez-Nelson 2000).

Being a limiting nutrient and controlling net carbon uptake, phosphorus plays a vital role in determining the function and productivity of some terrestrial (Tiessen *et al.,* 1984; Roberts *et al.,* 1985) and many aquatic (Conley, 2009) ecosystems. The main source of phosphorus in nature is soil weathering, which makes the release of phosphorus from apatite dissolution a crucial control of ecosystem productivity (Tiessen et al., 1984; Roberts *et al.,* 1985). Likewise, the weathering of phosphorus and its transport from the terrestrial system is the only appreciable source of phosphorus to aquatic systems. On greater time scales, this supply of phosphorus also limits the total amount of primary production in the ocean (Holland, 1978; Filippelli and Delaney, 1994).

Due to the restraints of phosphorus availability in nature, phosphorus is generally recycled to various degrees in ecosystems. This process is called "the phosphorus cycle". The phosphorus cycle is the biogeochemical cycle that describes the movement of phosphorus in nature. It differs significantly from biogeochemical cycles of other important nutrients, such as nitrogen, carbon, sulfur, and oxygen. Unlike the other elements, phosphorus does not have a major gaseous form in the natural environment. Although phosphine (PH_3) may be produced via the anaerobic enzymatic reduction of phosphate and thereafter transported into the atmosphere (Glindermann *et al*., 1996), it quickly reverts to phosphate in an aerobic environment. This limits the contribution of the atmosphere to the phosphorus cycle. Due to the lack of an atmospheric deposition of phosphorus to the

oceans, phosphorus limitation is more likely than nitrogen limitation on geological time scales (Karl *et al.,* 2001). Sediments (i.e., crustal rocks and soil > 60 cm deep and marine sediments), soils (0-50 cm), biomass phosphorus, surface and deep-ocean, and mineable phosphorus are greater phosphorus reservoirs (Ruttenberg, 2003; Jasinski, 2009; Smit *et al.,* 2009). However, the greatest portion of readily available phosphorus is held in soils in a variety of forms. The most common phosphorus form in nature is an apatite mineral. Apatite minerals contain phosphate oxyanions linked by Ca^{2+} cations to form a hexagonal framework. However, chemistries and structures of different apatite minerals vary widely and depend on the environment. Overall, apatite minerals could be formed in igneous, metamorphic, sedimentary and biogenic environments, which determine an elemental composition at the corners of the hexagonal cell (McClellan and Lehr, 1969). Igneous fluorapatite (FAP) and sedimentary carbonate fluorapatite (CFA) are the most abundant of the igneous apatite minerals (Filippeli, 2008).

The phosphorus global cycle consists of four main steps: (a) tectonic uplift and exposure of apatite minerals to the elements of weathering; (b) weathering and subsequent erosion of the parent rocks resulting in the formation of soils with soluble and particulate phosphorus; (c) transport of the soluble and particulate components to streams, lakes, and oceans; and, (d) sedimentation and subsequent lithification of deposited sediments into new rocks. The cycle then repeats itself starting with the tectonic uplift (Ruttenberg, 2003). Schematicaly, the phosphorus cycle is presented in Fig. 1.2.

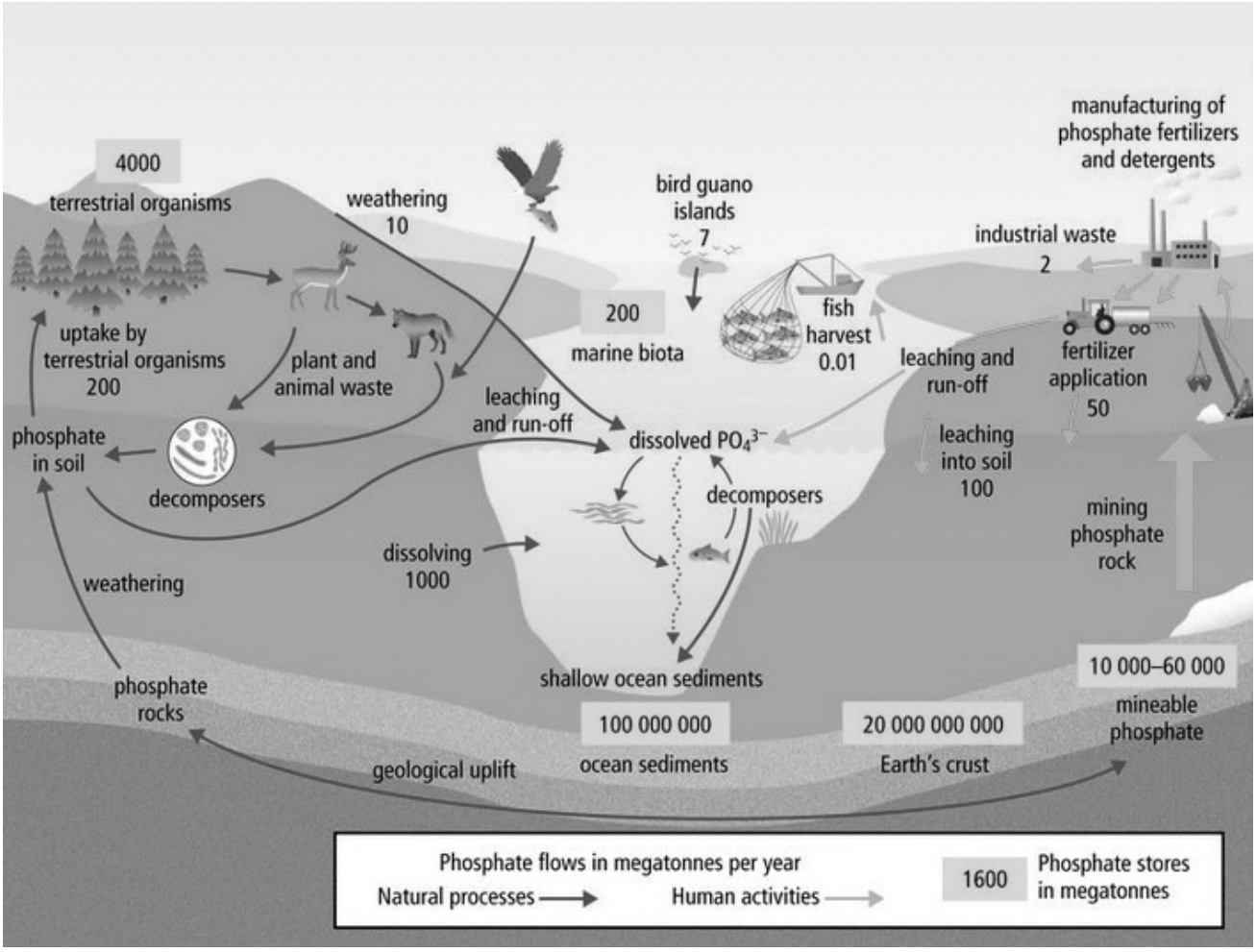


Fig. 1.2. Phosphorus global cycle (McGraw Hill Education, 2008).

1.2.1. Terrestrial Phosphorus Cycle

The release of phosphorus within the phosphorus cycle starts from weathering of apatite minerals, where apatite minerals weather congruently as a result of reaction with dissolved carbon dioxide in the form of carbonic acid:

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

Weathering reactions are driven by the exposure of minerals to acids mainly derived from microbial activity (Jurinak *et al.,* 1986). Additionally, the reduced pH formed by the decomposition of DOM can dissolve apatite minerals and release phosphorus to soil pore spaces (Schlesinger, 1997).

Phosphate solubilized during weathering becomes available for terrestrial plants. However, due to the phosphorus sorption by various soil constituents (particularly oxyhydroxides of ferric iron and aluminum), phosphates in soil-pore solutions are found in low concentrations. Moreover, much of the available phosphorus in soils is in organic matter, which is not directly accessible for plant nutrition. To increase the supply of phosphorus to roots, plants have evolved specific tactics. Some plants can increase root volume and its surface area to optimize uptake potential. Alternatively, other plants have developed symbiotic relationships with fungal mycorrhizae, which secrete phosphatase and other organic acids into the surrounding soil to cleave the phosphodiester bonds in organic matter, thereby releasing phosphorus to plants' root channels. Phosphorus is consumed by plants and then returned to the soil by the decay of dead plant material (Antibus *et al.,* 1981; Dodd *et al.,* 1987; Ruttenberg, 2003; Filippeli, 2008). Plants also minimize phosphorus loss by resorbing from their leaves much of their phosphorus prior to litterfall, and by efficient recycling of phosphorus from fallen litter. In extremely unfertile soils, phosphorus recycling is so efficient that topsoil contains virtually no phosphorus, as it is all tied up in biomass (Ruttenberg, 2003). When plants are consumed by animals, phosphorus returns to the cycle through the animal waste and manure.

1.2.2. Phosphorus in Soil

In soil, phosphorus exists in many different chemical forms, including both organic and inorganic phosphorus pools. Two major categories of phosphorus present in soil are particulate phosphorus and dissolved phosphorus (Logan, 1982).

1.2.2.1. Particulate Phosphorus

Particulate phosphorus is a form of phosphorus that consists of solid particulate and colloidal inorganic and organic phosphorus that can be captured by a filter (Carlson and Simpson, 1996). It can be composed of many physical forms, including, living organisms, mineral formations, and dead particulate organic matter (Logan, 1982; Jacobson *et al.,* 2000; Wetzel, 2001; Ruttenberg, 2003). In living organisms, phosphorus is present in nucleic acids (DNA and RNA), phosphoproteins, low-molecular-weight esters, such as enzymes and vitamins, energy storage molecules such as ATP and ADP, and a significant number of other compounds that have not been identified (Kovar and Pierzynski, 2009). Mineral forms of particulate phosphorus in soil could 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 the most abundant orthophosphate-containing form of minerals. It is not a single mineral, but a group of orthophosphate-containing minerals with chemical formula $Ca_{10}(PO_4)_3(X)_2$, where X represents OH^-, F^-, Cl^- or Br^- ions. Conditional on the ion present in the structure, apatite minerals are grouped into hydroxyapatite, fluorapatite,

chlorapatite and bromapatite. However, depending on the environment, hydroxyl, fluorine, and chlorine atoms may exist in a structure together or replace each other. In some cases, Ca^{2+} can be substituted by various other Group 1, Group 2, or transitional elements, such as manganese, strontium and rare-earth elements (Jacobson *et al.,* 2000). Some examples of apatite minerals and their formulas are presented in Table 1.5.

Table 1.5. Apatite minerals and their formulas (Ingall *et al.,* 2011).

Apatite	Apatite formula
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:

Non-apatite calcium phosphate minerals are a precursor phase for apatite formation in natural settings. Like apatite minerals, non-apatite calcium phosphate minerals vary in their structure and contain different cations and anions conditional on the environment. Some examples of non-apatite minerals and their formulas are presented in Table 1.6.

Table 1.6. Non-apatite minerals and their formulas (Ingall *et al.,* 2011).

Non-apatite mineral	Formula
Anapaite	$Ca_2Fe(PO_4)_2 \cdot 4H_2O$
Herderite	$CaBe(PO_4)F$
Messelite	$Ca_2(Mn,Fe^{2+})(PO_4)_2 \cdot 2H_2O$
Monetite	$CaHPO_4$
Scholzite	$CaZn_2(PO_4)_2 \cdot 2H_2O$
Whiteite	$(Ca,Fe,Mg)2Al_2(PO_4)_4(OH)_2 \cdot 8H_2O$

Aluminum Phosphate Minerals:

Aluminum phosphate minerals are formed when both alum and soluble phosphates are present in soil. Alum binds to phosphorus and sequesters soluble phosphorus into relatively insoluble aluminum phosphates. In agriculture, alum addition to fertilized soils can reduce the solubility of phosphorus by as much as 99% by forming different aluminum phosphates. Some examples of aluminum phosphate minerals and their formulas are presented in Table 1.7.

Table 1.7. Aluminum phosphate minerals and their formulas (Ingall *et al.,* 2011).

Aluminum phosphate mineral	Formula
Augelite	$Al_2(PO_4)(OH)_3$
Brazilianite	$NaAl_3(PO_4)_2(OH)_4$
Childrenite manganoan	$(Mn,Fe)Al(PO_4)(OH)_2 \cdot H_2O$
Eosphorite	$MnAl(PO_4)(OH)_2 \cdot H_2O$
Lazulite	$(Mg,Fe)Al_2(PO_4)_2(OH)_2$
Montebrasite	$(Li,Na)Al(PO_4)(OH,F)$
Variscite	$AlPO_4 \cdot 2H_2O$
Wardite	$NaAl_3(PO_4)_2(OH)_4 \cdot 2(H_2O)$

Iron and Manganese Phosphate Minerals:

Oxidized iron and manganese phosphate minerals are usually formed from the weathering of parent rocks. Due to the formation of anoxic and oxic zones in soil, iron and manganese phosphate minerals are often dissolved or modified when iron and manganese are reduced by changes in the redox potential. While phosphorus is not bioavailable in minerals containing oxidized iron and manganese, it becomes bioavailable in minerals containing reduced iron and manganese once appropriate conditions favor release of phosphorus (Ruttenberg and Berner, 1993; Ingall *et al.,* 2011). Some examples of iron and manganese phosphate minerals and their formulas are presented in Table 1.8.

Table 1.8. Iron and manganese phosphate minerals and their formulas (Ingall *et al.,* 2011)

Mineral	Formula
Childrenite manganoan	$(Mn,Fe)Al(PO_4)(OH)_2$
Eosphorite	$MnAl(PO_4)(OH)_2 \cdot H_2O$
Hureaulite	$Mn_5(PO_3OH)_2(PO_4)_2 \cdot 4H_2O$
Lazulite	$(Mg,Fe)Al_2(PO_4)_2(OH)_2$
Zwieselite	$(Fe,Mn)_2(PO_4)F$
Heterosite	$FePO_4$
Heterosite with Mn	$(Fe,Mn)(PO_4)$
Strengite	$FePO_4 \cdot 2H_2O$

In addition to being grouped by the origin and chemical speciation, particulate phosphorus in soil could be grouped by its bioavailability. Two major groups representing soil phosphorus are refractory (not readily available) and labile (readily bioavailable)

(Filippeli, 2008). The labile forms include water-soluble and loosely-bound fractions; whereas not readily available forms include metal-oxide-bound and calcium-bound fractions.

Water-Soluble Phosphorus:

The water-soluble fraction is the most bioavailable and mobile form of phosphorus in soil. It is mostly represented by orthophosphate and polyphosphate species (Hanief, 2011). Water-soluble phosphorus can be easily dissolved by water and transferred to plants or aquatic systems.

Loosely-Bound Phosphorus:

The loosely-bound phosphorus is represented by NH_4Cl-P. This fraction may consist of porewater phosphorus, phosphorus released from apatite minerals, and phosphorus from decaying cells of bacteria and plants that is loosely attached to the soil particles (Gonsiorczyk *et al.*, 1998).

Metal-Oxide-Bound Phosphorus:

The metal-oxide-bound phosphorus fraction (or metal-bound phosphorus) is represented by NaOH-P (Kaiserli *et. al.,* 2002). In this fraction phosphorus is associated with aluminum and iron. For ferrous phosphates, Ksp = 1.07×10^{-29}. For aluminum phosphates, Ksp = 9.84×10^{-21}. Metal-oxide-bound phosphorus is important for the evaluation of both short-term and long-term available phosphorus (Zhou *et al.,* 2001). This fraction is not directly available for the plants, but bioavailable phosphorus can be released under anoxic conditions (Kaiserli *et al.*, 2002).

Calcium-Bound Phosphorus:

Calcium-bound phosphorus is assumed to consist mainly of apatite minerals. This fraction is sensitive to low pH, but is overall stable and represents the immobile phosphorus form (Kaiserli *et. al.*, 2002). Apatite phosphorus is least available to plants since it is strongly sequestered within the soil (Golterman, 2001). Ksp = 6.8×10^{-37}.

Organic Phosphorus:

Organic phosphorus in soil is also divided into its labile, moderately-labile, and non-labile forms. Labile organic phosphorus is a form of phosphorus that is directly available to plants. Moderately-labile organic phosphorus is a form of phosphorus that is not directly available to plants but might become available in specific conditions (pH, presence/absence of oxygen, etc.). Non-labile organic phosphorus is a non-bioavailable form of phosphorus.

1.2.2.2. Dissolved Phosphorus

Dissolved phosphorus is a result of the interaction of phosphoric acid and soil porewater. This reaction is a stepwise dissociation of phosphoric acid that could be described as a series of the following reactions as shown by Karl and Yanagi (1997):

$$H_3PO_4(s) + H_2O(l) \rightleftharpoons H_3O^+(aq) + H_2PO_4^-(aq)$$

$$H_2PO_4^-(aq) + H_2O(l) \rightleftharpoons H_3O^+(aq) + HPO_4^{2-}(aq)$$

$$HPO_4^{2-}(aq) + H_2O(l) \rightleftharpoons H_3O^+(aq) + PO_4^{3-}(aq)$$

Dissolved phosphorus is especially important for microbes and plants as they can only absorb phosphorus in solutions.

1.2.3. Transport of Phosphorus from Continents to the Aquatic Environment

Phosphorus from soil can be removed by biological uptake. However, unused phosphorus has the potential of being transported to aquatic systems. Two major pathways of phosphorus transport to rivers are: (a) surface runoff and erosion and (b) subsurface flow and tile drainage (Oenema and Roest, 1998).

1.2.3.1. Runoff and Erosion

Runoff is a lateral movement of water over or just below the soil surface, which causes the short-term increase in water levels at the outlet of an area (Haygarth and Sharpley, 2000). Such movements are strongly related to rainfall, snow melt, and storm events. When precipitation/rainfall strikes the surface of the agricultural land, some portion of it infiltrates while another portion runs off. The runoff water moves downslope, eroding soil. Selective removal of clay-sized mineral particles and organic matter, both rich in phosphorus, occurs during the erosion and sediment transport process (Logan, 1982). Additionally, dissolved phosphorus can be removed from soil by runoff (Sims *et al.,* 1998). As a result, runoff water is enriched with soil phosphorus by the time it reaches streams and rivers. The infiltrating portion percolates within the shallow zone of soil (i.e., 1-5 cm) (Hansen *et al.,* 2002), where it reacts with the dissolved phosphorus held in soil pores before also leaving the soil in the form of runoff. The concentration of phosphorus leaving the soil by surface runoff and erosion is determined by the equilibrium between the

sediment and dissolved phosphorus (Logan, 1982). Phosphorus transport by surface runoff and erosion is illustrated in Fig. 1.3.

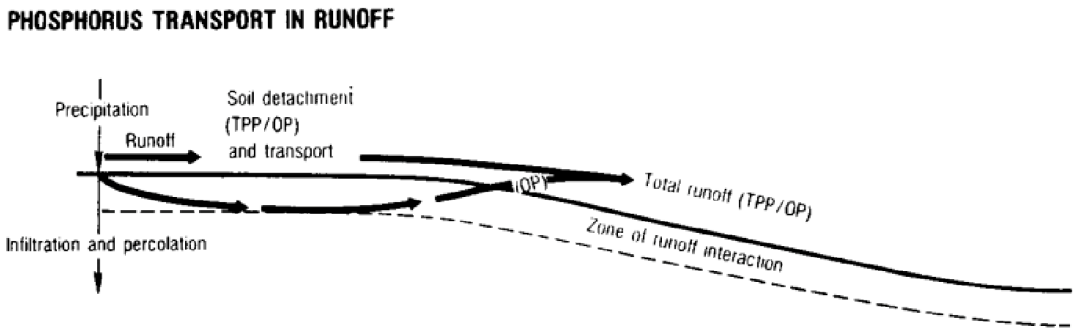


Fig. 1.3. Phosphorus transport by runoff (Logan, 1982).

1.2.3.2. Subsurface Flow and Tile Drainage

Phosphorus vertical transport occurs as a function of several processes (Magid *et al.,* 1999): (a) phosphorus desorption from the soil matrix; (b) phosphorus release via reductive dissolution of iron oxide particles; (c) phosphorus dissolution from phosphorus-enriched particles, and subsequent entry into preferential pathways (macropores); and (d) *in situ* generation and colloid-mediated phosphorus transport. The amount of phosphorus and the rate of phosphorus vertical transport depend on the source and forms of phosphorus (Makris *et al.,* 2006). When phosphorus is released, it percolates towards the underground drainage systems and/or groundwater. From there it is carried to streams and rivers. Artificial drainage systems, as they tend to accelerate drainage water transport to surface water bodies and to decrease the interaction of drainage water of subsoil, are a high environmental concern related to phosphorus loss through leaching (Sims *et al.,* 1998). Even though runoff and erosion account for approximately 60% of annual

phosphorus losses and the concentrations of phosphorus observed in surface runoff are 10.9 times higher than those found in subsurface drainage waters (Enright, 2004), the export of phosphorus from agricultural land by artificial drainage systems may be detrimental for the environment. The concentrations of phosphorus observed in subsurface drainage waters often exceed the values associated with eutrophication of surface waters (Bolton *et* al., 1970; Hanway and Laflen, 1974; Nicholls and MacCrimmon, 1974; Dils and Heathwaite, 1998; Enright, 2004). As much as 1.56 kg/ha of phosphorus can be annually lost from a typical Ontario cultivated field through tile drainage systems (Nicholls and MacCrimmon, 1974).

1.3. Research Rationale and Hypotheses

From the phosphorus cycle in nature, it becomes obvious that the form of phosphorus plays a great role in phosphorus' availability for biological uptake, and therefore, its effect in the environment. For that reason, while evaluating potential impact from phosphorus-containing biosolids, it is vital to gain full knowledge of the forms of phosphorus present in the biosolids. As phosphorus forms tend to change due to biochemical processes, while assessing the potential risks of land application of biosolids, it is also important to understand the behavior of phosphorus in soils amended by them.

Determination of different phosphorus forms in soil assists with the assessment of soil phosphorus bioavailability and provides the basis for the comparison of the amended and non-amended soils or soils amended with different fertilizers. Numerous studies have been done to evaluate phosphorus forms in amended soils.

Sharpley *et al.* (1984) reported that land application of cattle feedlot manure resulted in increased soil total phosphorus, in both inorganic and organic phosphorus fractions. Later, Sharpley and Moyer (2000) analyzed different types of manure and established that most manure phosphorus exists in inorganic form. The parallel study also specified that most of it is present in available (soluble) form and is susceptible to runoff loss after land application (Dou *et al.*, 2000). Hao *et al.* (2008) demonstrated that continuous, long-term application of manure in excess of crop nutrient demand led to a large accumulation of bioavailable phosphorus in soil, which, in turn, posed a threat to surface water quality. Results of the described studies provided a better understanding of the manure influence on soil phosphorus content and its potential effect on water bodies.

Penn and Sims (2002) analyzed phosphorus forms in biosolids-amended soils and losses in runoff. Their study illustrated that the change in phosphorus forms in soil after biosolids application is directly related to the type of biosolids and the type of soil. Overall biosolids application to the agricultural land was demonstrated to cause an increase in all forms of soil phosphorus. The study also showed that the runoff related losses of phosphorus from soil to surface water increased with biosolids application, and that soil type affected phosphorus concentrations in run-off. Sandy soils with low organic content were less able to retain phosphorus applied as biosolids. However, Penn and Sims did not evaluate potential risk to receiving water bodies from phosphorus losses.

Hanief (2011) analysed phosphorus fractions in biosolids and biosolids-amended soils. The biosolids used in this study were representative of those produced through anaerobic digestion along with the addition of ferric chloride in the tertiary treatment process. This is

the predominant type of biosolids that are produced in the province of Ontario. His results demonstrated, that such biosolids are especially rich in metal-bound phosphorus and are low in organic phosphorus content. The comparison of biosolids-amended soils to reference soils showed that addition of the biosolids led to at least a threefold increase in the total and inorganic phosphorus contents of soils and a fivefold increase in the organic phosphorus content of mineral soils. The timeframe of 120 days of the experiment indicated that bioavailable soil phosphorus decreased, whereas metal-bound phosphorus content significantly increased in biosolids-amended soils. However, Hanief used a formulated reference soil that initially had very little organic matter. That might be the main reason why it was possible to measure an increase in phosphorus concentrations relative to background. Ontario's agricultural soil, on the other hand, is relatively fertile, which would affect the retention and transformations of phosphorus. Therefore, in a scenario more probable for Ontario, the increase in phosphorus concentration might not be as significant as was shown by Hanief (threefold and fivefold increase). Furthermore, Hanief showed that substantial amounts of phosphorus from biosolids-amended soils were lost via surface runoff and that nutrient input from the surface runoff and tile water increased algal blooms in mesocosms, receiving runoff. Nonetheless, Hanief's experimental setup represented a worst-case scenario, where the maximum permissible loading rate was used for biosolids application on maximum permissible slope. Such experimental setup represents a very rare case for Southern Ontario, where slopes are seldom that great. Additionally, a large precipitation event was simulated soon after the application, the drainage of which entered directly into surface water. In real circumstances, however, this is unlikely to be the case.

The study of Hanief covers many existent gaps in the knowledge about Ontario biosolids effects on agricultural soils and surrounding water bodies. However, it would be useful to have a study simulating a more probable scenario, where soils are not on slope and where rainfall does not create large amounts of erosion and runoff immediately after application, and where phosphorus migrates vertically to drainage tile systems rather than horizontally with run-off.

Runoff and erosion can be an important pathway of soil phosphorus loss (Hanway and Laflen, 1974; Randall *et.al.,* 2000), and the majority of studies on phosphorus loss from agricultural land are devoted to them (Makris *et. al.,* 2006). Nevertheless, these losses represent only horizontal migration of phosphorus. On the other hand, vertical migration of phosphorus through the soil layers, in some cases, was proved to contribute to the phosphorus escape from agricultural lands (Hansen *et al.,* 2002). It is especially significant in areas with little slope, areas with shallow ground water, dry areas, or areas with a low phosphorus-sorbing capacity (Harris *et al.,* 1996; Simard *et al.,* 2000; Lu and O'Connor, 2001; Elliott *et al.,* 2000; Hansen *et al.,* 2002). Yet, the number of studies performed on the vertical migration of phosphorus from agricultural soil (e.g. Lucero *et al.,* 1995; Sims *et al.,* 1998; Gachter *et al.,* 1998; Magid *et al.,* 1999; Turner *et al.,* 2000; Markis *et al.,* 2006) is much smaller than on the horizontal (surface runoff) migration, despite the fact that vertical migration is likely the norm for many agricultural soils. The most relevant among these later studies was done by Nelson *et al.* (2005), who evaluated phosphorus vertical migration in soils amended with swine manure. The study demonstrated that long-term application of swine manure resulted in high soil phosphorus concentrations and substantial vertical movement in the upper horizons. It also indicated that phosphorus

desorption occurring in these horizons contributed up to 50% of phosphorus leaching at the depth of 45 cm from the surface. The hypotheses of the current research were framed by Nelson's work. However, the behaviour of phosphorus in swine manure amended soils might differ greatly from the behaviour of the phosphorus in biosolids-amended soil; therefore, the vertical transport of phosphorus in biosolids-amended soils must be studied separately.

To understand the likely impact of biosolids on phosphorus migration to surface waters, it is critically important to understand vertical migration of phosphorus in its various forms from biosolids through the soil profile, to tile drainage that leads to surface water. This represents the probable route for phosphorus loss in many agricultural areas, yet only few studies are known to having considered vertical phosphorus migration generally, and none that have considered vertical phosphorus migration of the fractions relevant to land application of biosolids. The absence of a study on the vertical phosphorus transport in biosolids-amended soils became the basis of the current project.

1.3.1. Research Hypotheses

Main hypothesis: Biosolids amendment of agricultural soil would lead to an increase in soil phosphorus concentrations compared to the phosphorus concentrations in non-amended soils.

Sub-hypothesis 1: Phosphorus from the biosolids-amended surface will migrate vertically through the soil profile towards tile drainage systems, with greater vertical migration of phosphorus in biosolids-amended soils relative to reference soils.

Sub-hypothesis 2: Phosphorus concentrations will be greater in leachate from biosolids-amended soils than in leachate from reference soils.

Sub-hypothesis 3: The contribution to surface water eutrophication of leachate from biosolids-amended soils will be greater than that of reference soils.

2. Materials and Methods

2.1. Experimental Setup

In order to conduct an experiment on vertical transport of phosphorus in biosolids-amended soils, a series of eight plastic vertical columns were set up in an indoor laboratory at Ryerson University. The dimensions of each column were 7.6 cm in diameter and 65 cm in length. Such setup and dimensions provided sufficient depth to simulate vertical transport of phosphorus applied in a manner consistent with field application (i.e., as a slurry incorporated into the upper 15 cm throughout upper soil horizons). The bottoms of the columns were sealed with rubber caps. To provide drainage of the columns, a plastic funnel was placed at the bottom of each column in such a way that the funnel's stem exited the column through a small hole in the rubber cap. Also, the bottom 10 cm of the column (immediately above the funnel) was filled with gravel to improve the drainage of the leachate percolating through the soil and to prevent soil escape through the funnel. All columns were then fixed by two horizontal wooden planks to increase stability of the setup. The light source (XTRABRITE VITALUX, ME-DTC) was positioned 30 cm above the columns to imitate a natural light cycle. Twelve hours a day,

Fig. 2.1: Experimental setup

the columns were exposed to the light with the intensity of 82 μmol photons m^{-2} s^{-1} at the soil surface.

After setup, each column was filled with 40 cm of sandy loam soil. Two weeks were given for the soil to settle, and the level was again brought to 40 cm. Of the eight columns, four were randomly chosen for biosolids application (see 2.2 "Biosolids application"), and the other four were chosen to be reference columns.

Two months after biosolids application, soya (*Glycine max*) seeds were planted within the top 4 cm of soil in each column (three seeds per column).

A series of six aquariums (8 L) were set up in order to evaluate the effect, if any, of the columns' leachate on surface water. Each aquarium was filled with 2 L of water collected from Lake Ontario and covered with clear plastic (Film-Gard, 3.0 m x 2.0 m, clear polyethylene) to minimize evaporation. The water level was monitored and brought back to 2 L whenever required using distilled water. Periodically, over the duration of the experiment (five months), leachate collected from the columns was added in equal amounts to each aquarium. Leachate from the biosolids-amended columns was added to three of the aquariums and leachate from the reference columns was added to the remaining aquariums. The volume of leachate varied over time, depending on the volume of leachate collected from the columns. However, at a given time point, the volume added to each aquarium was the same.

2.2. Biosolids Application

The biosolids used in the current research were produced at a Southern Ontario

wastewater treatment plant with working capacity of approximately 16000 m^3 and 11000 m^3 for its primary and secondary digestion respectively. The biosolids formation process utilizes a conventional secondary activated sludge process with chemical phosphorus removal and anaerobic sludge digestion. Secondary treatment involves phosphorus removal via precipitation with iron (in form of ferric chloride) followed by the addition of sodium hypochlorite to the treated water as a disinfectant. Consequently, precipitated Fe_2PO_3 becomes a constituent of the activated sludge, and ultimately the biosolids. Anaerobic digestion of the sludge occurs in airtight reactors over a two-week period. Biosolids produced at this wastewater treatment plant are either used on agricultural lands or dewatered and landfilled (Region of Waterloo, 2010).

To evaluate the solids content of the biosolids, 10 ml of biosolids was weighed and dried at 80 ^{0}C for 4 hours. The residue was weighed on analytical balances. The following equation was used to calculate the solids content:

$$Solids\ content\ (\%) = \frac{Mass\ of\ dried\ residue\ (g)}{Mass\ of\ biosolids\ sample\ (g)} * 100\%$$

The solids content was found to be 1.4% (see Appendix A). Within the province of Ontario, 22 dry tonnes of biosolids per ha per 5 years of land is the maximum permissible application (CCME, 2010). However, in Ontario's practice, 8 dry tonnes of biosolids per ha of land per 5 years is the common application rate. The application of biosolids in this experiment was also 8 dry tonnes of biosolids per ha of land. This was calculated (see Appendix A) to be 3.65 g of dry biosolids. Accounting the solids content and the density of used biosolids, 260 ml of biosolids slurry was applied per column. This volume (260 ml) of

biosolids was added on top of the soil in each column selected for biosolids application and incorporated into the top 5 cm. Reference columns were watered with the same volume of distilled water (260 ml).

2.3. Sample Collection and Storage

2.3.1. Sample Collection

At each sampling period, soil samples were collected from two different depths within the soil columns (3 cm from the top and 35 cm from the top) by drilling a hole in the side of each column. The holes were later covered with pieces of plastic cut from an extra column and glued into place.

An initial set of soil samples was collected for analysis before biosolids application to allow the evaluation of phosphorus content changes relative to the initial content (time zero). The biosolids were applied to the columns on Aug 16th, 2013. Subsequent sample collections were performed with decreasing periodicity until the last sample was collected at the end of a simulated growing season (assuming maximum growing season ~ 5-6 months for Southern Ontario) (Table 2.1).

Table 2.1. Soil sample collection schedule.

Serial number	Time passed from biosolids application
1	0
2	14 days
3	30 days
4	60 days
5	90 days
6	150 days

The leachate from the columns was collected periodically, usually the day following column watering. Watering was conducted when required (when soil surface appeared dry). The schedule of leachate sample collection, including the volumes of leachate subsequently added to the aquariums, is presented in the Table 2.2.

Table 2.2. Leachate sample collection schedule.

Serial number	Time passed from biosolids application	Size of an aliquot added to an aquarium (ml)
1	1 day	15
2	14 days	40
3	45 days	80
4	60 days	100
5	80 days	100
6	140 days	70

2.3.2. Sample Storage

Soil samples collected from the columns were placed in sealed test tubes and stored in a refrigerator at 5 ^{0}C until the time of the evaluation. This method of storage does not affect quantities of phosphorus subsequently extracted by different reagents as does air drying of samples (Sparling *et al.,* 1985; Turner *et al.,* 2005; Condor and Newman, 2010). It is furthermore known not to cause the transformation of labile compounds compared to the freeze-drying method (Martin *et al.,* 1987) which demonstrates a 14% decrease in the recovery of extractable phosphorus (Condor and Newman, 2010). Directly before the fractionation procedure, samples were removed from the fridge and oven-dried for 6 hours at 85 ^{0}C.

From each leachate sample collected from a column, 10 mL was taken for phosphorus evaluation. This portion was filtered through a 0.22 μm filter, and analyzed for soluble reactive phosphorus immediately to prevent any exchange of particles that may occur in the sample container. The rest of the sample was added to the aquariums.

2.4. Internal Standard Preparation

To validate methods used for soil sample analysis, an internal soil standard was prepared. A bucket of the same soil that was used for the columns was dried at 80 ^{0}C for 24 hours with periodic mixing. Dried soil was crushed using a mortar and pestle to a fine powder and stored in a sealed plastic container until needed. When soil samples from the columns were analyzed, one sample from the internal standard was added to the analysed set.

2.5. Sample Analysis

2.5.1. Analysis of Biosolids, Reference soils, and Biosolids-amended soils

Biosolids, reference soils, and biosolids-amended soils were analyzed using sequential fractionation. This method is based on the differential solubilities of the various phosphorus forms in various extracts. The procedure for the analysis was adopted from Kovar and Pierzynski (2009) and then adjusted to better fit research needs.

2.5.1.1. Inorganic Phosphorus Fractionation

The complete inorganic phosphorus fractionation procedure is outlined in Fig. 2.2.

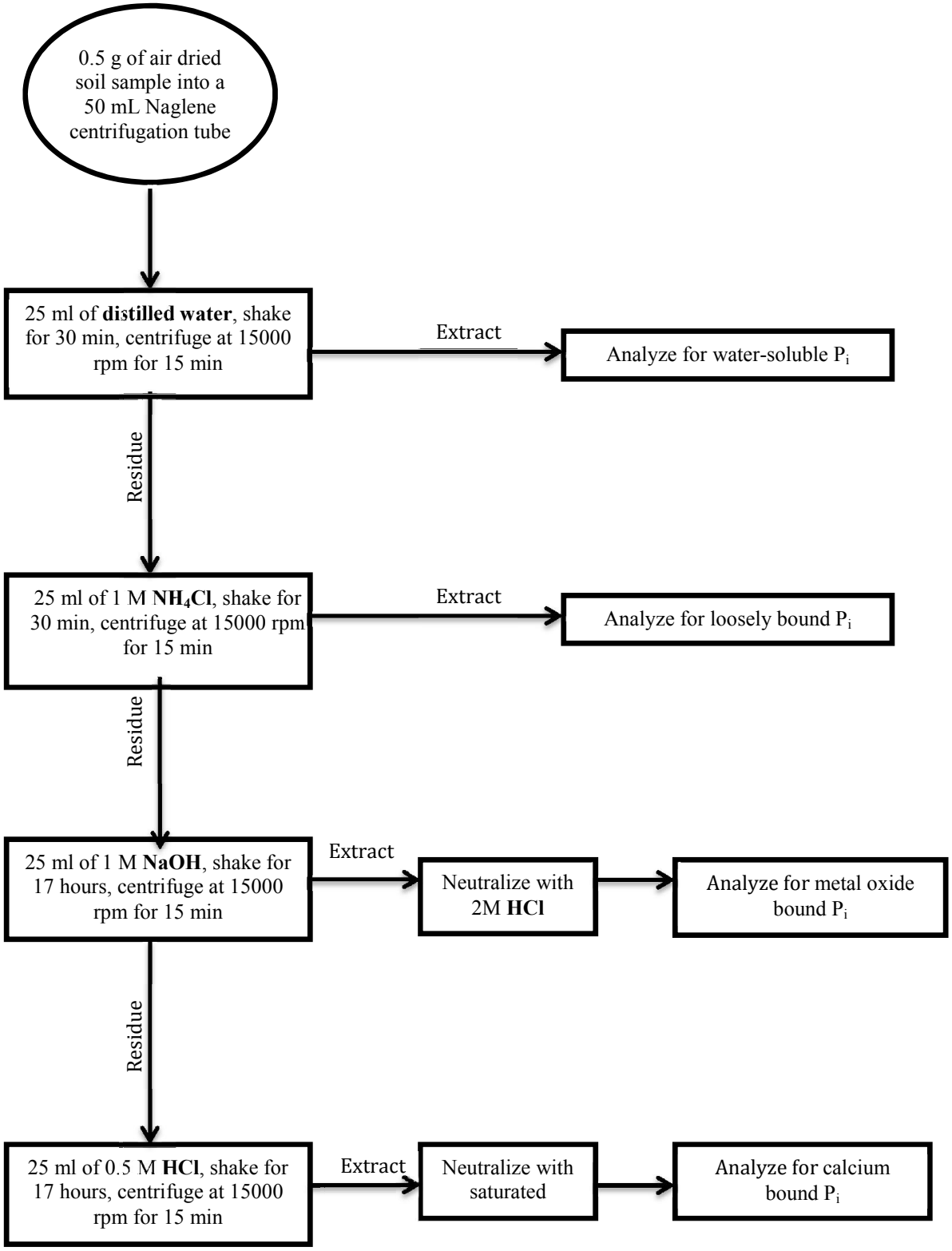


Fig. 2.2. Inorganic phosphorus fractionation.

Step 1. Water-Soluble Inorganic Phosphorus

0.5 g of soil sample (dry weight) was placed into a 50 mL Nalgene centrifuge tube and 25 mL of distilled water was added. The suspension was shaken for 30 min on a 3-D shaker and then centrifuged at 15000 rpm for 15 min. The supernatant was separated from the solid residue, filtered through a 0.22 μm filter (paper filter), and analysed colorimetrically using the ascorbic acid - molybdate method (see 2.5.1.5). The residual soil was kept for the next fractionation step.

Step 2. Loosely-Bound Inorganic Phosphorus

25 mL of 1 M NH_4Cl was added to the residue from step 1, and the suspension was then shaken for 30 min on a 3-D shaker and centrifuged at 15000 rpm for 15 min. The supernatant was separated from the solid residue, filtered through a 0.22 μm filter (paper filter), and analysed colorimetrically using the ascorbic acid - molybdate method. The residual soil was kept for the next fractionation step.

Step 3. Metal-Bound Inorganic Phosphorus

25 mL of 1 M NaOH was added to the residue from step 2, and the suspension was then shaken for 17 hours on a 3-D shaker and centrifuged at 15000 rpm for 15 min. The supernatant was separated from the solid residue and filtered through a 0.22 μm filter (paper filter). The pH of the extract was adjusted using 2M HCl to neutral or close to neutral (pH=6-7). The filtrate was thereafter analysed colorimetrically using the ascorbic acid - molybdate method. The volume of the added acid was accounted for in the dilution

factor when the phosphorus concentration was calculated. The residual soil was kept for the last fractionation step.

Step 4. Calcium-Bound Inorganic Phosphorus

25 mL of 0.5 M HCl was added to the residue from step 3, and suspension was therafter shaken for 17 hours on a 3-D shaker and centrifuged at 15000 rpm for 15 min. The supernatant was separated from the solid residue and filtered through a 0.22 μm filter (paper filter). The pH of the extract was adjusted using 18.5M NaOH and 2M NaOH solutions. The filtrate was then analysed colorimetrically using the ascorbic acid - molybdate method. The volume of the added base was accounted for in the dilution factor when the phosphorus concentration was calculated. The residual soil was discharged.

2.5.1.2. Organic Phosphorus Fractionation

The complete organic phosphorus fractionation procedure is outlined in Fig. 2.2.

Step 1. Organic Labile Phosphorus

0.5 g of soil sample (dry weight) was placed into a 50 mL Nalgene centrifuge tube and 25 mL 0.5 M $NaHCO_3$ was added to it. The suspension was shaken for 16 hours on a 3-D shaker and centrifuged at 15000 rpm for 15 min. The supernatant was separated from the solid residue and filtered through a 0.22 μm filter (paper filter). The extract was divided into two aliquots. The first aliquot was analysed for the inorganic labile phosphorus colorimetrically using the ascorbic acid -molybdate method. The second aliquot was digested using strong

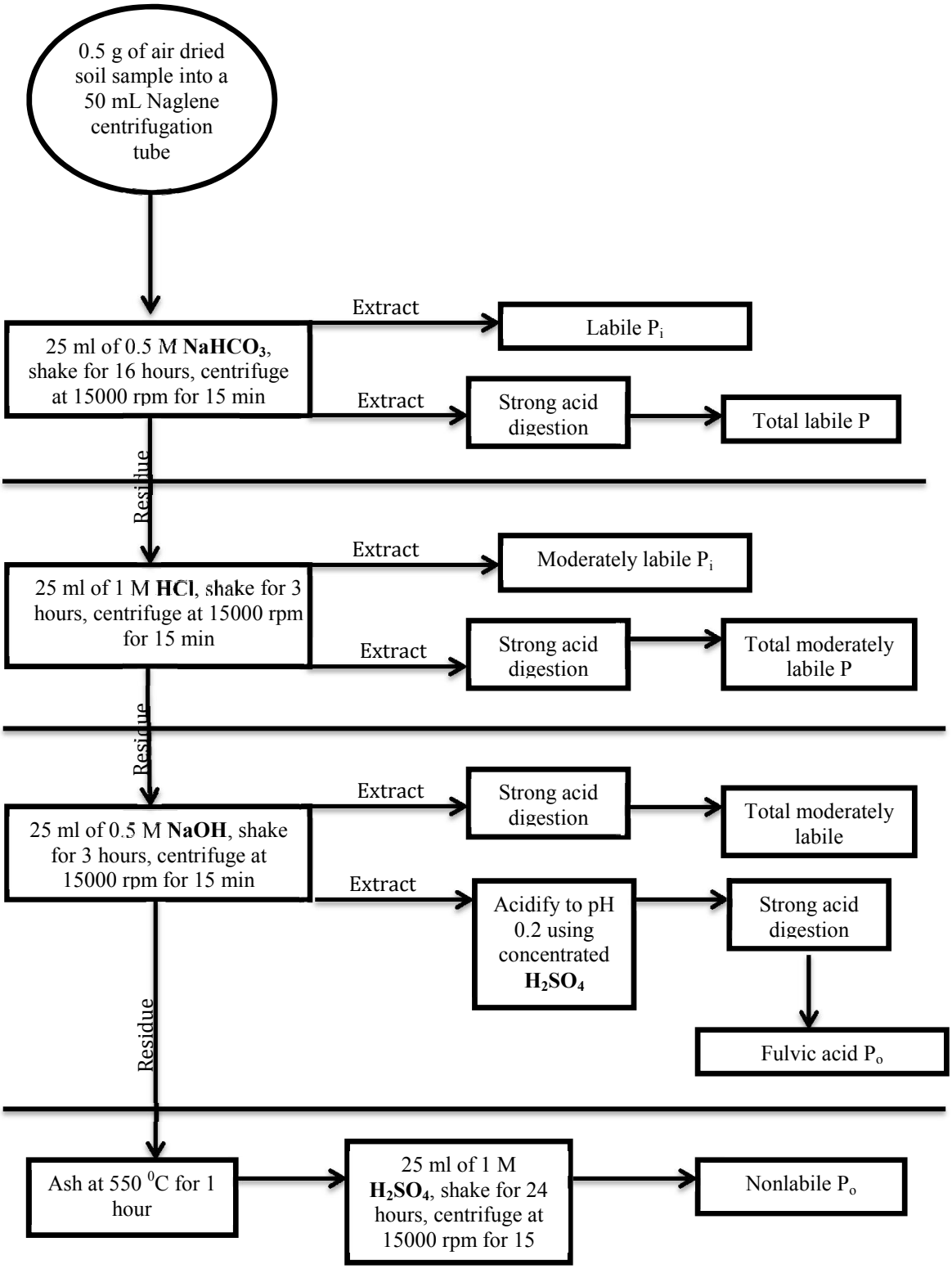


Fig. 2.3. Organic phosphorus fractionation.

acid digestion (see 2.5.1.3). The digested filtrate was then analyzed colorimetrically for the total labile phosphorus. The organic labile phosphorus was calculated as a difference between the total labile phosphorus and the inorganic labile phosphorus. The residual soil was kept for the next fractionation step.

Step 2. Organic Moderately-Labile Phosphorus

25 mL of 1 M HCl was added to the residue from step 1. The suspension was shaken for 3 hours on a 3-D shaker and centrifuged at 15000 rpm for 15 min. The supernatant was separated from the solid residue and filtered through a 0.22 μm filter (paper filter). The extract was divided into two aliquots. The pH of the first aliquot was adjusted to neutral or close to neutral using 18.5M NaOH and 2M NaOH solutions. The filtrate was then analysed for the inorganic moderately-labile phosphorus colorimetrically using the ascorbic acid - molybdate method. The volume of the added base was accounted for in the dilution factor when the phosphorus concentration was calculated. The second aliquot was digested using strong acid digestion. The digested filtrate was then analyzed colorimetrically for the total moderately-labile phosphorus. The organic moderately-labile phosphorus was calculated as a difference between the total moderately-labile phosphorus and the inorganic moderately-labile phosphorus. The residual soil was kept for the next fractionation step.

Step 3. Fulvic Acid Phosphorus (Moderately-Labile) and Humic Acid Phosphorus (Non-Labile)

25 mL of 0.5 M NaOH was added to the residue from step 2. The suspension was shaken for 3 hours on a 3-D shaker and centrifuged at 15000 rpm for 15 min. The supernatant was separated from the solid residue and filtered through a 0.22 μm filter (paper filter). The

extract was divided into two aliquots. The first aliquot was digested using strong acid digestion. The digested filtrate was then analysed for the total moderately-labile phosphorus colorimetrically using the ascorbic acid - molybdate method. The second aliquot was acidified to pH 0.2 using concentrated H_2SO_4 and centrifuged at 15000 rpm for 10 min. The supernatant was separated from the solid residue (humic acid phosphorus) and then also digested using strong acid digestion. The digested filtrate was then analyzed colorimetrically for the fulvic acid phosphorus. The humic acid phosphorus was calculated as a difference between the total moderately-labile phosphorus found in this fractionation step and the fulvic acid phosphorus. The fulvic acid phosphorus was then added to the organic moderately-labile phosphorus determined in step 2, and the humic acid phosphorus was later added to the non-labile phosphorus determined in step 4. The residual soil was kept for the last fractionation step.

Step 4. Organic Non-Labile Phosphorus

The residue from step 3 was ashed at 550 ^{0}C for 3 hours and then dissolved in 25 mL of 1 M H_2SO_4. The suspension was shaken for 24 hours on a 3-D shaker and centrifuged at 15000 rpm for 15 min. The supernatant was separated from the solid residue and filtered through a 0.22 μm filter (paper filter). The pH of the extract was adjusted using 18.5M NaOH and 2M NaOH solutions. The filtrate was then analyzed colorimetrically using the ascorbic acid - molybdate method. The volume of the added base was accounted for in dilution factor when the phosphorus concentration was calculated. The residual soil was discharged.

2.5.1.3. Strong Acid Digestion

A 10 mL aliquot of a supernatant sample was transferred into a 100 mL digestion flask and 1 mL of concentrated H_2SO_4 was added to it. The sample was then gently heated on a hot plate for 10 min. After, 1 mL of concentrated HNO_3 was added and the sample was again heated until it stopped producing brown smoke (usually ~ 5 min). The addition of HNO_3 was repeated two more times. The digested sample was then cooled to room temperature, and the pH of the digested extract was adjusted to neutral or close to neutral (pH=6-7) using 18.5M NaOH and 2M NaOH solutions. The volume of the sample was brought back to the initial 10 mL.

2.5.1.4. Total Phosphorus Determination

0.5 g of soil sample (dry weight) was placed into a ceramic crucible, ashed at 550 ^{0}C for 3 hour, and then dissolved in 25 mL of 1 M H_2SO_4. The suspension was shaken for 24 hours on a 3-D shaker and centrifuged at 15000 rpm for 15 min. The supernatant was separated from the solid residue and filtered through a 0.22 μm filter (paper filter). The pH of the digested extract was adjusted to neutral or close to neutral (pH=6-7) using 18.5M NaOH and 2M NaOH solutions. The filtrate was then analyzed colorimetrically using the ascorbic acid - molybdate method. The volume of the added base was accounted for in dilution factor when the phosphorus concentration was calculated.

2.5.1.5. Ascorbic Acid – Molybdate Method

An aliquot of 5 mL of each extract was transferred to a 10 mL test tube and 1 mL of ascorbic acid - molybdate solution (American Public Health Association, 1992) was added

All tubes containing extracts mixed with the reagent were left at room temperature for 30-40 min for color to develop. The extracts were then analyzed on a spectrometer Lambda 40 at 890 nm. As the detecting limits of a spectrometer vary significantly from one model to another, the top and the bottom detecting limits of the spectrometer used in research were tested by analysing standard phosphorus solutions. Solutions containing 3, 1, 0.75, 0.5, 0.25, 0.1, 0.05, 0.01, 0.005, and 0.001 ppm of phosphorus were tested. The analysis of the solution containing 3 ppm of phosphorus demonstrated the absorbance slightly exceeding 1 (1.1355). The analysis of the solution containing 1 ppm of phosphorus demonstrated the absorbance lower than 1 (0.5572). Therefore the top detectible limit for the current research was established to be 1 ppm for the concentration of phosphorus. All solutions produced during the experiment that were expected to have or actually had concentration higher then 1 ppm had to be diluted. The analysis of the solution containing 0.005 ppm of phosphorus demonstrated a detectable absorbance (0.0025). The analysis of the solution containing 0.001 ppm of phosphorus, however, demonstrated very low absorbance (0.0004) that was to close to 0 to be reliable. The bottom detectible limit for the current research was established to be 0.005 ppm for the concentration of phosphorus. During the analysis of experimental samples, the phosphorus concentration was found (in ppm) using the calibration curve plotted for standard phosphorus solutions. Accounting for the detectible limit of the spectrometer, these concentrations were accurate to 0.005 ppm and were rounded to the third decimal place. To find the concentration in mg of phosphorus per g of analyzed soil, the following equation was used:

$$Conc.of\ P\ \left({}^{mg}/_{g\ of\ soil}\right) = \frac{Conc.of\ P\ \left(\frac{mg}{L}\right) * Volume\ of\ extractant\ (L)}{Mass\ of\ soil\ sample\ (g)}$$

The concentrations found in mg/L of sample and in mg/g of soil were also rounded to the third decimal place for the agreement among obtained numbers.

2.5.1.6. Olsen Phosphorus Analysis

Olsen phosphorus was determined using the procedure described by Kovar and Pierzynski (2009) in order to predict soil response to fertilizer (biosolids) application.

1.0 g of soil sample (dry weight) was placed into a 50 mL Nalgene centrifuge tube and 20 mL of 0.5M $NaHCO_3$ (the pH of $NaHCO_3$ solution was adjusted to 8.5 using 18.5M NaOH) was added. The suspension was shaken for 30 min on a 3-D shaker, and 0.2g of charcoal was added in order to obtain colourless supernatant. The supernatant was filtered through Whatman # 42 (paper filter), and analysed colorimetrically using the ascorbic acid - molybdate method (see 2.5.1.5).

2.5.2. Analysis of Leachate and Surface Water

2.5.2.1. Phosphorus Analysis

The leachate from the columns was analyzed for the soluble reactive phosphorus (SRP) according to the procedure described by Kovar and Pierzynski (2009). In this part of the experiment, the leachate was collected from the outlets (funnels' stems) at the bottom of each column, immediately filtered through a 0.22 μm filter (paper filter), and analyzed colorimetrically using the ascorbic acid - molybdate method. Soluble reactive phosphorus was determined in samples collected from each column, and also in composite samples

from each treatment (where leachate from all reference columns or leachate from all biosolids-amended columns was combined into one sample).

Water from the aquariums, representing surface water, was also tested for soluble reactive phosphorus using the procedure described above.

2.5.2.2. Carbon Analysis

Surface water samples were tested for the total organic carbon. There are two different types of total organic carbon measurement methods: one is differential and the other is direct. As concentrations of inorganic carbon in samples were expected to be significantly lower than concentrations of total carbon, the differential method was used for the current research. In this method each sample was analyzed on a TOC-Vcsh analyzer (SHIMADZU, Model: TOC-VCSH) for inorganic carbon and total carbon concentrations.

To measure the total carbon, the TOC-Vcsh analyzer injected a small aliquot of a sample into a heated combustion tube packed with an oxidation catalyst. The water was then vaporized and all carbon was converted to CO_2. The carbon dioxide, in its turn, was carried with the carrier gas stream from the combustion tube to a NDIR (non-dispersive infrared gas analyzer), where its concentration was measured. The total carbon concentration of the sample was obtained by using the calibration curve prepared with standard solutions.

To measure the inorganic carbon, the TOC-Vcsh analyzer injected a second small aliquot of a sample into a reaction chamber filled with phosphoric acid solution. The inorganic carbon was then converted to carbon dioxide, and the concentration of CO_2 was measured with a NDIR. The inorganic carbon concentration of the sample was obtained by using the

calibration curve prepared with standard solutions. The organic carbon concentration in each sample was found by subtracting the inorganic carbon concentration from the total carbon concentration.

2.5.2.3. Chlorophyll Analysis

The presence of algae in surface water was evaluated by analyzing its chlorophyll content. Samples of water collected from the aquariums were filtered through glass fiber filters (1.6 μm) to concentrate algal cells. The filters were placed in capped 50 mL tubes and dissolved in 10 mL of 90% acetone. To ensure thorough extraction, tubes were left in the dark at room temperature for 16 hours. After extraction, the supernatant was centrifuged at 7000 rpm for 10 minutes to pellet particulate residue from the filter. The supernatant separated from particulate residue was analyzed on the spectrometer Lambda 40 at three different wavelengths (664, 647, and 630 nm) to determine the concentrations of chlorophyll *a*. To calculate the concentrations of the pigment, absorbance values were entered into the following equation (US EPA, 1997[b]):

$$C_{E,a} = 11.85 * (Abs\ 664) - 1.54 * (Abs\ 647) - 0.08 * (Abs\ 630)\ ,$$

where:

$C_{E,a}$ = concentration (mg/L) of chlorophyll *a* in the extraction solution analyzed.

The concentration of pigment in the whole water sample was calculated using the following equation:

$$Cs\ (\frac{mg}{L}) = \frac{CE\ \left(\frac{mg}{l}\right) * extract\ volume(L)}{sample\ volume\ (L)}$$

2.5.3. Plant Analysis

2.5.3.1. Germination Test

Before soya seeds were planted into the soil columns, a germination test was conducted in order to evaluate their germination efficiency. Twenty randomly selected seeds received from the supplier were planted for a three-week period in a plastic bucket filled with the same soil that was used for the columns. After that, germination ratio was calculated using the following equation:

$$Germination\ Ratio = \frac{Number\ of\ germinated\ seeds}{Number\ of\ planted\ seeds} * 100\%$$

2.5.3.2. Phosphorus Analysis in Plants

Phosphorus in soya seeds and produced soya plants was determined in order to evaluate an effect of biosolids soil amendment on plant phosphorus content.

Soya seeds received from the supplier were randomly divided into two groups. The first group (24 seeds) was planted in the soil columns (3 seeds in each column). The second group was analyzed for the total phosphorus. Seeds were crushed using a mortar and pestle, and four 0.5 g samples were weighed into four separate ceramic crucibles. The samples were ashed at 550 ^{0}C for 3 hours and then dissolved in 25 mL of 1 M H_2SO_4. The suspension was thereafter shaken for 24 hours on a 3-D shaker and centrifuged at 15000 rpm for 15 min. The supernatant was separated from the solid residue and filtered through a 0.22 μm filter (paper filter). The pH of the extract was adjusted to neutral or close to neutral (pH=6-7) using 18.5 NaOH and 2M NaOH solutions. The filtrate was then analyzed

colorimetrically using the ascorbic acid - molybdate method. The volume of the added base was accounted for in the dilution factor when the phosphorus concentration was calculated.

Soya plants grown in the soil columns were collected and dried at 65 ^{0}C for 10 hours. After that, dried material (above ground biomass) was also analyzed for total phosphorus, using the procedure described above. The belowground biomass was not analyzed due to the difficulty of retrieving thin root filaments from the soil. Plants collected from different columns were analyzed separately.

2.6. Statistical analyses

Statistical analyses were performed using virtual application SAS Enterprise Guide 5.1 for PC. Differences in soil phosphorus concentrations between treatments (amended and reference soils), locations (top or bottom), time, and treatment over time were analyzed using multi-way ANOVA. Differences in phosphorus concentrations in plants, leachate, and receiving waters, as well as differences in organic carbon and chlorophyll *a* concentrations, were analyzed using 1-way ANOVA. In all cases, statistical differences were accepted when probability was less than 0.05.

3. Results and Discussion

3.1 Biosolids Effects on Phosphorus Concentrations in Soil, Leachate, and Receiving Surface Waters

3.1.1 Phosphorus in Biosolids and Initial Soil

Soil (Fig. 3.1) and biosolids (Fig. 3.2) were analyzed for different phosphorus forms using sequential fractionation (Appendix B, Appendix C), prior to amendment of soil with biosolids.

The results of the initial soil phosphorus analysis revealed that the total phosphorus concentration was 0.226 mg/ g of soil. Readily available phosphorus fractions, such as inorganic water-soluble and organic labile fractions, are represented in Figure 3.1. However, these values were below the nominal detection limit of the method (<0.005 mg/g). Inorganic loosely-bound (0.055 mg/ g of soil) and organic moderately-labile fractions (0.022 mg/ g of soil) represented minor pools of phosphorus. Cumulative contribution of bioavailable fractions to the total phosphorus concentration in initial soil was less then 32%. Relatively unavailable phosphorus fractions, such as inorganic calcium-bound (0.019 mg/g of soil) and organic non-labile (0.049 mg/g of soil) fractions were also minor. Their cumulative contribution to the total phosphorus concentration in initial soil was approximately 13%. The other 43% of the initial total phosphorus concentration in soil was represented by the metal-bound phosphorus fraction (0.115 mg/ g of soil), a fraction that is not directly available for the plants, but that can release bioavailable phosphorus under anoxic conditions.

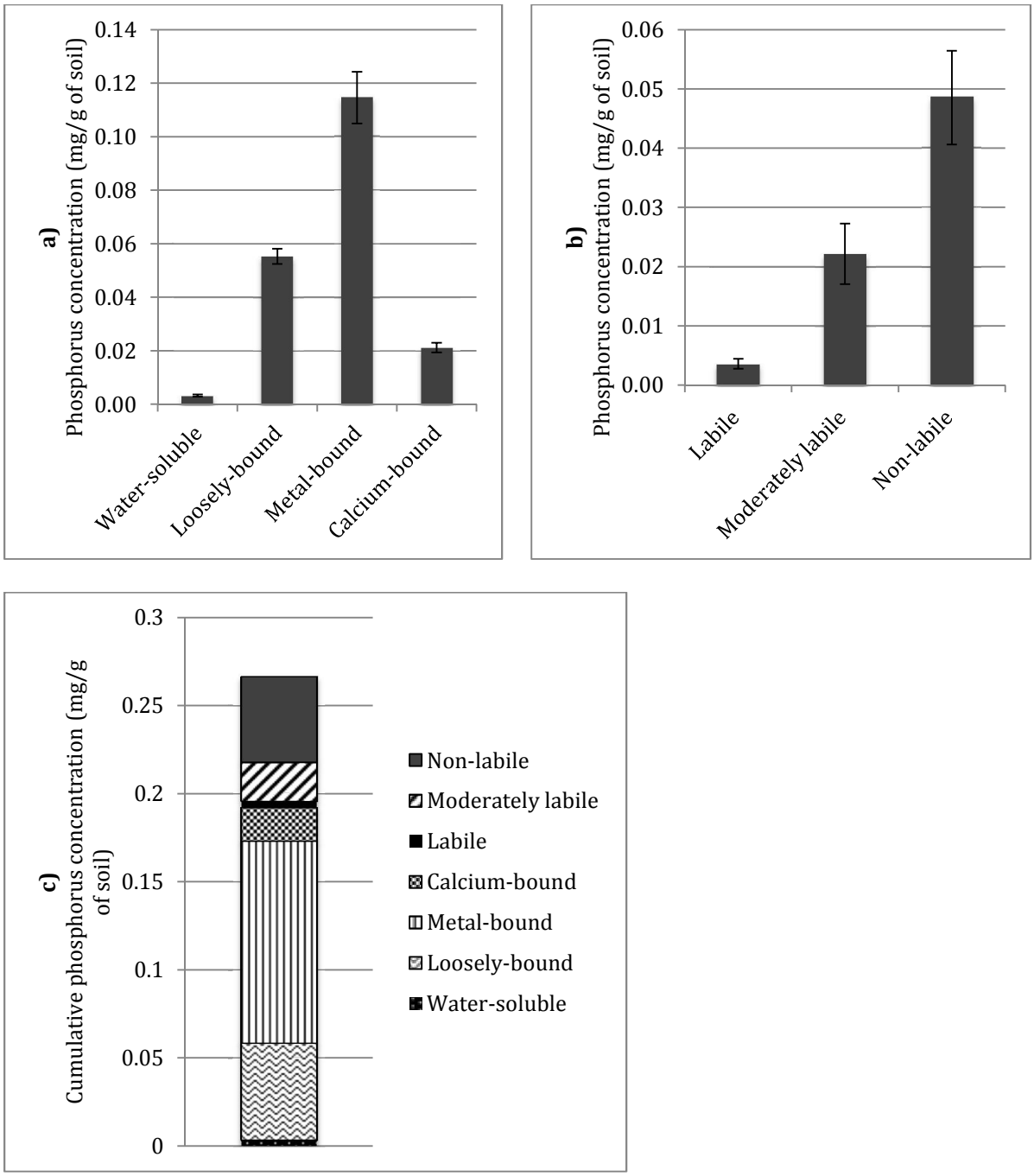


Fig. 3.1. Phosphorus in soil: a) initial inorganic phosphorus fractions in soil, b) initial organic phosphorus fractions in soil, and c) initial cumulative phosphorus concentrations in soil. The error bars represent standard deviation among replicate samples.

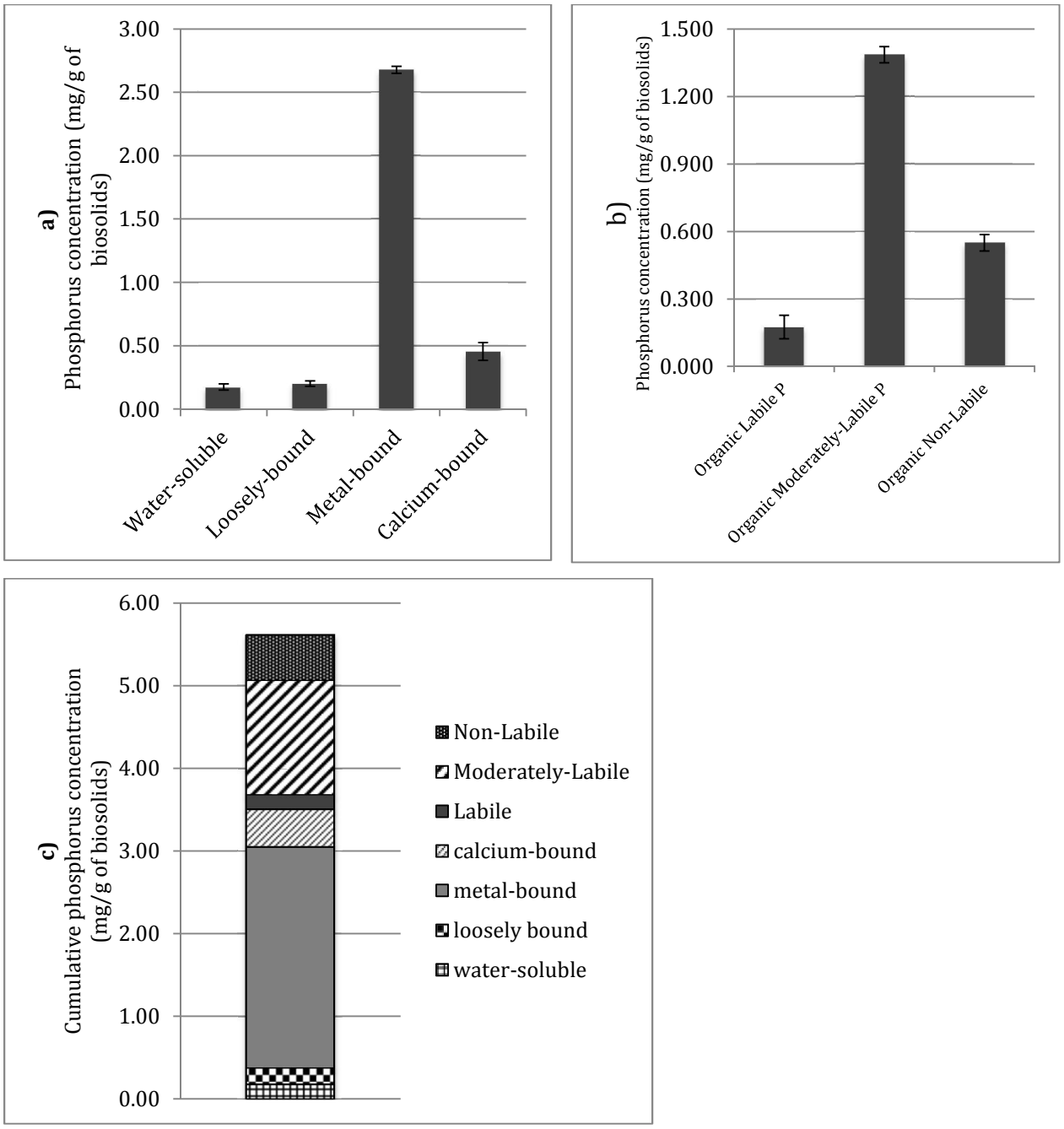


Fig. 3.2. Phosphorus in biosolids: a) inorganic phosphorus fractions in biosolids, b) organic phosphorus fractions in biosolids, and c) cumulative phosphorus concentrations in biosolids. The error bars represent standard deviation among replicate samples.

The Olsen phosphorus was determined to be ~0.06 mg/g of soil or 60 ppm. That suggested that the soil used in the experiment was representative of soil relatively rich in phosphorus in Ontario. The range of bioavailable phosphorus in agricultural soils determined through the Phosphorus Soil Test (Olsen phosphorus test) varies greatly (from 10 ppm to 60 ppm) across Ontario (OMAFRA, 2012b; Legg, 2013). "Low Response" (21-30 ppm of phosphorus) or "No Response" (31-60 ppm of phosphorus) soil test ratings (OMAFRA, 2009), mean that these soils are not generally phosphorus limited, and plant growth does not respond to fertilization by phosphorus. This type of soil is capable of producing high-yielding agricultural crops with little or now additional fertilizer (OMAFRA, 2009), but might increase the potential of phosphorus migration to surrounding water bodies (Legg, 2013). Table 3.1 illustrates a dependence of soil responsiveness to fertilizer application from the concentration of Olsen phosphorus. An addition of a fertilizer to "No response" soils typically has minimal benefit, but might increase the potential for phosphorus migration to surrounding water bodies (Legg, 2013). The choice of "No response" soil for the current research, therefore, provided the greatest opportunity to observe phosphorus migration within the constructed columns.

Table 3.1. Dependence of soil responsiveness to fertilizer application from the concentration of bioavailable phosphorus.

Concentration of Olsen Phosphorus (ppm)	Responsiveness to Fertilizer Application
0-9	High response
10-20	Moderate response
21-30	Low response
31-60	Rare or no response

Biosolids analysis revealed that the total phosphorus concentration (5.617 mg/g of biosolids) in biosolids was 21 times higher than the total phosphorus concentration in initial soil. Individual phosphorus fractions were also greatly exceeding those in initial soil (54 times for water-soluble phosphorus, 4 times for loosely-bound phosphorus, 23 times for metal-bound phosphorus, 24 times for calcium-bound phosphorus, 49 times for organic labile phosphorus, 63 times for organic moderately-labile phosphorus, and 11 times for organic non-labile phosphorus). The distribution of the different phosphorus fractions contributing to the total phosphorus concentration, however, was similar to the distribution observed for soil prior to amendment. The cumulative contribution of relatively unavailable phosphorus fractions, such as inorganic calcium-bound (0.454 mg/g of biosolids), and organic non-labile (0.550 mg/g of biosolids) was 17%. The metal-bound phosphorus fraction (2.677 mg/g of biosolids) represented the biggest phosphorus pool in biosolids and contributed 47% to the total phosphorus concentration. Readily available water-soluble (0.174 mg/g of biosolids), inorganic loosely-bound (0.201 mg/g of biosolids), and organic labile (0.175 mg/ g of biosolids) fractions in biosolids also represented minor pools of phosphorus. Their cumulative contribution to the total phosphorus concentration was 10%. The greatest difference between biosolids and the soil in the relative contribution to the total phosphorus was found for the moderately-labile organic fraction: 1.385 mg/g of biosolids (25% of total phosphorus) versus 0.022 mg/g of soil (8% of total phosphorus).

The biosolids used in this study were representative of typical biosolids produced through anaerobic digestion along with the addition of ferric chloride in the tertiary treatment process. Such treatment usually results in higher phosphorus content in produced biosolids

compared to all other stabilization treatments (Maguire *et al.,* 2001) except biological nutrient removal. Biological nutrient removal treatment would result in a higher percentage of readily available phosphorus, but plants with such treatment are not typical for Ontario. Therefore, the selection of the anaerobically digested biosolids provided the greatest opportunity to observe phosphorus migration within the constructed columns. However, concentrations of phosphorus in biosolids vary over treatments, and therefore one batch of anaerobically digested biosolids might have greater total phosphorus or a relatively larger percentage in labile fractions than another, affording greater opportunity for phosphorus migration. The total phosphorus content in biosolids used in the current research, for example, was much smaller than the total phosphorus concentration in biosolids used by Hanief (5.617 mg/g of biosolids vs. 33 mg/g of biosolids), although both batches were obtained from the same wastewater treatment plant.

Previously conducted studies have demonstrated that an addition of iron during the stabilization process resulted in low concentration of bioavailable phosphorus (Penn and Sims, 2002) and in a high concentration of metal-bound phosphorus (Huang *et al.*, 2008). The distribution of inorganic phosphorus fractions in biosolids produced through anaerobic digestion along with the addition of iron could be presented as follows: calcium-bound phosphorus > metal-bound phosphorus > loosely-bound phosphorus > water-soluble phosphorus (He *et al.,* 2010). The findings of the current research generally supported the pattern of the different fractions' contribution to the total phosphorus concentration in biosolids; however, the contributions of calcium-bound and metal-bound phosphorus fractions were reversed. This might have occurred due to the addition of different amounts of lime ($Ca(OH)_2$) during sludge formation processes. As lime is used for

a pH adjustment and some metal removal, its amount in a wastewater treatment process is regulated by the quality of wastewater and can vary significantly from one batch to another even within a plant (National Lime Association, 2010). The less lime added during the treatment, the lower the concentration of phosphorus that precipitates in a form of complex calcium phosphates (calcium-bound phosphorus).

3.1.2 Expected Phosphorus Concentration Increase After Biosolids Application

Based on the results of initial soil and biosolids phosphorus analysis, biosolids application to the soil columns was expected to cause some increase in the concentration of different soil phosphorus fractions, following the addition of biosolids and incorporation into soil. The increases in phosphorus were expected to be greatest near the surface, as biosolids were incorporated into the top 5 cm of soil. A set of simple calculations were performed to predict soil phosphorus increases after biosolids application (Appendix D) both in the upper layer of soil (top 5 cm) and in a column as a whole.

According to the model (Fig. 3.3a), an increase in almost all phosphorus fractions was expected in the top 5 cm of the soil columns immediately following biosolids application (67% increase for water-soluble, 4% increase for loosely-bound phosphorus, 29% increase for metal-bound phosphorus, 27% increase for calcium-bound phosphorus, 60% increase for organic labile phosphorus, 77% increase for organic moderately-labile phosphorus, and 24% increase for organic non-labile phosphorus). However, the expected increase in most pools was near or below the nominal limit of detection for phosphorus analysis. When phosphorus enrichment to the entire column was considered, the relative increase in each

fraction was smaller still (Figure 3.3b), with absolute increases below the detection limit for all pools except metal-bound phosphorus (Table 3.2). Therefore, this model predicted

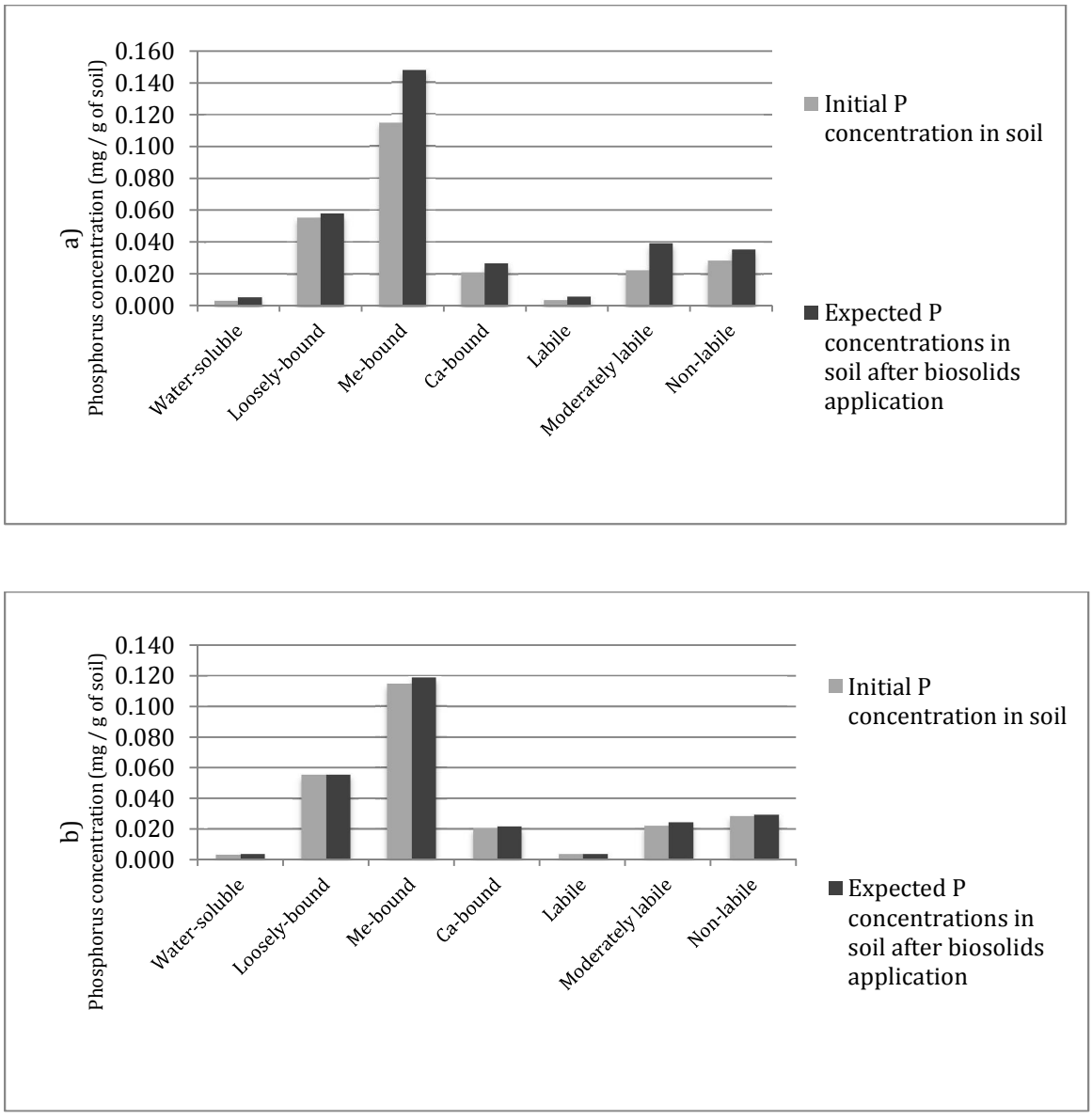


Fig. 3.3. Predictions of phosphorus increase: a) expected phosphorus concentration increases in top 5 cm of the soil columns within 1 day after biosolids application, b) expected phosphorus concentration increases in entire length of the soil columns within 1 week from biosolids application.

that application of biosolids with the moderately high total phosphorus concentration to a fertile soil would result in no measureable increase in phosphorus pools for the integrated soil column, and only marginally discernable increases in the top 5 cm, where the material is most concentrated. Moreover, the relative increase in various phosphorus fractions due to biosolids amendment should decay over time, and even a measurable increase in certain phosphorus fractions in the upper 5 cm may be short-lived.

Table 3.2. Quantitative phosphorus concentration increases expected in the entire length of the soil columns within 1 week from biosolids application.

	Inorganic Fractions				Organic Fractions		
	Water-soluble P	Loosely-bound P	Metal-bound P	Calcium-bound P	Labile P	Moderately-labile P	Non-labile P
Increase in P concentration (mg of P/ g of soil)	0.0003	0.0003	0.0041	0.0007	0.0003	0.0021	0.0008

The prediction of a minimal increase in phosphorus fractions was consistent with the findings of Hanief (2011). In that study, biosolids with much higher total phosphorus content were added to a synthetic mineral soil, a scenario ideal for increasing the relative concentration of various phosphorus fractions. Even under that scenario, the measured increase in most phosphorus fractions was barely measureable against a nominal detection limit of 0.005 mg/g of soil (Table 3.3). Moreover, Hanief's measured increases were consistent with those predicted by the current model if one accounts for the approximately five-fold greater total phosphorus (and in most phosphorus fractions) in the biosolids used by Hanief, relative to the biosolids used in this study. Further, a study by Ippolito *et al.* (2007), using biosolids with similar phosphorus concentrations to those in the current

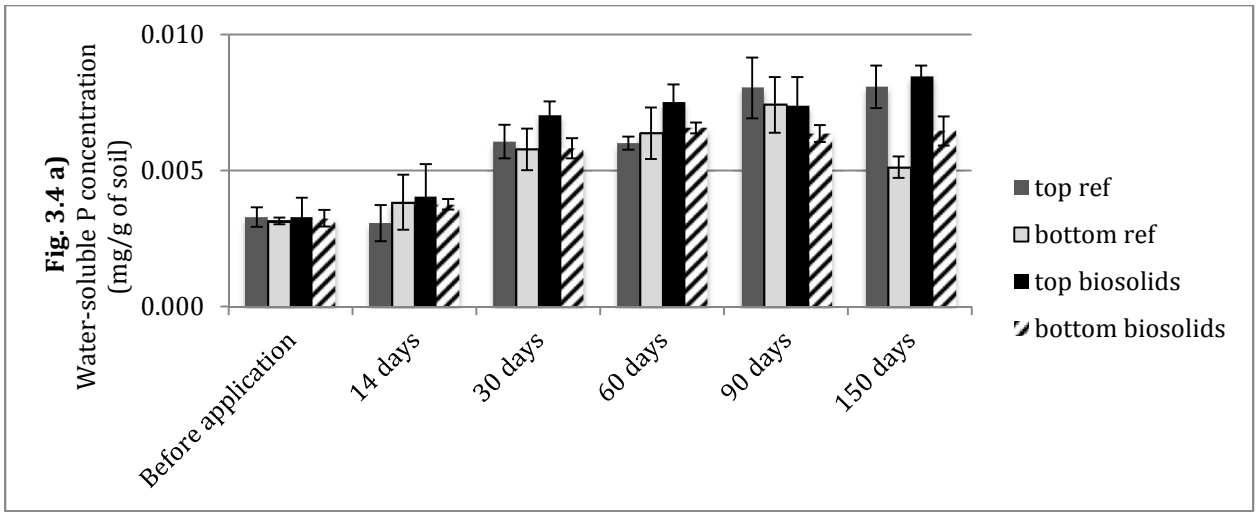
study, demonstrated increased soil phosphorus concentrations similar to those predicted by the model.

Table 3.3. Phosphorus concentration increases caused by the application of biosolids (Hanief, 2011).

	Inorganic fractions				Organic fractions		
	Water-soluble P	Loosely-bound P	Metal-bound P	Calcium-bound P	Labile P	Moderately-labile P	Non-labile P
Increase in P concentration (mg of P/ g of soil) caused by biosolids from Kitchener	0.0090	0.0040	0.0680	0.0000	0.0070	0.0280	0.0070
Increase in P concentration (mg of P/ g of soil) caused by biosolids from Guelph	0.0090	0.0100	0.1040	0.0100	0.0070	0.0360	0.0080

3.1.3 Phosphorus in Soil

Soil samples were analyzed for different phosphorus fractions with decreasing periodicity, starting 2 weeks after biosolids application until the end of a simulated growing season (5 months after biosolids application) (Fig. 3.4, Appendix E).



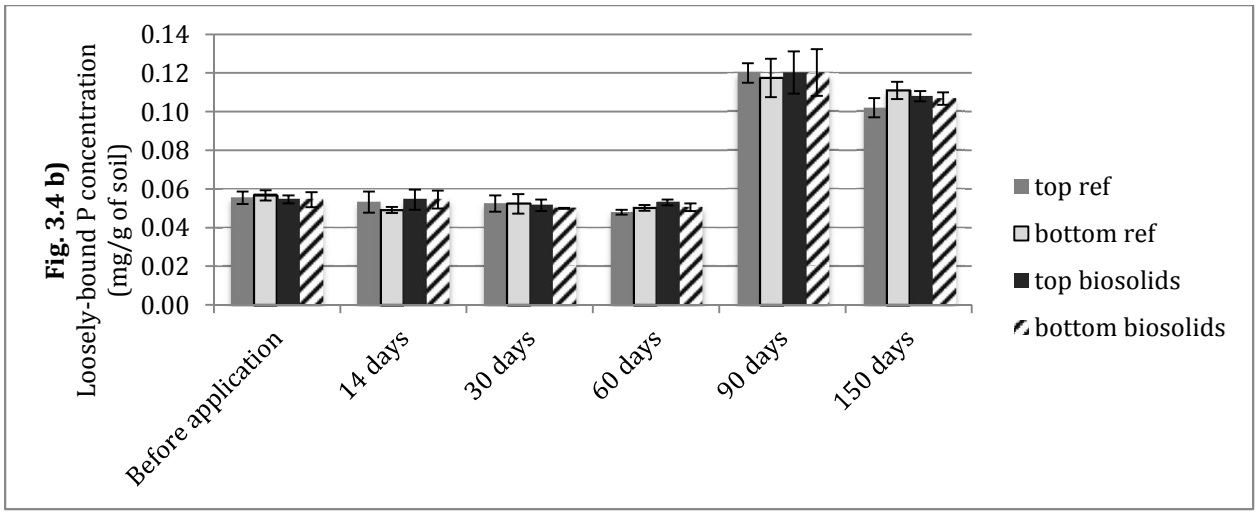


Fig. 3.4 b)
Loosely-bound P concentration (mg/g of soil)
0.14
0.12
0.10
0.08
0.06
0.04
0.02
0.00
Before application
14 days
30 days
60 days
90 days
150 days
top ref
bottom ref
top biosolids
bottom biosolids

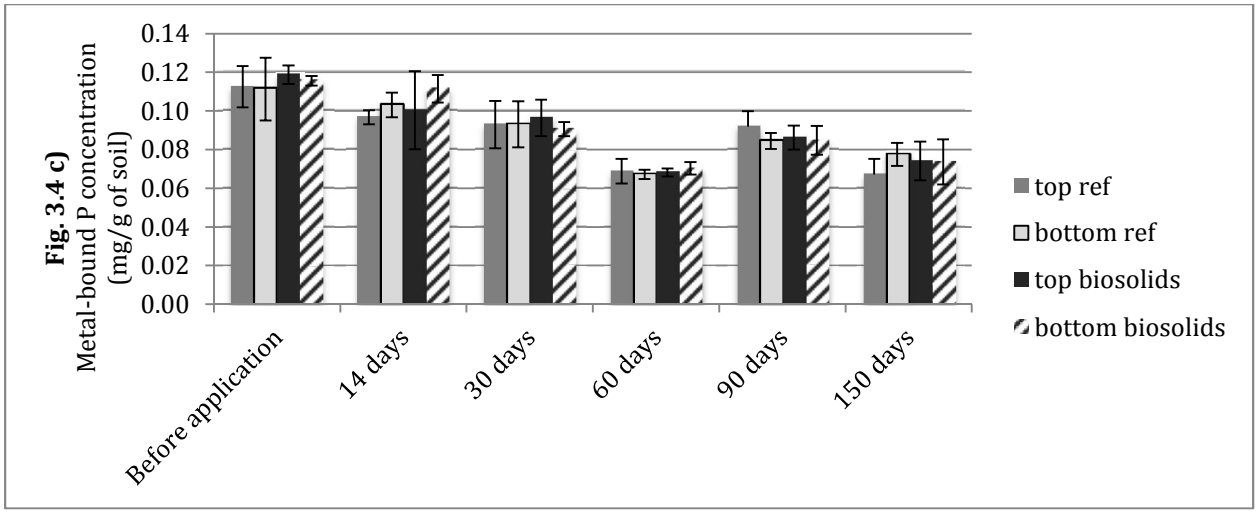


Fig. 3.4 c)
Metal-bound P concentration (mg/g of soil)
0.14
0.12
0.10
0.08
0.06
0.04
0.02
0.00
Before application
14 days
30 days
60 days
90 days
150 days
top ref
bottom ref
top biosolids
bottom biosolids

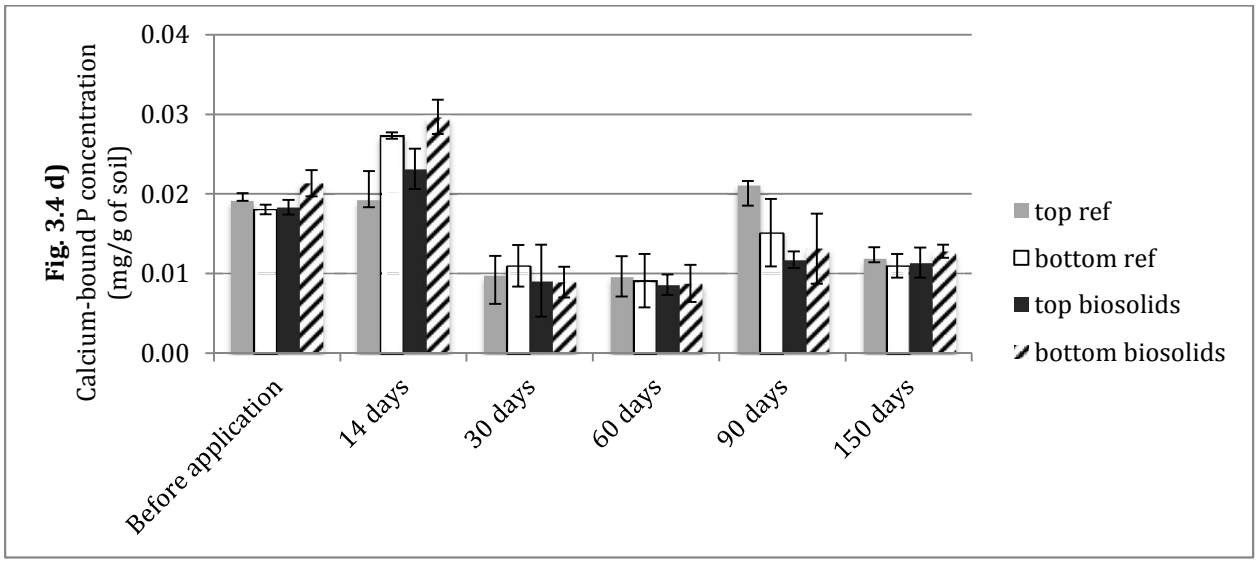
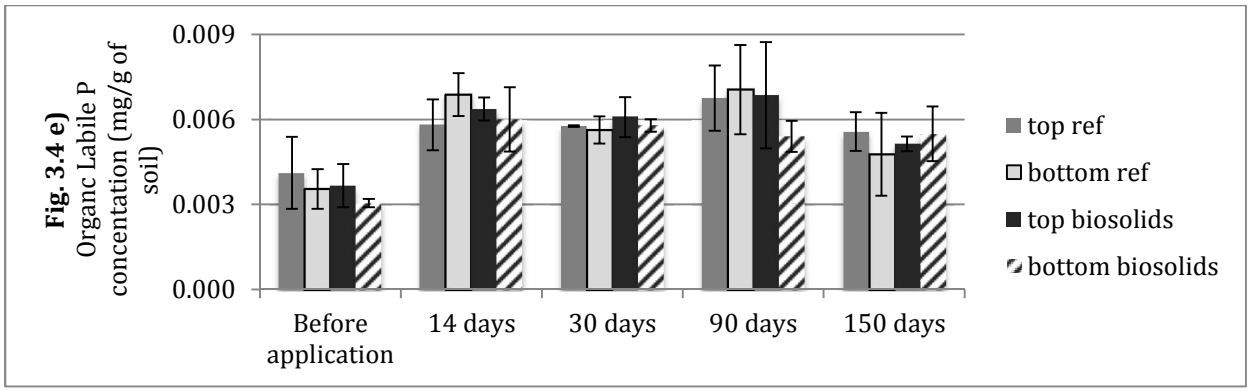
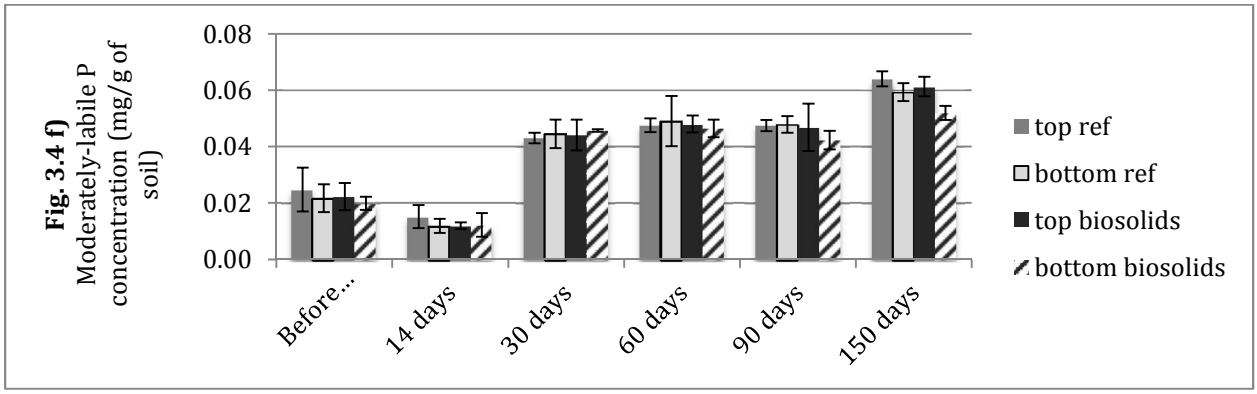


Fig. 3.4 d)
Calcium-bound P concentration (mg/g of soil)
0.04
0.03
0.02
0.01
0.00
Before application
14 days
30 days
60 days
90 days
150 days
top ref
bottom ref
top biosolids
bottom biosolids





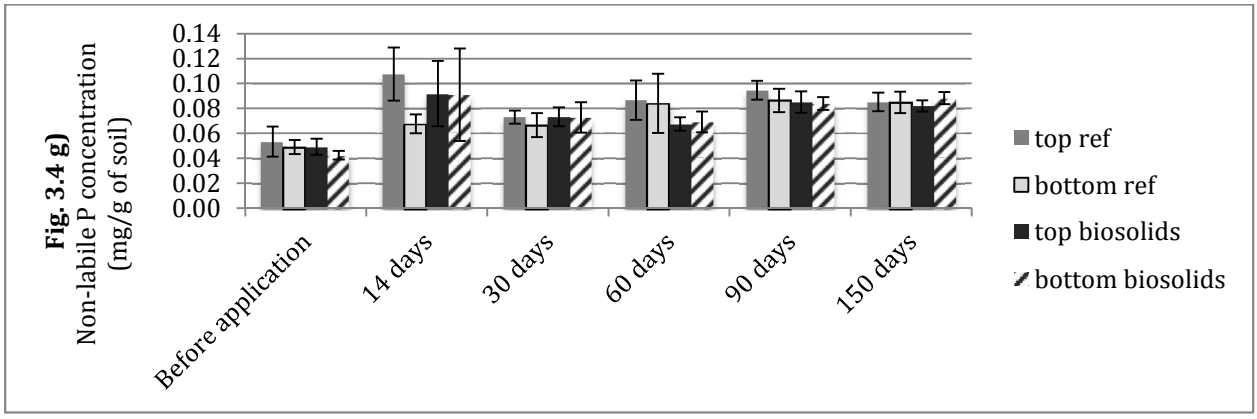


Fig. 3.4. Phosphorus in soil: a) inorganic water-soluble phosphorus in the soil columns, b) inorganic loosely-bound phosphorus in the soil columns, c) inorganic metal-bound phosphorus in the soil columns, d) inorganic calcium-bound phosphorus in the soil columns, e) organic labile phosphorus in the soil columns, f) organic moderately-labile

phosphorus in the soil columns, and g) organic non-labile phosphorus in the soil columns. The error bars represent standard deviation among replicate samples.

The water-soluble phosphorus fraction changed over time ($F_{5,83}$=142.3, p<0.0001), approximately doubling over the five-month period of the study. However, there was no difference in concentrations between biosolids-amended soils and reference soils ($F_{1,83}$=3.41, p=0.068), nor did the increase in water-soluble phosphorus over time differ between treatments (time*treatment effect, $F_{5,83}$=1.59, p=0.171). Moreover, no effect of location (i.e., top or bottom) was observed for the water-soluble phosphorus fraction ($F_{1,83}$=3.41, p=0.068). Although the increase over time was statistically significant, it was not meaningful, increasing by less than 0.005 mg/g of soil (Fig. 3.4a), the detection limit for the analysis.

For loosely-bound phosphorus, there was no difference in concentrations between biosolids-amended soils and reference soils ($F_{1,79}$=0.59, p=0.443), nor did the increase in loosely-bound phosphorus over time differ between treatments (time*treatment effect, $F_{5,79}$=0.35, p=0.879). Moreover, no effect of location (i.e., top or bottom) was observed for loosely-bound phosphorus ($F_{1,79}$<0.01, p=0.978). The loosely-bound phosphorus, however, did increase over time for both treatments and both locations ($F_{5,79}$=449.3, p<0.0001). Concentrations were constant for the first two months, and then increased by 50% at the three months (Fig. 3.4b). This observation was unexpected and in contrast to the findings of Ippolito *et al.* (2007). However, as the increase in loosely-bound phosphorus concentrations was observed for both reference and biosolids-amended soils, it could be explained in part by a 30% decrease in the metal-bound phosphorus fraction (Fig. 3.4c)

rather than by biosolids application. The metal-bound phosphorus decreased over time for both treatments and both locations ($F_{5,83}$=58.0, p<0.0001). There was no difference in metal-bound phosphorus concentrations between biosolids-amended soils and reference soils ($F_{1,83}$=0.82, p=0.3686), nor did the decrease in metal-bound phosphorus over time differ between treatments (time*treatment effect, $F_{5,83}$=0.76, p=0.5841). Furthermore, no effect of location was observed for the metal-bound phosphorus fraction ($F_{1.83}$=0.05, p=0.822). The transformation of the metal-bound phosphorus to the loosely-bound phosphorus through the reduction mechanism, therefore, could have occurred under anoxic conditions inside of the soil columns.

For calcium-bound phosphorus, there was no difference in concentrations between biosolids-amended soils and reference soils ($F_{1,73}$=0.5916, p=0.689). Moreover, no effect of location was observed for the calcium-bound phosphorus ($F_{1,73}$=2.7, p=0.1048). The calcium-bound phosphorus, however, did decrease over time for both treatments and both locations ($F_{5,73}$=78.9, p<0.0001). Over five-month period, the concentration of calcium-bound phosphorus decreased by 35% (Fig. 3.4d). This phosphorus may have been transformed, contributing (along with the metal-bound phosphorus) to the measured increase in loosely-bound phosphorus.

Although a statistically significant increase was observed in the organic labile phosphorus fraction ($F_{5,69}$=14.1, p<0.0001), it was less than 0.005 mg/g of soil (Fig. 3.4e), below the nominal detection limit. Therefore, no meaningful conclusion can be drawn regarding the change in this pool.

The organic moderately-labile phosphorus concentrations also increased over the five-month period ($F_{5,83}$=192.6, p<0.0001) (Fig. 3.4f). However, as with all other fractions, it did not demonstrate any statistically significant difference between locations ($F_{1,83}$=2.56, p=0.113) or between biosolids-amended soils and reference soils ($F_{1,83}$=2.56, p=0.113).

Finally, a statistically significant increase was observed in organic non-labile phosphorus fraction over time ($F_{1.83}$=10.8, p<0.0001). This fraction also illustrated no significant difference between locations ($F_{1,83}$=1.06, p=0.306) or between biosolids-amended soils and reference soils ($F_{1,83}$=0.22, p=0.644), nor did the changes differ between treatments over time (time*treatment effect, $F_{5,83}$=2.73, p=0.125). No explanation for the changes in organic moderately-labile and organic non-labile phosphorus concentrations was found.

No measureable increase occurred in any phosphorus fraction as a result of biosolids application (Fig 3.4). The main hypothesis, stating that biosolids amendment of agricultural soil would lead to a significant increase in soil phosphorus concentrations compared to the concentrations in reference soils, was therefore rejected. The soil used in this experiment was phosphorus-rich and amendment with biosolids increased the relative abundance of the phosphorus fractions less than would have occurred in phosphorus-poor soil (Table 3.4). However, the absolute increase for each fraction as a result of biosolids application would be the same, regardless of soil type. As predicted by the model, the absolute increase for most fractions was near or below detection limit.

The data demonstrated no difference for any phosphorus fraction between the upper 5 cm of the soil column and the bottom 5 cm, suggesting very limited (i.e., not measureable) vertical transport of phosphorus. The soil and biosolids used in this experiment

represented a kind of worst-case scenario; phosphorus-rich anaerobically digested biosolids were applied to soil that was also phosphorus-rich, providing conditions under which the phosphorus might be expected to migrate through the soil towards the drainage system rather than being immobilized (Legg, 2013). This was not observed. Accordingly, it is unlikely that phosphorus vertical migration would be observed following application to more phosphorus-poor soils where the phosphorus is more likely to be immobilized, or in soils receiving biosolids produced by other methods that are less rich in phosphorus. Therefore, the hypotheses that phosphorus from the biosolids-amended surface might migrate vertically in the soil towards tile drainage systems and that phosphorus vertical migration in biosolids-amended soils would occur at a higher rate than phosphorus migration in reference soils were rejected (sub-hypothesis 1).

Table 3.4. Relative increases in bioavailable phosphorus concentrations for different types of soil in conditions otherwise similar to the current research.

Type of Soil	Initial Concentration of Bioavailable Phosphorus (mg/g of soil)	Increase in Phosphorus Concentration (Caused by Biosolids Application) (%)
Biosolids (used in the current reseach)	0.375	N/A
High responsive	0 – 0.009	> 46.2
Moderate responsive	0.01 – 0.02	23.1 – 46.2
Lowly responsive	0.02 – 0.03	15.4 – 23.1
Not responsive (soil used in the current research)	0.05	8.4

3.1.4 Phosphorus in Leachate

Based on the evaluation of phosphorus vertical migration in constructed soil columns, phosphorus concentrations in the leachate produced by the amended soils were predicted not to differ from leachate produced by reference soils. Leachate samples collected from both biosolids-amended and reference soil columns were analyzed for soluble reactive phosphorus in order to test this assumption.

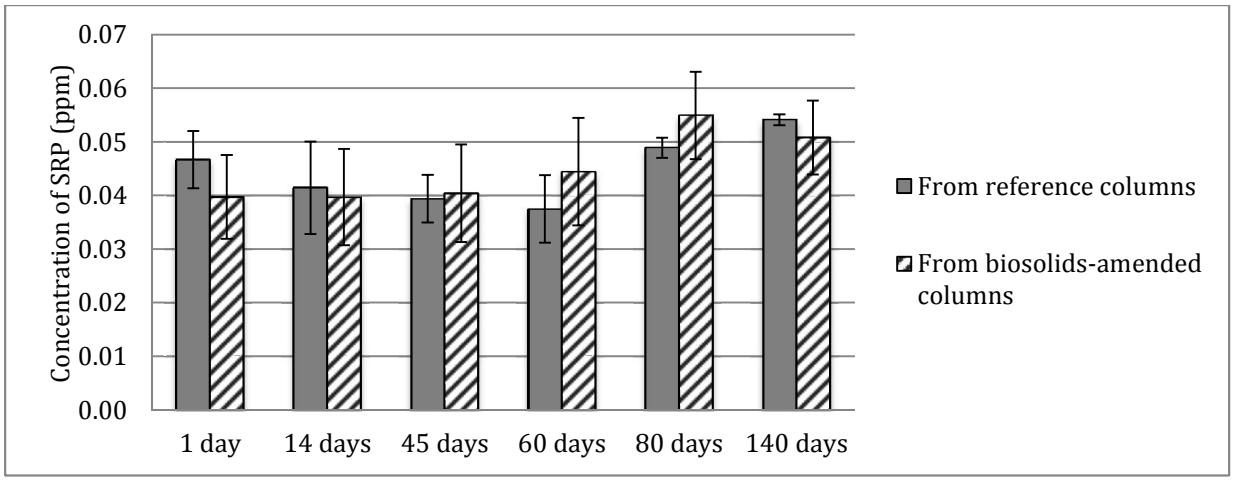


Fig. 3.5. Concentrations of soluble reactive phosphorus in analyzed leachate. The error bars represent standard deviation among replicate samples.

The concentrations of soluble reactive phosphorus in leachate samples collected from biosolids-amended soil columns was noted to be very close to the concentrations of soluble reactive phosphorus in leachate samples collected from reference soil columns (Fig. 3.5). No significant difference was observed between samples (t_{46}=0.31, p=0.758). However, the absence of a significant difference in phosphorus concentrations between leachate from biosolids-amended columns and leachate from reference columns could be once again

attributed to the initial enrichment of the reference soil in phosphorus. Within the five months of the research, leachate from both biosolids-amended soil columns and reference soil columns demonstrated phosphorus concentrations around 0.039-0.054 ppm or 39-54 mg/m^3 (Appendix F). These concentrations were much smaller than the concentrations of phosphorus measured in run-off from biosolids-amended soils (Sharpley 1991; Cox and Hendricks 2000; Andraski and Bundy 2003; Quilbe *et al.* 2005; White *et al.* 2010, Hanief, 2011), but still exceeded concentrations suggested as optimal for limiting eutrophication potential (below 25 mg/m^3 in streams and 10 mg/m^3 in lakes) (Smith *et al.,* 1999). Therefore, the contribution of the leachate from both biosolids-amended columns and reference columns could possibly contribute to the eutrophication of receiving waters. This potential contribution to leaching is related to the soil used in the study rather than to biosolids amendment of that soil. The level of contribution would depend on the dilution factor (the ratio between receiving water body's volume and leachate volume). The hypothesis that phosphorus concentrations in the leachate produced by the amended soils would be higher than those produced by reference soils (sub-hypothesis 2) was rejected for soils initially rich in phosphorus. Leachate from soil less rich in phosphorus, however, would likely contain lower phosphorus concentrations. In these soils, the effect of biosolids application on leachate phosphorus concentrations might be measureable, however as noted above, the absolute quantity of phosphorus added to these soils in the form of biosolids would be the same, and these soils would be more likely to immobilize phosphorus.

3.1.5 Impact of Leachate on Receiving Waters

At the end of five-month period, no signs of eutrophication were visually observed in aquariums mimicking receiving waters. Nonetheless, water from aquariums was analyzed for soluble reactive phosphorus, organic carbon, and chlorophyll *a* in order to objectively test the effects of leachate on eutrophication (biosolids-amended vs. reference).

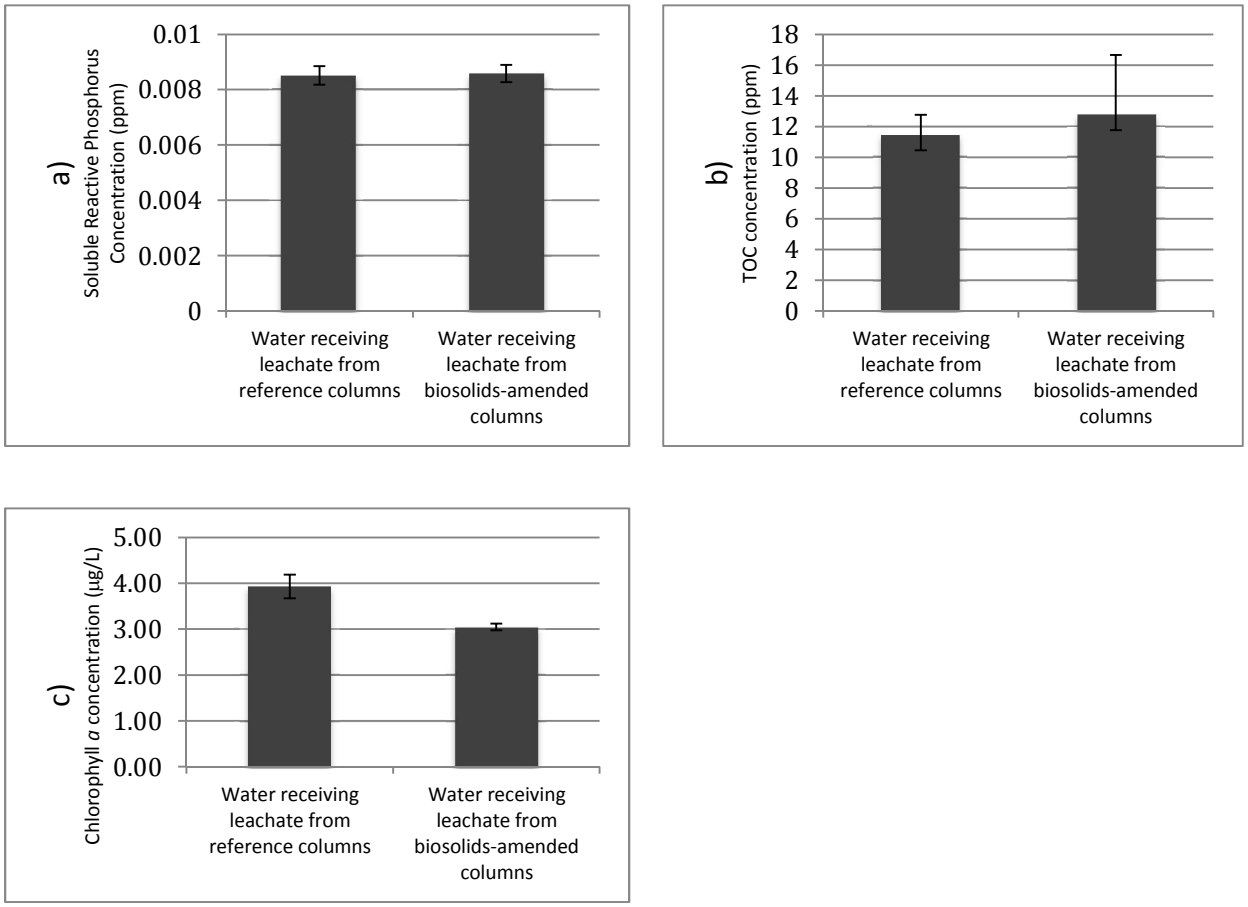


Fig. 3.6. Eutrophication's analysis: a) concentrations of soluble reactive phosphorus in receiving waters, b) concentrations of total organic carbon in receiving waters, and c) concentrations of chlorophyll *a* in receiving waters. Values plotted as mean ± standard deviation.

The concentration of soluble reactive phosphorus (Fig. 3.6a) in aquariums receiving leachate from biosolids-amended columns was not significantly different (t_4=0.27, p=0.800) from the concentration of soluble reactive phosphorus in aquariums receiving leachate from reference columns. Both values were determined to be around 9 mg/m^3. If a soil less rich in phosphorus were used in the experiment, the risk of the contribution to receiving water eutrophication would be even smaller. If biosolids with higher phosphorus content were used, the leachate produced by the amended columns might result in greater phosphorus concentrations in receiving waters and a higher risk of the contribution to eutrophication. However, based on Hanief (2011) where biosolids with approximately 5 times greater total phosphorus were applied, the increases in most phosphorus fractions were barely greater than the methodological ability to measure these increases. Therefore, it appears that soil type rather than biosolids application is the greater determinant in the contribution of leachate to eutrophication.

The concentration of total organic carbon (Fig. 3.6b) in aquariums receiving leachate from biosolids-amended columns also did not differ from the concentration of total organic carbon in aquariums receiving leachate from reference columns (t_4=1.64, p=0.176). Both TOC in aquariums receiving leachate from reference columns (11.46 ppm or 11.46 mg/L) and TOC in aquariums receiving leachate from biosolids-amended columns (12.76 ppm or 12.76 mg/dm^3) were close to the values of TOC content in natural waters (~10 mg/L) (Niemirycz *et al.,* 2006). Even though a slight increse was observed for TOC concentrations in aquariums, they were still far from a three-fold increase expected to be observed in eutrophied lakes (Pełechaty *et al.,* 2003). This suggested that neither leachate from

reference columns nor leachate from biosolids-amended columns caused eutrophication of receiving waters.

The concentrations of chlorophyll *a* (Fig. 3.6c) in aquariums receiving leachate from biosolids-amended columns (3.0 µg/L) and in aquariums receiving leachate from reference columns (3.9 µg/L) were low and belonged to the chlorophyll *a* range typical of oligotrophic lakes (Mitchell, 1990). There was no difference in chlorophyll *a* concentrations in aquariums receiving leachate from biosolids-amended columns compared to aquariums receiving leachate from reference columns (t_4=-0.36, p=0.7403). This observation suggested that neither leachate from reference columns nor leachate from biosolids-amended columns caused eutrophication of receiving waters.

According to the results for three analyzed parameters (phosphorus concentration, total organic carbon concentration, and chlorophyll *a* concentration), tile drainage from phosphorus-rich soils amended with anaerobically digested biosolids moderately rich in phosphorus does not lead to eutrophication of receiving water bodies in conditions where dilution factor is five or more, and sub-hypothesis 3 is rejected.

3.1.6 Phosphorus Findings Summary

A summary of the findings on phosphorus in biosolids, biosolids-amended soils, and reference soils is provided in Table 3.5.

Table 3.5. Phosphorus Findings Summary

Initial Data		
Soil	Representative of a typical "Non response" (rich in phosphorus) soil of Ontario	TP=0.226 mg/g of soil; Bioavailable P = 0.05 mg/g of soil; WSP=0.003 mg/g; LBP=0.05 mg/g; MBP=0.11 mg/g; CBP=0.02 mg/g; LP=0.003 mg/g; MLP=0.02 mg/g; NLP=0.05 mg/g.
Biosolids	Typical anaerobically digested biosolids of Ontario moderately rich in phosphorus	TP=5.6 mg/g of biosolids WSP=0.17 mg/g; LBP=0.20 mg/g; MBP=2.7 mg/g; CBP=0.45 mg/g; LP=0.18mg/g; MLP=1.4 mg/g; NLP=0.55 mg/g.
Results		
Phosphorus in soil	The concentrations of phosphorus have changed significantly over time; however, these changes were not caused by biosolids application (no significant difference between treatments). Additionally, no phosphorus migration was observed over time for either biosolids-amended soil or for reference soil (no significant difference between locations).	WSP=0.007 mg/g; LBP=0.12 mg/g; MBP=0.07 mg/g; CBP=0.01 mg/g; LP=0.005mg/g; MLP=0.06 mg/g; NLP=0.09 mg/g.
Phosphorus in leachate	Biosolids application did not cause an increase of phosphorus concentration in leachate (no significant difference between treatments). However, both leachate from reference columns and leachate from biosolids-amended columns exceeded concentrations suggested as optimal for limiting eutrophication potential reduction. Therefore, both types of leachate could contribute to the receiving water eutrophication depending on the dilution factor.	SRP=0.039 – 0.054 ppm
Eutrophication	No eutrophication was apparent in water receiving leachate from reference columns or in water receiving leachate from biosolids-amended columns (no significant difference between treatments).	SRP = 0.0085 ppm TOC = 11.5 – 12.8 ppm Chlorophyll *a* = 0.003 – 0.004 mg/L

3.2 Biosolids Effects on Soil Productivity

Before soya seeds were planted into the soil columns, a germination test was conducted in order to evaluate their germination efficiency. It was established that purchased soya seeds

had 65% germination ratio. In other words, each planted seed had a 65% chance to germinate. Such germination ratio is low and is not typical for commercially sold seeds.

The total phosphorus concentration was also evaluated in soya seeds (Appendix H). It was found to be 0.31 mg/g of biomass. Three soya seeds were planted in each soil column. The weight of each seed was ~0.23 g. Therefore, ~ 0.212 mg of phosphorus was added to each column in the form of seeds, which is approximately 100 times less than the total phosphorus added in the form of biosolids (~20.46 g).

At the end of the mimicked vegetation season, only four out of eight columns produced soya plants. Of four columns that produced plants, three were biosolids-amended columns and one was a reference column. However, accounting low germination ratio of the seeds, it is hard to conclude whether such an outcome had any meaning or was just a coincidence. Therefore, plant experiment was accounted to be a failure of the research. However, presence of plants in some columns did not affect the concentrations of any phosphorus forms (for water soluble phosphorus: $F_{1,27}$=0.77, p=0.3875; for loosely-bound phosphorus: $F_{1,27}$=2.17, p=0.1519; for metal-bound phosphorus: $F_{1,27}$=0.87, p=0.3594; for calcium-bound phosphorus: $F_{1,23}$=1.18, p=0.2892; for organic labile phosphorus: $F_{1,27}$=0.50, p=0.4882, for organic moderately-labile phosphorus: $F_{1,27}$=0.50, p=0.4842; and for organic non-labile phosphorus: $F_{1,27}$=0.04, p=0.8398). Additional information about plants produced by the columns is provided in Appendix I.

Even though biosolids application to soils rich in phosphorus might not be beneficial in terms of a crop yield increase, it might still be considered environmentally sustainable, as it demonstrated no negative environmental impact (i.e., increased phosphorus leaching and impact of leachate on receiving water) in the conditions of minimal or no slope, where phosphorus transport would be by vertical migration. Under such conditions, the results of this study suggest that as much as 8 tonnes per hectare of biosolids can be land applied without risk to receiving waters via migration to tile drainage.

3.3. Setup and Methods Efficiency

The setup created for the current research was found to be efficient for the evaluation of phosphorus vertical migration. The diameter of the constructed soil columns eliminated horizontal movement of phosphorus and provided for an easy collection of the total of six soil samples at any depth. The selected gravel did not affect the pH of leachate (both the pH of soil collected at the bottom of the columns and the pH of leachate were neutral or close to neutral), and therefore did not interfere with the experimental results, while still improving the drainage of the leachate and preventing soil escape from the system. The depth of the soil columns represented the minimum depth at which tile drainage systems would be expected, as tiles are located below tillage depth and typically below the depth of frost penetration. As these systems transfer soil leachate to nearby surface waters, it was vital to evaluate phosphorus migration towards them. Though most commonly tile drainage systems are located at the depth of 60-90cm, evaluation of phosphorus migration within the minimal depth is crucial, as it represents the worst scenario. As the depth of a drainage system increasing, less phosphorus reaches it being partially caught by soil and

clay particles. This experimental set-up created the minimum practicable path-length for vertical migration. Yet results found no evidence of increased phosphorus in leachate. As no migration of phosphorus was observed through the top 40 cm of soil, it would be fair to assume that no migration would be observed bellow this depth as well. A constructions of taller columns, in this case would be wasteful. Finally, the constructed system was built in a way that ensured a convenient access to the columns at any time.

The standard method described by Kovar and Pierzynski (2009) was used in the current research for phosphorus fractionation in soil samples. The method was found to be efficient for the analysis of inorganic phosphorus fractions. The organic phosphorus sequential fractionation, however, revealed inappropriate results. According to Kovar and Pierzynski, a persulfate digestion should be used to determine total labile phosphorus and total moderately-labile phosphorus. Determined concentrations of total labile phosphorus and total moderately-labile phosphorus should be greater than concentrations of inorganic labile phosphorus and inorganic moderately-labile phosphorus, respectively. Organic labile phosphorus and organic moderately-labile phosphorus are then calculated as the difference between the total and the inorganic phosphorus fractions. However, when applied to the soil samples in this study, persulfate digestion resulted in concentrations of total labile phosphorus and total moderately-labile phosphorus lower than concentrations of inorganic labile phosphorus and inorganic moderately-labile phosphorus. Such results might have been a consequence of an interference of residual persulfate with the ascorbic acid - molybdate solution; the color may not have developed properly in the digested samples, leading to an underestimation of total phosphorus in the labile and moderately-

labile fractions. To avoid these problems, strong acid digestion (commonly used for the liquid samples and also described by Kovar and Pierzynski) was used instead of persulfate digestion during all experiments. Other methods used in the current research posed no difficulties and were found efficient.

3.4. Future Studies Recommendations

The timeframe of the experiment allowed testing of short-term effects of biosolids on soil phosphorus, leachate, and surrounding environment. If long-term effects were tested, results might differ. Metal-bound phosphorus present in biosolids at high levels might become available in time, and therefore might be used by plants or negatively affect nearby receiving water bodies. Therefore, a long-term study on vertical phosphorus migration in biosolids-amended soils is suggested.

Additionally, the set-up used in the current research precluded aeration of soil by earthworms and potential increase in vertical migration associated with worms. If such a study is repeated in the future, it might use larger diameter columns to permit survival and activity of worms in the columns.

Finally, it might be recommended that the findings of the current study be tested through a field study.

4. Conclusions

Biosolids land application has been a controversial practice, particularly within broader society, but also among scientists. One of the environmental concerns related to this practice is an over application of nutrients, specifically phosphorus, and their subsequent transfer to aquatic ecosystems. The current study was conducted in order to evaluate the effect of biosolids land application on phosphorus behavior in soil profiles and leachate. Soil, representing typical "Non response" (i.e., rich in phosphorus) Ontario soil, was amended with biosolids, moderately rich in phosphorus and anaerobically digested, at a rate of 8 tonnes (dry weight) per hectare. Over five months, soil samples from two different depths were collected and sequentially fractionated to determine various inorganic and organic phosphorus pools in order to evaluate phosphorus vertical migration within a soil profile. Soil leachate was analyzed for soluble reactive phosphorus and added to aquariums mimicking receiving surface waters to determine the likely contribution of leachate to eutrophication.

The results of the soil analysis revealed that concentrations of several phosphorus fractions changed significantly over time. However, these changes were not caused by biosolids application, as no significant difference in phosphorus concentrations was observed between amended and reference soils. Therefore, it can be concluded that an amendment of phosphorus-rich soil with anaerobically digested biosolids under the conditions simulated within the current research does not lead to a significant increase in soil phosphorus concentrations. Additionally, no phosphorus migration was observed for either biosolids-amended soils or for reference soils, as suggested by the absence of any

significant difference in phosphorus concentrations between locations (i.e., top or bottom) over time. Therefore, it can be concluded that phosphorus from the biosolids-amended surface does not measurably migrate through the soil profile towards underground tile drainage systems under the conditions simulated within the current research. This is consistent with results of leachate analysis that showed that biosolids application did not cause an increase of phosphorus concentration in leachate. Leachate from both reference columns and biosolids-amended columns exceeded phosphorus concentrations suggested as optimal for eutrophication potential reduction. Therefore, both types of leachate could contribute to the receiving water eutrophication. Nonetheless, a five-fold dilution of leachate in receiving water led to no apparent eutrophication for either reference soils or biosolids-amended soils.

Therefore, biosolids application to the land at specified rates, and at no or minimal slope would not be expected to increase the risk of eutrophication of surrounding water bodies.

Overall, the study demonstrated that biosolids amendment of soil under the conditions simulated within the current research possesses no measurable phosphorus-related eutrophication risk to surface waters. This is true provided that the pathway for migration of phosphorus is vertical to tile drainage. The results presented are in contrast with some previous studies (e.g., Hanief, 2011), which demonstrated land application of biosolids could contribute to eutrophication. However, those previous studies simulated a very different scenario in which biosolids were applied to soil on a slope, with heavy rainfall immediately following application. The results presented here suggest that under a different scenario (i.e., no slope, incorporation of material into soils), biosolids can be safely

land applied. Under this scenario, concerns over leaching of phosphorus derived from biosolids, and potential for leachate to contribute to eutrophication, do not appear warranted. Land application may be safely practiced either as a way to fertilize soil poor in nutrients or simply as a biosolids disposal method.

Appendix A: Determining Biosolids Concentration and Biosolids Application Rate for Constructed Columns

Determining Biosolids Concentration

	Trial 1	Trial 2	Trial 3	Average Value
Mass of an empty crucible (g)	25.60	20.67	39.07	
Mass of a crucible with wet biosolids (g)	45.68	36.22	55.32	
Mass of wet biosolids (g)	20.08	15.55	16.25	
Mass of a crucible with dried biosolids (g)	25.90	20.90	39.30	
Mass of dried biosolids (g)	0.30	0.23	0.23	
Biosolids concentration (g/g)	0.0149	0.0148	0.0142	0.0146
Density of biosolids (g/mL)	0.9561	0.9561	0.9561	
Biosolids concentration (g/mL)	0.0143	0.0141	0.0135	0.0140

Determining Biosolids Application Rate for Constructed Columns

Parameter	Formula	Value	Metric	Converted Value	Metric
Column's diameter (D)		3	inch	0.0762	m
Column's radius (r)	r = D/2	0.0381	m		
Column's surface area (A)	A = π * r * r	0.0046	m^2		
Biosolids application rate (R)		8	tonnes/ha	0.8	kg/m^2
Biosolids required for column's surface area (dry weight) (M)	M = R * A	0.0036	kg	3.6464	g
Biosolids concentration (C)		0.0146	g/g	0.0140	g/mL
Biosolids required for column's surface area (wet weight) (V)	V = M/C	261	mL		

Appendix B: Phosphorus Fractions in Biosolids

Inorganic Phosphorus Fractions in Biosolids

Inorganic Water-soluble P

	Sample mass (g)	Volume of water (L)	Dilution factor	Absorbance	P Concentration (ppm)	P Concentration (mg/L)	P Concentration (mg/g)
Trial 1	0.25	0.025	4	0.3149	0.526	2.103	0.210
Trial 2	0.25	0.025	4	0.2587	0.415	1.660	0.166
Trial 3	0.25	0.025	4	0.2512	0.401	1.603	0.160
Trial 4	0.25	0.025	4	0.2512	0.401	1.603	0.160

Inorganic Loosely-Bound P

	Sample mass (g)	Volume of NH_4Cl (L)	Dilution factor	Absorbance	P Concentration (ppm)	P Concentration (mg/L)	P Concentration (mg/g)
Trial 1	0.25	0.025	6	0.2410	0.382	2.291	0.229
Trial 2	0.25	0.025	6	0.2140	0.333	1.996	0.200
Trial 3	0.25	0.025	6	0.2118	0.329	1.972	0.197
Trial 4	0.25	0.025	6	0.1943	0.298	1.787	0.179

Inorganic Metal-bound P

	Sample mass (g)	Volume of NaOH (L)	Dilution factor	Absorbance	P Concentration (ppm)	P Concentration (mg/L)	P Concentration (mg/g)
Trial 1	0.25	0.025	80	0.2173	0.339	27.083	2.708
Trial 2	0.25	0.025	80	0.2126	0.330	26.410	2.641
Trial 3	0.25	0.025	80	0.2151	0.335	26.768	2.677
Trial 4	0.25	0.025	80	0.2154	0.335	26.811	2.681

Inorganic Calcium-bound P

	Sample mass (g)	Volume of HCl (L)	Dilution factor	Absorbance	P Concentration (ppm)	P Concentration (mg/L)	P Concentration (mg/g)
Trial 1	0.25	0.025	12	0.2226	0.348	4.177	0.418
Trial 2	0.25	0.025	12	0.2745	0.445	5.344	0.534
Trial 3	0.25	0.025	12	0.2045	0.316	3.789	0.379
Trial 4	0.25	0.025	12	0.2538	0.406	4.869	0.487

Fraction	Average P Concentration (mg/g)	Standard Deviation
Water-soluble P	0.174	0.024
Loosely-bound P	0.201	0.021
Metal-bound P	2.677	0.028
Calcium-bound P	0.455	0.070

Organic Phosphorus Fractions in Biosolids

Organic Labile P

	With digestion							Without digestion					Organic P
	Sample mass (g)	Volume of $NaHCO_3$ (L)	Dilution factor	Absorbance	P Concentration (ppm)	P Concentration (mg/L)	P Concentration (mg/g)	Dilution factor	Absorbance	P Concentration (ppm)	P Concentration (mg/L)	P Concentration (mg/g)	P Concentration (mg/g)
Trial 1	0.25	0.025	40	0.1628	0.244	9.766	0.977	40	0.1424	0.211	8.422	0.842	0.134
Trial 2	0.25	0.025	40	0.1583	0.237	9.466	0.947	40	0.1309	0.192	7.681	0.768	0.179
Trial 3	0.25	0.025	40	0.1872	0.286	11.422	1.142	40	0.1504	0.224	8.945	0.894	0.248
Trial 4	0.25	0.025	40	0.177	0.268	10.723	1.072	40	0.1562	0.233	9.327	0.933	0.140

Organic Moderately-Labile P

	With digestion							Without digestion					Organic P
HCL extraction	Sample mass (g)	Volume of HCl (L)	Dilution factor	Absorbance	P Concentration (ppm)	P Concentration (mg/L)	P Concentration (mg/g)	Dilution factor	Absorbance	P Concentration (ppm)	P Concentration (mg/L)	P Concentration (mg/g)	P Concentration (mg/g)
Trial 1	0.25	0.025	20	0.2377	0.376	7.514	0.751	5	0.2987	0.493	2.465	0.247	0.505
Trial 2	0.25	0.025	20	0.2488	0.396	7.927	0.793	5	0.2956	0.487	2.434	0.243	0.549
Trial 3	0.25	0.025	20	0.2447	0.389	7.773	0.777	5	0.3067	0.509	2.546	0.255	0.523
Trial 4	0.25	0.025	20	0.2421	0.384	7.677	0.768	5	0.3178	0.532	2.659	0.266	0.502
	Humic Acid + Fulveic Acid (With digestion)							Fulvic Acid (Without digestion)					Humic Acid
NaOH extraction	Sample mass (g)	Volume of NaOH (L)	Dilution factor	Absorbance	P Concentration (ppm)	P Concentration (mg/L)	P Concentration (mg/g)	Dilution factor	Absorbance	P Concentration (ppm)	P Concentration (mg/L)	P Concentration (mg/g)	P Concentration (mg/g)
Trial 1	0.25	0.025	30	0.298	8.936	0.894	0.8936	30	0.185	0.282	8.453	0.845	0.048
Trial 2	0.25	0.025	30	0.322	9.658	0.966	0.9658	30	0.1786	0.271	8.124	0.812	0.153
Trial 3	0.25	0.025	30	0.324	9.711	0.971	0.9711	30	0.1912	0.292	8.774	0.877	0.094
Trial 4	0.25	0.025	30	0.336	10.070	1.007	1.0070	30	0.2006	0.309	9.266	0.927	0.080

	HCL extracted P (mg/g)	Fulvic Acid P (mg/g)	Total Moderately-Labile P (mg/g)
Trial 1	0.505	0.845	1.350
Trial 2	0.549	0.812	1.362
Trial 3	0.523	0.877	1.400
Trial 4	0.502	0.927	1.428

Organic Non-Labile P

Ashing + H2SO4 extraction							
	Sample mass (g)	Volume of H_2SO_4 (L)	Dilution factor	Absorbance	P Concentration (ppm)	P Concentration (mg/L)	P Concentration (mg/g)
Trial 1	0.25	0.025	10	0.2268	0.356	3.557	0.356
Trial 2	0.25	0.025	10	0.2152	0.335	3.348	0.335
Trial 3	0.25	0.025	10	0.234	0.369	3.689	0.369
Trial 4	0.25	0.025	10	0.2346	0.370	3.700	0.370
Strong acid digestion							
	Sample mass (g)	Volume of acid (L)	Dilution factor	Absorbance	P Concentration (ppm)	P Concentration (mg/L)	P Concentration (mg/g)
Trial 1	0.25	0.01	10	0.1596	0.239	2.388	0.096
Trial 2	0.25	0.01	10	0.1588	0.237	2.375	0.095
Trial 3	0.25	0.01	10	0.1745	0.264	2.638	0.106
Trial 4	0.25	0.01	10	0.1639	0.246	2.460	0.098

	Humic Acid P (mg/g)	H_2SO_4 extracted P (mg/g)	Strong acid extracted P (mg/g)	Total Non-Labile P (mg/g)
Trial 1	0.048	0.356	0.096	0.500
Trial 2	0.153	0.335	0.095	0.583
Trial 3	0.094	0.369	0.106	0.568
Trial 4	0.080	0.370	0.098	0.549

Fraction	Average P Concentration (mg/g)	Standard Deviation
Organic Labile P	0.175	0.052
Organic Moderately-Labile P	1.385	0.036
Organic Non-Labile	0.550	0.036

Total Phosphorus

	Sample mass (g)	Volume of H_2SO_4 (L)	Dilution factor	Absorbance	P Concentration (ppm)	P Concentration (mg/L)	P Concentration (mg/g)
Trial 1	0.25	0.025	140	0.473	66.238	6.624	0.473
Trial 2	0.25	0.025	140	0.394	55.146	5.515	0.394
Trial 3	0.25	0.025	140	0.460	64.337	6.434	0.460
Trial 4	0.25	0.025	140	0.454	63.571	6.357	0.454
Average							6.232
Standard Deviation							0.491

Appendix C: Initial Phosphorus Fractions in Soil

Inorganic Phosphorus Fractions

Inorganic Water-soluble P

Sample *	Sample mass (g)	Volume of water (L)	Dilution factor	Absorbance	P Concentration (ppm)	P Concentration (mg/L)	P Concentration (mg/g)
1a	0.5	0.025	1	0.0504	0.061	0.061	0.003
1b	0.5	0.025	1	0.0533	0.064	0.064	0.003
2a	0.5	0.025	1	0.0432	0.052	0.052	0.003
2b	0.5	0.025	1	0.049	0.059	0.059	0.003
3a	0.5	0.025	1	0.0518	0.062	0.062	0.003
3b	0.5	0.025	1	0.0547	0.066	0.066	0.003
4a	0.5	0.025	1	0.0518	0.062	0.062	0.003
4b	0.5	0.025	1	0.0539	0.065	0.065	0.003
5a	0.5	0.025	1	0.0533	0.064	0.064	0.003
5b	0.5	0.025	1	0.0507	0.061	0.061	0.003
6a	0.5	0.025	1	0.0702	0.085	0.085	0.004
6b	0.5	0.025	1	0.0607	0.073	0.073	0.004
7a	0.5	0.025	1	0.0631	0.076	0.076	0.004
7b	0.5	0.025	1	0.0506	0.061	0.061	0.003
8a	0.5	0.025	1	0.0534	0.064	0.064	0.003
8b	0.5	0.025	1	0.0521	0.063	0.063	0.003

* 1-8 – number of the columns (odd number columns are columns chosen to be reference columns, even number columns are columns chosen for biosolids application)
a – sample collected from the top of a column
b – sample collected from the bottom of a column

Inorganic Loosely-Bound P

Sample	Sample mass (g)	Volume of NH_4Cl (L)	Dilution factor	Absorbance	P Concentration (ppm)	P Concentration (mg/L)	P Concentration (mg/g)
1a	0.5	0.025	1	0.6448	1.122	1.122	0.056
1b	0.5	0.025	1	0.662	1.165	1.165	0.058
2a	0.5	0.025	1	0.6477	1.130	1.130	0.056
2b	0.5	0.025	1	0.6058	1.028	1.028	0.051
3a	0.5	0.025	1	0.6244	1.072	1.072	0.054
3b	0.5	0.025	1	0.6176	1.056	1.056	0.053
4a	0.5	0.025	1	0.6438	1.120	1.120	0.056
4b	0.5	0.025	1	0.6702	1.186	1.186	0.059
5a	0.5	0.025	1	0.6732	1.194	1.194	0.060
5b	0.5	0.025	1	0.6556	1.149	1.149	0.057
6a	0.5	0.025	1	0.6126	1.044	1.044	0.052
6b	0.5	0.025	1	0.6045	1.025	1.025	0.051
7a	0.5	0.025	1	0.6142	1.048	1.048	0.052
7b	0.5	0.025	1	0.6619	1.165	1.165	0.058
8a	0.5	0.025	1	0.6225	1.068	1.068	0.053
8b	0.5	0.025	1	0.6429	1.118	1.118	0.056

Inorganic Metal-bound P

Sample	Sample mass (g)	Volume of NaOH (L)	Dilution factor	Absorbance	P Concentration (ppm)	P Concentration (mg/L)	P Concentration (mg/g)
1a	0.5	0.025	50	0.0398	0.048	2.377	0.119
1b	0.5	0.025	50	0.0423	0.051	2.530	0.127
2a	0.5	0.025	50	0.0407	0.049	2.432	0.122
2b	0.5	0.025	50	0.0397	0.047	2.371	0.119
3a	0.5	0.025	50	0.0376	0.045	2.243	0.112
3b	0.5	0.025	50	0.037	0.044	2.206	0.110
4a	0.5	0.025	50	0.0467	0.056	2.801	0.140
4b	0.5	0.025	50	0.0403	0.048	2.408	0.120
5a	0.5	0.025	50	0.0374	0.045	2.231	0.112
5b	0.5	0.025	50	0.0344	0.041	2.048	0.102
6a	0.5	0.025	50	0.0343	0.041	2.042	0.102
6b	0.5	0.025	50	0.0382	0.046	2.279	0.114
7a	0.5	0.025	50	0.0361	0.043	2.152	0.108
7b	0.5	0.025	50	0.0356	0.042	2.121	0.106
8a	0.5	0.025	50	0.0374	0.045	2.231	0.112
8b	0.5	0.025	50	0.0368	0.044	2.194	0.110

Inorganic Calcium-bound P

Sample	Sample mass (g)	Volume of HCl (L)	Dilution factor	Absorbance	P Concentration (ppm)	P Concentration (mg/L)	P Concentration (mg/g)
1a	0.5	0.025	1.1	0.2706	0.371	0.408	0.020
1b	0.5	0.025	1.1	0.2626	0.359	0.394	0.020
2a	0.5	0.025	1.1	0.2631	0.359	0.395	0.020
2b	0.5	0.025	1.1	0.3267	0.464	0.511	0.026
3a	0.5	0.025	1.1	0.2641	0.361	0.397	0.020
3b	0.5	0.025	1.1	0.2635	0.360	0.396	0.020
4a	0.5	0.025	1.1	0.2549	0.346	0.381	0.019
4b	0.5	0.025	1.1	0.3019	0.422	0.465	0.023
5a	0.5	0.025	1.1	0.2893	0.402	0.442	0.022
5b	0.5	0.025	1.1	0.2745	0.378	0.415	0.021
6a	0.5	0.025	1.1	0.2707	0.372	0.409	0.020
6b	0.5	0.025	1.1	0.3097	0.435	0.479	0.024
7a	0.5	0.025	1.1	0.2854	0.395	0.435	0.022
7b	0.5	0.025	1.1	0.256	0.348	0.383	0.019
8a	0.5	0.025	1.1	0.282	0.390	0.429	0.021
8b	0.5	0.025	1.1	0.279	0.385	0.423	0.021

Fraction	Water-soluble P	Loosely-bound P	Metal-bound P	Calcium-bound P
Average P Concentration	0.003	0.055	0.115	0.021
Standard Deviation	0.000	0.003	0.010	0.002

Organic Phosphorus Fractions

Organic Labile P

Sample	Sample mass (g)	Volume of $NaHCO_3$ (L)	Dilution factor	Absorbance	P Concentration (ppm)	P Concentration (mg/L)	P Concentration (mg/g)	Dilution factor	Absorbance	P Concentration (ppm)	P Concentration (mg/L)	P Concentration (mg/g)	P Concentration (mg/g)
1a	0.5	0.025	1	0.0971	0.120	0.120	0.006	1	0.0414	0.050	0.050	0.002	0.004
1b	0.5	0.025	1	0.0877	0.108	0.108	0.005	1	0.0312	0.037	0.037	0.002	0.004
2a	0.5	0.025	1	0.0868	0.107	0.107	0.005	1	0.0366	0.044	0.044	0.002	0.003
2b	0.5	0.025	1	0.0828	0.101	0.101	0.005	1	0.0337	0.040	0.040	0.002	0.003
3a	0.25	0.025	1	0.0728	0.089	0.089	0.009	1	0.0307	0.036	0.036	0.004	0.005
3b	0.45	0.025	1	0.0846	0.104	0.104	0.006	1	0.0373	0.044	0.044	0.002	0.003
4a	0.5	0.025	1	0.1046	0.130	0.130	0.006	1	0.0402	0.048	0.048	0.002	0.004
4b	0.5	0.025	1	0.089	0.109	0.109	0.005	1	0.0415	0.050	0.050	0.002	0.003
5a	0.25	0.025	1	0.0804	0.098	0.098	0.010	1	0.0396	0.047	0.047	0.005	0.005
5b	0.5	0.025	1	0.0866	0.106	0.106	0.005	1	0.0413	0.049	0.049	0.002	0.003
6a	0.5	0.025	1	0.0968	0.120	0.120	0.006	1	0.051	0.061	0.061	0.003	0.003
6b	0.45	0.025	1	0.0888	0.109	0.109	0.006	1	0.0476	0.057	0.057	0.003	0.003
7a	0.5	0.025	1	0.0895	0.110	0.110	0.006	1	0.0482	0.058	0.058	0.003	0.003
7b	0.3	0.025	1	0.085	0.104	0.104	0.009	1	0.042	0.050	0.050	0.004	0.005
8a	0.3	0.025	1	0.088	0.108	0.108	0.009	1	0.0451	0.054	0.054	0.005	0.005
8b	0.5	0.025	1	0.0929	0.115	0.115	0.006	1	0.0415	0.050	0.050	0.002	0.003

Organic Moderately-Labile P

	HCL extraction												
	With digestion							Without digestion					Organic P
Sample	Sample mass (g)	Volume of HCl (L)	Dilution factor	Absorbance	P Concentration (ppm)	P Concentration (mg/L)	P Concentration (mg/g)	Dilution factor	Absorbance	P Concentration (ppm)	P Concentration (mg/L)	P Concentration (mg/g)	P Concentration (mg/g)
1a	0.5	0.025	1	0.1259	0.158	0.158	0.008	1	0.0346	0.041	0.041	0.002	0.006
1b	0.5	0.025	1	0.1311	0.165	0.165	0.008	1	0.0342	0.041	0.041	0.002	0.006
2a	0.5	0.025	1	0.1177	0.147	0.147	0.007	1	0.0307	0.036	0.036	0.002	0.006
2b	0.5	0.025	1	0.1318	0.166	0.166	0.008	1	0.0343	0.041	0.041	0.002	0.006
3a	0.25	0.025	1	0.109	0.136	0.136	0.014	1	0.0375	0.045	0.045	0.004	0.009
3b	0.45	0.025	1	0.127	0.160	0.160	0.009	1	0.0318	0.038	0.038	0.002	0.007
4a	0.5	0.025	1	0.1597	0.205	0.205	0.010	1	0.0343	0.041	0.041	0.002	0.008
4b	0.5	0.025	1	0.1345	0.170	0.170	0.008	1	0.0304	0.036	0.036	0.002	0.007
5a	0.25	0.025	1	0.1143	0.143	0.143	0.014	1	0.0478	0.057	0.057	0.006	0.009
5b	0.5	0.025	1	0.1259	0.158	0.158	0.008	1	0.055	0.066	0.066	0.003	0.005
6a	0.5	0.025	1	0.149	0.190	0.190	0.009	1	0.0487	0.058	0.058	0.003	0.007
6b	0.45	0.025	1	0.1536	0.196	0.196	0.011	1	0.0348	0.041	0.041	0.002	0.009
7a	0.5	0.025	1	0.1311	0.165	0.165	0.008	1	0.0477	0.057	0.057	0.003	0.005
7b	0.3	0.025	1	0.1104	0.138	0.138	0.011	1	0.0469	0.056	0.056	0.005	0.007
8a	0.3	0.025	1	0.1077	0.134	0.134	0.011	1	0.0379	0.045	0.045	0.004	0.007
8b	0.5	0.025	1	0.1283	0.162	0.162	0.008	1	0.0316	0.038	0.038	0.002	0.006

NaOH extraction													
	Humic Acid + Fulveic Acid (With digestion)							Fulvic Acid (Without digestion)					Humic Acid
Sample	Sample mass (g)	Volume of NaOH (L)	Dilution factor	Absorbance	P Concentration (ppm)	P Concentration (mg/L)	P Concentration (mg/g)	Dilution factor	Absorbance	P Concentration (ppm)	P Concentration (mg/L)	P Concentration (mg/g)	P Concentration (mg/g)
1a	0.5	0.025	2	0.1381	0.175	0.350	0.017	2	0.0997	0.123	0.247	0.012	0.005
1b	0.5	0.025	2	0.1296	0.163	0.327	0.016	2	0.1054	0.131	0.262	0.013	0.003
2a	0.5	0.025	2	0.1275	0.160	0.321	0.016	2	0.1074	0.134	0.267	0.013	0.003
2b	0.5	0.025	2	0.1163	0.145	0.291	0.015	2	0.1047	0.130	0.260	0.013	0.002
3a	0.25	0.025	2	0.1196	0.150	0.300	0.030	2	0.0883	0.109	0.217	0.022	0.008
3b	0.45	0.025	2	0.1393	0.177	0.353	0.020	2	0.1076	0.134	0.268	0.015	0.005
4a	0.5	0.025	2	0.15	0.191	0.383	0.019	2	0.1036	0.129	0.257	0.013	0.006
4b	0.5	0.025	2	0.1054	0.131	0.262	0.013	2	0.0974	0.120	0.241	0.012	0.001
5a	0.25	0.025	2	0.1255	0.158	0.315	0.032	2	0.0955	0.118	0.236	0.024	0.008
5b	0.5	0.025	2	0.1611	0.207	0.414	0.021	2	0.1014	0.126	0.251	0.013	0.008
6a	0.5	0.025	2	0.1556	0.199	0.398	0.020	2	0.1068	0.133	0.265	0.013	0.007
6b	0.45	0.025	2	0.1128	0.141	0.281	0.016	2	0.1069	0.133	0.266	0.015	0.001
7a	0.5	0.025	2	0.1083	0.135	0.269	0.013	2	0.1007	0.125	0.249	0.012	0.001
7b	0.3	0.025	2	0.126	0.158	0.317	0.026	2	0.1057	0.131	0.263	0.022	0.005
8a	0.3	0.025	2	0.1238	0.155	0.311	0.026	2	0.1061	0.132	0.264	0.022	0.004
8b	0.5	0.025	2	0.1589	0.204	0.408	0.020	2	0.0966	0.119	0.239	0.012	0.008

Sample	HCL extracted P (mg/g)	Fulvic Acid (mg/g)	Total Moderately-Labile P (mg/g)
1a	0.006	0.012	0.018
1b	0.006	0.013	0.019
2a	0.006	0.013	0.019
2b	0.006	0.013	0.019
3a	0.009	0.022	0.031
3b	0.007	0.015	0.022
4a	0.008	0.013	0.021
4b	0.007	0.012	0.019
5a	0.009	0.024	0.032
5b	0.005	0.013	0.017
6a	0.007	0.013	0.020
6b	0.009	0.015	0.023
7a	0.005	0.012	0.018
7b	0.007	0.022	0.029
8a	0.007	0.022	0.029
8b	0.006	0.012	0.018

Organic Non-Labile P

H2SO4 extraction							
Sample	Sample mass (g)	Volume of H_2SO_4 (L)	Dilution factor	Absorbance	P Concentration (ppm)	P Concentration (mg/L)	P Concentration (mg/g)
1a	0.5	0.025	1.5	0.501	0.752	0.038	0.501
1b	0.5	0.025	1.5	0.500	0.749	0.037	0.500
2a	0.5	0.025	1.5	0.564	0.847	0.042	0.564
2b	0.5	0.025	1.5	0.541	0.812	0.041	0.541
3a	0.25	0.025	1.5	0.371	0.556	0.056	0.371
3b	0.45	0.025	1.5	0.573	0.859	0.048	0.573
4a	0.5	0.025	1.5	0.482	0.722	0.036	0.482
4b	0.5	0.025	1.5	0.566	0.849	0.042	0.566
5a	0.25	0.025	1.5	0.373	0.560	0.056	0.373
5b	0.5	0.025	1.5	0.556	0.834	0.042	0.556
6a	0.5	0.025	1.5	0.644	0.966	0.048	0.644
6b	0.45	0.025	1.5	0.543	0.815	0.045	0.543
7a	0.5	0.025	1.5	0.561	0.842	0.042	0.561
7b	0.3	0.025	1.5	0.387	0.581	0.048	0.387
8a	0.3	0.025	1.5	0.405	0.608	0.051	0.405
8b	0.5	0.025	1.5	0.395	0.593	0.030	0.395

Sample	Humic Acid P (mg/g)	H2SO4 extracted P (mg/g)	Total Non-Labile P (mg/g)
1a	0.501	0.752	0.038
1b	0.500	0.749	0.037
2a	0.564	0.847	0.042
2b	0.541	0.812	0.041
3a	0.371	0.556	0.056
3b	0.573	0.859	0.048
4a	0.482	0.722	0.036
4b	0.566	0.849	0.042
5a	0.373	0.560	0.056
5b	0.556	0.834	0.042
6a	0.644	0.966	0.048
6b	0.543	0.815	0.045
7a	0.561	0.842	0.042
7b	0.387	0.581	0.048
8a	0.405	0.608	0.051
8b	0.395	0.593	0.030

Fraction	Labile	Moderately-labile	Non-labile
Average P concentration (mg/g)	0.004	0.022	0.049
Standard Deviation	0.001	0.005	0.008

Olsen P

Sample	Sample mass (g)	Volume of $NaHCO_3$ (L)	Dilution factor	Absorbance	Concentration (ppm)	Concentration (mg/L)	Concentration (mg/g)
Trial 1	1	0.02	8	0.2951	0.41	3.29	0.07
Trial 2	1	0.02	8	0.2881	0.40	3.20	0.06
Trial 3	1	0.02	8	0.2948	0.41	3.29	0.07
Trial 4	1	0.02	8	0.2842	0.39	3.15	0.06
						Average	0.06

Appendix D: Expected Phosphorus Concentrations Increase After Biosolids Application

Expected Phosphorus Concentrations Increase in Top 5 cm of The Soil Columns (within 1 day after biosolids application)

Parameter	Formula	Value	Metric
Column's diameter (D)		7.62	cm
Column's radius (r)	r = D/2	3.81	cm
Column's surface area (A)	A = π * r * r	45.5805	cm^2
Heights of top layer (h)		5	cm
Soil volume (V)	V = A * h	227.9027	cm^3
Soil density (d)		1.3	g/cm^3
Mass of soil (M)	M = V * d	296.2736	g

Parameter	Formula	Inorganic fractions				Organic fractions		
		Water-soluble P	Loosely-bound P	Metal-bound P	Calcium-bound P	Labile P	Moderately-Labile P	Non-Labile P
P concentration in biosolids (mg of P/ g of biosolids)		0.174	0.201	2.677	0.455	0.175	1.385	0.545
Amount of P applied to each column (g)	= P concentration in biosolids * Mass of biosolids	0.635	0.733	9.761	1.657	0.638	5.051	1.987
Increase in P concentration (mg of P/ g of soil)	= Amount of P applied / Mass of soil	0.002	0.002	0.033	0.006	0.002	0.017	0.007
Initial P concentration in soil (mg of P/ g of soil)		0.003	0.055	0.115	0.021	0.004	0.022	0.029
Expected P concentration in soil after application (mg /g)	= Increase in P concentration + Initial P concentration in soil	0.005	0.058	0.148	0.027	0.006	0.039	0.035
Increase (%)	= (Expected P concentration - Initial P concentration) / Initial P concentration	67.00%	4.48%	28.75%	26.51%	59.86%	76.79%	23.54%

Expected Phosphorus Concentrations Increase in Entire Length of The Soil Columns (within 1 week from biosolids application)

Parameter	Formula	Value	Metric
Column's diameter		7.62	cm
Column's radius	r = D/2	3.81	cm
Column's surface area	A = π * r * r	45.5805	cm^2
Heights of top layer		40	cm
Soil volume	V = A * h	1823.2221	cm^3
Soil density		1.3	g/cm^3
Mass of soil	M = V * d	2370.1888	g

Parameter	Formula	Inorganic fractions				Organic fractions		
		Water-soluble P	Loosely-bound P	Metal-bound P	Calcium-bound P	Labile P	Moderately-Labile P	Non-Labile P
P concentration in biosolids (mg of P/ g of biosolids)		0.174	0.201	2.677	0.455	0.175	1.385	0.545
Amount of P applied to each column (g)	= P concentration in biosolids * Mass of biosolids	0.635	0.733	9.761	1.657	0.638	5.051	1.987
Increase in P concentration (mg of P/ g of soil)	= Amount of P applied / Mass of soil	0.000	0.000	0.004	0.001	0.000	0.002	0.001
Initial P concentration in soil (mg of P/ g of soil)		0.003	0.055	0.115	0.021	0.004	0.022	0.029
Expected P concentrations in soil after application (mg /g)	= Increase in P concentration + Initial P concentration in soil	0.003	0.056	0.119	0.022	0.004	0.024	0.029
Increase (%)	= (Expected P concentration - Initial P concentration) / Initial P concentration	8.38%	0.56%	3.59%	3.31%	7.48%	9.60%	2.94%

Appendix E: Phosphorus Fractions in Soil Columns

Inorganic Phosphorus Fractions

Inorganic Water-soluble P within 14 days after biosolids application

Sample *	Sample mass (g)	Volume of water (L)	Dilution factor	Absorbance	P Concentration (ppm)	P Concentration (mg/L)	P Concentration (mg/g)
1a	0.5	0.025	1	0.0357	0.043	0.043	0.002
1b	0.5	0.025	1	0.058	0.070	0.070	0.004
2a	0.5	0.025	1	0.0582	0.070	0.070	0.004
2b	0.5	0.025	1	0.0634	0.077	0.077	0.004
3a	0.5	0.025	1	0.0608	0.074	0.074	0.004
3b	0.5	0.025	1	0.0602	0.073	0.073	0.004
4a	0.5	0.025	1				
4b	0.5	0.025	1	0.0641	0.078	0.078	0.004
5a	0.5	0.025	1	0.0536	0.065	0.065	0.003
5b	0.5	0.025	1	0.0861	0.106	0.106	0.005
6a	0.5	0.025	1	0.0881	0.108	0.108	0.005
6b	0.5	0.025	1	0.0575	0.069	0.069	0.003
7a	0.5	0.025	1	0.0538	0.065	0.065	0.003
7b	0.5	0.025	1	0.0487	0.058	0.058	0.003
8a	0.5	0.025	1	0.0533	0.064	0.064	0.003
8b	0.5	0.025	1	0.0634	0.077	0.077	0.004

Inorganic Water-soluble P within 30 days after biosolids application

Sample *	Sample mass (g)	Volume of water (L)	Dilution factor	Absorbance	P Concentration (ppm)	P Concentration (mg/L)	P Concentration (mg/g)
1a	0.25	0.025	1				
1b	0.25	0.025	1	0.0431	0.060	0.060	0.006
2a	0.25	0.025	1	0.0459	0.064	0.064	0.006
2b	0.25	0.025	1	0.0442	0.061	0.061	0.006
3a	0.25	0.025	1	0.0487	0.068	0.068	0.007
3b	0.25	0.025	1	0.0475	0.066	0.066	0.007
4a	0.25	0.025	1	0.0529	0.074	0.074	0.007
4b	0.25	0.025	1	0.0413	0.057	0.057	0.006
5a	0.25	0.025	1	0.0405	0.056	0.056	0.006
5b	0.25	0.025	1	0.0422	0.058	0.058	0.006
6a	0.25	0.025	1	0.0503	0.070	0.070	0.007
6b	0.25	0.025	1	0.0388	0.053	0.053	0.005
7a	0.25	0.025	1	0.0424	0.059	0.059	0.006
7b	0.25	0.025	1	0.0345	0.047	0.047	0.005
8a	0.25	0.025	1	0.0536	0.075	0.075	0.007
8b	0.25	0.025	1	0.0442	0.061	0.061	0.006

Inorganic Water-soluble P within 60 days after biosolids application

Sample *	Sample mass (g)	Volume of water (L)	Dilution factor	Absorbance	P Concentration (ppm)	P Concentration (mg/L)	P Concentration (mg/g)
1a	0.25	0.025	1	0.0432	0.060	0.060	0.006
1b	0.25	0.025	1	0.0553	0.077	0.077	0.008
2a	0.25	0.025	1	0.0486	0.067	0.067	0.007
2b	0.25	0.025	1	0.0453	0.063	0.063	0.006
3a	0.25	0.025	1	0.0412	0.057	0.057	0.006
3b	0.25	0.025	1	0.046	0.064	0.064	0.006
4a	0.25	0.025	1	0.0528	0.073	0.073	0.007
4b	0.25	0.025	1	0.0483	0.067	0.067	0.007
5a	0.25	0.025	1	0.0442	0.061	0.061	0.006
5b	0.25	0.025	1	0.0415	0.057	0.057	0.006
6a	0.25	0.025	1	0.0595	0.083	0.083	0.008
6b	0.25	0.025	1	0.048	0.067	0.067	0.007
7a	0.25	0.025	1	0.0452	0.063	0.063	0.006
7b	0.25	0.025	1	0.0412	0.057	0.057	0.006
8a	0.25	0.025	1	0.055	0.077	0.077	0.008
8b	0.25	0.025	1	0.0479	0.066	0.066	0.007

Inorganic Water-soluble P within 90 days after biosolids application

Sample *	Sample mass (g)	Volume of water (L)	Dilution factor	Absorbance	P Concentration (ppm)	P Concentration (mg/L)	P Concentration (mg/g)
1a	0.25	0.025	1	0.0625	0.087	0.087	0.009
1b	0.25	0.025	1	0.0628	0.088	0.088	0.009
2a	0.25	0.025	1	0.0614	0.086	0.086	0.009
2b	0.25	0.025	1	0.0482	0.067	0.067	0.007
3a	0.25	0.025	1	0.0494	0.069	0.069	0.007
3b	0.25	0.025	1	0.0496	0.069	0.069	0.007
4a	0.25	0.025	1	0.05	0.069	0.069	0.007
4b	0.25	0.025	1	0.0432	0.060	0.060	0.006
5a	0.25	0.025	1	0.0655	0.092	0.092	0.009
5b	0.25	0.025	1	0.0541	0.075	0.075	0.008
6a	0.25	0.025	1	0.0561	0.078	0.078	0.008
6b	0.25	0.025	1	0.0471	0.065	0.065	0.007
7a	0.25	0.025	1	0.0527	0.073	0.073	0.007
7b	0.25	0.025	1	0.0465	0.064	0.064	0.006
8a	0.25	0.025	1	0.0443	0.061	0.061	0.006
8b	0.25	0.025	1	0.0453	0.063	0.063	0.006

Inorganic Water-soluble P within 150 days after biosolids application

Sample *	Sample mass (g)	Volume of water (L)	Dilution factor	Absorbance	P Concentration (ppm)	P Concentration (mg/L)	P Concentration (mg/g)
1a	0.25	0.025	1				
1b	0.25	0.025	1	0.0336	0.046	0.046	0.005
2a	0.25	0.025	1	0.0569	0.079	0.079	0.008
2b	0.25	0.025	1	0.0489	0.068	0.068	0.007
3a	0.25	0.025	1	0.0614	0.086	0.086	0.009
3b	0.25	0.025	1	0.0389	0.054	0.054	0.005
4a	0.25	0.025	1	0.0588	0.082	0.082	0.008
4b	0.25	0.025	1	0.0414	0.057	0.057	0.006
5a	0.25	0.025	1	0.0538	0.075	0.075	0.007
5b	0.25	0.025	1	0.0347	0.048	0.048	0.005
6a	0.25	0.025	1	0.0622	0.087	0.087	0.009
6b	0.25	0.025	1	0.0454	0.063	0.063	0.006
7a	0.25	0.025	1	0.0584	0.082	0.082	0.008
7b	0.25	0.025	1	0.0416	0.057	0.057	0.006
8a	0.25	0.025	1	0.0642	0.090	0.090	0.009
8b	0.25	0.025	1	0.0507	0.070	0.070	0.007

Inorganic Loosely-Bound P within 14 days after biosolids application

Sample	Sample mass (g)	Volume of NH_4Cl (L)	Dilution factor	Absorbance	P Concentration (ppm)	P Concentration (mg/L)	P Concentration (mg/g)
1a	0.5	0.025	1	0.6681	1.181	1.181	0.059
1b	0.5	0.025	1	0.5828	0.974	0.974	0.049
2a	0.5	0.025	1	0.6465	1.127	1.127	0.056
2b	0.5	0.025	1	0.6351	1.098	1.098	0.055
3a	0.5	0.025	1	0.6436	1.119	1.119	0.056
3b	0.5	0.025	1	0.575	0.956	0.956	0.048
4a	0.5	0.025	1	0.6775	1.205	1.205	0.060
4b	0.5	0.025	1	0.6819	1.216	1.216	0.061
5a	0.5	0.025	1	0.5653	0.934	0.934	0.047
5b	0.5	0.025	1	0.58	0.968	0.968	0.048
6a	0.5	0.025	1	0.6232	1.069	1.069	0.053
6b	0.5	0.025	1	0.6026	1.020	1.020	0.051
7a	0.5	0.025	1	0.6	1.014	1.014	0.051
7b	0.5	0.025	1	0.6058	1.028	1.028	0.051
8a	0.5	0.025	1	0.5746	0.955	0.955	0.048
8b	0.5	0.025	1	0.6024	1.020	1.020	0.051

Inorganic Loosely-Bound P within 30 days after biosolids application

Sample	Sample mass (g)	Volume of NH_4Cl (L)	Dilution factor	Absorbance	P Concentration (ppm)	P Concentration (mg/L)	P Concentration (mg/g)
1a	0.25	0.025	1	0.3158	0.528	0.528	0.053
1b	0.25	0.025	1	0.3415	0.581	0.581	0.058
2a	0.25	0.025	1				
2b	0.25	0.025	1				
3a	0.25	0.025	1	0.334	0.565	0.565	0.057
3b	0.25	0.025	1				
4a	0.25	0.025	1	0.3258	0.548	0.548	0.055
4b	0.25	0.025	1	0.3031	0.502	0.502	0.050
5a	0.25	0.025	1				
5b	0.25	0.025	1	0.301	0.498	0.498	0.050
6a	0.25	0.025	1	0.2993	0.494	0.494	0.049
6b	0.25	0.025	1	0.3018	0.499	0.499	0.050
7a	0.25	0.025	1	0.2927	0.481	0.481	0.048
7b	0.25	0.025	1	0.2967	0.489	0.489	0.049
8a	0.25	0.025	1	0.3034	0.502	0.502	0.050
8b	0.25	0.025	1	0.3011	0.498	0.498	0.050

Inorganic Loosely-Bound P within 60 days after biosolids application

Sample	Sample mass (g)	Volume of NH_4Cl (L)	Dilution factor	Absorbance	P Concentration (ppm)	P Concentration (mg/L)	P Concentration (mg/g)
1a	0.25	0.025	1	0.2859	0.468	0.468	0.047
1b	0.25	0.025	1	0.3081	0.512	0.512	0.051
2a	0.25	0.025	1	0.3096	0.515	0.515	0.052
2b	0.25	0.025	1	0.2903	0.476	0.476	0.048
3a	0.25	0.025	1	0.2891	0.474	0.474	0.047
3b	0.25	0.025	1	0.2945	0.485	0.485	0.048
4a	0.25	0.025	1	0.3175	0.531	0.531	0.053
4b	0.25	0.025	1	0.307	0.510	0.510	0.051
5a	0.25	0.025	1	0.2999	0.495	0.495	0.050
5b	0.25	0.025	1	0.31	0.516	0.516	0.052
6a	0.25	0.025	1	0.3141	0.524	0.524	0.052
6b	0.25	0.025	1	0.3078	0.511	0.511	0.051
7a	0.25	0.025	1	0.2933	0.482	0.482	0.048
7b	0.25	0.025	1	0.2999	0.495	0.495	0.050
8a	0.25	0.025	1	0.3267	0.550	0.550	0.055
8b	0.25	0.025	1	0.3129	0.522	0.522	0.052

Inorganic Loosely-Bound P within 90 days after biosolids application

Sample	Sample mass (g)	Volume of NH_4Cl (L)	Dilution factor	Absorbance	P Concentration (ppm)	P Concentration (mg/L)	P Concentration (mg/g)
1a	0.25	0.025	3	0.2593	0.416	1.248	0.125
1b	0.25	0.025	3	0.252	0.402	1.207	0.121
2a	0.25	0.025	3	0.237	0.374	1.123	0.112
2b	0.25	0.025	3	0.2343	0.369	1.108	0.111
3a	0.25	0.025	3	0.2383	0.377	1.130	0.113
3b	0.25	0.025	3	0.2649	0.427	1.280	0.128
4a	0.25	0.025	3	0.2662	0.429	1.288	0.129
4b	0.25	0.025	3	0.2588	0.415	1.246	0.125
5a	0.25	0.025	3	0.2509	0.400	1.201	0.120
5b	0.25	0.025	3	0.2452	0.390	1.169	0.117
6a	0.25	0.025	3	0.2317	0.365	1.094	0.109
6b	0.25	0.025	3	0.2776	0.451	1.354	0.135
7a	0.25	0.025	3	0.2541	0.406	1.219	0.122
7b	0.25	0.025	3	0.2222	0.347	1.042	0.104
8a	0.25	0.025	3	0.2691	0.435	1.305	0.130
8b	0.25	0.025	3	0.233	0.367	1.101	0.110

Inorganic Loosely-Bound P within 150 days after biosolids application

Sample	Sample mass (g)	Volume of NH_4Cl (L)	Dilution factor	Absorbance	P Concentration (ppm)	P Concentration (mg/L)	P Concentration (mg/g)
1a	0.25	0.02	2.5	0.309	0.416	1.248	0.125
1b	0.25	0.02	2.5	0.3389	0.402	1.207	0.121
2a	0.25	0.02	2.5	0.3154	0.374	1.123	0.112
2b	0.25	0.02	2.5	0.3095	0.369	1.108	0.111
3a	0.25	0.02	2.5	0.3184	0.377	1.130	0.113
3b	0.25	0.02	2.5	0.3279	0.427	1.280	0.128
4a	0.25	0.02	2.5	0.3306	0.429	1.288	0.129
4b	0.25	0.02	2.5	0.3285	0.415	1.246	0.125
5a	0.25	0.02	2.5	0.28984	0.400	1.201	0.120
5b	0.25	0.02	2.5	0.3142	0.390	1.169	0.117
6a	0.25	0.02	2.5	0.3189	0.365	1.094	0.109
6b	0.25	0.02	2.5	0.3198	0.451	1.354	0.135
7a	0.25	0.02	2.5	0.311	0.406	1.219	0.122
7b	0.25	0.02	2.5	0.3347	0.347	1.042	0.104
8a	0.25	0.02	2.5	0.3217	0.435	1.305	0.130
8b	0.25	0.02	2.5	0.3173	0.367	1.101	0.110

Metal-Bound P within 14 days after biosolids application

Sample	Sample mass (g)	Volume of NaOH (L)	Dilution factor	Absorbance	P Concentration (ppm)	P Concentration (mg/L)	P Concentration (mg/g)
1a	0.5	0.025	50	0.0299	0.036	1.776	0.089
1b	0.5	0.025	50	0.0356	0.042	2.121	0.106
2a	0.5	0.025	50	0.0325	0.039	1.933	0.097
2b	0.5	0.025	50	0.0472	0.057	2.832	0.142
3a	0.5	0.025	50	0.0307	0.036	1.824	0.091
3b	0.5	0.025	50	0.0335	0.040	1.994	0.100
4a	0.5	0.025	50	0.0332	0.040	1.975	0.099
4b	0.5	0.025	50	0.0352	0.042	2.097	0.105
5a	0.5	0.025	50	0.0345	0.041	2.054	0.103
5b	0.5	0.025	50	0.0357	0.043	2.127	0.106
6a	0.5	0.025	50	0.0368	0.044	2.194	0.110
6b	0.5	0.025	50	0.033	0.039	1.963	0.098
7a	0.5	0.025	50	0.035	0.042	2.085	0.104
7b	0.5	0.025	50	0.0337	0.040	2.006	0.100
8a	0.5	0.025	50	0.0324	0.039	1.927	0.096
8b	0.5	0.025	50	0.034	0.040	2.024	0.101

Metal-Bound P within 30 days after biosolids application

Sample	Sample mass (g)	Volume of NaOH (L)	Dilution factor	Absorbance	P Concentration (ppm)	P Concentration (mg/L)	P Concentration (mg/g)
1a	0.25	0.025	9	0.0706	0.099	0.894	0.089
1b	0.25	0.025	9	0.0619	0.087	0.779	0.078
2a	0.25	0.025	9	0.0862	0.123	1.104	0.110
2b	0.25	0.025	9	0.0807	0.114	1.029	0.103
3a	0.25	0.025	9	0.076	0.107	0.966	0.097
3b	0.25	0.025	9	0.082	0.116	1.047	0.105
4a	0.25	0.025	9	0.0648	0.091	0.818	0.082
4b	0.25	0.025	9	0.0731	0.103	0.928	0.093
5a	0.25	0.025	9	0.0709	0.100	0.898	0.090
5b	0.25	0.025	9	0.07	0.098	0.886	0.089
6a	0.25	0.025	9	0.0784	0.111	0.998	0.100
6b	0.25	0.025	9	0.066	0.093	0.833	0.083
7a	0.25	0.025	9	0.0753	0.106	0.957	0.096
7b	0.25	0.025	9	0.0794	0.112	1.012	0.101
8a	0.25	0.025	9	0.0737	0.104	0.936	0.094
8b	0.25	0.025	9	0.0659	0.092	0.832	0.083

Metal-Bound P within 60 days after biosolids application

Sample	Sample mass (g)	Volume of NaOH (L)	Dilution factor	Absorbance	P Concentration (ppm)	P Concentration (mg/L)	P Concentration (mg/g)
1a	0.25	0.025	9	0.0584	0.082	0.734	0.073
1b	0.25	0.025	9	0.0532	0.074	0.666	0.067
2a	0.25	0.025	9	0.0529	0.074	0.662	0.066
2b	0.25	0.025	9	0.0537	0.075	0.672	0.067
3a	0.25	0.025	9	0.0558	0.078	0.700	0.070
3b	0.25	0.025	9	0.0575	0.080	0.722	0.072
4a	0.25	0.025	9	0.0543	0.076	0.680	0.068
4b	0.25	0.025	9	0.0573	0.080	0.719	0.072
5a	0.25	0.025	9	0.051	0.071	0.637	0.064
5b	0.25	0.025	9	0.0468	0.065	0.583	0.058
6a	0.25	0.025	9	0.0534	0.074	0.669	0.067
6b	0.25	0.025	9	0.0562	0.078	0.705	0.070
7a	0.25	0.025	9	0.0545	0.076	0.683	0.068
7b	0.25	0.025	9	0.0571	0.080	0.717	0.072
8a	0.25	0.025	9	0.057	0.079	0.715	0.072
8b	0.25	0.025	9	0.0568	0.079	0.713	0.071

Metal-Bound P within 90 days after biosolids application

Sample	Sample mass (g)	Volume of NaOH (L)	Dilution factor	Absorbance	P Concentration (ppm)	P Concentration (mg/L)	P Concentration (mg/g)
1a	0.25	0.025	9	0.0788	0.112	1.004	0.100
1b	0.25	0.025	9	0.0707	0.100	0.896	0.090
2a	0.25	0.025	9	0.0656	0.092	0.828	0.083
2b	0.25	0.025	9	0.0681	0.096	0.861	0.086
3a	0.25	0.025	9	0.075	0.106	0.953	0.095
3b	0.25	0.025	9	0.0681	0.096	0.861	0.086
4a	0.25	0.025	9	0.069	0.097	0.873	0.087
4b	0.25	0.025	9	0.0641	0.090	0.808	0.081
5a	0.25	0.025	9	0.0715	0.101	0.906	0.091
5b	0.25	0.025	9	0.0637	0.089	0.803	0.080
6a	0.25	0.025	9	0.0636	0.089	0.802	0.080
6b	0.25	0.025	9	0.0745	0.105	0.946	0.095
7a	0.25	0.025	9	0.0647	0.091	0.816	0.082
7b	0.25	0.025	9	0.0649	0.091	0.819	0.082
8a	0.25	0.025	9	0.0744	0.105	0.945	0.094
8b	0.25	0.025	9	0.0616	0.086	0.776	0.078

Metal-Bound P within 150 days after biosolids application

Sample	Sample mass (g)	Volume of NaOH (L)	Dilution factor	Absorbance	P Concentration (ppm)	P Concentration (mg/L)	P Concentration (mg/g)
1a	0.25	0.025	9	0.0604	0.084	0.760	0.076
1b	0.25	0.025	9	0.0587	0.082	0.738	0.074
2a	0.25	0.025	9	0.0533	0.074	0.667	0.067
2b	0.25	0.025	9	0.0655	0.092	0.827	0.083
3a	0.25	0.025	9	0.0488	0.068	0.609	0.061
3b	0.25	0.025	9	0.0572	0.080	0.718	0.072
4a	0.25	0.025	9	0.0524	0.073	0.656	0.066
4b	0.25	0.025	9	0.0615	0.086	0.774	0.077
5a	0.25	0.025	9	0.0573	0.080	0.719	0.072
5b	0.25	0.025	9	0.0673	0.095	0.851	0.085
6a	0.25	0.025	9	0.0686	0.096	0.868	0.087
6b	0.25	0.025	9	0.0454	0.063	0.565	0.057
7a	0.25	0.025	9	0.0479	0.066	0.597	0.060
7b	0.25	0.025	9	0.063	0.088	0.794	0.079
8a	0.25	0.025	9	0.0615	0.086	0.774	0.077
8b	0.25	0.025	9	0.0619	0.087	0.779	0.078

Calcium-Bound P within 14 days after biosolids application

Sample	Sample mass (g)	Volume of HCl (L)	Dilution factor	Absorbance	P Concentration (ppm)	P Concentration (mg/L)	P Concentration (mg/g)
1a	0.5	0.025	1	0.236	0.317	0.317	0.016
1b	0.5	0.025	1	0.3767	0.552	0.552	0.028
2a	0.5	0.025	1	0.3366	0.481	0.481	0.024
2b	0.5	0.025	1	0.3978	0.591	0.591	0.030
3a	0.5	0.025	1	0.324	0.460	0.460	0.023
3b	0.5	0.025	1				
4a	0.5	0.025	1	0.2921	0.406	0.406	0.020
4b	0.5	0.025	1	0.3767	0.552	0.552	0.028
5a	0.5	0.025	1				
5b	0.5	0.025	1				
6a	0.5	0.025	1	0.3488	0.502	0.502	0.025
6b	0.5	0.025	1				
7a	0.5	0.025	1	0.2763	0.381	0.381	0.019
7b	0.5	0.025	1	0.3704	0.541	0.541	0.027
8a	0.5	0.025	1				
8b	0.5	0.025	1	0.4228	0.638	0.638	0.032

Calcium-Bound P within 30 days after biosolids application

Sample	Sample mass (g)	Volume of HCl (L)	Dilution factor	Absorbance	P Concentration (ppm)	P Concentration (mg/L)	P Concentration (mg/g)
1a	0.25	0.025	1	0.0824	0.117	0.117	0.012
1b	0.25	0.025	1	0.0793	0.112	0.112	0.011
2a	0.25	0.025	1				
2b	0.25	0.025	1	0.0819	0.116	0.116	0.012
3a	0.25	0.025	1	0.0604	0.084	0.084	0.008
3b	0.25	0.025	1	0.1008	0.145	0.145	0.014
4a	0.25	0.025	1	0.046	0.064	0.064	0.006
4b	0.25	0.025	1	0.0511	0.071	0.071	0.007
5a	0.25	0.025	1	0.0842	0.120	0.120	0.012
5b	0.25	0.025	1	0.0597	0.083	0.083	0.008
6a	0.25	0.025	1	0.0998	0.143	0.143	0.014
6b	0.25	0.025	1	0.0584	0.082	0.082	0.008
7a	0.25	0.025	1	0.0504	0.070	0.070	0.007
7b	0.25	0.025	1	0.0699	0.098	0.098	0.010
8a	0.25	0.025	1	0.0476	0.066	0.066	0.007
8b	0.25	0.025	1	0.063	0.088	0.088	0.009

Calcium-Bound P within 60 days after biosolids application

Sample	Sample mass (g)	Volume of HCl (L)	Dilution factor	Absorbance	P Concentration (ppm)	P Concentration (mg/L)	P Concentration (mg/g)
1a	0.25	0.025	1	0.0439	0.061	0.061	0.006
1b	0.25	0.025	1	0.0403	0.056	0.056	0.006
2a	0.25	0.025	1	0.054	0.075	0.075	0.008
2b	0.25	0.025	1	0.053	0.074	0.074	0.007
3a	0.25	0.025	1	0.065	0.091	0.091	0.009
3b	0.25	0.025	1	0.0527	0.073	0.073	0.007
4a	0.25	0.025	1	0.0637	0.089	0.089	0.009
4b	0.25	0.025	1	0.0631	0.088	0.088	0.009
5a	0.25	0.025	1	0.0788	0.112	0.112	0.011
5b	0.25	0.025	1	0.0734	0.104	0.104	0.010
6a	0.25	0.025	1	0.055	0.077	0.077	0.008
6b	0.25	0.025	1	0.0845	0.120	0.120	0.012
7a	0.25	0.025	1	0.0838	0.119	0.119	0.012
7b	0.25	0.025	1	0.0921	0.132	0.132	0.013
8a	0.25	0.025	1	0.0729	0.103	0.103	0.010
8b	0.25	0.025	1	0.0493	0.068	0.068	0.007

Calcium-Bound P within 90 days after biosolids application

Sample	Sample mass (g)	Volume of HCl (L)	Dilution factor	Absorbance	P Concentration (ppm)	P Concentration (mg/L)	P Concentration (mg/g)
1a	0.25	0.025	1				
1b	0.25	0.025	1	0.1363	0.201	0.201	0.020
2a	0.25	0.025	1				
2b	0.25	0.025	1				
3a	0.25	0.025	1	0.1450	0.215	0.215	0.021
3b	0.25	0.025	1	0.1120	0.162	0.162	0.016
4a	0.25	0.025	1	0.0863	0.123	0.123	0.012
4b	0.25	0.025	1	0.1231	0.180	0.180	0.018
5a	0.25	0.025	1				
5b	0.25	0.025	1	0.0699	0.098	0.098	0.010
6a	0.25	0.025	1	0.0873	0.124	0.124	0.012
6b	0.25	0.025	1	0.0666	0.093	0.093	0.009
7a	0.25	0.025	1	0.1407	0.208	0.208	0.021
7b	0.25	0.025	1	0.1003	0.144	0.144	0.014
8a	0.25	0.025	1	0.0746	0.105	0.105	0.011
8b	0.25	0.025	1	0.0849	0.121	0.121	0.012

Calcium-Bound P within 150 days after biosolids application

Sample	Sample mass (g)	Volume of HCl (L)	Dilution factor	Absorbance	P Concentration (ppm)	P Concentration (mg/L)	P Concentration (mg/g)
1a	0.25	0.025	1	0.091	0.130	0.130	0.013
1b	0.25	0.025	1	0.0632	0.089	0.089	0.009
2a	0.25	0.025	1	0.0708	0.100	0.100	0.010
2b	0.25	0.025	1	0.0852	0.121	0.121	0.012
3a	0.25	0.025	1	0.0771	0.109	0.109	0.011
3b	0.25	0.025	1	0.0851	0.121	0.121	0.012
4a	0.25	0.025	1	0.0736	0.104	0.104	0.010
4b	0.25	0.025	1	0.097	0.139	0.139	0.014
5a	0.25	0.025	1	0.0741	0.105	0.105	0.010
5b	0.25	0.025	1	0.0775	0.110	0.110	0.011
6a	0.25	0.025	1	0.0985	0.141	0.141	0.014
6b	0.25	0.025	1	0.0905	0.129	0.129	0.013
7a	0.25	0.025	1	0.0927	0.132	0.132	0.013
7b	0.25	0.025	1	0.084	0.119	0.119	0.012
8a	0.25	0.025	1	0.078	0.110	0.110	0.011
8b	0.25	0.025	1	0.0864	0.123	0.123	0.012

Organic Phosphorus Fractions

Organic Labile P within 30 days after biosolids application

Sample	Sample mass (g)	Volume of $NaHCO_3$ (L)	Dilution factor	Absorbance	P Concentration (ppm)	P Concentration (mg/L)	P Concentration (mg/g)	Dilution factor	Absorbance	P Concentration (ppm)	P Concentration (mg/L)	P Concentration (mg/g)	P Concentration (mg/g)
1a	0.25	0.025	1	0.0972	0.120	0.120	0.012	1	0.0418	0.058	0.058	0.006	0.006
1b	0.25	0.025	1	0.0987	0.122	0.122	0.012	1	0.032	0.044	0.044	0.004	0.008
2a	0.25	0.025	1	0.0934	0.115	0.115	0.012	1	0.0361	0.050	0.050	0.005	0.007
2b	0.25	0.025	1	0.0978	0.121	0.121	0.012	1	0.0343	0.047	0.047	0.005	0.007
3a	0.25	0.025	1	0.0903	0.111	0.111	0.011	1	0.0328	0.045	0.045	0.004	0.007
3b	0.25	0.025	1	0.0954	0.118	0.118	0.012	1	0.0337	0.046	0.046	0.005	0.007
4a	0.25	0.025	1	0.0923	0.114	0.114	0.011	1	0.0405	0.056	0.056	0.006	0.006
4b	0.25	0.025	1	0.0917	0.113	0.113	0.011	1	0.0409	0.056	0.056	0.006	0.006
5a	0.25	0.025	1	0.0914	0.113	0.113	0.011	1	0.0393	0.054	0.054	0.005	0.006
5b	0.25	0.025	1	0.0973	0.120	0.120	0.012	1	0.041	0.057	0.057	0.006	0.006
6a	0.25	0.025	1	0.0998	0.124	0.124	0.012	1	0.0408	0.056	0.056	0.006	0.007
6b	0.25	0.025	1	0.09	0.111	0.111	0.011	1	0.0462	0.064	0.064	0.006	0.005
7a	0.25	0.025	1	0.0902	0.111	0.111	0.011	1	0.0473	0.066	0.066	0.007	0.005
7b	0.25	0.025	1	0.0965	0.119	0.119	0.012	1	0.0417	0.058	0.058	0.006	0.006
8a	0.25	0.025	1	0.0983	0.122	0.122	0.012	1	0.0417	0.058	0.058	0.006	0.006
8b	0.25	0.025	1	0.0986	0.122	0.122	0.012	1	0.0428	0.059	0.059	0.006	0.006

Organic Labile P within 60 days after biosolids application

Sample	Sample mass (g)	Volume of $NaHCO_3$ (L)	Dilution factor	Absorbance	P Concentration (ppm)	P Concentration (mg/L)	P Concentration (mg/g)	Dilution factor	Absorbance	P Concentration (ppm)	P Concentration (mg/L)	P Concentration (mg/g)	P Concentration (mg/g)
1a	0.25	0.025	1	0.0871	0.124	0.124	0.012	1	0.0478	0.066	0.066	0.007	0.006
1b	0.25	0.025	1	0.0877	0.125	0.125	0.012	1	0.0492	0.068	0.068	0.007	0.006
2a	0.25	0.025	1	0.0886	0.126	0.126	0.013	1	0.0437	0.060	0.060	0.006	0.007
2b	0.25	0.025	1	0.0882	0.126	0.126	0.013	1	0.0501	0.070	0.070	0.007	0.006
3a	0.25	0.025	1	0.0864	0.123	0.123	0.012	1	0.0473	0.066	0.066	0.007	0.006
3b	0.25	0.025	1	0.0846	0.120	0.120	0.012	1	0.0507	0.070	0.070	0.007	0.005
4a	0.25	0.025	1	0.0898	0.128	0.128	0.013	1	0.0438	0.061	0.061	0.006	0.007
4b	0.25	0.025	1	0.0856	0.122	0.122	0.012	1	0.0475	0.066	0.066	0.007	0.006
5a	0.25	0.025	1	0.0864	0.123	0.123	0.012	1	0.0473	0.066	0.066	0.007	0.006
5b	0.25	0.025	1	0.0886	0.126	0.126	0.013	1	0.0469	0.065	0.065	0.006	0.006
6a	0.25	0.025	1	0.0859	0.122	0.122	0.012	1	0.0501	0.070	0.070	0.007	0.005
6b	0.25	0.025	1	0.0877	0.125	0.125	0.012	1	0.0476	0.066	0.066	0.007	0.006
7a	0.25	0.025	1	0.0876	0.125	0.125	0.012	1	0.0482	0.067	0.067	0.007	0.006
7b	0.25	0.025	1	0.0853	0.121	0.121	0.012	1	0.0462	0.064	0.064	0.006	0.006
8a	0.25	0.025	1	0.0861	0.122	0.122	0.012	1	0.0471	0.065	0.065	0.007	0.006
8b	0.25	0.025	1	0.0829	0.118	0.118	0.012	1	0.0415	0.057	0.057	0.006	0.006

Organic Labile P within 90 days after biosolids application

Sample	Sample mass (g)	Volume of $NaHCO_3$ (L)	Dilution factor	Absorbance	P Concentration (ppm)	P Concentration (mg/L)	P Concentration (mg/g)	Dilution factor	Absorbance	P Concentration (ppm)	P Concentration (mg/L)	P Concentration (mg/g)	P Concentration (mg/g)
1a	0.25	0.025	1	0.1361	0.200	0.200	0.020	1	0.0823	0.117	0.117	0.012	0.008
1b	0.25	0.025	1	0.1261	0.184	0.184	0.018	1	0.0796	0.113	0.113	0.011	0.007
2a	0.25	0.025	1	0.1124	0.163	0.163	0.016	1	0.0743	0.105	0.105	0.010	0.006
2b	0.25	0.025	1	0.1041	0.150	0.150	0.015	1	0.0678	0.095	0.095	0.010	0.005
3a	0.25	0.025	1	0.1051	0.151	0.151	0.015	1	0.0605	0.085	0.085	0.008	0.007
3b	0.25	0.025	1	0.1394	0.206	0.206	0.021	1	0.0809	0.115	0.115	0.011	0.009
4a	0.25	0.025	1	0.1354	0.199	0.199	0.020	1	0.0817	0.116	0.116	0.012	0.008
4b	0.25	0.025	1	0.1044	0.150	0.150	0.015	1	0.0635	0.089	0.089	0.009	0.006
5a	0.25	0.025	1	0.1057	0.152	0.152	0.015	1	0.0637	0.089	0.089	0.009	0.006
5b	0.25	0.025	1	0.0955	0.137	0.137	0.014	1	0.0508	0.071	0.071	0.007	0.007
6a	0.25	0.025	1	0.095	0.136	0.136	0.014	1	0.0632	0.089	0.089	0.009	0.005
6b	0.25	0.025	1	0.0871	0.124	0.124	0.012	1	0.0538	0.075	0.075	0.007	0.005
7a	0.25	0.025	1	0.0973	0.139	0.139	0.014	1	0.0594	0.083	0.083	0.008	0.006
7b	0.25	0.025	1	0.1064	0.153	0.153	0.015	1	0.0712	0.100	0.100	0.010	0.005
8a	0.25	0.025	1	0.135	0.199	0.199	0.020	1	0.08	0.113	0.113	0.011	0.009
8b	0.25	0.025	1	0.1014	0.146	0.146	0.015	1	0.0675	0.095	0.095	0.009	0.005

Organic Labile P within 150 days after biosolids application

Sample	Sample mass (g)	Volume of $NaHCO_3$ (L)	Dilution factor	Absorbance	P Concentration (ppm)	P Concentration (mg/L)	P Concentration (mg/g)	Dilution factor	Absorbance	P Concentration (ppm)	P Concentration (mg/L)	P Concentration (mg/g)	P Concentration (mg/g)
1a	0.25	0.025	1	0.1241	0.181	0.181	0.018	1	0.0854	0.121	0.121	0.012	0.006
1b	0.25	0.025	1	0.0962	0.138	0.138	0.014	1	0.0782	0.111	0.111	0.011	0.003
2a	0.25	0.025	1	0.1134	0.164	0.164	0.016	1	0.0778	0.110	0.110	0.011	0.005
2b	0.25	0.025	1	0.1099	0.159	0.159	0.016	1	0.0705	0.099	0.099	0.010	0.006
3a	0.25	0.025	1	0.1021	0.147	0.147	0.015	1	0.0709	0.100	0.100	0.010	0.005
3b	0.25	0.025	1	0.107	0.154	0.154	0.015	1	0.0717	0.101	0.101	0.010	0.005
4a	0.25	0.025	1	0.0957	0.137	0.137	0.014	1	0.0635	0.089	0.089	0.009	0.005
4b	0.25	0.025	1	0.0936	0.134	0.134	0.013	1	0.0664	0.093	0.093	0.009	0.004
5a	0.25	0.025	1	0.1199	0.175	0.175	0.017	1	0.0792	0.112	0.112	0.011	0.006
5b	0.25	0.025	1	0.123	0.179	0.179	0.018	1	0.0832	0.118	0.118	0.012	0.006
6a	0.25	0.025	1	0.1075	0.155	0.155	0.016	1	0.0738	0.104	0.104	0.010	0.005
6b	0.25	0.025	1	0.1085	0.157	0.157	0.016	1	0.0704	0.099	0.099	0.010	0.006
7a	0.25	0.025	1	0.1145	0.166	0.166	0.017	1	0.0794	0.112	0.112	0.011	0.005
7b	0.25	0.025	1	0.1037	0.149	0.149	0.015	1	0.0712	0.100	0.100	0.010	0.005
8a	0.25	0.025	1	0.1202	0.175	0.175	0.018	1	0.0865	0.123	0.123	0.012	0.005
8b	0.25	0.025	1	0.1178	0.171	0.171	0.017	1	0.0775	0.110	0.110	0.011	0.006

Organic Moderately-Labile P

	HCL extraction within 14 days of biosolids application												
	With digestion							Without digestion					Organic P
Sample	Sample mass (g)	Volume of HCl (L)	Dilution factor	Absorbance	P Concentration (ppm)	P Concentration (mg/L)	P Concentration (mg/g)	Dilution factor	Absorbance	P Concentration (ppm)	P Concentration (mg/L)	P Concentration (mg/g)	P Concentration (mg/g)
1a	0.3	0.025	1	0.148	0.189	0.189	0.016	1	0.0356	0.042	0.042	0.004	0.012
1b	0.5	0.025	1	0.1877	0.245	0.245	0.012	1	0.034	0.040	0.040	0.002	0.010
2a	0.4	0.025	1	0.1535	0.196	0.196	0.012	1	0.0263	0.031	0.031	0.002	0.010
2b	0.5	0.025	1	0.1872	0.244	0.244	0.012	1	0.0459	0.055	0.055	0.003	0.009
3a	0.25	0.025	1	0.123	0.154	0.154	0.015	1	0.0354	0.042	0.042	0.004	0.011
3b	0.5	0.025	1	0.1961	0.257	0.257	0.013	1	0.0538	0.065	0.065	0.003	0.010
4a	0.25	0.025	1	0.1307	0.165	0.165	0.016	1	0.0369	0.044	0.044	0.004	0.012
4b	0.2	0.025	1	0.1286	0.162	0.162	0.020	1	0.0329	0.039	0.039	0.005	0.015
5a	0.2	0.025	1	0.12	0.150	0.150	0.019	1	0.0318	0.038	0.038	0.005	0.014
5b	0.4	0.025	1	0.1652	0.213	0.213	0.013	1	0.0394	0.047	0.047	0.003	0.010
6a	0.4	0.025	1	0.1637	0.211	0.211	0.013	1	0.045	0.054	0.054	0.003	0.010
6b	0.4	0.025	1	0.1583	0.203	0.203	0.013	1	0.0478	0.057	0.057	0.004	0.009
7a	0.35	0.025	1	0.157	0.201	0.201	0.014	1	0.0478	0.057	0.057	0.004	0.010
7b	0.5	0.025	1	0.2109	0.279	0.279	0.014	1	0.0505	0.061	0.061	0.003	0.011
8a	0.35	0.025	1	0.1511	0.193	0.193	0.014	1	0.0385	0.046	0.046	0.003	0.010
8b	0.5	0.025	1	0.1858	0.242	0.242	0.012	1	0.0378	0.045	0.045	0.002	0.010

NaOH extraction within 14 days of biosolids application													
	Humic Acid + Fulveic Acid (With digestion)							Fulvic Acid (Without digestion)					Humic Acid
Sample	Sample mass (g)	Volume of NaOH (L)	Dilution factor	Absorbance	P Concentration (ppm)	P Concentration (mg/L)	P Concentration (mg/g)	Dilution factor	Absorbance	P Concentration (ppm)	P Concentration (mg/L)	P Concentration (mg/g)	P Concentration (mg/g)
1a	0.3	0.025	1	0.1228	0.154	0.154	0.013	1	0.0095	0.011	0.011	0.001	0.012
1b	0.5	0.025	1	0.1158	0.145	0.145	0.007	1	0.0083	0.010	0.010	0.000	0.007
2a	0.4	0.025	1	0.1733	0.224	0.224	0.014	1	0.0076	0.009	0.009	0.001	0.013
2b	0.5	0.025	1	0.1600	0.205	0.205	0.010	1	0.0104	0.012	0.012	0.001	0.010
3a	0.25	0.025	1	0.1612	0.207	0.207	0.021	1	0.0773	0.094	0.094	0.009	0.011
3b	0.5	0.025	1	0.1508	0.192	0.192	0.010	1	0.0115	0.014	0.014	0.001	0.009
4a	0.25	0.025	1	0.1216	0.152	0.152	0.015	1	0.0127	0.015	0.015	0.001	0.014
4b	0.2	0.025	1	0.1283	0.162	0.162	0.020	1	0.0209	0.025	0.025	0.003	0.017
5a	0.2	0.025	1	0.1111	0.138	0.138	0.017	1	0.0121	0.014	0.014	0.002	0.016
5b	0.4	0.025	1	0.1505	0.192	0.192	0.012	1	0.0096	0.011	0.011	0.001	0.011
6a	0.4	0.025	1	0.1520	0.194	0.194	0.012	1	0.0283	0.034	0.034	0.002	0.010
6b	0.4	0.025	1	0.1172	0.147	0.147	0.009	1	0.0102	0.012	0.012	0.001	0.008
7a	0.35	0.025	1	0.1400	0.177	0.177	0.013	1	0.0106	0.012	0.012	0.001	0.012
7b	0.5	0.025	1	0.1539	0.197	0.197	0.010	1	0.0765	0.093	0.093	0.005	0.005
8a	0.35	0.025	1	0.1879	0.245	0.245	0.018	1	0.0093	0.011	0.011	0.001	0.017
8b	0.5	0.025	1	0.1717	0.222	0.222	0.011	1	0.0118	0.014	0.014	0.001	0.010

	HCL extraction within 30 days of biosolids application												
	With digestion							Without digestion					Organic P
Sample	Sample mass (g)	Volume of HCl (L)	Dilution factor	Absorbance	P Concentration (ppm)	P Concentration (mg/L)	P Concentration (mg/g)	Dilution factor	Absorbance	P Concentration (ppm)	P Concentration (mg/L)	P Concentration (mg/g)	P Concentration (mg/g)
1a	0.25	0.025	1	0.1331	0.196	0.196	0.020	1	0.0095	0.013	0.013	0.001	0.018
1b	0.25	0.025	1	0.135	0.199	0.199	0.020	1	0.0083	0.011	0.011	0.001	0.019
2a	0.25	0.025	1	0.1163	0.169	0.169	0.017	1	0.0076	0.010	0.010	0.001	0.016
2b	0.25	0.025	1	0.1398	0.206	0.206	0.021	1	0.0104	0.014	0.014	0.001	0.019
3a	0.25	0.025	1	0.1175	0.171	0.171	0.017	1	0.0089	0.012	0.012	0.001	0.016
3b	0.25	0.025	1	0.1517	0.226	0.226	0.023	1	0.0115	0.016	0.016	0.002	0.021
4a	0.25	0.025	1	0.1453	0.215	0.215	0.022	1	0.0127	0.017	0.017	0.002	0.020
4b	0.25	0.025	1	0.1514	0.225	0.225	0.023	1	0.0209	0.028	0.028	0.003	0.020
5a	0.25	0.025	1	0.1183	0.172	0.172	0.017	1	0.0121	0.016	0.016	0.002	0.016
5b	0.25	0.025	1	0.1107	0.160	0.160	0.016	1	0.0096	0.013	0.013	0.001	0.015
6a	0.25	0.025	1	0.1562	0.233	0.233	0.023	1	0.0283	0.039	0.039	0.004	0.019
6b	0.25	0.025	1	0.1121	0.162	0.162	0.016	1	0.0102	0.014	0.014	0.001	0.015
7a	0.25	0.025	1	0.119	0.173	0.173	0.017	1	0.0106	0.014	0.014	0.001	0.016
7b	0.25	0.025	1	0.1332	0.196	0.196	0.020	1	0.0207	0.028	0.028	0.003	0.017
8a	0.25	0.025	1	0.1441	0.213	0.213	0.021	1	0.0193	0.026	0.026	0.003	0.019
8b	0.25	0.025	1	0.1144	0.166	0.166	0.017	1	0.0118	0.016	0.016	0.002	0.015

NaOH extraction within 1 month of biosolids application													
	Humic Acid + Fulveic Acid (With digestion)							Fulvic Acid (Without digestion)					Humic Acid
Sample	Sample mass (g)	Volume of NaOH (L)	Dilution factor	Absorbance	P Concentration (ppm)	P Concentration (mg/L)	P Concentration (mg/g)	Dilution factor	Absorbance	P Concentration (ppm)	P Concentration (mg/L)	P Concentration (mg/g)	P Concentration (mg/g)
1a	0.25	0.025	2	0.1154	0.167	0.335	0.033	2	0.0944	0.135	0.270	0.027	0.006
1b	0.25	0.025	2	0.1208	0.176	0.352	0.035	2	0.1002	0.144	0.288	0.029	0.006
2a	0.25	0.025	2	0.1067	0.154	0.308	0.031	2	0.0721	0.102	0.203	0.020	0.010
2b	0.25	0.025	2	0.1086	0.157	0.314	0.031	2	0.0906	0.129	0.259	0.026	0.006
3a	0.25	0.025	2	0.1068	0.154	0.308	0.031	2	0.0968	0.139	0.277	0.028	0.003
3b	0.25	0.025	2	0.1202	0.175	0.350	0.035	2	0.0695	0.098	0.195	0.020	0.015
4a	0.25	0.025	2	0.1316	0.193	0.386	0.039	2	0.0999	0.143	0.287	0.029	0.010
4b	0.25	0.025	2	0.1270	0.186	0.372	0.037	2	0.0916	0.131	0.262	0.026	0.011
5a	0.25	0.025	2	0.1226	0.179	0.358	0.036	2	0.0943	0.135	0.270	0.027	0.009
5b	0.25	0.025	2	0.1185	0.172	0.345	0.034	2	0.0885	0.126	0.252	0.025	0.009
6a	0.25	0.025	2	0.1237	0.181	0.361	0.036	2	0.0959	0.137	0.275	0.027	0.009
6b	0.25	0.025	2	0.1168	0.170	0.339	0.034	2	0.1078	0.156	0.311	0.031	0.003
7a	0.25	0.025	2	0.1039	0.150	0.299	0.030	2	0.0875	0.125	0.249	0.025	0.005
7b	0.25	0.025	2	0.1444	0.214	0.428	0.043	2	0.1149	0.167	0.333	0.033	0.009
8a	0.25	0.025	2	0.1183	0.172	0.344	0.034	2	0.0914	0.130	0.261	0.026	0.008
8b	0.25	0.025	2	0.1283	0.188	0.376	0.038	2	0.1071	0.155	0.309	0.031	0.007

	HCL extraction within 60 days of biosolids application												
	With digestion							Without digestion					Organic P
Sample	Sample mass (g)	Volume of HCl (L)	Dilution factor	Absorbance	P Concentration (ppm)	P Concentration (mg/L)	P Concentration (mg/g)	Dilution factor	Absorbance	P Concentration (ppm)	P Concentration (mg/L)	P Concentration (mg/g)	P Concentration (mg/g)
1a	0.25	0.025	1	0.1786	0.271	0.271	0.027	1	0.0116	0.0157	0.0157	0.0016	0.0255
1b	0.25	0.025	1	0.1477	0.219	0.219	0.022	1	0.0096	0.0130	0.0130	0.0013	0.0206
2a	0.25	0.025	1	0.1662	0.250	0.250	0.025	1	0.0098	0.0132	0.0132	0.0013	0.0237
2b	0.25	0.025	1	0.1497	0.222	0.222	0.022	1	0.0095	0.0128	0.0128	0.0013	0.0210
3a	0.25	0.025	1	0.1621	0.243	0.243	0.024	1	0.011	0.0149	0.0149	0.0015	0.0228
3b	0.25	0.025	1	0.2077	0.321	0.321	0.032	1	0.0094	0.0127	0.0127	0.0013	0.0309
4a	0.25	0.025	1	0.1414	0.209	0.209	0.021	1	0.0113	0.0153	0.0153	0.0015	0.0194
4b	0.25	0.025	1	0.1487	0.221	0.221	0.022	1	0.0098	0.0132	0.0132	0.0013	0.0208
5a	0.25	0.025	1	0.1663	0.250	0.250	0.025	1	0.0102	0.0138	0.0138	0.0014	0.0236
5b	0.25	0.025	1	0.1845	0.281	0.281	0.028	1	0.0096	0.0130	0.0130	0.0013	0.0268
6a	0.25	0.025	1	0.1457	0.216	0.216	0.022	1	0.0102	0.0138	0.0138	0.0014	0.0202
6b	0.25	0.025	1	0.1405	0.207	0.207	0.021	1	0.0114	0.0154	0.0154	0.0015	0.0192
7a	0.25	0.025	1	0.1621	0.243	0.243	0.024	1	0.0108	0.0146	0.0146	0.0015	0.0228
7b	0.25	0.025	1	0.1613	0.242	0.242	0.024	1	0.0114	0.0154	0.0154	0.0015	0.0226
8a	0.25	0.025	1	0.1755	0.266	0.266	0.027	1	0.0103	0.0139	0.0139	0.0014	0.0252
8b	0.25	0.025	1	0.1711	0.258	0.258	0.026	1	0.0114	0.0154	0.0154	0.0015	0.0243

NaOH extraction within 60 days of biosolids application													
	Humic Acid + Fulveic Acid (With digestion)							Fulvic Acid (Without digestion)					Humic Acid
Sample	Sample mass (g)	Volume of NaOH (L)	Dilution factor	Absorbance	P Concentration (ppm)	P Concentration (mg/L)	P Concentration (mg/g)	Dilution factor	Absorbance	P Concentration (ppm)	P Concentration (mg/L)	P Concentration (mg/g)	P Concentration (mg/g)
1a	0.25	0.025	2	0.1190	0.173	0.346	0.035	2	0.0785	0.111	0.222	0.022	0.012
1b	0.25	0.025	2	0.0972	0.139	0.279	0.028	2	0.0823	0.117	0.234	0.023	0.005
2a	0.25	0.025	2	0.0881	0.125	0.251	0.025	2	0.0782	0.111	0.221	0.022	0.003
2b	0.25	0.025	2	0.0858	0.122	0.244	0.024	2	0.0762	0.108	0.215	0.022	0.003
3a	0.25	0.025	2	0.1111	0.161	0.322	0.032	2	0.0906	0.129	0.259	0.026	0.006
3b	0.25	0.025	2	0.0964	0.138	0.276	0.028	2	0.0943	0.135	0.270	0.027	0.001
4a	0.25	0.025	2	0.1080	0.156	0.312	0.031	2	0.0978	0.140	0.280	0.028	0.003
4b	0.25	0.025	2	0.1154	0.167	0.335	0.033	2	0.0984	0.141	0.282	0.028	0.005
5a	0.25	0.025	2	0.1088	0.157	0.314	0.031	2	0.0917	0.131	0.262	0.026	0.005
5b	0.25	0.025	2	0.1106	0.160	0.320	0.032	2	0.0985	0.141	0.283	0.028	0.004
6a	0.25	0.025	2	0.1064	0.153	0.307	0.031	2	0.0916	0.131	0.262	0.026	0.005
6b	0.25	0.025	2	0.1068	0.154	0.308	0.031	2	0.0919	0.131	0.262	0.026	0.005
7a	0.25	0.025	2	0.0927	0.132	0.265	0.026	2	0.0753	0.106	0.213	0.021	0.005
7b	0.25	0.025	2	0.0848	0.121	0.241	0.024	2	0.0593	0.083	0.166	0.017	0.008
8a	0.25	0.025	2	0.1023	0.147	0.294	0.029	2	0.0953	0.136	0.273	0.027	0.002
8b	0.25	0.025	2	0.0970	0.139	0.278	0.028	2	0.0867	0.123	0.247	0.025	0.003

	HCL extraction within 90 days of biosolids application												
	With digestion							Without digestion					Organic P
Sample	Sample mass (g)	Volume of HCl (L)	Dilution factor	Absorbance	P Concentration (ppm)	P Concentration (mg/L)	P Concentration (mg/g)	Dilution factor	Absorbance	P Concentration (ppm)	P Concentration (mg/L)	P Concentration (mg/g)	P Concentration (mg/g)
1a	0.25	0.025	1	0.1481	0.220	0.220	0.022	1	0.0105	0.014	0.014	0.001	0.021
1b	0.25	0.025	1	0.1665	0.250	0.250	0.025	1	0.0109	0.015	0.015	0.001	0.024
2a	0.25	0.025	1	0.1259	0.184	0.184	0.018	1	0.0116	0.016	0.016	0.002	0.017
2b	0.25	0.025	1	0.171	0.258	0.258	0.026	1	0.0123	0.017	0.017	0.002	0.024
3a	0.25	0.025	1	0.1608	0.241	0.241	0.024	1	0.012	0.016	0.016	0.002	0.022
3b	0.25	0.025	1	0.1831	0.279	0.279	0.028	1	0.012	0.016	0.016	0.002	0.026
4a	0.25	0.025	1	0.1877	0.286	0.286	0.029	1	0.0119	0.016	0.016	0.002	0.027
4b	0.25	0.025	1	0.1236	0.180	0.180	0.018	1	0.0126	0.017	0.017	0.002	0.016
5a	0.25	0.025	1	0.1524	0.227	0.227	0.023	1	0.0133	0.018	0.018	0.002	0.021
5b	0.25	0.025	1	0.1614	0.242	0.242	0.024	1	0.0127	0.017	0.017	0.002	0.022
6a	0.25	0.025	1	0.1656	0.249	0.249	0.025	1	0.0135	0.018	0.018	0.002	0.023
6b	0.25	0.025	1	0.17	0.256	0.256	0.026	1	0.0138	0.019	0.019	0.002	0.024
7a	0.25	0.025	1	0.1652	0.248	0.248	0.025	1	0.0119	0.016	0.016	0.002	0.023
7b	0.25	0.025	1	0.1617	0.242	0.242	0.024	1	0.012	0.016	0.016	0.002	0.023
8a	0.25	0.025	1	0.1627	0.244	0.244	0.024	1	0.0134	0.018	0.018	0.002	0.023
8b	0.25	0.025	1	0.1539	0.229	0.229	0.023	1	0.0124	0.017	0.017	0.002	0.021

NaOH extraction within 90 days of biosolids application													
	Humic Acid + Fulveic Acid (With digestion)							Fulvic Acid (Without digestion)					Humic Acid
Sample	Sample mass (g)	Volume of NaOH (L)	Dilution factor	Absorbance	P Concentration (ppm)	P Concentration (mg/L)	P Concentration (mg/g)	Dilution factor	Absorbance	P Concentration (ppm)	P Concentration (mg/L)	P Concentration (mg/g)	P Concentration (mg/g)
1a	0.25	0.025	2	0.1209	0.176	0.352	0.035	2	0.1016	0.146	0.292	0.029	0.006
1b	0.25	0.025	2	0.0974	0.140	0.279	0.028	2	0.0898	0.128	0.256	0.026	0.002
2a	0.25	0.025	2	0.0815	0.116	0.231	0.023	2	0.0651	0.091	0.183	0.018	0.005
2b	0.25	0.025	2	0.0970	0.139	0.278	0.028	2	0.0745	0.105	0.210	0.021	0.007
3a	0.25	0.025	2	0.1083	0.156	0.313	0.031	2	0.0805	0.114	0.228	0.023	0.008
3b	0.25	0.025	2	0.1266	0.185	0.370	0.037	2	0.0860	0.122	0.245	0.024	0.013
4a	0.25	0.025	2	0.1172	0.170	0.341	0.034	2	0.0955	0.137	0.273	0.027	0.007
4b	0.25	0.025	2	0.1188	0.173	0.346	0.035	2	0.0753	0.106	0.213	0.021	0.013
5a	0.25	0.025	2	0.1224	0.178	0.357	0.036	2	0.0896	0.128	0.256	0.026	0.010
5b	0.25	0.025	2	0.0908	0.130	0.259	0.026	2	0.0888	0.127	0.253	0.025	0.001
6a	0.25	0.025	2	0.0967	0.139	0.277	0.028	2	0.0835	0.119	0.237	0.024	0.004
6b	0.25	0.025	2	0.0868	0.124	0.247	0.025	2	0.0697	0.098	0.196	0.020	0.005
7a	0.25	0.025	2	0.1132	0.164	0.328	0.033	2	0.0884	0.126	0.252	0.025	0.008
7b	0.25	0.025	2	0.1141	0.165	0.331	0.033	2	0.0752	0.106	0.212	0.021	0.012
8a	0.25	0.025	2	0.1079	0.156	0.312	0.031	2	0.0993	0.143	0.285	0.029	0.003
8b	0.25	0.025	2	0.0989	0.142	0.284	0.028	2	0.0772	0.109	0.218	0.022	0.007

	HCL extraction within 150 days of biosolids application												
	With digestion							Without digestion					Organic P
Sample	Sample mass (g)	Volume of HCl (L)	Dilution factor	Absorbance	P Concentration (ppm)	P Concentration (mg/L)	P Concentration (mg/g)	Dilution factor	Absorbance	P Concentration (ppm)	P Concentration (mg/L)	P Concentration (mg/g)	P Concentration (mg/g)
1a	0.25	0.025	1	0.2434	0.386	0.386	0.039	1	0.0105	0.013	0.013	0.001	0.037
1b	0.25	0.025	1	0.2061	0.319	0.319	0.032	1	0.0109	0.015	0.015	0.002	0.030
2a	0.25	0.025	1	0.2396	0.379	0.379	0.038	1	0.0116	0.015	0.015	0.002	0.036
2b	0.25	0.025	1	0.2052	0.317	0.317	0.032	1	0.0123	0.015	0.015	0.001	0.030
3a	0.25	0.025	1	0.2452	0.390	0.390	0.039	1	0.012	0.013	0.013	0.001	0.038
3b	0.25	0.025	1	0.2353	0.371	0.371	0.037	1	0.012	0.014	0.014	0.001	0.036
4a	0.25	0.025	1	0.2306	0.363	0.363	0.036	1	0.0119	0.018	0.018	0.002	0.034
4b	0.25	0.025	1	0.2039	0.315	0.315	0.031	1	0.0126	0.013	0.013	0.001	0.030
5a	0.25	0.025	1	0.2248	0.352	0.352	0.035	1	0.0133	0.013	0.013	0.001	0.034
5b	0.25	0.025	1	0.2346	0.370	0.370	0.037	1	0.0127	0.010	0.010	0.001	0.036
6a	0.25	0.025	1	0.2351	0.371	0.371	0.037	1	0.0135	0.014	0.014	0.001	0.036
6b	0.25	0.025	1	0.1834	0.279	0.279	0.028	1	0.0138	0.015	0.015	0.001	0.026
7a	0.25	0.025	1	0.2231	0.349	0.349	0.035	1	0.0119	0.016	0.016	0.002	0.033
7b	0.25	0.025	1	0.1865	0.284	0.284	0.028	1	0.012	0.010	0.010	0.001	0.027
8a	0.25	0.025	1	0.2412	0.382	0.382	0.038	1	0.0134	0.015	0.015	0.002	0.037
8b	0.25	0.025	1	0.2	0.308	0.308	0.031	1	0.0124	0.016	0.016	0.002	0.029

NaOH extraction within 150 days of biosolids application													
	Humic Acid + Fulveic Acid (With digestion)							Fulvic Acid (Without digestion)					Humic Acid
Sample	Sample mass (g)	Volume of NaOH (L)	Dilution factor	Absorbance	P Concentration (ppm)	P Concentration (mg/L)	P Concentration (mg/g)	Dilution factor	Absorbance	P Concentration (ppm)	P Concentration (mg/L)	P Concentration (mg/g)	P Concentration (mg/g)
1a	0.25	0.025	2	0.1187	0.173	0.345	0.035	2	0.1045	0.151	0.301	0.030	0.004
1b	0.25	0.025	2	0.1004	0.144	0.288	0.029	2	0.0976	0.140	0.280	0.028	0.001
2a	0.25	0.025	2	0.0978	0.140	0.280	0.028	2	0.0735	0.104	0.207	0.021	0.007
2b	0.25	0.025	2	0.1002	0.144	0.288	0.029	2	0.0762	0.108	0.215	0.022	0.007
3a	0.25	0.025	2	0.1103	0.160	0.319	0.032	2	0.0843	0.120	0.240	0.024	0.008
3b	0.25	0.025	2	0.1201	0.175	0.350	0.035	2	0.0900	0.128	0.257	0.026	0.009
4a	0.25	0.025	2	0.1175	0.171	0.342	0.034	2	0.0943	0.135	0.270	0.027	0.007
4b	0.25	0.025	2	0.1200	0.175	0.349	0.035	2	0.0823	0.117	0.234	0.023	0.012
5a	0.25	0.025	2	0.1245	0.182	0.364	0.036	2	0.1067	0.154	0.308	0.031	0.006
5b	0.25	0.025	2	0.1012	0.145	0.291	0.029	2	0.0918	0.131	0.262	0.026	0.003
6a	0.25	0.025	2	0.1011	0.145	0.291	0.029	2	0.0884	0.126	0.252	0.025	0.004
6b	0.25	0.025	2	0.0995	0.143	0.286	0.029	2	0.0781	0.110	0.221	0.022	0.006
7a	0.25	0.025	2	0.1198	0.174	0.349	0.035	2	0.1008	0.145	0.290	0.029	0.006
7b	0.25	0.025	2	0.1167	0.170	0.339	0.034	2	0.0974	0.140	0.279	0.028	0.006
8a	0.25	0.025	2	0.1100	0.159	0.318	0.032	2	0.1008	0.145	0.290	0.029	0.003
8b	0.25	0.025	2	0.1034	0.149	0.298	0.030	2	0.0872	0.124	0.248	0.025	0.005

Organic Moderately-Labile P in 14 days after biosolids application

Sample	HCL extracted P (mg/g)	Fulvic Acid (mg/g)	Total Moderately-Labile P (mg/g)
1a	0.012	0.001	0.013
1b	0.010	0.000	0.011
2a	0.010	0.001	0.011
2b	0.010	0.001	0.010
3a	0.011	0.009	0.021
3b	0.010	0.001	0.010
4a	0.012	0.001	0.014
4b	0.015	0.003	0.018
5a	0.014	0.002	0.016
5b	0.010	0.001	0.011
6a	0.010	0.002	0.012
6b	0.009	0.001	0.010
7a	0.010	0.001	0.011
7b	0.011	0.005	0.016
8a	0.011	0.001	0.011
8b	0.010	0.001	0.011

Organic Moderately-Labile P in 30 days after biosolids application

Sample	HCL extracted P (mg/g)	Fulvic Acid (mg/g)	Total Moderately-Labile P (mg/g)
1a	0.018	0.027	0.045
1b	0.019	0.029	0.048
2a	0.016	0.020	0.036
2b	0.019	0.026	0.045
3a	0.016	0.028	0.044
3b	0.021	0.020	0.041
4a	0.020	0.029	0.048
4b	0.020	0.026	0.046
5a	0.016	0.027	0.043
5b	0.015	0.025	0.040
6a	0.019	0.027	0.047
6b	0.015	0.031	0.046
7a	0.016	0.025	0.041
7b	0.017	0.033	0.050
8a	0.019	0.026	0.045
8b	0.015	0.031	0.046

Organic Moderately-Labile P in 60 days after biosolids application

Sample	HCL extracted P (mg/g)	Fulvic Acid (mg/g)	Total Moderately-Labile P (mg/g)
1a	0.026	0.022	0.048
1b	0.021	0.023	0.044
2a	0.024	0.022	0.046
2b	0.021	0.022	0.042
3a	0.023	0.026	0.049
3b	0.031	0.027	0.058
4a	0.019	0.028	0.047
4b	0.021	0.028	0.049
5a	0.024	0.026	0.050
5b	0.027	0.028	0.055
6a	0.020	0.026	0.046
6b	0.019	0.026	0.045
7a	0.023	0.021	0.044
7b	0.023	0.017	0.039
8a	0.025	0.027	0.052
8b	0.024	0.025	0.049

Organic Moderately-Labile P in 90 days after biosolids application

Sample	HCL extracted P (mg/g)	Fulvic Acid (mg/g)	Total Moderately-Labile P (mg/g)
1a	0.021	0.029	0.050
1b	0.024	0.026	0.049
2a	0.017	0.018	0.035
2b	0.024	0.021	0.045
3a	0.022	0.023	0.045
3b	0.026	0.024	0.051
4a	0.027	0.027	0.054
4b	0.016	0.021	0.038
5a	0.021	0.026	0.046
5b	0.022	0.025	0.048
6a	0.023	0.024	0.047
6b	0.024	0.020	0.043
7a	0.023	0.025	0.048
7b	0.023	0.021	0.044
8a	0.023	0.029	0.051
8b	0.021	0.022	0.043

Organic Moderately-Labile P in 150 days after biosolids application

Sample	HCL extracted P (mg/g)	Fulvic Acid (mg/g)	Total Moderately-Labile P (mg/g)
1a	0.037	0.030	0.067
1b	0.030	0.028	0.058
2a	0.036	0.021	0.057
2b	0.030	0.022	0.052
3a	0.038	0.024	0.062
3b	0.036	0.026	0.061
4a	0.034	0.027	0.061
4b	0.030	0.023	0.054
5a	0.034	0.031	0.065
5b	0.036	0.026	0.062
6a	0.036	0.025	0.061
6b	0.026	0.022	0.049
7a	0.033	0.029	0.062
7b	0.027	0.028	0.055
8a	0.037	0.029	0.066
8b	0.029	0.025	0.054

Organic Non-Labile P

H2SO4 extraction 14 days after biosolids application							
Sample	Sample mass (g)	Volume of H_2SO_4 (L)	Dilution factor	Absorbance	P Concentration (ppm)	P Concentration (mg/L)	P Concentration (mg/g)
1a	0.3	0.025	1.5	0.4602	0.711	1.066	0.089
1b	0.5	0.025	1.5	0.5370	0.871	1.307	0.065
2a	0.4	0.025	1.5	0.4768	0.744	1.117	0.070
2b	0.5	0.025	1.5	0.5269	0.850	1.274	0.064
3a	0.25	0.025	1.5	0.4754	0.742	1.112	0.111
3b	0.5	0.025	1.5	0.5053	0.803	1.205	0.060
4a	0.25	0.025	1.5	0.4872	0.766	1.149	0.115
4b	0.2	0.025	1.5	0.4393	0.670	1.005	0.126
5a	0.2	0.025	1.5	0.3989	0.593	0.889	0.111
5b	0.4	0.025	1.5	0.4321	0.656	0.984	0.061
6a	0.4	0.025	1.5	0.4059	0.606	0.909	0.057
6b	0.4	0.025	1.5	0.5400	0.878	1.317	0.082
7a	0.35	0.025	1.5	0.4254	0.643	0.964	0.069
7b	0.5	0.025	1.5	0.4462	0.683	1.025	0.051
8a	0.35	0.025	1.5	0.4427	0.676	1.015	0.072
8b	0.5	0.025	1.5	0.4151	0.623	0.935	0.047

H2SO4 extraction 30 days after biosolids application							
Sample	Sample mass (g)	Volume of H_2SO_4 (L)	Dilution factor	Absorbance	P Concentration (ppm)	P Concentration (mg/L)	P Concentration (mg/g)
1a	0.25	0.025	1.5	0.2845	0.465	0.697	0.070
1b	0.25	0.025	1.5	0.2344	0.370	0.554	0.055
2a	0.25	0.025	1.5	0.2714	0.439	0.659	0.066
2b	0.25	0.025	1.5	0.2937	0.483	0.725	0.072
3a	0.25	0.025	1.5	0.2639	0.425	0.637	0.064
3b	0.25	0.025	1.5	0.2675	0.432	0.648	0.065
4a	0.25	0.025	1.5	0.2393	0.379	0.568	0.057
4b	0.25	0.025	1.5	0.3072	0.510	0.765	0.077
5a	0.25	0.025	1.5	0.2848	0.465	0.698	0.070
5b	0.25	0.025	1.5	0.2391	0.378	0.567	0.057
6a	0.25	0.025	1.5	0.2465	0.392	0.588	0.059
6b	0.25	0.025	1.5	0.2505	0.400	0.599	0.060
7a	0.25	0.025	1.5	0.2711	0.439	0.658	0.066
7b	0.25	0.025	1.5	0.2106	0.327	0.490	0.049
8a	0.25	0.025	1.5	0.2991	0.494	0.741	0.074
8b	0.25	0.025	1.5	0.2368	0.374	0.561	0.056

H2SO4 extraction 60 days after biosolids application							
Sample	Sample mass (g)	Volume of H_2SO_4 (L)	Dilution factor	Absorbance	P Concentration (ppm)	P Concentration (mg/L)	P Concentration (mg/g)
1a	0.25	0.025	1.5	0.2428	0.385	0.578	0.058
1b	0.25	0.025	1.5	0.2606	0.419	0.628	0.063
2a	0.25	0.025	1.5	0.2392	0.378	0.568	0.057
2b	0.25	0.025	1.5	0.2732	0.443	0.664	0.066
3a	0.25	0.025	1.5	0.3471	0.593	0.890	0.089
3b	0.25	0.025	1.5	0.4130	0.741	1.111	0.111
4a	0.25	0.025	1.5	0.2792	0.454	0.682	0.068
4b	0.25	0.025	1.5	0.2356	0.372	0.558	0.056
5a	0.25	0.025	1.5	0.2917	0.479	0.719	0.072
5b	0.25	0.025	1.5	0.2424	0.384	0.577	0.058
6a	0.25	0.025	1.5	0.2635	0.424	0.636	0.064
6b	0.25	0.025	1.5	0.3057	0.507	0.761	0.076
7a	0.25	0.025	1.5	0.3777	0.660	0.990	0.099
7b	0.25	0.025	1.5	0.3447	0.588	0.882	0.088
8a	0.25	0.025	1.5	0.2804	0.457	0.685	0.069
8b	0.25	0.025	1.5	0.2610	0.419	0.629	0.063

H2SO4 extraction 90 days after biosolids application							
Sample	Sample mass (g)	Volume of H_2SO_4 (L)	Dilution factor	Absorbance	P Concentration (ppm)	P Concentration (mg/L)	P Concentration (mg/g)
1a	0.25	0.025	1.5	0.3463	0.591	0.887	0.089
1b	0.25	0.025	1.5	0.3010	0.498	0.746	0.075
2a	0.25	0.025	1.5	0.2909	0.477	0.716	0.072
2b	0.25	0.025	1.5	0.2923	0.480	0.720	0.072
3a	0.25	0.025	1.5	0.3080	0.512	0.768	0.077
3b	0.25	0.025	1.5	0.3388	0.576	0.863	0.086
4a	0.25	0.025	1.5	0.3503	0.600	0.900	0.090
4b	0.25	0.025	1.5	0.3107	0.517	0.776	0.078
5a	0.25	0.025	1.5	0.3339	0.565	0.848	0.085
5b	0.25	0.025	1.5	0.3257	0.548	0.822	0.082
6a	0.25	0.025	1.5	0.3250	0.547	0.820	0.082
6b	0.25	0.025	1.5	0.3151	0.526	0.789	0.079
7a	0.25	0.025	1.5	0.3685	0.640	0.959	0.096
7b	0.25	0.025	1.5	0.3026	0.501	0.751	0.075
8a	0.25	0.025	1.5	0.3145	0.525	0.788	0.079
8b	0.25	0.025	1.5	0.3045	0.505	0.757	0.076

H2SO4 extraction 150 days after biosolids application							
Sample	Sample mass (g)	Volume of H_2SO_4 (L)	Dilution factor	Absorbance	P Concentration (ppm)	P Concentration (mg/L)	P Concentration (mg/g)
1a	0.25	0.025	2	0.2579	0.413	0.827	0.083
1b	0.25	0.025	2	0.2289	0.360	0.719	0.072
2a	0.25	0.025	2	0.2216	0.346	0.693	0.069
2b	0.25	0.025	2	0.2415	0.383	0.765	0.077
3a	0.25	0.025	2	0.2646	0.426	0.853	0.085
3b	0.25	0.025	2	0.2498	0.398	0.796	0.080
4a	0.25	0.025	2	0.2370	0.374	0.749	0.075
4b	0.25	0.025	2	0.2602	0.418	0.836	0.084
5a	0.25	0.025	2	0.2505	0.400	0.799	0.080
5b	0.25	0.025	2	0.2579	0.413	0.827	0.083
6a	0.25	0.025	2	0.2601	0.418	0.835	0.084
6b	0.25	0.025	2	0.2531	0.404	0.809	0.081
7a	0.25	0.025	2	0.2220	0.347	0.694	0.069
7b	0.25	0.025	2	0.2675	0.432	0.864	0.086
8a	0.25	0.025	2	0.2490	0.397	0.793	0.079
8b	0.25	0.025	2	0.2549	0.408	0.816	0.082

Non-Labile P within 14 days after biosolids application

Sample	Humic Acid P (mg/g)	H2SO4 extracted P (mg/g)	Total Non-Labile P (mg/g)
1a	0.012	0.089	0.101
1b	0.007	0.065	0.072
2a	0.013	0.070	0.083
2b	0.010	0.064	0.073
3a	0.011	0.111	0.123
3b	0.009	0.060	0.069
4a	0.014	0.115	0.129
4b	0.017	0.126	0.143
5a	0.016	0.111	0.127
5b	0.011	0.061	0.073
6a	0.010	0.057	0.067
6b	0.008	0.082	0.091
7a	0.012	0.069	0.081
7b	0.005	0.051	0.056
8a	0.017	0.072	0.089
8b	0.010	0.047	0.057

Non-Labile P within 30 days after biosolids application

Sample	Humic Acid P (mg/g)	H2SO4 extracted P (mg/g)	Total Non-Labile P (mg/g)
1a	0.006	0.070	0.076
1b	0.006	0.055	0.062
2a	0.010	0.066	0.076
2b	0.006	0.072	0.078
3a	0.003	0.064	0.067
3b	0.015	0.065	0.080
4a	0.010	0.057	0.067
4b	0.011	0.077	0.088
5a	0.009	0.070	0.079
5b	0.009	0.057	0.066
6a	0.009	0.059	0.067
6b	0.003	0.060	0.063
7a	0.005	0.066	0.071
7b	0.009	0.049	0.058
8a	0.008	0.074	0.082
8b	0.007	0.056	0.063

Non-Labile P within 60 days after biosolids application

Sample	Humic Acid P (mg/g)	H2SO4 extracted P (mg/g)	Total Non-Labile P (mg/g)
1a	0.012	0.058	0.070
1b	0.005	0.063	0.067
2a	0.003	0.057	0.060
2b	0.003	0.066	0.069
3a	0.006	0.089	0.095
3b	0.001	0.111	0.112
4a	0.003	0.068	0.071
4b	0.005	0.056	0.061
5a	0.005	0.072	0.077
5b	0.004	0.058	0.061
6a	0.005	0.064	0.068
6b	0.005	0.076	0.081
7a	0.005	0.099	0.104
7b	0.008	0.088	0.096
8a	0.002	0.069	0.071
8b	0.003	0.063	0.066

Non-Labile P within 90 days after biosolids application

Sample	Humic Acid P (mg/g)	H2SO4 extracted P (mg/g)	Total Non-Labile P (mg/g)
1a	0.006	0.089	0.095
1b	0.002	0.075	0.077
2a	0.005	0.072	0.076
2b	0.007	0.072	0.079
3a	0.008	0.077	0.085
3b	0.013	0.086	0.099
4a	0.007	0.090	0.097
4b	0.013	0.078	0.091
5a	0.010	0.085	0.095
5b	0.001	0.082	0.083
6a	0.004	0.082	0.086
6b	0.005	0.079	0.084
7a	0.008	0.096	0.104
7b	0.012	0.075	0.087
8a	0.003	0.079	0.081
8b	0.007	0.076	0.082

Non-Labile P within 150 days after biosolids application

Sample	Humic Acid P (mg/g)	H2SO4 extracted P (mg/g)	Total Non-Labile P (mg/g)
1a	0.004	0.083	0.087
1b	0.001	0.072	0.073
2a	0.007	0.069	0.077
2b	0.007	0.077	0.084
3a	0.008	0.085	0.093
3b	0.009	0.080	0.089
4a	0.007	0.075	0.082
4b	0.012	0.084	0.095
5a	0.006	0.080	0.085
5b	0.003	0.083	0.086
6a	0.004	0.084	0.087
6b	0.006	0.081	0.087
7a	0.006	0.069	0.075
7b	0.006	0.086	0.092
8a	0.003	0.079	0.082
8b	0.005	0.082	0.087

Appendix F: Soluble Reactive Phosphorus in Leachate

	Column number	1	2	3	4	5	6	7	8
1 day after biosolids application	Absorbance	0.0426	0.0387	0.0408	0.0369	0.0402	0.0336	0.0327	0.0242
	Concentration (ppm)	0.051	0.046	0.049	0.044	0.048	0.040	0.039	0.029
15 days after biosolids application	Absorbance	0.0346	0.0435	0.0416	0.0341	0.0379	0.0277	0.0250	0.0280
	Concentration (ppm)	0.041	0.052	0.050	0.041	0.045	0.033	0.030	0.033
45 days after biosolids application	Absorbance	0.0281	0.0390	0.0366	0.0393	0.0349	0.0341	0.0328	0.0232
	Concentration (ppm)	0.033	0.047	0.044	0.047	0.042	0.041	0.039	0.027
60 days after biosolids application	Absorbance	0.0244	0.0270	0.0341	0.0407	0.0252	0.0359	0.0259	0.0259
	Concentration (ppm)	0.033	0.037	0.047	0.056	0.034	0.049	0.035	0.035
80 days after biosolids application	Absorbance	0.0348	0.0386	0.0346	0.0477	0.0375	0.0391	0.0354	0.0339
	Concentration (ppm)	0.048	0.053	0.048	0.066	0.052	0.054	0.049	0.047
140 days after biosolids application	Absorbance	0.0387	0.0439	0.0391	0.0352	0.0389	0.0359	0.0403	0.0326
	Concentration (ppm)	0.053	0.061	0.054	0.048	0.054	0.049	0.056	0.045

	1 day	15 days	45 days	60 days	80 days	140 days
Average phosphorus concentration from reference columns (ppm)	0.047	0.041	0.039	0.038	0.049	0.054
Average phosphorus concentration from biosolids-amended columns (ppm)	0.040	0.040	0.040	0.044	0.055	0.051
Standard Deviation (reference columns) (ppm)	0.005	0.009	0.005	0.006	0.002	0.001
Standard Deviation (Biosolids-amended columns) (ppm)	0.008	0.009	0.009	0.010	0.008	0.007

Appendix G: Receiving Waters Analysis

Soluble Reactive Phosphorus

	Absorbance	P concentration (ppm)	Average P Concentration (ppm)	Standard Deviation (ppm)
Reference 1*	0.0064	0.009	0.009	0.000
Reference 2	0.0065	0.009		
Reference 3	0.0060	0.008		
Biosolids 1**	0.0066	0.009	0.009	0.000
Biosolids 2	0.0061	0.008		
Biosolids 3	0.0064	0.009		

* Leachate from non-amended soil columns were added to the reference aquariums during the time period of 5 months
** Leachate from biosolids-amended soil columns were added to the so-called biosolids aquariums during the time period of 5 months

Organic Carbon

	Total carbon (ppm)	Total Inorganic Carbon (ppm)	Total Organic Carbon (ppm)	Average Organic Carbon Concentration (ppm)	Standard Deviation (ppm)
Reference 1	34.75	21.87	12.88	11.46	1.31
Reference 2	36.62	26.33	10.29		
Reference 3	29.31	18.1	11.21		
Biosolids 1	34.77	22.42	12.35	12.76	0.41
Biosolids 2	41.45	28.68	12.77		
Biosolids 3	41.95	28.78	13.17		

Chlorophyll *a*

	Absorbance			Chlorophyll *a* (mg/L)	Average Chlorophyll *a* concentration (mg/L)	Standard Deviation
	at 630 nm	at 647 nm	at 664 nm			
Reference 1	0.0145	0.2060	0.0616	0.0041	0.393	0.026
Reference 2	0.0000	0.0000	0.0000	0.0000		
Reference 3	0.0000	0.0052	0.0323	0.0037		
Biosolids 1	0.0037	0.0054	0.0260	0.0030	0.305	0.007
Biosolids 2	0.0010	0.0035	0.0266	0.0031		
Biosolids 3	0.0000	0.0000	0.0000	0.0000		

Appendix H: Plants' Phosphorus

Total Phosphorus Concentrations in Soya Seeds

	Sample mass (g)	Volume of H_2SO_4 (L)	Dilution factor	Absorbance	P Concentration (ppm)	P Concentration (mg/L)	P Concentration (mg/g)
Trial 1	0.25	0.025	12.5	0.1932	0.253	3.161	0.316
Trial 2	0.25	0.025	12.5	0.1835	0.239	2.985	0.299
Trial 3	0.25	0.025	12.5	0.1874	0.245	3.056	0.306
						AVERAGE	0.307

Total Phosphorus Concentrations in Soya Plants

	Sample mass (g)	Volume of H_2SO_4 (L)	Dilution factor	Absorbance	P Concentration (ppm)	P Concentration (mg/L)	P Concentration (mg/g)
Biosolids-amended column #1	0.25	0.025	10	0.1663	0.214	2.142	0.214
Biosolids-amended column #2	0.25	0.025	10	0.1928	0.252	2.523	0.252
Reference column	0.25	0.025	10	0.1482	0.189	1.888	0.189
Biosolids-amended column #3	0.25	0.025	10	0.1789	0.232	2.322	0.232

Appendix I: **Plants' Analysis**

Even though only one reference column produced a soya plant, the characteristics of that plant did not differ much from the characteristics of plants produced by biosolids-amended columns. Overall characteristics of the produced plants are presented in a table below.

Table I.1. Characteristics of the plants produced by the soil columns.

	Number of produced plants	Stem length (cm)	Root length (cm)	Root to shoot ratio (%)	Number of leaves	Above ground biomass weight (g)
Biosolids-amended column #1	2	121.9	15.2	12.5	27	6.84
		111.7	15.0	13.4	24	
Biosolids-amended column #2	2	55.9	7.6	13.6	12	4.44
		106.7	13.4	12.6	24	
Biosolids-amended column #3	1	81.3	10.2	12.5	24	4.77
Reference column	1	81.5	9.4	11.5	21	4.23

Phosphorus analysis of plants demonstrated that the total phosphorus concentration per gram of biomass decreased compared to the total phosphorus concentration per gram of biomass observed in soya seeds, for both biosolids-amended and reference columns (Fig. I.1.). The decrease was similar for plants produced by biosolids-amended columns and for the plant produced by the reference column (t_2=1.99, p=0.185). The total phosphorus in

above ground biomass ranged between 1.07 and 1.40 mg in the three biosolids-amended columns and was 0.76 mg in the reference column. Therefore, the biosolids amendment of soil may have had a small effect on phosphorus content of plants growing in this soil, although there is not enough data to draw a conclusion. It is recognized that values above under-represent total biomass phosphorus, as root biomass was not included. However, 18.5-30% of the total phosphorus in above ground biomass could be supplied by the seed itself, with 70-81.5% (or 0.62-0.875 mg) supplied to plants from soil.

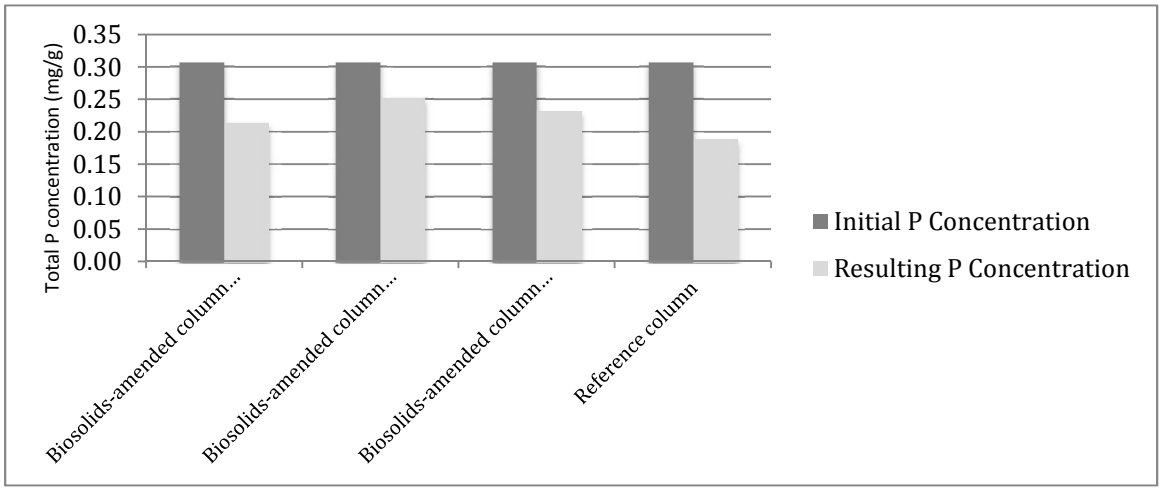


Fig. I.1. Total phosphorus concentrations in soya biomass.

Considering both physical characteristics and the phosphorus content of the produced plants, total above ground phosphorus notwithstanding, application of biosolids did not appear to increase soil fertility. Nonetheless, such an outcome was predictable, as soil used in the experiment was initially rich in phosphorus, and therefore might not be responsive to addition of a phosphorus fertilizer. If the soil was initially low in phosphorus, an addition of biosolids might result in a greater effect on soil fertility. A study conducted by Lagae *et*

al. (2009) demonstrated that soil rich in phosphorus had no response ($p<0.10$) to an application of 9 tonnes/ha of biosolids. An application of the same amount of biosolids to soil with lower phosphorus levels, however, resulted in a significant wheat yield response (2.1 tonnes/ha from the amended soil vs. 1.5 tonnes/ha from the non-amended soil). Furthermore, a study conducted by Warman and Termeer (2005) revealed that application of 10.7 tonnes/ha of anaerobically digested biosolids to soil low in phosphorus resulted in a significant increase in dry matter yields for grass (5.05 tonnes/ha from the amended soil vs. 3.38 tonnes/ha from the non-amended soil) and corn (14.71 tonnes/ha from the amended soil vs. 11.63 tonnes/ha from the non-amended soil). Therefore, the effect of biosolids application on soil productivity strongly relates to the initial soil phosphorus content.

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