

Phosphorus Non-Point Pollution from Equestrian Wastes and the Need for Recycling

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Abstract

South Florida and much of the rest of the World suffers from harmful algal blooms (HABs) and controls of both nitrogen (N) and phosphorus (P) pollution are required to curtail the onset, spread and/or expansion of these blooms.

This report covers our studies on several aspects of equestrian waste (viz. horse manure) aimed at yielding an overview of phosphorus and its pollution stemming from non-point horse manure sources in portions of Palm Beach County Florida. Methods included a modified Hedley extraction sequence, emphasizing 'easily extractable phosphorus' (EEP), and ³¹P nuclear magnetic resonance (NMR) spectroscopic identification of organic phosphorus (P_o) species.

Samples included fresh and aged horse manure, pasture soils, horse feed and pasture grasses, and canal waters adjacent to equestrian or agricultural fields.

Easily extractable Phosphorus (EEP) averaged about 54-77% of the total horse manure phosphorus. Total phosphorus ranged from 13,020 – 22,300 mg per kilogram dry weight. (≈60-100 lbs. P₂O₅ / ton and on a wet weight basis, this equates to 4,000 to 14,818 grams-P/ U.S. ton or 8.8 to 32.6 pounds of phosphorus (≈ 20-75 lb. P₂O₅) per wet weight ton of horse manure. Considering the values of EEP in fresh samples from a single horse, we found a range of 8,000 – 17,000 mg-P/kg (8-17 g-P/kg) dry weight horse manure.

Soil samples yielded the highest P in the NaOH extract of the Hedley sequence. This equates to the Al, Fe and ester forms.

Phosphorus (viz. EEP) runoff is viewed here as a non-point P pollution source.

Keywords: algal blooms, equestrian activities, horse manure, phosphorus, pollution, recycling

1. Introduction

The pollution of surface waters with primary plant nutrients, notably nitrogen and phosphorus, is increasing worldwide (Carpenter et al., 1998; Elser et al., 2007; Guignard et al., 2017; Smith et al., 1999). Nutrients can be and often are addressed within watershed management programs through processes termed best management practices (BMPs: FDACS, 2011; FDEP, 2013; Gunsalus et al., 1992; Mullins et al., 2005; Sims et al., 2000; USEPA, 2005) or decision support tools (DST: Drohan et al., 2019). It is noted that lag-times between implementation of BMPs and detection of impacts can range from years to decades (Meals et al., 2010).

In the October 2016 United States Environmental Protection Agency report entitled "National Nonpoint Source Program", it was stated "Of all the waterbodies across the nation that have been assessed and a possible source of impairment identified, 85% of rivers and streams and 80% of lakes and reservoirs are polluted by nonpoint sources." (USEPA, 2016).

Under the United States Clean Water Act Section 502, General Definitions, it states: "The term 'point source' means any discernible, confined and discrete conveyance, including but not limited to any pipe, ditch, channel,

tunnel, conduit, well, discrete fissure, container, rolling stock, concentrated animal feeding operation, or vessel or other floating craft, from which pollutants are or may be discharged. This term does not include agricultural stormwater discharges and return flows from irrigated agriculture.” (USEPA, 2019).

It is the last sentence in the above definition that generates the idea of ‘non-point’ sources (NPS) from agricultural operations. That is, “Nonpoint source pollution on the other hand comes from dispersing overland flow associated with rain events.” (Pierce et al., 1998). Nonpoint nitrogen and phosphorus pollution is a well-known worldwide problem (Carpenter et al., 1998; Lankoski and Ollikainen, 2013; Xia et al., 2020).

Pollution due to the leaching of manure derived nitrogen and phosphorus is well known (e.g. Carpenter, 2008; Hart and Cornish, 2012; Horta and Torrent, 2007; Liu et al., 2018; McDowel et al., 2020; Vadas et al., 2007). Many process-based mathematical models exist and utilize input data including nutrient concentrations, water flow, soil types, weather and numerous other parameters in order to develop and/or characterize BMP efficiencies. Such programs include SWAT (Soil and Water Assessment Tool) developed by the United States Department of Agriculture (Arnold et al., 1998; Neitsch et al., 2011; Shen et al., 2015). Several other models also exist (see e.g. Liu et al., 2019; Tuo et al., 2015) for the assessment of basin and catchment scale studies.

South Florida has been and still is experiencing nutrient (N, P) excesses in surface waters and sediments in Lake Okeechobee (Fisher et al., 2005; Havens, 1995; Havens and East, 1997; Havens and Thomas, 2005; Pollman and Thomas, 2011;), coastal estuaries (Duersch and Louda, 2017; Liu et al., 2009; Pant and Reddy, 2001; Philips et al., 2002) and the Greater Everglades (Bruland et al., 2007; Childers et al., 2003; Coralles et al., 2014; Louda et al., 2015; Reddy et al., 2011). Sources include sewerage, notably septic systems (aka OSTDS, Onsite Sewerage Treatment and Disposal Systems: Badruzzaman et al., 2012; FDOH, 2013; Lapointe et al., 2017; Meeroff et al., 2014), agricultural operations (Boggess et al., 1997; Duersch et al., 2020; Entry and Gottlieb, 2014; Stuck et al., 2001) and a growing equestrian industry (Cintron and Louda, 2007; Louda et al., 2019; Osetek and Louda, 2004).

Equestrian waste, notably manure, has received considerable attention pertaining to its use as agricultural fertilizer (e.g. Antonious et al., 2019; Flores et al., 2007; Motavalli and Miles, 2002; Shtangeeva et al., 2004; Tadesse et al., 2020; Zhu et al., 2012). Horse manure has also been studied for its role in N and P pollution (Cintron and Louda, 2007; Edwards et al., 1999; Liu et al., 2018; Louda et al., 2019; Paglari et al., 2012; Parvage et al., 2013; Narvanen et al., 2008).

The growth of the equestrian industry in south Florida, reaching 17-20,000 horses just in and around the Village of Wellington in Palm Beach County (Fig. 1), has been discussed frequently in local news media (e.g. Capone, 2019; Webb, 2019) and environmental news (FlaSpec, 2017). It is reported that about 500,000 horses, including 134,000 racing industry and 207,000 thoroughbreds, are in Florida year round (HorsePropNet, 2020). Much of the seasonal (Fall-Winter) horse population in Palm Beach County is involved in several Wellington horse shows including; the Wellington Horse Show, the Winter Equestrian Festival, Olympic level competitions, the Adequan Global Dressage Festival, and several Grand Prix events (WER, 2020). A single horse produces about 50 pounds of manure / waste per day, equating to over 9 tons per year (FDACS, 2011; FDEP, 2013; Krogman et al., 2006). Not including urine and manure contaminated bedding material wastes, it is reported that over 180-200,000 tons of horse manure is generated in Palm Beach County each year (FlaSpec, 2017).

Phytate (aka Phytic Acid, inositol hexakisphosphate (IUPAC nomenclature is 1R,2S,3r,4R,5S,6s-cyclohexane-1,2,3,4,5,6-hexayl hexakis-dihydrogen phosphate) is the major storage form of phosphorus in plants (Cosgrove, 1980; Skoglund et al., 2009; Xie et al., 2020) and is generally not digested by non-ruminant animals lacking the enzyme phytase (Dersjant-Li et al., 2015). As ruminants, horses are highly capable of releasing phosphate from phytate with efficiencies reported at 93-95% (Fowler et al., 2015; Lavin et al., 2013) due to phytase activity mainly in the lower large intestine (Matsui et al., 1999).

The current report covers several investigations aimed at providing an overview of phosphorus pollution stemming from non-point horse manure sources in portions of Palm Beach County Florida. Interest in this problem arose after hearing about and viewing the extreme overuse of horse manure and urine/manure soaked bedding on the nurseries and sugar cane fields in Palm Beach County. Agricultural landowners that receive equestrian waste (manure and bedding) receive “tipping fees” from the waste haulers. Therefore, the financial advantage of taking more than is needed appears drive this abuse.

Controls of both nitrogen (N) and phosphorus (P) pollution are required to curtail the onset, spread and/or expansion of micro- / macro- algal and cyanobacterial blooms (Paerl 1988, Paerl 2008, Paerl et al. 2011, Gobler et al. 2016; Paerl et al., 2016, Jankowiak et al., 2019, Levy 2017, Lu et al. 2019). Alterations of the watershed C:N:P values, the Redfield ratio (Redfield, 1934, 1958), are known to occur from agriculture field runoff (Smith et al., 2019).

We analyzed fresh and aged manure, various horse feed materials, surface and sub-surface soils, and canals that received surface / subsurface runoff of agricultural fields.



Figure 1. Map of Florida counties with the location of the Village of Wellington and the Town of Loxahatchee Groves indicated by the arrow pointing to a star

Emphasis was placed on the ‘easily extractable phosphorus’ (EEP) which consists of the phosphorus that is extracted with water and mild (0.1M sodium bicarbonate, NaHCO_3). This is also termed “Olsen P” (Olsen et al., 1954; Horta and Torrent, 2007; McDowell et al., 2020) or “soil-test phosphorus” (Hart and Cornish, 2012). Water extractable phosphorus (WEP) is often taken to mimic dissolved reactive phosphorus (DRP) in soil surface runoff (McDowell et al., 2020). WEP is the main part of the EEP and forms the first extract of the well-known Hedley fractionation scheme (Dou et al., 2000; Hedley et al., 1982).

Though phosphorus accretion from equestrian activities is but one part of the larger overall P and N surface water pollution problem, identifying areas where recycling can enter and enhance BMPs should contribute significantly to environmental remediation efforts.

2. Materials and Methodologies

2.1 – Materials

Horse manure was collected from fields and roads within the Town of Loxahatchee Groves and the Village of Wellington Florida by hand using inverted 1-gallon Ziploc® bags. In addition to horse manure, soil, and water analyses, we also examined selected samples of horse feed. This included pasture grasses, Timothy and Alfalfa (T&A), and Purina Pride 100® and Nutrena Proforce Senior® horse chows.

Surface soil samples were made using a stainless steel scoop and the samples placed into sealed sample jars (e.g. wide mouth HDPE, Thermo Scientific™ #3110500). Soil samples for depth studies were collected using a WILDCO® hand-driven core sediment sampler (WILDCO #77258) with a stainless steel nosepiece. Cores were kept at ice / refrigerator temperatures ($\sim 4^\circ\text{C}$) until sectioning and extractions were undertaken. The soil core was extruded from the core liner and sectioned into 1.2 cm sample layers.

Water samples were collected from bridges with a WILDCO 1120-G40 horizontal alpha water sampler of 2.2 L capacity. Water samples from canal or lake banks were collected using a 1.0-Liter rectangular amber HDPE bottle attached to an aluminum pole using a Castaloy™ chain clamp. In both cases, water was then transferred to well-rinsed amber HDPE rectangular bottles and, as with manure and soil samples, placed on ice, if not being analyzed within 2-3 hours, and transported to the lab for analyses.

2.2 Methodologies

2.2.1 Phosphate – limestone study

The limestone related removal of phosphates from natural canal waters or manure extracts in HPLC grade water

was examined by mixing with either fine grained (Fisher Scientific C64-500) or solid chunk (ACROS AC403790020) calcium carbonate (aka calcite; CaCO_3) added.

2.2.2 Phosphorus extractions and quantitation

The modified Hedley fractionation scheme (ex. Dou et al., 2000; cf. Hedley et al., 1982) for the extraction of phosphate from various phosphorus forms is given as Table 1. Water alone extracts free soluble reactive phosphorus (SRP) also commonly termed ortho-phosphate (PO_4^{3-}) or water extractable phosphorus (WEP), as described in the Introduction. Mild aqueous bicarbonate releases SRP from easily labile phosphates, notably pyrophosphate and polyphosphates. The sodium hydroxide solution releases SRP from amorphous-crystalline Al and Fe phosphates as well as phosphorus mono- and di-esters. The hydrochloric acid extraction frees SRP from Ca and Mg bound states. Lastly, digestion with strong acids releases SRP from organic phosphates. Each extraction includes 16 hours shaking.

Table 1. Modified Hedley fractionation scheme (Dou et al., 2000; Hedley et al., 1982)

Extractant	Phosphorus type(s)
water	Soluble Reactive Phosphorus (SRP)
0.5 M NaHCO_3 pH =8.5	Easily labile phosphates
0.1 M NaOH	Al- & Fe- and mono- / di-ester phosphates
1 M HCl	Stable Ca & Mg bound phosphates
60% HClO_4 : H_2SO_4 , 2:1, v/v	Organic phosphates (Po)

EEP = Easily Extractable Phosphorus = sum of water and mild bicarbonate extracts.

Ortho-phosphate, also known as soluble reactive phosphorus (SRP), present in and/or released from each fraction was measured by the molybdenum blue method. Reactive phosphorus (SRP) analyses were performed using HACH Chemical company reagent sets as follows: TNT #2742545 (0.06-5.00 mg PO_4^{3-} L⁻¹), TNT-Plus # 843 (0.15 - 4.5 mg PO_4^{3-} L⁻¹) and TNT-Plus #844 (1.5 - 15.0 mg PO_4^{3-} L⁻¹). Total phosphorus (TP) analyses were made with HACH Chemical company reagent sets as follows: TNT #2742645 (0.06-3.50 mg PO_4^{3-} L⁻¹), TNT-Plus # 843 (0.15 - 4.5 mg PO_4^{3-} L⁻¹) and TNT-Plus #844 (1.5 - 15.0 mg PO_4^{3-} L⁻¹). Phosphate data was multiplied by 0.3621 to convert to phosphorus (P) values or read directly as such. Total phosphate (TP) digestions were performed using HACH DRB200 reactors.

Spectrophotometric measurements were made using HACH DR5000 and DR3900 spectrophotometers by the molybdenum blue methods given above. Zero concentration was determined by testing HACH deionized water with each method and subtracting the method background from the observed reading during each analysis.

In all cases, standardization versus NIST-traceable standards gave $y \approx 1x$ with $R^2 \approx 1.00$. That is: DR5000 ($y = 1.0713x$, $R^2 = 0.9931$); DR3900 ($y = 0.9539x$, $R^2 = 0.9997$). The NIST traceable phosphate standards were (HACH #27428) from 1mg/L (HACH #256949), 15mg/L (HACH # 1424342), and a mixed-parameter standard with phosphate-P at a level of 1.0 mg/L (SRP = 3.066mg/L: HACH # LCA721). These standards were then diluted using HACH deionized water (#27248).

Total phosphorus was also determined in certain samples by Inductively-Coupled Plasma Mass Spectrometry (ICP-MS). ICP-MS analyses were contracted to the Florida Atlantic University Water Analysis Laboratory FAU-WAL) located in Davie, Florida (Dr. T. Root, director).

2.2.3 Organic phosphorus characterization

Sequential fractionation methods, such as the Hedley fractionation discussed above, measure P pools centered on the solubility of extractants and do not determine specific inorganic or organic P compounds (Negassa and Leinweber, 2009; Condon and Newman, 2011). Therefore, in order to assess the organic phosphorus species in horse manure, we incorporated ^{31}P Nuclear Magnetic Resonance spectroscopy (^{31}P -NMR). A 400 MHz Bruker NMR with a phosphorus probe was used to analyze whole samples (see Duersch, 2020). Samples were frozen in liquid nitrogen and subsequently lyophilized. Freeze-dried samples were reconstituted in 400 μL D_2O , 400 μL 0.05 M EDTA / 0.25 M NaOH solution, and 200 μL 1.0 M NaOH to ensure pH above 12 in accord with Cade-Menun (2015). The solutions were agitated for one minute and then centrifuged at 4,000 x g for 5 min. The solution was then placed in a 5 mm NMR tube and immediately analyzed. The NMR instrumental parameters were set as follows:

frequency at 161.98 MHz; T_1 relaxation delay time at 5 sec.; pulse angle 90° ; acquisition time at 1.0 sec. Data were analyzed using TopSpin 3.6.2[®] software. P compounds were identified by their chemical shifts (ppm) relative to the orthophosphate peak set to 6.00 ppm.

3. Results and Discussion

3.1 Results

3.1.1 Phosphorus in Horse Manure

A wide variety of horse manure, fresh and aged, was tested over the course of these studies. Here we present representative results for the Hedley fractionation of phosphorus types in various different manure samples. "Fresh" manure is defined here as that was excreted within 6-12 hours of collection. "Aged" manure (CC#2 Aged) was in the field for several days to weeks. It should be noted that the aged samples were collected during the dry season. That is, only small amounts of rain occurred during that period.

Sample CC#1 was aged *in vitro* at room temperature from CC#1-Fresh (Table 2) without the application of external water. In this case, Easily Extractable Phosphorus (EEP) was found to have decreased in percentage in concert with increases in the relative amount of Al and Fe bound P increasing by 58% (2.6 to 4.1 g-P/kg).

The yields of Hedley phosphorus fractions (groups), both as phosphate and phosphorus in mg per gram dry weight of manure, are given in Table 2. This data is also presented graphically as Figure 2.

Table 2. Representative Hedley fractionation results for fresh and aged (several weeks) horse manure. "EEP" = Easily Extractable Phosphorus = SRP plus labile forms

Sample	Extractant	Fraction	Yield (dry wt.)		Yield (wet wt.)		Percent
			mg- PO_4^{3-} / kg	mg-P / kg	mg-P / kg	% P	
CC#1 Fresh 57.1% wet wt	H ₂ O	SRP	17,380	5,666	3,333	43.5	62.7
	NaHCO ₃	labile-P	7,660	2,497	1,469	19.2	
	NaOH	Al & Fe & P-esters	8,010	2,611	1,536	20.0	
	HCl	Ca & Mg bound-P	1,010	329	194	2.5	
	Digestion	organic-P	5,890	1,920	1,129	14.7	
		TOTAL	39,950	13,024	7,661		
CC#1 Aged 38.8% wet wt.	H ₂ O	SRP	16,240	5,294	2,052	36.4	54.2
	NaHCO ₃	labile-P	7,940	2,588	1,003	17.8	
	NaOH	Al & Fe & P-esters	12,620	4,114	1,595	28.3	
	HCl	Ca & Mg bound-P	970	316	123	2.2	
	Digestion	organic-P	6,840	2,230	864	15.3	
		TOTAL	44,610	14,543	5,637		
CC#2 Aged 27.1% wet wt.	H ₂ O	SRP	23,380	7,622	2,066	46.9	72.3
	NaHCO ₃	labile-P	12,660	4,127	1,118	25.4	
	NaOH	Al & Fe & P-esters	9,750	3,179	861	19.5	
	HCl	Ca & Mg bound-P	870	284	77	1.7	
	Digestion	organic-P	3,240	1,056	286	6.5	
		TOTAL	49,900	16,267	4,409		
CC#3 FRESH 73.0% wet wt.	H ₂ O	SRP	42,900	13,985	10,208	62.7	77.3
	NaHCO ₃	labile-P	10,000	3,260	2,380	14.6	
	NaOH	Al & Fe & P-esters	11,900	3,879	2,832	17.4	
	HCl	Ca & Mg bound-P	500	163	119	0.7	
	Digestion	organic-P	3,100	1,011	738	4.5	
		TOTAL	68,400	22,298	16,276		

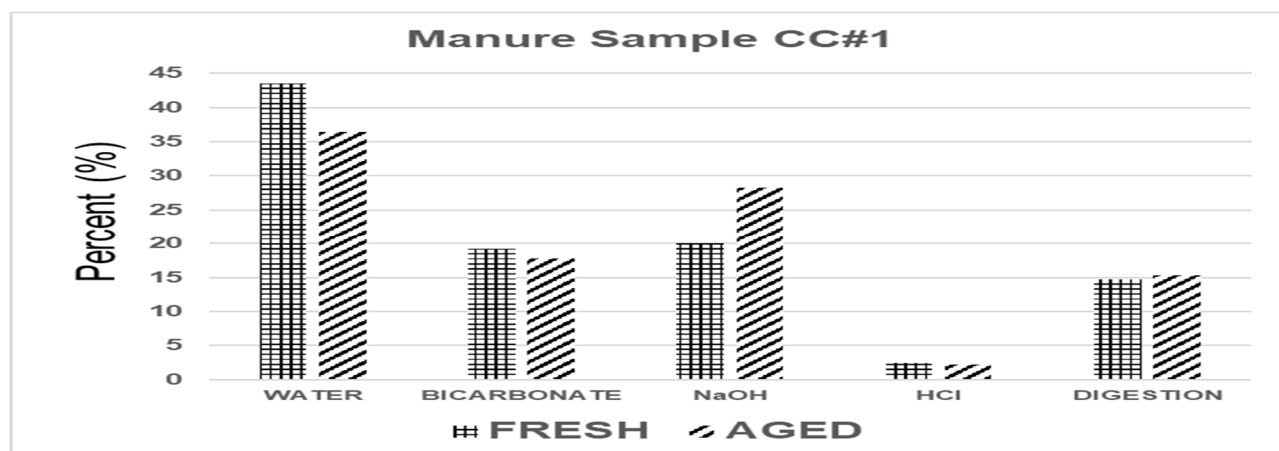


Figure 2. Graphic representation of phosphorus fractions in fresh and aged horse manure

3.1.2 Variability Study of SRP Yields in Horse Manures

All data presented above was arrived at by taking a large amount of the manure and mixing well (homogenizing) and then sub-sampling. For the variability test given here, we analyzed three separate sub-samples from each of three separate 'road apples' and in order to investigate the variability within fresh manure from a single animal deposited at the same time.

The samples varied in water weight from 59-82%, equaling 41-18% dry weight. Here we centered on the EEP (Easily Extracted Phosphorus) by extracting with water and 0.5M NaHCO₃. Sample sizes were small (0.5 -1.7 grams wet weight) in order to examine variability within the manure. The two larger fresh samples given above (Table 2) had quite variable EEP values of 8,163 mg/kg-dry wt. (CC#1-fresh) and 17,245 mg-P/kg-dry wt. (CC#3-fresh). The samples studied for within manure variability here (Table 3) had an overall mean value of 11,098 mg-P/kg dry wt. with a standard deviation of 1,392 mg-P/kg-dry wt. Across the samples from the three separate manure pieces (aka 'road apples'), the range of EEP (aka WEP, Olsen-P) was found to be 9,970 to 13,173 mg-P/kg (~ 10-13 g-P/kg) dry wt.

Considering the values of EEP for the fresh samples in Table 2 and the samples in the variability study, we can surmise a range of 8,000 – 17,000 mg-P/kg (8-17 g-P/kg) dry weight horse manure. Agricultural reports list 0.03-0.05 lb.-P₂O₅/lb. (≈ 60-100 lb./ton) horse manure (e.g. NOLA, 2020; UMin, 2020) and using P = 44% of P₂O₅, that would equate to about 13-22 g-P/kg-wet weight horse manure. Using an average percent water of 65%, that would give 4.6-7.7 g-P/kg-dry weight.

Table 3. Water extracted, Hedley Fraction #1, Soluble Reactive Phosphorus (SRP) in three separate fresh horse manure pieces ('road apples'). "a, b & c" are sub-samples of samples #1, 2 or 3

SAMPLE	WET	mg-P / kg manure				
		Mean	StdDev	DRY	Mean	StdDev
RA-1a	3,420	3,309	338	9,970	9,645	986
RA-1b	3,577			10,428		
RA-1c	2,929			8,538		
RA-2a	2,448	2,972	459	9,753	11,840	1,830
RA-2b	3,161			12,593		
RA-2c	3,306			13,173		
RA-3a	2,869	3,248	374	10,431	11,809	1,359
RA-3b	3,258			11,846		
RA-3c	3,616			13,149		
Overall means		3,176	390		11,098	1,392

3.1.3 Organic Phosphorus Forms in Horse Manure

Characterizing the molecular speciation of individual P_o compounds in natural environments is one of the more difficult analytical challenges in modern biogeochemistry (Ruttenberg, 2014; Schlesinger and Bernhardt, 2013; Sharp, 2002). The speciation and characterization through mass spectrometric techniques of samples with low concentrations of organic P, as well as those with complex backgrounds, has proven to be difficult (Masoom et al., 2013). ^{31}P -NMR spectroscopy is a technique used to both identify and quantify complex P-containing molecules that have eluded other standard methods such as mass spectrometry and colorimetric techniques (Cade-Menun et al., 2006). P_o within a sample is detected because the ^{31}P isotope is the only naturally occurring P isotope giving a $\frac{1}{2}$ spin. ^{31}P NMR is the optimal choice for the analysis of P_o in environmental samples as it is a non-destructive technique and P yields a high gyromagnetic ratio ($\gamma = 10.829 \times 10^7$ rad/T/sec) with 100% natural abundance.

The peaks in the spectra of ^{31}P NMR analysis represent the detected P-31 nuclei found in P_o compounds. The spectra are generally acquired with ^1H decoupling, meaning that spin-spin couplings are not observed. ^{31}P spectra of various P_o compound standards that have been ^1H ^1H coupled are shown in Figure 3. Splitting of the peaks for a single P_o compounds occurs due to the J -coupling caused by nearby P nuclei for an individual compound. An advantage of ^1H coupling is improved identification of P_o species. However, it can slightly decrease the signal to noise as well as quantitative aspects (Cade-Menun, 2015; Cade-Menun and Liu, 2014). Chemical shifts of signals were determined in parts per million (ppm) relative to an external ortho-phosphoric acid standard.

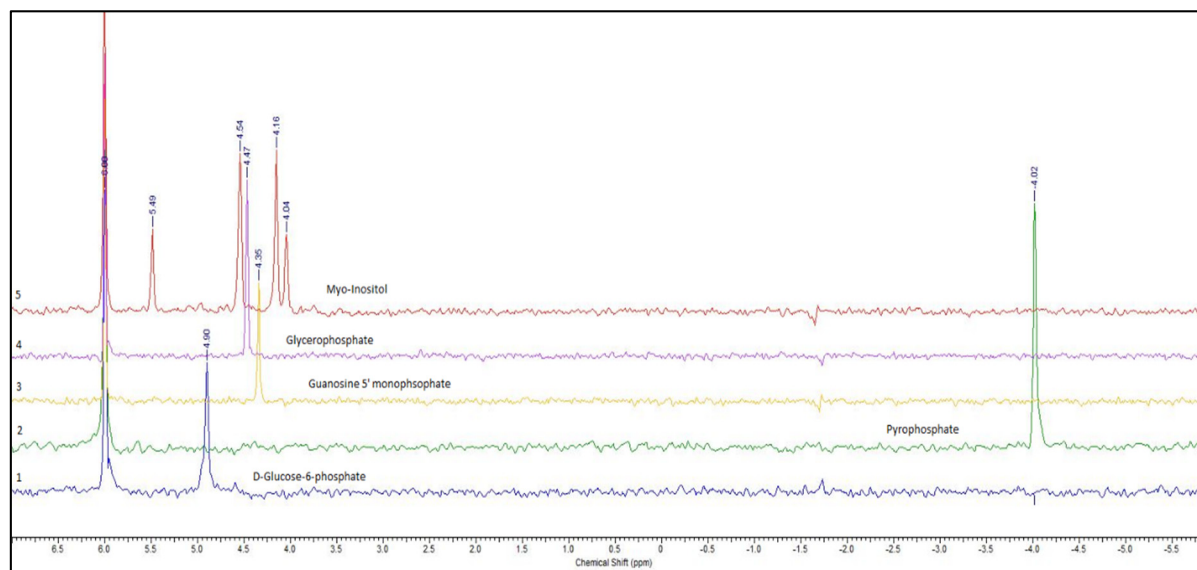


Figure 3. Proton decoupled ^{31}P NMR spectra of different P compound standards and their respective peak shifts. “Myo-inositol” = myo-inositol-hexakisphosphate (phytic acid). Orthophosphate was the reference at 6.00 ppm

Figure 4 is a ^1H decoupled ^{31}P NMR spectrum of the various phosphorus species in a fresh horse manure of the current study. The air-dried sample was extracted with an alkaline solution (NaOH-EDTA). Therefore, this extract equals the sum of the first three Hedley fractions, not just EEP. That is, SRP was released from Al, Fe and ester bound states.

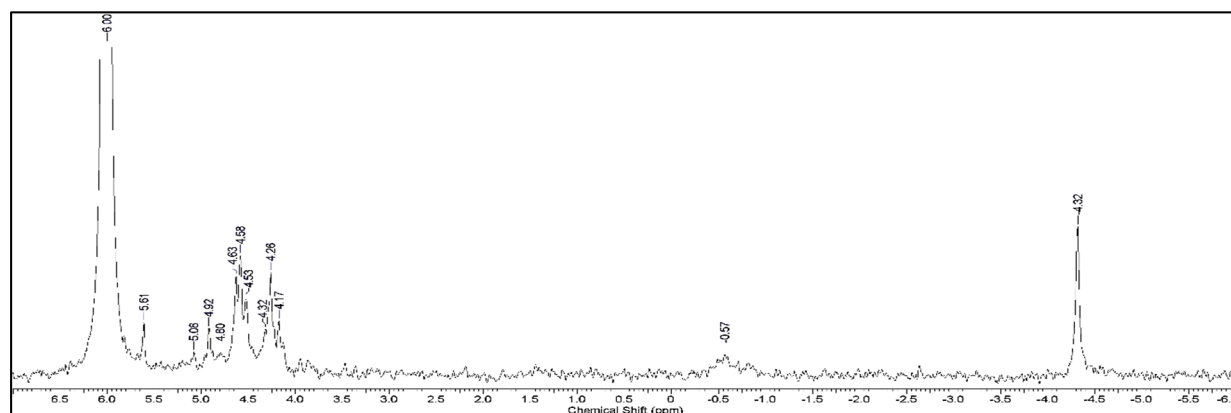


Figure 4. Proton decoupled ^{31}P NMR spectrum of phosphorus compounds in freshly excreted horse manure

3.1.4 Phosphorus in various feed types

Feed samples were analyzed for phosphorus using the Hedley fractionation scheme, as given earlier (Table 1). The horse feeds analyzed were Timothy and Alfalfa, Purina Pure Pride 100 horse chow, and Pasture Grasses as shown in Table 3.

These analyses gave P percentages (Table 3) in the range of 0.8 – 1.0% P, dry weight. The pasture grasses that we analyzed had about 0.94% P which is higher than a variety of grasses (0.23-0.44%-P) reported in Oklahoma and Virginia grasses (OKState, 2017; VTech, 2006).

The easily extracted phosphorus (EEP = water and bicarbonate extractant groups) of the feeds and pasture grasses was 46-63% of the total phosphorus content. The percent distribution of P in the various Hedley extractant fractions are given in Table 4 and graphically as Figure 5.

Table 4. Hedley fractionation analyses of certain equestrian feed materials

Sample	Extractant	Fraction	mg- PO_4^{3-} /kg	mg-P / kg	% P	"EEP"
						mg-P / kg
Timothy & Alfalfa	H_2O	SRP	11,700	3,814	33.2	51.8%
	NaHCO_3	labile	6,500	2,119	18.6	
	NaOH	Al & Fe & esters	6,200	2,021	17.6	
	HCl	Ca & Mg bound	300	98	0.9	
	Digestion	organic	8,700	2,836	29.7	
		TOTAL	33,400	10,888		
Purina Pure Pride-100	H_2O	SRP	13,100	4,271	49.5	62.8%
	NaHCO_3	labile	3,500	1,141	13.3	
	NaOH	Al & Fe & esters	5,000	1,630	18.9	
	HCl	Ca & Mg bound	300	98	1.0	
	Digestion	organic	3,000	978	17.3	
		TOTAL	24,900	8,117		
Pasture Grasses	H_2O	SRP	9,900	3,227	32.2	45.6%
	NaHCO_3	labile	4,100	1,337	13.4	
	NaOH	Al & Fe & esters	11,800	3,847	38.5	
	HCl	Ca & Mg bound	700	228	2.3	
	Digestion	organic	2,300	750	13.7	
		TOTAL	28,800	9,389		

EEP = Easily Extracted Phosphorus (= SRP plus labile).

Table 5. Comparison of the phosphorus species (Hedley fractions) between various feedstocks and horse manure

	Percentages					
	Water	Bicarb	NaOH	HCl	Digest	EEP
T&A	33.2	18.6	17.6	0.9	29.7	51.8
Purina-100	49.5	13.3	18.9	1.0	17.3	62.8
Grasses	32.2	13.4	38.5	2.3	13.7	45.6
Manure Avg.	39.8	18.4	24.4	2.3	15.1	58.2

EEP = Easily Extractable Phosphorus = Water plus mild aqueous bicarbonate

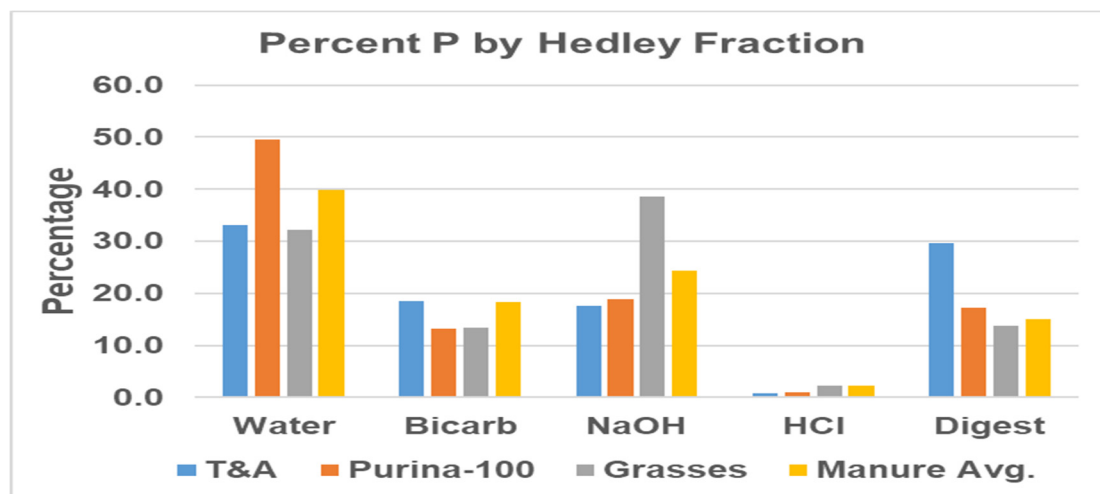


Figure 5. Graphic representation of phosphorus species (Hedley fractions) between feed stock and horse manure

An additional feed sample (Nutrena Proforce Senior®) was analyzed in triplicate only for total phosphorus using ICP-OES and it was found to have $6,340 \pm 300$ mg/kg-P, dry weight.

Given that this was a limited sample of horse feeds, TP can be noted to range from about 6,000 – 11,000 mg-P (6-11 g-P; 0.6 -1.1%) per kilogram of various feed materials.

3.1.5 Horse paddock soil phosphorus with depth

Certain manure samples (“CC”) were taken from a horse field where we also investigated phosphorus quantity and fractions versus soil depth. This field was in the Town of Loxahatchee Groves and the soils sampled are of the Riviera Association and have been described as being “nearly level, poorly drained sandy soils that have a loamy subsoil starting at around 2 feet (McCollum et al., 1978). The loamy subsoil was below our deepest (12 cm) sample level. We compared soil phosphorus fractions (Table 5) with the average values of manure samples CC#1-fresh and CC#1-aged (see Table 2-above). It is noted here that the soil cores studied (Table 6) were not directly under a manure pile. That is, this was a site within an actively horse occupied field and the history of manure immediately at the core site is unknown.

Table 6. Mass (mg-P / g soil) of phosphorus in Hedley fractions of sandy soil, including comparison to the average of fresh and aged horse manure samples

	mg-P / g					
	H ₂ O	NaHCO ₃	NaOH	HCl	Digest	TOTAL
(Manure)	6.087	2.824	3.730	0.358	2.303	15.302
0.0-1.2	0.067	0.254	1.827	0.013	0.119	2.280
1.2-2.3	0.066	0.217	1.328	0.091	0.169	1.871
2.3-3.4	0.067	0.105	0.143	0.005	0.029	0.348
3.4-4.5	0.040	0.070	0.091	0.004	0.065	0.270
4.5-5.6	0.058	0.142	0.304	0.015	0.120	0.638
5.6-6.7	0.076	0.148	0.329	0.005	0.023	0.580
6.7-7.8	0.053	0.166	0.332	0.008	0.044	0.604
7.8-8.9	0.067	0.105	0.323	0.009	0.074	0.578
8.9-11.0	0.074	0.113	0.345	0.016	0.134	0.681
11.0-12.0	0.062	0.092	0.253	0.007	0.068	0.482

From both the raw data (Tables 5 & 6) and their graphical representations (Figures 6a-b), it can be seen that there was a rapid decline in the amount of soil phosphorus below 2.3 cm. The largest decline was found to be in the sodium hydroxide extracted fraction, which contains Al and Fe bound phosphate plus mono- and di-ester phosphates (Dou et al., 2000; Hedley et al., 1982).

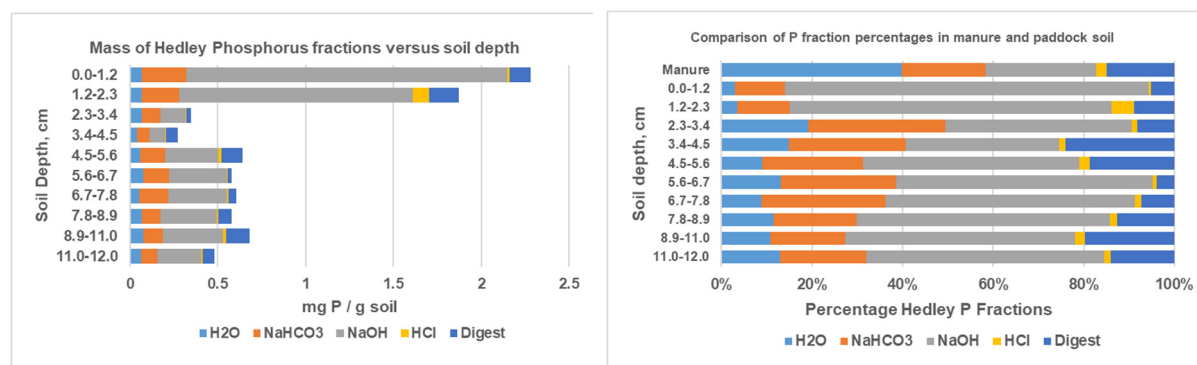


Figure 6. Phosphorus fractions in soil. (a) Mass versus depth.

(b) Hedley P fraction percentages versus depth and compared to horse manure

3.1.6 Tests for Calcium Sequestration of Phosphate

As phosphorus runoff and/or percolation from equestrian and agricultural lands will enter the numerous canals in south Florida, the influence of limestone will affect its mobility and/or sequestration. We performed a simple test to see how two different forms of calcite (CaCO_3), fine grained or chunks, alters SRP solubility. Manure samples were placed into 100.0 mL of either deionized or Loxahatchee Groves canal water and then steeped with the added calcite in the dark for one week. The P-polluted canal water had 3.728 mg-P/L (3,728 ppb-P) before the addition of manure. The resultant data are given in Table 7.

Table 7. Reaction of phosphate (SRP, ortho-phosphate) with calcium carbonate (Calcite)

Sample	100mL Deionized water		100mL Canal water	
	plus 10.01g "JO" manure		plus 10.01g "JO" manure	
	SRP mg-P / L	Percent	SRP mg-P / L	Percent
Plain	6.793	100.0%	10.519	100.0%
10g Fine	0.409	6.0%	0.489	46.0%
25g Fine	0.319	4.7%	0.369	3.5%
10g chunk	5.276	77.7%	8.361	79.5%
25g chunk	5.218	76.8%	7.984	75.9%
100g chunk	3.748	55.2%	7.289	69.3%

3.1.7 Phosphorus in Canal Waters

Neighboring the Village of Wellington to the north is the Town of Loxahatchee Groves Florida ($26^{\circ}42'56''\text{N}$ x $80^{\circ}16'23''\text{W}$) which has over 29 miles of canals within its 12.5 square mile area and these drain into the Palm Beach Canal (SFWMD #C-51). The C-51 canal moves water to the west towards South Florida Water Management's stormwater treatment areas (STAs) and the Greater Everglades and to the east towards Lake Worth Lagoon.

Study of phosphorus pollution in these canals, as well as certain other sites in south Florida, was driven by observations of excess utilization of horse manure and urine/manure soaked stall pine wood chip bedding on agricultural properties. During routine agricultural fertilization the N:P:K contents of industrial fertilizers are known and their application can occur in line with BMPs and minimally required additions. However, the use of horse manure and bedding occurs with unknown N:P:K concentrations and often unregulated spreading. That is, agricultural operations are allowed to receive manure / bedding and in so doing also receive a 'tipping fee'. That is, the hauler pays the receiver a certain amount per load and often the receiver takes much more than is actually needed to just supply good nutrient levels for plant growth. Figures 7a-b are presented to emphasize this point. The swales alongside the dirt roads drain into the canals discussed here.



Figure 7. Excessive use of equestrian manure and bedding. (a) A palm tree nursery that received and spread equestrian bedding and manure several feet thick over entire property and into adjacent drainage swale. (b) Close-up of equestrian waste in the swale adjacent to a road with obvious horse manure circled

(Photographs by senior author).

Well over a dozen canal sites were randomly sampled. Looking only at the SRP (EEP) values, September samples ranged from 10-268 with a mean of 56 ppb-P and October samples ranged from 47-262 ppb with a mean of 103 ppb-P. The lower values equated to sites receiving back-pumped waters from the C-51 canal during the equestrian

off-season months. However, once the equestrian season got well underway and wastes were received from nearby equestrian activities the range went to 78-797 ppb-P with a mean of 238 ppb-P. We sampled the water coming from an outflow pipe of a nursery that had 3-4 feet of equestrian wastes piled up on a 20 acre site. In this case, SRP was 7,958 ppb-P (24,410 ppb-PO₄) or 8 mg-P / L. Finding such a high level led to a resampling a few weeks later. In that case SRP was 4.84 mg / L (4,840 ppb-P) and TP was 4.96 mg / L (4,960 ppb-P). SRP was therefore 97.6% of TP. Summing nitrate and nitrite values in these outflow waters gave an atomic N:P ratio of <1:1. Water from a site about 1 mile from this outflow pipe had SRP at 340 ppb-P and an atomic N:P ratio of ~7:1. Such high P pollution certainly would favor diazotrophic (nitrogen-fixing) cyanobacteria. Indeed, we microscopically identified *Lyngbya* sp. in these canals. During a different year, we sampled 24 sites within the 24 miles of canals (i.e. 1 sampling per mile) of Loxahatchee Groves and found a range of 32 – 1,670 ppb as phosphate (= 10 – 604 ppb P) in the canal waters. This reinforces the concept that non-point sourcing of P pollution needs attention if such pollution is ever to be controlled.

3.2 Discussion

Overall, horse manure yielded easily extracted phosphorus (EEP) that was 54-77% of the total phosphorus. Total phosphorus ranged from 13,020 – 22,300 mg per kilogram dry weight. That is, 13-22 grams of P per kilogram of manure. That equates to 6 – 10 grams of P per pound and 12,000 – 20,000 grams (26 – 44 pounds) of P per ton of manure (~60-100 lbs. P₂O₅ / ton), dry weight. Looking at wet weight values, this equates to 4.4 to 16.3 g-P/kg manure, wet weight. Therefore, on the wet weight basis, this equates to 4,000 to 14,818 grams-P/ U.S. ton or 8.8 to 32.6 pounds of phosphorus (~ 20-75 lb. P₂O₅) per wet weight ton of horse manure. These values overlap the horse manure yield of 0.03-0.05 lb.-P₂O₅/lb. (~ 60-100 lb./ton) reported by others (e.g. NOLA, 2020; UMin, 2020).

Given that EEP and organic phosphorus species comprise the vast majority (~80%) of P in horse manure, the potential for rain and irrigation extraction and runoff / percolation can easily account for a highly significant “non-point” source of P pollution in surficial aquifers and open water systems.

Overall, values of about 5 to >20 g-P/kg- horse manure dry weight with about 55-75% (2 to >11g-EEP/kg dry wt.) of that being Easily Extractable Phosphorus can be concluded. Additionally, it can be stated that rather high variability in the amount of total (TP) and easily extractable (EEP) phosphorus exists when analyzing horse manure. This likely stems from the diet of the horse, the horse type and age, the freshness of the samples, the moisture content, and a myriad of other factors.

The fractionation of TP pools can be coupled with P speciation through ³¹P NMR spectroscopy to give a much more in-depth understanding of the biogeochemical dynamics of P, which distinguish individual P compounds in the fractionated P pools from horse manure (Turner and Leytem, 2004; McDowell and Stewart, 2006).

Along with mineral (Al, Fe, Ca, Mg) bound P, organic P (P_o) is a P source that is available for future bio-/geo-chemical conversions to SRP and release for immediate *in situ* bioavailability and/or movement into surface waters. Ortho-phosphate (SRP) was found to be the vastly major (80.8%) component with □-glycerol-phosphate (5.7%), phytic acid (4.8%) pyrophosphate (4.0%) and DNA (2.7%) also being present. SRP is regraded as the easily leachable / mobile phosphorus with respect to the P pollution of ground water and nearby surface waters (canals, lakes etc.). It is noted here that □-glycerol-phosphate and pyrophosphate can be hydrolyzed to ortho-phosphate (=SRP).

Given that we analyzed a limited sample of horse feeds, TP can be noted to range from about 6,000 – 11,000 mg-P (6-11 g-P; 0.6 -1.1%) per kilogram of various feed materials.

The large yield and percentage of the Al-, Fe- and mono-/di-ester phosphates (NaOH extract) in the soil, especially in the top several centimeters, may reflect retention of those forms from field grasses / roots (see feed results: Table 5, Fig. 6) and/or sequestration of SRP by Al and Fe in the soil. Compared to the horse manure (Figure 6b), considered here to be a major P source by leaching / runoff transport, the percent loss of SRP in the surface 2.3 cm can be envisioned as being due to surface runoff and/or sequestration by Al and Fe. The minor amounts and percentages of Ca and Mg bound P (HCl extract) reflects the low Ca / Mg content of the Rivera Association sandy soils in this field. Future studies should compare wet and dry seasons to obtain information of the mobilization of SRP and the leaching and/or breakdown of the easily labile P forms, such as pyro-phosphate. This area of Florida normally averages about 60 inches of rain per year (FCC, 2020) and, given the majority of that occurring in the wet season (May – October), significant runoff can be expected. Future studies of P input to soils from overlying manure needs to consider soil geochemistries and rain history.

Fined grained calcium carbonate, a surrogate for powdered ‘limestone’, was found to effectively (avg. ~ 95-96%)

remove phosphate from pure as well as high COD canal waters. This contrasts with the calcite chunks which, even at a 4-fold mass increase (viz. 100 vs. 25 g), removed only 30-45% of the SRP. This difference (50-60%) reflects the difference in available surface area between the fine-grained (high area/g) and chunk (low area/g). Future studies should repeat this test with native limestones. It is suggested here that limestone pebbles, that is neither powder nor large chunks, be utilized to fill a trough around equestrian paddocks and agriculture fields in order to intercept and immobilize phosphate that leaches from the field, forming a non-point source. The use of limestone pebbles to form a “French Drain” at the front of manure / bedding collection bins (Figure 8) would capture significant SRP runoff and could enhance current and planned best management practices (BMPs).



Figure 8. Picture of a manure / barn bedding collection / storage area established to lessen rain induced leeching. Limestone pebbles form a “French Drain” at the front

The Village of Wellington (Ordinance 2012-12 Amended Chapter 30, Article V), the Town of Loxahatchee Groves Ordinance 2010-003) and Palm Beach County (Ordinance 2017-005) have all passed ordinances covering equestrian waste haulers and dumping. These efforts certainly help and best management practices (BMPs) are evolving. However, the spreading of manure and bedding, such as occurred on thousands of acres of U.S. Sugar lands with “little resultant agricultural benefit” (as cited in Palm Beach Post 9/21/2020, p. 5B), is not the answer. Rather, recycling such that the manure and urine organic matter plus plant nutrient elements (N, P, K, Fe etc.) are pelletized and sold as fertilizer with a known N:P:K value and the wood bedding chips are cleaned and resold as bedding is the ultimate way to handle the hundreds of thousands of tons of equestrian waste. Palm Beach County recently approved a recycling facility to be operated by a private company on land leased from the Palm Beach County Solid Waste Authority (Bukley, 2020; Morse, 2020).

We need to point out another aspect of recycling. That is, it needs to be as close to the major sites of generation as possible in order to cut down on the addition of excess carbon dioxide and sulfur oxides from heavy diesel truck traffic. The average mileage of a class 8a diesel dump truck is 3-7mpg (US-DOE, 2015). Using the median value of 5 mpg, one truck puts 22.2 pounds of carbon dioxide per mile into the atmosphere. That is 111 pounds of carbon dioxide per 25 mile trip. Hence, shorter trips to and from major sites of generation would cut down on climate change. Additionally, diesel soot is heavy in particulate matter of the 2.5-micron size (PM_{2.5}) which is a lung problem, especially for asthmatics and the young (Balmes, 2011; Ris, 2007; Salvi et al., 1999).

4. Conclusions

The extremely high soluble reactive P (SRP), aka easily extracted phosphorus (EEP), concentrations found in horse manure, shown in Tables 2-3, leads to large amounts of P pollution from water runoff. This we found in Palm Beach County canal waters when equestrian activities and the indiscriminant waste spreading on adjacent lands was high (cf. Giles et al., 2015). As high amounts of P leaching into water sources occurs, eutrophic conditions and large micro-/ macro-algal and higher plant aquatic blooms may follow. Additionally, Al and Fe seem to play a role as P short-term storage while Ca and Mg lead to somewhat longer-term storage for P (Table 6). Organic phosphorus (P_o) species made up from 4.5 to 15.3% of the total phosphorus in several horse manure samples (Table

2). These P_o species are also water mobile and, through hydrolysis, give rise to highly soluble ortho-phosphate or are then bound to metals (Fe, Al, Ca, Mg). Through nutrient analyses and P speciation studies, future studies should be able to determine how P types are transported through the ecosystems that feed the Everglades, Lake Worth Lagoon, and adjacent bodies of water. Plant uptake is the main factor in the fate of labile P (Duersch et al., 2020 and references therein). Knowing the different P pools present in the environment should help guide the management of P containing wastes and soil amendments (Condrón and Newman, 2011). However, it may take years to remove substantial amounts of the so-called ‘legacy’ phosphorus (Gatiboni et al., 2020; Havens and Thomas, 2005; Lou et al., 2018; Pavinato et al., 2020) out of the soil to the point where dissolved P in runoff is effectively reduced (McCollum, 1991; Meals et al., 2010; Zhang et al., 2004; Sharpley et al., 2013).

The quite often excessive utilization of animal waste, equestrian in the present study, as landfill / fertilizer does not allow accurate tracing of N:P:K soil amendment and also leads to significant nutrient pollution of adjacent waters. Recycling of equestrian waste whereby the wood bedding chips are cleaned and reused and the manure / urine materials are processed into pellets with known N:P:K concentrations can then allow for enhanced fertilization best management practices.

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