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Factors Influencing Water Extractable Phosphorus Reduction in Poultry Litter by Chitosan Treatment

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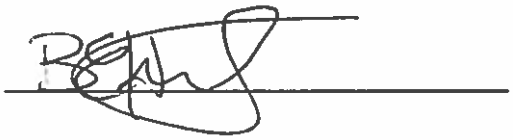

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ABSTRACT

Phosphorus is known to be a chief factor in the eutrophication of freshwaters. Phosphorus in land applied poultry litter can runoff and pollute these freshwaters. Chitosan, the deacetylated form of the biopolymer chitin, has been shown to have an effect on reducing water extractable phosphorus (WEP) in poultry litter when applied as a powder. The intent of this study was to measure the effect that acetic acid and incubation time have on chitosan's ability to reduce WEP in poultry litter. The results were that the presence of poultry litter treatment (PLT) in the litter inhibits chitosan's ability reduce WEP. Chitosan dissolved in acetic acid does not decrease WEP after any amount of incubation time. Chitosan in a powder form reaches its full effectiveness after three weeks of incubation.

INTRODUCTION

Phosphorus (P) has been a concern for water quality because it is considered to be one of the primary factors limiting algal growth and influencing eutrophication (Parry, 1998; Correll, 1998). The enrichment of freshwaters causes increased primary production (i.e. algal growth), leading to changes in aquatic communities (Smith, 1998; Swingle, 1966), diurnal changes in dissolved oxygen (Alabaster, 1959; Alabaster, 1961; Floyd, 1992), anoxic bottom waters during lake and reservoir stratification (Diaz and Rosenberg, 2008; Floyd, 1992) and even taste and odor issues in drinking water supplies (Walker, 1983). Phosphorus and other nutrients enter freshwaters through defined discharges and diffuse sources from the landscape.

The diffuse sources are transported during rainfall-runoff events from the landscape, including agricultural fields and urban development. The agricultural sources include P stored in soils and that applied to the landscape in fertilizers and animal manures. In northwest Arkansas, poultry production and application of poultry litter (manure plus bedding) represent an important diffuse source of P in watersheds. Several studies have shown that the WEP content of poultry litter is positively correlated to P concentrations in runoff during rainfall simulation studies (Haggard et al., 2005; Kleinman and Sharpley, 2003; Kleinman et al., 2007; Vadas et al., 2004). This relation has prompted research on ways to minimize the WEP content of poultry litter; for example, aluminum sulfate (alum) has been shown to reduce WEP in poultry litter (Dao, 1999) and therefore reduce P concentrations in runoff from field plots (Moore et al., 2000; Shreve et al., 1995; Smith et al., 2001).

A biologically derived coamendment, in the form of chitosan, has also been researched for its ability to reduce WEP in animal manures (Bailey, 2012) among its other uses (Garcia et al., 2009; Kumar and Majeti, 2000; Rabea et al., 2003; Rinaudo, 2006). The preliminary lab studies have shown that WEP in poultry litters was reduced when chitosan was applied at 1-10% rates, and chitosan was as effective as alum at the 1-5% application rates (Bailey et al., 2014). To further understand the ability of chitosan to reduce WEP content in poultry litter, the goal of this study is to evaluate factors that alter WEP reduction

in poultry litter treated with chitosan. We hypothesized that chitosan delivered in acetic acid solution will produce a significantly greater reduction of WEP content in poultry litter than dry application of chitosan powders. We also hypothesized that there is greater reduction of WEP content in poultry litter as incubation time progresses, especially with the dry application of chitosan powders.

MATERIALS AND METHODS

Poultry litter was collected from the stacking barn and compost at University of Arkansas poultry facilities, which grows birds under contract for Simmons Foods. These poultry facilities used Poultry Litter Treatment (PLT, sodium bisulfate, $NaHSO_4$) during bird production to reduce ammonia (NH_3) volatilization, and PLT also influences litter chemistry (Pope and Cherry, 2000; Sweeney et al., 1996). In the first experiment using PLT treated litter, a control and four different application rates (percent on dry weight basis) were used for each delivery method, that is delivery as a powder or dissolved in dilute (2%) acetic acid solution. The PLT treated litter was homogenized and divided into 20 g samples (dry weight equivalent), mixed with the treatment, and incubated at room temperature for two weeks. The treatments consisted of a control (untreated), a control treated with only dilute acetic acid, four application rates of chitosan in powder form (i.e., 0.5, 1.5, 3 and 5% dry weight equivalent, g chitosan g^{-1} poultry litter) and then chitosan delivered as dissolved in acetic acid (0.05, 0.1, 0.2, and 0.5% dry weight equivalent, g chitosan g^{-1} poultry litter); for each treatment, 4 replicates were used. After incubation, the poultry litter samples were extracted for water extractable phosphorus (WEP) using a 1:100 dry litter to water ratio (Kleinman et al., 2007) and then the filtrate was analyzed using the inductively coupled argon plasma optical emission spectrometry (ICP-OES) at the University of Arkansas Soil Diagnostic Lab. WEP_{ICP} content was compared across treatments using analysis of variance (ANOVA) with mean separation (Least Significant Difference, LSD) at an alpha level of 0.05. The filtrate was also analyzed using the ascorbic acid method for soluble reactive P to measure WEP_{SRP}.

In the second experiment, a new source of poultry litter that was not treated with PLT was collected from the University of Arkansas experimental poultry facilities at Arkansas Agricultural Research and Extension Center. This litter was handled as previously described in experiment one, and then both litters (PLT and non-PLT amended) were used in the next experiment. Four different types of chitosan were used in this experiment (Table 1), including the one used in first experiment and the same three used in a previous study (Bailey, 2012; Bailey et al., 2014). A control and four treatments (each chitosan form applied at 10 percent on dry weight basis) were used for each litter source, where the chitosan was applied in powder form not dissolved in dilute acetic acid. Five replicates were used for each control and treatment, where 6 g dry weight equivalent poultry litter was incubated. The treatments were applied; the litter was mixed, incubated for 8-weeks and then WEP was measured on subsamples after 1, 4 and 7 weeks. After the selected incubation time, up to 2 g (dry weight) of the samples were extracted to measure WEP (Kleinman et al., 2007) as modified. The WEP solutions were filtered using a Whatman-40 filter via gravity filtration (primary filtration) and the filtrate was analyzed for soluble reactive phosphorus (SRP) using the modified ascorbic acid reduction method, which is analogous to WEP_{SRP}.

In the third experiment, only the non-PLT litter source was used based on the results from experiments one and two. Approximately, 8 g of poultry litter (dry weight equivalent) were separated into containers. This experiment featured the following treatments: a control, a control with just acetic acid (approximately 0.8 mL), 10% (dry weight basis) chitosan in powder form, and varying application rates of chitosan delivered in a dilute acetic acid solution (i.e., 0.05, 0.10, 0.20 and 0.50% chitosan on a dry weight basis, g chitosan g^{-1} poultry litter). The chitosan used was just the medium molecular weight chitosan, and incubation times were set ranging from 1 week to 3 weeks for all treatments. The treatments were sampled at the selected incubation times, and then extracted following the same process as in experiment two and analyzed for WEP_{SRP}.

Table 1. A list of chitosan types used in experiment 2.

Number	Type of Chitosan
1	ChitoClear®, provided by Dr. Zaharoff.
2	≥75% deacetylated chitosan
3	Practical grade chitosan
4	Medium molecular weight chitosan

RESULTS AND DISCUSSION

Experiment 1

The results from the first experiment were unexpected since the WEP_{ICP} content of the poultry litter samples treated with chitosan in powder form and the samples treated with chitosan dissolved in acetic acid were not significantly different from the control (3942 mg kg^{-1}, Table 2). The PLT litter treated with 0.20 and 0.50% (dry weight basis) chitosan dissolved in acetic acid had WEP_{ICP} content (3986 mg kg^{-1} and 4143 mg kg^{-1}, respectively) numerically greater than the control and significantly different from WEP_{ICP} content of some of the other chitosan treatments. These results were contrary to the observations made in previous studies (Bailey, 2012; Bailey et al., 2014), which showed that chitosan applied to poultry litter in powder form significantly reduced WEP_{ICP} content.

Table 2. Water extractable phosphorus (WEP_{ICP}) in poultry litter amended with Poultry Litter Treatment (PLT) after mixing with chitosan delivered as powder or dissolved in acetic acid and incubated at room temperature for two weeks (Experiment 1).

Treatment	Replicates	WEP_{ICP} (mg kg^{-1} dry litter)		
		Mean	Standard Deviation	Homogeneous Groups[a]
Control	4	3942	247	AB
AA Control[b]	4	3769	77	B
0.5% Powder[c]	4	3774	32	B
1.5% Powder	4	3867	95	B
3.0% Powder	4	3869	244	B
5.0% Powder	4	3904	167	AB
0.05% Dissolved[d]	4	3761	210	B
0.10% Dissolved	4	3859	165	B
0.20% Dissolved	4	3986	90	AB
0.50% Dissolved	4	4143	245	A

[a]Homogenous groups based on means separation using Least Significant Difference.
[b]AA designates acetic acid, where this treatment received the same volume of AA without chitosan.
[c]Chitosan applied as a dry powder.
[d]Chitosan applied dissolved in acetic acid.

The first experiment was repeated to follow Bailey (2012), where WEP was measured using ICP-OES at the University of Arkansas System's Division of Agriculture Soil Diagnostic Lab (i.e., following Kleinman et al., 2007). The filtrate was also analyzed for SRP using a colorimetric method, which is designated as WEP_{SRP}. These two methods differ, where WEP_{ICP} represents the total P measured in the filtrate whereas WEP_{SRP} represents the reactive P measured in the filtrate. However, analysis of the same samples using both analytical methods showed a significant, positive correlation between WEP_{ICP} and WEP_{SRP} (Figure 1). Since both analyses were comparable and SRP analysis was more practical in the laboratory, SRP using spectrometry analysis was used for the rest of the experiments.

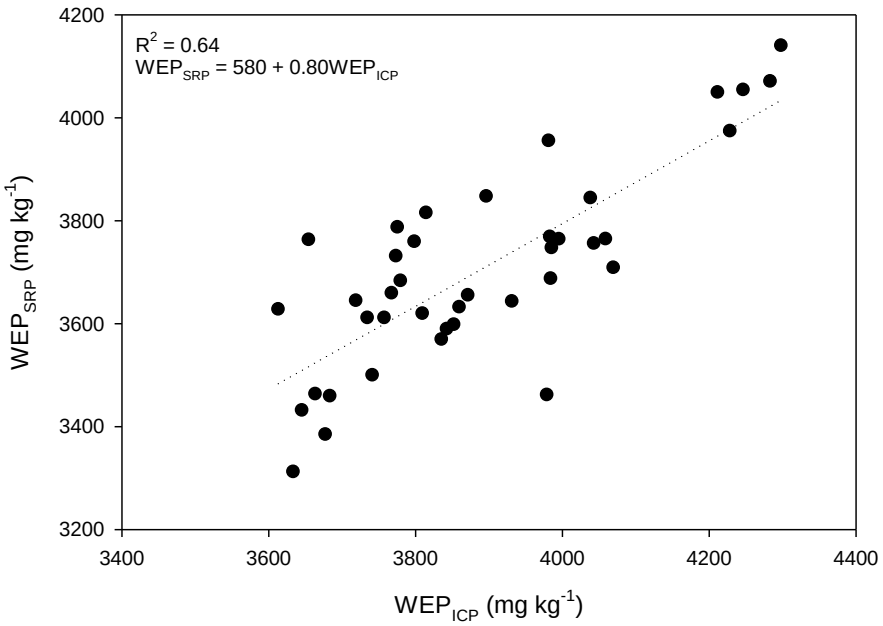

Figure 1. Comparison of Water Extractable Phosphorus (WEP) content by spectrometry (WEP_{SRP}) and by ICP-OES (WEP_{ICP}) for samples from experiment 1.

Experiment 2

Since the first experiment showed such unexpected results, several factors were called into question: the source of the poultry litter, the source of chitosan used, and also the length of the incubation. Experiment 1 used poultry litter that had been treated with PLT, which is chemically sodium bisulfate ($NaHSO_4$), and is used in commercial poultry production to reduce ammonia volatilization. The bisulfate, HSO_4^-, reduces litter pH which reduces ammonia volatilization and therefore improves bird health (Sweeney et al., 1996). This chemical amendment was suspected to have an effect on chitosan's ability to reduce WEP in the litter. In order to examine its effect, a new source of poultry litter that had not been treated with PLT was obtained for the second experiment.

To test whether the source of chitosan played a role in the first experiment's results, three sources of chitosan, all used by Bailey (2012), were included in the second experiment. The second experiment tested the new sources of chitosan and the original source on both sources of poultry litter (PLT and non-

PLT treated) at a rate of 10% (dry weight basis), which was shown to be effective at reducing WEP_{ICP} (see also Bailey et al., 2014).

For the poultry litter that had been treated with PLT, the results after a 4 week incubation showed that WEP_{SRP} of PLT treated litter treated by all sources of chitosan were not significantly different than WEP_{SRP} of the control (4172 mg kg^{-1}, Table 3). The samples treated with chitosan had numerically greater amounts of WEP_{SRP} than that of the control samples. These results show that none of the sources of chitosan that had been shown to reduce WEP by Bailey (2012) were able to have a similar effect on the litter treated with PLT. This suggests that chitosan was not effective at reducing WEP, when poultry litters were treated with PLT.

The results for the poultry litter not treated with PLT were much different. The WEP_{SRP} content of the control non-PLT litter (4448 mg kg^{-1}) was significantly greater than the WEP_{SRP} content of the four chitosan treatments. These results match with the results seen by Bailey (2012), which showed that WEP_{ICP} was significantly reduced by chitosan application. This proved that the chitosan source used in the first experiment reduced WEP_{SRP}, and it was not the factor that resulted in the lack of chitosan effect.

Experiment 2 also showed that incubation time has an effect on chitosan's ability to reduce to WEP_{SRP}. Subsamples from the non-PLT litter source were extracted after 1, 4, and 7 weeks of incubation. The amount of WEP_{SRP} removed across all chitosan treatments compared to the control is illustrated in Figure 2. While chitosan had some effectiveness after 1 week of incubation, its performance appeared to peak after 4 weeks of incubation and remained about the same for the rest of its incubation. The experiments performed by Bailey (2012) used incubation times that exceeded 4 weeks, based on the time incubated in the lab and then analyzed at the Soil Diagnostic Lab for WEP_{ICP}. So, it can be concluded from experiment 2 that chitosan reduced WEP in poultry litters not treated with PLT and that it needs to be mixed with litter for 4 weeks to maximize the reduction.

Table 3. Water Extractable Phosphorus (WEP_{SRP}) from two sources of poultry litter treatment with various sources of chitosan at a 10% dry weight basis application rate (Experiment 2) following a four week incubation.

Application Rate	Litter Source	Chitosan Source	WEP_{SRP} (mg kg^{-1} dry litter) Mean	Standard Deviation	Homogeneous Groups[a]
	PLT[b]		4172	393	A
10%	PLT	1	4527	385	A
10%	PLT	2	4466	378	A
10%	PLT	3	4559	170	A
10%	PLT	4	4566	408	A
	Non-PLT[c]		4448	70	A
10%	Non-PLT	1	3833	68	B
10%	Non-PLT	2	3830	67	B
10%	Non-PLT	3	3841	81	B
10%	Non-PLT	4	3918	42	B

[a]Homogenous groups, based on means separation with Least Significant Difference within a litter source.

[b]Poultry litter that has been treated with Poultry Litter Treatment (PLT).

[c]Poultry litter that has not been treated with PLT.

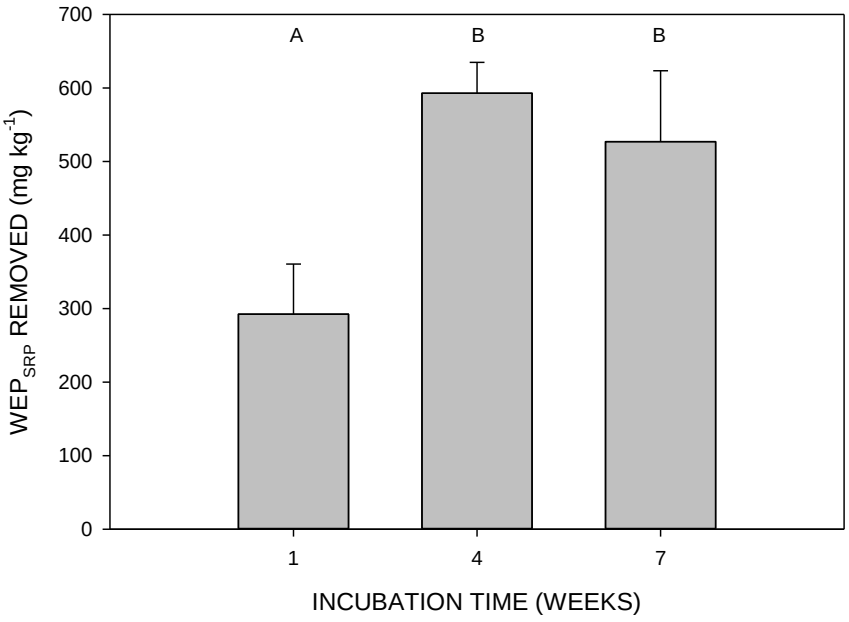

Figure 2. Comparison of removal ability of WEP_{SRP} for all chitosan treatments compared to the control after various incubation times for experiment 2.

Experiment 3

Having determined that the treatment of PLT to poultry litter has an effect on chitosan's ability to reduce WEP_{SRP} in the second experiment, the third experiment was a modified version of the first experiment that excludes the presence of PLT. The source of the litter used was the non-PLT litter in the second experiment. This allowed us to investigate the effect that dissolving chitosan into acetic acid has on its ability to reduce WEP. Since the second experiment showed that the sources of chitosan used did not produce significantly different results, which source of chitosan to use was not heavily considered.

After one week of incubation, the results showed that the chitosan powder (4354 mg kg^{-1}, Table 4) was the only treatment to reduce WEP_{SRP} in comparison to the control (4586 mg kg^{-1}); WEP_{SRP} content in the litter treated with chitosan powder was significantly different from the control, but it was applied at a rate an order of magnitude greater than the chitosan dissolved in acetic acid. The four chitosan dissolved in acetic acid treatments (0.05%, 4895 mg kg^{-1}; 0.10%, 4796 mg kg^{-1}; 0.20%, 4848 mg kg^{-1}; 0.50%, 4840 mg kg^{-1}) all had WEP_{SRP} contents numerically greater than the control, and only the WEP_{SRP} content of the 0.10% treatment was significantly not different from the control. Interestingly, the control with just acetic acid applied (4730 mg kg^{-1}) was also numerically greater than the control, but not significantly different.

Three weeks of incubation had results with the same trend as discussed above (Table 5). The 10% powder treatment (4372 mg kg^{-1}) had the least WEP_{SRP} content and was significantly different from all of the treatments. The next lowest WEP_{SRP} content was found in the control (4757 mg kg^{-1}). Of the treatments that involved acetic acid, only the 0.50% chitosan dissolved in acetic acid (4993 mg kg^{-1}) was significantly not different than the control. The 0.50% treatment was also the only one that was significantly different from the control with acetic acid (5334 mg kg^{-1}). The other three chitosan dissolved in acetic acid treatments (0.05%, 5171 mg kg^{-1}; 0.10%, 5306 mg kg^{-1}; 0.20%, 5202 mg kg^{-1}) were not significantly different from the acetic acid control nor the 0.50% treatment.

These results are evidence against the hypothesis that chitosan in acetic acid would have a greater effect on the reduction of WEP_{SRP} in poultry litter. The presence of acetic acid appears to actually increase WEP_{SRP}. The results of the chitosan powder treatment resemble that of the second experiment; chitosan powder has a peak effectiveness on reducing WEP_{SRP} after 3 weeks. Thus, it does not seem beneficial to dissolve chitosan into acetic acid when applying to poultry litter. However, acetic acid would likely reduce litter pH and therefore inhibit ammonia volatilization but it would increase WEP and the potential loss of P during rainfall runoff events. It would also be worthwhile analyzing the poultry litter treated with 0.50% chitosan in AA at seven weeks of incubation for WEP_{SRP}. Since this treatment was actually not significantly different from the control at three weeks of incubation, this treatment could provide a positive effect at the longer incubation time.

Table 4. Water Extractable Phosphorus (WEP_{SRP}) from poultry litter (without PLT) treated with chitosan as a powder or dissolved in acetic acid (Experiment 3) and incubated at room temperature for 1 week.

Treatment[a]	WEP_{SRP} (mg kg^{-1} dry litter)		
	Mean	Standard Deviation	Homogeneous Groups[b]
Control	4596	215	B
AA Control[c]	4730	91	AB
10% powder	4354	213	C
0.05% Dissolved[d]	4895	146	A
0.10% Dissolved	4796	191	AB
0.20% Dissolved	4848	87	A
0.50% Dissolved	4840	159	A

[a]Chitosan used is 4 in table 1.

[b] Homogenous groups based on means separation using Least Significant Difference.

[c] AA designates acetic acid, where this treatment received the same volume of AA without chitosan.

[d]Chitosan applied dissolved in acetic acid.

Table 5. Water Extractable Phosphorus (WEP_{SRP}) from poultry litter (without PLT) treated with chitosan as a powder or dissolved in acetic acid (Experiment 3) and incubated at room temperature for 3 weeks.

Treatment[a]	WEP_{SRP} (mg kg^{-1} dry litter)		
	Mean	Standard Deviation	Homogeneous Groups[b]
Control	4757	66	C
AA Control[c]	5334	280	A
10% powder	4372	277	D
0.05% Dissolved[d]	5171	113	AB
0.10% Dissolved	5306	191	A
0.20% Dissolved	5202	200	AB
0.50% Dissolved	4993	281	BC

[a]Chitosan used is 4 in table 1.

[b] Homogenous groups based on means separation using Least Significant Difference.

[c] AA designates acetic acid, where this treatment received the same volume of AA without chitosan.

[d]Chitosan applied dissolved in acetic acid

CONCLUSION

Chitosan's ability to reduce WEP is inhibited by the presence of PLT in the poultry litter. The source of poultry litter must be untreated with PLT in order for chitosan to have its desired effect. Application of chitosan dissolved in acetic acid proved to be ineffective and the presence of acetic acid alone even increases WEP. The time of incubation did have an effect on the reduction of WEP; chitosan's effectiveness peaks after 3 weeks of incubation. Future studies may find alternative methods of applying chitosan to poultry litter to improve effectiveness, such as using a different acid solution in place of acetic acid.

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