Effects of activated carbon on the growth of *Chlorella vulgaris* in an aqueous solution

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Abstract

Algal blooms, if left unmanaged, can negatively impact lake ecosystems. An unexplored method of removing excess nutrients from lakes, and therefore reducing algal blooms, is through the use of biochar. We hypothesize that due to the adsorptive characteristics of pyrolyzed material such as biochar and activated carbon, its presence would reduce the nutrient availability within aqueous solutions, therefore reducing algal growth. This experiment was conducted in an aqueous solution containing COMBO growth medium with and without the presence of activated carbon, studied under four conditions: 5 mg/L, 10 mg/L, 20 mg/L, and 50 mg/L phosphorous. We measured the fluorescence of the samples over a 12 day period to quantify algal growth. An analysis of covariance followed by a Tukey's HSD test demonstrated a significant difference between the mean amount and rate of algal growth in samples containing activated carbon, compared to samples without, with samples containing activated carbon showing less growth ($p < 0.0001$). Further, the concentration of both phosphorus and nitrogen in samples containing activated carbon were lower than in samples without, though these results were inconclusive. Our study demonstrates that activated carbon has the capacity to be used to reduce algal growth, likely due to its capacity to adsorb nutrients such as phosphorous and nitrogen. This suggests that both activated carbon, as well as its more adsorptive counterpart, biochar, have the potential to be used in mitigating algal blooms and, more importantly, reducing the effects of anthropogenic eutrophication in aqueous environments.

Keywords: Biochar; activated carbon; eutrophication; phosphorous adsorption

Introduction

Algal blooms, if left unmanaged, can have serious negative impacts on lake ecosystems (Conley et al. 2009; Michalak et al. 2012). Anthropogenic pollution has led to an increase in the amounts of phosphorous and nitrogen entering waterways, encouraging the growth of algal blooms, with phosphorous acting as the main limiting nutrient for algal growth (Conley et al. 2009; Paerl 2009; Lewis 2011). Algae, such as *Chlorella vulgaris*, flourish in nutrient-rich environments, resulting in an increase in their abundance (Paerl 2009). Increases in algal abundance within lakes reduces the amount of light that reaches the lake bed, resulting in benthic species being unable to photosynthesize, creating an anoxic environment and reducing lake biodiversity (Hautier et al. 2009; Ansari & Singh Gill 2014).

Evidence of eutrophication has been apparent in the Great Lakes from the 1960s (Scavia et al. 2014). The Great Lakes Water Quality Agreement of 1972 has helped manage one of the main causes of eutrophication: rising levels of phosphorous (Hartig et al. 1990). The management initiative's objective was to control algal blooms in the lower lakes and to maintain the oligotrophic conditions of the upper

lakes (Hartig et al. 1990). Despite management initiatives, eutrophication in the Great Lakes has increased since 1990 (Scavia et al. 2014). This eutrophication has created hypoxic conditions which threatens macroinvertebrates, many of which are benthic prey species that are important food sources for higher trophic levels (Scavia et al. 2014). These conditions reduce the water quality, effectively eliminating the conditions that are vital to the macroinvertebrates and the biodiversity found within the Great Lakes cold-water habitat (Scavia et al. 2014). Local communities and government stakeholders are investigating means of reducing eutrophication and returning nutrient concentrations to moderate levels (Schindler and Vallentyne 2008; Ruiz et al. 2011; Michalak et al. 2012). Doing so would prevent algal growth from reaching adverse levels, maintaining biodiverse communities within the aquatic ecosystem and improving the water quality upon which many non-aquatic species rely (Ansari and Singh Gill 2009).

An unexplored method of removing excess nutrients from lakes is through the use of biochar. Biochar is a substance created from biomass that has been pyrolyzed

under anaerobic conditions (Brown 2009). It is most commonly used as a soil additive for increasing plant growth and soil quality, which it does by enriching soils through its adsorptive capabilities, ultimately reducing nutrient leaching (Warnock et al. 2007). Its ability to adsorb pollutants in soil has also proven effective for removing pollutants from agricultural farm runoff (Beck et al. 2011; Knowles et al. 2011). Biochar has also shown promise as a means of reducing chemical contamination, particularly as a treatment for wastewater (Kimetu et al. 2014).

While several studies have investigated the potential uses of biochar as a method of filtering wastewater and for use in soil (Chen et al. 2011; Dalahmeh et al. 2012; Fang et al. 2014), very little of the research considers the potential environmental effects of the application of biochar to aquatic environments (Morales et al. 2013; Chintala et al. 2014; Soinne et al. 2014; Xu et al. 2014). Of the studies previously conducted, the use of biochar within wastewater filtration systems demonstrates that biochar has the capacity to adsorb phosphorous and nitrogen (Chen et al. 2011; Dalahmeh et al. 2012; Fang et al. 2014). These studies focus on the use of biochar in the filtration of human wastewater, which benefits humanity by developing and improving wastewater treatment practices (Chen et al. 2011; Dalahmeh et al. 2012; Fang et al. 2014). However, throughout the current scientific literature, there is a lack of focus on the use of biochar as a filtration system within the natural environment.

This raises the question of how biochar and its nutrient adsorbing qualities could be used within natural water systems, in particular, if it could affect the growth of algae through interactions with aqueous phosphorous. Previous studies suggest that biochar is able to adsorb both nitrogen and phosphorous (Beck et al. 2011; Berger 2012), but because phosphorous is usually the limiting agent for algal growth, we will focus our study upon this nutrient. Our research study aims to address how biochar could be used within aquatic environments to reduce algal growth. Biochar has been shown to have a greater adsorptive capacity than other pyrolyzed material, such as activated carbon, and is also less costly and labour intensive to make (Huggins et al. 2016). Despite these superior qualities, biochar is not easily accessible for use due to its novelty and the need for ongoing research on its chemical properties and potential applications (Berger 2012; Kimetu et al. 2016). Due to the lack of knowledge and availability of biochar, activated carbon has been substituted for biochar in our experiment as it has similar, though less effective, adsorptive characteristics.

Based on activated carbon's capacity to adsorb phosphorous, we hypothesized that the growth of algae would be impeded by the addition of activated carbon. Thus, solution samples without the addition of activated carbon

will contain higher levels of phosphorous compared to samples which have had activated carbon added.

Methods and Materials

To test our hypothesis, we manipulated levels of phosphorous and the presence of activated carbon in a diluted algal growth medium to observe their effects on algal growth. The single-celled green algae, *Chlorella vulgaris*, was used in this study due to its growth being dependent upon the presence of phosphorous (Ruiz et al. 2011). *C. vulgaris* was also chosen due to the ease with which changes in population abundance can be observed through its ability to fluoresce, with higher fluorescence readings corresponding with larger populations (Tang et al. 2016). Fluorescence measurements were used to determine algal growth over time, with data readings collected at 405 μm . To observe potential relationships of algal growth to nutrient availability, we also recorded phosphorous and nitrogen readings using API aquarium nutrient tests. Phosphorous and nitrogen readings were taken on the first day of the experiment to give baseline readings of the nutrient concentrations in each treatment, and as well as on the fourth and last days of the experiment (Aquarium Pharmaceutical Incorporated: Nebraska, U.S.). The API tests involved taking a 5 mL measure from each sample and adding drops of the respective phosphate and nitrogen test solutions to these measures. The resulting colour change of the 5 mL measure and test solution mixture was then compared against a chart of colours included with the tests, which indicated the concentrations of phosphorous or nitrogen within that sample.

Forty 1-L beakers were used, into which 2 mL of *C. vulgaris* inoculant, 500 mL of diluted algal growth medium, and 12.5 mg of nitrogen (25 mg/L) were added. Before being added, the inoculant was agitated by stirring to ensure an equal amount of algal cells would be present in each addition. This nitrogen concentration was chosen and added to all treatments to ensure that nitrogen would not act as the limiting growth factor, as 25 mg/L is well above the threshold required for algal blooms to form (Paerl, 2009; The 2012 Canada-US Great Lakes Water Quality Agreement, Annex 4). The COMBO algal growth medium was diluted to a concentration of 10% with distilled water to ensure minimum nutrient levels were established (Kilham et al., 1998). The phosphorous values were chosen to be above the threshold at which algal blooms are known to occur, 5 $\mu\text{g/L}$ (Paerl, 2009; The 2012 Canada-US Great Lakes Water Quality Agreement, Annex 4). Based upon this average, we set up four experimental concentrations of added phosphorous (0 mg/L, 5 mg/L, 20 mg/L, 50 mg/L). Ten beakers per each concentration treatment were created, with

each set of ten being further subdivided into five beakers of each treatment containing AquaClear aquarium filter activated carbon and five without.

The amount of activated carbon used in this experiment was modified from a study conducted by Beck et al. (2011), which used a ratio of 7% by mass of biochar to volume of the rainwater runoff solution in litres. We replicated this ratio based on our experimental volumes, and then further reduced the amount of activated carbon due to the size restriction of the tea bags used to hold the activated carbon. Tea bags were used to prevent the solutions from being clouded by particles of the activated carbon, which would interfere with the fluorometer readings. Empty tea bags were added to the treatments without the activated carbon to standardize all treatments and reduce the presence of confounding variables. The beakers were kept under wide-spectrum LED lights, which provided photosynthetic active radiation at wavelengths 400 nm to 700 nm, to encourage algal growth, and were covered with plastic film to reduce the loss of solution through evaporation and to prevent contamination. Each solution was stirred uniformly each day before the fluorometer readings were taken, to ensure that the algae remained suspended and did not develop uneven growth within the samples.

A fluorescence spectrophotometer, at wavelength 405 nm, was used to compare algal growth every day for two weeks, from October 17th to 31st. Data was lost from October 17th to 19th as well as October 30th due to equipment failure, and so the data analysis compares data collected from October 20th to October 31st, excluding October 30th; therefore, day 1 to 12, excluding day 11. The equipment failure was due to incorrect calibration by previous users of the spectrophotometer, and was resolved for our further use.

Using the replications of our data for the treatment variables, JMP software (SAS Institute Inc. 2017) was used to conduct the statistical analyses on the data. The independent variables - presence of activated carbon, P concentration, and day - were compared and contrasted using an analysis of covariance (ANCOVA) to determine their individual and combined effects on the fluorescence readings for each treatment. Due to its conservative nature (Steinberg 2011), the Tukey's HSD test was used as a post-hoc test following the ANCOVA, which excluded data collected the 11th day of our experiment due to equipment failure. An α -value of 0.05 was used to determine significance in these analyses. Treatments with a phosphorous level of 50 mg/L was also excluded after an initial analysis (**Table 1**) which demonstrated an obvious divergence in this data from the trend of the other treatment types. We believe this may have resulted due to the phosphorus concentration reaching a high enough level that the algal carrying capacity was reached at a

faster rate than the other samples, therefore levelling off sooner than the other samples, though this needs to be confirmed through further experimentation.

Results

With the exclusion of day 11 data and data for the treatment containing 50 mg/L of phosphorous, the results of the ANCOVA for fluorometer readings were significant for all variables - time (day), presence of activated carbon, and treatment type - and their combinations ($p < 0.0001$; **Figure 1, Table 1**). There was a visibly noticeable difference in the amount of algal growth between treatments with the presence versus absence of activated carbon during early readings in the experiment (**Figure 2**), though this difference decreased towards the end of data collection (**Figure 2**). The Tukey's HSD test demonstrated a significant difference between the means of samples containing activated carbon compared to samples without; samples with activated carbon had a lower mean than those without in each treatment type, supporting our hypothesis ($p < 0.05$; **Table 3**).

Additional ANCOVA analyses were performed for the phosphorous and nitrogen readings. When analyzing all treatments with and without activated carbon, most variables had a significant effect on nitrogen readings, in which samples with activated carbon had lower nitrogen levels than those without ($p < 0.05$) (**Figure 3**). Exceptions to this were the combined effects of "activated carbon*treatment type" and "time*activated carbon*treatment type" (**Figure 3, Table 4**). Similarly, most variables had a significant effect on phosphorous readings ($p < 0.05$) with the exception of combined effects for "time*activated carbon" and "treatment type*activated carbon" ($p = 0.6037$ and $p = 0.2344$ respectively; **Figure 3, Table 5**). Phosphorous readings were significantly different between samples with and without activated carbon for the conditions of 0 mg/L treatment with effect of "day" ($p = 0.0279$), 20 mg/L treatment with effect of "day" ($p = 0.0001$), and 50 mg/L treatment with effect of "day" ($p = 0.0295$).

Discussion

Our hypothesis that the growth of algal blooms will be impeded by the addition of activated carbon was supported by our findings. The addition of activated carbon reduces the growth of *C. vulgaris* through, as we have interpreted from our results, the reduction of available nutrients, notably phosphorous and nitrogen (Dempster et al. 2012; Tang et al. 2016).

Based upon the experiment's findings and the findings in the current scientific literature, it is evident that biochar would likely be capable of reducing levels of

nitrogen and phosphorous in aqueous solutions (Beck et al. 2011; **Supplementary Table 2, Supplementary Table 3**). The algal growth rates differed between treatments with and without activated carbon, with the algae growing at a slower rate in treatments containing activated carbon (**Figure 2**). This follows our hypothesis, that algal growth would be limited by the presence of activated carbon.

The phosphorous and nitrogen readings, excluding irregular results due to equipment failure, showed that the presence of activated carbon impacted the levels of these nutrients, though individual treatment analyses were not statistically significant for all variables. Firstly, while the level of nitrogen unexpectedly increased over the course of the experiment, treatments with activated carbon contained lower levels of nitrogen than those without (**Figure 3**). While it appears that the nitrogen levels increased, it is possible that the presence of algae skewed the results of the nitrogen tests. Algae, whose dry mass has been shown to be composed of 10% nitrogen (Ferrera-Lorenzo et al. 2013), releases nitrogen as it decomposes, and so may have resulted in an increase in the recorded nitrogen levels as algal cells began to die off. Through preventing the growth of algae, the presence of activated carbon indirectly resulted in a lower level of nitrogen due to the reduced amount of algae available for decomposition when compared to solutions without its presence. More studies should be conducted to further confirm this effect. This is observable in **Figure 3**, which demonstrates a lower increase in nitrogen levels in treatments containing activated carbon compared to those without. Further, the ANCOVA shows that all data for the effects of variables and their combinations on the nitrogen readings were significant ($p < 0.05$) when all treatment types were considered, excluding the combined effects of “activated carbon*treatment type” and “time*activated carbon*treatment type” (**Table 4**). Interestingly, significance was not found when considering each treatment type individually and the effects of variables “activated carbon*day”, “activated carbon”, and “day” (**Supplementary Figure 1**).

Secondly, the phosphorus readings were also influenced by the presence of activated carbon. Though the phosphorous readings decreased in solutions with and without activated carbon - excluding treatments 0 mg/L with and without activated carbon and 5 mg/L with activated carbon - the solutions with activated carbon had a greater decrease than those without (**Supplementary Figure 2**). The ANCOVA shows all data for the effects of variables and their combinations on the phosphorous readings were significant ($p < 0.05$), except for the combined effects of “time*activated carbon” and “activated carbon*treatment type” (**Table 4**). Interestingly, when the treatment types were analyzed in isolation, only the “day” variable was significant

for treatment type 0 mg/L, 20 mg/L, and 50 mg/L (**Supplementary Figure 2**).

The nitrogen and phosphorous tests compared the solution to a chemical indicator colour chart by eye, and so human error along with algal colour interference may account for these results, particularly the inclining slopes of best fit for nitrogen readings (**Figure 3**). The colour of the solutions was affected by the presence of the algae, which gave the solutions a light green hue. Human error may have occurred due to the solutions being similar in colour to the phosphorous colour chart, which used shades of blue and green, potentially skewing the results towards higher phosphorous levels. The API nutrient tests are intended to be used to roughly estimate nutrient levels in home aquariums, and so while they provided a valuable dimension to our experiment, the results we gathered using them should be taken with some scepticism. Using more sophisticated equipment for measuring nitrogen and phosphorous would provide a better gradient of nutrient levels over the course of the experiment, and therefore more accurate measurements of adsorption by the activated carbon. Further, when performing analyses for each treatment individually, five replicates per treatment may have been too small a sample size to accurately show potential changes in phosphorous or nitrogen quantities within the samples. Future studies should use larger sample sizes along with more sensitive nutrient level reading equipment to determine if these results are accurate, or if they may have been due to other factors such as human error. More studies should be conducted to further confirm the adsorptive effects of activated carbon on nitrogen and phosphorous.

Recent studies suggest that rather than reductions of phosphorous alone, simultaneous reductions of both nitrogen and phosphorous are more successful at reducing anthropogenic eutrophication within inland lakes (Conley et al. 2009; Paerl 2009; Lewis 2011). Phosphorous is the largest limiting factor of algal blooms, however if phosphorous concentrations are low, nitrogen can become the limiting agent (Conley et al. 2009; Paerl 2009). Procedures have been implemented to reduce phosphorous levels in hyper-eutrophic lakes, such as reducing water runoff from agricultural practices and treating wastewater prior to discharge (Schindler and Vallentyne 2008). Unfortunately, these proactive measures do not effectively reduce the impacts of nitrogen and phosphorous already present in lakes (Liu and Qui 2007). Developing novel, more efficient methods of removing excess phosphorous and nitrogen can benefit the conservation and rehabilitation of lake ecosystems. Our study demonstrates that activated carbon has the capacity to be used for the adsorption of phosphorous, presenting positive implications for the use of biochar to remedy anthropogenic eutrophication.

Biochar is similar to activated carbon, but has been shown to be more effective at adsorbing nutrients in both soil and aqueous solutions (Berger 2012; Morales et al. 2013; Huggins et al. 2016). This suggests that biochar could be more effective than activated carbon for reducing levels of anthropogenic nutrients in inland lakes, and therefore, a better management tool for conserving lake ecosystems. From our findings, we can speculate that biochar would be more effective at adsorbing phosphorous than activated carbon, and so future studies should focus on investigating the potential for biochar to reduce the growth of algae. Future studies should also conduct the study for a longer period to ensure the algae populations within each sample condition reaches its maximum abundance. Our graph readings of fluorescence did not reach asymptotic conditions, which is indicative of an organism reaching carrying capacity (Parkhurst 2006). This would enable a comparison not only of the rate of algal growth occurring as a result of the addition of biochar, but also the maximum abundance in each condition as well.

Alternative methods could be used in future studies, including the use of different algae species and treatment conditions. For instance, the effect of zooplankton on the population densities of algae may aid in understanding the impacts of biochar in a more natural environment. Further studies could consider the use of biochar in natural conditions, such as aquatic basin ecosystems, and how they react to the presence of activated carbon or biochar. The possible impacts that the addition of biochar may have upon an environment, such as changes to substrate carbon levels and levels of waterborne nutrients other than phosphorous and nitrogen, also need to be explored.

In conclusion, our study highlights the adsorptive capabilities of activated carbon, as well as the interactions it has with *C. vulgaris* algae. This carries implications for the future use of activated carbon, and similarly biochar, as a means of reducing anthropogenic eutrophication and its larger impact on aqueous environments.

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Figures and Tables

Figure 1. Boxplots Displaying Differences in Algae Fluorescence Readings. Boxplots of fluorescence values demonstrate algal concentrations over time in the solutions, with the initial algal concentration having been standardized at the beginning of the experiment. This figure demonstrates the correlation between fluorescence readings and the treatment type (phosphorous mg/L) and presence of activated carbon. The absence of activated carbon is denoted by a 0, while the presence of activated carbon is denoted by 1. The graph excludes data collected on October 30th, and data collected from treatments containing 50 mg/L of phosphorous. The figure refers to nitrogen and phosphorous information from table 2.

Figure 2. Display of Beakers Showing Colour Differential from October 21st (left) and November 1st (right). Treatments on the top row contain activated carbon, and treatments on the bottom do not. From left to right: treatments containing 0 mg/L of phosphorous (control), 5 mg/L of phosphorous, 20 mg/L of phosphorous, and 50 mg/L of phosphorous.

Figure 3. Scatterplots Based on Nitrogen and Phosphorous Values for Treatment Types with and without Activated Carbon. Scatterplots graphing the nitrogen and phosphorous readings over time for both the presence and absence of activated carbon. API Aquarium nitrogen and phosphorus tests were used to quantify values of these elements in the solutions (Aquarium Pharmaceutical Incorporated: Nebraska, U.S). Lines of best fit traversing the data values provide an estimate of the change in nitrogen and phosphorous readings over the collection period. The figure refers to nitrogen and phosphorous data isolating treatment types, compared to Table 4 and 5 which combine all treatment types with the analyses. Results conveyed by the scatterplots are expanded upon within the supplementary information.

Table 1. ANCOVA Summary Table of Fluorescence Values. The table references data presented in Figure 2. Table outlining significance of main and interactive effects of the treatment conditions, data collection days, and presence or absence of activated carbon on the fluorescence readings. The “*” is used to represent interactions between the variables while “DF” denotes the degrees of freedom for each analysis of covariance. The table excludes data collected on October 30th.

Source	DF	Sum of Squares	F Ratio	Prob > F
Activated Carbon	1	0.5346632	110.398	<0.0001*
Treatment (mg/L)	1	2.4326984	502.3068	<0.0001*
Time (Day)	1	4.8050256	992.1482	<0.0001*
Time*Activated Carbon	1	0.0783251	16.1727	<0.0001*
Treatment*Activated Carbon	1	0.1876575	39.8956	<0.0001*
Time*Treatment*Activated Carbon	1	0.0893151	18.4419	<0.0001*

Table 2. ANCOVA Summary Table of Fluorescence Values, Excluding Those from October 30th and the 50 mg/L phosphorous-Containing Treatments. The table references data in Figure 2. Table outlining significance of main and interactive effects of the treatment conditions, data collection days, and presence or absence of activated carbon on the fluorescence readings. The “*” is used to represent interactions between the variables while “DF” denotes the degrees of freedom for each analysis of covariance. The graph excludes data collected on October 30th, and data collected from treatments containing 50 mg/L of phosphorous.

Source	DF	Sum of Squares	F Ratio	Prob > F
Activated Carbon	1	0.7276080	783.2511	<0.0001*
Treatment (mg/L)	1	5.0284369	2706.491	<0.0001*
Time (Day)	1	6.0835479	6548.781	<0.0001*
Time*Activated Carbon	1	0.1536958	165.4496	<0.0001*
Treatment*Activated Carbon	1	0.6366330	342.6594	<0.0001*
Time*Treatment*Activated Carbon	1	0.1393236	74.9891	<0.0001*

Table 3. Tukey's HSD Test for Presence of Activated Carbon and Treatment Type. The table refers to information in Figure 2 and Table 2. Tukey's HSD test is a post-hoc test for the ANCOVA which excludes the raw data from October 30th and treatments containing 50 mg/L of phosphorous. The Tukey's HSD test demonstrates a positive confidence quantile between samples with compared to without activated carbon, concluding there is significant difference between data sets. The α -value of 0.05 was used to determine significant differences.

For Activated Carbon		
Measurement	Source	Value
Confidence Quantile		1.96237
Ordered Differences Report (p-value)	Y and N activated carbon	6.0835479
For Treatment Type		
Measurement	Source	Tukey's HSD Value
Confidence Quantile		1.96237
Ordered Differences Report (p-value)	Treatment 0 mg/L by Treatment 20 mg/L Treatment 0 mg/L by Treatment 5 mg/L Treatment 5 mg/L by Treatment 20 mg/L	< 0.0001* < 0.0001* < 0.0001*

Table 4. ANCOVA Summary Table of Nitrogen Readings Values, Excluding Those from October 30th and the 50 mg/L phosphorous-Containing Treatments. The table refers to information from Figure 3. The table demonstrates significance for variables (treatments, days, and activated carbon presence or absence) and their combinations. The "*" is used to represent interactions between the variables while "DF" denotes the degrees of freedom for each analysis of covariance. All data were significant ($p < 0.05$) except for the combined effects of "activated carbon*treatment type" and "time*activated carbon*treatment type."

Source	DF	Sum of Squares	F Ratio	Prob > F
Activated Carbon	1	1558.8021	8.6539	<0.0040*
Treatment (mg/L)	1	7957.1007	44.1751	<0.0001*
Time (Day)	1	2880.0000	15.9888	0.0001*
Time*Activated Carbon	1	845.0000	4.6911	<0.0324*
Treatment*Activated Carbon	1	160.3706	0.8903	<0.3474
Time*Treatment*Activated Carbon	1	38.0453	0.2112	<0.6467

Table 5. ANCOVA Summary Table of Phosphorous Readings Values, Excluding Those from October 30th and the 50 mg/L phosphorous-Containing Treatments. The table refers to information from Figure 3. The table demonstrates significance for variables and their combinations. The “*” is used to represent interactions between the variables while “DF” denotes the degrees of freedom for each analysis of covariance. All data were significant ($p < 0.05$) except for the combined effects of “time*activated carbon” and “treatment type*activated carbon.”

Source	DF	Sum of Squares	F Ratio	Prob > F
Activated Carbon	1	0.00003063	8.1974	0.0050*
Treatment (mg/L)	1	0.00077828	208.2977	<0.0001*
Time (Day)	1	0.00039383	105.4042	0.0001*
Time*Activated Carbon	1	0.00000101	0.2710	0.6037
Treatment*Activated Carbon	1	0.00000582	23.6805	0.2144
Time*Treatment*Activated Carbon	1	0.00001562	4.1798	0.0433*

Supplementary Information

Figure 1. Scatterplots Based on Nitrogen Values for Treatment Types with and without Activated Carbon. Scatterplots graphing the nitrogen readings over time for both the presence and absence of activated carbon. API Aquarium nitrogen tests were used to quantify values of nitrogen in the solutions (Aquarium Pharmaceutical Incorporated: Nebraska, U.S). Lines of best fit traversing the data values provide an estimate of the change in nitrogen readings over the collection period. The figure refers to nitrogen data isolating treatment types, compared to Table 4 of the main text, which combines all treatment types with the analyses.

0 mg/L Treatment Type with Activated Carbon:
Comparing Phosphorous Readings and Day

0 mg/L Treatment Type without Activated Carbon:
Comparing Phosphorous Readings and Day

5 mg/L Treatment Type with Activated Carbon:
Comparing Phosphorous Readings and Day

5 mg/L Treatment Type without Activated Carbon:
Comparing Phosphorous Readings and Day

Figure 2. Scatterplots Based on Phosphorous Values for Treatment Types with and without Activated Carbon. Scatterplots graphing phosphorous readings over time for both the presence and absence of activated carbon. API Aquarium phosphorus tests were used to quantify values of this element in the solutions (Aquarium Pharmaceutical Incorporated: Nebraska, U.S). Lines of best fit traversing the data values provide an estimate of the change in phosphorous readings over the collection period. The figure refers to phosphorous data isolating treatment types, compared to Table5 of the main text, which combine all treatment types within the analyses.

Table 1. ANCOVA of Initial Raw Data Values. The table outlines significance of variables and interactions between them on the fluorescence readings. The analysis includes all raw data, including those from October 30th and the 50 mg/L phosphorous treatment which were excluded in other analyses of covariance. The “*” is used to represent interactions between the variables while “DF” denotes the degrees of freedom for each analysis of covariance. The “time*biochar” analysis demonstrates less significance, likely due to the error from the October 30th data.

Source	DF	Sum of Squares	F Ratio	Prob > F
Activated Carbon	1	0.4574166	81.9863	<0.0001*
Treatment (mg/L)	1	5.5238264	330.0260	<0.0001*
Time (Day)	1	1.7293554	309.9657	<0.0001*
Time*Activated Carbon	1	0.0392870	7.0419	0.0001*
Treatment*Activated Carbon	1	0.9103578	54.3902	<0.0001*
Time*Treatment*Activated Carbon	1	0.1638753	9.7909	<0.0001*

Table 2. Meta-Analysis Raw Data. A raw data table of the information used in the meta-analysis of studies researching phosphorous sorption by biochar or activated carbon in aqueous solutions. The effect size was found from these studies as the phosphorous sorption ($\text{mg}\cdot\text{kg}^{-1}$) over time (days).

Study	Study #	Environment	Sample Size	Effect Size	Direction of Effect	Study Size
Dalahmeh	6	Aqueous	2	0.0338	neutral	0.0676
Fang et al.	7	Aqueous	9	15.14	positive	136.26
Fang et al.	7	Aqueous	9	15.24	positive	137.16
Fang et al.	7	Aqueous	9	15.48	positive	139.32
Fang et al.	7	Aqueous	9	15.98	positive	143.82
Fang et al.	7	Aqueous	9	16.08	positive	144.72
Fang et al.	7	Aqueous	9	16.5	positive	148.5

Table 3. Meta-Analysis Raw Data. A raw data table of the information used in the meta-analysis of studies researching phosphorous sorption by biochar or activated carbon in both aqueous solutions and soil mixtures. The effect size was found from these studies as the phosphorous adsorption ($\text{mg} \cdot \text{kg}^{-1}$) over the equilibrium solution ($\text{mg} \cdot \text{L}^{-1}$).

Study	Study #	Environment	Sample Size	Effect Size	Direction of Effect	Study Size
Morales et al.	1	Soil	4	51.43	positive	205.72
Morales et al.	1	Soil	4	14.26	positive	57.04
Morales et al.	1	Soil	4	1.13	positive	4.52
Morales et al.	1	Soil	4	10.55	positive	42.2
Morales et al.	1	Soil	4	10.37	positive	41.48
Morales et al.	1	Soil	4	1	positive	4
Morales et al.	1	Soil	4	19.88	positive	79.52
Morales et al.	1	Soil	4	19.82	positive	79.28
Morales et al.	1	Soil	4	2.07	positive	8.28
Morales et al.	1	Soil	12	12.12	positive	145.44
Xu et al.	2	Soil	8	16.575	positive	132.6
Xu et al.	2	Soil	8	30.5	positive	244
Xu et al.	2	Soil	8	86.675	positive	693.4
Chintala et al.	3	Soil	7	100	positive	700
Chintala et al.	3	Soil	7	14.4	positive	100.8
Chintala et al.	3	Soil	7	86.36	positive	604.52
Soinne et al.	4	Soil	5	0.903	neutral	4.514
Soinne et al.	4	Soil	5	1.239	neutral	6.196
Soinne et al.	4	Soil	5	0.844	neutral	4.219
Chen et al.	5	Aqueous	14	1.28	positive	17.92
Chen et al.	5	Aqueous	14	1.60	positive	2240
Chen et al.	5	Aqueous	14	224	positive	3136
Chen et al.	5	Aqueous	14	8.37	positive	117.18
Chen et al.	5	Aqueous	14	460	positive	6440