8-2022

Allelopathic Effect of Cereal Straw Extracts on Growth of Raphidocelis subcapitata and Microcystis aeruginosa

Holly Wren
University of Arkansas, Fayetteville

Allelopathic Effect of Cereal Straw Extracts on Growth of *Raphidocelis subcapitata* and *Microcystis aeruginosa*

A thesis submitted in partial fulfillment
of the requirements for the degree of
Master of Science in Crop, Soil, and Environmental Sciences

by

Holly Wren
University of Arkansas
Bachelor of Science in Environmental, Soil, and Water Science, 2018

August 2022
University of Arkansas

This thesis is approved for recommendation to the Graduate Council.

Mary C. Savin, Ph.D.
Thesis Director

Brian Haggard, Ph.D.
Committee Member

Wen Zhang, Ph.D.
Committee Member

Amanda Ashworth, Ph.D.
Committee Member

ABSTRACT

Harmful algal blooms are increasing in size, duration, and intensity around the globe. For several decades, cereal straws have been recognized as a viable algal control method, though the mechanisms by which cereal straws inhibit algae remain a topic of research. The objectives of this study were to evaluate the effectiveness of decomposing cereal straw extract, particularly rice (*Oryza sativa)* and barley (*Hordeum vulgare*) straw extract, to inhibit the green alga *Raphidocelis subcapitata* or cyanobacteria *Microcystis aeruginosa* in bioassays and to determine if the effectiveness of decomposing rice straw extract is altered by the presence of natural organic matter or microbial communities in pond water. In rice and barley straw extracts of the same concentration, total phenolic and flavonoid concentrations were also similar; however, rice straw was more effective than barley straw at inhibiting the growth of both *R. subcapitata* and *M. aeruginosa*. Combined with a lack of significant changes in H_2O_2 between cereal straw treatments, results support the hypothesis that algal inhibition cannot be attributed to H_2O_2 alone but depends on the release of specific phenolic and polyphenolic compounds from decomposing cereal straw. In media-based assays, both *M. aeruginosa* and *R. subcapitata* were inhibited by 5.0 g/L rice straw extract ($P<0.05$). In pond water bioassays, 10 g/L rice straw extract showed the greatest inhibitory effect in cultures with and without bacteria. *M. aeruginosa* showed signs of recovery 15 days following treatment with 2.5 g/L in media and 5.0 g/L rice straw extract in pond water. For reliable long-term suppression, greater concentrations of rice straw are necessary, possibly due to the instability of allelochemicals. In pond water, variability in responses between the controls (with and without bacteria) and 5.0 g/L treatments with bacteria versus without bacteria show that the microbial community within the context of abiotic conditions of natural waters (pH, nutrient concentrations, sediment, and temperature, etc.) is important in determining bloom behavior and treatment response. Though rice straw was an

effective method for the control of *M. aeruginosa* and *R. subcapitata*, complex interactions between the algae, the microbial community, and abiotic factors found in natural waters make the determination the of the inhibitory mechanism a challenge.

ACKNOWLEDGEMENTS

First, I would like to thank Dr. Mary Savin for her patience, guidance, and teaching throughout my academic career at the University of Arkansas. I would also like to thank Dr. Brian Haggard and Dr. Brad Austin for their help and discussion with methods, letting me use their lab equipment, and for undertaking autoclave maintenance. Thank you to Nathaniel Elliot who assisted with culture isolation and maintenance and who provided the grow lights and shelves with which these experiments were conducted and thank you to the members of the microbial ecology lab for your positivity and technical support. Lastly, I would like to thank my partner, Ian Dexter, who delivered countless homecooked meals to the lab without complaint and who provided endless encouragement, support, and bad jokes.

DEDICATION

This thesis is dedicated to my parents Keith and Amy Wren. I admire and appreciate you both for your incredibly hard work, for encouraging me to explore my interests, and for teaching me the value of patience and persistence.

TABLE OF CONTENTS

LIST OF FIGURES

CHAPTER 2

LIST OF TABLES

CHAPTER 2

CHAPTER 1. INTRODUCTION AND LITERATURE REVIEW

Cyanobacteria

Cyanobacteria (blue-green algae) are some of the Earth's oldest photoautotrophic prokaryotes with evidence of their presence as early as 2.5 to 2.7 million years ago (Mya) (Summons et al., 1999; Brocks et al. 1999). Over the evolutionary history of Earth, cyanobacteria have been exposed and adapted to a large range of eco-physiological stresses, allowing the prokaryotes to proliferate even in extreme conditions (Paerl, 2014). Having evolved in nitrogen deficient environments, certain cyanobacterial genera have adapted with an ability to fix dinitrogen (N_2). As a result, cyanobacteria can dominate aquatic ecosystems. Some cyanobacteria also form harmful algal blooms (CyanoHABs), particularly in anthropogenically modified waterbodies (Paerl and Otten, 2013).

Nutrient inputs

Historically, low nitrogen-to-phosphorus ratios (N:P) were used as indicators of CyanoHAB risk. More recent studies suggest that total nutrient (N and P) concentrations or existing biomass are better determinants of CyanoHAB formation (Downing et al., 2001), though complex nutrient cycling, differing nutrient species, and environmental conditions within systems make thresholds difficult to determine (Gilbert, 2017).

Watson et al. (1992) and Downing et al. (2001) discovered that total P can predict an aquatic system's total algal biomass with cyanobacteria assuming larger proportions, indicating greater bloom risk, as total P increases. Total P has since been regarded as the key limiting nutrient for cyanobacteria in freshwater systems. Support for this hypothesis has been demonstrated through long-term nutrient reduction studies. These long-term studies have shown that in the absence of

inorganic nitrogen loads, P alone is enough to sustain cyanobacterial populations (Schindler et al., 2008).

As a result, implementation of total maximum daily loads (TMDL) for P in aquatic systems has been the primary large-scale control method for CyanoHABs (Schindler et al., 2008), with minimal emphasis placed on N reduction. However, high levels of N can also stimulate CyanoHAB formation amongst non-N-fixing genera, along the freshwater-to-saltwater continuum, or in aquatic systems with sufficient indigenous P (Paerl & Otten, 2013; Paerl & Huisman, 2009; Paerl & Piehler, 2008). Loose regulation of N is especially problematic as N inputs are disproportionately increasing through expansion of fertilizer use, wastewaters, and groundwater and stormwater discharge (Gilbert, 2017).

The effects of increased nutrient loads are compounded by certain ecosystems' climatically altered sensitivities to nutrient inputs (Rabalais, 2002; Porter et al., 2013). Nutrient limitations can vary spatially and temporally with co-limitation occurring in many systems (Conley et al., 2009). Consequently, the most effective way to reduce the risk of CyanoHABs along the freshwater-to-saltwater continuum will be through reduction of both N and P inputs (Paerl, 2009; Elmgren & Larsson, 2001).

Effect of climate on CyanoHABs

The ability of cyanobacteria to dominate aquatic ecosystems is exacerbated further by climatically altered global mean precipitation and temperature trends as well as low flow rates due to abstraction or drought (Paerl & Huisman, 2009; Johnk et al., 2008). The projected increase in extreme precipitation events will likely result in large nutrient load depositions into

aquatic systems, where seasonal or environmental conditions influencing flow rates and water residence times may favor CyanoHAB formation.

Rising temperatures consistent with climate change trends can heat surface waters above optimal conditions for eukaryotic organisms and allow for vertical stratification of the water column. Intensified stratification enables buoyant cyanobacteria to proliferate at the water's surface and reduces the light and oxygen available to benthic communities (Huisman et al., 2004).

Impact of eutrophication on environment and ecology

Eutrophic and hypereutrophic waterbodies prone to CyanoHABs are likely to suffer from poor water quality. In eutrophic freshwater systems, excess macronutrients stimulate primary production with cyanobacteria and other phytoplankton comprising the majority of biomass (Perez-Ruzafa et al., 2019; Gilbert et al., 2017; Smith, 2003). Abundant cyanobacteria induce turbid conditions, reducing light available to other photoautotrophic organisms.

As the CyanoHABs senesce, decomposition and respiration by microbes creates or exacerbates hypoxia and results in the release of phosphorus from sediment (Paerl & Otten, 2013; Watson et al., 2016). Combined, turbidity and hypoxia can affect the overall distribution, abundance, diversity, and physiological state of benthic communities (Meyer-Reil & Köster, 2000; Zeng et al., 2019; Petkuviene at al., 2016).

The disruptions to nutrient cycling through bloom development can have complex and long-lasting effects on aquatic ecosystems. Due to the stoichiometric plasticity of primary producers, the altered availability of nutrients can change the elemental composition of algae, reducing the quality of algae as a food source. When consumers are deterred from a food source

(algae) by way of reduced quality or toxin production, imbalances occur within the ecosystem's food web, potentially affecting biodiversity or community ecology through bottom-up interactions. These imbalances may favor opportunistic cyanobacterial growth or allow the invasion of non-native species (Gilbert, 2017).

Cyanotoxins in the environment

Mass fish and animal kills are one of the most visible signs a community's food web has been altered by hypoxic conditions or through the release of toxic compounds. Many CyanoHABs release secondary metabolites collectively referred to as cyanotoxins. Cyanotoxins are observed in nearly half of all blooms throughout the world, though the conditions upon which these toxins are released are not yet understood.

Microcystins comprise the most prevalent and studied group of cyanotoxins and are produced by several cyanobacteria genera including *Anabaena, Hapalosiphon, Nostoc, Phormidium, Planktothrix, and Microcystis* spp., though certain strains within species have lost the toxin production gene (Sivonen, 2009). Microcystins and closely related nodularins are protein phosphatase enzyme inhibitors and can induce necrosis or hemorrhaging in the liver and death upon exposure. Microcystin-LR (MC-LR) and Microcystin-LA (MC-LA), named for the amino acids at positions 2 and 4 along the toxins' cyclic structure, are the most toxic of the group with an LD50 between 50 and 1000 µg/kg (Laskin et al., 2009).

In addition to acute exposures to microcystins, humans are exposed to these compounds over time through ingestion of contaminated domestic and recreational water supplies, seafood, or dietary supplements. Microcystins have even been found in vegetables irrigated with contaminated water supplies (Mohamed & Al Shehri, 2009), leading to the development of

chronic health concerns in humans. The Working Group for the International Agency for Research on Cancer in 2006 found evidence supporting the presence of tumor promoting mechanisms of MC-LR and recognized the compound as a potential carcinogen to humans. The World Health Organization has since set maximum concentrations for microcystin at 1 µg/L in drinking water and between 4 and 20 µg/L in recreational waters (Grosse et al., 2006; Hardy, 2008).

Cyanotoxins are regularly linked to mass fish and animal mortality events and have prominent impacts on both aquatic and terrestrial communities. In Doñana National Park, a wildlife refuge and special protection area in Spain, a 2004 CyanoHAB led to the demise of thousands of fish and over 6000 birds, including the endangered marbled teal (Lopez-Rodas et al., 2008). This mass mortality event occurred in the wake of one of the worst environmental disasters in European history after 5 million cubic meters of acidic mining waste decimated the park in 1998 (Pain et al., 1998). In Kruger National Park, South Africa, a dam harboring toxic CyanoHABs was drained following the death of 33 wildebeests, 19 zebra, 16 white rhinoceroses, two lions, and two cheetahs out of concern for the affected endangered species (Bengis et al., 2016).

Though effective in Kruger National Park, the removal of eutrophic water is not practical or possible in many aquatic systems. Without systemic reduction of nutrients into waterbodies, the number, duration, and pervasiveness of CyanoHABs will continue to rise, increasing the risk of ecologically and environmentally devastating outcomes.

Methods to control CyanoHABs

The risks and deleterious health effects associated with CyanoHABs are of justifiable concern as understanding of algal blooms develop. In conjunction with the risks to human, environmental, and ecological health, harmful algal blooms can also cause undesirable odors and interfere with recreational or aesthetic activities. CyanoHABs can be minimized through the reduction of nutrient inputs or the removal of low-flow conditions or impoundments. Where these measures are not feasible, physical, chemical, or biological methods must be employed to reduce CyanoHAB formation and sustain ecosystem health.

Physical control methods

Physical control methods involve mechanically removing and killing algae throughout the water column or limiting the extent a bloom may inhabit (Kidwell, 2015). Though many physical control methods exist, the physical methods deemed viable for the National Oceanic Atmospheric Administration's (NOAA) harmful algal bloom prevention program include flocculation, sediment burial and removal, cell harvesting and removal, and water column mixing (Kidwell, 2015). While physical control of algal populations can be effective, these methods often require routine maintenance and quickly become cost inhibitive. Physical control methods may also negatively impact benthic communities (Yu et al., 2017; Pan et al., 2012; Kidwell, 2015; Meyer-Reil and Koster, 2000)

Flocculation and coagulation

Flocculants such as clay applied to the water body form aggregates, known as flocs, through collisions with algae and phosphorus. As the flocs grow through repetitive collisions, they precipitate out of the photic zone, resulting in the death of photosynthetic algae. The

accumulation of flocs (flocculation capping) often prevents the rerelease of phosphorus from sediment.

Though montmorillonite was once regarded as best, this clay possesses low flocculating efficiency and requires applications of 110-400 t/km² (Shirota, 1989) for algal control. In 1994, Yu et al. (a, b) found that when compared to three-layer (-Si-Al-Si-) montmorillonites, two layer (-Al-Si-) kaolinites had greater flocculating efficiency with CyanoHAB cells due to lesser surface negative charge and greater Van der Waals forces.

Using this logic, clays are often modified to increase the flocculating efficiency. Clay modifiers change negative surface charges to positive, allowing for greater electrostatic attraction between clay and CyanoHAB cells. Common inorganic modifiers include polyaluminum chloride (PAC), aluminum chloride, and aluminum sulfate and show greater stability in seawater when compared to organic modifiers such as starch or chitosan (Yu et al., 2017).

The flocculating efficiency of a clay also depends on the targeted species. Nonmotile phytoplankton are more easily controlled by clay application than flagellated species, which may require the application of an additional oxidant. Following these discoveries, modified clays now constitute the primary HAB mitigation method in China with a required dosage of 4-10 t/km² (Yu et al., 2017) for algal control.

In addition to removing algal biomass, modified clays adsorb phosphorus in the water column and at the water-sediment interface through the creation of flocculation caps. The adsorption of phosphorus reduces the risk of secondary eutrophication stimulated by nutrients released from algal detritus, diffused from anoxic sediments, or through resuspension (Pan et al., 2012; Lu et al., 2016; Yu et al., 2017).

In some circumstances, resuspension of sediment may serve as a natural flocculant or to translocate dormant, thick-celled algae (algal cysts) into anoxic sediments, thereby preventing secondary blooms (Kidwell, 2015). However, resuspension of sediment stimulated by benthic shear stress, waves, wind, or anthropogenic disturbances may serve to release flocs, nutrients, or toxins into the water column, stimulating secondary eutrophication or blooms. When selecting a modified clay, the benthic environment, flow conditions, and algal variant should be taken into consideration to reduce resuspension of phosphorus or algal cells from flocculant caps (Pan et al., 2012; Beaulieu et al., 2005). Despite known environmental and benthic effects, modified clays remain a popular method for algal control.

Sediment burial and removal

In aquatic systems where sediments are laden with high concentrations of algal cysts or phosphorus, burial and removal of the sediment may be a beneficial management option. If resuspension of flocculated material and the release of phosphorus from sediment is a concern, uncontaminated allochthonous or autochthonous material may be placed over the existing sediment. Sediments harboring algal cysts or elevated phosphorus concentrations may also be dredged and disposed of ex-situ.

Cell harvesting and removal

The harvesting of algal cells removes algae and the nutrients contained therein, reducing the extent of blooms and the nutrients that are redelivered to the environment upon bloom decay. Harvesting works in conjunction with flotation or sedimentation. Flocculated particles in a water body are subjected to microbubbles formed by supersaturating water with air and delivered through high-pressure nozzles. The microbubbles attach to the flocculates and bring them to the surface for harvest (Pandhal et al., 2017).

Devices such as the fluidic oscillator are under development as an energetically favorable alternative for use in the natural environment. Pandhal et al. (2017) demonstrated the ability of this method to reduce cyanobacterial presence, allowing for reestablishment of a diverse microbial community. Upon removal, harvested biomass can then be used as fertilizers, in biomethane production, or refined and converted to biodiesel, industrial chemicals, or feed.

Water column mixing

Oxygen depletion in aquatic systems (hypoxia) may be induced or exacerbated by stratification (Visser et al., 2015). Phosphate-iron binding is inefficient in the absence of oxygen and can result in the release of phosphate from sediment under hypoxic conditions. During hypolimnetic oxygenation, oxygen is introduced to the hypolimnion, the layer below the thermocline. Oxygenation of the hypolimnion maintains stratification while reversing bottom-water hypoxia and minimizing the release of phosphate from sediment.

To eradicate stratification, artificial mixing of the water column is required. As the water column is mixed, buoyant, photosynthetic cyanobacteria no longer possess a competitive advantage over green algae and diatoms. This allows for the reestablishment of beneficial phytoplankton communities. Sustained cyanobacterial suppression through water column mixing is difficult to achieve as success is dependent upon mixing rates, depth of mixing and light availability, the complexity of the bathymetry, and the extent of induced turbulence (Visser et al., 2015).

Chemical control methods

Unlike physical removal methods, chemical control of algae works by interfering with cellular growth. Of the chemical control methods available, those deemed viable for use in

NOAA's proposed action plan include the use of native macroalgae and isolates, biosurfactants, copper, silica, isolated algicidal compounds, hydrogen peroxide, and barley straw (Kidwell, 2015). The efficacy or dosage of chemical control methods are often dependent on variables such as pH, light, temperature, nutrient concentrations, and water chemistry.

Native macroalgae and isolated algicidal compounds

Macroalgae such as *Spirogyra* spp., *Cladophora* spp., *Corallina* spp., and *Ulva* spp., have been known to limit CyanoHAB formation through nutrient competition or through release of allelopathic chemicals (allelochemicals) (Trochine et al., 2010; Jeong et al., 2000; Lu et al., 2011; Nan et al., 2008; Wang et al., 2007). Allelochemicals are naturally occurring chemicals produced by an organism that are either harmful or beneficial to the affected species and may be algistatic, inhibiting the growth of algae, or algicidal, resulting in the death of the targeted species.

The allelochemicals derived as secondary metabolites from macroalgae are increasingly considered for their inhibitory properties, especially for control of red tides in marine environments, and include monoterpenes, phenylpropanoids, and polyphenols, among others (Sun et al., 2019). Though isolates maintain allelopathic properties, the difficult identification and isolation of the compounds as well as rapid degradation encourage the use of the whole macroalgae specimen for sustained control (Sun et al., 2019; Kidwell, 2015).

Biosurfactants

Biosurfactants are amphiphilic, surface-active compounds released by fungi, bacteria, and yeasts. These compounds reduce surface tension, allowing for greater interaction between opposing phases and alteration of cell surfaces. As such, biosurfactants are often antimicrobial, antiviral, or algicidal. These compounds are of growing interest as they are environmentally

favorable, less toxic, and more biodegradable relative to their synthetic counterparts (Pacwa-Plociniczak et al., 2011).

Sophorolipid, a glycolipid biosurfactant derived in high yield from yeast *Candida bombicola,* is of developing interest due to its superior algicidal capabilities and rapid biodegradation half-life of less than 24 hours (Baek et al., 2003; Sun et al., 2010). Baek et al. (2003) found that sophorolipid concentrations of 5-20 mg/L are sufficient for CyanoHAB inhibition without great risk to non-target organisms.

Other bacteria in genera *Cryptophaga, Saprospira, Psuedoalteromonas,* and *Alteromonas* have also shown algicidal effects, with strains of *Bacillus* suppressing cyanobacteria *M. aeruginosa, Nostoc* spp., *and Anabaena* spp., through the release of bacilysin (Wu et al., 2014). Though biosurfactants have shown promising algicidal effects in laboratory settings, more research is needed regarding the ecological responses to each compound at concentrations required for in-situ CyanoHAB suppression.

Copper

Copper sulfate has been used as an effective and affordable algicide for decades. Copper sulfate controls algae and macrophytic vegetation in surface waters through alteration of photosynthetic pathways. Despite the efficacy of the compound, doses required for algal control are often ten to 100 times the lethal doses for aquatic organisms, promoting the death of many non-target species (Salam & El-Fadel. 2008).

Copper ion found in concentrations greater than 0.16 mg/L can result in lysis of *Microcystis spp.* (Wu et al., 2017). Microcystins released upon cell rupture are not subject to degradation by copper sulfate and pose a secondary threat to the aquatic ecosystem. Additionally, applied copper is non-biodegradable and has been shown to remain in the water

column for two months after application (Salam & El-Fadel, 2008; Van Hullebusch et al., 2003). Copper sulfate is also known to accumulate in sediment and is prone to remobilization upon disturbance or changes in water chemistry.

Silica

Silica is a major cell wall component and limiting nutrient in diatoms and rice straw (Van Soest, 2006). It is hypothesized that fortifying waterbodies with dissolved silica may allow diatoms to compete with and minimize populations responsible for CyanoHABs, though laboratory results are inconclusive (Kidwell, 2015; Burkholder & Marshall, 2012; Meyer-Reil & Koster, 2000).

The relatively high silica content of rice straw serves to protect the straw from degradation or hydrolysis. As such, the recovery of silica from untreated rice straw is negligible. However, recent studies have shown improved degradation efficiencies of rice straw upon microbial, mechanical, or chemical pretreatment (Zheng et al., 2020; Khaleghian et al., 2017). Improved degradation efficiencies of cereal straws could enhance the release of allelopathic (phenolic) compounds, as well as silica in rice straw. Silica released from rice straw may then promote species that compete with species responsible for CyanoHABs.

Hydrogen peroxide

Owing to the chemical's selectivity and benign byproducts in water, hydrogen peroxide (H_2O_2) is an effective and environmentally favorable method for the prevention and degradation of CyanoHABs. Within the chloroplasts of eukaryotes under high light conditions, oxygen is used as an excess electron acceptor creating superoxide (O^-), a reactive oxygen species (ROS).

Superoxide is then converted into H_2O_2 using superoxide dismutase and transformed into water by way of catalase or ascorbate peroxidase.

In cyanobacteria under similar conditions, flavoproteins aid in the transfer of electrons into water molecules, excluding the formation of superoxide and H_2O_2. This pathway in cyanobacteria mitigates the need for large quantities of anti-ROS enzymes like catalase or ascorbate peroxidase (Giannuzzi et al., 2016). Consequently, eukaryotic organisms are equipped for higher oxidative stress when compared to prokaryotes, rendering H_2O_2 a selective cyanocide in low concentrations (Weenink et al., 2015; Matthijs et al., 2012). Additionally, H_2O_2 has been shown to degrade microcystins upon cyanobacterial lysis, a previously unaddressed concern (Matthijs et al., 2012; Cornish et al., 2000; Bandala et al., 2004).

When applied within concentrations of 2.5 mg/L to 5 mg/L, H_2O_2 allows for long-term selective suppression of prokaryotic organisms such as cyanobacteria. When H_2O_2 is applied in concentrations less than 5 mg/L, higher plants and eukaryotes remain unharmed allowing for restoration of diverse phytoplankton communities (Weenink et al., 2015). Despite rapid degradation in highly reductive waters, sensitive taxa exposed to concentrations over 5 mg/L for any length of time exhibit increased mortality.

To minimize the risk of cyanobacterial resurgence, Weenink et al. (2015) suggested that initial H_2O_2 dosages be large enough to sustain concentrations at or above 2 mg/L for a minimum of 5 hours. In natural waters, H_2O_2 is decomposed more readily through oxidation of organic matter and by anti-ROS enzymes within non-target organisms. To account for these reactions in natural systems, a minimum homogenous application of 2.3 mg/L is recommended. However, elevated levels of organic matter or solutes in the water will require larger concentrations before cyanobacteria are affected. Wang et al. (2019) have recently shown that repetitive applications of

H_2O_2 in highly reductive waters can prolong residence time without exceeding detrimental concentrations.

Barley or rice straw

For several decades, barley straw has been gaining recognition as an economical and accessible algistat. Several studies have confirmed that the aerobic, microbial decomposition of lignin, the parent material of degradation products, is responsible for activating a cereal straw's algistatic effects (Pillinger et al., 1994; Iredale et al., 2012; Murray et al., 2010). Despite these findings, the primary compounds and pathways contributing to inhibition have not been determined definitively.

Iredale et al. (2012) discovered increased algal suppression upon photodegradation of barley straw, suggesting that the photochemical production of H_2O_2 plays a role in the inhibitory abilities of cereal straws. Irradiation of natural organic matter in water has been known to produce H_2O_2; however, barley straw was found to produce greater quantities of H_2O_2 and algal suppression when compared to rice or wheat straw or natural organic matter (Zhang et al., 2012; Ma et al., 2018).

Relative to wheat and barley, rice straw has a high silica-to-lignin ratio within the epidermis, contributing to its recalcitrance and resistance to microbial colonization (Agbagla-Dohnani et al., 2003). However, silica and lignin content alone cannot account for algal control. Wheat and barley have a similar silica-to-lignin ratio, yet wheat straw exhibits the lowest H_2O_2 production when compared to rice and barley (Van Soest et al., 2006). It is hypothesized that the variable H_2O_2 production amongst cereal straw is due to the unique microbial community and enzymatic activities responsible for decomposition. It is suspected that the community

responsible for barley straw decomposition transforms the decomposition products into protein-like structures with much greater potential for hydrogen peroxide formation relative to rice or wheat (Ma et al., 2015).

Polyphenols from cereal straws

By no coincidence, the optimal conditions for algal inhibition from decomposing cereal straw mirror ideal conditions for H_2O_2 production from polyphenols (Akagawa et al., 2003; Iredale et al., 2012). However, it is unclear if H_2O_2 can be considered the primary mechanism of algal inhibition. Multiple studies have concluded that polyphenolic compounds are the primary allelochemicals mediating inhibition, but the specific polyphenolic structures and mechanisms by which inhibition occurs have not yet been determined (Ma et al., 2015; Murray et al., 2010; Everall et al., 1996 and 1997; Pillinger et al., 1994; Waybright et al., 2009).

A plethora of phenolic compounds have been identified in decomposing straw extracts (Everall & Lees, 1996 & 1997), though few compounds exhibit algicidal or algistatic effects in concentrations found following field applications. For example, isolated benzaldehyde, 2-phenyl-phenol, and *p*-cresol from rotting barley straw showed greatest algistatic effects at 100 µg/L, 150 µg/L, and 150 µg/L, but are found in maximum concentrations of 10 µg/L, 10 µg/L, and 100 µg/L, respectively, upon straw degradation in water (Murray et al., 2010).

However, depending on the chemical structure (the number and position of hydroxyl groups), the availability of oxygen, pH, and presence of metal ions and UV light, polyphenols can act as pro-oxidants by forming ROS and H_2O_2 (Akagawa et al., 2003; Zhang et al., 2012). In natural waters, this process is likely aided by the microbial community. The microbial decomposition of cereal straw alters the photochemical properties of the soluble lignin so that

H_2O_2 production is stimulated by UV irradiation (Ma et al., 2018; Iredale et al., 2012; Pillinger et al., 1994). Together, these factors may alter the inhibitory concentrations of polyphenols in natural waters.

Additionally, the presence of organic matter in a waterbody may contribute to the photoproduction of H_2O_2 or ROS but may also consume any redox reactions by H_2O_2 or other ROS before the targeted algal species is affected. Therefore, inhibitory concentrations are difficult to determine as the results/treatment likely depend on the abiotic and biotic conditions during the time of decomposition. As such, additional experiments should be conducted to verify inhibitory concentrations in natural waters and to aid in understanding the mechanism by which inhibition occurs (Zhang et al., 2012; Iredale et al., 2012; Maris, 2019).

Research objectives, hypotheses, and experimental approach

The goal of this research was to determine the efficacy of barley and rice straw as algal inhibitors through evaluation of allelopathic (phenolic) compounds released during decomposition compared to H_2O_2.

Objectives

1. Evaluate the effectiveness of decomposing cereal straw extract, particularly rice and barley straw extract, to inhibit the green alga *Raphidocelis subcapitata* (Korshikov) Nygaard, Komárek, J.Kristiansen & O.M.Skulberg, or *Microcystis aeruginosa* (Kützing) Kützing cyanobacteria in bioassays.
2. Determine if the effectiveness of decomposing rice straw extract to inhibit growth of a green alga or cyanobacterium is altered by the presence of “natural” organic matter in pond water.

3. Determine if the effectiveness of decomposing rice straw extract is altered by the presence of microbial communities.

Hypotheses

1. Extracts of aerobically decomposed barley and rice straws will be more effective than hydrogen peroxide at inhibiting growth of green or cyanobacterial algae and in reducing microcystin concentrations due to the production of allelopathic compounds.
2. Photoaerobically biodecomposed rice straw extract will be more effective than the single reactive oxygen species, hydrogen peroxide, in inhibiting growth of green or cyanobacterial algae and in reducing microcystin concentrations, regardless of the presence of natural organic matter, due to the suite allelopathic (phenolic) compounds produced.
3. The ability of photoaerobically biodecomposed rice straw extract to inhibit green or cyanobacterial algae will not be affected by the presence of a microbial community in pond water.

References

Akagawa, M., Shigemitsu, T., & Suyama, K. (2003). Production of hydrogen peroxide by polyphenols and polyphenol-rich beverages under quasi-physiological conditions. *Bioscience, Biotechnology, and Biochemistry, 67*(12), 2632–2640. https://doi.org/10.1271/bbb.67.2632

Appel, H. M. (1993). Phenolics in ecological interactions: The importance of oxidation. *Journal of Chemical Ecology, 19*(7), 1521-1552. doi:10.1007/bf00984895

Bandala, E. R., Martínez, D., Martínez, E., & Dionysiou, D. D. (2004). Degradation of Microcystin-LR toxin by fenton and photo-fenton processes. *Toxicon, 43*(7), 829-832. doi:10.1016/j.toxicon.2004.03.013

Beaulieu, S. E., Sengco, M. R., & Anderson, D. M. (2005). Using clay to control harmful algal blooms: Deposition and resuspension of clay/algal flocs. *Harmful Algae, 4*(1), 123-138. doi:10.1016/j.hal.2003.12.008

Bengis, R., Govender, D., Lane, E., Myburgh, J., Oberholster, P., Buss, P., Prozesky, L.,Keet, D. (2016). Eco-epidemiological and pathological features of wildlife mortality events related to cyanobacterial bio-intoxication in the Kruger National Park, South Africa. *Journal of the South African Veterinary Association, 87*(1). doi:10.4102/jsava.v87i1.1391

Brocks, J., Logan, G., Buick, R., & Summons, R. (1999). Archean molecular fossils and the early rise of eukaryotes. *Science, 285*(5430), 1033-1036.

Conley, D. J., Paerl, H. W., Howarth, R. W., Boesch, D. F., Seitzinger, S. P., Havens, K. E., Likens, G. E. (2009). Controlling eutrophication: nitrogen and phosphorus. *Science, 323*(5917), 1014-1015. doi:10.1126/science.1167755

Cornish, B. J., Lawton, L. A., & Robertson, P. K. (2000). Hydrogen peroxide enhanced photocatalytic oxidation of microcystin-LR using titanium dioxide. *Applied Catalysis B: Environmental, 25*(1), 59-67. doi:10.1016/s0926-3373(99)00121-6

Elmgren, R. and Larsson, U. (2001) Nitrogen and the Baltic Sea: Managing nitrogen in relation to phosphorus. In *Optimizing Nitrogen Management in Food and Energy Production and Environmental Protection*: Proceedings of the 2nd International Nitrogen Conference on Science and Policy. *TheScientificWorld* 1(2), 371-377.

Everall N., Lees D. (1996) The use of barley-straw to control general and blue-green algal growth in a Derbyshire reservoir. *Water Research* 30: 269-276.

Everall N., Lees D. (1997) The identification and significance of chemicals released from decomposing barley straw during reservoir algal control. *Water Research* 31: 614-620.

Farasat, M., Khavari-Nejad, R-A, Nabavi, S.M.B., Namjooyan, F. (2014) Antioxidant activity, total phenolics and flavonoid contents of some edible green seaweeds from northern coasts of the Persian Gulf. *Iranian journal of pharmaceutical research*: IJPR 13: 163.

Gilbert, P. M. (2017). Eutrophication, harmful algae and biodiversity — Challenging paradigms in a world of complex nutrient changes. *Marine Pollution Bulletin, 124*(2), 591-606. doi:10.1016/j.marpolbul.2017.04.027

Grosse, Y., Baan, R., Straif, K., Secretan, B., Ghissassi, F. E., & Cogliano, V. (2006). Carcinogenicity of nitrate, nitrite, and cyanobacterial peptide toxins. *The Lancet Oncology, 7*(8), 628-629. doi:10.1016/s1470-2045(06)70789-6

Giannuzzi, L., Krock, B., Minaglia, M. C., Rosso, L., Houghton, C., Sedan, D., Malanga, G., Espinosa, M., Andrinolo, D., &Hernando, M. (2016). Growth, toxin production, active oxygen species and catalase activity of *Microcystis aeruginosa* (cyanophyceae) exposed to temperature stress. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology,189*, 22-30. doi:10.1016/j.cbpc.2016.07.001

Hardy, J. (2008). *Washington State recreational guidance for microcystins (provisional) and anatoxin-a (interim/provisional)* (pp. 5-6) (Environmental Health Assessments). Olympia, WA: Washington State Dept. of Health.

Herald, T. J., Gadgil, P., & Tilley, M. (2012). High-throughput micro plate assays for screening flavonoid content and DPPH-scavenging activity in sorghum bran and flour. *Journal of the Science of Food and Agriculture*, *92*(11), 2326–2331. https://doi.org/10.1002/jsfa.5633

Hua, Q., Liu. Y-g., Yan, Z-l., Zeng, G-m., Liu, S-b., Wang, W-j., Tan, X-f., Deng, J-q., Tang, X., Wang, Q-p. (2018) Allelopathic effect of the rice straw aqueous extract on the growth of Microcystis aeruginosa. *Ecotoxicology and Environmental Safety* 148: 953-959. Iredale R.S., McDonald A.T., Adams D.G. (2012) A series of experiments aimed at clarifying the mode of action of barley straw in cyanobacterial growth control. *Water research* 46: 6095-6103.

Huisman, J., Sharples, J., Stroom, J.M., Visser, P.M., Kardinaal, W.E.A., Verspagen, J.M.H., & Sommeijer, B. (2004), Changes in turbulent mixing shift competition for light between phytoplankton species. *Ecology*, 85: 2960-2970. doi:10.1890/03-0763

Iredale R.S., McDonald A.T., Adams D.G. (2012) A series of experiments aimed at clarifying the mode of action of barley straw in cyanobacterial growth control. *Water research* 46: 6095-6103.

Jeong, J., Jin, H., Sohn, C., Suh, K, & Hong, Y.K. (2000). Algicidal activity of the seaweed *Corallina pilulifera* against red tide microalgae. *Journal of Applied Phycology*, 12, 37-43.

Jöhnk, K. D., Huisman, J., Sharples, J., Sommeijer, B., Visser, P. M., & Stroom, J. M. (2008). Summer heatwaves promote blooms of harmful cyanobacteria. *Global Change Biology,* 14(3), 495-512. doi:10.1111/j.1365-2486.2007.01510.x

Khaleghian, H., Molaverdi, M., & Karimi, K. (2017). Silica removal from rice straw to improve ITS hydrolysis and ethanol production. *Industrial & Engineering Chemistry Research,* 56(35), 9793-9798. doi:10.1021/acs.iecr.7b02830

Kidwell, D. M. (2015). *Programmatic Environmental Assessment for the Prevention, Control, and Mitigation of Harmful Algal Blooms Program* [Pdf]. Silver Spring: National Oceanic and Atmospheric Association (NOAA).

Laskin, A. I., Sariaslani, S., & Gadd, G. M. (2009). Bioremediation of cyanotoxins. In *Advances in Applied Microbiology* (Vol. 67, pp. 109-129). London: Academic Press.

Lopez-Rodas, V., Maneiro, E., Lanzarot, M. P., Perdigones, N., & Costas, E. (2008). Mass wildlife mortality due to cyanobacteria in the Donana National Park, Spain. *Veterinary Record, 162*(10), 317-318. doi:10.1136/vr.162.10.317

Lu, G., Song, X., Yu, Z., & Cao, X. (2016). Application of PAC-modified kaolin to mitigate *Prorocentrum donghaiense*: Effects on cell removal and phosphorus cycling in a laboratory setting. *Journal of Applied Phycology, 29*(2), 917-928. doi:10.1007/s10811-016-0992-3

Lu, H., Xie, H., Gong, Y., Wang, Q., & Yang, Y. (2011). Secondary metabolites from the seaweed *Gracilaria lemaneiformis* and their allelopathic effects on *Skeletonema costatum*. *Biochemical Systematics and Ecology*, *39*(4-6), 397-400. doi:10.1016/j.bse.2011.05.015

Ma, H., Huang, L., Zhang, J., Shi, D., & Yang, J. (2018). Optical properties of straw-derived dissolved organic matter and growth inhibition of *Microcystis aeruginosa* by straw-derived dissolved organic matter via photo-generated hydrogen peroxide. *Environmental Pollution, 242*, 760-768. doi:10.1016/j.envpol.2018.07.052

Margraf T., Karnopp, A.R., Rosso, N.D., Granato, D. (2015) Comparison between Folin-Ciocalteu and Prussian Blue assays to estimate the total phenolic content of juices and teas using 96-well microplates. *Journal of food science* 80: C2397-C2403.

Matthijs, H. C., Visser, P. M., Reeze, B., Meeuse, J., Slot, P. C., Wijn, G., Talens, R., &Huisman, J. (2012). Selective suppression of harmful cyanobacteria in an entire lake with hydrogen peroxide. *Water Research, 46*(5), 1460-1472. doi:10.1016/j.watres.2011.11.016

Meyer-Reil, L., & Köster, M. (2000). Eutrophication of marine waters: Effects on benthic microbial communities. *Marine Pollution Bulletin, 41*(1-6), 255-263. doi:10.1016/s0025-326x(00)00114-4

Murray, D., Jefferson, B., Jarvis, P., & Parsons, S. (2010). Inhibition of three algae species using chemicals released from barley straw. *Environmental Technology, 31*(4), 455-466. doi:10.1080/09593331003663294

Nan, C., Zhang, H., Lin, S., Zhao, G., & Liu, X. (2008). Allelopathic effects of *Ulva lactuca* on selected species of harmful bloom-forming microalgae in laboratory cultures. *Aquatic Botany, 89*(1), 9-15. doi:10.1016/j.aquabot.2008.01.005

Pacwa-Płociniczak, M., Płaza, G. A., Piotrowska-Seget, Z., & Cameotra, S. S. (2011). Environmental applications of biosurfactants: Recent advances. *International Journal of Molecular Sciences, 12*(1), 633-654. doi:10.3390/ijms12010633

Pain, D., Sánchez, A., & Meharg, A. (1998). The Doñana ecological disaster: Contamination of a world heritage estuarine marsh ecosystem with acidified pyrite mine waste. *Science of The Total Environment, 222*(1-2), 45-54. doi:10.1016/s0048-9697(98)00290-3

Pan, G., Dai, L., Li, L., He, L., Li, H., Bi, L., & Gulati, R. D. (2012). Reducing the recruitment of sedimented algae and nutrient release into the overlying water using modified soil/sand flocculation-capping in eutrophic lakes. *Environmental Science & Technology, 46*(9), 5077-5084. doi:10.1021/es3000307

Pandhal, J., Choon, W. L, Kapoore, R. V., Russo, D. A., Hanotu, J., Grant Wilson, I. A., Desai, P., Bailey, M., Zimmerman, W. J., &Ferguson, A. (2017). Harvesting environmental microalgal blooms for remediation and resource recovery: A laboratory scale investigation with economic and microbial community impact assessment. *Biology, 7*(1), 4. doi:10.3390/biology7010004

Paerl, H.W. (2014). Mitigating harmful cyanobacterial blooms in a human- and climatically-impacted world. *Life, 4*(4), 988-1012. doi:10.3390/life4040988

Paerl, H. W., & Huisman, J. (2009). Climate change: A catalyst for global expansion of harmful cyanobacterial blooms. *Environmental Microbiology Reports, 1*(1), 27-37. doi:10.1111/j.1758-2229.2008.00004.x

Paerl, H.W., Otten, T.G. (2013). Harmful cyanobacterial blooms: causes, consequences, and controls. *Microbial Ecology* 65, 995–1010 https://doi.org/10.1007/s00248-012-0159-y

Paerl H.W., Piehler MF (2008) Nitrogen and marine eutrophication. In: D.G, Capone, M. Mulholland, E. Carpenter (eds.) *Nitrogen in the marine environment*, vol. 2. Academic Press,Orlando, pp. 529–567

Pérez-Ruzafa, A., Campillo, S., Fernández-Palacios, J. M., García-Lacunza, A., García-Oliva, M., Ibañez, H., Navarro-Martinez, P. C., Perez-Marcos, M., Perez-Ruzafa, I. M., Quispe-Becerra, J. I., Sala-Mirete, A., Sanchez, O., &Marcos, C. (2019). Long-term dynamic in nutrients, chlorophyll a, and water quality parameters in a coastal lagoon during a process

of eutrophication for decades, a sudden break and a relatively rapid recovery. *Frontiers in Marine Science, 6*. doi:10.3389/fmars.2019.00026

Petkuviene, J., Zilius, M., Lubiene, I., Ruginis, T., Giordani, G., Razinkovas-Baziukas, A., & Bartoli, M. (2016). Phosphorus cycling in a freshwater estuary impacted by cyanobacterial blooms. *Estuaries and Coasts, 39*(5), 1386–1402. https://doi.org/10.1007/s12237-016-0078-0

Pillinger J., Cooper J., Ridge I. (1994) Role of phenolic compounds in the antialgal activity of barley straw. *Journal of Chemical Ecology* 20: 1557-1569.

Porter, E. M., Bowman, W. D., Clark, C. M., Compton, J. E., Pardo, L. H., & Soong, J. L. (2013). Interactive effects of anthropogenic nitrogen enrichment and climate change on terrestrial and aquatic biodiversity. *Biogeochemistry* 114**,** 93–120..https://doi.org/10.1007/s10533-012-9803-3

Pueyo, I.U., Calvo M.I. (2009) Assay conditions and validation of a new UV spectrophotometric method using microplates for the determination of polyphenol content. *Fitoterapia* 80: 465-467.

Salam, D., & El-Fadel, M. (2008). Mobility and availability of copper in agricultural soils irrigated from water treated with copper sulfate algaecide. *Water, Air, and Soil Pollution, 195*(1-4), 3-13. doi:10.1007/s11270-008-9722-z

Savin, M. (2020). 2020 expt_ algal control with straw or H2O2

Schindler, D. W., Hecky, R. E., Findlay, D. L., Stainton, M. P., Parker, B. R., Paterson, M. J., Beaty, K. G., Lyng, M., & Kasian, S. E. (2008). Eutrophication of lakes cannot be controlled by reducing nitrogen input: results of a 37-year whole-ecosystem experiment. *Proceedings of the National Academy of Sciences of the United States of America*, *105*(32), 11254–11258. https://doi.org/10.1073/pnas.0805108105

Shirota, A. (1989). Red tide problem and countermeasures (II). *International Journal of Aquaculture and Fisheries Technology, 1,* 195-223.

Sivonen, K. (2009). Cyanobacterial toxins. *Encyclopedia of Microbiology,* 290-307. doi:10.1016/b978-012373944-5.00005-5

Summons, R.E., Jahnke, L.L., Hope, J.M., & Logan, G.A. (1999). 2-methylhopanoids as biomarkers for cyanobacterial oxygenic photosynthesis. *Nature, 400(6744), 554-557. doi:10.1038/23005*

Sun, X., Kim, E., & Sun, S. (2010). Laboratory study on the ecological impact of sophorolipid used for harmful algae elimination. *Chinese Journal of Oceanology and Limnology, 28*(6), 1240-1247. doi:10.1007/s00343-010-9962-9

Sun, Y., Dong, S., Zhou, W., Guo, L., Guo, G., & Zhang, X. (2019). A comprehensive review of secondary metabolites with antialgal activity from marine macroalgae against red tide microalgae. *Journal of Coastal Research, 93*(Sp1), 475. doi:10.2112/si93-062.1

Trochine, C., Guerrieri, M., Liboriussen, L., Meerhoff, M., Lauridsen, T. L., Søndergaard, M., & Jeppesen, E. (2010). Filamentous green algae inhibit phytoplankton with enhanced effects when lakes get warmer. *Freshwater Biology, 56*(3), 541-553. doi:10.1111/j.1365-2427.2010.02521.x

United States Environmental Protection Agency (USEPA). Office of Research and Development. Potter, B. B., & Wimsatt, J. C. (2009). Method 415.3 Determination of total organic carbon and specific UV absorbance at 254 nm in source water and drinking water. Cincinnati, Ohio.

Van Hullebusch, E., Deluchat, V., Chazal, P. M., & Froissard, D. (2003). Copper accumulation in a reservoir ecosystem following copper sulfate treatment (St. Germain Les Belles, France). *Water, Air, and Soil Pollution, 150,* 3-22. doi: 10.1023/A:1026148914108

Van Soest, P. (2006). Rice straw, the role of silica and treatments to improve quality. *Animal Feed Science and Technology, 130*(3-4), 137-171. doi: 10.1016/j.anifeedsci.2006.01.023

Visser, P. M., Ibelings, B. W., Bormans, M., & Huisman, J. (2015). Artificial mixing to control cyanobacterial blooms: A review. *Aquatic Ecology, 50*(3), 423-441. doi:10.1007/s10452-015-9537-0

Watson, S., Mccauley, E., & Downing, J. A. (1992). Sigmoid relationships between phosphorus, algal biomass, and algal community structure. *Canadian Journal of Fisheries and Aquatic Sciences, 49*(12), 2605-2610. doi:10.1139/f92-288

Watson, S. B., Miller, C., Arhonditsis, G., Boyer, G. L., Carmichael, W., Charlton, M. N., Confesor, R., Depew, D. C., Höök, T. O., Ludsin, S. A., Matisoff, G., McElmurry, S. P., Murray, M. W., Peter Richards, R., Rao, Y. R., Steffen, M. M., & Wilhelm, S. W. (2016). The re-eutrophication of Lake Erie: Harmful algal blooms and hypoxia. *Harmful Algae, 56,* 44–66. https://doi.org/10.1016/j.hal.2016.04.010

Wang, Y., Yu, Z., Song, X., Tang, X., & Zhang, S. (2007). Effects of macroalgae *Ulva pertusa* (chlorophyta) and *Gracilaria lemaneiformis* (rhodophyta) on growth of four species of bloom-forming dinoflagellates. *Aquatic Botany, 86*(2), 139-147. doi:10.1016/j.aquabot.2006.09.013

Waybright T.J., Terlizzi D.E., Ferrier M.D. (2009) Chemical characterization of the aqueous algistatic fraction of barley straw (*Hordeum vulgare*) inhibiting *Microcystis aeruginosa*. *Journal of Applied Phycology* 21: 333-340.

Weenink, E. F., Luimstra, V. M., Schuurmans, J. M., Herk, M. J., Visser, P. M., & Matthijs, H. C. (2015). Combatting cyanobacteria with hydrogen peroxide: A laboratory study on the

consequences for phytoplankton community and diversity. *Frontiers in Microbiology, 6.* doi:10.3389/fmicb.2015.00714

Yu, Z., Zou, J., & Ma, X. (1994a). Application of clays to removal of red tide organisms I. Coagulation of red tide organisms with clays. *Chinese Journal of Oceanology and Limnology, 12*(3), 193-200. doi:10.1007/bf02845163

Yu, Z., Zou, J., & Ma, X. (1994b). Application of clays to removal of red tide organisms II. Coagulation of different species of red tide organisms with montmorillonite and effect of clay pretreatment. *Chinese Journal of Oceanology and Limnology, 12*(4), 316-324. doi:10.1007/bf02850491

Yu, Z., Song, X., Cao, X., & Liu, Y. (2017). Mitigation of harmful algal blooms using modified clays: Theory, mechanisms, and applications. *Harmful Algae, 69*, 48-64. doi:https://doi.org/10.1016/j.hal.2017.09.004

Zhang, Y., Del Vecchio, R., & Blough, N. V. (2012). Investigating the mechanism of hydrogen peroxide photoproduction by humic substances. *Environmental Science & Technology, 46*(21), 11836-11843. doi:10.1021/es3029582

Zeng, J., Jiao, C., Zhao, D., Xu, H., Huang, R., Cao, X., Yu, Z., & Wu, Q. L. (2019). Patterns and assembly processes of planktonic and sedimentary bacterial community differ along a trophic gradient in freshwater lakes. *Ecological Indicators, 106*, 105491. https://doi.org/10.1016/j.ecolind.2019.105491

Zheng, G., Yin, T., Lu, Z., Boboua, S. Y., Li, J., & Zhou, W. (2020). Degradation of rice straw at low temperature using a novel microbial consortium LTF-27 with efficient ability. *Bioresource Technology, 304*, 123064. doi:10.1016/j.biortech.2020.123064

CHAPTER 2. ALLELOPATHIC EFFECT OF CEREAL STRAW EXTRACTS ON GROWTH OF *RAPHIDOCELIS SUBCAPITATA* AND *MICROCYSTIS AERUGINOSA*

Abstract

Harmful algal blooms are increasing in size, duration, and intensity around the globe. For several decades, cereal straws have been recognized as a viable algal control method, though the mechanisms by which cereal straws inhibit algae remain a topic of research. The objectives of this study were to evaluate the effectiveness of decomposing cereal straw extract, particularly rice (*Oryza sativa)* and barley (*Hordeum vulgare*) straw extract, to inhibit the green alga *Raphidocelis subcapitata* or cyanobacteria *Microcystis aeruginosa* in bioassays and to determine if the effectiveness of decomposing rice straw extract is altered by the presence of natural organic matter or microbial communities in pond water. In rice and barley straw extracts of the same concentration, total phenolic and flavonoid concentrations were also similar; however, rice straw was more effective than barley straw at inhibiting the growth of both *R. subcapitata* and *M. aeruginosa*. Combined with a lack of significant changes in H_2O_2 between cereal straw treatments, results support the hypothesis that algal inhibition cannot be attributed to H_2O_2 alone but depends on the release of specific phenolic and polyphenolic compounds from decomposing cereal straw. In media-based assays, both *M. aeruginosa* and *R. subcapitata* were inhibited by 5.0 g/L rice straw extract ($P<0.05$). In pond water bioassays, 10 g/L rice straw extract showed the greatest inhibitory effect in cultures with and without bacteria. *M. aeruginosa* showed signs of recovery 15 days following treatment with 2.5 g/L in media and 5.0 g/L rice straw extract in pond water. For reliable long-term suppression, greater concentrations of rice straw are necessary, possibly due to the instability of allelochemicals. In pond water, variability in responses between the controls (with and without bacteria) and 5.0 g/L treatments with bacteria versus without bacteria show that the microbial community within the context of abiotic conditions of natural waters (pH, nutrient concentrations, sediment, and temperature, etc.) is important in determining bloom behavior and treatment response. Though rice straw was an

effective method for the control of *M. aeruginosa* and *R. subcapitata*, complex interactions between the algae, the microbial community, and abiotic factors found in natural waters make the determination the of the inhibitory mechanism a challenge.

1. Introduction

Cyanobacteria (blue-green algae) are some of the Earth's oldest photoautotrophic prokaryotes with evidence of their presence as early as 2.5 to 2.7 million years ago (Mya) (Summons et al., 1999; Brocks et al. 1999). Over the evolutionary history of Earth, cyanobacteria have been exposed and adapted to a large range of eco-physiological stresses, allowing the prokaryotes to proliferate even in extreme conditions (Paerl, 2014). As a result, cyanobacteria can dominate aquatic ecosystems. Some cyanobacteria also form harmful algal blooms (CyanoHABs), particularly in anthropogenically modified waterbodies (Paerl and Otten, 2013).

For several decades, barley straw has been gaining recognition as an economical and accessible algistat. Several studies have confirmed that the aerobic, microbial decomposition of lignin, the parent material of degradation products, is responsible for activating a cereal straw's algistatic effects (Pillinger et al., 1994; Iredale et al., 2012; Murray et al., 2010). Despite these findings, the primary compounds and pathways contributing to inhibition have not been determined definitively.

Iredale et al. (2012) discovered increased algal suppression upon photodegradation of barley straw, suggesting that the photochemical production of hydrogen peroxide ($H_2O_{2)}$ plays a role in the inhibitory abilities of cereal straws. Irradiation of natural organic matter in water has been known to produce H_2O_2; however, barley straw was found to produce greater quantities of H_2O_2 and algal suppression when compared to rice or wheat straw or natural organic matter (Zhang et al., 2012; Ma et al., 2018).

Owing to the chemical's selectivity and benign byproducts in water, H_2O_2 is an effective method for the prevention and degradation of CyanoHABs. Within the chloroplasts of eukaryotes under high light conditions, oxygen is used as an excess electron acceptor creating

superoxide (O^{-}), a reactive oxygen species (ROS), that is then converted into H_2O_2 using anti-ROS enzymes. In cyanobacteria under similar conditions, flavoproteins aid in the transfer of electrons into water molecules, excluding the formation of superoxide and H_2O_2 and mitigating the need for large quantities of anti-ROS enzymes (Giannuzzi et al., 2016). Consequently, eukaryotic organisms are equipped for higher oxidative stress when compared to prokaryotes, rendering H_2O_2 a selective cyanocide in low concentrations (Weenink et al., 2015; Matthijs et al., 2012). Additionally, H_2O_2 has been shown to degrade microcystins upon cyanobacterial lysis, a previously unaddressed concern (Matthijs et al., 2012; Cornish et al., 2000; Bandala et al., 2004).

Other studies have concluded that polyphenolic compounds are the primary allelochemicals mediating inhibition, but the specific polyphenolic structures and mechanisms by which inhibition occurs have not yet been determined (Ma et al., 2018; Murray et al., 2010; Everall et al., 1996 and 1997; Pillinger et al., 1994; Waybright et al., 2009). Depending on the chemical structure (the number and position of hydroxyl groups), the present microbial community, the availability of oxygen, pH, and presence of metal ions and UV light, polyphenols can act as pro-oxidants by forming ROS and H_2O_2 (Akagawa et al., 2003; Zhang et al., 2012). Additionally, the presence of organic matter in a waterbody may contribute to the photoproduction of ROS but may also consume any redox reactions before the targeted algal species is affected. Therefore, inhibitory concentrations in natural waters are difficult to determine as the results/treatment likely depend on the abiotic and biotic conditions during the time of decomposition (Zhang et al., 2012; Iredale et al., 2012; Maris, 2019).

When applied within concentrations of 2.5 mg/L to 5 mg/L, H_2O_2 allows for long-term selective suppression of prokaryotic organisms such as cyanobacteria. When H_2O_2 is applied in concentrations less than 5 mg/L, higher plants and eukaryotes remain unharmed allowing for

restoration of diverse phytoplankton communities (Weenink et al., 2015). Despite rapid degradation in highly reductive waters, sensitive taxa exposed to concentrations over 5 mg/L for any length of time exhibit increased mortality.

To minimize the risk of cyanobacterial resurgence, Weenink et al. (2015) suggested that initial H_2O_2 dosages be large enough to sustain concentrations at or above 2 mg/L for a minimum of 5 hours. In natural waters, H_2O_2 is decomposed more readily through oxidation of organic matter and by anti-ROS enzymes within non-target organisms. To account for these reactions in natural systems, a minimum homogenous application of 2.3 mg/L is recommended. However, elevated levels of organic matter or solutes in the water will require larger concentrations before cyanobacteria are affected. Wang et al. (2019) have shown that repetitive applications of H_2O_2 in highly reductive waters can prolong residence time without exceeding detrimental concentrations.

The objectives of this research were to evaluate the effectiveness of decomposing cereal straw extract, particularly rice straw extract, to inhibit the green alga *Raphidocelis subcapitata* or *Microcystis aeruginosa* in bioassays and to determine if the effectiveness of decomposing rice straw extract is altered by the presence of natural organic matter or a microbial community in pond water.

2. Materials and methods

2.1 Rice and barley straw extracts

Rice straw was obtained from a farmer in central Arkansas and barley straw was purchased from Amazon (Seattle, WA). Both were washed three times with deionized water, air-dried at room temperature, and stored in bags at room temperature until used in decomposition experiments. Straw (40 g/L) was cut into 10-cm pieces and decomposed aerobically 30 days in an aquarium at room temperature (Ma et al., 2015; Iredale et al., 2012). Aeration was controlled

using an aquarium pump and light was maintained on a 12 hr:12 hr light:dark cycle using full daylight spectrum (6400K), 54 watt (initial 5000 lumens) T5 high output fluorescent tubes. After 30 days, the straw extracts were filtered through a REXEED-25S (Asahi Kasei Medical Co., Ltd, Chiyoda-ku, Tokyo) ultrafilter using a peristaltic pump maintained at 31 kPa. The extract was stored for up to 6 months at 4°C. Before use in bioassays, extracts were filter-sterilized using Nalgene® Rapid-Flow™ PES membrane filter units.

2.2 Maintenance of Raphidocelis subcapitata *and* Microcystis aeruginosa *algal cultures.* Sterilized Bristol medium (Table A1) was inoculated with *Raphidocelis subcapitata* (https://www.atcc.org/products/all/22662.aspx#generalinformation) and placed on a shaking incubator at 40 rpm, 23 °C, and under full daylight spectrum (6400K), 24 watt (initial 2000 lumens) T5 high output fluorescent tubes on a 12 hr:12 hr light:dark cycle. *Microcystis aeruginosa* (https://utex.org/products/utex-lb-2385) was obtained and grown in blue-green (BG-11) medium (Table A2, A3) with shaking at 40 rpm, 23 °C, and under full daylight spectrum (6400K), 24 watt (initial 2000 lumens) T5 high output fluorescent tubes on a 12 hr:12 hr light:dark cycle. Fresh media was inoculated with cultures at peak density every 14 to 21 days. Approximate cell counts were determined using a Bulldog-Bio 4-chip disposable hemocytometer.

2.3 *Bioassays*

Objectives were achieved using laboratory incubations of cultures grown in pre-sterilized 1-L Erlenmeyer flasks with treatments added as shown in Table 1 (n=3). For experiment 1, media-based cultures were created from 325 mL of 2x sterile Bristol or BG-11 culture medium and 325 mL sterile MilliQ water or appropriately diluted treatment plus 14 mL of *Raphidocelis*

subcapitata or *Microcystis aeruginosa* inoculated at an initial density approximating 5.8 x 10^5 cells/mL (similar to Hua et al., 2018).

For experiment 2 and 3, pond water was collected from Lake Fayetteville and filtered through a 5-µm pore size cellulose nitrate filter. Pond water used in experiment 3 was then filter-sterilized using a 0.2 µm pore-size Nalgene® Rapid-Flow™ PES membrane filter unit. Following filtration, pond water-based cultures were created from 487.5 mL of pond water and 162.5 mL sterile MilliQ water or appropriately diluted treatment plus 14 mL of each respective algal inoculum.

Experimental flasks were randomly arranged on metal storage racks under T5 high output fluorescent tubes maintained at 2000 lux on a 12 hr:12 hr light:dark cycle. The flasks were swirled and rearranged every three days to minimize edge effects throughout the 28-day incubation period.

Samples (20 mL total) during all three experiments were collected at time 0, 1, 4, 8, 15, and 28 days for measurement of chlorophyll-a, pH (Table B1, B2), oxidation-reduction potential (ORP; Table B3, B4), dissolved organic carbon, total nitrogen, total phenolics and flavonoids, and H_2O_2 in pond water experiments. In media and pond water experiments, samples (1 mL total) were collected at hour 0, 1, 4, and 24 for measurement of H_2O_2. Samples collected from *M. aeruginosa* cultures on day 0, 4, and 15 were analyzed for microcystin concentrations.

2.4 Determination of chlorophyll-a content

First, each sample was filtered through a 0.7-µm pore size Whatman GF/F filter, using enough volume to color the filter paper and with rinsing the filter syringe with MilliQ water between each sample. Without disturbing the filter residue, the filter was folded and transferred to a plastic screw-top centrifuge tube. The tubes were wrapped in foil to preclude light and stored

in the freezer until one day before analysis. One day before analysis, 7 mL of 90% acetone were added to each vial and returned to the freezer to steep for 23 – 25 hours. The fluorescence was measured using a calibrated Turner fluorometer (Turner Designs, Sunnyvale, CA). To quantify pheophytin, fluorescence was measured a second time, 90 seconds after the addition of 0.1 mL of 0.1 N HCl.

2.5 Determination of total flavonoid content

Total flavonoid content was measured using a method modified from Farasat et al. (2014). To determine total flavonoid content, 25 µL of sample was added to a clear, flat bottom 96-well polystyrene microplate followed by 100 µL of a 1:1 mixture of 10% $AlCl_3$ and 1M sodium acetate. Then, 180 µL of DI water was added to the wells. The plate was covered with a transparent lid and allowed to react for 30 minutes at room temperature. The absorbance was measured against a blank at 415 nm using a SpectraMax iD3 microplate reader (Molecular Devices, San Jose, CA). Calibration curves were created using quercetin as a standard at 0.5 to 25 µg/mL. Standards were measured throughout analysis for quality control.

2.6 Determination of total phenolic content

Total phenolic content was measured using Prussian Blue assays in clear, flat bottom 96-well polystyrene microplates (Margraf et al., 2015; Pueyo and Calvo, 2009). Gallic acid standards were prepared by dissolving the gallic acid in the minimal volume of ethanol and brought to volume with MilliQ water in concentrations from 0.5 to 25 µg/mL. To determine total phenolic content of samples, 100 µL of 0.50 mM $FeCl_3.6H_2O$ in 0.01 N HCl was added to 100 µL of appropriately diluted sample (1:40 to 1:50 v/v in MilliQ water) and left to react for two minutes. Next, 100 µL of 0.50 mM potassium ferricyanide ($K_3Fe(CN)_6$) was added to each well and allowed to react for 15 minutes in the dark at 25 °C. Absorbance was measured at 725 nm

using a SpectraMax iD3 microplate reader. Standards were measured throughout analysis for quality control.

2.7 Determination of hydrogen peroxide

Hydrogen peroxide (H_2O_2) was measured following a reaction with cerium (IV) sulfate (Putt and Pugh, 2013). First, 100 µL of sample was added to a clear, flat bottom 96-well polystyrene microplate. Then, 100 µL of 10 mM cerium (IV) sulfate was added to the wells and the absorbance was immediately read at 400 nm. The cerium (IV) sulfate concentration was determined using the standard curve. To determine the H_2O_2 concentration (mM) of the sample, the final cerium (IV) sulfate concentration was subtracted from the initial cerium (IV) sulfate concentration and divided by two.

To create the standard curve, 100 µL of DI water was added to a clear, flat bottom 96-well polystyrene microplate followed by 100 µL of 0.01 to 10 mM cerium (IV) sulfate in 10% (v/v) sulfuric acid/MilliQ water. DI water (200 µL) was added to wells to serve as a blank and the absorbance was read at 400 nm.

2.5 Data analysis

The chlorophyll-a concentrations were used to calculate the growth inhibition for each algal population as a percentage of the control and to indicate inhibition in the presence of each straw concentration over time. Treatment averages were analyzed by repeated measures analysis of variance (ANOVA, $P<0.05$; SigmaPlot 11.0, San Jose, CA) with a Bonferroni post hoc test to separate the means where appropriate.

3. Results

3.1 Experiment 1 – Growth in culture media

3.1.1 Chlorophyll-a

In *M. aeruginosa* cultures on day 15, 2.5 and 5.0 g/L rice straw extract showed significant inhibition of 96% and 97% of biomass as measured by chlorophyll-a, respectively, when compared to the no treatment control ($P<0.05$; Figure 1). On day 28, 5.0 g/L rice straw extract was the only significantly inhibitory treatment, with growth inhibited by 98% ($P<0.05$). On day 28, cultures treated with 2.5 g/L barley straw extract showed increased growth in flasks when compared to the no treatment control.

In R. subcapitata cultures on day 15, 5.0 g/L barley straw extract significantly inhibited growth by 58% and 5.0 g/L rice straw extract by 91% compared to the no treatment control ($P<0.05$; Figure 2). On day 28, 5.0 g/L barley straw extract significantly inhibited growth by 62% and 5.0 g/L rice straw extract by 94% ($P<0.05$). On day 28, these two treatments were significantly different from each other and the control ($P<0.05$). Growth in flasks treated with either 2.5 g/L rice or barley straw extract showed no difference from the untreated control ($P<0.05$).

3.1.2 Phenolics and flavonoids

In *M. aeruginosa* cultures on day 0, flasks treated with 5.0 g/L rice straw extract contained the greatest concentration of flavonoids followed by 5.0 g/L barley straw extract ($P<0.05$; Figure 3). Flasks treated with 2.5 g/L rice or barley straw extract contained the next greatest concentrations of flavonoids followed by the control with the smallest concentration ($P<0.05$). From day 0 to 15, there was an increase in flavonoid concentration in all treatments and in the control ($P<0.05$). From day 15 to 28, greater concentrations were maintained in the

control, 2.5 g/L barley and rice straw extracts and 5.0 g/L barley straw extracts and flavonoids decreased in the 5.0 g/L rice straw extract treatment group until concentrations were similar to those found on days 0 through 4 ($P<0.05$). Over the incubation period, flavonoids increased the most in the control (3.7 µg/mL) and the least in the 5.0 g/L rice straw extract treatment group (0.14 µg/L).

From day 0 to 15, phenolic concentrations were greatest in flasks treated with 5.0 g/L rice and barley straw extracts ($P<0.05$; Figure 4). Total phenolics were smallest in the control on day 4 and 15, and on day 28 phenolics were smaller in the control than in flasks treated with 5.0 g/L rice straw extract ($P<0.05$). Over the incubation period, total phenolic concentrations increased in the control and with 2.5 g/L rice and barley straw extracts until there was no difference between the 2.5 and 5.0 g/L rice and barley straw treatment groups ($P<0.05$).

In *R. subcapitata* cultures, flavonoid concentrations significantly increased from day 0 to 28 in all treatment groups and in the control ($P<0.05$; Figure 5). On day 0, flavonoid concentrations were significantly greater in flasks treated with 5.0 g/L rice or barley straw extract than in flasks treated with 2.5 g/L barley straw extract ($P<0.05$). By day 28, there was no difference in flavonoids between the 2.5 and 5.0 g/L rice and barley straw treatments ($P<0.05$). Again, the no treatment control contained the smallest flavonoid concentrations ($P<0.05$).

On day 0, phenolic concentrations in *R. subcapitata* cultures were greatest with 5.0 g/L rice and barley straw treatments ($P<0.05$; Figure 6). Between day 0 and 4, phenolics in flasks treated with 5.0 g/L rice and barley straw extracts decreased until they were no different from 2.5 g/L treatments or the control ($P<0.05$). After day 4, phenolics concentrations remained small in all treatment groups and in the control. The H_2O_2 concentrations were measured for 24 hours

following inoculation but showed no significance over time or amongst treatments ($P<0.05$, data not shown).

3.2 Experiment 2 and 3 – Growth in pond water with and without bacteria present

3.2.1 Chlorophyll-a

Chlorophyll-a concentrations in pond water-based *M. aeruginosa* cultures increased from day 15 to 28 in control with bacteria and in the flasks treated with 5.0 g/L rice straw extract without bacteria (Figure 7). On day 28, growth in flasks was greatest in the flasks treated with 5.0 g/L rice straw extract without bacteria and in the control with bacteria.

In *R. subcapitata* cultures from day 8 to day 28, growth was greatest in the sterile control followed by the control with bacteria (Figure 8). From day 0 to 15, *R. subcapitata* had very little to no growth in the presence of all treatment groups. Between day 15 and 28, there was growth in flasks treated with 5.0 g/L rice straw extract with bacteria.

3.2.2 Phenolics and flavonoids

In pond water *M. aeruginosa* cultures on day 0 through 28, flasks treated with 10 g/L rice straw extract, with and without bacteria, contained the greatest concentration of flavonoids followed by 5.0 g/L rice straw extract with and without bacteria (Figure 9). Flavonoids increased from day 1 to 28 in the sterile 5.0 g/L rice straw extract treatment group ($P<0.05$). On day 28, concentrations in this treatment group were similar to concentrations in flasks treated with 10 g/L rice straw extract (with bacteria; $P<0.05$). Flavonoids also increased from day 1 to day 4 in the control. The control and H_2O_2 treatments, with and without bacteria, had the smallest concentration of flavonoids on all days except for day 15 ($P<0.05$). On day 15, the control had a greater concentration of flavonoids than flasks treated with 50 mg/L H_2O_2, with and without bacteria ($P<0.05$).

In pond water *M. aeruginosa* cultures on day 0 through 28, phenolic concentrations decreased in flasks treated with 10 g/L rice straw extract (with and without bacteria) and 100 mg/L H_2O_2 with bacteria (Figure 10). Total phenolics were greatest in both 10 g/L rice straw extract treatment groups over the duration of incubation ($P<0.05$).

In pond water-based *R. subcapitata* cultures, flavonoid concentrations increase between day 15 and 28 in the control without bacteria and decreased in flasks treated with 10 g/L rice straw extract without bacteria (Figure 11). Similar to *M. aeruginosa* pond water experiments, 10 g/L rice straw extract treatment groups with and without bacteria contained the greatest flavonoid concentrations followed by 5.0 g/L rice straw extract, with and without bacteria ($P<0.05$). The smallest flavonoid concentrations were found in the controls and all H_2O_2 treatments ($P<0.05$).

Phenolic concentrations in pond water-based *R. subcapitata* cultures decreased in all rice straw extract treatments over time (($P<0.05$; Figure 12). After day 4, phenolics were detected only in flasks treated with 10 g/L rice straw extracts (with and without bacteria) and continued to decrease until there was no difference between treatment groups on day 28 ($P<0.05$).

3.2.3 Hydrogen peroxide

In pond water *M. aeruginosa* experiments, H_2O_2 (mg/L) was measured for 28 days following inoculation. Over the incubation period, flasks treated with 100 mg/L H_2O_2 maintained the greatest H_2O_2 concentrations. Flasks treated with 50 mg/L H_2O_2, with and without bacteria, had the second greatest concentrations from day 0 to day 15. After day 15, H_2O_2 decreased in the 50 mg/L H_2O_2 (with bacteria) treatment group until concentrations were no different from the rice straw treatments or sterile control ($P<0.05$).

From day 1 to 15 in *M. aeruginosa* cultures, H_2O_2 increased in flasks treated with 10 g/L rice straw with bacteria, 5.0 g/L rice straw extract treatments with and without bacteria, and 100 mg/L H_2O_2 without bacteria (Figure 13a, b). The 10 g/L rice straw extract (with bacteria) treatment group was the only treatment with a greater H_2O_2 concentration after 28 days. On day 28, the 10 g/L rice straw treatment (with bacteria) contained more H_2O_2 than the control with bacteria ($P<0.05$).

In pond water *R. subcapitata* experiments, H_2O_2 was measured for 24 hours following inoculation (Figure 14a, b). Throughout the 24 hours, flasks treated with 100 mg/L H_2O_2 with and without bacteria had the highest H_2O_2 concentration followed by 50 mg/L H_2O_2 with and without bacteria and then 25 mg/L H_2O_2 without bacteria ($P<0.05$).

4. Discussion

4.1 Algal inhibition by rice and barley straw

In media-based cultures, the growth of *R. subcapitata* was inhibited by 5.0 g/L barley straw extract and further inhibited by 5.0 g/L rice straw extract, whereas the growth of *M. aeruginosa* was inhibited only by 5.0 g/L rice straw extract and was stimulated or uninhibited by the addition of barley straw extract. This pattern of inhibition is supported by previous studies in which cereal straws were found to be algistatic or stimulatory at low concentrations and algicidal at greater concentrations (Xiao et al., 2010; Choe and Jung, 2002; Hua et al., 2018). In a 2014 study, Su et al. found that rice straw administered at low concentrations (0.5 to 2.0 g/L) can temporarily stimulate algal growth by increasing the membrane potential, nutrient uptake, and metabolic activity of *M. aeruginosa*, whereas concentrations greater than 4.0 g/L rice straw caused a decrease in membrane potential that triggered autolysis. If cellular damage did occur

after treatment at low concentrations, *M. aeruginosa* was able to recover within seven to eight days (Su et al., 2014; Xiao et al., 2010).

However, the stimulatory versus inhibitory thresholds were determined in culture media after no more than 15 days (Su et al., 2014; Iredale et al., 2012). The results of this study suggest that algae can recover following treatment with slightly greater concentrations (2.5 g/L) after 15 days, and the algistatic versus algicidal threshold may occur at greater concentrations (5.0 g/L) when administered in pond water. The conditions of the natural environment must be considered as allelochemical stability is dependent on oxidative reduction potential, pH, temperature, light availability, existing biomass, grazing and bioaccumulation, or sorption, among other processes (Zhang et al., 2020; Delle Site, 2001; Li et al., 2021). As a result, inhibitory concentrations derived from laboratory media cultures may not be reliable or effective for in-situ implementation. Therefore, the effects of naturally occurring organic matter or a microbial community on stimulatory versus inhibitory thresholds need to be evaluated so that accurate recommendations can be made for long term, in-situ algal suppression.

4.2 Relationships between flavonoids, total phenolics, and H_2O_2

Evidence of allelochemical degradation was apparent in the decline of total phenolic compounds in *R. subcapitata* cultures. *R. subcapitata* are inhibited by phenolic compounds such as catechol and 3,4-dihydroxyphenylacetic acid (Fiorentino et al., 2003), but in practice *R. subcapitata* and microalgae are regularly used in wastewater systems for their ability to scavenge, transform, or remove phenolic compounds (Lindner and Pleissner, 2019; Zhang et al., 2020; Al-Dahhan et al., 2018). In this study, *R. subcapitata* showed an ability to degrade phenolic compounds derived from barley and rice straw extracts; however, the metabolic

consequences of phenolic degradation of specific compounds and concentrations as found in cereal straw extracts requires further investigation.

In flasks where algae did grow or recover, the release of phenolics compounds by *M. aeruginosa* and *R. subcapitata* counteracted a decline in total concentrations (Ferdous and Balia Yusof, 2021; Jin et al., 2021; Żyszka-Haberecht et al., 2018). In media-based *M. aeruginosa* cultures, the production of flavonoids and phenolics resulted in a net increase in concentrations over 28 days. In pond water *M. aeruginosa* cultures, flavonoid production led to stable concentrations over time except in treatments where algal biomass was stimulated; in *R. subcapitata* this trend was restricted to flavonoids. A decline in total phenolic concentration was also recorded in *M. aeruginosa* pond water cultures treated with 10 g/L rice straw extract with and without bacteria. At this treatment concentration algal growth was clearly inhibited and the pattern of phenolic degradation was similar in treatments with bacteria versus without bacteria over the 28-day experiment. Therefore, the decrease in total phenolics in these treatment groups can likely be attributed to abiotic degradation in pond water.

The lack of significant changes in H_2O_2 results combined with significant phenolic and flavonoid concentrations between treatments support the hypotheses that algal inhibition cannot be attributed to H_2O_2 alone. The flavonoids and phenolic compounds released during the decomposition of cereal straw contribute to inhibition of green algae and cyanobacteria. It is clear, based on the results of this and other studies, that total extract concentration is an important factor for inhibition of *R. subcapitata* and *M. aeruginosa*. However, even at similar total extract, phenolic, and flavonoid concentrations, rice straw was more effective than barley straw at inhibiting the growth of both *R. subcapitata* and *M. aeruginosa,* further supporting the

hypothesis that inhibition can be attributed to the release of specific phenolic and polyphenolic compounds from decomposing cereal straw (Choe and Jung et al., 2002; Park et al., 2006).

While the inhibitory effect of total rice and barley straw extract concentrations did support previous findings, total flavonoid and phenolic concentrations were not helpful in explaining the effects of rice and barley straw. Identification of specific compounds released during the aerobic photobiodegradation of cereal straws and the fate of these compounds in cultures over time would be beneficial in understanding the algistatic or algicidal ability of rice straw.

4.3 Microbial community on effectiveness of treatments

In *R. subcapitata* pond water cultures, the control with bacteria experienced less algal growth than the control without bacteria. In *M. aeruginosa* pond water cultures, the control with bacteria surpassed the control without bacteria between day 15 and 28. While analyzing the microbial community before, during, and after a dinoflagellate harmful algal bloom, Zhou et al. (2020) found complex interactions between microorganisms including competition, predation, parasitism, and cross-feeding. Though the microbial community of the pond water used in this study was not analyzed, the suppression of algal growth in the green algae bacterial control and the variability in responses between treatments of the same concentration with and without bacteria show that the microbial community is important in determining bloom behavior and treatment response.

In an effort to predict and mitigate harmful algal blooms, affected waterbodies are routinely monitored for environmental variables. However, a study by Needham and Fuhrman

(2016) found that phytoplankton had a stronger correlation with prokaryotic communities than with water or air temperature, salinity, pH, precipitation, wind speed, or wave height preceding a bloom event. While evaluating the effects of different H_2O_2 concentrations on cyanobacteria and non-target species in natural waters, Lusty and Gobler (2020, 2023) and Piel et al. (2021) found that H_2O_2 did reduce cyanobacterial biomass; however, green algae and fecal bacteria indicators consistently and significantly increased in response to the treatment. Likewise, the results of this study support the hypothesis that the microbial community is a vital component of algal bloom dynamics and must be considered when deciding on treatment compounds, concentrations, and application methods. Therefore, the effectiveness of in-situ rice straw applications cannot be considered without regard for the original microbial community.

5. Conclusion

Rice and barley straw are effective at controlling the growth of *M. aeruginosa* and *R. subcapitata* in media. At similar concentrations, rice straw extract was more effective than barley straw at suppressing the growth of both *R. subcapitata* and *M. aeruginosa*. For reliable long-term suppression, greater than 2.5 g/L rice straw extract in media and 5.0 g/L rice straw extract in pond water is necessary, possibly due to the instability of allelochemicals. The lack of significance in H_2O_2 between treatments supports the hypothesis that inhibition is related to the release of other allelochemicals, such as flavonoids or other phenolic compounds.

However, total concentrations were not helpful in determining the mechanism(s) causing the inhibitory effect of cereal straws. Rather, individual compounds produced from photoaerobically biodecomposing rice straw should be identified to determine the algistatic effect on algal species and on the original microbial community at concentrations found in decomposing rice straw extracts. Additionally, the original microbial community composition

within the context of abiotic conditions of natural waters (pH, nutrient concentrations, sediment, and temperature, etc.) alters algal dynamics and response to treatment. Therefore, these conditions must be considered when choosing treatment methods and concentrations.

Rice straw waste is predominately managed through soil incorporation or through burning that contributes to serious respiratory conditions, greenhouse gas emissions, and soil and nutrient losses (Skaug, 2017; Bhattacharyya et al., 2021). In contrast to many current algal control methods, cereal straws have been recognized as environmentally safe algal inhibitors, are safe for non-target species, and can easily be applied with little to no additional management (Zhu et al., 2021). Harnessing the potential energy of rice straw in bio-based decomposition products, such as algal inhibitors, could incentivize less harmful agricultural waste management practices and promote environmentally conscious algal control methods.

Acknowledgements

The authors would like to thank Nathaniel Elliot and members of the Soil Biology and Microbial Ecology lab for technical assistance. The authors would also like to thank the USGS 104B grant 003501-00001A through the Arkansas Watershed Resources Center for funding this work. This project was partially funded by USDA-NIFA Hatch project 1024383, and the University of Arkansas System Division of Agriculture.

Literature Cited

Akagawa, M., Shigemitsu, T., & Suyama, K. (2003). Production of hydrogen peroxide by polyphenols and polyphenol-rich beverages under quasi-physiological conditions. *Bioscience, Biotechnology, and Biochemistry*, *67*(12), 2632–2640. https://doi.org/10.1271/bbb.67.2632

Al-Dahhan M., Al-Ani, F., Al-Saned, A. (2018) Biodegradation of phenolic components in wastewater by micro algae: a review. *MATEC Web of Conferences,* vol. 162. EDP Sciences, pp. 05009.

Bandala, E. R., Martínez, D., Martínez, E., & Dionysiou, D. D. (2004). Degradation of Microcystin-LR toxin by fenton and photo-fenton processes. *Toxicon, 43*(7), 829-832. doi:10.1016/j.toxicon.2004.03.013

Bhattacharyya, P., Bisen, J., Bhaduri. D., Priyadarsini, S., Munda, S., Chakraborti, M., Adak, T., Panneerselvam, P., Mukherjee, A., Swain, S.L. (2021) Turn the wheel from waste to wealth: Economic and environmental gain of sustainable rice straw management practices over field burning in reference to India. *Science of the Total Environment* 775: 145896.

Brocks, J., Logan, G., Buick, R., & Summons, R. (1999). Archean molecular fossils and the early rise of eukaryotes. *Science, 285*(5430), 1033-1036.

Choe, S., Jung, I. (2002) Growth inhibition of freshwater algae by ester compounds released from rotted plants. *Journal of Industrial and Engineering Chemistry* 8: 297-304.

Cornish, B. J., Lawton, L. A., & Robertson, P. K. (2000). Hydrogen peroxide enhanced photocatalytic oxidation of microcystin-LR using titanium dioxide. *Applied Catalysis B: Environmental, 25*(1), 59-67. doi:10.1016/s0926-3373(99)00121-6

Delle Site, A. (2001) Factors affecting sorption of organic compounds in natural sorbent/water systems and sorption coefficients for selected pollutants. A review. *Journal of Physical and Chemical Reference Data* 30: 187-439.

Everall N., Lees D. (1996) The use of barley-straw to control general and blue-green algal growth in a Derbyshire reservoir. *Water Research* 30: 269-276.

Everall N., Lees D. (1997) The identification and significance of chemicals released from decomposing barley straw during reservoir algal control. *Water Research* 31: 614-620.

Farasat, M., Khavari-Nejad, R-A, Nabavi, S.M.B., Namjooyan, F. (2014) Antioxidant activity, total phenolics and flavonoid contents of some edible green seaweeds from northern coasts of the Persian Gulf. *Iranian journal of pharmaceutical research*: IJPR 13: 163.

Ferdous, U.T., Balia Yusof, Z.N. (2021) Insight into potential anticancer activity of algal flavonoids: current status and challenges. *Molecules* 26: 6844.

Fiorentino, A., Gentili, A., Isidori, M., Monaco, P., Nardelli, A., Parrella, A., Temussi, F. (2003) Environmental effects caused by olive mill wastewaters: toxicity comparison of low-molecular-weight phenol components. *Journal of Agricultural and Food Chemistry* 51: 1005-1009.

Giannuzzi, L., Krock, B., Minaglia, M. C., Rosso, L., Houghton, C., Sedan, D., Malanga, G., Espinosa, M., Andrinolo, D., &Hernando, M. (2016). Growth, toxin production, active oxygen species and catalase activity of *Microcystis aeruginosa* (cyanophyceae) exposed to temperature stress. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology,189,* 22-30. doi:10.1016/j.cbpc.2016.07.001

Hua, Q., Liu, Y.-g., Yan, Z.-l., Zeng, G.-m., Liu, S.-b., Wang, W.-j., Tan, X.-f., Deng, J.-q., Tang, X., & Wang, Q.-p. (2018). Allelopathic effect of the rice straw aqueous extract on the growth of Microcystis aeruginosa. *Ecotoxicology and Environmental Safety, 148*, 953-959.

Iredale R.S., McDonald A.T., Adams D.G. (2012) A series of experiments aimed at clarifying the mode of action of barley straw in cyanobacterial growth control. *Water research* 46: 6095-6103.

Jin, H., Wang, Y., Zhao, P., Wang, L., Zhang, S., Meng, D., Yang, Q., Cheong L-Z, Bi Y., Fu, Y. (2021) Potential of Producing Flavonoids Using Cyanobacteria As a Sustainable Chassis. *Journal of Agricultural and Food Chemistry* 69: 12385-12401. Ma, H., Zhang, J., Tong, L., & Yang, J. (2015). Photochemical production of hydrogen peroxide from natural algicides: decomposition organic matter from straw. *Environmental Science: Processes & Impacts*, *17*(8), 1455-1461.

Li, J., Cao, L., Guo, Z., An, G., Li, B., Li, J. (2021) Time-and dose-dependent allelopathic effects and mechanisms of kaempferol on toxigenic Microcystis growth. *Ecotoxicology and Environmental Safety* 222: 112508.

Lindner, A.V., Pleissner, D. (2019) Utilization of phenolic compounds by microalgae. *Algal Research* 42: 101602. Ma, H., Huang, L., Zhang, J., Shi, D., & Yang, J. (2018). Optical properties of straw-derived dissolved organic matter and growth inhibition of *Microcystis aeruginosa* by straw-derived dissolved organic matter via photo-generated hydrogen peroxide. *Environmental Pollution, 242,* 760-768. doi:10.1016/j.envpol.2018.07.052

Lusty, M.W., Gobler, C.J. (2020) The efficacy of hydrogen peroxide in mitigating cyanobacterial blooms and altering microbial communities across four lakes in NY, USA. *Toxins* 12: 428. Margraf, T., Karnopp, A. R., Rosso, N. D., & Granato, D. (2015). Comparison between Folin-Ciocalteu and Prussian Blue assays to estimate the total phenolic content of juices and teas using 96-well microplates. *Journal of Food Science, 80*(11), C2397-C2403.

Lusty, M.W., Gobler, C.J. (2023) Repeated hydrogen peroxide dosing briefly reduces cyanobacterial blooms and microcystin while increasing fecal bacteria indicators in a eutrophic pond. *Journal of Environmental Sciences* 124: 522-543.

Maris, J. (2019). Evaluating rice straw as a substitute for barley straw in inhibiting algal growth in farm ponds. *Crop, Soil and Environmental Sciences Undergraduate Honors Theses* Retrieved from https://scholarworks.uark.edu/csesuht/19

Matthijs, H. C., Visser, P. M., Reeze, B., Meeuse, J., Slot, P. C., Wijn, G., Talens, R., &Huisman, J. (2012). Selective suppression of harmful cyanobacteria in an entire lake with hydrogen peroxide. *Water Research, 46*(5), 1460-1472. doi:10.1016/j.watres.2011.11.016

Murray, D., Jefferson, B., Jarvis, P., & Parsons, S. (2010). Inhibition of three algae species using chemicals released from barley straw. *Environmental Technology, 31*(4), 455-466. doi:10.1080/09593331003663294

Needham, D.M., Fuhrman, J.A. (2016) Pronounced daily succession of phytoplankton, archaea and bacteria following a spring bloom. *Nature microbiology* 1: 1-7.

Paerl, H.W. (2014). Mitigating harmful cyanobacterial blooms in a human- and climatically-impacted world. *Life, 4*(4), 988-1012. doi:10.3390/life4040988

Paerl, H.W., Otten, T.G. (2013). Harmful cyanobacterial blooms: causes, consequences, and controls. *Microbial Ecology* 65, 995–1010 https://doi.org/10.1007/s00248-012-0159-y

Park, M.H., Han, M.S., Ahn, C.Y., Kim, H.S., Yoon, B.D., Oh, H.M. (2006) Growth inhibition of bloom-forming cyanobacterium Microcystis aeruginosa by rice straw extract. *Letters in Applied Microbiology* 43: 307-312.

Piel, T., Sandrini, G,, Muyzer, G., Brussaard, C.P., Slot, P.C., van Herk, M.J., Huisman, J., Visser, P.M. (2021) Resilience of microbial communities after hydrogen peroxide treatment of a eutrophic lake to suppress harmful cyanobacterial blooms. *Microorganisms* 9: 1495.

Pillinger J., Cooper J., Ridge I. (1994) Role of phenolic compounds in the antialgal activity of barley straw. *Journal of Chemical Ecology* 20: 1557-1569.

Pueyo, I. U., & Calvo, M. I. (2009). Assay conditions and validation of a new UV spectrophotometric method using microplates for the determination of polyphenol content. *Fitoterapia, 80*(8), 465-467.

Putt, K.S., Pugh, R.B. (2013) A high-throughput microtiter plate based method for the determination of peracetic acid and hydrogen peroxide. *Plos one* 8: e79218.

Skaug W (2017) Agricultural Burning in NE Arkansas: What It's Costing Us. The Children's Clinic.

Su, W., Hagström, J.A., Jia, Y., Lu, Y., Kong, F. (2014) Effects of rice straw on the cell viability, photosynthesis, and growth of Microcystis aeruginosa. *Chinese Journal of Oceanology and Limnology* 32: 120-129.

Summons, R.E., Jahnke, L.L., Hope, J.M., & Logan, G.A. (1999). 2-methylhopanoids as biomarkers for cyanobacterial oxygenic photosynthesis. *Nature, 400(6744), 554-557. doi:10.1038/23005*

Wang, B., Song, Q., Long, J., Song, G., Mi, W., & Bi, Y. (2019). Optimization method for *Microcystis* bloom mitigation by hydrogen peroxide and its stimulative effects on growth of chlorophytes. *Chemosphere, 228*, 503-512.

Waybright T.J., Terlizzi D.E., Ferrier M.D. (2009) Chemical characterization of the aqueous algistatic fraction of barley straw (*Hordeum vulgare*) inhibiting *Microcystis aeruginosa*. *Journal of Applied Phycology* 21: 333-340.

Weenink, E. F., Luimstra, V. M., Schuurmans, J. M., Herk, M. J., Visser, P. M., & Matthijs, H. C. (2015). Combatting cyanobacteria with hydrogen peroxide: A laboratory study on the consequences for phytoplankton community and diversity. *Frontiers in Microbiology, 6.* doi:10.3389/fmicb.2015.00714

Xiao, X., Chen, Y-x., Liang, X-q., Lou, L-p., Tang, X-j. (2010) Effects of Tibetan hulless barley on bloom-forming cyanobacterium (Microcystis aeruginosa) measured by different physiological and morphologic parameters. *Chemosphere* 81: 1118-1123.

Zhang, C., Wang, X., Ma, Z., Luan, Z., Wang, Y., Wang, Z., Wang, L. (2020) Removal of phenolic substances from wastewater by algae. A review. *Environmental Chemistry* Letters 18: 377-392.

Zhang, Y., Del Vecchio, R., & Blough, N. V. (2012). Investigating the mechanism of hydrogen peroxide photoproduction by humic substances. *Environmental Science & Technology, 46*(21), 11836-11843. doi:10.1021/es3029582

Zhu, X., Dao, G., Tao, Y., Zhan, X., Hu, H. (2021) A review on control of harmful algal blooms by plant-derived allelochemicals. *Journal of Hazardous Materials* 401: 123403.

Żyszka-Haberecht, B., Niemczyk, E., Lipok, J. (2019) Metabolic relation of cyanobacteria to aromatic compounds. *Applied microbiology and biotechnology* 103: 1167-1178.

Table 1. Each chemical (hydrogen peroxide or decomposing straw extract) treatment added to construct microcosms for bioassays for analysis of inhibition of cyanobacteria and green algae.

Experiment 1		Experiments 2 and 3	
Treatment[1]	Aqueous solution (mL solution added)	Treatment	Treatment addition[2] (mL of treatment)
Control	325 MQ + 325 2x media	Pond water control	487.5 PW + 162.5 MQ
2.5 g/L BSE	325 BSE + 325 2x media	Bacteria control	487.5 PW(b) + 162.5 MQ
5.0 g/L BSE	325 BSE + 325 2x media	25 mg/L H_2O_2, no bacteria	487.5 PW + 162.5 H_2O_2
2.5 g/L RSE	325 RSE + 325 2x media	25 mg/L H_2O_2 with bacteria	487.5 PW(b) + 162.5 H_2O_2
5.0 g/L RSE	325 RSE + 325 2x media	50 mg/L H_2O_2, no bacteria	487.5 PW + 162.5 H_2O_2
		50 mg/L H_2O_2 with bacteria	487.5 PW(b) + 162.5 H_2O_2
		100 mg/L H_2O_2, no bacteria	487.5 PW + 162.5 H_2O_2
		100 mg/L H_2O_2 with bacteria	487.5 PW(b) + 162.5 H_2O_2
		5 g/L RSE, no bacteria	487.5 PW + 162.5 RSE
		5 g/L RSE, with bacteria	487.5 PW(b) + 162.5 RSE
		10 g/L RSE, no bacteria	487.5 PW + 162.5 RSE
		10 g/L RSE, with bacteria	487.5 PW(b) + 162.5 RSE

[1]BSE is barley straw extract, RSE is rice straw extract, H_2O_2 is hydrogen peroxide, MQ is Milli-Q water, PW is pond water, (b) is bacteria included from collected pond water

[2]mL of treatment pond water added at the same filtration pore size as the background matrix

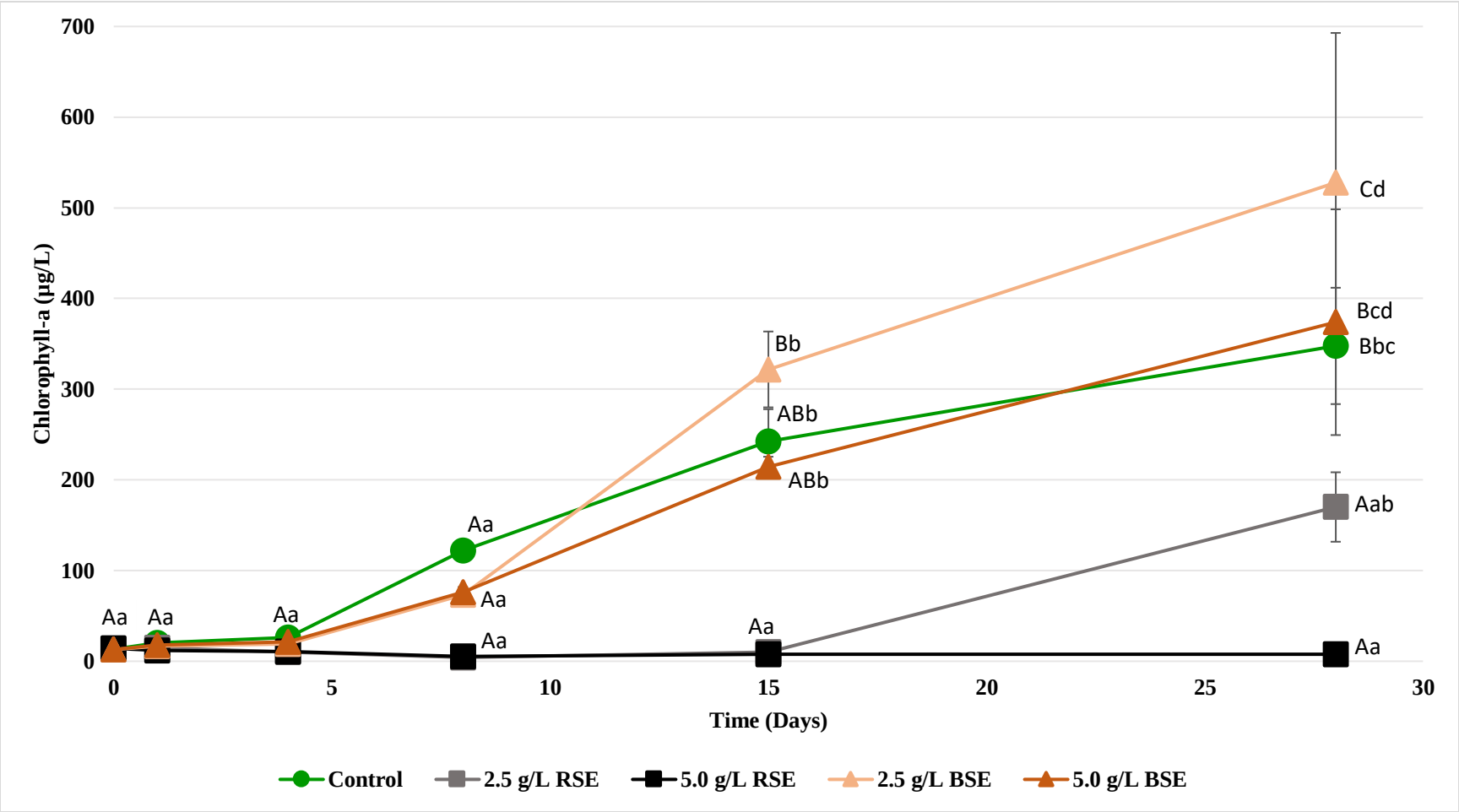


Figure 1. Chlorophyll-a concentrations (µg/L) in media based *Microcystis aeruginosa* cultures for 0 to 28 days following treatment with rice straw extract (RSE), barley straw extract (BSE), or no treatment (control) (n=3). Sampling times followed by similar uppercase letters within a treatment represent lack of significant differences within that treatment over time ($P > 0.05$). Similar lowercase letters represent lack of significant differences between the treatments ($P < 0.05$).

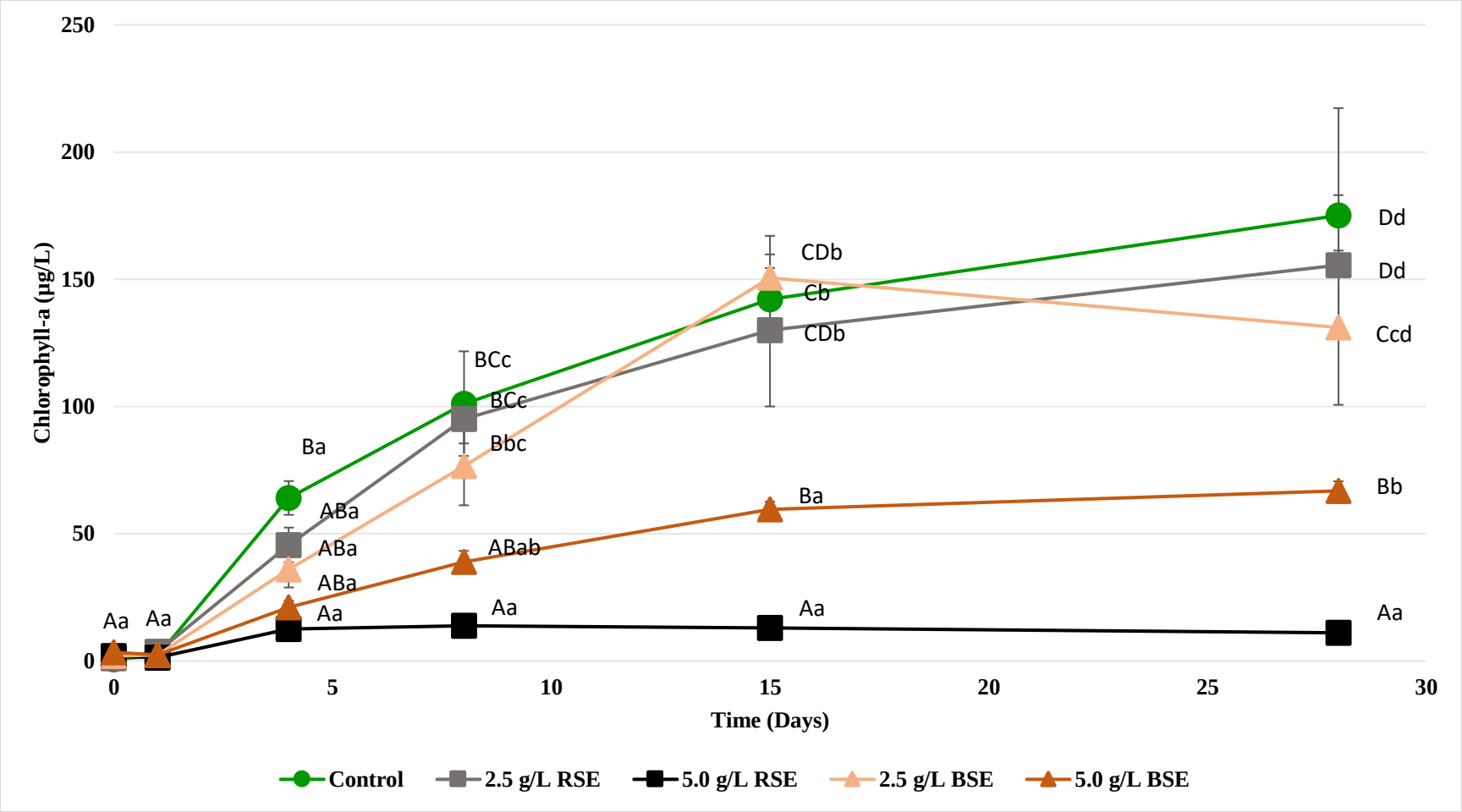


Figure 2. Chlorophyll-a concentrations (µg/L) in media based *Raphidocelis subcapitata* cultures for 0 to 28 days following treatment with rice straw extract (RSE), barley straw extract (BSE), or no treatment (control, C) (n=3). Sampling times followed by similar uppercase letters within a treatment represent lack of significant differences within that treatment over time ($P > 0.05$). Similar lowercase letters represent lack of significant differences between the treatments ($P < 0.05$).

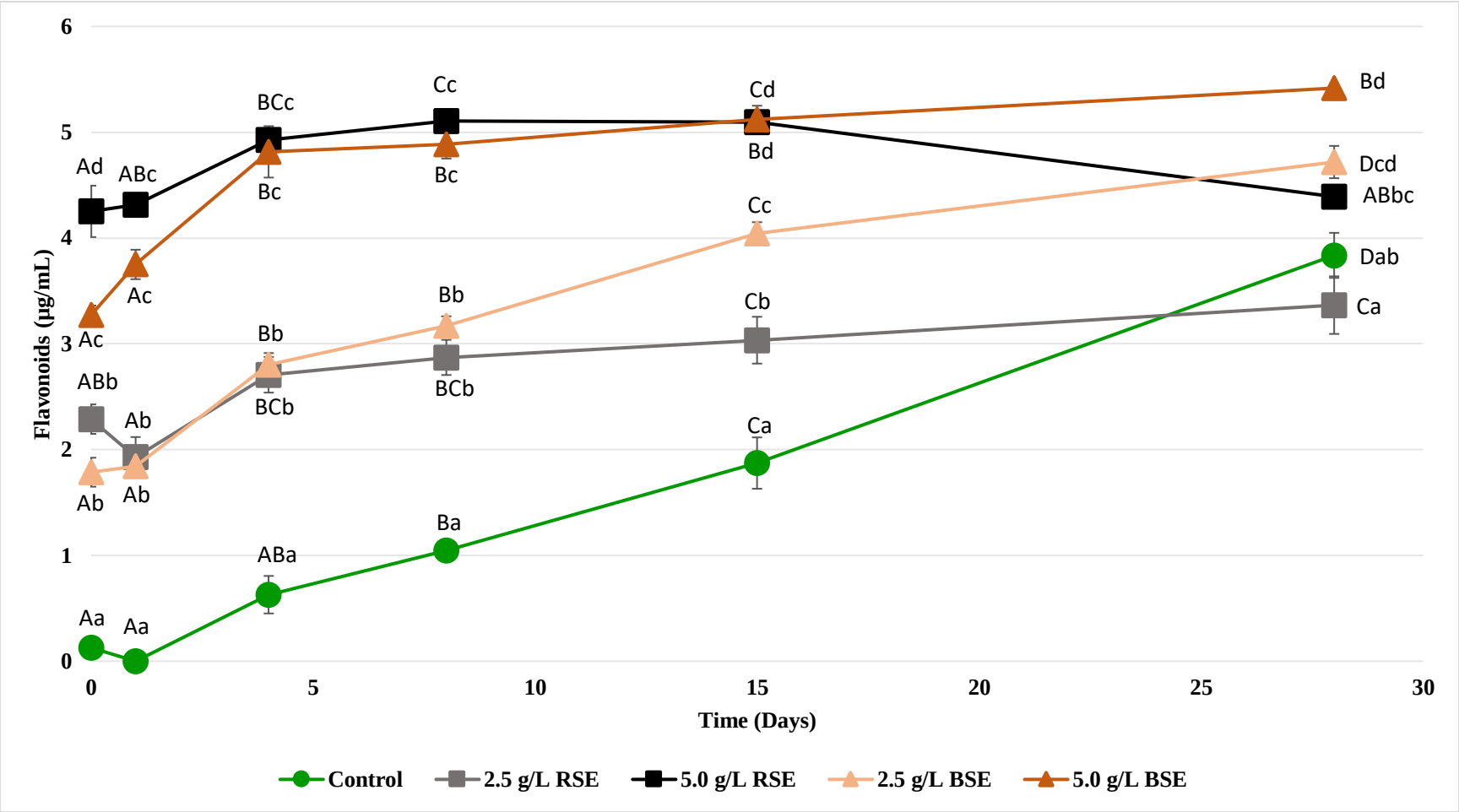


Figure 3. Flavonoid concentrations (µg/mL) in quercetin equivalents in media based *Microcystis aeruginosa* cultures for 0 to 28 days following treatment with rice straw extract (RSE), barley straw extract (BSE), or no treatment (control) (n=3). Sampling times followed by similar uppercase letters within a treatment represent lack of significant differences within that treatment over time ($P > 0.05$). Similar lowercase letters represent lack of significant differences between the treatments ($P < 0.05$).

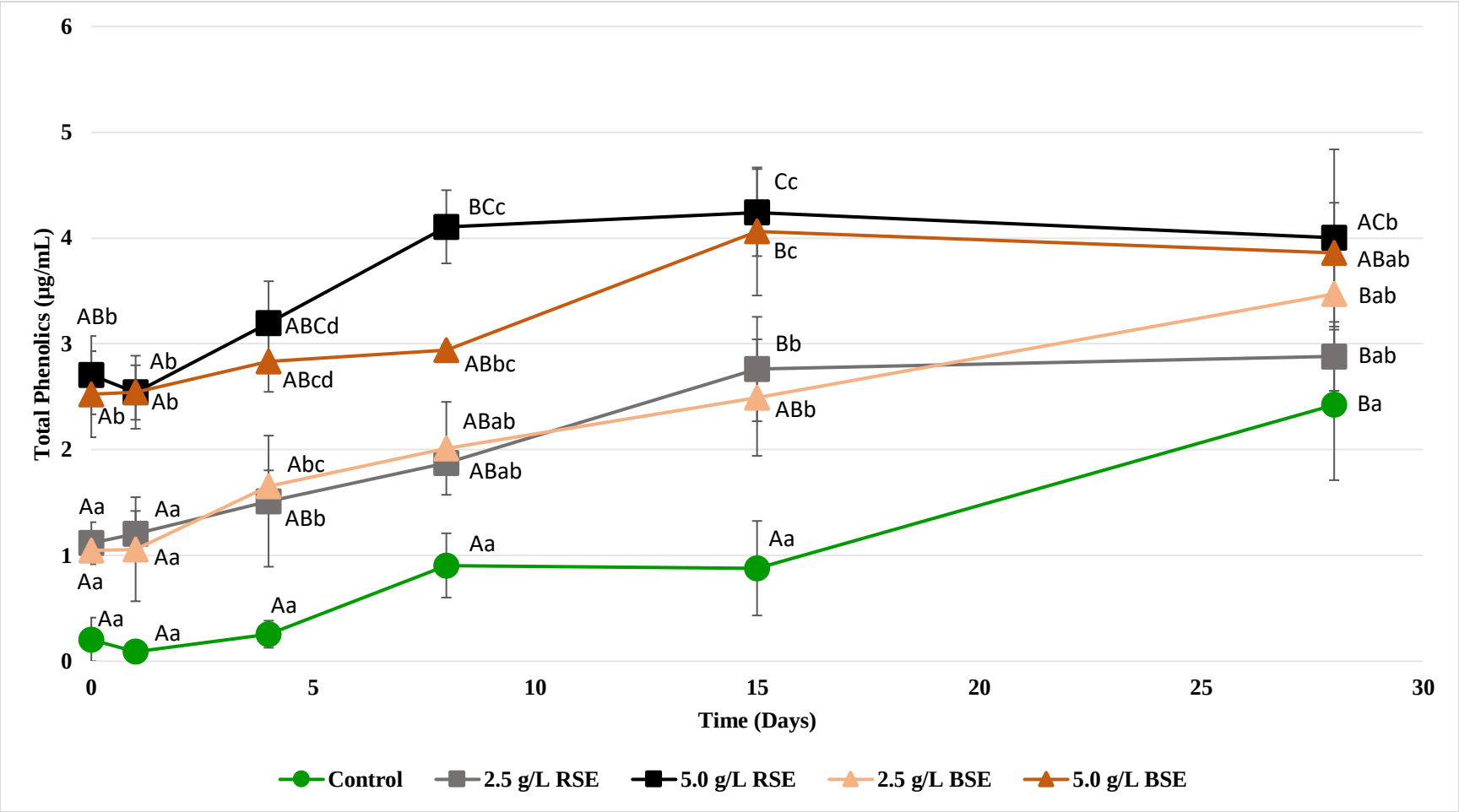


Figure 4. Total phenolic concentrations (µg/mL) in gallic acid equivalents in media based *Microcystis aeruginosa* cultures for 0 to 28 days following treatment with rice straw extract (RSE), barley straw extract (BSE), or no treatment (control, C) (n=3). Sampling times followed by similar uppercase letters within a treatment represent lack of significant differences within that treatment over time ($P > 0.05$). Similar lowercase letters represent lack of significant differences between the treatments ($P < 0.05$).

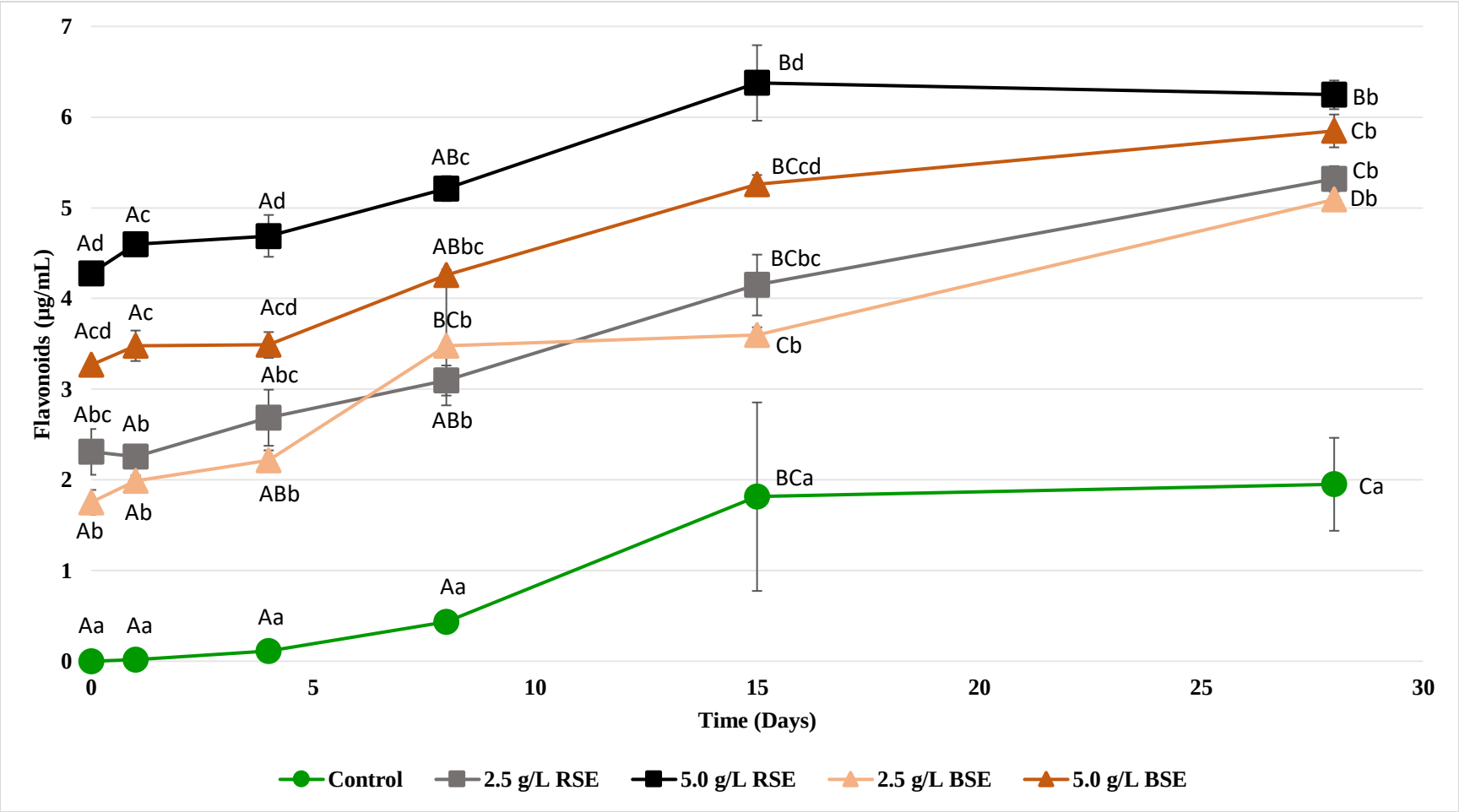


Figure 5. Flavonoid concentrations (µg/mL) in quercetin equivalents in media based *Raphidocelis subcapitata* cultures for 0 to 28 days following treatment with rice straw extract (RSE), barley straw extract (BSE), or no treatment (control, C) (n=3). Sampling times followed by similar uppercase letters within a treatment represent lack of significant differences within that treatment over time ($P > 0.05$). Similar lowercase letters represent lack of significant differences between the treatments ($P < 0.05$).

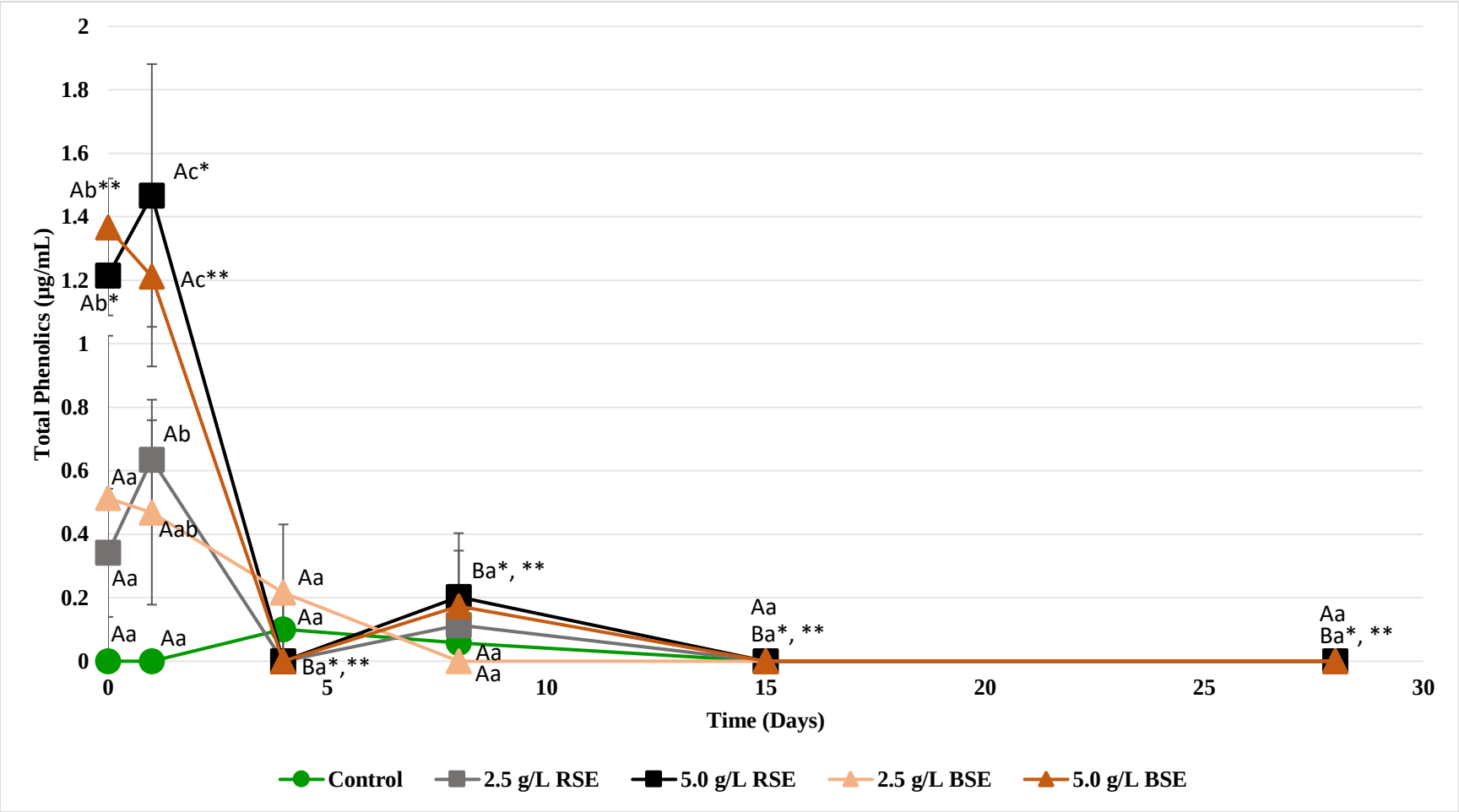


Figure 6. Total phenolic concentrations (µg/mL) in gallic acid equivalents in media based *Raphidocelis subcapitata* cultures for 0 to 28 days following treatment with rice straw extract (RSE), barley straw extract (BSE), or no treatment (control, C) (n=3). Sampling times followed by similar uppercase letters within a treatment represent lack of significant differences within that treatment over time ($P > 0.05$). Similar lowercase letters represent lack of significant differences between the treatments ($P < 0.05$).

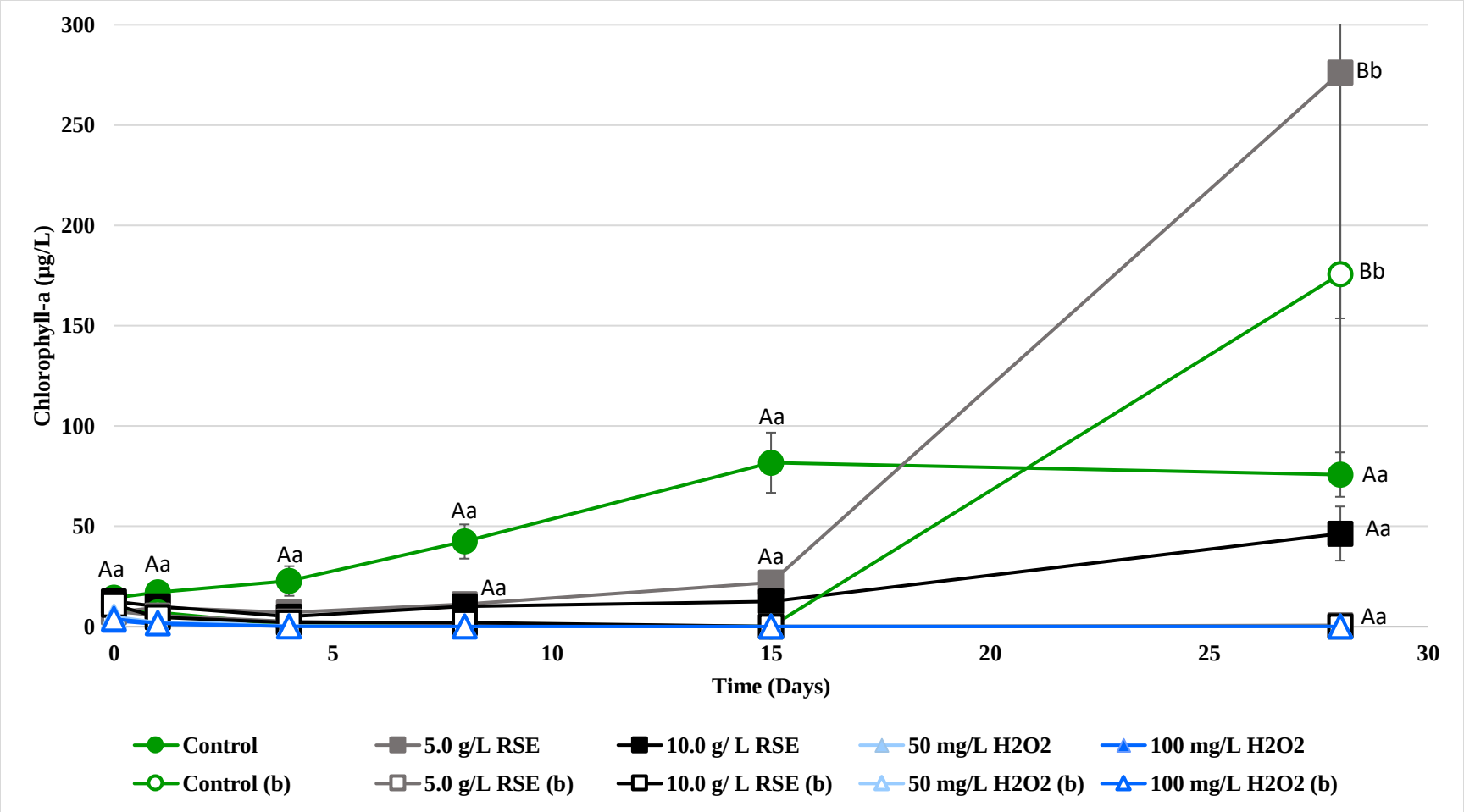


Figure 7. Chlorophyll-a concentrations (µg/L) in pond water based *Microcystis aeruginosa* cultures for 0 to 28 days following treatment with rice straw extract (RSE), hydrogen peroxide (H_2O_2), or no treatment (control) and with bacteria (b) or without bacteria (n=3). Sampling times followed by similar uppercase letters within a treatment represent lack of significant differences within that treatment over time ($P > 0.05$). Similar lowercase letters represent lack of significant differences between the treatments ($P < 0.05$).

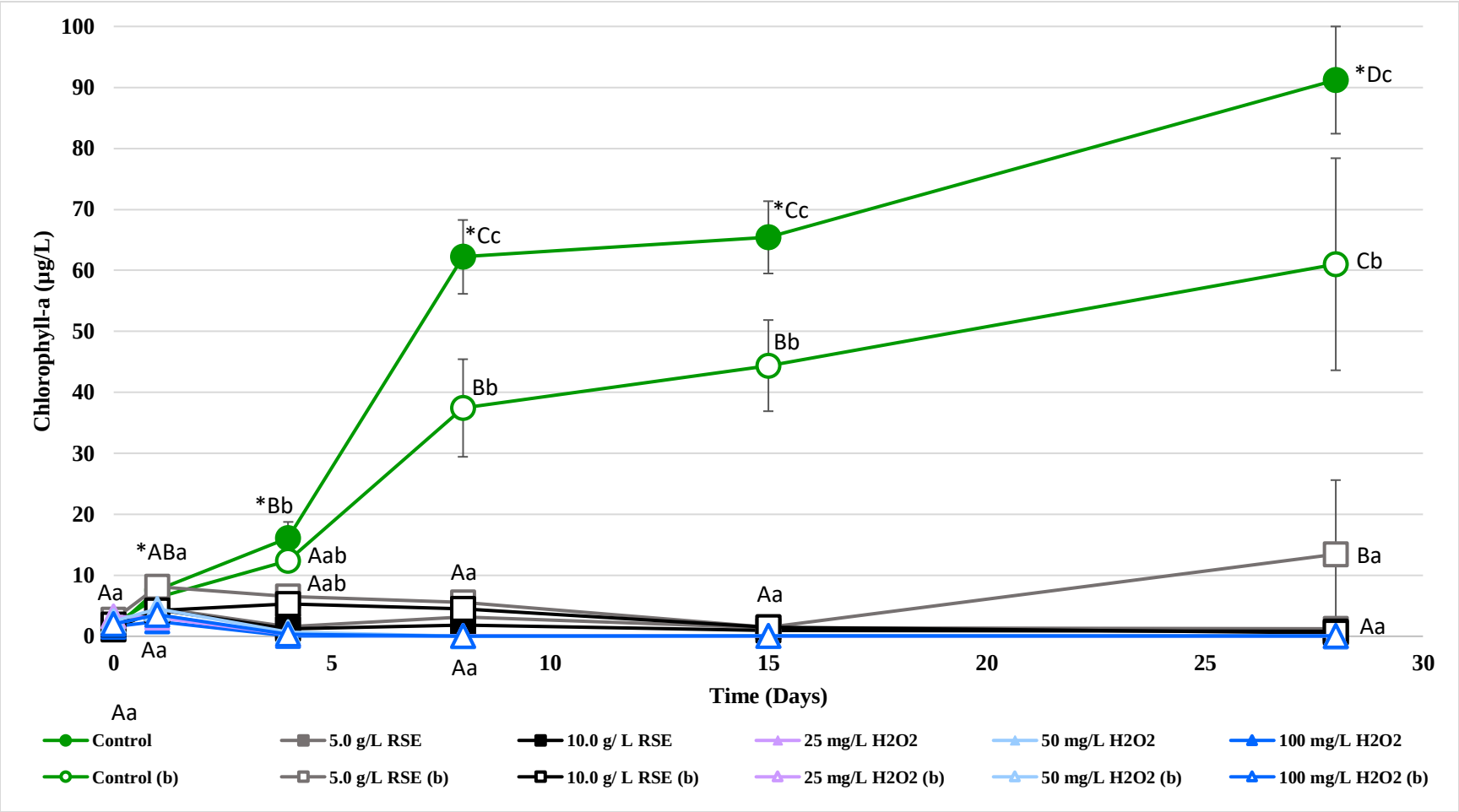


Figure 8. Chlorophyll-a concentrations (µg/L) in pond water based *Raphidocelis subcapitata* cultures for 0 to 28 days following treatment with rice straw extract (RSE), hydrogen peroxide (H_2O_2), or no treatment (control) and with bacteria (b) or without bacteria (n=3). Sampling times followed by similar uppercase letters within a treatment represent lack of significant differences within that treatment over time ($P > 0.05$). Similar lowercase letters represent lack of significant differences between the treatments ($P < 0.05$).

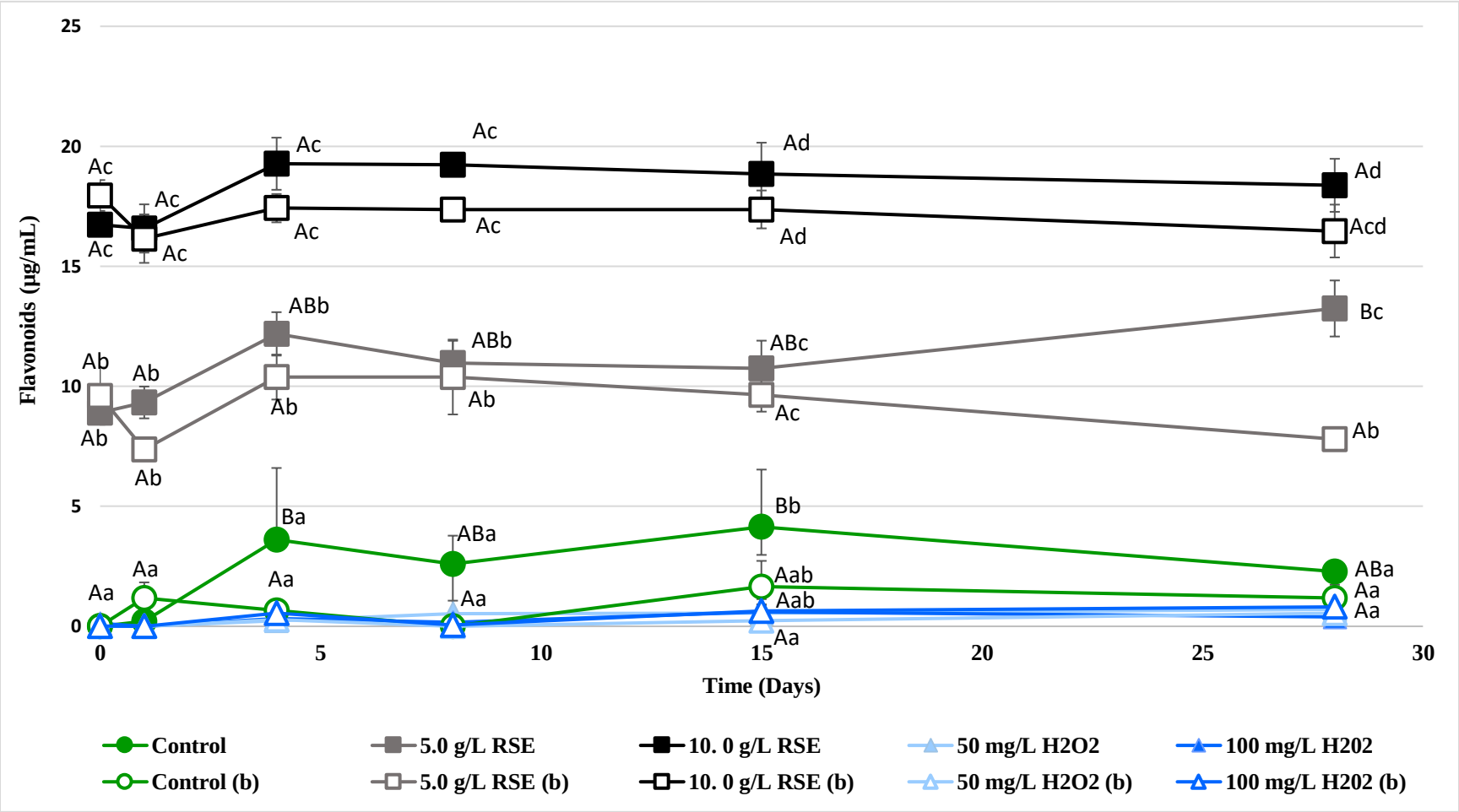


Figure 9. Flavonoid concentrations (µg/mL) in quercetin equivalents in pond water based *Microcystis aeruginosa* cultures for 0 to 28 days following treatment with rice straw extract (RSE), hydrogen peroxide (H_2O_2), or no treatment (control) and with bacteria (b) or without bacteria (n=3). Sampling times followed by similar uppercase letters within a treatment represent lack of significant differences within that treatment over time ($P > 0.05$). Similar lowercase letters represent lack of significant differences between the treatments ($P < 0.05$).

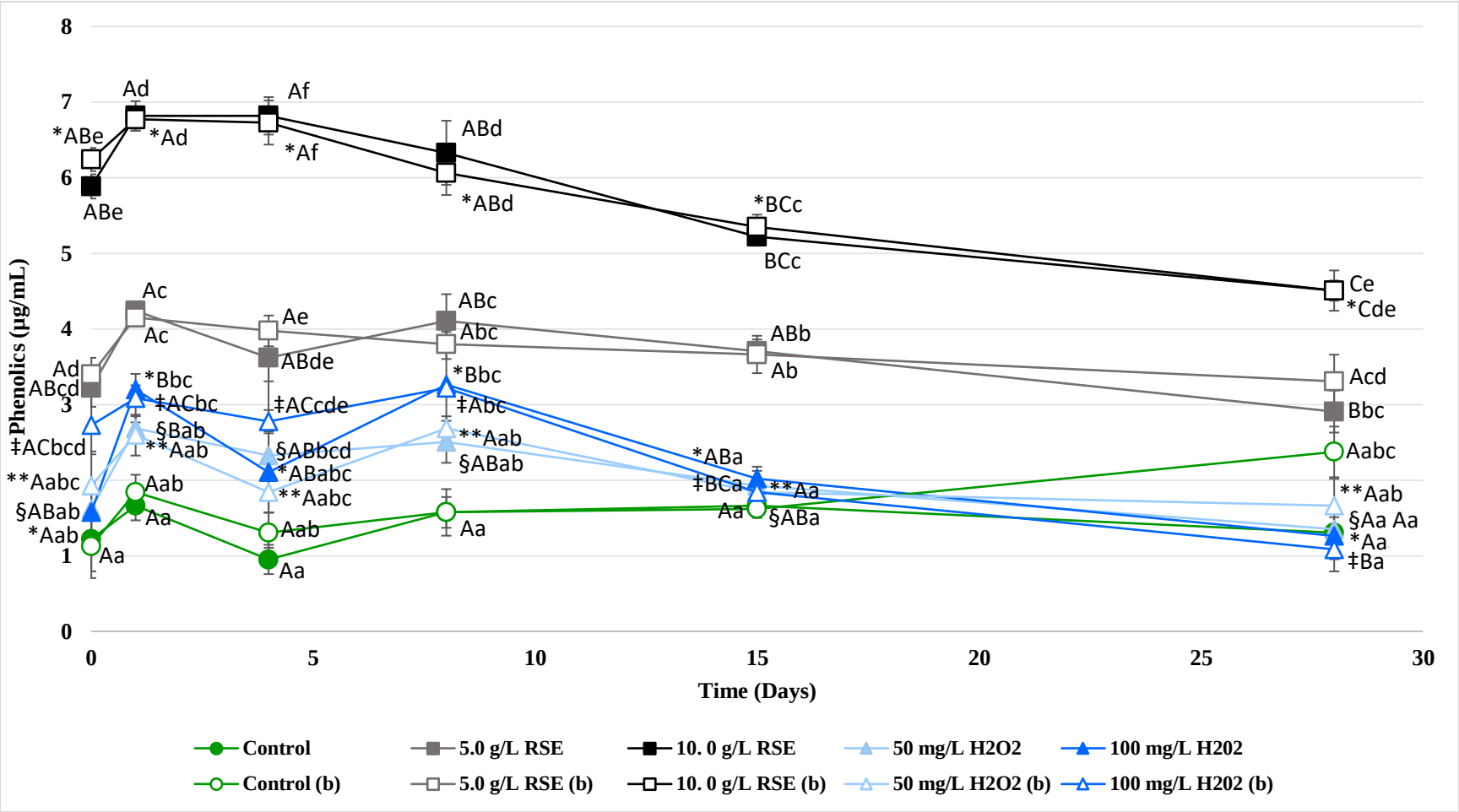


Figure 10. Phenolic concentrations (µg/mL) in gallic acid equivalents in pond water based *Microcystis aeruginosa* cultures for 0 to 28 days following treatment with rice straw extract (RSE), hydrogen peroxide (H_2O_2), or no treatment (control) and with bacteria (b) or without bacteria (n=3). Sampling times followed by similar uppercase letters within a treatment represent lack of significant differences within that treatment over time ($P > 0.05$). Similar lowercase letters represent lack of significant differences between the treatments ($P < 0.05$).

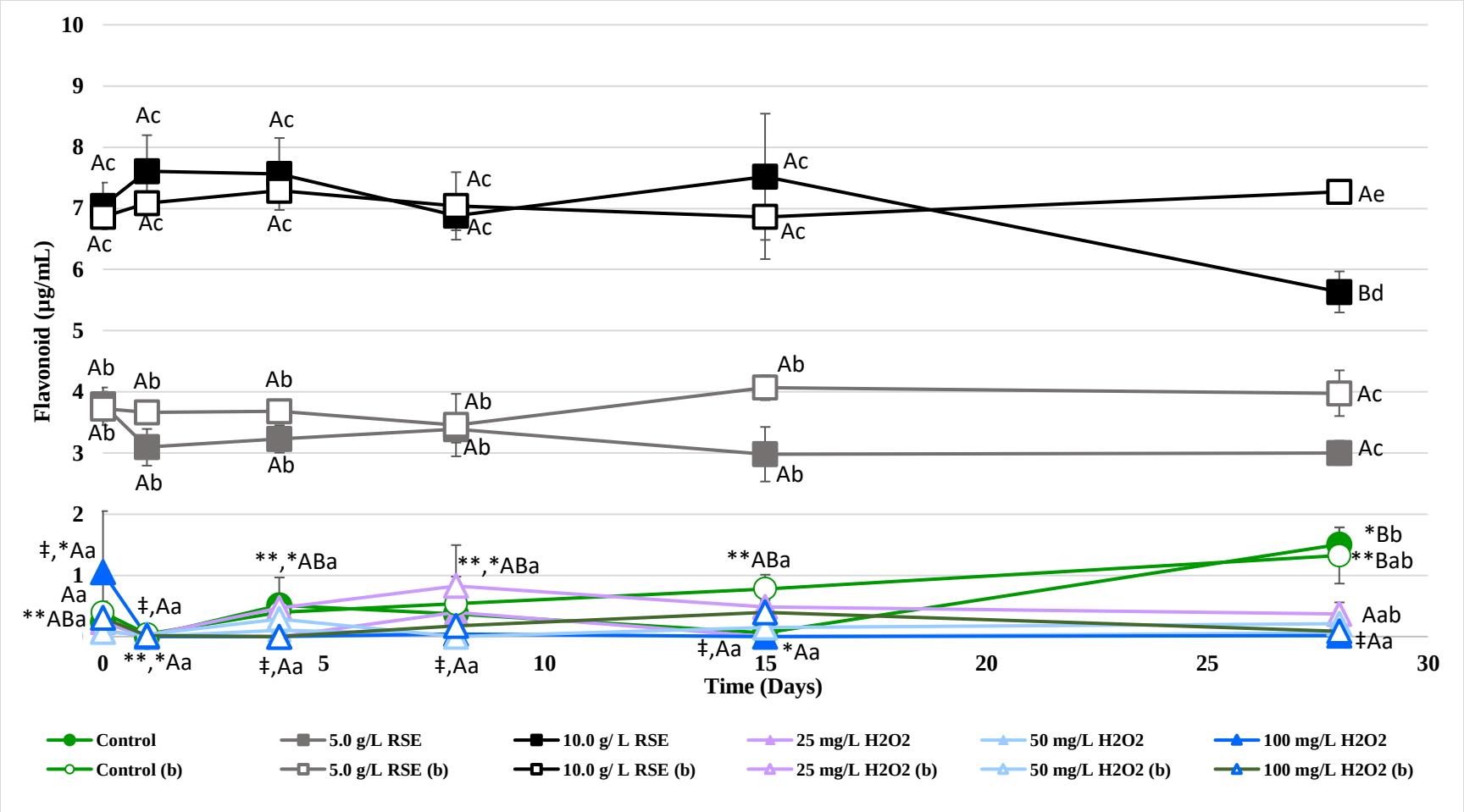


Figure 11. Flavonoid concentrations (µg/mL) in quercetin equivalents in pond water based *Raphidocelis subcapitata* cultures for 0 to 28 days following treatment with rice straw extract (RSE), hydrogen peroxide (H_2O_2), or no treatment (control) and with bacteria (b) or without bacteria (n=3). Sampling times followed by similar uppercase letters within a treatment represent lack of significant differences within that treatment over time ($P > 0.05$). Similar lowercase letters represent lack of significant differences between the treatments ($P < 0.05$).

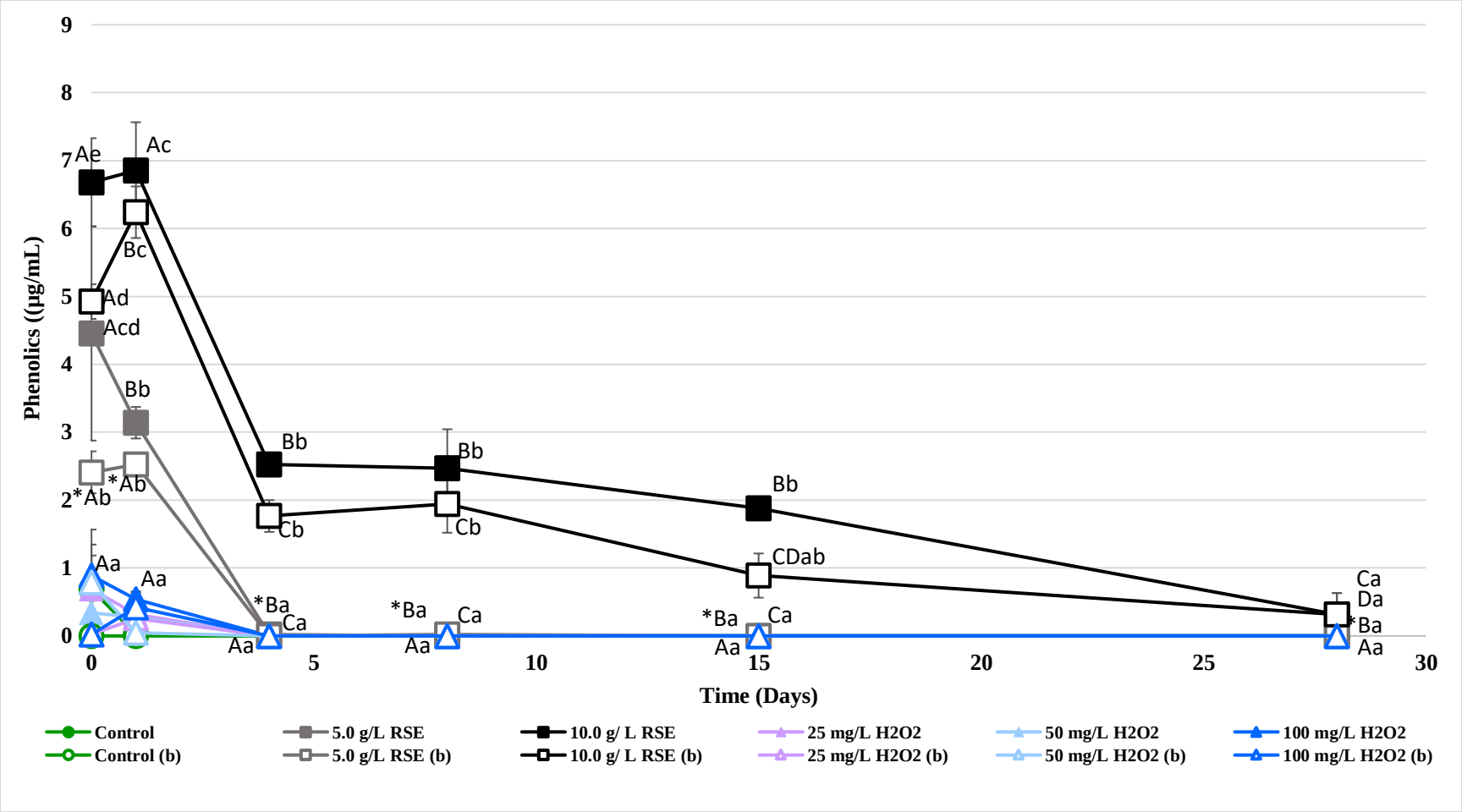
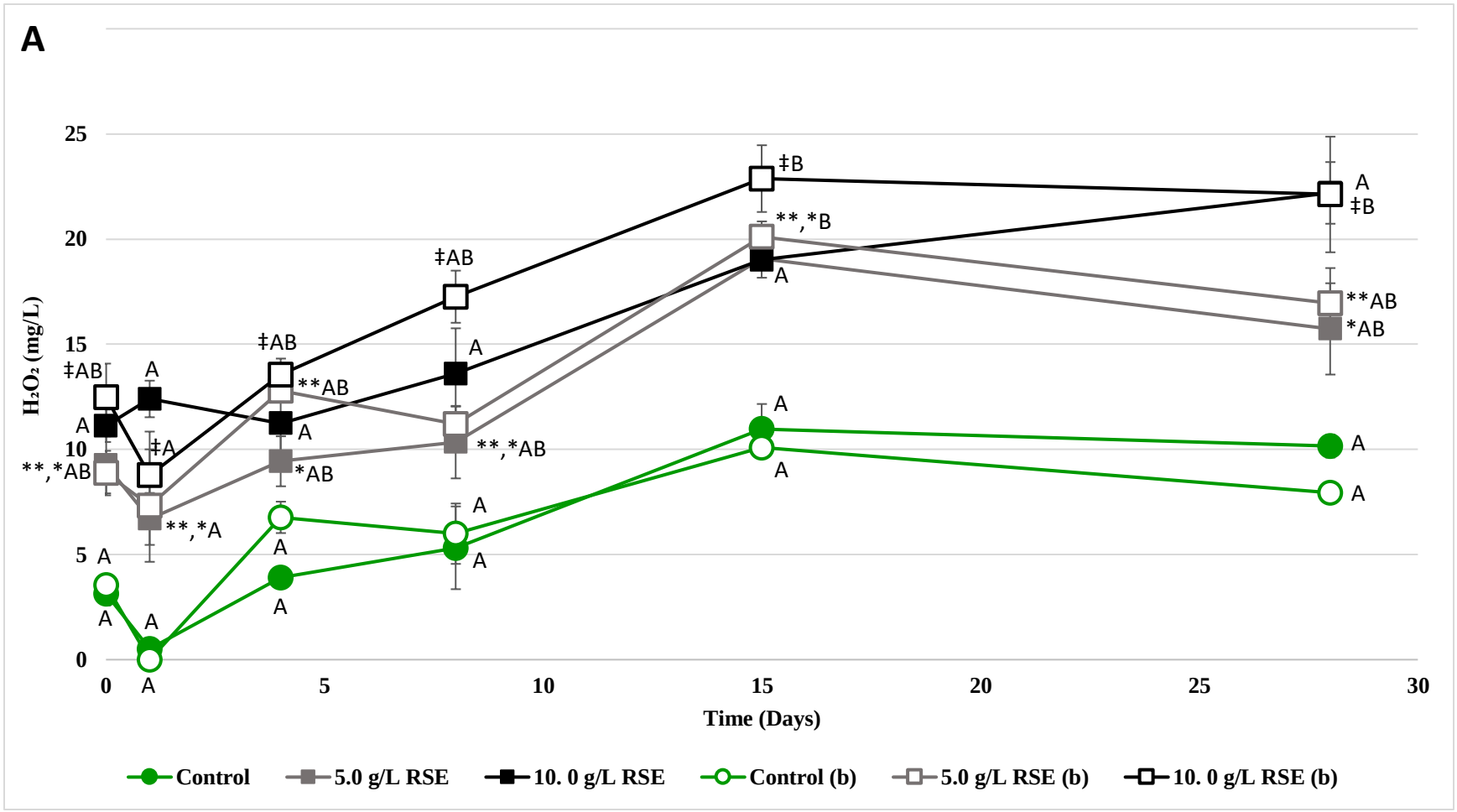


Figure 12. Phenolic concentrations (µg/mL) in gallic acid equivalents in pond water based *Raphidocelis subcapitata* cultures for 0 to 28 days following treatment with rice straw extract (RSE), hydrogen peroxide (H_2O_2), or no treatment (control) and with bacteria (b) or without bacteria (n=3). Sampling times followed by similar uppercase letters within a treatment represent lack of significant differences within that treatment over time ($P > 0.05$). Similar lowercase letters represent lack of significant differences between the treatments ($P < 0.05$).


A
25
20
15
10
5
0
H₂O₂ (mg/L)
0
5
10
15
20
25
30
Time (Days)
‡AB
A
‡A
**,*AB
**,*A
‡AB
**AB
A
*AB
‡AB
A
**,*AB
‡B
**,*B
A
A
A
A
A
A
A
A
A
A
A
A
A
A
A
‡B
A
**AB
*AB
Control
5.0 g/L RSE
10. 0 g/L RSE
Control (b)
5.0 g/L RSE (b)
10. 0 g/L RSE (b)

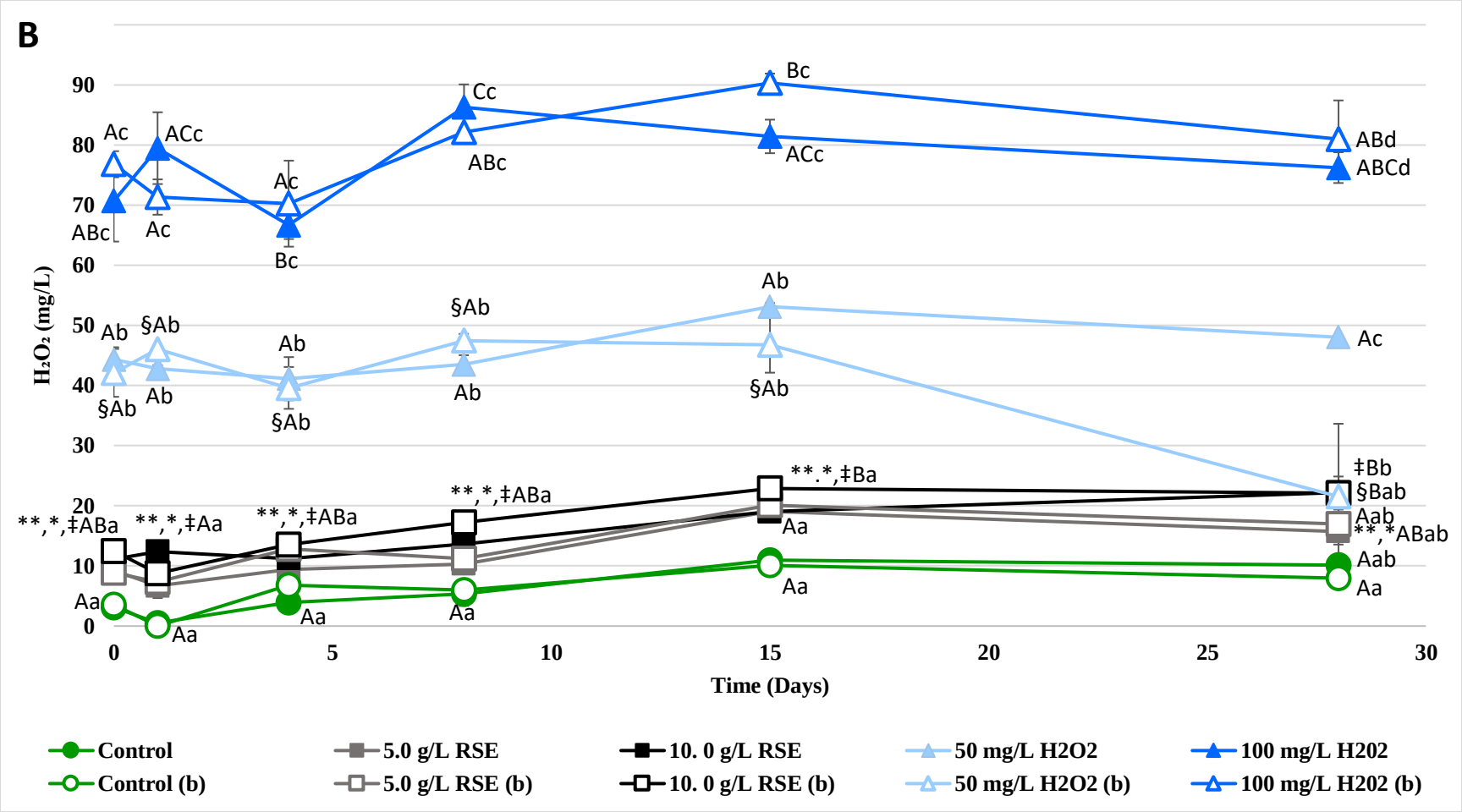
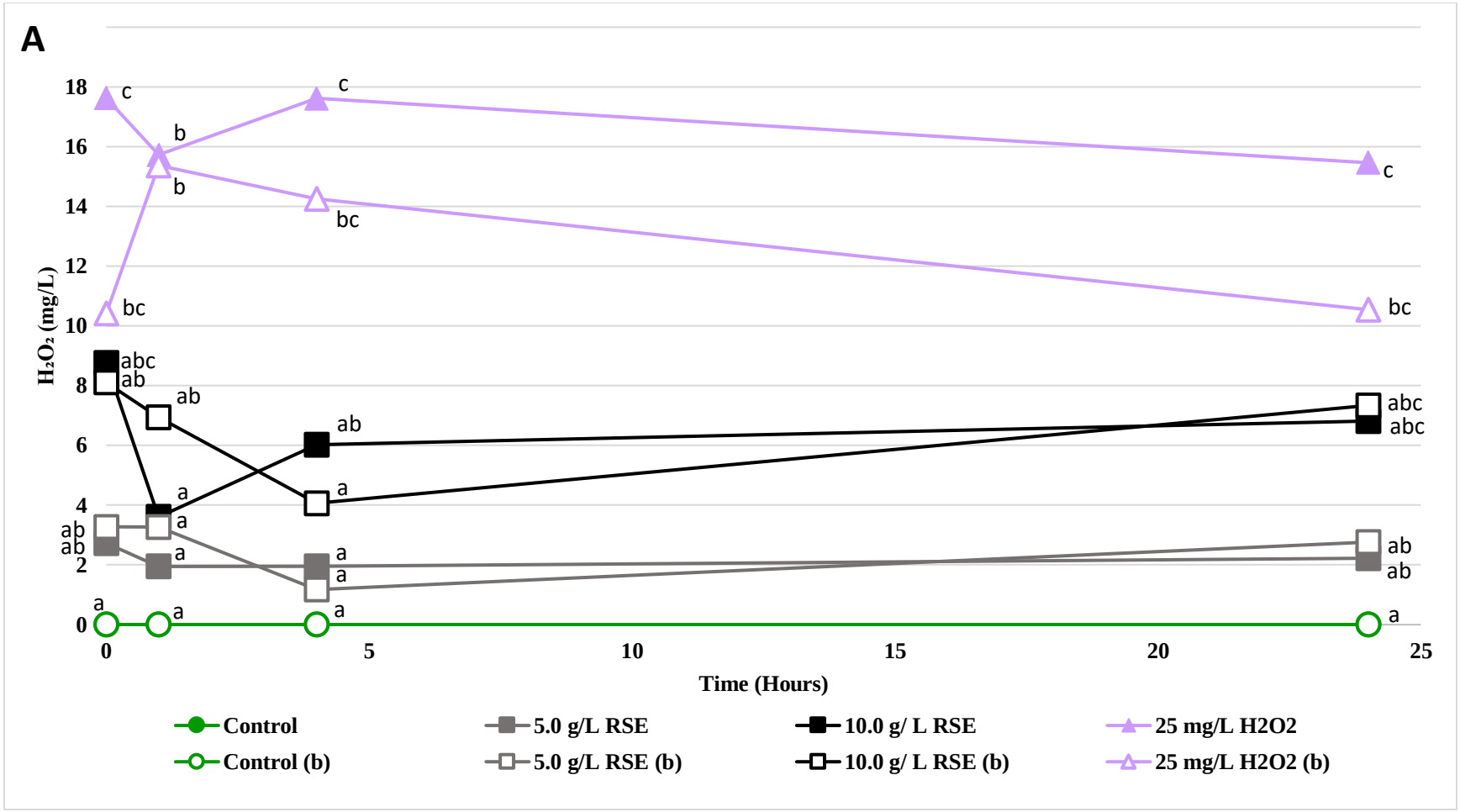


Figure 13 a-b. Hydrogen peroxide concentrations (mg/L) in pond water based *Microcystis aeruginosa* cultures for 0 to 28 days following treatment with rice straw extract (RSE), hydrogen peroxide (H_2O_2), or no treatment (control) and with bacteria (b) or without bacteria (n=3). Sampling times followed by similar uppercase letters within a treatment represent lack of significant differences within that treatment over time ($P > 0.05$). Similar lowercase letters represent lack of significant differences between the treatments ($P < 0.05$).


A
18
16
14
12
10
8
6
4
2
0
H₂O₂ (mg/L)
0
5
10
15
20
25
Time (Hours)
Control
5.0 g/L RSE
10.0 g/ L RSE
25 mg/L H2O2
Control (b)
5.0 g/L RSE (b)
10.0 g/ L RSE (b)
25 mg/L H2O2 (b)

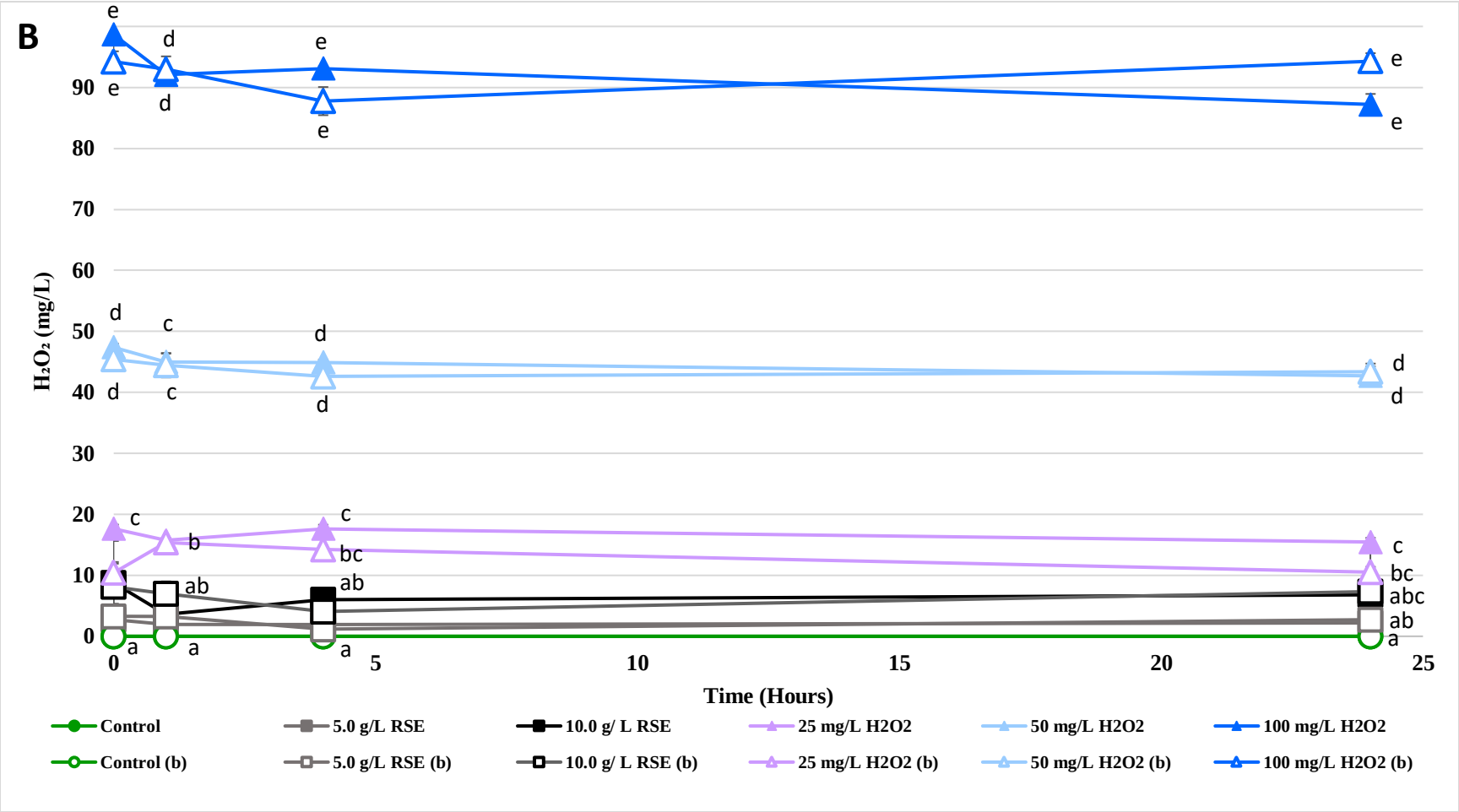


Figure 14 a-b. Hydrogen peroxide concentrations (mg/L) in pond water based *Raphidocelis subcapitata* cultures for 0 to 24 hours following treatment with rice straw extract (RSE), hydrogen peroxide (H_2O_2), or no treatment (control) and with bacteria (b) or without bacteria (n=3). Sampling times followed by similar uppercase letters within a treatment represent lack of significant differences within that treatment over time ($P > 0.05$). Similar lowercase letters represent lack of significant differences between the treatments ($P < 0.05$).

CHAPTER 3. CONCLUSION

Conclusions

Rice and barley straw are effective at controlling the growth of *M. aeruginosa* and *R. subcapitata* in media. At similar concentrations, rice straw extract was more effective than barley straw at suppressing the growth of both *R. subcapitata* and *M. aeruginosa*. For reliable long-term suppression, greater than 2.5 g/L rice straw extract in media and 5.0 g/L rice straw extract in pond water is necessary, possibly due to the instability of allelochemicals. The lack of significance in H_2O_2 between treatments supports the hypothesis that inhibition is related to the release of other allelochemicals, such as flavonoids or other phenolic compounds.

However, total concentrations were not helpful in determining the mechanism(s) causing the inhibitory effect of cereal straws. Rather, individual compounds produced from photoaerobically biodecomposing rice straw should be identified to determine the algistatic effect on algal species and on the original microbial community at concentrations found in decomposing rice straw extracts. Additionally, the original microbial community composition within the context of abiotic conditions of natural waters (pH, nutrient concentrations, sediment, and temperature, etc.) alters algal dynamics and response to treatment. Therefore, these conditions must be considered when choosing treatment methods and concentrations.

Rice straw waste is predominately managed through soil incorporation or through burning that contributes to serious respiratory conditions, greenhouse gas emissions, and soil and nutrient losses (Skaug, 2017; Bhattacharyya et al., 2021). In contrast to many current algal control methods, cereal straws have been recognized as environmentally safe algal inhibitors, are safe for non-target species, and can easily be applied with little to no additional management (Zhu et al., 2021). Harnessing the potential energy of rice straw in bio-based decomposition

products, such as algal inhibitors, could incentivize less harmful agricultural waste management practices and promote environmentally conscious algal control methods.

Appendix A

Media components and stock solutions

Table A1. Bristol medium stock solutions and final concentrations.

#	Component	Amount	Stock Solution Concentration	Final Concentration
1	$NaNO_3$	10 mL/L	25 g/L MilliQ	2.94 mM
2	$CaCl_2{\cdot}2H_2O$	10 mL/L	2.5 g/L MilliQ	0.17 mM
3	$MgSO_4{\cdot}7H_2O$	10 mL/L	7.5 g/L MilliQ	0.3 mM
4	K_2HPO_4	10 mL/L	7.5 g/L MilliQ	0.43 mM
5	KH_2PO_4	10 mL/L	17.5 g/L MilliQ	1.29 mM
6	NaCl	10 mL/L	2.5 g/L MilliQ	0.43 mM

Table A2. BG-11 medium stock solutions and final concentrations.

#	Component	Amount	Stock Solution Concentration	Final Concentration
1	$NaNO_3$	10 mL/L	150 g/L MilliQ	17.6 mM
2	K_2HPO_4	10 mL/L	4 g/L MilliQ	0.23 mM
3	$MgSO_4{\bullet}7H_2O$	10 mL/L	7.5 g/L MilliQ	0.3 mM
4	$CaCl_2{\bullet}2H_2O$	10 mL/L	3.6 g/L MilliQ	0.24 mM
5	Citric Acid$\bullet H_2O$	10 mL/L	.6 g/L MilliQ	0.031 mM
6	Ferric Ammonium Citrate	10 mL/L	.6 g/L MilliQ	0.021 mM
7	$Na_2EDTA{\bullet}2H_2O$	10 mL/L	.1 g/L MilliQ	0.0027 mM
8	Na_2CO_3	10 mL/L	2 g/L MilliQ	0.19 mM
9	BG-11 Trace Metals Solution	1 mL/L		
10	Sodium Thiosulfate Pentahydrate (agar media only,sterile)	1 mL/L	249 g/L MilliQ	1 mM

Table A3. BG-11 trace metals stock solution.

#	Component	Amount	Final Concentration
1	H_3BO_3	2.86 g/L	46 mM
2	$MnCl_2{\bullet}4H_2O$	1.81 g/L	9 mM
3	$ZnSO_4{\bullet}7H_2O$	0.22 g/L	0.77 mM
4	$Na_2MoO_4{\bullet}2H_2O$	0.39 g/L	1.6 mM
5	$CuSO_4{\bullet}5H_2O$	0.079 g/L	0.3 mM
6	$Co(NO_3)_2{\bullet}6H_2O$	49.4 mg/L	0.17 mM

Appendix B

Table B1. pH averages in pond water *Microcystis aeruginosa* cultures for 0, 1, 4, 8, 15 and 28 days following inoculation.

	Day 0		Day 1		Day 4		Day 8		Day 15		Day 28	
Treatment[1]	**Mean**	**SE**	**Mean**	**SE**	**Mean**	**SE**	**Mean**	**SE**	**Mean**	**SE**	**Mean**	**SE**
Control	8.07	0.13	8.14	0.10	7.76	0.08	8.19	0.07	8.30	0.02	8.14	0.06
5.0 g/L RSE	8.31	0.01	8.40	0.02	7.91	0.05	8.12	0.06	8.55	0.08	8.83	0.32
10.0 g/ L RSE	8.29	0.02	8.43	0.02	7.85	0.10	8.22	0.04	8.80	0.02	8.75	0.09
50 mg/L H_2O_2	8.21	0.06	8.29	0.02	8.17	0.05	8.24	0.03	8.19	0.05	8.34	0.03
100 mg/L H_2O_2	8.21	0.01	8.26	0.03	8.18	0.04	8.28	0.04	8.24	0.02	8.26	0.02
Control (b)	8.05	0.06	7.45	0.15	8.01	0.03	8.04	0.02	8.26	0.01	8.66	0.56
5.0 g/L RSE (b)	7.96	0.13	7.93	0.12	8.28	0.04	8.28	0.07	8.27	0.11	8.34	0.10
10.0 g/L RSE (b)	8.13	0.05	7.96	0.08	8.36	0.03	8.45	0.02	8.55	0.12	8.46	0.02
50 mg/L H2O2 (b)	8.09	0.16	8.24	0.01	8.17	0.03	8.28	0.01	8.30	0.01	8.31	0.02
100 mg/L H2O2 (b)	8.23	0.02	8.23	0.01	8.14	0.02	8.24	0.02	8.26	0.01	8.35	0.02

[1]RSE is rice straw extract, H_2O_2 is hydrogen peroxide, (b) is bacteria included from collected pond water, SE is standard error.

Table B2. pH averages in pond water *Raphidocelis subcapitata* cultures for 0, 1, 4, 8, 15 and 28 days following inoculation.

	Day 0		Day 1		Day 4		Day 8		Day 15		Day 28	
Treatment[1]	**Mean**	**SE**	**Mean**	**SE**	**Mean**	**SE**	**Mean**	**SE**	**Mean**	**SE**	**Mean**	**SE**
Control	8.14	0.01	8.19	0.08	8.31	0.03	8.29	0.00	8.36	0.02	8.25	0.01
5.0 g/L RSE	8.52	0.06	8.56	0.02	8.54	0.01	8.57	0.02	8.61	0.03	8.65	0.03
10.0 g/ L RSE	8.63	0.02	8.78	0.04	8.73	0.01	8.72	0.03	8.78	0.02	8.90	0.04
25 mg/L H2O2	8.14	0.04	8.32	0.03	8.31	0.02	8.15	0.07	8.36	0.03	8.33	0.02
50 mg/L H2O2	8.09	0.06	8.30	0.01	8.30	0.02	8.25	0.01	8.36	0.01	8.33	0.01
100 mg/L H2O2	8.16	0.02	8.24	0.04	8.28	0.02	8.32	0.01	8.32	0.00	8.30	0.02
Control (b)	8.04	0.08	8.12	0.10	8.20	0.03	8.27	0.05	8.28	0.05	8.25	0.04
5.0 g/L RSE (b)	8.03	0.05	8.25	0.07	8.06	0.05	8.24	0.06	7.40	0.08	8.26	0.08
10.0 g/L RSE (b)	8.21	0.03	8.36	0.05	8.53	0.09	8.46	0.05	7.40	0.05	8.51	0.07
25 mg/L H_2O_2 (b)	8.17	0.03	8.28	0.01	8.23	0.09	8.33	0.02	8.25	0.05	8.41	0.01
50 mg/L H_2O_2 (b)	8.17	0.01	8.27	0.02	8.24	0.00	8.31	0.01	8.29	0.02	8.40	0.01
100 mg/L H_2O_2 (b)	8.21	0.00	8.21	0.06	8.27	0.02	8.30	0.01	8.31	0.02	8.43	0.01

[1]RSE is rice straw extract, H_2O_2 is hydrogen peroxide, (b) is bacteria included from collected pond water, SE is standard error.

Table B3. Redox averages in pond water *Microcystis aeruginosa* cultures for 0, 1, 4, 8, 15 and 28 days following inoculation.

	Day 0		Day 1		Day 4		Day 8		Day 15		Day 28	
Treatment[1]	**Mean**	**SE**	**Mean**	**SE**	**Mean**	**SE**	**Mean**	**SE**	**Mean**	**SE**	**Mean**	**SE**
Control	185	10.40	187	7.42	222	7.86	223	17.70	227	4.93	200	2.52
5.0 g/L RSE	148	4.36	147	8.09	156	26.21	171	4.70	220	2.33	181	8.82
10.0 g/ L RSE	136	3.46	118	5.67	154	7.57	143	8.74	186	2.33	176	9.29
50 mg/L H_2O_2	239	3.06	229	4.51	223	3.51	143	4.93	197	7.42	215	2.85
100 mg/L H_2O_2	226	3.18	227	3.51	215	2.52	187	27.09	202	2.89	216	2.08
Control (b)	169	3.93	184	9.50	195	6.17	155	3.53	197	2.89	186	18.45
5.0 g/L RSE (b)	151	4.91	154	7.80	150	1.86	147	11.15	184	3.61	195	9.94
10.0 g/L RSE (b)	142	2.73	140	3.71	137	5.24	114	12.86	159	6.93	162	7.02
50 mg/L H_2O_2 (b)	230	0.00	228	2.85	225	1.86	158	5.61	218	0.67	175	4.33
100 mg/L H_2O_2 (b)	231	1.45	225	0.88	215	1.76	222	8.17	212	2.08	220	2.08

[1]RSE is rice straw extract, H_2O_2 is hydrogen peroxide, (b) is bacteria included from collected pond water, SE is standard error.

Table B4. Redox averages in pond water *Raphidocelis subcapitata* cultures for 0, 1, 4, 8, 15 and 28 days following inoculation.

	Day 0		Day 1		Day 4		Day 8		Day 15		Day 28	
Treatment[1]	**Mean**	**SE**	**Mean**	**SE**	**Mean**	**SE**	**Mean**	**SE**	**Mean**	**SE**	**Mean**	**SE**
Control	210	20.09	189	4.67	167	21.83	154	5.49	152	4.04	157	9.40
5.0 g/L RSE	151	14.89	143	1.86	115	2.40	158	5.93	140	6.94	125	9.54
10.0 g/ L RSE	135	11.06	134	12.20	115	7.45	138	12.58	114	3.38	124	1.76
25 mg/L H_2O_2	235	4.18	237	0.33	206	0.33	213	0.00	210	1.67	212	1.20
50 mg/L H_2O_2	230	4.67	240	3.84	213	3.71	212	1.76	218	4.84	221	0.67
100 mg/L H_2O_2	244	4.18	240	0.88	210	0.67	210	1.53	217	0.00	229	5.36
Control (b)	190	0.58	214	7.94	149	9.50	150	5.03	166	19.52	172	6.36
5.0 g/L RSE (b)	164	3.61	170	10.60	118	6.81	130	8.29	165	7.84	167	0.58
10.0 g/L RSE (b)	138	4.41	143	0.88	101	6.66	102	4.33	178	13.35	138	5.51
25 mg/L H_2O_2 (b)	218	26.03	240	0.88	114	11.41	131	5.67	135	9.24	122	4.16
50 mg/L H_2O_2 (b)	248	0.88	243	2.31	187	28.20	213	1.20	219	0.67	219	14.01
100 mg/L H_2O_2 (b)	241	2.08	239	1.45	215	1.20	214	0.88	221	0.67	234	2.89

[1]RSE is rice straw extract, H_2O_2 is hydrogen peroxide, (b) is bacteria included from collected pond water, SE is standard error.