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Impact of Copper-Containing Products and Irrigation On Hybrid Bermudagrass (Cynodon Dactylon (L.) Pers X C. Transvaalensis Burtt-Davy)

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IMPACT OF COPPER-CONTAINING PRODUCTS AND IRRIGATION ON HYBRID BERMUDAGRASS (*Cynodon dactylon* (L.) *Pers* x *C. transvaalensis* Burtt-Davy)

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ABSTRACT

Copper (Cu) is an often-seen component in various turf industry products including fungicides, algaecides, and colorants. Though an essential micronutrient in plants, excessive levels of Cu has been shown in various plant commodities to cause phytotoxicity and plant death. With the increasing use of pigments on hybrid bermudagrass [*Cynodon dactylon* (L.) Pers x. *C. transvaalensis* Burtt-Davy] putting greens to replace overseeding practices during traditional dormant periods combined with regular applications of fungicide, algaecide and spray additives containing Cu, the objective of this study was to investigate the potential impact of individual and combined copper-containing treatments on hybrid bermudagrass and possible remediation options for turfgrass managers dealing with toxic levels of Cu in soil.

A turf colorant, fungicide, and algaecide were selected for testing alone and in combination for 13-week field trials conducted in 2019 and 2020 on hybrid bermudagrass prior to, and during, Spring transition: PAR (copper phthalocyanine pigment), Junction DF (copper hydroxide + mancozeb), 0.25 ppm and 2.0 ppm copper sulfate ($CuSO_4$) by volume, PAR + 0.25 ppm $CuSO_4$, PAR + 2.0 ppm $CuSO_4$, Junction DF + 0.25 ppm $CuSO_4$, Junction DF + 2.0 ppm $CuSO_4$, PAR + Junction DF + 0.25 ppm $CuSO_4$, and PAR + Junction DF + 2.0 ppm $CuSO_4$. All treatments were also used in two separate greenhouse studies, in addition to weekly PAR, 50, 100, and 200 ppm by volume of $CuSO_4$ treatments, investigating levels of Cu necessary to negatively impact the growth of hybrid bermudagrass.

In field trials, treatments containing the colorant PAR exhibited limited increases in turf quality, normalized difference vegetative index, and coverage compared to other treatments. In field trials, turf areas treated with PAR alone experienced the worst change of turf quality over time with an average change of -0.0453 and 0.2157 per week in studies one and two, respectively. Applications of Junction DF resulted in net soil Cu concentrations exceeding non-Junction treatments in studies one and two with applications of PAR + Junction DF resulting in statistically higher net Cu concentrations in soil each year of 7.846 and 4.511 kg Cu ha^{-1}. In greenhouse trials, irrigation treatments containing 50, 100, and 200 ppm $CuSO_4$ had the greatest impact on turf measurements with turf quality decreasing in 2022 study at rates of -0.1054, -0.1631, and -0.2585 per week, respectively. The continual application of PAR, alone or in combination, resulted in decreases of phytotoxicity over time in both greenhouse studies except for PAR + Junction DF and PAR + Junction DF + 2.0 ppm $CuSO_4$ in 2022 study. Applications of Junction DF, alone or in combination, resulted in leaf tissue concentrations of copper exceeding concentrations of 50 ppm $CuSO_4$ treated pots which averaged 544 mg Cu kg^{-1} leaf tissue concentration in 2020 greenhouse study and greater than 316 mg Cu kg^{-1} in 2022 tissue concentration with irrigation treatments of 200 ppm $CuSO_4$. Results of field and greenhouse studies suggest labeled rates of Cu-containing products should not cause decreases in turf quality or appearance initially, however, continued use of products may lead to excessive Cu concentrations over time.

In soils, Cu adsorbs tightly with organic matter and clay soil particles resulting in potentially excessive accumulation in thatch layers and native soil turf areas. To test where (depth) Cu may accumulate in soil and if soil Cu could be displaced with various compounds, greenhouse pot plugs were obtained from a 1-year-old renovated hybrid bermudagrass research green built to USGA specifications. Plugs were treated over 13 weeks with previously listed greenhouse study treatments and Cu concentrations were measured in thatch layers, 0 – 2.54 cm below thatch, and 2.54 – 5.08 cm below thatch for all pots. The thatch layer contained the greatest amount of copper with 635 kg Cu ha^{-1} compared to subsequent two layers with 11.26 and 6.84 kg Cu ha^{-1}, respectively. This suggests mechanical removal of thatch layer could reduce overall soil Cu concentration. However, mechanical removal of thatch and subsoil leads to temporary decreases in aesthetic and playability of turf surfaces, rendering these methods as potentially unacceptable for some turf managers. The use 1 N ammonium sulfate ($(NH_4)_2SO_4$) (AMS) solution, 1 N calcium nitrate ($(Ca)(NO_3)_2$) solution, 1 N gypsum ($CaSO_4{\cdot}2H_2O$), and tap water were investigated for potential to leach Cu from soil treated with 1,000 ppm Cu-$CuSO_4$. Exposure to 1 filtration of AMS resulted in the removal of approximately 2,064 mg Cu kg^{-1} which was similar or greater than all of treatments cumulative effects after four filtrations. Results suggest ammonium sulfate may render Cu into a soluble state allowing for its removal from root zone sites in areas where mechanical removal of thatch is not possible.

Visual characteristics for turf quality were not consistently impacted negatively by any products applied at industry labeled rates and timings in individual studies, though early evidence indicates the potential for excessive accumulation of Cu when applied frequently. In situations of excess Cu concentrations, soil flushes with AMS may serve to facilitate leaching of Cu ions from rootzone in areas where mechanical removal is not possible. Identification of minimal AMS quantity necessary for efficient Cu flushing and irrigation requirement should be focus of future investigation in addition to identifying soil adsorption potential of copper species in USGA and native soil greens.

DEDICATION

I dedicate this work to my wife, Autumn, whose sacrifices have allowed me to pursue a dream, and to my parents, Donnie and Debbie, who put me on every golf course and sports field, and never pulled me away from a mower.

ACKNOWLEDGMENTS

For his time, expertise, and patience, I express sincere appreciation to my major professor, Dr. Bert McCarty. Without his guidance, I would not have near the professional success that I do, nor the confidence to delve further into the turfgrass industry.

I also greatly appreciate Dr. Virgil Quisenberry, Dr. William Bridges, and Dr. Philip Brown for serving on my graduate committee. Dr. Quisenberry’s one-on-one class time brought me greater understanding of soil physics and fertility, as well as a better ability to laugh at myself. Dr. Bridges ability to simplify even the most complex statistical questions is unsurpassed in my eyes, and his ability to do so with easy-to-grasp explanations brought greater confidence to an area that many struggle. Dr. Brown served as the initial investigator on the precursor study to this work and offered great advice on improvements, not only on the study itself but navigating graduate work. Without any of these great men, I surely would have floundered.

I am grateful for all the assistance I have received along the way from my fellow graduate students, Jacob Taylor and Tee Stoudemayer, as well as student intern, Seth Conover. Their presence and experience throughout this study made the hard days a little easier.

I also express great thanks to my family for providing nothing but encouragement on the days of doubt and reasons to smile when adversity would appear.

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CHAPTER ONE

INTRODUCTION

Hybrid Bermudagrass

Hybrid bermudagrass (*Cynodon dactylon* (L.) *Pers.* x *C. transvaalensis* Burtt-Davy) is the most prevalent warm-season turfgrass in the southeastern United States in both sports turf and golf course turf areas. This is due to its ability to withstand the high temperature and humidity associated with the climate as well as its aggressive rhizomatous and stoloniferous growth habits. These characteristics allow bermudagrass to create dense turf stands and superior damage recovery compared to many other turfgrass species even at very low mowing heights (3.2 to 4 mm) (Turgeon, 2008; Stier et al., 2013). Bermudagrass (*Cynodon dactylon* (L.) Pers) is native to the hot and dry climates near the Indian Ocean and has been commonly used on golf course playing surfaces since the mid-1940's (McCarty, 2018).

Bermudagrass is a C_4 plant and experiences a state of dormancy when exposed to frost or low temperatures, ceasing active growth and exhibiting an aesthetically undesirable yellow to brown appearance. This is normally associated with temperatures consistently below 10°C (50°F) (McCarty, 2018) with additional potential for chilling and freezing damage depending on the rate of temperature drop and continued length of sub-freezing temperatures. Additionally, ultradwarf bermudagrasses cultivars such as Tifdwarf, Sunday, and TifEagle which are associated with golf course putting greens, are typically less drought tolerant due to a shallow root system (McCarty and Canegallo, 2005). This suggests the need for heavier and more frequent irrigation during periods of drought and prolonged heat on these putting green varieties (Christians, et al., 2016).

Role of Copper in Plants and Soil

Copper (Cu) is one of the 17 essential elements for plant growth. In normal function, Cu is a requirement for specific enzymes controlling the conversion of amino acids to proteins, cell wall metabolism, electron transport, and other processes (Yruela, 2009; Christians, et. al., 2016). This performance within a plant is due, in large part, to the oxidation-reduction potential of Cu – its ability to exist with a valence charge of +1, +2, or +3. This ability, however, may also lead to potential plant decline as excessive cycling between +1 and +2 charges can create free radicals via Fenton reactions damaging essential plant structures (Printz et al., 2016; Yruela, 2009). Excessive Cu concentrations can result in lipid peroxidation, DNA and protein damage, reduced photosynthesis and growth rate, and impact nutrient absorption (Drzewiecka et al., 2017; De Freitas et al., 2015; Drkiewicz, et al., 2004). Santos Silva and group noted foliar applications of Cu significantly altered physiological, biochemical, and molecular characteristics of *Theobroma cacao* including leaf gas exchange and proline accumulation in young leaves (Dos Santos Silva et al, 2020). Healthy turfgrass leaves typically display sufficient levels of Cu between 5 to 38 ppm depending on grass species with toxicity symptoms becoming pronounced with tissue levels of 20 to 30 ppm (Robson & Reuter, 1981; Jones, 1980). Previous work at Clemson University indicated weekly spray applications containing 250 ppm of Cu could reduce turf quality in as few as 28 days (Gore et al., 2021).

In soils, Cu is adsorbed to surfaces of clay and iron (Fe), aluminum (Al), and manganese (Mn) oxides using oxygen as an intermediary in these surface bonds. It forms similar bonds to clay minerals (Mitra, 2015). Cupric copper (Cu^{2+}) is absorbed by roots either individually in solution or as a component in natural or synthetic complexes though absorption is greatly affected by soil pH, cation exchange capacity (CEC), and percentage of organic content (Havlin et al, 2005). Though absorption processes are not fully understood, it is theorized a combination of Cu transporter proteins, P-type ATPases, Zrt- and Irt-like proteins, and zinc-regulated transporters aid in the movement of Cu into root tips and throughout the plant. Copper will often be bound by nitrogen in a histidine side chain or by sulfur in cysteine or methionine depending on its oxidative state (Shabbir et al., 2020; Mitra, 2019). Copper becomes more immobile as soil pH, CEC, or organic matter content increase (Maier et al, 2000). Due to its high density, Cu is likely to accumulate in the topsoil (Araujo et al., 2019). Soils contained in 32.5 cm columns exposed to the equivalent of 50 kg Cu ha^{-1} followed by eight soil pore volume water replacements yielded less than 3% of total Cu in leachate. Further, it was observed up to 98% of total Cu remained in the upper 5 cm of the soil columns for those containing a greater percentage of organic matter (Sun et al., 2019).

Plant species naturally evolve to cope with elevated heavy metal levels in soils using one of two routes: exclusion or enrichment. Fu and colleagues (2015) noted plant transpiration, as well as structural characteristics were two important factors governing whether plants accumulate or exclude Cu.

USGA Golf Green Profile

Since 1960, the United States Golf Association (USGA) has provided recommendations for the construction of golf greens (USGA, 2018) which currently uses a layered soil profile consisting of 30 cm (12 inches) sand mix root zones, 10 cm (4 inches) of underlying gravel, with a drainage system at bottom of the profile (USGA, 2018). This construction method, and the recommend soil particle sizes, are designed to be resistant to wear, provide rapid internal drainage, and provide a growing medium for putting green turf. Recommendations contain physical properties such as a total porosity of 35-55% and a saturated hydraulic conductivity equal to or greater than 6 inches per hour (150 mm hr^{-1}) (USGA, 2018).

The root zone mixture is recommended to consist predominately (≥60%) of coarse and medium sands with a particle diameter of 0.5-1.0 mm and 0.25-0.5 mm, respectively. The remaining particle types consist of ≤20% fine sand (0.15-0.25 mm diameter), ≤10% very coarse sand (1.0 - 2.0 mm diameter), ≤5% very fine sand (0.05-0.15 mm diameter), ≤5% silt (0.002-0.05 mm diameter), and ≤3% clay (0.05-0.15 mm diameter) with the combined total of very fine sand, silt, and clay being ≤10%. Additives such as peat, compost, or other porous amendments are sometimes incorporated to the root zone mixture in effort to increase water and nutrient retentions (USGA, 2018).

Selecting predominately silicon dioxide (SiO_2) sands have been investigated for use due to their resistance to chemical decomposition and change over time. However, sand containing other minerals such as feldspar are used and therefore are subject to decomposition and weathering over time by reactions such as hydrolysis (Earle, 2019; USGA, 2018).

With reports of hybrid bermudagrass failing to successfully exit winter dormancy on golf course greens treated with various Cu-containing industry products, this research investigated the potential impact of sequential applications of various industry Cu-containing products, as well as determine potential remediation techniques on soils heavily impacted by excessive soil Cu concentrations.

CHAPTER TWO

RESPONSE OF TIFEAGLE BERMUDAGRASS TO COPPER-CONTAINING PRODUCTS

Introduction

Use of Copper-Containing Products

The use of copper (Cu) in horticulture dates to 1885 in Bordeaux, France with the usage of copper sulfate ($CuSO_4$) in vineyards to prevent the development of downy mildew, with this becoming known as the ‘Bordeaux mixture’ (McBride et al., 1981). The continued use of Cu-based fungicides is the largest contributor of Cu to soil within the agriculture industry (McBride et al.,1981). Due to its record of use as both a fungicide and algaecide, Cu is regularly used in organic agriculture with the USDA including several Cu-based substances in ‘The National List of Allowed and Prohibited Substances’ in organic agriculture production (Coelho et al., 2020).

Copper is also a vital component in various colorants and pigments commonly used in turfgrass management. Applying colorant and pigments to playing areas is an increasing trend to provide desirable winter color in place of overseeding mainly with perennial ryegrass (*Lolium perenne* L.) and roughstalk bluegrass (*Poa trivialis* L.) (Shearman et al., 2005). Applications of pigmented products often enhance spring green-up when compared to traditional overseeding practices and dormant turf, due in part to the increased soil and air temperatures (Liu et al., 2007). Many of the pigments have a molecular structure similar to chlorophyll, though the pigment-centered molecules are Cu ions instead of magnesium as in chlorophyll. The similar structure of pigment molecules to chlorophyll is considered as a possible means of increasing a plant's photosynthetic efficiency. McCarty et al (2017) noted foliar applications of Cu-containing pigments increased tissue concentrations of Cu 5 to 25 times of untreated creeping bentgrass [*Agrostis stolonifera* L. var. *palustris* (Huds.)]. Areas previously receiving repeated applications of pigmented products often have continued persistence of products within soil profile even years after ending treatments (Figure 2-1). Additionally, Cu-based products, predominately $CuSO_4$ or chelated Cu, are historically used for the control of various algae in irrigation ponds and water ways, though the release of toxins, such as microcystin-LR, as a result Cu use has been a topic of extensive research (Jones and Orr, 1994; Kennefick et al., 1993).

Irrigation Practices and Water Movement within Golf Greens

During summer months, hybrid bermudagrass has a mean evapotranspiration rate of 0.84 to 2.10 inches (2.1 to 5.3 cm) per week, the lower end of the range is representative of bermudagrass grown in more humid areas (McCarty, 2018). Irrigation water quality is an ever-increasing issue for turf managers as the demand on potable water increases (Devitt et al., 2004). This has led to increased use of effluent water sources which is often of lesser quality, containing varying levels of soluble salts, carbonates, pH, and nutrient contents (McCarty, 2018).

Brown et al (2019) described 100% sand mixes combined at USGA recommendations possessed a saturated hydraulic conductivity (K_{sat}) of 269 cm hr^{-1} (106 in hr^{-1}), however the introduction of only 20% fines (<0.05 mm) decreased K_{sat} to 4.67 cm hr^{-1}. Pure sand mixes were also found to possess lower volumetric water content (θ_v) throughout the soil profile, with a θv ranging from 0.06 to 0.22 $cm^3\ cm^{-3}$ from surface to 10 cm (4 in) depth, respectively, when soil was at "field capacity" (Brown et al., 2019).

Digital Image Analysis

When evaluating turfgrass quality, a visual rating scale of 1 to 9, with 9 representing the highest standard of turf, is often used. However, this can be subjective and provides opportunity for bias on the part of the rater (Karcher & Richardson, 2003). This potential for bias and variability, combined with inconsistency between multiple evaluators on similar plots, has led to increasing use of digital image analysis to quantify turfgrass color, density, and overall quality with increasing accuracy (Karcher & Richardson, 2003; Richardson et al., 2001). With the continued development of analysis software, such as the ability to calibrate for turf shadow, digital image analysis can accurately detect weedy plant populations in turf stands (Hoyle et al., 2013).

Some limitations in digital image analysis have been noted with Leinauer et al (2014) reporting traditional visual assessments more accurately detecting varietal differences. Kowalewski and team (2023) noted image analysis provided the most consistent method for measuring the percentage of turf coverage and seed establishment. This suggests a combination of measurements may be necessary in accurately evaluating turfgrass quality.

Research was conducted at Clemson University with the objective to assess possible negative impact of applications of Cu-containing products to hybrid bermudagrass in dormancy and breaking dormancy.

FIGURE 2-1. Green pigment in underlying soil profile of former creeping bentgrass (*Agrostis stolonifera* L. var. *palustris* (Huds.)) putting green treated with pigmented products seven years prior.

Materials and Methods

Field Trials

Field research was conducted in Clemson, SC at the Clemson University Turfgrass Research Facility on a 23-year-old TifEagle bermudagrass putting green, constructed to USGA specifications (USGA Green Section Staff, 1993) with the objective to assess possible negative impact of applications of Cu-containing products to hybrid bermudagrass in dormancy and breaking dormancy. Research was conducted from 9 March to 9 June 2020 and 23 March to 15 June 2021. Treatments consisted of an untreated control and various Cu-containing products, rates, and combinations (Table 2-1). Due to the content of mancozeb in Junction DF, mancozeb (Fore 80WP) (Dow

Agrosciences LLC., Indianapolis, IN) was applied at 0.75 oz 1,000 ft^{-2} (2.29 kg ha^{-1}) to all plots not containing Junction DF as a treatment (Figure 2-2).

Treatments containing copper phtalocyanine pigment (PAR) were applied every 14 days for twelve weeks at labeled rates, treatments containing $CuSO_4$ were applied every 7 days for twelve weeks, and treatments containing Junction DF were applied every 14 days for 6 weeks according to labeled instructions using a CO_2 backpack sprayer delivering 20 gal $acre^{-1}$ (187.3 L ha^{-1}). Plots were 4.92 x 4.92 ft (1.5 x 1.5 m) and replicated 4 times in each experiment using a randomized completed block design. Putting green was maintained at 0.125 in (3.175 mm) height with fertilization practices mimicking industry standards (McCarty, 2018). Linear regressions of visual qualities and vegetative measurements were compared among treatments using Analysis of Variance (ANOVA) followed by Fisher’s LSD (alpha=0.05) using JMP statistical software (SAS Institute, Cary, NC). Means of tissue analysis were compared among treatments in similar fashion. Due to high variability probability of soil chemical and physical characteristics within a given area as well the potential impact of Type II errors in environmental studies, means of soil variables were compared among treatments using Analysis of Variance followed by Fisher’s LSD (alpha=0.10) (Pennock, 2004; Peterman, 1990).

Turf Quality

Two measurements were recorded to quantify and assess treatments. Visual turfgrass quality (VTQ) was measured weekly (1-9, 9=best) with rating impacted by turf color, uniformity, density, and presence of pest. Normalized Difference Vegetation Index

(NDVI) was measured weekly using a Field Scout Turf Color Meter (Spectrum Technologies, Plainfield, IL). NDVI is estimated by Field Scout device by measuring red and near-infrared light (NIR) reflected off a plant's surface. A "greener" surface is indicated by a higher NDVI ratio ([NIR-Red light]/[NIR+Red light]) (Bremer et al, 2011) measured on a 0-1 scale, with 1 indicating a denser, darker green color.

Turf Coverage

Density of turfgrass was calculated via digital image analysis with the image processing software TurfAnalyzer (Green Research Services, LLC., Fayetteville, AR) (Karcher et al., 2017). Pictures of individual plots were taken weekly using iPhone SE 2nd Generation smartphone (Apple, Inc., Cupertino, CA) with 12 MP wide camera with aperture of *f*1.8, and then processed from HEIC file format to JPEG format so only treatment areas were in frame. Pictures were then processed via TurfAnalyzer based on user-defined thresholds hue, saturation, and brightness. This processing uses these thresholds to determine what is considered healthy, intact turfgrass, then compares the pixel quantity of what is interpreted as plant tissue to overall pixel count for turf coverage calculation which is then given as a percentage (Figure 2-3).

Nutrient Analysis

Soil nutrient analysis was performed pre- and post-trial by extracting 5 cores (2 cm diameter x 15 cm deep) per plot, blending each plot's respective cores, then placing blended soil into individual paper bag. Tissue analysis was performed by obtaining clippings from each plot using a standard walk-behind greens mower with bucket attachment. Three passes were made on each plot before removing and placing clippings into paper bags. Heavy metal analysis for both tissue and soil samples were performed by Clemson University Agricultural Service Laboratory using a Mehlich 1 extractant for soil samples.

Greenhouse Trials

Two separate 13-week studies were conducted in greenhouses located in the Clemson University greenhouse facility in Clemson, SC to determine the impact of Cu contained within industry products and irrigation water on bermudagrass. TifEagle bermudagrass plugs (10 cm diameter x 10 cm deep) (4 x 4 in) were removed from the Clemson University Turfgrass Research Facility and placed into 100% sand rootzone contained within 15.24 cm diameter x 15.24 cm (6 x 6 in) deep greenhouse pots. Turf was established for 3 weeks within greenhouse facility at a temperature of 23.9°C (75°F) until reaching 15 cm (5.9 in) diameter and 13 mm (0.5 in) height. To reduce the potential for localized effect of pot placement within greenhouse, pot positions were rerandomized weekly.

Treatments used in greenhouse trial consisted of products used in field trials, however additional treatments were present and application methods of $CuSO_4$ differed compared to field trial. Greenhouse treatments (Table 2-2) differing from the field trials included copper phtalocyanine pigment (PAR) applied weekly and biweekly at 0.37 oz 1,000 ft^{-2} (1.17 L ha^{-1}), and 1-inch (2.54 cm) equivalents of irrigation water containing $CuSO_4$ (copper sulfate pentahydrate, LabChem, Zelienople, PA) at 0.25 parts per million of solution (ppm) (0.25 mg $CuSO_4$ L^{-1}) (referred to as low rate), 2.0 ppm of solution (2.0 mg $CuSO_4$ L^{-1}) (referred to as high rate), 50 ppm of solution (0.05 g $CuSO_4$ L^{-1}), 100 ppm of solution (0.1 g $CuSO_4$ L^{-1}), and 200 ppm of solution (0.2 g $CuSO_4$ L^{-1}) (Table 2-2). Due to the content of mancozeb in Junction DF, mancozeb (Fore 80 WP) (Dow Agrosciences LLC., Indianapolis, IN) was applied at 0.75 oz 1,000 ft^{-2} (2.29 kg ha^{-1}) to all pots not containing Junction DF as a treatment (Figure 2-4). All pots weekly received 454 ml of water, representing 1 inch (2.54 cm) of irrigation water, however pots containing $CuSO_4$ received irrigation water containing respective amounts of $CuSO_4$.

Linear regressions of visual qualities and vegetative measurements and means of root and tissue analysis were compared, respectively, among treatments using Analysis of Variance (ANOVA) followed by Fisher's LSD (alpha=0.05) using JMP statistical software. Due to high variability probability of soil chemical and physical characteristics within a given area as well the potential impact of Type II errors in environmental studies, means of soil variables were compared among treatments using Analysis of Variance followed by Fisher's LSD (alpha=0.10) (Pennock, 2004; Peterman, 1990).

Turf Quality

Three measurements were recorded to quantify and assess treatments on the vegetative quality, similar to measurements in the field trial. Visual turfgrass quality (VTQ) was measured weekly (1 to 9, 9=best). Normalized Difference Vegetation Index (NDVI) was measured weekly using a Field Scout Turf Color Meter (Spectrum Technologies, Plainfield, IL). Phytotoxicity of turfgrass was measured weekly on a 0-100% (100%=full discoloration).

Turf Coverage

Density of turfgrass was calculated using image processing software TurfAnalyzer (Green Research Services, LLC., Fayetteville, AR). Pictures of individual pots were taken weekly, and then processed so only treatment areas were in frame. Pictures were then processed via program analyzing color of individual pixels for content of red, green, and blue content. This processing then compares what is interpreted as plant tissue to overall pixel count for density calculation.

Nutrient Analysis

Soil nutrient analysis was performed pre- and post-trial by extracting five cores (2 cm diameter x 15 cm deep) per pot, blending each plot's respective cores, and then placing the blended soil into individual paper bags. Tissue analysis was performed by removing all above ground growth from each pot and placing clippings into paper bags. Additionally, roots were collected by washing all soil from below a 0.5-inch (1.77 cm) thatch layer and clipping roots beneath thatch layer. Roots were collected and placed into paper bags. Heavy metal analysis for tissue and soil samples were performed by Clemson University Agricultural Service Laboratory using a Mehlich 1 extractant for soil samples.

Root and Shoot Weights

Prior to submitting vegetative and root tissue for nutrient analysis, plants were allowed to dry at ambient air temperature for seven days. Tissue and roots were then sieved separately to remove any remaining soil particles and weighed.

FIGURE 2-2. Dormant TifEagle bermudagrass (*Cynodon dactylon* (L.) Pers. X *C. transvaalensis* Burtt-Davy) appearance following foliar application of various copper-containing products.

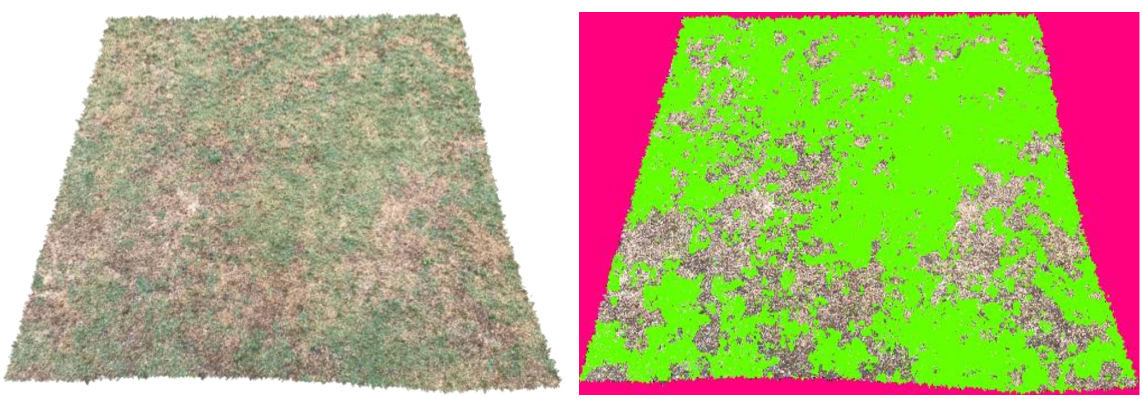


FIGURE 2-3. Field plot image edited to contain only treated area (left) is then processed via TurfAnalyzer with bright green area representative of turf coverage (right). Fuchsia color represents frame and is used to set boundaries of analyzed image.

FIGURE 2-4. TifEagle bermudagrass (*Cynodon dactylon* (L.) Pers. X *C. transvaalensis* Burtt-Davy) pots varied in appearance after treatment with various copper-containing products.

TABLE 2-1. Treatments and rates applied to TifEagle bermudagrass putting green field studies in 2020 and 2021 at Clemson University, Clemson, SC.

Treatment	**Rate**
Phtalocyanine pigment (PAR)	0.37 oz 1,000 ft^{-2} (1.17 L ha^{-1})[A]
Copper hydroxide + mancozeb (Junction DF)	4.0 oz 1,000 ft^{-2} (12.21 kg ha^{-1})[C]
0.25 ppm Copper sulfate	0.25 ppm solution (0.25 mg $CuSO_4$ L^{-1})[B]
2.0 ppm Copper sulfate	2.0 ppm of solution (2.0 mg $CuSO_4$ L^{-1})[B]
PAR + 0.25 ppm copper sulfate	0.37 oz 1,000 ft^{-2} (1.17 L ha^{-1})[A] + 0.25 ppm solution[B]
PAR + 2.0 ppm copper sulfate	0.37 oz 1,000 ft^{-2} (1.17 L ha^{-1})[A] + 2.0 ppm solution[B]
Junction DF + 0.25 ppm copper sulfate	4.0 oz 1,000 ft^{-2} (12.21 kg ha^{-1})[C] + 0.25 ppm solution[B]
Junction DF + 2.0 ppm copper sulfate	4.0 oz 1,000 ft^{-2} (12.21 kg ha^{-1})[C] + 2.0 ppm solution[B]
PAR + Junction DF	0.37 oz 1,000 ft^{-2} (1.17 L ha^{-1})[A] + 4.0 oz 1,000 ft^{-2} (12.21 kg ha^{-1})[C]
PAR + Junction DF + 0.25 ppm copper sulfate	0.37 oz 1,000 ft^{-2} (1.17 L ha^{-1})[A] + 4.0 oz 1,000 ft^{-2} (12.21 kg ha^{-1})[C] + 0.25 ppm solution[B]
PAR + Junction DF + 2.0 ppm copper sulfate	0.37 oz 1,000 ft^{-2} (1.17 L ha^{-1})[A] + 4.0 oz 1,000 ft^{-2} (12.21 kg ha^{-1})[C] + 2.0 ppm solution[B]

All treatments not including Junction DF received mancozeb (Fore 80 WP) (Dow Agrosciences LLC., Indianapolis, IN) at a rate of oz 1,000 ft^{-2} (2.29 kg ha^{-1}).

[A] Applied bi-weekly

[B] Applied weekly

[C] Three applications applied bi-weekly

TABLE 2-2. Treatments and rates applied to 'TifEagle' hybrid bermudagrass for greenhouse trials in 2020 and 2022 at Clemson University, Clemson, SC

Treatment	Rate
Phtalocyanine pigment (PAR) - biweekly	0.37 oz 1,000 ft^{-2} (1.17 L ha^{-1})[A]
PAR (2X) - weekly	0.37 oz 1,000 ft^{-2} (1.17 L ha^{-1})[B]
Copper hydroxide + mancozeb (Junction DF)	4.0 oz 1,000 ft^{-2} (12.21 kg ha^{-1})[C]
0.25 ppm Copper sulfate	0.25 ppm solution irrigation (0.25 mg $CuSO_4$ L^{-1})[B]
2.0 ppm Copper sulfate	2.0 ppm of solution irrigation (2.0 mg $CuSO_4$ L^{-1}) [B]
50 ppm Copper sulfate	50.0 ppm of solution irrigation (0.05 g $CuSO_4$ L^{-1}) [B]
100 ppm Copper sulfate	100.0 ppm of solution irrigation (0.1 g $CuSO_4$ L^{-1}) [B]
200 ppm Copper sulfate	200.0 ppm of solution irrigation (0.2 g $CuSO_4$ L^{-1}) [B]
PAR + 0.25 ppm copper sulfate	0.37 oz 1,000 ft^{-2} (1.17 L ha^{-1}) [A] + 0.25 ppm solution irrigation [B]
PAR + 2.0 ppm copper sulfate	0.37 oz 1,000 ft^{-2} (1.17 L ha^{-1}) [A] + 2.0 ppm solution irrigation [B]
Junction DF + 0.25 ppm copper sulfate	4.0 oz 1,000 ft^{-2} (12.21 kg ha^{-1}) [C] + 0.25 ppm solution irrigation [B]
Junction DF + 2.0 ppm copper sulfate	4.0 oz 1,000 ft^{-2} (12.21 kg ha^{-1}) [C] + 2.0 ppm solution irrigation [B]
PAR + Junction DF	0.37 oz 1,000 ft^{-2} (1.17 L ha^{-1}) [A] + 4.0 oz 1,000 ft^{-2} (12.21 kg ha^{-1}) [C]
PAR + Junction DF + 0.25 ppm copper sulfate	0.37 oz 1,000 ft^{-2} (1.17 L ha^{-1}) [A] + 4.0 oz 1,000 ft^{-2} (12.21 kg ha^{-1}) [C] + 0.25 ppm solution irrigation [B]

PAR + Junction DF + 2.0 ppm copper sulfate	0.37 oz 1,000 ft^{-2} (1.17 L ha^{-1}) [A] + 4.0 oz 1,000 ft^{-2} (12.21 kg ha^{-1}) [C] + 2.0 ppm solution irrigation [B]

All treatments not including Junction DF received mancozeb (Fore 80 WP) (Dow Agrosciences LLC., Indianapolis, IN) at a rate of 0.75 oz 1,000 ft^{-2} (2.29 kg ha^{-1}).
[A] Applied bi-weekly
[B] Applied weekly
[C] Three applications applied bi-weekly

Results and Discussion

Field Trials

Due to the variability between studies, results were analyzed individually and are presented as such.

Turf Quality

As bermudagrass breaks dormancy and begins new growth, visual quality normally improves over time. Over a 13-week span, the average rate of change was highest in untreated bermudagrass plots in both study 1 and 2 with a visual quality increase of 0.1744 and 0.2981 per week, respectively.

In study 1, PAR + Junction, Junction + $CuSO_4$ 0.25 ppm, and PAR + Junction + $CuSO_4$ 0.25 ppm, increase at similar rates of 0.125, 0.1154, and 0.0948, respectively (Figure 2-5) (Table 2-3). PAR + $CuSO_4$ 0.25 ppm, with an average increase of 0.0879, was similar to Junction + $CuSO_4$ 0.25 ppm and PAR + Junction + $CuSO_4$ 0.25 ppm treatments. Copper sulfate 2.0 ppm and Junction + $CuSO_4$ 2.0 ppm were similar to Junction + $CuSO_4$ 0.25 ppm, PAR + Junction + $CuSO_4$ 0.25 ppm, and PAR + $CuSO_4$ 0.25

ppm, with an average increase 0.0728 and 0.0618, respectively. Similar to $CuSO_4$2.0 ppm and Junction + $CuSO_4$ 2.0 ppm, $CuSO_4$ 0.25 ppm and Junction treatments had an average increase of 0.0495 and 0.0467, respectively. PAR + Junction + $CuSO_4$ 2.0 ppm was similar to the previous two treatments with an average increase of 0.0234. PAR + $CuSO_4$ 2.0 ppm and PAR treatments were similar with negative changes in time of -0.0137 and -0.0453, respectively.

In study 2, Junction + $CuSO_4$ 0.25 ppm, $CuSO_4$ 0.25 ppm, PAR + Junction + $CuSO_4$ 0.25 ppm, and $CuSO_4$ 2.0 ppm had similar rates of 0.2665, 0.2624, 0.2582, and 0.25, respectively (Figure 2-7) (Table 2-5). PAR + $CuSO_4$ 2.0 ppm was similar to PAR + Junction + 0.25 ppm and $CuSO_4$ 2.0 ppm, with an average increase of 0.2418. Similar to $CuSO_4$ 2.0 ppm and PAR + $CuSO_4$ 2.0 ppm, PAR + Junction and Junction increased at a rate of 0.2390 and 0.2376, respectively. Similar to the previously two mentioned treatments, PAR + $CuSO_{4\ 0.25}$ and Junction + $CuSO_4$ 2.0 ppm both increased at a rate of 0.2239. PAR, with an average increase of 0.2157, was similar to PAR + $CuSO_4$ 0.25 and Junction + $CuSO_4$ 2.0 ppm. PAR + Junction + $CuSO_4$ 2.0 ppm increased exhibited the lowest increase in visual quality at a rate of 0.1964.

Means were compared over time, within treatments, by curve fitting sigmoidal four parameter log curve. Due to the variability of data, some treatment R^2 values are low, however curve fitting does provide for observational notes on timing of any changes. In study 1, apart from PAR treatment, increases in turf quality for all treatments were observed after week 9 (Figure 2-6) (Table 2-7). In study 2, all treatments experienced a similar timing of quality increase (Figure 2-8) (Table 2-6).

Overall, results suggest the use of pigmented products such as PAR may mask turfgrass problems and cause turfgrass areas to appear in better than actual condition. Data also suggest the use of colorants can provide a temporary increase in aesthetic appearance, they, however, do not enhance turf quality once improved growing conditions, including increased temperatures and day length, occur. Increasing treatment Cu concentration did not have a consistent effect on visual quality.

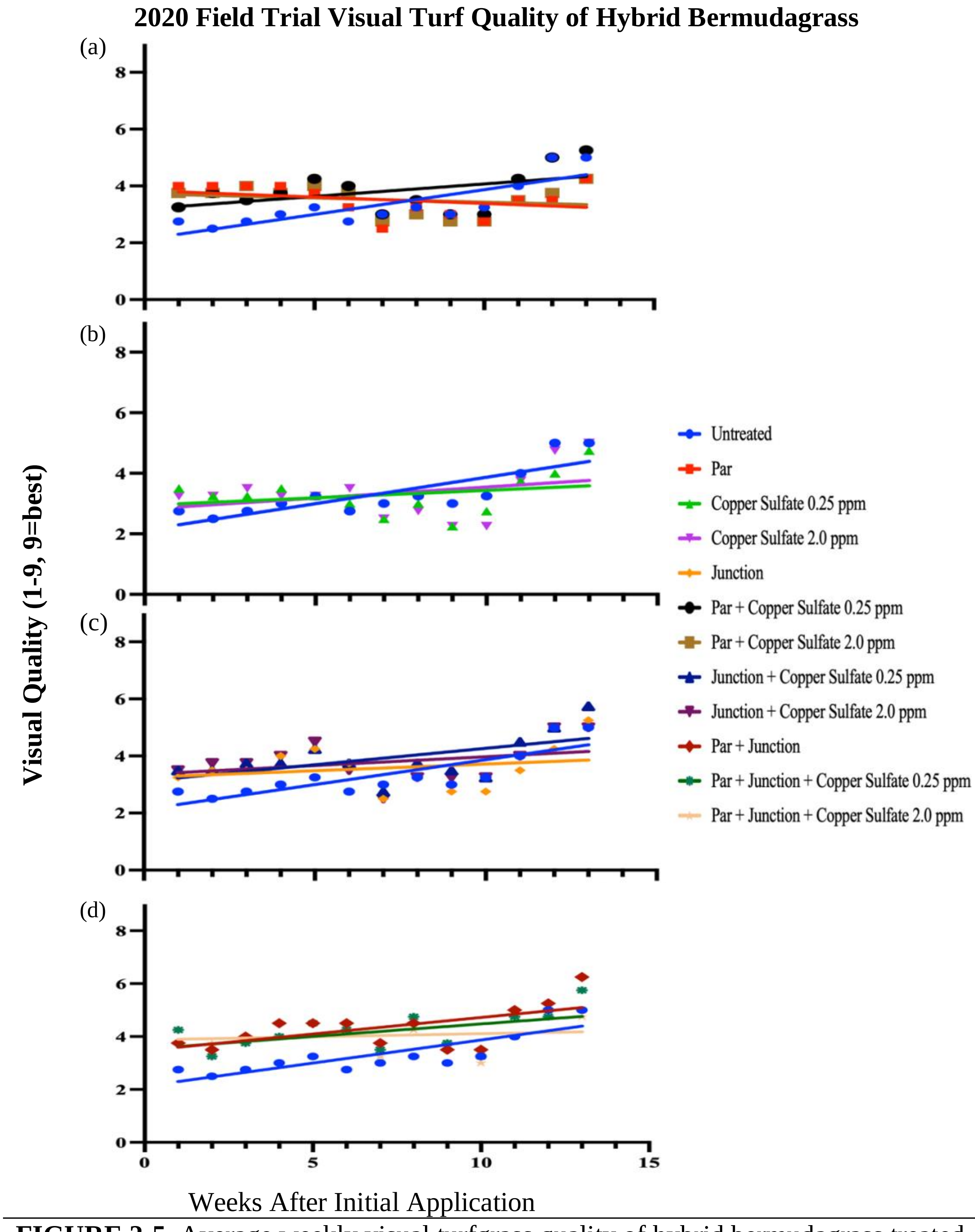


FIGURE 2-5. Average weekly visual turfgrass quality of hybrid bermudagrass treated with various copper-containing products beginning in March 2020 with trendline representing individual treatment progression over trial length of (a) PAR and PAR + $CuSO_4$ treatments, (b) $CuSO_4$ treatments, (c) Junction and Junction + SO_4 treatments, and (d) PAR + Junction and PAR + Junction + $CuSO_4$ treatments.

TABLE 2-3. Trendline slope (improvement) of hybrid bermudagrass visual turf quality treated with copper-containing products in 2020 field trial.

Treatment	Trendline Slope
Untreated	0.1744a ± 0.03
PAR	-0.0453g ± 0.03
Copper Sulfate 0.25 ppm	0.0495ef ± 0.03
Copper Sulfate 2.0 ppm	0.0728de ± 0.03
Junction	0.0467ef ± 0.03
PAR + Copper Sulfate 0.25 ppm	0.0879cd ± 0.03
PAR + Copper Sulfate 2.0 ppm	-0.0137g ± 0.03
Junction + Copper Sulfate 0.25 ppm	0.1154bc ± 0.03
Junction + Copper Sulfate 2.0 ppm	0.0618de ± 0.03
PAR + Junction	0.125b ± 0.03
PAR + Junction + Copper Sulfate 0.25 ppm	0.0948bcd ± 0.03
PAR + Junction + Copper Sulfate 2.0 ppm	0.0234f ± 0.03

Note: Within columns, means followed by the same letter are not significantly different according to LSD (0.05).

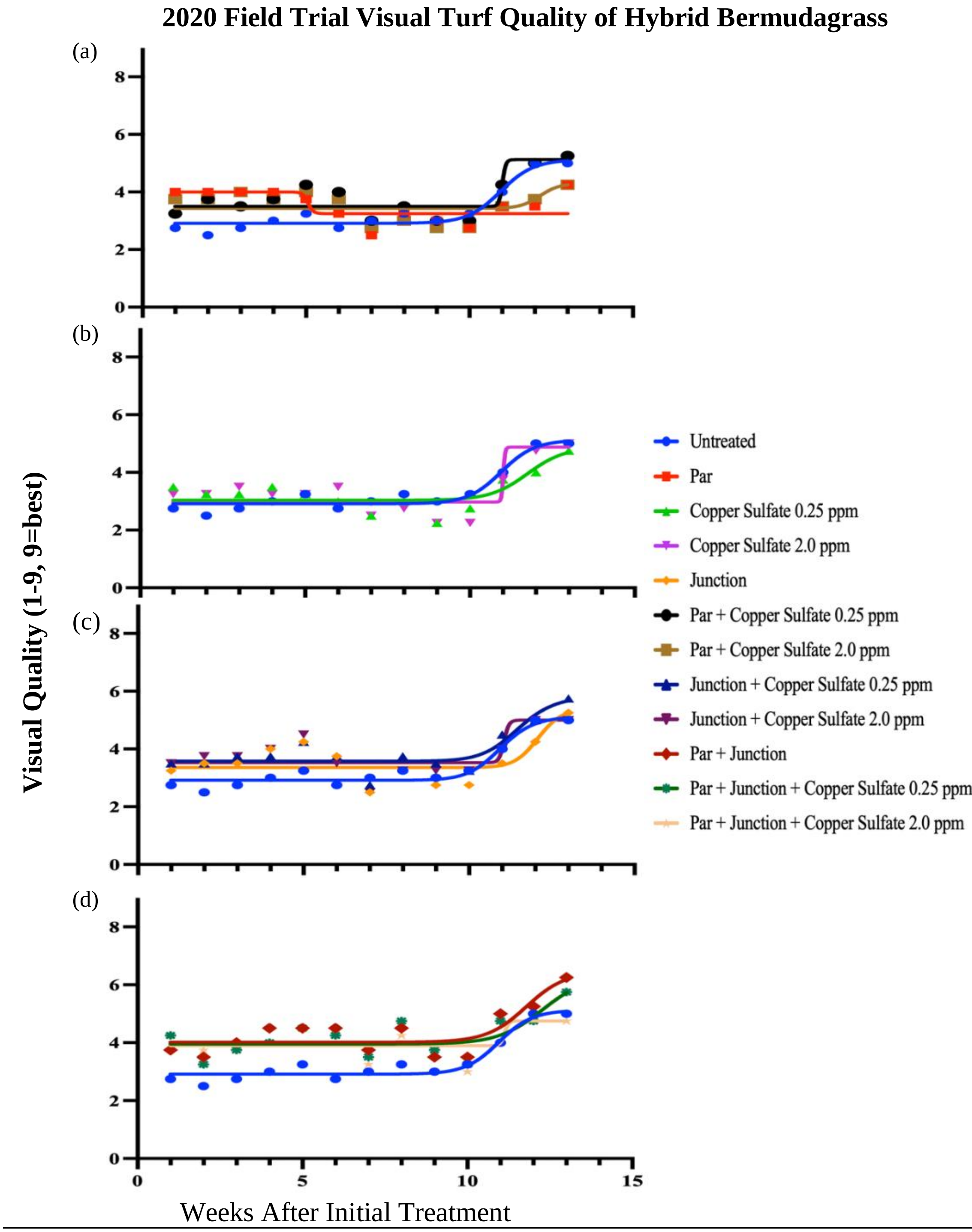


FIGURE 2-6. Average weekly visual turfgrass quality of hybrid bermudagrass treated with various copper-containing products beginning in March 2020 with sigmoidal curve representing individual treatment progression over trial length of (a) PAR and PAR + $CuSO_4$ treatments, (b) $CuSO_4$ treatments, (c) Junction and Junction + SO_4 treatments, and (d) PAR + Junction and PAR + Junction + $CuSO_4$ treatments.

TABLE 2-4. Treatments with respective 2020 field trial visual turf quality inflection points and R^2 values when fit to a logistic four parameter sigmoidal curve.

Treatment	**Inflection**	$\mathbf{R^2}$
	-----Week-----	
Untreated	10.949	0.561
PAR	5.030	0.139
Copper Sulfate 0.25 ppm	11.746	0.328
Copper Sulfate 2.0 ppm	11.047	0.524
Junction	12.068	0.353
PAR + Copper Sulfate 0.25 ppm	11.020	0.460
PAR + Copper Sulfate 2.0 ppm	11.755	0.103
Junction + Copper Sulfate 0.25 ppm	11.485	0.586
Junction + Copper Sulfate 2.0 ppm	11.083	0.380
PAR + Junction	11.782	0.420
PAR + Junction + Copper Sulfate 0.25 ppm	12.276	0.337
PAR + Junction + Copper Sulfate 2.0 ppm	11.142	0.115

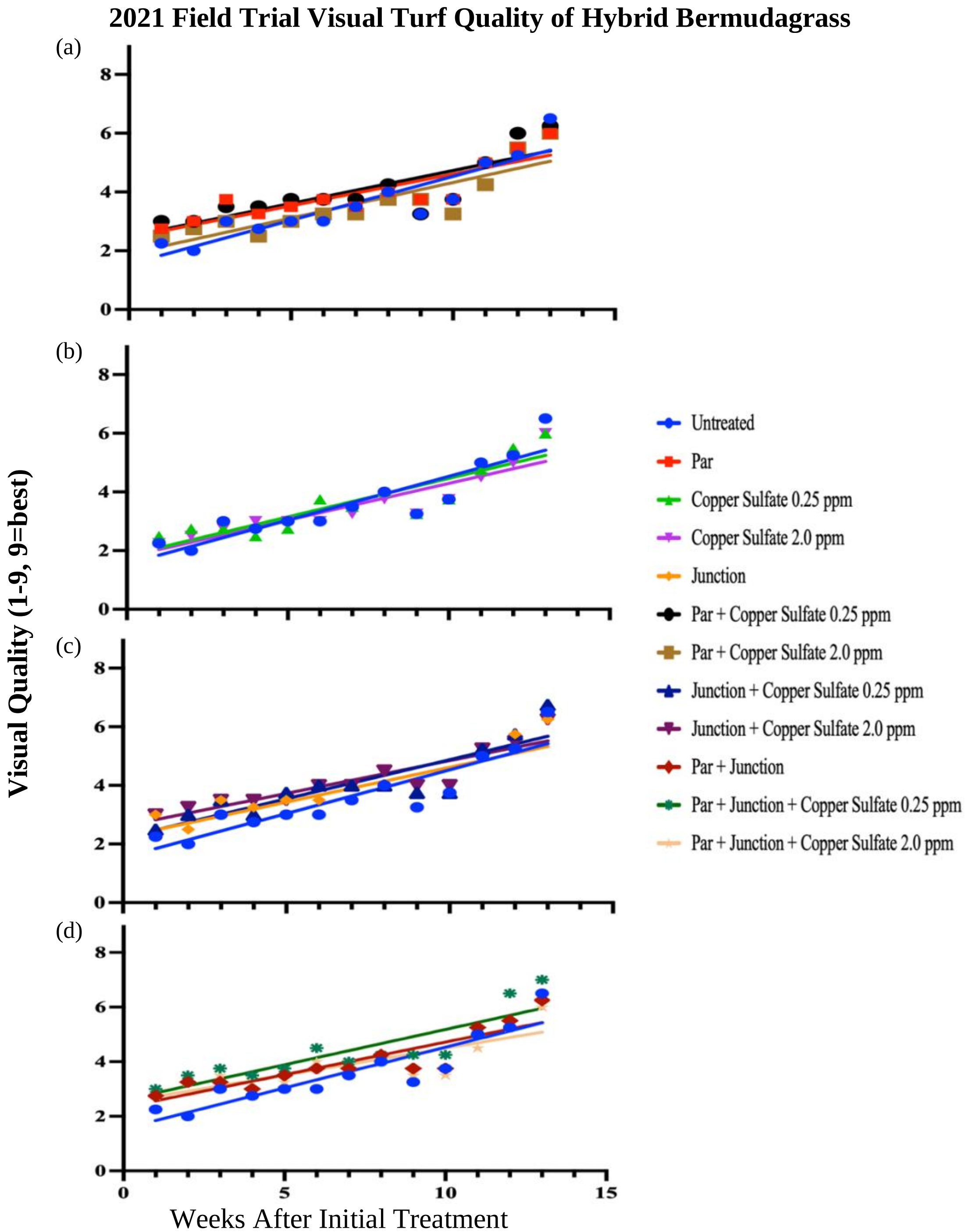


FIGURE 2-7. Average weekly visual turfgrass quality of hybrid bermudagrass treated with various copper-containing products beginning in March 2021 with trendline representing individual treatment progression over trial length of (a) PAR and PAR + $CuSO_4$ treatments, (b) $CuSO_4$ treatments, (c) Junction and Junction + SO_4 treatments, and (d) PAR + Junction and PAR + Junction + $CuSO_4$ treatments.

TABLE 2-5. Trendline slope (improvement) of hybrid bermudagrass visual turf quality treated with copper-containing products in 2021 field trial.

Treatment	**Trendline Slope**
Untreated	0.2981a ± 0.03
PAR	0.2157f ± 0.03
Copper Sulfate 0.25 ppm	0.2624b ± 0.03
Copper Sulfate 2.0 ppm	0.25bcd ± 0.03
Junction	0.2376de ± 0.03
PAR + Copper Sulfate 0.25 ppm	0.2239ef ± 0.02
PAR + Copper Sulfate 2.0 ppm	0.2418cd ± 0.03
Junction + Copper Sulfate 0.25 ppm	0.2667b ± 0.03
Junction + Copper Sulfate 2.0 ppm	0.2239ef ± 0.03
PAR + Junction	0.2390de ± 0.03
PAR + Junction + Copper Sulfate 0.25 ppm	0.2582bc ± 0.03
PAR + Junction + Copper Sulfate 2.0 ppm	0.1964g ± 0.02

Note: Within columns, means followed by the same letter are not significantly different according to LSD (0.05).

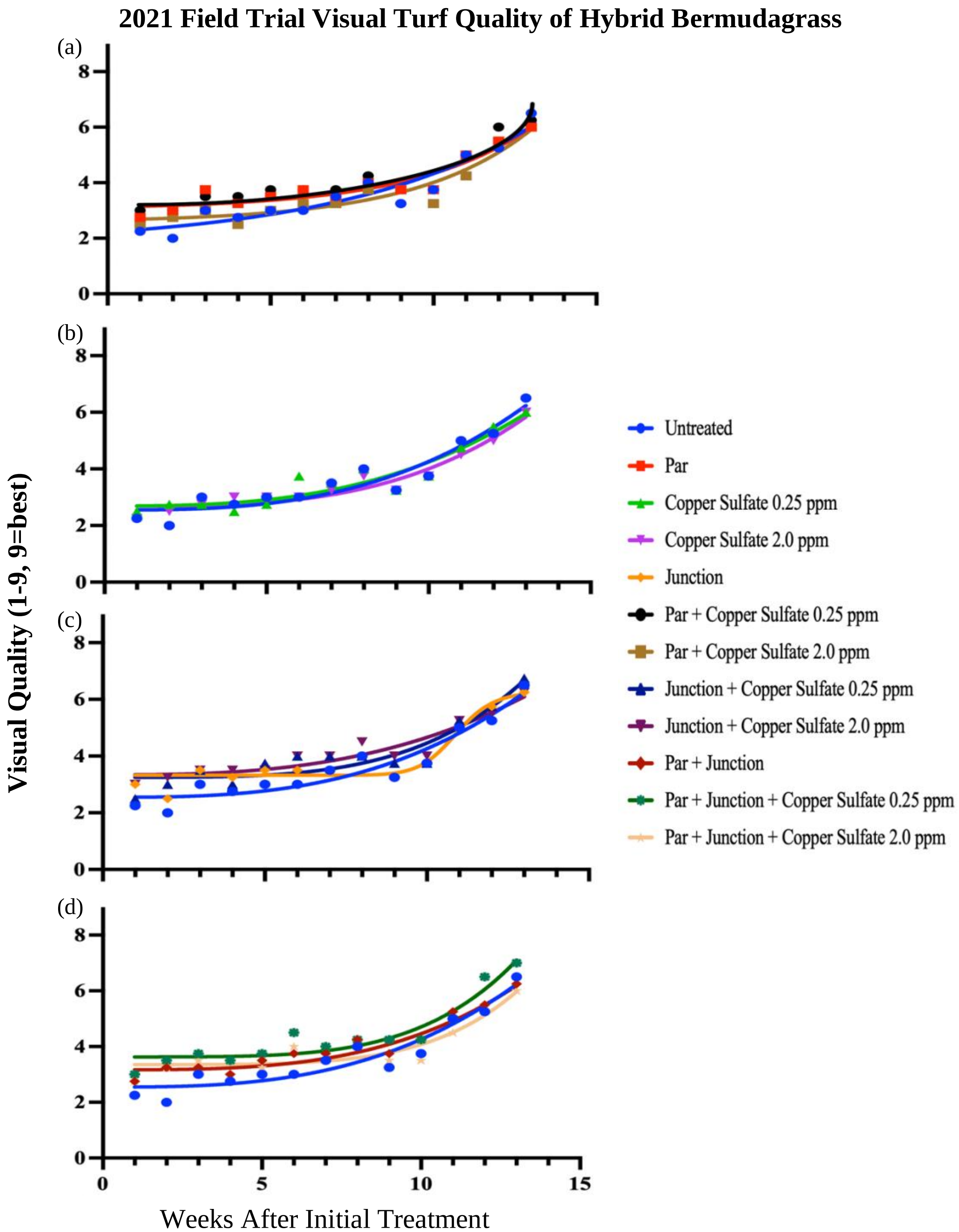


FIGURE 2-8. Average weekly visual turfgrass quality of hybrid bermudagrass treated with various copper-containing products beginning in March 2021 with sigmoidal curve representing individual treatment progression over trial length of (a) PAR and PAR + $CuSO_4$ treatments, (b) $CuSO_4$ treatments, (c) Junction and Junction + SO_4 treatments, and (d) PAR + Junction and PAR + Junction + $CuSO_4$ treatments.

TABLE 2-6. Treatments with respective 2021 field trial visual turf quality inflection points and R^2 values when fit to a logistic four parameter sigmoidal curve.

Treatment	Inflection	R^2
	-----Week-----	
Untreated	71.343	0.820
PAR	64.577	0.551
Copper Sulfate 0.25 ppm	73.917	0.710
Copper Sulfate 2.0 ppm	58.915	0.693
Junction	10.921	0.761
PAR + Copper Sulfate 0.25 ppm	67.173	0.779
PAR + Copper Sulfate 2.0 ppm	56.630	0.597
Junction + Copper Sulfate 0.25 ppm	48.704	0.727
Junction + Copper Sulfate 2.0 ppm	69.170	0.535
PAR + Junction	66.166	0.700
PAR + Junction + Copper Sulfate 0.25 ppm	52.600	0.814
PAR + Junction + Copper Sulfate 2.0 ppm	51.923	0.687

Normalized Difference Vegetative Index

In study 1, $CuSO_4$ 2.0 ppm exhibited the greatest normalized difference vegetation index (NDVI) rate of increase 0.0222 (Figure 2-9) (Table 2-7). NDVI of $CuSO_4$ 0.25 ppm and Junction + $CuSO_4$ 0.25 ppm increased at similar rates of 0.0210 and 0.0207, respectively. Junction + $CuSO_4$ 2.0 ppm, untreated, and Junction increased at similar rates of 0.0193, 0.0193, and 0.0191, respectively. PAR + Junction + $CuSO_4$ 0.25 ppm and PAR + Junction were similar with rates of 0.0130 and 0.019, respectively. PAR + $CuSO_4$ 0.25 ppm, PAR + $CuSO_4$ 2.0 ppm, and PAR + Junction + $CuSO_4$ 2.0 ppm increased at similar rates of 0.0114, 0.0110, and 0.0105, respectively, while PAR was similar to the latter two with an average change of 0.0102.

In study 2, $CuSO_4$ 2.0 ppm, Junction, Junction + $CuSO_4$ 0.25 ppm, and untreated areas increased NDVI at similar rates of 0.0137, 0.0130, 0.0130, and 0.0129, respectively (Figure 2-11) (Table 2-9). Copper sulfate 0.25 ppm and Junction + $CuSO_4$ 2.0 ppm were similar with rates of 0.0115 and 0.0113, respectively. PAR + Junction + $CuSO_4$ 2.0 ppm, PAR + Junction + $CuSO_4$ 0.25 ppm, PAR + Junction were similar at rates of 0.0097, 0.0094, and 0.0089, respectively. Similar to PAR + Junction, PAR + $CuSO_4$ 0.25 ppm increased at a rate of 0.0081, which was similar to PAR with an average increase of 0.0077. PAR + $CuSO_4$ 2.0 ppm was similar to PAR with a rate of 0.0069.

Means were compared over time, within treatments, by curve fitting sigmoidal four parameter log curve. Due to the variability of data, R^2 values are low, however curve does provide for observational notes on timing of any changes. In study 1, treatments containing PAR appear to remain at consistent levels, with PAR + Junction treatment increasing after week 8 (Figure 2-10) (Table 2-8). Treatments not containing PAR appear to increase as consistent rates beginning after week 3. In study 2, all treatments experienced increase in NDVI between weeks 2 and 6 with variances in rate of change (Figure 2-12) (Table 2-10).

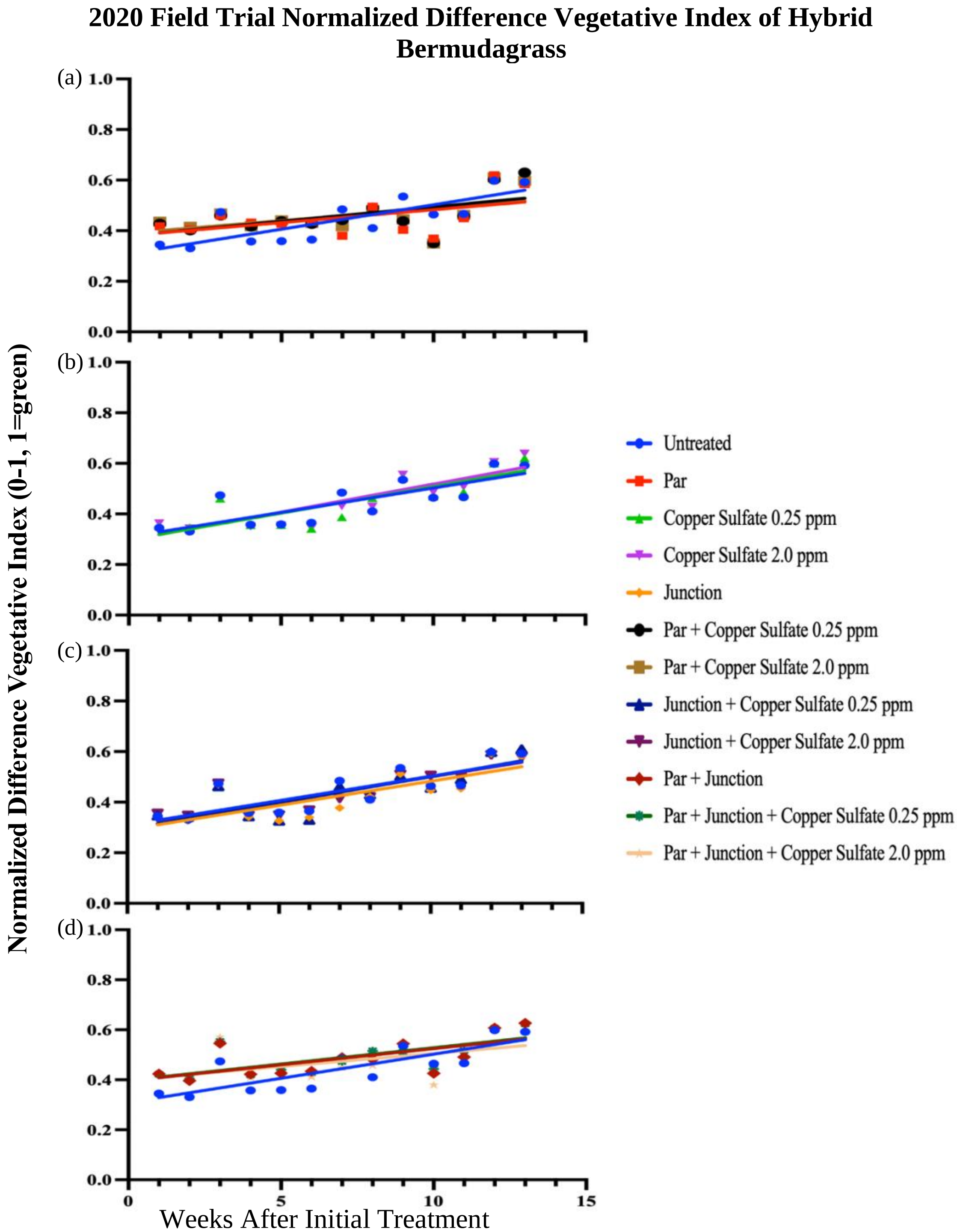


FIGURE 2-9. Average normalized difference vegetative index of hybrid bermudagrass treated with various copper-containing products beginning in March 2020 with trendline representing individual treatment progression over trial length of (a) PAR and PAR + $CuSO_4$ treatments, (b) $CuSO_4$ treatments, (c) Junction and Junction + SO_4 treatments, and (d) PAR + Junction and PAR + Junction + $CuSO_4$ treatments.

TABLE 2-7. Trendline slope (improvement) of hybrid bermudagrass normalized difference vegetative index treated with copper-containing products in 2020 field trial.

Treatment	**Trendline Slope**
Untreated	0.0193c ± 0.002
PAR	0.0102f ± 0.003
Copper Sulfate 0.25 ppm	0.0210b ± 0.002
Copper Sulfate 2.0 ppm	0.0222a ± 0.003
Junction	0.0191c ± 0.002
PAR + Copper Sulfate 0.25 ppm	0.0114e ± 0.003
PAR + Copper Sulfate 2.0 ppm	0.0110ef ± 0.003
Junction + Copper Sulfate 0.25 ppm	0.0207b ± 0.002
Junction + Copper Sulfate 2.0 ppm	0.0193c ± 0.002
PAR + Junction	0.0130d ± 0.002
PAR + Junction + Copper Sulfate 0.25 ppm	0.0130d ± 0.002
PAR + Junction + Copper Sulfate 2.0 ppm	0.0105ef ± 0.003

Note: Within columns, means followed by the same letter are not significantly different according to LSD (0.05).

2020 Field Trial Normalized Difference Vegetative Index of Hybrid Bermudagrass

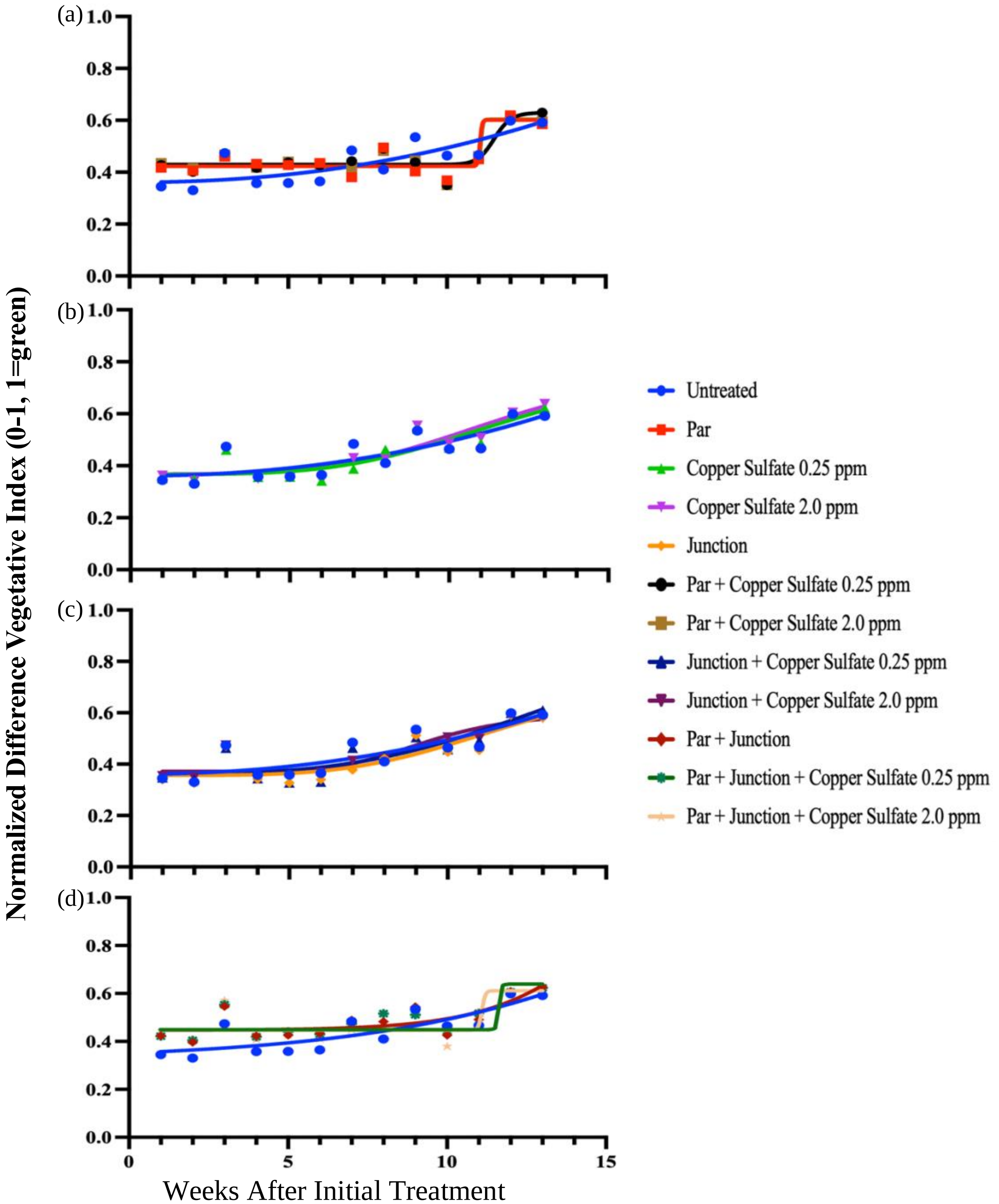


FIGURE 2-10. Average normalized difference vegetative index of hybrid bermudagrass treated with various copper-containing products beginning in March 2020 with sigmoidal curve representing individual treatment progression over trial length of (a) PAR and PAR + $CuSO_4$ treatments, (b) $CuSO_4$ treatments, (c) Junction and Junction + SO_4 treatments, and (d) PAR + Junction and PAR + Junction + $CuSO_4$ treatments.

TABLE 2-8. Treatments with respective 2020 field trial normalized difference vegetative index inflection points and R^2 values when fit to a logistic four parameter sigmoidal curve.

Treatment	Inflection	R^2
	-----Week-----	
Untreated	98.516	0.593
PAR	11.178	0.636
Copper Sulfate 0.25 ppm	11.576	0.712
Copper Sulfate 2.0 ppm	11.119	0.691
Junction	11.365	0.630
PAR + Copper Sulfate 0.25 ppm	11.496	0.722
PAR + Copper Sulfate 2.0 ppm	11.102	0.531
Junction + Copper Sulfate 0.25 ppm	12.722	0.704
Junction + Copper Sulfate 2.0 ppm	9.032	0.641
PAR + Junction	42.864	0.484
PAR + Junction + Copper Sulfate 0.25 ppm	11.171	0.556
PAR + Junction + Copper Sulfate 2.0 ppm	11.149	0.501

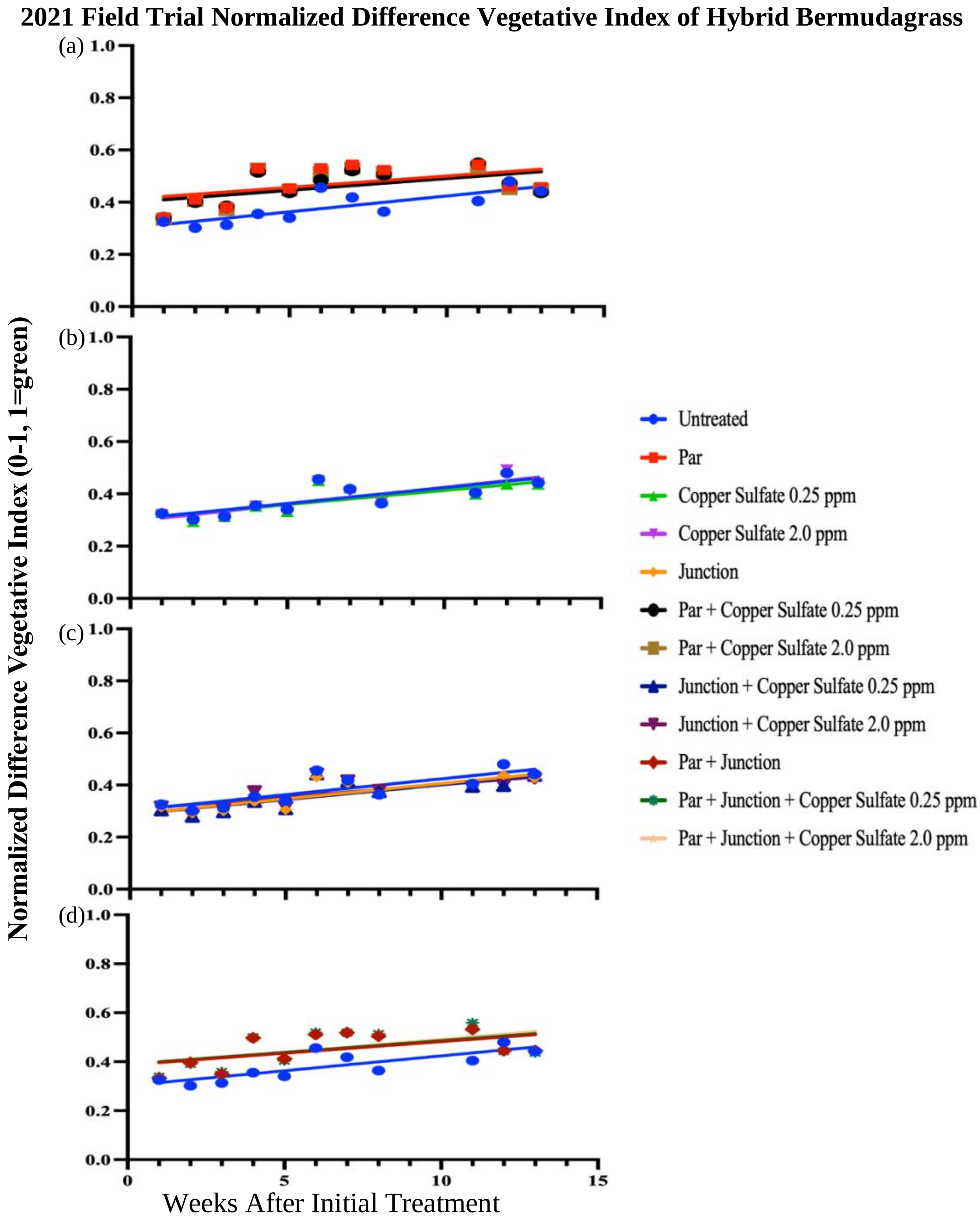


FIGURE 2-11. Average normalized difference vegetative index of hybrid bermudagrass treated with various copper-containing products beginning in March 2021 with trendline representing individual treatment progression over trial length of (a) PAR and PAR + $CuSO_4$ treatments, (b) $CuSO_4$ treatments, (c) Junction and Junction + SO_4 treatments, and (d) PAR + Junction and PAR + Junction + $CuSO_4$ treatments.

TABLE 2-9. Trendline slope (improvement) of hybrid bermudagrass normalized difference vegetative index treated with copper-containing products in 2021 field trial.

Treatment	**Trendline Slope**
Untreated	0.0129a ± 0.002
PAR	0.0077ef ± 0.002
Copper Sulfate 0.25 ppm	0.0115b ± 0.002
Copper Sulfate 2.0 ppm	0.0137a ± 0.002
Junction	0.0130a ± 0.002
PAR + Copper Sulfate 0.25 ppm	0.0081de ± 0.002
PAR + Copper Sulfate 2.0 ppm	0.0069f ± 0.003
Junction + Copper Sulfate 0.25 ppm	0.0130a ± 0.002
Junction + Copper Sulfate 2.0 ppm	0.0113b ± 0.002
PAR + Junction	0.0089cd ± 0.002
PAR + Junction + Copper Sulfate 0.25 ppm	0.0094c ± 0.003
PAR + Junction + Copper Sulfate 2.0 ppm	0.0097c ± 0.003

Note: Within columns, means followed by the same letter are not significantly different according to LSD (0.05).

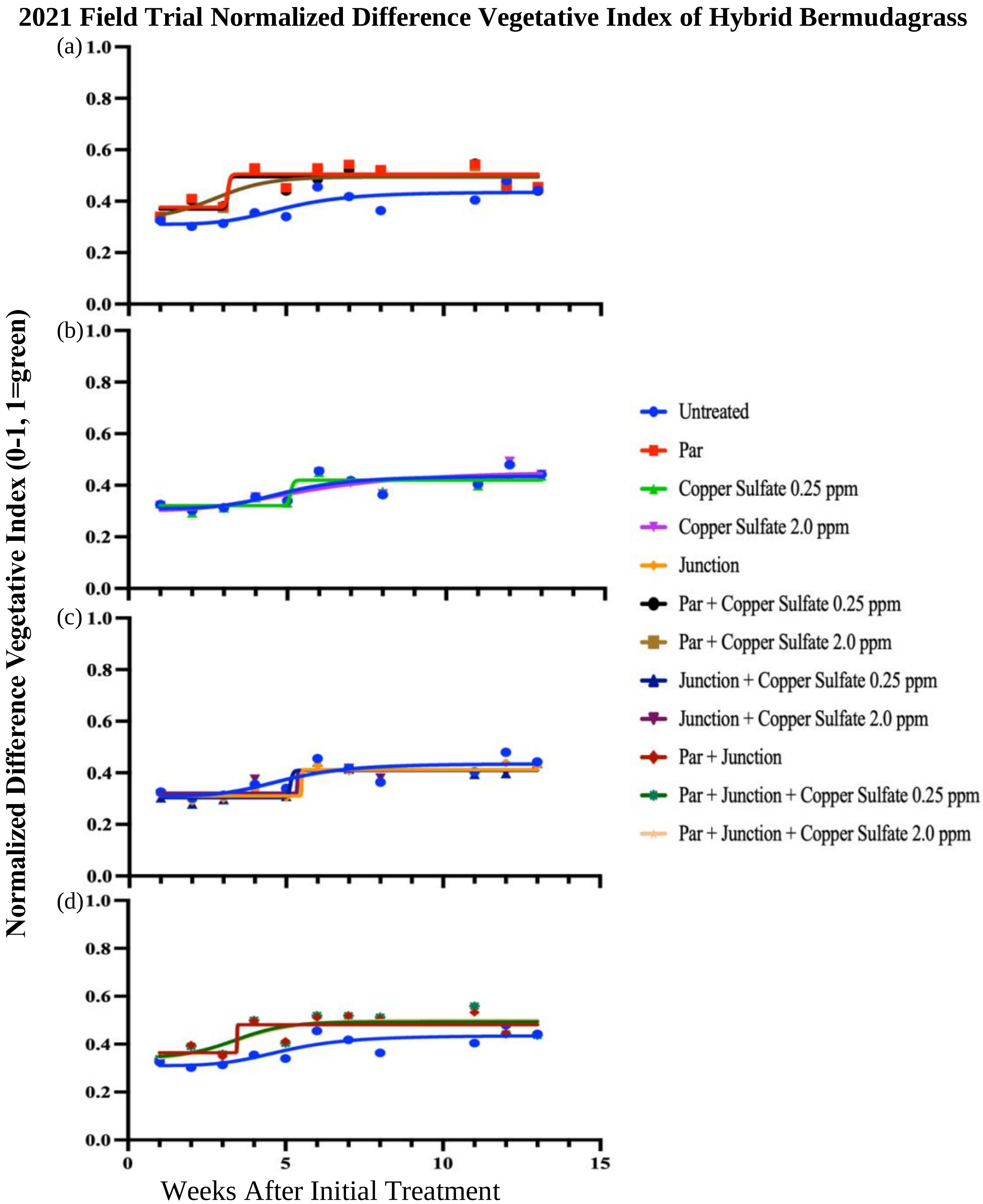


FIGURE 2-12. Average normalized difference vegetative index of hybrid bermudagrass treated with various copper-containing products beginning in March 2021 with sigmoidal curve representing individual treatment progression over trial length of (a) PAR and PAR + $CuSO_4$ treatments, (b) $CuSO_4$ treatments, (c) Junction and Junction + SO_4 treatments, and (d) PAR + Junction and PAR + Junction + $CuSO_4$ treatments.

TABLE 2-10. Treatments with respective 2021 field trial normalized difference vegetative index inflection points and R^2 values when fit to a logistic four parameter sigmoidal curve.

Treatment	**Inflection**	$\mathbf{R^2}$
	-----Week-----	
Untreated	4.751	0.536
PAR	3.135	0.568
Copper Sulfate 0.25 ppm	5.113	0.613
Copper Sulfate 2.0 ppm	4.788	0.514
Junction	5.646	0.534
PAR + Copper Sulfate 0.25 ppm	3.105	0.426
PAR + Copper Sulfate 2.0 ppm	-58.668	0.276
Junction + Copper Sulfate 0.25 ppm	5.138	0.609
Junction + Copper Sulfate 2.0 ppm	5.168	0.598
PAR + Junction	3.363	0.497
PAR + Junction + Copper Sulfate 0.25 ppm	3.264	0.409
PAR + Junction + Copper Sulfate 2.0 ppm	3.077	0.380

Turf Coverage

In study 1, untreated and $CuSO_4$ 2.0 ppm plots increased coverage over 13 weeks at the greatest rates of 1.195% and 1.096%, respectively (Figure 2-13) (Table 2-11). Copper sulfate 0.25 ppm increased at a rate of 0.609%. PAR + Junction + $CuSO_4$ 0.25 ppm, Junction + $CuSO_4$ 0.25 ppm, and PAR + Junction turf coverage produced similar changes of -0.056, -0.130, and -0.216%, respectively. The latter two treatments were similar in change over time to Junction + $CuSO_4$ 2.0 ppm at -0.52% which was similar to PAR + Junction + $CuSO_4$ 2.0 ppm rate of -0.664%. PAR + $CuSO_4$ 0.25 ppm, PAR + $CuSO_4$ 2.0 ppm, and Junction exhibited similar rate changes with -1.127, -1.365, and -1.401%, respectively. PAR had the worst rate of change, decreasing coverage over 13 weeks at a rate of -1.840% per week.

In study 2, $CuSO_4$ 2.0 ppm increased plot coverage at rate of 4.106% per week (Figure 2-9) (Table 2-8). Untreated plots increased coverage at average of 3.779% weekly. Copper sulfate 0.25 ppm increased at a rate of 3.273%. Junction, Junction + $CuSO_4$ 0.25 ppm, and Junction + $CuSO_4$ 2.0 ppm had similar coverage increase rates of 2.307, 2.096, and 2.066%, respectively. PAR and PAR + $CuSO_4$ 2.0 ppm plot coverage increased at similar rates of 0.990% and 0.895%, respectively. PAR + $CuSO_4$ 0.25 ppm, PAR + Junction + $CuSO_4$ 0.25 ppm, and PAR + Junction increased turf coverage 0.598, 0.557, 0.51%, and 0.352%, respectively, whereas PAR + Junction + $CuSO_4$ 0.25 ppm was similar to PAR + Junction, increasing at a rate of 0.293% weekly.

Means were compared over time, within treatments, by curve fitting sigmoidal four parameter log curve. Due to the variability of data, R^2 values are low, however curve does provide for observational notes on timing of any changes. In study 1, most treatments containing a colorant decreased at some point within the trial with the exception of PAR + Junction + 0.25 ppm $CuSO_4$ (Figure 2-14) (Table 2-12). Treatments not containing a colorant experienced increases between weeks 3 and 8. In study 2, treatments containing PAR maintained consistent coverage throughout trial while other treatments experienced consistent increases at similar timings (Figure 2-16) (Table 2-14).

The presence of pigments such as PAR may give the appearance treated areas are covered by actively growing grass, resulting in little change over time of perceived coverage, hence their appeal for usage in place of overseeding. However, the presence of these products covering leaf tissue for long periods of time may inhibit growth process of plants breaking dormancy resulting in decreased rates of change of an area’s coverage as observed in Study 1.

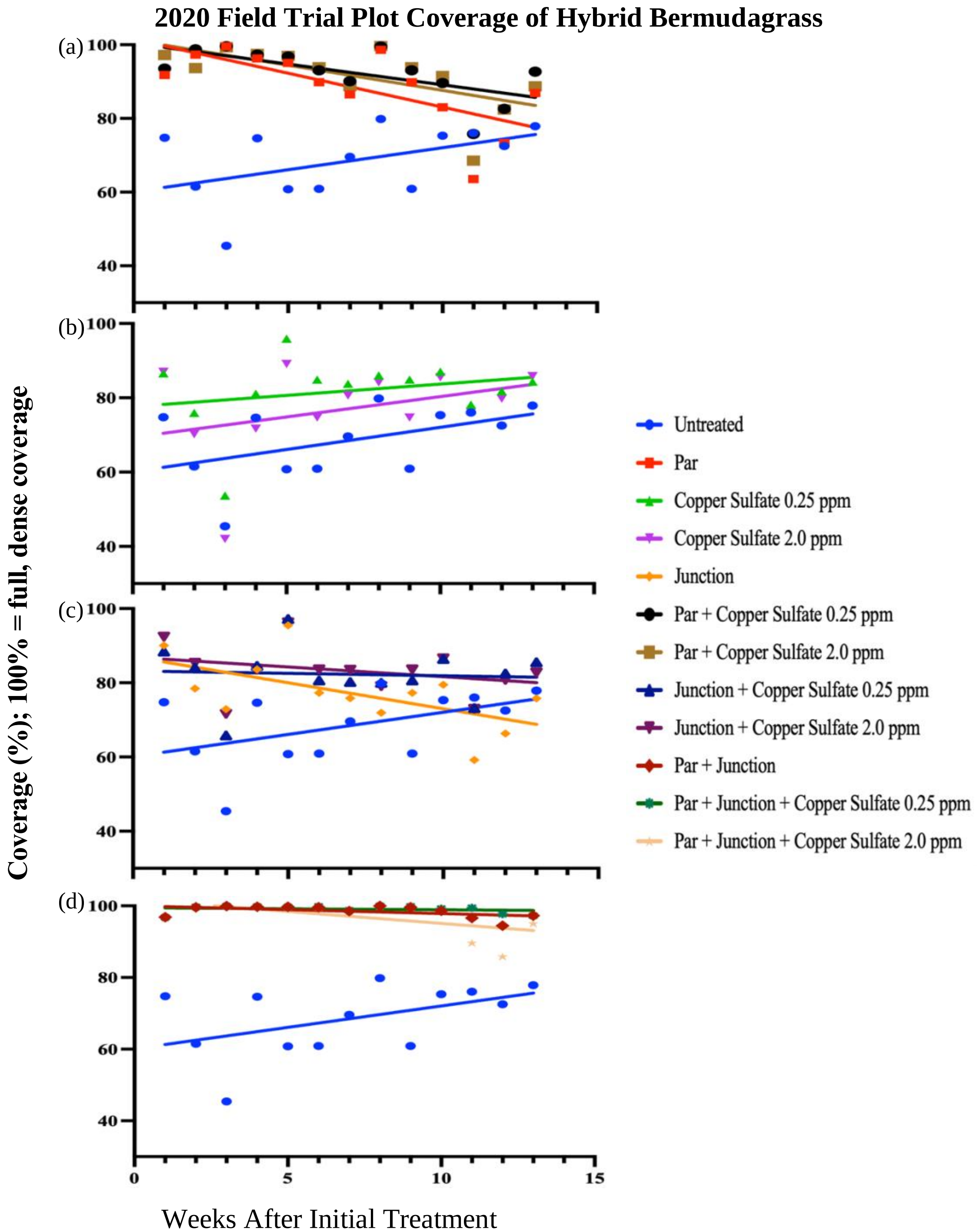


FIGURE 2-13. Average weekly coverage of hybrid bermudagrass plots treated with various copper-containing products beginning in March 2020 with trendline representing individual treatment progression over trial length of (a) PAR and PAR + $CuSO_4$ treatments, (b) $CuSO_4$ treatments, (c) Junction and Junction + SO_4 treatments, and (d) PAR + Junction and PAR + Junction + $CuSO_4$ treatments.

TABLE 2-11. Trendline slope (improvement) of hybrid bermudagrass plot coverage treated with copper-containing products in 2020 field trial.

Treatment	Trendline Slope
Untreated	1.195a ± 0.53
PAR	-1.840g ± 0.39
Copper Sulfate 0.25 ppm	0.609b ± 0.44
Copper Sulfate 2.0 ppm	1.096a ± 0.62
Junction	-1.401f ± 0.48
PAR + Copper Sulfate 0.25 ppm	-1.127f ± 0.22
PAR + Copper Sulfate 2.0 ppm	-1.365f ± 0.30
Junction + Copper Sulfate 0.25 ppm	-0.130cd ± 0.37
Junction + Copper Sulfate 2.0 ppm	-0.527de ± 0.43
PAR + Junction	-0.216cd ± 0.08
PAR + Junction + Copper Sulfate 0.25 ppm	-0.056c ± 0.04
PAR + Junction + Copper Sulfate 2.0 ppm	-0.664e ± 0.21

Note: Within columns, means followed by the same letter are not significantly different according to LSD (0.05).

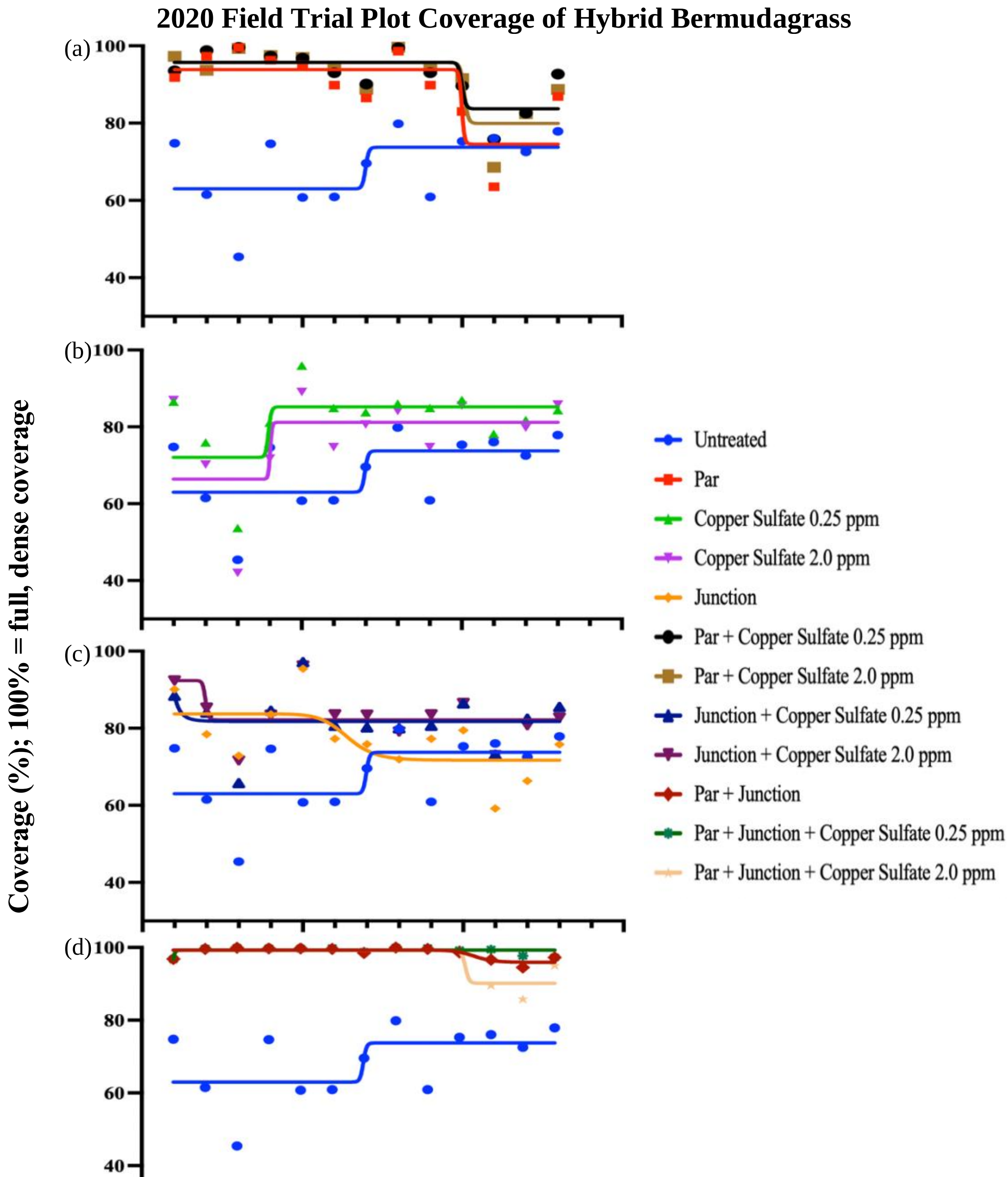


FIGURE 2-14. Average weekly coverage of hybrid bermudagrass plots treated with various copper-containing products beginning in March 2020 with sigmoidal curve representing individual treatment progression over trial length of (a) PAR and PAR + $CuSO_4$ treatments, (b) $CuSO_4$ treatments, (c) Junction and Junction + SO_4 treatments, and (d) PAR + Junction and PAR + Junction + $CuSO_4$ treatments.

TABLE 2-12. Treatments with respective 2020 field trial turf coverage inflection points and R^2 when fit to a logistic four parameter sigmoidal curve.

Treatment	**Inflection**	$\mathbf{R^2}$
	-----Week-----	
Untreated	6.996	0.124
PAR	9.988	0.436
Copper Sulfate 0.25 ppm	3.931	0.214
Copper Sulfate 2.0 ppm	4.037	0.141
Junction	7.667	0.163
PAR + Copper Sulfate 0.25 ppm	9.998	0.507
PAR + Copper Sulfate 2.0 ppm	10.047	0.477
Junction + Copper Sulfate 0.25 ppm	1.205	0.033
Junction + Copper Sulfate 2.0 ppm	1.926	0.057
PAR + Junction	10.494	0.377
PAR + Junction + Copper Sulfate 0.25 ppm	11.92	0.323
PAR + Junction + Copper Sulfate 2.0 ppm	10.217	0.393

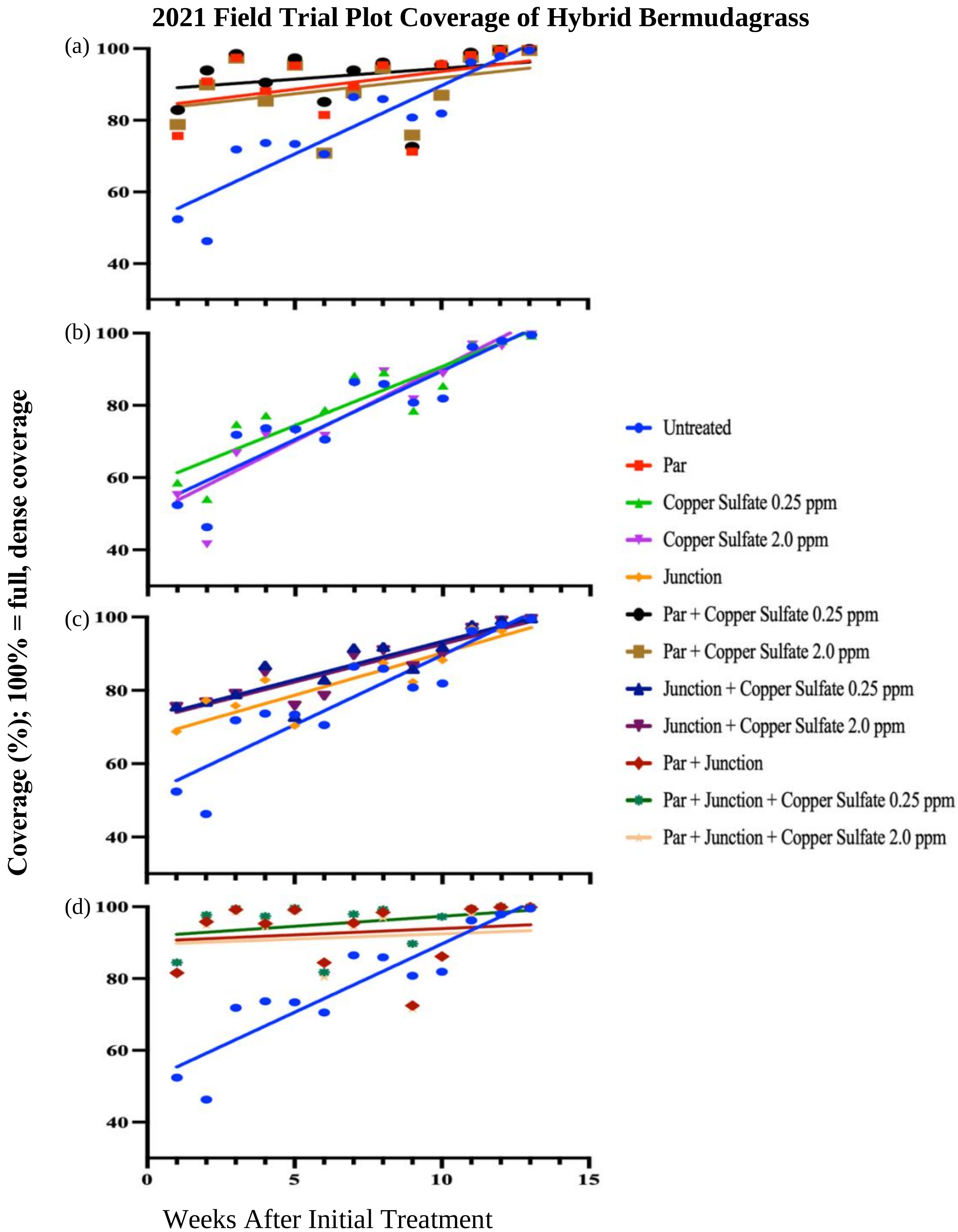


FIGURE 2-15. Average weekly coverage of hybrid bermudagrass plots treated with various copper-containing products beginning in March 2021 with trendline representing individual treatment progression over trial length of (a) PAR and PAR + $CuSO_4$ treatments, (b) $CuSO_4$ treatments, (c) Junction and Junction + SO_4 treatments, and (d) PAR + Junction and PAR + Junction + $CuSO_4$ treatments.

TABLE 2-13. Trendline slope (improvement) of hybrid bermudagrass plot coverage treated with copper-containing products in 2021 field trial.

Treatment	Trendline Slope
Untreated	3.779b ± 0.35
PAR	0.99e ± 0.39
Copper Sulfate 0.25 ppm	3.273c ± 0.36
Copper Sulfate 2.0 ppm	4.106a ± 0.31
Junction	2.307d ± 0.46
PAR + Copper Sulfate 0.25 ppm	0.598f ± 0.31
PAR + Copper Sulfate 2.0 ppm	0.895e ± 0.43
Junction + Copper Sulfate 0.25 ppm	2.096d ± 0.28
Junction + Copper Sulfate 2.0 ppm	2.066d ± 0.41
PAR + Junction	0.352fg ± 0.40
PAR + Junction + Copper Sulfate 0.25 ppm	0.557f ± 0.31
PAR + Junction + Copper Sulfate 2.0 ppm	0.293g ± 0.44

Note: Within columns, means followed by the same letter are not significantly different according to LSD (0.05).

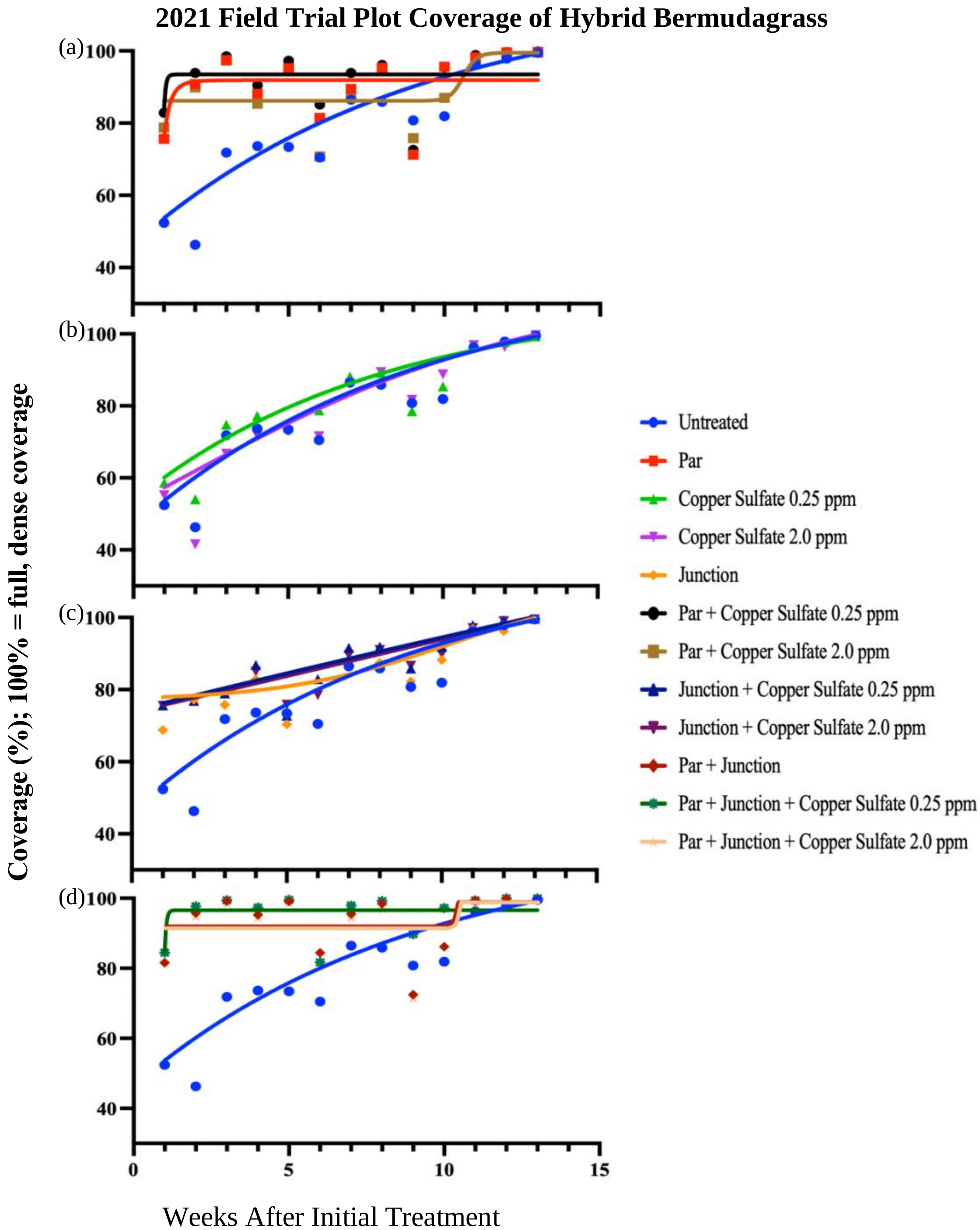


FIGURE 2-16. Average weekly coverage of hybrid bermudagrass plots treated with various copper-containing products beginning in March 2021 with sigmoidal curve representing individual treatment progression over trial length of (a) PAR and PAR + $CuSO_4$ treatments, (b) $CuSO_4$ treatments, (c) Junction and Junction + SO_4 treatments, and (d) PAR + Junction and PAR + Junction + $CuSO_4$ treatments.

TABLE 2-14. Treatments with respective 2021 field trial turf coverage inflection points and R^2 values when fit to a logistic four parameter sigmoidal curve.

Treatment	Inflection	R^2
	-----Week-----	
Untreated	-134.363	0.709
PAR	-1.094	0.378
Copper Sulfate 0.25 ppm	-111.111	0.636
Copper Sulfate 2.0 ppm	1.136	0.809
Junction	9.926	0.350
PAR + Copper Sulfate 0.25 ppm	1.798	0.562
PAR + Copper Sulfate 2.0 ppm	10.625	0.204
Junction + Copper Sulfate 0.25 ppm	9.809	0.540
Junction + Copper Sulfate 2.0 ppm	10.675	0.345
PAR + Junction	10.827	0.125
PAR + Junction + Copper Sulfate 0.25 ppm	0.939	0.145
PAR + Junction + Copper Sulfate 2.0 ppm	10.914	0.126

Tissue Copper Concentration

Due to the lack of comparable samples from limited vegetative growth, statistical analysis of Cu concentration within plant tissue could not be performed in study 1. However, areas treated with Junction, alone or in combination with other treatments, appeared to have higher tissue concentration of Cu than other treatments (Figure 2-17).

In study 2, Junction + $CuSO_4$ 2.0 ppm, PAR + Junction + $CuSO_4$ 2.0 ppm, Junction + $CuSO_4$ 0.25 ppm, PAR + Junction, and Junction had similar copper concentrations of 48.3145, 37.2328, 33.6725, 32.8238, and 29.933 mg Cu kg^{-1}, respectively (Figure 2-18). Except for Junction + $CuSO_4$ 2.0 ppm, these treatments were also similar to PAR +

Junction + $CuSO_4$ 0.25 ppm, PAR + $CuSO_4$ 0.25 ppm, and PAR + $CuSO_4$ 2.0 ppm with concentrations of 24.1593, 19.124, and 19.0765 mg Cu kg^{-1}, respectively. Copper sulfate 2.0 ppm, $CuSO_4$ 0.25 ppm, PAR, and untreated had Cu concentrations of 17.2383, 17.2293, 16.9918, and 16.6213 mg Cu kg^{-1}, respectively, which were similar to all trial treatments except for Junction + $CuSO_4$ 2.0 ppm and PAR + Junction + $CuSO_4$ 2.0 ppm.

Results suggest the absorption or usage of copper by plants may vary depending on copper species and compound, with areas treated with copper hydroxide (Junction) exhibiting higher copper concentrations in leaf tissue than areas treated foliarly with $CuSO_4$ or phthalocyanine compounds.

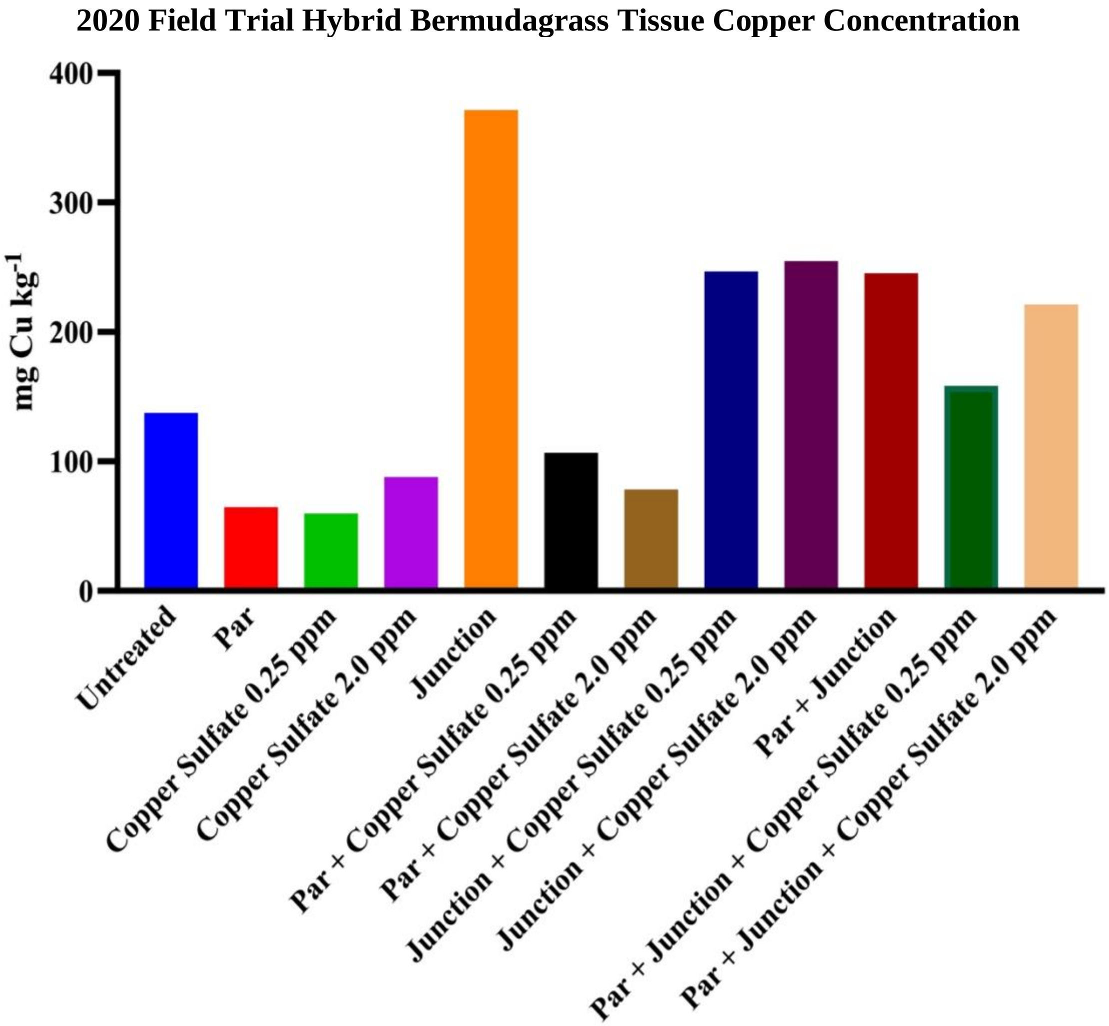


FIGURE 2-17. Observed plant tissue copper concentration for field trial beginning March 2020 following applications of various copper-containing products to hybrid bermudagrass.

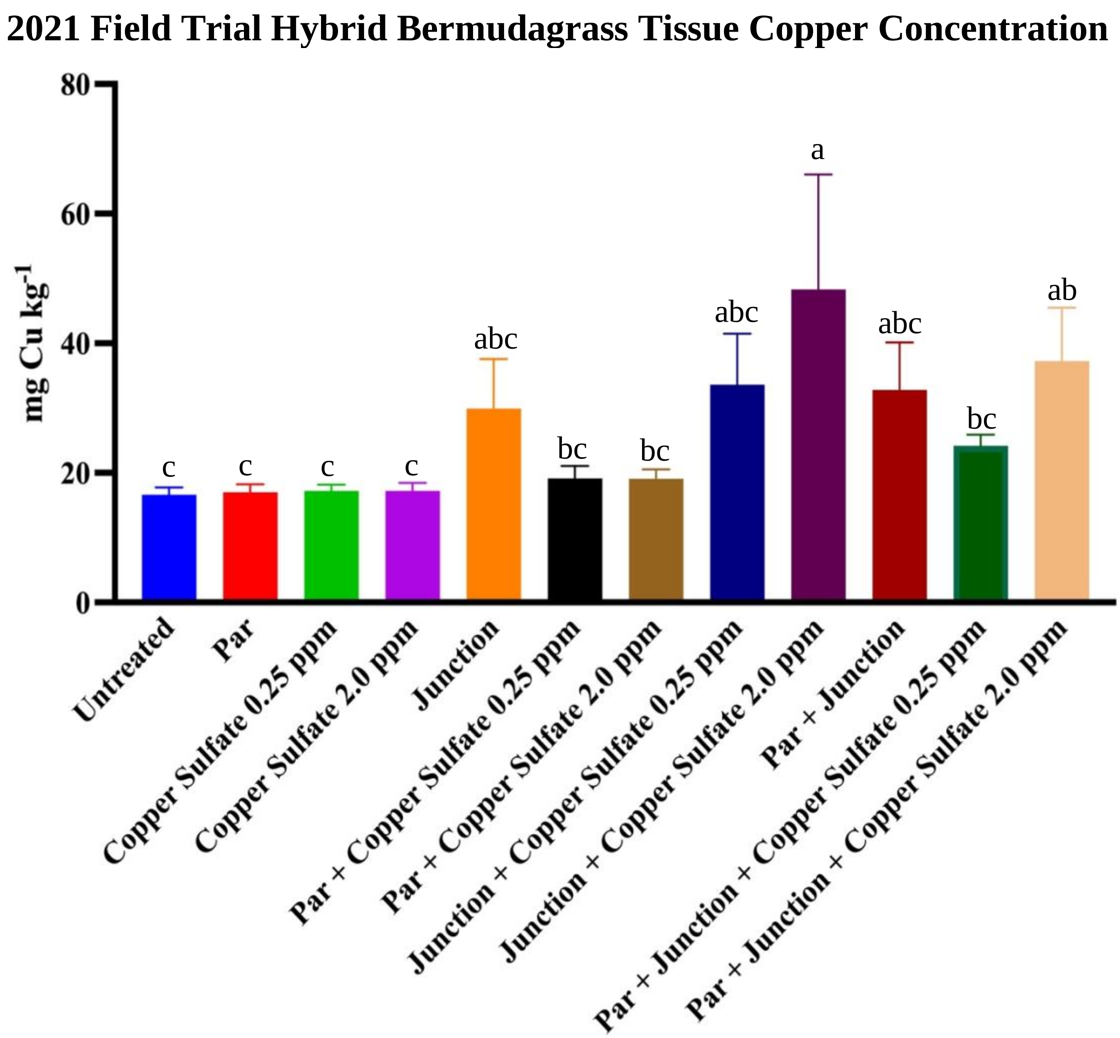


FIGURE 2-18. Plant tissue copper concentration for field trial beginning March 2021 following applications of various copper-containing products to hybrid bermudagrass. Vertical bars represent standard errors. Different letters indicate significant differences between treatments within study by LSD test ($p \leq 0.05$).

Soil Copper Concentration

In study 1, net soil Cu concentrations were statistically highest in areas treated with PAR + Junction, Junction + 0.25 ppm $CuSO_4$, and Junction, with net Cu concentrations of 7.846, 6.697, and 5.800 kg Cu ha^{-1}, respectively, however the latter two treatments were not different from PAR + Junction + 2.0 ppm $CuSO_4$ or PAR + Junction + 0.25 ppm

$CuSO_4$ with increases of 5.492 and 4.820 kg Cu ha^{-1}, respectively (Figure 2-19). Junction + 2.0 ppm $CuSO_4$ with a change of 3.222 kg Cu ha^{-1}, was also similar to PAR + Junction + 2.0 ppm $CuSO_4$ and PAR + Junction + 0.25 ppm $CuSO_4$. PAR + 0.25 ppm $CuSO_4$, PAR, and PAR + 2.0 ppm $CuSO_4$ exhibited similar changes to Junction + 2.0 ppm $CuSO_4$ with net concentrations of 1.849, 1.009, and 0.953 kg Cu ha^{-1}, respectively. These three treatments were also similar to 0.25 ppm $CuSO_4$, untreated areas, and 2.0 ppm $CuSO_4$ with net concentrations of 0.869, 0.280, and -0.084 kg Cu ha^{-1}, respectively.

In study two, the largest net increases in soil copper concentration were the result of PAR + Junction + 2.0 ppm $CuSO_4$, Junction + 2.0 ppm $CuSO_4$, PAR + Junction, and PAR + Junction + 0.25 ppm $CuSO_4$ with increases of 7.229, 5.688, 4.511, and 4.147 kg Cu ha^{-1} (Figure 2-20). Similarly, Junction + 0.25 ppm $CuSO_4$, 0.25 $CuSO_4$, Junction, untreated, and 2.0 ppm $CuSO_4$ areas increased soil Cu concentrations by 1.597, 0.953, 0.925, 0.785, and 0.476 kg Cu ha^{-1}, respectively. PAR, PAR + 0.25 ppm, and PAR + 2.0 ppm had net concentrations of -0.056, -0.224, and -0.505 kg Cu ha^{-1}, which were not statistically significant when compared to PAR + Junction + 2.0 ppm $CuSO_4$ and Junction + 2.0 ppm $CuSO_4$.

With copper's affinity for adsorbing to organic matter, and thatch commonly developing on golf greens, an increase of soil Cu can be expected, however the results of both studies suggest copper hydroxide ($Cu(OH)_2$) may maintain longer soil residual presence compared to $CuSO_4$.

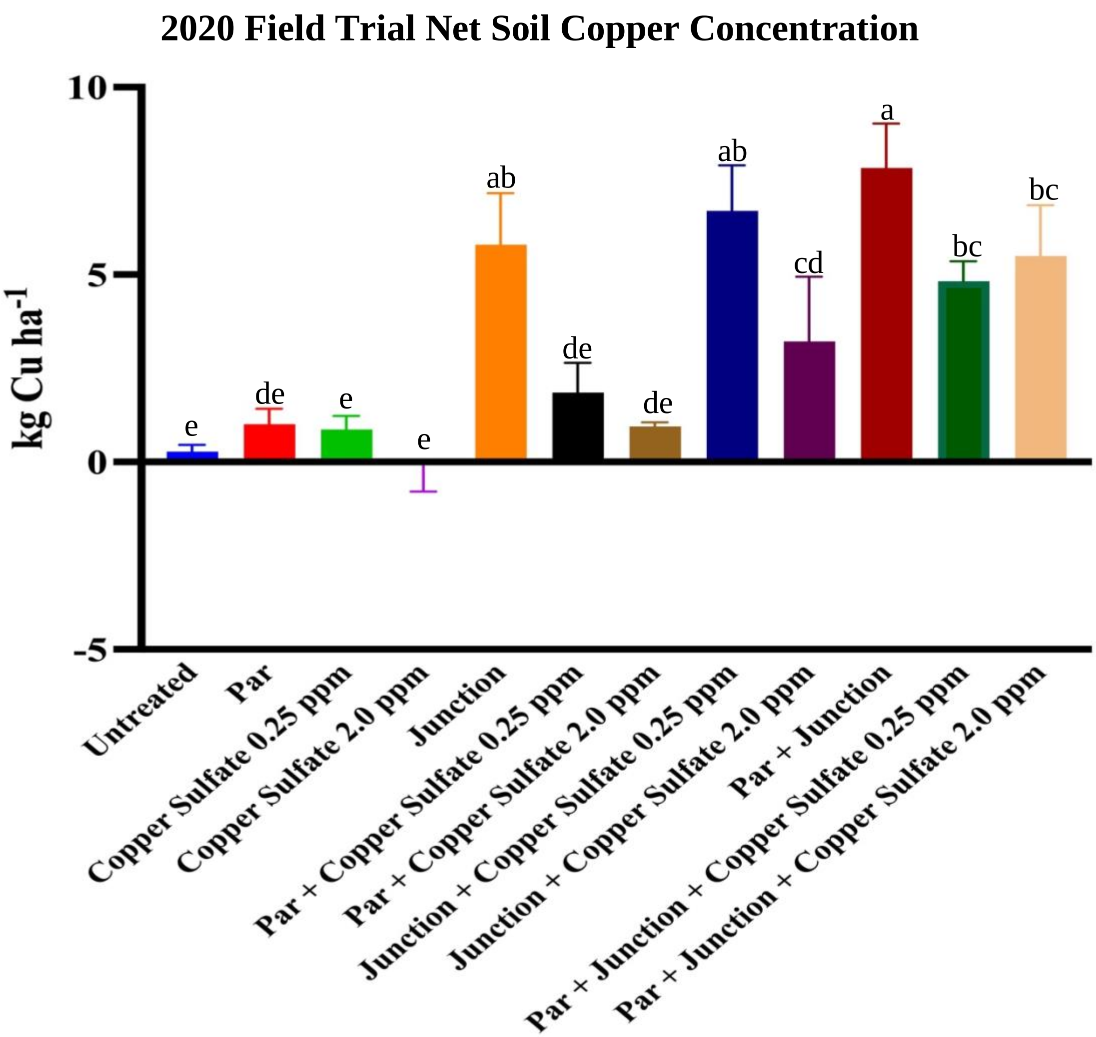


FIGURE 2-19. Net change in soil copper concentration for field trial beginning March 2020 following applications of various copper-containing products to hybrid bermudagrass on USGA spec green. Vertical bars represent standard errors. Different letters indicate significant differences between treatments within study by LSD test ($p \leq 0.10$).

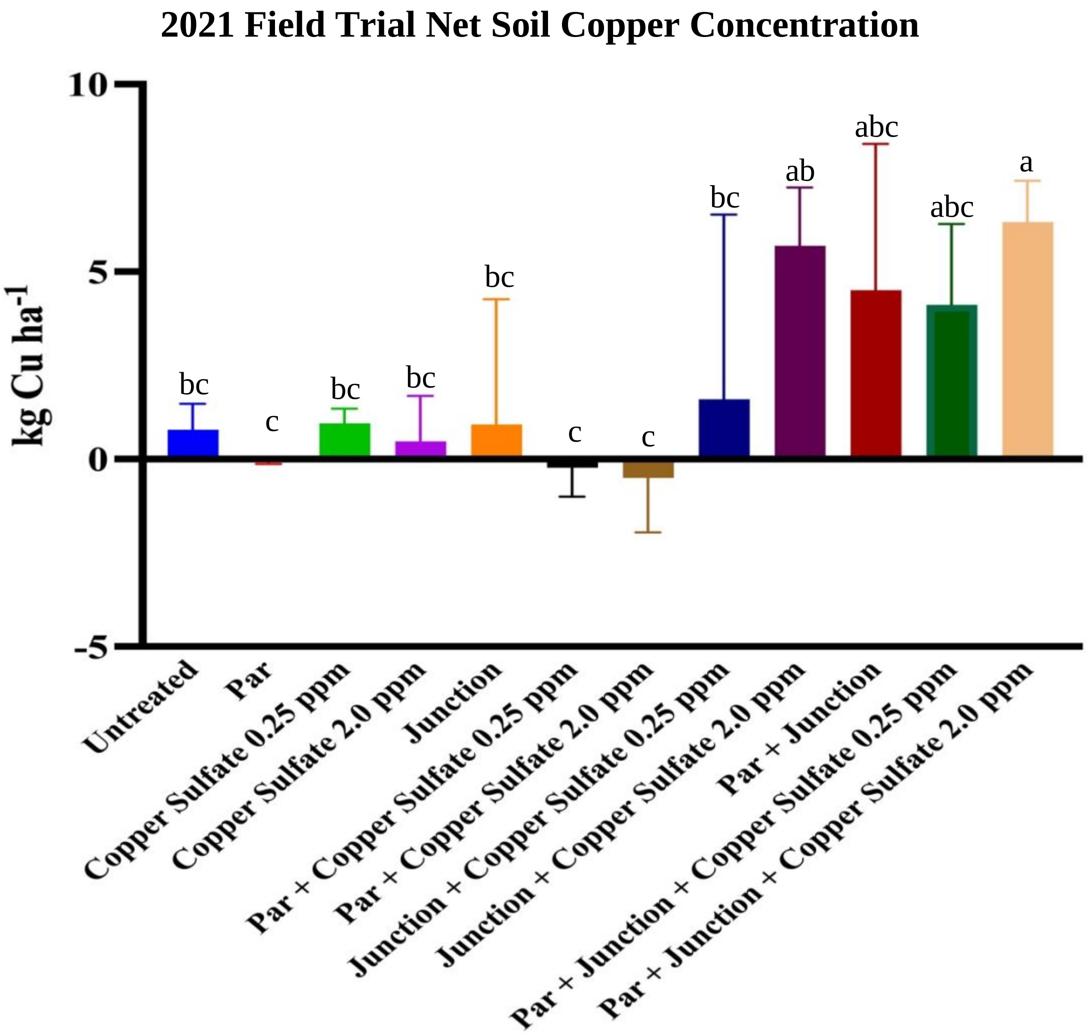


FIGURE 2-20. Net change in soil copper concentration for field trial beginning March 2021 following applications of various copper-containing products to hybrid bermudagrass on USGA spec green. Vertical bars represent standard errors. Different letters indicate significant differences between treatments within each study by LSD test ($p \leq 0.10$).

Greenhouse Trials

Due to the variability between studies, results were analyzed individually and are presented as such.

Visual Turf Quality

In trial 1, Junction + $CuSO_4$ 2.0 ppm was the only treatment exhibiting a positive rate of turf quality change over 12 weeks with an average rate of 0.0121 (Figure 2-21) (Table 2-15). Copper sulfate 0.25 ppm, PAR + Junction + $CuSO_4$ 0.25 ppm, $CuSO_4$ 2.0 ppm, and Junction + $CuSO_4$ 0.25 ppm had similar turf quality change rates of -0.0217, -0.0230, -0.0333, and -0.0352, respectively, with the latter two also similar to PAR + $CuSO_4$ 0.25 ppm with a rate of -0.0557. Similar to PAR + $CuSO_4$ 0.25 ppm, $CuSO_4$ 200 ppm, PAR + Junction, PAR + $CuSO_4$ 2.0 ppm, and PAR exhibited -0.0653, -0.0689, -0.0718, and -0.740 average rates of weekly change, respectively, which were similar to untreated and PAR + Junction + $CuSO_4$ 2.0 ppm rates of -0.0914 and -0.0925, respectively. Copper sulfate 50 ppm with a rate of -0.0997 was similar to $CuSO_4$ 100 ppm rate of -0.1279, which was similar to weekly PAR treatments (PAR (2x)) and Junction with rates of -0.1389 and -0.1415, respectively.

In trial 2, pots treated with Junction + $CuSO_4$ 0.25 ppm, PAR + $CuSO_4$ 2.0 pm, PAR + Junction + $CuSO_4$ 0.25 ppm, PAR (2x), and Junction, exhibited the greatest turf quality increase over 12 weeks with 0.1054, 0.0826, 0.0822, 0.0780, and 0.0712, respectively, with the latter two treatments also similar to PAR's rate of 0.0445 (Figure 2-23) (Table 2-17). Similar to PAR, PAR + $CuSO_4$ 0.25 ppm and PAR + Junction, increased turf quality at a rate of 0.0217 and 0.0167, respectively, which were similar to $CuSO_4$ 2.0 ppm, Junction + copper sulfate 2.0 ppm, untreated, and PAR + Junction + $CuSO_4$ 2.0 ppm with rates of 0.0075, -0.0000, -0.0028, and -0.0135, respectively. Latter three treatments were similar to copper sulfate 0.25 ppm with a rate of -0.0281. Copper sulfate 50 ppm, $CuSO_4$ 100 ppm, and $CuSO_4$ 200 ppm shared no similarities with other treatments with turf quality rates of -0.1054, -0.1631, and -0.2585, respectively.

Means were compared over time, within treatments, by curve fitting sigmoidal four parameter log curve. Due to the variability of data, R^2 values are low, however curves do provide for observational notes on timing of any changes. In both studies, irrigation treatments containing 50, 100, and 200 ppm of $CuSO_4$ experienced decreased quality within 3 weeks. In study 1, all treatments exhibited some decline in quality, though those treatments experiencing decline near end of trial may be partially resulting from the presence of rhodesgrass mealybugs (*Antonina graminis* (Maskell)) (Figure 2-22) (Table 2-16). In study 2, irrigation treatments containing only $CuSO_4$ exhibited declines at or below a turf quality of 6 while all other treatments remained or increased to above 6, including treatments combined with $CuSO_4$ irrigation (Figure 2-24) (Table 2-18).

Irrigation treatments containing 50, 100, and 200 ppm of $CuSO_4$ consistently were associated with some of the steepest decline in the visual quality over the trial lifespan. While some decline in study 1 may be contributed to the presence of a turf pest, the early decline of these irrigation treatments compared to others suggests irrigation water containing 50 ppm $CuSO_4$ or greater can be detrimental to turfgrass in a short period.

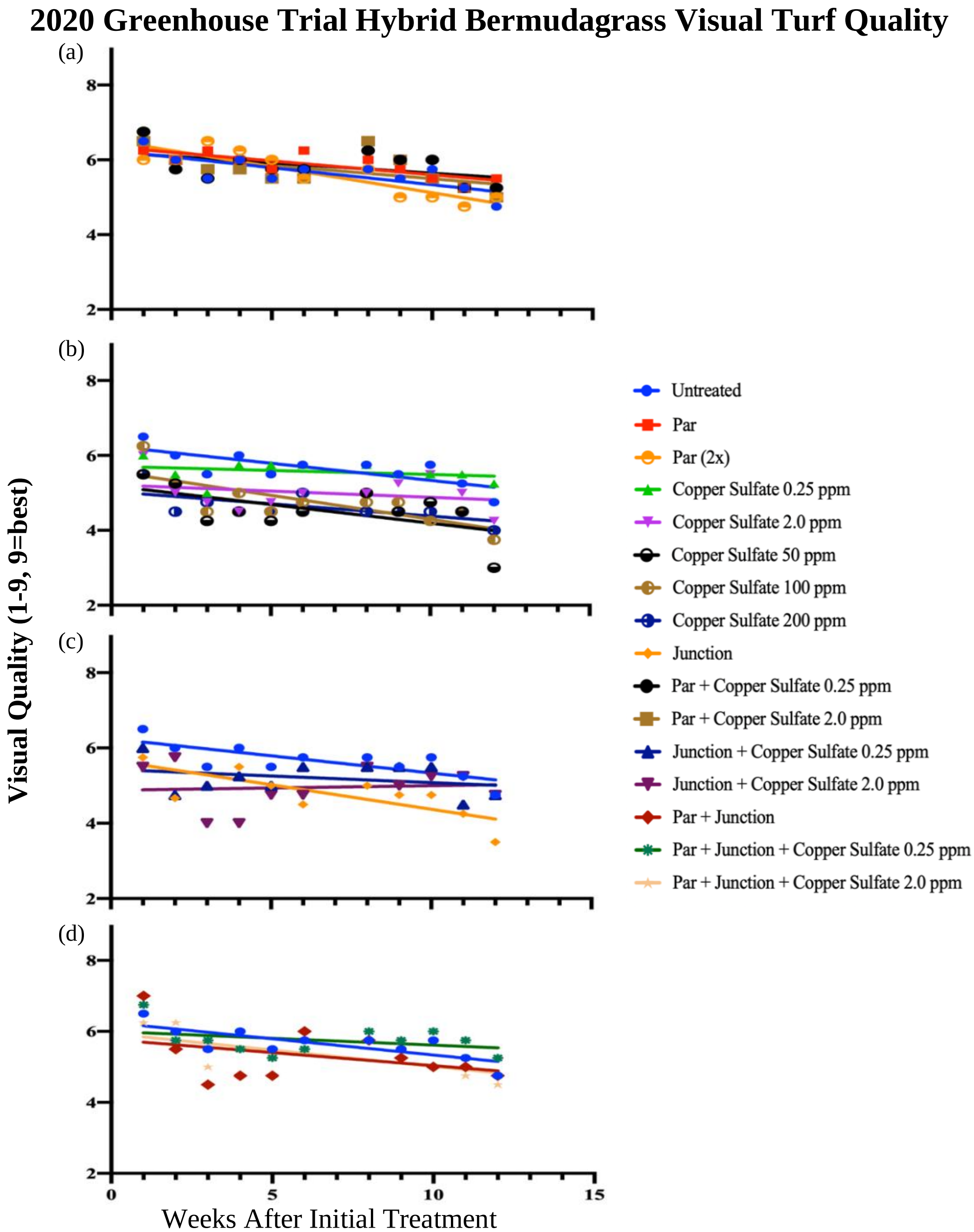


FIGURE 2-21. Average weekly visual turfgrass quality of hybrid bermudagrass treated with various copper-containing products in 2020 greenhouse trial with trendline representing individual treatment progression over trial length of (a) PAR and PAR + $CuSO_4$ treatments, (b) $CuSO_4$ treatments, (c) Junction and Junction + SO_4 treatments, and (d) Par + Junction and Par + Junction + $CuSO_4$ treatments.

TABLE 2-15. Trendline slope (improvement) of hybrid bermudagrass visual turf quality treated with copper-containing products in 2020 greenhouse trial.

Treatment	Trendline Slope
Untreated	-0.0914ef ± 0.05
PAR	-0.0740def ± 0.03
PAR (2x)	-0.1389h ± 0.03
Copper Sulfate 0.25 ppm	-0.0217b ± 0.04
Copper Sulfate 2.0 ppm	-0.0333bc ± 0.05
Copper Sulfate 50 ppm	-0.0997fg ± 0.04
Copper Sulfate 100 ppm	-0.1279gh ± 0.04
Copper Sulfate 200 ppm	-0.0653de ± 0.04
Junction	-0.1415h ± 0.03
PAR + Copper Sulfate 0.25 ppm	-0.0557cd ± 0.03
PAR + Copper Sulfate 2.0 ppm	-0.0718def ± 0.04
Junction + Copper Sulfate 0.25 ppm	-0.0352bc ± 0.04
Junction + Copper Sulfate 2.0 ppm	0.0121a ± 0.05
PAR + Junction	-0.0689de ± 0.04
PAR + Junction + Copper Sulfate 0.25 ppm	-0.0229b ± 0.05
PAR + Junction + Copper Sulfate 2.0 ppm	-0.0925ef ± 0.04

Note: Within column, means followed by the same letter are not significantly different according to LSD (0.05).

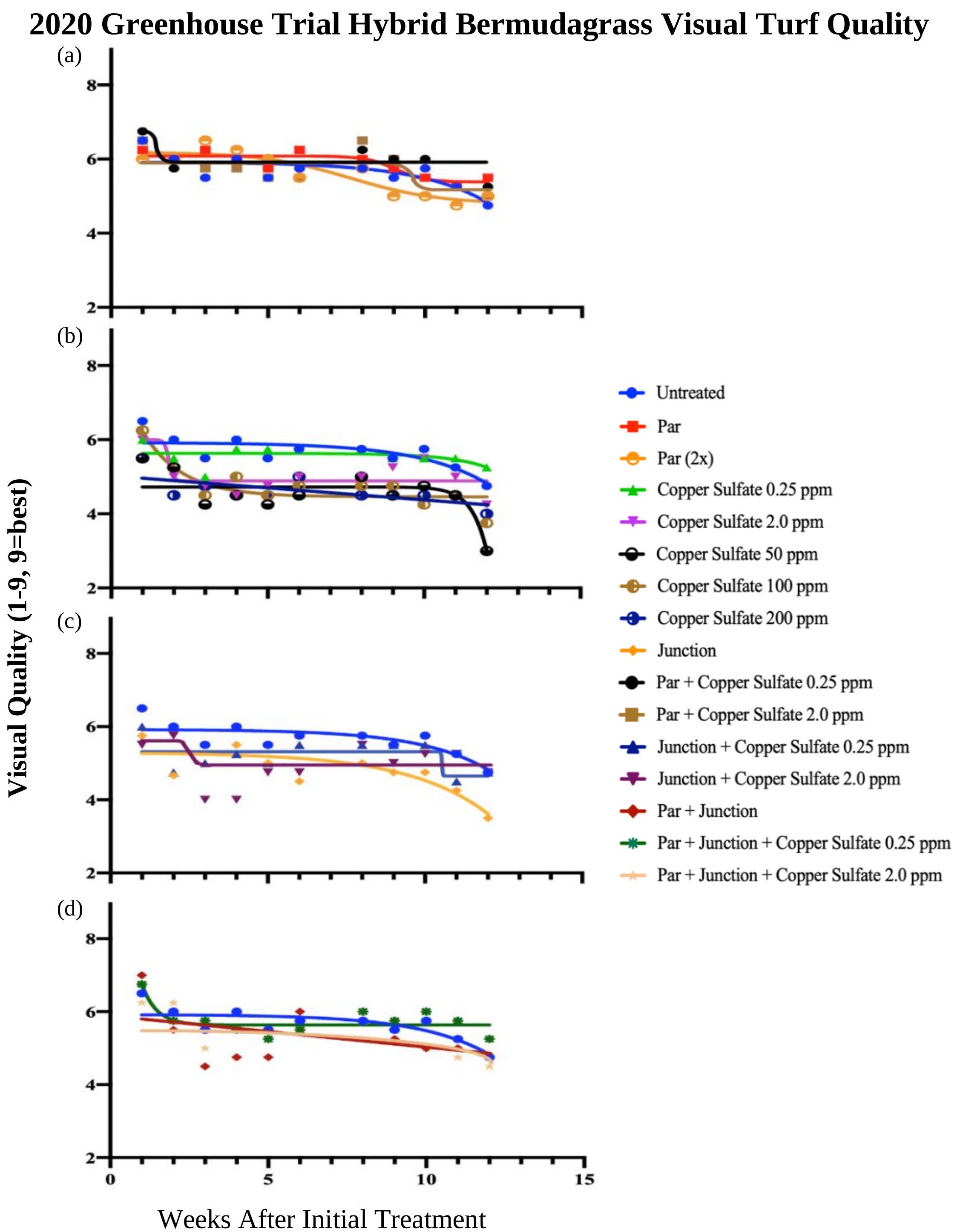


FIGURE 2-22. Average weekly visual turfgrass quality of hybrid bermudagrass treated with various copper-containing products in 2020 greenhouse trial with sigmoidal curve representing individual treatment progression over trial length of (a) PAR and PAR + $CuSO_4$ treatments, (b) $CuSO_4$ treatments, (c) Junction and Junction + SO_4 treatments, and (d) PAR + Junction and PAR + Junction + $CuSO_4$ treatments.

TABLE 2-16. Treatments with respective 2020 greenhouse trial turf visual quality inflection point and R^2 value when using a sigmoidal logistic four parameter curve fitting.

Treatment	**Inflection**	**R^2**
	-----Week-----	
Untreated	44.351	0.084
PAR	9.053	0.119
PAR (2x)	7.624	0.423
Copper Sulfate 0.25 ppm	29.514	0.016
Copper Sulfate 2.0 ppm	1.739	0.072
Copper Sulfate 50 ppm	11.014	0.275
Copper Sulfate 100 ppm	-12.079	0.239
Copper Sulfate 200 ppm	-2244.088	0.057
Junction	43.155	0.306
PAR + Copper Sulfate 0.25 ppm	1.438	0.122
PAR + Copper Sulfate 2.0 ppm	9.995	0.09
Junction + Copper Sulfate 0.25 ppm	10.166	0.076
Junction + Copper Sulfate 2.0 ppm	2.429	0.088
PAR + Junction	9.999	0.069
PAR + Junction + Copper Sulfate 0.25 ppm	0.232	0.092
PAR + Junction + Copper Sulfate 2.0 ppm	57.947	0.138

2022 Greenhouse Trial Hybrid Bermudagrass Visual Turf Quality

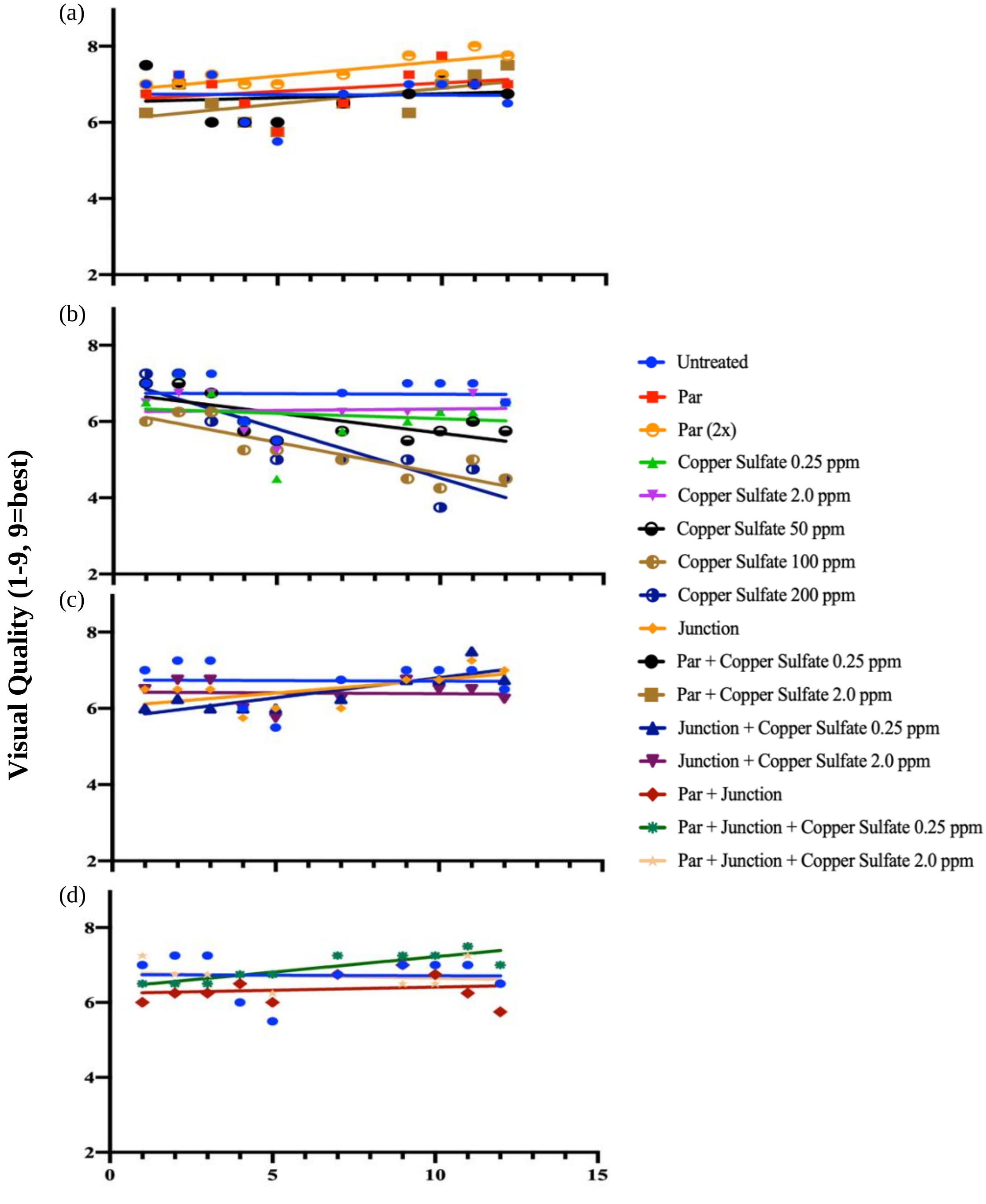


FIGURE 2-23. Average weekly visual turfgrass quality of hybrid bermudagrass treated with various copper-containing products in 2022 greenhouse trial with trendline representing individual treatment progression over trial length of (a) PAR and PAR +

$CuSO_4$ treatments, (b) $CuSO_4$ treatments, (c) Junction and Junction + SO_4 treatments, and (d) PAR + Junction and PAR + Junction + $CuSO_4$ treatments.

TABLE 2-17. Trendline slope (improvement) of hybrid bermudagrass visual turf quality treated with copper-containing products in 2022 greenhouse trial.

Treatment	Trendline Slope
Untreated	-0.0028de ± 0.03
PAR	0.0445bc ± 0.03
PAR (2x)	0.0780ab ± 0.03
Copper Sulfate 0.25 ppm	-0.0281e ± 0.04
Copper Sulfate 2.0 ppm	0.0075d ± 0.04
Copper Sulfate 50 ppm	-0.1054f ± 0.03
Copper Sulfate 100 ppm	-0.1631g ± 0.03
Copper Sulfate 200 ppm	-0.2585h ± 0.04
Junction	0.0712ab ± 0.04
PAR + Copper Sulfate 0.25 ppm	0.0217cd ± 0.04
PAR + Copper Sulfate 2.0 ppm	0.0826a ± 0.04
Junction + Copper Sulfate 0.25 ppm	0.1054a ± 0.03
Junction + Copper Sulfate 2.0 ppm	-0.0000de ± 0.04
PAR + Junction	0.0167cd ± 0.05
PAR + Junction + Copper Sulfate 0.25 ppm	0.0823a ± 0.03
PAR + Junction + Copper Sulfate 2.0 ppm	-0.0135de ± 0.04

Note: Within column, means followed by the same letter are not significantly different according to LSD (0.05).

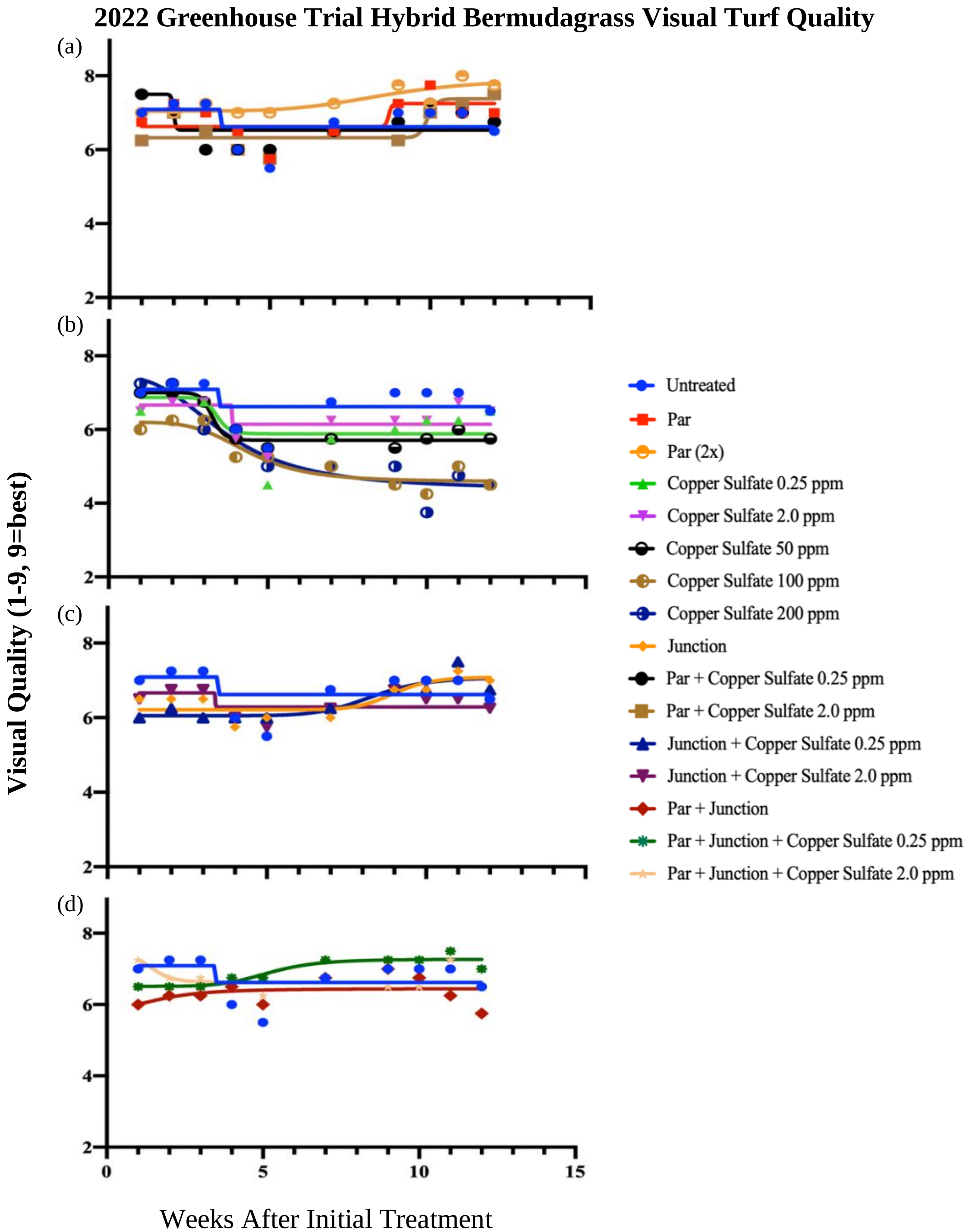


FIGURE 2-24. Average weekly visual turfgrass quality of hybrid bermudagrass treated with various copper-containing products in 2022 greenhouse trial with sigmoidal curve representing individual treatment progression over trial length of (a) PAR and PAR + $CuSO_4$ treatments, (b) $CuSO_4$ treatments, (c) Junction and Junction + SO_4 treatments, and (d) PAR + Junction and PAR + Junction + $CuSO_4$ treatments.

TABLE 2-18. Treatments with respective 2022 greenhouse trial turf visual quality inflection point and R^2 value, when using a sigmoidal logistic four parameter curve fitting.

Treatment	**Inflection**	**R^2**
	-----Week-----	
Untreated	44.351	0.084
PAR	8.262	0.137
PAR (2x)	7.765	0.212
Copper Sulfate 0.25 ppm	3.574	0.233
Copper Sulfate 2.0 ppm	3.382	0.085
Copper Sulfate 50 ppm	3.302	0.464
Copper Sulfate 100 ppm	4.099	0.466
Copper Sulfate 200 ppm	3.163	0.684
Junction	8.958	0.132
PAR + Copper Sulfate 0.25 ppm	2.015	0.191
PAR + Copper Sulfate 2.0 ppm	10.746	0.214
Junction + Copper Sulfate 0.25 ppm	8.371	0.291
Junction + Copper Sulfate 2.0 ppm	3.407	0.043
PAR + Junction	1.535	0.011
PAR + Junction + Copper Sulfate 0.25 ppm	5.217	0.225
PAR + Junction + Copper Sulfate 2.0 ppm	0.197	0.050

Turf Coverage

In trial 1, PAR + Junction + $CuSO_4$ 0.25 ppm and bi-weekly applications of PAR exhibited similar rates of coverage increase with 0.575% and 0.189%, respectively (Figure 2-25) (Table 2-19). Bi-weekly PAR coverage change was similar to PAR + Junction, PAR + Junction + $CuSO_4$ 2.0 ppm, and PAR + $CuSO_4$ 2.0 ppm with 0.106%, 0.105%, and -0.181%, respectively. Treatments $CuSO_4$ 0.25 ppm, $CuSO_4$ 2.0 ppm, and

Junction + $CuSO_4$ 0.25 ppm were similar in average weekly coverage change with -1.194%, -1.214%, and -1.575%, respectively. Copper sulfate 50 ppm, Junction, and untreated pots exhibited similar rates of change with -2.396%, -2.545%, and -2.687%, respectively. Irrigation treatments containing $CuSO_4$ 100 and 200 ppm were similar with average change of -3.218% and -3.246%, respectively.

In trial 2, Junction + $CuSO_4$ 0.25 ppm had the greatest average rate of coverage increase with 1.015% (Figure 2-27) (Table 2-21). Copper sulfate 2.0 ppm was similar to Junction + $CuSO_4$ 2.0 ppm which was also similar to Junction, PAR + $CuSO_4$ 0.25 ppm and PAR + Junction + $CuSO_4$ 0.25 ppm pots with rates of 0.695%, 0.574%, and 0.292%, respectively. PAR + $CuSO_4$ 2.0 ppm, bi-weekly PAR, untreated, PAR + Junction + $CuSO_4$ 2.0 ppm, and $CuSO_4$ 50 ppm exhibited similar average coverage change of 0.279%, 0.21%, 0.204%, 0.088%, and 0.059%, respectively. PAR (2X), $CuSO_4$ 0.25 ppm, and $CuSO_4$ 100 ppm experienced similar coverage change rates -0.127%, -0.326%, and -0.402%, respectively, with latter two also similar to PAR + Junction with -0.595%. Pots receiving irrigation treatments containing $CuSO_4$ 200 ppm had the worst coverage change rate of -1.038%.

Means were compared over time, within treatments, by curve fitting sigmoidal four parameter log curve. Due to the variability of data, R^2 values are low, however curves do provide for observational notes on timing of any changes. In both studies, irrigation treatments containing 50, 100, and 200 ppm of $CuSO_4$ experienced decreased coverage within 3 weeks if application. In study 1, only treatments contain the PAR colorant remained above 70% turf coverage of pot area (Figure 2-26) (Table 2-20). Treatments not containing PAR began a decline in coverage by week 9, potentially owing partially to presence of rhodesgrass mealybugs. In study 2, irrigation treatments containing 50, 100, and 200 ppm $CuSO_4$ decreased below 80% turf coverage 3 weeks after initial treatment (Figure 2-28) (Table 2-22). All other treatments remained above 80%.

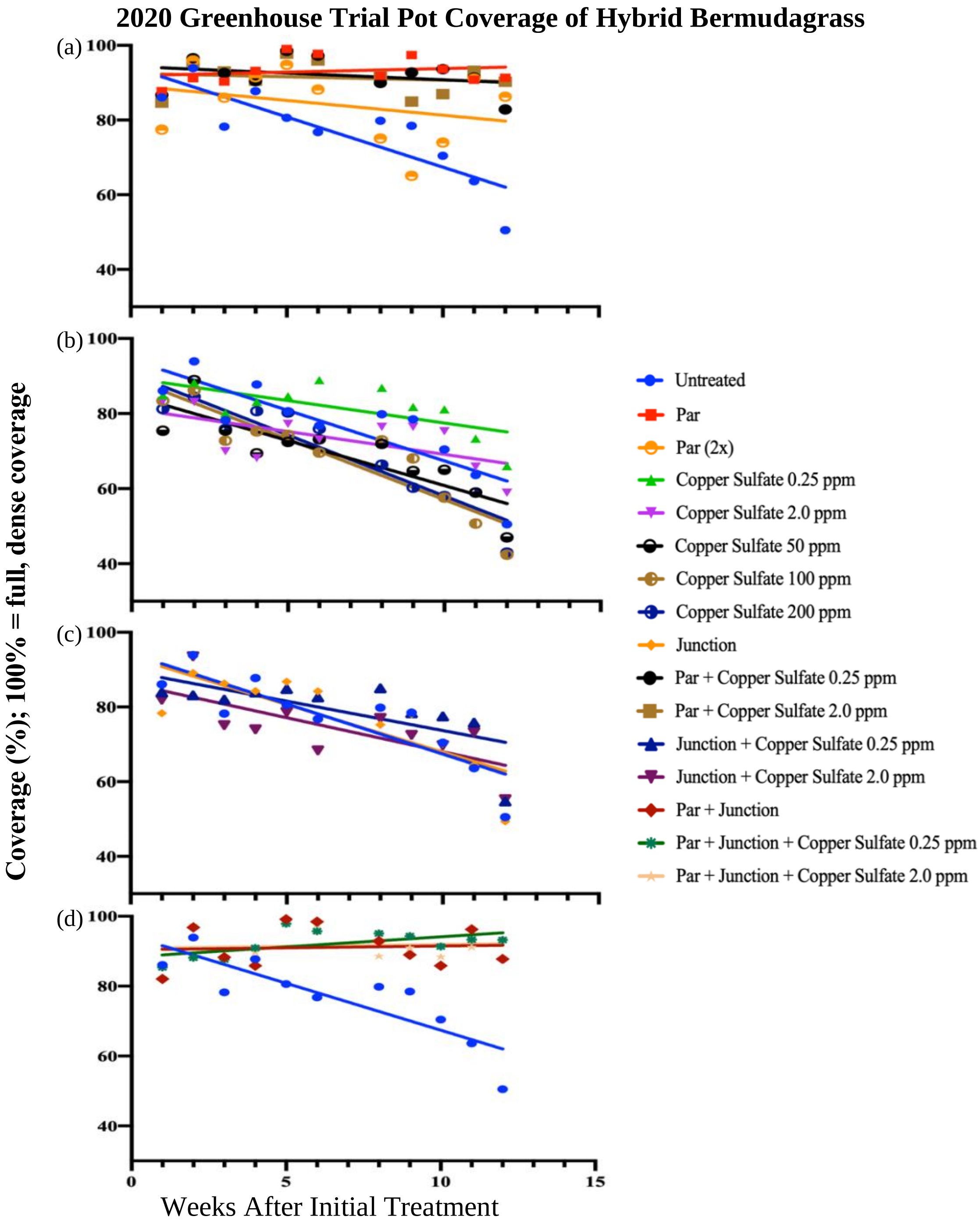


FIGURE 2-25. Average weekly coverage of greenhouse grown hybrid bermudagrass pots treated with various copper-containing products in 2020 with trendline representing individual treatment progression over trial length of (a) PAR and PAR + $CuSO_4$ treatments, (b) $CuSO_4$ treatments, (c) Junction and Junction + SO_4 treatments, and (d) PAR + Junction and PAR + Junction + $CuSO_4$ treatments.

TABLE 2-19. Trendline slope (improvement) of hybrid bermudagrass turf coverage treated with copper-containing products in 2020 greenhouse trial.

Treatment	Trendline Slope
Untreated	-2.687g ± 0.52
PAR	0.189ab ± 0.24
PAR (2x)	-0.790d ± 0.54
Copper Sulfate 0.25 ppm	-1.194e ± 0.38
Copper Sulfate 2.0 ppm	-1.214e ± 0.68
Copper Sulfate 50 ppm	-2.396g ± 0.52
Copper Sulfate 100 ppm	-3.218h ± 0.44
Copper Sulfate 200 ppm	-3.246h ± 0.46
Junction	-2.545g ± 0.49
PAR + Copper Sulfate 0.25 ppm	-0.356c ± 0.25
PAR + Copper Sulfate 2.0 ppm	-0.181bc ± 0.29
Junction + Copper Sulfate 0.25 ppm	-1.575ef ± 0.54
Junction + Copper Sulfate 2.0 ppm	-1.821f ± 0.5
PAR + Junction	0.106b ± 0.31
PAR + Junction + Copper Sulfate 0.25 ppm	0.575a ± 0.29
PAR + Junction + Copper Sulfate 2.0 ppm	0.105b ± 0.29

Note: Within column, means followed by the same letter are not significantly different according to LSD (0.05).

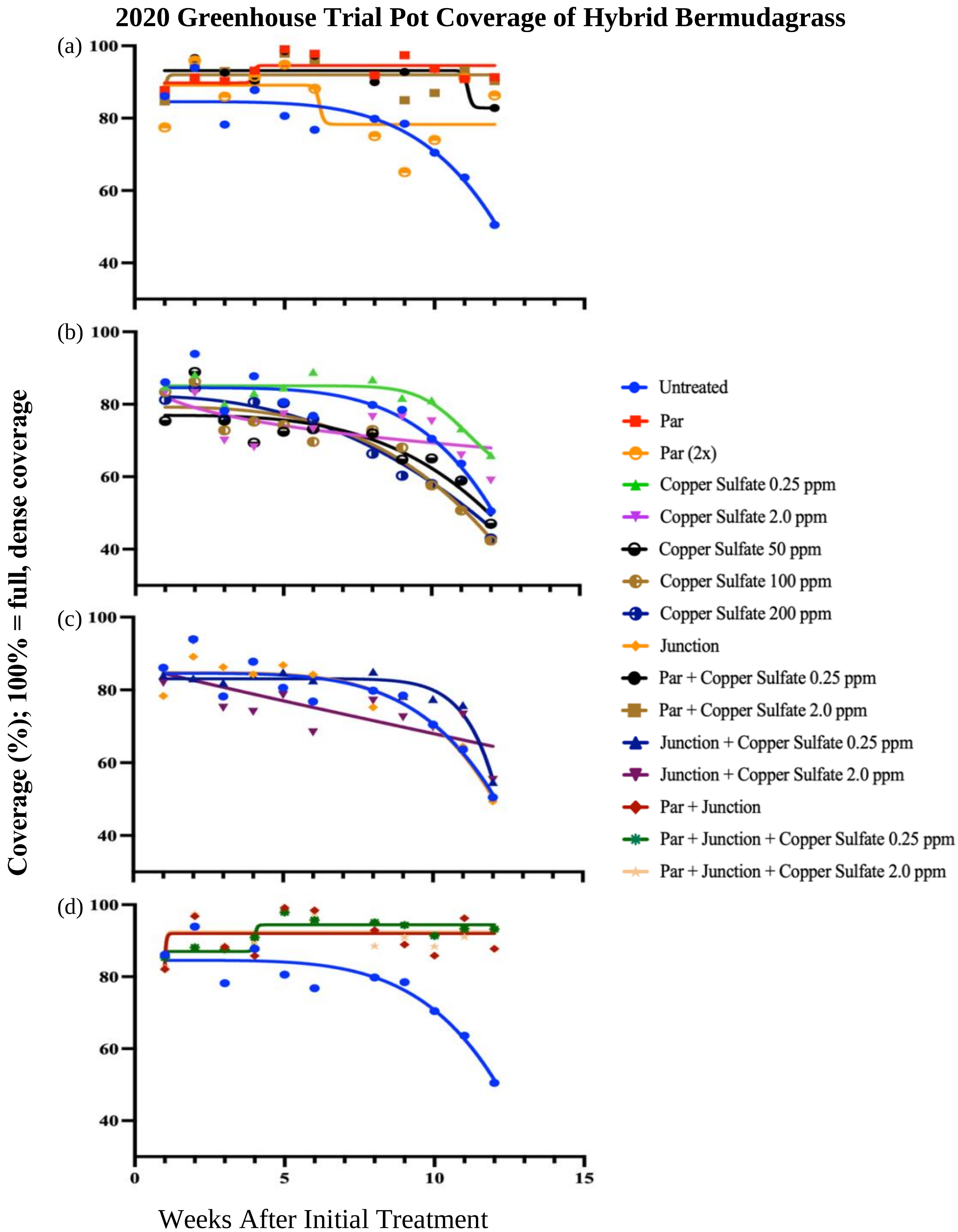


FIGURE 2-26. Average weekly coverage of greenhouse grown hybrid bermudagrass pots treated with various copper-containing products in 2020 with sigmoidal curve representing individual treatment progression over trial length of (a) PAR and PAR + $CuSO_4$ treatments, (b) $CuSO_4$ treatments, (c) Junction and Junction + SO_4 treatments, and (d) PAR + Junction and PAR + Junction + $CuSO_4$ treatments.

TABLE 2-20. Treatments with respective 2020 greenhouse trial turf coverage inflection point and R^2 value when using a sigmoidal logistic four parameter curve fitting.

Treatment	**Inflection**	**R^2**
	-----Week-----	
Untreated	44.763	0.475
PAR	3.633	0.141
PAR (2x)	6.103	0.169
Copper Sulfate 0.25 ppm	11.088	0.380
Copper Sulfate 2.0 ppm	10.908	0.109
Copper Sulfate 50 ppm	58.057	0.374
Copper Sulfate 100 ppm	68.796	0.613
Copper Sulfate 200 ppm	20.804	0.584
Junction	42.972	0.552
PAR + Copper Sulfate 0.25 ppm	1.438	0.122
PAR + Copper Sulfate 2.0 ppm	0.812	0.094
Junction + Copper Sulfate 0.25 ppm	26.911	0.345
Junction + Copper Sulfate 2.0 ppm	113.995	0.241
PAR + Junction	1.091	0.157
PAR + Junction+ Copper Sulfate 0.25 ppm	3.999	0.212
PAR + Junction+ Copper Sulfate 2.0 ppm	-4.304	0.162

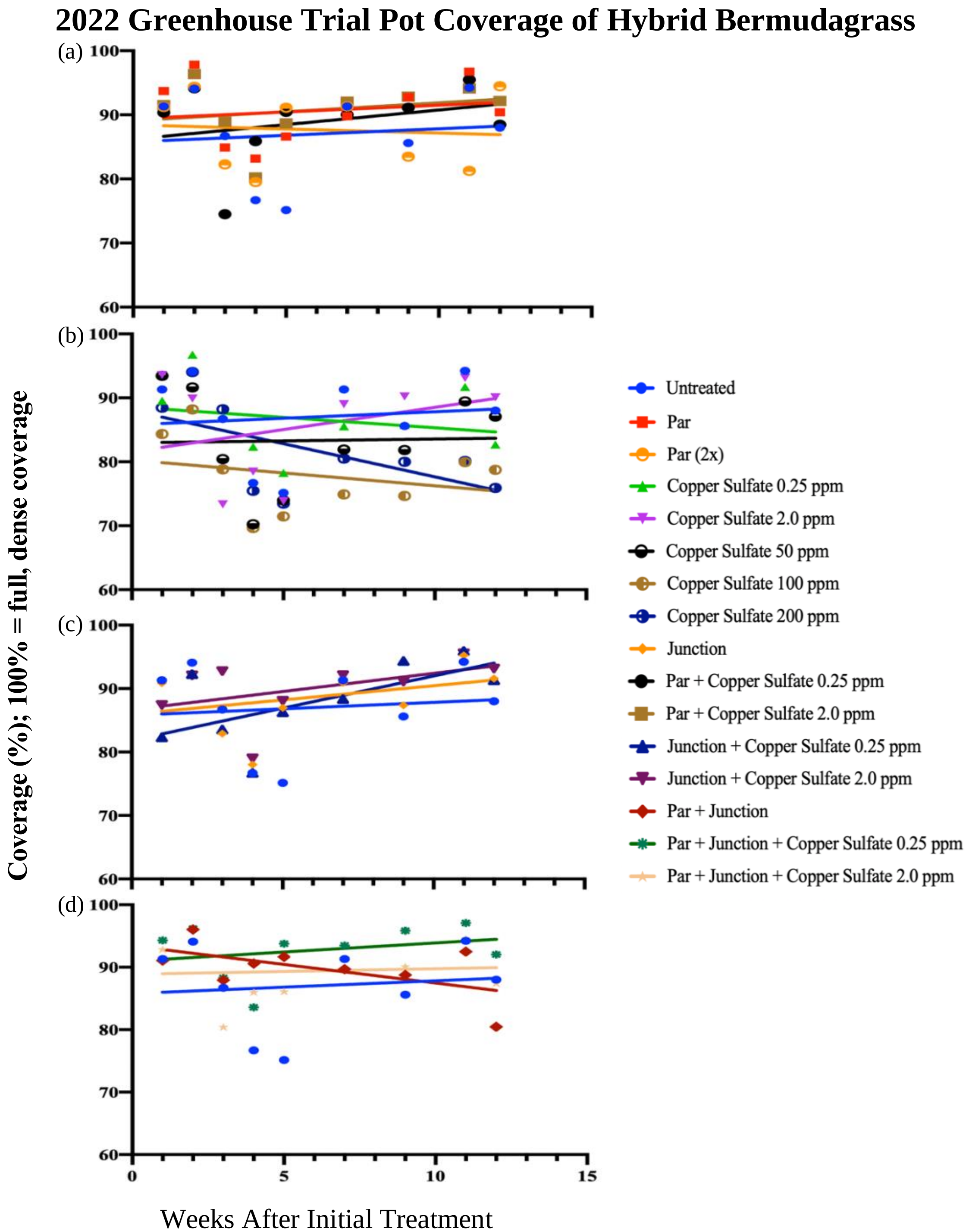


FIGURE 2-27. Average weekly coverage of greenhouse grown hybrid bermudagrass pots treated with various copper-containing products in 2022 with trendline representing individual treatment progression over trial length of (a) PAR and PAR + $CuSO_4$ treatments, (b) $CuSO_4$ treatments, (c) Junction and Junction + SO_4 treatments, and (d) PAR + Junction and PAR + Junction + $CuSO_4$ treatments.

TABLE 2-21. Trendline slope (improvement) of hybrid bermudagrass turf coverage treated with copper-containing products in 2022 greenhouse trial.

Treatment	Trendline Slope
Untreated	0.204de ± 0.48
PAR	0.210de ± 0.32
PAR (2x)	-0.127fg ± 0.4
Copper Sulfate 0.25 ppm	-0.326gh ± 0.4
Copper Sulfate 2.0 ppm	0.695b ± 0.5
Copper Sulfate 50 ppm	0.059ef ± 0.43
Copper Sulfate 100 ppm	-0.402gh ± 0.39
Copper Sulfate 200 ppm	-1.038i ± 0.43
Junction	0.448bcd ± 0.38
PAR + Copper Sulfate 0.25 ppm	0.456bcd ± 0.4
PAR + Copper Sulfate 2.0 ppm	0.279de ± 0.28
Junction + Copper Sulfate 0.25 ppm	1.015a ± 0.33
Junction + Copper Sulfate 2.0 ppm	0.574bc ± 0.32
PAR + Junction	-0.595h ± 0.32
PAR + Junction + Copper Sulfate 0.25 ppm	0.292cde ± 0.26
PAR + Junction + Copper Sulfate 2.0 ppm	0.088ef ± 0.31

Note: Within column, means followed by the same letter are not significantly different according to LSD (0.05).

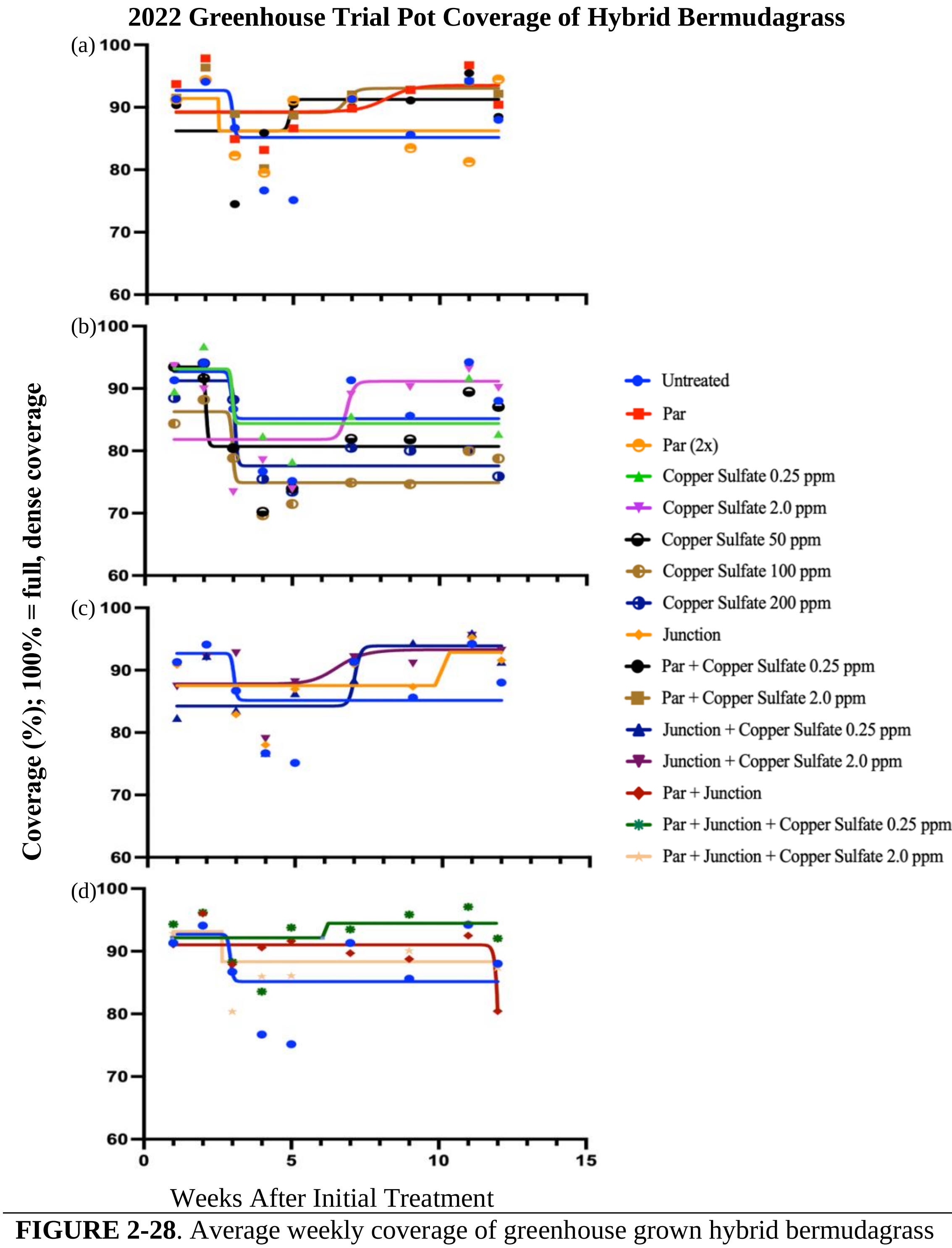


FIGURE 2-28. Average weekly coverage of greenhouse grown hybrid bermudagrass pots treated with various copper-containing products in 2022 with sigmoidal curve representing individual treatment progression over trial length of (a) PAR and PAR + $CuSO_4$ treatments, (b) $CuSO_4$ treatments, (c) Junction and Junction + SO_4 treatments, and (d) PAR + Junction and PAR + Junction + $CuSO_4$ treatments.

TABLE 2-22. Treatments with respective 2022 greenhouse trial turf coverage inflection point and R^2 value when using a sigmoidal logistic four parameter curve fitting.

Treatment	**Inflection**	**R^2**
	-----Week-----	
Untreated	2.845	0.085
PAR	8.191	0.073
PAR (2x)	2.470	0.100
Copper Sulfate 0.25 ppm	2.882	0.161
Copper Sulfate 2.0 ppm	6.890	0.153
Copper Sulfate 50 ppm	2.112	0.280
Copper Sulfate 100 ppm	2.991	0.296
Copper Sulfate 200 ppm	3.074	0.346
Junction	10.114	0.091
PAR + Copper Sulfate 0.25 ppm	4.893	0.074
PAR + Copper Sulfate 2.0 ppm	6.970	0.088
Junction + Copper Sulfate 0.25 ppm	7.109	0.298
Junction + Copper Sulfate 2.0 ppm	6.635	0.124
PAR + Junction	19.211	0.195
PAR + Junction + Copper Sulfate 0.25 ppm	6.050	0.083
PAR + Junction + Copper Sulfate 2.0 ppm	2.459	0.162

Normalized Difference Vegetative Index

In trial 1, PAR (2x) had the statistically greatest rate of NDVI change with 0.0095. PAR + Junction and PAR + Junction + $CuSO_4$ 2.0 ppm showed similar rates of 0.0050 and 0.0047, respectively, and were significantly better than PAR + Junction + $CuSO_4$ 0.25 ppm and PAR + $CuSO_4$ 2.0 ppm with rates of 0.0026 and 0.0023, respectively (Figure 2-18) (Table 2-13). PAR was similar to PAR + $CuSO_4$ 0.25 ppm averaging

0.0006 and -0.0003, respectively. Copper sulfate 2.0 ppm and Junction + $CuSO_4$ 2.0 ppm both averaged a weekly NDVI change of -0.0074. Copper sulfate 0.25 ppm, Junction + $CuSO_4$ 0.25 ppm, Junction, $CuSO_4$ 200 ppm, $CuSO_4$ 100 ppm, and $CuSO_4$ 50 ppm produced similar rates ranging from -0.0090 to -0.0102. Untreated plants exhibited the greatest negative rate of change, averaging a weekly change of -0.0151.

In trial 2, PAR + copper sulfate 0.25 ppm had an average NDVI change of 0.0026 which was similar to Junction + $CuSO_4$ 0.25 ppm with a rate of 0.0021, which was also similar to PAR + Junction + $CuSO_4$ 2.0 ppm which averaged 0.0011 (Figure 2-31) (Table 2-25). PAR + Junction + $CuSO_4$ 0.25 ppm, Junction, PAR (2x), PAR + $CuSO_4$ 2.0 ppm, $CuSO_4$ 50 ppm, and PAR exhibited similar rates of weekly change ranging from 0.0001 to 0.0095. Junction + copper sulfate 2.0 ppm, $CuSO_4$ 0.25 ppm, $CuSO_4$ 2.0 ppm, and PAR + Junction progressed at similar rates of -0.0002, -0.0006, -0.0009, and -0.0009, respectively. Similar to the latter two treatments, untreated pots progressed at a rate of -0.0018 which was similar to copper sulfate 100 ppm rate of -0.0025. Copper sulfate 200 ppm expressed the worst average weekly NDVI change of -0.0039.

When fit to a sigmoidal four parameter log curve, R^2 values were low due to variability of data, however curve does provide for observational notes on timing of any changes. In study 1, treatments containing the PAR colorant experienced little to no decrease in NDVI measurement with weekly applications of PAR resulting in increases in green color, noticeably between weeks 3 and 5 (Figure 2-30) (Table 2-24). The untreated pots began earliest decrease in green color starting between weeks 3 and fours with $CuSO_4$ irrigation and Junction treatments reaching similar levels in shorter amounts of time near the end of the study. In study 2, all treatments maintained average NDVI equal to or greater than study 1, with any decreases occurring between weeks 3 and 5 of the study (Figure 2-32) (Table 2-26). Any increases of individual treatment NDVI were gained between weeks 5 and 7.

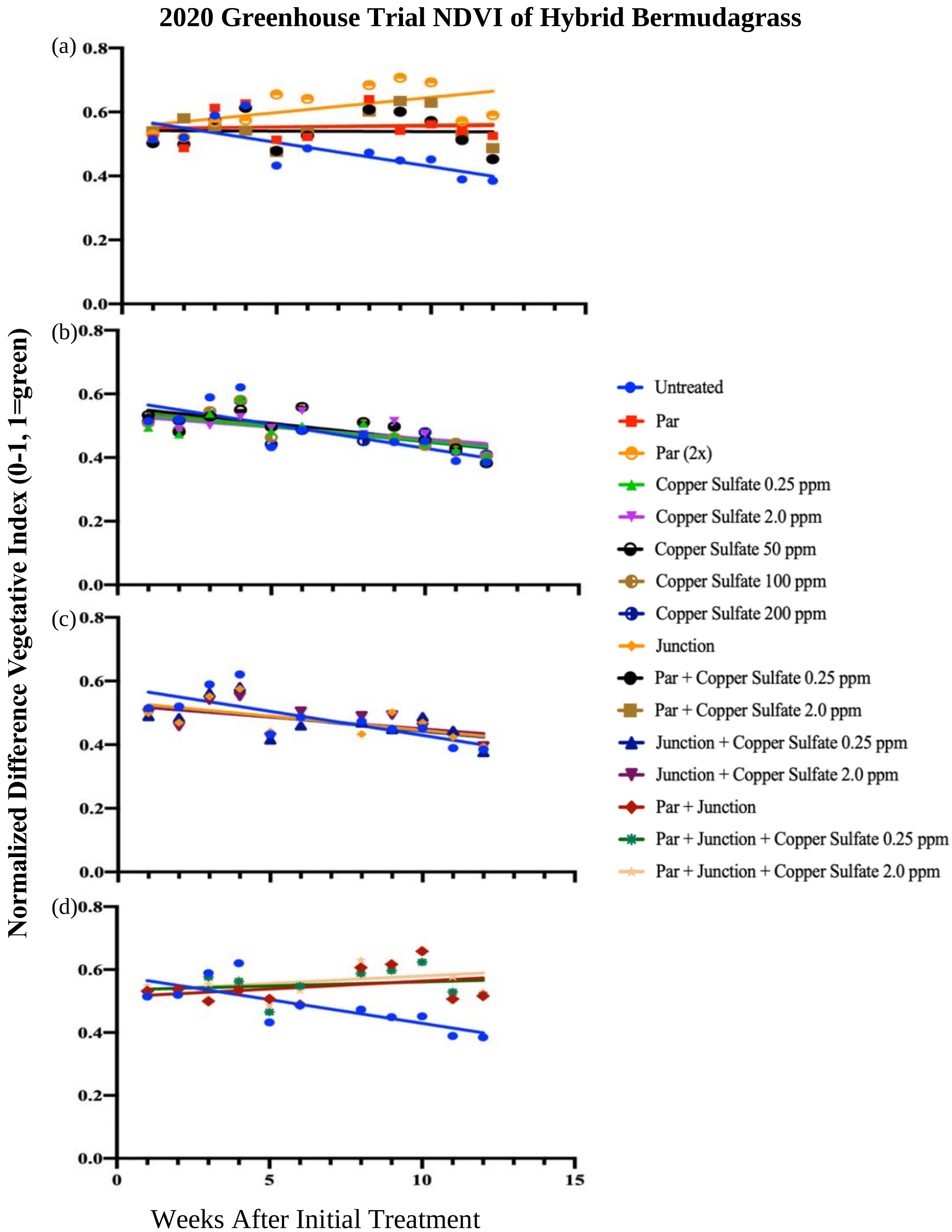


FIGURE 2-29. Average weekly normalized difference vegetation index of greenhouse grown hybrid bermudagrass treated with various copper-containing products in 2020 with trendline representing individual treatment progression over trial length of (a) PAR and PAR + $CuSO_4$ treatments, (b) $CuSO_4$ treatments, (c) Junction and Junction + SO_4 treatments, and (d) PAR + Junction and PAR + Junction + $CuSO_4$ treatments.

TABLE 2-23. Trendline slope (improvement) of hybrid bermudagrass normalized difference vegetative index treated with copper-containing products in 2020 greenhouse trial.

Treatment	**Trendline Slope**
Untreated	-0.0151g ± 0.003
PAR	0.0007d ± 0.002
PAR (2x)	0.0095a ± 0.003
Copper Sulfate 0.25 ppm	-0.0090f ± 0.002
Copper Sulfate 2.0 ppm	-0.0074e ± 0.002
Copper Sulfate 50 ppm	-0.0102f ± 0.002
Copper Sulfate 100 ppm	-0.0098f ± 0.002
Copper Sulfate 200 ppm	-0.0097f ± 0.002
Junction	-0.0093f ± 0.002
PAR + Copper Sulfate 0.25 ppm	-0.0004d ± 0.003
PAR + Copper Sulfate 2.0 ppm	0.0021fc ± 0.003
Junction + Copper Sulfate 0.25 ppm	-0.0092f ± 0.003
Junction + Copper Sulfate 2.0 ppm	-0.0074e ± 0.002
PAR + Junction	0.0050b ± 0.003
PAR + Junction + Copper Sulfate 0.25 ppm	0.0026c ± 0.003
PAR + Junction + Copper Sulfate 2.0 ppm	0.0047b ± 0.002

Note: Within column, means followed by the same letter are not significantly different according to LSD (0.05).

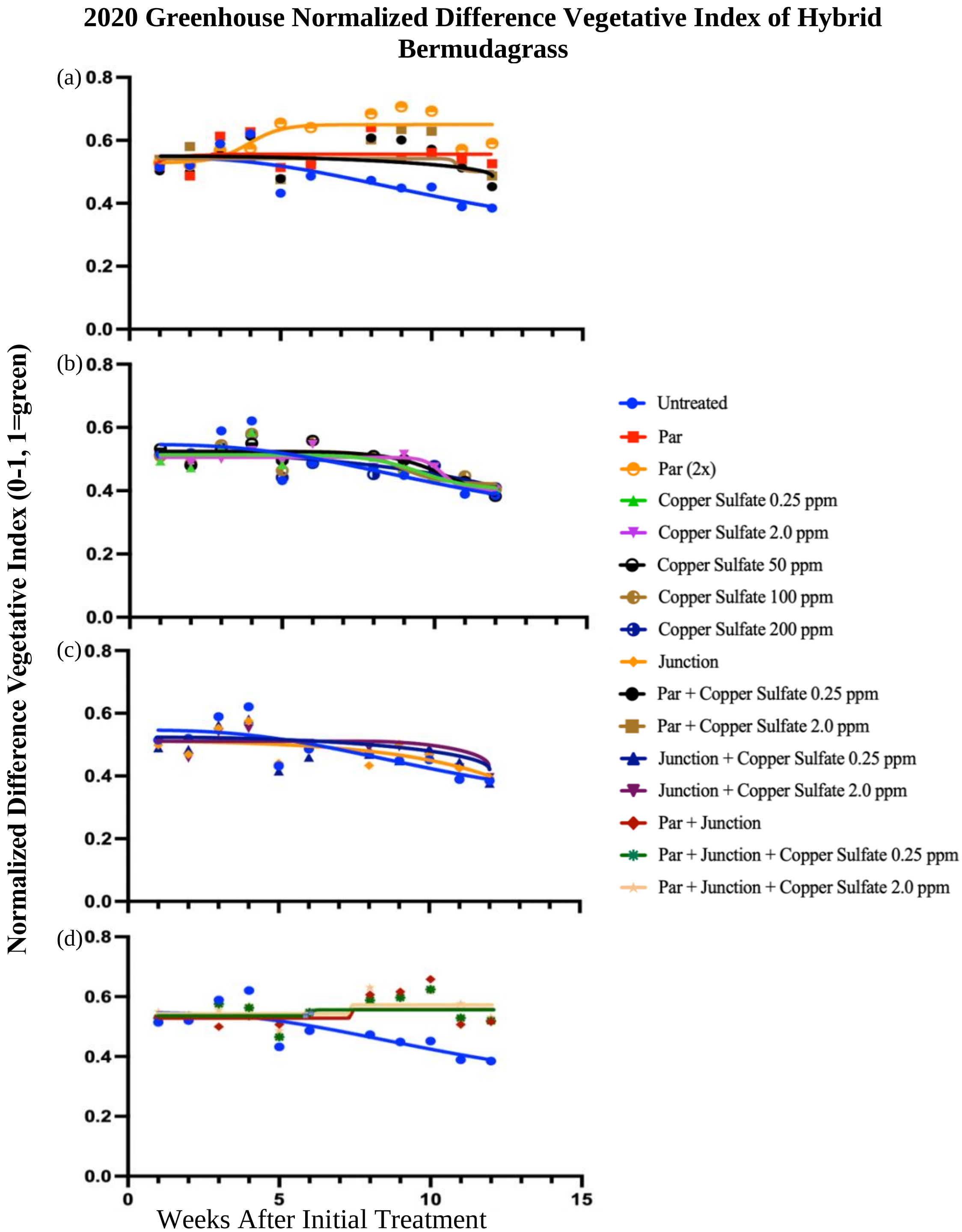


FIGURE 2-30. Average weekly normalized difference vegetation index of greenhouse grown hybrid bermudagrass treated with various copper-containing products in 2020 with sigmoidal curve representing individual treatment progression over trial length of (a) PAR and PAR + $CuSO_4$ treatments, (b) $CuSO_4$ treatments, (c) Junction and Junction + SO_4 treatments, and (d) PAR + Junction and PAR + Junction + $CuSO_4$ treatments.

TABLE 2-24. Treatments with respective 2020 greenhouse trial normalized difference vegetation index and R^2 value, when using a sigmoidal logistic four parameter curve fitting.

Treatment	**Inflection**	**R^2**
	-----Week-----	
Untreated	9.614	0.484
PAR	-2.106	0.08
PAR (2x)	3.936	0.470
Copper Sulfate 0.25 ppm	9.534	0.462
Copper Sulfate 2.0 ppm	10.264	0.446
Copper Sulfate 50 ppm	10.574	0.481
Copper Sulfate 100 ppm	8.869	0.397
Copper Sulfate 200 ppm	70.065	0.284
Junction	62.882	0.340
PAR + Copper Sulfate 0.25 ppm	46.086	0.093
PAR + Copper Sulfate 2.0 ppm	11.036	0.131
Junction + Copper Sulfate 0.25 ppm	56.340	0.246
Junction + Copper Sulfate 2.0 ppm	10.608	0.294
PAR + Junction	7.049	0.243
PAR + Junction + Copper Sulfate 0.25 ppm	6.045	0.085
PAR + Junction + Copper Sulfate 2.0 ppm	6.978	0.259

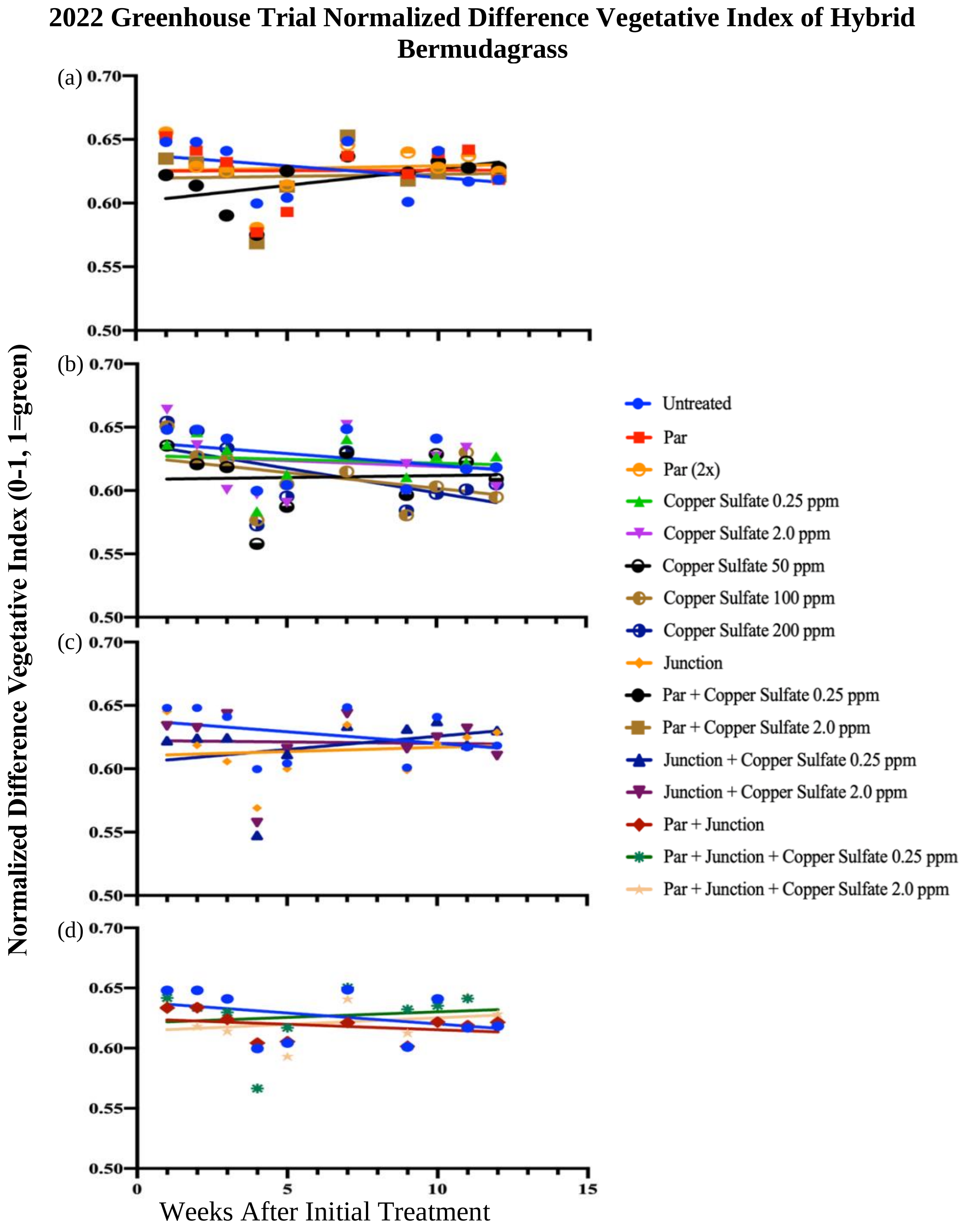


FIGURE 2-31. Average weekly normalized difference vegetation index of greenhouse grown hybrid bermudagrass treated with various copper-containing products in 2022 with trendline representing individual treatment progression over trial length of (a) PAR and PAR + $CuSO_4$ treatments, (b) $CuSO_4$ treatments, (c) Junction and Junction + SO_4 treatments, and (d) PAR + Junction and PAR + Junction + $CuSO_4$ treatments.

TABLE 2-25. Trendline slope (improvement) of hybrid bermudagrass normalized difference vegetative index treated with copper-containing products in 2022 greenhouse trial.

Treatment	**Trendline Slope**
Untreated	-0.0018hi ± 0.001
PAR	0.0001defg ± 0.002
PAR (2x)	0.0004cdef ± 0.001
Copper Sulfate 0.25 ppm	-0.0006fg ± 0.001
Copper Sulfate 2.0 ppm	-0.0009gh ± 0.001
Copper Sulfate 50 ppm	0.0003cdef ± 0.001
Copper Sulfate 100 ppm	-0.0025i ± 0.001
Copper Sulfate 200 ppm	-0.0039j ± 0.001
Junction	0.0007cde ± 0.001
PAR + Copper Sulfate 0.25 ppm	0.0026a ± 0.001
PAR + Copper Sulfate 2.0 ppm	0.0003cdef ± 0.001
Junction + Copper Sulfate 0.25 ppm	0.0021ab ± 0.001
Junction + Copper Sulfate 2.0 ppm	-0.0002efg ± 0.001
PAR + Junction	-0.0009gh ± 0.001
PAR + Junction + Copper Sulfate 0.25 ppm	0.0010cd ± 0.001
PAR + Junction + Copper Sulfate 2.0 ppm	0.0011bc ± 0.001

Note: Within column, means followed by the same letter are not significantly different according to LSD (0.05).

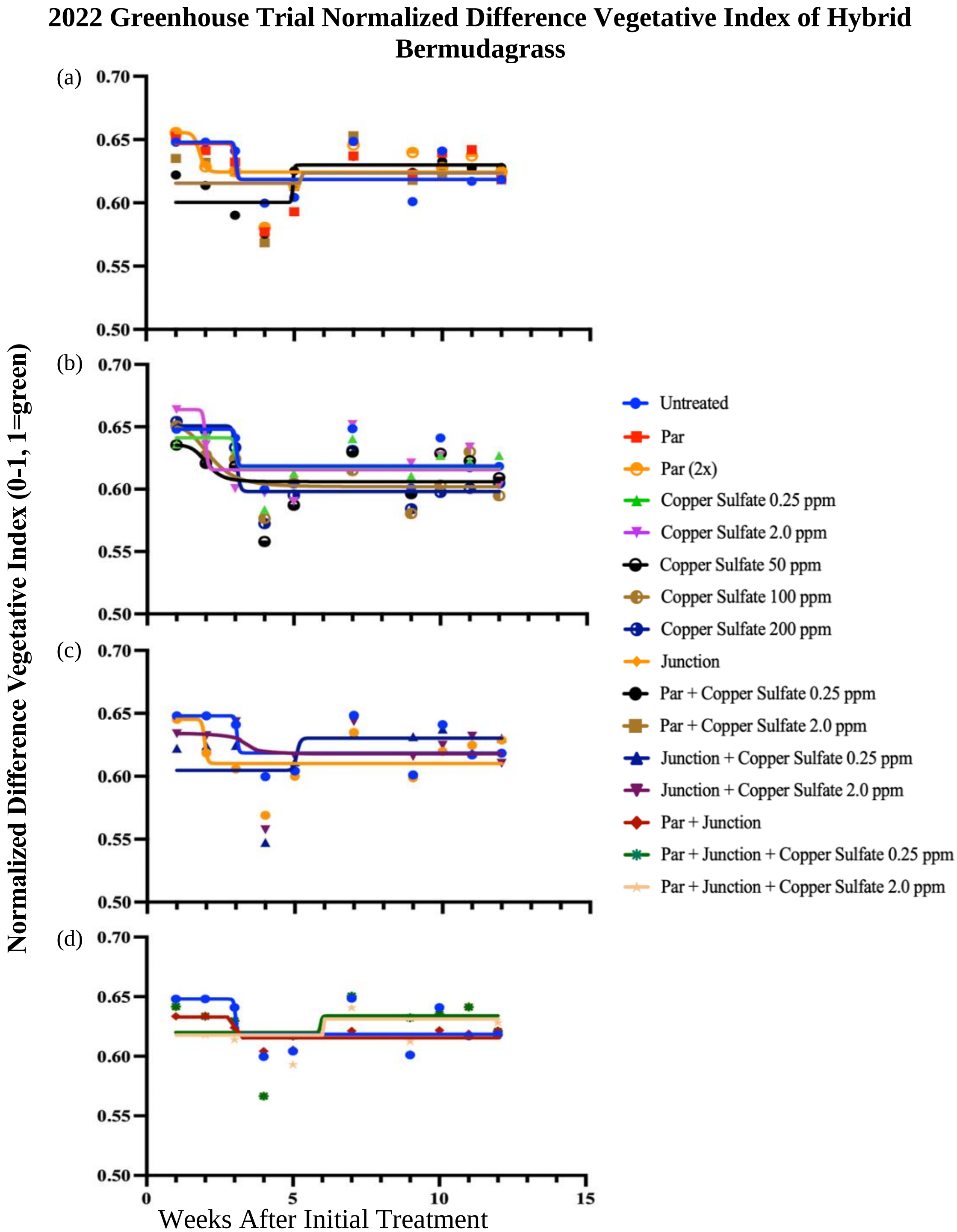


FIGURE 2-32. Average weekly normalized difference vegetation index of greenhouse grown hybrid bermudagrass treated with various copper-containing products in 2022 with sigmoidal curve representing individual treatment progression over trial length of (a) PAR and PAR + $CuSO_4$ treatments, (b) $CuSO_4$ treatments, (c) Junction and Junction + SO_4 treatments, and (d) PAR + Junction and PAR + Junction + $CuSO_4$ treatments.

TABLE 2-26. Treatments with respective 2022 greenhouse trial NDVI inflection point and R^2 value when using a sigmoidal logistic four parameter curve fitting.

Treatment	**Inflection**	**R^2**
	-----Week-----	
Untreated	3.143	0.170
PAR	3.013	0.137
PAR (2x)	1.588	0.089
Copper Sulfate 0.25 ppm	3.030	0.161
Copper Sulfate 2.0 ppm	1.975	0.203
Copper Sulfate 50 ppm	3.033	0.111
Copper Sulfate 100 ppm	2.156	0.308
Copper Sulfate 200 ppm	3.047	0.409
Junction	1.900	0.122
PAR + Copper Sulfate 0.25 ppm	4.855	0.150
PAR + Copper Sulfate 2.0 ppm	5.311	0.061
Junction + Copper Sulfate 0.25 ppm	5.061	0.105
Junction + Copper Sulfate 2.0 ppm	2.861	0.068
PAR + Junction	3.021	0.111
PAR + Junction + Copper Sulfate 0.25 ppm	5.694	0.075
PAR + Junction + Copper Sulfate 2.0 ppm	5.840	0.100

Phytotoxicity

A positive rate of change for phytotoxicity represents increasing yellow, and discolored turf. In study one, untreated pots had statistically the highest rate of phytotoxicity change with an average increase of 0.0140 per week (Figure 2-33) (Table 2-27). Treatments receiving treatments of 100 ppm $CuSO_4$, 200 ppm $CuSO_4$, Junction + 0.25 ppm $CuSO_4$, and Junction + 2.0 ppm $CuSO_4$ exhibited similar rates of change with

0.0061, 0.0035, 0.0035, and .0023, respectively. Junction, 50 ppm $CuSO_4$ and 0.25 ppm $CuSO_4$ showed similar rates of change with 0.0002, -0.0002, and -0.037, respectively. Similar to 0.25 ppm $CuSO_4$, treatments PAR + 2.0 ppm $CuSO_4$, 2.0 ppm $CuSO_4$, PAR + Junction + 2.0 ppm $CuSO_4$, PAR, PAR + Junction, and PAR (2x) decreased phytotoxicity weekly rates ranging from -0.0054 to -0.072. PAR + Junction + 2.0 ppm $CuSO_4$ and PAR + 0.25 ppm $CuSO_4$ exhibited the largest average weekly decreases of phytotoxicity with -0.0097 and -0.0113, respectively.

In study two, irrigation treatments containing 100, 200, and 50 ppm $CuSO_4$ were statistically different from all treatments, with an increase in weekly phytotoxicity of 0.0321, 0.0260, and 0.0111, respectively (Figure 2-31) (Table 2-29). PAR + Junction + 2.0 ppm $CuSO_4$ and PAR + Junction had similar rates, increasing weekly by 0.0061, and 0.0041, respectively. PAR + 0.25 ppm $CuSO_4$, 0.25 ppm $CuSO_4$, Junction + 2.0 $CuSO_4$, PAR, untreated, PAR + Junction + 0.25 ppm $CuSO_4$, PAR (2x), 2.0 ppm $CuSO_4$, and PAR + 2.0 ppm $CuSO_4$ decreased weekly at rates ranging from -0.0004 to -0.0035. Junction + 0.25 ppm $CuSO_4$ and Junction had similar rates of -0.0045 and -0.0054, respectively, which was also similar to PAR + Junction + 0.25 ppm $CuSO_4$, PAR (2x), 2.0 ppm $CuSO_4$, and PAR + 2.0 ppm $CuSO_4$.

Means were compared over time, within treatments, by curve fitting sigmoidal four parameter log curve. Corresponding R^2 values were low due to variability of data, however curve does provide for observational notes on timing of any changes. In study 1, treatments containing the PAR colorant exhibited some phytotoxicity at trial onset however were all were below 10% at conclusion, whereas pots treated with Junction DF without PAR and irrigation treatments containing 50, 100, and 200 ppm $CuSO_4$ maintained or increase phytotoxicity through duration of study (Figure 2-34) (Table 2-28). In study 2, irrigation treatments of 0.25, 50, 100, and 200 ppm $CuSO_4$ increased phytotoxic symptoms by at least 10% by week 5 (Figure 2-36) (Table 2-30). PAR + Junction DF and Par + Junction DF increased phytotoxicity approximately 9% between weeks 5 and 12.

Results suggest hybrid bermudagrass will exhibit foliar stress to excessive Cu levels within four weeks, however the use of colorants may mask this foliar stress with sequential applications.

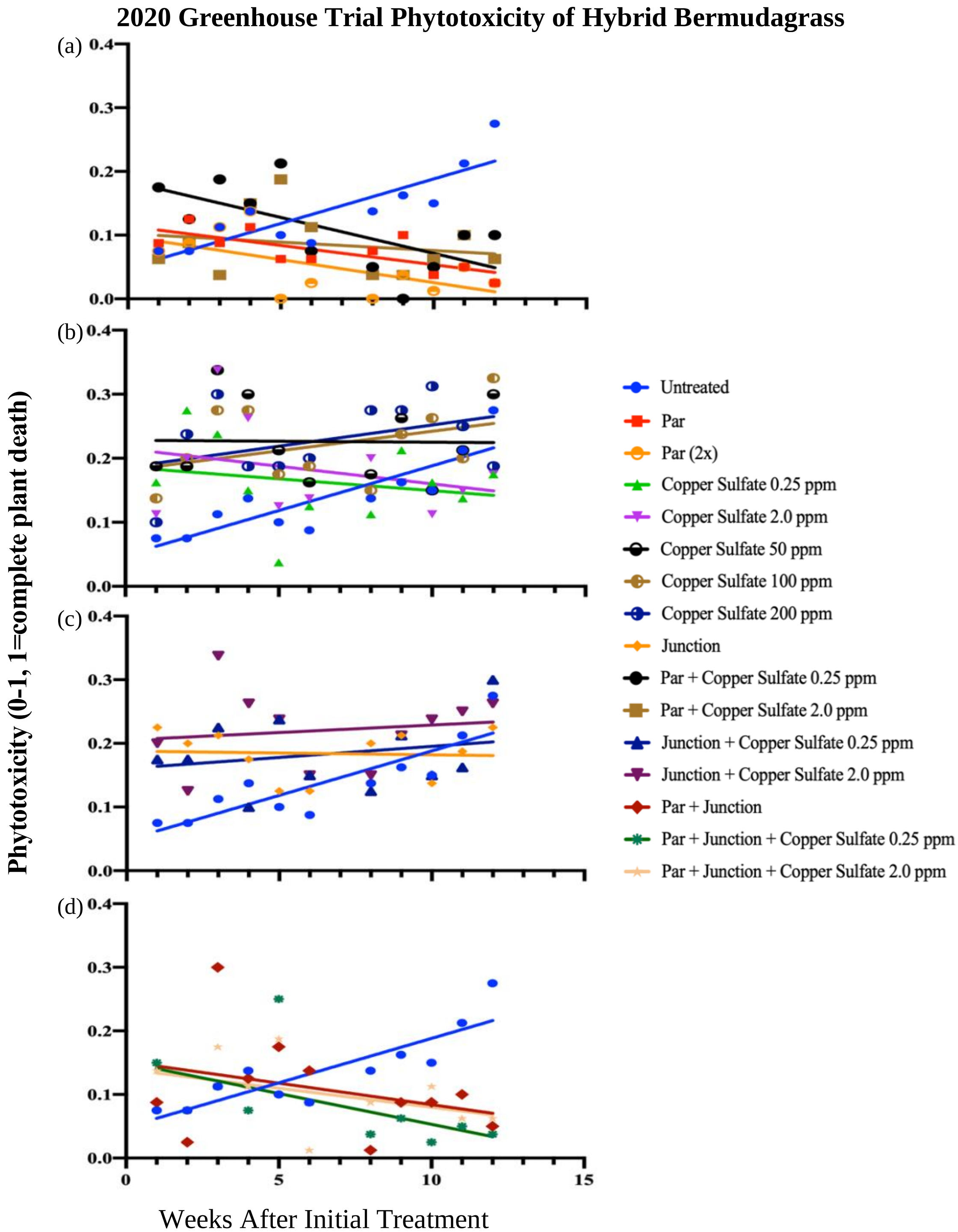


FIGURE 2-33. Average weekly phytotoxicity of greenhouse grown hybrid bermudagrass treated with various copper-containing products in 2020 with trendline representing individual treatment progression over trial length of (a) PAR and PAR + $CuSO_4$ treatments, (b) $CuSO_4$ treatments, (c) Junction and Junction + SO_4 treatments, and (d) PAR + Junction and PAR + Junction + $CuSO_4$ treatments.

TABLE 2-27. Trendline slope (improvement) of hybrid bermudagrass phytotoxicity treated with copper-containing products in 2020 greenhouse trial.

Treatment	Trendline Slope
Untreated	0.0140a ± 0.005
PAR	-0.0060ef ± 0.003
PAR (2x)	-0.0072efg ± 0.003
Copper Sulfate 0.25 ppm	-0.0037de ± 0.004
Copper Sulfate 2.0 ppm	-0.0055ef ± 0.006
Copper Sulfate 50 ppm	-0.0002cd ± 0.005
Copper Sulfate 100 ppm	0.0061b ± 0.005
Copper Sulfate 200 ppm	0.0035bc ± 0.005
Junction	-0.0005cd ± 0.004
PAR + Copper Sulfate 0.25 ppm	-0.0113g ± 0.003
PAR + Copper Sulfate 2.0 ppm	-0.0054e ± 0.004
Junction + Copper Sulfate 0.25 ppm	0.0035bc ± 0.005
Junction + Copper Sulfate 2.0 ppm	0.0023bc ± 0.005
PAR + Junction	-0.0062ef ± 0.004
PAR + Junction + Copper Sulfate 0.25 ppm	-0.0097fg ± 0.004
PAR + Junction + Copper Sulfate 2.0 ppm	-0.0060ef ± 0.004

Note: Within column, means followed by the same letter are not significantly different according to LSD (0.05).

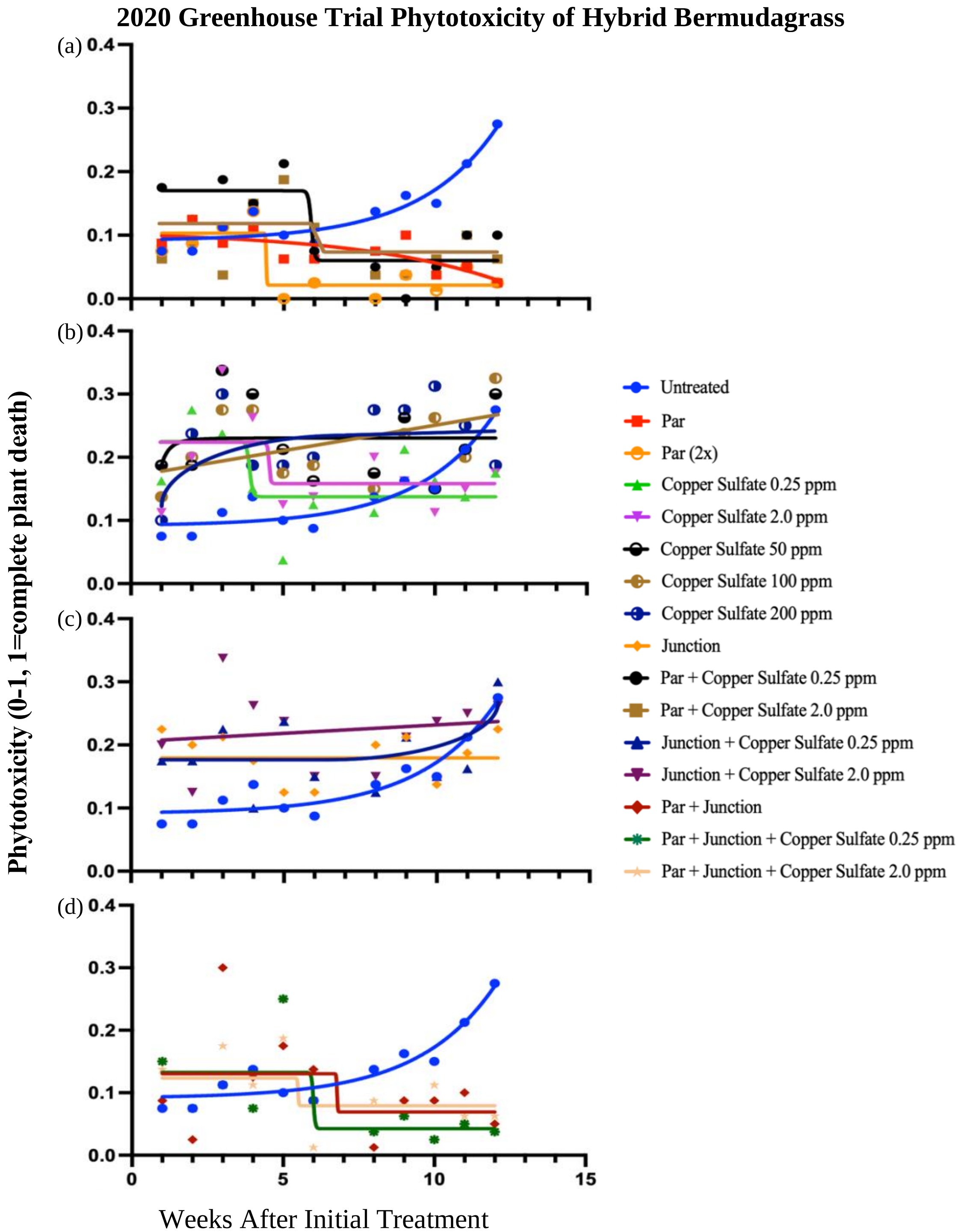


FIGURE 2-34. Average weekly phytotoxicity of greenhouse grown hybrid bermudagrass treated with various copper-containing products in 2020 with sigmoidal curve representing individual treatment progression over trial length of (a) PAR and PAR + $CuSO_4$ treatments, (b) $CuSO_4$ treatments, (c) Junction and Junction + SO_4 treatments, and (d) PAR + Junction and PAR + Junction + $CuSO_4$ treatments.

TABLE 2-28. Treatments with respective 2020 greenhouse trial phytotoxicity inflection point and R^2 value when using a sigmoidal logistic four parameter curve fitting.

Treatment	**Inflection**	**R^2**
	-----Week-----	
Untreated	46.342	0.174
PAR	66.783	0.090
PAR (2x)	4.886	0.275
Copper Sulfate 0.25 ppm	3.879	0.173
Copper Sulfate 2.0 ppm	4.740	0.061
Copper Sulfate 50 ppm	-5.121	0.01
Copper Sulfate 100 ppm	-151.161	0.034
Copper Sulfate 200 ppm	-17.215	0.062
Junction	67.182	0.000
PAR + Copper Sulfate 0.25 ppm	5.864	0.358
PAR + Copper Sulfate 2.0 ppm	6.073	0.095
Junction + Copper Sulfate 0.25 ppm	36.144	0.072
Junction + Copper Sulfate 2.0 ppm	-17.779	0.006
PAR + Junction	6.313	0.117
PAR + Junction + Copper Sulfate 0.25 ppm	6.000	0.172
PAR + Junction + Copper Sulfate 2.0 ppm	5.588	0.153

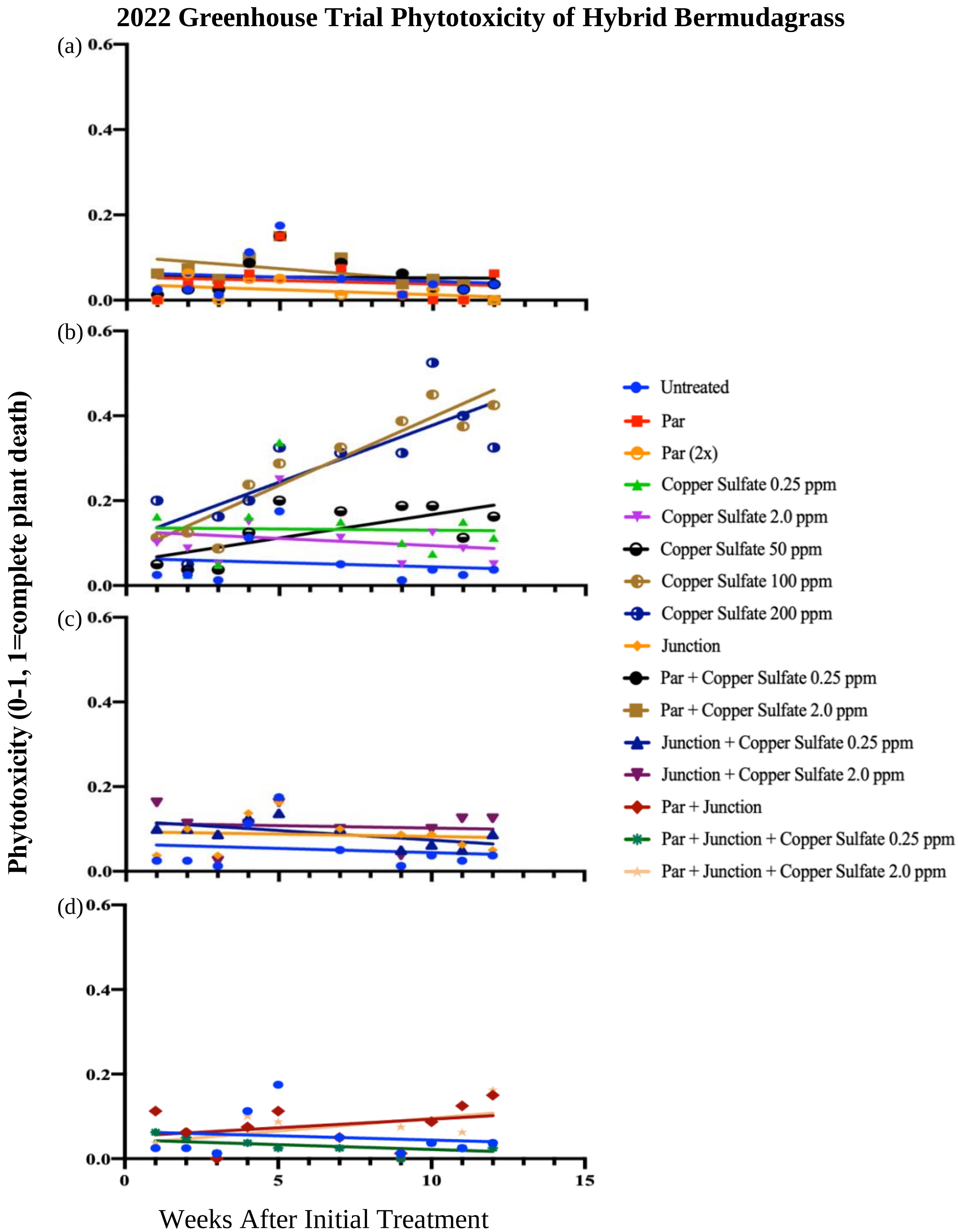


FIGURE 2-35. Average weekly phytotoxicity of greenhouse grown hybrid bermudagrass treated with various copper-containing products in 2022 with trendline representing individual treatment progression over trial length of (a) PAR and PAR + $CuSO_4$ treatments, (b) $CuSO_4$ treatments, (c) Junction and Junction + SO_4 treatments, and (d) PAR + Junction and PAR + Junction + $CuSO_4$ treatments.

TABLE 2-29. Trendline slope (improvement) of hybrid bermudagrass phytotoxicity treated with copper-containing products in 2022 greenhouse trial.

Treatment	Trendline Slope
Untreated	-0.0020ef ± 0.003
PAR	-0.0016ef ± 0.002
PAR (2x)	-0.0024efg ± 0.002
Copper Sulfate 0.25 ppm	-0.0006e ± 0.006
Copper Sulfate 2.0 ppm	-0.0033efg ± 0.004
Copper Sulfate 50 ppm	0.0111c ± 0.003
Copper Sulfate 100 ppm	0.0321a ± 0.004
Copper Sulfate 200 ppm	0.0260b ± 0.005
Junction	-0.0054g ± 0.004
PAR + Copper Sulfate 0.25 ppm	-0.0004e ± 0.002
PAR + Copper Sulfate 2.0 ppm	-0.0035efg ± 0.004
Junction + Copper Sulfate 0.25 ppm	-0.0045fg ± 0.003
Junction + Copper Sulfate 2.0 ppm	-0.0012e ± 0.004
PAR + Junction	0.0041d ± 0.004
PAR + Junction + Copper Sulfate 0.25 ppm	-0.0023efg ± 0.002
PAR + Junction + Copper Sulfate 2.0 ppm	0.0061d ± 0.003

Note: Within column, means followed by the same letter are not significantly different according to LSD (0.05).

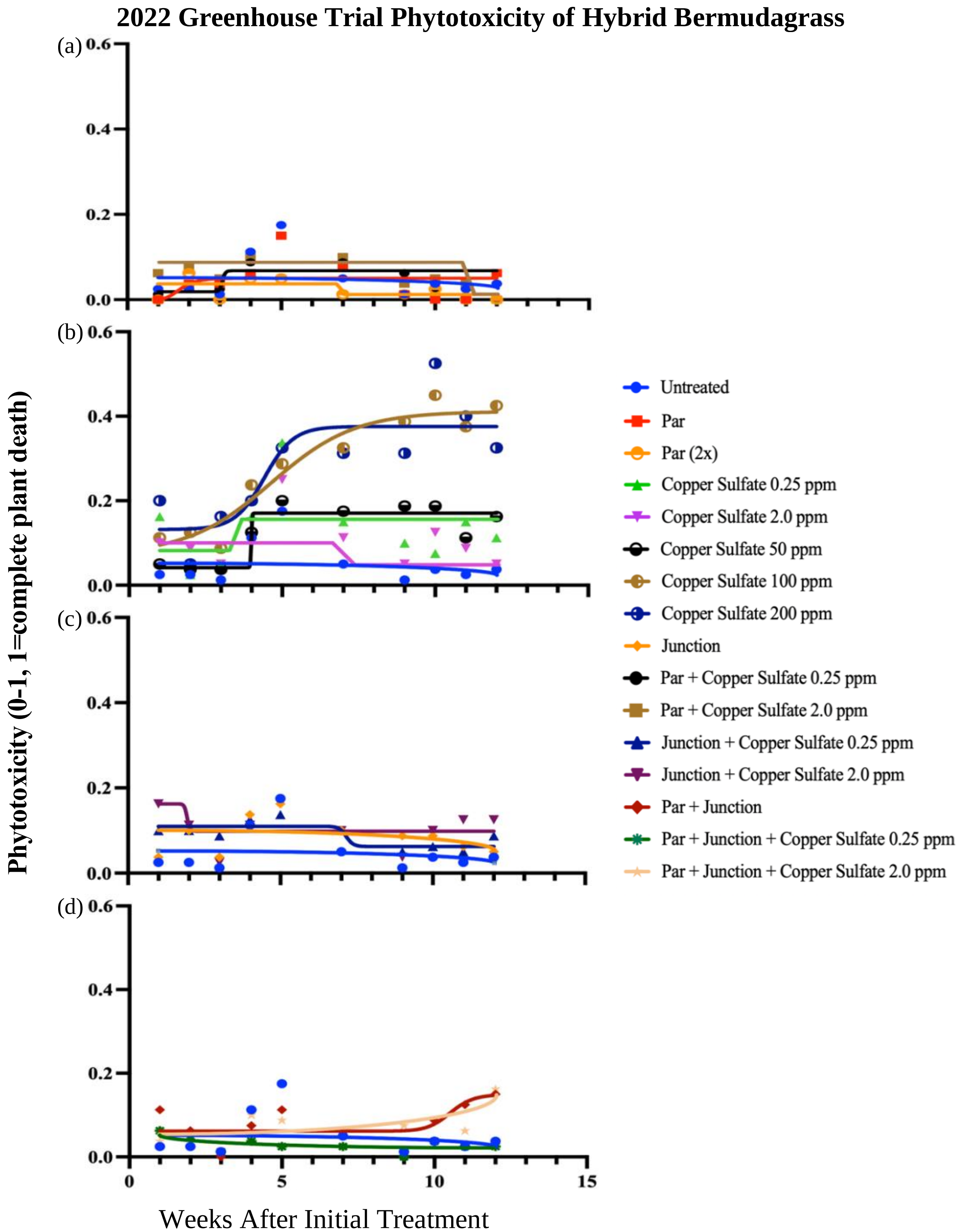


FIGURE 2-36. Average weekly phytotoxicity of greenhouse grown hybrid bermudagrass treated with various copper-containing products in 2022 with sigmoidal curve representing individual treatment progression over trial length of (a) PAR and PAR + $CuSO_4$ treatments, (b) $CuSO_4$ treatments, (c) Junction and Junction + SO_4 treatments, and (d) PAR + Junction and PAR + Junction + $CuSO_4$ treatments.

TABLE 2-30. Treatments with respective 2022 greenhouse trial phytotoxicity inflection point and R^2 value when using a sigmoidal logistic four parameter curve fitting.

Treatment	**Inflection**	**R^2**
	-----Week-----	
Untreated	25.451	0.032
PAR	1.502	0.102
PAR (2x)	6.955	0.077
Copper Sulfate 0.25 ppm	3.548	0.096
Copper Sulfate 2.0 ppm	7.187	0.116
Copper Sulfate 50 ppm	3.957	0.445
Copper Sulfate 100 ppm	4.468	0.663
Copper Sulfate 200 ppm	4.195	0.507
Junction	69.983	0.049
PAR + Copper Sulfate 0.25 ppm	3.141	0.187
PAR + Copper Sulfate 2.0 ppm	10.960	0.137
Junction + Copper Sulfate 0.25 ppm	7.195	0.114
Junction + Copper Sulfate 2.0 ppm	1.825	0.038
PAR + Junction	10.834	0.130
PAR + Junction + Copper Sulfate 0.25 ppm	-33.532	0.131
PAR + Junction + Copper Sulfate 2.0 ppm	25.895	0.121

Tissue Copper Concentration

In trial one, treatments of irrigation water containing 200 and 100 ppm $CuSO_4$ resulted in plant tissue containing 1475.58 and 1348.37 mg Cu kg^{-1}, respectively (Figure 2-37). Similar to 100 ppm $CuSO_4$, Junction + 2.0 ppm $CuSO_4$ and Junction + 0.25 ppm $CuSO_4$ resulted in Cu tissue concentrations of 1095.57 and 1090.10 mg Cu kg^{-1}, respectively. Similar to the previously mentioned Junction treatments, PAR + Junction + 0.25 ppm

$CuSO_4$, PAR + Junction + 2.0 ppm $CuSO_4$, PAR + Junction, and Junction resulted in tissue concentrations of 943.65, 938.64, 922.98, and 858.87 mg Cu kg^{-1}, respectively. Irrigation water containing 50 ppm $CuSO_4$ resulted in a Cu tissue concentration of 543.93 mg Cu kg^{-1}. PAR + 2.0 ppm $CuSO_4$, PAR + 0.25 ppm $CuSO_4$, PAR applied weekly, PAR (2x), irrigation water containing 0.25 ppm $CuSO_4$, irrigation water containing 2.0 ppm $CuSO_4$, and untreated had similar results ranging from 44.73 – 132.65 mg Cu kg^{-1}.

In trial two, PAR + Junction + 2.0 ppm $CuSO_4$ and PAR + Junction + 0.25 ppm $CuSO_4$ had similar tissue concentrations of 591.33 and 522.11 mg Cu kg^{-1}, respectively (Figure 2-23). Similar to PAR + Junction + 0.25 ppm $CuSO_4$, Junction + 2.0 ppm $CuSO_4$ and Junction + 0.25 ppm $CuSO_4$ had Cu concentrations of 500.90 and 477.19 mg Cu kg^{-1}, respectively. Junction, PAR + Junction, and 200 ppm $CuSO_4$ had similar Cu concentrations of 383.79, 338.79, and 315.91 mg Cu kg^{-1}, respectively, with the latter also similar to 100 ppm $CuSO_4$ with a Cu concentration of 247.53 mg Cu kg^{-1}. Treatments of 50 ppm $CuSO_4$, PAR (2x), PAR, PAR + 2.0 ppm $CuSO_4$, PAR + 0.25 ppm $CuSO_4$, 0.25 ppm $CuSO_4$, untreated, and 2.0 ppm $CuSO_4$ all exhibited similar tissue concentrations ranging from 34.16 – 90.34 mg Cu kg^{-1}.

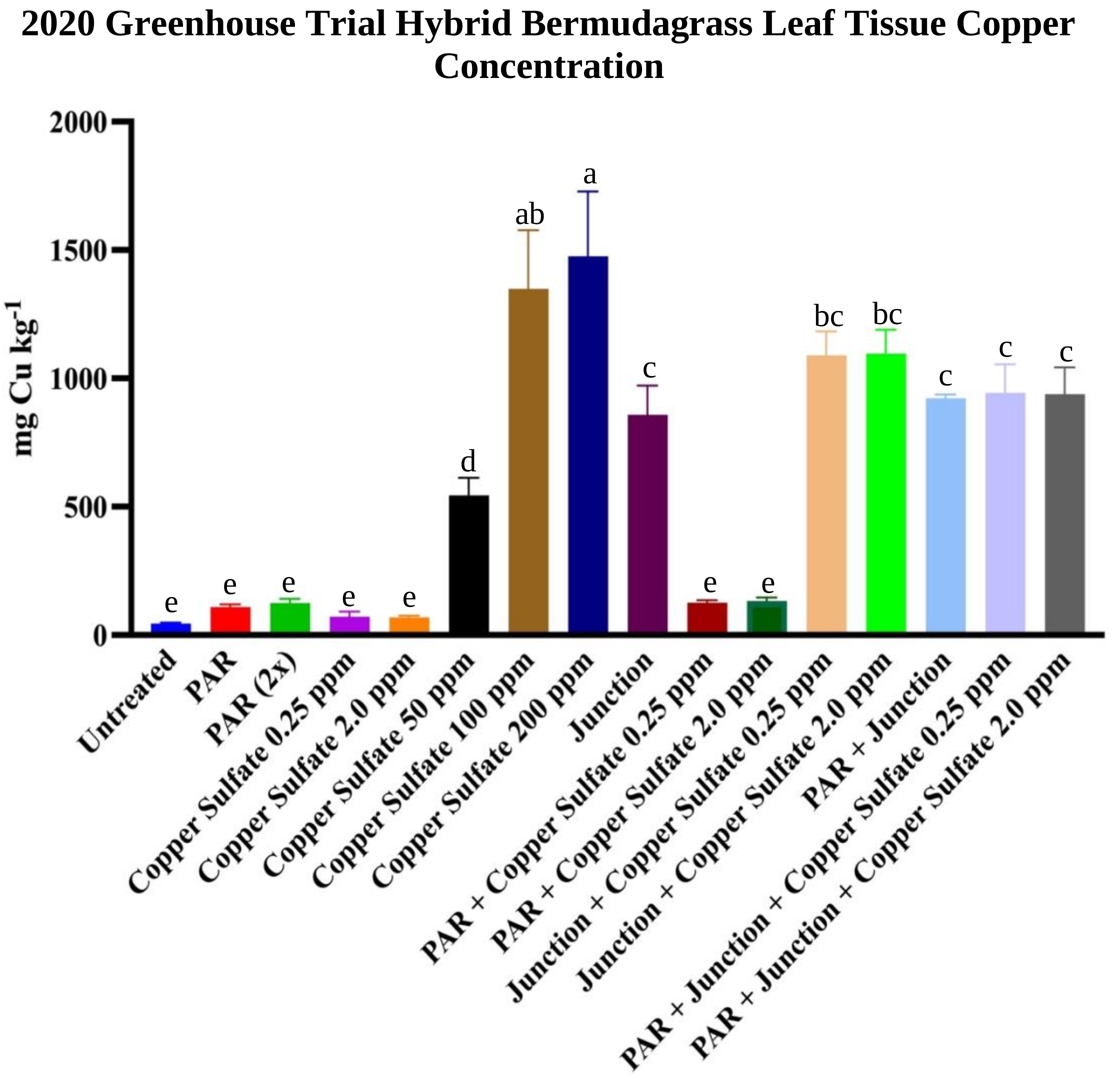


FIGURE 2-37. Plant tissue copper concentration for 2020 greenhouse trial following applications of copper-containing products to hybrid bermudagrass. Vertical bars represent standard errors. Different letters indicate significant differences between treatments by Fishers LSD ($\alpha = 0.05$).

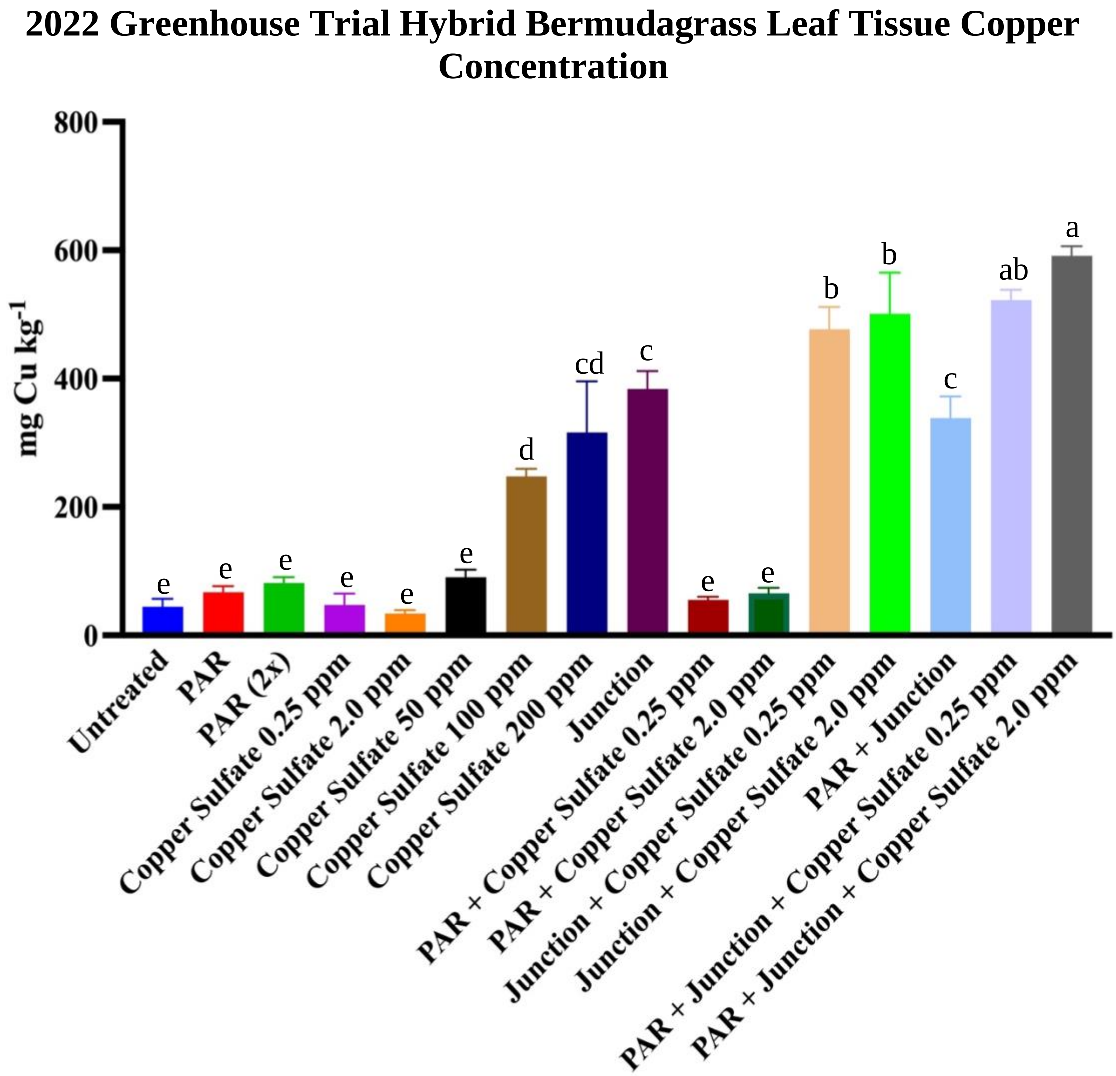


FIGURE 2-38. Plant tissue copper concentration for 2022 greenhouse trial following applications of copper-containing products to hybrid bermudagrass. Vertical bars represent standard errors. Different letters indicate significant differences between treatments using Fishers LSD ($\alpha = 0.05$).

Root Copper Concentration

In trial one, treatments of irrigation water containing 200 and 100 ppm $CuSO_4$ resulted in plant root concentrations 877.50 and 574.08 mg Cu kg^{-1}, respectively (Figure 2-24). Statistically similar to 100 ppm Cu, treatments of 50 ppm $CuSO_4$, PAR + Junction + 0.25

ppm $CuSO_4$, Junction, and Junction + 2.0 ppm $CuSO_4$ resulted in Cu concentrations of 479.73, 393.11, 229.83, and 187.42 mg Cu kg^{-1}, respectively. PAR + Junction + 2.0 ppm $CuSO_4$, Junction + 0.25 ppm $CuSO_4$, PAR + 2.0 ppm $CuSO_4$, and 2.0 ppm $CuSO_4$ resulted in similar root concentrations of 165.00, 125.50, 107.27, and 101.20 mg Cu kg^{-1}, respectively. Treatments of PAR + Junction, PAR + 0.25 ppm $CuSO_4$, 0.25 ppm $CuSO_4$, PAR (2x), untreated, and PAR had similar root concentrations ranging from 30.21 – 72.16 mg Cu kg^{-1} but were also similar to all other treatments except for irrigation treatments containing 50, 100, and 200 ppm $CuSO_4$.

In trial two, irrigation water containing 200 ppm $CuSO_4$ resulted in root concentration of 1167.16 mg Cu kg^{-1} (Figure 2-25). Irrigation water containing 100 ppm $CuSO_4$ with Cu concentration of 538.46 mg Cu kg^{-1} was similar to Junction + 2.0 ppm $CuSO_4$ with a concentration of 275.53 mg Cu kg^{-1} which was similar to all other treatments ranging from 21.26 – 223.45 mg Cu kg^{-1}. The presence of Cu at elevated concentrations can be deleterious over time, though specific tolerance levels exhibit wide variability between grass cultivars (Hull, 2002)

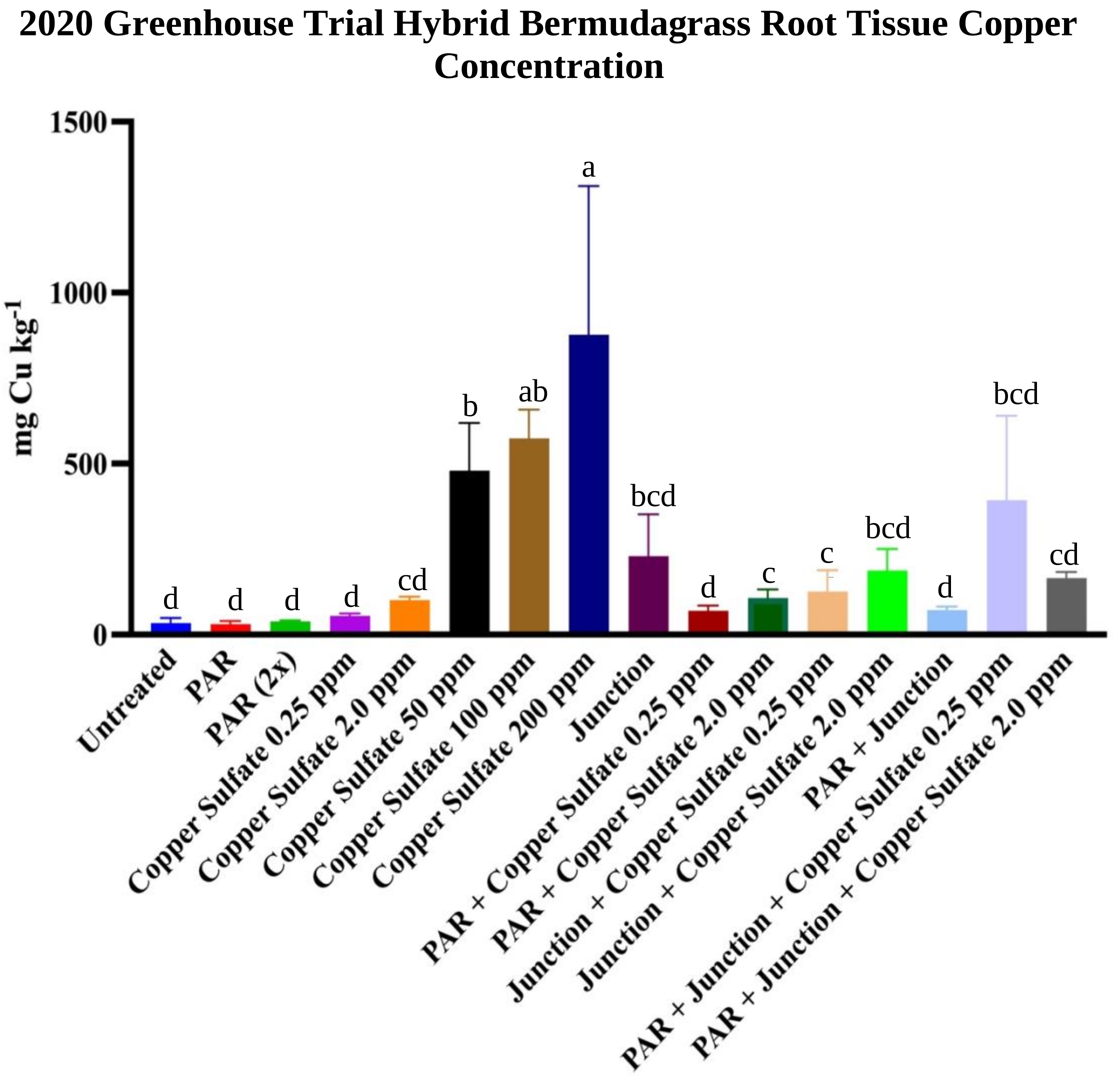


FIGURE 2-39. Root tissue copper concentration for 2020 greenhouse trial following applications of copper-containing products to hybrid bermudagrass. Vertical bars represent standard errors. Different letters indicate significant differences between treatments using Fishers LSD ($\alpha = 0.05$)

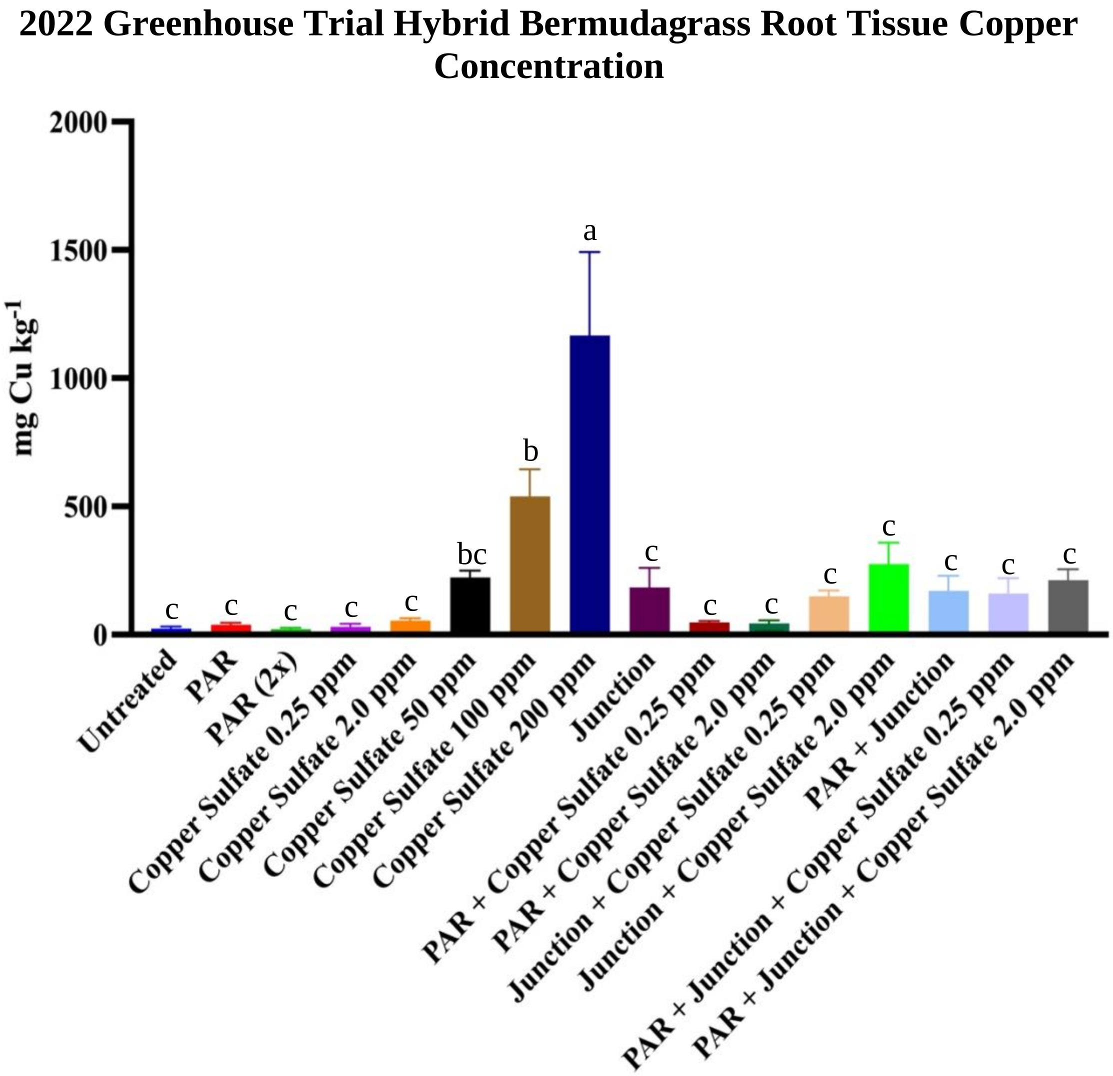


FIGURE 2-40. Root tissue copper concentration for 2022 greenhouse trial following applications of copper-containing products to hybrid bermudagrass. Vertical bars represent standard errors. Different letters indicate significant differences between treatments using Fishers LSD ($\alpha = 0.05$)

Soil Copper Concentration

In trial one, treatment of irrigation water containing 200 ppm $CuSO_4$ resulted in soil Cu concentrations of 431.98 kg Cu ha^{-1} (Figure 2-41). Irrigation treatment with 100 ppm $CuSO_4$ resulted in Cu concentrations of 220.11 kg Cu ha^{-1}. Treatment with 50 ppm

$CuSO_4$ resulted in soil Cu concentrations of 100.37 kg Cu ha^{-1}. All other treatments resulted in statistically similar soil Cu concentrations ranging from 3.47 – 34.69 kg Cu ha^{-1}.

In trial two, irrigation water containing 200 ppm $CuSO_4$ resulted in soil concentration of 148.09 kg Cu ha^{-1} (Figure 2-42). Irrigation water containing 100 ppm $CuSO_4$ with copper concentration of 55.82 kg Cu ha^{-1} which was similar to 50 ppm $CuSO_4$ treatments with a concentration of 23.62 kg Cu ha^{-1}. The latter treatment was similar to all other treatments ranging from 1.65 – 11.40 kg Cu ha^{-1}.

Variability of copper concentration between years may be due to age of greens initial plugs were taken from with 2020 trial plugs being from a 23-year-old TifEagle bermudagrass green and 2022 trial plugs being from a 1-year-old TifEagle bermudagrass green. This age allows for greater accumulation of organic matter content over time which could increase Cu adsorption in older plugs. Sampling of varying ages of golf greens for Cu analysis and organic matter content can serve to confirm this.

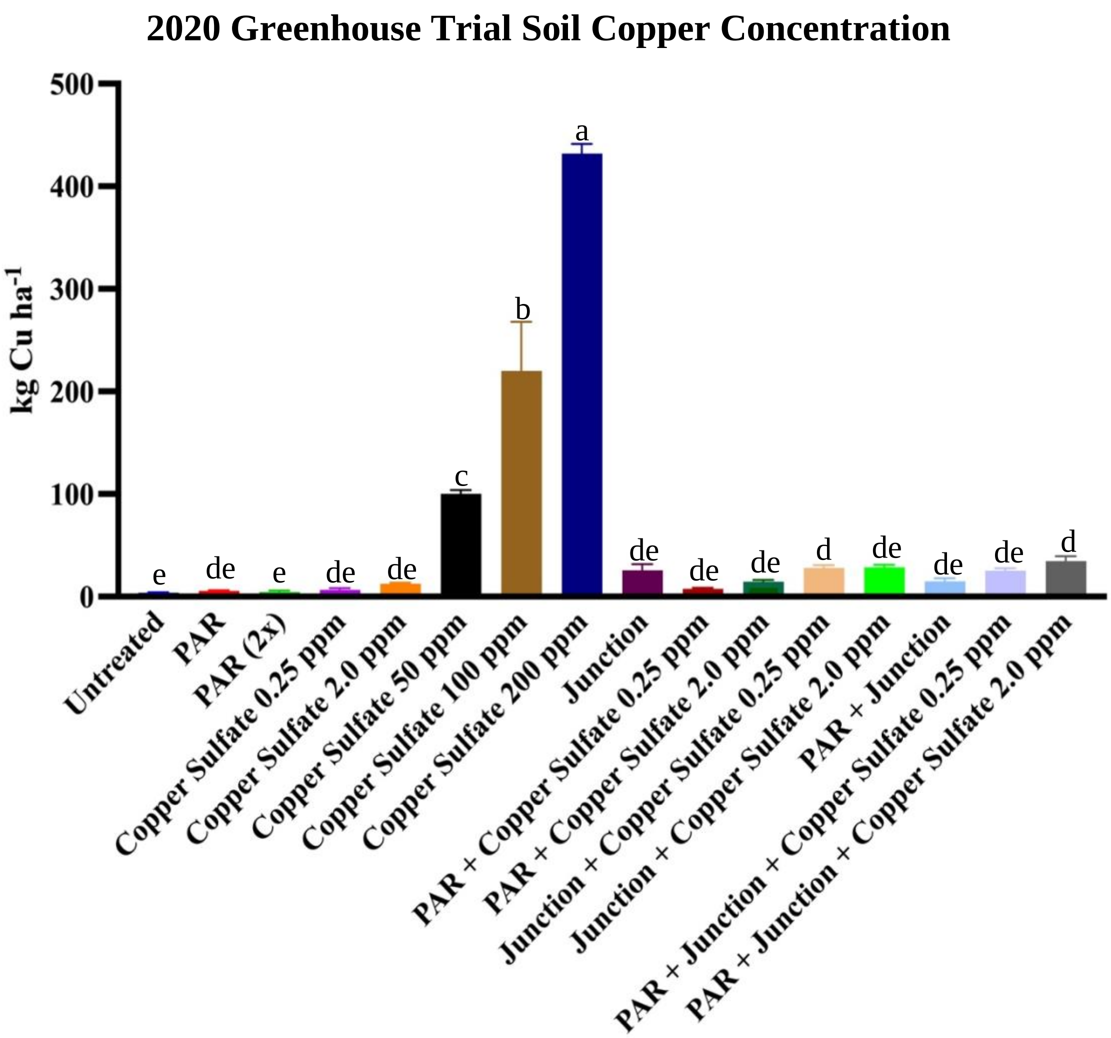


FIGURE 2-41. Soil copper concentration for 2020 greenhouse trial following applications of copper-containing products to hybrid bermudagrass. Vertical bars represent standard errors. Different letters indicate significant differences between treatments using Fishers LSD (0.10).

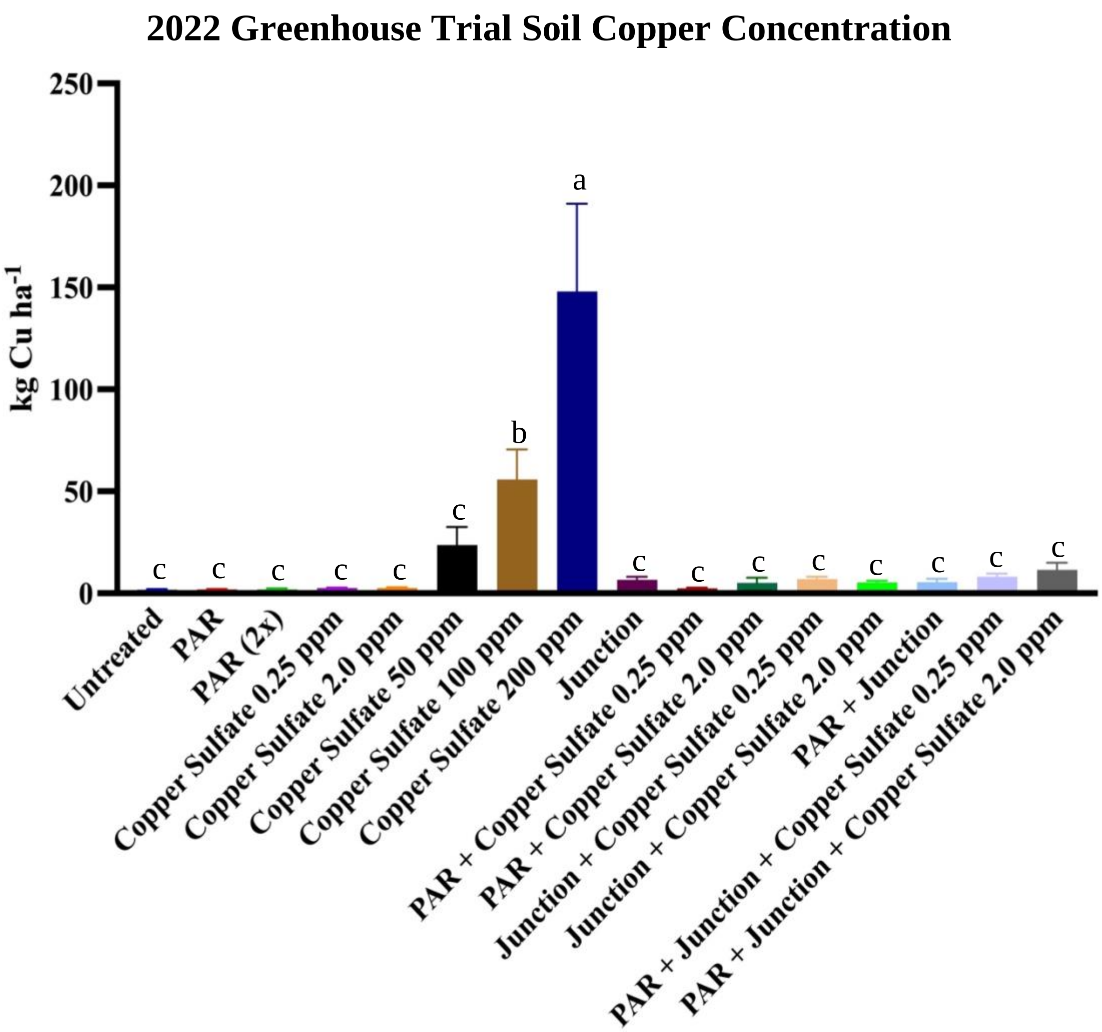


FIGURE 2-42. Soil copper concentration for 2022 greenhouse trial following applications of copper-containing products to hybrid bermudagrass. Vertical bars represent standard errors. Different letters indicate significant differences between treatments using Fishers LSD (0.10).

Conclusion

Pigmented products, such as PAR, provide turf managers the opportunity for better visual aesthetics in dormant and shoulder seasons, however no benefit or detriment was consistently observed between trials from the use of these products. The potential risks of using colorants during those times prior to and during spring transition, include products may unintentionally mask problems requiring more rapid attention.

Copper species and compounds appear to have an impact in the adsorption, absorption, and longevity of the metal within a system. Junction DF consists approximately 46.1% of $Cu(OH)_2$, which is not readily soluble in water resulting in minimal dissociation of the compound. This could be the cause for treatments containing Junction DF consistently having elevated Cu concentrations within tissue, root, and soil throughout field and greenhouse trials. In 1980, Jones (1980) described appropriate Cu concentrations in plant tissue as 5 – 20 ppm. Concern is warranted in this regard as multiple treatments resulted in higher than desired Cu concentration within plant tissue in field and greenhouse trials. The persistence of elevated Cu levels in soil, as shown with irrigation treatments containing 50 ppm or greater of $CuSO_4$, also suggest the survivability of hybrid bermudagrass may be more heavily impacted by continued root exposure than foliar application.

Additionally, though not a focus of this research, some morphological differences were observed between plants (Figure 2-43). This could be the result of Cu toxicity occurring within a plant causing stunting and altered leaf shape, however there is potential for grasses to alter physical appearance as a result of environmental conditions leading to the observance of ‘off-types’ (Reasor, 2019). TifEagle bermudagrass is an induced mutant, being artificially exposed to cobalt radiation to increase its dwarf habits (McCarty, 2021), thus the potential for heavy metals to induce mutation.

(a)

(b)

FIGURE 2-43. Observed variation in hybrid bermudagrass with (a) grass exposed to low copper input via pigmented product displaying elongated internodes and more rounded leaf tips compared to (b) grass exposed to highest amount of copper exhibiting shorter internodal spacing, distorted leaf margins, and some phytotoxicity on older leaves.

CHAPTER THREE

REMEDIATION OF SOIL-BOUND COPPER

Introduction

Phytoremediation

Due to its long history of production from, and use for, various anthropogenic activities including agricultural, mining, smelting, and fossil fuel pursuits, coupled with copper's propensity for adsorption to clay soil particles and organic substrates within the soil profile, the concentration of the heavy metal has reached hazardous levels possibly posing a considerable threat to biological health in addition to crop production (Bokhari et al., 2016; Jan et al., 2015). Methods commonly used for remediation of contaminated soil including the excavation and burial of the contaminated soils as well as the offsite cleansing or stripping of the soil using various chelating and acid products, however these carry with them a hefty economical price (Hazrati et al., 2020; Leštan et al., 2008). Additionally, these practices can result in the deterioration of the structure and fertility of soil (Ghazaryan et al., 2019).

The use of various hyperaccumulator plant species for the remediation of contaminated soils is considered more sustainable and cost-effective compared to previously used techniques (Goswami & Das, 2016). Hyperaccumulator plants are capable of absorbing high quantities of heavy metals from soil medium and quickly translocate these ions to shoots and leaves at concentrations as high as 1000x of other plants (Muszyńska & Hanus-Fajerska, 2015). Kavousi et al. in 2020 noted common mullein (*Verbascum thapsus* L.) was able to accumulate almost 500 mg Cu kg^{-1} dry weight in shoots when subjected to hydroponic growing solution containing 375 mg L^{-1} Cu concentration. The use of chemical amendments to aid in the absorption and transference of copper is sometimes necessary with Ghazaryan and company finding the application of ammonium nitrate (NH_4NO_3), when combined with citric or malic acid, increased the phytoextraction of copper by wormwood (*Artemisia absinthium* L.) (Ghazaryan et al., 2022).

Practical Soil Management Approaches

Accumulation of various ions to toxic levels and the buildup of different organic and inorganic substrates are not new problems for turfgrass managers with issues of soil salinity, pH, and thatch management being long dealt with (McCarty, 2018). When tackling issues of salinity, whether caused by poor irrigation water quality, natural soil occurrence, or various product applications, applications of organic substrates including humic acid and calcium-containing products such as gypsum ($CaSO_4{\cdot}2H_2O$) have been evaluated for ability to improve turfgrass quality as well as for ability to flush and/or bind sodium ions which otherwise may cause deflocculation (Bello et al., 2021; Rahayu et al., 2019; Sekar, 2016).

The removal of thatch by the processes of verticutting or hollow tine aerification is common practice by turfgrass managers as they strive to maintain only a thin layer of living and dead stems, leaves, and roots of grass between the actively growing shoots of grasses and the soil surface (Atkinson et al., 2012). In 1986, Danneberger and Turgeon (1986) reported core cultivation and vertical mowing could reduce the overall content of a thatch layer while causing an increase in the overall cation exchange capacity (CEC) on a per volume basis.

Materials and Methods

Copper Profile Accumulation

TifEagle bermudagrass plugs (10 cm diameter x 10 cm deep) were removed from the Clemson University Turfgrass Research Facility and placed into a 100% sand rootzone contained within 15.24 cm diameter x 15.24 cm deep greenhouse pots. Turf was established for 3 weeks within greenhouse facility at temperature of 23.89°C (75°F) until reaching 15 cm diameter and 13 mm height. Pots were then treated over the span of 13 weeks with varying species, compounds, and concentrations of Cu. Treatments consisted of four replications of products listed in Table 2-2 applied in similar form described previously in Chapter 2 Greenhouse Studies.

At the conclusion of the 13-week study, 5 cores (2 cm diameter x 15 cm deep) were extracted from each pot and were divided by depths: thatch (approximately 0.5 inch (1.27 cm), 0 - 2 inches (0 - 5.08 cm) below the thatch layer, and 2-4 inches (5.09 – 10.16 cm). Cores were submitted to Clemson University Ag Services Laboratory for Cu concentration analysis. Due to high variability probability of soil chemical and physical characteristics within a given area as well the potential impact of Type II errors in environmental studies, means of soil analysis were compared among treatments using Analysis of Variance followed by Fisher's LSD (alpha=0.10) (Pennock, 2004; Peterman, 1990) using JMP statistical software (SAS Institute, Cary, NC).

Copper Adsorption Curve

Using a Cecil sandy loam soil, 5 g of soil (approximately 4 cm^3) were placed into 50 ml test tubes with 15 ml of solution containing either 1, 10, 50, 100, 200, or 1,000 ppm $CuSO_4$-Cu (copper sulfate pentahydrate, LabChem, Zelienople, PA) with 3 replications. Solution and soil were then mixed at a constant rate of 180 oscillations per minute with a 3-inch stroke for 24 hours. Contents were then placed in a Damon/IEC HN-SII centrifuge (Thermo Scientific, Waltham, MA) at a rate of 1500 revolutions per minute for 1 hour. Supernatant was then extracted and analyzed for Cu concentration by Clemson University Agricultural Services Lab (Figure 3-1).

Adsorption amount (q_e) (mg kg^{-1}) of Cu solutions was calculated from mass balance equation using the following equation:

$$q_e = \left(\frac{(C_i - C_e)V}{W_d}\right)1000$$

where V is solution volume (L), C_i and C_e are initial and equilibrium concentrations of the copper solution (mg L^{-1}), respectively, and W_d is weight of dry soil (g).

Copper Replacement with Industry Products

Five g (approximately 4 cm^3) of Cecil sandy loam previously treated with 1,000 ppm $CuSO_4$-Cu in a manner consistent with methods described in *Adsorption Curve,* was exposed to treatments consisting of 20 ml of tap water, 20 ml 1 N gypsum ($CaSO_4 \bullet 2H_2O$) (SoftCal Pellets, Austinville Limestone Co., Austinville, VA), 20 ml 1 N calcium nitrate ($CaNO_3$)(15.5-0-0)(Hi-Yield, Voluntary Purchasing Groups, Bonham, TX), and 20 ml 1 N ammonium sulfate ($(NH_4)_2SO_4$)(21-0-0)(Hi-Yield, Voluntary Purchasing Groups, Bonham, TX)(AMS). Soil and solutions were shaken for 5 minutes at a rate of 180 oscillations per minute with a 3-inch stroke and then placed in a Damon/IEC HN-SII centrifuge (Thermo Scientific, Waltham, MA) at a rate of 1500 revolutions per minute for 1 hour. Supernatant was then extracted for analysis and soil was then retreated with 20 ml of same solution, shaken, centrifuged, and extracted 3 more times. Supernatants were analyzed for Cu concentration by Clemson University Agricultural Services Lab. All treatments had 4 replications.

Due to high variability probability of soil chemical and physical characteristics within a given area as well the potential impact of Type II errors in environmental studies, means of soil analysis were compared among treatments using Analysis of Variance followed by Fisher's LSD (alpha=0.10) (Pennock, 2004; Peterman, 1990).

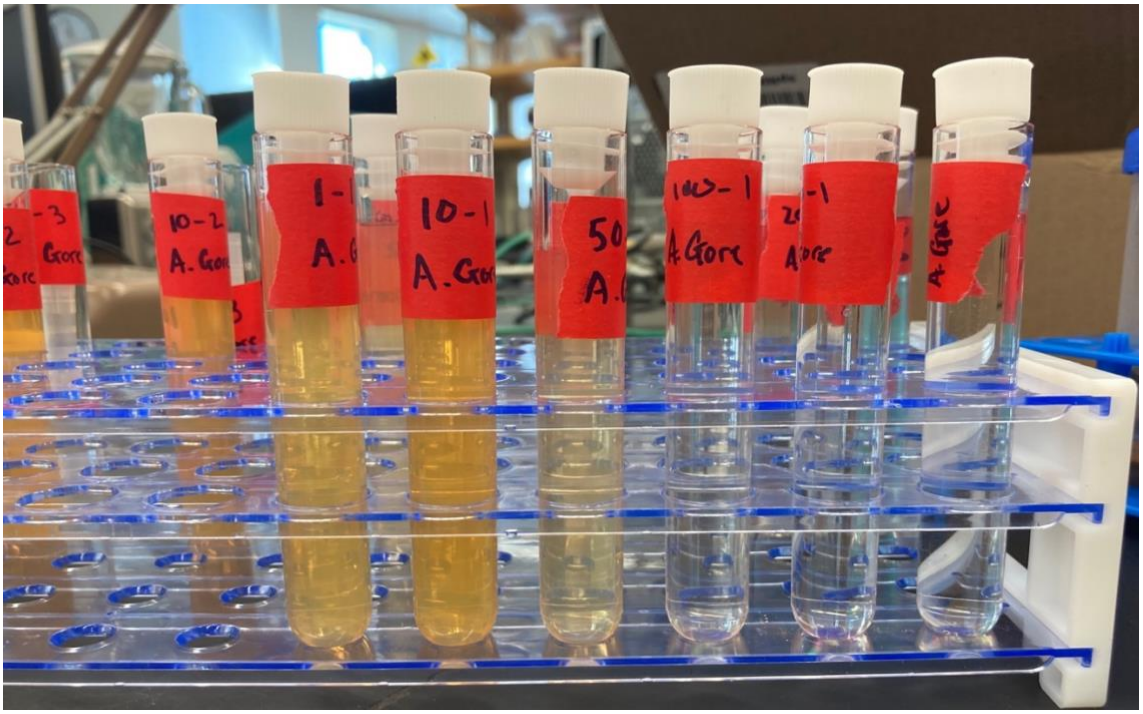


FIGURE 3-1. Supernatant of centrifuged treatments after being exposed to 5 mg of Cecil sandy loam for 24 hours. Original concentrations of treatments (foreground, from left to right): 1, 10, 50, 100, 200, and 1,000 ppm $CuSO_4$-Cu.

Results and Discussion

Copper Profile Accumulation

Statistical differences were present between thatch and underlying soil depths with average Cu concentrations of 634.7, 11.26, and 6.842 kg ha^{-1} for the thatch, 0 - 2.54 cm, and 2.54 - 5.08 cm intervals, respectively (Figure 3-2). Within each interval, irrigation treatments containing 200 and 100 ppm $CuSO_4$ had greater Cu accumulation than all other treatments, but statistically different from one another in 0 – 2.54 cm and 2.54 – 5.08 cm depth intervals below thatch layer (Figure 3-3). Irrigation water containing 50 ppm $CuSO_4$ exhibited the third highest Cu concentration in thatch layer with 1952.29 kg ha^{-1} but was similar to all other treatments in two lower soil layer intervals.

This data supports similar findings in vineyards where Cu was found at highest concentrations higher in soil profile where organic matter content was greatest (Sonoda, et al., 2019; Duplay, et al., 2014). Thatch is a collection of dead and decaying organic material which provides preferred binding sites for copper. At greater depths within a golf green built to USGA specifications, there is potential for other binding sites depending on the usage of organic substrates such as peat moss during construction process, however pure sand greens may lack this organic material thus Cu adsorption would likely rely on various compound complexes (Duplay et al, 2014).

With the thatch layer possessing greatest concentrations of Cu within the profile, management practices aimed at reduction of the thatch layer, such as hollow tine aerification and vertical mowing, may also result in reduction of Cu in soil profile. Aggressive aerification has been reported to reduce thatch content by approximately 10% (Atkinson et al., 2012).

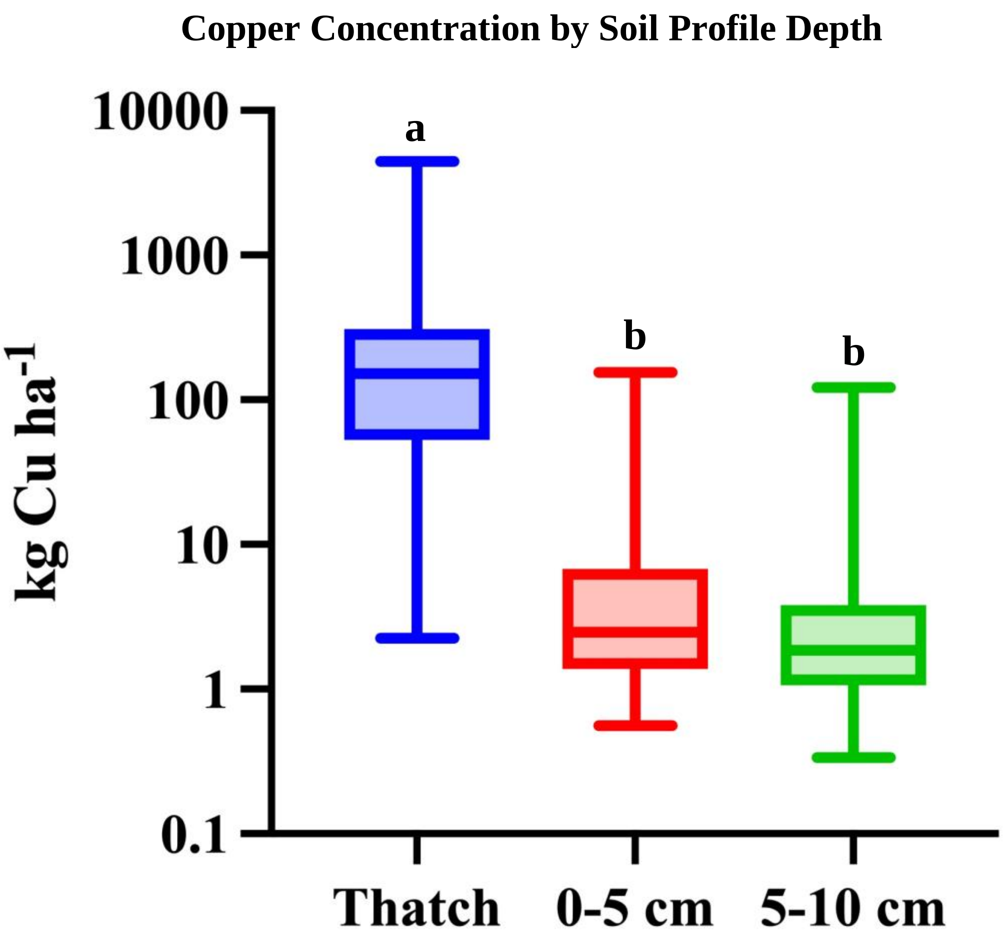


FIGURE 3-2. Concentration of copper within subsurface layers following 13 weeks of treatments with various copper-containing products. Different letters indicate significant differences between treatments using Fishers LSD (α = 0.10)

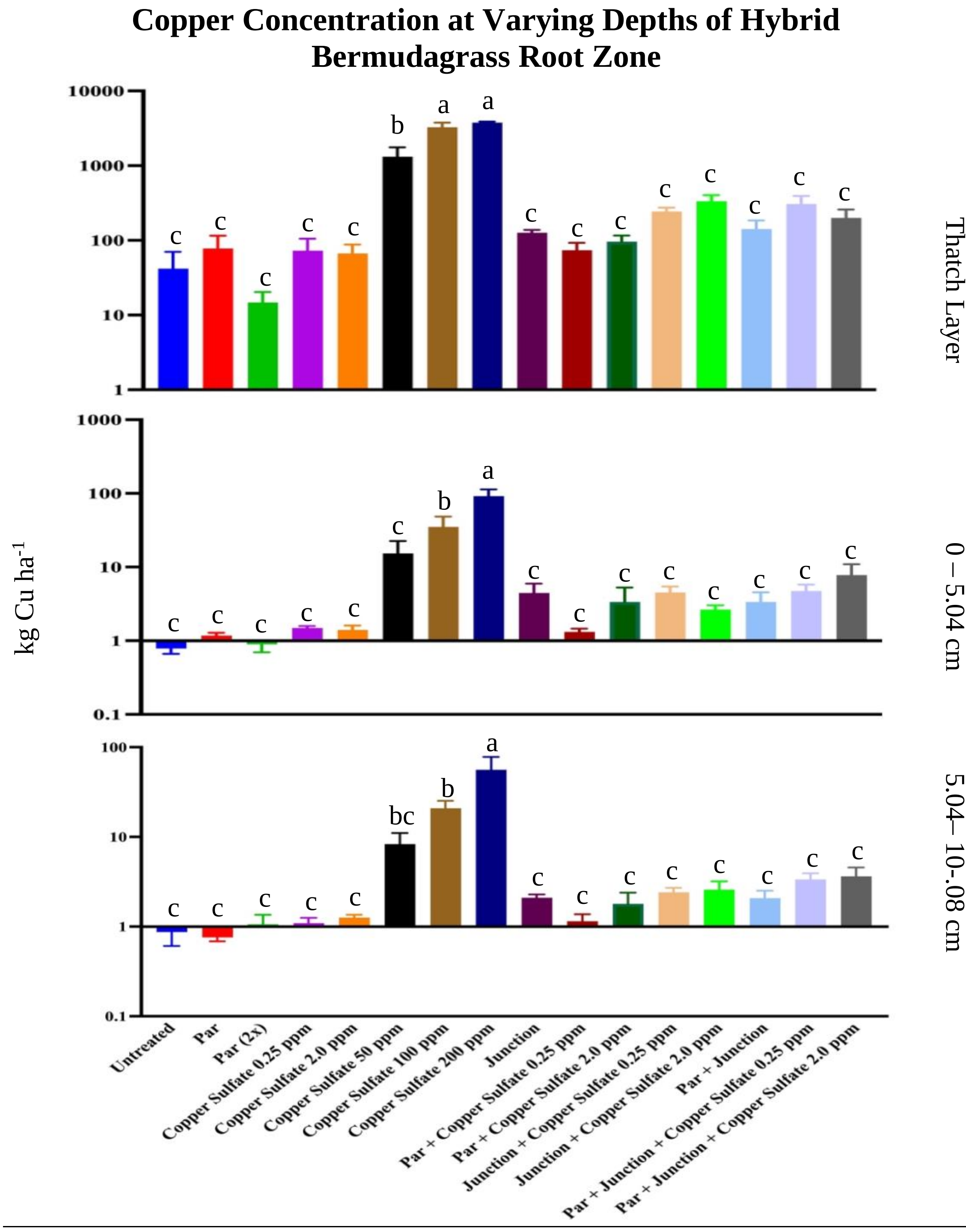


FIGURE 3-3. Copper concentration of hybrid bermudagrass rootzone at varying depths after being exposed to copper-containing products. Vertical bars represent standard errors. Different letters indicate significant differences between treatments within depths using Fishers LSD ($\alpha = 0.10$)

Copper Adsorption Curve

In 2014, Komy et al (2014) described the impact of pH and humic acids on Cu^{2+} adsorption on kaolinite and hematite, with increased pH and the presence of humic substrates with carboxylic and phenolic groups resulting in maximum adsorption. Using a Cecil sandy loam with a reported pH of 4.6, a maximum adsorption capacity may not have been met with observed maximum of 1942 mg kg^{-1} (Figure 3-4).

Upon analysis determining adsorption quantity of copper, samples were exposed in four sequential events to 1 N $CaNO_3$ or Mehlich I. Due to lack of replications, no statistical comparison can be made, thus observations are only discussed. Use of Mehlich I resulted in removal of all known quantities of Cu on soil exposed to at least 100 ppm $CuSO_4$-Cu, whereas the use of $CaNO_3$ resulted in less efficient removal and did not remove all known Cu content at any time (Figure 3-5). Future related studies may be required to test for consistency in use of Mehlich I for extraction of heavy metals from golf course putting green situations. Faust and Christians reported in 1999 the use of AB-DTPA and Mehlich III soil analysis resulted in higher copper extractions in non-sand-based soil media, thus may be more suitable for push-up greens (those greens using native soil as underlying soil profile) and heavy organic matter situations, though use of Mehlich I in USGA specific green soil profile is less clear and researched.

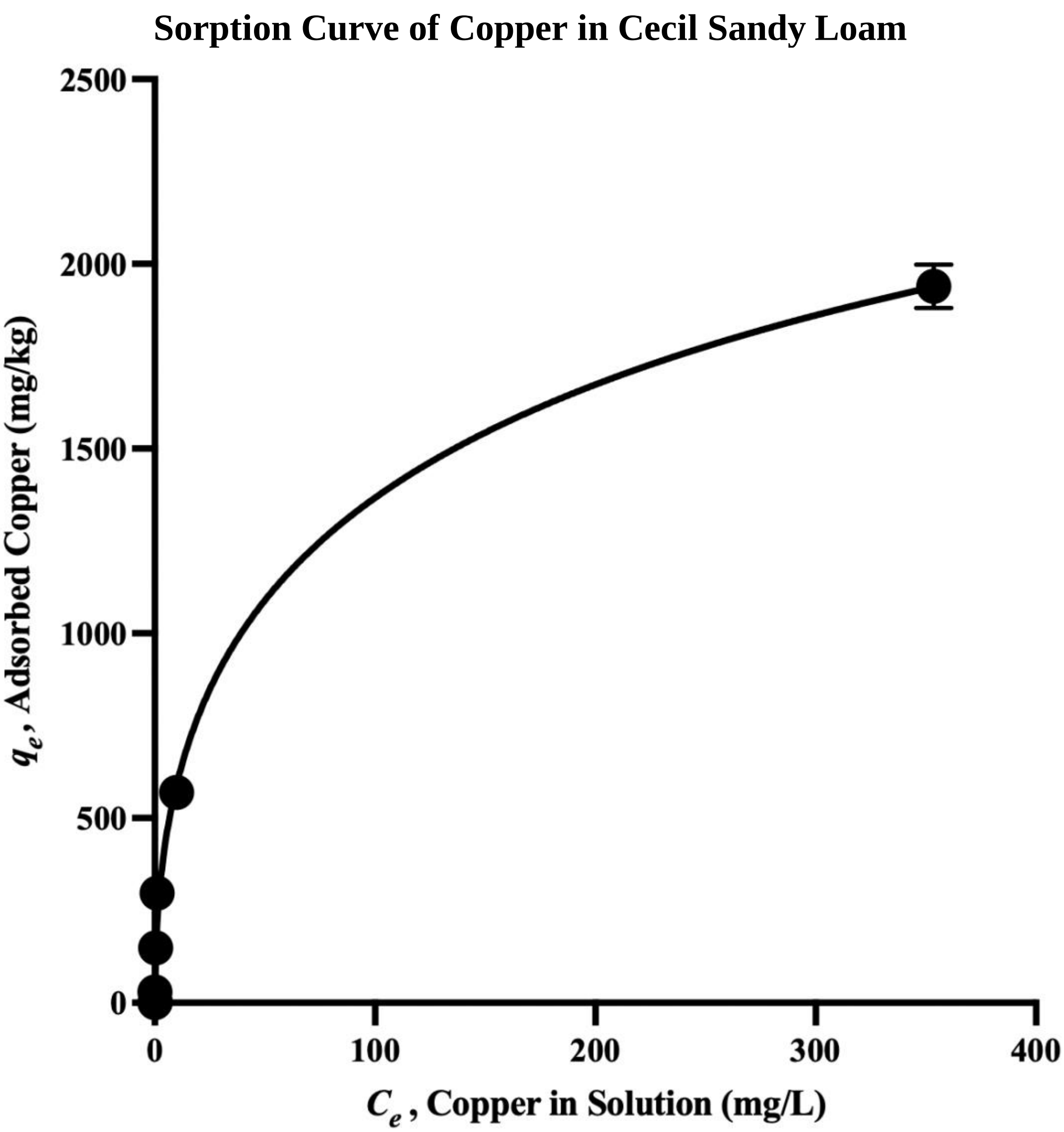


FIGURE 3-4. Copper adsorption curve of Cecil sandy loam soil with pH 4.6.

Cumulative Copper Desorption from Cecil Sandy Loam Using Sequential Filtrations

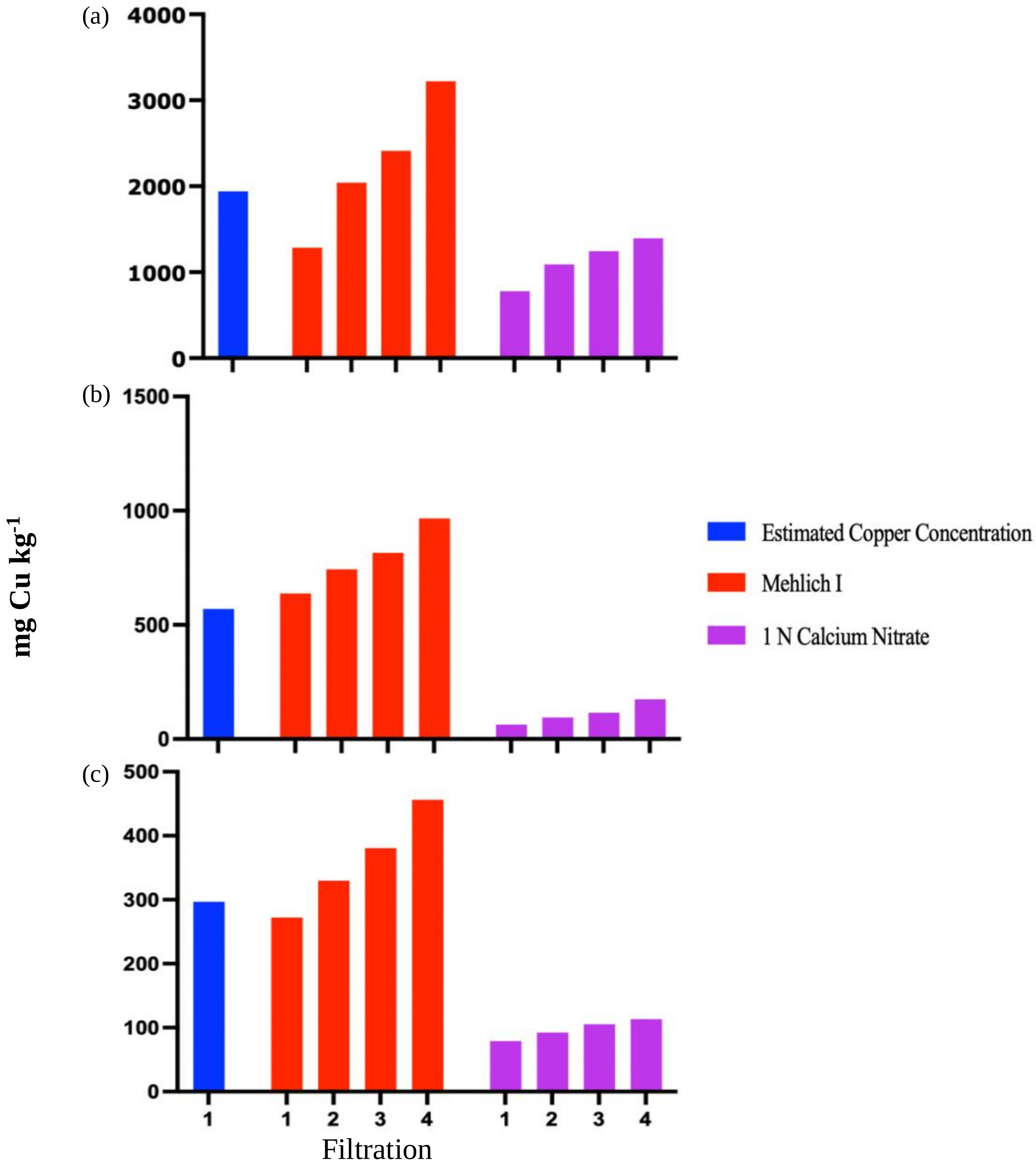


FIGURE 3-5. Cumulative copper desorption in Cecil sandy loam upon exposure to sequential filtration treatments after previous exposure to (a) 1000, (b) 200, and (c) 100 ppm $CuSO_4$-Cu.

Copper Replacement with Industry Products

Replacement treatments were analyzed by effect of individual treatment filtration and cumulative impact of sequential filtrations. Individually, first filtration of ammonium sulfate resulted in the greatest concentration of Cu removed from soil, extracting an average 2064.36 mg kg^{-1} (Figure 3-6). First filtration of $CaNO_3$ was similar to first filtration of gypsum removing 958.02 and 714.88 mg kg^{-1}, respectively. With the exception of water treatment, first filtrations resulted in the greatest amount of displaced copper.

Ammonium sulfate (AMS) resulted in the greatest cumulative impact on removing copper with cumulative effect of 2, 3 and 4 filtration cycles being statistically greater than all other treatments and their respective cumulative impact (Figure 3-7). A single filtration of AMS was statistically similar to all cumulative AMS amounts, and also to four filtrations of $CaNO_3$ and gypsum, and 3 filtrations of $CaNO_3$. Water by itself resulted in the lowest Cu displacement with only one filtration yielding 495.92 mg kg^{-1} of Cu.

With AMS providing for the potential displacement of Cu from a soil profile, turf managers may have a viable option for removal of copper if more aggressive practices such as aerification are impractical at the time. More evaluation is needed to determine minimum necessary quantity of AMS required for efficient removal of Cu to avoid any potential phytotoxic effects associated with large amount of soluble nitrogen fertilizer as well as evaluate any potential environmental issues related to possible N run-off or leaching. The presence of a thatch layer may aid in reducing the leaching potential of excessive nitrogen content in an AMS treatment, but is not necessary (Engelsjord et al., 2004). Additionally, water carrier volume must be evaluated to assess the necessary volume of water for displacement. One filtration cycle in present study represents a water volume four times greater than treated soil, equivalent to using 651,702 gallons (2,466,960.4 L) to flush 1 acre (0.01 ha) of ground 6 inches (15.24 cm) deep.

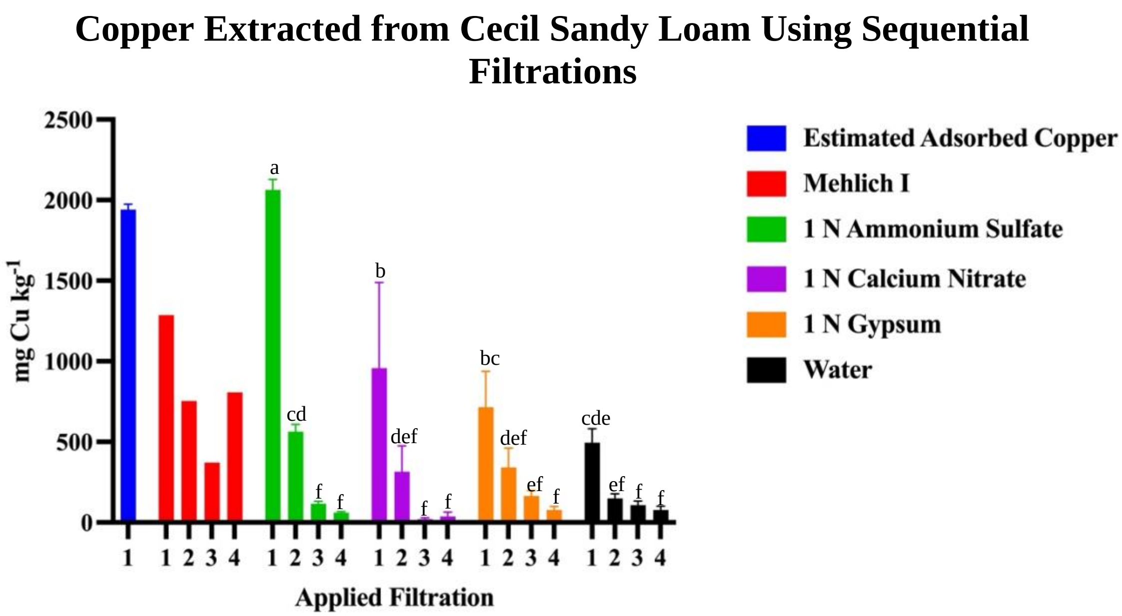


FIGURE 3-6. Copper concentration of sequential filtration treatments to Cecil sandy loam previously exposed to 1000 ppm $CuSO_4$-Cu. Vertical bars represent standard errors. Different letters indicate significant differences between treatments using Fishers LSD ($\alpha = 0.10$)

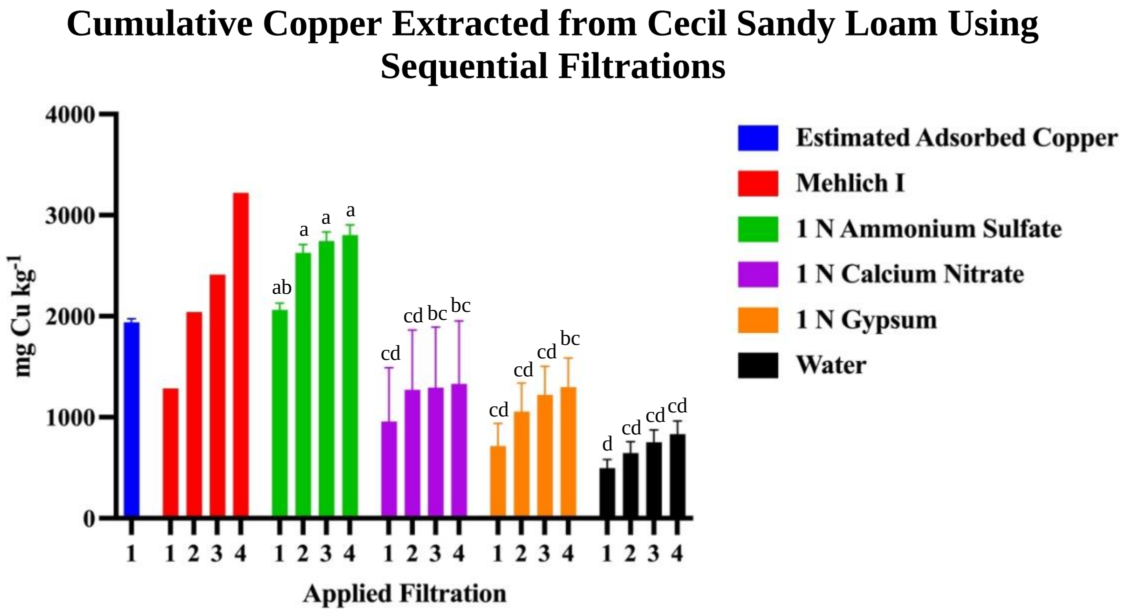


FIGURE 3-7. Cumulative extracted copper concentration of sequential filtration treatments to Cecil sandy loam previously exposed to 1000 ppm $CuSO_4$-Cu. Vertical bars represent standard errors. Different letters indicate significant differences between treatments using Fishers LSD ($\alpha = 0.10$)

Conclusions

Results suggest turf areas exhibiting Cu toxicity may be best served through the use of aerification, vertical mowing, and other thatch management practices so as to remove preferred Cu binding sites. These aggressive agronomic practices can be impractical for golf course superintendents and other turf managers at times due to the potential temporary damage to a facility's aesthetic and playability. In situations where mechanical removal is impractical or rootzones contain higher percentages of clay particles, the use of fertilizer products such as ammonium sulfate may serve as an alternative to aid in flushing heavy metals from turfgrass rootzones in practice similar to sodium flushing.

CHAPTER FOUR

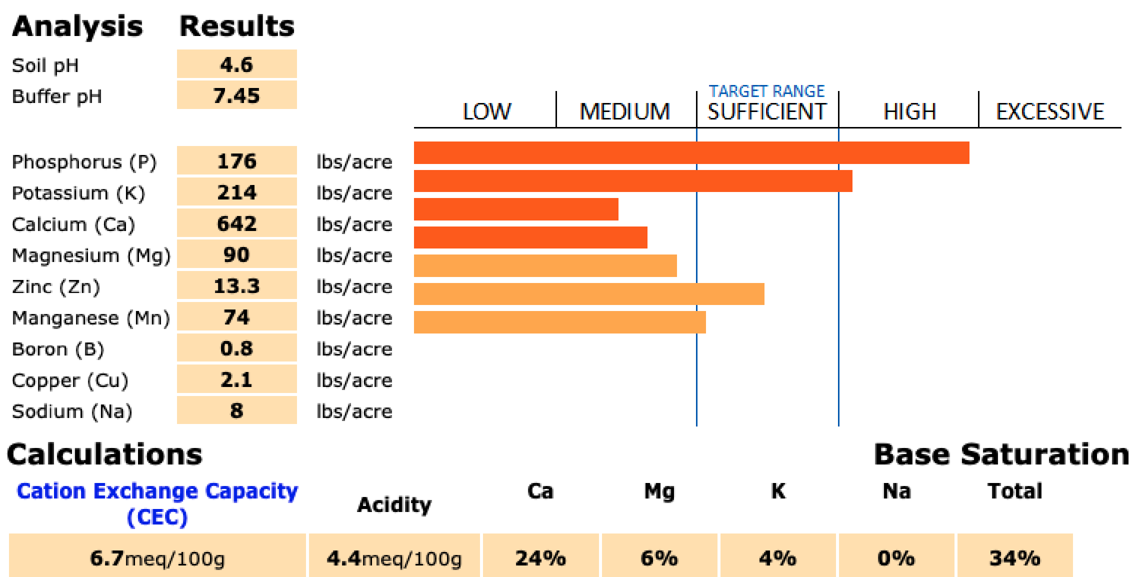
SUMMARY

Copper has the potential to negatively impact hybrid bermudagrass growth and visual quality in as few as three weeks when weekly irrigation contains of 50 ppm $CuSO_4$ or more. Potentially masking these issues, colorants used in combination may temporarily improve visual quality of turf areas while concealing more serious issues. Usage of $Cu(OH)_2$ product resulted in tissue and root Cu concentrations similar to that of 50 ppm $CuSO_4$ in greenhouse studies and were associated with highest concentrations of Cu concentration in field study soils when applied three times throughout individual trial.

Accumulation of Cu is greatest in thatch layers compared to soil below suggesting that mechanical and cultural practices designed for reduction of thatch may also serve to reduce Cu concentration within system. Push-up greens containing greater quantities of clay particles and greens containing organic substrates within soil profile could be at greater risk of excessive Cu accumulation toxicity. The use of AMS may serve to flush excessive Cu ions out of soil system when more aggressive and aesthetically damaging practices are not possible.

Future research should investigate Cu absorption dynamics in dormant grasses to determine critical thresholds prior to plant death. Further research should build upon the potential use of AMS to aid in removal of Cu ions from soil profiles by investigating the minimum required AMS to avoid turfgrass damage in addition to irrigation water volume necessity.

Appendix A
Soil Analysis for Cecil Sandy Loam



Analysis	Results	
Soil pH	4.6	
Buffer pH	7.45	
Phosphorus (P)	176	lbs/acre
Potassium (K)	214	lbs/acre
Calcium (Ca)	642	lbs/acre
Magnesium (Mg)	90	lbs/acre
Zinc (Zn)	13.3	lbs/acre
Manganese (Mn)	74	lbs/acre
Boron (B)	0.8	lbs/acre
Copper (Cu)	2.1	lbs/acre
Sodium (Na)	8	lbs/acre

Calculations / **Base Saturation**

Cation Exchange Capacity (CEC)	Acidity	Ca	Mg	K	Na	Total
6.7meq/100g	4.4meq/100g	24%	6%	4%	0%	34%

Figure A-1. Soil analysis report for Cecil sandy loam used for copper adsorption, desorption, and replacement studies.

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