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Health Risk and Impacts of Microcystins Irrigation of B*rassica oleraceae* L., L*actuca sativa* L. and A*maranthus hybridus L.* with Contaminated Water from River Kaduna, Nigeria

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Abstract

Cyanobacteria are photosynthetic prokaryotes that synthesize natural toxins harmful to various organisms and are primarily found in freshwater environments worldwide. Water contaminated with microcystins (MCs) or other cyanotoxins is widely used in agriculture in many developing countries, with no policies or management strategies in place. This activity has detrimental effects on numerous plant species. This study aimed to assess the risks associated with using MCs-contaminated water from the Kaduna River for the irrigation of Brassica oleracea L. (cabbage), Lactuca sativa L. (lettuce), and Amaranthus hybridus L. (spinach) in 2018 and 2019. The results showed that MCs-contaminated water positively impacted the growth and productivity of the vegetables. However, it was also observed that the cyanotoxins bioaccumulated in the plants. Additionally, lettuce and spinach exhibited aberrant leaf shape, lesions, and color changes, indicating the impact of MCs on irrigated vegetables. High bioaccumulation of MCs in vegetables resulted in significantly higher estimated daily intake per kilogram of body weight, surpassing the maximum acceptable limits set by the World Health Organization (0.04 µg kg-1 body mass). Using microcystin-contaminated water to irrigate cabbage, lettuce, and spinach poses potential acute and chronic health risks to humans who consume these vegetables.

Keywords: Microcystin; Cabbage; Lettuce; Spinach, Wastewater; Phytotoxicity

INTRODUCTION

Harmful algal blooms (HABs), often composed of cyanobacteria, pose a real threat to aquatic ecosystems, particularly in freshwater environments, where they are referred to as cyanobacterial harmful blooms (CHBs) (Gobler 2020) Climate change and anthropogenic pollution, such as increased nutrient loads, further contribute to the frequency and intensity of CHBs occurrences (Igwaran *et al.* 2024) Cyanobacteria, a group of photosynthetic prokaryotes, are of great concern in aquatic environments due to their potential to contaminate and poison wildlife and humans (Buratti *et al.* 2017) Certain cyanobacterial strains produce potent toxins called microcystins, which pose a substantial threat to the environment and human health when found in water used for various activities (Catherine *et al.* 2016).

Microcystins are bioactive metabolites produced non ribosomally through an enzyme complex consisting of peptide synthetases, polyketide synthases (PKS), and modifying enzymes (Chia *et al.* 2019) With over 300 congeners, these toxins have been linked to human cancer cases and deaths resulting from direct contact with contaminated water and the consumption of contaminated food (Chia *et al.* 2019, 2022) Their bioactivity is associated with the inhibition of phosphatases of serine/threonine-protein 1 (PP1) and 2A (PP2A), disrupting intracellular homeostasis and leading to cell cytoskeleton disarray, cell death, intrahepatic haemorrhage, and mortality in exposed organisms (Bittencourt-Oliveira *et al.* 2015, 2016; Buratti *et al.* 2017) To mitigate health risks, the World Health Organization (WHO) recommends a maximum daily intake of microcystins not exceeding 0.04 µg Kg^{-1} of body mass (USEPA 2015; WHO 1998).

Microcystins have been associated with various deleterious effects on plants, including decreased growth, tissue necrosis, inhibition of photosynthesis, and metabolic changes, leading to impaired crop productivity and economic losses (Cao *et al*. 2018, 2019) These toxins can also adversely affect soil ecosystems by reducing the abundance of ammonia-oxidizing bacteria and important plant growth-promoting rhizobacteria, resulting in decreased nitrification potential and compromised plant growth (Zhang *et al*. 2023).

In northern Nigeria, including Kaduna, irrigation farming is a common practice, and during the dry season, untreated water is often used for irrigating vegetables such as *Brassica oleraceae* L. (cabbage), *Lactuca sativa* L. (lettuce), and *Amaranthus hybridus* L. (spinach) (Abdullahi *et al*. 2022; Chia *et al*. 2019) These vegetables are consumed raw and cooked by residents in Kaduna State and the southern states of Nigeria, where they are exported. Consequently, the contamination of these crops with microcystins may present a long-term health crisis for the entire country.

Previous studies have shown the presence of microcystins in vegetables sold in markets and obtained from irrigation farms in Kaduna State, indicating their contamination (Abdullahi *et al*. 2022; Chia *et al*. 2019) It is important to note that the significant sources of cyanobacterial and microcystin contamination of irrigated vegetables are water bodies, including lakes, streams, and agricultural drainage, which collect water from areas rich in fertilizers and herbicides (Redouane *et al*. 2023) These factors contribute to the excessive proliferation of cyanobacteria, underscoring the need to investigate the public health risk associated with irrigation using water from these contaminated points. Therefore, this study aimed to assess the potential risk of irrigating cabbage, lettuce, and spinach with water from the Kaduna River.

MATERIALS AND METHODS

The characteristics of the water, soils, and the microcystin concentration of the water used for irrigation of experimental and control plants were evaluated. The concentrations of microcystins detected in the water (three sampling stations, Kaduna River stretch) and utilized for vegetable irrigation ranged from 2 to 12 µg L^{-1} in 2018 and 2019. Inorganic growth nutrients (NPK and Urea) were applied at 0.40 Kg per 5 Kg soil, simultaneously, once to both plants, irrigated with contaminated river water and uncontaminated borehole water.

Experimental materials and experimental design

Seeds of *Brassica Oleraceae* L (Hybrid white cabbage, variety oxylus lot 103132942), *Latuca Sativa* L. (Hybrid lettuce variety Oxylus lot 201344292), and *Amaranthus hybridus* L. (spinach) were obtained from the Institute of Agricultural Research (IAR), Ahmadu Bello University, Zaria, Nigeria. The experiment was conducted in the Botanical Garden of the Department of Biological Sciences, Kaduna State University, by planting the seeds in 10 Kg planting bags containing 5 Kg of loamy soil and watering for 10 weeks with 200 mL of contaminated and uncontaminated water from the Kaduna State University general borehole. The contaminated water was obtained from stations A, B, and C along the River Kaduna stretch, where many agricultural activities occur (Figure 1). These points receive surface runoff directly from farmland in the river catchment. Water was collected weekly from the sampling stations and analyzed for toxin content to confirm total microcystin concentration.

The water samples from the three sampling stations were thoroughly mixed to provide composite water for irrigation. The vegetable controls received uncontaminated water from the Kaduna State University general borehole, free of microcystin contamination, while the treatments received 200 mL of microcystin-contaminated water, allowing the excess water to spill into the loamy soils. Each treatment was replicated three times, with each replicate consisting of two plant stands, and the experiment was conducted over two consecutive years (2018 and 2019) to assess the repeatability of the results.

The cyanotoxin levels were confirmed using the procedure described below before irrigation with microcystin-contaminated water from the Kaduna River channel. To determine the effects of microcystin on irrigated vegetables, samples were collected weekly for growth changes, plant height, leaf area, number of leaves, number of leaf falls, and leaf length measurement.

Water and vegetable samples collection, processing, and handling for microcystin and morphological analyses

During the ten-week vegetable-growing phase, 2 liters of water samples were collected biweekly and stored in clear polyethylene terephthalate (PETC) sample containers. Leaf samples of each vegetable for microcystin analysis were collected weekly for three weeks, specifically from weeks eight to ten, when the vegetables were matured enough to be harvested and consumed.

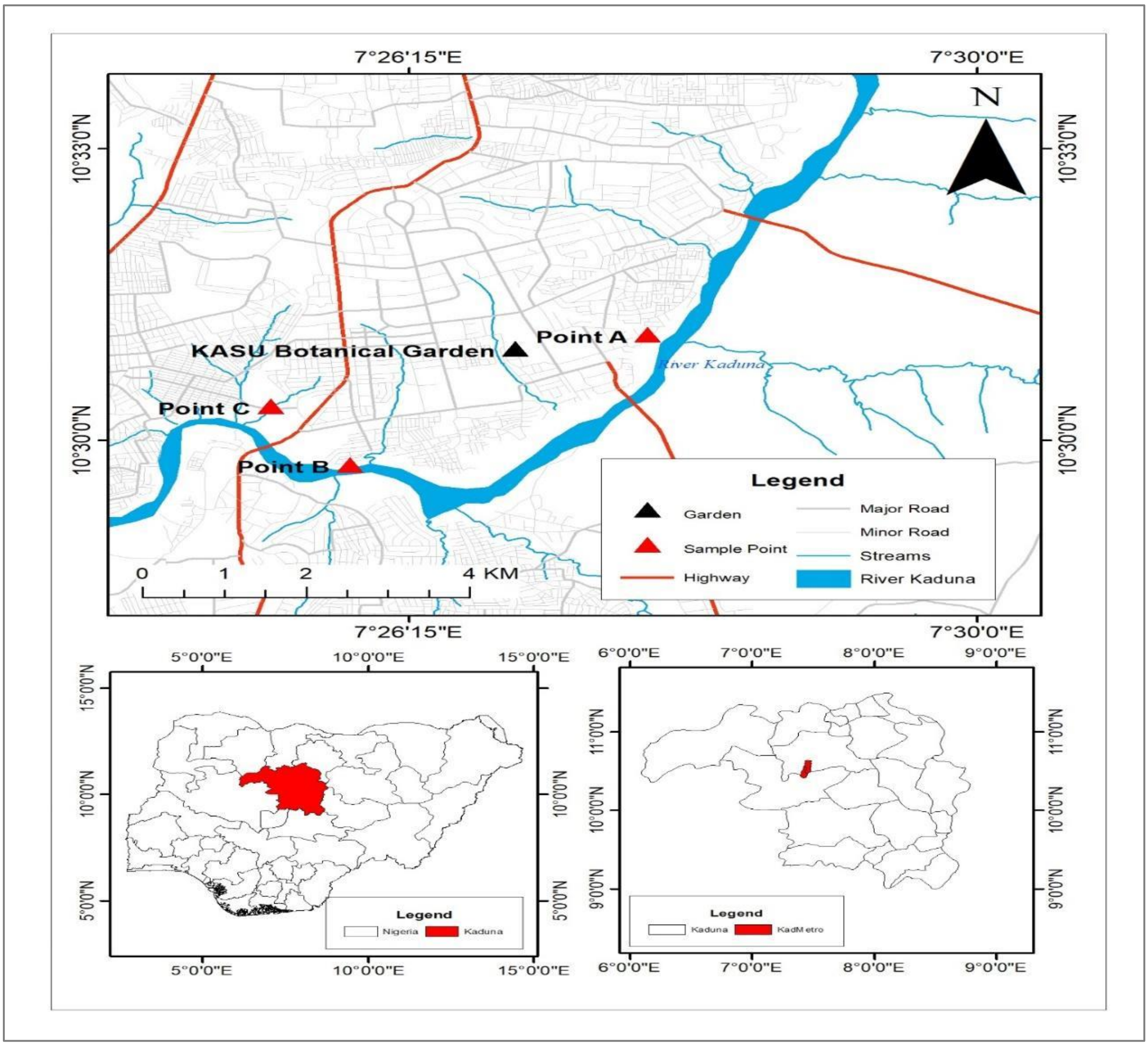

Figure 1. Map of Kaduna metropolis showing the location of KASU and waste water collection points (GIS Dept. 2018)

To perform a detailed morphological analysis of cabbage, lettuce, and spinach, samples were collected weekly starting from the beginning of the experimental period until the end of the growing phase. The parameters measured included plant height, leaf area, number of leaves, leaf length, and the number of leaf falls. A caliper was used to measure leaf length and width accurately. Plant height was measured from the base to the tip of the tallest leaf. Leaf area was calculated by tracing the leaf on graph paper and counting the squares, and the number of leaves per plant was recorded.

The leaves were chopped into smaller pieces with a razor blade, washed with distilled water to remove microcystin residues from their surfaces, weighed to 2 g, and stored at -40^{0}C until microcystin examination. The vegetable samples were homogenized in a ceramic mortar and pestle with 80 % methanol, centrifuged for 20 minutes at 4000 rpm, and the supernatants collected and frozen for microcystin analysis (Chia *et al.* 2019). According to the manufacturer's instructions, the extract and water samples were analyzed for microcystins using a 96-well Abraxis Microcystins-ADDA Enzyme-Linked Immunosorbent Assay (ELISA) kit. The limit of detection for the assay, based on MC-LR, was 0.10 µg L^{-1}, and the limit of quantification was 5.00 µg L^{-1}. The absorbance of the color reaction at the end of the ELISA procedure was measured at 450 nm in a Bio-Rad iMark™ Microplate reader (Bio-Rad Laboratories, Inc., Hercules, CA, USA), and the concentrations were expressed per cell quota of potential microcystin-producing species (Chia *et al.* 2019).

Data Analysis

After evaluating the data for homogeneity and normality, the statistical significance of changes in microcystin accumulation in vegetables, total microcystin levels, growth, and morphological parameters was determined using analysis of variance (ANOVA). When significant differences existed, Tukey's post hoc test was used to perform multiple comparisons. For all analyses, the significance level was set at $p < 0.05$. The total daily intake (TDI) limit of 0.04 µg Kg^{-1} body weight, determined by the World Health Organization, was used to estimate the risk of eating contaminated vegetables (USEPA 2015; WHO 1998). The first part of the analysis was based on the premise that an adult weighing 60 kg consumes at least 40 g of vegetables per day and that the microcystin content threshold in vegetable tissues is 60 µg kg^{-1}.

$$TDI_{vegetable} = MCs_{40g^{-1}}/BM \qquad \text{Equation 1}$$

Note: MCs_{40g}^{-1} is the concentration of MCs per 40g of vegetables, and BM is an adult's body mass weighing at least 60 kg.

RESULTS

The total MCs levels in water and irrigated vegetables

Microcystin concentrations recorded in the irrigation water were generally less than 10.00 µg L^{-1} in 2018 and 2019 (Figure 2). In 2018, the levels of microcystins in irrigated cabbage and lettuce were similar, while those in spinach were the lowest (Fig. 3A). The trend was different in 2019 because cabbage recorded the highest accumulation of microcystins with 11.257 µg g^{-1} fresh weight, while the levels in spinach lettuce and spinach were similar and not significantly different (Fig. 3B). The temporal changes in bioaccumulated microcystins followed the same pattern as the overall results. Specifically, spinach had the lowest levels of microcystin bioaccumulation from week one to three in 2018. There was an initial increase in microcystin levels in all three vegetables from the first to the second week. However, there was a drop in accumulated microcystin levels in all the irrigated vegetables in the third week (Fig 3A).

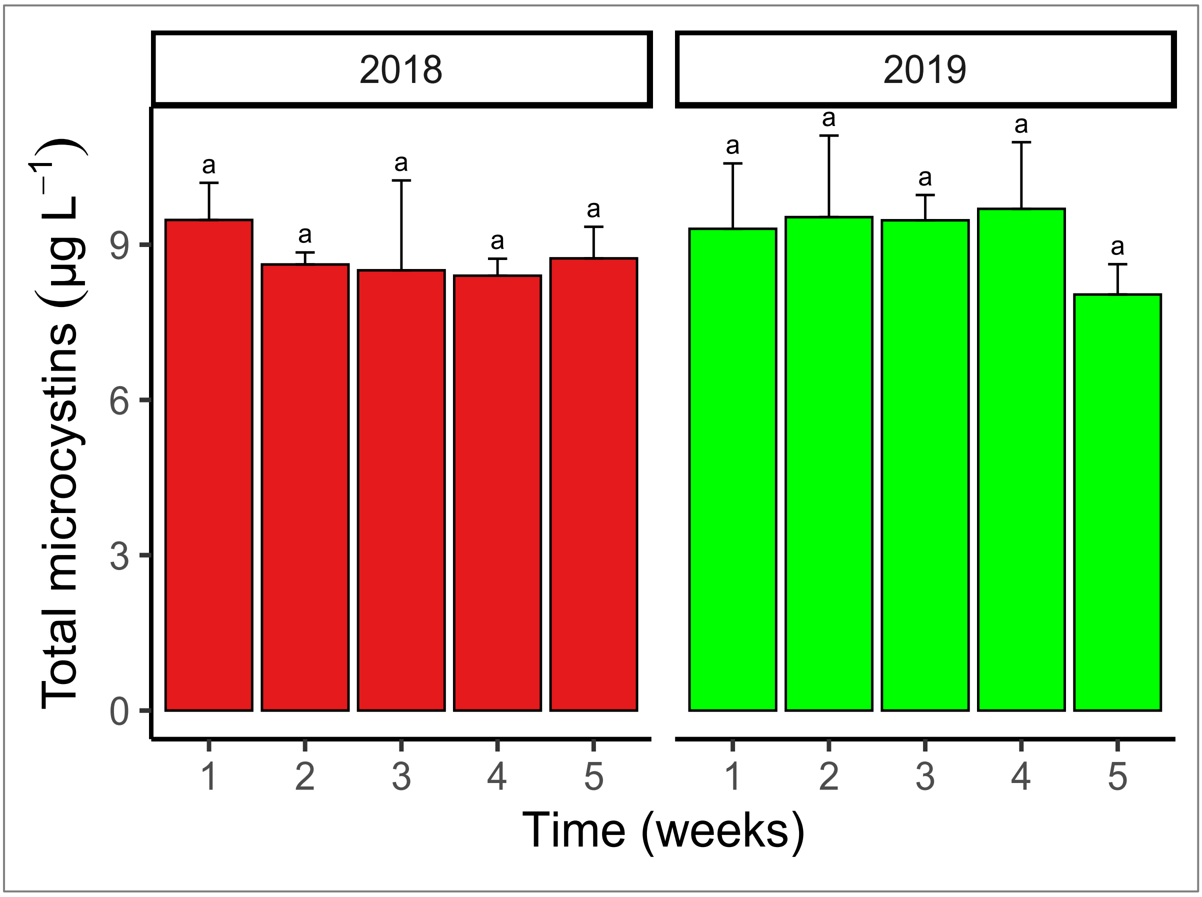

Fig. 2: Changes in microcystins concentrations in water collected from the Kaduna River and used for irrigation. Values are means for *n* = 4. Means with different alphabets are significantly different at p < 0.05.

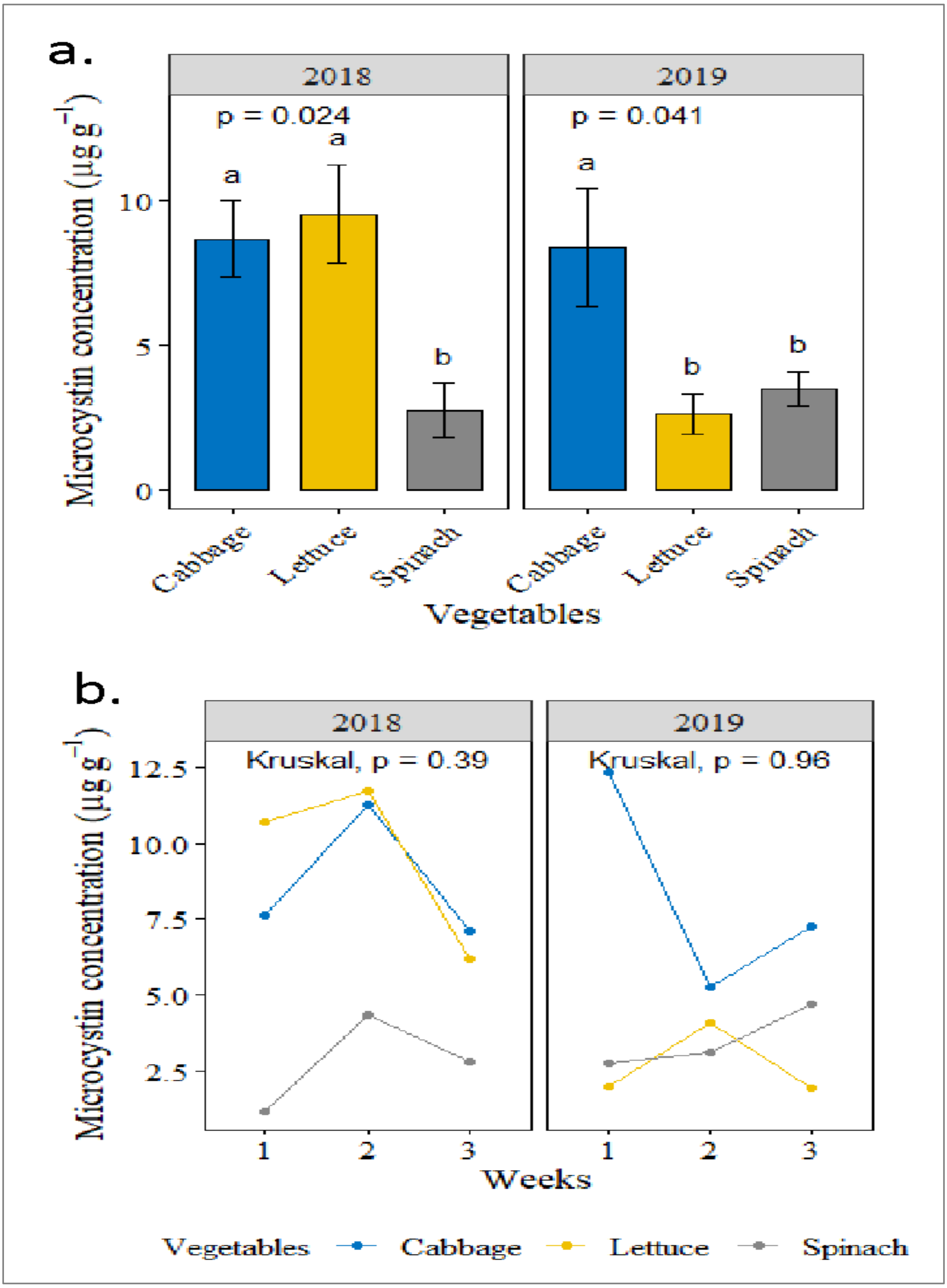

Fig. 3: Changes in microcystin concentrations in cabbage, lettuce, and spinach plant leaves following irrigation with microcystins-contaminated water. Values are mean for *n* = 4. Means with different alphabets are significantly different at $p < 0.05$.

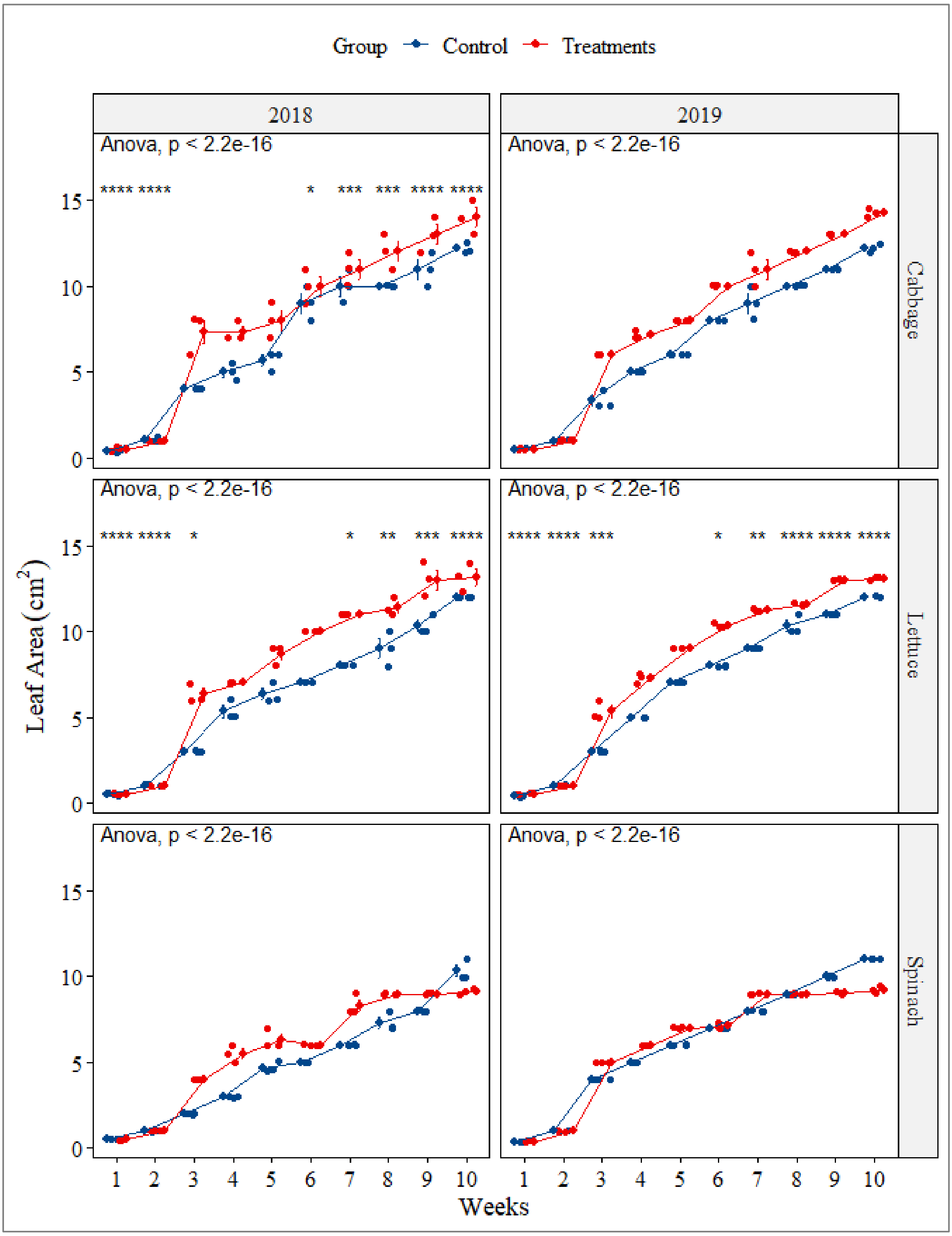

Fig. 4: Changes in leaf length (cm2) of vegetables irrigated with microcystins-contaminated water in 2018 and 2019. Treatments are significantly different from the control at $p < 0.05$.

Total daily intake (TDI) for microcystins in the vegetables for adults and children

Changes in TDI of microcystins by adults in 2018 revealed that lettuce consistently had higher values from week one to two, at 7.130 and 7.800 µg Kg^{-1}, respectively as shown in Table 1. Throughout the study, spinach had the lowest TDI values for adults and children. However, the highest TDI of microcystins in children was observed in cabbage exposed to microcystins for 2 weeks (Table 1).

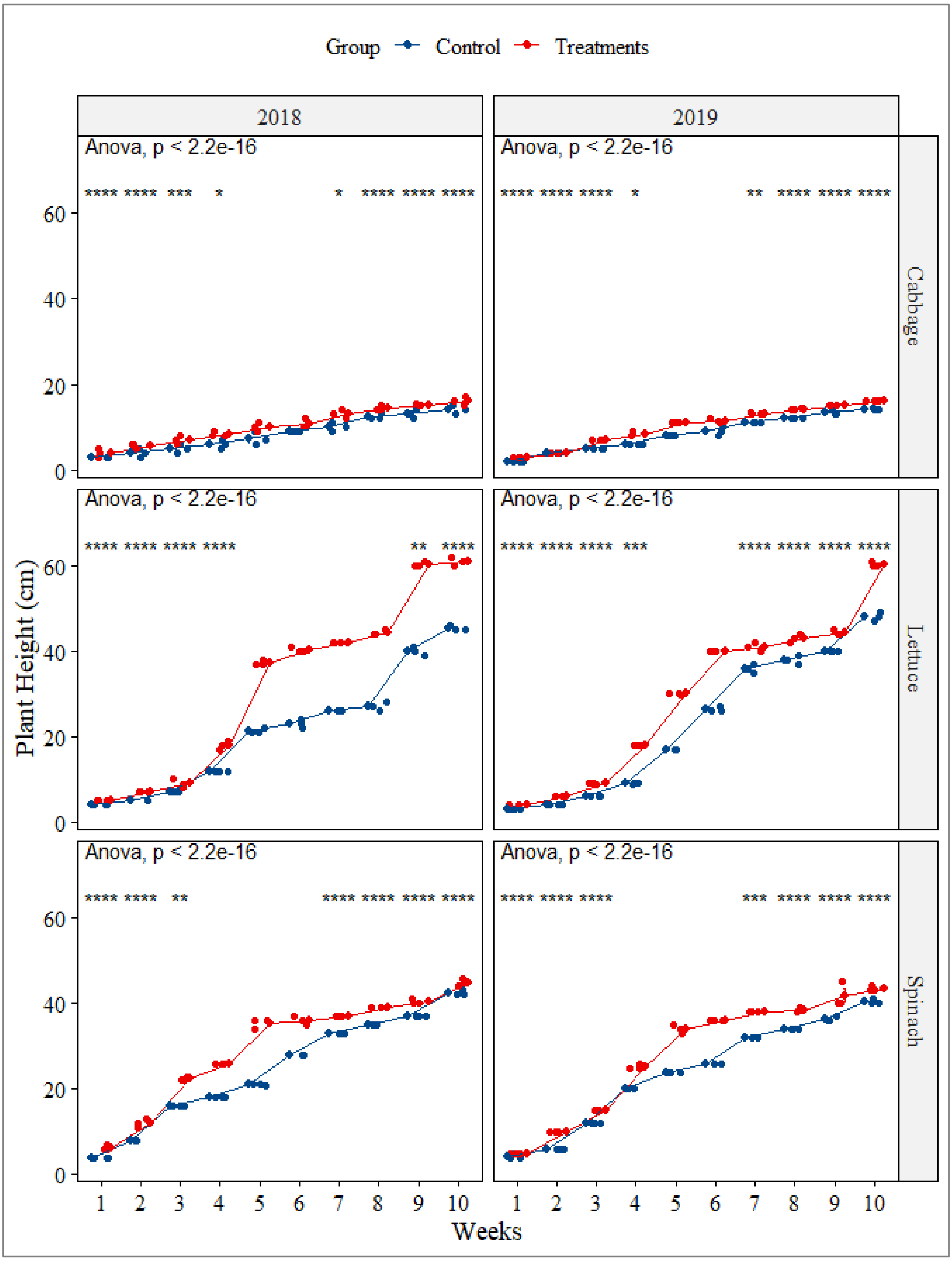

Fig. 5: Changes in plant height (cm) of vegetables irrigated with microcystins-contaminated water in 2018 and 2019. Treatments are significantly different from the control at $p < 0.05$.

The TDI of microcystin in vegetables irrigated with water in 2019 ranged from 1.311 to 8.210 µg Kg^{-1} for adults as shown in Table 1. The highest and lowest values were found in lettuce samples collected at harvest weeks two and one, respectively (Table 1). The estimated TDI values for children ranged from 0.786 to 4.926 µg Kg^{-1}, with the highest TDI observed in cabbage at week one and the lowest in lettuce at harvest week one as shown in Table 1.

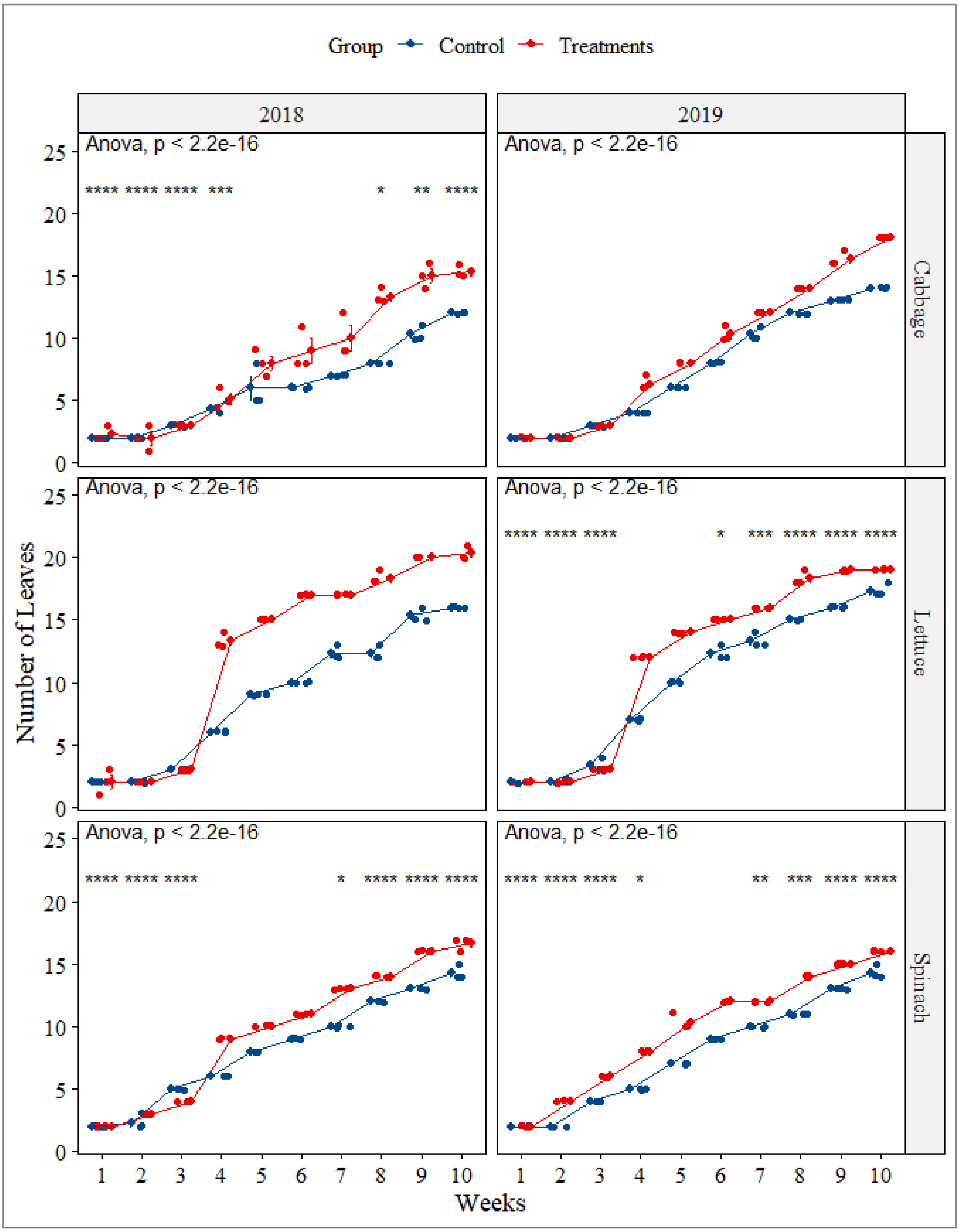

Fig. 6: Changes in the number of leaves of vegetables irrigated with microcystins-contaminated water in 2018 and 2019. Treatments are significantly different from the control at $p < 0.05$.

Morphological characteristics of the three vegetables

From the third week of irrigation, cabbage and lettuce plants irrigated with microcystins-contaminated water supported larger leaf areas than controls, regardless of the year of cultivation as shown in Fig. 4. Changes in the leaf area of spinach varied between the treatment and control. However, there was a common result for spinach. From weeks nine to ten, the controls had higher leaf areas than the plants exposed to microcystins-contaminated water in 2018 and 2019. For most of the remainder of the exposure period, the plants irrigated with contaminated water had a larger leaf area index than controls. The ANOVA results confirmed that there is significant differences ($p < 0.05$) in leaf area between plants irrigated with microcystin-contaminated water and controls in both years of the investigation.

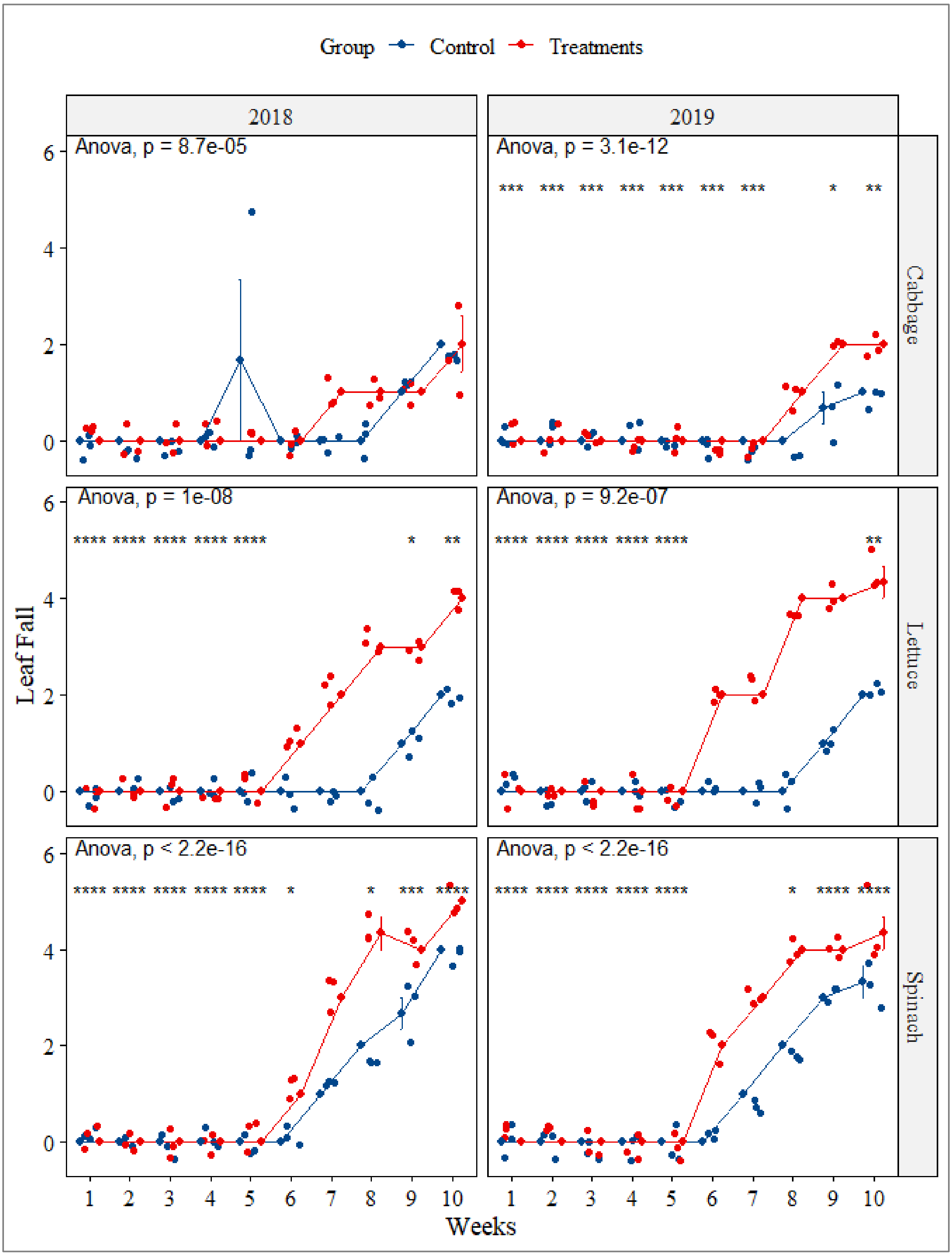

Fig. 7: Number of leaf-fall of vegetables irrigated with microcystins-contaminated water in 2018 and 2019. Treatments are significantly different from the control at $p < 0.05$.

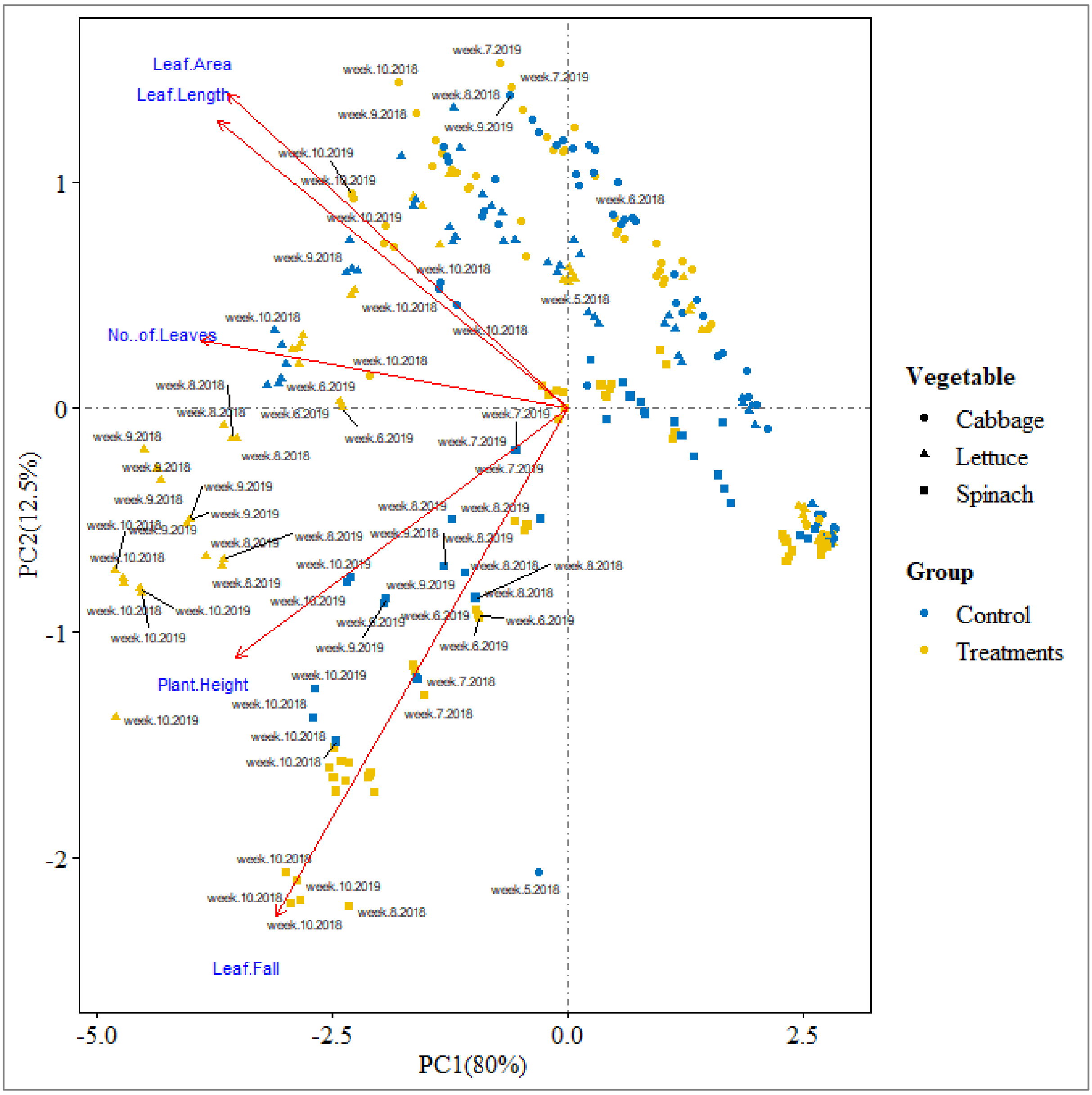

Fig. 8: PCA bi-plot showing the interrelationship between the vegetable growth index (leaf area, leaf length, number of leaves, plant height, and leaf fall) of three vegetables irrigated using contaminated water from the river Kaduna. Parameters grouped on the same orthogonal axis are positively correlated, while those on the opposite axis have a negative correlation.

Table 1: Estimation of total daily intake (TDI) of microcystins in vegetables for adults and children

Vegetable/Sampling time	2018		2019	
	Adult (μg Kg^{-1})	Child (μg Kg^{-1})	Adult (μg Kg^{-1})	Child (μg Kg^{-1})
Cabbage				
Week 1	6.397	3.838	8.21	4.926
Week 2	7.505	4.503	3.685	2.211
Week 3	4.729	2.837	4.759	2.856
Lettuce				
Week 1	7.130	4.278	1.311	0.786
Week 2	7.800	1.950	7.800	4.680
Week 3	4.106	2.464	4.106	2.464
Spinach				
Week 1	0.761	0.457	1.830	1.098
Week 2	2.904	1.742	2.04	1.248
Week 3	1.870	1.122	3.123	1.874

Plant height and the number of leaves per plant for the vegetables followed a similar trend to the leaf area data (Figs. 5 and 6). However, there was a minor difference. Specifically, for most of the investigation, from weeks three to ten, all the plants irrigated with contaminated water had significantly ($p < 0.05$) taller plants than the controls.

The first three weeks of cultivation showed that the controls had more leaves per cabbage and lettuce plant than in the 2018 and 2019 treatments. However, this trend was observed only in spinach in 2018, as in 2019, the treatment-exposed plants had a higher number of leaves than the controls from the second week onward.

The leaf fall data varied substantially between the treatment and control and among the different investigated vegetables (Fig. 7). In 2018, the control cabbage stands had higher leaf fall at weeks five and ten than the treatments. The changes in 2019 were more pronounced; from week one to seven, there were no differences in leaf fall, whereas from week eight to ten, the number of leaves falling was higher in the treated plants than in the in the control. For lettuce and spinach, the number of leaf falls was the same for the control and treated plants from week one to five. However, the number of leaves falling from week six to ten was higher in the treatments than in the control.

Principal Component Analysis (PCA) showing the interrelationship between the vegetable growth index

The spinach sample from week 7 to 10 for both treated and the control in the years 2018 and 2019 had a strong positive correlation with leaf fall and plant height, and a weak positive correlation with leaf area, leaf length, and the number of leaves (Fig. 8). The lettuce samples collected from week 8 to 10 for both control and treated in the years 2018 and 2019 had a strong positive correlation with the number of leaves, leaf length and leaf area, while having a weak positive correlation with plant height and leaf fall. The cabbage samples for weeks 6 to 10 for both control and treatment in 2018 and 2019 showed a strong positive correlation with each other and with the number of leaves, leaf length, and leaf area, while showing a weak positive correlation with plant height and leaf fall. The first two PCA components of the irrigated vegetable data for the two years accounted for 92.5% of the total variance, with PC1 accounting for 80% and PC2 for 12.5% (Fig. 8).

DISCUSSION

The contamination of vegetables with microcystins through irrigation with water from River Kaduna raises important concerns about human health risks. Microcystins are potent toxins produced by cyanobacteria in water, and their bioaccumulation in vegetables poses a potential hazard to consumers. Understanding the extent of microcystin contamination in these commonly consumed vegetables is crucial for assessing the magnitude of associated health challenges. Higher concentrations of microcystins were detected in cabbage (*Brassica oleraceae* L) and lettuce (*Lactuca sativa* L) than in spinach (*Amaranthus hybridus*). This may be due to the larger area of cabbage and lettuce leaves than that of spinach. The leaf size influences the rate of microcystin uptake and bioaccumulation in irrigated vegetables and plants with larger leaves tend to retain a higher concentration of microcystins than those with smaller leaves (Campos *et al*. 2021; Cao *et al*. 2023; Cordeiro-Araújo *et al*. 2016) Also, the leaves of irrigated vegetables, probably because they are the first to come in contact with contaminated water, tend to have higher microcystins than other plant parts. This is because the method of irrigation for the vegetables was surface irrigation, in which water frequently comes into contact with the plant leaves. Additionally, these variations depend on the concentrations of microcystins in the environment, the timing of exposure, the type and size of the vegetables' leaves, and their physiological and biochemical responses to microcystin exposure (Bakr *et al*. 2022; Wijewickrama and Manage 2019; Xiang *et al*. 2019).

Estimation of total daily intake (TDI) of microcystins in vegetables for adults and children

The estimation of the TDI of microcystins in vegetables for adults and children has significant implications for general public health, given the well-known fact that these metabolites can adversely affect the normal functioning of various organs in the human body, such as the liver, kidney, and skin (Igwaran *et al*. 2024) The concentration of microcystins bioaccumulated in vegetables is directly proportional to the TDI by humans (reference). Therefore, higher microcystins concentrations in the vegetables in this study suggested higher TDI values for both adults and children, surpassing the FAO/WHO recommended daily intake limit of 0.04 µg/kg body weight per day, thereby posing risks to human health (USEPA 2015; WHO 1998) This study directly demonstrates the risks associated with using water from River Kaduna to irrigate vegetables. Moreover, these risks are even higher when exposure is consistent and frequent over an extended period (Melaram *et al.* 2022) Since microcystins concentration in irrigation water are positively correlated with those in irrigated vegetables, chronic toxic effects from exposure to microcystins through food are possible, especially when vegetables such as cabbage, lettuce, and spinach are consumed daily as sources of vitamins and mineral salts in this region.

The results of the present study highlight the potential for chronic exposure when the consumption of contaminated vegetables is not controlled due to a lack of an effective monitoring program. This chronic human exposure issue is further compounded because these vegetables are consumed both raw and cooked, and cooking increases the risk of exposure to microcystins,s heat tends to enhance the extraction of the cyanotoxin (Drobac Backović and Tokodi 2024). Specifically, MC-LR is known to be resistant to heat and is stable at temperatures as high as 300 °C.

The findings from the present study emphasize the need to develop a robust management program and to implement policies to mitigate the risks of using untreated water for irrigation. By implementing adequate monitoring and management practices, the risks identified in this study can be minimized, ensuring that the water source used to irrigate vegetables is safe for animal and human consumption.

Changes in morphological parameters of the irrigated vegetables

Despite the higher yield and improved growth performance observed in plants grown with river-contaminated water, specific adverse effects were evident, including delayed or reduced germination, leaf lesions, and leaf deformations compared with the control group. This finding is consistent with the studies that reported that microcystins have negative impacts on the fresh weight, leaf shape, leaf colour, root and shoot length of exposed seedlings (Bakr *et al.* 2022; Bittencourt-Oliveira *et al.* 2016; Cordeiro-Araújo *et al.* 2016; Wijewickrama and Manage 2019) Additionally, microcystins have been associated with several deleterious effects, such as decreased growth, tissue necrosis, inhibition of photosynthesis, and metabolic changes in plants, leading to impairment of crop productivity and economic losses (Drobac Backović and Tokodi 2024; Lad *et al.* 2022; Melaram *et al.* 2022).

The severity of these effects varies considerably and strongly depends on the concentrations of microcystins to which the plants are exposed. In addition to impairing plant development and productivity, using microcystin-contaminated irrigation water may also contaminate food produced by these crops and pose a risk of human exposure to the toxins. Therefore, it is crucial to implement measures to monitor and manage the use of contaminated water for irrigation to safeguard crop productivity and human health.

The increase in leaf fall recorded in the vegetables beyond week five, especially in spinach, may be attributed to the effects of microcystins in the River Kaduna water used to irrigate the vegetables. This also highlights the differences in the susceptibility of leaves from different leafy vegetables to fall when irrigated with microcystin-contaminated water. Using contaminated irrigation water worldwide has significant economic implications, leading to reduced germination rates and alterations in crop quality and productiviy (Melaram et al., 2022). Thus, the harmful effects of dissolved microcystins on agricultural plants pose ecological, ecophysiological, socio-economic, and sanitary risks that can ultimately lead to leaf fall or plant stress. It is plausible that these adverse effects are causing plant stress, leading to the observed leaf fall in week five of our study.

CONCLUSION

Due to the crucial role that cabbage, lettuce, and spinach play in the human diet, any contamination poses a significant threat to human health. Although there were variations in microcystins concentrations among the different vegetable species, factors such as sampling and harvest times, as well as physiological and morphological adaptations, influence their presence in specific plant species at certain times. This study demonstrates that all three vegetables irrigated with water from the River Kaduna in 2018 and 2019 exceeded the FAO/WHO permissible limits. The findings of this study underscore the necessity for legislation to safeguard irrigation water from sources with high microcystin concentrations. As a result, robust protective measures are imperative to ensure the safety of irrigation water and, subsequently, protect human health from the harmful effects of microcystin contamination.

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