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Ivermectin, an antiparasitic medication, affects the pigment level of the hydrophyte *Lemna minor*

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ABSTRACT
Ivermectin (IVM) is a potent antiparasitic drug commonly used to treat parasitic diseases in plants and animals. It is widely used in aquaculture to control parasites, but it can also find its way into water bodies, where it persists and poses a risk to non-target organisms such as hydrophytes. Exposure to elevated levels of IVM can adversely affect the physiology of hydrophytes, affecting the health and balance of entire aquatic ecosystems.In this study, we investigated the effects of IVM at varying concentrations (1, 10, 100, 1000, and 10000 µg L^{-1}) on the physiology of the hydrophyte Lemna minor. We found leaf size remained normal at 1 µgL^{-1} (0.14 cm^2) but decreased significantly to 0.13 cm^2 at 10 µgL^{-1} and 0.11 cm^2 at 10000 µgL^{-1}. Chlorophyll a levels were most affected at 100 µg L^{-1}, where they decreased from a control value of 1.2 mg g^{-1} to 0.1 mg g^{-1}, followed by 10 µg L^{-1} (0.25 mg g^{-1}) and 1 µg L^{-1} (0.6 mg g^{-1}). Chlorophyll b levels were most affected at 1000 µgL-1 (0.05 mg g^{-1}), followed by 1 µgL^{-1} (0.7 mg g^{-1}) and 1000 µg L^{-1} (1.2 mg g^{-1}). Total chlorophyll levels were most affected at 10000 µgL-1 (0.5 mg g^{-1}), followed by 1 µg L^{-1} and 10 µg L^{-1} (0.7 mg g-1), and were highest at 1000 µg L^{-1}(1.2mg g^{-1}).Our results show that IVM can threaten hydrophytes and the entire aquatic ecosystem, even at low concentrations.

KEYWORDS:*Pesticides, aquatic macrophytes, pollution, chlorophyll, aquatic ecosystem*

1. Introduction

In the late 1970s, Ivermectin was discovered as a dihydro derivative of avermectin, which was isolated solely from a microorganism at the Kitasato Institute in Tokyo, Japan (Omura & Crump, 2014). Initially introduced as a veterinary drug toxic to a wide range of internal and external parasites of commercial livestock and companion animals, it has also been used to treat several other human diseases. However, due to its adaptability, usefulness, and off-label availability, it is sometimes used illegally to treat fish lice in the aquaculture industry (Tisato et al., 2021). As a result of its extensive use, Ivermectin ends up in aquatic ecosystems, where non-target organisms, such as aquatic macrophytes or hydrophytes, are exposed and affected to varying degrees.

Previous researchers have primarily focused on developing standardized test methodologies (mesocosm) between water and sediment (Sanderson et al., 2007), investigating the environmental fate of Ivermectin in an aerobic sediment/water system (Prasse et al., 2009), evaluating the fate and effects of Ivermectin on soil invertebrates (Förster et al., 2011) and in terrestrial/aquatic environments (Förster et

al., 2011; Rath et al., 2016). However, the impact of the antiparasitic drug on hydrophytes is not well-known. Hydrophytes are a diverse assemblage of macroscopic plants whose life cycle occurs entirely or periodically in the aquatic environment (Lesiv et al., 2020). They play critical roles in the primary productivity of water bodies, which can be measured by the level of pigments in them.

Pigments aid hydrophytes in photosynthesis, absorbing and transferring light into their photosynthetic machinery. The primary pigments, chlorophyll a and b are within the blue and red spectrum, respectively (Raven et al., 2019). Hydrophytes also contain other pigments, such as carotenoids and phycobilins, that enable them to absorb light from a broader range of the spectrum, allowing them to photosynthesize in conditions of low light penetration, leading to an increase in photosynthetic activity, growth, and reproduction in the aquatic environment (Kolber & Falkowski, 1993).

In addition to aiding in photosynthesis, pigments shield hydrophytes from UV ray damage and excessive light. By absorbing damaging light wavelengths, such as UV rays, pigments protect the plant's photosynthetic apparatus, which is crucial in aquatic habitats due to the vast variations in light quality and intensity (Raven et al., 2017). *Lemna minor* (duckweed) is one of the smallest of all angiosperms, or flowering plants, in the plant kingdom (Correll & Correll, 1972). *Lemna* is an ideal candidate for ecotoxicity studies due to its role in the environment as a primary producer, its small size, rapid growth, and its establishment of clonal lineages (Cedergreen et al., 2009). Therefore, in light of the scarcity of adequate information on the effect of Ivermectin on hydrophytes, the objective of the present study is to evaluate the impact of Ivermectin on the growth and pigment content of *L. minor*.

2. Materials and Methods

2.1*Lemna minor* culture.

One hundred eighty fresh samples of *L. minor*were obtained from the Department of Biology, Ahmadu Bello University, Zaria, Kaduna State.*L. minor* was cultured in a plastic container containing 500ml of 20×-AAP medium (OECD 2002) and maintained under laboratory conditions of 100 µmol m^{-2} s^{-1}photosynthetically active radiation. Beforeuse in the maintenance of stock and experimental cultures, the culture medium was adjusted to 7.5±0.1 and sterilized by autoclaving at 121°C for 15 minutes. The growth medium was replaced every week.

2.3 *Lemna minor* assay.

2. 3. 1.Stock solution for the preparation of *L. minor.*

100mg of *L. minor* was weighed and transferred into a 100mlmeasuring cylinder, and 50ml of acetone was added to the above flask, dissolved, and sonicated for 15 min. The volume was made up to 100ml with 80% acetone.

2.4 Exposure experiment.

To evaluate the effect of Ivermectin on the pigment of *L. minor,*180 fresh fronds of *L.minor*were selected as experimental material. The assay was conducted in a controlled environment with the same illumination as the stock culture. Test vessels were transparent plastic containers having 48 wells. Each well contained three*L. minor* fronds; six wells constituted one treatment. The plants were exposed to 1, 10, 100, 1000, and 10000µgL^{-1}ivermectin. The controls were maintained in AAP medium containing 0.04 % acetone and devoid of ivermectin. Cultures were incubated for six days following ISO guidelines (ISO/WD/20079, 2001).

Following 72h of exposure to various concentrations of ivermectin, *L. minor*was harvested to determine changes in the surface area using Adobe Photoshop Pixel Analyzer. Photographs were taken alongside a 1×1cm white plastic square, and the frond surface area was determined to

monitor growth.

2.5 Pigment quantification.

Chlorophyll content extraction was done using acetone. Three randomly selected fronds from the different treatment conditions wereweighed and macerated in 3 ml 100% acetone. The container was wrapped with foil paper and allowed to extract at -20^{0}C for 24h. After refrigeration, it was centrifuged for 4 minutes, the supernatantswere decanted into a cuvette,and absorbance readings were taken at 470, 653, and 666 nm with a UV-VIS Spectrophotometer (B. Bran Scientific and Instrument Company, England). Pigment concentrations were determined using the equation provided by Ritchie *et al.* (2006).

2.6**Statistical analysis**

The acute toxicity of effluent was assessed at different concentrations using the EC50, which was calculated using the USEPA probit analysis software version 2.0. A one-way analysis of variance (ANOVA) was conducted to determine the significant differences among the treatments. For treatments that showed significant differences, Tukey's HSD post-hoc test was used to separate means. The experiment was performed with a significance level of $p < 0.05$.

3. **Results**

The leaf area of *L. minor* hydrophyte shows no significant differencebetween the control and the 1 µgL^{-1}ivermectin treatment. In comparison, there was a gradual decrease in the leaf area from 10 µgL^{-1} and 100 µgL^{-1} at 0.13 cm^2 to 10000 µgL^{-1} at 0.12cm^2(Fig. 1).The 1000 µgL^{-1}treatment resulted in the highest decrease in leaf area with 0.11cm^2. The changes in leaf area as a function of ivermectin exposure were significant ($p< 0.05$).

The chlorophyll content of *L.minor* showed varying results as the effect of ivermectin on chlorophyllawas higher at 1 µgL^{-1} at 0.6mg g^{-1}, followed by 1000 µgL^{-1}at 0.4mgg^{-1}, 10 µgL^{-1}, 10000 µgL^{-1} and 100 µgL^{-1} at 0.4, 0.25, 0.2, and 0.1 mg g^{-1}, respectively (Fig. 2). Variations in chlorophyll a content of the macrophyte were significant ($p<0.05$).The concentrations of chlorophyll bwere 0.6, 0.7, and 0.5 mg g^{-1} in cultures exposed to 10,1,and 10000 µgL^{-1}ivermectin (Fig.3).Total chlorophyll results were similar to those of chlorophyll b because the highest ivermectin concentration led to the lowest total chlorophyll content (Fig.4).

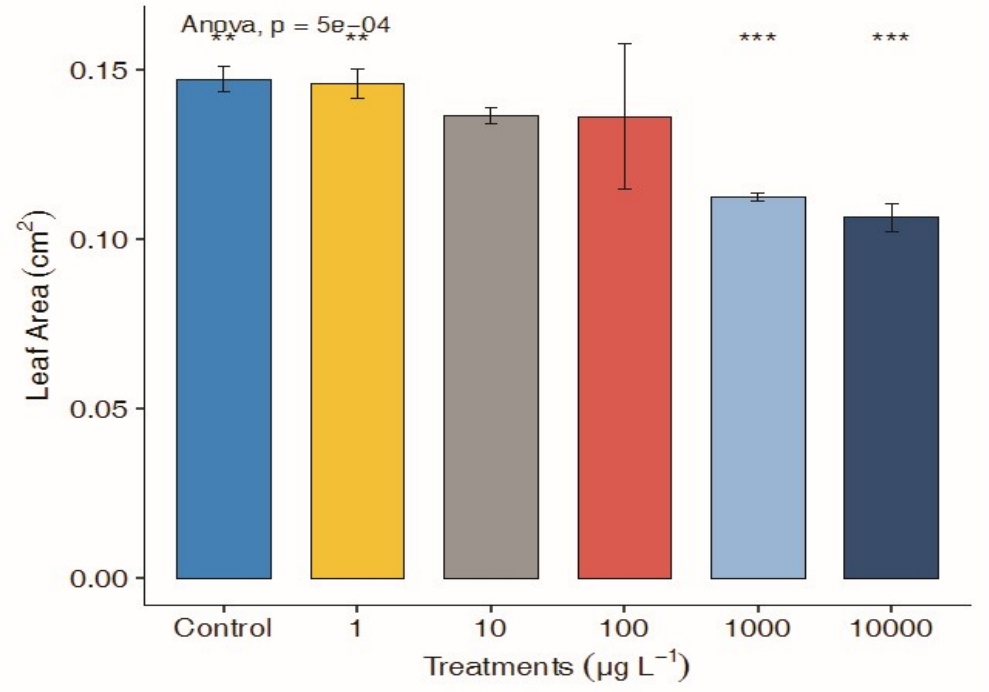

Fig. 1: Changes in leaf Area (cm^2)at different concentrations of ivermectin (IVM).Bars with asterisks (** $p < 0.05$, *** $p<0.01$) are significantly different compared to the control (0 gL^{-1}). Bars with the same * are not significantly different at $p < 0.05$. Error bars are standard deviation for $n = 3$

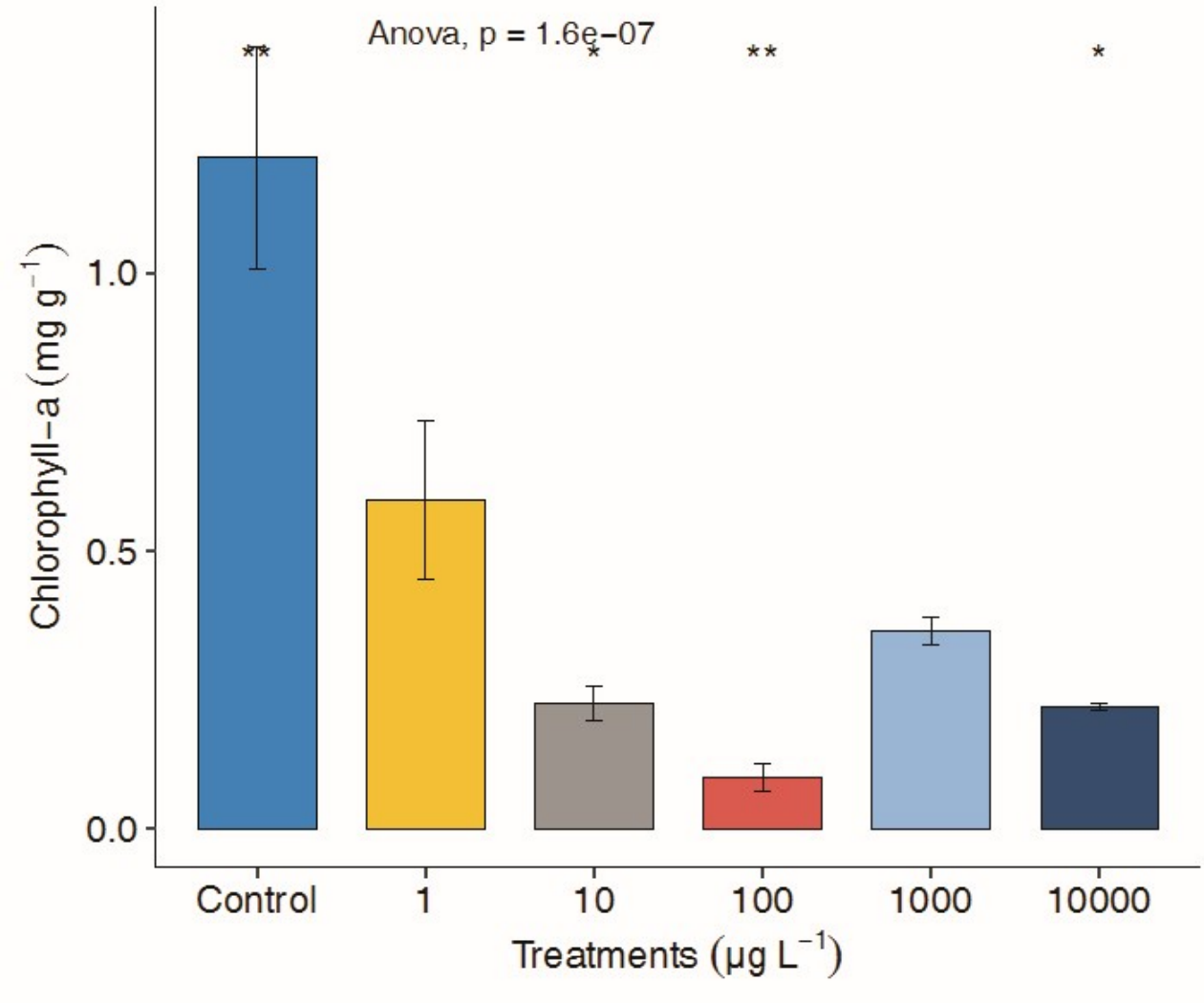

Fig. 2: Chlorophyll a at different concentrations of ivermectin (IVM). Bars with asterisks (** $p < 0.05$ *** $p<0.01$) are significantly different compared to the control (0 gL^{-1}). Bars with the same * are not significantly different at $p < 0.05$. Error bars are standard deviation for $n = 3$

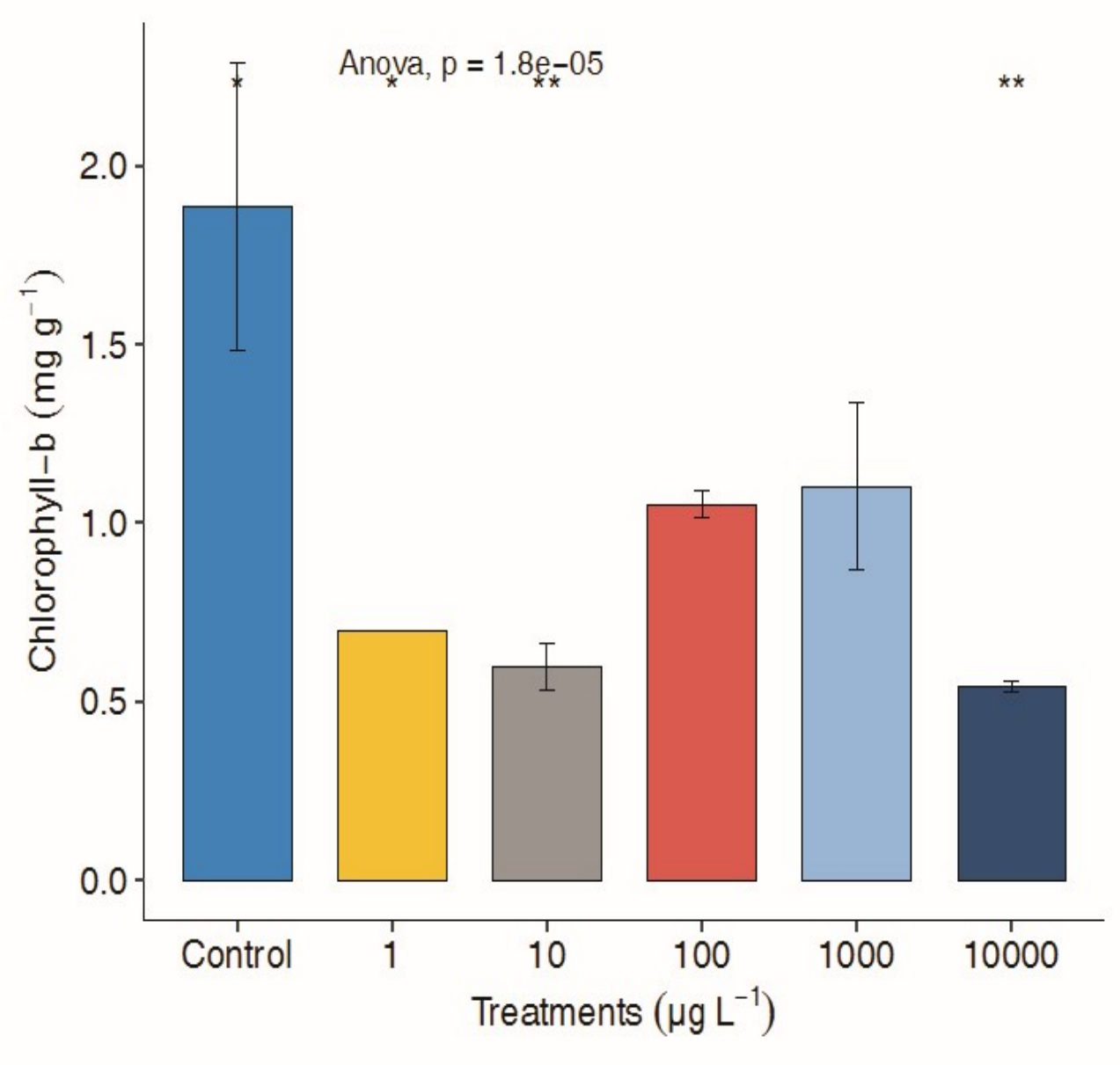

Fig. 3: Chlorophyll b at different concentrations of ivermectin (IVM). Bars with asterisks (** $p < 0.05$ *** $p<0.01$) are significantly different compared to the control (0 gL^{-1}). Bars with the same * are not significantly different at $p < 0.05$. Error bars are standard deviation for $n = 3$

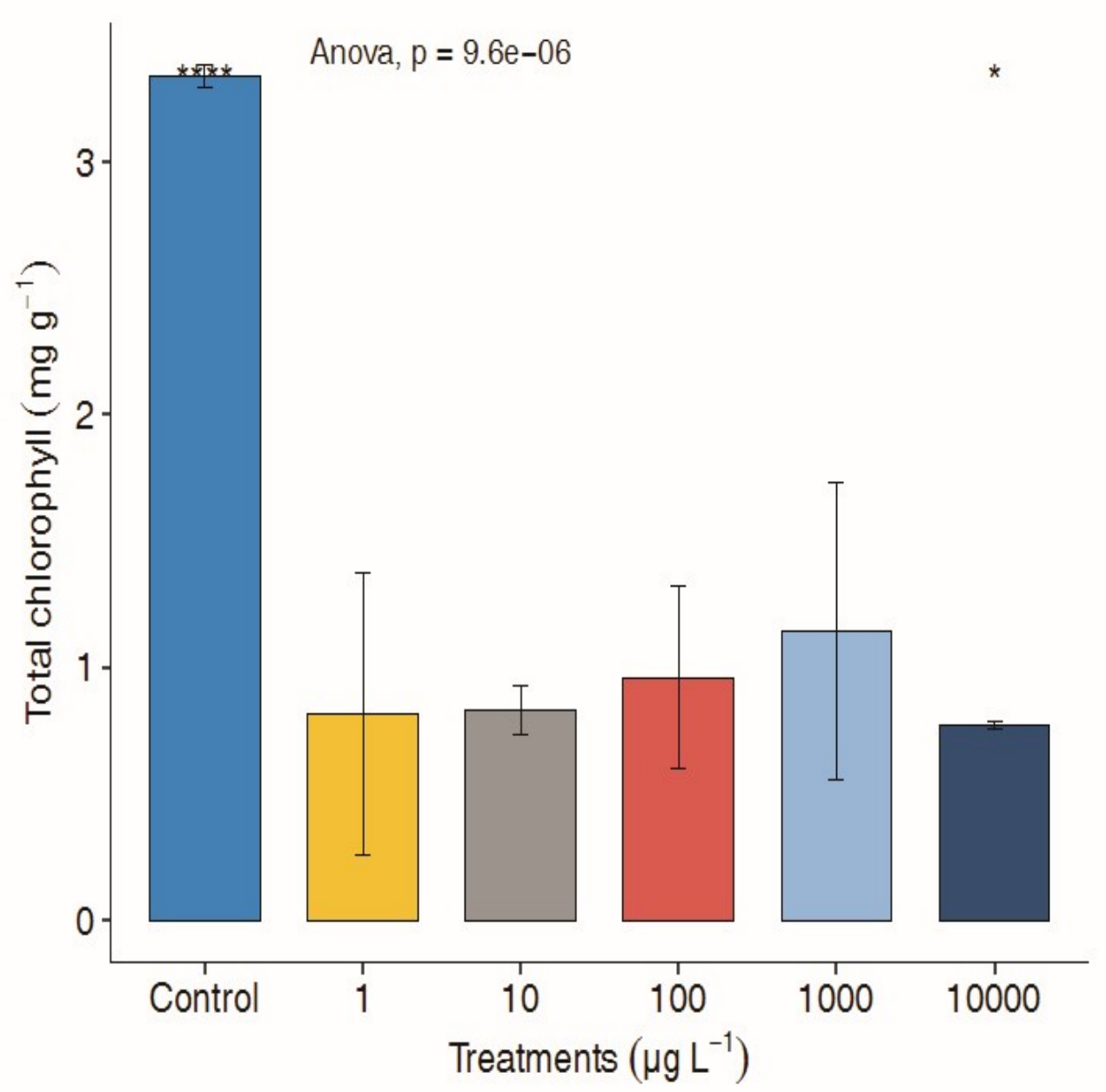

Fig. 4: Total chlorophyllat different concentrations of ivermectin (IVM). Bars with asterisks (** $p < 0.05$ *** $p<0.01$) are significantly different compared to the control (0 gL^{-1}). Bars with the same * are not significantly different at $p < 0.05$. Error bars are standard deviation for $n = 3$

4. Discussion

Aquaculture chemicals can harm non-target organisms, altering the population structure of surrounding ecosystems (Burridge et al., 2010). The results of this study (Cardona et al., 2018; Larkum et al., 2012) suggest that these chemicals pose a risk to hydrophytes, such as *L. minor*, and other macrophytes found in aquaculture facilities and nearby aquatic ecosystems.

As the concentration of ivermectin increased, the leaf area of L. minor decreased, likely due to the chemical's impact on the plant's physiology. Ivermectin may disrupt the cell division and differentiation process, leading to a decrease in size. It can also accumulate reactive oxygen species, disrupting cell function and potentially leading to cell death. However, the reduction in leaf size may also indicate the plant's phytoremediation abilities, as L. minor has been shown to remediate various organic and inorganic compounds (Abraham et al., 2019).

The chlorophyll content of L. minor also decreased significantly with exposure to ivermectin, with reductions in chlorophyll a, b, and total chlorophyll ($p = 1.6e-07$, $p = 1.8e-05$, $p = 9.6e-06$, respectively). This reduction in chlorophyll content is likely due to the plant's sensitivity to pollution exposure (Fekete-Kertész et al., 2015) and the decrease in leaf area, which reduces the plant's photosynthetic area. This reduction in the photosynthetic area ultimately hinders the plant's ability to photosynthesize and produce energy and nutrients, hindering the synthesis of biomolecules and the creation of energy required for biological functions (Cardona et al., 2018; Larkum et al., 2012). The decrease in chlorophyll levels was not concentration-based. It may indicate that the aquatic plant's photosynthetic system is being inhibited by ivermectin, resulting in a reduction in carbon dioxide fixation. This reduction in sugar levels and chlorophyll content provides an indirect but accurate estimate of the plant's nutritional status (Paul et al., 2017). While the decrease in chlorophyll a was not as drastic as that of chlorophyll b or total chlorophyll, there was a significant reduction in all chlorophyll content compared to the control.

5. Conclusion

It is evident that Ivermectin finds its way into water bodies through feces and aquaculture pool overflow, and its presence can have detrimental effects on the hydrophyte *L. minor*. Specifically, the research demonstrates that Ivermectin negatively impacts leaf size and chlorophyll pigment levels in *L. minor*, which can ultimately result in the death of the hydrophytes and have broader impacts on the aquatic ecosystem. While information on the effects of Ivermectin on *L. minor* pigment levels was previously scarce, this study highlights the need for further research to better understand the overall impact of Ivermectin on *L. minor*.

References

Burridge, L., Weis, J. S., Cabello, F., Pizarro, J., & Bostick, K. (2010). Chemical use in salmon aquaculture: A review of current practices and possible environmental effects. *Aquaculture*, *306*(1–4), 7–23. https://doi.org/10.1016/j.aquaculture.2010.05.020

Campbell, W. C., Fisher, M. H., Stapley, E. O., Albers-Schönberg, G., & Jacob, T. A. (1983). Ivermectin: A potent new antiparasitic agent. *Science*, *221*(4613), 823–828. https://doi.org/10.1126/science.6308762

Cardona, T., Shao, S., & Nixon, P. J. (2018). Enhancing photosynthesis in plants: The light reactions. *Essays in Biochemistry*, *62*(1), 85–94. https://doi.org/10.1042/EBC20170015

Crump, A., & Omura, S. (2011). Ivermectin, " Wonder drug" from Japan: The human use perspective. *Proceedings of the Japan Academy, Series B*, *87*(2), 13–28. https://doi.org/10.2183/pjab.87.13

Halling-Sørensen, B., Nors Nielsen, S., Lanzky, P. F., Ingerslev, F., Holten Lützhøft, H. C., & Jørgensen, S. E. (1998). Occurrence, fate and effects of pharmaceutical substances in the environment- A review. *Chemosphere36*(2), 357–393. https://doi.org/10.1016/S0045-6535(97)00354-8

Kirk, J. T. O. (1994). *Light and photosynthesis in aquatic ecosystems* (2nd ed). Cambridge University Press.

Stephen, C. (2021). Human poisoning in South Africa – the knowledge gap. *Southern African Journal of Critical Care*, *37*(1), 4. https://doi.org/10.7196/SAJCC.2021.v37i1.493

Wang, D., Han, B., Li, S., Du, X., Cao, Y., & Lu, T. (2020). Assessment of the fate and effect of ivermectin in a simulated aquaculture ecosystem. *Aquaculture Research*, *51*(2), 535–541. https://doi.org/10.1111/are.14398