EFFECT OF HEAVY METALS AND NUTRIENTS ON *LEMNA MINOR* AND *SALVINIA NATANS*

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Abstract

A research investigation explored the impact of heavy metals and nutrients on the viability and chlorosis of *Lemna minor* and *Salvinia natans*. Fresh samples of *Lemna minor* and *Salvinia natans* were gathered from different regions of Dal Lake, Wular Lake, and Nigeen Lake. Various concentrations of heavy metals (Pb, Cd, Cu, Zn, Hg) ranging from higher to lower levels (5.0, 3.0, 2.8, 2.6, 2.4, 2.2, 2.0, 1.8, 1.6, 1.4, 1.2, 1.0, 0.8, 0.6, 0.4 mg/l per liters of water) were introduced into 15-liter containers, each representing eutrophic, mesotrophic, and oligotrophic conditions, with one control group. These setups were observed for 15 days to assess the effects. The findings consistently demonstrated a decline in survival rates with prolonged exposure and increased heavy metal concentrations across all trophic conditions for both plant species. *Lemna minor* displayed the highest survival rates at 1.0 ppm of heavy metals, while *Salvinia natans* exhibited greater tolerance with maximum survival at 0.4 ppm. Conversely, chlorosis levels rose with extended exposure, indicating heightened stress on the plants. The study highlights the susceptibility of aquatic plants to heavy metal pollution, emphasizing the necessity for monitoring and mitigation strategies to safeguard aquatic ecosystems.

Key Words: *Lemna minor* and *Salvinia natans*, Dal Lake, Wular Lake, and Nigeen Lake.
Heavy metals, Trophic condtions, Survival Rate, Chlorosis

Introduction

Aquatic plants play a critical role in maintaining the equilibrium of aquatic ecosystems, offering habitat, oxygenation, nutrient cycling, and erosion control. However, human activities, notably the release of heavy metals into water bodies, pose a growing threat to these essential organisms. Heavy metals like cadmium, lead, copper, and zinc, known for their persistence and harmful effects on aquatic life, accumulate in water through industrial, agricultural, and urban runoff, endangering aquatic flora and fauna. Among these, *Lemna minor* and *Salvinia natans* are significant due to their wide distribution and ecological importance. These floating plants, commonly found in freshwater habitats, efficiently absorb nutrients and pollutants.

Understanding how *Lemna minor* and *Salvinia natans* respond to heavy metal pollution is crucial for assessing aquatic ecosystem health and devising effective conservation strategies. This study explores the survival and chlorosis rates of these plants under different trophic conditions and varying heavy metal concentrations. By examining their reactions to pollution across diverse environments, we aim to understand their tolerance levels and susceptibility. This research contributes to our comprehension of the ecological impacts of heavy metal pollution in aquatic ecosystems and guides management efforts to safeguard these critical habitats.

Review of Literature

Research on the survival rates and chlorosis dynamics of *Lemna minor* and *Salvinia natans* has been extensively documented, offering crucial insights into how these aquatic plants respond to environmental stressors. Lee, Kim, and Park (2015) investigated the acute toxicity of copper, zinc, and mercury on *Lemna minor*, their finding revealed concentration-dependent declines in survival rates with significant variations across different concentrations. Cui et al. (2018) observed a negative correlation between cadmium concentration and *Lemna minor* survival. Mahamuni and Shinde (2014) studied nickel's phytotoxic effects on *Lemna minor*, reporting reductions in both survival rates and chlorophyll content. Haq and Hussain (2012) revealed a concentration-dependent increase in chlorosis levels in *Lemna minor* exposed to aluminum. Li et al. (2019) and Wang et al. (2017) highlighted chlorosis responses in *Lemna minor* and *Salvinia natans* to zinc and cadmium contamination, respectively. Shrivastava et al. (2017) observed chlorosis induction in *Salvinia natans* exposed to heavy metals. Gopal et al. (2018) explored combined heavy metal effects on *Salvinia natans*, noting concentration-dependent declines in survival rates and increased chlorosis levels. Jia and Liang (2018) stressed the need for further research on *Lemna minor* and *Salvinia natans*' responses to heavy metal pollution and their role in phytoremediation. Overall, these studies offer valuable insights into the ecological implications of heavy metal pollution on *Lemna minor* and *Salvinia natans* survival and chlorosis dynamics, emphasizing the importance of conserving these species and their habitats for ecosystem health.

Material and Methods

The identification of macrophytes collected from these water bodies was done with the help of available literature (Needham and Needham, 1966; Zutshi, 1975; Kak, 1989; Cook, 1996). From these lakes, selected macrophytes (in healthy condition) were collected and transferred to the laboratory in polythene bags. After collection, these macrophytes (Plate 1, 2and 3) were flapped and washed a number of times with tap in order to eliminate dirt and soil. For experimental purpose, plants of approximately same size and weight were selected.
The desired quantities of Zinc sulphate Heptahydrate ($ZnSo_4.7H_2o$ M.W= 287.54), Lead (II) Sulphate ($PbSo_4$ M.W= 303.26), Cadmium Chloride Monohydrate $CdCl_2.H_2o$ M.W=201.33), Mercuric Chloride ($HgCl_2$ M.W=271.50), Cupric Sulphate Pentahydrate (M.W= 249.68 $CuSo_4.5H_2o$), Potassium Chloride (Kcl M.W= 74.55), Potassium Nitrate (KNo_3 M.W=101.10), and Potassium Dihydrogen Orthophosphate (KH_2PO_4 M.W=136.09) salts were dissolved in distilled water separately to achieve the appropriate contamination levels of Cd, Pb, Hg, Cu, Zn- labeled as priority pollutants with different nutrient levels of potassium, nitrogen and phosphorous. Working metal and standard solutions with varied concentrations of each metal for survival rates from higher concentrations to lower concentrations (5.0, 3.0, 2.8, 2.6, 2.4, 2.2, 2.0, 1.8, 1.6, 1.4, 1.2, 1.0, 0.8,

0.6, 0.4, mg/l per liters of water) and three trophic conditions of Eutrophic (N= 2.5 ppm, P= 1.25 ppm, K= 2.50 ppm, Mesotrophic (N= 1.0 ppm, P= 0.125 ppm, K= 1.0 ppm, and Oligotrophic (N= 0.25 ppm, P= 0.025 ppm, K= 0.125 ppm) were formulated by diluting the corresponding stock solution (1000 mgL-1) using deionized water.

To fulfill the objectives of this current investigation, various concentrations of five metallic chemicals and three nutrient chemicals were employed to evaluate the survival rates and Chlorosis of two free-floating aquatic plants: *Lemna minor* and *Salvinia natans.*

Methods

The dose-response study on aquatic plants was experimental and entirely relied on the development of the research protocol. Several studies (Aziz and Wantanabe 2005); Shahate et al., 2011); Rifat et al., 2017); Akhtar et al., 2007); Raja et al., 2012)] were reviewed to formulate the protocol. Attempts were made to determine the nominal dosage rate by utilizing a range of concentrations, with trials primarily focusing on concentrations exceeding the nominal dosage.

Following the establishment of the protocol, the subsequent procedure was adopted to conduct the dose-response study. Fresh samples of *Lemna minor* and *Salvinia natans*, were collected from various areas of Dal Lake, Wular Lake, and Nigeen Lake. Different concentrations of the heavy metals collectively (Pb, Cd, Cu, Zn, Hg) from higher concentrations to lower concentrations (5.0, 3.0, 2.8, 2.6, 2.4, 2.2, 2.0, 1.8, 1.6, 1.4, 1.2, 1.0, 0.8, 0.6, 0.4, mg/l per liters of water) in different trophic conditions such as eutrophic, mesotrophic and oligotrophic conditions were taken in 15 litre tubs with one control each, and were allowed to stand for an exposure period of 2 weeks (15 days).

Results and Discussion

Survival rate of cultured Macrophytes in laboratory conditions:

In the present study the survival rates of the selected aquatic plants were worked out during the growing season of 2018-19. The survival rates were checked in different nutrient conditions representing different trophic levels. The results of the survival trials are discussed below.

As it is evident in (Fig. 1.1), the survival rate of *Lemna minor* declined notably with increasing exposure duration. The highest survival rate, 95%, was consistently observed at a concentration of 1.0 ppm of heavy metals throughout the study period. Conversely, the lowest survival rate of 0% was noted on the 9th, 10th, 11th, 13th, and 15th days when treated with 5.0, 3.0, 2.8, 2.6, 2.4 and 2.2 ppm of heavy metals respectively. The concentration of 2.0, 1.8, 1.6, 1.4 and 1.2 ppm of heavy metals resulted in 5%, 20%, 40%, 60% and 80% survival rate of *Lemna minor* respectively in eutrophic conditions.

As it is observed in (Fig. 1.2), *Lemna minor* exhibited a gradual decrease in survival rates across all concentrations of heavy metals in mesotrophic conditions over varying time intervals. The highest survival rate of 95% was observed at a concentration of 1.0 ppm on the 14th day. Conversely, the lowest survival rates, 0%, were recorded on the 8th, 9th, 11th, 12th, 14th, and 15th days when treated with 5.0, 2.8, 2.6, 2.4, 2.2, 2.0 ppm concentration of heavy metals. The concentration of 1.8, 1.6, 1.4 and 1.2 ppm of heavy metals accounted to 15%, 35%, 55% and 75% survival rate of *Lemna minor* respectively in mesotrophic conditions during the entire study period.

As revealed in (Fig. 1.3), a steady decrease in the survival rate of *Lemna minor* was observed across all concentrations of heavy metals in oligotrophic conditions over different treatment periods. The highest survival rate of 90% was noted at a concentration of 1.0 ppm on the 15th day. Conversely, the lowest survival rates of 0% were observed on the 8th, 9th, 11th, 12th, 14th, and 15th days when treated with 5.0, 2.8, 2.6, 2.4, 2.2, 2.0 ppm concentration of heavy metals.The concentration of 1.8, 1.6, 1.4 and 1.2 ppm of heavy metals accounted to 10%, 30%, 50% and 70% survival rate of *Lemna minor* respectively during the study period.

The above findings resembles with the findings of Lee, Kim, and Park (2015), Cui et al. (2018) and Mahamuni and Shinde (2014) investigated the acute toxicity heavy metals on *Lemna minor*, their finding revealed concentration-dependent declines in survival rates with significant variations across different concentrations, reporting reductions in both survival rates and chlorophyll content.

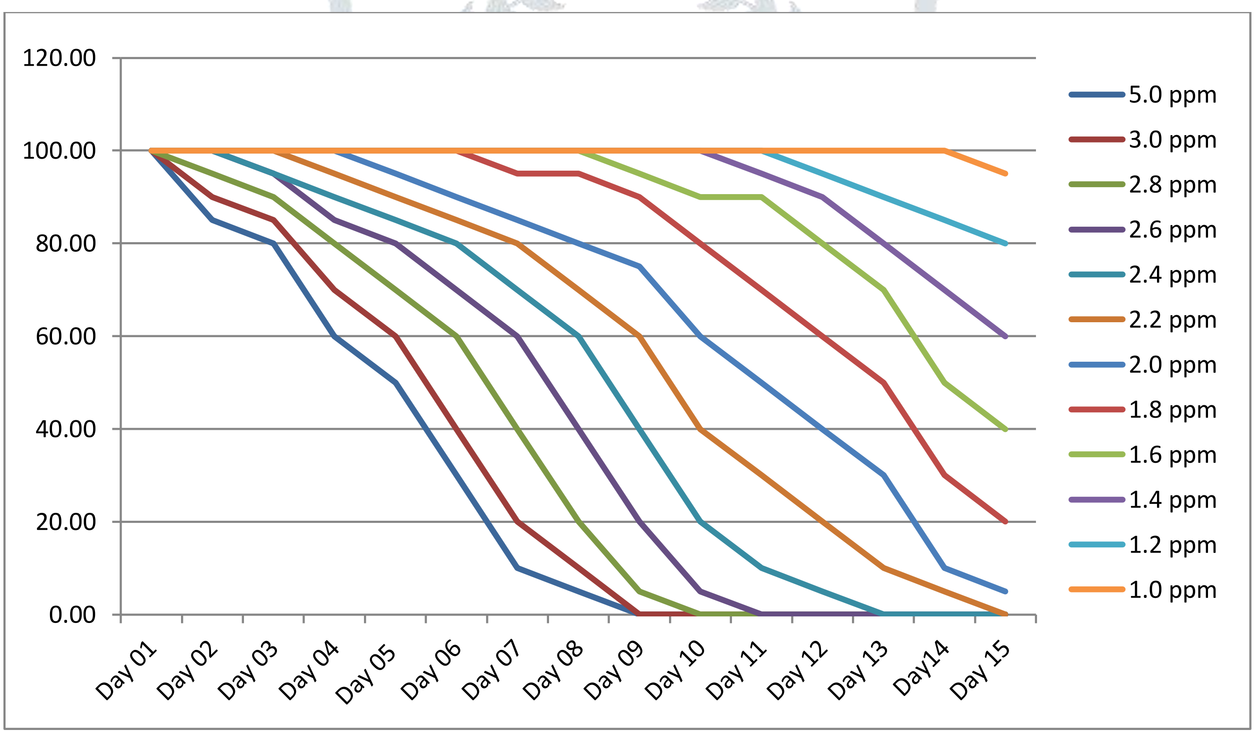

Figure 1.1 showing the effect of heavy metals in eutrophic conditions on the survival rate of *Lemna minor* in different concentrations.

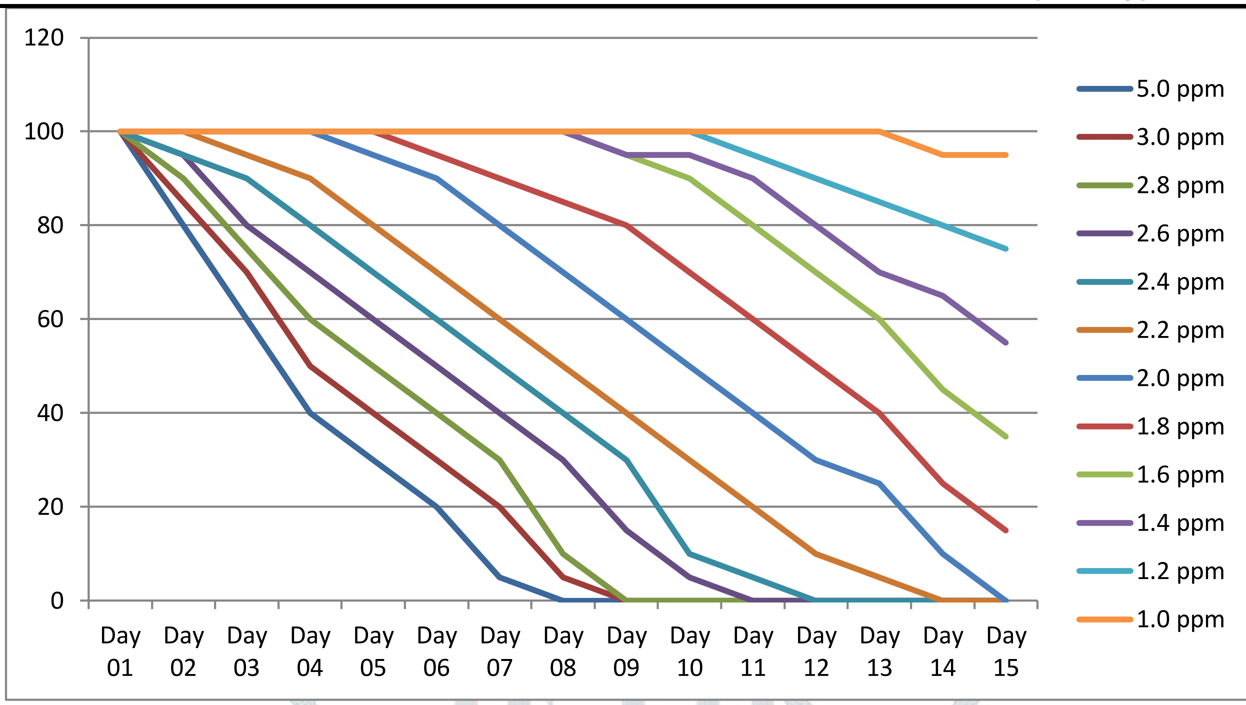

Figure 1.2 showing the effect of heavy metals in mesotrophic conditions on the survival rate of *Lemna minor* in different concentrations.

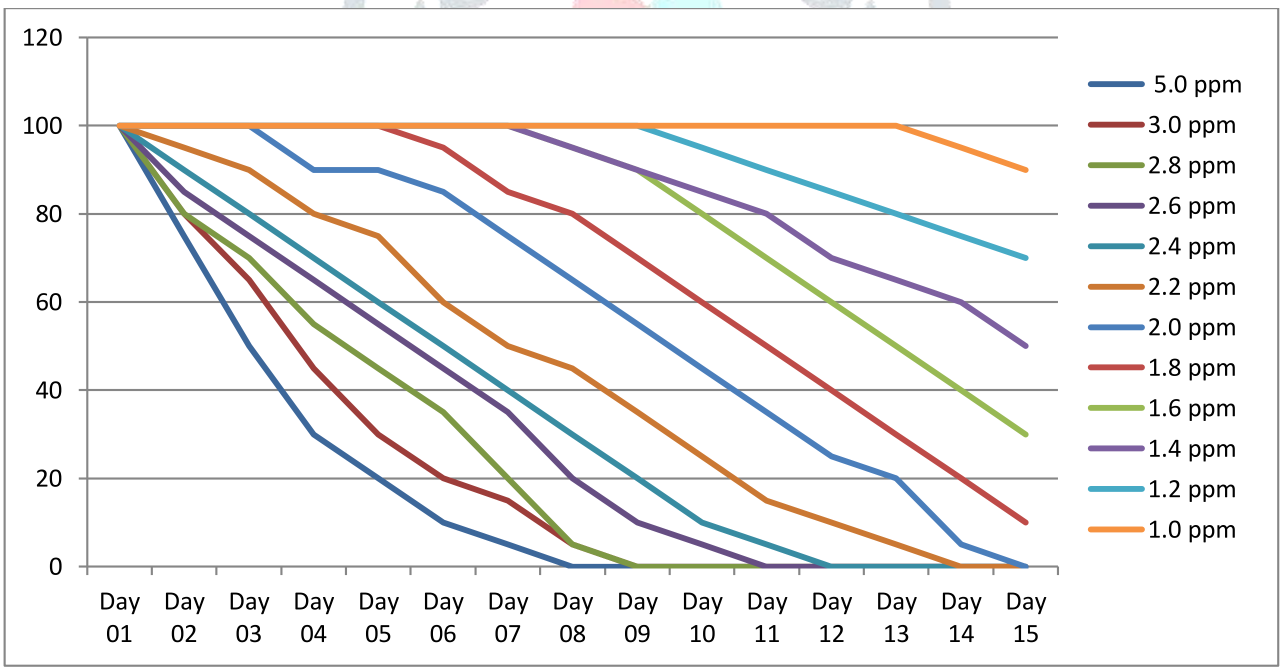

Figure 1.3 Showing the effect of heavy metals in oligotrophic conditions on the survival rate of *Lemna minor* in different concentrations.

As it is evident from the (Fig. 1.4), a decrease is observed in the survival rate of *Salvinia natans* across all concentrations of heavy metals over time. Notably, the highest survival rate of 95% was recorded at 0.4 ppm on the 14th day of treatment, while the lowest survival rate of 0% occurred on days of 10^{th}, 11^{th}, 12^{th} and 14^{th} day treated with 5.0, 2.8, 2.6, 2.4 and 2.2 ppm concentration of heavy metals. Heavy metals concentrations of 2.0, 1.8, 1.6, 1.4, 1.2, 1.0, 0.8 and 0.6 ppm accounted for 10%, 20%, 30%, 40%, 50%, 65%, 80% and 85% survival rate of *Salvinia natans* respectively in eutrophic conditions during the entire study period.

It is observrved in (Fig. 1.5), indicates a declining trend in *Salvinia natans* survival rates across varying concentrations of heavy metals over time. The highest survival rate of 90% was observed at 0.4 ppm on the 15th day of treatment, whereas the lowest survival rates of 0% were recorded on days 9th, 10th, 11th, 12th and 15th day treated with 5.0, 3.0, 2.8, 2.6, 2.4 and 2.2 ppm concentration of heavy metals. Heavy metals concentrations of 2.0, 1.8, 1.6, 1.4, 1.2, 1.0, 0.8 and 0.6 ppm accounted to 05%, 10%, 20%, 30%, 45%, 65%, 75% and 80% survival rate of *Salvinia natans* respectively in mesotrophic conditions during the entire study period.

It is revealed in (Fig. 1.6), demonstrates a consistent decline in *Salvinia natans* survival rates across all concentrations of heavy metals over time. The highest survival rate of 90% was observed at 0.4 ppm on the 15th day of treatment, while the lowest survival rates of 0% were observed on days 9th, 10th, 11th, 12th and 15th day treated with 5.0, 3.0, 2.8, 2.6, 2.4 ppm concentration of heavy metals. A survival rate of 5%, 15%, 30%, 40%, 60%, 70% and 80% was observed for the concentrations of 1.8, 1.6, 1.4, 1.2, 1.0, 0.8 and 0.6 ppm respectively in oligotrophic conditions throughout the study period.

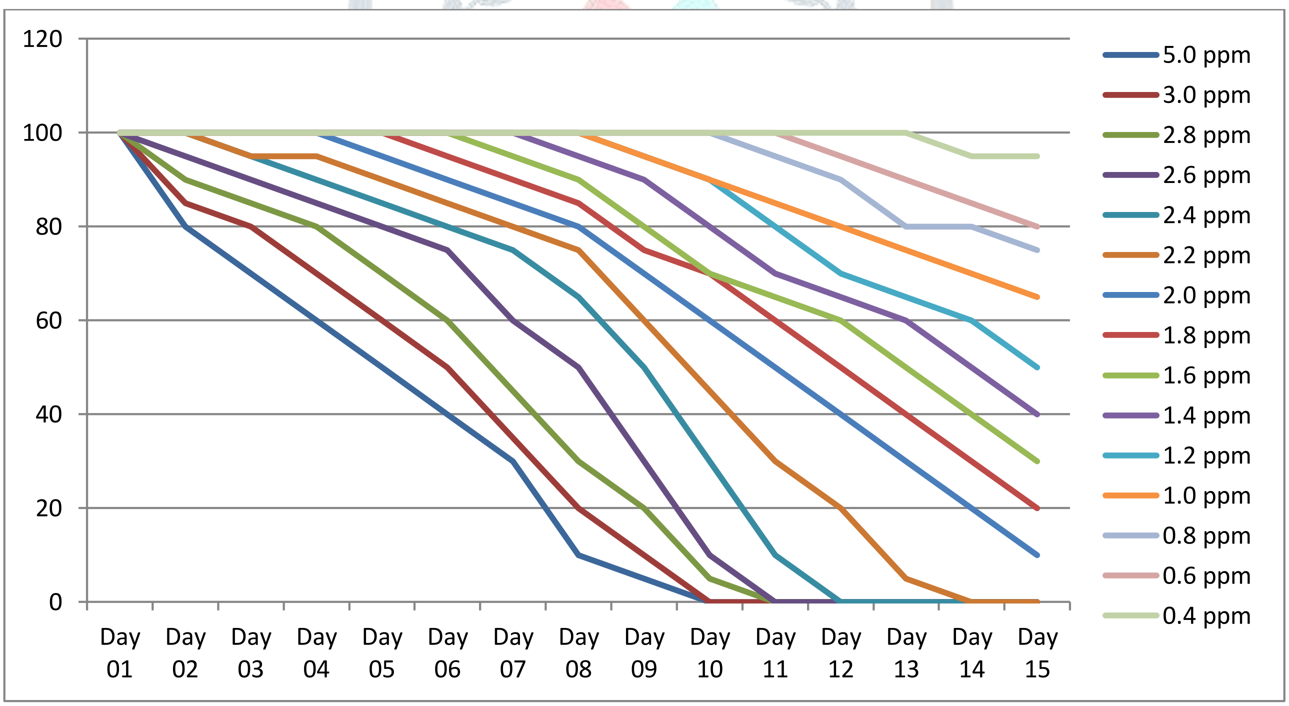

Figure 1.4 showing the effect of heavy metals in eutrophic conditions on the survival rate of *Salvinia natans* in different concentrations.

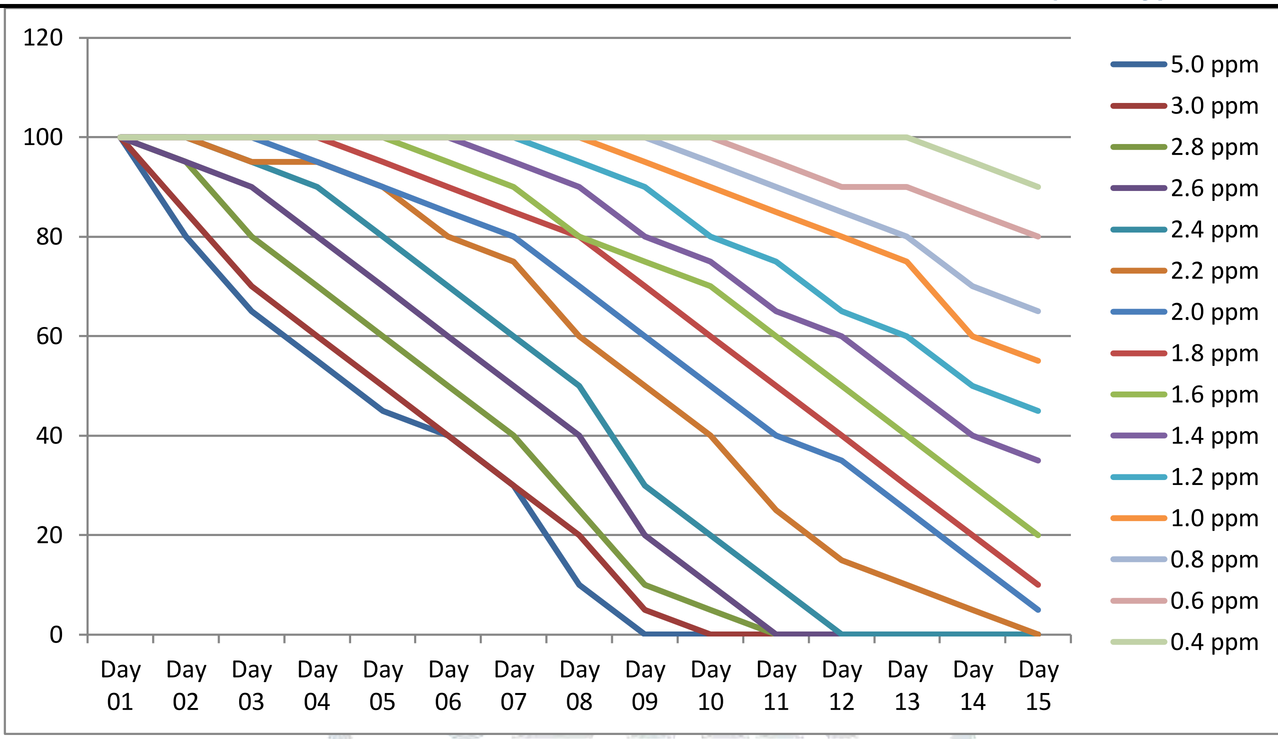

Figure 1.5 showing the effect of heavy metals in mesotrophic conditions on the survival rate of *Salvinia natans* in different concentrations.

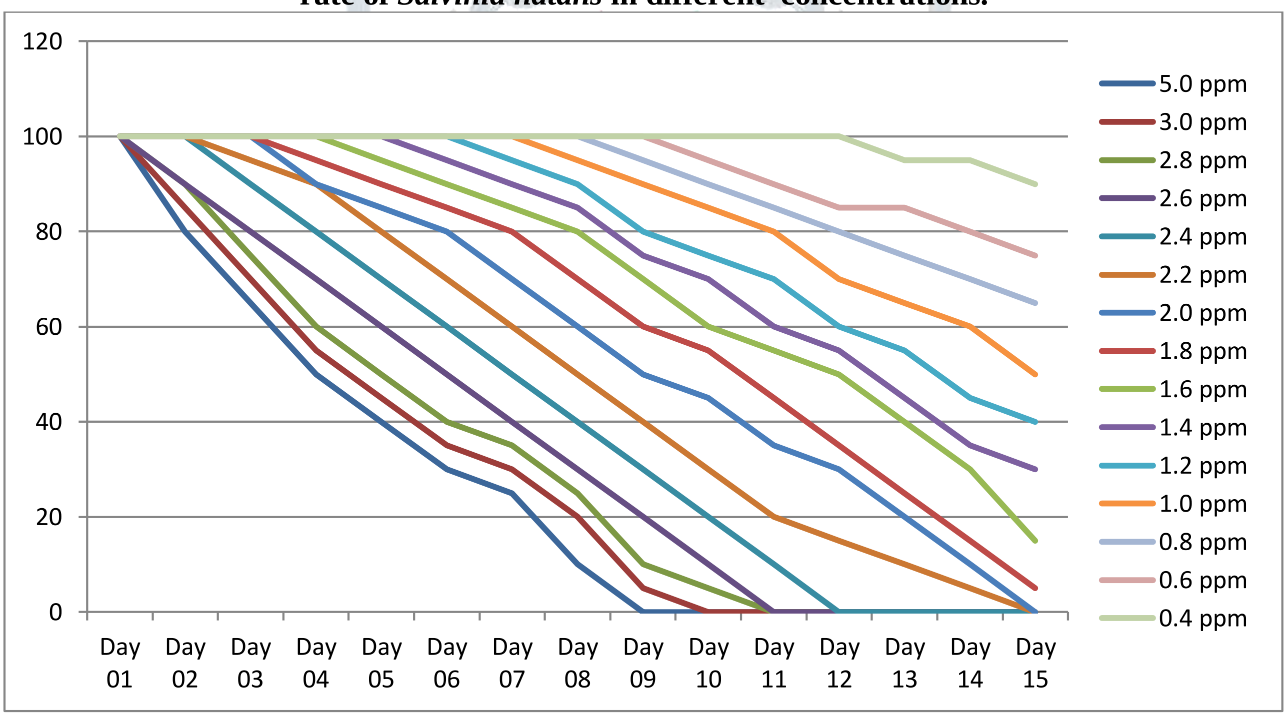

Figure 1.6 showing the effect of heavy metals in oligotrophic conditions on the survival rate of *Salvinia natans* in different concentrations.

Chlorosis of cultured Macrophytes in laboratory conditions:

In the present study the rate of chlorosis of the selected study plants were worked out during the growing season of 2018-19. The rates of chlorosis were checked simultaneously with the survival rates in different nutrient conditions representing different trophic levels.

As is obseerved from the (Fig. 2.1), illustrates a significant increase in *Lemna minor* chlorosis with extended treatment periods. The highest chlorosis levels (100%) were recorded at heavy metal concentrations of 5.0, 3.0, 2.8, 2.6, 2.4, and 2.2 ppm, with 2.0 ppm resulting in 95% chlorosis. Conversely, the lowest chlorosis (5%) persisted at 1.0 ppm of heavy metals throughout the study. Under eutrophic conditions, concentrations of 1.8, 1.6, 1.4, and 1.2 ppm induced chlorosis rates of 80%, 60%, 40%, and 20%, respectively, in *Lemna minor*.

As revealed by (Fig. 2.2), a similar trend is observed, with *Lemna minor* chlorosis increasing notably with prolonged treatment durations. The highest chlorosis levels (100%) were registered at heavy metal concentrations of 5.0, 3.0, 2.8, 2.6, 2.4, 2.2, and 2.0 ppm, while 1.8 ppm resulted in 85% chlorosis. Notably, the lowest chlorosis (5%) was seen on the 14th day with 1.0 ppm of heavy metals. Mesotrophic conditions yielded chlorosis rates of 65%, 45%, and 25% at concentrations of 1.6, 1.4, and 1.2 ppm, respectively, in *Lemna minor*.

As it is observed in (Fig. 2.3), *Lemna minor* demonstrated a pronounced increase in chlorosis rates with prolonged treatment durations. The highest chlorosis levels (100%) were observed at heavy metal concentrations of 5.0, 3.0, 2.8, 2.6, 2.4, 2.2, and 2.0 ppm, with 1.8 ppm resulting in 90% chlorosis. Conversely, the lowest chlorosis (10%) was recorded on day 15 with 1.0 ppm of heavy metals. Under oligotrophic conditions, concentrations of 1.6, 1.4, and 1.2 ppm led to chlorosis rates of 70%, 50%, and 30%, respectively, in *Lemna minor*.

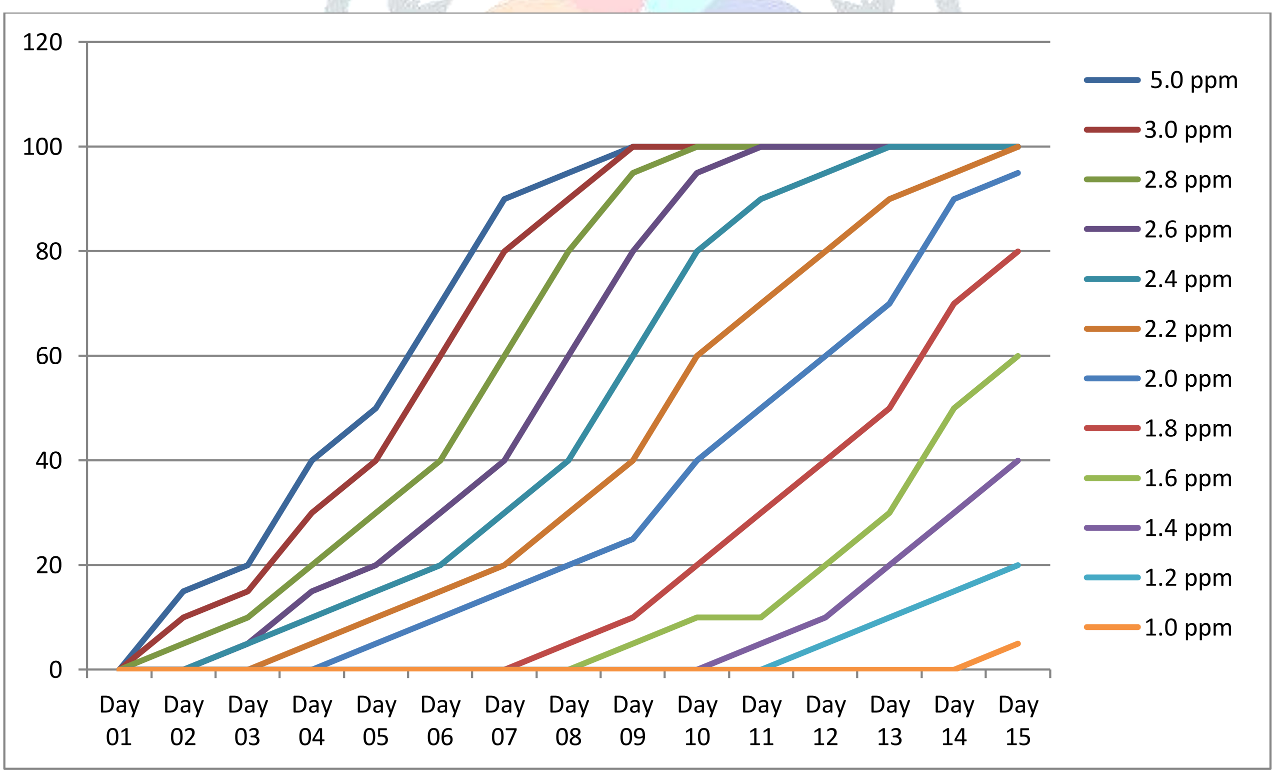

Figure 2.1 showing the effect of heavy metals in eutrophic conditions on the chlorosis of *Lemna minor* in different concentrations

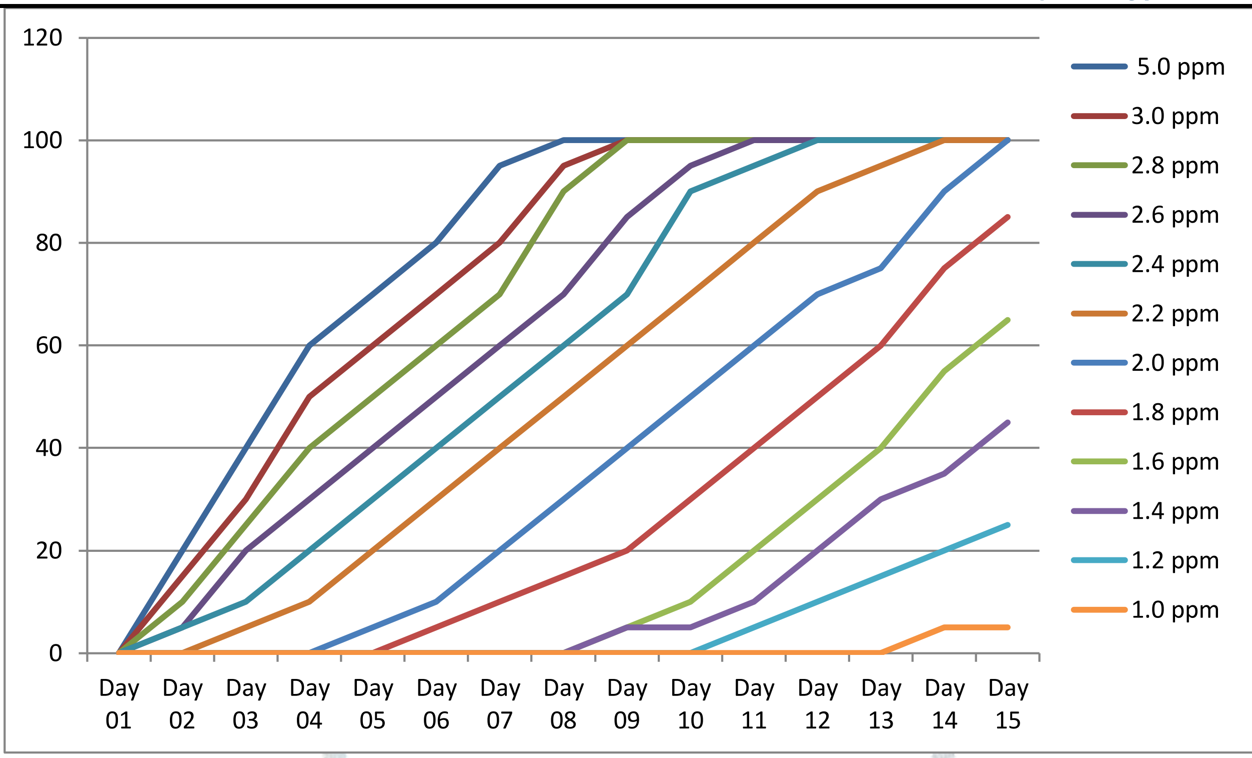

Figure 2.2 showing the effect of heavy metals in mesotrophic conditions on the chlorosis of *Lemna minor* in different concentrations.

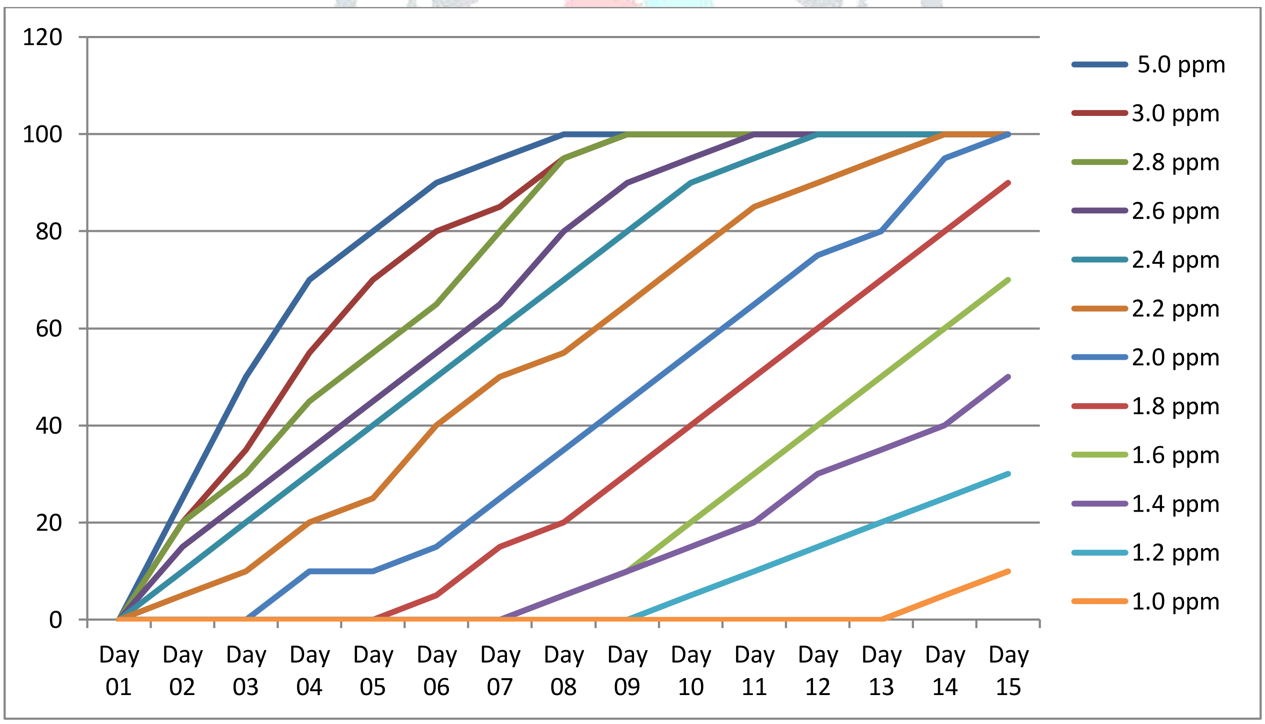

Figure 2.3 showing the effect of heavy metals in oligotrophic conditions on the chlorosis of *Lemna minor* in different concentrations.

A perusal of data in (Fig.2.4) indicates a notable increase in chlorosis in *Salvinia natans* corresponding to longer treatment periods. The highest level of chlorosis, reaching 100%, was observed with heavy metal concentrations of 5.0, 3.0, 2.8, 2.6, 2.4, and 2.2 ppm, followed by 2.0 ppm which resulted in 90% chlorosis. Conversely, the lowest chlorosis rate of 5% occurred on the 14th day of treatment with 0.4 ppm of heavy metals throughout the study. The chlorosis rate of 80%, 70%, 60%, 50%, 35%, 20% and 15% was observed in concentration of 1.8, 1.6, 1.4, 1.2, 1.0, 0.8 and 0.6 ppm of *Salvinia natans* respectively in eutrophic conditions.

As it is evident in (Fig.2.5), illustrates a significant increase in chlorosis of *Salvinia natans* with prolonged treatment duration. The highest chlorosis level, reaching 100%, was observed with heavy metal concentrations ranging from 5.0 to 2.2 ppm, followed by 2.0 ppm resulting in 95% chlorosis. The lowest chlorosis rate of 10% was recorded on the 14th day with 0.4 ppm of heavy metals over the study period. The concentration of 1.8, 1.6, 1.4, 1.2, 1.0, 0.8 and 0.6 ppm of heavy metals in mesotrophic conditions accounted to 90%, 80%, 65%, 45%, 35%, 25% and 20% rate of chlorosis in *Salvinia natans* respectively during the study period.

As observed in (Fig.2.6), highlights a significant increase in chlorosis in *Salvinia natans* with prolonged treatment. The highest chlorosis, reaching 100%, was observed with heavy metal concentrations ranging from 5.0 to 2.0 ppm, followed by 1.8 ppm resulting in 95% chlorosis. The lowest chlorosis rate of 5% occurred on the 13th day with 0.4 ppm of heavy metals throughout the study. The concentration of 1.6, 1.4, 1.2, 1.0, 0.8 and 0.6 ppm of heavy metals resulted in 85%, 70%, 60%, 40%, 30% and 20% of chlorosis in *Salvinia natans* respectively in oligotrophic conditions.

The above findings resembles with the findings of Haq and Hussain (2012), Li et al. (2019) and Wang et al. (2017), Shrivastava et al. (2017), and Gopal et al. (2018) explored combined heavy metal effects on *Salvinia natans*, noting concentration-dependent declines in survival rates and increased chlorosis levels. Thus these findings reveal that the concentration of heavy metals increases, resulting the decrease in the survival rates and increases the chlorosis rate of these aquatic plants.

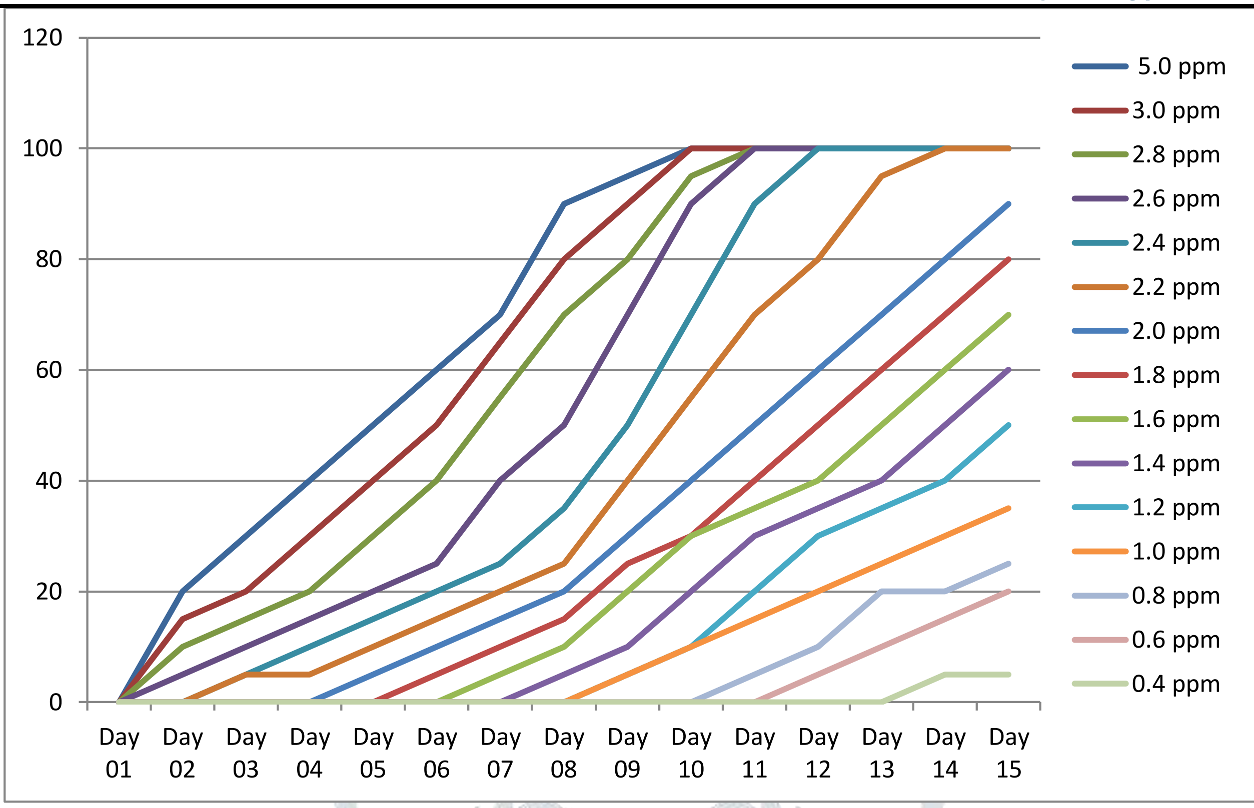

Figure 2.4 showing the effect of heavy metals in eutrophic conditions on the chlorosis of *Salvinia natans* in different concentrations.

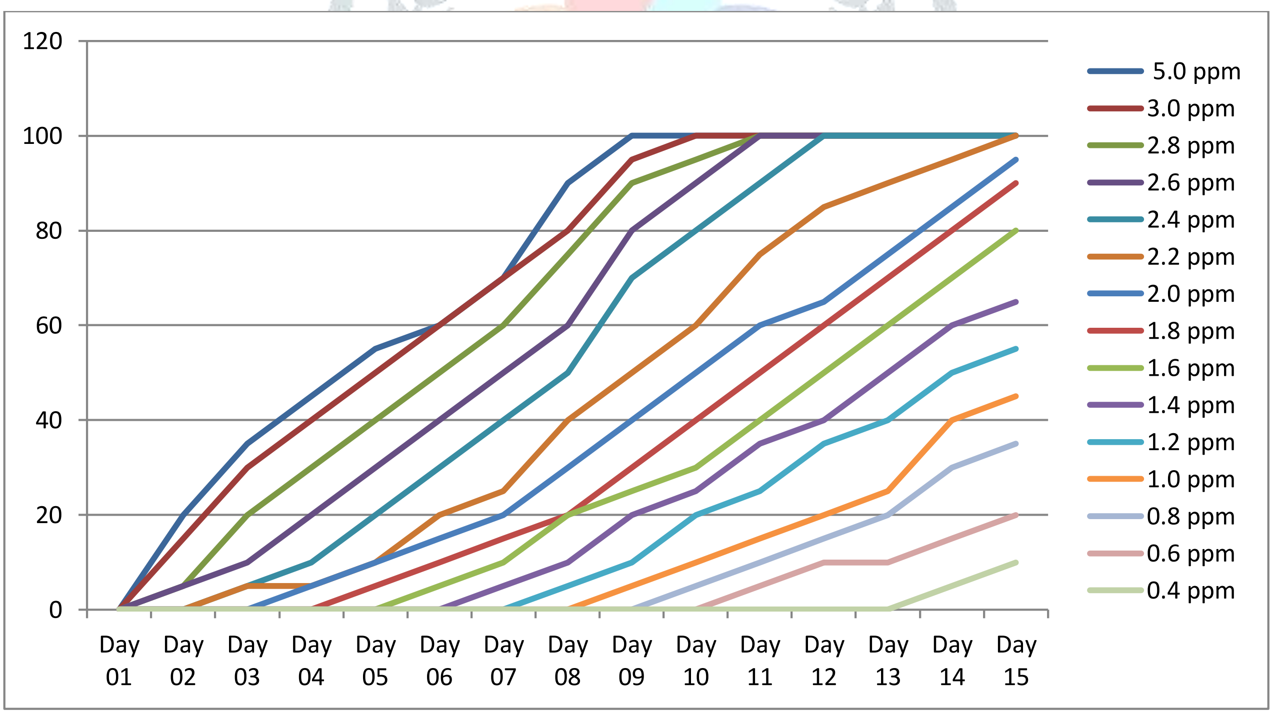

Figure 2.5 showing the effect of heavy metals in mesotrophic conditions on the chlorosis of *Salvinia natans* in different concentrations.

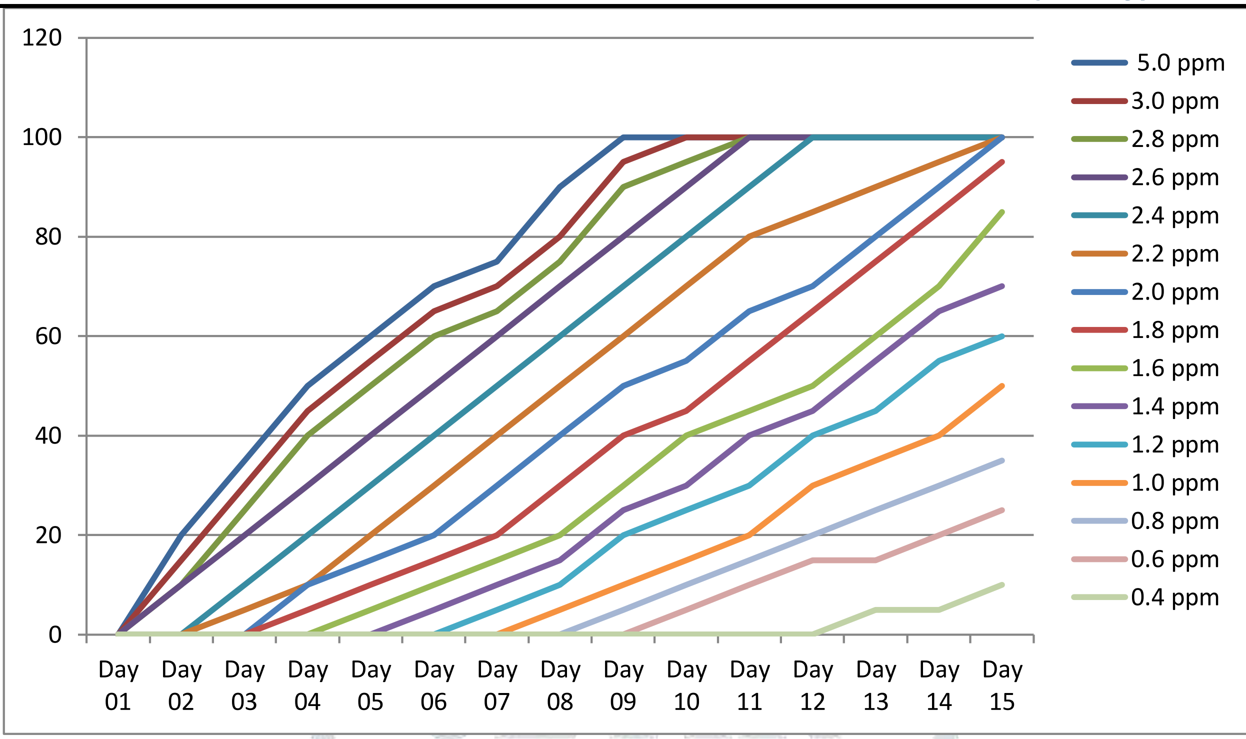

Figure 2.6 showing the effect of heavy metals in oligotrophic conditions on the chlorosis of *Salvinia natans* in different concentrations.

Conclusion

The findings of this study underscore the significant impact of heavy metal pollution on the survival and chlorosis rates of aquatic plant species, specifically *Lemna minor* and *Salvinia natans*, under varying trophic conditions. Across eutrophic, mesotrophic, and oligotrophic environments, both species exhibited a consistent decline in survival rates as the concentration of heavy metals and exposure duration increased.

In eutrophic conditions, *Lemna minor* displayed the highest survival rate of 95% at a heavy metal concentration of 1.0 ppm, while *Salvinia natans* exhibited a similar trend with a 95% survival rate at 0.4 ppm. However, as heavy metal concentrations rose, survival rates declined sharply, reaching 0% in some cases, indicating the susceptibility of these species to heavy metal toxicity.

Under mesotrophic conditions, *Lemna minor* and *Salvinia natans* also experienced a decline in survival rates with increasing heavy metal concentrations, albeit with slight variations in response. While *Lemna minor* showed a gradual decrease in survival rates across all concentrations, *Salvinia natans* exhibited a more pronounced decline, particularly at higher concentrations. Similarly, in oligotrophic conditions, both species displayed reduced survival rates as heavy metal concentrations increased, with *Lemna minor* showing a steady decrease and *Salvinia natans* exhibiting a consistent decline. Furthermore, the chlorosis rates of *Lemna minor* and *Salvinia natans* increased significantly with prolonged exposure to heavy metals, indicating physiological stress and damage caused by metal toxicity. This underscores the detrimental effects of heavy metal pollution on the health and vitality of aquatic plant species.

In conclusion, this study highlights the importance of monitoring and mitigating heavy metal pollution in aquatic ecosystems to preserve the biodiversity and ecological integrity of these vital habitats. Further research is warranted to explore the long-term effects of heavy metal exposure on aquatic plant communities and to develop effective conservation strategies aimed at minimizing the impacts of pollution on aquatic flora and fauna.

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