

Zinc Uptake Potential in Water Lettuce (*Pistia Stratiotes*, Linn)

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Abstract: The macrophyte *Pistia stratiotes* Linn. was exposed to the of Zinc (Zn) viz. 1 ppm, 2 ppm, 3 ppm, 4 ppm and 5 ppm and studied its potential of Zn absorption at different time periods with the interval of 5 days for continuous thirty days, arranging simultaneously a set of control without Zn for the comparison in same environment with similar all other operating conditions. It is observed that Zn absorption was higher at initial period of exposure and gradually reached to the saturation in all the exposed Zn concentrations. The absorption of Zn was higher at 3 ppm and all above exposure concentrations. The absorption potential was higher in roots than the absorption potential of shoots at all concentrations of Zn. The Transfer Factor (TF) was highest (0.270) in Set 4 having 3.0 ppm Zn concentration after 10 days. The study concluded that, *Pistia stratiotes* L. is capable to absorb Zn at higher concentrations and is hyper accumulator of Zn. Therefore, it may be used in Natural Treatment Systems of Phytoremediation for Pollution remediation using innovative constructed wetlands like floating wetland reactor systems.

Keywords: Constructed wetland, Floating wetland, *Pistia stratiotes*, Ecotoxicity, Metal concentrations, Macrophyte, Floating plant, Metal Uptake, Phytoremediation, Water Lettuce, Water Pollution, Zn absorption.

1. Introduction

The *Pistia stratiotes* L. belongs to Araceae family of aquatic plants and is vernacularly known as water cabbage or water lettuce or shellflower. Its native or region of origin is uncertain and was first noticed from Nile near Victoria Lake in Africa and hence also named as Nile cabbage. It is present in the major water bodies of all tropical and subtropical regions and is considered invasive species. *Pistia stratiotes* L. is one of the invasive plants in India. Conceptually, we, the human beings move the organisms and life-forms around all the time. Sometimes, we bring a non-native species of plant or organism into a new area where the species takes over and spreads rapidly and widely throughout the area and such spread causes major harm to native ecosystem dwellers due to alterations, we consider such species as invasive species, among these, *Pistia stratiotes* L. is a major one.

Pistia stratiotes L. is world's one of the most productive fresh water macrophyte. It is highly tolerant to water contaminants and high load of nutrients from sewage, industrial effluents or other polluted waters. It grows luxuriously in the forms of large mats which block the penetration of light by its shading and drastically affect the submerged plant communities. Moreover, its overgrowth is problematic for water flow systems. It can block gas exchanges between water and atmospheric air and lead to anoxic conditions, provides sheltered mosquito breeding centers, creates anaerobic environment and can cause fouling of water, mortality of aquatic animals including fishes.

Presence of *Pistia stratiotes* L. disrupts the submersed animals and plant communities, greatly reduces, biological diversity of both submersed and emergent plants, decreases dissolved oxygen concentrations, causing fish kills, decreases in planktonic diversity (Ramey, 2001; Dray and Center, 2002; Cai, 2006). Structurally, *Pistia stratiotes* L.

macrophyte plays important role in miniaturization of plankton volume (Cai, 2006); its floating mats change the community architecture, fishes respond to changes in architecture (USFS-PIER). 2010). It results in the increased siltation, nutrient loading, alkalinity, and thermal stratification; reduced dissolved oxygen (Dray and Center, 2002). Its dense canopy decreases light penetration; increases siltation and causes thermal stratification and hence the biodiversity decreases especially, the planktonic structure, diversity decreases (Cai, 2006). It decreases in dissolved oxygen concentration, pH and permanganate index (Cai, 2006); increase in siltation, transparency, nitrate, ammonium, total nitrogen, total phosphorous, and total bacteria (Drya and Center, 2002; Cai, 2006). It provides ecological benefits such as it increases water clarity (Cei, 2006); provides habitat for macroinvertebrates (Albertoni, 2007; Arimoro, 2007) and is useful in wastewater treatment (Lu et.al., 2010, 2011; Zimmels, 2006; Akinbile and Yusoff, 2012; Vesely et.al., 2011; Mufarree et.al., 2010; Prasertsup and Ariyakanon. 2011; Momtaz et.al., 2010; Mukherjee and Kumar. 2005; Odjegba and Fasidi, 2004; Aoi and Hayashi, 1996); ethno-medicinal plant (Tripathi et.al., 2010). Simultaneously, it possesses the risk of release and population expansion outweighs benefits of use and provides favorable breeding ground for mosquitoes (Kengne et.al., 2003). It can be removed from the water body by Hand pulling or seining (GISD, 2005; AP, 2018).

Zinc ranks 23rd among the most abundant element in the Earth's crust. Its concentrations in the environment are rising unnaturally, almost exponentially due to addition of zinc through human activities. Almost in all parts of the world, Zn is one of the major pollutants of both aquatic and terrestrial ecosystems. Most of the zinc is added during the industrial activities, such as mining, coal and solid waste combustion, hazardous waste disposals, and wastes from processing units like steel processing. Zinc is one of the important trace elements that play a vital role in the physiological and metabolic process of many organisms

(Aboud and Nandini, 2009). It plays an active role in a variety of enzyme systems which contribute to energy metabolism, transcription and translation (Abassi *et al.*, 1998). Zinc salts have many applications routinely. They are commonly used in the preservation of wood, fertilizers, catalysts, nutritional or medicinal supplements, in textiles, rubber and ceramic industries and it is also used as galvanising agent to prevent corrosion of metals. Zinc chloride ($ZnCl_2$) is used in smoke bombs for dispersal of crowds and also in fire fighting exercises. Zinc is used in various chemical compound forms like nitrate, chloride, sulphate, oxide, and sulphide for medical and dental purposes. Furthermore, zinc chloride ($ZnCl_2$) and zinc sulfate ($ZnSO_4$) are commonly used in the production of chemical fertilizers for agriculture and also in the manufacturing of herbicides and pesticides (Roney *et al.*, 2005).

Zinc can be released by natural processes, but mostly results from anthropogenic activities. Zinc in different forms routinely used in several industries like galvanizations, paints, batteries, smelting processes, fertilizer productions and pesticide manufacturing, combustion of fossil fuels, various pigments, polymer stabilizers, etcetera, and the wastewater released from these industries is polluted with zinc, due to its presence in large quantities (GISD, 2005; AP, 2018). Most of the times this wastewater is not purified satisfactory and ultimately, one of the consequences is that rivers are depositing zinc-polluted sludge on their banks.

Any surface water in any natural water bodies can be impacted by discharges of metal manufacturing and chemical industrial wastes, and also by the run-off following precipitation on soils high in zinc, either due to the natural setting or human applications, including use of zinc fertilizer on agricultural soils. Particles released from vehicle tyres and brake linings are a major source of zinc in the environment (WHO, 2001). It is known that zinc (Zn) is one of the essential elements to human and plant growths (Jordao *et al.*, 2002). Recent studies indicated that Zn is also involved in bone formation. However, increased intake of Zn causes muscular pain and intestinal haemorrhage (Honda *et al.*, 1997; Jordao *et al.*, 2002). Even if it is present in less quantity and enters in human's body, it adversely affects considerably the human's health. Although humans can handle large extent of zinc in routine life, too much of it can still cause eminent health problems (Gakwisiri *et al.*, 2012).

According to Rajappa *et al.* (2010), zinc shows fairly low concentrations in water samples due to its restricted mobility from the place of rock weathering or from the natural sources. In lakes and rivers, some zinc remains dissolved in water or as fine suspended particles, while other zinc settles to the bottom in association with heavier particles. Rajkovic *et al.* (2008) asserted that elevated zinc levels as toxic to some species of aquatic life. From an environmental pollution standpoint, zinc is generally considered as a toxic element and has the potential to bioaccumulate in the food chain (Abassi *et al.*, 1998; WHO, 2004).

2. Experimental Procedures

Water Lettuce (*Pistia stratiotes*, Linn) macrophytes of same size were collected from a local water-body. The plant samples collected were rinsed with tap water to remove any epiphytes and insect larvae grown on entire plants. Plastic tubs of 20 liters capacity were used for the study. The sampled plants were placed in plastic tubs filled with tap water and placed under natural sunlight for one week to let them to adapt to the anthropogenic new environment, then the plants of the same size were selected for further experimental studies. A stock solution (1000 mg/L) of zinc was prepared in distilled water with analytical grade $ZnSO_4 \cdot 7H_2O$ which was later diluted as per experimental requirement. The Water Lettuce (*Pistia stratiotes*, Linn) macrophytes were maintained in tap water supplemented with aqueous solutions having 1, 2, 3, 4 and 5 ppm (mg/L) concentrations of Zn. Plants those were not exposed to Zn metal solution served as controls for the reference and comparison. All sets of experiments were performed in triplicate in same environment with similar all other operating conditions. The total test durations were 0 days to 30 days with 5 days intervals for the needful intermediate analysis. Tap water was added daily to compensate for water lost through plant transpiration, sampling and evaporation. The Water Lettuce (*Pistia stratiotes*, Linn) macrophyte plant samples from each experimental set were harvested after each of the test durations at the interval of 5 days. They were separated into shoots and roots, and were analyzed for respective uptake potential of Zn accumulation.

The water Lettuce (*Pistia stratiotes*, Linn) macrophyte sampled plants were washed with tap water and then rinsed with distilled water to wipe out adhering substances, if any. The root and body of plants were separated, softly make water-free by tissue papers and slowly crushed, blended to homogenize and then these were dried in oven (60°C for 24 hrs). The all dried samples were pulverized to fine powder which was sieved (0.15 mm) following the procedure described by Kalra (1998). Separately prepared powder of plant parts (shoots and roots) were directly digested with nitric and perchloric acid till a transparently clear solution was obtained. It was further diluted to 50 ml with double distilled water (APHA, 1998) to make up the volume. Zinc (Zn) contents were determined by EDTA Complexometry-Back Titration method (Nicolaysen, 1941) and confirmed (Islam and Ahmed, 2013). The analytical procedure was confirmed by analyzing 2 blanks considering all laboratory and analytical conditions being equal. The translocation factor for Zn was determined using following formula;

$$\text{Translocation factor (TF)} = \text{MCS/MCR}$$

Where, MCS stands for Metal Concentration in Shoot and MCR stands for Metal Concentration in Root.

3. Results and Discussions

Zinc is readily absorbed in the macrophytes including the *Pistia stratiotes* L. as it is one of the 17 essential micronutrients required by the plants for their healthy growth. Zn is one of the important nutrients not only growth but also for various other developmental and physiological

processes occurring in plants. It is one among the 8 essential elements required by the plants for performing various physiological and metabolic mechanisms (Das et.al., 2013). Ultimately, it has led in the healthy growth of *Pistia stratiotes L.* in the all sets arranged in present investigations. Moreover, it is absorbed and magnified in the all plants in all sets arranged irrespective of the exposed various Zn concentrations. Although, Zn is an essential micronutrient and is required by the plants for performing various physiological processes but at elevated levels Zn may prove toxic and hence cause phyto-toxicity in plants. The threshold level of Zn toxicity depends on the type of plant species, growth media or concentrations of Zn contamination in growth media and the time of exposure. In plants, Zn toxicity occurs at concentration range between 400-500 µg/g in the leaves with the visible sign of excess level of Zn in plants such as chlorosis or necrosis of young leaf (Sidhu, 2016). But, no such noticeable phyto-toxic effect was observed in present studies. It reveals that the macrophyte *Pistia stratiotes L.* is tolerant to the higher concentrations of Zn and hence is a hyper accumulator for Zn. Paivoke and simola (2001) reported that the decrease in the growth rate of *Pistia stratiotes L.* (water lettuce) is a typical plant response to As exposure at high concentrations which has not observed in the present study for zinc exposure in any of the concentrations studied indicating that the plant is tolerant to Zn exposure even at high concentrations.

The results presented in Table 1 and Table 2 indicates that the zinc tends to accumulate more in the roots of *Pistia*

stratiotes L. than its shoots. It was also noticed that the there was retarding of elongation and growth of roots as compared with the control set. The effect was more visible at the exposure of higher concentrations of Zn. These observations are in good agreement with the observations and conclusion of Bayer and his co-workers (1913). In plants exposed to higher concentrations, Zn toxicity reduced the growth of main root and also retarded the growth of lateral roots as has been described by Ren and his co-researchers (Ren et.al., 1993).

Absorption of Zn in the roots of *Pistia stratiotes L.* was relatively rapid in the initial periods in all the experimental sets for 10 days and was slowed down thereafter. The absorption rate was turned to almost study state after 25 days of exposure in almost all sets. There was no change in the Zn concentration of plant samples in control set (Fig.1).

Table 1: Absorption potential in the Roots of water Lettuce (*Pistia stratiotes*, Linn) exposed to various concentrations of Zinc at different time intervals

Zinc conc. (ppm)	Exposure period (Days)						
0	0	5	10	15	20	25	30
0.0(Set 1) Control	0.01	0.01	0.01	0.01	0.01	0.01	0.01
1.0 (Set 2)	0.00	3.4	8.8	12.5	14.3	15.2	16.0
2.0 (Set 3)	0.00	5.0	10.4	15.0	17.2	18.3	19.1
3.0 (Set 4)	0.00	7.1	12.1	16.9	20.2	22.2	23.0
4.0 (Set 5)	0.00	8.4	15.2	19.3	21.8	23.0	24.7
5.0 (Set 6)	0.00	9.3	17.2	21.5	23.7	25.5	25.6

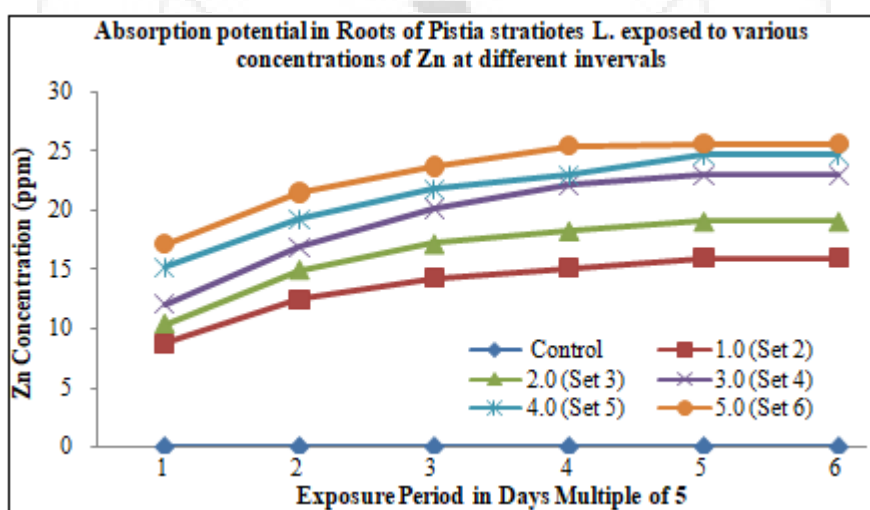


Figure 1: Zn absorption in the Roots of *Pistia stratiotes L.* exposed to various concentrations for different periods in Days multiple of 5

The rate of absorption in shoots was lower than the absorption in roots of *Pistia stratiotes L.* In all the Zn exposed sets. The Zn concentrations for entire experimental period in control were nil indicating that the absorption in all other sets was due to the exposed concentrations of Zn. The trend of absorption of Zn in shoots in all experimental sets other than control were almost similar to the trend in roots, with less absorbed quantities (Fig.2). Rates of Zn absorption were higher at higher exposure concentrations and were increasing at initial period and were tending to steady state after 25 days.

Table 2: Absorption potential in the Shoots of water Lettuce (*Pistia stratiotes*, Linn) exposed to various concentrations of Zinc at different time intervals

Zn Conc. ppm)	Exposure period (Days)						
0	0	5	10	15	20	25	30
0.0 (Set 1) Control	0.00	00	00	00	00	00	00
1.0 (Set 2)	0.00	0.4	1.0	2.9	3.7	3.9	4.0
2.0 (Set 3)	0.00	1.1	2.4	3.0	4.1	4.5	4.7
3.0 (Set 4)	0.00	1.9	2.7	4.1	5.1	5.4	5.5
4.0 (Set 5)	0.00	2.1	4.1	4.8	5.1	5.3	5.7
5.0 (Set 6)	0.00	2.2	4.2	5.0	5.3	5.9	6.2

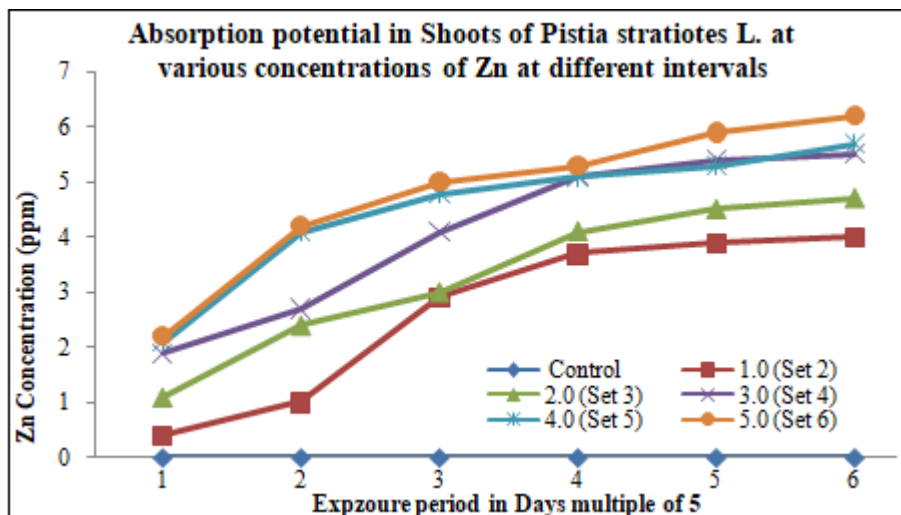


Figure 2: Zn absorption in the Shoots of *Pistia stratiotes L.* exposed to various concentrations for different periods in Days multiple of 5

Aquatic plants have naturally developed the process for the acquisition of relatively less abundant micronutrients such as Cu, Zn, Ni and Mn from the water which are sometimes highly reactive and potentially toxic to plants. Therefore, the uptake, transport and accumulation of these micronutrients are highly coordinated and regulated by necessity in plants. Some of these macrophytes are tolerant to specific metals and are capable to accumulate these metals in substantial quantities in their biomass due to their effective uptakes and translocation abilities. Such plants with effective transfer and translocation can concentrate metals in different parts from low to great water concentrations and are regarded as accumulators. The uptake and transfer of heavy metals from water to plant parts such as roots, shoot, stem and leaves etcetera is a process of significant importance and determining of transfer factor (TF) as a key parameter to assess the availability of elements and hyper-accumulation capacity of the aquatic plant. Therefore, a detailed study on the metal transfer from water to plant and metal translocation to its parts could possibly reflect more light on the metal accumulation potentials of aquatic macrophyte plant species and their phytoremediation potentials (Ogunkunle, 2014). Therefore, transfer factor for Zn translocation in shoot of *Pistia stratiotes Linn.* from its root in each set was determined. It is observed that the TF was highest (0.270) in set 4 after 10 days of absorption indicating high rate of transfer. The TF was found relatively increased

with the absorption period in all sets except control. TF indicates a plant's ability to translocate metals from the roots to the shoots (Baker, 1981). Higher value indicates higher transfer. The transfer factor greater than 1 ((TF > 1) signifies that the plant is efficient in translocation of the heavy metal from root to the shoot (Baker and Brook, 1989). The calculated values of TF factor in present investigation are relatively higher in all exposure concentration in all sets and are comparatively higher than TF factor in *Echhornia crassipes* as published elsewhere (Shingadgaon and Chavan, 2018).

Table 3: TF factor of *Pistia stratiotes L.* exposed to various concentrations of zinc at different time intervals.

Zn Conc. ppm)	Transfer Factor at Different Exposure periods (Days)						
	0	5	10	15	20	25	30
(Set 1) Control	0.00	00	00	00	00	00	00
1. 0 ppm (Set 2)	0.00	0.117	0.114	0.232	0.259	0.257	0.250
2. 0 ppm (Set 3)	0.00	0.220	0.231	0.200	0.238	0.246	0.246
3. 0 ppm (Set 4)	0.00	0.268	0.223	0.243	0.252	0.243	0.239
4. 0 ppm (Set 5)	0.00	0.250	0.270	0.249	0.224	0.230	0.231
5. 0 ppm (Set 6)	0.00	0.237	0.244	0.233	0.224	0.231	0.242

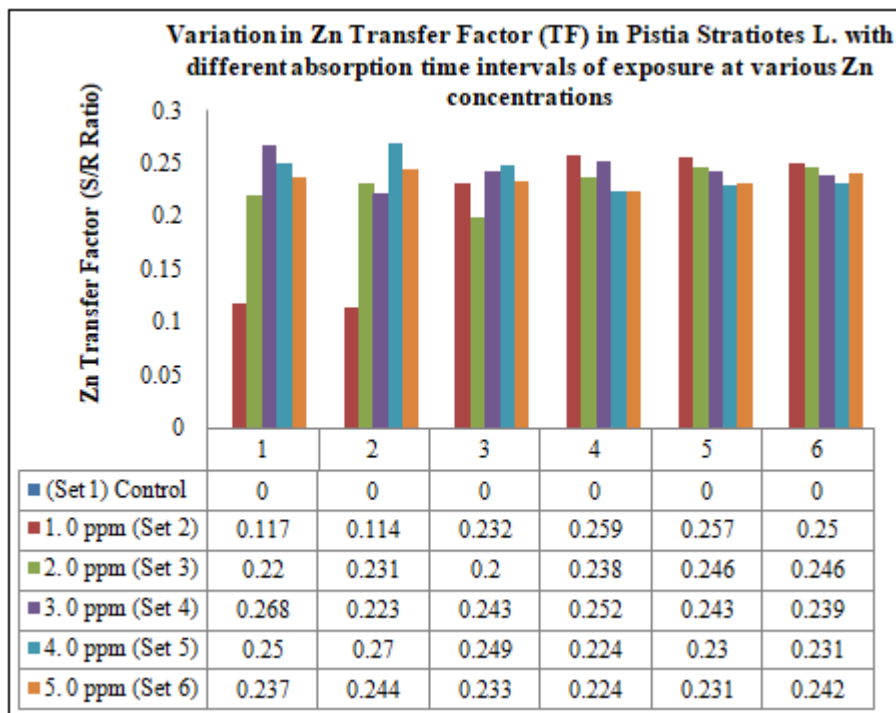


Figure 3: Variation in Zn Transfer Factor (TF) in *Pistia stratiotes* Linn. with different absorption time intervals of exposure at various Zn concentrations

The *Pistia stratiotes* Linn has good potential for Zn absorption as observed in the present investigation. Similar observations on absorption potential and translocation ability of *Pistia stratiotes* L. in its roots and shoots have been reported by many workers including Reddy and Debusk (1985), Aoi and Hayashi (1996), Sridhar (1986), Sen et al, (1987), Gumbricht (1993), Reddy (1983), Lu *et al.* (2011), Makhopadhyay *et al.* (2007), Alam *et al.* (1995), De *et al.* (1985), O'Keefe *et al.* (1984), Haidar *et al.* (1984), Chigbo *et al.* (1984), Liao and Chang (2004), Wang *et al.* (2002), Zayed *et al.* (1998), Greenfield *et al.* (2007), Chandra and Kulshreshtha (2004), Lindsey and Hirt (1999), Singhal and Rai (2003), Aoi and Ohba, (1995), Karpiskak *et al.* (1994) and El-Gendy *et al.* (2005). Many scientific studies have also pointed out that heavy metals are not only retained in the roots and transferred to the shoots. These also get deposited in the leaves. This deposition is at the concentrations 100 to 1000 folds higher than those found in the non-hyper accumulating plant species (Rascio and Izzo, 2012; Mansauri *et al.*, 2012; Kumar *et al.*, 2008; Naseem and Tahir, 2001). Therefore, such macrophytes can be used effectively for phytoremediation in Natural Treatment Technologies for any wastewater containing Zn as one of the contaminants. The floater constructed wetland systems would be more appropriate for its use in phytoremediation. It is advisable from the present studies that the *Pistia stratiotes* L. (commonly known as water lettuce) can be recommended for phytoremediation technology in industrial waste water treatment units having Zn as major polluting constituent as it is effective, efficient, ecofriendly, better and tolerant absorber of Zn.

4. Conclusions

The present investigation has led to conclude that, *Pistia stratiotes* L. (Water lettuce) is not only a good absorber of Zinc but also is tolerant hyper accumulator of Zn. Therefore,

it may be used in Natural Treatment System of Phytoremediation as an Eco-technology for Pollution control with in situ design of constructed wetlands like floating wetland reactor systems that can resolve the problem of zinc contamination.

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