*Original Research Article*

Bioremediation Potentials of *Chlorella Vulgaris* on Water Samples from Ikose Stream ( Tributary of Oba River) Ogbomoso, Nigeria

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ABSTRACT

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| **Aim:** The aim of the study is to evaluate the potentials of *Chlorella vulgaris* as a phycoremediation tools of water samples collected from Ikose stream, a tributary of Oba river in Ogbomoso.  **Study design:** The study employed an experimental study design.  **Place and Duration of Study:** Samples were collected in November 2021 and the study was conducted between December 2021 to May 2022. The research was carried out in the Microbiology Central Research Laboratory of Pure and Applied Biology Department, Ladoke Akintola University of Technology, Ogbomoso, Oyo State, Nigeria.  **Methodology:** The water sample was collected from Ikose water reservoir in Ogbomoso and the dried biomass of *Chlorella vulgaris* for the adsorption study was obtained from microbiology laboratory, LAUTECH. Three different concentrations of dried biomass of *chlorella vulgaris* were prepared by weighing 10mg, 50mg and 100mg into 80ml of the water sample with pH 5.0 and 8.0 in different bottles, the contact time was at an internal of 24h, 48h and 72h. At the end of each time duration, heavy metal concentration was determined by inductively coupled plasma- Mass Spectrometry (ICP-MS).  **Results:** The ICP-MS analysis which revealed the presence of five different heavy metals which are Manganese, Nickel, Zinc, Lead, Iron. Iron shows the highest concentration of 9.789mg/L while manganese shows the lowest at 1.0977mg/L.  **Conclusion:** The result obtained for the bioabsorption of heavy metals in this study shows that *C. vulgaris* was efficient in the bioabsorption of most of the heavy metals identified from the water sample collected from the reservoir patients. These predictors, however, need further work to validate reliability. |

*Keywords:* Heavy metals, Bioadsorption, Phycoremediation, *Chlorella Vulgaris,* Biomass concentration

1. INTRODUCTION

Water pollution represents a major global threat to health of human and animal. Water bodies such as freshwater bodies which are commonly used for domestic purposes are getting polluted daily by several anthropogenic activities. Approximately, two billion people have reported using contaminated water for drinking purposes (WHO, 2019) which results in over two million deaths per year globally, because it is linked to numerous diseases (e.g., salmonella, typhoid, skin infections, trachoma, cancer, cholera, and polio) (WHO, 2016). Furthermore, the deterioration of water quality has caused an increase in the scarcity of water-based resources, which is leading to global water stress (Mohita *et al.,* 2022). A study estimated that about sixty percent (60%) of the global population will suffer from water stress by 2025 (Khalid *et al.,* 2019). Municipalities and industrial activities point source is one of the greatest sources that pose a serious risk to the water bodies, other activities like Agricultural practice, mining, and urbanization also have an impact on water quality. All these mentioned acts caused the presence of toxic contaminants in the environment particularly, water bodies (Eduardo *et al.,* 2012). Excessive exposure to heavy metals which are one of the most water-toxic contaminants can lead to dangerous impacts (Abdullah *et al.,* 2020). Heavy metals such as arsenic cadmium, lead mercury, and chromium are toxic even at trace levels, and they are non-degradable can enter the food chain and bioaccumulate in major human body systems posing a serious risk (Zak, 2012). Apart from human health, these contaminants have a hazardous impact on flora and fauna (Luciene *et al.,* 2015).

Several conventional physiological methods like ion exchange, electro-winning, electro-coagulation, cementation, and reserve osmosis are available for the removal of heavy metals from contaminated water. However, all these methods are highly expensive and require skilled technicians (priyadarshani *et al.,* 2012). To overcome this current situation bioremediation especially, phytoremediation has been proven as an effective, eco-friendly, affordable technology for the removal of heavy metals (Lukhan *et al.,* 2022). Phycoremediation is the term used to describe the use of algae in the clean-up of water contaminants. Microalgae has outstanding biological characteristic such as photosynthetic efficiency and heavy metals reduction efficiency and also can grow under extreme environmental conditions (Olguin, 2023).

Metal remediation through standard physicochemical techniques is simple, fast, efficient, and operative; however, these techniques are expensive, unsuitable for large contaminated areas, energy requirements, generate significant amounts of toxic sludge, and are not practical with a low metals content nor are they environmentally friendly (Liu *et al.,* 2020). Microbial remediation involves using microorganisms (e.g., bacteria, microalgae, yeast) to remove/immobilize, transform, or detoxify HMs from the environment (Hadiani *et al.,* 2018). While in phytoremediation, the use of plants and associated soil microbes involves the extraction, stabilization, and volatilization of HMs from the contaminated substrates (Yanitch *et al.,* 2020). The biological strategies exploit the mechanisms used by endogenous or exogenous microorganisms or plants to cope with HMs, such as extracellular/intracellular sequestration, production of metal chelators, precipitation, enzymatic detoxification, and volatilization, to remediate HM-contaminated environments (Cepoi *et al.,* 2022).

Although several studies attest to the high ability of *Chlorella vulgaris* to remove heavy metals from wastewater, *Chlorella vulgaris* has been investigated for its potential to phycoremediate effluent wastewater. According to a study by Hameed and Ebrahim (2007), 14 different species of microalgae have great potential for heavy metals removal. They identified *C. vulgaris* as one of the efficient types of microalgae for biological removal of a variety of heavy metals, because *C. vulgaris* has alginate compounds in its cell wall. The effectiveness of *Chlorella vulgaris* in treating swine wastewater and the connections between microalgae development and the removal of contaminants have both been described in studies. It was determined how effectively nutrients were being used (Hasan *et al.,* 2014). *Chlorella vulgaris* was used to treat a wastewater discharge sample. The disclosed outcomes demonstrate that 69.23% less phosphate was present, and over 60% of the phosphate had within ten days been eliminated by *Chlorella vulgaris*. Additionally, the water's nitrate concentration *Chlorella vulgaris* treatment resulted in a sample's 84% reduction and a 247.83% increase in *Chlorella vulgaris* treatment resulted in the discovery of dissolved oxygen in the water sample (Hasan *et al.,* 2014).

2. material and methods

**2.1 Description of Water sample collection site**

Ogbomoso reservior is situated at Ikose, Orire local Government area, Oyo state, Nigeria. The Reservoir lies within the geographical coordinate 8•10'5.50''N, 4•11'37.62''E, and 8•11'53.33"N, 4•12'12.09"E, the physical characteristics of the Ogbomoso reservoir were described according to Awogbade, (2013). The Oba River which is the main source of Ogbomoso Reservoir is severely polluted by the activities of farmers that cultivate the land along its length. These farmers make used of Nitrogenous fertilizers to increase their farm yield. Little or less of these fertilizers were later carried back into the river through runoff.

**2.2 Sample collection**

The water sample used for this research was collected from Ogbomoso reservoir at ikose, into a sterile five litre (5L) plastic container, and then transported immediately to the laboratory for further studies. The dried biomass of *Chlorella vulgaris* used for the bioadsorption of heavy metals was obtained from the Microbiology Laboratory of the Department of Science Laboratory Technology, Ladoke Akintola University of Technology Ogbomoso.

**2.3 Evaluation of Heavy Metals in the water sample**

Heavy metals from collected water sample were evaluated by inductively coupled plasma-mass spectrometry.

**2.4 Bioadsorption Experiment**

The potential hydrogen (pH) of the water sample was determined to be 5.2 using a pH meter (calibrated with buffer pH 4.7 and 7) and then adjusted to pH 5.0 and 8.0 using HCl and NAOH respectively. Thereafter, 80ml of the stabilized water sample was measured into various polyethylene bottles that has already been washed and rinsed with distilled water. Three (3) concentrations of dried Biomass of *Chlorella vulgaris* (10, 50 and100mg) were measured. Each of these concentrations were then added to 80ml of water with pH values of 5.0 and 8.0, which were placed in separate bottles and agitated together using a shaker at 150rpm for 4hrs. The contact time was at an interval of (24, 48 and 72hrs). The mixture was then filtered using Whatman filter No. 1 at the end of each duration and the concentration of heavy metals were evaluated using ICP-MS.

3. results and discussion

**Evaluation of heavy metals in the water sample**

The inductively coupled plasma mass spectrometry (ICP-MS) analysis of the collected water sample revealed the presence of five different heavy metals, which are iron, manganese, Nickel, lead, and zinc. Iron shows the highest metal concentration of 9.7890mg/L, while lead has the lowest metal concentration at 0.1998mg/L, The heavy metal concentrations in the water sample are shown in Table 1.

**Table 1: Concentration of heavy metals in water sample collected from Ogbomoso reservoir**

|  |  |  |
| --- | --- | --- |
| S\N | Heavy Metals | Concentration (mg/L) |
| 1 | Iron | 9.7890 |
| 2 | Manganese | 1.0977 |
| 3 | Nickel | 0.2230 |
| 4 | Lead | 0.1998 |
| 5 | Zinc | 0.5960 |

**Bioadsorption of heavy metals using *Chorella vulgaris***

Under various operational conditions, including pH(5 and 8),absorbent concentration (10, 50 and 100mg),and contact time (24, 48 and 72hrs) .The bioadsorption of the heavy metals by dried biomass of *Chlorella vulgaris* was analyzed and the results revealed the drastic or little reduction in each metal concentrations at various level at each contact time . In this current study, the removal efficiency of (5) heavy was calculated from the following equation.

**Bioadsorption of iron at pH (5 and 8)**

After 24, 48, and 72 hours, the effect of the absorbent at 10, 50, and 100mg on iron at pH 5 demonstrate that there was a substantial variance in the reduction of iron concentration when compared to the initial concentration of iron. The obtained results indicate that 50mg of the absorbent at 24hours showed the greatest affinity for the reduction in iron concentration (1.030mg/L), followed by 100mg at 24hours with a reduction in concentration of 1.200mg/L. Figure 1a shows how this is depicted. At pH 8.0, the reduction in concentration reached its maximum point at 1.0900/L after 48hours with 10mg of the absorbent, and 1.300mg/L after 72hours with 100mg of the absorbent. Despite the fact that, there was a drop in iron concentration at all absorbent masses and contact time, as shown in figure 1b, the pattern of the reduction was not time-nor-concentration-dependent.

**Bioadsorption of lead at pH (5 and 8)**

At pH 5, with 10 mg of the absorbent, lead concentrations decreased over the course of 24, 48, and 72 hours, peaking at 0.1420 mg/L, which is marginally significant compared to the initial concentration of 0.1998 mg/L. Similarly, at 100 mg of the absorbent, there is an increase in the reduction in lead concentration at all contact times, peaking at 0.1354 mg/L; however, at 50 mg of the absorbent after 24 hours, the highest reduction in lead concentration was obtained, 0.0965 mg/L; there was a decline in the reduction in the metal's concentration after that, falling to 0.1693 and 0.1271 after 48 and 72 hours, respectively. Figure 2a

Figure 2.b shows that,at pH 8, 10 mg of the absorbent causes a gradual increase in the reduction in lead concentration at 24 and 48 hours (0.1236 mg/L and 0.0873 mg/L, respectively), followed by a drop at 72 hours (0.1393 mg/L). When using 50 mg of the absorbent, a similar reduction in lead concentration was seen at 24 and 48 hours. After 72 hours, it continued to decline marginally. In total, 100 mg of the absorbent after 24 hours (0.0366mg/L)

**Bioadsorption of Manganese at pH (5 and 8)**

The result of the manganese concentration is shown in Figure 3a. The results show that for different pH values, contact times, and absorbent masses, the manganese concentration dramatically decreases as compared to the starting concentration. Manganese shows a progressive substantial concentration decline for pH 5 at 10 mg of the absorbent, going from an initial concentration of 1.0977 mg/L to 0.2750, 0.2191, and 0.0952 mg/L after 24, 48, and 72 hours. This is the metal's highest concentration reduction for pH 5. While the lowest amount of metal concentration was reduced at 50 mg of absorbent after 48 hours with 0.4337 mg/L

Similarly, at pH 8 with 10 mg of the absorbent, the concentration of manganese gradually decreases after 24, 48, and 72 hours (0.3252, 0.1862, and 0.1475 mg/L). However, the largest decrease in manganese concentration was seen with 100 mg of the absorbent after 24 hours with 0.0659 mg/L, and there was a subsequent decrease in the metal's concentration at 48 and 72 hours respectively (Fig. 3)

**Bioadsorption of Nickel at pH (5 and 8)**

At pH 5, the maximum reduction in nickel concentration was attained with 100 mg of the absorbent after 24 hours (0.1012 mg/L), as shown in Figure 4a. This was followed by a slight decrease in nickel reduction at the same absorbent mass after 48 hours (0.1455 mg/L), and 72 hours (0.1451), which demonstrates that there was no discernible difference in the reduction in nickel concentration after 48 and 72 hours.

The concentration of nickel at 100 mg of the absorbent decreased to 0.0713 mg/L after 24 hours at pH 8, significantly different from the initial concentration of 0.2230 mg/L. This is the largest drop in nickel concentration at pH 8 overall. After 24 hours, the nickel levels dropped by the least amount, 0.1711 mg/L (Figure 4)

**Bioadsorption of zinc at pH (5 and 8)**

The results for zinc at pH 5 are shown in Figure 5a; the highest reduction in zinc concentration at this pH was observed when 50 mg of the absorbent were applied, resulting in a reduction in concentration of 0.2254 mg/L, while the lowest reduction in zinc concentration was obtained when 10 mg of the absorbent were used, resulting in a reduction in concentration of 0.5225 mg/L. At 100 mg of the absorbent, the zinc content was dramatically reduced during all periods of contact, with a reduction of 0.2392.

The graphic depiction of zinc for pH 8 is shown in Figure 5b when the results of various absorbent masses at various contact times were compared to the initial concentration of zinc,the result indicated a decrease in the concentration of heavy metals. 0.147, 0.3900, and 0.3747 mL were recovered at 100 mg of the absorbent after 24, 48, and 72 hours, respectively. After 24, 48, and 72 hours, it was possible to achieve 0.2897, 0.2776, and 0.2672 mL at 50 mg, while at 10mg, it was possible to obtain 0.3220, 0.2663, and 0.2747 mL of metal concentration reduction.

**A B**

**Fig. 1: (A) Trend of reduction in concentration of Iron at pH 5 (B) at pH 8**

**A B**

**Fig.2: (A) Trend of reduction in concentration of Lead at pH 5 (B) at pH 8**

**A B**

**Fig.3: (A) Trend of reduction in concentration of Manganese at pH 5 (B) at pH 8**

**A B**

**Fig.4: (A) Trend of reduction in concentration of Nickel at pH 5 (B) at pH 8**

**A B**

**Fig.5: (A) Trend of reduction in concentration of Zinc at pH 5 (B) at pH 5**

**Table 2: Table of efficiency of heavy metal reduction**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Heavy metals | pH | Concentration (mg/L) | Time (hours) | Max. Efficiency (%) |
| Iron | 5 | 10 | 24, 48, 72 | 3.05, 65.37, 39.32 |
| 50 | 24, 48, 72 | 89.48, 75.89, 28.39 |
| 100 | 24, 48, 72 | 87.74, 24.92, 24.81 |
| 8 | 10 | 24, 48, 72 | 31.86, 88.87, 51.99 |
| 50 | 24, 48, 72 | 55.66, 23.89, 86.31 |
| 100 | 24, 48, 72 | 77.62, 56.58, 88.46 |
| Lead | 5 | 10 | 24, 48, 72 | 9.71, 12.56, 28.96 |
| 50 | 24, 48, 72 | 51.96, 15.27, 36.37 |
| 100 | 24, 48, 72 | 11.91, 26.38, 32.23 |
| 8 | 10 | 24, 48, 72 | 38.14, 56.31, 30.28 |
| 50 | 24, 48, 72 | 43.39, 20.87, 59.01 |
| 100 | 24, 48, 72 | 81.68, 23.57, 63.91 |
| Manganese | 5 | 10 | 24, 48, 72 | 74.95, 80.04, 91.23 |
| 50 | 24, 48, 72 | 81.09, 60.49, 0.79 |
| 100 | 24, 48, 72 | 81.19, 79.34, 82.85 |
| 8 | 10 | 24, 48, 72 | 70.37, 83.04, 86.55 |
| 50 | 24, 48, 72 | 84.29, 75.54, 75.00 |
| 100 | 24, 48, 72 | 93.99, 87.43 90.79 |
| Nickel | 5 | 10 | 24, 48, 72 | 25.61, 40.54, 40.40 |
| 50 | 24, 48, 72 | 39.37, 25.69, 37.94 |
| 100 | 24, 48, 72 | 54.62, 34.75, 34.93 |
| 8 | 10 | 24, 48, 72 | 23.27, 35.11, 40.22 |
| 50 | 24, 48 , 72 | 33.99, 31.08, 32.51 |
| 100 | 24, 48, 72 | 68.03, 33.14, 28.74 |
| Zinc | 5 | 10 | 24. 48, 72 | 32.48 12.33, 50.54 |
| 50 | 24, 48, 72 | 62.18, 18.05, 55.29 |
| 100 | 24, 48, 72 | 41.02, 57.35, 59.87 |
| 8 | 10 | 24, 48, 72 | 45.97, 55.32, 53.91 |
| 50 | 24, 48, 72 | 51.39, 53.42, 55.15 |
| 100 | 24, 48, 72 | 75.34, 34.56, 37.13 |

**DISCUSSION**

Heavy metals are refractory in the environment, poisonous, and non-biodegradable. About half of them are released into the environment in proportions that are dangerous to the environment and have a detrimental impact on human health. Anthropogenic activities are largely responsible for the release of heavy metals into the environment such as Agricultural practice, mining and pharmaceutical industries (Martin-Gonzalez *et al.,* 2006). Different convectional, advanced nanomaterials-based and biological method has been employed for the treatment of heavy metals. Among the biological method, microalgae is an important group of micro-organism that have numerous environmental application and can remediate heavy metals from contaminated water (Zohooriam *et al*., 2020). This research work aimed to reduce the health and environmental impact of these heavy metals. In this investigation, the bioabsorption ability of dried biomass of *chlorella vulgaris* to completely or partially remove the five heavy metals (iron manganese, nickel, lead, and zinc) found in water sample collected from the Reservoir was examined. Furthermore, this study explores the effect of various parameters such as contact time, pH, and biomass dosage on the bioabsoption process. From the result obtained in this study, the concentration of iron (Fe) was found to be more prevalent than the permissible limit as acceptable by WHO, which could pose a huge threat to human health and the natural environment. The concentration of iron (1.0300 mg/L) discovered, was above recommended maximum concentration 0.03 mg/L by WHO (Iyama *et al.,* 2018). The presence of these metals in higher concentration is an indicator of heavy metal contamination as reported by Mansourri and Madani, (2016). The presence of iron, manganese, lead, nickel, and zinc in the water sample indicate high level of toxicity as they have been reported to cause damages of respiratory, renal, skeletal and cardiovascular systems as well as development of cancers of the lungs, kidneys, prostate and stomach (WHO, 2011). In this study, the maximum percentage reduction obtained for iron concentration at pH5 is (1.0300 with 50mg of dried biomass of *Chlorella vulgaris* at 24) at pH 8 (1.1300, 100mg at 72hrs); Manganese at pH 5 (0.0952, 10mg at 72hrs) ,at pH 8 (0.0659,100mg at 24hrs ); Nickel at pH 5 (0.1012, 100mg at 24hrs) ,at pH 8 (0.0713, 100mg at 24hrs); lead at pH 5 (0.0965, 50mg at 24hrs), pH 8 (0.0366,100mg at 24hrs); Zinc at pH 5 (0.2252,50mg at 24hrs),at pH 8 (0.1470, 100mg at 24hrs). Comparing all these metals concentration to the initial concentration (Table 1), it can be deducted that remediation of contaminated water is possible by using *Chlorella vulgaris.* The result obtained for the bioabsorption of heavy metals in this study shows that *C. vulgaris* was efficient in the bioadsorption of most of the heavy metals identified from the water sample obtained from the Ogbomoso reservoir (Oyo state)

For the bioabsorption of iron at pH 5, *C. vulgaris* had the highest efficiency of 89.48 % at 50 mg after 24 hours, whereas at pH 8, maximum efficiency was obtained 100 mg after 72 hour (88.46 %). This result shows the bioadsorption of iron is not concentration and time dependent.

For the bioabsorption of manganese at pH 5, *C. vulgaris* had the highest efficiency of 91.23 % at 10 mg after 72 hours, whereas as at pH 8 at 100 mg after 24 hours the maximum efficiency of 93.99 % was obtained. From the obtained result, it was observed that adsorption of manganese was concentration dependent but not time dependent.

For the bioadsorption of zinc at pH 5*, C. vulgaris* had the highest efficiency of 62.18 % at 50 mg after 24 hours, whereas as at pH 8 at 100 mg after 24 hours the maximum efficiency of 75.34 % was obtained. From the obtained result, it was observed that adsorption of Zinc was concentration dependent but not time dependent because at 24 huours maxium efficiency was obtained.

For the bioadsorption of lead at pH 5, *C. vulgaris* had the highest efficiency of 30.28 % at 100 mg after 72 hours, whereas as at pH 8 at 100 mg after 24 hours the maximum efficiency of 81,68 % was obtained, this shows the impact of pH on the adsorption of lead with maximum adsorption achieved under basic environment.

For the bioadsorption of nickel at pH 5, *C. vulgaris* had the highest efficiency of 54.62 % at 100 mg after 24 hours, whereas as at pH 8 at the same condition (100 mg; 24 hours) the maximum efficiency of 68.03 % was obtained, this shows the impact of pH on the adsorption of cobalt with maximum absorption achieved under basic environment.

The result of bioabsorption of the heavy metals obtained from this study is in agreement with the work of Bahiru, (2020) who determine the concentration and toxicological implications of heavy metals six metals (lead, cadmium, zinc, copper, chromium and iron) in effluents coming out Eastern Industrial Zone (EIZ). Similarly, this study also conform with the work of Khan *et al*., (2018) who reported significant amounts of heavy metals (Cr, Cu, Ni, Pb, Zn, and Cd) in wastewater-irrigated soils and food crops in Beijing, China. Similarly Indhumathi *et al*., (2018) use *Chlorella vulgaris* for bioadsorption of arsenic, copper, chromium iron and zinc with a recovery of 90.3% under pH 7, 105 min of contact time and 20 mg/L of initial copper concentration From this present investigation, it is clear that the dried biomass of *Chlorella vulgaris* effectively removed the heavy metals found in the water sample. *Chlorella vulgaris* is an efficient microalgal for remediation of diverse water contaminants also for waste water treatment. The culture duration was 5 days, the temperature was 25°C, and the initial lead and nickel ion concentrations were taken into account as being 112 and 203 g/L, respectively. In the case of lead metal, *C. vulgaris* has a higher capacity therefore the maximum lead removal after 5 days is 94% for *C. vulgaris* and 88.3% for *N. oculata.* However, in the case of nickel, the two microalgae performed similarly, with the maximum effectiveness of nickel removal for *C. vulgaris* being recorded at 94.9% and *N. oculata* being comparable to 93.4%.Therefore, *C. vulgaris* was proven in this current investigation as an efficient phycoremediation tool for the removal of few heavy metals found in water sample collected from ogbomoso reservoir.

4. Conclusion

[*Chlorella vulgaris* is an effective phycoremediation tool used in eradicating water contaminants such as heavy metals. The ICP-MS analysis carried out in this research revealed the presence of five (5) heavy metals namely, Zinc, Iron, Manganese, Lead and Nickel. All of these contaminants get into the reservoir through several human activities which include domestic wastes, Market waste (solid/liquid), farming activities like application of fertilizer, pesticides in that region. All of these activities contributed to the high concentration of heavy metals found in the water sample obtained. Application of dry biomass of *Chlorella vulgaris* which adsorbed and reduces the concentration of all metals discovered in the water sample. Highest metal removal is manganese (99.93%), followed by iron (89.48%) the pH of the culture medium was 8 and 5 respectively, the results obtained shows the efficiency and how effective *chlorella vulgaris* is in removing heavy metals significantly on biomass concentration.

**RECOMMENDATION**

This research on evaluation and concentration of heavy metals was limited only to some metals Zinc, Iron, Manganese, Lead and Nickel in the water sample. Further research work is needed to be carried out on the concentration of other trace metals, in rainy season, and determine other conditions that were not investigated in this research work.

**Disclaimer (Artificial intelligence)**

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

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