CHANGES IN THE CONCENTRATIONS OF SIZE-FRACTIONED IRON AND RELATED ENVIRONMENTAL FACTORS IN THE NORTHEASTERN PART OF LAKE DIANCHE (CHINA)

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SUMMARY

To observe changes in the concentrations of size-fractionated iron and related environmental factors, experiments were conducted in the northeastern part of the shallow eutrophic lake Dianchi (China) from March 2003 to February 2004. Iron concentrations were measured for three size fractions: particulate iron (φ>0.22 µm), colloidal iron (φ = 0.025-0.22 µm) and soluble iron (φ < 0.025 µm), and environmental factors (physicochemical and biological factors) were synchronously analyzed. Results showed that size-fractionated iron and the related environmental factors all varied with season. Colloidal iron accounted for only 5-9% of total iron, while particulate and soluble iron each accounted for 40-50% of total iron. The results suggested that size-fractionated iron can transform into each other, especially the highly reactive colloidal iron. Significant linear correlations were found between iron in different size fractions, and significant correlations were also obtained between chlorophyll a and environmental factors, such as TN, TP and secchi depth. No significant correlation between iron and chlorophyll a was found in this study.

KEYWORDS: particulate iron, colloidal iron, soluble iron, chlorophyll a, lake Dianchi.

INTRODUCTION

In recent years, cyanobacterial blooms have been reported from a number of lakes in many countries, resulting from eutrophication of water, in which bloom-forming cyanobacterial species thrive and dominate composition of phytoplankton [1, 2]. Cyanobacteria, especially Microcystis, Anabaena and Aphanizomenon, can cause serious water pollution and public health problems, such as death of commercially cultivated fish due to decreased dissolved oxygen, production of volatile odor compounds in the drinking water supply, and contamination by toxic materials, such as microcystins [1-3].

It is known that phosphorus is a limiting factor in formation of cyanobacterial bloom in freshwater, so the concentration of phosphate in water is the key in the control of cyanobacterial blooms and the restoration of lakes and water reservoirs [4]. However, we should not ignore the effects of trace metals in the formation of algal bloom, especially iron. Iron is the fourth most abundant element in the continental crust [5], but bioavailable iron concentration is very low in water because of its low solubility. Iron is essential in the electron-transport chains in photosynthesis and respiration, reduction of nitrate and nitrite, nitrogen fixation [6], and the synthesis route of chlorophyll [7]. Recent studies on metal availability to phytoplankton have focused on iron enrichment and iron limitation in laboratory and field [8-12]. However, the understanding of iron dynamics is still hampered by the complex forms of iron. In order to solve these problems, several attempts had been made based on size separation [13]. In this study, we operationally define “soluble iron” as the <0.025 µm iron fraction, “colloidal iron” as the 0.025-0.22 µm iron fraction (calculated from <0.025 µm and <0.22 µm data), and “particulate iron” as the >0.22 µm iron fraction [14-17]. This iron size separation work was done for the first time in the northeastern part of lake Dianchi (China). It aims to explore and discuss seasonal change in size-fractionated iron and related environmental factors, correlations between different forms of iron, and relationships between iron and environmental factors in the northeastern part of lake Dianchi. We hope this first work may contribute to the control of cyanobacterial blooms in lake Dianchi.
MATERIALS AND METHODS

Sampling Sites

Lake Dianchi is located in a plateau area in the southwestern part of China, with water surface of 306 km², mean depth of 4.4 m, watershed of 2920 km², surface length (N-S) of 114 km, width (W-E) of 25.6 km on average. Its altitude is 1886 m above sea level, in a climate of subtropical, humid, monsoon type, average temperature 14.7 °C, average precipitation 797~1007 mm, and frost-free 227 days. The lake is too shallow for a distinct normal-mode thermal stratification model. It has long been a multi-functioning lake that mainly provides water for drinking and agricultural use, and is also an important habitat for fish and birds. Experimental area, consisting of Macun Bay and Haidong Bay, is located in the northeastern part of lake Dianchi with an area of 6.01 km², where fishes breed, primarily Hypophthalmichthys molitrix and Aristichthys nobilis. We used the experimental area to obtain the distribution and seasonal change in concentrations of iron and other parameters through monthly monitoring, in order to gain insight into the mechanism of cyanobacterial bloom. Water samples were collected at ten sites shown in Figure 1. Sites 1-4 are in Macun Bay and sites 5-10 in Haidong Bay.

The experiment was carried out from March 2003 to February 2004. We defined March, April and May as spring; June, July and August as summer; September, October and November as autumn; December, January and February as winter. This definition was suitable for local climate. Seasonal values shown in the text were average values of three months (mean) and standard deviation (SD).

Determination of size-fractionated iron concentration

Before sampling, polyethylene bottles were initially washed with eradicator, rinsed with distilled water, soaked for 3 days in 5% (v/v) HCl at room temperature, rinsed with Milli-Q water, soaked for 3 days in 0.5 M HNO₃, and rinsed again with Milli-Q water. In addition, 0.22 µm (Millipore, Cat. No. GSWP04700) and 0.025 µm (Millipore, Cat. No. VSWP04700) MF-Millipore membrane filters were heated at about 80-90 °C for 1 h in 3% (v/v) HCl solution, rinsed with Milli-Q water, and stored in Milli-Q water until used for sample filtration [14-17]. The 0.22 µm and 0.025 µm-filtered water samples were acidified approx. to pH 2 with 6 M HCl and stored in pre-cleaned polyethylene bottles. Concentrations of size-fractionated iron were determined by the method of Stookey [18].

Determination of biological factor (chlorophyll a)

Samples were passed through Whatman GF/C filters (Whatman, Cat. No.1822047), and the filters were ground with 90% acetone, extracted for 24 h in the refrigerator at 4 °C, and centrifuged at 3000-4000 rpm for 10 min. Contents of chlorophylla were determined spectrophotometrically at four wavelengths, 630, 645, 663 and 750 nm. Chlorophylla values were calculated using the following equation:

\[ \text{Chlorophyll a (mg/L)} = \frac{[11.64 \times (D_{663} - D_{750}) - 2.16 \times (D_{645} - D_{750}) + 0.10 \times (D_{630} - D_{750})] \times V_1}{V \times \delta} \]

In this equation, V is sample volume (mL), D is absorbency, V₁ is the last volume of extraction (mL), and δ is the width of glass cuvette (cm) [19].
Determinations of physicochemical factors

Dissolved oxygen (DO), pH and water temperature were measured with a DO-meter DO-24P and a pH-meter HM-20P (DKK-TOA Corporation, Tokyo, Japan). The other factors were determined using the methods of SPEAC as standard methods [19].

RESULTS

The seasonal change in size-fractionated iron concentrations

Total iron concentrations varied with the seasons in the two bays (Table 1, Figure 2). In Macun Bay, the descending order of total iron concentrations was summer, winter, autumn and spring. The values ranged from 0.683 mg/L to 2.358 mg/L. In Haidong Bay, the highest concentration appeared in winter rather than summer. Total iron concentrations in Haidong Bay ranged from 0.909 mg/L to 2.141 mg/L. The concentrations of total iron at sites near drainage ditches (sites 2 and 8) were higher than at other sites.

Particulate iron concentrations also varied with the seasons, and its order was the same as that of total iron concentration. The ranges of particulate iron were 0.285 mg/L-1.131 mg/L in Macun Bay and 0.373 mg/L-1.050 mg/L in Haidong Bay. Particulate iron, accounting for 40%-50% of total iron, was the dominant iron during the whole year. The higher values also appeared at the sites near drainage ditches.

### TABLE 1 - Changes in concentrations of size-fractionated iron, chemical parameters, physical parameters, and chlorophyll a with season at sampling sites in Macun Bay and Haidong Bay. Data shown are means and standard deviations (S.D.) of three months.

<table>
<thead>
<tr>
<th>Sites</th>
<th>Season</th>
<th>TFe</th>
<th>PFe</th>
<th>CFe</th>
<th>SFe</th>
<th>NO$_3^-$</th>
<th>NH$_4^+$</th>
<th>TN</th>
<th>TP</th>
<th>COD$_{Mn}$</th>
<th>DO</th>
<th>pH</th>
<th>S</th>
<th>TFe</th>
<th>Chl-a</th>
</tr>
</thead>
<tbody>
<tr>
<td>Macun Bay</td>
<td>1#</td>
<td>Spring</td>
<td>0.922</td>
<td>0.387</td>
<td>0.061</td>
<td>0.408</td>
<td>0.259</td>
<td>0.260</td>
<td>4.260</td>
<td>0.197</td>
<td>18.837</td>
<td>9.067</td>
<td>9.003</td>
<td>35.000</td>
<td>20.033</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Summer</td>
<td>1.977</td>
<td>0.954</td>
<td>0.030</td>
<td>0.028</td>
<td>0.046</td>
<td>0.062</td>
<td>0.981</td>
<td>0.040</td>
<td>3.744</td>
<td>1.082</td>
<td>0.078</td>
<td>5.000</td>
<td>3.288</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Autumn</td>
<td>1.976</td>
<td>0.952</td>
<td>0.029</td>
<td>0.029</td>
<td>0.239</td>
<td>0.762</td>
<td>2.167</td>
<td>0.112</td>
<td>21.341</td>
<td>0.147</td>
<td>0.213</td>
<td>4.660</td>
<td>7.032</td>
</tr>
<tr>
<td></td>
<td>2#</td>
<td>Winter</td>
<td>1.643</td>
<td>0.871</td>
<td>0.117</td>
<td>0.763</td>
<td>0.286</td>
<td>0.462</td>
<td>2.695</td>
<td>0.283</td>
<td>15.360</td>
<td>9.490</td>
<td>8.557</td>
<td>35.000</td>
<td>11.900</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Autumn</td>
<td>1.597</td>
<td>0.688</td>
<td>0.125</td>
<td>0.676</td>
<td>0.008</td>
<td>0.125</td>
<td>0.233</td>
<td>4.897</td>
<td>0.130</td>
<td>19.807</td>
<td>8.317</td>
<td>5.176</td>
<td>20.000</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Winter</td>
<td>1.597</td>
<td>0.688</td>
<td>0.125</td>
<td>0.676</td>
<td>0.008</td>
<td>0.125</td>
<td>0.233</td>
<td>4.897</td>
<td>0.130</td>
<td>19.807</td>
<td>8.317</td>
<td>5.176</td>
<td>20.000</td>
</tr>
</tbody>
</table>

| Haidong Bay | 2# | Spring | 0.922 | 0.387 | 0.061 | 0.408 | 0.259 | 0.260 | 4.260 | 0.197 | 18.837 | 9.067 | 9.003 | 35.000 | 20.033 | 0.198 |
| | | Summer | 1.977 | 0.954 | 0.030 | 0.028 | 0.046 | 0.062 | 0.981 | 0.040 | 3.744 | 1.082 | 0.078 | 5.000 | 3.288 | 0.098 |
| | | Autumn | 1.976 | 0.952 | 0.029 | 0.029 | 0.239 | 0.762 | 2.167 | 0.112 | 21.341 | 0.147 | 0.213 | 4.660 | 7.032 | 0.080 |
| | | Winter | 1.643 | 0.871 | 0.117 | 0.763 | 0.286 | 0.462 | 2.695 | 0.283 | 15.360 | 9.490 | 8.557 | 35.000 | 11.900 | 0.070 |

5# Spring | 0.920 | 0.384 | 0.065 | 0.414 | 0.259 | 0.460 | 2.144 | 0.177 | 15.597 | 8.087 | 8.997 | 33.333 | 19.867 | 0.119 |
| | Summer | 2.650 | 0.905 | 0.035 | 0.130 | 0.010 | 0.191 | 0.612 | 0.040 | 2.787 | 0.573 | 0.064 | 5.774 | 3.044 | 0.068 |
| | Autumn | 1.502 | 0.724 | 0.091 | 0.602 | 0.454 | 0.291 | 4.076 | 0.330 | 17.973 | 8.407 | 8.520 | 20.000 | 21.300 | 0.167 |
| | Winter | 0.649 | 0.360 | 0.031 | 0.357 | 0.724 | 0.233 | 3.129 | 0.046 | 5.110 | 0.686 | 0.213 | 3.660 | 0.400 | 0.059 |
| | Autumn | 0.963 | 0.413 | 0.076 | 0.408 | N.D. | 0.465 | 7.005 | 0.180 | 18.867 | 9.000 | 8.757 | 21.667 | 17.600 | 0.148 |
| | Winter | 0.173 | 0.903 | 0.023 | 0.074 | 0.008 | 0.843 | 3.805 | 0.101 | 4.461 | 1.000 | 0.157 | 5.774 | 3.775 | 0.047 |
| | Spring | 1.887 | 0.926 | 0.130 | 0.781 | 0.155 | 0.523 | 2.397 | 0.193 | 11.910 | 9.430 | 8.763 | 4.667 | 12.367 | 0.068 |
| | | Autumn | 0.511 | 0.198 | 0.056 | 0.259 | 0.014 | 0.025 | 0.870 | 0.070 | 3.938 | 1.103 | 0.100 | 7.638 | 2.967 | 0.082 |

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TABLE 1 – continued.

<table>
<thead>
<tr>
<th>Season</th>
<th>TFe (mg/L)</th>
<th>PFe (mg/L)</th>
<th>CFe (mg/L)</th>
<th>SFe (mg/L)</th>
<th>NO3 (mg/L)</th>
<th>NH4 (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Spring</td>
<td>Autumn</td>
<td>Summer</td>
<td>Winter</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>1.007</td>
<td>1.340</td>
<td>1.507</td>
<td>1.890</td>
<td>2.333</td>
<td>2.333</td>
</tr>
<tr>
<td>S.D.</td>
<td>0.167</td>
<td>0.199</td>
<td>0.335</td>
<td>0.714</td>
<td>0.667</td>
<td>0.667</td>
</tr>
</tbody>
</table>

**Notes:**
- TFe: total iron (mg/L); PFe: particulate iron (mg/L); CFe: colloidal iron (mg/L); SFe: soluble iron (mg/L); NO3: nitric plus nitrous nitrogen (mg/L); NH4: ammonia nitrogen (mg/L); TN: total nitrogen (mg/L); TP: total phosphorus (mg/L); COD_{bod}: dissolved oxygen (mg/L); pH; S: secchi depth (cm); Tw: water temperature (°C); Chl.a: chlorophyll a (mg/L).

**Figure 2:** Concentration changes of size-fractionated iron in different seasons at Macun Bay and Haidong Bay.

**Graph:**
- Total iron
- Particulate iron
- Colloidal iron
- Soluble iron

**Legend:**
- Macun Bay
- Haidong Bay

**Values:**
- Spring: 0.0, 1.0, 2.0, 2.5
- Summer: 0.0, 1.0, 1.5, 2.0
- Autumn: 0.0, 0.5, 1.0, 1.5
- Winter: 0.0, 0.5, 1.0, 1.5

**Seasonal Trends:**
- Spring: Concentration increases from Macun Bay to Haidong Bay.
- Summer: Concentration decreases from Macun Bay to Haidong Bay.
- Autumn: Concentration stabilizes from Macun Bay to Haidong Bay.
- Winter: Concentration decreases from Macun Bay to Haidong Bay.

**Conclusion:**
- Iron concentration varies significantly between different seasons and locations, with highest concentrations observed in Winter and lowest in Summer.

**References:**
Small colloidal iron concentrations were very low in the ten sampling sites and only accounted for 5-9% of total iron. The ranges were 0.048 mg/L-0.145 mg/L in Macun Bay and 0.063 mg/L-0.149 mg/L in Haidong Bay. Although the concentrations of colloidal iron were low, there were still seasonal changes. Interestingly, the higher values also appeared at the sites near drainage ditches.

Soluble iron concentrations were very similar to particulate iron, and accounted for 40-50% of total iron. The concentrations of soluble iron ranged between 0.307 mg/L-1.084 mg/L in Macun Bay and 0.368 mg/L-0.886 mg/L in Haidong Bay. The higher values appeared at the sites near drainage ditches in Macun Bay, but not in Haidong Bay. The values in summer and winter were all high, and no obvious seasonal changes of soluble iron were observed.

Changes in concentrations of chemical factors

Concentrations of nitrate + nitrite (NO$_3^-$+NO$_2^-$) had shown remarkable variations with the seasons, being higher in summer and lower in autumn, and almost undetectable in autumn at Haidong Bay, but at sites 4 and 6, the highest concentrations appeared in spring, and not in summer. The range of NO$_3^-$+NO$_2^-$ was N.D. (not detected) – 0.600 mg/L.

The higher concentrations of ammonium (NH$_4^+$) in Macun Bay and Haidong Bay appeared in autumn and winter, respectively. The lower concentrations of NH$_4^+$ appeared in spring in Macun Bay, except for site 2 in summer, and in summer in Haidong Bay, except for site 8 in spring. The values ranged from 0.247 mg/L to 0.761 mg/L.

Changes in total nitrogen (TN) were similar to those of NH$_4^+$, but the lower concentrations appeared in spring, except for sites 1, 3 and 8, that appeared in winter. The TN levels were found to be 1.993 mg/L-12.231 mg/L, and the highest value was observed in autumn at site 10, Haidong Bay.

Total phosphate (TP) ranged from 0.153 mg/L to 0.463 mg/L. The higher values appeared at the sites near drainage ditches, such as sites 2 and 8. In Macun Bay, the seasonal change of TP was higher in summer and lower in spring. In Haidong Bay, the changes were similar to those in Macun Bay, but the lower values at sites 5 and 8 were in winter, not in spring.

Chemical oxygen demand (COD) was slightly higher in autumn than in summer. The COD values ranged from 11.910 mg/L to 30.367 mg/L.

DO had no seasonal changes in the two bays, and the values ranged from 7.783 mg/L to 10.480 mg/L.

The pH values were higher in spring in both bays, with the exception of site 2 in summer.

Changes in physical factors and chlorophyll a

The descending order of secchi depth was generally winter, spring, autumn and summer. The higher values appeared at the sites near the center of lake Dianchi, the ranging from 15.000 cm to 41.667 cm.

Water temperature and atmospheric temperature (not presented) were very similar among the sampling sites. The ranges of water and atmospheric temperature were 11.9-21.5 °C and 5.0-25.0 °C, respectively.

The concentrations of chlorophyll a varied with the seasons between 0.061-0.336 mg/L. The ascending order is winter, spring, summer and autumn, except for sites 3, 4 and 5. The higher values appeared at the sites near drainage ditches, such as site 2.

 DISCUSSION

Size-fractionated iron and related environmental factors

Iron distribution in lakes is complex, and this complexity reflects the highly dynamic nature of lakes where inputs, mixing, and removal processes might vary on short-time scales. It is known that iron forms in lakes are mostly affected by the chemical composition of the lake water, iron inputs and removal processes, and internal recycling [20, 21]. The chemical composition of the northeastern part of lake Dianchi is shown in Table 1, and expatiated more detailed in results. Based on these results, we knew that factors are far higher than those in normal lakes, especially in summer or autumn. Data of chemical composition suggested that the northeastern part of lake Dianchi is a super-eutrophic area. Eutrophication complicates iron forms and distribution. Iron inputs included eolian dust, rain precipitation, wastewater from drainage ditches, polluted water used for life, agriculture and industry. In this study, iron concentrations in eolian dust and precipitants were not determined in lake Dianchi. In spite of the large quantity of eolian iron, it seemed that this type has a minor impact on iron behavior in the lake. The reason might be related to the low solubility and reactivity of the atmospheric dust. Similar observations were made previously in the open ocean and near-shore aquatic environments [22, 23]. Iron contribution by direct rain in lake Dianchi was relatively small, although the local annual rainfall was 797~1007 mm, because average concentrations of size-fractionated iron in Haidong Bay were higher in winter and not during the summer-rainy season. The great contributors of total and size-fractionated iron were mainly waste and polluted water, which flowed into Macun Bay and Haidong Bay through streams. There are three streams, named Dongbai-sha, Baoxiang and Maliao, in which domestic, industrial and agricultural wastewater were discharged during spring and summer, thus sampling sites 2 and 8 seriously polluting. The Spearman rank correlation test was performed between iron in different size fractions and environmental factors. Significant correlations were obtained between iron in different size fractions and TP and pH. Surprisingly, there was a significant correlation between colloidal iron, temperature.
which possibly promotes the formation of cyanobacterial bloom. But no significant correlation was obtained between iron and chlorophyll \(a\), suggesting that concentrations of size-fractionated iron have no marked influence on phytoplankton.

**Relationships between iron in different size fractions**

The redox transformations at the oxic-anoxic boundary affect the distribution of iron and its cycle in lakes because of the different solubility between ferrous and ferric one [24]. In well-oxygenated waters, ferric Fe is the stable oxidation state, and at neutral pH it forms highly insoluble oxides and hydroxides [25]. Ferrous iron is stable in anoxic waters, and in many freshwater systems it exists usually in the form of dissolved ions, but at high carbonate, sulfide, and orthophosphate concentrations it forms insoluble salts [26]. The physicochemical speciation of Fe, which profoundly influences its bioavailability, depends on the relative importance of various competing processes including adsorption-desorption, precipitation-dissolution, ion exchange, complexation-dissociation, and redox reactions. Results indicated that experimental area is very shallow and eutrophic, so it has no stratification. And under these conditions, phytoplankton grew fast accumulating on the water surface, resulting in the higher concentrations of particulate and soluble iron in water.

Of all the size fractions of iron, the concentration of small colloidal iron remained low throughout the study period. Meanwhile, the concentrations of particulate and soluble iron were high during the twelve months. These results suggested that the highly reactive colloidal iron may either form larger particles, or become soluble. In addition to controlling the solubility, the formation of colloidal and larger, more refractory iron particles provides a mechanism for removing dissolved iron and other trace metals from the water [26]. The mechanism was related to chemical/physical aggregation, physical adsorption to phytoplankton cell surfaces, and/or phytoplankton biological uptake processes [16]. The linear correlations of particulate iron against soluble iron \((R^2=0.9526, n=40)\), particulate iron against colloidal iron \((R^2=0.8605, n=40)\), and colloidal iron against soluble iron \((R^2=0.8394, n=40)\) were presented in Figures 3a-c. Such correlations indicate dynamic transformations of iron between different size fractions and the changes in size-fractioned iron varied with season.

**Relationships between chlorophyll \(a\) and physicochemical factors**

It is known that iron affects the productivity and species composition of phytoplankton in lakes [13]. The above data indicate that the sources and bioavailability of iron were so abundant that iron-limited algal growth could not

![Figure 3](image-url)

**FIGURE 3** - The linear correlation of (a) particulate iron against soluble iron \((R^2=0.9526, n=40)\), (b) particulate iron against colloidal iron \((R^2=0.8605, n=40)\), and (c) colloidal iron against soluble iron \((R^2=0.8394, n=40)\). Every seasonal concentration is an average of three months.
appear in the northeastern part of lake Dianchi. So we can come to a conclusion that the seasonal changes in chlorophyll a were mostly influenced by environmental factors in Macun Bay and Haidong Bay. The Spearman rank correlation test was performed between chlorophyll a and environmental factors. Significant correlations were observed between chlorophyll a and TN, TP, COD Mn, secchi depth and water temperature (P<0.01) (Table 2).

There are some reasons for the high chlorophyll a concentration that appeared in autumn. As light intensity of the sun in summer is very strong and the light can penetrate water very deeply, however, lake Dianchi lies in the plateau area, ultraviolet radiation is so violent possibly restraining algal growth. In addition, higher concentrations of NH₄⁺, TN and COD Mn in autumn contributed to the increase of chlorophyll a. It is known that phosphate is a limiting factor for the growth of phytoplankton, and high P concentrations may lead to high chlorophyll a levels. However, in this study higher TP and NO₃⁻+NO₂⁻ amounts were found in the northeastern part of lake Dianchi in summer, but not in autumn. We speculate that ultraviolet radiation in autumn is less than that in summer, when biomass of phytoplankton (chlorophyll a) could reach its maximal value.

In conclusion, there are many factors influencing the concentrations of size-fractioned iron, and all factors varied with season. Our results showed that different sizes of iron can transform into each other, but unexpectedly, no significant correlation was found between iron and chlorophyll a. The work was done in lake Dianchi for the first time, and more experiments need to be carried out in the future.

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