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The use of epiphytic lichen as a biomonitor on air quality, nitrogen dioxide and sulphur dioxide deposition in mab ta phut industrial estate, Rayong province

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Abstract Lichens are co-occurring organisms including fungi and algae that used as the environmental biomornitor or biomarker extensively. The results of air pollution to lichen diversity in surrounding area of Mab Ta Phut industrial estate, Rayong province was recorded. The duration of study was in October 2012 to September 2013. The kind and frequency of lichen using the frequent surveying flame (20 x 50 cm^2) on mango trees of 110 was studied by collected nitrogen dioxide and sulphur dioxide deposition using the method of atmospheric collection with the tube of passive sampling and was measured gas deposition by ion chromatography technique. Result found of 11 families, 20 genera and 26 species including the foliose lichens of 6 species and the crustose lichens of 20 species, the most lichens were in genera of Arthonia, Dirinaria, Lecanora and Physcia, and the presented lichen species of *Physcia poncinsii* Hue. and *Pyxine cocoes* (Swartz) Nyl. were highly frequency and found in all areas. The measurements showed that a value of nitrogen dioxide deposition of 0.28-5.08 ppbv and sulphur dioxide deposition of 0.52-7.60 ppbv. The analysis of correlation between lichen diversity of biomarker and gas deposition in each study area was using the correlation coefficient of Pearson value found that both of nitrogen dioxide and sulphur dioxide deposition had negatively significant correlation with lichen diversity index at 95% confidence interval ($r = -0.245$, $p < 0.05$) and ($r = -0.081$, $p < 0.05$), respectively. There were demonstrated that nitrogen dioxide and sulphur dioxide deposition increasingly that affected to lichen diversity, and there was possible used lichen as the atmospheric biomonitor.

Keywords: Lichen, Diversity, Nitrogen Dioxide, Sulphur Dioxide

Introduction

Using of lichen as a biomonitor in Mab Ta Phut area that was the method could make rapidly and could predict the health impact on human (Nimis *et al*., 2002). The lichen was one of the most sensitive organisms in nature among the different elements of biodiversity, sensitive to the chemical composition of

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environment, as well as climate change. The lichen bioindicator communities had been shown to exhibit correlation with climatic factors of an area (Duman *et al*., 2015; Bolshunova *et al*., 2017; Bajpai *et al*., 2018). All the factors influence sensitive organisms such as lichens, either directly or indirectly through modifications in their habitats, so that ecophysiology, growth, biomass, community structure and distribution in ecosystem could all change in space and time (Insarov and Insarova, 1996). The lichen had slowly growth rate, had a long life (Seminara *et al*., 2018), that were able to use as an indicator of atmospheric pollution or could use the indication of environmental condition (Ristic *et al*., 2017; Cristofolini *et al*., 2018) including to still have rapidly response to concentration level of atmospheric pollution (Szczepaniak and Biziuk, 2003) and as a bioindicator of air quality (Boutabia *et al*., 2018). The environmental monitoring that should have investigated all of chemical, physical and biological simultaneously, in order that obtained both of quantitative and quanlitative data which were acquired increasingly complete monitoring, accuracy and believable results (Banfield *et al*., 1999). The atmospheric pollution of monitoring in that area was not able to use only lichen diversity it must apply the monitoring data of atmospheric pollution in along with the area (Saipunkaew *et al*., 2005). The lichen species showed differing degree of sensitivity to air pollution, but were generally adversely affected by sulphur dioxide and nitrogen oxide (Duman *et al*., 2015) as at an industrial area (Danesh *et al*., 2011). This research that had been had biological monitoring with using lichen as a biomarker (Jayalal *et al*., 2017) and had been investigated on physical chemistry by measurement of nitrogen dioxide and sulphur dioxide deposition that used the method of passive sampling (Plaisance *et al*., 2002; Olszowski, 2007; Salem *et al*., 2009) for indicated the qualitative and quantitative data simultaneously. The study was started from October 2012 to September 2013.

Materials and methods

The research was studied lichen on the mango trees (*Mangifera indica* L.) as a common plant species in the study area. The lichen appressed to the bark, significantly profit from nutrients dissolved from the substrate and were thus affected by the bark chemistry and pH (Favero-Longo and Piervittori, 2010). The bark of mango trees had not too low pH or high pH value suitable to education on growth. To select the mango trees with a circumference more than 50 cm, with straight stems and an inclination should not exceed 5 degrees in opening area, had not the building to eclipse daylight which may influence on the growth of lichens in the study area, and to select the mango trees were not disturbed by human or destruction by herbivore (VDI, 1995). The study was explored to species and frequency of lichen richness on the stem of the mango trees as the method was applied from the Lichen Diversity (Verein Deutscher Ingenieure (VDI) method), which adjusted suitable with the study area in Thailand in accordance with Saipankaew *et al*. (2005). The method was used a survey frame of dimension of 20×50 cm^2 that divided into 10 sub-squares of dimension of 10×10 cm^2 put on the stem of the mango trees with the below edge of the survey frame was high from the ground of 100 cm (Saipankaew *et al*., 2005).

The study was classified collection of lichens sampling using the external structure such as thallus type, thallus color, and reproductive structure, etc (Jayalal *et al*., 2013). These were performed section of internal structure for spores study including used to a chemical substances of dripping test such as potassium hydroxide solution, Lugol's iodine solution and calcium hypochlorite solution (Vitikainen, 2001; Jagadeesh Ram and Sinha, 2011), and were tested chemical matters in lichens with the Thin Layer Chromatography (TLC) method in accordance with White and James (1985) and Elix (1996) methods that to classify the lichen species.

Cartography on presentation of air quality using frequency of lichens: The Air Quality Index (AQI) was determined. The mean was calculated the total lichen frequencies that found on the tree in one square according to the formula as follows:-Standard deviation of square meters, Classification was comp, uted low-high value and highest value, where i = the mango trees in each square meters, j = the square numbers of surveying, Fij = the total summation of the lichen frequencies on the surveying trees, nj = the mango trees number of surveying in each square, Sj = standard deviation, L1j, L2j = the minimum and maximum layers of air quality index with the value between L2-L1, tj = the quantity of t-square with the distribution study.

The quantitative analysis of nitrite ion (NO_2^-) and sulfate ion (SO_4^{2-}) content was recorded. The quantitative analysis of nitrogen dioxide and sulfur dioxide deposition in the air were found the quantitative analysis of nitrite ion (NO_2^-) and sulfate ion (SO_4^{2-}) contents by ion chromatography using suitable condition of device as shown in table 4.5 (Shakya, 2004; Pomphueak, 2005; Wiriya, 2008). The quantitative analysis of the correlation between lichen diversity index and the deposition of nitrogen dioxide and sulfur dioxide were recorded in each study area and usiung Pearson's correlation coefficient (Pearson Correlation: r) (Jayalal *et al*., 2017) with SPSS (Statistical Package for social Science) program for window Version 18.0.

Results

Lichen species in a total study of 22 areas were surveyed at the village in surrounding area of Mab Ta Phut industrial estate. They had been studied from February to May 2013 that explored 110 of mango trees were found the total lichens of 11 family, 20 genera and 26 species. The lichens were the foliose lichens of 4 genera and 6 species and the crustose lichens of 16 genera and 20 species. Most of the lichens were found in genus *Arthonia*, *Dirinaria*, *Lecanora* and *Physcia*. A few of the lichens were found in genus Buellia, Pyrenula and Rinodina as shown in table 1. Moreover, *Physcia poncinsii* Hue and *Pyxine cocoes* (Swartz) Nyl were also found in high frequencies and found in all study areas. Lichen species were often found and high frequencies such as *Chrysothrix xanthina* (L.) Laundon, *Arthonia tumidula* (Ach.) Ach. and *Hyperphysia adglutinata* (Florke) H. Mayrh. & Poel that were discovered in 15 study sites from the total area of 22 sites as shown in table 2. The lichens were discovered of minimum that only found in one study area and low frequencies i.e. *Anisomeridium* sp. at Ban Nong Wai Soam village and *Caloplaca* sp. Cartography on presentation of air quality in Map Ta Phut Industrial Estate area using frequencies of lichens was reported. The lichen samples and frequencies of lichens were collected on the mango trees. After finding the lichens frequencies found on each mango tree in each table, then calculated the sum of the frequencies of lichens in each table, Air Quality Index (AQI), Standard Deviation (S), Lower Limit (L1), and Upper Limit (L2) as shown in Table 3.

The isoline was calculated from the air quality index by divided into three areas which were 5.4, 10.8 and 16.2. The area of the isoline boundary of 5.4 consisted of air pollution highly and replaced it with red color as within the middle region of Map Ta Phut Industrial Estate. The area of the isoline limit of 10.8 was prospective to high up to very high. And the isoline extent was 16.2 as the expected area to comprise high air pollution and instead of yellow color that was located of most of the area in Map Ta Phut Industrial Estate as shown in figure 1.

The gas deposition by tube of passive sampling was measured. Measurement of nitrogen dioxide and sulfur dioxide deposition in the air of total study areas of 22 areas were collected air samples of dry season during April 25 to 29, 2013. In each study area was set a tube air sampling that contained in protected box with 4 tubes / area by hanging of the tube air sampling in the area for 5 days. The width values of the atmospheric quality layers of nitrogen dioxide in the study site was recorded by sequenced the air quality layers of nitrogen dioxide with dividing to 4 layers as shown in table 4.

Table 1. List of lichens on rocks as finding in Map Ta Phut area

Groups	Families	Genera	Species
Crustose	Arthoniaceae	*Arthonia*	*Arthonia antillarum*)Fée (Nyl.
			Arthonia tumidula)Ach (.Ach.
			Arthonia sp.2
		Cryptothecia	*Cryptothecia* sp.
	Chrysothricaceae	*Chrysothrix*	*Chrysothrix xanthina*) L (.Laundon
	Graphidaceae	*Graphina*	*Graphina lapidicola* Mull .Arg
		Graphis	*Graphis sp.*
		Opegrapha	*Opegrapha rufescens* Pers.
	Lecanoraceae	*Lecanora*	*Lecanora leprosa* Fee
			Lecanora sp.
	Monoblastiaceae	*Anisomeridium*	*Anisomeridium* sp.
	Physciaceae	*Buellia*	*Buellia* sp.
		Rinodina	*Rinodina* sp.
	Pyrenulaceae	*Pyrenula*	*Pyrenula* sp.
	Ramalinaceae	*Bacidia*	*Bacidia* sp.
	Roccellaceae	*Lecanographa*	*Lecanographa* sp.
	Teloschistaceae	*Caloplaca*	*Caloplaca* sp.
	Trypetheliaceae	*Laurera*	*Laurera subbenguelensis* Upreti & Ajay Singh .
		Trypethelium	*Trypethelium tropicum*) Ach (.Müll .Arg .
			Trypethelium eluteriae Spreng.
Foliose	Physciaceae	*Dirinaria*	*Dirinaria applanata*)Fee (D.D .Awasthi
			Dirinaria picta) Sw (.Schaer .ex Clem.
		Hyperphyscia	*Hyperphyscia adglutinata*)Florke (H .Mayrh .& Poel
		Physcia	*Physcia dimidiata*)Arnold (Nyl.
			Physcia poncinsii Hue.
		Pyxine	*Pyxine cocoes*)Swartz (Nyl.

Table 2. Frequencies of each lichen species in the study area

species	Frequencies of lichens in each area																					
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
A .antillarum		10	21																			
A .tumidula	10	21		7	10	9	6	5	2	1		13	1	12		19	6	1	12	1		
Anisomeridium sp.														3								
Arthonia sp.2			3					3				7				11					5	
Bacidia sp.	10			14				11							8		3			5		4
Buellia sp.			12		34		4	10	11		16		11	23								
C .xanthina	8	46	25	11	25			20	41	10	18	5	10	21			15	8		25	25	6
Caloplaca sp.																	6					
Cryptothecia sp.					7					16					32			23		18		
D .applanata	22		26	3										23			15	11	9	15		
D .picta			24	12	3		9	2	16	14	21	12	12	3		9	2	11				9
G .lapidicola							51					25				16						
G .librata					6	7	2								5			25				
H .adglutinata	46	20		5		9		12	16	9	26	7		12	17	12		15		20	16	31
L .leprosa		5		3	7	16	20		14	26	14	10	7	20	37	15				21		
L .subbenguelensis	10			16				14				26	35	8		15				22	31	
Lecanographa sp.		3	3		3							12										
Lecanora sp.	5	2	3		4			21		6	8	22					17	21	14	3		
O .rufescens	1			3					15				4								6	
P .cocoes	55	2	35	19	12	24	4	24	19	6	1	12	6	6	1	3	1	17	13	6	21	24
P .dimidiata					3	1	17		3				11			16	3					
P .poncinsii	43	17	63	32	8	20	6	16	7	27	16	35	13	17	18	17	12	45	43	22	18	47
Pyrenula sp.		3					5			14			13									
Rinodina sp.	21	14		28	19				13	11	15					18		6				
T .eluteriae	14				15					17									24	16	13	28
T .tropicum	8	6		7			20		13									15	28			22

Table 3. Frequencies of lichens, Air Quality Index, Standard Deviation, Lower Limit and Upper Limit values

Areas	**the sum of the lichen frequencies on the mango trees**										**AQI**	**S**	**L_1**	**L_2**
	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10				
Plot1	17	14	12	11	27	20	7	5	11	13	13.7	6.4	9.1	18.3
Plot2	27	20	22	15	18	5	19	21	17	11	17.5	6.1	13.1	21.9
Plot3	18	20	24	34	19	20	15	20	13	14	19.7	6.0	15.4	24.0
Plot4	10	16	15	14	37	36	2	12	18	10	17	11.2	9.0	25.0
Plot5	14	16	16	24	5	4	7	10	2	22	12	7.6	6.6	17.4
Plot6	19	9	11	12	9	19	8	3	0	5	9.5	6.2	5.1	13.9
Plot7	14	5	1	28	21	13	3	17	9	13	12.4	8.3	6.4	18.4
Plot8	8	14	16	11	9	11	14	19	20	11	13.3	4.1	10.4	16.2
Plot9	11	13	8	9	4	14	16	6	11	6	9.8	3.9	7.0	12.6
Plot10	5	8	30	18	19	26	10	5	6	2	12.9	9.7	5.9	19.9
Plot11	17	19	15	5	12	18	0	1	0	32	11.9	10.4	4.4	19.4
Plot12	26	11	12	8	16	13	5	10	16	5	12.2	6.2	7.8	16.6
Plot13	9	8	26	10	19	4	15	5	3	18	11.7	7.5	6.3	17.1
Plot14	24	10	10	25	7	15	7	20	12	14	14.4	6.6	9.7	19.1
Plot15	7	29	41	7	23	5	10	12	19	17	17	11.4	8.8	25.2
Plot16	11	0	0	3	5	18	14	10	6	0	6.7	6.3	2.2	11.2
Plot17	8	12	13	3	14	4	15	17	9	26	12.1	6.7	7.3	16.9
Plot18	3	8	13	7	3	3	17	24	14	11	10.3	6.9	5.4	15.2
Plot19	0	5	15	17	17	3	3	7	18	26	11.1	8.6	5.0	17.2
Plot20	19	8	27	36	5	5	5	0	0	0	10.5	12.6	1.5	19.5
Plot21	4	10	3	2	3	12	2	0	3	0	3.9	4.0	1.0	6.8
Plot22	0	8	7	1	3	28	23	14	28	21	13.3	11.0	5.4	21.2

Table 4. Total standard deviations, means of total number of surveyed mango trees, means of total standard deviations in the surveyed area and width of atmospheric quality layer

Standard optimization	Result of calculation
Sum of total standard deviations	167.8
means of total number of surveyed mango trees	10
means of total standard deviations in the surveyed area	7.6
width of atmospheric quality layer	5.4

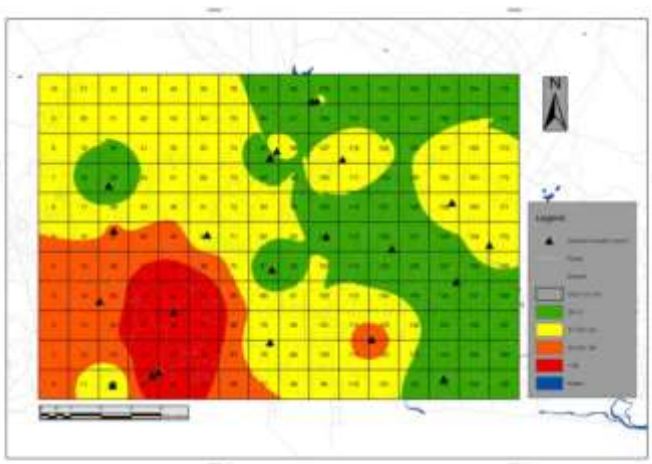

Figure 1. Air quality map by measurement of nitrogen dioxide deposition using tube of passive sampling method

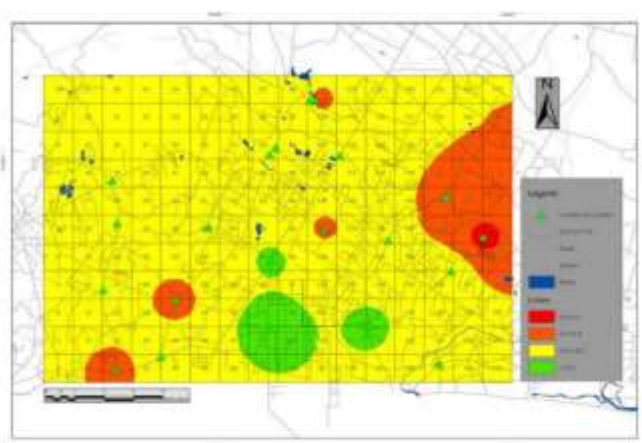

Figure 2. Air quality map using lichens as biomonitor in locality of Map Ta Phut Industrial Estate, Rayong province

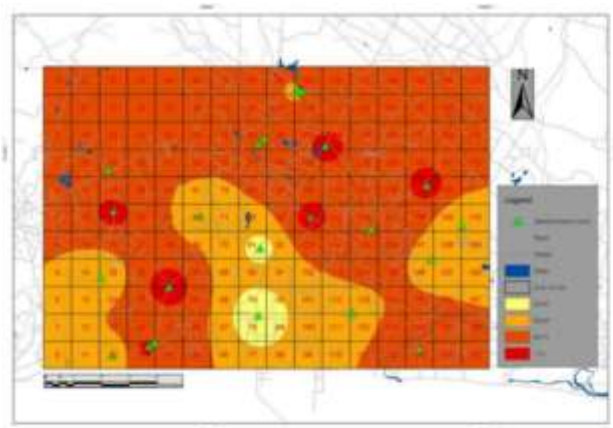

Figure 3. Air quality map by measurement of sulfur dioxide deposition using tube of passive sampling method

The comparison on the mean concentration in the air of nitrogen dioxide and sulfur dioxide in each study site by one-way ANOVA(analysis of variance) showed that the mean concentration of nitrogen dioxide in each study area was significantly different at 95% confidence intervals ($F^* = 2.818$, $p < 0.05$), similarly the mean concentration of sulfur dioxide in each study site was significantly different at 95% confidence intervals ($F^* = 12.032$, $p < 0.05$) and compared to the mean concentration of nitrogen dioxide and sulfur dioxide for examination by ANCOVA (analysis of covariance) found that the mean concentration of nitrogen dioxide and sulfur dioxide were significantly different at 95% confidence intervals ($F = 1.824$, $p <0.05$). Mean concentration of nitrogen dioxide was analyzed between the correlation and lichen diversity index in each study area using Pearson's correlation coefficient. It found that the mean concentration of nitrogen dioxide was negative correlation with the lichen diversity index significantly at the level of 95% confidence intervals ($r = - 0.245$, $p <0.05$). The 22 areas surveying were found most of common lichens for example, *Pyxine cocoes* (Swartz) Nyl and *Physcia poncinsii* Hue. that really discovered of 7 areas, next below as *Derminaria picta* (Sw.) Schaer. Clem., *Arthonia tumidula* (Ach.) Ach. and *Physcia poncinsii* Hue. The study area of plot 5 (Ban Mab Ya village) was showed the highest lichen diversity index of 2.36 and the highest lichens diversity of 9 species, next below as plot 12 (Ban Wat Sophon village), plot16 (Ban Charoen Pattana village), plot 4 (Ban Bon village) and plot10 (Ban Chak look Ya village). However, the study area at plot 15 (Ban Nhong Feab) had the lowest lichen diversity index of 1.64 and there was only 3 species of lichen diversity. As for evenness value of lichens in each area had ranged values from 0.55 to 0.72 and the similarity value of lichen was in the range of 0.43 to 0.76. The sequence of the air quality layers in study area could classify air quality of 4 layers and replaced it with different colors for observed the difference in air quality as shown in Figure 2. It was showed the areas with air pollution highly by representing with red color that only found Map Ta Phut Industrial Estate in the west direction of finding 1 area at plot 21 (in the area of Phadaeng Industrial Estate area), then plot16 (Ban Charoen Pattana village), plot 6 (Ban Pa village), plot 9 (Ban Map Ta Phut village), plot 18 (Ban Ta Auon Aao Pradu village) and plot 20 (in Map Ta Phut Industrial Estate area). These were the zones with air pollutant condition by high to very high and replaced with orange color. There were 12 areas with high air pollution in place of yellow color – namely plot 1 (Ban Nong Wai Ni Kom village), plot 5 (Ban Mab Ya village), plot 7 (Ban Wat Mab Ta Phut village), plot 8 (Ban Islam village), plot10 (Ban Chak look Ya village), plot 11 (Ban Mab Cha lood village), Plot 12 (Ban Wat So Phon village), plot 13 (Ban Nhong Nam Yen village), plot 14 (Ban Pa Yoon village), plot 17 (Ban Ruam Pattana village), plot 19 (Ban Klong Nam Hu village) and plot 22 (in the east of Map Ta Phut Industrial Estate area). These areas consisted of middle air pollution instead of green color as plot 4 (Ban bon village), plot 15 (BanNong Fab village), plot 2 (Ban Talad Huay Pong village) and plot 3 (Ban Huay Pong Nai village). The mean concentration of nitrogen dioxide in each study area was found that ranged values from 28 to 58 ppbv by the lowest measured values of 1 area at plot 1 (Ban Nong Wai Ni kom village) and the highest value of 1 area at plot 12 (Ban Wat So Phon village). While the deposition of sulfur dioxide was the mean concentration of 52 to 76 ppbv. The lowest measured values was discovered at area of plot 3 (Ban Huay Pong Nai village) and the highest value was found of 2 area at plot 6 (Ban Bon village) and plot 8 (Ban Islam village).

Discussion

The species diversity of lichens around the area of Map Ta Phut Industrial Estate at Rayong province was studied by survey of species and lichens sampling on barks of the mango trees. It was totally classified to 11 families, 20 genera and 26 species which most of common lichens found in families Physciaceae, Arthoniaceae, Trypetheliaceae and Graphidaceae. These were in accordance with the study of Saiphunkaew *et al*. (2005). The lichens of family Physciaceae was abandant in the plateau wild or the highlands of northern in Thailand. The survey of lichen diversity found that Ban Mab Ya village had the maximum of diversity index and richness of lichens, which located on the east side of Map Ta Phut Industrial Estate. While Ban Nong Faeb village had the minimum of diversity index and richness of lichens, the area was situated in the southwest of Map Ta Phut Industrial Estate. The survey of wind direction during measurement on the lichen diversity found that the waft of westward wind was away from Map Ta Phut Industrial Estate. By monitoring the deposition of nitrogen dioxide and sulfur dioxide in the atmosphere found both of nitrogen dioxide and sulfur dioxide deposition at the detection time that presented the most content of gas in the northward and showed the least content in the northwestern from Map Ta Phut Industrial Estate. When they were analyzed the data with correlation between lichen diversity index and the deposition of nitrogen dioxide and sulfur dioxide in the atmosphere of study area using Pearson's correlation coefficient, it found that there was significantly a negative correlation with lichen diversity index at the 95% confidence level ($r = -0.245$, $p < 0.05$) and ($r = -0.081$, $p < 0.05$), respectively. The research was able to conclude that the deposition of nitrogen dioxide and sulfur dioxide by affecting of lichen diversity and pH of the bark in study area. Both of nitrogen dioxide and sulfur dioxide had increased which causing the lichen diversity index value in the area was tendency to decrease possibly.

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