

ความแตกต่างของแอนไอออนอนินทรีย์ที่สะสมในไลเคน *Parmotrema tinctorum* จากทลัสต์ส่วนต่างๆ

VARIATION OF INORGANIC ANIONS ACCUMULATED IN THE LICHEN *Parmotrema tinctorum* FROM DIFFERENT SECTION OF THALLUS

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บทคัดย่อ: เปรียบเทียบความแตกต่างในการสะสมปริมาณแอนไอออนอนินทรีย์ที่บริเวณขอบของทลัสต์(thallus margin) และที่บริเวณส่วนกลางของทลัสต์ (thallus center) ของไลเคน *Parmotrema tinctorum* จากอุทยานแห่งชาติเขาใหญ่ โดยนำวิธีไอออนโครมาโทกราฟีมาใช้ในการวิเคราะห์ แอนไอออน ที่ศึกษา ได้แก่ ฟลูออไรด์ คลอไรด์ ไนไตรต์ โบรไมด์ ไนเตรต ฟอสเฟต ซัลเฟต และ ออกซาลेट โดยใช้คอลัมน์ IonPac AS12A ตัวชะคือสารผสมของ 2.7 mM โซเดียมคาร์บอเนต และ 0.3 mM โซเดียมไบคาร์บอเนต ด้วยระบบการชะแบบไอโซคราติก ตรวจวัดด้วยการวัดค่าการนำไฟฟ้าที่ใช้ระบบออโตซัพเพรสชันด้วย ASRS-300 (4 mm) ได้ทดสอบความน่าเชื่อถือของวิธีการวิเคราะห์ทางปริมาณของเจือไนโครมาโทกราฟี(method validation) ในเทอมขีดจำกัดของการตรวจวัด ความเที่ยงและความสัมพันธ์เชิงเส้น พบว่าค่าขีดจำกัดของการตรวจวัดไอออนทุกชนิดต่ำกว่า 0.27 mg/L ค่าความเที่ยง(%RSD) ในการวิเคราะห์ซ้ำ 7 ครั้งต่ำกว่า 3.14% ความสัมพันธ์เชิงเส้น (r^2) มีค่ามากกว่า 0.995 ผลการวิเคราะห์พบว่าปริมาณไอออน ฟลูออไรด์ คลอไรด์ ฟอสเฟต และ ออกซาลेट มีอยู่ที่ส่วนกลางมากกว่าที่ขอบของทลัสต์ส่วน ไนเตรต และ ซัลเฟต มีอยู่ที่ขอบมากกว่าส่วนกลางของทลัสต์ การใช้ไลเคนตรวจสอบคุณภาพอากาศจึงควรใช้ทุกส่วนของทลัสต์

Abstract: Comparison of the accumulation of inorganic anions in the lichen *Parmotrema tinctorum* inhabited Khao Yai National Park between thallus margin and central areas of thalli was studied by using ion chromatography. The studied anions were F^- , Cl^- , NO_2^- , Br^- , NO_3^- , PO_4^{3-} , SO_4^{2-} and $C_2O_4^{2-}$. The column used was IonPac AS12A. The isocratic elution using a mixture of 2.7 mM Na_2CO_3 and 0.3 mM $NaHCO_3$ was employed. The detection was autosuppression conductivity with ASRS-300 (4 mm). The chromatographic conditions were validated in terms detection limit, precision and linearity. It was found that the detection limit for all analytes were below 0.27 mg/L, and precision in seven repetitions(%RSD) were less than 3.14%. The linearity was obtained with correlation coefficient (r^2) more than 0.995. The results show that F^- , Cl^- , PO_4^{3-} , and $C_2O_4^{2-}$ were

accumulated more in the central area of thallus than the margin, whereas NO_3^- and SO_4^{2-} were opposite. Therefore, the whole part of lichen thallus should be used to monitor air quality.

Introduction: Amounts of inorganic anions in lichen tissues can be used to assess air quality because they absorbed elements directly from their surroundings by exchanging water and gas over the entire thalli. Despite lacking of roots the atmosphere and from water seeping over substrate surfaces. Thus, their tissue content largely reflects atmospheric sources of nutrients and contaminants. Therefore lichens are more sensitive to air pollution than plants and animals, which have better protective covering and selectively obtain their nutrients from the soil or from eating other organisms[1]. Several factors contribute to this sensitivity. Many elements in the air and rainfall are easily absorbed and metabolized as nutrients for growth of the lichens. Some elements are quickly absorbed and immediately leach out of the lichens, whereas others can be concentrated, or bioaccumulate, in lichen tissues[2]. The older part of lichen thalli, central areas, which has longer exposure to the environment should accumulate more elements than those from the young margin areas. This study was designed to test the hypothesis that the old central area of lichen thallus accumulated more elements from the environment than the young thallus margin. The lichen *Parmotrema tinctorum*, which commonly found in every forest type at Khao Yai National Park was used as the study material (Figure 1).

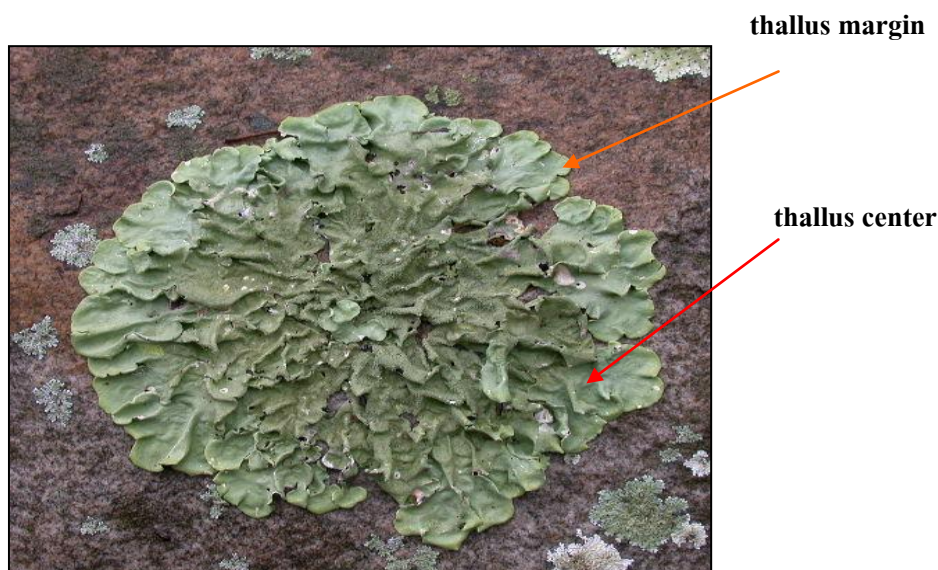


Figure 1. The lichen *Parmotrema tinctorum* commonly inhabits every forest type of Khao Yai National Park.

Methodology: The lichen *Parmotrema tinctorum* were collected from Khao Yai National Park on January 2009. Analysis of anions were performed by the following procedures. A DX500 IC system (Dionex, Sunnyvale, CA, USA) was used in this experiment. It consist of a quaternary gradient pump (GP50), LC20 chromatography enclosure equipped with a Rheodyne Model 9126 injection valve with 50 μL sample loops, CD20 conductivity detector with an ASRS-300 (4 mm) suppression, a Peaknet 5.1 chromatography workstation was used for instrument control, data collection and processing. The IonPacAS12A analytical column (250 x 4 mm I.D.) coupled with IonPac AG12A guard column (50 x 4 mm I.D.) was used for analysis, the eluent was a mixture of 2.7

mM Na₂CO₃ and 0.3 mM NaHCO₃ at flow rate 1.0 ml/min [3]. Eluents and all standard solutions were prepared by using deionized water which has specific resistance > 18.0 MΩ-cm. It was prepared by an Easy Pure RF Compact ultrapure water system. The mixture of standard ions for analyses were prepared daily from stock standard solutions of each ion. Lichen samples were cleaned from dust and soil. Thalli at the center were separated from the margin, and ground both portions in liquid nitrogen. The grounded lichen samples of 0.1 g were weighted in polyethylene bottles, 10 ml of DI water were added and then sonicated in an ultrasonic bath for 10 minutes. The sonicated samples were filtered and subsequently cleaned up by passing through SPE ODS cartridges to eliminate organic contaminations. Through the 0.2 μm nylon membrane the samples were filtered. The filtrates were kept in polyethylene bottles at 4°C until analysis.

Results, Discussion and Conclusion: Optimized chromatographic conditions by using a mixture of 2.7 mM Na₂CO₃ and 0.3 mM NaHCO₃ as eluent were validated in terms detection limit, precision and linearity. The results showed the reliability of the IC method. The method validation data was shown in table 1. Analysis of the lichen samples demonstrates the central thallus contained larger amount of F⁻, Cl⁻, PO₄³⁻, and C₂O₄²⁻ than the margin, except NO₃⁻ and SO₄²⁻ (Table 2). They were significantly difference at 95% confident limit for all ions, except NO₂⁻ and Br⁻, which could not be detected. The central part of thallus, which was older should accumulate more ions than the young thallus margin, unless the ions are metabolized by plants. Lower amount of NO₃⁻ and SO₄²⁻ at central thallus implied that these ions may be transferred to the young active area of the thallus at the margin, which the lichens utilized them as nutrients.

Table 1. The validation data of inorganic anions on ion chromatographic method

Ions	LOD(mg/L)	Precision (%RSD) peak area	Conc. Range	R ²
Fluoride	0.009	0.513	0.15 - 1.75	0.9997
Chloride	0.019	2.392	0.25 - 2.50	0.9951
Nitrite	0.034	0.861	1.20 - 13.00	0.9980
Bromide	0.023	3.139	1.20 - 13.00	0.9985
Nitrate	0.272	1.098	0.80 - 8.00	0.9983
Phosphate	0.100	1.892	1.20 - 13.00	0.9994
Sulfate	0.033	0.718	1.20 - 13.00	0.9981
Oxalate	0.140	1.896	1.20 - 13.00	0.9993

Table 2. The amount of inorganic anions in lichen thallus margin and thallus center in $\mu\text{g/g}$ (N = 5)

Ions	$\bar{x} \pm SD$	
	Margin	Center
Fluoride	0.581 ± 0.001	0.972 ± 0.214
Chloride	1.500 ± 0.006	1.856 ± 0.359
Nitrite	N.D.	N.D.
Bromide	N.D.	N.D.
Nitrate	0.616 ± 0.023	0.539 ± 0.036
Phosphate	0.916 ± 0.009	1.593 ± 0.953
Sulfate	2.126 ± 0.005	1.542 ± 0.160
Oxalate	3.164 ± 0.025	4.235 ± 0.806

In conclusion, two main points can be drawn from this experiment. Firstly, differences in amounts of anions accumulated at the center and margin of lichen thallus may responsible for variation of ions accumulated in lichens that report elsewhere. Secondly, whole part of lichen thallus should be used to determine the amount of ions to assess air quality.

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Keywords: Ion chromatography, Inorganic anions, Lichen *Parmotrema tinctorum*, Thallus margin , Thallus center

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