

Comparison of moss bag and native moss technique in monitoring airborne particulate and toxic elements

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ABSTRACT

Introduction: In Vietnam, the government has invested in monitoring stations in a few big cities like Hanoi and Ho Chi Minh City, which have transportation centers and industrial zones, to assess and predict levels of air pollution. However, the main disadvantage of installing monitoring stations is the cost of investment for operations, maintenance, and equipment. It is also time-consuming to collect and analyze the results. Therefore, it is generally not suitable for the country as a whole.

Methods: Using mosses to monitor air quality brings qualitative and quantitative data with simple, environmentally-friendly economic methods. Mosses have particular biological characteristics that make them very suitable adsorbents for a wide variety of chemical elements. When used as transplants like moss bags, allow them to monitor a highly dense sampling network of any site easily. Mosses are bioindicators, plants with artificial roots. **Results:** In this study, moss bag and native moss were the two methods used to evaluate the accumulation of trace elements in air through *Barbula Indica*. Observations showed that both methods could detect the same elements: Al, Si, P, S, Ca, Ti, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, Br, Rb, Y, Sb, Ba, Pb, and U. However, the accumulation of the elements in native moss is higher than in moss bag. The main reason is that the absorption efficiency of native moss in air-deposited elements is higher than in moss bags. **Conclusion:** Moss bags have been used most extensively and successfully in urban areas, where vegetation samples are either unobtainable or are poorly located to the source. These areas can lack moss, or the native moss simply does not grow during the dry season.

Key words: Air monitoring, air pollution, *Barbula Indica*, moss bag, native moss

INTRODUCTION

Biomonitor represents a good solution for air quality monitoring. Vuković, *et al.*¹ has shown that bioindicator monitoring is a simple and cost-effective method to evaluate pollutants in the atmosphere. Biomonitor organisms like lichens and moss can be considered bioindicators or bio accumulators.

The moss bag technique was introduced by Goodman and Roberts². Since then, it has been a valuable technique for detecting airborne contaminants, such as heavy metals and nonmetals.

The moss bag technique is helpful in doing a detailed survey of polluted urban areas, where moss cannot grow naturally³. Moreover, this technique does not require maintenance or a source of electricity to continue operation. Another essential difference between the moss bag technique and instrumental measurements is sampling time⁴. Instrumental measurements are usually done in a short period, providing daily pollutant concentrations. On the other hand, Moss bags play a long-term role in the collection of statistical pollutants. Long-term sampling is a prerequisite for assessing the cumulative exposure to a given

contaminant and its harmful effect on human health. Since 2014, Vietnam has participated in projects with European countries to investigate environmental pollution through metal deposition in the air. Together, they have developed research directions on air pollution on the moss indicator. Initially, works related to air pollution by the *Barbula Indica* technique were published⁵⁻⁸.

In this study, two methods are used: native and moss bag. The method of detection is Total Reflection X-ray Fluorescence (TXRF), a multi-element analysis technique. It is used to determine qualitative and quantitative elements from the air in the town of Lac Duong.

MATERIALS — METHODS

Sampling area

Barbula Indica was used in native moss and moss bag. The time of the survey was from the beginning of November, 2020 to February, 2021. The research was conducted in Lac Duong, Lamdong, Vietnam. Moss bag exposure was at 2 meters high, the same height as collected fresh moss. **Table 1**, and **Figure 1** show the sampling area.

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History

- Received: 2021-03-13
- Accepted: 2021-05-05
- Published: 2021-05-15

DOI : 10.32508/stdj.v24i2.2531



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Cite this article : Sang N T M, Khiem L H, Son N A. **Comparison of moss bag and native moss technique in monitoring airborne particulate and toxic elements** . *Sci. Tech. Dev. J.*; 24(2):1967-1974.

Table 1: The local moss samples network

No.	Sampling site	Place	Latitude	Longitude
1	LD01	Lac Duong	12.003704	108.412293
2	LD02	Top of Langbiang moutain	12.046528	108.440146
3	LD03	Langbiang tourist area	12.020075	108.419331
4	LD04	Strawbery farm, Lac Duong	12.000362	108.434013
5	LD05	Van Xuan - DT723	12.020963	108.443454



In this study, 5 sites in Lac Duong were selected to evaluate the effects of different possible pollution sources, specifically:

- Location LD01: the gateway to Lac Duong, which is adjacent to the tourist city of Da Lat. Here, many vehicles are travelling to the next popular tourist destination, the top of Langbian mountain.
- Location LD02: the peak of Langbian mountain, a famous tourist destination of Lac Duong, with an altitude of 2167 m above sea level. From this peak, one can evaluate the diffusion ability of chemical elements in the air in Lac Duong from different sources of pollution, such as agriculture and transportation.
- Location: LD03, the tourist area at the bottom of Langbian mountain, with many vehicles and a few brocade handicraft villages.

- Location LD04: a place of pure agricultural production, including high-tech farming and strawberry farms.
- Location LD05: a new, uninhabited, residential area.

Native moss preparation

After collection, only the green part of the moss is used for analysis. Therefore, Moss must be handled carefully, avoiding contamination from soil or rock, or other plants. In order to ensure the exact sampling location and minimize the external impact on the collected moss, it is necessary to prepare gloves and zip bags and use GPS to determine the location. Moss sampling points are selected according to pollution sources and clean areas, with a 3 to 5 kilometer distance between the nearest two sampling points. Moss samples are treated before being put into experimental measurements. The process of treating moss










No.	Describe	Picture	No.	Describe	Picture
1	<ul style="list-style-type: none"> - Determine the type of moss before sampling, taking care not to confuse different types of moss. - Determine the coordinates and sampling location. - Create a specific description of the surrounding area. 		6	<ul style="list-style-type: none"> - After washing 2-3 times with normal water, soak the sample in distilled water. - In a glass, water will have removed one part of the soil and rock, so the water is clearer. - Continue to pick out the mosses to obtain the cleanest sample. - Clean samples thoroughly by washing with distilled water 4 times. 	
2	<p>Tools for sample handling:</p> <ul style="list-style-type: none"> - Glass cups for washing samples. - Pan top off, or use the plastic pan to avoid unwanted impact. - Glass dish for storing samples. - Filter paper to dry samples. 		7	<ul style="list-style-type: none"> - After soaking in distilled water, take out the filter paper and let dry. 	
3	<ul style="list-style-type: none"> - Cut moss, taking only the stem and leaves. - Use scissors to cut more samples easily. 		8	<ul style="list-style-type: none"> - Place all dried samples in the oven at 30 °C. - Dry for 3-4 days. 	
4	<ul style="list-style-type: none"> - Sample after removing the roots, taking only the green part. 		9	<ul style="list-style-type: none"> - After drying, put the sample in a weight-weight zip bag for storage. 	
5	<ul style="list-style-type: none"> - Wash the sample with water. - When putting the sample in the cup to wash, stir the soil and let it settle on the bottom. - Then transfer the algae sample to a glass dish or some other glass container. 				

Figure 2: The process of sample treatment.

samples (from collection to drying) is shown below in Figure 2.

Moss bag preparation

The studies show that the selection of moss species in the algae bag technique depends on the degree of deformation of the type of algae in the study area and the ability of the algae to absorb and adapt to pollution in that area⁹. The composition of the chemical elements in old shoots and young buds of moss is different¹⁰, and the absorption of the moss bags is also different from that of fresh ones¹¹. In this study, *Barbula Indica* moss was selected as a biological indicator in a moss bag. The steps were as follows:

- First, *Barbula Indica* moss is chosen in a definite area, less affected by pollution, like moss in the mountains of Dung K'No, belonging to Bidoup Nui Ba National Park (Lam Dong, Vietnam). The location of collecting a moss sample has longitude 12.188447, latitude 108.463527. Many mosses are deep in the primary forest.
- After collecting the moss, perform the steps from 1 to 8 in Figure 3.
- Then, put and spear moss - natural moss collection samples in a bag.



Figure 3: Moss bag.

TXRF technique

This study used a microwave digestion digester MARS 6 (Figure 4) to prepare samples in TXRF technique and homogenize the moss sample. The manipulations were carried out on the MARS 6 microwave digestion machine for moss samples: mix 0.5 g of the dried moss with 10 ml of HNO₃ (Merck) (65%) in the digestion flask. The digestion time includes 15 minutes for heat-

ing to 220 °C, 25 minutes for annealing, then natural cooling to room temperature. After finishing the microwave digestion, the moss was completely liquefied.



Figure 4: MARS 6 microwave digestion.

For analysis on TXRF, the liquefied moss requires an internal standard solution. In this study, the internal standard of Galium was used, with a concentration of 1 ppm.

To create a uniform surface for the sample, 10 µl of silicone should be applied to the sample carrier (plate), then dried at 40 °C for 20 minutes. The specimen carrier used is a quartz glass disc, which has many advantages: high purity, low background, and easy cleaning¹². After the dish is dried, add 10 µl of the sample solution to the measured center of the dish - the diameter of the drop should not exceed 10 mm - and continue to dry at 35 °C for 40 minutes.

In addition to the size requirement for the diameter, the droplet, after being dripped onto the sample carrier and dried, must also satisfy the thickness requirement - namely, not to exceed 100 µm (Figure 5).

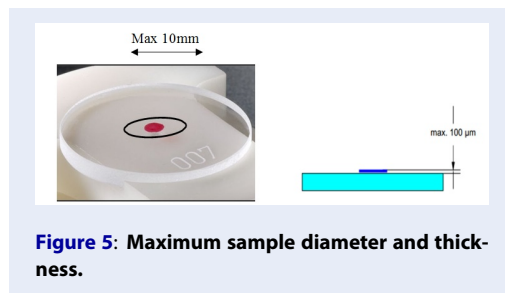


Figure 5: Maximum sample diameter and thickness.

After the sample preparation, continue to place the carriers' sample on the dryer to allow the sample to

dry completely. The measuring system in the TXRF analysis is a S2 PICOFOX™ device. Steps to create moss samples for the TXRF measurement method can be shown in Figure 6.

No.	Steps	Picture
1	Pipette 1.35 ml of homogeneous moss sample solution into the sample flask.	
2	Add 0.15 ml of the internal standard Galium 10 ppm together with the moss sample.	
3	Sample homogenization with the internal standard of Galium.	
4	Drop 10 µl of the sample onto a quartz carrier.	
5	Dry the quartz carrier at 40 °C with a dryer.	
6	Put the test sample in the sample changer cassette.	

Figure 6: Steps to create moss samples for TXRF.

TXRF Type S2 PICOFOX™ is a semi-automatic analytical system, qualitative and quantitative analysis of multiple elements, detection threshold to ppb (µg/kg), wide-range analysis of elements from Al to U. Creation of the system includes: X-ray tube (Molipden target), emitted 17.5 keV energy, working at a voltage of 50 kV, current of 1000 µA. The single-function filter is a multilayer crystal made of copper metal. X-ray acquisition detector is a semiconductor detector of SDD type. The system consists of the main components shown in Figure 7.

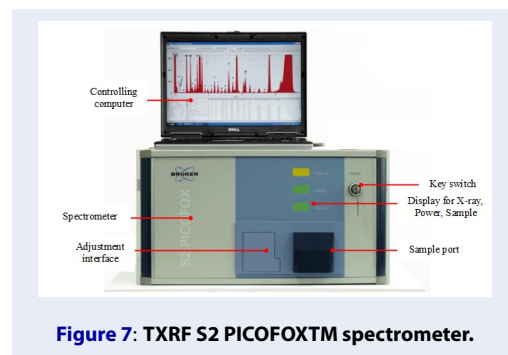


Figure 7: TXRF S2 PICOFOX™ spectrometer.

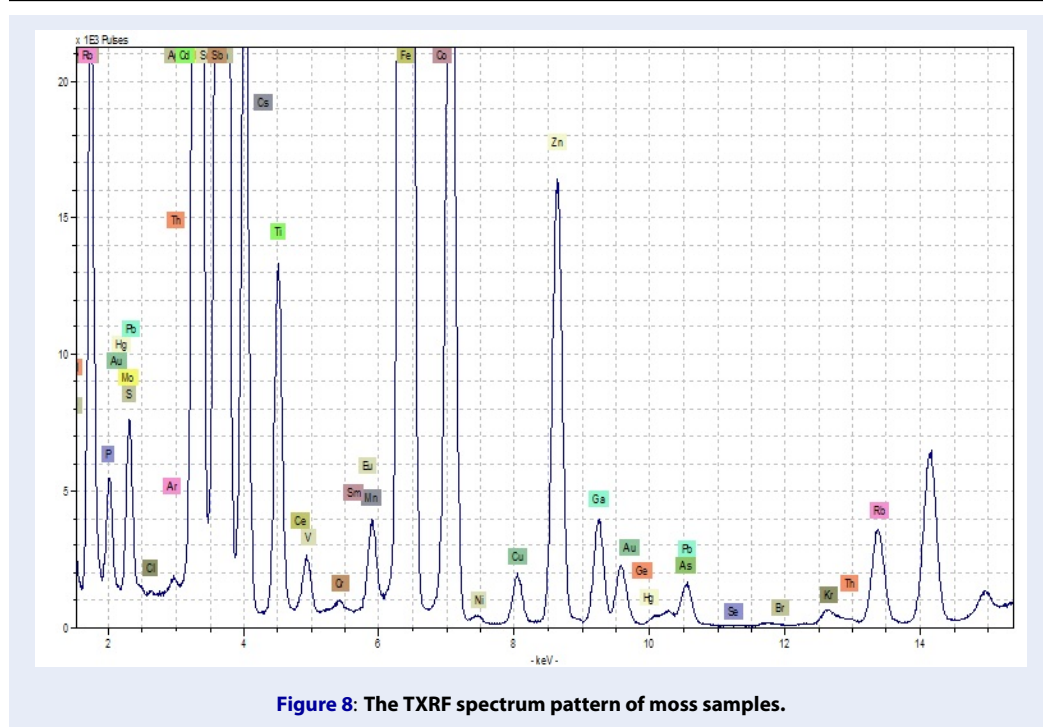


Figure 8: The TXRF spectrum pattern of moss samples.

RESULTS

TXRF measurement samples were conducted on an S2 PICOFOXTM spectrometer. The measuring time was 600 seconds per sample. The fit quality is a statistical parameter, which allows conclusions about the quality of the deconvolution. The standardized square sum of the differences between the measured and the calculated, deconvoluted intensities is calculated for all channels. Preferably, the value for the fit quality should be less than 10. High values (>10) indicate misidentified or non-identified elements respectively for an inaccurate gain correction. The following function is used to fit the following equation:

$$\chi^2 = \frac{1}{n_2 - n_1} \sum_{i=n_1}^{n_2} \frac{1}{\delta_i^2} (y_{i+1} - y_i)^2 \quad (1)$$

where n_1 is the first channel of the peak i (the left channel); n_2 is the end channel of the peak i (the right channel); y_{i+1} the counts of channel $i+1$; y_i the counts of channel i .

$$\delta_i = \sqrt{N_i + 2N_{BG}} \quad (2)$$

where δ_i is the standard deviation for the peak area; N_i is net peak area for the element i ; N_{BG} is the background area.

The TXRF method has identified 21 elements, including Al, Si, P, S, Ca, Ti, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, Br, Rb, Y, Sb, Ba, Pb, and U. **Figure 5**, **Table 2** and **Table 3** present the measurement results.

DISCUSSION

The chemical elements deposited in the air were conducted by two methods: native moss and active moss in Lac Duong town (Lam Dong Province). The results showed that:

- Using TXRF analyze system, both methods have similar chemical elements;
- For the most part, the concentrations of deposited elements in the air measured by the moss bag are much smaller than with the fresh one. This illustrates that the absorption efficiency of native moss in air-deposited elements is higher than in moss bag. However, the result also shows that some elements such as Si, Ca, Cu, Zn, Br, and U detected by the moss bag technique have approximate values. Moreover, some of these values are slightly higher than using native moss methods. The reason for this is that elements from the earth's crust can influence these elements: the moss bag can be filled with dust from the street (elements Si, Ca, U). The remaining elements can be evaporated from agrochemicals, such as fertilizers and pesticides (elements: Cu, Zn, Br);
- The concentration of elements deposited in the air in Lac Duong was compared with a previously published paper in Hanoi, Hochiminh,

Table 2: The concentration of chemical elements in the native moss (mg/kg)

No.	Sam- ple Ele.	LD01		LD02		LD03		LD04		LD05		Mean
		Con.	±Δ	Con.	±Δ	Con.	±Δ	Con.	±Δ	Con.	±Δ	
1	Al	1837	74	1870	75	4031	161	1773	71	1442	58	2191
2	Si	2825	113	4585	183	9970	399	4700	188	3698	148	5156
3	P	287	12	922	37	738	30	520	21	531	21	600
4	S	482	19	1216	49	1286	52	896	36	1090	44	994
5	Ca	781	31	742	30	763	31	770	31	714	29	754
6	Ti	107	4	122	5	194	8	206	8	105	4	147
7	V	6.86	0.37	8.45	0.44	6.69	0.37	10.82	0.53	4.93	0.30	7.55
8	Cr	5.19	0.31	5.37	0.31	10.38	0.52	11.18	0.55	5.10	0.30	7.44
9	Mn	121.00	4.96	61.00	2.53	89.00	3.66	51.00	2.14	53.00	2.21	75.00
10	Fe	3955	158	2400	96	4456	178	2565	103	1928	77	3061
11	Co	1.39	0.16	0.70	0.13	1.24	0.15	0.57	0.12	0.58	0.12	0.90
12	Ni	0.95	0.14	1.28	0.15	2.66	0.21	1.88	0.18	0.76	0.13	1.51
13	Cu	10.86	0.53	10.51	0.52	14.33	0.67	5.95	0.34	9.49	0.48	10.23
14	Zn	76	3	188	8	155	6	52	2	138	6	122
15	Br	2.99	0.22	2.05	0.18	6.05	0.34	2.27	0.19	2.31	0.19	3.13
16	Rb	24.11	1.06	6.86	0.37	15.66	0.73	16.90	0.78	12.23	0.59	15.15
17	Y	24.82	1.09	1.13	0.15	1.72	0.17	6.93	0.38	0.69	0.13	7.06
18	Sb	0.33	0.11	0.07	0.10	0.38	0.12	0.04	0.10	0.14	0.11	0.19
19	Ba	2.22	0.19	23.39	1.04	20.05	0.90	3.17	0.23	21.44	0.96	14.05
20	Pb	4.77	0.29	5.49	0.32	6.81	0.37	4.68	0.29	2.83	0.21	4.92
21	U	3.12	0.27	1.67	0.15	1.21	0.11	1.47	0.13	1.95	0.17	1.88

Ele.: Elements

Con.: Concentration

Hoi An and Hue city (T. T. Doan Phan et al, 2018; Khiem L. H. et al., 2020). The results showed that the concentration of deposition elements is very low in Lac Duong, which is not affected much by traffic, agriculture and industry.

CONCLUSION

This study used moss bags and native moss to investigate the deposition of chemical elements in the air in Lac Duong town. The results of analysis by TXRF technique identified 21 elements. The results showed that the concentration of element deposition in the Lac Duong atmosphere was quite low compared to other studies in a few big cities in Vietnam.

This method can be applied through the moss bag technique when making biological observations to survey the air-deposited elements in the urban area – where there is a lack of native moss, or where native moss is difficult to grow. Because of low cost, this technique is also suitable for the assessment of air quality, regardless of environment, location, or topography. Additionally, it is very convenient to build environmental monitoring networks to have an overview of future environmental change.

COMPETING INTERESTS

The authors commit that they have no competing interests.

Table 3: The concentration of chemical elements in the active moss (mg/kg)

No.	Sam- ple Ele.	LD01		LD02		LD03		LD04		LD05		Mean
		Con.	±△	Con.	±△	Con.	±△	Con.	±△	Con.	±△	
1	Al	1580	110	1613	113	3363	235	1327	93	1277	89	1832
2	Si	2995	170	5335	276	10831	580	4522	245	4284	229	5593
3	P	227	17	731	55	566	43	357	27	432	33	462
4	S	448	29	1134	73	1160	75	725	47	1044	67	902
5	Ca	843	47	804	45	799	44	723	40	794	44	793
6	Ti	79	6	91	7	139	11	133	11	80	6	104
7	V	4.64	0.41	5.73	0.51	4.39	0.39	6.36	0.56	3.43	0.30	4.91
8	Cr	4.81	0.31	4.99	0.32	9.32	0.60	9.01	0.58	4.86	0.32	6.60
9	Mn	106	7	54	4	76	5	39	3	48	3	65
10	Fe	3000	237	1826	144	3279	259	1693	134	1506	119	2261
11	Co	1.30	0.08	0.66	0.04	1.13	0.07	0.47	0.03	0.56	0.04	0.82
12	Ni	0.83	0.06	1.13	0.08	2.27	0.15	1.44	0.10	0.69	0.05	1.27
13	Cu	11.79	0.65	11.45	0.63	15.10	0.83	5.62	0.31	10.62	0.59	10.91
14	Zn	81	5	200	11	159	9	48	3	151	9	128
15	Br	3.55	0.18	2.44	0.12	6.98	0.35	2.35	0.12	2.83	0.14	3.63
16	Rb	16.30	1.45	4.65	0.41	10.27	0.91	9.94	0.88	8.52	0.76	9.93
17	Y	16.38	1.49	0.75	0.07	1.10	0.10	3.98	0.36	0.47	0.04	4.54
18	Sb	0.18	0.02	0.04	0.00	0.20	0.02	0.02	0.00	0.08	0.01	0.10
19	Ba	2.19	0.13	23.13	1.41	19.18	1.17	2.72	0.17	21.77	1.32	13.80
20	Pb	3.75	0.29	4.33	0.33	5.19	0.40	3.20	0.24	2.29	0.17	3.75
21	U	3.39	0.19	1.82	0.10	1.27	0.07	1.39	0.08	2.18	0.12	2.01

Ele.: Elements

Con.: Concentration

ACKNOWLEDGEMENTS

This research is supported by Dalat University under the project from 2021.

AUTHORS CONTRIBUTIONS

L. H. Khiem proposed the experimental plan, implemented the experiment. N. A. Son, N. T. M. Sang performed the experiments and literature review, compiled the data and manuscript preparation.

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