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DIMINUTIONS OF FLUORINE CONTENTS IN LICHENS DUE TO A REGRESSION OF POLLUTION IN AN ALPINE VALLEY (MAURIENNE, SAVOIE, FRANCE) from 1975 to 1985

by Gladys BELANDRIA,* Juliette ASTA * and Jean Pierre GARREC**

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RESUME.- L'analyse du fluor a été effectuée dans plusieurs espèces de lichens récoltés durant 11 années (1975 à 1985) dans la vallée de l'Arc (Maurienne, Savoie, France). On observe une diminution générale de la concentration en fluor des lichens en particulier de 54 à 21 ppm chez les Parméliacées, de 106 à 12 ppm chez *Peltigera canina* et de 124 à 44 ppm chez *Umbilicaria cylindrica*. Nous mettons en évidence que cette diminution de la concentration en fluor des lichens reflète bien les variations du niveau de pollution fluorée de l'atmosphère entre 1975 et 1985. Nous confirmons que la teneur en fluor des Parméliacées et de *Peltigera canina* est respectivement plus élevée que celle des lichens fruticuleux filamenteux et que celle de *Cladonia* gr. *pyxidata*, que la teneur en fluor des lichens est moins élevée dans les stations éloignées des sources de pollution mais dépend aussi beaucoup des courants aériens dans la vallée. Nous constatons également que les concentrations en fluor des supports (écorce et sol) sont toujours plus élevées que celles des lichens. Chez *Usnea muricata*, *Peltigera canina* et *Cladonia* gr. *pyxidata*, la concentration en fluor mesurée en 1985 ne dépasse pas celle des mêmes espèces analysées en régions non contaminées mais la difficulté d'obtenir des valeurs de référence rigoureuse est discutée.

MOTS-CLES.- Maurienne, vallée alpine, pollution fluorée, lichen, bioaccumulateur.

SUMMARY.- Fluorine analyses were made for different species of lichens collected in the course of 11 years (from 1975 to 1985) in the Arc valley (Maurienne, Savoie, France). A general decrease of fluorine contents in lichens was observed for instance from 54 to 21 ppm for *Parmeliaceae*, from 106 to 12 ppm for *Peltigera canina* and from 124 to 44 ppm for *Umbilicaria cylindrica*. This decrease of the fluorine contents for lichens reveals perfectly well the variations of the level of fluorine pollution for the atmosphere from 1975 to 1985. We confirm here that the fluorine contents of *Parmeliaceae* and of *Peltigera canina* are respectively higher than those of fruticulous filamentous lichens and of those of *Cladonia* of the *pyxidata* group, that the fluorine contents of lichens are lower in places distant from the sources of pollution, but depend also very much on the air currents in the valley. Fluorine contents of substrates (bark and ground), are always higher than those of the lichens. For *Usnea muricata*, *Peltigera canina* and *Cladonia* gr. *pyxidata*, the fluorine contents measured in 1985 in that valley are not higher than those found for lichens of the same kinds living in places where there is no fluoride contamination, but the difficulty to obtain rigourous reference data is discussed.

KEYS-WORDS.- Maurienne, alpine valley, fluoride pollution, lichen, bioaccumulator.

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INTRODUCTION AND OBJECTIVE OF THE PRESENT WORK

The use of lichens as pollution indicators for acid pollution (especially SO_2) was dealt with in many papers (FERRY, BADDELEY and HAWKSWORTH, 1973; KERSHAW, 1985). But the part played by these plants in the detection of fluoride pollution has been much less studied. Nevertheless certain studies concern the action of fluor on the morphology, the anatomy, the cytology or the physiology of lichens (SCHÖNBECK, 1969; LEBLANC, COMEAU & RAO, 1971; NASH III, 1971; COMEAU & LEBLANC, 1972; BÖRTITZ & RANFT, 1972; DE WIT, 1976; STEUBING, 1977; SWIEBODA & KALEMBA, 1978; CLERC & ROH, 1979 & 1980; HOLOPAINEN, 1986). Other investigators were interested by the aptitude to accumulate fluorine in the thalli; they made isopollution maps or studied the impact of pollution on lichenic vegetation (MARTIN & JACQUARD, 1968; GILBERT, 1971 & 1985; AUREAU & BEDENEAU, 1974; ROBERTS & THOMPSON, 1980; PERKINS & *al*., 1980, 1987 a & b; OLECH & *al*., 1981 a & b; BATIC & MARTINCIN, 1982; DAVIES, 1982 & 1986).

In the French Alps, studies concerning the impact of fluoride pollution on lichens were carried on more and more actively, especially after 1975 (ASTA, 1980 & 1981; ASTA & GARREC, 1980; BELANDRIA, 1986; BELANDRIA & ASTA, 1987). These studies were made in three alpine valleys (Romanche valley, Arly valley, Maurienne) where fluoride pollution is due to the presence of aluminium factories. Two reasons made us choose the Arc valley (Maurienne) to carry on research work (ASTA & GARREC, 1980) : the first one is the presence in the Arc valley of an atmospheric fluoride pollution, due to the proximity of three aluminium factories; the second one is the fact that extensive work concerning the impact of this pollution on phanerogamic vegetation was made there (GARREC & BATTAIL, 1976).

The classical methods of pollution cartography made with the use of lichens did not appear to us to be adaptable to the present investigations, on account of the most different characters of the environment. Indeed it was impossible to use the sensivity scale of HAWKSWORTH & ROSE (1970) adapted to pollution by SO_2, nor the IAP method, which can be applied to areas showing only slight topographical differences and in which can be found several "homologous sites" (DE SLOOVER, 1964), having almost similar microclimates. Moreover, the territory considered here is a montainous valley, showing important ecological differences (topography with accused relief, variable altitude, climate of a mountainous continental type, with important microclimatic changes). Besides, no investigations on the flora and the lichenic vegetation were made before the factories were built at the beginning of the last century (1907); so that no comparison could be made between a former healthy condition and the present one, as it has been done in England by GILBERT (1985) and in Wales by PERKINS & MILLAR (1987 a & b); these last authors carried on their work for a long period during which they could follow the changes of the flora and of the lichenic vegetation before the launching of the factories, during the period of releasing of fluorine and after the closing of the factories; this made it possible for these authors to observe a recolonisation by the lichenic flora. Finally, in the Alps, as the ecological factors, and therefore the vegetation, showed great changes from one valley to the next, it was difficult for us to compare in a satisfactory way the Maurienne and another valley.

That is why we believed it would be interesting in this peculiar case to investigate the fluorine contents for a number of species of corticolous, terricolous and saxicolous lichens and to follow the changes of these contents during several years. A first investigation, carried on during years 1975 to 1977 (ASTA, 1980; ASTA & GARREC, 1980), made it possible to show that the fluorine contents in the thalli were different from one species to another, but also reflected the changes of the pollution level in the atmosphere both in space and time, as it had been observed for the fluorine content of phanerogamic plants (GARREC & PLEBIN, 1975). We had moreover showed that corticolous lichens were perfect indicators for the seasonal and climatic changes of the level of atmospheric pollution.

This first investigation was continued in the Maurienne until 1985 (BELANDRIA, 1986) by sampling lichens and by making fluorine analyses. Owing to the fact that, between 1975 and 1985, the pollution level of the atmosphere had progressively become lower and lower, it was especially interesting to follow the consequential effects of the lowering of the fluorine contents of the atmosphere on the accumulation of fluorine in the different lichens.

The investigation presented here can therefore be used as an example of uninterrupted observations on a steadily diminishing fluoride pollution with the help of lichens, the evaluations of the results obtained spreading over eleven years, from 1975 to 1985.

PRESENTATION OF THE CONCERNED AREA : THE MAURIENNE

Location (fig. 1).

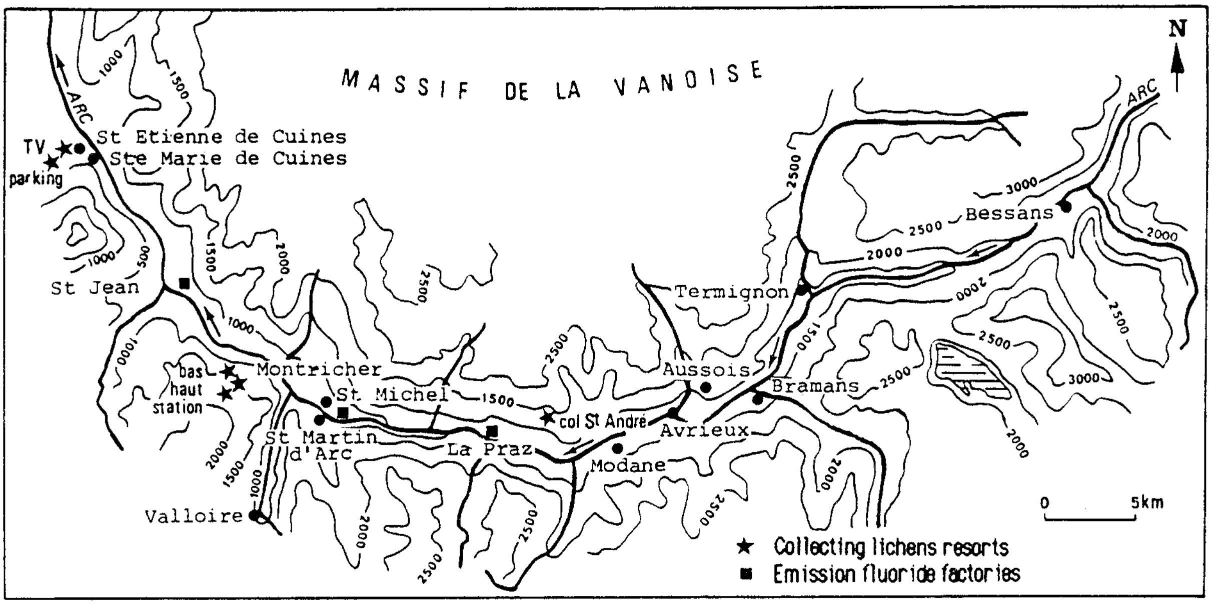

Fig. 1.- Arc Valley (Maurienne). Location map of the study area. Contour lines are at a distance of 500 m.

The Maurienne, or Arc valley, is located in the Northern Alps, in the Savoie department, at the foot of the Vanoise Massif. In this valley, three parts may be differentiated. Going up the valley, we find : a) a low area, below Saint-Etienne de Cuines, or Low-Maurienne, oriented NS and at an altitude inferior to 500 m; b) a middle area, or Middle-Maurienne, extending from Saint-Etienne de Cuines to Modane and oriented NW-SE; c) a high area or High-Maurienne, above Modane, oriented SW-NE and at an altitude greater than 1000 m.

Climate.

Winds. The "Centre de Météorologie Nationale" does not have ciphered information on the variations of the wind in that valley, but the wind pattern was studied between 1970 and 1974 (GARREC, PLEBIN & BOSSAVY, 1976). As for the other alpine valleys, the diurnal air currents are ascending, while nocturnal currents go down the valley. In most cases, the recorded winds blow from the west and go up the valley.

Precipitations (tabl. I). The annual average rainfalls do not exceed 1160 mm. So that the Maurienne is the driest valley among the three which were subject to investigation. The annual average rainfalls in Middle-Maurienne do not exceed 1006 mm (FOURNIER,1985) and they diminish very rapidly when altitude increases.

STATIONS	1975	1976	1977	1978	1979	1980	1981	1982	1983	1984	1985
Avrieux 1 100 m	440	477	530	529	184	368	895	471	377	496	502
Ste-Marie de-Cuines 900 m	843	625	1037	1192	1147	980	1482	1057	1107	975	840
St-Michel de Maurienne 800 m	868	755	1145	1185	1094	776	1441	867	903	890	1005

Table I.- Maurienne. Annual precipitation in mm (period from 1975 to 1985). (Météorologie Nationale, Département de Savoie, France).

Fluoride pollution.

Origins. The Maurienne has, in its middle part, three aluminium factories (Pechiney) of differing capacities established there after 1907. These factories are distributed along 25 km of the Middle-Maurienne : Saint-Jean de Maurienne factories (Les Plans), Saint-Michel de Maurienne factories (La Saussaz) and Le Freyney factories (La Praz). In 1975, the production of aluminium was of about 4000 metric tons per year for the La Praz factory, of 12000 metric tons per year for La Saussaz and of 40 000 metric tons per year for the Saint-Jean de Maurienne factory.

Fluoride pollution made by these factories is added to other gaseous effluents cast into the atmosphere by the 18 factories which are in the Maurienne between Aiguebelle and Avrieux (steel, cement, phosphates, paper, compounds containing chlorine and sodium). In 1973 Pechiney dispatched an important programme in order to fight against atmospheric pollution and to modernize equipment, thus obtaining an important reduction of fluoride emissions. On account of the crisis, Pechiney decided, in 1981, to stop the using of the Söderberg tanks. In 1984, the factories of La Saussaz and of La Praz were braught to a standstill. Work to extend the factory of Saint-Jean de Maurienne then began and the installation of new tanks, which were unpolluting at 99% (personal information given by M.LEDRU, Saint-

Table II.- Maurienne. Fluoride emissions (t/an).

ANNEES	St-Jean	La Saussaz	La Praz	TOTAL
1975	409	180	90	679
1976	410	90	40	540
1977	400	90	40	530
1978	391	90	35	516
1979	315	90	35	440
1980	285	90	35	410
1981	293	94	20	407
1982	200	58	20	278
1983	130	56	15	201
1984	128	36	0	164
1985	148	0	0	148

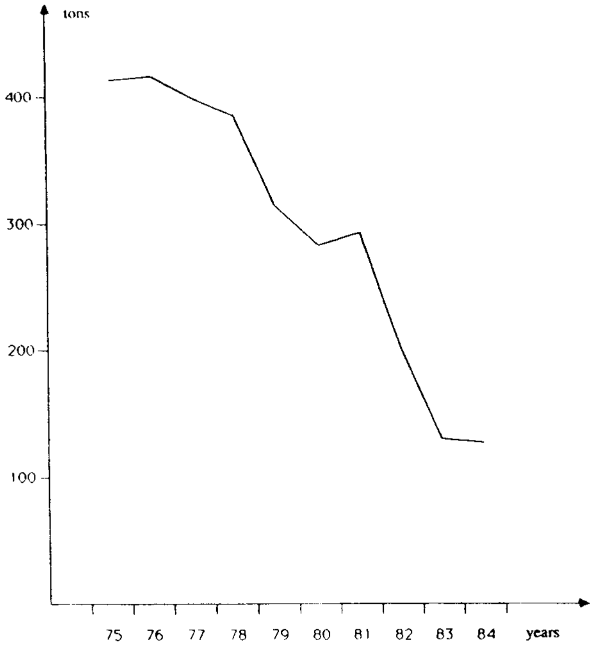

Fig. 2.- Successive changes of the fluoride emissions made by Saint-Jean de Maurienne factory.

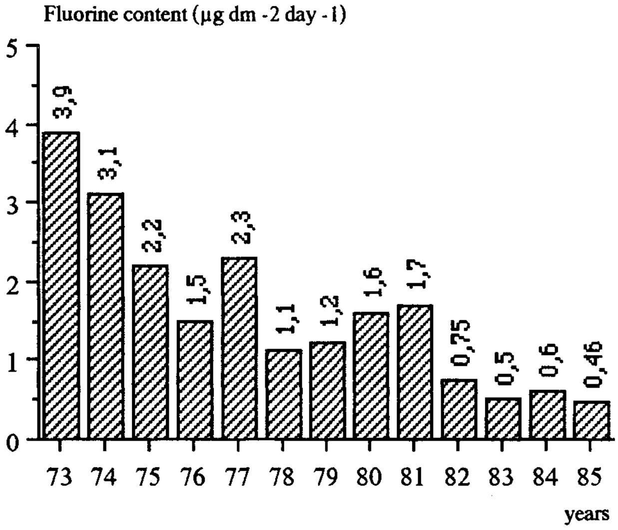

Fig. 3.- Maurienne Valley. Successive changes in the average atmospheric fluorine content measured on lime-paper (average on 14 posts between 1973 and 1985) (Documents INRA).

Jean de Maurienne factory). According to table II and to figure 2, it can be seen that the fluoride atmospheric emissions have deminished, between 1975 to 1984, of about 60% at Saint-Jean de Maurienne, and of more than 80%, if we consider the factories altogether.

Pollution appears in two different forms fluoride gas (mainly HF) and submicronic dust composed chiefly of a sodium aluminium fluoride, and also of calcium fluoride.

Fluoride pollution level. Ambient air fluorine levels have been measured using the lime paper method during active vegetation season, from April to October, in 14 resorts in Moyenne-Maurienne (fig.3). We note that the yearly average of the air pollution level has decreased of more than 85 % between 1973 and 1985 (from 3,9 to 0,46 g F dm^{-2} day^{-1}). This decrease began in 1973, when the first collecting devices for fluoride smoke were established. It continued until 1978, with a higher level in 1977. From 1978 on, a slight increase could be noted; it lasted until 1981, when the augmentation of fluoride emissions was well established. After that period, a progressive and very important lowering of the pollution level is ascertained.

MATERIAL AND METHODS

In the field.

The sites at which investigations were made are those in which the first samples were collected from 1975 to 1977. These sites were chosen close to the polluting factories, in the upper and in the lower part of the valley, at high altitude and in places chosen according to natural features. For the recording of the changes, we kept the Middle-Maurienne sites, but did not investigate those of Upper-Maurienne, because this segment of the valley was considered after 1977 (ASTA & GARREC, 1980) as permanently outside the reach of pollution. We specify, for each site, the altitude and the exposure, and also its distance to the Saint-Jean de Maurienne factory, the latter being the main source of atmospheric pollution (tabl. III).

Table III.- Middle-Maurienne. Collecting sites for the investigated lichens.

SITES	Distance of the site to St-Jean de Maurienne km	Altitude m	Aspect
St-Etienne-de-Cuines TV	12	800	NE
St-Et. de Cuines Parking	12,5	900	NE
Montricher bas	5	1 070	NNE
Montricher haut	5,5	1 400	NNE
Montricher station de ski	6	1 550	NNE
Buste Mougin	13	1 450	N
Col St-André	25	1 350	SSW
Chambaran	94	600	NNE

In each site, lichens are collected in the following way. The corticolous lichens are all taken from the same tree, when it is possible, and put into paper bags. The terricolous and saxicolous lichens are gathered within an area of 10 to 20 m^2, also kept in paper bags until they reach the laboratory. Simultaneously to the collecting of the corticolous and terricolous lichens, are gathered samples of bark and of soil. The collections were made twice a year in April and in September from 1975 until 1985 (except between spring 1981 and autumn 1983). On bark were taken : *Hypogymnia physodes* L. Nyl., *Pseudevernia furfuracea* (L.) Zopf., *Usnea muricata* Mot. and *Bryoria fuscescens* (Gyel.) Brodo & Hawksw. The terricolous species for which samples were collected were : *Cladonia* gr. *pyxidata* (L.) Hoffm., *Peltigera canina* (L.) Willd., *P. aphthosa* (L.) Willd. and *P. horizontalis* (Huds.) Baumg. A few saxicolous lichens were also gathered : *Umbilicaria pustulata* (.) Hoffm., *U. cylindrica* (L.) Del. ex Duby and *Parmelia conspersa* (Ehrh. ex Ach.) Ach.

In order to obtain a comparison, samples of the same species were collected in an unpolluted locality : Fond de France, at 1500 m in the Belledonne massif and in the Isère department. We must emphasize that one of the difficulties with investigations of this kind, requiring continuous collecting of specimens during a long period, is in the risk of exhausting lichens at the sites. For instance in Montricher, which is a ski site and where populations of *Peltigera canina* were followed from 1975 to 1977, this species was not found again later (tabl. V).

In the laboratory.

Fluorine contents were determined in lichen thalli, in the barks and in the soil. The preparation of the samples and the quantitative analyses of the fluorine were made according to the procedures described in the preceding paper (ASTA & GARREC, 1980). The method consists in a mineralising in a Schöniger oxygen flask and thereafter in a quantitative analysis of the fluorine obtained in an adsorbing solution by the means of a specific electrode (LEVAGGI, OYUNG & FELDSTEIN, 1971). The advantages of this method are many. Among them its great rapidity (6 minutes instead of half a day when a double distillation is required) renders it possible to make a large number of analyses. Its accuracy (7%) is satisfactory and above all its great sensibility gives the possibility to detect until 0,1 ppm, which means 100 g for about 1 kg of dry material. A unique quantitative analysis of fluorine is made for the crushed sample of one species in each site.

These results we obtained were made more precise by making statistical tests. First we determined what samples were normal with a test of asymetry and of flattening (SNEDECOR & COCHRAN, 1971). When the distribution proved normal, we used the STUDENT-NEWMAN-KEULS's test. When the distribution of value series was abnormal, in order to compare the average values, we were abliged to use an unparametrical test. We must recall here that, to compare two average values, we used MANN and WHITNEY's test, and that, to compare a number of average values superior to two, we used KRUSKALL and WALLIS's test.

RESULTS AND DISCUSSION

Concentration of fluorine in investigated lichens according to the weather.

<u>Concentration of fluorine in corticolous lichens</u> (tabl. IV & fig. 4).

Annual changes. We noted a regular and very important decreasing (fig.4A) of the fluorine content in thalli from 1975 to 1985, both for the *Parmeliaceae* (of about 60 ppm to 25 ppm) and for the fruticulose filamentous lichens (decrease of 50 ppm to 10 ppm).

In the latter case, *Bryoria fuscescens* stores more fluorine than does *Usnea muricata*. If the annual decrease is regular for *Bryoria*, it was, on the other hand, important for *Usnea* until 1978, but much less so afterwards and appears to be stabilized at present (fig.4B). If we consider the *Parmeliaceae*, the decrease of the fluorine content in the thalli of *Hypogymnia physodes* and of *Pseudevernia* goes on in a different way until 1971, but from this year on, almost parallel changes occur for the two species and the fluorine content settles on a regular level (fig. 4C).

Seasonal changes. Seasonal changes, some being very important, could be observed until 1980; their importance decreased later. Moreover, these changes do not always go the same way (for instance, in spring 1978 and in autumn of the same year, a lowering of the fluorine content was noted for fruticulose filamentous lichens, but at the same time was observed a rise for the *Parmeliaceae*). The amounts in fluorine were not regularly lower or higher than those noted the following autumn.

For the *Parmeliaceae*, the changes in fluorine content of the two species involved do not always go the same way. Concerning the fruticulose lichens, *Usnea muricata* always has a lower content than that of *Bryoria fuscescens*, except for an important rise in autumn 1977.

In contrast to their substratum, lichens do not act only as physical adsorbants. Indeed, they are able to accumulate pollutants and appear to be excellent indicators of atmospheric pollution, but they are also very good integrators for other characters of the environment, such as the rainfall. We had already shown (ASTA & GARREC, 1980), that a very significant rise of the amount of fluorine could appear in thalli of corticolous lichens submitted to a fluoride pollution, when precipitation was less than 50 mm/month; a lowering of this amount could be observed, when rainfall was more than 50 mm/month during the two months just before the period during which the samples were collected. The amount of fluorine of the corticolous lichens therefore was submitted to seasonal changes related to precipitations and those changes showed an evident parallelism with those of the amount of fluorine in phanerogamous plants (Epicea and Larch leaves); but the amount of fluorine for terricolous lichens is not subject to such changes and seems to be under the influence of other factors. Thus seasonal changes noted here for corticolous lichens could be realated to climate factors.

Table IV.- Maurienne. Annual and seasonal successive changes of the fluorine content (ppm) for thalli of *Hypogymnia physodes* and of *Pseudevernia furfuracea*, and for thalli of *Usnea muricata* and of *Bryoria fuscescens*. Annual and seasonal average for the two *Parmeliaceae* and for the two *Usneaceae*. Annual average for bark. (A, autumn; P, spring; L, lichen ; E, bark).

	1975	1976		1977		1978		1979		1980		1981	1983	1984		1985	
Hypogymnia physodes	A	P	A	P	A	P	A	P	A	P	A	P	A	P	A	P	A
Montricher (station de ski)	-	-	-	52	74	61	58	-	-	54	33	-	22	45	33	34	27
St-Etienne de Cuines (parking)	54	83	63	39	73	39	14	-	20	16	4	-	33	25	4	11	20
Seasonal average	54	83	63	46	74	50	36	-	20	35	19	-	28	35	19	23	24
Annual average	54	73		59		43		20		27		-	28	27		23	
Bark : Annual average	64	85		62		60		-		57		20	41	27		17	
Pseudevernia furfuracea																	
Montricher (station de ski)	-	-	-	52	71	57	81	-	52	38	51	-	21	27	37	16	35
St-Etienne de Cuines (parking)	-	-	45	24	61	7	24	-	-	34	0	19	18	16	6	8	11
Seasonal average	-	-	45	38	66	32	53	-	52	36	26	19	20	22	22	12	23
Annual average	-	45		52		42		52		36		19	20	26		17	
Bark : Annual average	-	63		66		26		-		31		28	22	26		12	
Parméliacées																	
Seasonal average	54	83	54	42	70	41	45	-	36	35	22	19	24	29	20	17	23
Annual average	54	64		56		43		36		29		19	24	24	21	15	
Usnea muricata	-	-	51	29	70	35	24	-	17	18	3	-	8	3	5	1	4
Bryoria fuscescens	-	-	-	-	50	43	42	-	37	51	14	-	23	21	20	12	20
Seasonal average	-	-	51	29	60	39	33	-	27	35	9	-	16	12	13	7	12
Annual average		51		45		36		27		22		-	16	12		9	
Bark : Annual average	-	116		112		47		-		66		-	30	36		24	

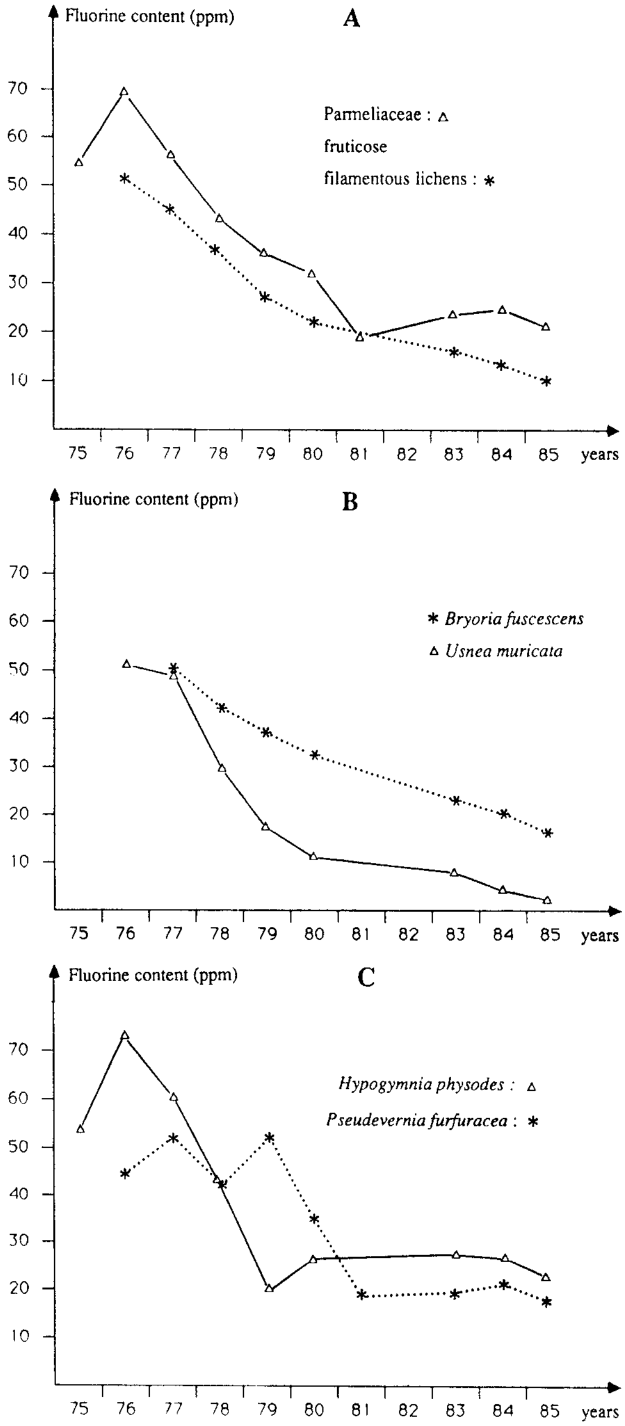

Fig. 4.- Annual successive changes in the fluorine content (ppm) for corticolous lichens in Maurienne.

Concentration of fluorine in terricolous lichens (tabl. V & fig. 5).

Annual changes. We note a very distinct general lowering of the fluorine content for *Cladonia* gr. *pyxidata* and for *Peltigera canina* from 1975 to 1985. Besides, an annual increase of the concentration is obvious in 1979 for *Cladonia*, and also in 1979 and 1980 for *Peltigera*. It could be noticed also that, in 1985, representatives of these two same species had the same amount of fluorine, amount which as a matter of fact is that of healthy specimens (about 5 ppm).

Seasonal changes. They reflect also the general decrease of the amount of fluorine in the thalli, but they show fluctuations and peaks which are more or less accentuated according to the species involved. Nevertheless, if we examine the detail of the information, it is not possible to determine which is the season (spring or autumn) the most favorable for the accumulation of fluorine in lichen thalli. In contrast to what happens for corticolous lichens, the seasonal changes of the amount of fluorine do not seem to be under the influence of climatic factors.

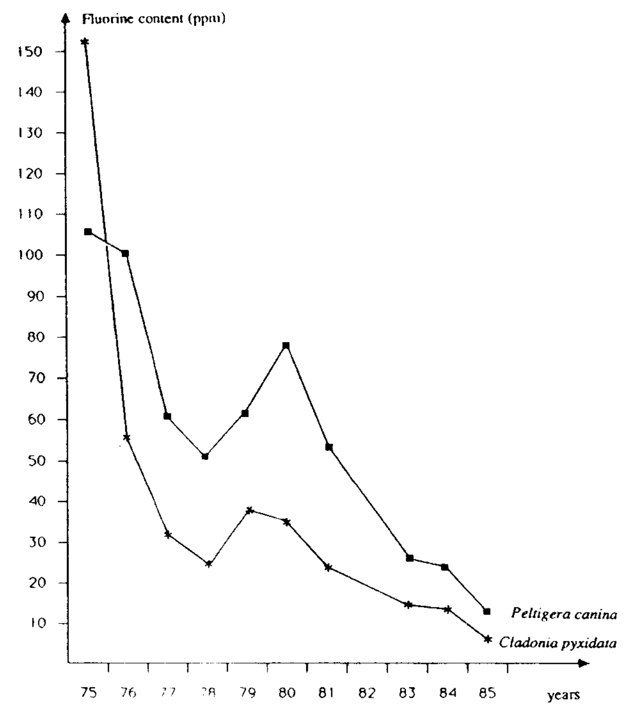

Fig. 5.- Annual successive changes in fluorine content (ppm) for terricolous lichens in Maurienne.

Concentration of fluorine in saxicolous lichens (tabl. VI & fig. 6).

We note also a general lowering of the fluorine content from 1975 to 1985 for the saxicolous lichens *Parmelia conspersa*, *Umbilicaria cylindrica* and *U. pustulata*, but with fluctuations from 1979 to 1985. The two species of *Umbilicariaceae* showed an almost similar series of changes of their fluorine content. Moreover, for these two species, after 1980, there was a new increase of content, but the latter decreased after 1984.

Table V.- Maurienne. Annual and seasonal successive changes of the fluorine content (ppm) for thalli of *Peltigera canina* and of *Cladonia* gr. *pyxidata*. Annual and seasonal average of the fluorine content (ppm) of the soil. (A, autumn; P, spring; L, lichen; S, soil).

	1975	1976		1977		1978		1979		1980		1981	1983	1984		1985	
Peltigera canina	A	P	A	P	A	P	A	P	A	P	A			P	A	P	A
Col St-André	-	-	-	35	39	108	-	-	-	-	58	-	23	50	50	31	-
Buste Mougin	-	118	87	23	55	44	-	-	-	43	20	46	0	11	42	6	19
Montricher (station de ski)	66	68	108	77	-	-	-	-	-	-	-	-	-	-	-	-	-
Montricher - Haut	-	-	-	44	56	95	32	90	-	75	123	53	18	18	9	4	13
Montricher - Bas 1	97	169	89	160	56	50	48	51	-	85	177	67	22	35	7	17	10
Montricher - Bas 2	156	230	52	113	87	162	-	126	55	135	117	99	72	30	30	20	28
St-Etienne de Cuines (parking)	-	41	72	29	37	12	3	5	-	13	7	18	37	16	0	1	1
St-Etienne de Cuines (TV)	-	75	-	32	79	35	33	45	-	53	122	32	13	-	7	6	0
Seasonal average	106	117	82	64	58	72	29	68	55	67	89	53	26	27	21	12	12
Annual average	106	100		61		51		62		79		53	26	24		12	
Soil: Annual average	331	366		273		148		183		153		256	340	200		156	

	1975	1976		1977		1978		1979		1980		1981	1983	1984		1985	
Cladonia pyxidata	A	P	A	P	A	P	A	P	A	P	A			P	A	P	A
Col St-André	-	65	45	28	31	52	25	25	-	39	58	23	13	22	16	12	8
Buste Mougin	-	-	35	18	21	76	23	23	123	31	31	-	12	10	13	2	8
Montricher (station de ski)	-	115	43	26	64	21	16	-	22	17	44	-	25	29	11	4	8
Montricher - Haut	-	-	-	36	80	32	30	60	-	71	44	27	10	9	9	1	5
Montricher - Bas	-	-	-	-	-	-	0	20	-	34	37	29	25	17	5	0	5
St-Etienne de Cuines (parking)	157	44	13	9	28	5	10	2	-	26	14	17	11	8	0	0	2
St-Etienne de Cuines (TV)	146	87	19	9	32	17	10	19	-	22	23	-	12	21	24	19	13
Seasonal average	152	78	31	21	43	34	16	25	73	34	36	24	15	17	11	5	7
Annual average	152	52		32		24		37		35		24	15	14		6	
Soil: Annual average	270	422		297		366		301		228		271	232	352		167	

Table VI.- Maurienne. Annual successive changes of fluorine contents (ppm) for saxicolous lichens : 1, St-Etienne de Cuines T.V.; 2, Col Saint-André ; 3, Montricher station de ski.

	1975	1976	1977	1978	1979	1980	1981	1983	1984	1985
Umbilicaria pustulata (1)	78	44	30	18,5	5	13	33	25	30,5	21,5
Umbelicaria cylindrica (2)		124	115	60		39		43	65	36,5
Parmelia conspersa (1 et 3)	110	102	94	73	29	67	42	81	85	55

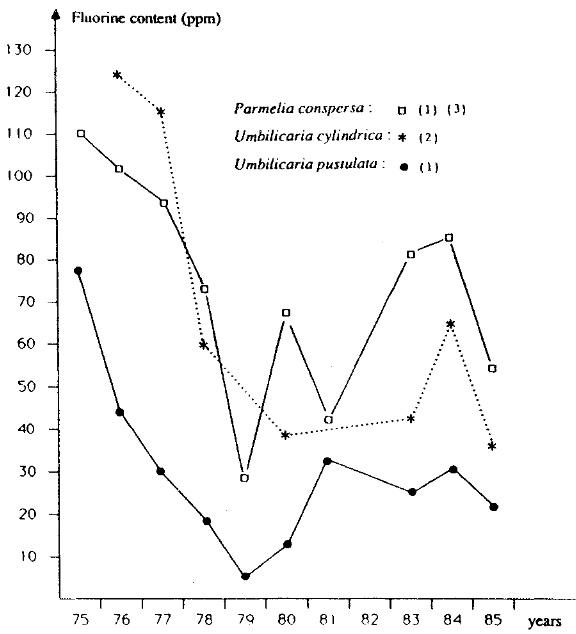

Fig. 6.- Annual successive changes of fluorine content (ppm) for saxicolous lichens in Maurienne : 1, Saint-Etienne de Cuines T.V.; 2, Col Saint-André; 3, Montricher station de ski.

Comparison of the fluorine contents between different species of lichens (tabl. VII)

The average fluorine contents for the different species of lichens considered indicates that, among the corticolous species, the *Parmeliaceae* accumulate the most fluorine (*Hypogymnia physodes* and *Pseudevernia furfuracea*) while fruticulose filamentous lichens retain rather less (*Bryoria fuscescens* and *Usnea muricata*). If we consider the terricolous species, *Peltigera canina* accumulates more fluorine than *Cladonia* gr. *pyxidata*.

Table VII.- Maurienne. Average of the fluorine contents (ppm) for corticolous and terricolous lichens. Sites of Middle-Maurienne and unpolluted area.

Species	Mean ± SE (95%)	Unpolluted area 1976	1984
	Corticolous lichens *Parmeliaceae*		
Hypogymnia physodes	38 ± 8,6	19 ± 11	≤ 8
Pseudevernia furfuracea	33 ± 8,6	24 = 6	≤ 5
	Fruticulose filamentous lichens		
Bryoria fuscescens	30 ± 8,4	13 ± 4	0
Usnea muricata	21 ± 8,2	14 ± 8	≤ 4
	Terricolous lichens		
Peltigera canina	55 ± 9,3	15 ± 5	≤ 5
Cladonia gr. *pyxidata*	28 ± 5,8	22 ± 9	≤ 8

With statistical tests made using the techniques given in preceding pages, we note a highly significant difference between the average fluorine contents in the 6 investigated species of lichens (H= 29,24; ρ < 10-4 after χ^2 =5). For corticolous lichens, we noted a significant difference between the amounts of fluorine in the two *Parmeliaceae* (average of 35 ppm) and in the two fruticulose filamentous lichens (average of 12,5) used for comparison (ρ = 0,0291). The test showed that there was no significant difference between the average amounts of fluorine in *Hypogymnia* and in *Pseudevernia* (ρ = 0,03693) and also of the amounts between *Usnea muricata* and *Bryoria fuscescens* (ρ = 0,1045). For terricolous lichens, the difference between the average amounts of fluorine in *Peltigera* (55 $\pm$ 9,3 ppm) and in *Cladonia* (28 $\pm$ 5,8 ppm) is highly significant (ρ < 10-4).

These results maybe could be explained by the anatomy of the lichens considered. It is possible that the stratified structure of the *Parmeliaceae* is more favorable for the accumulation of fluor than the radiate structure of filamentous thalli. For the same reason, the foliaceous thalli of *Peltigera* may be more favorable for the storage of fluorine than the scyphae of *Cladonia* gr. *pyxidata*.

Comparison between the fluorine contents of lichens and the levels of atmospheric pollution.

There was a general lowering of the content of fluorine accumulated in lichens and in their substratum (from 1975 to 1985). This general lowering is even more distinct, if we consider the average fluorine content measured for the whole of the lichens investigated from 1975 to 1985 (tabl. IV to V, fig. 4 to 7). This decrease reflects almost exactly the lowering of the content of fluorine in the atmosphere established by means of physico-chemical measures made with lime paper during the same period (fig. 3).

Comparison of the fluorine contents between polluted lichens and control samples (tabl. VII).

Lichens from the unpolluted control sites (Fond de France, Isère), had very low fluorine contents (≤ 8ppm).

As the average values of fluorine contents in terricolous and corticolous lichens remain important compared to those of control samples, it appears that, in *Usnea muricata* (tabl. IV) and in the two species of terricolous lichens, at least in certain resorts (tabl. V), the fluorine contents measured in 1985 are not higher than those in control samples.

Nevertheless the concentrations obtained for control samples in the present survey are less than those measured from 1975 to 1977 (between 11 and 24 ppm). We obtained results identical to those we had in the Romanche valley and in the Val d'Arly by taking as control samples lichens from the Bois de Chambaran (Isère) : 8 - 14 ppm in 1976, 2 - 6 ppm in 1984 (ASTA, 1980; BELANDRIA, 1986). Thus this confirms that, even in areas chosen considered by us as unpolluted, the natural fluorine contents of lichens appear to be lower in 1984 than in 1976. Consequently, the facts show that the chosen area (forêt de Chambaran) was not completely protected from some pollution, though it is at a long distance from industrial plants producing fluor. Today, it seems possible to confirm that this area can be considered as a good reference - zone, as measures made in other protected areas in France (LALLEMANT, personal communication) gave concentrations very close to ours for control samples.

We therefore realize once more the difficulty to find healthy areas, even at a distance from pollution sources, and consequently to obtain reference values. It is in order to try to partly resolve this problem that the supervisory "stations" for pollution were conceived by the European Community as, for instance, the station of Casset, (Hautes-Alpes, France), which is inside the protected zone of the Parc National des Ecrins.

Table VIII.- Maurienne. Annual average of fluorine contents (ppm) for *Peltigera canina* and *Cladonia* gr.*pyxidata*. Sites of Saint-André pass, Montricher (low, high, ski resort) and St-Etienne de Cuines (parking and T.V.).

		1975	1976	1977	1978	1979	1980	1981	1983	1984	1985
Peltigera canina	Col St-André	-	-	37	108	-	58	-	23	50	31
	Montricher	106	119	85	77	85	109	73	37	2	17
	St-Etienne de Cuines	-	63	44	21	27	49	25	25	8	3
Cladonia pyxidata	Col St-André	-	55	30	39	25	49	23	13	19	10
	Montricher	-	79	52	20	34	41	28	20	13	4
	St-Etienne de Cuines	152	41	20	11	11	21	17	12	13	9

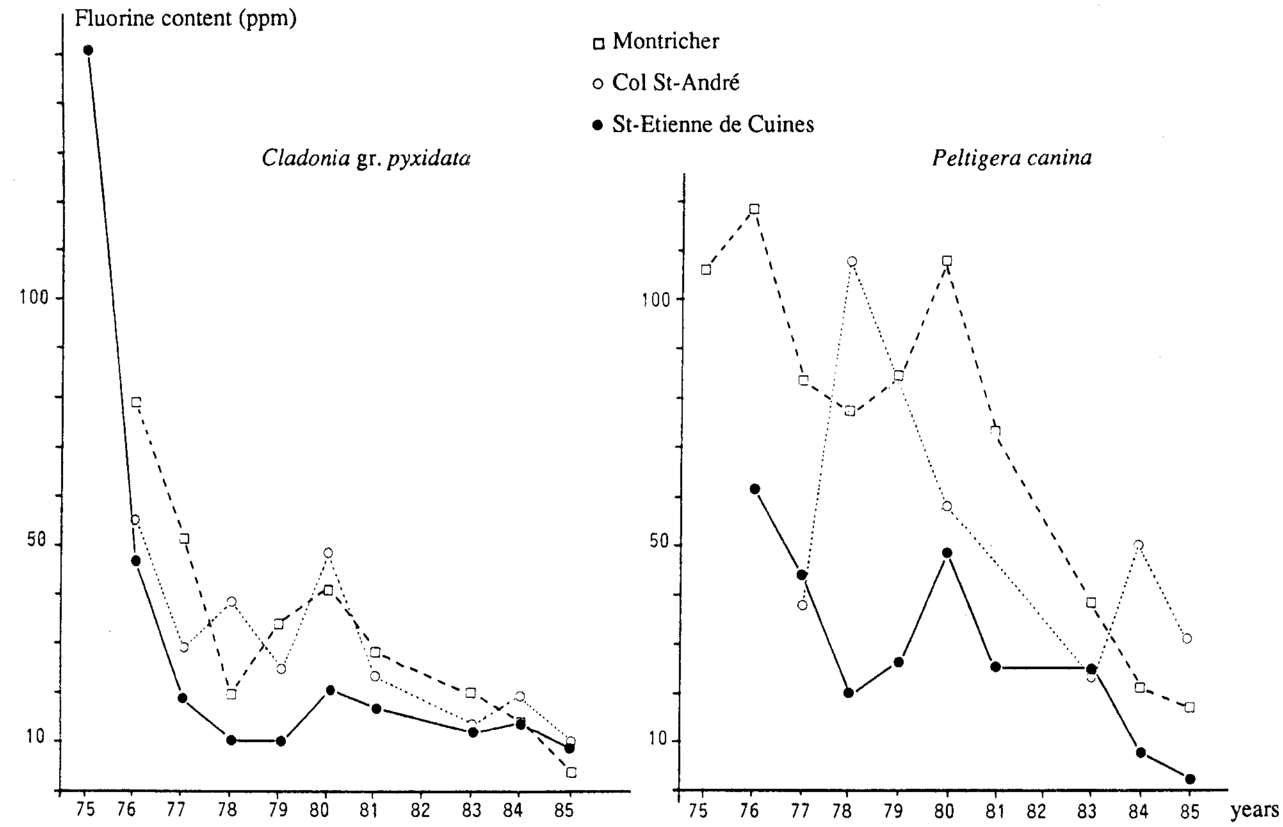

Fig. 7.- Maurienne. Successive changes of the average fluorine content (ppm) for *Cladonia* gr. *pyxidata* and *Peltigera canina*. Sites of Saint-André pass, Montricher (low, high, ski resort) and St-Etienne de Cuines (parking and T.V.).

- Relationship between the fluorine content in the lichens and the location of the sites in which collecting was made (tabl. VIII & fig.7)

The average fluorine content was calculated in the two terricolous species which were considered according to the distance of their respective resorts to the sources of pollution, which is mainly at Saint-Jean de Maurienne. We thus observe the changes of the annual content for three groups of resorts Montricher (ski-resort, high and low), Saint-Etienne de Cuines and Saint-André pass, resorts which are respectively at 6 km, 12 km and 25 km from Saint-Jean de Maurienne.

We note that :

- the fluorine content measured in lichens at Saint-Etienne de Cuines is always less than it is in specimens at Montricher, which is normal, as this site it further than the first one from the pollution-source; as it has already been observed by ASTA & GARREC (1980) for the Haute-Maurienne, the percentage of fluorine in lichen-thalli is more important for resorts being close to pollution-sources.
- the changes of the pollution during years 1975 to 1985 in the two sites Montricher and Saint-Etienne de Cuines were parallel for *Cladonia* gr. *pyxidata* and *Peltigera canina* (a hollow between 1978 and 1979 and a peak in 1980).
- except for two concentrations values, the pollution at Saint-Etienne de Cuines is also less than at Saint-André pass ; nevertheless, this last site is much further from the pollution-sources - and this should contradict the preceding statement-, but it is above the source of rejection. The wind, meteorological factor, plays a very important part in the dispersion of atmospheric pollution and, in the present case, predominant diurnal aerial currents help the upward motion of pollutants towards the high part of the valley.

The changes in the annual fluorine-content in terricolous lichens collected at Saint-André pass are moreover much nearer to those in the annual fluorine-content in lichens collected at the site of Montricher than to those for lichens gathered at the site of Saint-Etienne de Cuines. Similar facts can also be ascertained for saxicolous lichens of the genus *Umbilicaria* (fig. 6). The species collected at Saint-André pass (*U. cylindrica*) has always a content of accumulated fluor superior to that observed in specimens of *U. pustulata* found at Saint-Etienne de Cuines. Nevertheless, this difference might be inherent in lichenic species, but, as the two species do not grow together in the two areas, a verification of the facts could not be made.

Comparison between the fluorine content in lichens and in the corresponding substrate.

Results of fluorine contents in the bark and in the soil are given on tables IV & V. Annual average contents of the soil are generally much higher than they are in the bark. Although they vary very much from one year to the next, they are clearly lower in 1985 than in 1975. The question which one could ask would be to know whether the changes of the fluorine content in the substrate supporting the lichens would run parallel to those of the fluorine content in the lichens themselves. There is a highly significant correlation (SPEARMAN correlation coefficient) between *Hypogymnia physodes* and the bark on which it grows (0,7909; $\rho < 0{,}01$) and also between *Pseudevernia furfuracea* and its supporting bark (0,6998; $\rho < 0{,}01$). For *Bryoria fuscescens*, we found a significant correlation between the lichen and its substrate (0,6762; $\rho < 0{,}05$). On the other hand the correlation is not significant between *Usnea muricata* and its support (0,4601 ; $\rho > 0{,}05$). Concerning the terricolous lichens and their respective substrate, the correlation is highly significant for *Cladonia* (0,4367; $\rho < 0{,}01$) as well as for *Peltigera* (0,5082; $\rho < 0{,}01$).

These results show that the fluorine contents of the substrates are in most cases correlated to those of the lichens growing on them, but this does not at all prove that the lichens can augment their content by being in contact with their support. Indeed barks as well as soils may receive a new supply of fluor braught by rain or snow, or on the contrary may be submitted to a washing by these same elements, so that their pollutant-content may have a great variability, the importance of which is greater when the source of pollution is close to the site. Previous investigations (ASTA, 1980) showed with some evidence an important lowering of the fluorine content for terricolous lichens in resorts of Haute-Maurienne until a constant basic level was reached and this whatever was the fluorine content of the soil. This might show the lack of influence, or at least the smallness of it, of the fluorine content of the soil on that of the terricolous lichens. In actual fact, only physiological experiences made is laboratories could really demonstrate that the fluorine content of lichens is completely independant of the fluorine content of the soil.

CONCLUSION

The detection and the recording of fluoride pollution in an alpine valley (Maurienne, Savoie, France) were realized from 1975 to 1985 by using lichens as bioaccumulators. When we started our investigations in 1975, we did not have any information concerning the condition of the lichen vegetation before the factories were built (1907) and it was therefore impossible for us to make assessment and comparisons with a more recent condition of the vegetation. In the Maurienne, we based our results only on the analyses of the fluorine accumulated in thalli of lichens. We made measured fluorine contents not only for corticolous lichens, but also for terricolous and saxicolous ones. Besides these analyses were made twice a year, in the spring and in autumn (except during 1982).

The results obtained show a general decrease in the fluorine-content of the thalli of the lichens investigated in the Arc Valley from 1975 to 1985, concerning as well corticolous lichens (from 54 to 21 ppm for *Parmeliaceae*), as terricolous lichens (from 106 to 12 ppm for *Peltigera canina*), and as saxicolous lichens (from 124 to 44 ppm for *Umbilicaria cylindrica* for instance). In a parallel way, physico-chemical measures of fluoride pollution were made from 1973 to 1985 by using methods indicated previously. They show important lowering of the level of fluoride pollution.

The decrease of the fluorine content in thalli of lichens reflects clearly the lowering of fluoride pollution in the atmosphere. This result confirms those obtained by similar investigations made in the Romanche valley (Isère, France) between 1976 and 1984 (BELANDRIA & ASTA, 1987).

Among corticolous lichens, the *Parmeliaceae* store more fluorine than do fruticulose filamentous lichens (*Usnea, Bryoria*) for instance, over the 11 years during which the investigations were made, the average fluorine-content is of 38 ppm for *Hypogymnia physodes* and of 21 ppm for *Usnea muricata.* Likewise, for terricolous lichens, which usually store more fluorine than do corticolous ones, the fluorine content of *Peltigera canina* (55 ppm) is higher than that of *Cladonia* gr *pyxidata* (28 ppm).

We show here, as did ASTA (1980), GILBERT (1985) and PERKINS & MILLAR (1987) that the fluorine content of lichens is usually higher in sites close to the source of pollution. Nevertheless, in the site at Saint-André pass, which is at a distance of 25 km above the aluminium factories, the fluorine content of lichens remains high. This can be explained by the part played by air currents, which help the ascending of pollutants in the valley.

If the average value of the fluorine content of terricolous and corticolous lichens remains high compared with the value of this content for control samples of lichens, for *Usnea muricata, Peltigera canina* and *Cladonia* gr. *pyxidata,* the amount measured in 1985 in certain sites, is not greater than that obtained with control samples. In connection with this, we want to point out the difficulty to obtain rigorous reference values.

The analyses of fluorine in the bark and in the soil give concentrations usually much larger than those obtained for lichens. We statistically showed that the fluorine contents of the support usually go the same way as those of the corresponding lichens, but this does not prove that the lichens can enrich themselves by contact with their support.

If in the present investigation on the Maurienne and in that made in the Romanche valley (BELANDRIA & ASTA, 1987), the lowering of the fluorine-content of lichens appears to be distinctly related to the lowering of the atmospheric pollution level, it was not the same in the Val d'Arly (Savoie), in which we noted in 1984 a lowering of the fluorine content for lichens just collected compared to that noted for specimens in 1976, lowering which was not in relation with the atmospheric pollution level, the latter having remained high (BELANDRIA, 1986). In the present case, the results are to be considered as directly related to precipitation; indeed, for lichens which have been collected after a long period of precipitations, the decrease of the fluorine content noted for them can be due to an important washing of the fluorine off the thalli.

While detection of acid pollution could be carried on, in certain cases, by observing lichen vegetation and by perfecting different qualitative methods, no standardization work could so far be made concerning fluoride pollution.

The quantitative method described here for fluorine analyses in lichen thalli appears to be rather easy to be used, but is at the same time sensitive and very precise. The precautions which are to be taken in the utilization of this method concern mainly the conditions realized in the open when the work is made choice of the species of lichens according to the characters of the area, to the period of the year and to the precipitations (gathering of the specimens after a dry period). It must be noted that few investigations were made on the consequential effects on the lichen flora of the lowering of an atmospheric pollution by fluorine (ASTA & GARREC, 1980; BELANDRIA & ASTA, 1987; GILBERT, 1985; PERKINS & MILLAR, 1987 a & b).

Therefore lichens seem to be among the very best bioindicators as far as pollution is concerned. Indeed, because of their phytosynthetical activity all the year round, of their slow metabolism, of their great possibility to accumulate and of their absence of defense possibilities, lichens are not only physical adsorbants, but also indicator plants, which are sensitive and reliable concerning the air quality; they are also good integrators for all the factors of the environment (especially for the precipitations). In the present survey (and also in the investigations on the Romanche valley (BELANDRIA & ASTA, 1987), they were used to detect a regression of a fluoride pollution; but they render us able to detect every rise, even a very small one, of the level of fluoride pollution. Therefore, we show that, without the help of any mesure apparatus for atmospheric pollution, lichens can be used to watch fluoride pollution in any kind of area and whatever is the direction of the changes in pollution.

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