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The fluoride effect (NaF) on germination and yield production of three local species of Poaceae fodder

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Abstract. The effect of fluoride (NaF) of three local species of rye grass (*Lolium multiflorum* Lamk***.,*** tall fescue *(Festuca arundinaceae*) and red canary grass (*Phalaris arundinaceae*) was analyzed at the concentration of 0; 10^{-1}M; 10^{-2} M and 10^{-3}M. The results showed that germination was effected by fluoride pollution. In fact, applying constant level of NaF caused considerable decrease in germinal capacity and power. At the concentration level of NaF solution over 2.33 g.l^{-1}. The process of germination was completely inhibited in the three studied species. Moreover, it is worth mentioning that fluoride was found to greatly affect fescue growth. In contrast, fluoride had a favorable effect on rye grass and reed canary grass growth. Such growth stimulation is explained by an elevation in the respiratory activity. On the other hand, biochemical estimations show that fluoride treatment disturb biosynthesis of both photoreceptor pigments and sugars. Finally, we can conclude that the response of these ecotypes to fluoride pollution seems to be relatively resistant to this type of pollution.

Keywords. Fluoride pollution – Germination – Growth – Chlorophyll – Sugars – Toxicity.

L'effect du fluorure (NaF) sur la germination et le rendement de trois espéces fourragères locales (Poaceae)

Résumé *Les effets de fluorures (NaF) sur trois graminées fourragères locale*s Lolium multiflorum *Lamk.,* Festuca arundinacea *Schreb.,* Phalaris arundinacea *L., ont été analysés aux concentrations suivantes: 0, 10^{-1}M, 10^{-2}M et 10^{-3}M. Les résultats obtenus montrent une réelle sensibilité de la germination à la pollution fluorée. En effet, l'application de doses croissantes de fluorures diminue considérablement la faculté et l'énergie germinative. Au delà de la concentration 2,33 g.l^{-1} de NaF, l'inhibition du processus de germination est totale chez les trois espèces. Par ailleurs, le fluor a affecté sensiblement la croissance de la fétuque et favorisé légèrement celle du ray-grass et l'alpiste, mais ces variations ne sont pas significatives. Concernant les paramètres biochimiques, on note que le fluor a nettement perturbé les voies de biosynthèse des sucres et les pigments photorécepteurs. Enfin on peut conclure que la réponse de ces écotypes au fluor se révèle relativement résistante à ce type de pollution.*

Mots-clés. *Pollution fluorée – Germination – Growth – Chlorophylles – Sucres-toxicité.*

I – Introduction

Fluoride is a phytotoxic pollutant. Its accumulation by plants and its migration to the periphery of the organs and the appearance of apical leaf necrosis are well known phenomena (Stevens *et al.*, 1998; Fornasciero, 2001). This element will accumulate in the cytoplasm of plant cells and is up to 200 mg. kg^{-1} in dry tissue (Mezghani *et al*., 2005). The Annaba's region is an important industrial pole which rejects waste nature and many air and waste gas including fluorine fall out to the soil surface causing serious disturbances in the plant and animal communities (Semadi, 1989; Miller, 1993; Tsiros et Haidouti, 1998; Salesse, 2008) such as fluorosis poisoning (Cerklewski, 1997). The purpose of this study is to assess the effect of fluorine on i) germination and growth of plants with certain biochemical markers and ii) the phytotoxity by accumulation to preserve the cattle from poisoning.

II – Materials

Germination test: The germination test was conducted under standard laboratory conditions and he soaked seeds were grown in Petri dishes of 8.5 cm on two pieces of filter paper (Whatman 5). The experiment was set up in a completely randomized design with 3 replications of 20 seeds per variety, and 4 concentrations of NaF was added to the germination medium (0, 10^{-1}M, 10^{-2}M, 10^{-3} M). Treatment with solutions containing fluoride was made at a rate of 25ml per concentration. The germination rate (GR) is expressed as percentage of seeds (Shirafew and Baker, 1996).

Trial biomass production: Sowing was carried out in pots containing soil with sandy clay loam texture. Mineral fertilization was made to correct the defect in fertility with an NPK (15-5-15). Treatment with fluoride solutions was made from the 50th day of growth with three sprays of 25 ml per week. The plant biomass was harvested twice, first cut was made at the 80th day of growth and a second cut at the 150th day of cycle. After the second harvest, the roots also were cut and separated from the soil by washing with water. For biochemical assays, chlorophyll was measured by method of Hicox and Israelsam (1979). Soluble sugars were measured using the method of Schields and Burnett (1960) and fluoride in the leaves using the ionometric T.IS.A.B-C.D.T.A amended method (Devilliers, in Semadi, 1989). Data from germination and dry matter yield was statistically analyzed by ANOVA 2 (Minitab Inc., 2003).

III – Results and discussion

1. Rate and speed of germination

The results (Table1) showed that seed treatment with fluorides at 10^{-1}M level inhibited completely the rate and speed of germination of three studied species. The canary grass was the most sensitive to fluoride since concentration10^{-2}M also inhibited germination, while the other two species (rye-grass and fescue) had the most sensitive to fluoride since concentration10^{-2}M also inhibited germination, while the other two species (ryegrass and fescue) had their germination rate reduced by 50%. A second germination test showed that the toxic threshold of inhibition was 33 g.l^{-1}.

Table 1. Rate and speed of germination

	Rye-grass		Tall fescue		Red canary grass	
	RG (%)	Vm (seed/day)	RG (%)	Vm (seed/day)	RG (%)	Vm (seed/day)
Control	100	11.1	59.7	5.4	50.0	3.1
10^{-1}M	–	–	–	–	–	–
10^{-2}M	33.30	3.70	20.0	2.1	–	–
10^{-3}M	83.30	9.2	31.1	3.5	31.7	1.7

2. DM biomass yield

The results showed that the biomass yield did not seem to be affected significantly (NS) neither the first cut nor the second cut by fluorine treatment (Table 2). Moreover, species responded differently to this type of pollution ($P<0.05$). On root biomass, the same observation was reported; fluorine did not exert significant negative effect from the statistical point of view. The content of 10^{-1}M had no effect on ryegrass root but reduced the weight of fescue and stimulated that of the canary grass. The doses of fluoride used did not exert any significant effect on the performance of dry matter (DM). The decreased biomass in the second cut was due to “cutting effect” and the low yield was explained by the negative fluorine action on hormone and enzymatic systems (Deruelle and Lallemant, 1983).

Table 2. DM production of biomass and belowground (g. pot^{-1})

	Rye-grass		Tall fescue		Red canary grass	
	1st cut	2^{ed} cut	1st cut	2^{ed} cut	1st cut	2^{ed} cut
Aerial biomass						
Control	2.1 ± 0.1	1.0 ± 0.2	1.7 ± 0.3	1.0 ± 0.4	1.8 ± 0.3	1.0 ± 0.4
10^{-1}M	2.1 ± 0.3	1.1 ± 0.1	0.7 ± 0.3	1.2 ± 0.3	2.9 ± 0.5	0.3 ± 0.1
10^{-2}M	2.8 ± 1.3	0.9 ± 0.3	1.2 ± 0.1	1.3 ± 0.5	2.2 ± 0.4	0.6 ± 0.2
10^{-3}M	2.9 ± 0.5	0.9 ± 0.3	1.4 ± 0.5	0.7 ± 0.1	2.0 ± 0.2	0.4 ± 0.2
Belowground biomass						
Control	0.4 ± 0.1		1.3 ± 0.1		0.4 ± 0.1	
10^{-1}M	0.4 ± 0.1		1.2 ± 0.2		0.5 ± 0.1	
10^{-2}M	0.7 ± 0.1		1.3 ± 0.2		0.4 ± 0.1	
10^{-3}M	0.5 ± 0.1		1.2 ± 0.1		0.4 ± 0.0	

3. Chlorophyll content

The effect of fluoride on chlorophyll content of leaves was observed. The results (Table 3) showed that the total amount of chlorophyll increased in all treated plants of rye grass. However, tall fescue showed reduced chlorophyll content in leaves was in canary grass, low doses of fluoride (10^{-2}M and 10^{-3}M) stimulated chlorophyll synthesis in leaves while the high dose (10^{-1}M) inhibited. During the second cut, it was observed that the fluorine always tend to raise the chlorophyll content. In fact, fluoride distrupted the synthesis pathways of chlorophyll. It was responsible for the content reduction by inhibition of the synthesis or by conversion into the corresponding pheophytins.

Table 3. Chlorophyll content in leaves (mg.g^{-1} of FM)

	Rye-grass		Tall fescue		Red canary grass	
	1st cut	2^{ed} cut	1st cut	2^{ed} cut	1st cut	2^{ed} cut
Control	1.07	6.35	1.61	0.93	1.65	1.30
10^{-1}M	0.13	1.01	0.67	1.30	0.81	4.20
10^{-2}M	0.70	1.20	1.07	1.54	1.08	1.60
10^{-3}M	0.74	1.93	1.13	1.25	1.12	4.95

4. Soluble sugar content

During the first period of growth, ryegrass contained more soluble sugars than the fescue and canary grass in the control plants (Table 4). In contrast,, fully grown this species first undergoes a drastic reduction and ends up with a rate two times lower than the other two species (tall fescue and red canary grass). However, in treated groups, fluorine distrupted metabolism carbohydrates. The highest concentration (10^{-1}M) causeda stimulation of the sugars synthesis. Carbohydrate metabolism was also affected by fluoride. At low doses, fluoride can stimulate soluble sugar content, while high concentrations of fluorine reduce the sugar content (Garrec *et al*., 1981).

Table 4. Soluble sugar content in leaves (mg.g^{-1} of FM)

	Rye-grass		Tall fescue		Red canary grass	
	1st cut	2^{ed} cut	1st cut	2^{ed} cut	1st cut	2^{ed} cut
Control	97.72	10.80	68.62	20.50	81.13	24.14
10^{-1}M	37.42	6.40	39.80	14.60	55.20	24.20
10^{-2}M	43.12	4.50	67.82	11.80	78.82	21.65
10^{-3}M	72.80	17.90	92.80	33.70	90.97	22.80

5. Leaves fluoride content

Fluoride content in the leaves (Table 5) showed that ryegrass and canary grass accumulate constant to relatively low levels during the two cuts. These vary between 17.5 and 21.04 µg.g^{-1} DM for the respective concentrations of Co (control) and 10^{-1}M. In contrast, fescue seems accumulate more fluoride (1.5) than the other species (34.80 µg.g^{-1}) with the treatment dose of 10^{-1}M. Fluoride accumulated in the leaves of the three species was greater than 10 µg.g^{-1}of DM. They were considered phytotoxic and can induce serious metabolic disturbances (chlorosis, necrosis). Furthermore, the ingestion of these forage by cattle would not cause the fluorosis disease, because the observed levels were below 40 µg.g^{-1}, threshold toxic to animals (Plebin and Garrec, 1986).

Table 5. Leaves fluoride content in (µg.g^{-1}of DM)

	Rye-grass		Tall fescue		Red canary grass	
	1st cut	2^{ed} cut	1st cut	2^{ed} cut	1st cut	2^{ed} cut
Control	–	–	–	–	–	–
10^{-1}M	18.80 ± 0.2	18.70 ± 0.1	34.8 ± 0.3	16.5 ± 0.3	21.04 ± 0.5	19.90 ± 01
10^{-2}M	18.12 ± 0.1	18.12 ± 0.3	29.6 ± 0.1	16.7 ± 0.5	20.60 ± 0.4	15.40 ± 0.2
10^{-3}M	17.50 ± 0.4	17.50 ± 0.3	26.2 ± 0.5	16.2 ± 0.4	20.20 ± 0.2	20.20 ± 0.2

IV – Conclusions

This work shows that fluoride reduces germination by acting on the system involved enzyme (degradation of reserves and production of the energy required for this phenomenon). The dose-effect is important. Growth biomass, production of DM was also weakened by fluoride, disrupting photosynthesis by inhibiting the metabolism of chlorophyll and sugars. Moreover, the accumulation of fluoride in the leaves shows that these levels are phytotoxic causing chlorosis and necrosis but below threshold (<40µg.g^{-1}DM) can cause fluorosis disease to cattle.

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