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# The influence of air pollution on the phyllosphere microflora composition of *Tillandsia* leaves (Bromeliaceae)

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**Abstract:** The effect of air pollution on total phyllospheric microflora from two species of the epiphytic neotropical genus *Tillandsia* (Bromeliaceae) was studied by comparing unpolluted plants living in a forest (Escazú, San José) with polluted ones from an urban site of Costa Rica (San José city). Dilutions of homogenized leaf samples were plated on media suitable for each microbial group. For each microorganism group, total counts were performed and purified strains of randomly chosen colonies were identified. There was a global reduction in the number of living microorganisms due to pollution effects, especially yeasts and bacteria, while nitrogen-fixing microorganisms and fungi were less affected. Our results showed that the phyllosphere microflora of *Tillandsia* plants living in a tropical urban environment changes in terms of number and species composition of yeasts and bacteria with respect to plants living in unpolluted environment.

**Key words:** *Tillandsia;* phyllosphere; air pollution; microflora

Air pollutants influence biological systems in different ways at a global level. At regional or local levels these effects are more significantly detectable, especially in urban and industrial areas or when the pollution is associated with roadways. Studies concerning air pollution impacts are difficult to undertake because of the different nature of the pollutants, the meteorology and the orography of the considered area. Even the complexity of the affected biological systems can be a cause of difficulty. The microbial ecosystems established on plant surfaces are strongly influenced by pollutants (Manning 1976, Dowding 1986, Fenn *et al.* 1989) and the capacity of these microbial populations to withstand environmental changes can influence their activity and consequently the whole host plant condition. The presence of such microbial populations on plant leaves and their role in global plant welfare are not well understood. It is known that some of these microorganisms fix atmospheric nitrogen (Ruinen 1974, Sucoff 1979, Murty 1983, Favilli and Messini 1990), produce plant growth regulators (Buckley and Pugh 1971), and can control plant parasites either by stimulating plants to synthesize phytoalexins (Last and Warren 1972) or by producing antibacterial (Mc Cormack *et al.* 1994) and/or antifungal compounds (Starmer *et al.* 1987). In some cases such a community can also produce air contaminants (Smith 1976).

*Tillandsia* (Bromeliaceae), a vascular neotropical epiphytic genus, represents a suitable model for phyllospheric studies because the microbial community that lives on these plant leaves, especially the nitrogen fixing species, greatly contributes to the plant nutrition (Brighigna *et al.* 1992). *Tillandsia* leaves are covered by characteristic trichomes, which

play a role in the absorption of water and nutrients for the plants. In addition, the trichomes conform an ecological niche that shelters a plentiful microbial population (Brighigna 1992) whose metabolic activity, together with the organic and inorganic nutrients coming from different sources (air, host leaching), play a fundamental role in the whole plant nutrition. This nutritional activity is especially important in *Tillandsia* plants that live in extreme conditions such as high-tension wires. In general the species of *Tillandsia* either lack roots or the roots are unable to absorb nutrients and serve only as an adhesion system. To date, investigations on phyllosphere organisms have mainly considered the effects of pollution on yeasts and fungi of different plant species (Manning 1976, Dowding 1986, Fenn *et al.* 1989). Since the phyllospheric microflora plays a fundamental role in *Tillandsia* nutrition, we have investigated the influences of air pollution on the total phyllospheric microflora of *Tillandsia* leaves, with the main purpose to evaluate how it affects the phyllosphere microbial composition, and trying to establish whether a relationship exists between the nutrition of the plants and the associated phyllospheric microflora. This first approach to the problem of the relationships between the phyllosphere composition in *Tillandsia* and the air pollution was the consequence of a direct observation, not foreseen in advance..

## MATERIALS AND METHODS

**Material.-** Visiting San Josè, the capital of Costa Rica, the authors observed that all the *Tillandsia* plants living into the urban area were, as a consequence of intense roadway traffic fumes, markedly covered by a dark coat made by dust and ash particles retained by trichomes. In contrast *Tillandsia* plants living in open air sites were not coated. Many *Tillandsia* species were present on the host tree, *Thabebuia* spp. (Bignoniaceae), but we prefer do not collect tank species, because of their capacity to retain water, prefering bulbous species as *Tillandsia caput-medusae* and the very common intermediate species *Tillandsia schiedeana*. Thus plants of *Tillandsia caput-medusae* Morr, and *Tillandsia schiedeana* Morr, were collected at the heigh of 3.0-3.5 m in two different areas: one, an urban area, with high level of air pollution (the Avenida Segunda of San José, close to the border line between the municipalities of San Josè and San Pedro) and the other one without pollution, an open air site 10 Km away from any urban area, factories and road trafic but with the same epiphyte-host combination (inside a wood; along the road to Escazu', 84° 05' W; 9° 56' N).

**Method.-** The sampling was limited to few plants only and the researchs carried out on a representative sample obtained homogenizing three plants collected in each site.

The collected plants were put in sealed polyethylene bags, stored at 0°C, in a portable refrigerator, and sent to the lab in Firenze (Italy) where they arrived 96 hours after the collection. Samples of each unpolluted and polluted *Tillandsia* species collected were prepared immediatly after the arrival of the plants in the lab, selecting leaves from the same position with the assumption that their age was comparable.

Chemical analisys of the leaves samples, carried out as reported elsewhere (Brighigna *et al.* 1997), collected in the urban area showed high concentration of heavy metals (169.0; 2.9; 69.0 $\mu g\ g^{-1}$ respectively for Pb, Cd, and Cu) indicating pollution by motor vehicles and industries while in the leaves collected in the open air site the concentration of the same heavy metals was negligible.

Each sample was obtained by homogenizing, in a mortar with sterile quartz sand and 9 ml of sterile saline (9 %.), one gram of fresh weight of leaves taken from each *Tillandsia* specimens collected. Samples were replicated three times.

The dilutions were plated on different media suitable for each microbial group. Developed colonies were counted and then randomly chosen and purified. The media used were: (1) tryptone yeast extract for bacteria (5 g tryptone, 3 g yeast extract, 15 g agar, with volume made up to 1 l with distilled water, pH 6.8); (2) caseinate-asparagine agar for actinomycetes

(2 g sodium caseinate, 0.1 g asparagine, 4 g sodium propionate, 0.5 g dipotassium phosphate, 0.1 g magnesium sulphate, 0.001 g ferrous sulphate, 15 g agar, with volume made up to 1 l with distilled water, pH 8.0); (3) potato dextrose agar supplemented with streptomycin for fungi and yeast (potato infusion from 200 g potatoes, 20 g dextrose, 15 g agar with volume made up to 1 l with distilled water, pH 5.6); (4) semisolid N-free Rennie medium modified replacing sucrose by glucose for nitrogen fixing bacteria (5 g glucose, 5 g mannitol, 0.5 g sodium lactate, 0.8 g dipotassium phosphate, 0.2 g potassium diphosphate, 0.2 g magnesium sulphate, 0.06 g calcium cloride, 0.1 g sodium cloride, 0.1 g yeast extract, 0.025 g sodium molibdate, 0.028 g sodium-ferrous EDTA, 5 $\mu$g biotine, 10 $\mu$g *p*-aminobenzoic acid, 1.75 g agar with volume made up to 1 l with distilled water, pH 7.0). Nitrogen-fixing bacteria were counted with the MPN method combined with the acetylene reduction assay (Burris 1974). The bacterial pellicle developed was diluted and plated on modified N-free solid Rennie medium (Brighigna *et al.* 1992). After 36 h of incubation several colonies were isolated. These bacteria were considered nitrogen fixing bacteria. All the bacterial strains isolated were identified using the criteria suggested by Krieg and Holt (1984) in Bergey's Manual of Systematic Bacteriology as well as by using either the API (Appareil et Proceides d'Identification API system S.A.A., France) tests 20 E, 20 NE (Rennie 1980) and 50 CHB (Anonimous 1979, Mc Carley and Rennie 1980, Logan and Berkeley 1981, 1984) or the BIOLOG method (Garland and Mills 1991). The yeasts were identified according to Kreger Van Rij (1984) and by using the API systems ID 32C, while the isolated fungi were identified using the criteria recommended by Domsh *et al.* (1980).

## RESULTS

The main effect of the pollution by heavy metals, retaines by the leaves on the phyllosphere microflora was the strong inibition of each group of microorganisms (Table 1). The degree of inibition was ranging from the 100% for yeasts and 97% for the bacteria in both Tillandsias to nearly 60% and 50% either for the fungal population or for the nitrogen fixing bacteria in *Tillandsia caput-medusae* and *Tillandsia schiedeana* respectively..

The species composition of the microflora living on *Tillandsia caput-medusae*, *Tillandsia schiedeana* polluted and unpolluted leaves is shown in Tables 2-3.

Table 1.

*Total counts of microorganisms on* Tillandsia *leaves (CFU/g fresh weight)*.*

| | *Tillandsia caput-medusae* | | *Tillandsia schiedeana* | |
|---|---|---|---|---|
| | Not polluted | Polluted | Not polluted | Polluted |
| Bacteria | $103 \times 10^4$ | $2.7 \times 10^4$ | $25 \times 10^6$ | $50 \times 10^5$ |
| $N_2$ Fixing Bacteria | $2.5 \times 10^4$ | $1.5 \times 10^4$ | $4.5 \times 10^4$ | $2.5 \times 10^4$ |
| Fungi | $18 \times 10^4$ | $5.5 \times 10^4$ | $5 \times 10^4$ | $2.4 \times 10^4$ |
| Yeasts | $66 \times 10^4$ | 0 | $1.6 \times 10^4$ | 0 |

* Average of six replicates of three sampling.

On *Tillandsia caput-medusae* and *Tillandsia schiedeana* there were no marked differences in the bacterial species composition. The same species with some small differences occur in both unpolluted leaves, while on polluted leaves Tillandsia *schiedeana* showed more bacterial species than *Tillandsia caput-medusae.*

Concerning the yeast and fungal species detected on both *Tillandsia* plants the differences between unpolluted and polluted is very relevant, but the unpolluted leaves of *Tillandsia schiedeana* showed more fungal species than *Tillandsia caput-medusae.*

TABLE 2

*Bacterial microflora living on leaves of* Tillansia caput-medusae *and* Tillandsia schiedeana*

| Species | *Tillandsia caput-medusae* | | *Tillandsia schiedeana* | |
|---|---|---|---|---|
| | Not polluted | Polluted | Not polluted | Polluted |
| *Aeromonas* spp. | | (5) | | (2) |
| *Agrobacterium radiobacter* | (10) | | (8) | |
| *Bacillus brevis* | (3) | | | |
| *Bacillus cereus* | (3) | (5) | | (3) |
| *Bacillus circulans* | | (3) | (3) | (3) |
| *Bacillus licheniformis* | | | (2) | (4) |
| *Bacillus pumilus* | | (2) | (3) | (3) |
| *Bacillus subtilis* | | (3) | | |
| *Bulkolderia cepacea* | (2) | | (5) | |
| *Enterobacter agglomerans* | (4) | (4) | (3) | |
| *Erwinia* spp. | | (4) | (2) | |
| *Flavobacterium* spp. | | | | (3) |
| *Flavobacterium odoratum* | | | (3) | |
| *Pseudomonas luteola* | (2) | | | (3) |
| *Pseudomonas maltophila* | (2) | | | (2) |
| *Pseudomonas paucimobilis* | | | (3) | |
| *Pseudomonas vesicularis* | | | (4) | |
| *Rhanella aquatilis* | | | | (2) |
| *Serratia liquefaciens* | | | | (2) |
| *Serratia marcescens* | | | | (2) |
| *Sphingobacterium* spp. | | | | (3) |
| *Sphingobacterium multivorum* | (2) | (4) | (2) | |
| *Yersinia aldovae* | | (2) | | |
| **Actinomycetes** N.I. | | (9) | | |

*() *number of strains isolated. N.I not identified*

TABLE 3

*Yeast and Fungi living on leaves of* Tillansia caput-medusae *and* Tillandsia schiedeana *

| | *Tillandsia caput-medusae* | | *Tillandsia schiedeana* | |
|---|---|---|---|---|
| Species | not polluted | polluted | not polluted | polluted |
| Yeasts | | | | |
| *Aureobasidium pullulans* | (2) | | (4) | |
| *Candida* spp. | (6) | | (2) | |
| *Cryptococcus* spp. | (2) | | (4) | |
| *Sporobolomyces* spp. | | | (4) | |
| Fungi | | | | |
| *Acremonium* spp. | | | (2) | |
| *Humicola* spp. | | | | (2) |
| *Mucor* spp. | | | (2) | |
| *Nigrospora* spp. | | | (2) | |
| *Penicillium* spp. | (5) | (3) | (16) | (9) |

*() *number of strains isolated.*

## DISCUSSION

Our investigations showed that air pollution influences phyllospheric microflora in terms of total microbial counts and species composition, reducing the number of bacteria and yeasts on exposed leaves in *Tillandsia-caput-medusae* and *T. schiedeana.* Fungi and nitrogen-fixing bacteria showed less sensitivity.

We also observed on both *Tillandsia* species, from the polluted site, a reduction of the number of the organisms in each of the considered microbial groups.

The reduction of the phyllospheric colonization by pollutans can be explained by the fact that leaves collected in the urban area have absorbed hight quantities of heavy metals, mainly lead, cadmiun and copper. Even if the effects of these metals on phyllosphere microbial population are poor understood, our results are comparable with previous investigations on the relative effects of lead, cadmiun and zinc on the microflora occurring on leaves of hawthorn,oak, willow, elm and some herbaceous plants showing that bacteria and yeasts seemed very sensitive to lead, while fungal population seemed less influenced, but the combined effect of the three metals exerts the main negative influence (Little 1973, Bewley 1979, Bewley and Campbell 1980).

Because of the hight content of the heavy metals in the *Tillandsia* leaves collected in the urban area, the strong reduction of the yeast and bacterial population observed can be the consequence of this pollutans. This finding is also consistent with other reports that suggest the use of leaf yeasts as indicators of air pollution (Dowding 1986, Dowding and Richardson 1990). Nevertheless, despite the significant reduction of the total bacterial population, the nitrogen-fixing bacteria, which represent an important source of fixed nitrogen for *Tillandsia* nutrition (Favilli *et al.* 1975, Favilli 1992, Brighigna *et al.* 1992), were not greatly reduced in the urban area. This fact is noteworthy, since is in agreement with the observed good health of the polluted *Tillandsia* samples. The fact that the fungal population was not greatly reduced in polluted samples is also surprising because other authors indicate that air pollutants and acid rains strongly reduce the phyllosphere fungi (Fenn 1989, Helander and Rantio-Lehtimäki 1990). The limited decrease in the fungal abundance could be a consequence of the change in the competitive relationships between microorganisms, caused by the strong reduction of bacteria.

Air pollution also influenced *Tillandsia caput-medusae* and *T. schiedeana* phyllospheric species diversity. Among polluted plants bacteria

there was a notable absence of the genera *Agrobacterium* and *Enterobacter* and the presence of *Aeromonas*. These changes suggest greater sensitivity to urban pollutants of the first two genera with respect to *Aeromonas* species. The absence of species belonging to the genus *Pseudomonas* on polluted *Tillandsia caput-medusae* does not seem to be correlated with air pollution effects because *Pseudomonas* is present even on *Tillandsia schiedeana* polluted leaves. The presence of more species belonging to the genus *Bacillus* on polluted plants is in agreement with the capacity to tolerate heavy metals that characterizes the spore-forming bacteria (Austin *et al.* 1977). Fungal species composition has shown a variation only on *Tillandsia schiedeana* plants: fungi belonging to the genera *Acremonium*, *Mucor* and *Nigrospora* have been shown to be more sensitive than the genus *Penicillium*, which was found also on polluted leaves of *Tillandsia caput-medusae*. The effects on actinomycetes are not easily explicable because this microbial group is absent on unpolluted *Tillandsia caput-medusae* plants and their presence on *Tillandsia schiedeana* may be an occasional event which is rarely recorded in the phyllosphere.

Further research has to be carried out on several other species of *Tillandsia* in order to establish which group of microoganisms, habitual members of the phyllospheric microflora of these epiphytic plants, is influenced by pollutants. Our preliminary results show that the phyllosphere microflora of *Tillandsia* plants living in a tropical urban environment changes in terms of number and species composition of yeasts and bacteria with respect to plants living in unpolluted environment, but despite of the high levels of metal pollution, the contaminated plants appear able to support a phyllosphere microflora. The relationship between microflora and heavy metals pollution may be more complex and furthemore, other environmental pollutants in the study area may have an influence as great as that of lead, cadmiun and copper.

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