EXPOSURE OF TILLANDSIA USNEOIDES AT SILVER NANOPARTICLES

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Abstract
Spanish moss (Tillandsia usneoides) is an epiphyte which absorbs nutrients and water from air and rainfall. Spanish moss has threadlike leaves with a length of 2.5-7.5 cm and a rough surface which makes it suitable for atmospheric monitoring.

T. usneoides, after pre-treatment with distilled water, was exposed to Ag-NPs through two spraying cycles (Ag-T1, Ag-T2) of a suspension at 1000 mg L⁻¹ in a special greenhouse.

Five replicates of treated and untreated plants were harvested, air dried and finely milled. 250 mg were weighted in PTFE vessels and mineralized in microwave oven (Start D 1200, Milestone) with HNO₃ Suprapure (Merck, Germany) and H₂O₂. The mineralized solution was brought to 20 mL with Milli-Q water. The major and trace elements were determined by ICP-OES (Arcos, Ametek). Some samples of leaves were placed in formalin 10% for analysis with ESEM-EDS.

Concentration of Ag in plant tissues depended from the time of exposure. In particular 0.1 mg Ag kg⁻¹ was the background level of untreated Tillandsia, while Ag-T1 was 2.7 mg kg⁻¹ and Ag-T2 was 3.2 mg kg⁻¹. The Ag accumulation in Tillandsia tissues were statistically significant with respect to background level (p<0.05).

The leaves of Tillandsia treated with Ag-NP had higher amounts of Ca, Na, P and S than the control; at the same time, Al and Fe concentrations were lower.

Electron microscopy investigations on the fate of Ag-NPs in T. usneoides leaves revealed the presence of Ag-NP on the leaves tissue.

Keywords: Nanoparticles, nanosilver, Tillandsia usneoides, biomonitor, SEM.

1. INTRODUCTION
The development of nanoscience and nanotechnology is an opportunity to produce superior materials for industrial and health applications [1]. The worldwide expansions of nanomaterials led to a huge impact on environment and human health; however the effects of large emissions of manufactured nanomaterials have not been fully explored yet.

Nanoparticles (NPs) represent the first block for nanomaterials building; particularly ultrafine particles (<100 nm) are well known to have a greater adverse impact on health than larger particles [2]. In addition, due to their antimicrobial potential [3] silver nanoparticles (Ag-NPs) are among the most common NPs in consumer goods: cosmetics, therapeutic agents, textile, household products and in agronomics pesticides (e.g. “Nanoargentum 10”, Fa Nanosys, Switzerland). Hence products containing engineered nanomaterials (ENMs) are already on the market and can be released into the environment during all their life cycle, from the production to the waste disposal (landfills and incinerator plants).
However, safety regulations for NPs use and disposal at the end of the life cycle have not been delivered yet by organizations for standards (ISO, OECD, etc.). Moreover, there is a lack of knowledge on the real release of engineered nanoparticles in the environment; this is a key problem because the risk of a molecule is determined by the effect as well as by the exposure. Currently, techniques to quantitatively monitor ENM emissions are lacking [4]. Therefore, the development of methods to measure and characterize ENMs in atmospheric, aquatic and terrestrial ecosystems is an important research priority.

Particularly, air pollution (presence of inorganic and organic toxic products) is now placed in relation to the risk for human health and environment. Air quality assessment by environmental biomonitoring, the study of atmospheric deposition on plants (higher plants, mosses and lichens), is well documented [5]. The use of biological materials for monitoring heavy metal air pollution was introduced 30 years ago. Since then, a variety of organisms and materials have been proposed for biomonitoring purposes. These include mosses, lichens, tree barks, tree rings, pine needles, grass, leaves and ferns [6]. However the use of mosses for the monitoring of NPs deposition has two main drawbacks: the interference of natural NPs makes difficult to determine the trace levels against a higher background of natural colloids; moreover mosses can't live in dry weather conditions. *Tillandsia usneoides* (Spanish moss), an epiphytic bromeliad plant which takes out nutrients and water from air and rainfall, seems to be a suitable biomonitor for atmospheric aerosol study. Indeed, it has no contact with soil thus it can take out contaminants from the atmosphere [7]. Spanish moss has threadlike leaves that are about 2.5 to 7.5 centimetres long and with a rough surface which makes it suitable for atmospheric monitoring. Scanning electron microscope observation analysis [8] suggested that pollutants associated with particulate matter are adsorbed by leaves surface, scales and stem.

Therefore this research aimed at evaluating the potentiality of *Tillandsia* as biomonitor for nanoparticles in the atmosphere. For this purpose *Tillandsia usneoides* samples were sprayed and exposed at Ag nanoparticles in a special greenhouse built for toxicological investigations of NPs and incubated for 2 months, then the Ag accumulation in *Tillandsia* was investigated both by mineralization in microwave oven and by ESEM-EDS analysis.

### 2. MATERIALS AND METHODS

#### 2.1 Exposure of NPs in greenhouse.

*Tillandsia* plants were washed for one week in the greenhouse of the Agricultural Science of the University of Bologna in order to eliminate the particulate stored on stem and leaves, then they were brought into a special greenhouse in Modena (Italy). The toxicological investigations of NPs on *Tillandsia* were performed in this greenhouse (10x4 m²) in which two cubicles were set up dividing the space for nanoparticles fumigation from a clean control area. The cubicle polluted with NPs was equipped with two certified NPs filtering devices in order to protect the external environment. The operators were protected with a mask connected through a tube to an external air generator (Draeger, Germany) and an electro filter to bring down the nanopollution [9]. For the evaluation of the acute effects of NPs, an aerosol system was designed to expose plants to airborne NPs. Aerosol generator emitted an aqueous suspension of NPs (1000 mg/L) at a rate of 0.3 g/min with a mean droplet diameter of 0.35 μm.

*Tillandsia* was exposed to Ag-NPs through two spraying cycles (Ag-T1, Ag-T2) of the suspension, while the control plants were sprayed with distilled water. The cycles were composed by 5 and 10 spraying operations, for Ag-T1 and Ag-T2 respectively, of 10 minutes every day, for one and two week respectively. The apparatus used to spray the *Tillandsia* is showed in Fig.1.
Fig. 1 Aerosol generator used to spray the Tillandsia in greenhouse.

2.2 Tillandsia usneoides analysis.

Five replicates of treated and untreated Tillandsia were harvested, air dried and finely milled using an agate mill in order to obtain a fine and homogeneous powder. 250 mg were weighted in PTFE vessels and mineralized in a microwave oven (Start D 1200, Milestone) with HNO₃ Suprapure (Merck, Germany) and H₂O₂ (ratio 4:1 v/v). The mineralized solution was brought to 20 mL with Milli-Q water. Ag as well as the major and trace elements concentrations were determined by Inductively Coupled Plasma with optical spectrometry (ICP-OES Arcos, Ametek, Germany).

In addition some Tillandsia plants were sampled and placed in formalin 10% for analysis with scansion electron microscopy coupled to X-ray probe (ESEM-EDS). Plants without any chemical fixation were directly mounted on stubs for chemical analysis.

3. RESULTS AND DISCUSSION

3.1 Concentration of Ag and macro elements in Tillandsia usneoides tissues.

The concentration values for Ag in T. usneoides tissues are shown in Tab.1.

<table>
<thead>
<tr>
<th>Control</th>
<th>Ag-T1</th>
<th>Ag-T2</th>
</tr>
</thead>
<tbody>
<tr>
<td>µg kg⁻¹</td>
<td>SD</td>
<td>SD</td>
</tr>
<tr>
<td>Ag</td>
<td>107.8</td>
<td>12.2</td>
</tr>
</tbody>
</table>

The T. usneoides stem and leaves were completely covered by scales, being responsible for the majority aerosol adsorption [10]. The concentrations of Ag in tissues of sprayed plants were higher than the control. The enrichment of Ag in T. usneoides treated in greenhouse with respect to the control was calculated by the following equation:
\[ EF = \frac{(CEa - CEc)}{CEc} \times 100 \]

Where: EF is the enrichment (\%) of the concentration of the element E; CEa is concentration of the element E in the sample; CEc is concentration of the element E in the control sample. The values of enrichment were were 200 times higher for both treatments, highlighting the efficacy of Ag-NPs treatments in greenhouse and the Tillandisia capability to collect the Ag-NPs on its leaves.

3.2 Concentration of major elements in T. usneoides tissues.

The concentration values for the analysed major elements are presented in Tab. 2.

Tab. 2 Results obtained in Tillandsia usneoides in greenhouse treatment with Ag-NPs spraying. The standard deviation (SD) of three replicates is also reported.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Ag-T1</th>
<th>Ag-T2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SD</td>
<td>µg kg(^{-1})</td>
<td>SD</td>
</tr>
<tr>
<td>Ca</td>
<td>5404.8</td>
<td>1037.3</td>
<td>10538.2</td>
</tr>
<tr>
<td>Fe</td>
<td>43.7</td>
<td>8.4</td>
<td>52.7</td>
</tr>
<tr>
<td>K</td>
<td>694.9</td>
<td>83.5</td>
<td>120.0</td>
</tr>
<tr>
<td>Mg</td>
<td>1543.7</td>
<td>227.5</td>
<td>3068.6</td>
</tr>
<tr>
<td>Na</td>
<td>1707.6</td>
<td>249.8</td>
<td>1607.9</td>
</tr>
<tr>
<td>P</td>
<td>460.3</td>
<td>64.1</td>
<td>526.6</td>
</tr>
<tr>
<td>S</td>
<td>1078.0</td>
<td>180.9</td>
<td>1520.0</td>
</tr>
</tbody>
</table>

The values of major elements concentration were lower than those found in T. usneoides exposed at atmospheric pollution in an urban environment [11]. The plants sprayed with Ag-NPs showed concentration of Ca, Mg, Na, P and S higher, while those of Al, Fe and K were lower than the control. The variation of major elements concentrations can be due to metabolic stress of T. usneoides after treatment with Ag-NPs.

3.3 Electron microscopy investigation

Analytical techniques associated with electron microscopy, like energy-dispersive X-ray analysis have been used to detect contaminant elements, helping the understanding of the process involved in nanoparticles accumulation by organisms. The morphological analysis showed that the surfaces of all shoots and leaves were completely covered by particles (Fig. 2a). The detail of leaves (Fig. 2b) showed the particles collected by scales. The particles were present on the leaves and their sizes were about 3-4 micrometres (Fig. 3a and 3b). These particles were probably aggregates of Ag nanoparticles sprayed, or nanoparticles collected from the atmosphere before the treatment. Nanoparticles of Ag were found on leaves (Fig. 4a and 4b).
Fig. 2a View of shoot of *T. usneoides* covered by particle

Fig. 2b Detail of leaves of *T. usneoides* in which particles are collected by scales.

Fig. 3a Enlargement of leaves

Fig. 3b Enlargement of stoma

Fig. 4a Ag nanoparticles found on *Tillandsia* leaves

Fig. 4b X-ray spectra of Ag-NPs
4. CONCLUSIONS

*Tillandsia usneoides* proved to be an excellent bioindicator for nanoparticles. In particular the simulation of NPs emission in greenhouse and SEM investigations could allow the determination of the potential incorporation of engineered nanoparticles in the tissues. Our research is in progress to determine the introduction processes of nanoparticle into tissues of plants and understand their potential risk.

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REFERENCES


