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STSM type: Regular (from Slovenia to Switzerland)

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#### Short Term Scientific Mission

STSM Topic: **Measuring isotopic composition of carbohydrates in leaves of stressed urban trees**

#### Introduction

Urban trees grow under the influence of many stress factors. Most common are higher temperatures, changed water availability and modified soil structure. Soil surface, covered with tarmac/asphalt, disables gas exchange and increases water run-off and as such directly causes drought stress to urban trees, more dependable on precipitation than to ground water. Our research question was to investigate how much influence have different types of covered soil on trees: 1) growing beside road, 2) between road and pavement and 3) on parking lots. To test this influence, the isotopic composition of carbohydrates in leaves of stressed urban trees was measured.

#### Purpose of the STSM:

To learn a method of extracting soluble organic material from bulk leaf and to measure its isotopic C12/C13 ratio.

#### Description of the work carried out during the STSM;

All the methods were part of standardized operation procedure, using techniques and infrastructure available at the Paul Scherrer Institute as the host institution. First, dry leaves were grinded, using a Retsch MM 2000 ball mill (Retsch GmbH, Haan, Germany) for 11 min. For each tree, around 50 mg of grinded leaf material was put in plastic vials, weight was noticed and added 1500 ml of purified water. Vials were well shaken with Vortex and put in warm water bath at 85 °C for 30 minutes. Then centrifuged at 12 000 RPM for 2 minutes. Out of solution, 1.2 ml was taken and put in separate capsules through filter. The solution was considered to be the water-soluble (exportable) fraction. Centrifuged again. From each capsule 0.15 ml was taken of the liquid and put into tin capsules for liquids. Samples were put to freezer at -20 °C for half an hour and then relocated to freeze-dry, at -54 °C overnight. Weight of the samples was again measured and noticed. Tin capsules of dried material were burned in an elemental-analyser and the resulting CO<sub>2</sub> analysed by Isotope-ratio mass spectrometry (IRMS).

Description of the main results obtained;

Results indicated, that trees growing with most covered soil suffered more stress (less negative values of C13 ratio) than trees with mean soil coverage. But the highest (least negative values) C13 ratio was observed for trees with the least soil coverage, which we believe is connected to higher photosynthesis rates. However, in the next phase of studying trees response to stress factors, results of carbon isotopic measurements from leaves or urban trees will be compared to results of soil moisture measurements, pre-dawn water potential and photosynthesis rates.

Description about how the results contribute to the Action aims

Trees response to temperature and drought stress can be measured using different tools. Isotopic composition in tree-rings, bulk leaf, and photosynthetic products provide useful information because isotope fractionation during CO<sub>2</sub> fixation is modulated by environmental conditions. Materials like tree-rings and bulk leaf record growth factors for a longer period of time, and for this reason their stress signal is averaged over the whole growing season or more. On the other hand, sugars or water soluble organic material as current photosynthetic products, are formed in a shorter time window, and are therefore under the influence of short-term environmental factors. Measuring isotopic composition of these photosynthetic products, can help us estimate the stress of trees in more detailed way. Combining support information on trees vitality, photosynthetic rates and isotopic composition of their products, offers better understanding of tree responses to extreme events.

This report may be posted on the Action website.