SHORT TERM Mobility Program 2016

Scientific Report

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Title of the Research Program: Evaluation of phytotoxicity in sediments of Lake Megantic- Chaudière River and in water samples of Lake Orta's rivers.

Specific Objectives of the Research Program: The project aims to evaluate toxicity of water and sediments samples by means of phytotoxicity test using three plant species representative of mono- and dicotyledons. Tests evaluating seed germination and root elongation on cress, cucumber, and sorghum, will be run according to the Italian Official Method UNI MU 1651:2003 protocol. We will test samples of water from three rivers of Lake Orta (Italy) and of bottom sediments and soils from shores of Lake Megantic- Chaudière River (Quebec, Canada).

Research completed during the visit: During these weeks, the activity focused on the discussion of results deriving from phytotoxicity test on Canadian sediments and on water samples of Lake Orta's rivers. Results highlighted the necessity of new samples from the Canadian river in order to improve the number of data to complete a sound statistical analysis, so we plan new samplings for the next year. In, addition we discuss the possibility to apply other techniques to evaluate the toxicity of Canadian river: e.g. test in situ with *Daphnia magna* or with the autochthonous species, test in situ with mosses.

Results on Canadian sediments

Seed germination – root elongation tests on Canadian sediments
The tests have been performed according to the Italian Official Method UNICHIM (2003) Water quality Determination of the inhibition of the seed germination and root elongation on *Cucumis sativus* L. (cucumber), *Lepidium sativum* L. (cress), *Sorghum saccharatum* Moench (sorghum) (Short-chronic toxicity test).

This seed germination— early growth inhibition test foresees the use of seeds of 3 higher plants (two monocotyledons, one monocotyledon), exposed in the dark at 25 °C for 72 h, with 4 replicates *plus* their respective negative controls for each species.

For each replicate, an amount of sample equivalent to 10 g dry weight has been placed on a Petri dish, covered with a Whatman No 1 filter paper, and wetted with 5 ml pure water (MilliQ). On top of the filter paper, 10 seeds of the respective plant species have been placed.

Since different toxicants can affect different metabolic pathways, the test has three different points: the number of germinated seeds, the lengths of roots (and shoots for sorghum, which presents separated emergency for root and shoot), and the Germination Index (number germinated seeds times the lengths, in percent relative to negative controls).

Negative controls
The seed normally contains enough reserve materials to allow the early stages of development.

Therefore, the seeds can germinate regularly when wetted with pure water (Milli Q). However, to provide closer conditions to those of the real samples, the negative controls can use also a solid reference material.

In this study, both plain sand (Fontainbleu) and a reference soil have been used. This artificial soil, prepared according to ISO11269-1 and ISO 11269-2 has the following composition:

	Percentage expressed on dry-mass basis	
Sphagnum peat finely ground and with no visible plant remains	10 %	
Kaolinite clay containing not less than 30 % kaolinite	20 %	
Industrial quartz sand (dominant fine sand with more than 50 % of particle size 0,05 mm to 0,2 mm)	69 %	

The germination and early growth of each plant species in these three negative controls are shown in figures 1 and 2.

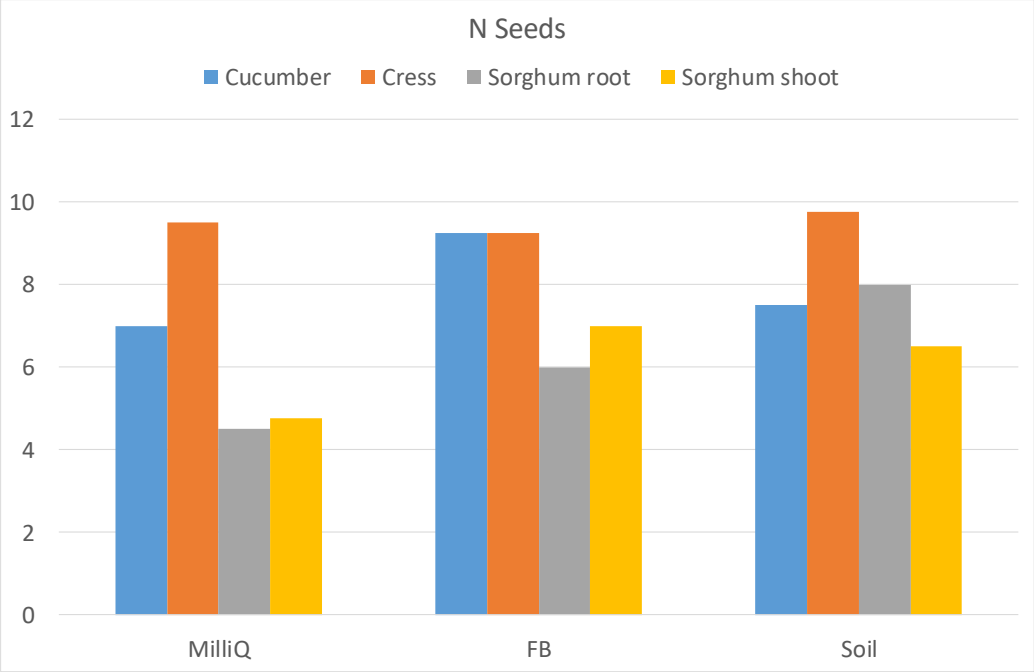

Fig. 1 – Number of germinated seeds in the three negative controls (pure water MilliQ, Fontainbleu sand, reference soil.

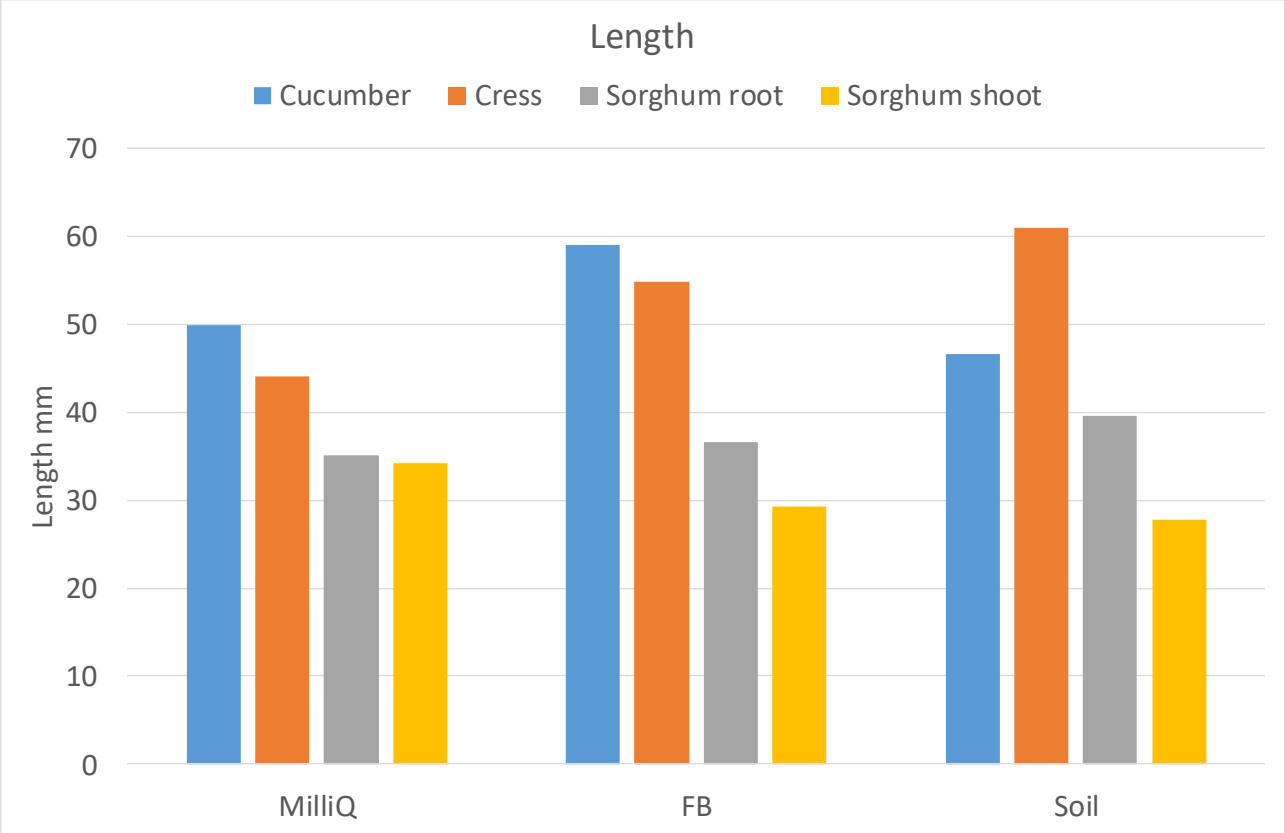

Fig. 2 – Length (mm) of roots (and shoot for sorghum) in the three negative controls (pure water MilliQ, Fontainbleu sand, reference soil.

Since the sediment samples contain a variable amount of organic matter (Carbon ranging from 0.23 to 10.86 %), and considering that both germination and elongation are rather similar for the three negative controls, the data for sediments in the following discussion will be referred to the artificial soil.

Germination

The results of seed germination are shown in figure 3.

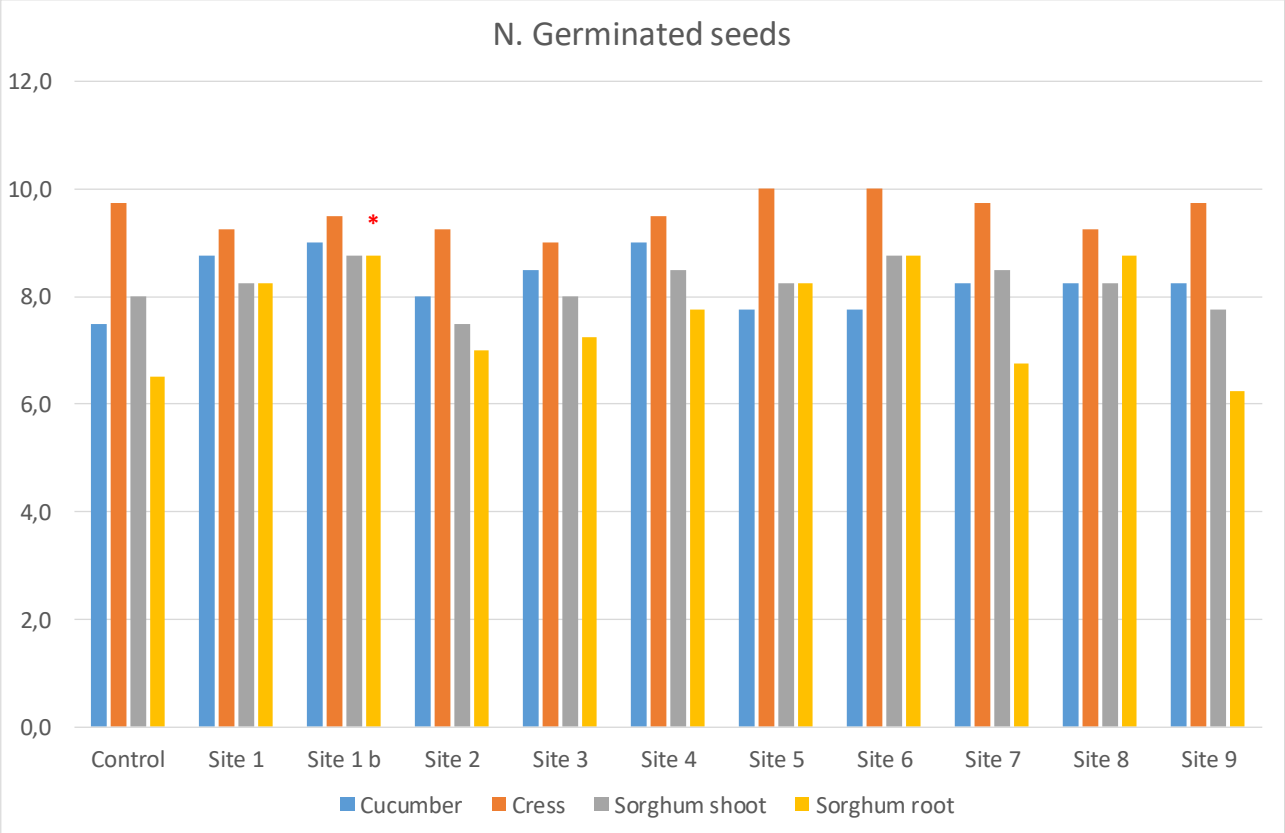

Fig. 3 – Seed germination

Only for sorghum, Site 1b shows a statistically significant ($P \leq 0.05$, marked with *) higher germination than the negative control (reference soil).
All other samples and plant species have similar germination for the three negative controls.

Elongation

Figure 4 reports the data for the early growth of the three plant species. Only Site 1b and Site 7 do not differ from the respective controls, whereas for all the other sites a statistically significant ($P \leq 0.05$, marked with *), or highly significant ($P \leq 0.01$, marked with **) increase of length, with respect to the relative negative controls (reference soil), has been detected for one or more of the tested plant species.

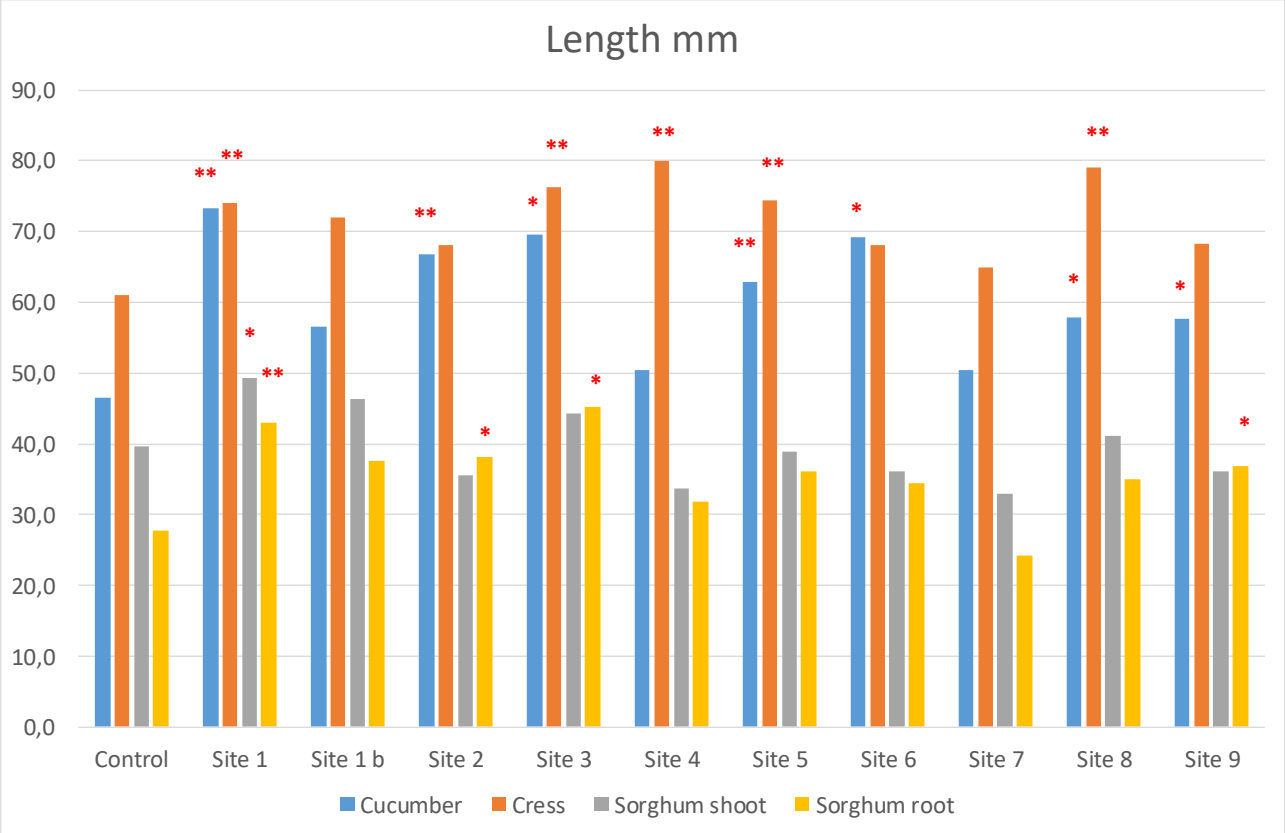

Fig. 4 – Length of roots (and shoots, for sorghum), compared with those of the respective negative controls (reference soil).

Germination Index %

Combining the seed germination with the length, the Germination Index, expressed as a percentage of the respective negative control (reference soil), has been calculated.

As shown in figure 5, sites from 1 to 6 display a statistically significant ($P \leq 0.05$, marked with *), or highly significant ($P \leq 0.01$, marked with **) increase of the GI%, with respect to the relative negative controls (reference soil) for one or more of the tested plant species.

Correlations

The chemical analyses of the sediments, provided by Laval University, have been used to look for possible correlations with the results of the seed germination – elongation tests.

Only for the Germination Index of sorghum some statistically significant correlation has been found, and precisely a direct one with Benzo(c) phénanthrène, C1-Acénaphtène, and C1-Naphtalène. This means that, for sorghum, an increase of the concentrations of these compounds results in an increase of the sorghum GI%. In addition, a reverse correlation has been found with D8-Naphtalène, meaning that increasing concentrations of this chemical decrease the GI%.

However, all these correlations are based on only 3 or 4 data, and with a rather limited range of concentrations. Therefore, these indications should be considered very cautiously, and preferably to be confirmed testing more sediment samples, with a larger range of concentrations of these chemicals.

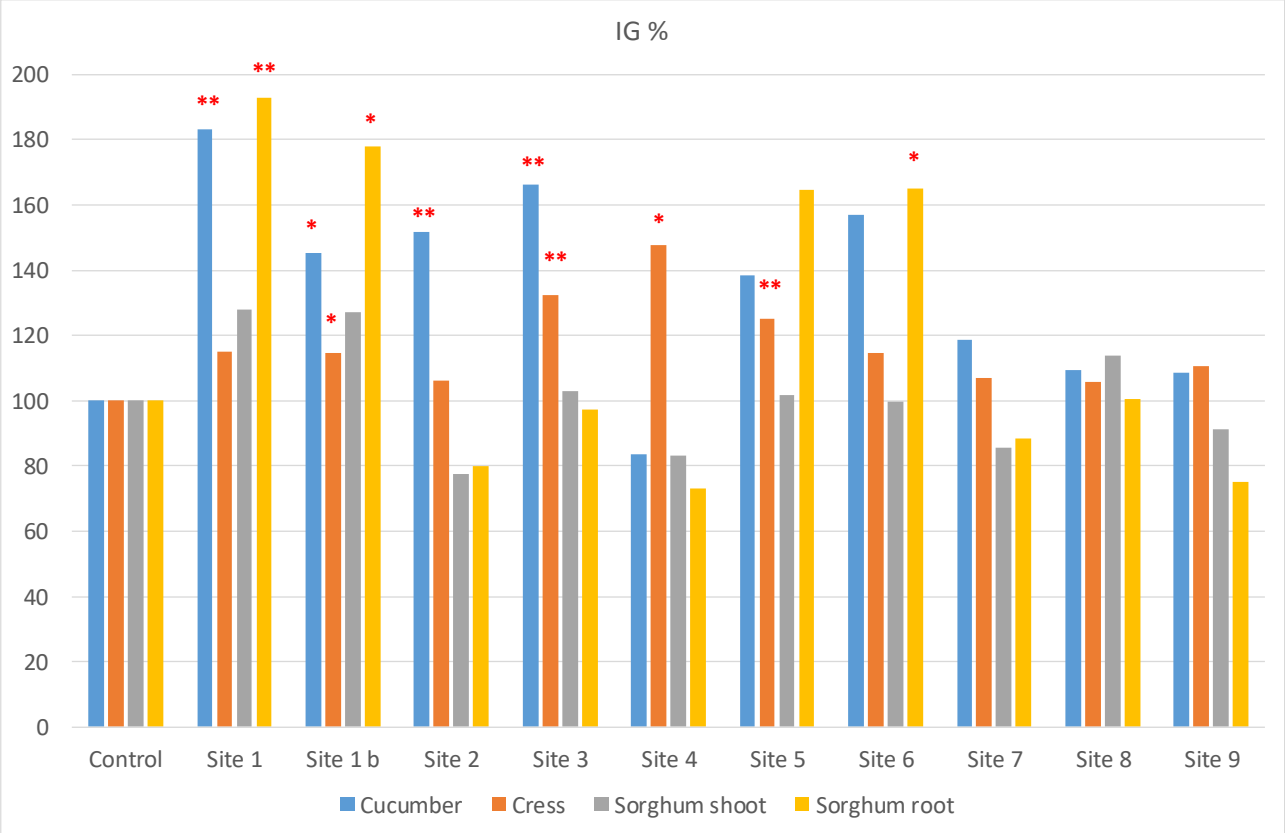

Fig. 5 - Germination Index, expressed as a percentage of the respective negative control (reference soil)

Conclusions

As general conclusion, none of the sediments proved to be definitely toxic for the three plant species. However, to various degree all sites altered the seed germination or the development or one or more of the plant species, inducing a bio stimulation (response higher than in the respective negative controls).

A common interpretation of this effect is called hormesis, it seems to be the first response to the exposure to one or more toxicants acting together: actually, the seeds response to the exposure to a slightly toxic sample is to increase their metabolism, hence apparently developing better and faster than in the negative controls, with the result that the toxicant(s) intake is “diluted” within a larger mass of plant tissue. In this way, the inner local concentrations are lowered and the seeds are able to better cope with the toxicant metabolic interferences.

Results on water samples of Italian sites.

Rivers (Pellino, Pellesino e Nigulglia) were sampled monthly from June 2015 to March 2016, test were performed as already described for Canadian sites and results compared with historical data (from May 2004 to May 2005). The chemical analyses, including those of metals, show a general improvement of the water quality from 2004 to 2016. All stations show an increase of the germination index for all species from 2004 to 2016. For Cucumis sativus the increase points to a shift from slight toxicity (Germination Index < 100 %) to a biostimulation (Germination Index > 100 %).

The water quality of the tributaries (Rivers Pellino and Pellesino) shows a slight increase of phosphorus and a decrease of Total Organic Carbon (TOC), with a further reduction of the metals loads, while the lake water (Qualba) points to a substantial stability (from 2004 to 2016). The outlet (River Niguglia) well records the improvement of the water quality of the whole lake. The results from the phytotoxicity tests confirm these general trends, proving that the lake conditions are still slowly changing for the best.

Products of the Mobility Grant visit:

During the visit, Rosa Galvez gave a seminar at ISE entitled "The human and environmental disaster at Lac Mégantic: the event, the impacts and the lessons to be learned ", about the accident occurred on the night of July 5, 2013, a freight train carrying light crude oil from North Dakota derailed in the town of Lac-Mégantic (Quebec, Canada), causing explosions, fires and the spill of 7000m^3 of oil. This catastrophe resulted in 47 human deaths, the destruction of the city's downtown and significant environmental pollution. Indeed, oil infiltrated soils and contaminated Lac-Mégantic (lake) and the Chaudière River. Floating oil was observed up to 186 km from Lac-Mégantic in the direction of the St. Lawrence River. This emergency is considered the worst case of an inland oil spill in North America. Remediation actions included excavation and biotreatment of contaminated urban soils and monitoring of contaminated water and sediments along the Chaudiere River.

A multidisciplinary group of researchers initiated a vast research program in order to use this case as a learning instrument for various environmental studies that included emergency actions and environmental impact evaluation. This presentation aims to present the emergency and some of the environmental quality monitoring results and research works to come.

On the basis of these preliminary results and on results deriving from next samplings we expect to accomplish at minimum one joint peer-reviewed publication. A tentative title and authors list is given below.

Galvez R., Beltrami M., Piscia R., Volta P., Baudo R.: Evaluation of phytotoxicity in sediments and water: a comparison between Canadian and Lake Orta's rivers.

Verbania, October 7th, 2016