Applicability of the Caenorhabditis elegans survival, growth and reproduction test to assess the effects of biosolids used in agriculture

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Applicability of the *Caenorhabditis elegans* survival, growth and reproduction test to assess the effects of biosolids used in agriculture

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1. Introduction

In the context of environmental sustainability, more and more materials are intended to be valuable instead of being wasted. Modern high productivity agriculture has led to an impoverishment of nutrients and organic carbon in field soils, and as a consequence plants nutrition complements like fertilisers or biosolids have to be used to keep high yield. These complex materials are a source of nutrients for plants; they could also be a source of pollutants depending on their origin. As these materials are applied in field, they can be potentially harmful to soil fauna, plants and humans. So there is a necessity to assess the environmental effects of these materials before field use.

In this study, environmental effects of biosolids were assessed on the survival, growth and reproduction of a microfauna organism of the nematoda family, *Caenorhabditis elegans*, following the standardized protocol ISO 10872 [1]. This widespread organism was chosen due to the possibility of testing soils and mixtures of soil and biosolids as well as water extracts of these matrices.

Performing tests with the ISO standard showed that protocols for sediment and water testing were clearly defined. These tests also demonstrated that the standard could be improved for testing soils and mixtures of soil and biosolids. Indeed, according to their respective water holding capacities (WHC), addition of growing media and food leads to a two phase system (e.g. water on top of the matrix). This can be explained by two reasons: soil moistening in the standardized protocol is based on soil dry weight and the food volume added to the wet sample is too important in comparison with the mass of the tested soil.

In order to harmonize test conditions and to allow comparison between results for soils and/or soils mixed with biosolids, optimization of the protocol aspects mentioned above were carried out: 1) a range of suitable percentage of water holding capacities was determined, to base sample moisture on WHC instead of dry weight; 2) food was inoculated at a higher concentration, to reduce the added volume. Then, different biosolids (limed sludge, manure ...) were tested using this protocol. An example for a limed sludge is given in this extended abstract.

2. Optimization of the test protocol concerning soil water input

Tests were conducted on five soils with different texture classes (Table 1). These soils were selected to cover a wide range of WHC. In addition to these experiments, extraction ratios on 75 and 1200 juveniles (Table 1) were carried out on the studied soils in optimized conditions, to ensure that the modification of test conditions didn't affect the recovery after the exposure period.

In order to determine if juveniles of *C. elegans* can develop in unsaturated as well as in saturated matrices, growth and reproduction of this nematode were recorded. The range from 40% to 100% of WHC was tested on each of the selected soils. Food (*Escherichia coli*) volume was decreased to be able to be included in the soil moistening volume. In the current protocol, growing media is added at 40% of soil dry weight and another volume of food is added. For comparison, expressions of results for soils moistened as the standardized protocol are given in Table 1 as a percentage of WHC of each of the selected soils.

To minimize the input of bacteria solution added to the soils, the volume was decreased by a factor of 10 and the concentration was increased by the same factor, to ensure that the same amount of food is provided as in standardized conditions. To determine if decreasing food volume can influence *C. elegans* endpoints responses, a comparison were made between the above mentioned soils, moistened according to the standardized method and moistened at 80% of their respective WHC, including food.

Table 1: Soils classification, WHC, extraction ratio and water addition for standardized conditions.

Soil	Soil classification	WHC (%)	Extraction ratio (mean %)		Standardized water addition (% of WHC)
			75 ind.	1200 ind.	
LUFA	Loamy sand	45	87	76	280
Agricultural	Sandy loam	40	93	85	350
Forest	Sandy clay loam	70	94	72	200
Garden 1	Sandy loam	65	77	81	215
Garden 2	Light clay	36	81	90	388

Regarding improvements of the test protocol, our results showed that:

1. In the range of soils moisture tested, 40% of WHC was not adapted for *C. elegans* reproduction. Growth was not impacted by this condition. Moreover the soils textures studied were shown to be suitable for *C. elegans* survival, growth and reproduction, as the test validity criteria for each soil were all satisfied.
2. The range from 60% to 100% WHC of the selected soils was suitable, 80% of WHC were selected to carry further tests on solid matrices. This condition allows to keep samples unsaturated and avoid soil drying during the exposure period.
3. Decreasing food volume and including it in the soil moistening volume didn't significantly affect neither *C. elegans* growth nor reproduction endpoints for the selected soils.

3. Assessing biosolids with *Caenorhabditis elegans*: an example of a limed sludge

To assess the effect of biosolids, different methods are available. As the studied biosolids are spread on agricultural field, it seems more logical to assess their effects based on their agricultural use rather than to determine a dose-effect relationship of the biosolid itself. In this example, the limed sludge application rate (3 $tons.ha^{-1}$, equivalent to 1X in figures) was used as a basis for the preparations of mixtures with standard soil (LUFA 2.2, control soil) and their respective water extract [2]. Different rates were tested, from one time to one hundred times the application rate. Results of *C. elegans* growth and reproduction in soil mixtures and in their water extracts are presented respectively in Figure 1 a and b. These results showed that reproduction is the most sensitive endpoint for both solid and liquid media. Indeed, it significantly decreased at a hundred times of the application rate on standard soil and from one fold this rate for water extracts. No significant differences were found for growth in solid matrices but it significantly decreased for the water extracts of soil and limed sludge mixtures. Surprisingly, water extracts of soil mixtures were more toxic than the mixtures themselves. These effects cannot be attributed to a pH increase.

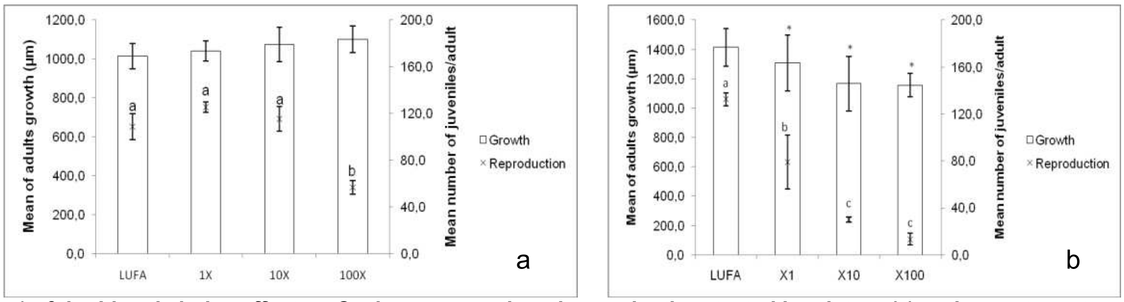

Figure 1a & b : Limed sludge effect on C. elegans growth and reproduction, tested in mixture (a) and as water extracts (b). a,b,c : significant differences among reproduction results, after ANOVA and Tukey HSD test (α=0.05); *: significant differences against LUFA for growth results after Kruskal-Wallis test (α=0.05).

4. Conclusions

There was a need to harmonize moisture conditions of soils and soil/biosolid mixtures to test this kind of materials with *Caenorhabditis elegans*. Growth and reproduction results for soils moistened based on their water holding capacities demonstrate that the range from 60% to 100% of samples WHC is suitable for the development of this invertebrate and 80% were chosen to run these tests and further ones. In order to determine if food volume can be lowered for *C. elegans* soil tests, it was included in the total volume used to moisten the soils and soil/biosolid mixtures.The endpoints results for these conditions were compared to those got for the same soils in standardized conditions and no significant differences were found between the two protocols. Moreover, soils textures studied had no effect on the endpoints studied. With regards of the effects of soils and biosolids mixtures on *C. elegans* growth and reproduction, moistening the samples on a WHC basis is adequate to assess this kind of materials. Endpoints results of *C. elegans* in solid and liquid phases demonstrated again the relevance of this organism to assess toxic effects on both media.

5. References

[1] ISO 10872. 2010. Water quality – Determination of the toxic effect of sediment and soil samples on growth, fertility and reproduction of *Caenorhabditis elegans* (Nematoda).

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