# THE USE OF AQUATIC BIOASSAYS IN THE RISK ASSESSMENT TO SURFACE AND GROUNDWATER FROM THE APPLICATION OF ORGANIC WASTES AS SOIL IMPROVERS


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## INTRODUCTION

Despite the benefits that can arise from the use of organic wastes in agriculture, a wide variety of undesired substances can be disseminated in the environment as a consequence of their application, such as potentially toxic trace elements (TEs) and organic contaminants, which can have adverse effects on the environment. That fact was highlighted by the Proposal for a Soil Framework Directive, which recognized that "soil degradation or soil improvements have a major impact on other areas, (…) such as surface waters and groundwater, human health, climate change, protection of nature and biodiversity, and food safety".

## AIM

**This study aims to assess the potential impact on surface and groundwater from the application of different organic wastes as soil improvers.**

## MATERIALS & METHODS

**THE ORGANIC WASTES SELECTED FOR THIS PURPOSE WERE:**

- sewage sludge (SS1 and SS2),
- agro-industrial sludge (AIS), from a wine producing plant;
- compost produced from sewage sludge and agricultural wastes (CSSAW),
- mixed municipal solid waste compost (MMSWC),
- compost produced from agricultural wastes (CAW), and
- pig slurry digestate (PSD).

**ORGANIC WASTES PHYSICOCHEMICAL CHARACTERIZATION:**

- pH (1:5), electrical conductivity (EC) (1:5), total organic matter content (OM), Kjeldahl nitrogen, total P, Na, K, Ca, Mg;
- Total TEs concentrations (Cd, Cr, Cu, Ni, Pb and Zn) by atomic absorption spectrometry (AAS) after digestion with aqua regia (ISO 11466).

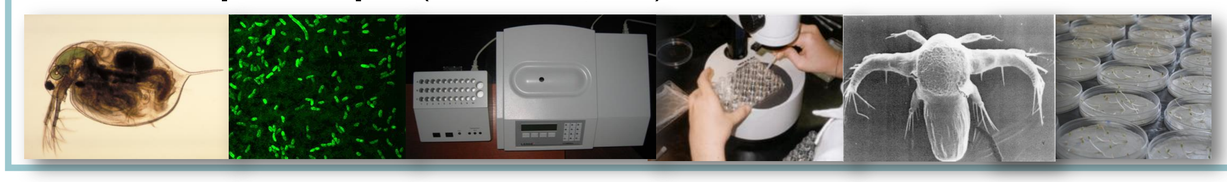
**ORGANIC WASTES ECOTOXICOLOGICAL CHARACTERIZATION:**

In order to assess the organic wastes' potential impact on surface and groundwater, a leachate was obtained using DIN 38414-S4 method (1984), which was characterized considering:

- pH, electrical conductivity, and TEs concentration, and, also using ....

**SEVERAL AQUATIC BIOASSAYS WERE USED IN ORDER TO ASSESS THE LEACHATE ECOTOXICITY:**

- Luminescence inhibition of the marine bacteria *Vibrio fischeri* (ISO 11348-2, 1998),
- *Daphnia magna* immobilization (ISO 6341, 1996),
- *Thamnocephalus platyurus* survival (Thamnotoxkit FTM),
- Seed germination (*Lactuca sativa*): relative seed germination (RSG) and relative root growth (RRG), were calculated, referring to distilled water as the negative control. Germination index (GI), was also determined GI=[RSG×RRG]/100 (Zucconi et al., 1985).



## RESULTS

### 1 - Physicochemical characteristics of the organic wastes

**Table 1 –** Physicochemical characteristics of the organic wastes (mean values, n=3).

| | pH | EC (mS/cm) | OM (%) | $N_{Keldahl}$ (%) | C/N | $P_{total}$ (% $P_2O_5$) | $K_{total}$ (g $K_2O$/kg) | Na (g/kg) | Ca (g/kg) | Mg (g/kg) |
|---|---|---|---|---|---|---|---|---|---|---|
| SS1 | 7.1 | 3.49 | 67.5 | 6.18 | 5.5 | 13.5 | 7.1 | 2.4 | 12.0 | 4.6 |
| SS2 | 7.4 | 1.23 | 74.3 | 6.18 | 6.0 | 6.9 | 14.4 | 1.6 | 28.1 | 7.9 |
| AIS | 12.2 | 9.76 | 37.6 | 2.00 | 9.4 | 5.6 | 1.6 | 1.3 | 223.2 | 8.7 |
| CSSAW | 5.8 | 6.37 | 87.2 | 3.16 | 13.8 | 2.1 | 29.0 | 20.1 | 9.2 | 4.8 |
| MMSWC | 7.8 | 7.19 | 39.5 | 2.13 | 9.3 | 3.7 | 19.2 | 32.1 | 83.3 | 14.0 |
| CAW | 8.3 | 6.12 | 41.3 | 1.78 | 11.6 | 3.5 | 45.9 | 29.8 | 29.5 | 37.5 |
| PSD | 6.4 | 7.95 | 60.8 | 2.56 | 12.0 | 19.3 | 26.3 | 4.4 | 107.9 | 18.1 |

## RESULTS (Cont.)

### 2 - Total potentially toxic TE concentrations

**Table 2.** Total TE concentrations (mean values, n=3) and threshold values of some regulatory documents.

| | | Cd (mg/kg) | Cr (mg/kg) | Cu (mg/kg) | Ni (mg/kg) | Pb (mg/kg) | Zn (mg/kg) |
|---|---|---|---|---|---|---|---|
| SS1 | | 1.0 | < LD | 140.8 | 22.6 | < LD | 757.2 |
| SS2 | | < LD | < LD | 155.8 | 22.5 | < LD | 581.1 |
| AIS | | 4.6 | < LD | 30.6 | 44.3 | 52.2 | 84.2 |
| CSSAW | | < LD | < LD | 31.9 | 7.3 | < LD | 91.1 |
| MMSWC | | 3.0 | 14.5 | 179.5 | 29.2 | 202.3 | 473.5 |
| CAW | | 1.1 | 99.1 | 54.9 | 360.5 | 17.4 | 412.8 |
| PSD | | 1.8 | 8.9 | 510.3 | 20.8 | 9.0 | 2033.0 |
| LC for TE in SS – EC Directive (*) | | 20-40 | none | 1000-1750 | 300-400 | 750-1200 | 2500-4000 |
| LC for TE in SS – Portuguese legislation (**) | | 20 | 1000 | 1000 | 300 | 750 | 2500 |
| British Specification for Composted Materials (***) | | 1.5 | 100 | 100 | 50 | 200 | 400 |
| 2nd draft - Compost LC (****) | Class 1 | 0.7 | 100 | 100 | 50 | 100 | 200 |
| | Class 2 | 1.5 | 150 | 150 | 75 | 150 | 400 |
| | Stabilized biowaste | 5 | 600 | 600 | 150 | 500 | 1500 |

LD(Cd) = 0.3 mg/kg DM; LD(Cr) = 6.7 mg/kg DM; LD(Pb) = 6.7 mg/kg DM;
(*): Limit concentrations for metals in the European Community, EC Directive 86/278/EEC (EC,1986);
(**): Limit concentrations for metals in the Portuguese Legislation for sewage sludge, Decreto-Lei n.º 276/2009;
(***): British Specification for Composted Materials, BSI PAS 100: 2005;
(****): Environmental quality classes for compost and stabilised biowaste suggested in the Working Document – Biological Treatment o Biowaste (2nd draft), European Commission Directorate-General Environment (DG Env.A.2., 2001).

### 3 – Sludge / Compost classification in accordance with the TE concentrations

**Table 3 -** Sludge / Compost classification.

| | SS EC Directive | SS Portuguese Legislation | BSI PAS 100: 2005 | Proposed EC Classes for compost (2nd draft) |
|---|---|---|---|---|
| SS1 | ✓ | ✓ | - | - |
| SS2 | ✓ | ✓ | - | - |
| AIS | ✓ | ✓ | - | - |
| CSSAW | ✓ | ✓ | ✓ | Class 1 |
| MMSWC | ✓ | ✓ | X (Cd, Cu, Pb and Zn) | Stabilized biowaste |
| CAW | ✓ | ✓ | X (Ni and Zn) | X (Ni) |
| PSD | ✓ | ✓ | X (Cd, Cu and Zn) | X (Zn) |

### 4 - Organic wastes' leachate chemical and ecotoxicological characterization

**Table 4 -** Leachate characterization (mean values, n=3).

| | pH | EC | Cd (mg/l) | Cr (mg/l) | Cu (mg/l) | Ni (mg/l) | Pb (mg/l) | Zn (mg/l) |
|---|---|---|---|---|---|---|---|---|
| SS1 | 8.6 | 1.44 | 0.018 | < LD | < LD | < LD | < LD | < LD |
| SS2 | 8.1 | 2.62 | 0.034 | < LD | < LD | < LD | < LD | < LD |
| AIS | 12.2 | 6.93 | 0.182 | 0.703 | 0.289 | 0.034 | < LD | 0.433 |
| CSSAW | 6.7 | 2.59 | 0.461 | < LD | < LD | < LD | < LD | 0.052 |
| MMSWC | 8.0 | 3.55 | 0.374 | < LD | < LD | < LD | < LD | < LD |
| CAW | 8.3 | 3.05 | 0.487 | < LD | < LD | < LD | < LD | 0.084 |
| PSD | 6.6 | 4.96 | 0.179 | < LD | 0.057 | < LD | < LD | 0.018 |

**Table 5 -** Results from the ecotoxic bioassays: EC50 and EC20 (% v/v) (mean values, n=4). $EC_{XX}$: leachate concentration, % v/v, at which a toxic effect on XX% of the exposed organisms can be observed.

| | | | SS1 | SS2 | AIS | CSSAW | MMSWC | CAW | PSD |
|---|---|---|---|---|---|---|---|---|---|
| *D. magna* immobilization | | $EC_{50}$ | <33(*) | 13.3 | 9.4 | 4.7 | n.t. | 75.4 | n.t. |
| *T. platyurus* survival | | $EC_{50}$ | 17.1 | 5.7 | 22.6 | 17.9 | 68.8 | 38.6 | 54.4 |
| Seed germination (*L. sativa*) | | $EC_{50}$ | 16.4 | 57.0 | 5.9 | 43.0 | n.t. | n.t. | n.t. |
| Luminescence inhibition of *V. fischeri* | 15 min | $EC_{20}$ | 3.6 | 6.1 | 15.5 | 3.6 | 16.3 | 8.1 | n.t. |
| | | $EC_{50}$ | 11.8 | 27.6 | 32.6 | 11.2 | 54.1 | 22.3 | n.t. |
| | 30 min | $EC_{20}$ | 4.0 | 10.1 | 6.6 | 3.9 | 21.4 | 7.7 | n.t. |
| | | $EC_{50}$ | 11.8 | 33.7 | 60.1 | 11.6 | n.t. | 24.8 | n.t. |

**Toxicity order: non-toxic (n.t.) ⇨ lower toxic effects ⇨ higher toxic effects**

***D. magna*:** MMSWC ≈ PSD < CAW < SS1 < SS2 < AIS < CSSAW
***T. platyurus*:** MMSWC < PSD < CAW < AIS < CSSAW < SS1 < SS2
***L. sativa*:** MMSWC ≈ PSD ≈ CAW < SS2 < CSSAW < SS1 < AIS
***V. fischeri*:** PSD ≈ SS1< MMSWC ≈ AIS < SS2 < CAW < CSSAW

## CONCLUSIONS

- **Despite the fact that some organic wastes are allowed to be land applied according to the existing regulations, their leachates can be very toxic towards some organisms.**
- **That toxicity is difficult to be predicted from the total TE concentrations, which are one of the main criterions to be accomplished when evaluating organic wastes risk – PSD and MMSWC were the organic wastes with overall higher total TE concentrations and were not identified as having higher toxicity.**
- **That is why bioassays are so important in the environmental risk assessment of the use of different organic wastes as soil amendments - they provide a more truthful and holistic response to the overall composition of the material.**
- **In conclusion, when assessing the environmental risk of the use of different organic wastes as soil amendments the results from chemical methods should be combined with results from bioassays.**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