Ecotoxicological characterization of end-of-life organic substrate

Caracterização ecotoxicológica de um substrato orgânico em fim de vida

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ABSTRACT

In recent years the use of substrates in horticultural protected crops has been increasing. Their use presents several advantages including a more efficient use of water and nutrients and a reduction in the use of phytopharmaceutical products. However, at the end-of-life of these materials, their disposal is still a matter of continuous research and controversial discussion. Due to the high organic matter content of organic substrates (OS), the incorporation of these residues in agricultural fields could be advantageous. Nevertheless, this practice requires a prior assessment of its environmental safety because of the risk of contamination and accumulation of bioactive molecules during cultivation cycles. The aim of this work was to make an ecotoxicological evaluation of one OS from soilless tomato production using standard protocols. A natural agricultural soil was amended with three increasing percentages of end-of-life OS (2.5, 5 and 10%). Effects of amended soils on seedling emergence and plant growth, earthworm reproduction rate and selected soil enzymatic activity were assessed. The preliminary results did not reveal major detrimental effects derived from the amendment of soil using end-of-life coconut substrate, at least for the percentages tested. In the future, more studies are necessary to evaluate possible impacts in freshwater resources.

Keywords: organic substrates, ecotoxicological assays, environmental risk assessment, waste valorisation, soil health

RESUMO

Nos últimos anos a utilização de substratos para a produção de frutos e hortícolas em estufa tem vindo a aumentar. Estes materiais apresentam várias vantagens, nomeadamente um uso mais eficiente da água e dos nutrientes e a redução da aplicação de produtos fitofarmacêuticos. No entanto, o destino destes substratos no fim da sua vida útil é um assunto controverso que necessita de aprofundamento científico. Devido ao alto teor de matéria orgânica, a incorporação de substratos orgânicos (OS) em solos agrícolas poderá representar uma mais-valia. Porém, a segurança ambiental desta prática necessita de ser avaliada devido ao risco de acumulação de contaminantes e moléculas bioativas durante os ciclos de produção. Assim, este trabalho teve como objetivo efetuar uma avaliação ecotoxicológica de um OS em fim de vida, proveniente da cultura de tomate, através da realização de ensaios padronizados. Três percentagens deste resíduo foram incorporadas num solo agrícola natural (2.5, 5 e 10%), tendo sido testados os efeitos na germinação de sementes, crescimento de plantas, reprodução de oligoquetas e atividade enzimática dos solos. Os resultados preliminares não revelaram impactos negativos preocupantes para as concentrações testadas. De futuro, são necessários estudos mais abrangentes, nomeadamente para avaliar os possíveis efeitos ecotoxicológicos em matrizes aquáticas.

Palavras-chave: substratos orgânicos, ensaios ecotoxicológicos, avaliação de risco ambiental, valorização de resíduos, saúde dos solos

INTRODUCTION

Soilless production systems are a form of intensive agriculture that relies on the use of growing media, which have the advantages of maximizing crop yield and product quality while reducing the use of phytopharmaceutical products (PPPs) particularly for soil disinfection (Asaduzzaman *et al.*, 2015; Atzori *et al.*, 2021). Organic substrates (OS) growing media offer several advantages in comparison with inorganic ones (e.g., nutrient efficiency, cost, end-of-life environmental impact), so their use have been continuously increasing (Raviv, 2011). Nevertheless, end-of-life (EL) OS disposal can still pose an environmental risk due to their potential to accumulate fertilizers, PPPs and salts.

Many studies have focused on promoting agricultural waste recycling, especially regarding crops residues and the production of more eco-friendly substrates (Diacono *et al.*, 2019). Even so, further research is still needed to promote the valorisation of agricultural wastes and to produce value-added products (O'Connor *et al.*, 2020). Currently, there is still a lack of proper solutions to EL-OS.

In fact, several farmers already perform the incorporation of EL-OS in agricultural soils considering that this practice increases the organic matter content of degraded soils, also contributing to fertilizing the soil (data not shown) (Figure 1).

To create adequate solutions and/or validate the use of EL-OS as a soil amendment, it is critical to evaluate the environmental safety of these residues. Therefore, the aim of this study was to assess the potential ecotoxicity of agricultural soils amended with different percentages EL-OS.

Figure 1 - End-of-life substrate accumulated in open field before incorporation in soil.

MATERIAL AND METHODS

An EL-OS, originally consisting of 100% coconut fibre, used in a commercial greenhouse for tomato soilless production, was collected at the Centre Region of Portugal. The substrate was dried and sieved and the fraction <4 mm was incorporated (2.5, 5 and 10%) in a natural agricultural soil (NAS) collected at Vairão Campus of the Faculty of Sciences, University of Porto. All assays were performed using standard methods. In all ecotoxicity assays, EL-OS-free, NAS was used as control.

NAS, OS and NAS-EL-OS pH and electrical conductivity (EC) were measured in a soil:water suspension (1:5 w/v) according to ISO (2021). Similarly, maximum water holding capacity (WHC_{max}) was determined following OECD (2016). Lastly, soil organic matter content (SOM) was measured by loss on ignition at 450°C for 8h.

Four seedling emergence and seedling growth tests were performed following ISO (2012a). Two dicotyledonous *Lactuca sativa* and *Solanum lycopersicum* and two monocotyledonous *Avena sativa* and *Zea mays* species were used as test plants. All seeds were purchased at a local supplier. Briefly, five pots were prepared for each percentage of NAS-EL-OS, including controls, and moisture was adjusted to 45% of WHC_{max}. Then, twenty seeds were sow per replicate. Pots were kept under optimal conditions of light and temperature (20 ± 2ºC; $16h^{L}:8^{D}$) and moisture was continuously monitored and adjusted. After 50% of the control group plants emerged, exposure lasted 14 days. At the end of the assay, seed emergence was recorded, plants were harvested, fresh and dry shoot weight were determined.

Eisenia fetida reproduction test was carried out accordingly to ISO (2012b). Four containers for each percentage of NAS-EL-OS, including controls, were prepared and moisture was adjusted to 60% of WHC_{max}. Ten adult earthworms, originated from synchronised laboratory culture, with well-developed clitellum, were randomly distributed for each container and kept under optimal conditions of light ($16h^{L}:8^{D}$) and temperature (20 ± 2ºC). Once a week, moisture content was adjusted and worms were fed. After 28 days, adults were removed, counted, weighed and the containers kept at the same experimental conditions for another additional 28 days. In the end of this period, juveniles were counted.

To assess the effects on soil enzymatic activity, three containers for each percentage NAS-EL-OS were incubated for 28 days at 20 ± 2ºC. Photoperiod was set for $16h^{L}:8^{D}$ and moisture was kept at 80% of $WHC_{max.}$ (n=3, including control group). In the end of incubation, the fraction <2mm was stored at -20ºC until further analyses. The activity of dehydrogenase (DHA), acid phosphatase (AP), urease (UR), cellulase (CL), nitrogen mineralization (NM) and potential nitrification (PN) were measured based on adaptations of Schinner *et al*. (1996) methodology as described by Bouguerra *et al*. (2022).

Statistical analyses were performed in IBM® SPPS® 26 Statistics software (IBM Corp Armonk, NY). Significant differences were calculated using one-way ANOVA followed by post-hoc comparisons using Dunnett test. Significant differences were accepted when $p<0.05$.

RESULTS AND DISCUSSION

NAS-EL-OS resulted in a decrease of pH values and increase in EC to a maximum value of 0.426 dS/m. Furthermore, WHC and SOM increased as the percentages of EL-OS increased in soil.

Regarding seedling growth and seedling emergence, none of the tests revelled valuable toxic effects for these endpoints. Moreover, *L. sativa* and *S. lycopersicum* showed to benefit with some of the tested percentages of EL-OS, increasing fresh and dry shoot weight.

E. fetida reproduction was not affected by any of the tested percentages of EL-OS incorporation.

When compared with control (NAS) the activity of DHA, AP, and PN showed a tendency to increase with the increase of the percentage of EL-OS incorporated. Contrariwise, UR and CL activities tended to decrease. NM activity did not change compared with the control.

Physical-chemical parameters results were expectable. pH and EC are a direct consequence of the intensive use of fertilizers required in these systems being extensively explained in literature (Robson, 2012). Although acidity and salinity can compromise crops quality and yield, the obtained values were within acceptable range for agriculture production (Van Beek *et al*., 2010).

Coconut fibre OS is recognized to have an apparent low density and high WHC and SOM (Louro & Reis, 2020). As expected, our results showed that the incorporation of NAS with EL-OS resulted in an increase of these parameters. Such alterations have the potential to improve aeration, $WHC_{max.}$, function and fertility of soils, especially in more degraded ones.

Improvements observed on plants biomass, although significantly positives, were not detected in all species, probably indicating that the bioavailability of the nutrients on the organic matter can be enhanced. Composting is well established solution to increase organic wastes fertilizing capacity (Zhang *et al*., 2019), possibly being a suitable approach for EL-OS. More importantly, plants and earthworms were not negatively affected, indicating that soils amended with EL-OS at the doses tested in this study were not toxic.

EL-OS amended soils increased the overall microbial metabolic activity as showed by the higher DHA activity. This can result of the increase of SOM content since it offers more substrate to support higher microbial biomass (Wolińska & Stępniewska, 2012). However, enzymatic activities translate complex relations between biotic and abiotic factors. Also, the C, N and P content and bioavailability that EL-OS can add to soil are important factors to interpret our results regarding nitrification and mineralization processes, as well as CL and UR activities. Overall, the quantified values obtained for EL-OS amended soils did not suggest major concerns regarding their safety.

CONCLUSIONS

Our results showed that soil amended with end-of-life coconut fiber substrate (up to 10%) obtained from tomato cultivation was safe for terrestrial matrices. Nevertheless, these results should be interpreted with caution because they may be dependent of the substrate origin (farming-specific differences), agronomic practices during cultivation, the OS incorporation rate and the type of soil. Different substrates constituents, crops cultivated, certification processes and countries regulations and policies are all factors that should be consider before validating the incorporation of end-of-life substrate in open fields. Further studies are needed, not only with substrates from different provenience but also to assess the safety for aquatic resources and organisms.

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