The role of earthworms in soil biochemistry: effects of valsartan on different enzymatic biomarkers

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ABSTRACT

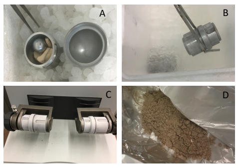
The antihypertensive valsartan is usually found in wastewater. This pharmaceutical cannot be completely eliminated in wastewater treatment plants, reaching high concentrations (0.06-0.6 µg/l) in effluents. Therefore, the use of reclaimed water in farmlands is a potential risk for human health, affecting soil quality and cropping. We hypothesise that the earthworm *L. terrestris* contributes to degradation of Valsartan and, mitigate its impact on soil microbial and biochemical processes. The experiment consisted in four conditions (Control soil, Soil+Worms, Soil+Valsartan and Soil+Worms+Valsartan), with the spiked soils with Valsartan at a concentration of 5 mg/kg of dry soil. We measured biomarkers of exposure in worms, where AChE showed no effect but Carboxylesterases and GST were strongly inhibited 7 days after exposure. Soil biomarkers showed that CE and alkaline phosphatase activity increased after 7 days of incubation, and the soil quality was higher in those conditions were worms were present. Pending of chemical analysis of residues of valsartan in soil. This study shows that *L. terrestris* could be a potential tool for the bioremediation of soil pollution.

MATERIAL AND METHODS

Experimental conditions:
- 22 – 25 % H
- 15 ºC (constant)
- Photoperiod 12h
- Natural soil
- 5 mg drug/kg dry soil

Tissue preparation: crio-grinding

A) The entire worm is placed in a stainless steel capsule.
B) The capsule is frozen in liquid N_2
C) The capsule is placed in a mixer and grinded for 1 m. 30 s. (hz)
D) The result was an homogenic dust



Enzymatic activity

Samples were sonicated with Phosphate buffer Tris 20 mM, pH=7.4.

Centrifugation 10000 rpm, 4ºC, 20 min. Extraction of the liquid supernatant. Keep at -80ºC.

25 µL of the sample were mixed with 200 µL of the Reaction Mixture (phosphate buffer 50 mM pH 7.4 and 1 mM of the substrate)

The 96 well microplate is measured with a Tecan Infinite 2000.

Results and Discussion

EARTHWORMS

GST strongly inhibited 7 days after exposure

No effect of valsartan on AChE activity

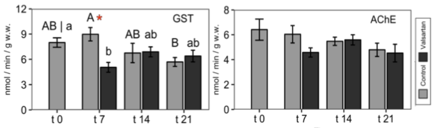


Figure 1. GST and AChE activity in nmol/min/g w.w. At time 0, 7, 14 and 21 days of exposure. Statistical differences for the control (A-B-C) and exposed (a-b-c) conditions

Different behaviour of CE isozymes under Valsartan exposure

No significant effect of valsartan on butyrate substrates

Acetate substrates were strongly inhibited by valsartan 7 days after exposure

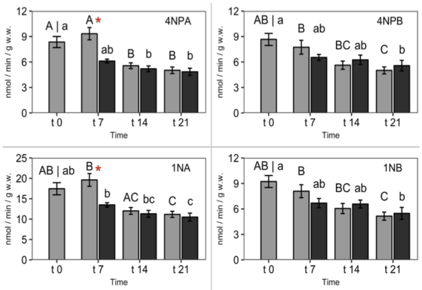


Figure 2. CE activity in nmol/min/g w.w. At time 0, 7, 14 and 21 days of exposure. Measured with the substrates 4NPA, 4NPB, 1NA and 2NB. Statistical differences between different times are indicated for the control (A-B-C) and exposed (a-b-c) conditions.

In Worms, the inhibition occur after the exposure and the enzymatic activity recovers to control values after 14 days of exposure.

SOIL

Carboxylesterase, Alkaline phosphatase and b-glucosidase activity in soil recovers and exceed the control values after 14 days in the pots containing earthworms. Figure 1.

Spiked soils without worms do not have significant changes of CE enzymatic activity

The presence of *L. terrestris* in clean and spiked soil increases enzymatic activities. The presence of valsartan together with worms stimulate CE activity in soil.

As CE activity in earthworm tissue is constant at times 14 and 21, the results suggest the existence of transferences of active CEs to soil from worms, as it only occurs in pots where they were present.

We hypothesise that this transfer is carried on by earthworm mucus, which is an external defence and, in fact, we observed that in the spiked soil earthworms secreted more mucus than in the non-spiked soil.

Soil quality was improved in those soils containing worms, either spiked or not.

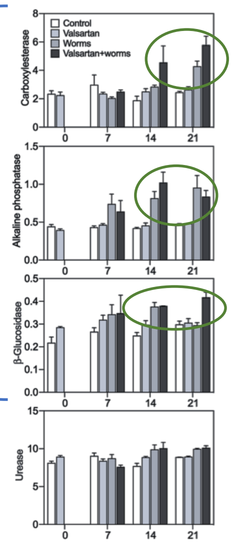


Figure 3. Carboxylesterase, Alkaline phosphatase, b-glucosidase and urease in soil, for the conditions control, valsartan, worms and worms+valsartan at times 0, 7, 14 and 21 days.

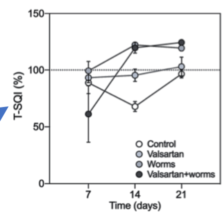


Figure 4. Treated-Soil Quality Index (T-SQI), in %, for the control, valsartan, worms and worms+valsartan conditions.

Conclusions:
- **The presence of the earthworm *L. terrestris* in farmlands improves soil quality.**
- ***L. terrestris* could be a tool for the bioremediation of the pollution caused by the use of effluent waters from wastewater treatment plants.**
- **The excretion of mucus by worms increases the enzymatic load to the soil that could confer xenobiotics metabolization.**
- **Despite an initial inhibition of esterase activity, the presence of Valsartan in soil stimulates the enzymatic activity.**

Next steps:
To complement this study, residuals of valsartan in the soil will be analysed, the method is currently being developed. Also, the changes in the microbiological community will be taken in account as a possible factor affecting the values of enzymatic activities.

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References:
Bartrons, M., Peñuelas, J., 2017. Pharmaceuticals and Personal-Care Products in Plants. Trends Plant Sci. 22, 194–203.
Gros, M., Rodríguez-Mozaz, S., Barceló, D., 2012. Fast and comprehensive multi-residue analysis of a broad range of human and veterinary pharmaceuticals and some of their metabolites in surface and treated waters by ultra-high-performance liquid chromatography coupled to quadrupole-linear ion trap tandem. J. Chromatogr. A 1248, 104–121.
Mekahlia, M.N., Tine, S., Menasria, T., Amieur, H., Salhi, H., 2016. In Vitro Biomarker Responses of Earthworm *Lumbricus terrestris* Exposed to Herbicide Sekator and Phosphate Fertilizer. Water. Air. Soil Pollut. 227.
Sanchez-Hernandez, J.C., Notario del Pino, J., Capowiez, Y., Mazzia, C., Rault, M., 2018. Soil enzyme dynamics in chlorpyrifos-treated soils under the influence of earthworms. Sci. Total Environ. 612, 1407–1416.