Irrigation with treated wastewater can impact soil quality: a sentinel invertebrate approach

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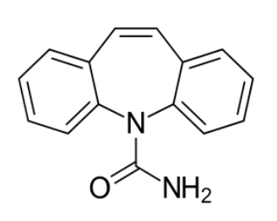
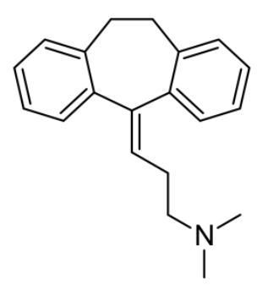
BACKGROUND

Toxic pollutants are often ingested, suggesting that soil rather than air is a prominent human exposure route. Active pharmaceutical ingredients (APIs) can be observed in terrestrial systems that are characterized by elevated human activities, mainly due to the application of treated water and/or sewage sludge[1]. Earthworms, one of the essential indicator species of soil health, accumulate biosolid fertilisers and wastewater contaminants that exert negative impacts on soil quality.

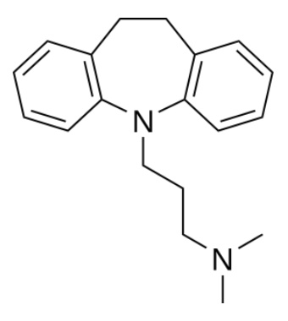
Carbamazepine



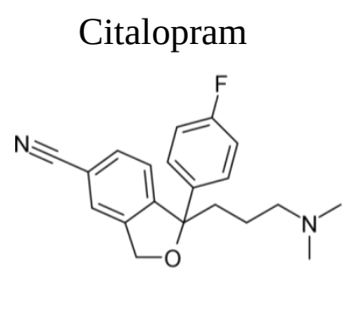
Amitriptyline

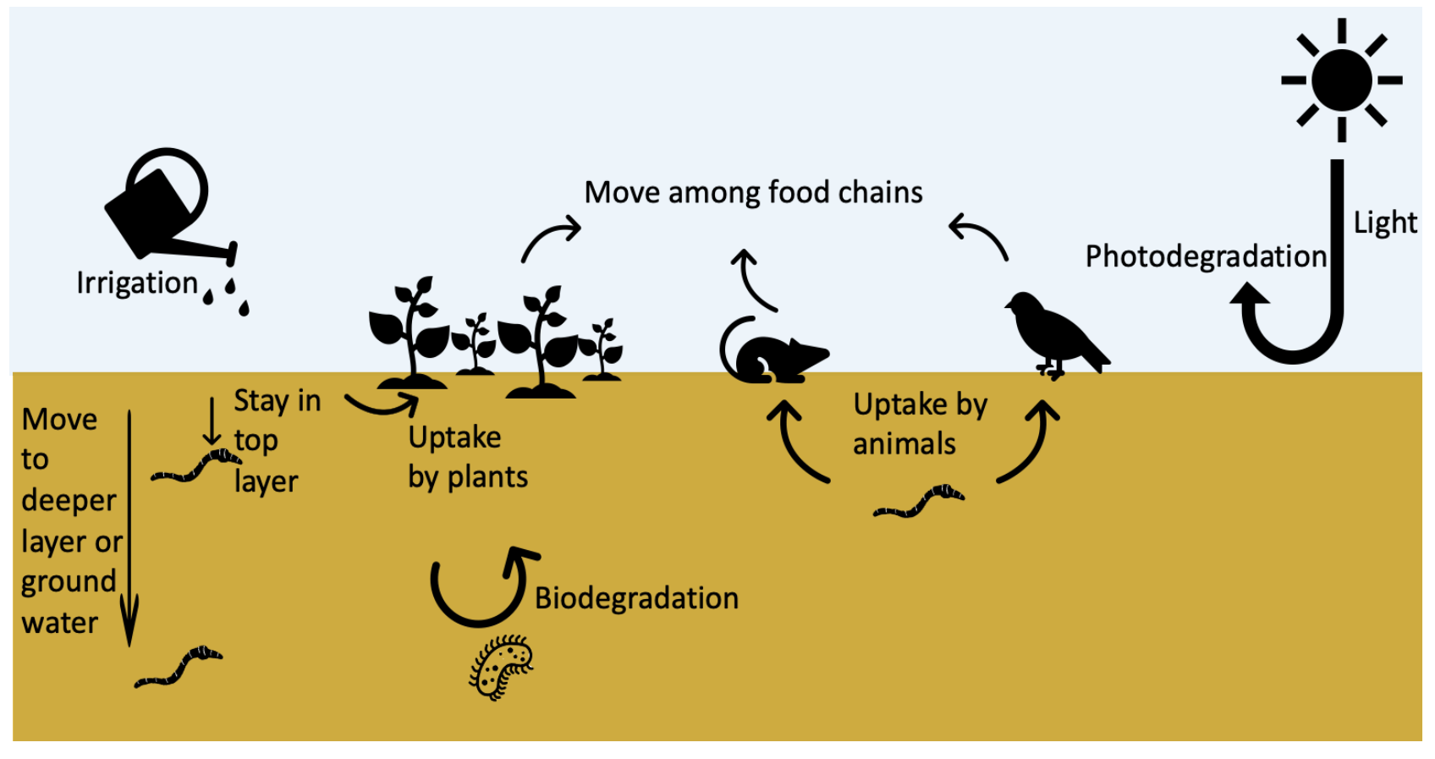


Imipramine



Citalopram

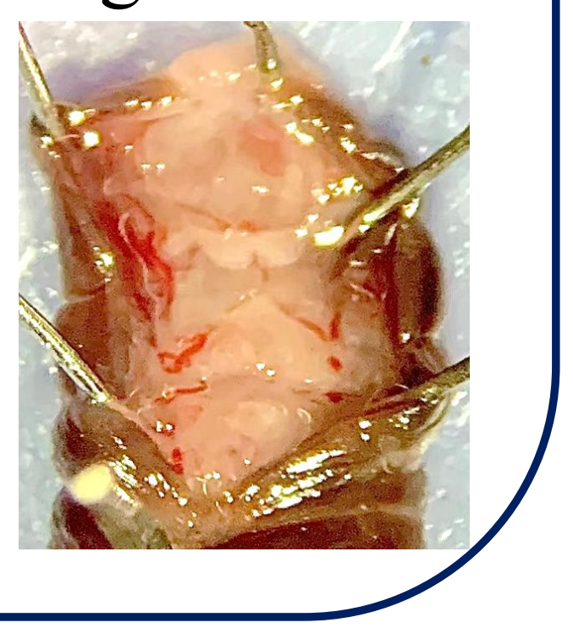




METHOD

RNA-Seq: *De novo* transcriptome assembly initially identified differentially expressed genes under Carbamazepine exposure, and gene ontology analysis was conducted with Trinity, RStudio and Panther. A list of high-altered genes were extracted and quantified by the quantitative-PCR to support following monitoring on earthworm's reactions.

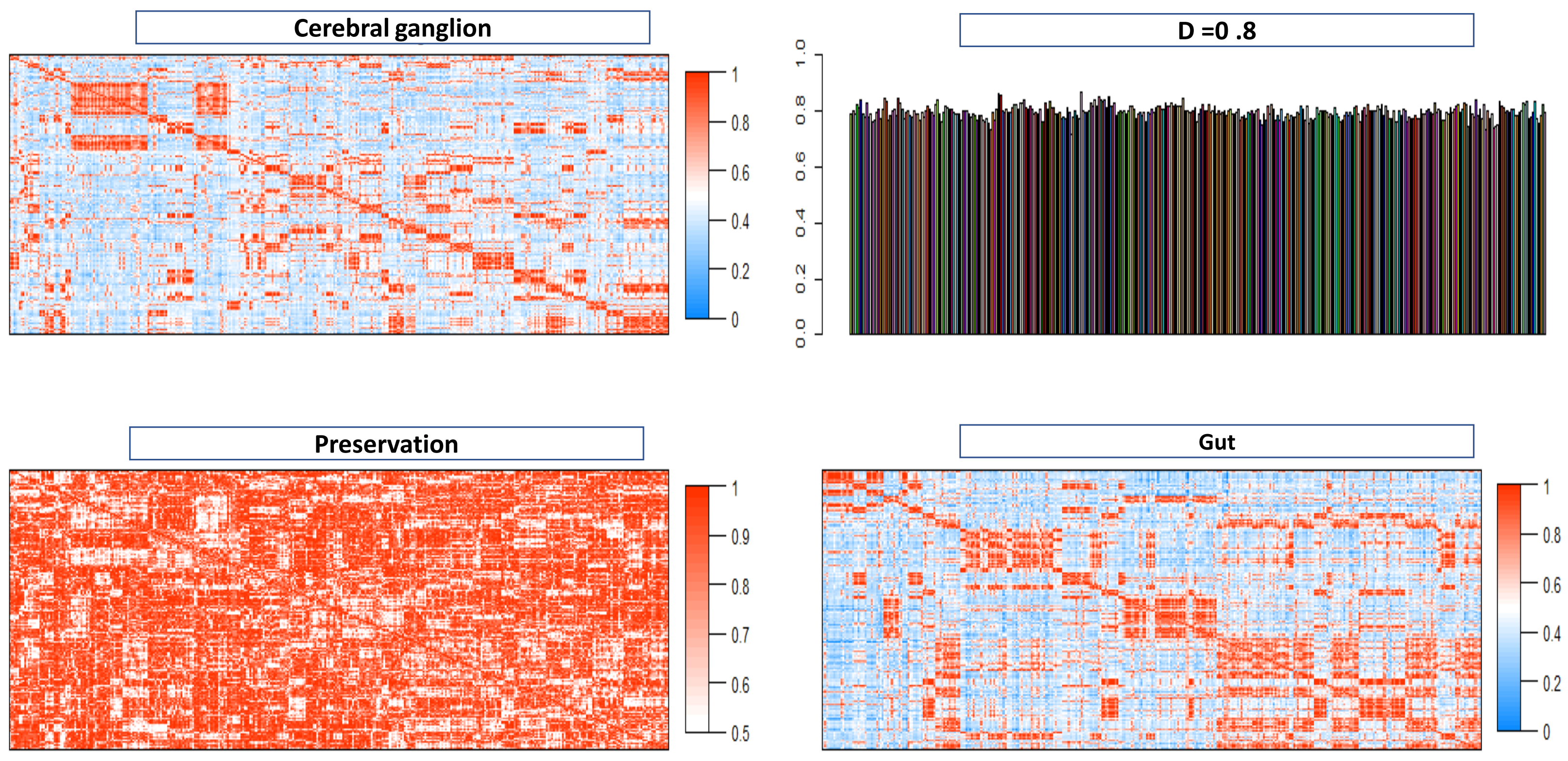
Lab-based mesocosm design: Soil columns (60cm height, 15cm diameter) with earthworms (3 species, occupying unique niches) were set up and irrigated with artificial drug mixture under 15 Degree Celsius. Worm and soil samples were collected in a 12-week time frame.



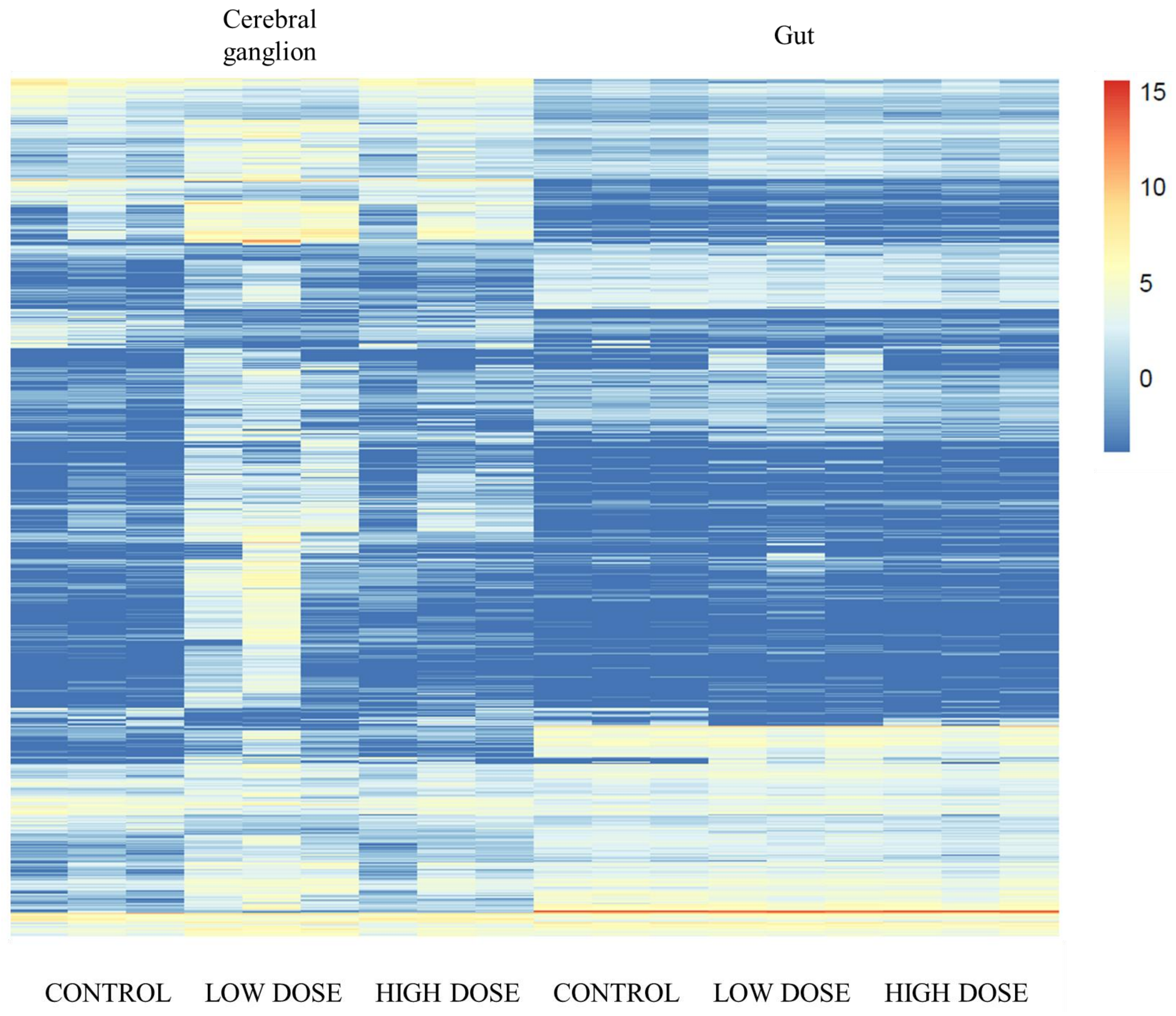
HPLC-MS/MS: Distribution of four antidepressants in the soil column and in worm bodies were analysed by HPLC-MS/MS, following sample-specific extraction strategies.

RESULTS

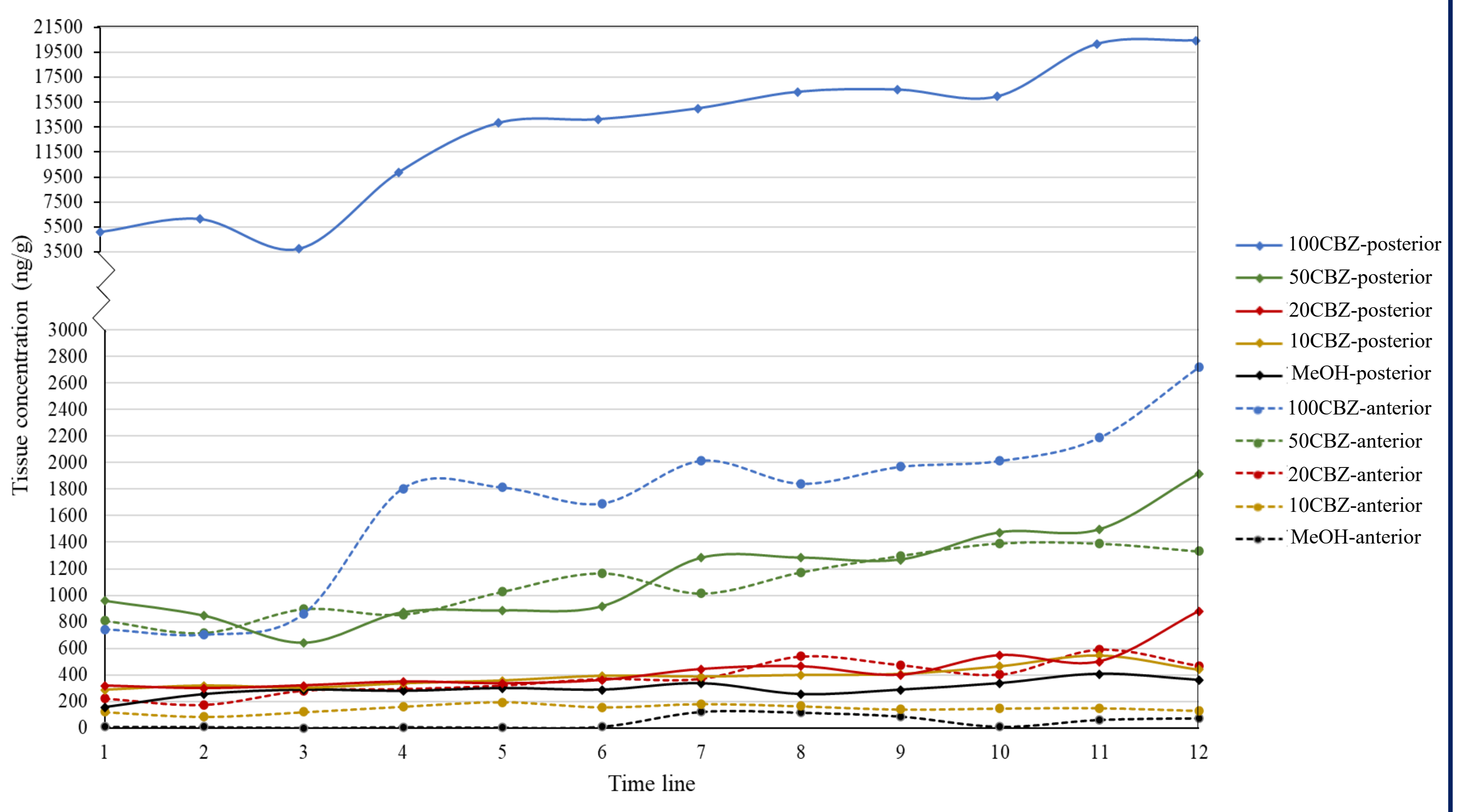
1. Tissue-specific differentially expressed genes in CBZ-exposed earthworms. Over 4000 significantly differentially expressed genes were identified, however only 41 genes were commonly regulated in all exposure conditions.



2. Two of the top upregulated genes in cerebral ganglion samples were Tubulin polymerization-promoting protein (TPPP) gene and stabilizer of axonemal microtubules 2 (Saxo2) which are both involved in neurodegeneration. Gene ontology analysis also identified several neurodegenerative disease related pathways, including phosphatidylinositol signalling.



3. In the 12-week column-exposed *Dendrobeana veneta*, CBZ was highly accumulated in the posterior tissues but also gradually delivered to anterior parts after intake.



4. In total 3 genes were successfully screened to work for all three earthworm species and their regulations differed significantly among species, exposure time and irrigation strategies.

		CBZ+CIT Exposure			CBZ+CIT+AMI+IMI Exposure		
time	species	TPPP1-3	NADH1	CYP2C	TPPP1-3	NADH1	CYP2C
WEEK 1	*D.veneta*	-41.4	7.1	-3.1	1.6	-2.2	4.8
	L.rubellus	13.3	-36.3	3.3	1.0	-2.2	2.2
	L.terrestris	-2.9	-112.7	-1.2	-2.0	-2.0	-2.0
WEEK 2	*D.veneta*	2.3	-1.2	10.4	4.4	9.1	1.7
	L.rubellus	2.3	-3.7	2.7	-2.8	-15.1	-1.7
	L.terrestris	1.9	4.7	2.1	5.1	-1.5	-1.9
WEEK 4	*D.veneta*	-2.4	-1.1	-21.8	6.4	-3.8	-18.8
	L.rubellus	10.0	-21.1	1.9	1.4	-6.0	21.6
	L.terrestris	1.6	-1.2	3.9	4.6	-1.1	-1.4
WEEK 6	*D.veneta*	-3.4	-1.7	-2.9	-1.7	-1.3	1.1
	L.rubellus	12.8	-121.9	2.2	-1.1	23.0	-1.3
	L.terrestris	-2.0	-4.9	-1.4	13.1	105.2	4.3
WEEK 8	*D.veneta*	-2.5	-4.8	2.5	-2.3	-1.6	-2.4
	L.rubellus	2.5	-358.7	1.6	-3.3	-5.1	-1.5
	L.terrestris	2.9	-5.7	-1.1	1.3	-7.0	2.4
WEEK 10	*D.veneta*	-5.4	-125.2	-5.4	-31.7	3.2	-5.5
	L.rubellus	1.7	-648.7	3.0	-2.9	-36.3	-1.5
	L.terrestris	-3.4	1.2	-1.1	1.3	1.5	2.1
WEEK 12	*D.veneta*	-32.6	-14.0	-7.0	-1.0	57.9	1.6
	L.rubellus	-3.2	-142.5	-2.1	-3.3	2.6	-2.4
	L.terrestris	-4.0	3.0	-1.1	1.3	2.9	-33.4

CONCLUSIONS

- In humans, CYP3A4 is the key response to CBZ exposure[2]. However, in earthworms this transcript was not identified, suggesting that alternative pathways might be involved in the metabolic process.
- For cerebral ganglion, a potential tissue-specific indicator for CBZ exposure could be TPPP as this gene is highly linked to Alzheimer, Parkinson and other neurodegeneration diseases.
- The different regulations of gene expression under antidepressants exposures among three earthworm species suggested potential species-specific responses. This findings altered the risk of over/under-estimations of APIs' toxicity with a certain worm species. A more detailed and flexible method would be required to predict the environmental and biological risks of emerging contaminants.

Reference

W. C. Li, "Occurrence, sources, and fate of pharmaceuticals in aquatic environment and soil," Environmental Pollution. 2014
Fuhr, L.M., Marok, F.Z., Hanke, N., Selzer, D. and Lehr, T., 2021. Pharmacokinetics of the CYP3A4 and CYP2B6 Inducer Carbamazepine and Its Drug–Drug Interaction Potential: A Physiologically Based Pharmacokinetic Modeling Approach. Pharmaceutics, 13(2), p.270.