Effect of earthworms, submitted to urban amendments in agricultural context, on nanoplastic distribution

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Context

Plastics are major contaminants in environment and their degradation produces micro and nano plastics (MPs and NPs) [1]. Moreover, plastics contain metals and release these metals during their degradation [2]. Recent study has demonstrated that **metals can act as a proxy to the contamination of MPs and NPs** [3].

The impact of plastic pays more and more attention but only few studies have focused on effects of MPs and NPs on **terrestrial environment**. As part of the agricultural amendments, particular attention is given to amendments of urban origin which can contain plastic. It is becoming urgent to improve knowledge on the effect of these plastic-rich amendments on soil functioning, and the effect of soil organisms, such as earthworms which are key actors on soil functioning, on plastic evolution and distribution.

Objectives

The aim of this study (CINAPE project, ADEME n°1806C0022) was to determine the dynamic of micro and nano plastics in soil, by the evaluation of nanoplastic in casts and earthworm body, applying the quantification of metal content; heterogeneity along earthworm body was assessed to identify if earthworm accumulate preferentially element in one part, e.g. before the male pore, at the clitellum part or after the clitellum part.

The evaluation of nanoplastic in the different compartments (cast, earthworm body) was possible due to the development of innovative analytical methods.

Microcosme approach

A microcosm approach was carried during 2 months.

Microcosms (30 cm high, 16 cm dia) were filled with soil (5 kg) from 2 sites (Orléans, France)

i) amended site with urban compost 15 to 30 years ago, rich in MPs and NPs; this soil was rich in metal linked to plastic [3] (tab. 1),

ii) no amended site (i.e. no contaminated site= control).

Earthworms (*Lumbricus centralis*) were sampled in both sites.

Table 1: Soil metal contents (µg/g of soil)

Element contents	Cd	Cr	Cu	Ni	Pb	Zn
soil control	0,075	23,26	15,65	5,62	27,66	22,13
contaminated soil	0,38	41,66	92,91	9,56	84,68	221,12

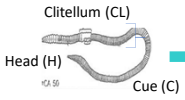
Controled parameters:
Temperature: 0°C night/12°C day;
Light exposure: 12h day/12h night;
Soil humidity: 30mL of water added each week (field capacity)
Food ressource: dried ray gras (0,077 g /g of earthworm) provided each week
Biological material: in each microcosm, 2 adults of *L. centralis* were introduced

Table 2: Microcosme description

Treatment	Species	Number of replicates	Microcosme composition
Control	*L. centralis*	5	earthworms from no amended soil introduced in no amended soil
M1	*L. centralis*	6	earthworm from no amended soil introduced in amended soil
M2	*L. centralis*	3	earthworm from amended soil introduced in amended soil

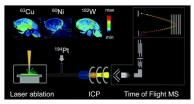
After 8 weeks
- earthworms were collected, weighed and stored (formaldehyde 4%) ,
- casts were collected on soil surface and stored (4°C)

Measurments of metal content in earthworm body



1. Cutting 3 parts (H,CL,C)

2. After freezing (isopropanol -80°C), earthworm parts were cut (50µm) with microtome and slices were placed on thin blade (Plateforme H2P2)



3. Thin blades were mailed to University Laval (Canada) and analysed on LA ICP MS

Measurments of metal content in earthworm casts

5 steps for cast analysis:
- Grinding cast sample
- Weighing (75 mg)
- Mineralisation of sample (microwave)
- *One tenth dilution (if necessary)*
- ICP MS analysis

Metals content in earthworm body

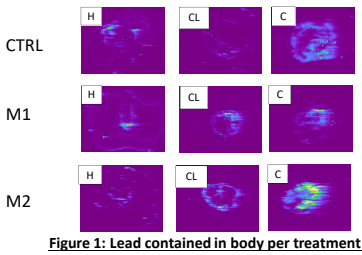


Figure 1: Lead contained in body per treatment in head (H), clitellium (CL) and cue (C)

Whatever treatment, accumulation of Pb is higher in Cue > Clitellum >Head

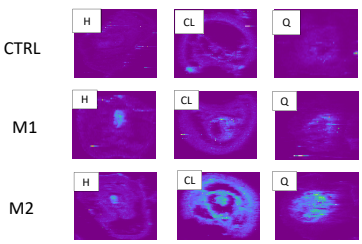


Figure 2: Copper contained in body per treatment in head, clitellium and cue

Whatever treatment, accumulation of Cu is higher in Clitellum > Cue > head

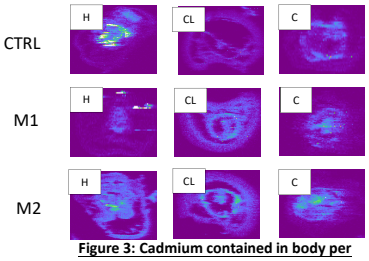


Figure 3: Cadmium contained in body per treatment in head, clitellum and cue

Dispite the low Cd concentration in soil (Tab 1), earthworm accumulate Cd in his body.

In treatments M1 and M2, accumulation of Cd is higher in clitellum ; in control, the accumulation is observed in the head.

Metals	Body part presenting higher accumulation	Higher concentration
Pb	Cue	M2
Cr	Cue	M2
Ni	Head	CTRL
Cd	Cue	M2
Cu	Clitellum	M2
Zn	Clitellum	M2

Metals are preferentially accumulated in clitellum and cue of earthworms [4].

For each metal, concentrations are higher in M2 > M1 > Control
→ Long term exposure (M2) leads to an accumulation of metals in body
→ In most of the case, 2 months of plastic contamination exposure (M1) is not enough to lead accumulation

Metals content in earthworm casts

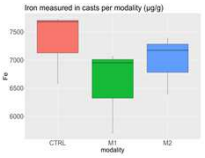


Figure 4: Iron measured in casts per treatment

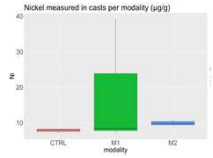


Figure 5: Nickel measured in casts per treatment

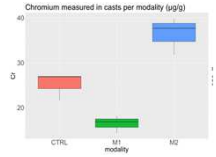


Figure 6: Chromium measured in casts per treatment

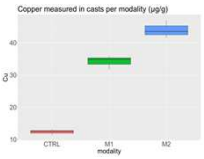


Figure 7: Copper measured in casts per treatment

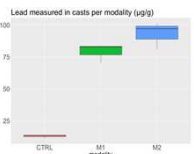


Figure 8: Lead measured in casts per treatment

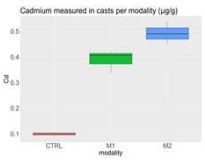


Figure 9: Cadmium measured in casts per treatment

→ Iron and Nickel accumulation: No effect of urban compost inputs

→ Chromium accumulation: Effect of urban compost inputs suspected

→ Copper, Lead and Cadmium accumulation: Effect of urban compost inputs

Conclusion

- Urban compost rich in MPs and NPs affect the presence of metals in earthworm body and casts, as other amendements e.g. sewage sludge [2].
- Earthworms accumulate metals in each part of their body, but depending on metals, the accumuluation is preferentially done in the head, clitellum or cue. However, this study has to be reinforced by analysing the response of other earthworm species as compartmention may depend of ecological category, during of exposition and toxicity of metals [2,4].
- The accumulation in casts of earthworms suggests that a part of metals is evacuated by biological processes[6] According to previous studies, it has been suggested that chloragogen cells were implicated in these processes[1].
- Analysing in parallele the accumultation in earthworm body and earthworm cast, our study highlights the complexity of the location of metal and MPs, NPS. Some metal, such as Iron, Nickel, Chromium, could be preferentially accumulated in body, while Coper, Lead and Cadmium are both accumulated in body and cast.
- Our study has to be considered as a first step to understand the evolution and transfer of metal, micro and nanoplastic due to earthworms. Complementary study, such as the destruction of earthworm body, followed by the analysis of metal content could reinforce these first results.

References

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