Short Communication

Agar as a medium for removing soil from earthworm guts

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Accepted 4 November 1999

Abstract

Earthworms were kept on a water–agar gel for 96 h at 20°C, after that time all soil had been voided from their guts. Earthworms treated in this way may be used for soil-free chemical analysis, as required in biomonitoring programmes for soil contamination. © 2000 Elsevier Science Ltd. All rights reserved.

Keywords: Biomonitoring; Earthworm guts; Lumbricidae; Soil invertebrates; Soil removal

Body residues of soil pollutants in earthworms are often determined in biomonitoring programmes to indicate bioavailability and toxicity risks (Van Straalen and Krivolutsky, 1996). Since bioavailability and toxicity are determined by the amount of pollutant in the body, one usually wishes to exclude any pollutant residues present in the gut. If the guts are not evacuated before conserving the worms, relatively complicated methods must be applied to correct for gut contents, for example using the acid-insoluble residue after digesting worms for trace metal analysis (Sta/C128ord and McGrath, 1986). Another approach is to keep the worms alive after sampling and evacuate the guts before conservation or chemical extraction.

Recently Dalby et al. (1996) suggested a “filter paper method” to remove soil from earthworm intestines and to standardize the water content of earthworm tissues. With this method, a small amount of soil remained in the gut after keeping the worms for 72 h on saturated filter paper. Although this varied from 0.2 to 1.5 mg of soil (0.5–2.5% of the dry weight), depending on the species, such residues could still influence a precise determination of soil pollutants in earthworm bodies, especially in the case of trace metals. Some years ago the Dnepropetrovsk University Group on Bioindication (Smirnov, Misjura and Philipenko 1987, personal communications) began to use dry starch for evacuating earthworm guts before chemical analysis. This method allowed them to replace soil for starch within a period of 2 or 3 days. The main disadvantage of the method is that it is convenient mainly for large specimens of earthworms due to the rapid water loss by small animals in dry starch.

Agar is known as a culture medium for soil animals such as protozoa and nematodes and it has also been used as a medium for experiments with enchytraeids (Westheide and Bethke-Beilfuss, 1987; Sustr and Chaplinsky, 1996). Preliminary experiments by ourselves (Pokarzhevskii and Semenov, 1996, unpublished data) showed that the earthworm Perionyx excavatus (Perrier) could live in an agar environment for 6 months with a minimum amount of food (oat flakes). We suggested that agar may be a good medium for removing earthworm gut content in experiments in which the chemical composition of the worms is very important.

For the experiments, 50 ml jars were filled with 30 ml of a 1.5% agar gel prepared with deionized water. After cooling in the jars the gel was cut in small pieces. Four species of earthworms were collected in sandy-clay soil near Beverwijk, The Netherlands, among
them 11 specimens of *Lumbricus rubellus* Hoffmeister, two of *Allolobophora longa* (Ude), eight of *A. caliginosa* (Savigny) and seven of *A. rosea* (Savigny). Collected animals were weighed (Mettler 4000, 1 mg precision), put individually in the jars and kept at 20°C in a constant temperature chamber. Animals were re-weighed after 24, 50 and 96 h. After every weighing animals were examined under a binocular microscope (Wild) to check for soil in their guts. After 96 h every animal was put into a separate Eppendorf tube and lyophilized during 24 h. After lyophilization animals were weighed (Sartorius, 0.1 mg precision) and put into ceramic pots for combustion in a muffle oven. Ashing was done for 1 h at 200°C, for another 1 h at 400°C and then for 5 h at 550°C. After combustion the pots were weighed with and without ash and the ash content was calculated for each worm.

The live weight of the earthworms increased during the first 50 h of incubation and then decreased towards the end of the experiment except for *Allolobophora longa* (Fig. 1). Practically all earthworms had soil in their guts during the first 50 h but after 96 h the guts...
were all filled with agar and the faeces were also composed of agar. The water and ash contents did not differ between the species (Figs. 2 and 3).

Our data are very similar to the data reported by Dalby et al. (1996) both for water content (82 versus 85% of live weight) and for ash content (6.3–10.2 versus 11–12% of dwt). Our experiment confirmed that during culture in agar earthworms loose all soil from their gut and may be used for chemical analysis without any disturbance from elements associated with soil particles. This method is more convenient for analytical aims than others in which the animals are exposed to water-saturated media without food. While eating agar, the peristaltic movements of the gut are maintained and this allows a more complete evacuation of the contents than can be achieved when worms are starved.

Acknowledgements

The study was carried out as part of a programme of collaboration between The Netherlands and the Russian Federation, supported by NWO, on “Pollution-induced changes in soil invertebrate food-webs”.

References

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Fig. 3. Ash content of different species of earthworms after 96 h of incubation in an agar gel (in % of dry weight). Means are shown with standard deviations.