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## Enchytraeid abundance in mixed Araucaria forest determined using four methods (cold and hot extraction, handsorting, formol application)

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Enchytraeids (Enchytraeidae, Oligochaeta) are found worldwide in soils where there is sufficient moisture and organic matter, however, information on their diversity, biology and ecology are still very scarce in tropics and subtropics. Enchytraeids are usually extracted from soil samples with wet-funnel based methods, with or without heat. The ISO guideline 23611-3 describes a simple cold wet extraction for enchytraeids. Our experience with this method in a subtropical zone in Brazil has shown frequent high mortality during the extraction process, which is critical for the determination of abundance and diversity. Therefore, between September 2011 and April 2012 we tested hot and cold extraction methods and compared them with handsorted samples and formalin extraction (two other methods that also yield enchytraeids) in a fragment of Mixed Araucaria Forest at Embrapa Forestry in Colombo (Paraná State), Brazil. Soil cores of 7 cm of depth x 5.1 diam. were collected and extracted. The period for cold extraction was 67 h in a room cooled to 17°C and hot extraction was performed for 3 h in a hot enchytraeid extraction device with adapted funnels and lamps. The extracted worms were immediately counted and the percentage of damaged worms was also recorded. Healthy worms were kept in a Petri dish with a thin layer of sediment and water from the extraction process in a cooled room and identified to genus level on the following 3 d. Each sample was replicated 8-16 times. Soil samples of 25 x 25 cm and 20 cm depth were handsorted and 5-L dilute (0.5%) formalin was applied over a nearby 50 x 50 cm surface and enchytraeids collected over a 10 min period. The first sampling resulted in ca. 12,000 enchytraeids m² extracted by hot or cold method. The second and third samplings resulted in statistically more enchytraeids by hot (11,565 and 5,507 m²) than cold (6,119 and 2,142 m²) method. Cold method showed 11-26% of damaged worms after extraction process. Furthermore, most of the worms were dead on the following day and identification was restricted to only 6% of the cold extracted worms. In contrast, the viability of the worms extracted by hot method was generally good even 3 d later. The genera Achaeta, Enchytraeus, Fridericia, Guaranidrilus and Hemienchytraeus were found.