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# Influence of low soil moisture on enchytraeids

Anneke BEYLICH & Rudolf K. ACHAZI

*Keywords: enchytraeids, soil moisture, reproduction, segment number*

**Abstract**

Former sewage irrigation sites in Berlin and Brandenburg are contaminated with heavy metals as well as PAH and PCB. At most of these sites enchytraeids have been found in low abundances and low species numbers only. The most obvious explanation for the low enchytraeid densities is the high concentration of heavy metals in the soil, often in combination with acidic conditions (pH 4.0 - 5.4). Furthermore, the soils were also exposed to extended periods of drought during the years of investigation. The objective of the present study therefore was to show in which way adverse soil moisture conditions contribute to the low numbers of enchytraeids found in the irrigation field soils.

Field studies included determination of enchytraeid abundance and species composition as well as measurement of soil water contents. In addition, laboratory experiments were performed concerning the reproduction and growth of *Enchytraeus buchholzi*, one of the dominant species of the studied sites. In the field, water contents below 5 % (DW) coincided with very low enchytraeid densities. In the laboratory, reproduction was reduced at water contents of 15 % (DW) and lower. Body length of the adults was higher at 20 % water content than at 5 % water content. It was concluded that enchytraeid densities as well as species composition are influenced by low soil moisture at the studied site.

## Introduction

Former sewage irrigation sites in Berlin and Brandenburg are contaminated with heavy metals as well as PAH and PCB. At most of these sites enchytraeids have been found in low abundances and low species numbers only (BEYLICH ET AL. 1996). One obvious explanation for these low densities is the high concentration of heavy metals in some of these sites, often in combination with acidic conditions (pH 4.0-5.4). However, enchytraeid densities are also low at sites where heavy metal concentrations amount only to approximately double background values (LABO 1995). Therefore, other abiotic factors had to be taken into consideration. During the years of investigation the soils were exposed to periods of drought several times. The aim of this investigation was therefore

[1] Freie Universität Berlin, Fachbereich Biologie, Institut für Ökotoxikologie und Biochemie, Ehrenbergstraße 26-28, D-14195 Berlin, Germany

to show in which way adverse soil moisture conditions contribute to the low numbers of enchytraeids found in the contaminated soils. So far there are hardly any data on drought-resistance of enchytraeids in contaminated soils.

For the investigation of drought effects laboratory experiments in addition to field investigations were performed. These studies were realized with *Enchytraeus buchholzi*, one of the dominant species of the studied sites. Specimen of *E. buchholzi* were collected in the field, taken into culture and used for reproduction tests and growth studies at different levels of soil moisture.

## Field studies

### *Sample sites*

Field studies were conducted from 1995 until 1998 on former irrigation fields near Berlin (Germany). These fields had been levelled and afforested with various tree species in 1985. Due to the irrigation or flooding with waste water during previous years, the sandy soils show considerable contamination with heavy metals. For the present study site RefB was selected as its degree of contamination was considered to be representative for the irrigation fields (Table 1). The mean annual rainfall in Berlin amounts to approximately 590 mm.

Table 1: Contents of heavy metals [mg/kg DW], pH [$CaCl_2$], organic matter content (loss on ignition, % DW) and vegetation of the study site

| *Site* | *Cd* | *Cu* | *Cr* | *Zn* | *Ni* | *Pb* | *pH* | *Organic matter* | *Vegetation* |
|---|---|---|---|---|---|---|---|---|---|
| RefB | 14 | 146 | 632 | 432 | 26 | 158 | 5.0 | 5.5% | fallow, mainly *Agropyron repens* |

### *Materials and methods*

Soil sampling in the field was done with a soil corer (∅ 5 cm), taking 5 replicates at each sampling date. Sampling depth was 16 cm for enchytraeid population studies and 8 cm for measurement of water content. Enchytraeids were extracted from soil by extraction without heating (DUNGER & FIEDLER 1997: 420) and counted and identified alive. Samples for the determination of soil water content and for enchytraeid densities were taken in immediate vicinity of each other. For the gravimetric determination of water content the soil material was dried over night at 105 °C. The maximum water holding capacity (WHC) was determined following SCHINNER ET AL. (1993). Samples were taken from November 1995 to April 1998 with monthly sampling in 1996 and less frequent sampling in the rest of the period. During winter (~ December to March) no samples were taken due to extended frost periods.

*Results*

The soil of irrigation field RefB is characterised by a very low population density and species number of enchytraeids (Table 2). Representatives of the genus *Enchytraeus* were dominant at most sampling dates.

Table 2: Species composition at the former sewage site RefB (15 sampling dates). Total abundance ranged from 510 to 12,250 ind./m$^2$.

| *Species* |
|---|
| *Enchytraeus buchholzi* |
| *Enchytraeus christenseni (=minutus)* |
| *Enchytraeus christenseni (=minutus) bisetosus* |
| *Enchytraeus lacteus* |
| *Henlea ventriculosa* |
| *Fridericia bulbosa* |
| *Oconnorella (= Marionina) cambrensis* |

Fig. 1 shows the population densities of enchytraeids and the water contents at two depths. The water content at 0-4 cm is always higher than at 4-8 cm as the upper layer comprises also the litter layer and therefore contains more organic matter. Minimum abundances of enchytraeids were found in November 1995, June and August 1996 and August 1997. These very low abundances coincided with soil water contents of 5 % (DW) or below in the mineral soil (4-8cm) at the sampling date or were preceded by an extended dry period (summer 1995) (Fig. 1). On the other hand, high soil moisture did not neccessarily coincide with peak enchytraeid densities (see May 1996, April 1998).

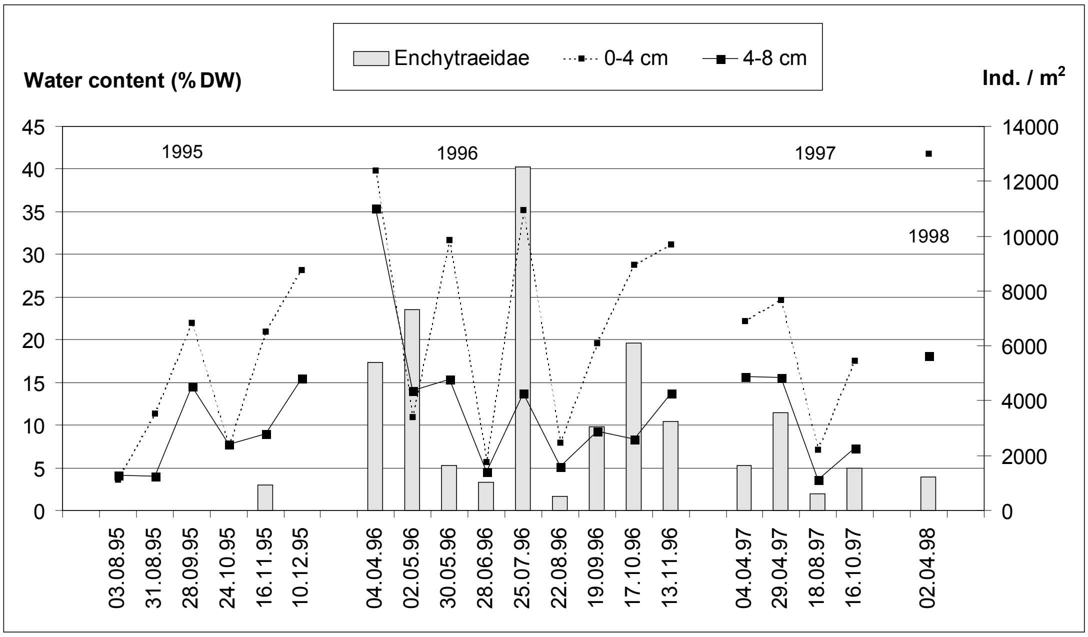


*Fig. 1: Population densities of enchytraeids (Ind. / m$^2$) and water contents (% DW) at two depths (0-4 cm and 4-8 cm) of the sampling site RefB*

There was no mathematical correlation between water content and enchytraeid abundance (Spearman Rank Order Correlation, SigmaStat, Jandel Scientific Software).

*Discussion*

The water contents at the site studied are rather low. Usually soil moisture did not exceed 20 % DW in the mineral soil (4-8 cm). Similar moisture levels were found by NIELSEN (1955 b) in a sandy soil in Denmark. However, there are only very few precise data on soil moisture in the literature. Among 26 long term field studies on Enchytraeidae browsed for soil moisture data, 42 % gave no information at all and 15 % contained only a general discription of soil moisture like „dry in summer“, „moor“ or the mean annual rainfall. 42 % of the studies contained detailed information. The results were nevertheless difficult to compare as investigators used different methods and studied various soils.

In comparing the species composition in the present study with the findings of other authors, an effect of the recurring low moisture contents seems probable. Three of the species collected at RefB, *Enchytraeus buchholzi, Fridericia bulbosa* and *Henlea ventriculosa,* were considered tolerant against low soil moisture by HEALY (1980). LAGERLÖF & STRANDH (1997) found the cocoons of *Enchytraeus, Fridericia* and *Henlea* to be more drought tolerant than those of other genera. On the other hand, most of the species found at RefB are known to inhabit rather slightly acid to neutral soils (HEALY 1980, BEYLICH ET AL. 1994), whereas the examined soil is medium to strongly acid. Living conditions in the RefB-soil therefore seem not to be especially favourable for these species. Although the decomposer community at RefB is rather poor of species and earthworms are entirely missing, it can be classified as an *Enchytraeion* according to GRAEFE (1993) which is typical for disturbed soils.

In agreement with our expectations, very low water contents coincide with low enchytraeid numbers, while high water contents did not always coincide with high abundances. Therefore, no mathematical correlation could been established between soil moisture and enchytraeid abundance. This is probably due to methodical problems: 1. Water content measurements give actual data for the sampling date, but little information on the moisture conditions over the last few weeks. On the other hand, enchytraeid abundance at the sampling date is the outcome of the population development during the last weeks or even months. 2. For technical reasons, enchytraeid counting and moisture measurement must be executed with different samples. As soil is inhomogeneous, especially at the studied site, the soil water content can differ considerably within centimeters and therefore may be different in two samples taken close to each other. Other authors, too, found it difficult or even impossible to correlate soil moisture or precipitation with total enchytraeid abundances (MELLIN 1989, RÖMBKE 1989, FEDERSCHMIDT & RÖMBKE 1995, VAN VLIET ET AL. 1997). The results of this investigation do not imply that enchytraeid populations are not controlled by soil moisture but rather that the methods applied were not really appropriate to demonstrate it.

The water content (% DW), gives us no suficcient information on the bioavailability of soil water and is unsuitable for the comparison of different soils. For the latter purpose, the water content given in % of the WHC is more adaquate.

Availability of soil water for soil organisms and plant roots can probably be estimated best by measuring the water tension (pF). According to RENGER et al. (1992) in the RefB soil a pF of 4.0 is reached at a water content of about 3 % (DW). NIELSEN (1955a) and GRÖNGRÖFT (1981) considered a soil moisture of pF 4 to be lethal to Enchytraeids. However, a tolerance of low soil moisture of around pF 4 is apparently also depending on the soil temperature (ABRAHAMSEN 1971, DÓZSA-FARKAS 1977, GRÖNGRÖFT 1981). In addition, the length of the dry period plays an important part.

The data presented here indicate that at some occasions the soil moisture at the sample site RefB decreases far enough to cause mortality among the enchytraeid population. The frequent occurrence of drought periods can be seen as one reason for the dominance of a few species. These species are apparently less sensitive to extremely low soil moisture compared to the majority of species. Further, some of them display fast population growth when living condidtions improve (colonizer-species of the genus *Enchytraeus*). As most of the species found at site RefB are not acid tolerant, the comparatively low abundances of enchytraeids are probably caused by an unfavourable combination of soil moisture, pH and heavy metal contents. The total contents of cadmium and copper found at the sample site would not prove to be toxic when tested separately in laboratory reproduction tests (BEYLICH et al. 1997). However, the combined effect of heavy metals under the field conditions given at RefB is not known. At water contents below 5 % (DW) the soil moisture seems to be the limiting factor on the studied site, while with increasing moisture other environmental factors become more influential.

## Laboratory investigations

The aim of this part of our studies was to determine the lowest water content that still allowed a) survival and b) reproduction of *Enchytraeus buchholzi* under laboratory conditions.

### *Materials and methods*

For laboratory reproduction tests the enchytraeid species *Enchytraeus buchholzi* was used. The culture originated from specimen collected at one of the former sewage sites. The animals were cultivated on agar-agar in petri dishes and fed with rolled oats. The tests were conducted with adult animals of approximately the same age. The soil material used for the tests was obtained from the LUFA (Landwirtschaftliche Untersuchungs- und Forschungsanstalt) Speyer, batch No. 2.2. The glass test vessels (with screw lid) contained soil equivalent to 20 g dry weight. Ten mature enchytraeids were added to each testvessel. During the test, the vessels were kept in an incubator at 15 ± 2 °C. Water loss and food were replenished if necessary during the test period. After 21 days the offspring and the surviving adults were counted. For two moisture levels (5 % and 20 % DW) the segment number of the surviving adults was counted.

Note: The culture of *Enchytraeus buchholzi* used in this investigation has previously been described in the Newsletter on Enchytraeidae No.5 (BEYLICH & ACHAZI 1996). There, two types were mentioned, differing in the possession of pale versus refractile granula in their lymphocytes. Some authors call the „pale“ type

*E. christenseni* (*=minutus*) and the „refractile“ type *E. buchholzi* while others do it the other way round. The status of these two types is currently under investigation (see SCHMELZ et al. 1999). The reproduction tests presented here were conducted with the „pale“ type.

*Results and discussion*

From 20 % up to 40 % water content *Enchytraeus buchholzi* showed no significant difference in reproduction (Fig. 2). Below 20 % and above 40 %, the number of offspring was reduced. No juveniles were found at 5 % water content, although adult survival was equal to higher moisture levels.

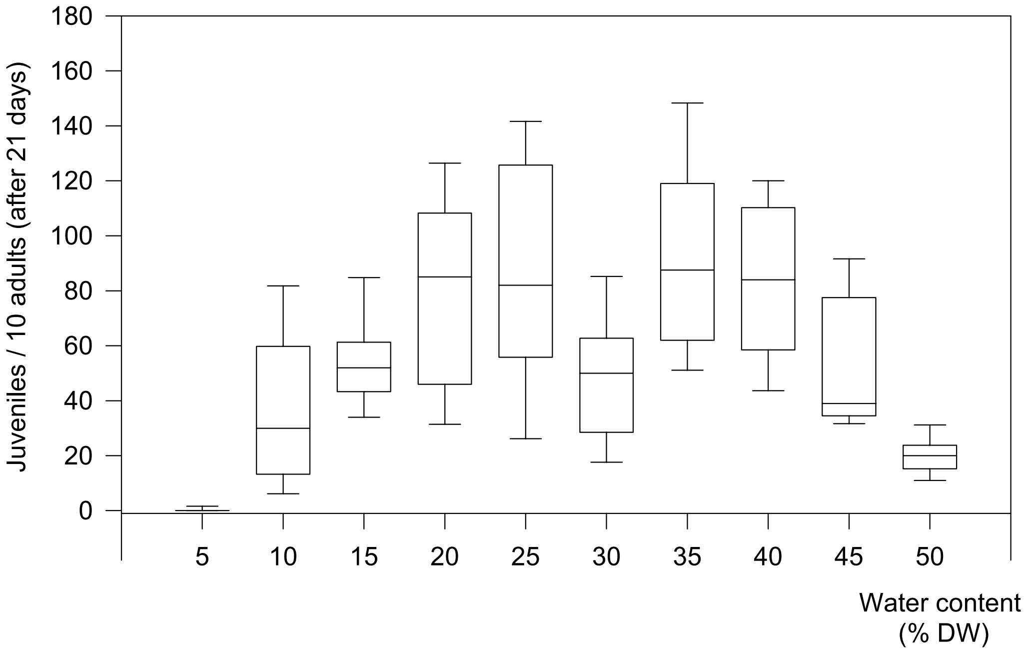


*Fig. 2: Reproduction of* Enchytraeus buchholzi *at different soil moisture regimes (n = 7)*

Reproduction was decreased at 30 % water content compared to 25% and 35% water content. It was conspicuous that at this moisture level the mixing of the water and the soil resulted in the formation of rather coarse crumbs. Crumbs were much smaller at lower moisture, while at higher moisture the soil material formed a smooth layer. Therefore, a test was conducted investigating the effect of soil structure on reproduction. The different soil structures were obtained by pouring water on top of the soil material and a) stirring the soil or b) letting it soak into the soil for several hours. Three different moisture levels were tested. At all three moisture levels tested this way, reproduction was significantly higher in the unmixed soil material with no crumbs (Fig. 3). The difference was increasing with increasing soil moisture and increasing crumb size. This proves, that apparently small variations of the test method can yield significantly differing results. In this case, the effects of soil structure exceeded the effects of soil moisture.

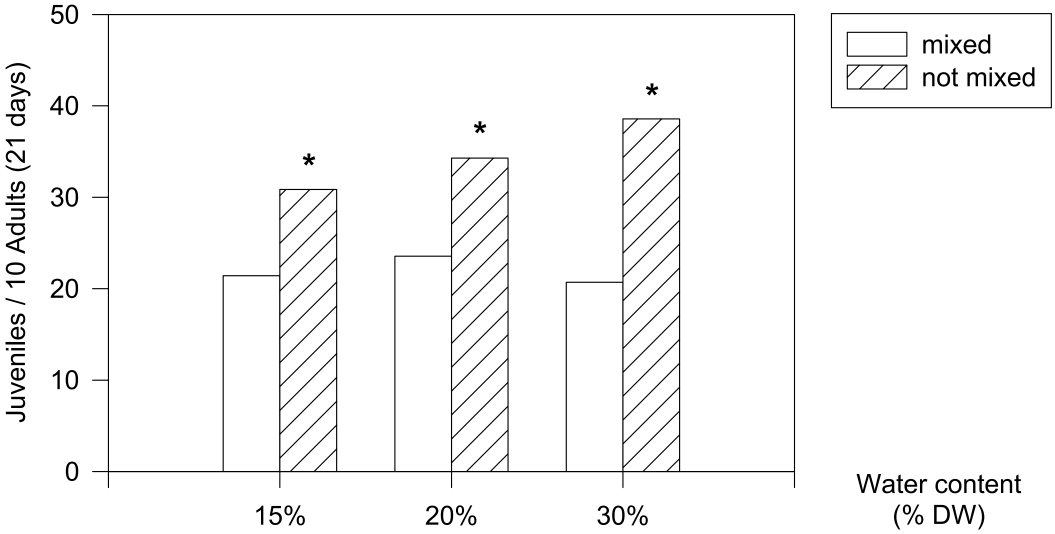


*Fig. 3: Effect of stirring the test substrate on the reproduction of* Enchytraeus buchholzi *(n = 7). Significant difference *: α = 0.05*

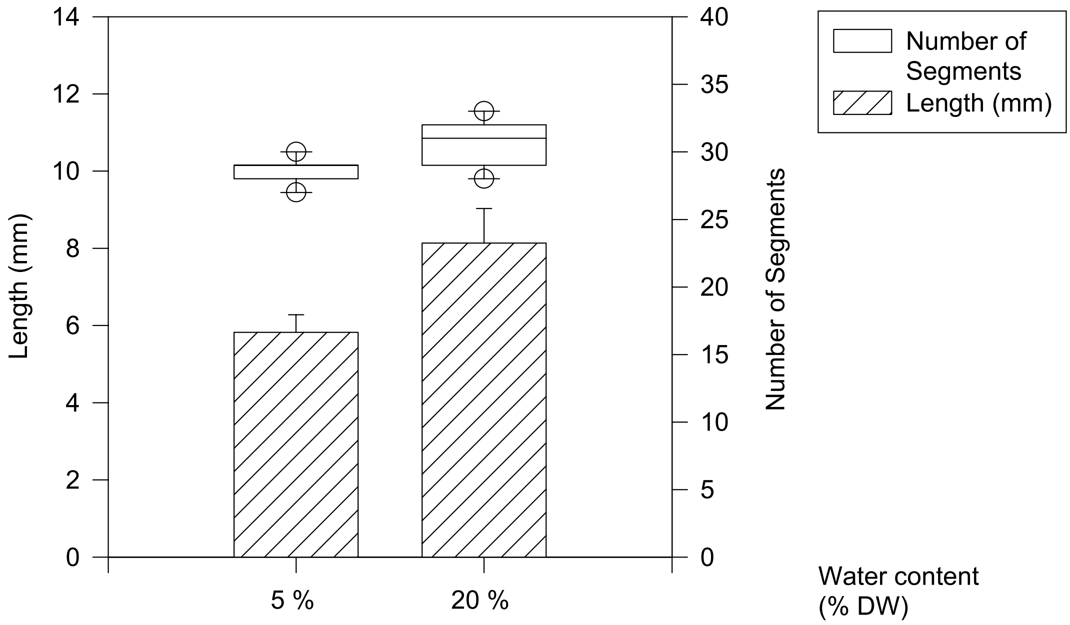


*Fig. 4: Effect of low soil moisture on body length of the parent generation after 21 days (n ≥ 30 ). Treatments differ significantly with α = 0.01*

Low soil moisture inhibited not only reproduction, but had also a negative effect on the growth of the parent generation. Fig. 5 shows a significantly smaller segment number and reduced body length (in mm) for individuals kept at 5 % water content than for those kept at 20 % for 21 days.

The WHC of the LUFA-Standardsoil was 53.2 % whereas the WHC of the RefB-soil from the irrigation field was 39.0% (Table 3). Moisture conditions that proved to be favourable for reproduction in laboratory (≥ 40 % WHC) were hardly ever reached in the mineral soil in the field (4-8 cm depth). DOZSÀ-FARKAS (1977) found that *Fridericia galba* was unable to survive in a mineral soil with water contents lower than 15 % water holding capacity for more than several hours, whereas according to ABRAHAMSEN (1971) *Cognettia sphagnetorum* tolerates a water content of 13 % WHC in raw humus material for months. This indicates that *Enchytraeus buchholzi* is at least equally tolerant towards low soil moisture: adults survived for three weeks at a water content of 9.4 % of the WHC in the present study.

Table 3: Water contents in % DW and in % WHC for RefB-soil and LUFA standard soil

| % DW | 5 | 10 | 15 | 20 | 25 | 30 | 35 | 40 | 45 | 50 |
|---|---|---|---|---|---|---|---|---|---|---|
| % WHC RefB | 12.8 | 25.6 | 38.5 | 51.3 | 64.1 | 76.9 | 89.7 | 109.6 | 115.4 | 128.2 |
| % WHC LUFA | 9.4 | 18.8 | 28.2 | 37.6 | 46.9 | 56.4 | 65.8 | 75.2 | 84.6 | 93.9 |

**Conclusions and outlook**

On the investigated site, a combination of unfavourable abiotic factors (moisture, pH, heavy metals) apparently restrict enchytraeid abundance and species diversity. Enchytraeids possibly do not play a role as important for decomposition as in other habitats where they occur in higher densities. Conceivable measures to improve the living conditions for the decomposer community could be liming, elevating the ground water table and reducing microclimate fluctuations by afforestation. Further work in the field of drought resistance of enchytraeids could include:

– Development or improvement of methods for measuring soil moisture in soil fauna (micro)habitats. For comparison of data it would be best to measure water potentials.
– Investigations into survival strategies of different enchytraeid species to cope with unfavourable moisture conditions: Do they improve their survival potential by vertical migration and/or by physiological adaptations? Are there different survival strategies for adult worms and cocoons?
– The effect of drought stress on the sensitivity against other stress factors should be considered. So far this problem has rarely been studied in detail (PUURTINEN & MARTIKAINEN 1997), although it might have implications for defining legal threshold values for hazardous substances.

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