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Optimal Conditions for the Wet Funnel Extraction of Enchytraeidae from Peat Soils of Moorlands in England

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영국 고원지대(Moorland)의 이탄 토양에서 애지렁이 추출을 위한 수분깔때기의 최적조건

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ABSTRACT

The effects of combinations of temperature, water column height and extraction time on the efficiency of wet funnel extraction methods for enumerating Enchytraeids in a blanket bog peat soil from Moor House, Cumbria, England were investigated. The optimal conditions for extracting enchytraeids from this study were found to be a water surface temperature of 35°C, with an extraction time of 6 hours, regardless of initial water temperature and water level in the extraction funnel. The original O'Connor method (40-45°C for 3 hours extraction and gradually increasing heating) yielded a high variation in the extraction efficiency, largely due to the comparatively higher temperature and shorter extraction time than this method. Attempts to extract without heat over longer periods showed very low extraction efficiencies for this highly organic blanket bog soil when compared with any of the heating wet funnel methods.

Key words: Soil fauna, Enchytraeid worms, Heating, Peat soil, Extraction method

INTRODUCTION

Enchytraeid worms are usually extracted by some modification of the wet funnel method devised by O'Connor (1955). He proposed that enchytraeids could be almost completely extracted from wet funnels by gradually increasing the surface water temperature to 45°C with a light bulb over a three hour extraction period. Further studies have confirmed the high efficiency of the wet method of O'Connor (O'Connor, 1962; Peachey 1962, Abrahamsen 1972) in which enchytraeids move downward through a sieve in the funnel to avoid heat from the bulb (O'Connor 1955, Nielson 1952, 1953). However, at a too great heating rate can worms be killed before they are able to escape from the sample soils; at a too low heating rate do not the worms respond to the treatment. Other variables included in the extraction procedure include the length of extraction time and the depth of water into which the sample is placed. Although it is necessary to ensure that optimal extraction conditions are determined for any soil type under study, there are few published comparative studies for blanket bog soils.

There have been a lot of different versions of the basic funnel technique published, frequently without providing the necessary background data to validate the changes proposed (see Didden *et al.* 1995). However, different conditions in the wet funnel method can produce very different results in the population estimates for Enchytraeidae. In this study, it was tried to optimize the method for use with peat soils from a blanket bog. This was achieved through a comparison of the extraction efficiencies at an assortment of extraction temperatures (surface temperatures 20 to 40°C), extraction times (3 hours to 14 days) and water depths. Since the development of temperature gradients is considered to be critical in the extraction of enchytraeids (O'Connor 1962), temperature gradients were monitored in the different extraction regimes using thermocouples and a data logger, to see if links existed with measured extraction efficiencies. The extraction efficiency was also compared in the 'no heating' wet funnel methods (Graefe 1973, 1984, Schauermann 1983, Didden *et al.* 1995).

MATERIALS AND METHODS

Soil sampling and enchytraeid extraction

Soils for this study were sampled in March 1998 from the peat soils of a blanket bog within the Moor House National Reserve in the UK. The sampling site is adjacent to the cabin used for routine monitoring within the Environmental Change Network (Sykes and Lane 1996) located on Hard Hill at an elevation of 560 m and National Grid Reference NY 751333. The vegetation at the site is dominated by heather (*Calluna vulgaris* L.), cotton grass (*Eriophorum* spp.) and moss (*Sphaguun* spp.). The enchytraeid species in this region have been extensively studied by Standen and Latter (1977), with *Cognettia sphagnetorum* being most abundant, and five other species being found, viz. *Marionina filiformis*, *Marionina clavana, Cognetti glandulosa, Cernossvitoviella brignata* and *Mesenchytraeus sanguineus* in this *Calluna-Eriophorum* mixed moor (Springett 1970). Detailed information about the Moor House National Reserve, including climatic and edaphic conditions at the site, together with a through assessment of the flora and fauna at the site is given in Heal and Smith (1978).

The upper layer of soil was sampled to a depth of 3.5 cm using a square (5 cm) cross-section peat corer. Samples were taken randomly within a designated area of 100 m^2, put into plastic bags and immediately transported to the laboratory. They were stored at room temperature (about 15°C) for 15-24 hours (O'Connor, 1962) and subsequently extracted. The extraction funnels were made of plastic with 74 holes of 2 mm diameter per 100 cm^2 in the bottom (Fig. 1) as an alternative to the original wire-gauze sieve used in the O'Connor method (1955, 1962). Soils were gently crumbled and placed on milk filters (R.J. Fallwood & Blan Ltd.). The milk filter could prevent soil particles from sinking into the sample, yet not inhibiting movement of the worms (see Results). Deionized water at room temperature was used for extraction and the extracted enchytraeid were counted within a day after extraction, using a Wild M7A microscope (Wild, Switzerland).

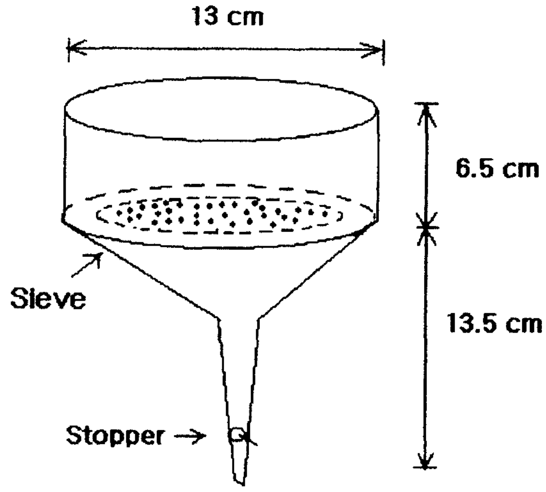

Fig. 1. The wet funnel used in the study.

Dead worms were not counted because there was a tendency for dead worms to fragment and a possibility of overestimation on worm numbers.

Temperature and time for extraction

Since there are few comparative studies on extraction efficiencies of the wet funnel using lower heat and longer extraction periods than those prescribed by O'Connor's (1955). Four lower temperatures (20, 25, 30 and 35°C) were used together with the recommended 40°C, for comparison. The target temperature was simply obtained by controlling the electric power supplied to 25 and 100 Watt bulbs and/or the distance from the funnel to the bulb (Gorny, 1975). Soils for this particular comparison were sampled in 4 March 1998. Four replicates were used for each extraction combination.

During the extraction of enchytraeids from the soils, temperature changes in the surface and bottom water of the wet funnel were monitored using a Delta-T data-logger (DL2, Cambridge, UK) equipped with thermistors (Betatherm 2252 ohms at 25°C; P/N 151-588 supplied by Farnell Components Ltd., Leeds, UK). A comparison of extraction times at each temperature was also made, using the 3 hours recommended by O'Connor (1955) and two longer extraction times of 6 and 24 hours. Water levels in the funnels for this experiment were maintained at 4.5 cm (Fig. 1).

Water level and time for extraction

On the basis of the results from the former experiment (see Results section) a further experiment was performed to compare extraction efficiencies over four extraction times (6, 8, 12 and 20 hours) at 35°C using two water levels (4.5 and

6.5 cm). Fresh soil samples for this comparison were taken on 11 March 1998 with four replicate soil samples for each experimental combination. The data of worms were log transformed (Peachey, 1963) and an ANOVA (SAS, ver. 6.03) was used to determine the effects of temperature, extraction time and water level on worm extraction efficiencies.

Comparison among no heating, constant heating and increasing heating

Again, on the basis of the results from the former experiments (see Results section) a further experiment was performed to compare extraction efficiencies among different procedures. The optimized procedure decided in the prior experiment, constant heating at 35°C temperature with 6 hours extraction and a water level at 6.5 cm, were compared with the original method (after O'Connor, 1955) of a gradual heat increase and the modified one of constant heating at ca. 40°C temperature, and a 'no heating' techniques as advocated by Schauerman (1983). Ten replicates were used for the comparison. Water levels were 6.5 cm throughout all the procedures. In the O'Connor method with increasing heating, temperature of the surface water was increased to 42°C after 3 hours by gradually increasing the voltage of the bulb in 30 minute intervals.

To clarify the reasons for the high variability (see Results section) of extraction efficiency at the ca. 40°C heating regime, the temperature changes at the increasing and constant heating with different water levels were continuously monitored using the data logger. The data of worms were log transformed, and difference among the extraction methods was analyzed by ANOVA and posteriorly by Duncan's multiple range test.

RESULTS

Optimization of extraction temperature and time

Initial trials showed the importance of using milk filters as a supporting medium for the soil during extraction, ensuring the higher efficiency in the longer extraction (Table 1). It was therefore concluded that the filters would be used in all subsequent extractions and comparisons. The development of the temperature profiles within the extraction funnels is shown in

Table 1. Enchytraeid worms extracted by the wet funnel technique with or without milk filters

Extraction time (hours)	n	Without the filter mean±SD	With the filter mean±SD	p-value (T-test)
0.5	4	4.0±3.7	2.5±3.3	0.567
3	4	13.8±10.6	18.0±4.8	0.498
6	4	23.5±10.5	39.5±0.6	0.023

Temperature of surface water in wet funnels after 6 hours of extraction was 32°C

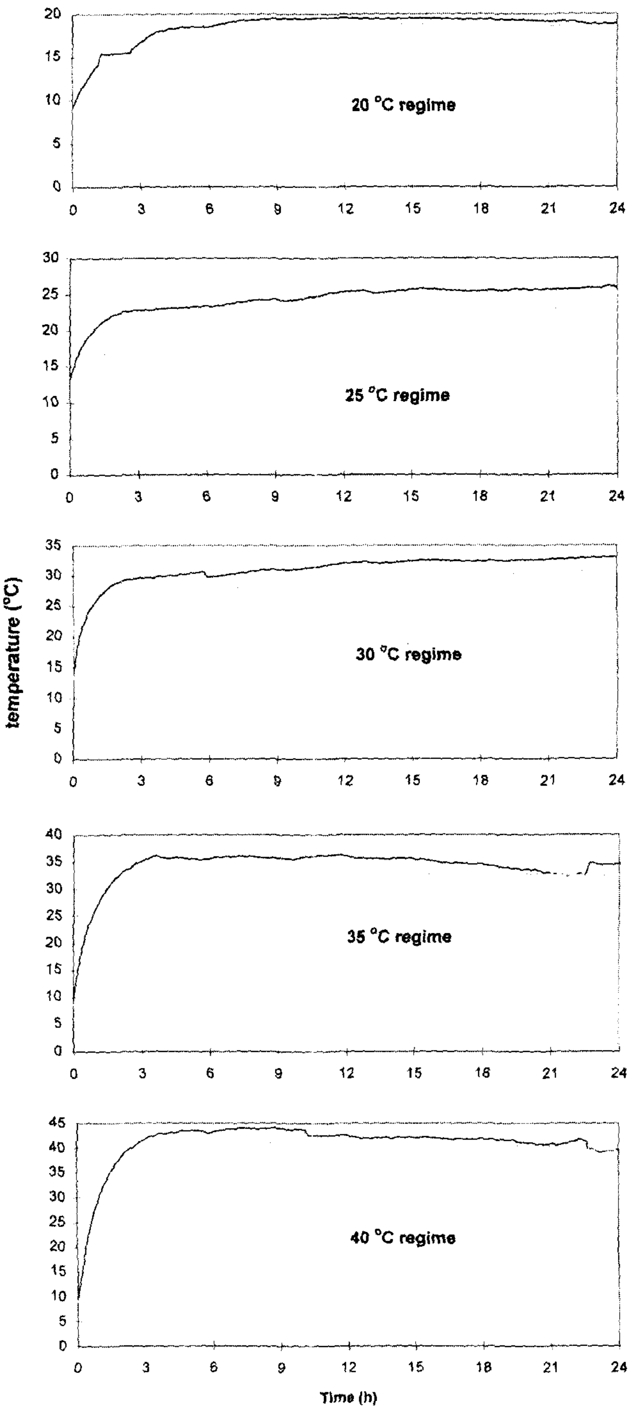

Fig. 2. Temperature changes of the surface and bottom water in the 5 different heat regimes. The top and bottom lines indicate temperature changes of the surface and bottom water, respectively.

Table 2. Analysis of variance on the effects of temperature, extraction time and their interactions on extraction of enchytraeids by the wet funnel method

Factor	DF	Sum of square	Mean square	F	p
Temperature	4	38.5572	9.6393	25.88	0.0001
Extraction time	2	8.6092	4.3046	11.56	0.0001
Temperature × Extraction time	8	10.8520	1.3565	3.64	0.0025

DF: Degree of freedom.

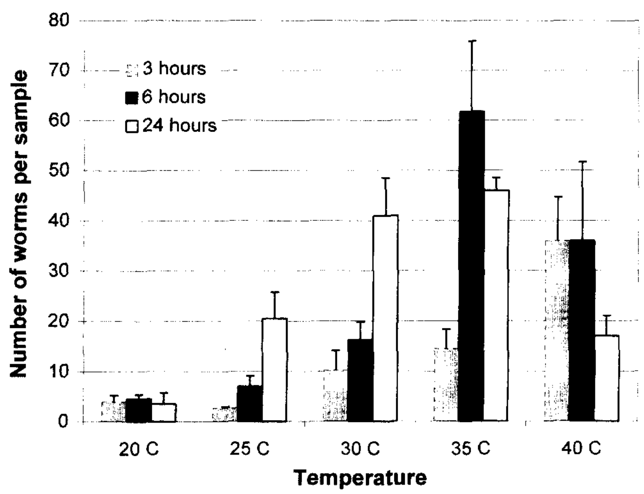

Fig. 3. Number of enchytraeid worms extracted by the wet funnels with the 5 different heat regimes and 3 different extraction times. Lines on the bar indicate one standard error (n = 4).

Fig. 2, which compares the water temperatures at the surface and bottom of the extraction funnels across the five different heating regimes. The surface water temperatures were usually reached to the target temperature within around 2 hours, with the exception of the 20°C treatment, which took around 6 hours to stabilize. Temperatures at the base of the extraction funnels lagged behind those at the surface, even after 24 hours, a gradient still existed in the 20, 25 and 30°C treatments. In contrast, the higher temperature (35 and 40°C) treatments showed that the temperatures had converged after ca. 21 hours. There was no clear relationship between the target temperatures and the gradients which developed.

It was clear that temperature and extraction time had significant influences on the extraction efficiency of the wet funnel method (Table 2, Fig. 3). There were significant ($P<0.01$) effects of extraction time, temperature and the interaction of these two factors on extraction efficiency, which varied markedly between treatments. Fig. 3 shows the consistent inefficiency of extraction at 20°C across all of three extraction periods with maximal numbers of worms being recovered in the 35°C treatment after 6 hours. It appears that heating to 20 °C failed to stimulate the downward movement of the earthworm, even though a substantial temperature gradient was maintained in the funnel up to about 3 hours (Fig. 2). A positive influence of extraction time on extraction rate was found in both the 25°C and the 30°C heating treatments. Surprisingly, the temperature gradients in these two treatments were maintained throughout the duration of the experiment, in contrast to the two higher temperatures, where the gradient collapsed after around 21 hours. At the higher temperatures of 35°C and 40°C, extraction efficiency of live worms over the 24 hours decreased with an increase in the proportion of dead animals, because dead worms were not counted (see Materials and Methods section). The number of worms extracted after 6 hours was significantly higher ($P<0.05$) than after 3 hours at 35°C, but no such differences at 40°C. Temperature gradients maintained in the 35°C heating treatment were consistently higher than those at 40°C (Fig. 2). These data suggest that the optimal conditions for enchytraeid extraction from the peat soils are 35°C for 6 hours.

Optimization of extraction water level and time

Fig. 4 shows the gradient in temperatures which developed in the funnels when different depths of extraction water were used. Surface target temperatures of 35°C were attained in both treatments within ca. 3 hours with the higher water level (6.5 cm) reaching target faster, which is probably due to having the water surface closer to the heat source. The deeper water level gave rise to a higher temperature gradient (Fig. 4) although this was not reflected in the number of worms extracted (Fig. 5, Table 3). There were no significant differences between the numbers of worms extracted in the two depths or across the sampling intervals, yet it was clear that extending extraction time over 6 hours tended to decrease numbers of live worms counted, confirming the reductions found over 24 hours in the previous experiment (Fig. 3), as dead worms increased in the longer extraction times. It is clear from this

Table 3. Analysis of variance on the effects of water depth and extraction time in extraction of enchytraeids by the wet funnel method with ca. 35°C of top water temperature at end of extraction

Factor	DF	Sum of square	Mean square	F	p
Water depth	1	0.0003	0.0003	0.00	0.9725
Extraction time	3	1.4560	0.4853	2.31	0.1013
Water depth × Extraction time	3	0.2703	0.2703	1.29	0.3008

DF: Degree of freedom.

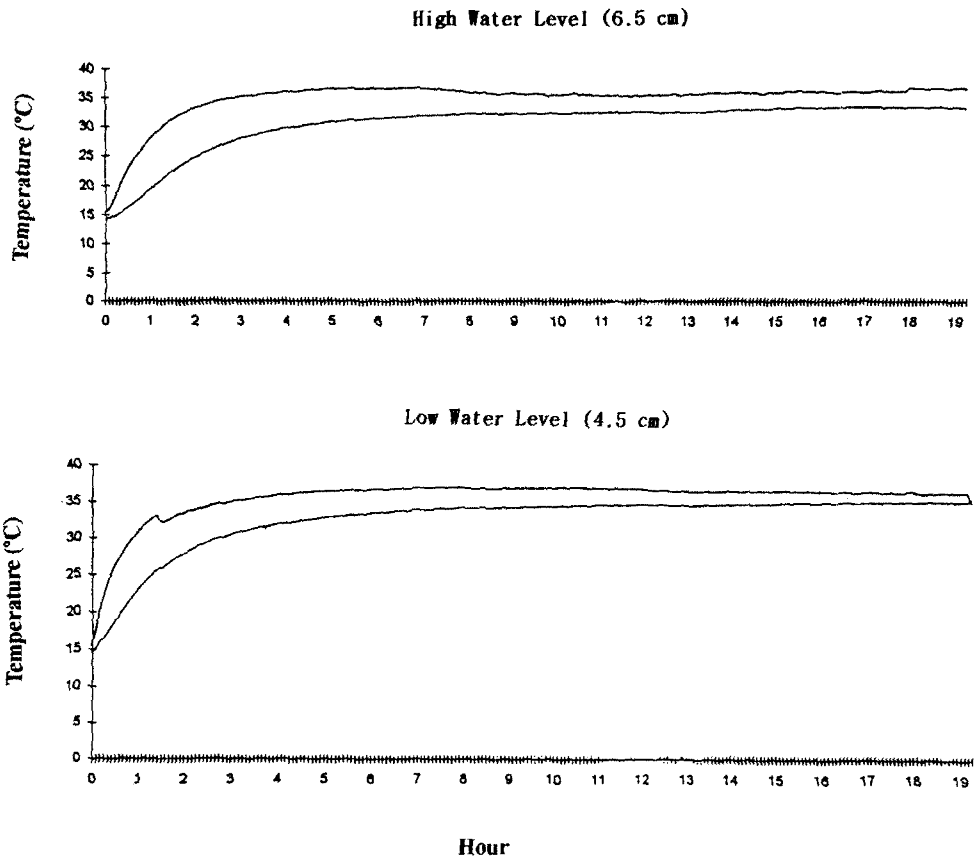

Fig. 4. Temperature change of the surface and bottom water in the wet funnels of ca. 35°C heat regimes according to the high or low water level. The top and bottom lines indicate temperature of the surface and bottom water, respectively.

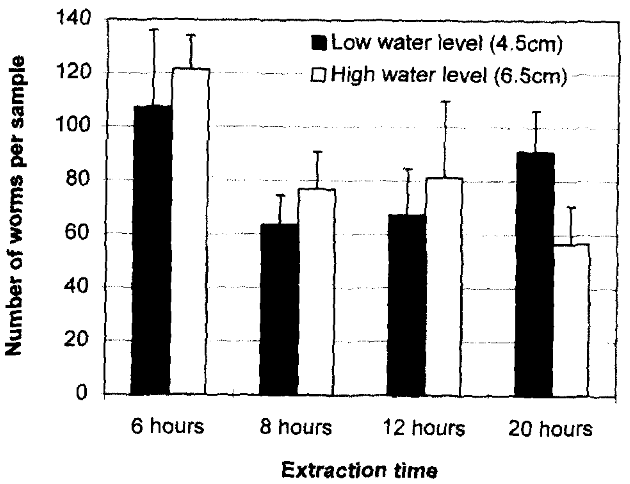

Fig. 5. Number of enchytraeid worms extracted at four different extraction times and two different water level in ca. 35°C temperature heat regime. Lines on the bar are represented as the same as Fig. 3.

experiment that it is best to limit extraction time to 6 hours at an extraction temperature of 35°C. Therefore, the data from this second experiment suggest that neither the depth of water (4.5 to 6.5 cm) nor the time of extraction have significant effects on final numbers counted, although trends in the data suggested that 6 hours extraction was optimal.

Comparison of no heating, constant heating and increasing heating

The importance of heating is clear from Fig. 6 which shows the results of the 5 different extraction protocols comparison. The highest extraction efficiencies were found for the 'optimal' and 'modified' O'Connor method, both being significantly different ($P < 0.05$) from the remaining three methods. Additionally, the no heating method over both sampling intervals gave considerably lower numbers of live worms than the other methods tested. In the modified O'Connor method with constant heat, there were more worms than in the original O'Connor's version with gradually increasing heat so that the gradually increasing heating did not increase extraction efficiency as O'Connor (1955) suggested.

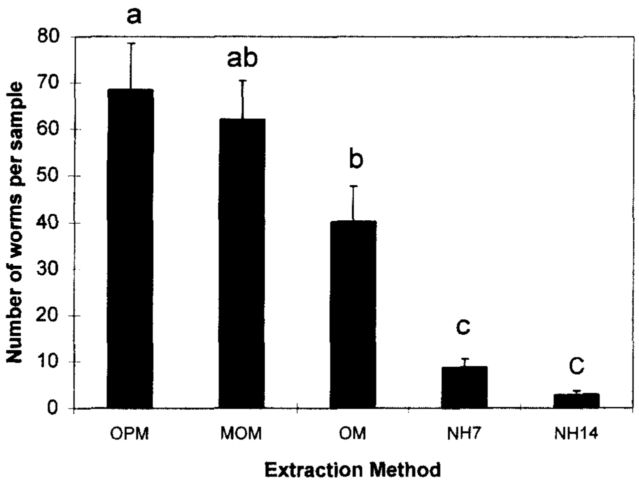

Fig. 6. Number of enchytraeid worms extracted at the five different wet funnel methods. Legends for methods are as follows. OPM: the optimal wet funnel method in this study (ca. 35°C temperature heat, constant heat and 6 hours of extraction time), MPM: the modified O'Connor method (ca. 42°C temperature, constant heat, and 3 hours of extraction time), OM: the O'Connor method (ca. 42°C temperature, increasing heat and 3 hours of extraction time), NH7: the wet funnel method without heat for 7 days of extraction time, and NH14: the wet funnel method without heat for 14 days of extraction time. Lines on the bars are represented as same in Fig. 3. Character on the bar indicates significant difference in the Duncan's multiple range test. The same characters on bars indicate that there are not any significant differences among the methods ($p > 0.05$).

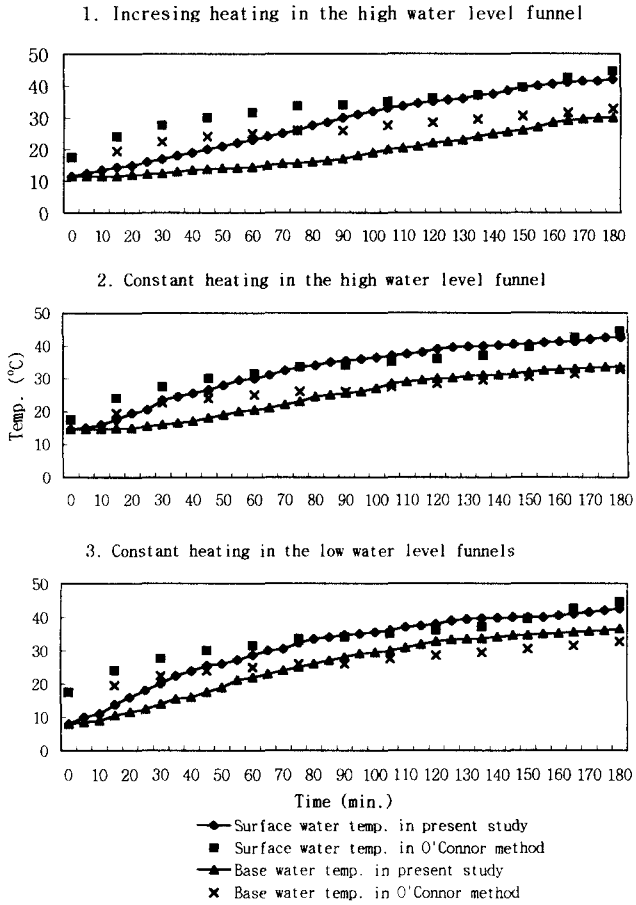

Fig. 7. Comparison of temperature changes of the surface and base water in the wet funnel between this study and the O'Connor' study (1962). Height of water column in the high and low water level was 6.5 cm and 4.5 cm, respectively.

DISCUSSION

The low efficiency in the wet funnel method without heating conflicts with other studies (Graefe 1973, 1984, Schauermann 1983, Didden *et al.* 1995). There were found numerous dead worms in this method. This abundant dead worms were partly associated with the low extraction efficiency, because the dead worms were not counted in this study for preventing overestimation of numbers due to dead fragmented bodies. High mortality of worms could be due to an oxygen deficit in the funnel (Didden *et al.* 1995) or higher water temperature in the funnel than in their original habitat. But even if dead worms were included, the worms extracted from the no heating regimes were not abundant as much as the three heating ones (Fig. 6). Reasons for such low extraction efficiency in this study are not clear. If there was high difference in temperatures between the laboratory and worm's original habitat, there would be abundant dead worms in funnel before extraction. The extraction regime without heat has another problem in its too long extraction time to be used in enumerating enchytraeids. It is likely to overestimate density of enchytraeids by juveniles hatched or worms fragmented during the prolonged extraction.

As shown in Figs. 3 and 6, the extraction efficiency varied greatly in the 40°C temperature heating regime with extraction time of 3 hours as suggested by O'Connor (1955). Changes in water temperature (Fig. 7) represent that heating method and water level would be important for extraction efficiency in this temperature. Low efficiency in the increasing heating method (Fig. 6) may be due to the longer cold period of the water temperature during extraction (Fig. 7). Compared with the O'Connor's temperature change (O'Connor 1962), temperature of surface water in the funnel was lower for 105 minutes and that of bottom water lower for the whole extraction period (Fig. 7). Temperature below 20°C can not stimulate movements of worms (Fig. 3). The cold period below 20°C in this study was 50 minutes in surface water and 105 minutes in bottom water, while that in the O'Connor's study (1962) was less than 15 minutes in both surface water and bottom water. In this study, an effective temperature period was too short to expel most of worms from the soil samples. The initial low temperature of water was an obviously main reason for the long cold period during extraction (Fig. 7). This suggests that the O'Connor's method could have low efficiency in winter for low water temperature and the water cooling prior to extraction could decrease extraction efficiency.

O'Connor (1962) reported that the rate of heating is the most important single factor influencing extraction efficiency in the wet funnel method. In this study, however, extraction efficiency in the constant heating regime is higher (t-test, $p = 0.08$, $n = 10$) than in the gradually increasing heating regime (see MOP and OP in Fig. 6). There were numerous small dead worms being not counted in the constant heating method but only few dead worms in the increasing heating method. Therefore, the real difference between two methods may be higher than the one shown in Fig. 6. Effect of increasing heating is to increase temperature gradient between surface water and bottom water in the funnel (Fig. 7) due to slower heating in bottom water than in surface water. In this study, however, the slow heating resulted in low extraction efficiency by increasing the ineffective cold period below 20°C.

High efficiency in the constant heating method (MOM of Fig. 6) would be due to the shorter cold period below 20°C, compared to the increasing heating method. The temperature change of surface water and bottom water was more close to that of O'Connor's than that of the increasing heat method (Fig. 7). In the constant heating method, water level are crucial for temperature gradient. As water level decreased, temperature gradient did. In low water level (Fig. 7), temperature change of surface water was similar to that of O'Connor's, but that of bottom water became higher at 105 minutes after extraction. Low temperature gradient may decrease worms' downward movement. After 150 minutes of extraction, temperature of bottom water increased over 35°C. This high temperature at the late extraction period may kill worms remained in the soil sample (Nielson 1952-1953). Difference in the water level may cause difference of extraction efficiency. This may be related with low efficiency of the 40°C heating regime in Fig. 3, because the low water level (4.5 cm) was used there.

In the 35°C heating regime, however, extraction efficiency was not affected by water level in the funnel (Fig. 5, Table 3). There was no significant decrease in worms even in the long extraction time of 20 hours (Table 3, Fig. 5). Accordingly this temperature would be relatively safe for worms. The consistent high extraction efficiency in the 6 hours extraction (Figs. 3, 5 and 6) proves that 6 hours are enough long to extract most of worms from the sample irrespective of initial water temperature. It is more robust to initial water temperature and water level for its lower safe temperature and longer extraction time, compared to that of the O'Connor's condition.

In the ca. 40°C heat regime suggested by O'Connor (1955, 1962), high efficiency may be obtained when water change of water temperature is similar to that of O'Connor's (1962; see Figs. 3, 6 and 7). In summary, the optimum conditions for heating water in funnel may be obtained as a trade-off of maximizing stimulation of worms' downward movement and minimizing their mortality in funnel. The ca. 40°C heating regime would need the more restricted conditions in terms of initial water temperature and water level for the higher heat and shorter extraction time than the optimized regime. The results indicate that the optimum conditions for the wet funnel are critical, and the diverse modified versions of it are not favorable for the study of Enchytraeidae.

The enchytraeids are supposed to be of arctic origin, and are the dominant soil animals in tundra, moorlands, and temperate coniferous soils (Dash 1990). It is very likely that enchytraeids may conduct the dominant roles for decomposing organic matters in litters and soils, and forming soils in the Korean coniferous forests. Since enchytraeids increase in acidic soils, their roles may be increased under the influence of acidic rain (Ineson, personal communication). As far as I know, there is no study on it in Korea. The studies of taxonomy and ecology of enchytraeids should be started.

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적 요

영국의 고원지대(Moorland)의 이탄토양(blanket bog peat soil)에 서식하는 애지렁이 정량조사를 위한 수분깔때기추출법의 효율에 미치는 수온, 물의 량, 추출시간의 복합적인

효과를 조사하였다. 이탄 토양에서 애지렁이 추출을 위한 최적조건은 물의 양과 초기 수온에 관계없이 표면수의 최종 온도는 35°C이고, 추출시간은 6시간이었다. O'Connor의 방법(최종 온도 40-45°C, 열의 점진적인 증가, 3시간 추출)은 비교적 높은 온도와 짧은 추출시간으로 인해, 추출효율에 변이가 심하였다. 열을 가하지 않고 장시간 추출하는 방식은 열을 가하는 방식에 비해 추출효율이 현저히 낮았다.

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