TISCIA 33, 3-7

A TULLGREN–TYPE EXTRACTOR FOR SAMPLING SPRINGTAILS POPULATIONS FROM SMALL VOLUME SOIL CORES IN HIGH SAMPLE SIZE

M. Dombos

Dombos, M. (2002): A Tullgren–type extractor for sampling springtails populations from small volume soil cores in high sample size. — *Tiscia 33, 3-7.*

Abstract. I investigated the accuracy and precision of a Tullgren–type extractor, modified to sample springtails populations from small volume soil cores in high sample size. Efficiency of the extractor was tested in two types of running procedures by putting known number of *Folsomia candida* (Willem) in the soil cores. The accuracy and precision depended highly on the running procedures, one of the loading types had sufficient reliability, whereas other conditions did make high variance in the efficiency. In the loading methods, when the temperature was slightly increased, both the accuracy and precision of the census technique was higher compared to that one, where temperature was enhanced abruptly. The construction of the extractor is detailed.

Key–words: sampling methods, Tullgren–type extractor, Collembola

M. Dombos, Department of Ecology, University of Szeged, H-6701 Szeged, POB. 51, Hungary,
Present address: Department of Ecology, Szent István University, H-1400 P.O.Box 2 Budapest, Hungary

Introduction

There are several methods to estimate population size of microarthropods, among others of springtails. Like in all other measurements, the feasibility of these census techniques depends on its accuracy — how close a population estimate is to the true population size — and its precision — how close a population estimate is to its expected value. For sampling euedafic collembolan populations one of the most popular technique is the extraction method. The Tullgren-type extraction procedure (Tullgren 1918, Macfadyen 1953) is the simplest one, in which soil animals are forced by a temperature gradient to move from the soil cores to the vials. This technique is based on the behavior of soil animals, therefore it has a variability of its accuracy. Under different conditions the accuracy (efficiency in other papers) depended not only on the technical setting up, but on external factors, such as soil type, species and age (van Straalen and Rijninks 1982). There are some other works dealing with technical modifications (Hassal *et al.* 1988, Crossley and Blair 1991), which are improving both the cleanly of the samples, the practical laboratory serviceableness, the heating and cooling systems, as well.

According to the reviews of Edwards and Fletcher (1971) and Edwards (1991), although the extraction method has high accuracy compared to other techniques, the estimation of its precision has been neglected. The precision is reduced when springtails have to be sampled from small soil cores, like in analysis of spatial patterns of soil springtails. On the other hand, such an analysis requires relatively high number of samples at which precision is increased. My goal was to build up an extractor complying with such requirements.

The aim of this paper is (1) to present this extractor modified for the above demands with respect to its accuracy and precision under two different extracting procedure to estimate the sensitivity of the apparatus, and (2) to detail the materials used by the construction of the extractor, available in Hungary.

The extractor

The construction of the extractor is similar to that one built by Rijninks (van Straalen and Rijninks 1982).

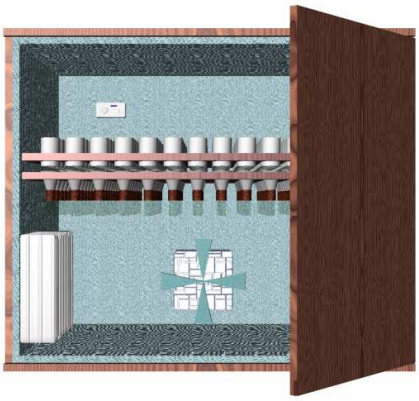

Fig. 1. The view of the extractor.

The cabinet made of plywood has 1.5 m^2 basic area (external dimension: 228x66x100cm) and is isolated on the inner side with polyurethane (thickness = 2cm, Fig.1). It can be opened by 2x2 doors, which split the frame into two sides. The inside of the extractor is also horizontally subdivided into two parts, one for the heating and one for the cooling system. The racks of cores and funnels are equipped in between the two parts, mounted on four sliding drawers. The upper drawers are made of polyurethane, which are hard enough to hold the 103 and 112 soil samples. They isolate the upper side from the lower one at the same time. These are perforated by a steel cylinder (Ø = 5.1cm) rendering the core holders to slide up from the racks possible. The core holder's dimensions are 5cm diameter and 8cm height, provided 137cm^3 inner volume and have a sieve at the bottom (mesh size: = 1mm). Under the sieve there are two perforated disks, which are twisted so that their holes do not overlap. This is an important detail, because these disks prevent the preservative samples from becoming dirty during the extraction. The core holders are covered with a fine-meshed gauze. The lower drawers consist of polyurethane too and are perforated like the upper ones to hold the funnels. The vials (Ø: 2.1cm, height: 5.6 cm) are joined to the ends of plastic funnels (upper Ø: 5cm, lower Ø: 2.1cm, angle: 31°C) with rubber tubes. They have to have the same diameter, for easy attaching, and because any obstacle for the moving of animals in this part would diminish the efficiency of the extractor (Merchant and Crossley 1970). This type of contact has other advantages, as it is easy and fast to work with, and it prevents the preservative material to evaporate from the vials during the extraction.

The heating system is equipped on the top of the inner side of the canister consisting of two 150W infra satin bulbs and a thermostat unit (IMIT, reliability: 0.2°C) to control the temperature. Below the bulb there is a plate to decrease the direct radiation of heat to the core samples.

The cooling system is mounted on the bottom of the cabinet. If the extractor works on room temperature, the cooling system is made up of a refrigerator unit, but if it works in cool room (10–15°C), it is enough to build in a simple ventilator. Other technical details are available on request.

Methods

Extraction

The soil cores with known number of animals (see below) was placed in the extractor. Two types of running procedure were completed. In the first experiment the temperature was set at 20°C the first day and was increased with 5°C the second and third days, so from the third to the sixth days the cores were extracted on 30°C. In the second one the thermostat unit was set at 30°C at start and remained on this temperature.

Measurements of temperature and humidity

Temperature was recorded with a thermistor (LOGIT) in the two compartments of the extractor and in the environment permanently throughout the extraction period.

Relative humidity of the soil samples was estimated by choosing randomly 5 samples from each drawer every day during the extraction and was determined according to the thermo-gravimetric method.

Testing accuracy and precision

Accuracy was measured by the efficiency, where efficiency [%] was defined as the number of collembolans in the soil core at the time/in the start]x100. The explicit efficiency was estimated by giving known number of *Folsomia candida* to the soil cores. 50 specimens were put in different age in each of the 50–50 cores on each rack. Precision was estimated by standard deviation and standard error of the number of springtails caught during the procedures. The soil used was defaunated by freezing at –20°C (Bengtsson *et al.* 1994).

The possible environmental heterogeneity in the cabinet can provide differences of the efficiency among the samples. Furthermore, differences in airing can also contribute to this systematic error. For this reason efficiencies were measured on the five different parts of racks in five groups and it was tested whether the extractor on different parts of the rack has different efficiency. Five parts were selected on each drawer, four in the corners and one in the middle of the drawers. Each group consisted of four core samples. The number of animals captured in the vials was counted every day.

Statistics were calculated using the software package StatSoft, Inc. (1995). Means ± standard deviations are presented, standard errors are indicated as SE

Results

Temperature profiles

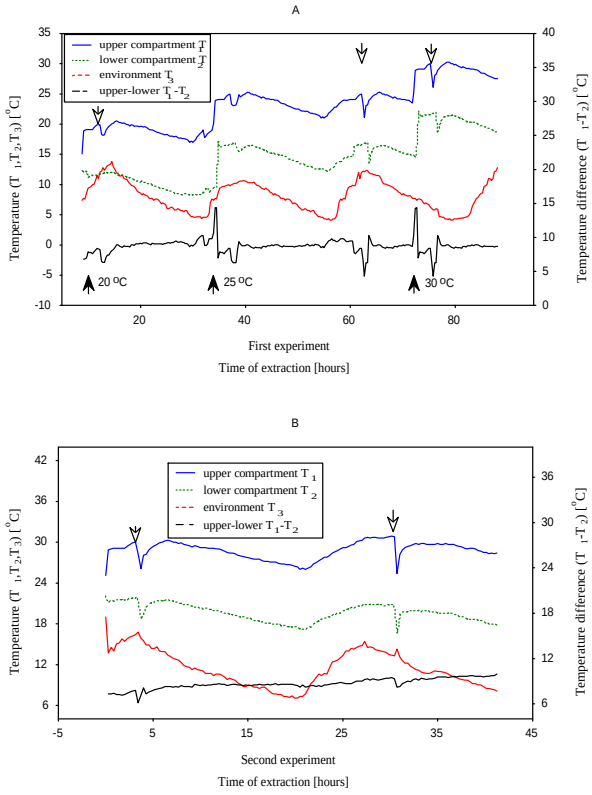

Fig.2. Temperature profiles. Footnotes: Thick arrows indicate setting time, Open arrows show, when the cabinet was opened.

In the first experiment the temperature was set at 20, 25, 30°C (Fig. 2a). The temperature of the environment ranged from 4 to 13.8°C with a mean 8.1±0.3°C, the inner temperature varied with the environment, but the gradient remained considerably stable. The difference between the upper and lower compartments of the extractor was 8.7±0.1°C.

Humidity profile

In the first experiment the cores were dried up more softly, compared to the second one, where after two days the relative humidity decreased sharply to 30% (Fig. 3). Higher values of the standard deviation in the second experiment indicated that the conditions were more uncontrolled. At the end of both experiment all of the soil cores, sampled from different core holders had low humidity (12.3 ± 0.8 % and 12.7± 2.2 %).

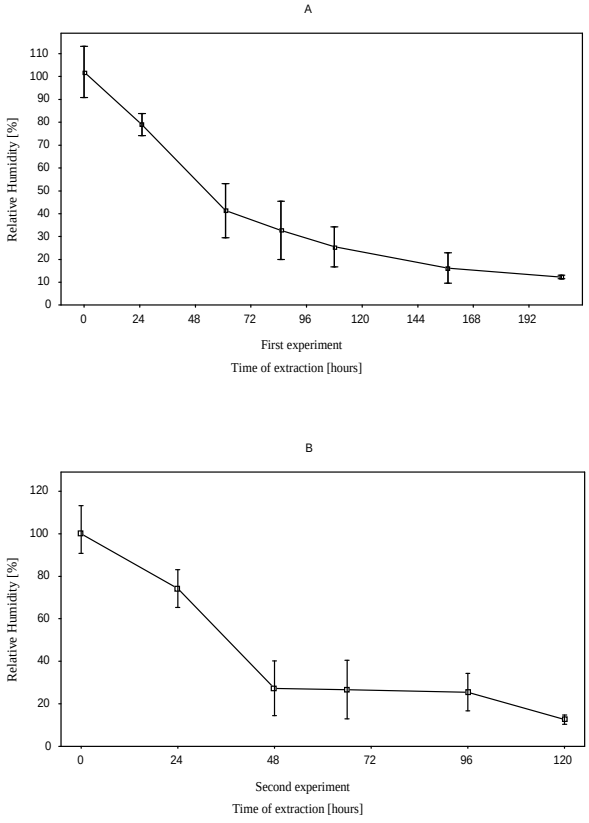

Fig. 3. Relative humidity of soil cores during the extraction. Footnotes: squares: means, whiskers: ± standard deviation

Accuracy and Precision

85.3±3 percent of the springtails has been recaptured in the first, and 72.1±9.4% in the second experiment. There was a significant difference

between the efficiency of the two procedures (t(38) = 2.8 (p=0.008)). There was no difference between the efficiency of the two last samples showing that no more animals would have been alive. The first procedure had not only higher efficiency then the second one, but provided lower and more stable variance during the experiment, compared to the second one, suggesting, that the first experiment had not only higher accuracy, but also it was more reliable, because it had higher precision. In the second procedure some soil cores could be found with extremely low efficiency (range = 68%).

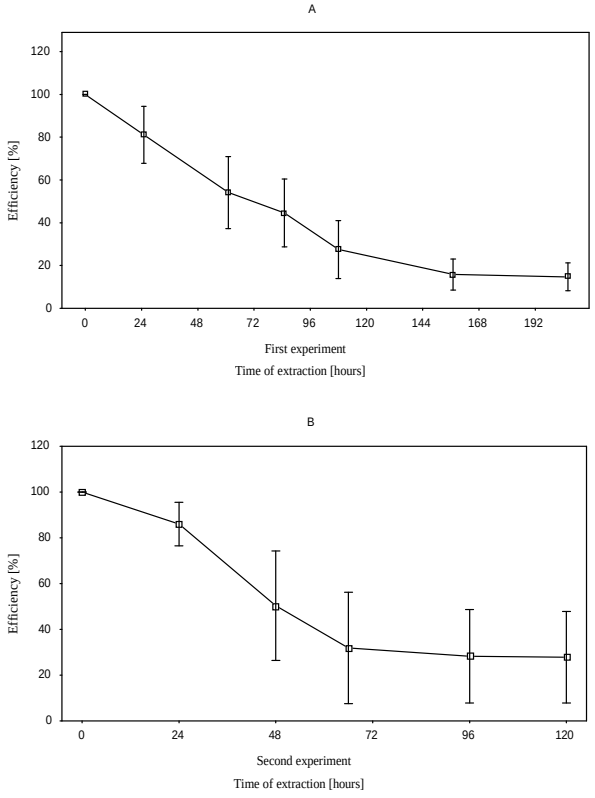

Fig. 4. The efficiency of the extractor during the extraction. Footnotes: squares: means, whiskers: ± standard deviation

There were considerable differences between the means of groups' efficiencies in both experiments (Table 1). In the first procedure the highest difference was 11%, whereas in the second one it was 26%. One way ANOVA demonstrated significant differences between the means of groups' efficiencies in the first experiment, but could not distinguish between the means of groups in the second one, because of high variances. If we regard the groups in both experiments, non of them behaved in the same manner, giving higher or lower efficiency consistently.

Discussion

Regarding the technical details we can conclude that the thermostat and the heating unit could not control the inner temperature with adequate sensitivity, because of the vulnerability of the heating unit. Because the compartments of the cabinet could keep approx. 8°C gradient, the isolation can be regarded proper. There have been many attempts to minimise the amount of soil and debris that falls into the collecting tube (von Torne 1962, Murphy 1962), but it always decreased the efficiency of the extraction. In our case the two perforated disks under the sieve had such a task, although we do not know how it reduced the efficiency.

In the second experiment not only the efficiency, but also the reliability of the extractor has to be regarded as insufficient. The cores could dry out immediately and therefore increased the probability of animals dying in situ. The results obtained in the first experiment has given an appropriate set of temperature and extraction time, non of the core's efficiency fell bellow 72 %.

The examination of the efficiency of the extractor was based upon giving known number of springtails to the soil cores, which technique is considered as a minimal estimate of efficiency, because laboratory animals are sometimes injured, or behave abnormally (Petersen 1978). The comparisons of different apparatus, given by van Straalen *et al.* (1982) suggested, that estimates of efficiency can vary between 62–90% and its efficiency is significantly lower, than passive technique, like hand–sorting or flotation–type technique.

The technical facilities available rendered possible to build up such a construction in that the heating and cooling system could provide relatively stable and homogeneous environment to the soil cores. Both accuracy and precision can be improved by further development, especially in heating system.

In ecological examinations, where high sample size employed sampling procedures require sampling error estimates. The extraction methods render possible to estimate absolute census or population number indexes on soil microarthropods, of which biases depend on the technique used. If the ecological analysis is more sophisticated, demographic, marking or other topics are investigated, further accuracy and precision estimates, for example age–specific aspect of efficiency have to be conducted.

Table 1. The efficiency of the extractor among the soil cores groups.

Experiments:	First			Second		
		Efficiency			Efficiency	
Groups	Mean	Std. Error	Range	Mean	Std. Error	Range
1	91	3.3	14	61.5	5.9	26
2	80	3.2	14	87.5	6.0	26
3	86	1.2	4	69	7.9	34
4	83	4.1	20	76.5	6.1	24
5	86.5	2.2	10	66	18.0	68

Differences in means between groups:

$F(4,15) = 3.68$; $p<.028$ $F(4,15) = 1.05$; $p<.414$

Acknowledgments

The extractor was built up by the author. I gratefully acknowledge to I. Szalkai (Gransprint 97 Ltd.) for making spares for no charge and F. Szentesi (Trio–Plus Ltd.) for his help by the acquisition. The author is indebted to Dr. Péter Kabai for comments on the manuscript. The investigations were supported by the OTKA F/020105 Grant.

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