Comparison of Extraction Efficiency between Winkler and Tullgren Extractors for Tropical Leaf Litter Macroarthropods

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Abstract

Winkler and Tullgren extractors are commonly used to extract soil and litter arthropods for ecological surveys and community studies all over the world. These two devices differ in their extraction efficiency. In this study, we investigated the extraction efficiency of Winkler and Tullgren extraction devices. We collected paired samples and extracted them under the same conditions in both types of devices. Tullgren extraction was more effective for extracting soil and litter macroarthropods than Winkler extraction both qualitatively and quantitatively. The number of specimens collected by Tullgren extraction was consistently higher than Winkler extraction for all macroarthropod groups. Qualitatively, Tullgren extraction yielded more beetle families and pselaphine beetle species than Winkler extraction. Winkler extraction sampled only dominant groups of soil and litter macroarthropods collected. Thus for more complete species inventories in ecological and taxonomic studies, Tullgren extraction method is more effective than Winkler.

Keywords: soil, litter, animals, qualitative, quantitative, sampling, berlese funnel

Introduction

Soil arthropods are among the most species rich guilds in terrestrial ecosystems (Giller, 1996), but are poorly understood (Hall, 1996). Soil fauna is important to the functioning of the ecosystem processes of decomposition, nutrient cycling and maintenance of soil fertility (Lavelle et al., 1997). Most soil and litter animals are tiny, numerous, and can nit be seen easily with the naked eye. To study soil animals, special techniques are needed to extract the animals from soil and litter. Many specialized extractors have been developed to assess animal diversity in soil and litter including Tullgren (Berlese) funnels, high-gradient funnels, and Winkler bags (André et al., 2002; Chung and Jones, 2003).

One of the best known devices for extracting arthropods is the Tullgen funnel. The apparatus was first invented by A. Berlese and later modified by A. Tullgren. Thus, the apparatus is also called Berlese funnel or Berlese-Tullgren funnel (Southwood, 1978). The principle mechanism of the extraction is that the funnel creates warm and dry condition at the upper part by a lighting source equiped on the top, which leads the litter and soil dwelling invertebrates to move down the funnel away from the light source and finally fall out to collecting bottle (André et al., 2002; Barnard, 1995; Sutherland, 1996; Vargo, 2000).

Winkler extraction is also frequently used in ecological surveys and functional studies of soil and litter micro and macroarthropod communities (Hammond, 1990; Chung et al., 2000). This is a portable device using the similar principle of extraction as Tullgren extractor but depends on the natural drying by hanging it in the field. The apparatus is sometimes called as Winker/Moczarski or Moczarski/Tullgren extractor (Besuchet et al., 1987; Wheeler and McHugh, 1987).

These two devices have advantages and disadvantages in their application and extraction efficiency. Different authors have argued about the efficiency of these two extraction devices, e.g. Fisher (1999), Kalif and Moutinho (2000), Longino et al., (2002).

In this study, we evaluated the extraction efficiency of Winkler and Tullgren extractors for extraction periods of 3 hours to 7 days. We compared the extraction efficiency and taxonomic bias of Winkler and Tullgren extraction at ordinal level for soil insects, and for non insect groups Arachnida, Chilopoda, Isopoda and Diplopoda. At the family, to access familial diversity we compared relative beetle abundance in each family, and we used pselaphine beetles (Staphylinidae: Pselaphinae) for comparison at the species level.

Materials and Methods

Sampling Site

Samples were collected from a relatively undisturbed moist evergreen forest in the Khao Soi Dao Wildlife Sanctuary (KSD), Chanthaburi province, (N 13° 01' 08" and E 102° 12' 46", 329 m elevation) on July 11, 2007. This area is one of the largest protected areas in eastern Thailand. The study site is mountainous and the climate has high relative humidity with annual rainfall in 2006 of 3800 mm. The sampling plots were located in moist evergreen forest dominated by *Dipterocarpus* alatus Roxb.ex G. Don, Mallotus peltatus (Geiseler.) Mull. Arg, *Shorea quiso* (Blanco) Blume and *Strombosia javanica* Blume.

Model of Winkler Extractor

The Winkler extractors have an internal frame consisting of two wire rectangles (30 × 25 cm) set 50 cm apart between upper and lower wire frames. Below the lower wire frame, the Winkler gab is funnel-shaped and empties into a collecting bottle. The soil sample is placed in a mesh bag (38×25 cm, 2 mm^2 mesh size) suspended inside the Winkler bag from the upper wire frame. A maximum of four mesh bags can be suspended in one Winkler bag. For more details of Winkler bag construction, see Chung and Jones (2003).

Model of Tullgren Extractor

The model of Tullgren extractors used in this study was designed by the second author for extracting soil beetles. It was made of tinplate steel and consisted of three removable parts: funnel-shaped cover, main canister and funnel-shaped bottom. The dimensions of each part are (diameter ×height), 38×17 cm, 38×26 cm and 38×17 cm respectively. Inside the main canister is a 34×17 cm (diameter×height) internal stainless steel basket (with cover of 1×1 cm wire mesh net) placed in its main part. All removable parts were well tight-fitting.

Samples and Extraction Procedures

Ten paired 1 m^2 samples of leaf litter and soil (10 replicates for each extraction) were randomly taken at moist evergreen forest in Khao Soi Dao Wildlife Sanctuary. The litter and surface soil was scraped up to 2cm depth (by hand). Each sample (weighing approximately 1.5-2 kg) was sifted through a wire sieve of 1 m mesh size to exclude the larger elements such as leaves, twigs and stones. After sifting, each sample was stored in ventilated cloth bag and transferred to a field station where ten Tullgren extractors and ten Winkler extractors were set up.

Ten samples were loaded into a single mesh net basket and placed into a Tullgren extractor. Each Tullgren extractor had a 60W incandescent light positioned above the soil sample. The 60 W incandescent light was turned on throughout the extraction period. A collecting bottle containing 80% ethanol was placed under each Tullgren extractor to collect the falling arthropods. The second sample from each pair was loaded into three mesh bags and was suspended in a Winkler extractor. A collecting bottle containing 80% ethanol was attached to the Winkler cloth bag to collect the falling soil arthropods during extraction.

The extractions were conducted at room temperature over 7 days. The collecting bottles under Tullgren and Winkler extractors were replaced with new 80% ethanol bottles after 3 h, 6h, 12h, 1 d, 2d, 3d, 4d, 5d 6d and 7d. During the extraction periods, temperature and moisture of soil samples inside Tullgren extractors were measured at 0 h (the start time after loading the sample), 1d, 2d, 3 d, 4d, 5d, 6d, and 7d. Temperature and moisture of soil samples in Winkler extractors were only measured twice: at the start time (0 h) and 7d due to the difficulty of accessing the soil samples in Winkler bags.

Target Groups

All insects extracted with Winkler and Tullgren extractors were counted and identified. Macroarthropods in the class Arachnida, Chilopoda, Malacostraca (Isopoda) and Diplopoda were also separated. These groups are among the most abundant in litter samples (IBOY, 2000). Acari (mites and ticks) and Collembola were excluded from this study because these two groups are extremely abundant and would require sub-sampling of litter quadrats to limit the number of samples. Comparisons of extraction efficiency were done for all soil and litter arthropods: (1) major arthropod class, (2) beetle families, (3) beetle species using pselaphine beetles (Staphylinidae: Pselaphinae) as a reference taxon.

Results and Discussion

Extraction Efficiency between Tullgren and Winkler Extractors

After seven days, the number of specimens in each class extracted with Tullgren funnels was higher than the numbers extracted by Winkler bags (Figure 1). Both methods extracted arthropods in the similar proportions (Figure 2). Ants were the most abundant arthropod group extracted by both Winkler and Tullgren methods in the class Insecta followed by Coleoptera, Hemiptera, and Lepidoptera, respectively. Among the non-insects, Arachnida was the most abundant group extracted by both methods followed by Diplopoda, Isopoda, and Chilopoda (Figure 1).

The number of specimens extracted by Tullgren and Winkler extractors were very different, 5,904 ants specimens were extracted by Tullgren funnels, while 1521 specimens (25.76%) were extracted by Winkler bags, adult beetles were extracted 1,820 (Tullgren) and 275 (15.11%,Winkler), Arachnida was extracted 481 (Tullgren) and 116 (24.12%, Winkler), Diplopoda 205 (Tullgren) and 68 (33.17%,

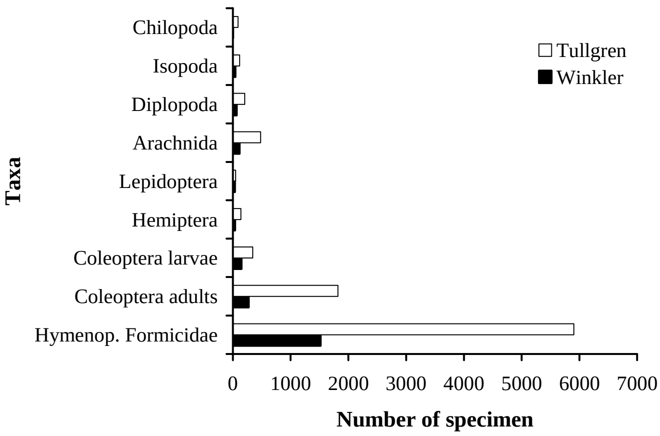

Figure 1 Number of soil and litter macroarthropod specimens extracted from 10 m^2over 7 days by Winkler and Tullgren extractors.

Winkler) and all other groups of arthropods in this study had shown similar extraction trends. Winkler had shown low efficiency for extracting Chilopoda. Our data showed that only 4 centipedes were extracted by Winkler extractors, and 88 were extracted by Tullgren extractors.

Composition of Higher Taxonomic Level

The composition of specimens changed as extraction time increased (Figure 2). Ants and adult beetles were always high in proportion of extracted specimens regardless of extraction time, and the proportion of these groups gradually decreased with increasing extraction time but was still higher than other groups. Adult Diptera, Hemiptera, and Coleoptera larvae increased in proportion as extraction time increased in both Winkler and Tullgren extractions. Chilopoda, Diplopoda, and Arachnida comprised a small proportion of the sample immediately after extraction began, but increased in proportion as extraction progressed (Figure 2). The proportion of macro-arthropods extracted with these two methods was quite similar throughout the time course of extraction.

Extraction and Composition at Beetle Families

Twenty-two families were collected in this study (Table 1). We counted beetles in subfamily Pselaphinae separately from other Staphylinidae, as this group is abundant in forest litter (Chandler, 2001) and the focus of our taxonomic study. Winkler extractors were inefficient for collecting

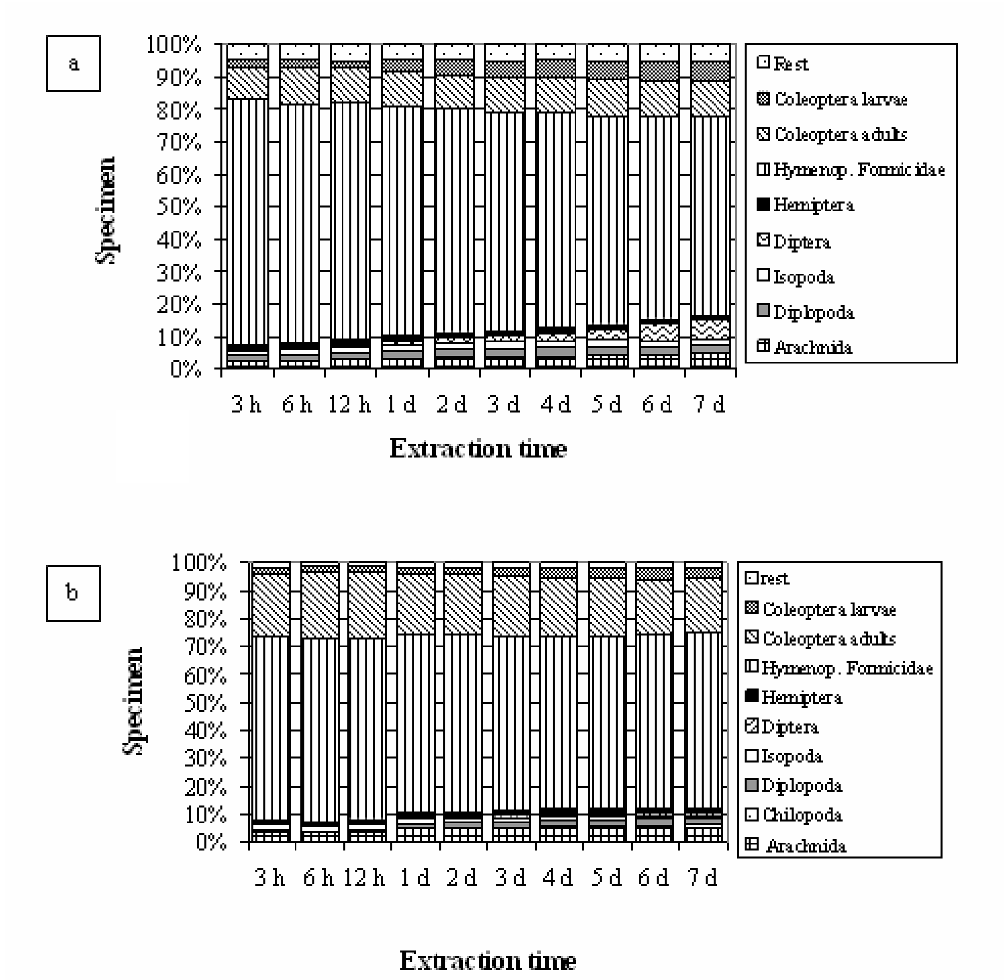

Figure 2 Proportion of soil-litter macro-arthropod groups among the extracted specimens from samples after varying periods of time by Winkler extractors (a); Tullgren extractors (b).

soil and litter beetles, Only 275 adult and 150 larval of beetles were extracted. Tullgren funnels extracted 1,820 adult and 344 larval Coleoptera (Figure 3). Fifteen beetle families were collected with Winkler extractors, while 22 families were collected with Tullgren extractors. Beetles in families Staphylinidae, Scydmaenidae, Scaphidiidae, Ptiliidae, and in the staphylinid subfamily Pselaphinae were among the top ten most abundant taxa (Figure 3). The proportion of beetle families extracted by Winkler and Tullgren extractors was similar (Figure 4). The families Silvanidae, Throscidae, Salpingidae, Eucnemidae, Discolomidae, Heteroceridae, Hydrophilidae, Leiodidae and Nitidulidae were only extracted by Tullgren extractors. Tenebrionidae was the third most abundant beetle family extracted with Tullgren extraction, but few individuals were extracted with Winkler extractors (Figure 3).

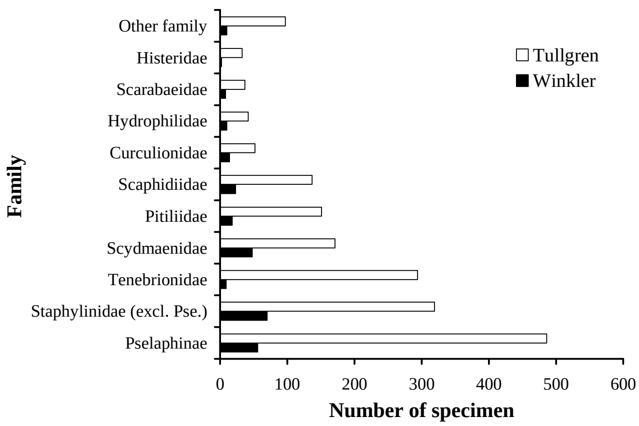

Figure 3 Number of specimens of beetle families extracted from 10 m^2 over 7 days by Winkler and Tullgren extractors.

Taxonomic Bias: Species Collecting

Specimen accumulation curves for abundant beetle families are given in Figure 5. Hydrophilidae, Scarabaeidae, Curculionidae, Scaphidiidae and Histeridae were completely extracted after three days and Scydmaenidae were completely extracted on the day four with Tullgren funnels (Figure 5b). Only specimens in the family Hydrophilidae showed saturation by Winkler extractors after three days (Figure 5a). Other abundant families were not completely extracted with Winkler bags at the end of the extraction period (Figure 5a).

Pselaphine beetles are the most dominant group of soil and litter beetles. They are abundant (56/275 beetles via Winkler extraction; 486/1,820 via Tullgren extraction) (Table 2). Nine and 19 pselaphines species were extracted with Winkler and Tullgren extractors respectively (Figure 6). In this set of comparisons at the species level, *Plagiophorus* sp. 1, *Pseudophanias* sp. 1 were the dominant species collected by both methods. *Apharina* sp. 1 and *Batraxis doriae* were two additional dominant species extracted with Tullgren extractors. For the other species, Tullgren funnels extracted more pselaphine species as well

Table 1 Number of beetle specimens in selected families after 7 days extraction with Winkler and Tullgren extractors. Averages are mean±SD (N = 10).

Family	Extraction method			%
	Trophic guild[1/]	Winkler	Tullgren	
SF. Pselaphinae	Pr	56	486	25.87
Staphylinidae (excl. Pselaphinae)	Pr, S	70	319	18.57
Tenebrionidae	F, S, H	9	294	14.46
Scydmaenidae	Pr	48	171	10.45
Pitiliidae	S, F	18	151	8.07
Scaphidiidae	F	23	137	7.64
Curculionidae	X, H	14	52	3.15
Hydrophilidae	Pr, S	10	42	2.48
Scarabaeidae	S, X, H	8	37	2.15
Histeridae	Pr	2	33	1.67
Scolytidae	X, F	9	26	1.67
Discolomidae	F		16	0.76
Salpingidae	?F		12	0.57
Crytophagidae	?F	2	10	0.57
Leiodidae	F, S		9	0.43
Carabidae	Pr	4	7	0.53
Coccinellidae	Pr, H	1	5	0.29
Silvanidae	F, S		4	0.19
Acanthoceridae	F		4	0.19
Nitidulidae	F, S		2	0.10
Chrysomelidae	H	1	1	0.10
Eucnemidae	F, X		1	0.05
Throseidae	?F, ?X		1	0.05
Coleoptera larvae		150	344	
Coleoptera adults		275	1820	
Total specimens		425	2164	
Average specimens in 1 m^2		42.5 ± 15.6	216.4 ± 61.2	

[1/] Most of tropic guilds assigned to each family are after Hammmond (1990). Abbreviation of trophic guilds: Pr, predacious; F, fungivorous; H, herbivorous; Pa, parasitic; S, saprophagous; X, xylophagous.

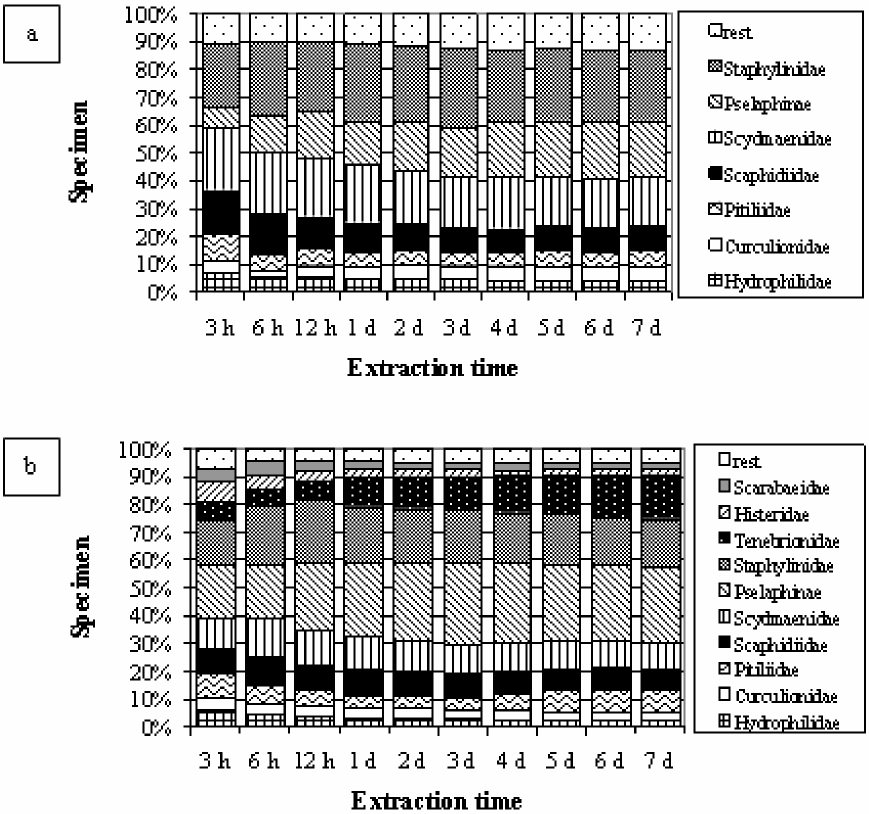

Figure 4 Proportion of beetle families extracted among the extracted specimens from samples after varying periods of time by Winkler extractors (a); Tullgren extractors (b). Note: Staphylinidae was excluded Pselaphinae.

as other soil and litter beetle families. The species accumulation curve for pselaphine beetles for Winkler and Tullgren samples approached saturation on the first day for Winklers and on the second day for Tullgren samples (88.88% and 94.73%, respectively) and both methods extracted all pselaphines by day four. However, pselaphine species and specimens collected by these two methods were quite different (Figure 6; Table 2).

Conclusions

Tullgren extraction is a more efficient method for extracting soil and litter macroarthropods than Winkler extraction, both qualitatively and quantitatively. The number of specimens collected by Tullgren extraction is higher than the number collected via Winkler extraction for all arthropod groups except Lepidoptera larvae, which differed little between traps (n = 37, Winkler; 46, Tullgren).

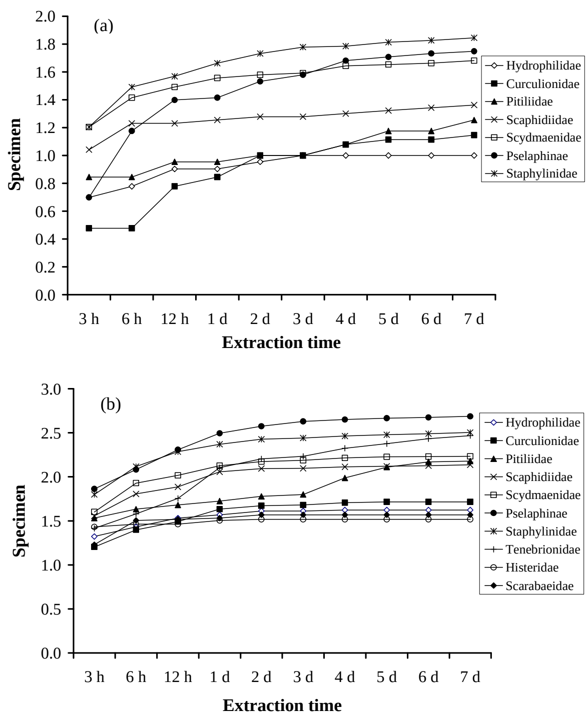

Figure 5 Specimen accumulation curve ($\log_{10}$) of dominant beetle families extracted after varying extraction time with Winkler extractors (a); Tullgren extractors (b). Note: Staphylinidae was excluded Pselaphinae.

Table 2 Number of pselaphine beetle specimens collected after 7 days in Winkler and Tullgren extractors. Average is mean±SD (N=10).

Family Staphylinidae Subfamily Pselaphinae	Extraction method	
	Winkler	Tullgren
Plagiophorus sp. 1	36	399
Pseudophanias sp. 1	11	18
Batraxis doriae	3	15
Apharina sp. 1	1	12
Pseudoplectus sp. 1	1	7
Euplectus sp. 1	1	5
Parapyxidicerus sp. 1		5
Articerodes sp. 1	1	4
Leptoplectus sp. 1		4
Euplectodina sp. 1		3
Pseudophanias sp. 2		3
Leptoplectus sp. 2		2
Philiopsis sp. 1		2
Plagiophorus sp. 2	1	2
Atenisodus sp. 1		1
Parapyxidicerus sp. 3		1
Tribasodites sp. 1		1
Hypochareus sp. 1	1	
Undet genera allied to *Hypochareus*		1
Hamophorus sp. 1		1
Total specimens	56	486
Average specimens in 1 m^2 (quadrat)	5.6±7.1	48.6±14.2

The number of pselaphine beetle species extracted with Winkler bags was half of the number of species extracted with Tullgren funnels and Winkler bags extracted fewer beetle families: 15 families, or 66% of the families extracted with Tullgren funnels (22 families). Not only did Tullgren extraction sample more individuals and greater diversity at every taxonomic level, but they also sampled more rare species than did Winklers.

The species accumulation curves for pselaphine beetles collected with Winklers and Tullgren funnels (Figure 6) were both saturated but the number of specimens and species were quite different. We would get lower estimates of species richness and diversity values if we used only the Winkler method, because some diversity indices employ the number of specimens (individuals) in their calculation e.g. Shannon diversity, Evenness, ACE, and Chao1 (Margurran, 1988; Colwell, 2005). In this study, only nine species of pselaphine beetles were extracted by the Winkler method, 19 species were extracted with Tullgren funnels in the same habitat.

The low efficiency of Winkler extraction could be explained by (1) Winkler extractors made the soil and litter arthropods stay in soil samples instead of failing into the alcohol bottles. This was supported by Scheerpeltz (1968) found that alcohol in collecting bottle makes beetles stay in the substratum rather than falling into the bottles. (2) The average soil moisture in Winkler bags at the start of extraction was 35.22% at 26.3°C and after 7d of extraction, the average soil moisture dropped slightly to 31.60% at 28.61°C (Figure 7). These small changes in microenvironmental conditions in Winkler extractors (cloth bag) may not have affected the organisms much, because the soil samples dried out slowly in the inner compartment, some organisms may have moved inside the apparatus. Thus, many arthropods in the Winkler extractor may still be alive after 7d. (3) Since the extraction of predators such as Arachnida and particularly Chilopoda are relatively slow with Winkler and Tullgren extractors, the influence of predation might be increased with longer extraction time, particularly for Winkler extraction, which was found to be inefficient for extracting Chilopoda (Figure 2). Krell et al. (2005)and Chung and Jones (2003) both reported as we did that Chilopoda and Araneae were very slowly extracted with the Winkler method at the beginning of the extraction interval, and were extracted more rapidly as time progress. This increasing the chances that these predators will prey on smaller organisms in the substrate. Additionally, predacious beetles (families Staphylinidae, Scydmaenidae, Hydrophilidae, Histeridae, etc) were the major guild of beetles in our soil and litter samples (58% of the total specimens), and they would feed on small organisms in Winkler bag (Table 1). Chung et al. (2000) also found that predacious beetles were the most diverse and abundant guild, representing more than 40% of soil beetles in forests in Sabah, Malaysia.

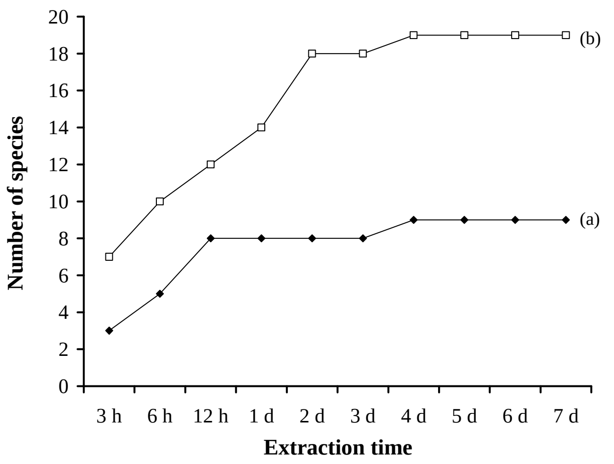

Figure 6 Species accumulation curves of pselaphine beetles (Staphylinidae: Pselaphinae) extracted after varying periods of time using Winkler extractors (a); Tullgren extractors (b).

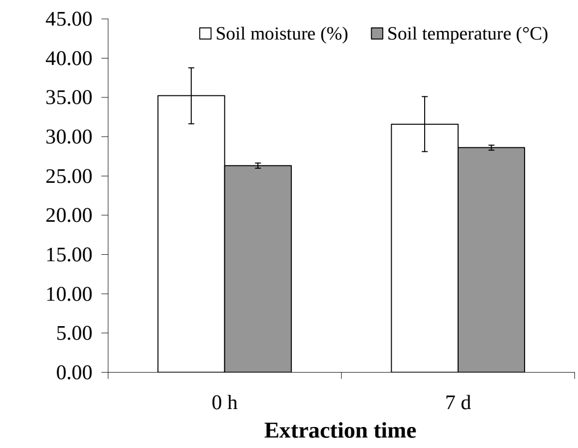

Figure 7 Change in soil/litter temperature and moisture of soil samples in Winkler extractors following extraction time from 0 h (starting) and 7 d. Bars shown are mean ± standard deviation (N=10).

Tullgren extraction works by the heat, the low light in a Tullgren extractor (60 W light) heats and dries the litter, which gradually changes the soil samples humidity and slightly increases the temperature. In our experiment, the starting temperature was 26.32°C at the beginning of the extraction and 42.12°C at the end. Litter moisture decreased from 37.46% to 3.62% at the end of 7d (Figure 8). Temperature and moisture gradients in Tullgren extractors were slowly change soil sample conditions, inducing arthropods to migrate through the soil sample to escape the increasing unfavorable conditions near the bulk. Some large-sized beetles in families Tenebrionidae, Carabidae and Scarabaeidae have hard elytra and strong body. They were found in relatively low numbers in Winkler bags. We found nine specimens of tenebrionids in our Winkler bags and 319 specimens in our Tullgren funnels (Table 1).

Although, Winkler extractions were less efficient at extracting soil arthropods, Winkler extraction might still an effective method if the propose of the study is to compare dominant soil-litter macro-arthropods. Krell et al. (2005) recommended three days for extraction to recover 70% of the individuals and nearly all species of ants from soil samples. Beetles were also extracted in their rank order abundance within the samples.

Tullgren extraction seems to be more suitable for community, diversity, and functional studies of soil macroarthropods that require both quantitative and qualitative data. Additionally, for taxonomic studies, Tullgren extraction is a better method to sample rare species. Longino et al. (2002) reported that Berlese extraction (one type of Tullgren funnel) is the most efficient method for sampling soil and litter ants. It collects many species that are not collected with any other method, including Winkler extraction, and many of the unique species (those species found only one sample) were either manually excavated from soil or were obtained in a Berlese (Tullgren) sample.

Both methods have advantages and disadvantages. Winkler extraction is a simple method which does not required any electricity in the extraction process itself making it suitable for remote field locations, while Tullgren extraction requires a constant power source. We observed fewer swift insects (e.g. ants, spiders, carabid beetles) escaped when loading soil samples into a Tullgren funnel than when loading soil samples into Winkler mesh bags.

Acknowledgments

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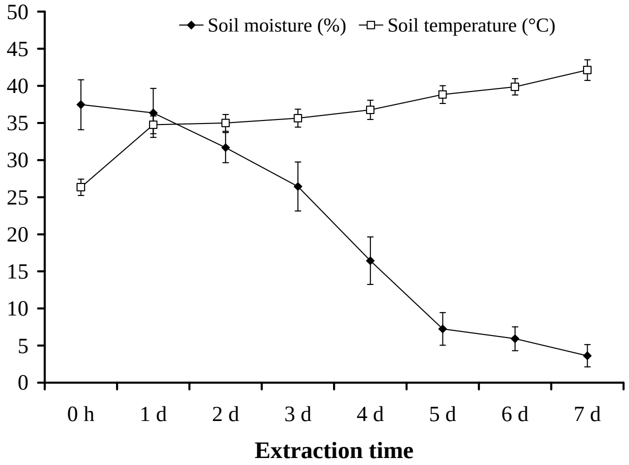

Figure 8 Change in soil/litter temperature and moisture of soil samples in Tullgren extractors following extraction time from 0 h (starting) up to 7 d. Bars shown are mean ± standard deviation (N=10).

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