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Development of a Simplified Sampling Technique for Soil Fauna Extraction

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ABSTRACT

Human activities such as intensive agriculture and industries could weaken the soil quality. Traditional approaches of soil quality evaluations are mainly based on the use of physical, chemical and microbiological indicators. The importance of including soil invertebrates in soil assessments has been recognized in the recent past because these organisms enable to evaluate another dimension of soil quality which may not be measured by using physical and chemical indicators alone. The reason is that the soil organisms are interlinked with the physical environment and the soil processes. Attempts have been made to assess the soil quality using soil invertebrates as the indicators. Any such indicator should be accurate and sensitive to changes. Further, the associated techniques should be accessible and convenient to a range of users including scientist, farmers and land managers. Visible invertebrates as earthworms, enchytraeids and insect larvae have already been used in soil quality evaluations. However, their sampling and identification are challenging. This study focused on simplifying the sampling process for hand-sorting by reducing the soil sample volume (within 0-10 cm soil depth). A 0.5 l core sampler and a soil block of 15 × 15 × 10 cm (2.25 l) volume was compared against a reference soil block of 30 × 30 × 10 cm (9 l) volume ($\alpha = 0.05$, t-test). Sampling was done in a Mahogany (Swietenia macrophylla) Plantation, Rubber (Hevea brasiliensis) plantation and a lawn dominated by Paspalum spp. in the low country wet zone of Sri Lanka. Sixteen samples each from 2.25 l block and 0.5 l core and four samples from 9 l block were taken from each system. Total count of the invertebrates visible under a hand lens (magnification- ×2/4) was recorded. There was no significant difference between 2.25 l block, and 83.33% of observations (p=0.643, 0.182, 0.063, 0.079, 0.052, 0.404, 0.356, 0.590, 0.125, 0.263) in the reference block. However, there was a significant difference between 0.5 l core sampler and the reference block in all the comparisons (p=0.000, 0.001, 0.000, 0.002, 0.005, 0.011, 0.015, 0.008, 0.000, 0.029, 0.002, 0.001). Therefore, it can be concluded that the soil block of 2.25 l can produce accurate data in soil fauna extraction. Therefore, time and effort required for sorting out soil fauna can be reduced by nearly four times by reducing the soil volume down to 2.25 l.

KEYWORDS: *Soil quality, Soil invertebrates, Soil sampling, Soil volume, Hand-sorting*

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1. INTRODUCTION

Soil quality is defined as the "capacity of a specific kind of soil to function within natural or managed ecosystem boundaries, to sustain plant and animal productivity, maintain or enhance water and air quality, and support human health and habitation" (Karlen *et al*., 1997). Soil quality may alter over time due to natural phenomena**.** However, various actions in agriculture and manipulation of the eco-systems have substantially degraded the soil throughout the world (Menta, 2012). Soil is a limited natural resource which is severely affected by improper land use (Keith and Kevan, 1963). Land-use changes and intensive agriculture cause severe degradation of soil and destruction of soil biota. Soil biota is the driving force of soil processes. Any combination of agricultural activities such as tillage, pesticide and fertilizer application, cropping patterns, soil compaction during the cultural operations and removal of plant biomass may affect the soil biota in terms of population size and community structure (Menta, 2012). Maintaining soil quality in agricultural fields is very important for overall sustainability. Further, soil quality monitoring is required to assess the long-term impact of the cultivation of the soil environment and optimize the management practices reducing the negative impacts.

According to the United State Department of Agriculture (USDA), soil quality indicators are classified into four categories as visual, physical, chemical and biological (Karlen *et al*., 1997). Use of living organisms for the evaluation of soil health is justified by the great potential to assess certain health-related parameters which are hard to assess by physical or chemical parameters alone. Biological indicators were mostly neglected in the soil quality evaluation processes in terms of count of micro and macro-organisms and their activities or functions. However, it has been repeated that the abundance and diversity of soil organisms are well correlated with beneficial soil functions (Parisi *et al*., 2005). These functions include storing and releasing of water, decomposition of plant and animal residues, sequestering and detoxifying organic toxicants, supporting plant health by suppressing plant pathogenic microbes and phytophagous fauna and transforming and recycling nutrients (Doran and Zeiss, 2000). Therefore, these soil organisms illustrate the chains of cause and effect that link and management decisions to ultimate productivity and health of plant and animals.

Biotic indices, based on invertebrate community parameters were recently developed as a promising tool in soil quality monitoring. Evaluation of population parameters of soil fauna possible to assess soil processes instead of evaluation of isolated parameters. These organisms are highly sensitive to natural and human disturbances and, are increasingly being recognized as a useful tool for assessing soil quality (Menta, 2012). Soil fauna makes a complex relationship with their ecological niches in the soil and their mobility is limited. For these reasons, their lack of capacity to leave from the soil environment and some

taxa (Collembola, Protura, Pauropoda) are particularly vulnerable to soil impact. Most soil fauna is particularly vulnerable to soil impact because the life cycle of these faunas highly depends on their immediate environment (Menta, 2012). Soil faunal communities considered as a bioindicator due to their communities can make a strong relationship with soil factors (Menta, 2012). When soil factor influences community structure, the structure of a community must contain information on the soil factors. For these reasons, soil fauna can be considered as an excellent bioindicator for evaluating the impact on soil quality. When evaluating soil quality, properties of soil faunal community structure such as species richness and diversity, distribution of species within the different soil layers (Van Straalen, 2004), distribution of body-size over species (Warwick, 1986), classification of species according to life-history attributes, classification of species according to ecophysiological preferences (Van Straalen and Verhoef, 1997) and structure of food webs (Pimm *et al*., 1991) can be used.

Different methods are available for soil fauna extraction depending on the activity of soil fauna during the extraction process (Meyer, 1996). The main three extraction methods are Mechanical (Passive) method, Dynamic (Active) method and Soil sectioning. There are several types of mechanical methods; hand sorting, floatation, sieving, sedimentation, elutriation, pit-fall- trapping, differential wetting, grease- film extraction, and centrifugal method. Dynamic methods include dry and wet funnel methods, and the dry funnel method using Berlese, Tullgren, and Modified Tullgren dry funnels are more commonly used (Domingo-Quero and Alonso-Zarazaga, 2010). Further, the use of molecular methods to examine environmental DNA (eDNA) in a broad range of soil invertebrate taxa on the global scale is a newly developed technique (Wu *et al*., 2011). However, all of above sampling techniques have a number of limitations. Hence, this study focuses on developing a simple and inexpensive sampling technique for soil quality evaluation using hand-sorting of soil invertebrates which is the standard method of soil fauna evaluation. The concept of the proposed sampling techniques was to reduce the extracted soil volume to 15 × 15 × 10 cm^3 from 30 × 30 × 10 cm^3 and reduce the extracted soil volume to 500 cm^3 of soil sampled by a specially fabricated soil core. This size range was selected envisioning to develop an effective sampling technique which can be practiced by a range of users including farmers, quality assurance personnel, or scientists.

2. METHODOLOGY

2.1 Study Area

This study was carried out in the research farm of the Faculty of Agriculture, University of Ruhuna from July to December 2016. The study area was located close to Kamburupitiya town in Matara district and, the area was classified as low country wet zone (WL_2) of Sri Lanka. Mean annual rainfall of the area was 2250 mm and it was received

during the south-west monsoon (May to September) and during the inter-monsoonal periods. The average ambient temperature ranges from 22-29°C with an average relative humidity of 80%. The dominant soil type in the area is Red Yellow Podzolic soil which is classified as Hapludults according to the USDA soil taxonomy (Mapa and Somasiri, 1999).

2.2 Experimental Design

A soil block of 30 × 30 × 10 cm volume was selected as the reference soil volume to test the selected sampling techniques and soil depth for the whole experiment was set to 10 cm as if most of the biological activities in the soil fauna (especially earthworms) were found in the first 10 cm (Keith and Kevan, 1963; Galli *et al*., 2014). Most of the soil organisms were found in a class or order of Haplotaxida, Coleoptera, Arachanida, Collembola, Diplura, Symphyla, Diplopoda and Chilopoda. The order of Isoptera and Isopoda were not considered for population assessment due to high abundance in some sampling points in some land-use systems. However, taxonomic classification was not considered for the analysis and soil organisms who are visible under the hand lens (2/4 ×) were sorted for this study.

Three land-use types were selected for this study. They were Mahogani (*Swietenia macrophylla*) plantation; Rubber (*Hevea brasiliensis*) plantation and a Lawn dominated by *Paspalum* spp. (*Paspalum renggeri* Steud). Sampling points were selected randomly and initially, the litter layer or any other ground cover was removed from the sampling points. Sampling area of 30 × 30 cm was selected and divided into four 15 × 15 cm areas (Fig.1). A 500 cm^3 core sampler (Fig.2) was driven into the middle of each 15 × 15 cm area by simultaneously turning and pushing with hand (Fig.3). The soil extracted by the core sampler was sorted separately by hand to extract the rest of the soil organisms. The rest of the soil in the 15 × 15 cm areas were collected separately and sorted by hand to extract the soil organisms. Complete sorting of four soil cores and the rest of the soil of four 15 × 15 cm areas completed the 30 × 30 × 10 cm soil volume. Sixteen 15 × 15 × 10 cm of soil blocks and 500 cm^3 core samples were extracted from each land use type. Therefore, the data became available for four 30 × 30 × 10 cm soil blocks from each land use type. Only the total number of organisms which were visible under the hand lens was hand-sorted and counted in the study.

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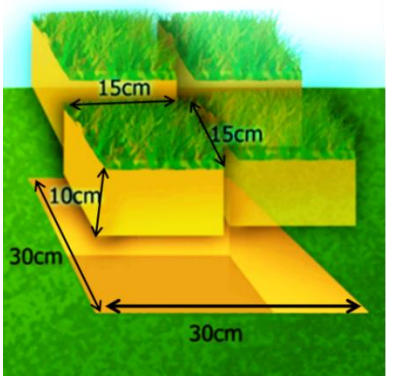

Figure 1: Soil extraction by 15 × 15 × 10 cm soil block

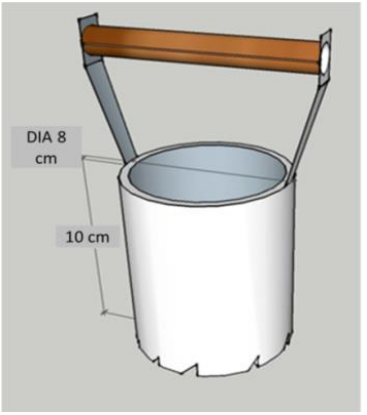

Figure 2: Soil extraction by 500 cm^3 soil core

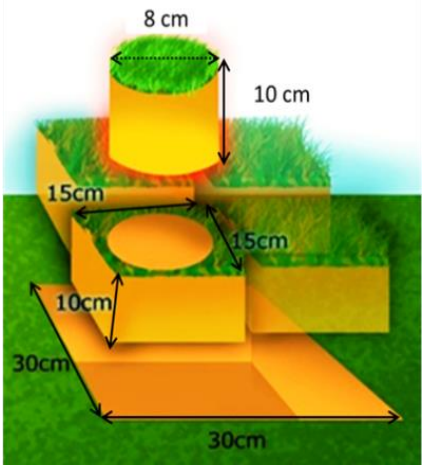

Figure 3: Soil extraction procedure

2.2 Data Analysis

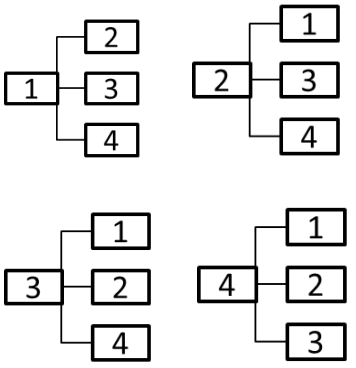

Figure 4: Combination of comparison of total number of individual in each soil block in each vegetation type

Minitab statistical software V.16 was used in the statistical analysis. Combinations for the comparisons were selected as indicated in Figure 4. For each land use type of the population mean was determined by averaging the total organism count of three 30 × 30 × 10 cm soil blocks. One 30 × 30 × 10 cm soil block was purposely excluded from the population mean calculation during the analysis. The data (total organisms count) obtained for the 500 cm^3 soil cores and the 15 × 15 × 10 cm soil volumes of the excluded soil block was compared with the population mean using one-sample t-test where $\alpha = 0.05$.

3. RESULTS

Considering the volume of 15 × 15 × 10 cm soil block, the highest number of total soil fauna was observed in the lawn (Total number of soil fauna in replicate 1, 2, 3, 4 = 39, 87, 57, 70 respectively and total fauna in four replicates = 253) (Fig. 5 c) and the lowest number was observed in Rubber plantation (Total number of soil fauna in replicate 1, 2, 3, 4 = 13, 21, 16, 10 respectively and the total number of soil fauna in four replicates = 60) (Fig. 5 b). The total number of soil fauna in the mahogany plantation was 143 (Total number of soil fauna in replicate 1, 2, 3, 4 = 42, 34, 34, 33) and it was higher than the total number of soil fauna in rubber plantation (60). The statistical analysis (one-sample t-test, $\alpha = 0.05$.) revealed

that, there was no significant difference between 15 × 15 × 10 cm (2.25 l) soil block and the reference block (30 × 30 × 10 cm) in 83.33% of the observations with reference to the total number of organisms in all the systems (Table 1).

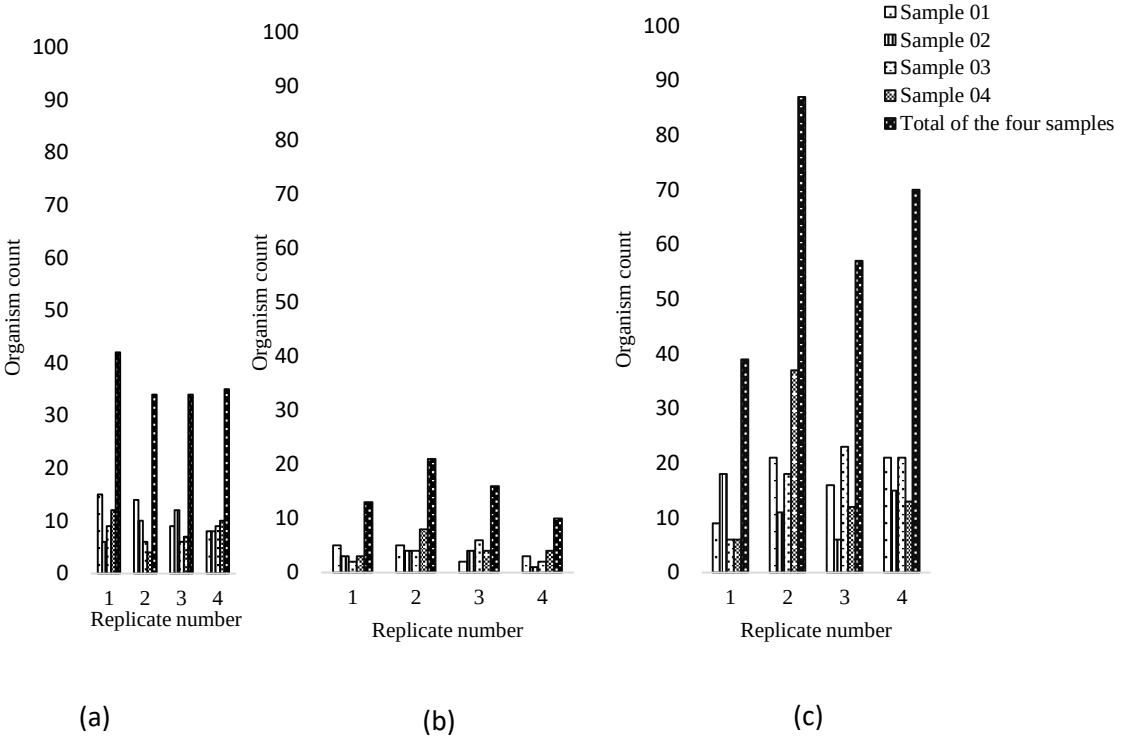

Figure 5: Distribution of total soil invertebrates counts in each 1, 2, 3, 4 replicates (a) Mahogany plantation (b) Rubber plantation (c) Lawn dominated by *Paspalum* spp.

Table 1: Total number of soil fauna in three land use systems compared with 2.25 l soil volume of block and Reference soil volume (9 l) of block.

	p value			
	Replicate 1	Replicate 2	Replicate 3	Replicate 4
Mahogany plantation	0.643*	0.182*	0.063*	0.079*
Rubber plantation	0.052*	0.404*	0.356*	0.018
Lawn	0.016	0.590*	0.125*	0.263*

*$p > 0.05$, not significant.

When extrapolated, the soil fauna count of the 500 cm^3 core sampler appeared to be similar to that of the reference soil block under mahogany plantation. However, similar values were not obtained for the other two land-use systems (Fig. 6). Despite that, statistical analysis showed a significant difference between the counts of organisms obtained by 500 cm^3 core sampler and the counts obtained from the reference soil block in all three systems considered (p = 0.000, 0.001, 0.000, 0.002, 0.005, 0.011, 0.015, 0.008, 0.000, 0.029, 0.002, 0.001) (Table 2)

Table 2- Total number of soil fauna in three land-use systems compared with 500 cm^3 soil core sampler and Reference soil volume (9 l) of block.

	p-value			
	Replicate 1	Replicate 2	Replicate 3	Replicate 4
Mahogany plantation	0.000*	0.001*	0.000*	0.002*
Rubber Plantation	0.005*	0.011*	0.015*	0.008*
Lawn	0.000*	0.029*	0.002*	0.001*

*$p < 0.05$, significant.

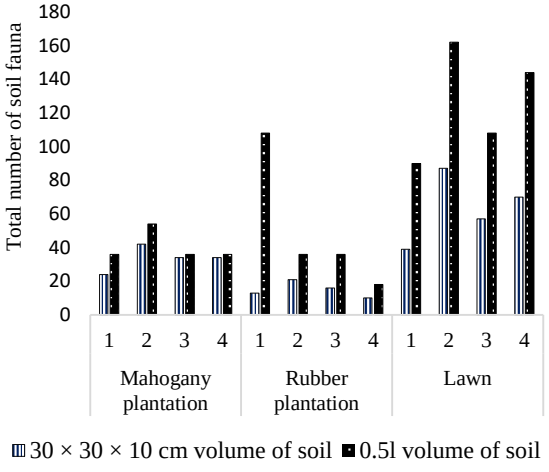

Figure 6: Total number of soil fauna in three land-use systems; comparison of 500 cm^3 soil core samplers and reference of soil volume (9 l).

4. DISCUSSION

4.1 Soil Volume

Sampling volume is one of the most important factors when evaluating the soil quality by using soil invertebrates which can influence the data quality and final conclusion of the experiment. The standard method was introduced by the Tropical Soil Biology and Fertility institute which is called TSBF method for the evaluation of soil macro fauna. During this process, soil blocks or monoliths of dimensions with the 25 cm length, 25 cm wide and 40 cm depth were dug and soil macro fauna were hand sorted from 25 cm× 25 cm square soil monoliths, divided into several layers of 10 cm along with the total depth of 40 cm. This method has been employed worldwide by a number of researches for the assessing of soil health. Major limitations of this method were time consumption and high labor requirement (Parisi *et al*., 2005). According to this study, there was no significant difference between 2.25 l block, and 83.33% of observations in the reference block. Therefore, 15× 15 × 10 cm (2.25 l) soil block was enough for accurate assessment of soil health based soil invertebrates. The main premise of this suggested method was the rapid assessment of soil health by using soil invertebrates. Other advantages of this method were less time requirement, less labor demand, non-requirement of sophisticated equipment, inability to address all the taxonomic groups of soil invertebrates and the consumption of a small soil volume compared to the standard method. The main disadvantages were higher proportion of damaged earthworms compared to the standard method, detection of some species were more difficult due to their slow movement or capture by hand was difficult due to rapid movement or fly (Smith *et al*., 2008). However, 15 × 15 × 10 cm sampling volume was enough to take a representative sample which used to determine soil health by using soil invertebrates. Because, when increasing the sampling volume required a longer time to processing (hand sorting) or more equipment needed (Berlese-Tullgren funnels/ Winkler bags) or would have to reduce the quantity of samples taken in the considered area (Smith *et al*., 2008).

Certain studies based on soil fauna evaluations have used different diameter and length (small soil volume) of core samplers or higher number of core samples in one location with different sampling extraction techniques (Gagnarli *et al*., 2015; Stevenson *et al*., 2002; Fountain and Hopkin, 2004; Wilcocks and Oliver, 1971). Whatever the extraction technique, soil volume is important for accurate assessment of soil fauna in soil quality evaluation. In accordance with the results of this study, small soil volume of core sample (500 cm^3) was not provided representative soil volume for soil fauna extraction. Collecting more number of soil samples with small volumes required more time for any soil fauna extraction method. Therefore, it is better to use representative soil volume (15 × 15 × 10 cm) instead of the use of small core volume (0.5 l). In addition to that, the extraction technique is focused on behavioral characters of soil fauna which

can be reason decrease the accuracy of the results. The major premise is a core sampler compacted the soil, which may lead to the mortality of some soil invertebrates (Barberena-Arias *et al*., 2012). Therefore, proper standards of soil volume should be developed for the advancement of the research related to soil faunal populations.

The present study was carried out with the samples taken from a few land-use systems (Mahogany and Rubber plantation and Lawn). If the sampling was done in different land-use systems, it could be much effective and efficient in testing the accuracy of the sample volume in the process of soil quality assessment which is done based on the soil invertebrates who are visible under the hand lens (2/4 ×).

4.2 Soil Depth

Different depths of soil were used for extraction of soil invertebrates in soil quality assessment according to their purposes of the study (Smith *et al*., 2008; Gagnarli *et al*., 2013; Wilcocks and Oliver, 1971; Weland, 2009). A common source of error is variation in the depth of soil. Most of the biological activity of soil meso fauna can be found in the first 20 cm of the soil which corresponds to plow layer in agricultural soil (Neher and Barbercheck, 1999). Some studies indicated sampling depth as a 10 cm for population assessment of soil invertebrates (Galli *et al*., 2014; Parisi *et al*., 2005; Smith *et al*., 2008). TSBF standard method was introduced several 10 cm soil layers down to the total soil depth (Parisi *et al*., 2005). In this experiment, soil depth was set to the 10 cm for easy assessment of soil invertebrates. When considering 10 cm of soil depth, there were some disadvantages such as soil organisms migrate on daily and seasonal basis example mite, springtails and isopods can move ranging from few millimetres to few centimetres towards the surface (Menta, 2012), symphyla that can go down as much as 40 cm and by sampling only to a depth of 10 cm, anecic earthworms, which burrow up to 1 m below the surface during the day, will be underestimated (Smith *et al*., 2008).

4.3 Target Soil Faunal Taxonomic Group

Assessment of the single taxon of soil fauna for soil quality evaluation is more common in previous studies (Iturrondobeitia *et al*., 1997; Paoletti, 1999; Paoletti and Hassall, 1999). Studies on the soil fauna often focus on single taxon only making a challenge for accurate assessment of soil fauna. Main limitations were due to difficulties in extracting organisms efficiently from the soil matrix (André *et al*., 2002; Decaëns *at el*., 2006; Smith *et al*., 2008), uncertainties in taxonomic identification, lack of expertise for identification and classification of soil fauna and requirement of sophisticated equipment for the identification of soil fauna. Some researches were also based on the general evaluation of soil micro arthropods (Parisi *et al*., 2005). This experiment was based on the hand sorting of soil invertebrates (macro and meso fauna) which were

visible under hand lens (Magnification-2/4 ×). This is because most of the soil faunal groups are sensitive indicators of soil quality apart from that, these soil invertebrates are occupied by more than 20 taxonomic groups and performed different roles in land ecosystems such as decomposing plant and animal residues, transforming and recycling nutrients, sequestering and detoxifying organic toxicants, suppression of noxious organisms, improve soil structure and water infiltration (Pereira *et al*., 2017; Lavelle *et al*., 2014). In addition to their ecological function, soil invertebrates are also used as indicators of soil quality or environmental disruptions where they are widely prominent and distributed, yet they are quite sensitive to the diverse human interventions made in the ecosystems (Pereira *et al*., 2017; Vasconcellos *et al*., 2013; Segat *et al*., 2015).

4. CONCLUSION

There was no significant difference between the 15 × 15 × 10 cm (2.25 l) volume of soil block and the 30 × 30 × 10 cm (9 l) volume of reference soil block in 83.33% of the observations. However, there was a significant difference between 500 cm^3 core samplers and the 30 × 30 × 10 cm reference block in all the comparisons. Therefore, it can be concluded that 2.25 l soil block can be safely used to extract soil organisms for population evaluation purposes and 500 cm^3 core samplers is not appropriate for population assessment of soil invertebrates. Time and effort required for sorting out soil fauna can be reduced nearly by four times by reducing the soil volume down to 2.25 l. Therefore, the results of the present study suggest that standardized soil volume is necessary for the rapid assessment of soil fauna for the assessment of soil quality. Moreover, this study was carried out only using few land-use systems in the wet zone of Sri Lanka. This study suggests that it could be better to reiterate this process for the Dry and Intermediate zones of the country to examine the accuracy of the sample volume in the process of soil quality assessment.

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