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Terrestrial invertebrates as bio indicators: Selecting the best Orders and the best methods

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ABSTRACT

The present study investigated the utility of soil, litter and arboreal invertebrates for monitoring the progress of restoration. Three different techniques were used for sampling various invertebrate orders; pitfall trap, vacuuming and litter sampling (Tullgren funnels). This study provides data to determine the most proper method for sampling different arthropods orders and the best order to use as a bio indicator. A total of 79,183 arthropods were sampled from the study area by the three sampling methods. The majority was from pitfall traps followed by vacuuming samples and litter ones. Comparisons between the three sampling methods indicated that pitfall traps most often captured taxa considered active at ground level, such as ants, carabids and spiders. Most of the arboreal invertebrates were collected by vacuuming and litter sampling most frequently succeeded in collecting certain groups of arthropods associated with moisture and sheltered areas, including beetles larvae and litter isopods. The pitfall trap method appeared ideal for quantitative estimates, while the suction method is ideal for qualitative estimates. Certain groups of invertebrates, notably hemipterans, beetles, ants and spiders are cost-effective to survey and potentially high in information content.

Key words: Bioindicators, terrestrial invertebrates, restoration, pitfall traps, vacuuming, litter sampling.

INTRODUCTION

Our world undergoes rapid changes and is faced with an increasing number of known and unknown pollutants, which combine with climate changes and losses of biodiversity to threaten almost all ecosystems (Market *et. al.,* 2003). The scale of human activities has become such that most of the ecosystems of the earth have been disturbed in some way (Ehrlich, 1993). More than 40% of the vegetated terrestrial surface of the earth has been directly disturbed (Daily, 1995) and its natural productive capacity has been diverted, reduced, or destroyed (Vitousek *et. al.,* 1986).

The area of land directly altered by mining industries is still relatively low in terms of the global inventory of degradation, but can represent considerable quantities on an individual ecosystem or country basis. Further, the scale of mining is increasing and the impacts are generally more severe than most other kinds of disturbance (Walker and Willig, 1999).

The direct impacts of mining to land surfaces are usually severe, with the likelihood of destruction of biodiversity within natural ecosystems as a result of removal of natural soils, with their associated plants and animals. These practices can cause erosion and soil damage, air pollution and contamination, and can have adverse effects on the surface and groundwater through processes such as salinization and acidification, as well as causing loss of flora, fauna and habitat. Mining can introduce diseases and pests (Knight, 1998).

The goal of minesite rehabilitation in Australia has increasingly moved from simple revegetation towards more comprehensive ecosystem restoration, with the aim of establishing sustainable ecosystems similar to those occurring naturally in the region (Unger and Milnes, 1992; Read, 1994; Finucane, 1995). Given the complex and intangible nature of many ecosystem processes and properties, attention has focused on the use of practical indicators that reflect the general state of the ecosystem in which they occur (McKenzie *et al*., 1992; Spellerberg, 1993).

Ecologists often use changes in species diversity to determine the effects of disturbance, because species diversity is an important aspect of any ecosystem (May, 1975; Hutchinson, 1978; Magurran, 1988). However, it is necessary to use appropriate species assemblages as indicators of the effect of disturbance on species diversity, as not all taxa are responsive to particular disturbances.

In terrestrial landscapes, invertebrates constitute a substantial proportion of species richness and biomass (> 65 percent) (Jeanneret, *et. al.,* 2003), and play a significant role in ecosystem functioning. Invertebrate community composition also offers insight into ecosystem function and interaction due to the wide range of functional groups represented (Pik, *et. al.,* 2002). Invertebrates are abundant medium-sized organisms that, as a generality, have growth rates and population turnover times lying midway between those of microorganisms and higher plants and animals. They also have effective active and passive dispersal mechanisms that allow wide dissemination and rapid recolonization of disturbed habitats. All these attributes make invertebrates highly suitable and the most likely choice as bio indicators of environmental change.

Invertebrates are useful, appropriate and often highly effective and informative indicators of other elements of biodiversity, ecosystem function and restoration, system health and associated threats, including invasive alien species (McGeoch, 2007). Invertebrates also provide sensitive, appropriate and logistically feasible target taxa for monitoring a wide cross section of protected area management objectives (McGeoch, 2011).

The use of arthropods as indicator species can provide highly sensitive advance warning of ecosystem changes (Holloway and Stork, 1991; Paoletti *et. al*., 2010; Santorufo *et. al*., 2012). Some species react quickly to

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environmental stressors and are ideally suited to act as bioindicators. Arthropods are environmental bio-indicators of habitat disturbance, pollution and climate change (Hawksworth and Ritchie, 1993).

The advantage of using arthropod species as indicators or candidates for ecosystem monitoring is that their tremendous ecological diversity provides a wide choice for designing appropriate assessment programs (Kremen *et. al.*, 1993) which can be applied for both short-term and long-term monitoring. The use of arthropods in ecosystem analysis is cost effective. Arthropods are easily, quickly, and cheaply sampled, thereby providing means to obtain timely, cost-effective ecosystem information. The aim of this study was to undertake a post priori evaluation of the performance of selected invertebrate taxa as bioindicators, and the best method to sample them, using restored bauxite minesites as a test situation.

MATERIALS AND METHODS

The study area

This study was undertaken at the Worsley Alumina bauxite mine near Boddington, Western Australia. The area has a typical Mediterranean climate with a hot dry summer and cool wet winter. The mine site has an average annual rainfall of 740 mm; 50% of rainfall occurs in winter, with less than 6% in the summer (Vlahos *et al.*, 1999).

The vegetation on the Darling Plateau is a dry sclerophyll forest dominated by *Eucalyptus marginata* (Jarrah) with varying proportions of *Corymbia calophylla* (Marri) and *Allocasuarina fraseriana* (Sheoak). *Eucalyptus wandoo* (Wandoo) forest and woodland occurs in the clay soil valleys and on some upper slopes throughout the eastern edge of the Darling Plateau.

The jarrah forest mid-storey is predominantly composed of trees and shrubs from the Proteaceae family (including *Banksia grandis*, *Persoonia longifolia*, *Banksia sessilis*, and *Hakea prostrata*), plus species of Allocasuarina and Melaleuca. Shrub and herb species from the Proteaceae, Papilionaceae, Myrtaceae, Mimosaceae and Asteraceae families constitute a major proportion of the understorey vegetation. Within the current mining envelope, the vegetation comprises ~400 species from 180 genera, with the distribution of these species being associated with land form and soil type (Worsley Alumina, 1985).

Site selection

A chronosequence of restored areas plus forest controls (representing varying forest vegetation types) were selected. Where possible, these were sites already assessed by Worsley Alumina for other taxa, so that comparisons could ultimately be made with other flora and vertebrate fauna monitoring programs. Sites were grouped into ‘young’ (4 sites; revegetated 3–7 years previously), ‘mid’ (3 sites; revegetated 9–13 years previously), and ‘old’ (3 sites; revegetated 15–19 years previously) (Table 1).

Sampling methods

A 100-m sampling transect was staked out in a representative area of each site at least 15m from the border, so that edge-effects were avoided. Along each transect, 10 plastic vials (43 mm diameter_110 mm depth) were used as pitfall traps, set at 10-m intervals. These were inserted into the ground so that the lip was flush with the soil surface and contained a 40-mL solution of three parts of 70% ethyl alcohol and one part 30% glycerol (see Samways *et. al.*, 1996). They were left open for seven consecutive days. Each transect was used as the starting point for vacuum sampling swathes, which ran at right angles to the transect. Each vacuum sample consisted of a 40-m walk away from the pitfall trap transect and a 40-m return walk, the latter aligned 2m to the side of the original traverse. Invertebrates vacuumed off plants were placed in containers of 70% alcohol for subsequent sorting. Leaf litter samples were also collected along pitfall-trap transects and placed in 3-kg polyethylene bags for subsequent extraction of Invertebrates using Tullgren funnels (the same amount of leaf litter was collected from each site). Sampling by each method was carried out for a period of fifteen months, at 3-monthly intervals. These three sampling methods provided a relative estimation of the abundance and species richness of Invertebrates on the ground (pitfall-traps), on the foliage (vacuum samples) and in the leaf litter (Tullgren funnels). Invertebrate samples were sorted in the laboratory to broad taxonomic levels, with the selected taxa further sorted to morpho-species level when represented by adult forms. These were then sent to relevant taxonomists for verification and allocation of generic and species names.

Data analysis

Invertebrate species abundance, richness and diversity were calculated using the PC-ORD program for Windows version 4.14 (McCune & Mefford, 1999).

Species richness was taken as the total number of species recorded. The mean of the total count of all individuals for each order collected at each site was used as a measure of abundance. Abundance of the invertebrates was used to determine the seasonal fluctuation during the study periods. For each sampling technique, differences in invertebrate abundances and richness per plot between sites were compared using one-way analysis of variance (ANOVA) (Zar, 1999) using the SPSS for Windows 12 statistical software package (SPSS, Inc. 1996). For testing the significant difference in various invertebrates abundance and richness between different techniques we used the Two-way analysis of variance (ANOVA) (type 3 sums-of-squares).

RESULTS

Arthropod assemblages:

Overall abundances, a total of 79,183 arthropods were sampled from the study area by the three methods during the study periods. The majority from pitfall traps

Table (1): Description, age and codes for restored and forest control sites.

Site Code	Treatment	Age class (years)	Age group
M02J	Forest control	n.a.	Control
M04J	Forest control	n.a.	Control
SSB02	Forest control	n.a.	Control
SSB09	Forest control	n.a.	Control
EP 86	Restored mine pit (1986)	19	Old
NP 86	Restored mine pit (1986)	19	Old
WP90	Restored mine pit (1990)	15	Old
WP92	Restored mine pit (1992)	13	Mid
EP93	Restored mine pit (1993)	12	Mid
NP96	Restored mine pit (1996)	9	Mid
NP 98B	Restored mine pit (1998)	7	Young
NP 98A	Restored mine pit (1998)	7	Young
NSS01	Restored mine pit (2001)	4	Young
NSS02	Restored mine pit (2002)	3	Young

n.a., not applicable

(49516 individuals), 26,405 from suctions samples and 3,262 specimens from litter samples. Among 28 ordinal sorted taxa identified (excluding springtails), ants were the most abundant with 11,766 individuals (8,514 from pitfall traps), followed by Hemiptera with 6,923 and Diptera with 5,062 individuals.

Pitfall Traps

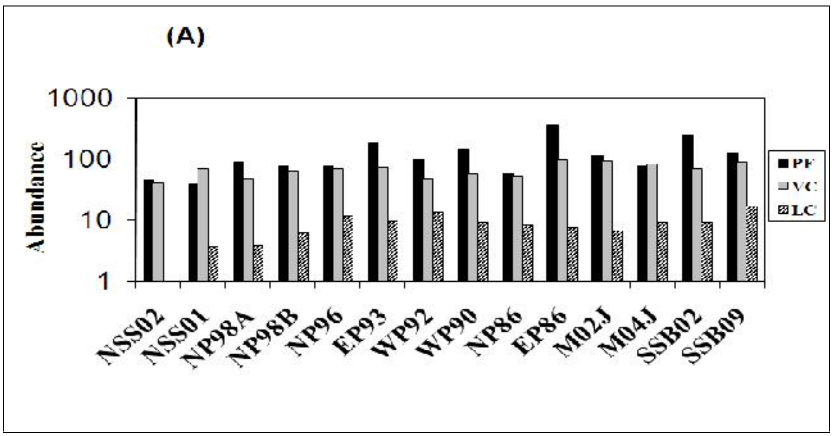
In pitfall traps, we collected 33,773 (68 %) individuals from restorations sites and 15,743 (32 %) individuals from the forest (control) sites of the most abundant taxa, 7396 (73 %) of ants were found at restoration sites and 1118 (27 %) were unique to forest sites; 2283 (59 %) of coleopteran were found at restoration sites and 622 (41 %) were found at forest sites; 39 % of springtails were found at restoration sites and 61 % were found at forest sites. (Fig. 1a and b). The highest diversity indices were 1.679 for Shannon (H) and 0.754 for Simpson (D) at restoration site NP98B. The restoration site NSS01 recorded the highest evenness (0.63) (Fig. 2 a).

Vacuuming samples

In vacuuming samples, we collected 26,405 individuals; 17.210 (65 %) individuals from restorations sites and 9,195 (35 %) individuals from forest sites. The most abundant groups were Hemiptera (6,125 specimens) of which 73 % were collected from restoration sites and only 27 % were found at forest sites. The second most abundant taxa was Diptera (5,060 individuals), 64 % at restoration sites and 36 % at forest sites. (Fig. 1a and b). The forest site M02J showed the highest value for Shannon, and Simpson indices and evenness (2.27, 0.875 and 0.758 respectively) fig. (2 B).

Litter Samples

A total of 3,262 individuals were collected, 2,095 (64%) from restoration sites and 1,176 (36 %) from forest sites.



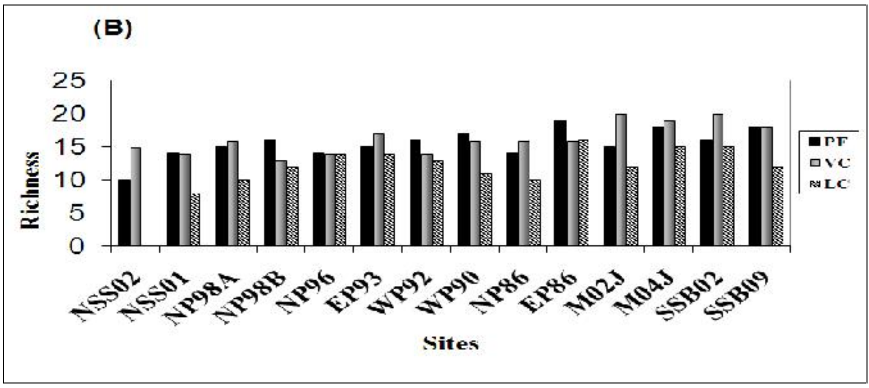


Figure (1): Invertebrate orders abundance (A) and richness (B) collected by; pitfall trap, (PF) vacuum sampling, (VC) and litter sampling (LC) for all sites together at the Worsley Alumina bauxite mine.

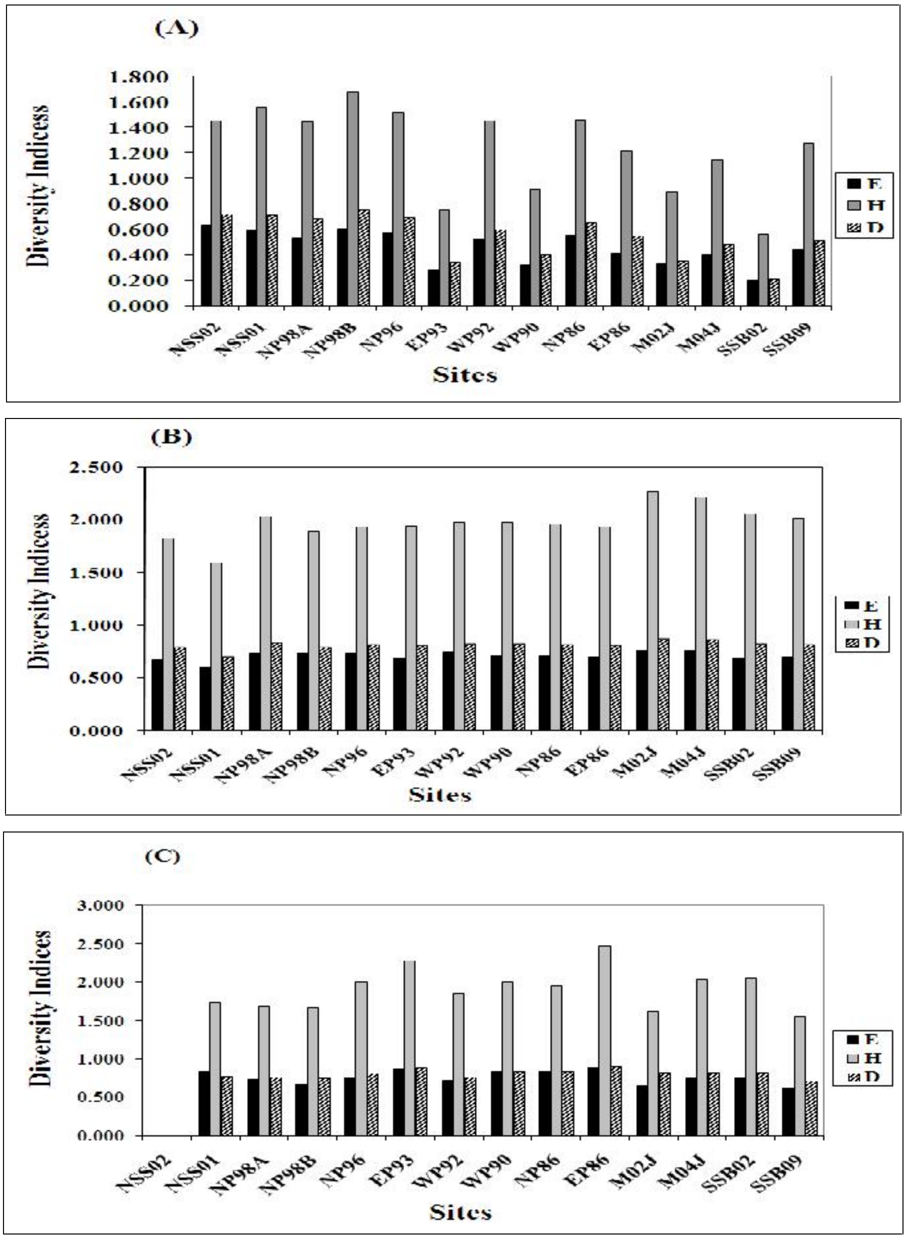


Figure (2): Shannon diversity index (H), Simpson index (D) and evenness (E) of invertebrate orders collected by pitfall trap, (A) vaccum sampling, (B) litter sampling (C) for all sites together at the Worsley Alumina bauxite mine

Collembola was the most dominant in litter collections (760, specimens); 72 % at restoration sites and 28 % at forest sites. Acarina was the second abundant taxa with 178 (33 %) individuals at restoration sites and 362 (67 %) found at forest sites (fig. 1a and b). In litter sampling the old restored site EP86 recorded the highest value for Shannon, Simpson indices and evenness 2.47, 0.9 and 0.89, respectively (Fig. 2 C).

One-way ANOVA's revealed no significant differences in total invertebrates richness between the restores sites and the forest sites for both pitfall trap method and litter collection. However, for vacuuming method there were significant differences (P< 0.01) between the restored sites and the forest sites.

For the invertebrates collected by both pitfall trap and vacuum method, there was no significant difference between the forest and restored sites in terms of taxa abundance; while in litter collection, the four young restored sites (NSS02, NSS02, NP 98 A and NP 98 B) differed significantly from both the mid restored sites and forest sites (P< 0.01). For invertebrates diversity indices Shannon and Simpson and evenness, the One-way ANOVA's showed significant differences only between the four young sites (NSS02, NSS02, NP 98 A and NP 98 B) and the forest sites in terms of Shannon diversity index only for the vacuum sampling. The litter collection showed non significant differences for both diversity indices and evenness between all the groups. Mean invertebrates richness and mean abundance differed significantly between the three different techniques at sites by the Two-way ANOVA without replication (df 2,13, F 10.19, 17,12 and P< 0.001, respectively) with the maximum number of invertebrates order collected by the vacuuming methods and the maximum mean abundance collected by pitfall trap methods.

Seasonal variation

Invertebrate's taxa showed seasonal maxima in specimen's numbers throughout the study period. For pitfall traps invertebrates taxa peaked in October 03 (spring), followed by April 03, then February 04 and finally July 03 (Winter season). For vacuuming, the month with the most species numbers was October 03 (spring), followed by April 04 and April 03 (autumn) then July 03 and February 04. For litter collection, October 03 (spring) was the best season for invertebrates collection, followed by July 03 (winter); then April 03, April 04, February 04 (summer) was the season with the lowest catch (fig. 3).

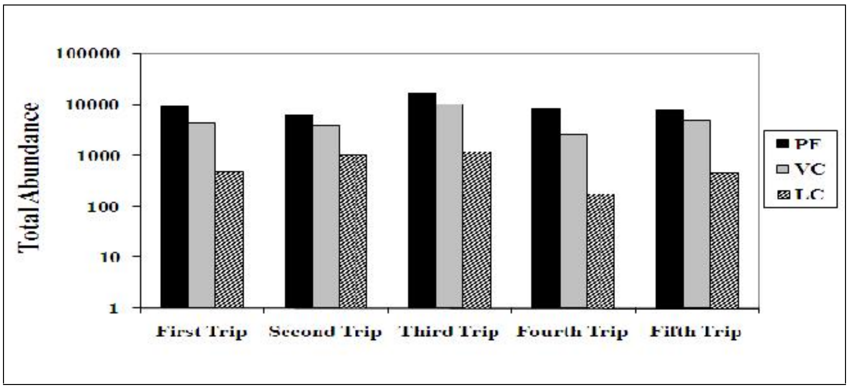


Figure (3): Invertebrates total abundance collected by pitfall trap samples (PF), vacuum samples (VC) and litter samples (LC) for all plots together during each of the five individual sample periods.

DISCUSSION

Results of this study foreground the relative utility of pitfall trapping, suction sampling and litter extraction methods for sampling small invertebrates. A pronounced divergence is found in the capture of the arthropod taxa among the three tested sampling methods, with the maximum number of invertebrate orders collected by the vacuuming method and the maximum mean abundance collected by pitfall trap method. These finding indicates that pitfall trapping is a useful standard arthropod collection method for ecological studies of ground - surface dwelling arthropods (South wood and Henderson, 2000; Work *et. al.*, 2002; Schmidt *et al.*, 2006) compared with the suction method and litter extraction method. Although pitfall trapping samples tend to include more ground-active searching species, however with appropriate adjustments would be the best method for comprehensive quantitative survey of ground-dwelling arthropods. Litter extraction is appropriate for collecting Coleoptera and litter Formicidae, for which it is a conventional method (Delabie *et. al.*, 2007; Underwood and Fisher, 2006) but not for ecological studies involving many arthropod groups. Regardless of the method tested, few individuals of the minor taxa (isopods and myriapods) were captured; and such an effect could be attributed to the low population densities of these taxa in the study area.

When cost and time constraints prescribe the confining of ground-dwelling arthropod sampling to one technique, the pitfall trap method appears ideal for quantitative estimates, and the suction method for qualitative estimates. Use of invertebrates in biological assessments must also take into account the practicality of sampling and identifying them, as well as the time taken to sample them, a feature that transcribes into expense of performing the survey. The following of a prescribed sampling protocol, such as the one used in this study (see Allen 1989 for further details) removes the need to design a sampling program each time a survey commences. Identification of the various invertebrate groups is dependent on availability of taxonomists and of the resources to reimburse them. Both can be problematic, although acquisition of the scientific names is not a prerequisite for the types of analyses performed here. Everything that was carried out in this study could have been done using morphospecies names, provided by parataxonomists rather than highly specialised taxonomists (Beattie and Oliver 1994), so cost and availability of taxonomists should not be regarded as a reason to not include invertebrates in environmental monitoring schedules.

Whichever approach is adopted, sampling must be performed at times of the year that maximise the number of species within the target taxonomic group. This differs depending on the group concerned. For instance, spring sampling maximises the species count of spiders, hemipterans, beetles and ants, while autumn and winter are better seasons for sampling Collembola. We therefore recommend that for comprehensive censusing of invertebrate species present in an area, sampling during each of the four seasons is highly desireable. Any reduction in periodicity of sampling will compromise the comprehensiveness of the survey. We conclude by stating that certain groups of invertebrates, notably hemipterans, beetles, ants and spiders are cost-effective to survey and potentially high in information content. Being among the most diverse members of the animal kingdom, their inclusion in restoration assessments can contribute to data on physical factors and plant and vertebrate assemblages in habitats. As well as strengthening the conclusions reached from measurements of the more commonly used variables, invertebrate data can provide an indication of the degree of re-establishment of ecosystem functioning. Although this study has been concerned with minesite restoration, it is likely that the conclusions drawn here will be relevant to other situations where biological assessments are carried out, such as of the conservation value of natural areas, of the degradation of exploited habitats or of the impact of pollution. Notable exceptions are the isopods and myriapodous groups, whose numbers were too low to reveal any interpretable tends in species richness or assemblage composition. We therefore do not recommend them for consideration at the assemblage level as ecological or biodiversity indicators. It may be that certain species within these groups prove to be useful environmental indicators, although we have no evidence as yet that this is the case.

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المفصليات كمؤشر حيوى: الطريقة المثلى والرتبة المثلى

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الملخـــص العربـــي

قامت هذه الدراسة ببحث إمكانية إستخدام المفصليات الأرضية والنباتية والموجودة فى أوراق الأشجار المتساقطة لرصد التقدم المحرز في إستعادة النظام البيئى. واستخدمت ثلاث تقنيات مختلفة لأخذ العينات من اللافقاريات المختلفة، المصايد الأرضية وتلك المتخصصة فى تجميع اللافقاريات من كلاً من النباتات و أوراق الأشجار المتساقطة. ووفرت هذه الدراسة البيانات اللازمة لتحديد الأسلوب الأكثر مناسبة لجمع العينات المختلفة من المفصليات وكذلك تحديد أيهما أفضل لإستخدامه كمؤشر حيوى. ولقد تم تجميع ٧٩١٨٣ عينة من المفصليات من منطقة الدراسة بإستخدام الطرق الثلاثة للتجميع. وقد تم تجميع معظم العينات بإستخدام المصايد الارضية ثم تلك الخاصة بكلاً من النباتات و أوراق الأشجار المتساقطة. وتوضح المقارنة بين طرق التجميع الثلاثة، بتخصص المصايد الارضية فى تجميع الكائنات الأرضية النشطة، مثل الخنافس والنمل والعناكب بينما تم تجميع اللافقاريات المرتبطة بالنباتات بواسطة الشفط و نجحت المصايد الخاصة بأوراق الأشجار المتساقطة في جمع فئات معينة من المفصليات المرتبطة الرطوبة والمناطق الظليلة، بما في ذلك يرقات الخنافس ومتماثلة الأرجل. وتعتبر المصايد الارضية مثالية فى التجميع الكمي، في حين أن طريقة الشفط مثالية فى التجميعات النوعية. ولقد نجحت مجموعات معينة من اللافقاريات، مثل الخنافس ونصفيات الجناح والنمل والعناكب وخاصة من حيث التكلفة للإستخدام فى عمليات المسح كمؤشر حيوى.