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# Ecotoxicological assessment of diffuse pollution using biomonitoring tool for sustainable land use in Thailand

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**Abstract**

As a developing country, Thailand has a significant issue with diffuse pollution of the soil ecosystem due to an indiscriminate use of agrichemicals and poorly regulated disposal of a wide variety of hazardous wastes. Practical risk assessment tools based on locally-occurring species are needed to assess the effects of diffuse pollutants on the soil ecosystem in Thailand because reliance on soil criteria developed for overseas conditions may provide inadequate protection. Native soil organisms in Thailand may be more or less sensitive to contaminants compared to overseas test species. This article described a biological indicator approach for ecological risk assessment of diffuse pollution in the soil ecosystem of Thailand from pesticide application with the aim of developing standardized protocols using native species and locally generated data to better evaluate the ecological risks of non-point source soil pollution. It was found that ecotoxicological assessment provided a better understanding of the ecological impacts that diffuse pollution induced on Thai environmental conditions. Thai soil biota species were more sensitive to soil contaminants than similar species overseas. Soil series also had an influence on the ecotoxicology of contaminants to soil biota. Collembolan, *Cyphoderus* sp., was demonstrated as a useful alternative test species to *Folsomia candida* (international test species) for terrestrial ecotoxicological testing of Thai soils. In addition, the soil biota activities such as soil respiration and earthworm avoidance including soil biodiversity and the litter bag decomposition technique are also good tools to assess the effects of diffuse pollution by pesticides on the soil ecosystem of Thailand.

**Key words**: ecotoxicological assessments; diffuse pollution; biomonitoring; soil ecosystem

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## Introduction

Rapid growth in the Thai economy and increasing human population have resulted in an increased demand for land, energy, agricultural products, raw materials and investment opportunities. Expansion of these activities has caused significant losses of biodiversity in Thailand. Hazardous waste disposal is a significant environmental issue in Thailand, related to the increased use of hazardous material and chemicals with the expansion of industrial and agricultural sectors (Pollution Control Department, 2006). Therefore, management of contaminated land has become an important issue for Thailand. Solid waste management is not an appropriate disposal technique for hazardous wastes in Thailand, because the potential release of hazardous chemicals into the soil ecosystem and transfer into the food chain threaten both human and ecological health. Moreover, the combined lack of practical risk management tools and relevant ecotoxicological data make ecological risk assessments a restricted activity in Thailand.

Although pesticide use and other contaminated land issues are significant impacts, the development of ecotoxicologically-based approaches to manage such issues is in its infancy and few studies regarding environmental distribution, toxic effects on soil organisms, or general impact on soil ecosystems have been undertaken (Iwai et al., 2007).

Therefore, the aim of this study was to undertake ecotoxicological assessments to develop diffuse pollution biomonitoring tools for sustainable land use management in Thailand. In particular, this study utilised a three-faceted approach that incorporates: (1) field based assessment of the effects of contamination from different land uses using a biological indicator approach; (2) semi-field based assessment of the effects of contaminants on biota in the soil ecosystem using a biological indicator approach; and (3) laboratory based ecotoxicological tests for contaminants with Thai soil biota and local soils. The data generated by these studies will provide useful information for the pollution control authorities to assist with adequate protection and management of soil quality in Thailand.

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## 1 Materials and methods

To assess and develop tools to measure the ecotoxicological effects of contaminants from diffuse pollution on the Thai soil ecosystems, different experimental sets of soils were subjected to various laboratory, semi-field and field experiments. The existing standardized test systems in term of test species, substrate and conditions were modified to reflect Thai soil ecosystems. The experiments of this study were divided into 3 parts: (1) field based assessment of the effects of contamination from different land uses using a biological indicator approach based on soil biodiversity index ($H'$) and soil functionality; (2) semi-field based assessment of the effects of contaminants on biota in the soil ecosystem using a biological indicator approach based on $H'$ and soil functionality; and (3) laboratory based ecotoxicological tests for contaminants with Thai soil biota and local soils.

### 1.1 Field sites

Nine field sites were selected to encompass a range of land uses, and associated levels of chemical contamination, in north-east Thailand within 50 km from the city of Khon Kaen, 450 km north-east from the capital Bangkok (Bell and Seng, 2004). The sub-region is part of the catchment of the Mekong River and represents 36.8% of Thailand's land area. The sub-region is characterized by the presence of high clay floodplain soil with broad lateritic features giving overall low organic carbon content in sediment (< 2%). There are three major soil types represented in this region, the Korat, Yasothon and Nampong soil series, all of which were represented within the 9 field sites. The annual rainfall and average temperature at Khon Kaen meteorological station (location: Lat 16°26′00″; Long 102°50′00″) are 1113 mm and 27.5°C, respectively. Average rainfall from May to September is 1000 mm, corresponding to the period of monsoonal activity, and may exceed 200 mm/month. The dry season is from October to April.

### 1.2 Soil sample collection and physico-chemical characterization

Soil samples were collected at the surface (0–15 cm) from the field sites by randomly collecting three samples per site during 2005–2006. Selected physical soil properties (soil type/series, soil texture (i.e., sand, silt, clay), bulk density, moisture (field capacity (FC), Permanent Wilting Point (PWP), Moisture content (MC) and porosity) and chemical soil properties (pH, electrical conductivity (EC), organic matter, cationic exchange capacity (CEC), total nitrogen, available phosphorus, exchangeable K, Na, Mg and Ca) were analyzed at the Khon Kaen University analytical laboratory (Abbott, 1985).

### 1.3 Ecotoxicological assessments

#### 1.3.1 Field study assessing soil biodiversity ($H'$) and functionality

The field experiment assessed the effects of soil contamination associated with the land uses of the field sites on soil biodiversity and functionality. Soil invertebrate diversity was assessed at each site using the following methods. For macroinvertebrate (i.e., visible) soil organisms, a hand sorting method was used to count the organisms found in an area of 1 $m^2$ to a depth of 5–10 cm. For microinvertebrate soil organisms, three of 1 kg soil samples from an area of 50 $cm^2$ to a depth of 5–10 cm were collected at each site (Anderson, 1994) and transported to the laboratory at Khon Kaen University. A Berlese-Tullgren apparatus was used for soil invertebrate extraction (Cornu et al., 1997). The extraction duration was 5 days and the extraction system was kept free from vibrations and other disturbance. The soil samples were carefully placed on the mesh above the funnel before inserting a bottle filled with 70% ethanol solution. The specimens were collected in 70% ethanol solution and then identified to the level of order and counted under a stereo microscope. The biodiversity of soil invertebrates (pooled macro- and microinvertebrate data) was determined by using the Shannon Wiener Diversity Index ($H'$) (Krebs, 1999), which was calculated using the following equation:

$$H' = \sum P_1 \log P_1 \quad (1)$$

where, $P_1 = n_1/N$, $n_1$ is the number of soil biota found in each order, and $N$ is the total number of soil biota found.

Soil functionality was assessed by evaluating litter decomposition at all sites using the litter bag technique (Blair and Crossley, 1988). Organic matter decomposition is a critical process in terrestrial ecosystems and responsible for the maintenance of soil fertility. The litter bag technique is designed to determine the effect of chemicals on the decomposition of organic matter in the field. Each nylon litter bag (10 cm × 15 cm) was filled with 10 g dried leaves of morning glory (*Ipomoea aquatica*; dried in oven at 50°C for 24 hr) and sealed using a sewing machine. At each site, 3 litter bags were buried within a soil depth of 5–10 cm, distributed randomly and left in the field for 2 months, after which they were collected and transported under ambient conditions to the laboratory for analysis. The litter bags were re-dried and re-weighed and the weight change of the organic matter was calculated as percent of initial dry weight at each sampling date.

#### 1.3.2 Semi-field study assessing $H'$ and soil functionality

The semi-field experiment assessed the effects of three chemicals of atrazine, carbofuran, and chlorpyrifos, on soil diversity and functionality, and was undertaken at the experimental station of the Faculty of Agriculture, Khon Kaen University. The experiment consisted of 8 treatments (i.e., 3 chemicals and a control and plots with plants (morning glory, *I. aquatica*) and without plants) with 3 replicates each. Each replicate plot covered an area of 5 m × 5 m. The chemical concentrations used for the experiment were based on the recommended application rate for farmers, as follows: Atrazine 90 WG (water dispersible granules), 300 g/80 L water/rai); carbofuran 3% GR (granules), 5 kg/rai; and chlorpyrifos 40% *W/V* (emulsifiable concentrate), 200 g/rai. The "rai" is Thai unit of land area, where 1 rai = 1600 $m^3$. The chemicals were sprayed over the plots by a manual insecticide sprayer. The same control plots were used for all three chemicals. Chemical residue levels were not measured.

For soil functionality analysis, 3 litter bags were distributed randomly over each of the plots, buried in the surface layer. The experiment lasted 2 months, after which soil biodiversity and soil functionality were measured for each treatment, as described above. The soil invertebrate density and $H'$, and the litter bag decomposition rates in the plots treated with chemicals were compared to the control plots.

### 1.3.3 Laboratory toxicity testing

**Test organisms**

Test organisms and culture conditions were developed for the soil laboratory toxicity testing. In these experiments, two different soil organisms were used: the springtail (or collembolan, *Cyphoderus* sp.) and woodlouse (*Porcellio laevis*). The test species were selected based on their wide distribution and common occurrence in several soil types in Thailand. The test species also met additional OECD-specified criteria for ecotoxicological test organisms (Garcia, 2004) in that they play an important ecological role in the soil ecosystem, live in direct contact with the substrate to ensure exposure to chemicals, and have high reproduction rates, thus permitting their maintenance in mass cultures.

**Toxicity test procedures**

The laboratory toxicity tests were used to assess the acute and chronic toxicity of chlorpyrifos, identified as a commonly used pesticide, on the two test species. The results of the acute tests were used to guide the further experiments with sub-lethal concentrations of pesticides. Two acute toxicity tests (one was preliminary or range-finding test and the other the actual test) were performed using each species. All tests were undertaken according to methods of the International Standardization Organization (ISO, 1999).

### 1.4 Data analysis

For the field and semi-field experiments, one-way analysis of variance (ANOVA) and two way ANOVA with Tukey's significance test ($\alpha = 0.05$; using Statistica V8) were used to assess the effects of the treatments (i.e., land use type for the field study, and pesticide used for the semi-field study) on the soil invertebrate density, $H'$ and decomposition rate. In addition, for the field study, regression analysis ($\alpha = 0.05$) was performed (using SPSS 12.0) to look for relationships between the biological responses and specific soil characteristics. For the laboratory toxicity tests, mortality data were subjected to probit analysis (Finney, 1971; using the program POLO; Robertson and Preisler, 1992) to determine $LC_{50}$ values and 95% confidence limits. Abbott's formula for the corrected control mortality was applied where necessary. The effects of chlorpyrifos on *Cyphoderus* sp. offspring production and adult *P. laevis* growth for the woodlouse were assessed using one-way ANOVA with Tukey's significance test (one tailed, $\alpha = 0.05$). Samples were normally distributed and the variances were homogeneous. The homogeneous variance was tested by Bartlett's Test.

## 2 Results and discussion

### 2.1 Soil characteristics and contaminant levels

Key physical and physico-chemical characteristics of the soils from the field sites showed clear differences in their contaminant history, while soil pH (2–6.8), moisture (1.5%–11%) and organic matter (0.07%–2.55%) also varied considerably between the sites. The physico-chemical characteristics of the three representative soil types (Table 1) showed that the key difference between the soils was the particle size distribution, with the Korat and Yasothon soils having a lower sand fraction and higher silt and clay fractions than the Nampong soil.

### 2.2 Field study assessing soil biodiversity ($H'$) and functionality

The results of the field study showed that the soil organism density, diversity and litter decomposition rate were statistically different between the different land uses (Table 2). The greatest biological activity (in terms of organism density, diversity and litter decomposition rate) was measured at the control (forest) site, followed by the organic horticulture site, both of which were known to be uncontaminated. Relatively poor biological activity was measured at all the other sites, which included one organic (uncontaminated) and a mix of conventional agricultural, as well as urban land use sites. The diversity of soil invertebrates was positively correlated ($P \leqslant 0.05$) with soil pH, moisture and organic matter (Fig. 1). This was also the case for soil organism density and litter decomposition rate ($P \leqslant 0.05$; regression data not shown). In contrast, there appeared to be a negative relationship between the

**Table 1** Physico-chemical characteristics of three representative natural soil types in Northeast of Thailand

| Soil properties | Korat soil series | Yasothon soil series | Nampong soil series |
|---|---|---|---|
| Chemical characteristics | | | |
| pH (1:2.5, soil:water, *W/V*) | 4.61 | 4.42 | 4.29 |
| EC (1:5, soil:water, *W/V*) | 0.10 | 0.13 | 0.19 |
| Organic matter (%) | 0.93 | 0.72 | 0.44 |
| Total nitrogen (%) | 0.06 | 0.03 | 0.01 |
| Available P (ppm) | 6.47 | 6.59 | 5.63 |
| CEC (cmol(+)/kg) | 5.75 | 5.23 | 3.96 |
| Exch. K (cmol(+)/kg) | 0.05 | 0.08 | 0.04 |
| Exch. Na (cmol(+)/kg) | 1.32 | 1.26 | 1.23 |
| Exch. Ca (cmol(+)/kg) | 0.65 | 0.58 | 0.53 |
| Exch. Mg (cmol(+)/kg) | 0.20 | 0.27 | 0.10 |
| Physical characteristics | | | |
| Sand (%) by weight | 71.61 | 74.42 | 92.50 |
| Silt (%) by weight | 19.83 | 17.78 | 5.60 |
| Clay (%) by weight | 8.56 | 7.79 | 1.90 |
| Texture class | Sandy loam | Sandy loam | Sand |
| FC (%) | 8.23 | 8.06 | 4.62 |
| PWP (%) | 3.39 | 3.04 | 2.83 |
| MC (%) | 0.51 | 0.48 | 0.29 |

EC: electrical conductivity; CEC: cation exchange capacity; Exch. K, Na, Ca and Mg: exchangeable K, Na, Ca and Mg; FC: field capacity; PWP: permanent wilting point; MC: moisture content.

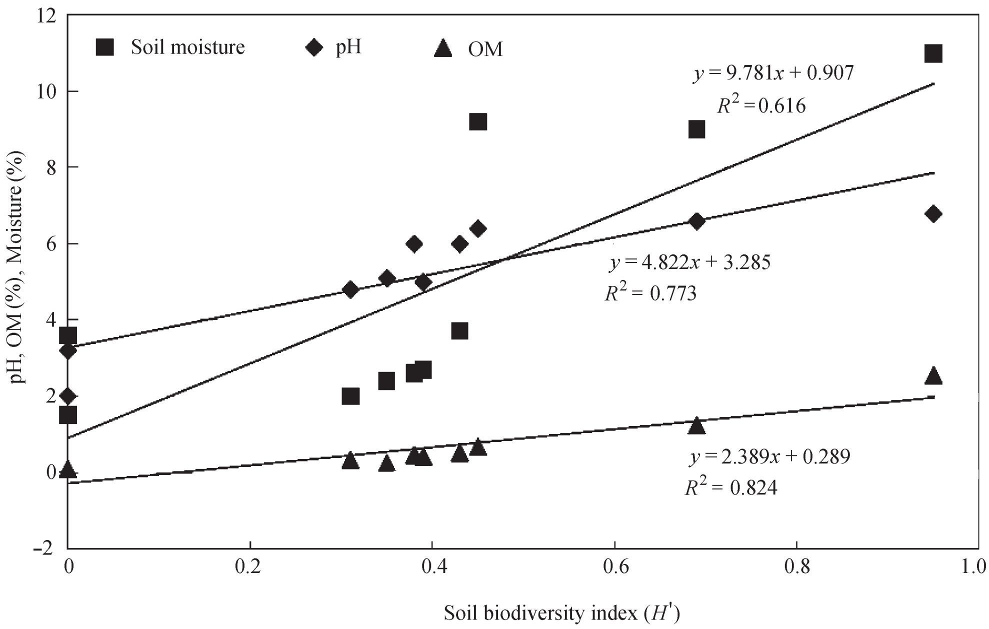


**Fig. 1** Relationship between the key soil characteristics of the different land uses and the soil biodiversity index ($H'$). All regression relationships were statistically significant ($P \leqslant 0.05$).

**Table 2** Soil invertebrate density, biodiversity ($H'$) and functionality (as litter decomposition rate) from field sites with different land-uses

| Type of Land Use | Soil invertebrates (number/m$^2$) | Biodiversity ($H'$) | Decomposition rate (%) |
|---|---|---|---|
| Forest | 68.06 ± 9.48 a | 0.95 ± 0.39 a | 98.67 ± 0.55 a |
| Cassava plot | 15.31 ± 1.47 d | 0.38 ± 0.12 b | 60.00 ± 4.58 b |
| Sugarcane plot (chemical) | 7.14 ± 0.52 f | 0.35 ± 0.20 b | 21.23 ± 5.88 e |
| Sugarcane plot (organic) | 18.66 ± 2.16 c | 0.45 ± 0.35 ab | 45.53 ± 5.75 d |
| Horticulture plot (chemical) | 11.02 ± 1.27 e | 0.39 ± 0.17 b | 48.60 ± 6.74 d |
| Horticulture plot (organic) | 54.44 ± 4.08 b | 0.69 ± 0.40 ab | 83.63 ± 5.08 c |
| Landfill and disposal site | 5.75 ± 0.41 g | 0.31 ± 0.11 b | 12.47 ± 4.23 e |
| Garage | 0 | 0 c | 0.7 ± 0.26 f |
| Petro-station | 0 | 0 c | 1.09 ± 0.31 f |

Data are expressed as mean ± SE ($n$ = 3). Values indicated by different letters (a–f) in the same column are significantly different ($P \leqslant 0.05$).

biological responses and the degree of soil contamination (Fig. 1).

### 2.3 Semi-field study assessing $H'$ and soil functionality

The results of the semi-field study are shown in Table 3. The greatest biological activity (in terms of organism density, diversity and litter decomposition rate) was measured in the control treatments. All three pesticides had a significant effect on soil organism density, reducing density by 60%–75% compared to the control plots with plants ($P \leqslant 0.05$). Only chlorpyrifos had a significant effect on litter decomposition rate ($P \leqslant 0.05$), while none of the pesticides had a significant effect on diversity ($P > 0.05$). Although not statistically significant, the pesticides did appear to result in slight to moderate 20%–40% reductions in diversity compared to the control plots. The presence of plant cover, in the form of *I. aquatica*, in the field plots had no significant effect on the toxicity of the pesticides ($P \leqslant 0.05$; based on 2-way ANOVA).

### 2.4 Laboratory toxicity testing

The $LC_{50}$ for chlorpyrifos to *Cyphoderus* sp. and *P. laevis* in the three soil types after 7 days and 14 days are shown in Table 4 and indicate marked increases in toxicity between day 7 and day 14 for both species. *Cyphoderus* sp. appeared to be more sensitive than *P. laevis* at both time, although this difference was less pronounced at day 14. The different soil types had some influence on chlorpyrifos toxicity, particularly for *Cyphoderus* sp., but this influence

**Table 3** Soil invertebrate density, biodiversity ($H'$) and functionality (as litter decomposition rate) in soils*

| Treatment | Soil invertebrates (number/m$^2$) | Biodiversity ($H'$) | Decomposition rate (%) |
|---|---|---|---|
| Control plot | 48.75 ± 24.88 ab | 0.55 ± 0.19 a | 83.53 ± 6.32 a |
| Control plot + plant | 57.50 ± 26.46 a | 0.55 ± 0.12 a | 84.70 ± 7.02 a |
| Atrazine | 23.25 ± 3.78 b | 0.41 ± 0.04 ab | 74.00 ± 7.00 a |
| Atrazine + plant | 21.00 ± 10.36 b | 0.41 ± 0.08 ab | 75.60 ± 10.02 a |
| Carbofuran | 13.50 ± 10.54 b | 0.31 ± 0.12 b | 72.60 ± 5.69 a |
| Carbofuran + plant | 13.25 ± 9.18 b | 0.31 ± 0.27 b | 73.80 ± 7.01 a |
| Chlorpyrifos | 15.25 ± 8.42 b | 0.33 ± 0.09 b | 65.00 ± 5.51 b |
| Chlorpyrifos + plant | 16.50 ± 15.42 b | 0.37 ± 0.21 b | 54.80 ± 5.51 b |

* The soil samples were collected from field plots at Khon Kaen University experimental station with or without plant (*Ipomoea aquatica*) cover, exposed to atrazine, carbofuran or chlorpyrifos for 30 days.
Data are expressed as mean ± SE ($n$ = 3). Values indicated by different letters (a–b) in the same column are significantly different ($P \leqslant 0.05$; based on one-way ANOVA; two-way ANOVA confirmed that the presence/absence of plants in the plots had no significant effect on any of the biological responses ($P \leqslant 0.05$).

**Table 4** Acute toxicity of chlorpyrifos to the springtail (*Cyphoderus* sp.) and woodlouse (*Porcellio laevis*) after exposure for 7 and 14 days

| Soil series | $LC_{50}$ (mg/kg dry soil) (95% confidence limits) | | | |
|---|---|---|---|---|
| | 7 days | | 14 days | |
| | *Cyphoderus* sp. | *P. laevis* | *Cyphoderus* sp. | *P. laevis* |
| Korat | 0.00475 (0.00119–0.01446) | 0.01150 (0.00425–0.02584) | 0.00029 (0.00006–0.00076) | 0.00080 (0.00009–0.00242) |
| Yasothon | 0.00055 (0.00014–0.00139) | 0.00976 (0.00418–0.01967) | 0.00013 (0.00002–0.00030) | 0.00091 (0.00017–0.00243) |
| Nampong | 0.00036 (0.00005–0.00110) | 0.00720 (0.00233–0.01686) | 0.00007 (0–0.00028) | 0.00079 (0.00008–0.00253) |

was also less pronounced at day 14 compared to day 7. At day 14, there was a maximum four-fold difference in the toxicity of chlorpyrifos to *Cyphoderus* sp. between the soil types (i.e., $LC_{50}$ range of 0.00007–0.00029 mg/kg dry soil), with toxicity being lowest and highest in the Korat and Nampong soils, respectively. The toxicity of chlorpyrifos between the soil types at day 14 to *P. laevis* was very similar (i.e., $LC_{50}$ range of 0.00079–0.00091 mg/kg dry soil).

### 2.5 Effectiveness of ecotoxicological assessments to monitor diffuse pollution

The results show how the biological indicator approach can be used to reveal the relationship between different land uses including contaminated land. The characteristics of the soils and the levels of pesticide and contaminant residues in each land use were comparable. The levels of pesticide and contaminant residues in the conventional farming soil were found to be higher for chemical agriculture when compared with the reference soil from organic farming. For other land uses, the contaminants such as some heavy metals were found but they are in low level. The degree of pollution was evaluated by interviewing land owners regarding kind and extent of pesticide use and viewing the management data for each particular land use to show pollution characteristics (contamination residues from agrochemical and diffuse sources of pollution). The results for the laboratory-based studies showed that diversity of soil invertebrates was statistically different between the different land uses as derived from interviewing the land owners. The diversity of soil invertebrates and the decomposition rate were positively correlated with habitat suitability but negatively correlated with the degree of pollution on soil. The results showed that diversity of soil invertebrates was statistically different between the different experimental plots.

## 3 Discussion and conclusions

Although contaminated land is assessed worldwide, each country, or at least region, has unique climatic and ecological characteristics that will determine the impact of soil contaminants. For this reason universal guidelines for soil contamination are often inappropriate to apply to local situations. Thus, to determine the impacts of contaminants, site-specific factors must be examined and comprehensive investigations of biotic and abiotic interactions are required. This study found that the broadening of the assessment approach for soil contamination from focusing just on chemical concentrations to also including ecotoxicological methods provided a better understanding of the ecological risks of contaminated sites that may be affected by diffuse pollution under Thailand's environmental conditions.

Field and semi-field assessments measuring community level structural and functional responses to contaminated soils were effective in detecting effects of different land uses and individual pesticides. In addition, two Thai soil species, the springtail, *Cyphoderus* sp. and woodlouse, *P. laevis*, were successfully used in laboratory-based toxicity assessments of the pesticide chlorpyrifos. These species are more ecologically relevant to Thai soils than international test species and also benefit from the fact that they are readily collectable, and easy to maintain and breed in culture. Moreover, they were found to be more sensitive to chlorpyrifos than the international test species, *F. candida*. However, additional endpoints, such as enzyme activity or avoidance, should be also further investigated. From this study, the growth of the woodlouse may not be a suitable endpoint for giving an early warning of exposure to pesticides. Overall, the results of this study suggested that the springtail, *Cyphoderus* sp., should be as a useful alternative test species to *F. candida* for terrestrial ecotoxicological testing in Thailand.

Chemical analysis alone is inadequate for comprehensively assessing the impact of diffuse pollution on soil biota. Integration of biological, physiological and chemical data can often yield significant advances in hazard assessment and act as a suitable baseline for making site-specific risk assessments.

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