# Earthworm: Potential Bioindicator for Monitoring Diffuse Pollution by Agrochemical Residues in Thailand.

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Chuleemas Boonthai Iwai<sup>1,2\*</sup>, Yupin Pratad<sup>1</sup>, Surasak Sereepong<sup>1</sup>, and Barry Noller<sup>3</sup>

# Abstract

The use of pesticides has long been a feature of conventional agricultural practice and their use has made it possible to increase crop yields and food production. However, the impact of many of these chemicals on ecosystem and human health has been of concern due to widespread impacts beyond crop protection. Economical monitoring procedure for assessing agrochemical non-point source pollution is needed. Earthworms are important components in agro- ecosystem. They play a significant role in soil functioning and soil fertility.

The purpose of this investigation was to assess the potential of earthworm as bioindicator of agrochemical residues in soil. The survey of earthworm population was conducted in the different land used management in Khon Kaen Province, Thailand during year 2006–2007. The physical soil properties (bulk density, moisture and porosity) and the chemical soil properties (pH, EC, organic matter, CEC, Total nitrogen, available phosphorus, Exchangeable K, Na, Mg and Ca) and pesticide residues were analyzed to correlate the results with the biological parameters. Ecotoxicological study of pesticide to earthworm has confirmed the results in the field. The results showed that the earthworm cast of *Pheretima* sp. per square meter in different land uses was significantly different (p<0.05) from each site and the earthworm casts in land used without using pesticide were higher than land use with using pesticide. The earthworm population was positively correlated with habitat suitability but negatively correlated with the degree of agrochemical residues in soil.

Keywords: Earthworm, Biomonitoring, Agrochemical

<sup>&</sup>lt;sup>1\*</sup> Department of Plant Sciences and Agricultural Resources, Land Resources and Environment Section, Faculty of Agriculture, Khon Kaen University, Khon Kaen 40002, Thailand

<sup>&</sup>lt;sup>2</sup>Research Centre for Environmental and Hazardous Substance Management, Khon Kaen University, Khon Kaen, 40002, Thailand

<sup>&</sup>lt;sup>3</sup>CMLR, University of Queensland, Brisbane, 4072 Australia

<sup>\*</sup>corresponding author, e-mail: chulee\_b@kku.ac.th

# Introduction

Dispersal of agrochemical residues into the environment has attracted a great deal of public interest over the past few decades. The widespread use and misuse of pesticides worldwide has resulted in their occurrence throughout the biosphere.

Contamination of land from indiscriminate use of agrochemicals has been a significant issue for Thailand. Therefore, the development of the assessment and monitoring techniques for appropriate local used is desirable, as is obtaining information for establishing the risks faced by the Thailand environment. Biomonitoring has been developed and used as a useful environmental management tool for monitoring diffuse pollution in many developed countries (Marinari, 2006; Rodri Guez-Castellanos and Sanchez-Hernandez, 2007)but its practice is very limited in Thailand.

Earthworms play a significant role in soil functioning and soil fertility. Their ecological and physiological features make them excellent indicators of soil pollution compared to other terrestrial invertebrates (Bunning, 2003) However, limited study is available for monitoring the ecological significance of the effects of diffuse pollution from agrochemical residues in Thailand soil ecosystem.

# Objective

The objective of the present study was: (i) to investigate the potential of earthworm as bioindicator of diffuse pollution from agrochemical residues in Thai soil ecosystem; and (ii) to propose a simple and cost effective methodology for the monitoring and assessment of soil pollution for Thailand and other developing country.

# **Material and Methods**

### Study area and sampling

Soils and earthworms were collected at a polluted field site and a reference site during 2006-2007. The study area was selected from paired farms, with one utilizing organic practices (as a reference site) and a second adjacent farm using conventional methods. (as a pollution site) Side-by-side comparisons of two study sites were investigated at the agro ecosystems in Northeast Thailand within 50 km from the city of Khon Kaen, 450 km NE from the capital Bangkok. The reference site was chosen because of its similarity to the polluted site with respect to the same pedological conditions and soil characteristics except agrochemical used, agricultural practices and management system. Soil samples were collected and used for analysis of pesticide residues (organochlorine, organophosphate, carbamate, and pyrethroid group) and measurement of soil physical and chemical properties. The survey of earthworm population was conducted at the different land use management sites. Ecotoxicological studies of pesticide to earthworm were conducted in the laboratory to confirm the field results.



Figure 1. Meteorological data of the study site during 2006–2007

# Table 1. Management practices employed on the conventionally and organically managed study sites

Specific annual inputs and practices used
Chemical ferlizer and Animal Manure
Organic matter input composted manureadded as fertilizer and mixed
in the upper layer of soil by disking.Green manuring of covercrops
residues

### Table 2. Pesticides used in organic farming

Botanical Pesticide	Scientific Name
Citronella Grass extract	Cymbopogon nardus Rendle
Fermented Plant Juice	
Heart-Leaved moonseed extract	Tinospora crispa (L.)
Neem	Azadirachta siamensis
Organic fertilizer	Animal Manure
Lotin	Derris elliptica Benth

# Table 3. Pesticide used in conventional farming

Common Name	Amount (Rate)
Detroid 35	2 Table Spoon/ 20L water
Furadan	33.3 Kg/hectar
chlorpyrifos,	
glyphosate	2 Table Spoon/ 20L water
Touchdown	
Grammoxone	2 Table Spoon/ 20L water
	Common Name Detroid 35 Furadan chlorpyrifos, glyphosate Touchdown Grammoxone

# Results

### Soil characteristics and pollutant levels

The characteristics of the soils were comparable. The levels of pesticide residues in the conventional farming soil were found higher compared with the reference soil from organic farming. Organochlorine (HCH, endosulfan and DDE) and carbamate (carbofuran and carbaryl) pesticide were found.

<b>Table 4.</b> The soil characteristics of the stud
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Study site				
Study site	Sand (%)	Silt (%)	Clay (%)	
Conventional Farming				
Long bean field with high used of pesticide	92.54	2.19	5.27	sand
Grass field with the history of pesticide used	87.76	6.42	5.82	sand
Galangal field with used of pesticide	92.52	2.49	4.99	sand
Organic Farming				
Long bean field with botanical pesticide	93.42	2.19	4.39	sand
Grass field with no used of pesticide	91.98	3.03	4.99	sand
Galangal field with no used of pesticide	91.45	3.86	4.69	sand

 Table 5.
 Pesticide residue in soil of the study sites

Pesticides Organochlorine	Organic Farming ( g/kg)	Conventional Farming ( g/kg)
Alpha-HCH	0.040	0.034
Beta-HCH	0.11	0.13
Gramma-HCH	0.16	0.075
Heptachlor	0.212	0.212
Dicofol	_	2.823
Heptachlor Epoxide	-	0.037
Alpha-Endosulfan	0.10	0.13
4,4-DDE	-	0.126
2,4-DDD	0.010	0.050
Beta-Endosulfate	_	0.274
2,4-DDT	0.039	0.123
Endosulfan sulfate	-	1.430
4,4-DDT	0.0615	1.100
Pesticides carbamate	Organic Farming ( g/kg)	Conventional Farming ( g/kg)
Carbofuran	0.00061	0.000921
Carbaryl	0.00253	0.005123

Study site	Soil Moisture	Bulk density	Porosity
	(%)	$(g/Cm^3)$	(%)
Conventional Farming			
Long bean field with high used of pesticide	15.42d	1.67a	36.83c
Grass field with the history of pesticide used	15.47d	1.54b	41.94b
Galangal field with used of pesticide	27.68b	1.12c	57.93a
Organic Farming			
Long bean field with botanical pesticide	16.12d	1.57b	40.78b
Grass field with no used of pesticide	19.18c	1.69a	36.22c
Galangal field with no used of pesticide	35.72a	1.24c	53.06a
F-test	**	**	**
C.V. (%)	4.21	2.58	3.90

 Table 6.
 The physical soil properties of the study sites

Data are expressed as mean se (n=10-20). Values indicated by different letters are significantly different (p < 0.05).

Table 7. 🗌	The chemical	soil	properties	of the	study sit	ies
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Study site	рН (1:2.5)	EC (1:5) (dS/m)	OM (%)	CEC (cmol(+) /kg)
Conventional Farming				
Long bean field with high used of pesticide	5.72 0.16	0.051 0.02b	1.41 0.35b	10.58 1.88b
Grass field with the history of pesticide used	5.48 0.31	0.028 0.01c	1.01 0.10de	10.80 1.95b
Galangal field with used of pesticide	5.78 0.19	0.061 0.04a	1.10 0.11cd	10.47 0.55b
Organic Farming				
Long bean field with botanical pesticide	5.26 0.10	0.045 0.02c	0.86 0.19e	10.81 0.90b
Grass field with no used of pesticide	5.39 0.34	0.025 0.01d	1.21 0.14bc	10.18 0.74b
Galangal field with no used of pesticide	5.58 0.04	0.060 0.01a	2.85 0.25a	15.51 2.41a
F-test	ns	**	**	**
C.V.(%)	3.93	2.27	5.59	6.29

Data are expressed as mean (n=10-20). Values indicated by different letters are significantly different  $(p \le 0.05)$ .

Study site	N (%)	P(ppm)	K(cmol(+)/kg)
Conventional Farming			
Long bean field with high used of pesticide	0.077 0.00b	34.04 9.64b	0.426 0.11a
Grass field with the history of pesticide used	0.062 0.01d	13.78 7.21e	0.220 0.16d
Galangal field with used of pesticide	0.069 0.01c	31.25 9.71c	0.210 0.13e
Organic Farming			
Long bean field with botanical pesticide	0.059 0.00d	26.80 16.06d	0.296 0.04b
Grass field with no used of pesticide	0.061 0.10d	6.33 1.05f	0.112 0.08e
Galangal field with no used of pesticide	0.107 0.01a	58.48 26.08a	0.248 0.05c
F-test	* *	* *	**
C.V. (%)	3.57	2.25	5.03

 Table 7.
 The chemical soil properties of the study sites (continued)

Data are expressed as mean  $\pm$  se (n=10-20).Values indicated by different letters are significantly different (p  $\leq 0.05$ ).

#### Earthworm population

The results showed that the earthworm cast of *Pheretima* sp. per square meter in different land uses was significantly different (p<0.05) (Table 8). The earthworm population was positively correlated with habitat suitability but negatively correlated with the degree of agrochemical residues in soil.

### **Ecotoxicology Study**

Ecotoxicological tests were conducted with Earthworm, *Pheretima posthuma* exposed to chlorpyrifos, carbofuran and glyphosate. The results showed that the 48-hour  $LC_{50}$  of chlorpyrifos, carbofuran and glyphosate on earthworm were 0.32, 89.17 and 12, 651.98 mg/ kg dry soil, respectively. Moreover, the pesticide avoidance behavior were found in earthworm exposed to chlorpyrifos, carbofuran and glyphosate between 0-50 % avoidance.

Table 8.The number of earthworm cast of<br/>Pheretima sp. per square meter in<br/>different land uses and agricultural<br/>practices.

Study site	Number of earthworm
Study site	cast per square meter
Conventional Farming	
Long bean field with high	$6.22\pm0.38\mathrm{b}$
used of pesticide	
Grass field with the history	8.22±1.58b
of pesticide used	
Galangal field with used	7.33±0.58b
of pesticide	
Organic Farming	
Long bean field with	14.44±3.83a
botanical pesticide	
Grass field with no used	12.55±1.35a
of pesticide	
Galangal field with no used	15.00±2.60a
of pesticide	
F-test	*
C.V. (%)	21.06

Data are expressed as mean±se (n=10-20). Values indicated by different letters are significantly different ( $p \le 0.05$ ).

Dostigido		(95 % Confider	nce Limits (CL)	Recommendation Rate
Testicide	$LC_{50}$ (mg/kg)	Lowest	Highest	(mg/kg soil dry wt.)
ChorPyrifos	0.32	3.463424E-21	2.02	0.41
CarboFuran	89.17	29.94	273.78	0.43
Glyphosate	12,651.98	9,611.98	14,314.86	973.43

Table 9. Acute Toxicity of selected pesticides on the earthworm at 48 hours

 Table 10.
 Avoidance behaviors of earthworm to chlorpyrifos.

Chlorpyrifos Concentrations	% avoid	% avoidance	
(mg/kg soil dry wt.)	Treated soil	Artificial soil	
0	0	0	
3	0	0	
12	10	20	
48	10	20	
192	30	30	
768	30	30	

 Table 11. Avoidance behaviors of earthworm to carbofuran

<b>Carbofuran Concentrations</b>	% avoidance	
(mg/kg soil dry wt.)	Treated soil	Artificial soil
0	0	0
0.01	30	0
0.1	20	10
1	50	20
10	50	30
100	50	30

Glyphosate Concentrations	% avoidance	
(mg/kg soil dry wt.)	Treated soil	Artificial soil
0	0	0
0.1	0	10
1	0	10
10	10	20
100	10	30
1,000	10	30

Table 12. Avoidar	ice behaviors of ear	thworm to glyphosate
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# Conclusion

The earthworm has a potential to be a soil bioindicator that can be used to assess and monitor soil pollution, soil health and ecosystem functioning under different land use systems and management practices. Chemical analysis of contaminated soil can be expensive and uninformative regarding environmental hazards associated with polluted soil. The use of biomonitoring to evaluate hazardous agrochemical contaminated sites provides a direct, inexpensive, and integrated estimate of the impact of the contaminant on the ecosystem. Therefore, biological assessment of contaminated land using earthworm bioindicator in support of chemical analysis should be possible and suitable for use in Thailand. However, further research and real data are needed. The cost benefit analysis and the comparison with other monitoring methodologies may provide valuable information for the implementation of the proposed methodology at a broader scale.

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