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Effect of organophosphorus pesticide pollution on soil animals

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Abstract: This paper studied the change in soil animal community in contaminated and irrigated area by organophosphorus pesticide waste water and its mechanism was discussed. The results showed that the species and quantities of soil animals decreased with organophosphorus pesticide pollution degree increasing. The species of soil animals decreased with average and rare population mainly decreasing, while the amount change was associated with that of the dominant population of Acarina, Collembola and Nematoda. Toxicity experiment demonstrated that the respiration intensity of soil animal was restrained obviously by pesticide pollution. After observation of SEM, the effect of pesticide pollution on the earthworm (*Pheretima robusta*) stomach intestinal mucosa damage has been observed, which showed the pesticide contamination may damage the earthworm's bodies. Ulcerous focus and perforation on stomach mucosa were usually seen. The stomach microvilli appeared atrophic and disordered especially in the bodies of these earthworms growing in seriously polluted soil, bad atrophic phenomena were seen not only on the stomach mucosa microvilli but also on the cilia of the intestinal mucosa which appeared swollen as a spheroid. Under TEM, RER and Golgi compound dilatation, chondrisome swell and ridge disappearance were observed on cells of stomach epithelium mucosa of *P. robusta* collected from heavy polluted area. By calculation, the safety concentration of earthworm in methamidophos pesticide is 0.2517 ml/L.

Key words: pesticide pollution; soil animal; respiration intensity; pathological changes; LC_{50}

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Introduction

The study of modern soil zoology has entered into a stage of biological productivity and the relation between human and environment. Pesticide is a chemical environmental pollutant and it was discovered that sensitive organism species decreased and anti-polluted species increased in farmland ecosystem due to pesticide overdone. Because soil animal is a kind of sensitive organism indicating soil pollution, many countries attached much importance to the study of utilizing soil animal monitoring soil pollution (Jeffrey, 1992; Bruce, 1992; Stroo, 1992; Eljsackers, 1983; Dunger, 1992). A series of meetings of international environmental organism monitoring were held: the 10th, the 11th, the 12th international soil animal meetings; the 5th, the 6th international ecology conference and the 4th, the 5th earthworm ecology symposium. All these meetings made it a special subject to discuss and concluded that soil pollution affected the soil animals. Briggle's experiments demonstrated that pesticide pollution has an effect on the metabolism of soil animal and quantities and hatch ability of eggs (Briggle, 1992). Edwards thought that pesticide pollution can influence structure and function of natural and agricultural ecosystem (Edwards, 1992), Lee held it can be regarded as a monitoring index of environment pollution degree using earthworm monitoring environment pollution and analysing content of heavy metal and pesticide in earthworm's tissues (Lee, 1992). Some investigation showed that overdone pesticide in farmland can lead 90% earthworms dead. As a result, the study of soil animal community structure and ecotoxicity in polluted area can be used to monitor soil pollution and appraise soil quality (Zhang, 1991; Wang, 1990; Chen, 1991), which is a field worthy of further research.

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1 Material and method

1.1 Environmental survey in pesticide polluted area and research method

Hunan pesticide factory had produced methamidophos, dichlorvos, parathionmethyl, etc. mainly and ejected a great deal of waste water and poisonous gas, which polluted surrounding soil, water and atmosphere during the course of production. The work set up 5 areas along the sewage irrigation ditches, which are 0.1 km (1 area), 0.5 km (2 area), 1 km (3 area), 2 km (4 area) and 4 km (5 area) away from pollution source. In each area we selected plots randomly, dug soil profile, laid and sampled according to 0—5 cm, 5—10 cm, 10—15 cm and 15—20 cm with soil circle-knife, then soil animals were severed indoors with *Tullgran appartus* and *Baermann appartus*, and the big soil animal were collected by hand. The soil animals samples are identified to family or genus generally (Yin, 1992; Junichi, 1973).

1.2 Determining of soil animal respiration intensity

Respiration intensity of big soil animal-earthworm was determined to test biological effect of pesticide pollution. In forest soil they were not affected by pollution, ripe *Pheretima guillelmi* of the same species were collected to be treated acutely by pesticide with method of contact filter paper. 6 different concentrations of methamidophos solution: 20 μl/L, 100 μl/L, 310 μl/L, 1000 μl/L, 3150 μl/L and 10000 μl/L are adopted, each treatment group has ten earthworms whose weights were determined by microscale and a repeat after 1, 2, 3, 4, 5, 6 h the change in earthworm respiration intensity was determined with skw-2 type respiration determining apparatus.

1.3 Preparation of specimen used for SEM

After a series of community investigations on pesticide polluted area, *Bimastus paevus* and *Pheretima robusta* were defined of dominant species, but the latter is bigger, as a result it was regarded as the object of our study. When collected, *Pheretima robusta* was cultivated hungrily in laboratory for three days, then dissected, washed the remains of digestive path using phosphate buffer solution (pH=7.3), and picked stomach-intestinal path behind the gizzard for specimen in the study. The specimen were double fixed with C_5H_3O-O_sO_4, dehydrated with series of alcohol and dried at critical point, jet-plated with platinum and gold in vacuum, then observed and photographed with a/s-450 Hitachi SEM.

1.4 Preparation of specimen used for TEM

Stomach-intestinal path of earthworm was washed many times with PBS (pH = 7.35), double-fixed with glutaraldehyde-osmic acid, dehydrated with a series of acetone and embodied with Epon 812. The specimen were cut with an ultramicrotone into 50—70 nm and double-dyed with uranium vranyl acetate and lead citrate. AH-600 Hitachi TEM was used for observation and photography.

1.5 Determining of soil physical and chemical property

Item and method: soil pH value (pH meter); soil organic substance ($K_2Cr_2O_7$ method), total exchangeable bases (EDTA magnesium salt); cation exchange capacity (EDTA ammoioumsalt); hydrolyzable N (diffusion and absorption method); total N ($HClO_4$-H_2SO_4 nitration); total P_2O_5 ($HClO_4$-molybdenum-Stibium sulfate colorimetry); total K (flame-photometry); soil bulk (circle-knife method); soil mechanical composition (densitometer method).

1.6 Determining of earthworm's LC_{50}

Earthworms (the species are *Pheretima californica* and *Pheretima robusta*) collected were divided into four groups, each of which has 23 individuals (about 17g). According to equal natural logarithm interval of 0.6, methamidophos pesticide solutions have four concentrations: 3.2 ml/L, 5.6ml/L, 10 ml/L and 18.2 ml/L. In every culture var, 100g soil and earthworm grouped were put into, then methamidophos pesticide solution (10 ml) of different concentration was spread, finally covered it with gauze, keep good windy condition, observe and record activity of earthworm.

2 Result and discussion

2.1 Effect of pesticide pollution on soil animal community structure

2.1.1 Effect of pesticide pollution on soil animal composition of species and quantities

Waste water with high concentration pesticide is the main water source of agricultural irrigation in the locality, which leads soil environment worsen and soil animal species decreasing with long-term affecting of waste water irrigation. After systemly investigations in a fixed plot on soil animal in waste water irrigation area, 7222 soil animal samples were collected, which belonged to 4 phylums, 12 classes, 68 kinds. Soil animal distribution has a close relation with polluted degree in polluted area. In heavy polluted 1, 2 area, soil animals have 21 and 22 kinds, covering 4.98% and 8.94% of the total quantity, respectively. In middle and light polluted 3, 4 area, soil animals have 35 and 47 kinds, covering 10.34% and 27.32% of the total; in 5 area far away from pollution source, 58 kinds, covers 48.1% of the total (Table 1), which is consistent with report that species and quantities of soil animal decreased with polluted degree increasing (Wang, 1992). Diversity index of species is an important one that judges the structure level and ecology feature of community. The calculating result showed, diversity index of soil animal is the highest in control area: 4.4404; in 4 area: 3.8242, in heavy polluted 1 and 2 areas: 3.3564 and 2.9448, respectively.

Table 1 Indexes of the soil animal community in pesticide polluted area

Index	No. of plots				
	1	2	3	4	5
Group number	21	22	35	47	58
Total number of individual	360	646	747	1973	3496
Diversity index	3.3564	2.9448	3.3293	3.8248	4.4404

2.1.2 Effect of pesticide pollution on dominant species of soil animal

In polluted area, dominant species (> 10% of the total) are Acarina, Collembola and Nematoda, whose numbers of individual are 34.58%, 29.36% and 16.96% of the total (Table 2). These wide distributed species can stand pollution, and are main part of soil animal community of polluted area, especially in heavy polluted areas, three species covered 95.54% of the total and had more high superiority, while in control area, they only covered 70.46% of the total and the superiority decreased, which showed that change in the soil animal quantity is main due to that of quantity of dominant species. But, of the 26 kinds of animals forming dominant species, kinds which distributed widely and are in abundance are *Oppiidae*, *Epilohmanniidae*, *Acaridae*, *Galumnidae* of *Acarina*; Hypogastruridae, Isotomidae, Onychiuridae of Collembodla and Plectidae, Rhobditidae, Dorylaimidae of Nematoda, whose whole quantity covers 87.64% of the total and are main parts of dominant species, while other 16 kinds of animals are sensitive to pesticide pollution, some kinds becoming average species or rare species, even death. For example, in heavy polluted area (1 area), 13 species are discovered, but light polluted area (4 area) have 23 species.

Table 2 Soil animal species and quantity distribution of the dominant population in the pesticide polluted area

Animal species	No. of plots				
	1	2	3	4	5
Nematoda					
Dorylaimidae	13	36	32	95	127
Mononchidae	4			3	1
Tylenuchidae	5	4	7	34	56
Heteroderidae				1	
Rhobditidae	8	25	45	60	156
Plectidae	8	19	21	70	265
Camacolaimidae		1	1		5
Mermithidae	6			23	1
Diplogasteridae		1	3	1	25
Steinernematidae	4	9	7	9	31
Oxyuroidae					2
Acarina					
Oppiidae	64	13	55	276	365
Haplozetidae			37	110	74
Gehypochthoniidae	46	45			
Epilohmanniidae			28	561	239
Phthiracaridae		9		18	64
Galumnidae			8	37	95
Acaridae	37	19	18	9	129
Bdelloidae				37	74
Eupodoidae				19	10
Collembodla					
Hypogastruridae	92	249	341	315	556
Onychiuridae	39	154		43	33
Sminthuride	6			34	11
Tomoceridae				6	28
Neanuridae		23	6	7	24
Isotomidae			21	45	87

2.1.3 Effect of pesticide pollution on average species and rare species of soil animal

The result of investigation showed that in pesticide polluted area, the decreasing of soil animal quantity is because of decreasing or disappearance of average and rare species: of the 42 kinds soil animal, *Achaeta*, *Lycosidae* of *Arachnida*, *Erigoninae* are average species (>10% of the total), which covered 11.21% of the total animals, other 39 kinds are rare species (<10% of the total), which only is 7.89% of the total quantity, which indicated many kinds of these species are especially sensitive to pesticide pollution, in heavy polluted area are difficult to exist and reproduce (You, 1994). Fox example: *Oligochaeta Opisthopora*, *Isopada*, *Chilopoda*, *Diplopoda* and *Diplura*, *Orthoptera*, *Psocoptera*, *Homoptera*, *Hemiptera*, *Thysanoptera*, *Lepidoptera* and *Dermaptera* etc. are hardly discovered in heavy polluted area, but existed everywhere in light polluted area and control area (Table 3).

Table 3 Composition and distribution of average population and rare population in the pesticide polluted area

	Animal species	No. of plots				
		1	2	3	4	5
Turbellaria	Tricadiada				6	
Oligochaeta	Achaeta	9	6	21	16	371
	Hemienchytraeus	2	3	1	1	3
	Bimastus parvus		8	21		29
	Pheretima robusta		11	3	11	
	Metaphire californica				9	
	Amynthas guillelmi				10	
	Drawida japonica			6		
	Amynthas morrisi		12			
Gastropoda	Limacidae		1		1	
Cruslacea	Cyprididae				9	
Arachnida	Lycosidae	4	4	15	35	209
	Tetragnathidae	2		4		19
	Erigoninae	5	10	3	2	99
	Linyphiinae			6	18	
	Theridiidae	5			25	
Malacostrace	Porcelliondae		4	27	30	
Chilopoda	Geophilidae			4	3	
	Scolopendridae				4	
	Lithobiidae	3		2	15	18
	Scutigeromorpha				12	
Diplopoda	Sphaerotheriida	1	8	2		
Symphyla	Scutigerellidae	1		4	6	19
Pauropoda	Pauropodidae			1	58	
Insecta	Protura				1	
	Campodeidae		1		3	2
	Japygidae		5			
	Grylliodae		2	8	4	
	Acridiodae			1		
	Trogiidae		1		2	
	Carabidae	2	2	3	5	
	Staphylinidae		1	2	35	
	Tenebrionodae			2	6	
	Elateridae	1				
	Bibionidea				1	
	Dimocenia austrina				1	
	Labiduridae				4	
	Homoptera		1	2	1	
	Hemiptera			1		
	Thysanoptera		1			
	Hymenoptera	1		4	7	11
	Lepidoptera			1		
	Total number	28	39	117	160	1038
	Number of species	9	8	21	23	34

2.1.4 Effect of change of soil physical and chemical property on soil animal

The result of analysis of soil physical and chemical indexes in the Yijiawang waste water irrigating area showed the content of organic substance is 7.64% in heavy polluted area, more than that in other areas. The content of N, P, K and cation exchange capacity are equal to other areas (Table 4), while the density of soil animal is only $28571/m^2$, much lower than that in other areas. We can say that the decreasing of soil animal in pesticide-polluted area is not because of acrid soil. Generally, the abundance of soil animal is an important index of soil fertility (Ulrik, 1992), while it is not in pesticide-polluted area. Long-term effect of waste water irrigation had made pesticide accumulating, soil environment worsened and ecobalance destroied. In heavy polluted area, pH value of soil is 4.45 and the soil soured; soil bulk density increased; total porosity is 49.71%, lower than that in other areas, which limited existence and reproducing of soil animal.

Table 4 The soil properties of physics, chemistry and soil animal density in pesticide polluted area

No. of plots	pH (H_2O)	O.S., %	CEC, μl/100g	Total N, %	Total P, %	Total K, %	Bulk density, g/cm^3	Porosity, %	Soil animal Species	Soil animal Density
1	4.45	7.64	13.15	0.11	0.22	0.26	1.2	49.71	21	28571
2	5.04	5.94	12.58	0.06	0.08	2.41	1.27	51.28	22	51269
3	5.39	6.55	12.99	0.16	0.30	2.39	1.07	59.28	35	59285
4	5.84	6.11	16.87	0.11	0.21	2.48	1.27	51.26	47	156887
5	7.02	6.49	17.11	0.18	0.35	2.90	1.05	59.96	59	277460

2.2 Effect of pesticide pollution on toxicity to soil animal

2.2.1 Effect of pesticide pollution on respiration intensity of soil animal

Since 1980, ecotoxicology research has gained very significant achievements (Debruim, 1976; Senapati, 1992; Hanmi, 1992; Stenersen, 1992; Wells, 1979) in exploring poisonous substance toxicity to ecosystem, organism community and species, and is very important to understand poisonous substance migration, transformation and end; define and forecast ecotoxicity; establish threshold standard of ecotoxicity; promote safe appraisal of chemical substance. Big soil animal—earthworm was regarded as materials for poisoning tests, because earthworm is a kind of annelid respirating with surface and an ideal respiration toxicity study object. The primary result indicated the higher of pesticide concentration and the longer of contact time, the weaker of earthworm's respiration intensity (Table 5), under the same concentration (1000 μl/L) and different contact time, earthworm's respiration intensity lowered with contact time prolonging, after 12h contact, most of earthworms can not live. In the meantime, the change in earthworm's respiration intensity is related with growth age and individual size. The experiment process showed the larva of earthworm died after 1h at concentration of 1000 μl/L, but growed earthworm can recover and big earthworm have more capacity of anti-poison. As a result, respiration toxicity research can be thought of as one of bases pesticide pollution monitoring and guiding pesticide using in farmland.

The formula of respiration intensity is as follows (Beijing Agricultural College, 1984):

$$Q = h \cdot k/m \cdot t = \frac{\frac{V_g \cdot 273}{T'} + V_t \cdot a}{P_0} \cdot h/m \cdot t,$$

where, h is the pressure gauge manometer reading; k the constant of reaction bottle; V_g the gas volume of reaction bottle; V_t the liquid bulk of reaction bottle; T' the absolute temperature of water-bath; P_0 the standard atmospheric pressure; a the gas solubility of reaction bottle under one standard atmospheric pressure at T'.

Table 5 Respiration intensity of earthworms contacting different concentration of methamidophos solution after the same hours

Concentration,	Fresh w.,	Volume,	Respiration intensity, μl/(g·h)					
μl/L	mg	ml	10'	20'	30'	40'	50'	60'
0	744.5	0.62	296.50	295.50	292.72	295.79	292.72	293.27
20	538.0	0.54	272.94	262.18	284.95	286.95	286.95	272.89
100	362.0	0.32	228.92	198.03	185.33	199.81	198.81	262.89
310	730.4	0.70	190.50	192.50	184.65	169.82	169.82	169.30
1000	514.2	0.50	158.58	159.13	148.75	153.83	153.83	158.23
3150	605.8	0.55	143.32	132.95	138.70	139.81	139.81	140.89
10000	744.5	0.74	11.26	11.23	10.25	-	-	-

2.2.2 SEM observation for stomach-intestinal mucosa injured by pesticide pollution

Through SEM observation of stomach-intestinal mucosa tissue of *Pheretima robusta*, it was found that surface structure is distinct, the stomach is made up of the same type epithelial cells like semi-spherical pillars which are arranged as a cluster. These clusters are divided into many small stomach areas. The sunken part between stomach areas is named gastric dent which is the exit of secretion of stomach gland cells. There are some short and dense microvillis on the dissociated end of stomach mucous cells, which maybe play a preliminary absorption role in digesting food. Intestinal mucosa is made up of folds which can increase area of intestinal cells, on which there were six-corner or five corner absorption cells and cilia cells. Free surface of absorption cells have short and dense microvilli; free end of cilia cells grows many long and thick cilias. These observations are in accord with those of digestive tract of *Pheretima califorica* using SEM (Guo, 1994).

In area II, under SEM microvillis on absorption cells of *Pheretima robusta* stomach intestinal mucosa, cells appeared atrophic or hard (Fig. 1), cilia cells on intestinal mucosa appear atrophic, and the cilias are disordered and fused (Fig. 2). Though the area is from polluted source (2 km), the remained toxin can make inner tissue-cell of earthworms change in structure and shape completely. The abnormal shape of microvilli and cilia reflects abnormal shape and functional obstruction of mirofibre and microtube within cell. In area I, under SEM of stomach-intestinal mucosa of *Pheretima robusta*, microvilli appear atrophic and hard too, cilia cells seem ulcerous, on which cilias are sparsed and disordered. The tip of atrophic cilia merges and swells as spherical (Fig. 3). Furthermore ulcerous focus and perforation on cilia cell are usually seen (Fig. 4). These ultrastructure alterations illustrated that the pesticide pollution is quite serious in area I. Soil accumulates residual pesticide for long time, and earthworms feed on organic substance in the mud. After food enters tomach-intestinal tract, it endangers surface structure of mucosa cells and makes surface turn pathological change. These results are very similar to the reported on stomach-intestinal mucosa cells damage of *Pheretima califonica* in heavy metal polluted area by authors (Guo, 1994).

2.2.3 Observation for TEM of effect of pesticide pollution on stomach intestinal mucosa cells

Under TEM, no abnormal change of the ultrastructure of stomach-intestinal mucosa. However in area II, mitochondrian in stomach mucosa of earthworm appeared condense and

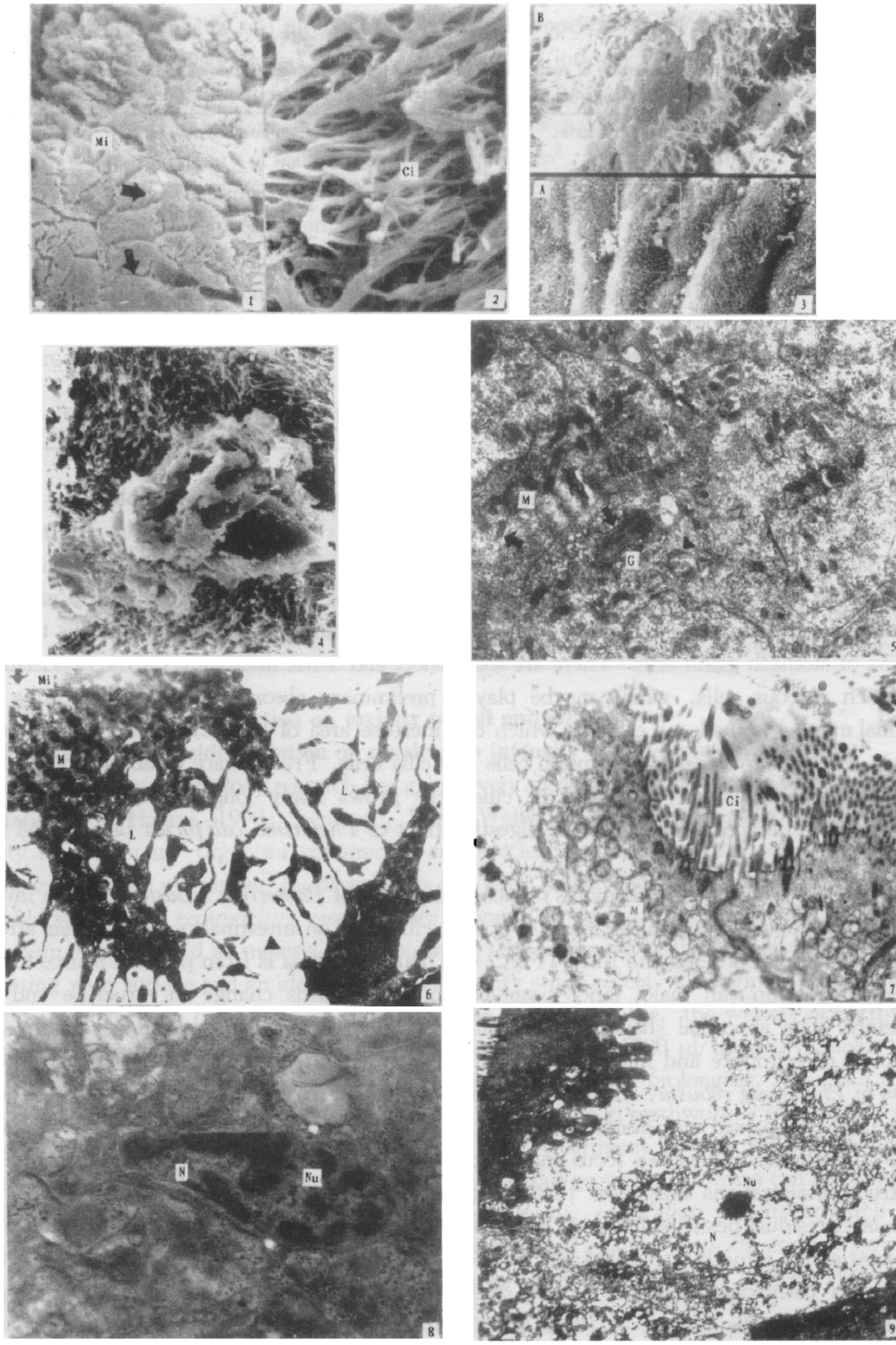

Fig. 1 The stomach mucosa cell of earthworm appeared atrophic in medial polluted area (⬅), 2000×

Fig. 2 The intestinal mucosa cell of earthworm appeared fused in medial polluted area, 7000×

Fig. 3 In heavy polluted area, epithelium of earthworm appeared ulcerous focus (B), magnification of lower micrograph (A): Cilla appeared strophic and disorder; the tip of cilia swellen to ball

A: 1000× B:2500×

Fig.4 The intestinal mucosa epithelium of earthworm appear ulcerous focus and perforated in heavy polluted area, 2000×

Fig.5 In the cell of stomach mucosa of earthworm growing in medial polluted area, Golgi apparatus swelled (⬅), mitochondria shrank (⬅), myelinated structure(▲), 5000×

Note: M (mitochondrion); G (golgi compound); Mi (microvillus); Ci (cilium)

Fig.6 In the cell of intestinal mucosa of earthworm of earthworm, growing in medial polluted area. The microveill appeared atrophic(⬅), lipid droplets fused to form bigger one(▲), mitochondria shrank(⬅)

Fig.7 In the cell of intestinal mucosa of earthworm growing in heavy polluted area, microvilli and cilia appeared swollen of atrophic; mitochondria swelled and vacuolated, cristae of Midisappear, 12000×

Fig.8 In the cell of stomach mucosa of earthworm, growing in heavy polluted area, perinuclear space enlarged, chromatin contributed near inner nuclear membrane, nucleolus broke, 8000×

Fig.9 In the cell of intestinal mucosa of earthworm, growing in heavy polluted area, mitochondria broke, endoplasmic reticulum enlarged, nuclear envelope broke, chromatin decreased, complasm vacuolated, 5000×

Note: N(nuclear); Nu(nucleoli); M(mitochondrion); Mi(microvillus); L(lipidoses); Ci (Cilium).

electron density in matrix increased obviously. Golgi compound and RER swell. Ribosomes come off RER, and polyribosome appeared depolymerized. Glycogen granules in cytoplasm increased greatly (Fig.5). Those showed remained poison could make mitochondrial substancial metabolization of epithelial cells disordered. Microvillis of cells also disappeared or atrophied and mitochondrions on cytoplasm became condense, where the demarcation line of lipid-drop vanished, and they fused together (Fig.6). Maybe it was metabolic block of RER suffered from pesticide toxicity which was related to excessive lipogenesis of absorption epithelial cells, while organochlorine pesticides were lipophilic which made the remained pesticides accumulate in lipid-drops by absorption cells. Pesticides are the main factor of fatty substance fusing together.

Under microscope, it can be seen that microvilli and cilia were disordered, short and swelled in the intestinal mucosa cells of *Pheretima robusta* from seriously polluted soil. Most of the mitochondrion in cytoplasm swelled, and its cristae disappeared and became vacuity, or ever lysis (Fig.7). Mitochondrion played a critical role in oxidative phosphorylation and electron transfer, and almost participated in all of metabolizaton in cytoplasm. Since remained pesticides could change the osmotic pressure of cell, make water and solute infiltrate into cell, enter into mitochondrion, lead mitochondrion swelling, its cristae disappearing, even becoming a large vacate without any visible structures (Xu, 1986). At the same time, nucleis of earthworm also became abnormal in intestinal mucosa cell in area I, which mainly manifested local expansion of perinuclear space between outer and inner nuclear membrane; chromatin appeared irregular block and gathered around the inner nuclear membrane (Fig.8). It can even be seen that the chromatin appeared sparse, the nuclear membrane broke, and the chromation flew out (Fig.9). These further showed the remained pesticides could make nuclear substances damaged, which was the morphological mark of cell necrosis.

2.2.4 LC_{50} of earthworm

LC_{50} is an important toxicity index and can reflex the poisoning situation. According to the simulating test, the death rates at different concentrations in different time were obtained (Table 6). On the base of death rates, using straight line method, we can know that the LC_{50} are 13.7 ml/L, 5.4 ml/L, 3.9 ml/L on 24h, 48h, 72h, respectively. The formula of safety concentration (Yang, 1990) that earthworms live in environment can be expressed as follows:

$$\text{Safety concentration} = \frac{48\text{TLM} \times 0.3}{(24\text{TLM}/48\text{TLM})} (\text{mg/L, ml/L})$$

In the formula, TLM is LC_{50}.

Table 6 Methamidophos pesticide concentration and the death rate of earthworm

C, ml/L	Death rate of earthworm in different time, h				
	24	48	72	96	120
3.2	0	6	23	91	100
5.6	0	51	94	100	
10	14	100			
18.2	100				

In the light of formula of safety concentration and LC_{50} calculated, safety concentration can be calculated, which is 0.2517 ml/L. The result showed that earthworm is extreme sensitive to methamidophos pesticide and can be regarded as an important indicator animal.

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