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Bioconcentration Effects of Heavy Metal Pollution in Soil on the Mucosa Epithelia Cell Ultrastructure Injuring of the Earthworm's Gastrointestinal Tract

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Heavy metal pollution of soil destroys agricultural ecological surroundings, affects agricultural production seriously, and changes the animal community in the soil greatly (Wang Zhenzhong 1989, 1994). Earthworm as a big animal in soil, is first affected. Many researchers (at home and abroad) have done much work on earthworm' s classification, ecology, physiology, biochemistry, and morphology. In recent years, many reports about pesticidae pollution on earthworm have been presented as pollution gets more and more serious , but there are few about heavy metal pollution on earthworm, the authors have made a series of studies on them (Chen Yi 1956;leaverach MC 1963;Thomnson A8 1971;Huang Fuyi 1982 ; Yin Wenying 1992 ; Wang Zhenzhong 1994; Guo Yongcan 1993, 1994, 1995), and this paper mainly reports the bioconcentration of heavy metals in earthworms and the effects of heavy metal pollution in soil on mucosa epithelial cell ultrastructure of earthworm' s gastrointestinal tract from the point of heavy metal toxicology.

MATERIALS AND METHODS

Zhuzhou smeltery was the source of polluton. Six sampletaking zones were chosen along the major wind respectively. Pheretima califonica, a dominant variety in the soil were collected as the experimental animals.

The fieldcollected earthworms were cleaned and put in a container to starve for 48hrs, random samples were dissected and washed with physiological saline water to get rid of dirt in the digestive tract, nitrify the samples with nitric acid - perchloric acid and determine Cd, Pb, Zn with atomic absorption spectrophotometry instrument(HITACHI 8080), nitrify As With nitric acid - sulphuric acid, and determine it through silver diethyldithio carbamate spectrophoto metric method, nitrify Hg with nitric acid - sulphuric acid, and determine it with cold - vapour atomic absorption instrument.

The collected earthworms *were* cleaned and then put in a container to starve for three days to excrete their excrement completely. The digestive tract was dissected and washed with phosphate buffer. The gastrointestinal tract tissue behind gizzard was fixed with 1% osmic acid following with 2.5 % glutaraldehydrade solution, dehydrated with acetone and embedded in Epon 812, the sample was sectioned (7000A thick) with LKB III Ultramicrotome. The sections were stained by uranium and lead citrate, and then observed under H - 600TEM The control pheretima califonica were collected in the nonpollution areas near the yuelu mountain.

RESULTS AND DISCUSSION

According to Guo Yongcan(1994), heavy metal pollution areas near Zhuzhou smeltery had

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been devided into six zones: serious pollution I , moderate pollution II and III , slight pollution IV and VI, and no pollution V . The earthworms from V and the control areas were all in the normal conditions and showed no changes in ultrastructure, but those from the other zones all showed pathological changes of different levels.

The earthworms belongs to complete soil annelid , it collects the heavy metal elements in soil through food chain and the contents in the body increases as the heavy metal pollution get more seriously . Further demonstration is provided by the calculation of Spearmen' s correlation coefficients of heavy metal contents in soil and in body of earthworm. thers is from 0.67-0.77(Table,l)

Table 1. Spearmen's correlation coefficients of heavy metal contents in soil and in body of earthworm.

Sampling place	Heavy metal contents (mg/kg) As		Cu		Cd		Pb		Zn		Hg	
	A	B	A	B	A	B	A	B	A	B	A	B
1	76.4	4.58	56.6	16.32	9.18	47.3	0.705	35.2	657.8	67.0	0.94	0.50
2	67.3	3.10	57.8	4.77	3.8	15.8	325.3	3.70	367.8	53.0	0.39	0.27
3	51.2	3.00	51.8	5.06	6.3	15.4	459.0	13.50	479.0	80.0	0.72	0.36
4	25.0	3.06	28.6	1.41	1.9	1.9	204.8	20.8	159.3	35.0	0.30	0.21
5	25.6	2.00	23.6	1.29	0.5	5.8	55.0	2.46	91.1	47.0	0.26	0.05
6	8.6	2.28	24.6	1.72	0.5	2.1	61.0	0.81	2220.8	41.0	0.74	0.11
Rs	0.68		0.77		0.71		0.77		0.77		0.67	

A : soil B : body of earthworm

Under TEM, it was found that short microfless spread on the dissociated surface of stomach mucosa epithelial cells of the earthworms from slight pollution zones, the mitochondria abundance in cytoplasm near dissoicated surface were long in shape and had no changes in the structure, the nucleus of epithelial cell rested on the base of the cell around which there were many RERs, there were lysosomes of different size between mitochondria and RERs, and the primary and secondary lysosomes increased in mumber (Fig1). It was also found that there were autophagy lysosmes whose contents were very complex. In addition to myelin structure, there were also destroyed mito chondria and fragments of other cytolasmic organelles (Fig2). The RER of stomach mucosa epithelial cells of earthworms from the moderate pollution zones swelled and took the shape of bubble. In addition, there was also a phenomenon of granules dropping from the RER. At the same time , it was discovered that the space between outer - membrane and inner - membrane of mitochondria was enlarged and the inner - membrane shrunk, which leaded to the mitochondria cristae disappearance and vacuolization. It could be seen that there was wooly structure or black sediments in the matrix of mitochondria (Fig3). In the seriously polluted zones, many mitochondria cristae of mucosa epithelial cellso earthworm' s stomach disappeared and the mitochondria had a wooly structure. The space of the cell nuclear membrane extended. Nuclear swelled to change shape. Mitochondrin agglutinated and trended to move towards the membrane. Nucleolus swelled to change shape. In some cell , the nucleus were almost blank, whose chromatin decreased and nucleolus disappeared and nuclear membrane split. Some mitochondria disintegrated and there were a lot of blank areas inside the cytoplasm (Fig4) .

Under TEM it could be seen that intestinal mucosa epithelial cells were single - layer pillar - like cells, which were made up of absrobing cells and glandcells. Microvillus spread over the dissociated surface of the absorbing sell, which could increase the absorbing areas of nutritious substances, It could also be seen that excluding absorbing cells, there were cilium cells with many cilia on the surfacein the intestine far away from the stomach, which was probably related to the exciting movement. Whether or not there were cilium cells in the intestine of other animal has not been reported. In cytoplasm of intestinal cells near cilia the mitochon

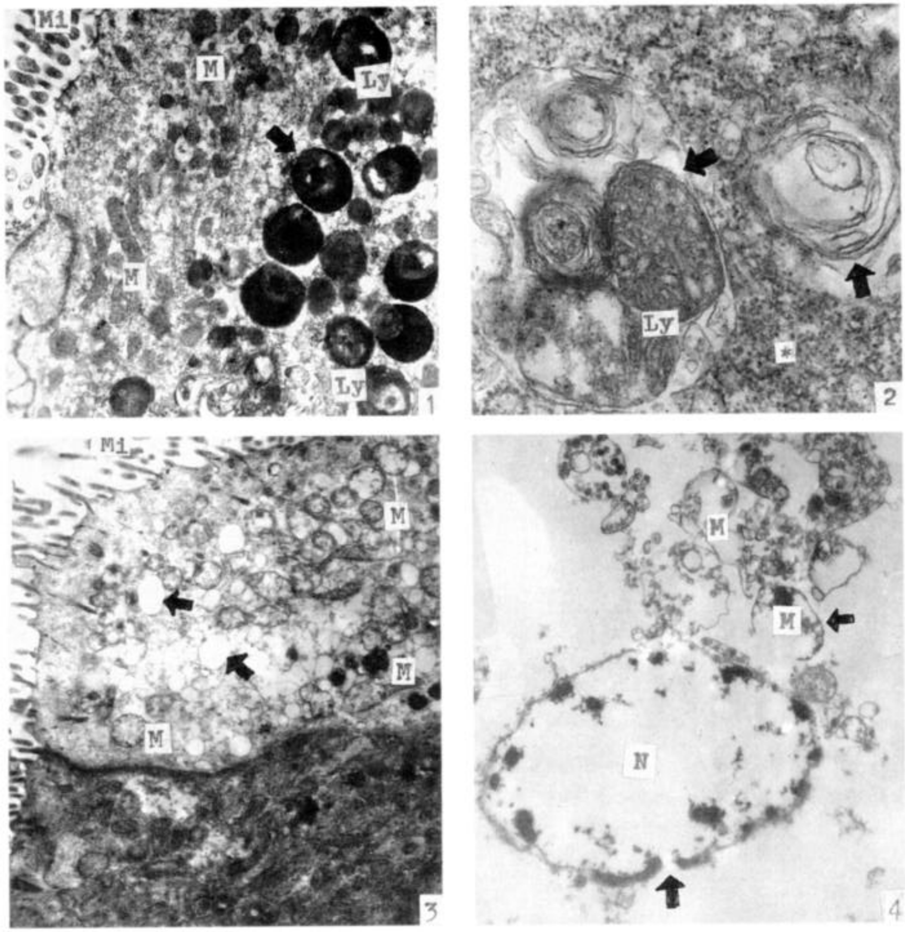

Figure 1. The stomach epithelial cell of the earthworm in the slighty polluted area : a large number of lysosomes(←) x 12K

Figure 2. The Stomach epithelial cell of the earthworm in slighty polluted area con - tained medullar and vacuolar structure of secondary lysosome(←) x 35K

Figure 3. The stomach mucosa epithelial cell of the earthworm in middle polluted area: mitochondrion swelled, contained black or wadding sediment(←), or mitochondri on curdle. x8K

Figure 4. The stomach mucosa epithelial cell in the seriously polluted area: chromatin losing, nucleoli disappearing, nuclear envelope broke and disintegrating. (←) a large number mitochondrion disintegrating(←). x10K

dria were abundant in number, which was probably related to supplying energy for cilium movement, but SER was often seen between mitochondria. Nucleus lay in the lower part of the cell and near membrane. Most Golgi body distributed above the nucleus. All the above mentioned was about normal cell structure. But in the heavy metal slight pollution zones, it could be seen that Golgi body swelled and lysosomes among the Golgi bodies increased, with the other structures unchanged , In moderate pollution zones , it was discoveried that SER increased obviously and overlopped. At the same time , Gogi body had the shape of a larg bubble. There were large fat granules around SER (Fig5) . In seriously polluted zone, it was found that mitochondria agglutinated, swelled or lacked cristae. In some mitochondria, matrix had wooly or bubble - like structures. The whole cilium or its ends swelled. There were also

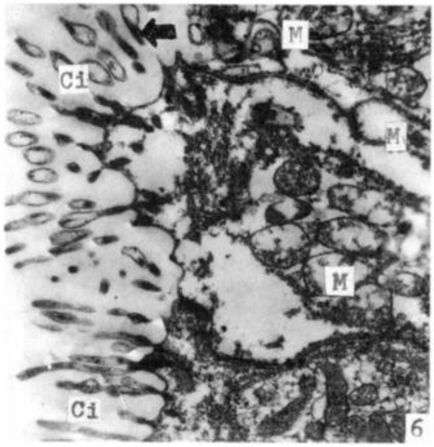

Figure 5. The intestinal epithelial cell of the earthworm in middle polluted area: SER enlarged lipid deposited. x8K

Figure 6. The intestinal epithelial cell of earthworm in the seriously polluted area: mi tochondrion vesiculated or mitochondrion to curdle, the cilia atrophiedorci cilia ut - most expension(←) . x15K

N(nuclear), Nu (nucleoli), M (mitochondrion). RER (rough endoplasmic reticulum), SER (smooth endoplasmic reticulum) . G (golgi complex), Ly (lysosome). Mi (microvillus) , Ci (cilium), Ri(ribosome) .

some shrunk cilia(Fig6). In the meantime, the perinuclear space of the cell expanded. Some nuclear membrane split to fragments. In the matrix of mitochondria there was black sediments. The cytoplasm further disintegrated and appeared blank.

According to Peng Peiqin (1991), the farmland near Zhuzhou smeltery industrial areas has suffered seriously from heavy metal pollution, in which Cd pollution is the most serious , Pb pollution more serious, Zn pollution serious, As and Cu pollution less serious. The concentration of heavy metals in soil is negatively related to the distance from the pollution source exponentially. Our sample - taking areas have been affected by air pollution. The dusts of pollution source drift with wind to the farmland and get in touch with the soil .Although the polluted soil can be cleaned by the absorption of animals, plants and micro organisms, excesssively accumulated poisonous substances in soil can still destroy the ecological balance in the soil. The earthworms live on the humus in soil . Therefore, after heavy metals touch the soil, they can easily enter the earthworm’s body with its food and be absorbed by the mucosa epithelial cells of earthworm’s intestine. Because there are a number of microvili on the free end of gastrointestinal tract epithelial cell to increase its absorbing areas, the poisonous substances can easily enter cell by means of simple diffusion, facilitied diffusion, pinocytosis, phagocytosis, and so on. The heavy metals do harms to living things chiefly by inhibiting the activities of enzymes. The actions of heavy metals to proteins have two main types: 1)action to some special groups such as imidazole group, carboxyl group and mercapto group. 2) action to special structures of amino acid residues. In addition, the phosphate groups of nuclear acid can also combine with positive ions of heavy metals into chelales (Douglas HKL, 1977).

Heavy metals getting into a cell stimulates it to produce a defensive stress - increasing lysosomes in the cell . The cells in the slightly polluted zones have the above reaction. After the heavy metals are concentrated in lysosome, the lysosome can relieve the poison of some heavy metals. Heavy metals’ poison can also destroy other organeles, such as mitochondria and SER. Lysosome digests these destroyed membranous structures in cells by autophegsome’ s action. So there were myelinsomes found in more destroyed ultrastructures. Hage Y (1973) suggested that the action between poisonous matters and proteins changes membrane permeability. For example, Pb poisoning is caused by Pb inhibiting the ativities of ATPase on the cell membrane which affects the distribution of K^+ , Na^+ ions controlled by ATPase so as to change cell membrane permeability and lead to a series of changes in ultrastructure. The combination of Cd and sulfohemoglobin on the membrane leads to changes in cell menbrane permeability. K^+exudation, Na^+infiltration and high concentration inhibites mitochondrium breathing, Mitochondrium is a organelle the most sensitive to poisonous substances in cell, whose structure destroying and function losing lead to lacking energy neccessary for the life activities , which causes cell necrosions in the end . The death caused by poisonous substances is related to the disoder of Ca^{2+} stable state. After poisonous substances gets into the organisms, they are converted metabolically catalyzed by enzymes. On the other hand, they can change the activities of enzymes in the cells. The authors of present paper have studied earthworm' s isozymes. The results shows that heavy metals can activate the activities of peroxidase isozymes and inbibits those of easterase isozymes . If the inhibition caused by poisonous substances is irreversible. it will lead to biochemical damages, which will in the end bring about organelle destroying and organization necrosis.

Organisms assimilate metal elements selectively as the part of their body , they also assimilate some other elements more or less which have no direct connection with organism. In normal organisms, there are certain ratios between essential and unessential elements, also among essential, once the enviroment get polluted, some poisonous elements are assimilated more than enough, organic functions will be hindered or lost. We determine the size of earthworms in various heavy metal polluted soils, the calculation shows the value of the concentration coefficient K of each element is in the order of Cd > Hg > As > Zn > Cu > Pb, the value of K of Cd > 1, it shows Cd is thickly collected . Because the bioconcentration quantity of heavy metal decides the injured degree of cell ultrastructure, it can be used as a index of heavy metal pollution (Czarnowska K, and Joopkiewicz K 1978). The earthworm which is an important experimental material can be used to control heavy metal pollution

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