

Aluminum Bioaccumulation in the Earthworm and Acute Toxicity to the Earthworm

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Abstract—Aluminum is a highly cytotoxic metal to plants, but its toxicity and accumulation to invertebrates in neutral soils is still unknown. This study is to determine the acute toxicity and bioaccumulation of aluminum to the earthworm. The ecotoxicity tests were based on the methods of international standardization of organization (ISO) and OECD guideline with some modification. The contents of aluminum were measured with Graphite Furnace Atomic Absorption Spectrometry. The experimental period of accumulation test was 32 days. The experiments indicated that aluminum had strongly toxic to earthworms with LC_{50} of 532.33 mg Al/kg dry soil at pH of 7.0. There were about 1.7 mg monomeric Al/kg dry soil and 2.5 mg water-soluble Al/kg dry soil. Earthworms had ability to enrich aluminum at the low contaminated soils doses and bioaccumulation attained maximum then biodegraded obviously. There was a negative correlation evidently between the bioconcentration factor and the dose contaminated soils by Al.

Keywords- aluminum; earthworm; bioaccumulation; acute toxicity

I. INTRODUCTION

Earthworms were common in vast varies of soils and represented 60% to 80% of the total soil biomass [1]. They played an important role in maintaining soil structure and promoting soil ecosystem processes and plant production. Moreover, earthworms can inhabit and survive in soils that were heavily contaminated by metals [2]. *Eisenia andrei* is one of the most commonly used species in the standard assays of acute and chronic ecotoxicity for industrial chemicals, which make it a key role in terrestrial ecotoxicological risk assessment [3].

Aluminum (Al) is the third most abundant element in the earth's crust. As a major constituent of mineral soils, it is present in the daily life of all organisms and may concentrate and transfer in food chain. But because of its low solubility in a solution under neutral condition, Al was regarded as a nontoxic element and was often neglected in soil, water, plant and many other samples. Its environmental and biological effects were not investigated until recently. Al was considered to be the most important growth-limiting factor for plants in the soil, even it was a highly cytotoxic metal to plants in acid soils [4]. No data were currently available to confirm that invertebrate could have Al stress tolerance or resistance, some plant species

did, as many genes in plant associated with Al accumulation [5]. Moreover, bioaccumulation and toxicity of Al was mainly restricted to plants in acid soils. Little study was known about invertebrate species in neutral soils.

The aim of this study was to determine the acute toxicity and bioaccumulation of Al to earthworms in the neutral soil. Presently, studies on Al toxicity to animals were mainly on freshwater fish, while few were on soil invertebrate species. Only several studies have determined the effects of Al on earthworms in acid soil [6, 7]. But Fischer and Rundgren used a highly organic substrate, composed of peat soil mixing with horse manure, which no doubt would have reduced Al bioavailability and toxicity. Because Al chemistry is extremely complex and it can form complexes with various organic compounds and inorganic ligands, most but not all of which are soluble [8]. In order to eliminate the effects of variable factors on toxicity, this study was carried out in optimal and stable artificial soils at the laboratory. Moreover, it determined the contents of monomeric and water-soluble Al which are the phytotoxic Al species.

As metal chloride always used in toxicity test, this study carried out with aluminum chlorin ($AlCl_3$).

II. MATERIALS AND METHOD

A. Chemicals

Chemicals of analytical grade and industrial analytical grade quartz sands were supplied by Shanghai chemical reagent company (Shanghai, China).

B. Earthworm treatment

The *Eisenia andrei* was purchased from a commercial breeder and kept in the dark at $20\pm 1^\circ C$ in a mixture of 50% cow manure for more than 4 weeks. Adults with well-developed clitellum were weight from 500 to 600 mg. They were voided for 24 h to clear the gut of debris, then exposed to the filter papers and artificial soils and kept in an artificial climate incubator at temperature $20\pm 1^\circ C$, humidity $75\pm 7\%$, illuminant 1333 lx.

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C. Soil preparation

The compositions of artificial soils were 69% industrial quartz (0.02 mm–0.5 mm in diameter), 20% kaolin clay, 10% sphagnum peat obtained from an uncontaminated plot. The mixture were dried and crushed. $AlCl_3$ was dissolved into distilled water and added into each replicate of dry soils. The pH was adjusted to 7.0 with calcium carbonate. Six replicates were set for each dose. The same preparation of soil only without $AlCl_3$ was used as control.

D. The acute toxicity test

The acute toxicity test was from the international standard of OECD-guideline No.207 (1984). The total Al concentrations were 347, 416, 500, 600, 720, 864, and 1036 mg Al/kg dry soil. The death rates and symptoms were recorded respectively after 7th day and 14th day in the artificial soil test.

E. The bioaccumulation test

The bioaccumulation test was from the international standard of OECD-guideline No.207 (1984). The total Al concentrations were 20, 34, 50 and 100 mg Al/kg dry soil. The contents of aluminum in the earthworms and the soils were measured with Graphite Furnace Atomic Absorption Spectrometry [9]. The experimental period of accumulation test was 32 days.

F. The determination of monomeric and water-soluble Al in artificial soils

A spectrophotometry based on the extraction of the 8-hydroxyquinoline complex (at pH 7.2-7.3) into butyl acetate was introduced to determine the monomeric and water-soluble Al in soil solutions [10]. The separation of very labile Al species (inorganic aqua, hydroxyl and sulphate Al cationic monomers) by 8-hydroxyquinoline method was based on kinetic strength discrimination, chelating solid phase extraction, and a single extraction procedure with 8-hydroxyquinoline [11]. Quantification of Al concentrations in these extracts can be achieved by fluorometry or UV/visible spectrometry.

G. Statistic analysis

LC_{50} of Al to earthworm was calculated using the Spearman-Kärber method [12]. All figures were prepared by EXCEL2000.

III. RESULTS

A. The toxicity test

All earthworms survived in the controls. The mortality rates of earthworms in the treatments were recorded in 7 d and 14 d after exposure to the doses of 347, 416, 500, 600, 720, 864, and 1036 mg Al/kg dry soils (Fig.1). At the pH value of 7.0, the points spread were “S” curves. The LC_{50} (with 95% confidence interval) values for the effect of Al on earthworm survival were calculated to be 532.33 (490.12-578.07) mg Al/kg dry soils at 14 d using Spearman-Kärber method.

With the increasing of Al in artificial soils the doses of monomeric and water-soluble Al in the soils were increasing

(Fig.2). The monomeric Al doses varied so little that the range was from 1.24 to 2.38 mg /kg dry soils which the content of the control was 0.93 mg /kg dry soils. The range of water-soluble Al was from 1.76 to 9.40 mg /kg dry soils. The curve of the mortality in artificial soils at 14 d and the different Al species was drawn in Fig.3. There were positive correlation evidently between the mortality and the doses of water-soluble Al.

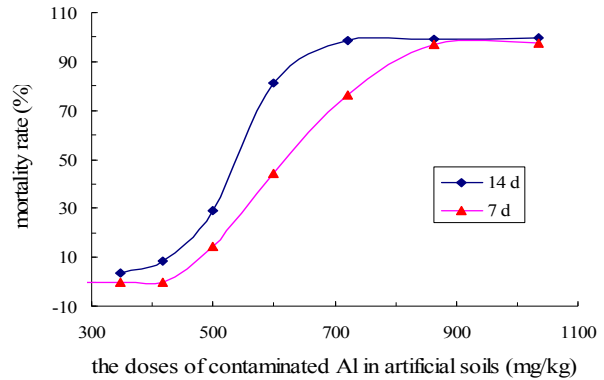


Figure 1. The mortality rate of earthworms in contaminated soils by Al (Values are means, n=6)

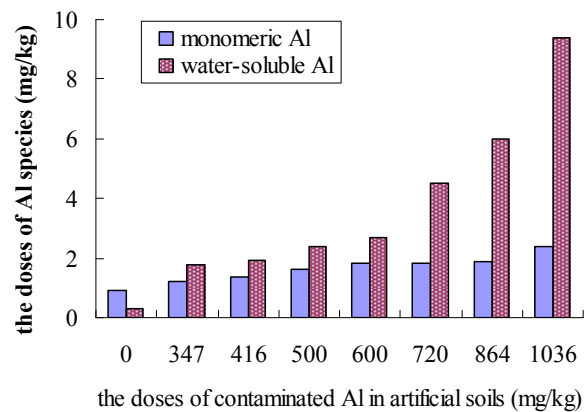


Figure 2. The curve of Al species in contaminated soils by Al. (Values are means, n=3).

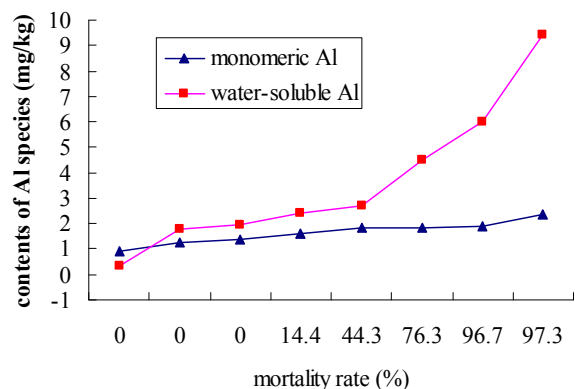


Figure 3. The content curves of Al in earthworms at different days. (Values are means, n=6.)

B. The bioaccumulation test

All earthworms survived in the test. The contents of aluminum in earthworms fluctuated with test days in Fig.4. The bioaccumulation attained maximum which was about 170 mg/kg, then descended obviously. It was shown that the contents did not have much direct relation with Al doses. The bioconcentration factor increased with decreasing of Al doses (Fig.5). There was a negative correlation evidently between the bioconcentration factor and the dose contaminated soils by Al.

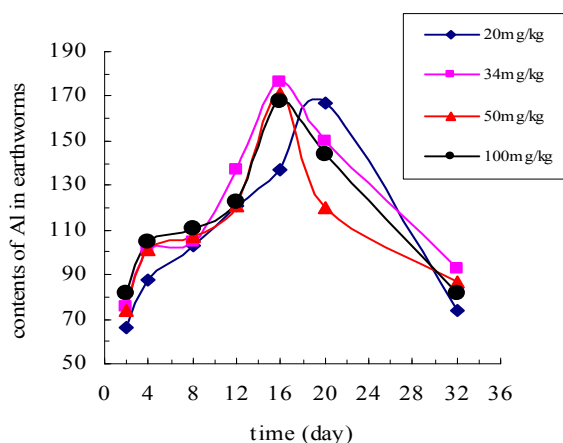


Figure 4. The content curves of Al in earthworms at different days. (Values are means, n=6.)

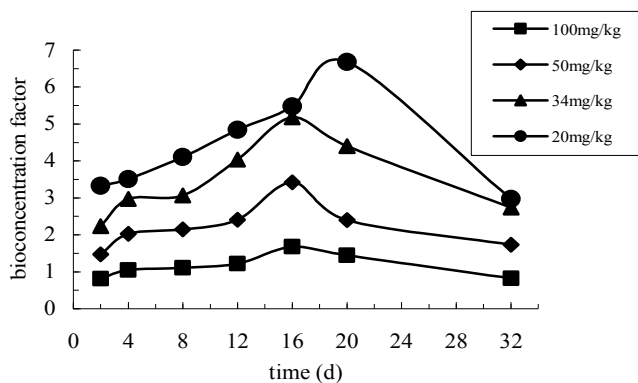


Figure 5. The bioconcentration factor curves of Al in earthworms at different days. (Values are means, n=6.)

With the increasing of Al in artificial soils the doses of water-soluble Al in the soils were increasing (Table.1).

TABLE I. CONTENTS OF WATER-SOLUBLE AL IN ARTIFICIAL SOILS (VALUES ARE MEANS, N=3.)

Exposed Al	The contents of Al (mg Al ³⁺ / kg dry soil)				
	0	20	34	50	100
Monomeric Al	0.45±0.09	0.59±0.04	0.79±0.02	0.83±0.09	0.87±0.07
Water-soluble Al	1.39±0.38	2.07±0.27	2.27±0.09	2.67±0.20	3.17±0.43

IV. DISCUSSION

It is difficult to assess the effects of toxicants since little information on Al in the soils is currently available. Moreover, as no environmental soil standards of Al currently, we compared LC₅₀ with those of CdCl₂ and CuCl₂, and use environmental quality standard for soils of China (1995) and soil contamination countermeasures law in Japan (1992) as the basis for a more thorough assessment. All data were shown in Table 2. The LC₅₀ values of Cd, Cu and Al were 875.06 mg/kg, 116.91 mg/kg, and 532.33 mg/kg respectively, so it was clearly indicated that the acute toxicity of Al to earthworms was stronger than Cd but weaker than Cu. There were precise prescripts for Cd and Cu of soil laws in China and Japan. Therefore, considering its acute toxicity, Al should be of highly concern.

TABLE II. ENVIRONMENTAL QUALITY STANDARD FOR SOILS OF CHINA, JAPAN AND THE RESULTS OF THIS TEST

metal	Environmental quality standard for soils (mg/kg)			Japan (JIS 46-1992)	This study (mg/kg)
	China (GB 15618-1995)		Artificial soil LC ₅₀		
	Dipole	Tertiary		pH >7.0	
	pH >7.5	pH 6.5-7.5	pH >6.5		
Cd	≤0.60	≤0.30	≤1.0	≤1.0	LC ₅₀ 875.05
Cu(cropland)	≤100	≤100	≤400	≤125	LC ₅₀ 116.91
Al	none	none	none	none	LC ₅₀ 532.33

Earthworms could accumulate Cd and Cu rapidly and transform to non-toxic form [13, 14]. In this bioaccumulation test it indicated that earthworms had ability to enrich aluminum at the low contaminated soils doses, but after the contents of Al attained maximum it was not to stay the same. It then descended obviously. The bioconcentration factor increased with decreasing of Al doses. It was difficult to interpret mechanism of toxicity and detoxification. The earthworm absorbed and digested Al through alimentary canal, this process was so slow that tolerance or resistance to the metal pressures might evolve.

The other reason may be attributed to the different pH values. It is generally assumed that aluminum toxicity is caused by labile monomeric aluminum species. When the pH of a solution is raised above 4.0, Al³⁺ forms the monomeric species AlOH²⁺, Al(OH)₃⁺, Al(OH)₃, Al(OH)₄⁻ and Al³⁺ undergoes repeated deprotonations to form insoluble Al(OH)₃ at pH 7.0 [15]. Al occurs in the various chemical species, of which the monomeric and water-soluble species were found to be the most crucial to evaluate its toxicity in the soils [16]. There were positive correlation evidently between the mortality and doses of monomeric and water-soluble Al species in the acute toxicity test. Furthermore, the OECD standard artificial soil contains 20% kaolin clay, which had a purity of approximately 50%. Considering that kaolin (Al₄(Si₄O₁₀)(OH)₈) on a mass basis contains 20.9% Al, it could be concluded that artificial

soil with 10% pure kaolin clay contains approximately 2% Al. Whereas it was generally considered that the speciation of $Al_4(Si_4O_{10})(OH)_8$ was innocuous and stable so that it is commonly used in artificial soil test. And the quantity of calcium carbonate added could lead to different results. Al toxicity could be counteracted by calcium, species of low calcium exhibited higher tissue metal concentrations compared with species of high calcium [17]. But that is not the major factor of toxicity. The behavior and physiology of earthworms could change physical properties of soil, which unfortunately affected the chemical's fate and bioavailability.

V. CONCLUSION

In the neutral soils, Al acute toxicity to earthworm falls between Cu and Cd. Therefore, Al toxicity must be a concern, particularly when Al has an adverse effect on ecosystem that may concentrate and transfer within the food chain, which subsequently affects animals of higher grade along the food chain. Moreover, Al has direct toxicity to earthworms, an interaction occurs that causes a twofold change in sensitivity (for juvenile production), while there is no apparent effect of conditions on the pattern of physiological responses.

Earthworms had ability to accumulate aluminum at the low contaminated soils doses and the contents of bioaccumulation attained maximum then biodegraded obviously. There was a negative correlation evidently between the bioconcentration factor and the dose contaminated soils by Al. The cause could be cytological and histological factors which need to interpret in further research.

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