EFFECTS OF LEAD AND HUMIC ACID ON DUCKWEED, *LEMNA MINOR*

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The effects of lead on total chlorophyll content, growth rate, multiplication rate, lead uptake and morphology on duckweed, *Lemna minor* were studied. *L. minor* was exposed to various concentrations of lead nitrate solutions (30, 50, 100 and 200 mg/L) for 12 days. Observations were done every three days. The results showed that the lower concentrations of lead (30-50 mg/L) did not have any effects on the total chlorophyll content, growth rate, multiplication rate and morphology of *L. minor*. However, the high concentrations of lead (100-200 mg/L) resulted in decreases of total chlorophyll content, growth rate, multiplication rate and morphology of *L. minor*. Morphological changes exhibited consisted of chlorosis, necrosis, breaking up of colonies and loss of buoyancy of plants. Lead uptake by *L. minor* was increased with the increase of lead nitrate concentration. The highest lead contents were observed on day 12 (30 mg/L) and day 6 (50, 100, 200 mg/L). The effects of lead and humic acid on total chlorophyll content, growth rate, multiplication rate, lead uptake and morphology of *L. minor* were also studied. The results showed that low concentrations of humic acid (10, 20, 40, 80 mg/L) did not have any effects on the toxicity of lead. Decreases in total chlorophyll content, growth rate , multiplication rate and changes in morphology of *L. minor* were still observed at the end of the period of exposure. However, the application of a high concentration of humic acid (160 mg/L) resulted in increases in total chlorophyll content, growth rate and multiplication rate of *L. minor* exposed to lead nitrate solution. The study on the effects of humic acid on lead uptake by *L. minor* showed that in the lead nitrate solution of 50, 100, 200 mg/L of lead without humic acid, the highest lead contents were found on day 6, then they decreased. Similar results were obtained from *L. minor* exposed to solutions of lead and humic acid at low concentrations (10, 20, 40, 80 mg/L). The application of a high concentration of humic acid (160 mg/L) resulted in a general decrease of lead contents in *L. minor* from day 3 to day 12.

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การศึกษาครั้งนี้เป็นการศึกษาถึงพิษของโลหะตะกั่วที่มีต่อพืชน้ำชนิดหนึ่ง คือ แหนเป็ด พารามิเตอร์ที่ใช้ในการศึกษาผลของโลหะตะกั่ว คือ ปริมาณคลอโรฟิลล์, การเจริญเติบโต, อัตราการแบ่งตัว, การดูดซับสารตะกั่ว และลักษณะภายนอกของแหนเป็ด โดยทำการวัดพารามิเตอร์ต่าง ๆ เหล่านี้ในวันที่ 3, 6, 9 และ 12 หลังจากที่เลี้ยงแหนในสารละลายตะกั่วที่มีความเข้มข้นต่าง ๆ กัน (30, 50, 100 และ 200 มิลลิกรัม/ลิตร) ผลการทดลองพบว่าสารละลายตะกั่วที่มีความเข้มข้นต่ำ ๆ (30 และ 50 มิลลิกรัม/ลิตร) ไม่มีผลต่อปริมาณคลอโรฟิลล์, การเจริญเติบโต, การแบ่งตัว และลักษณะภายนอกของแหน แต่ที่ความเข้มข้นของสารละลายตะกั่วสูง ๆ (100 และ 200 มิลลิกรัม/ลิตร) มีผลทำให้ปริมาณคลอโรฟิลล์, การเจริญเติบโต, การแบ่งตัวของแหนลดลง รวมทั้งมีผลต่อการเปลี่ยนแปลงลักษณะภายนอกของแหนที่พบคือ การมีสีซีดจางลง, การตายของเนื้อเยื่อ, การไม่สามารถจับตัวรวมกันเป็นโคโลนี และรวมไปถึงการสูญเสียความสามารถในการลอยตัวอยู่เหนือผิวน้ำ ปริมาณสารตะกั่วที่ถูกดูดซับเข้าไปในแหนเพิ่มขึ้นตามความเข้มข้นของสารละลายตะกั่วที่แหนสัมผัส ที่ความเข้มข้นของสารละลายตะกั่ว 30 มิลลิกรัม/ลิตร พบมีปริมาณสารตะกั่วในแหนสูงสุดในวันที่ 12 และที่ความเข้มข้นของสารละลายตะกั่ว 50, 100 และ 200 มิลลิกรัม/ลิตร พบมีปริมาณสารตะกั่วในแหนสูงสุด ในวันที่ 6 ของการทดลองและเมื่อศึกษาผลร่วมกันของโลหะตะกั่วกับกรดฮิวมิกโดยใช้พารามิเตอร์ต่าง ๆ เหมือนกับการศึกษาผลของโลหะตะกั่วต่อแหนเป็ดเป็นตัววัด พบว่าความเข้มข้นของกรดฮิวมิกต่าง ๆ คือ 10, 20, 40 และ 80 มิลลิกรัม/ลิตร ไม่มีผลทำให้พิษของตะกั่วต่อแหนเป็ดเปลี่ยนแปลง แต่พบว่าปริมาณคลอโรฟิลล์, อัตราการเจริญเติบโต, การแบ่งตัวของแหนลดลงในวันที่ 12 ของการทดลองรวมทั้งมีการเปลี่ยนแปลงลักษณะภายนอกของแหนด้วย อย่างไรก็ตามเมื่อทำการทดลองโดยใช้กรดฮิวมิกที่มีความเข้มข้นสูง (160 มิลลิกรัม/ลิตร) มีผลทำให้ปริมาณคลอโรฟิลล์, อัตราการเจริญเติบโต, การแบ่งตัวของแหนเพิ่มขึ้นเมื่อเปรียบเทียบกับสารละลายตะกั่วที่มีความเข้มข้น 50, 100 และ 200 มิลลิกรัม/ลิตร และเมื่อศึกษาผลของกรดฮิวมิกต่อการดูดซับสารตะกั่วของแหน พบว่าในสารละลายตะกั่วที่มีความเข้มข้น 50, 100 และ 200 มิลลิกรัม/ลิตรแหนจะมีปริมาณสารตะกั่วที่สูงสุดในวันที่ 6 ของการทดลอง จากนั้นจึงลดลงซึ่งก็คล้าย ๆ กับเมื่อทดลองโดยใช้สารละลายกรดฮิวมิก ที่ความเข้มข้นต่าง ๆ (10, 20, 40 และ 80 มิลลิกรัม/ลิตร) แต่เมื่อใช้สารละลายกรดฮิวมิกที่ความเข้มข้นสูง (160 มิลลิกรัม/ลิตร) มีผลทำให้ปริมาณสารตะกั่วในแหนลดลงจากวันที่ 3 ไปจนถึงวันที่ 12 ของการทดลองตามลำดับ

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CHAPTER I

INTRODUCTION

Lead is one of the oldest metals known to man and has been used in piping, building materials, solders, paint, tube metal ammunition and casting. In more recent years, lead has been used mainly in storage batteries, metal products, chemicals and pigments.

Emission of anthropogenic lead to atmosphere has increased sharply reaching a peak of $4,265 \times 10^3$ metric tons during 1970's. The acute effects of lead to invertebrates are usually reported at a concentration of 0.1 - 1mg/L, and a significant mortality may occur within a range of 0.002 - 670 mg/L (1). Cooper (2) reported that inorganic lead compound induced renal carcinoma in rats and mice and the embryonic effects of lead nitrate in rats depended on the day of the administration.

Lead is in the form of soluble and suspended in sediment in natural reservoirs by rain off from the atmosphere to soil and run off from soil to natural reservoirs with a residence time of 100-200 years. In natural reservoirs, soluble lead compounds are intermediate between hard and soft acid in their interaction towards oxygen and sulfur containing ligands. The behavior of lead in natural water is a combination of precipitation equilibrium and complexing.

Water pollution is one of the most serious problem faced by human today. So monitoring of environmental contamination becomes an important field. A large

number of biomonitoring methods were reported during the past year. Biological test methods for determining the dangerous potential of water and wastewater were reviewed. Plant bioassays were determined to be quick and simple methods for determining the genotoxicity of environmental pollutants (3) . Free-floating plants and emergent plants are most easily harvested and therefore are the types utilized almost exclusively for this purpose. Both of them also have great absorption capabilities in a relatively short period of time. Free-floating species have a tremendous advantage in that they can be harvested by merely skimming them off the water's surface. Partial harvest can be made on a periodic basis without disrupting the system in anyway. The two groups of floating plants most often used are water hyacinths and duckweeds but the duckweeds are easier to harvest than water hyacinths. Furthermore, they can tolerate much cooler temperature, which makes them suitable to be used in wider geographic area than water hyacinths (4) . Many studies have shown that duckweeds are an excellent candidate for phytotoxic test due to their sensitivity (5).

Members of the free-floating duckweed family (Lemnaceae) have shown a potential usefulness in the treatment of eutrophicated water system. They are also being used in some localities to reduce the concentration of excess natural water nutrients. Plants have the advantage that they absorb not only the essential nutrients but also other elements and even organic pollutants such as phenol. Mohan and Hosetti(6) reported that *Lemna minor* was very sensitive to heavy metals when compared with other plant species. It can be used as an indicator species for the assessment of ecotoxicological effects of wastewater and effluent monitoring. Huebert and Shay (7) concluded that *Lemna trisulca* had many excellent features for

toxicological studies of aquatic systems such as its small size, rapid growth rate and submerged habitat.

Although total lead is often found in high concentrations in aquatic plants particularly those growing in freshwater and receive mine or other industrial waste, but the degree of uptake is affected by the type of species, plant organ analyzed and by various environmental factors such as pH, temperature. The presence of multiple toxicants and organic matters can affect both the distribution of a metal between water and sediment, and the bioavailability of porewater contaminants (8).

Mohan *et al.* (9) reported that *L. minor* is sensitive to metal pollutants. Several workers have studied its potential use in phytometer method. Toxicity test to understand the toxic effects of lead on *L. minor* was conducted under laboratory conditions. This paper deals with the influence of organic matter (humic acid) on the bioindicative and accumulative properties of *L. minor*.

CHAPTER II

OBJECTIVES

The objectives of the present study are :

1. To study the effects of lead on total chlorophyll content, growth rate, multiplication rate and morphology of duckweed, *Lemna minor*.
2. To study the combined effects of lead and humic acid on total chlorophyll content, growth rate, multiplication rate and morphology of *L. minor*.
3. To determine the lead uptake by *L. minor*.
4. To determine the lead uptake by *L. minor* with the application of humic acid.

CHAPTER III

LITERATURE REVIEW

3.1 Lead

Lead is the 36th most abundant element in the earth crust, with an average concentration of 15 mg/kg. Lead occurs as a constituent of more than 200 minerals but concentrated in gauna (PbS), gelesite ($PbSO_4$) and cerusite ($PbCO_3$). The input of antropogenically derived lead to environment now outweighs all natural resources. The world production of lead was 1,700 x 10^3 metric tons in 1973 and increasing to 3,100 x 10^3 metric tons in 1980. Production in recent years has stayed around 3,100 x 10^3 metric tons , reflexing a concern about the health effects of lead, particularly in young children(9).

Lead continues to be used in large amounts in storage batteries , metal products, pigments, chemicals, buildings and constructions. Most anthropogenic lead emissions result from the mining, smelting, refining of lead, and other metal ores. Approximately 96% of all lead emissions originate from anthropogenic sources with the input into natural resources (Tables 3-1,3-2)

Table 3-1. Worldwide anthropogenic input of lead to freshwaters.

Source	Input (thousand metric tons/yr)
Atmospheric fall out	87-113
Manufacturing processes	
Metals	2.5-22
Chemicals	0.4-3.0
Pulp and paper	0.01-0.9
Petroleum products	0-0.1
Dumping of sewage sludge	2.9-16
Domestic waste water	
Central	0.9-7.2
Noncentral	0.6-4.8
Smelting and refining	
Nonferrous metals	1.0-6.0
Iron and steel	1.4-2.8
Steam eletrical production	0.2-1.2

Source : Nriagu and Pacyna (10).

Table 3-2. Worldwide emission of lead to the atmosphere.

Source	Input (thousand metric ton)
Leaded fuel combustion	248(approx.)
Pyrometallurgical nonferrous metal product	
Mining	1.7-3.4
Lead production	11.7-31.2
Copper-nickle production	11.1-22.1
Zinc-cadmium production	5.5-11.5
Secondary metal production	1.1-14.2
Steel and iron manufacturing	0.1-1.4
Cement production	<0.01-14.2
Coal combustion	
Electrical utilities	0.8-4.7
Industrial and domestic	1.0-9.9
Wood combustion	1.2-3.0
Refuse incinerator	
Municipal	1.4-2.8
Sewage sludge	<0.3
Phosphate fertilizer production	<0.3
Other emissions	5(approx.)
Total emissions	289-376

Source: Nriagu and Pacyna (10).

3.1.1 Chemistry of lead

Lead is a number of the group IV elements (C, Si, Ge, Sn and Pb). It has stable 2^+ , 4^+ oxidation states. In freshwater, lead forms a number of complexes of low solubility with many of the major anions, including hydroxides, carbonates, sulfides and sulfates. Lead also partitions favorably with humic and fulvic acids, forming moderately strong chelates, Approximately 75% of lead in rivers is in suspension and 25% in solution. Lead undergoes methylation in the environment to from several organic derivatives. The process is mediated by bacteria in sediments to form$(CH_3)_3Pb^+$ and related compounds. $(CH_3)_3Pb^+$ disproportionates slowly as follows:

$$(CH_3)_3PbX \longrightarrow 2(CH_3)_4Pb + PbX_2 + CH_3X$$

Chemical alkylation of lead has been reported under laboratory conditions, apparently in the absence of bacterial methylation.

Numerous organic complexes are found in the atmosphere and rain water due to use if lead in fuels has concomitantly broken down from the parent compound. Sorption to sediment plays a key role in the fate of lead complexes. The rate of absorption is initially rapid and follows the Freundlich Isothem, before reaching a plateau after 24-28 hours.

3.1.2 Lead in aquatic systems

The behavior of lead in natural waters is a combination of precipitation equilibria and complexing with inorganic ligands(11). The degree of mobility of lead depends on the physicochemical state of the complexes formed. Lead has a property to bind with inorganic and organic ligands, and particulate.

Binding to inorganic and organic ligands. Hydrolysis of precipitates (lead phosphate and lead sulphides) above pH 6 solubilizes lead as $Pb(OH)^+$. Insoluble lead is not formed until pH 10.0. At pH 8.5, $Pb(OH)^+$ is the only major species in the chloride concentration range (350-56,200 mgL^{-1}). The hydroxy ion is common in natural waters and its interaction with heavy metals alters their mobility. Lead forms moderately strong chelates with organic ligands containing S, N and O donor atoms. Lead also binds to different microbial growth media (10).

Binding to particulates. Wilson (12), reviewing the metal concentration in river waters around the world, reported that lead showed variable levels of binding (15-85%) association with suspended solids.

3.1.2.1 Transport in natural water

In freshwater , about 45% of the total lead was present in particulate forms. The calculated equilibrium distribution of ion exchangeable species shows that $PbCO_3$ accounts for about 80% with smaller contributions from Pb (+2) and Pb $(OH)^+$.Pb – organic complexes become significant at ligand concentrations > 10^{-6} . In the estuarine, 69% of lead is present in the particulate fraction. In the fileable fraction, about 54% is ion-exchangeable(13).

Organic matter at concentration > 10^{-5} M does not significantly influence speciation. It was shown that as river water entered the estuary, the concentration of lead increased. This was largely due to the increased particulate fractions in the estuary, reflecting resuspended sediments, atmospheric fall out and input from urban zone creeks(10).

3.1.2.2 Lead in sediments

Sorption of lead by river sediments is correlated to organic content and grain size. In the absence of soluble complexing species, lead is almost totally absorbed as precipitated species at pH > 6.00. In acidic media, humic acid absorbs lead stronger than clays. The trend is reversed at pH ≥ 6.5 where soluble Pb – humate complexes are formed (10).

3.1.2.3 Residues

Concentrations of soluble lead in uncontaminated freshwater are generally ≤ 3 mgL^{-1}. However, much higher levels often occur near highways and cities due to the combustion of gasoline. The discharge of liquid mine wastes may produce ≥ 500 $\mu g\ PbL^{-1}$ in receiving water. The total levels in precipitation generally range from 1 – 50 $\mu g\ L^{-1}$ (10).

The presence of Cu^{2+} , Zn^{2+} and other metals is likely to hinder the uptake of Pb^{2+}. Lead typically desorbs from sediments and suspended solids in estuaries, due to the competition with chlorides, producing an appreciable increase in residues in the water column (10).

3.1.2.4 Bioaccumulation

Total lead is often found in high concentration in aquatic plants, particularly those growing in freshwater and receiving mine or other industrial wastes. The rate of uptake of inorganic lead is generally rapid and increases with the exposure concentration. Sorption is generally suppressed by H^+ and humic acids. Concentration factors (plant residue/water residue) for inorganic lead are often high and variable (10). Wang *et al.* (14) reported that the corresponding concentration factors for the

freshwater green algae *Ankistrodesmus falcatus* exposed to triakyllead and diakyllead were only 100 and 2,000 , respectively.

3.1.2.5 Toxic effects on aquatic organisms

Plants. Inorganic lead is moderately toxic to aquatic plants. Under many test conditions, it is more toxic than chromium, manganese, barium, zinc and iron, but is less toxic than cadmium, mercury and copper. Since aquatic animals are generally more sensitive to lead than plants, any surface water quality value aimed at protecting animals will also protect plants.

Lead acts synergistically with combinations of copper and zinc, and H^+. As with most metals, complexation with humic acid and other organic molecules, and inorganic ligands, reduces toxicity to most plant species studied to date. It is generally assumed that organoleads particularly tetraethyllead, are more toxic to aquatic plants than either the methylated derivatives or inorganic compound(10). Mohan and Hosetti (6) reported that lead had the effect on enzymatic changes of *L.minor* by increasing the activity of catalase and protease which might be due to the formation of protein complex with lead.

Invertebrates. Althought there is a variability among species and test conditions among species of invertebrates, the toxicity of inorganic lead to freshwater invertebrates is generally less than cadmium, copper, mercury and zinc. An acute toxicity dose ranges from 0.5 – 5 mg/L. Chronic toxicity to freshwater invertebrates such as reducing reproduction capacity has been recorded at the concentration of 0.019 - 0.025 mg/L (1).

Fish. The LC_{50} for freshwater fish exposed to inorganic lead generally ranges from 0.5 – 10 mg/L , but the increase in water hardness may increase the resistance of freshwater fish. Chronic effects following long term exposure to inorganic lead have been reported at concentration < 0.01 mg/L of inorganic lead (11).

The chronic effects of inorganic lead include :

1. Impairment of calcium and skeleton calcification.
2. Retardation of ovarian maturation.
3. Inhibition of Na-K ATP-ase activity.
4. Extensive spinal deformities.

3.1.2.6 Human – health effects

Acute toxicity. The primary symptoms of acute poisoning of inorganic lead in food and drinking water are fatigue, colic anemia, neuritis, seizures and other neurological disorders.

Chronic toxicity. The symptoms of chronic poisoning of inorganic lead are

- loss of appetite
- constipation
- mallalic taste
- anemia
- weakness
- insomnia .
- muscle and joint pain
- colic anemia

and other effects especially to heart and peripheral nervous system. Organolead compounds, by virtue of their lipophilic properties, can penetrate the blood barrier, making them more neuro-toxic than inorganic compound, given the fact that most of lead in water is inorganic and less toxic than the organic forms (15).

3.2 Humic Substances

Humic substances, the end product of chemical and biological degradation of animal and plant residues, are the most widely distributed natural organic products on the surface of the earth. They occur in all aquatic and terrestrial environments (16).

Humic substances are dark-colored, acidic, polydispersed systems of relatively high molecular weight (17). Humic acids contained (w/w) 40-62% carbon, 3-6% nitrogen, 3-5 % hydrogen and 32-36% oxygen. Moreover, sulfur, phosphorus and different metal cations are always present in humic acids. Sulfur content constitutes a tenth of a percent while phosphorus constitutes a hundredth to a tenth of a percent. Metal cations are not the constituents of humic acids but their presence indicates the formation of simple or complex salts of humic acids. Operationally, humic materials are divided into three groups based on their solubility. These are :

1. humic acid, soluble in bases, insoluble in acids.

2. fulvic acid, soluble in both bases and acids and generally of lower molecular weight but of higher oxygen than humic acid.

3. humic, insoluble in both base and acids and of higher molecular weight than humic acid (18).

Oxygen is found in phenolic, hydroxylic, carbonyl and carboxyl moieties. These functional groups are responsible for the reactivity, acidity and polyelectrolyte behavior of humic substances.

The formation of humus may be considered as organic in moist warm soil attacked by a host of different soil organisms. Two major kinds of organic compound that tend to remain in soil after attacking are :

1. resistance compounds of higher plant origin such as oils, fats, waxes
2. new compounds such as polysaccharides and polyuronides.

Both of them will provide the humus forming. The process of formation of specific humic substances as a result of the transformation of the organic residues is called humification process (19).

There are several hypotheses that explain the formation of humus. The two most significant hypotheses are :

1. the condensation-polymerization hypothesis by Trusov and Kononova (20).
2. Hypothesis of oxidized acid formation by Aleksandrova (20).

According to Trusov and Kononova (20), humification process occurs in two stages (Fig. 3-1)

1. Disintegration of organic residues to monomers.
2. Condensation and polymerization leading to the formation of humic acid.

H_2O, CO_2, amino acid peptides are the products of breakdown and resynthesis during the decomposition of polysaccharides, proteins and lignins. The phenolic compounds which are the decomposition products of lignins, catechins and other

substance, and quinones condense with amino acids and peptides, producing dark colored prohumic substances.

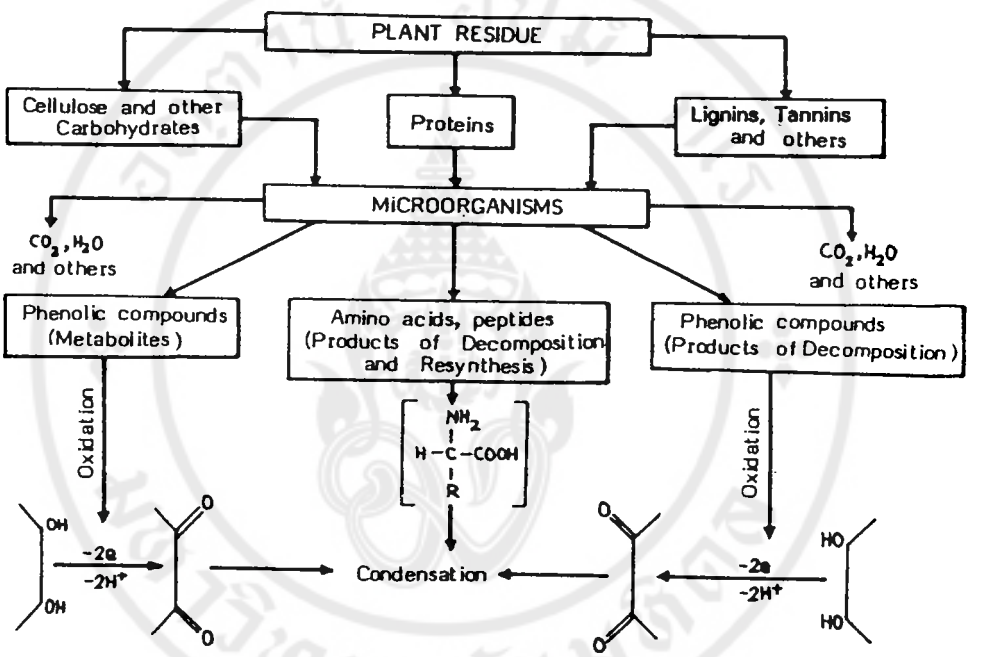


Fig. 3-1 scheme of humification process [after Trusov and Kononova (20)].

The humification hypothesis proposed by Aleksandrova (20) presents three stages of the process (Fig. 3-2).

1. Neoformation humus acid.
2. Their futher humification and condensaton.
3. Gradual and slow decomposition of humus acids.

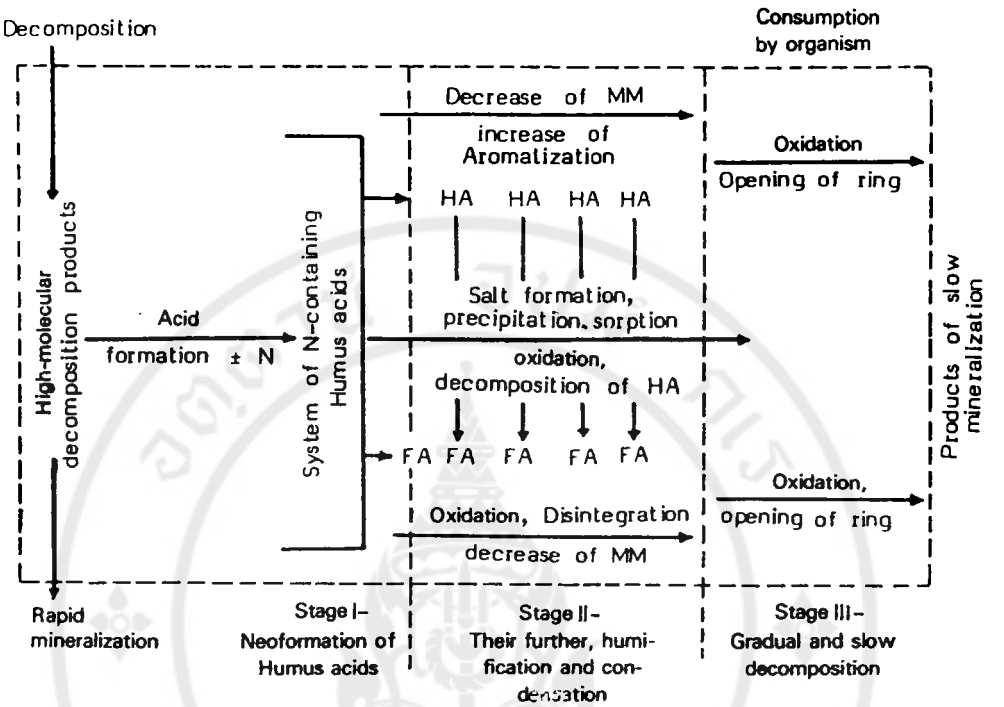


Fig. 3-2 The scheme of humification process [after Aleksandrova (20)].

The first process of neoformation of humic acid the formation of oxidized acid. Oxidatin takes place with the participation of oxidases and is achieved in several stages :

$$C \rightarrow COH \rightarrow CHO \rightarrow COOH$$

In these reactions, the high molecular weight compounds (plant residues and their large fragments) take part. With further humification their molecular weights decrease. In this stage the formation of the nitrogenous part of the molecular occurs.

The second stage of humification, according to Aleksandrova (20) are the formation of the nitrogenous part of the molecules of humic acids and further humification of newly formed humus acids.

Besides the enrichment of residues with corboxyl groups by humification, there are changes in the content of nitrogen-containing groups. During the humification of

plant residues rich in proteins, there occurs a gradual decrease in the nitrogen content in resultant humic acids. If substances poor in nitrogen are being humified, the nitrogen content in humification products increases gradually. Thus, according to Aleksandrova (20), either the partial loss of nitrogen or nitrogen accumulation are possible during humification. In further humification process of newly formed humus acids,the transformation of humus acid continuously occurs from the nucleation of the molecule to its complete mineralization.

After a prolong existence in soil, humic acids are either mineralized to the end products or form fragments participating in the synthesis of new molecules of humus acids. This is the third stage of the humification process.

3.2.1 Functional groups of humic substances

In natural environment, the formation of organomineral compounds occurs with a participation of different types of bonds. Humic substances contain about 15 different types of functional groups among which the most important are the carboxyl, phenol and amino groups.

3.2.2 Nature of binding of humic substances with mineral components

Chemical bonds play a great role causing the formation of the chemically stable multiatomic systems. They are subdivided into valence (ionic and covalent), nonvalent and coordination bonds. Ionic bonds play a significant role in the formation of organomineral compounds. Such a bond arises between the anions of humus acids and cations of alkali an alkaline earth metals.

Complex compounds are formed with a participation of coordination bonds. The complex compound consists of metal atom in definite valence state (central atom or central ion), which is bound with one or several molecules capable of independent existence. The molecules of ions linked with the central atom are called ligands.

Hydrogen bond plays an important role in the formation of organomineral compounds. This type of bond arises because the hydrogen atom bound with a highly electronegative atom does not have a symmetrical electron shell. The electron is pulled towards the electronegative atom on the opposite side and the hydrogen atom acquires some electropositive charge (21, 22, 23, 24, 25).

3.2.3 Adsorption complexes

Adsorption complexes are the products of interaction of humic substance with crystalline and amorphous soil minerals or organomineral products of adsorption of humus acid by minerals. Adsorption complexes are also called clay-humus complexes or organomineral compound.

Adsorption complexes are formed by intermolecular bonds and ionic or coordination bonds. Intermolecular forces act between practically any molecules, but this interaction is weak and the resultant organomineral derivatives are unstable. This type of bond is of great significance for nonpolar of neutral polar molecules. High molecular weight substances are absorbed more strongly. The ionic bond may arise in those cases where the organic compound is positively charged.

A large part of humic substances carries predominantly a negative charge which leads to the negative adsorption of anions. However, the negatively charged ions could

partially be held by electrostatic forces of the positive charge that may be present on the surfaces of crystals of alumino-silicates.

Hydrogen bonds are of great importance in the formation of organomineral compounds. Oxygen atoms on the surface of clay minerals and oxygen atom or nitrogen atoms of humic substance are the electronegative atoms between which the hydrogen bonds may form. Oxygen on the surface of clay minerals forms hydrogen bonds with the carboxyl groups of humus acids (21).

Miele and Ingram (26) studied the role of humic substances in the mobilization of mercury from watersheds. They found that the mercury concentration in water which was closely related to the color, probably reflected the concentration of humic and fluvic matter in the streams. The association of mercury with this parameter is most likely due to the complexation of mercury by humic material. The observation reported in this study extended the phenomenon to small headwater and streams, and clearly indicated that the geochemistry of mercury in streams and lakes was dominated by its interactions with humic material (26).

Narine and Guy (18) studied the binding of diquat and paraquat to humic acids in the aquatic environment and found that the rate of absorption of paraquat to humic acids showed the variation of binding paraquat as function of ionic strength. They also showed the variation of binding of diquat to humic acids as a function of increasing concentration of diquat. The binding was rapid and the equilibrium was reached within 3 hours (18).

3.3 Duckweeds

Duckweeds are the simplest and smallest flowering plants found in fresh – water worldwide. They are floating plants found at just below the surface of relatively still freshwater. There are four genera of duckweeds. They are *Lemma*, *Spiradela*, *Wolffia*, and *Wolffiella*. None have distinct leaves, but consist instead of flattened, minute, leaf-like structures called "fronds". These fronds are more or less oval and are a few millimeters across. Many of them lack roots. Their flowers (rare in many species) are so small that they are nearly invisible to the naked eye (27).

The remarkable trait of duckweeds is their power of living and flourishing in water which is so full of organic impurities that no other plants can survive in it. When they are introduced into the water with a bad smell, they can purify it until it is a fit habitation for small animals (28). Duckweeds have been widely used as an investigative tool in ecological, biological, and physiological studies (29). This is because they have three characteristics. The first is their vegetative growth habit. There are two meristems that alternately produce new fronds. Each frond produces new frond and so on. Although every frond finally dies, it yields 10 to 20 (or more) others before doing so. The second characteristic is that fronds do not remain attached indefinitely to form an increasingly large and complex structure. Instead, they break into colonies representing only a few vegetative generation. Thus, they maintain a constant relationship to their environment. This situation is analogous to that in an exponentially growing microbial culture , at least until surface crowding becomes severe. The third characteristic is an almost total lack of woody tissue (30).

The reason that duckweeds are more sensitive to effluent toxicity may be due to their faster growth and shorter life cycle (31). It is possible that the more life processes

the test organism is involved during the period, the more sensitive it responds to toxicity. Because each process may be inhibited by different toxicants or the same toxicant in different degrees, it is combined effects of all toxicants resulting in growth inhibition and death (5). Several species of duckweeds are used in recovering nutrients from the wastewater. It is thought that they absorb nutrients through both roots and lower leaves (32).

The other usefulness of duckweeds which is our interest is their sensitivity to water pollutants. With this characteristic, they are used in bioassay method for the determination of pollutants in water (3).

3.3.1 Reproduction of duckweeds

Almost all reproduction of duckweeds is vegetative ; sexual reproduction is rare. Duckweeds produce new (daughter) fronds from two pockets on each side of the narrower end of an older (mother) frond. Each daughter frond becomes a mother in its turn, usually while still attached to its mother. Such groups of attached fronds may be called colonies. Each mother frond produces a considerable number of daughters during its life time, but the exact number depends on various factors.

In an 18 – month study, the doubling time for *L. minor* fronds ranges from 1.3 – 2.8 days. Duckweeds cultured in the laboratory can grow indefinitely if plant nutrients, light, and water are provided (33).

3.3.2 Habitat of duckweeds

Duckweeds grow in still or slightly moving water ; more rapid motion sweeps them away unless they become entangled in anchored plants or debris. Growth may also continue for sometime out of the water on mud. All species appear to grow well

within the pH range of 4.5 – 7.5 with outer limits at 3.5 and 8.5. There may be a few differences in the pH tolerance of various species.

Duckweeds are found at all light intensities from full sunlight to below 50 foot candles , with considerable differences in the apparent preferences of various species. *L. minor* are notably sun-plants. Temperature requirements of various species differ particularly in the minimum temperature which they can survive. In general,the temperatures between 20° - 30° C are most favorable for them to grow (34). When either drought or low temperature bring conditions unfavorable for growth, many species of Lemnaceae are able to persist until growth is again possible. In some cases, this may be in the form of buds or as ordinary fronds. Many species also produce more or less specialized turion or resting stage, which are more resistant than normal frond (33).

Duckweeds are composed of two parts, the frond and the root. The plants are colonial and from aggregates of two or more fronds in a colony. They are very small. *L. minor* is 2-4 mm across. The plant size is so small that a large laboratory space is not required for culturing or testing. Yet the size is sufficient large enough to be visually observed, allowing non-destructive repeated observation (33).

3.3.3 Utilization of duckweeds

3.3.3.1 Biological filtration of water

One reason for growing aquatic plants is for use in removing nutrients from raw sewage and effluent of sewage treatment plants. Even efficient modern plants normally has concentrations of phosphorus, nitrogen and other plant nutrients that exceed those of natural water in the area. It is desirable and in some cases mandatory that these nutrients be removed prior to the return of the water to the environment.

Plants have the advantage that they absorb not only the essential nutrients, but other elements and even organic pollutants such as phenols (4). The capacity of aquatic plants to remove potential toxic heavy metals, including chromium from water is well documented (35). Rodgers *et al.* (32) reported that aquatic plants had the ability to take up heavy metals and survive in a highly stressed aquatic environment. They also found that duckweeds had the ability to accumulate thousand of times higher than the ambient chromium concentration to which they were exposed.

3.3.3.2. Animal feeds

The tiny free-floating fronds of duckweeds are especially valuable as feeds because they do not contain any woody elements. Because of their size and floating habit of growth, they require neither mechanical support nor conduction elements. Their reproductive and growth potentials are enormous and they are used as fertilizers or soil conditioners. For the most part, the chemical composition of aquatic plants is suitable for these uses but the same factors that interfere with the use of these plants as animal feeds make them impractical as soil amendments. The high water content results in high transportation costs and in anaerobic decomposition with its attendant nitrogen loss. The energy input required to dry the plants is too great to be practical for general purpose as fertilizer and plant nutrient content is too low on a fresh weight basis to be practical for field application (4).

Wang (37) studied the site specific of barium toxicity to common duckweeds *L.minor* and found that *L.minor* in different sites of water had differences in the net increase in nutrients of fronds. He also suggested that barium toxicity was

dependence on site specific and water quality characteristics. At the current water quality standard of 5 mg/L barium is considered sufficiently stringent to protect common duckweeds in all water tested.

Staves and Knaus (35) studied the chromium removal from water by three species of duckweeds *(Spirodela punctata, S. polurtriza, L. gibba)* and found that there were distinct differences in chromium tolerance among the three species tested, depending on the chromium concentration and length of exposure.

Glandon and Mc Nabb (38) reported that the laboratory data showed that the accumulation of carbon by *L. minor* was related to the environmental contamination. The ambient concentration of boron determined the total amount of boron taken up over one week interval. The tissue concentration at the end of experiments reflected different uptake rates . They also concluded that the ability to concentrate boron appeared to be a feature of six species within the genus *Lemna.*

Hangovan and Miranda (39) studied the uptake of lead by *L. gibba* which had the influence on specific growth rate and basic biochemical changes. They found that a significant rate of uptake was noticed during the initial 24 hours of growth. The low concentration of lead (30-50 mg/L) did not inhibit the specific growth rate of the plant under continuous illumination at 7 days of growth when compared to discontinuous illumination. No significant chlorotic symptoms were noticed in the plants treated with 30, 50, 100, and 200 mg/L of lead ,but were prominent at 500 mg/L resulting in reduced total chlorophyll concentration. They suggested that *L. gibba* could be used in tertiary treatment plants to remove heavy metals.

CHAPTER IV

MATERIALS AND METHODS

4.1 Preliminary Study

All experiments were performed with *Lemna minor* collected from natural ponds and kept in reservoirs in the greenhouse of the Faculty of Science, Mahidol University. The experiments were carried out in the laboratory under controlled conditions. The duckweed tests were conducted by using 200 ml circular mouth jars.

4.1.1 Preliminary test for for lead nitrate solution application

A series of lead nitrate solutions were prepared in various concentrations for the preliminary testing as follows:

Jar Number.	Lead nitrate solution concentration (mg/L)
1 (control)	0
2	10
3	30
4	50
5	100
6	200
7	400
8	500

Each experimental condition was done in three replicates and incubated at room temperatures (25° - 27° C) under a 40 watt cool-white fluorescent illumination for 12 days.

4.1.2 Preliminary testing for humic acid solution application

A series of combined humic acid and lead nitrate solutions were prepared in various concentrations for the preliminary testing as follows :

Jar number	Lead nitrate solution (mg/L)	Humic acid solution (mg/L)
1	50	5
2	50	10
3	50	20
4	50	40
5	50	80
6	50	160
7	100	5
8	100	10
9	100	20
10	100	40
11	100	80
12	100	160
13	100	5
14	200	10
15	200	20
16	200	40
17	200	80
18	200	160

All jars were incubated for 12 days under the same conditions as described previously for lead nitrate solution application.

Every three days, plant samples were collected to determine the effect of lead and combined effects of lead with humic acid on *L. minor* by frond counting. The

solution concentrations that gave a distinct effect on frond number were selected for the subsequent solution testing.

4.2 Cultivation of Plants

Plant specimens, *L. minor* were selected from the stock culture 24 hours before the test began. The selection criteria were that the plants were healthy-looking and had two fronds of approximately equal size.

L. minor were cultured in a modified Steenberg medium (40), diluted to one quarter strength and maintained at pH 6.0 ±0.5. This medium was used for all culturing and experimental stages. The composition of this medium was as follows:

KNO_3 0.72 mM	$MgSO_4 7H_2O$ 0.101 mM
$Ca(NO_3)_2 4H_2O$ 0.312 mM	KH_2PO_4 0.184 mM
H_3BO_3 4.9 mM	$MnCl_2H_2O$ 2.3 μM
$(NH_4)_6Mo_7O_{25}$ $4H_2O$ 0.071 mM	$CuSO_4.5H_2O$ 0.5 μM
$ZnSO_4 7H_2O$ 1.6 μM	EDTA-ferric monosodium 6.8 μM

4.3 Experimental Design

4.3.1 Combined effects of lead on *L. minor*

L. minor were taken from the stock culture and rinsed with distilled water in a sieve and placed on absorbent papers to remove excess water. Fifteen plants with thirty fronds were transferred to each jar, which contained 100 ml of a control (metal free) and 30, 50, 100 and 200 mg/L of lead nitrate solution. Three replicates were prepared for each concentration. The jars were placed under a 40 watt cool-white fluorescent continuous light. The medium in each jar was renewed every

48 hours in order to maintain both nutrient and lead concentrations, and the volume of testing solution was adjusted to 100 ml every day. The room temperature was 25 - 27° C. On the 3^{rd} , 6^{th} , 9^{th} and 12^{th} days, the samples were analyzed for total chlorophyll content, total fresh weight, total frond number and frond's visible symptoms were recorded. Then the samples were analyzed for lead content. The experimental period was 12 days.

4.3.2 Combined effects of lead and humic acid on *L. minor*

All conditions of this experiment were set up similar to those of 4.3.1, but each concentration (control, 50, 100, and 200 mg/L) of lead nitrate solution was combined with humic acid solution concentrations as follows:

Lead nitrate solution	Humic acid
50	10
50	20
50	40
50	80
50	160
100	10
100	20
100	40
100	80
100	160
200	10
200	20
200	40
200	80
200	160

On the 3rd , 6th , 9th , and 12th days, the samples were analyzed for total chlorophyll content, total fresh weight, total frond number and frond's visible symptoms were recorded. Then the samples were analyzed for lead content. The experimental period was 12 days.

4.4 Analytical Method

Samples collected from each observation were analyzed for the following parameters:

4.4.1 Total chlorophyll measurement

Chlorophyll from *L. minor* was extracted by grinding the leaves in a tissue grinder in 90% acetone. The homogenate was quantified by transferring to 3 ml centrifuge tube and centrifuged at 2,000 rpm for 10 minutes. The sample was filtered through a Whatman No. 42 filter paper. The filtered extract was transferred to a 3 ml tube and adjusted the volume to 3 ml with 90 % acetone.

The optical density of the extract was measured at 663 nm (D663) and 645 nm (D645), respectively, by using a quartz glass cuvette with 1 cm thickness by a spectrophotometer(BioSpec-1601). This position was in the spectrum where maximum absorption by chlorophyll a, b occurred. The instrument was set at 100 % transmittance with a tube containing 90% acetone. Readings were made with the solution of pigments. The cuvette was rinsed with 90% acetone after each use. According to Mackinney (41), the concentration of chlorophyll a and b in mg/L of sample was calculated by the formula

Chlorophyll a (mg/L)	=	12.7(A663) - 2.69(A645)
Chlorophyll b (mg/L)	=	22.9(A645) - 4.68(A663)
Total chlorophyll(mg/L)	=	8.02(A633) + 20.2(A645)

4.4.2 Growth rate

The calculation of growth rate (g/g/d) was done according to the method of Porath *et al.* (42)

$$R = \frac{\ln Wt/Wo}{T}$$

ln = natural logarithm

Wo = weight of the plant at the zero time of treatment

Wt = weight of the plant at time t

T = time of treatment

4.4.3 Multiplication rate (43)

$MR = 1000(\log f_t - \log f_o)/t$

f_t = number of frond at time t

f_o = number of frond at initial

t = time in days

4.4.4 Determination of lead content using hot concentrated acid extraction

The procedures for the decomposition of plant material were performed according to Knause and Keutz (44, 45) prior to the determination of trace metals by an atomic absorption spectrophotometer as follows:

- Transferred 200 mg of finely ground plant material into 10 ml Pyrex screw cap test tube containing 10 ml of 3:1 hydrochloric and nitric acid mixture.

- Placed the tube in 3/4 water filled beaker on a hot plate and heated below the boiling point about 4 hours, or until ensure that digestion is completed (generally indicated by a light colored residue).

- Removed the beaker from the hot plate and cooled to room temperature.

- Filtered sample through a Whatman No. 42 filter paper to remove the silicate and other insoluble materials that could clog the atomizer and adjusted volume to 50 ml.

- The sample was now ready for analysis with the atomic absorption spectrophotometer(PERKIN ELMER Atomic Absorbtion Spectrophotometer 3100).

CHAPTER V

RESULTS

5.1 Effects of Lead on *Lemna minor*

5.1.1 Total chlorophyll contents

The effects of lead on total chlorophyll contents of *L. minor* are shown in Table 5-1 and Figs. 5-1 to 5-4. In the control and at lead concentrations of 30, 50 mg/L, the total chlorophyll contents were subsequently increased ($P \leq 0.05$) from day 3 to day 12. They were 37.01 mg/g(w/w), 22.85 mg/g and 14.4 mg/g, in the control, 30 mg/L and 50 mg/L, respectively. At lead nitrate concentration of 100 mg/L, the total chlorophyll content was significantly decreased ($P \leq 0.05$) from 9.48 mg/g on day 9 to 4.07 mg/g on day 12. At lead concentration of 200 mg/L, the total chlorophyll content was significantly decreased ($P \leq 0.05$) from 5.81 mg/g on day 6 to 2.47 mg/g and 1.84 mg/g on day 9 and 12, respectively.

5.1.2 Growth rate

The effects of lead on the growth rate of *L. minor* are shown in Table 5-2 and Fig. 5-5. In the control and at lead concentrations of 30, 50 and 100 mg/L, the growth rates subsequently increased from day 3 to day 12. They were 0.14, 0.13, 0.13 and 0.08 g/g/d in the control, 30 mg/L, 50 mg/L and 100 mg/L, respectively. But at the concentration of 200 mg/L, the growth rate significantly decreased ($P \leq 0.05$)

from 0.04 on day 6 to 0.03 and –0.02 g/g/d on day 9 and 12, respectively. The lowest growth rate was found at the concentration of 200 mg/L (-0.02 g/g/d), and the highest was found in the control (0.14 g/g/d)

5.1.3 Multiplication rate

The effects of lead on the multiplication rate of *L. minor* are shown in Table 5-3 and Fig. 5-6. In the control and at lead concentrations of 30, 50 and 100 mg/L, the multiplication rate at every lead concentration was subsequently increased ($P \leq 0.05$) from day 3 to day 12. They were 391.33, 397.56 and 379.31 in the control, 30 mg/L and 50 mg/L, respectively. But at the concentrations of 100 mg/L and 200 mg/L, the multiplication rates were significantly decreased ($P \leq 0.05$) from day 3 to the minimum level on day 12. They were 72.89 and 42.72 in 100 and 200 mg/L, respectively. The highest multiplication rate was found in the control (391.33) and the lowest multiplication rate was found in the plants treated with 200 mg/L of lead nitrate on day 12 (42.72).

5.1.4 Lead uptake

The lead uptake by *L. minor* is shown in Table 5-4 and Fig. 5-7. The lead contents in *L. minor* increased with the increase of lead nitrate concentration ($P \leq 0.05$). At the concentration of 30 mg/L, the lead content increased to the maximum level on day 12 (2.21 and 4.28 mg/g, respectively). At the concentrations of 50, 100 and 200 mg/L, the lead contents increased to the maximum level (2.37, 7.19 and

22.10 mg/g, respectively) on day 6. The lead contents in the controls at all observation times were nearly zero.

5.1.5 Morphological changes

The multiplication of *L.minor* is shown in Fig. 5-8. The number of frond became double every three days, and within 12 days, they completely covered the water surface. *L. minor* in the control (Fig. 5-8), 30 mg/L (Fig. 5-9), 50 mg/L (Fig. 5-10) did not show any morphological changes. But at the concentration of 100 mg/L of lead nitrate, chlorosis occurred on day 9 especially on mature fronds (Fig. 5-11). It did not occur in younger fronds. In comparison, the number of chlorotic frond on day 12 was more than that on day 9 (Fig. 5-11). At the concentration of 200 mg/L, no morphological changes were observed on day 3 (Fig. 5-12). Most fronds became chlorotic on day 6 except for younger leaves which became chlorotic on day 12. A large number of colonies broke up and a loss of buoyancy was found in the fronds (Fig. 5-12).

5.2 Combined effects of Lead and Humic Acid on *L. minor*

5.2.1 Total chlorophyll contents

In lead nitrate (50 mg/L) and lead nitrate (50 mg/L) combined with humic acid (10, 20, 40, 80 and 160 mg/L), the total chlorophyll contents were increased ($P \leq 0.05$) to the maximum level on day 12. They were 18.9, 29.76, 32.74, 52.08, 37.40, 33.04 mg/g in 50/0, 50/10, 50/20, 50/40, 50/80 and 50/160 mg/L, respectively (Table 5-5, Figs. 5-13 to 5-16). In comparison, they were not significantly different ($P \leq 0.05$) at each concentration of humic acid on day 3, 6, 9 and 12.

At lead nitrate concentration of 100 mg/L combined with various concentrations of humic acid, the total chlorophyll contents were subsequently decreased ($P \leq 0.05$) from day 3 to the minimum level on day 12 (Table 5-6. Figs. 5-17 to 5-20). They were 4.07, 12.73, 10.28, 11.08, 9.46, 10.33 mg/g, in 100/0, 100/10, 100/20, 100/40, 100/80 and 100/160 mg/L, respectively (Table 5-6, Fig. 5-20). The comparison of every concentration on day 3, 6, 9 and 12 showed that the maximum total chlorophyll content was found at the concentration of 100/160 mg/L (17.81mg/g) on day 6 (Table 5-6, Fig. 5-18). The lowest total chlorophyll content (4.07 mg/g) was found in the control on day 12 (Table 5-6, Fig. 5-20).

At lead nitrate concentration of 200 mg/L, combined with various concentrations of humic acid (10, 20, and 40 mg/L), the total chlorophyll contents were subsequently decreased ($P \leq 0.05$) from day 3 to the minimum level on day 12 (Table 5-7, Figs. 5-21 to 5-24). They were 1.14, 2.51, 1.58 and 1.61 mg/g in 200/0, 200/10, 200/20 and 200/40 mg/L, respectively (Table 5-7, Fig 5-24). But at the

concentration of 200/80 mg/L, the total chlorophyll content was increased ($P \leq 0.05$) from 3.25 mg/g on day 3 to the maximum (12.13 mg/g) on day 12 (Table 5-7, Figs. 5-21 to 5-24). Similarly, the total chlorophyll content at 200/160 mg/L was increased from 8.07 mg/g on day 3 to the maximum level of 13.59 mg/g on day 12 (Table 5-7, Figs. 5-21 to 5-24).

5.2.2 Growth rate

At lead nitrate concentration of 50 mg/L, the growth rate of *L. minor* slowly decreased ($P \leq 0.05$) from 0.3 g/g/d on day 3 to the minimum of 0.12 g/g/d on day 12 (Table 5-8, Fig 5-25). This is significantly similar to those of lead nitrate combined with various concentrations of humic acid whose growth rates slowly increased ($P \leq 0.05$) to the maximum level on day 12. They were 0.18, 0.21, 0.2 g/g/d, at 50/10, 50/20 and 50/40 mg/L, respectively (Table 5-8, Fig. 5-25). The highest growth rates were found at the concentration of 50/160 mg/L. They were 0.23, 0.22 g/g/d, on day 6 and day 9, respectively (Table 5-8).

At lead nitrate concentration of 100 mg/L, most of the growth rates of *L. minor* slowly decreased from day 3 to day 12. They were 0.10, 0.08, 0.09, 0.08 g/g/d, at the 100/10, 100/20, 100/40, 100/80 and 100/160 mg/L, respectively (Table 5-9, Fig 5-26). The highest growth rate (0.16 g/g/d) was found at the concentration of 100/160 mg/L on day 3 (Table 5-9).

At lead nitrate concentration of 200 mg/L, most of the growth rates of *L. minor* subsequently decreased ($P \leq 0.05$) to the minimum level on day 12. They were –0.02, 0.02, 0.00, 0.02, 0.02 g/g/d, in 200/0, 200/10, 200/20, 200/40 and 200/80

mg/L, respectively (Table 5-10, Fig. 5-27). But at the concentration of 200/160 mg/L, the growth rate was increased ($P \leq 0.05$) to the maximum (0.14 g/g/d) on day 6 (Table 5-10). The comparison of every concentration on day 6, 9, 12 showed the highest growth rates at the concentration of 200/160 mg/L. They were 0.14, 0.13, 0.07 g/g/d on day 6, 9 and 12, respectively (Table 5-10).

5.2.3 Multiplication rate

At lead nitrate concentration of 50 mg/L, the multiplication rates at every concentration of lead combined with humic acid was subsequently increased ($P \leq 0.05$) from day 3. They were 326.11, 405.22, 443, 417.22, 337.22 and 396.18 in 50/0, 50/10, 50/20, 50/40, 50/80, and 50/160 mg/L, respectively (Table 5-11, Fig. 5-28). The comparison of every concentration showed that the multiplication rates were not significantly different ($P \leq 0.05$) on day 3, 6, 9 and 12 (Table 5-11, Fig 5-28).

At lead nitrate concentration of 100 mg/L, the multiplication rates subsequently decreased ($P \leq 0.05$) from day 3 to the minimum level on day 12. They were 72.89, 71.08, 69.81, 28.11, 64.08 and 75.31 in 100/0, 100/10, 100/20, 100/40, 100/80 and 100/160 mg/L, respectively (Table 5-12, Fig. 5-29).

At lead nitrate concentration of 200 mg/L, the multiplication rates at every concentration of humic acid slowly increased ($P \leq 0.05$) to the maximum level on day 6. They were 85.17, 101.14, 96.78, 96.17, 90 and 115.44 in 200/0, 200/10, 200/20, 200/40, 200/80 and 200/160 mg/L, respectively (Table 5-28, Fig. 5-30). The comparison of every concentration on day 6, 9, 12 showed the highest multiplication rates at the concentration of 200/160 mg/L. They were 115, 91.81 and 71.44 on day 6, 9 and 12, respectively (Table 5-13, Fig. 5-30).

5.2.4 Lead uptake

At lead nitrate concentration of 50 mg/L, most of lead content in *L.minor* was increased ($P \leq 0.05$) to the maximum level on day 6. They were 2.34, 1.43, 1.57, 1.8, 1.19 mg/g in 50/0, 50/10, 50/20, 50/40, and 50/80 mg/L respectively (Table 5-14, Fig. 5-31). The comparison of every concentration on day 6 and 9 showed the lowest lead content at the concentration of 50/160 mg/L. They were 0.44 mg/g and 0.58 mg/g on day 6 and 9, respectively (Table 5-14).

At lead nitrate concentration of 100 mg/L, most of lead content in *L. minor* was increased ($P \leq 0.05$) to the maximum level on day 6. They were 7.19, 4.57, 3.14, 4.75 mg/g in 100/0, 100/10, 100/20, 100/40 mg/L, respectively (Table 5-15, Fig. 5-32).Then they were decreased to 1.82, 2.28, 2.07 and 3.44 mg/g on day 12 (Table 5-15). The comparison of every concentration on day 3, 6, 9, 12 showed the highest lead content (7.19 mg/g) in the control on day 6 (Table 5-15). The lowest lead content on day 6, 9 and 12 (1.48, 1.64, 1.91 mg/g, respectively) was found at the concentration of 100/160 mg/L (Table 5-25, Fig. 5-32).

At lead nitrate concentration of 200 mg/L, the lead content was subsequently increased ($P \leq 0.05$) from day 3 to the maximum level on day 12. They were 25.29, 43.52, 42.92, 23.18 and 7.95 mg/g in 200/0, 200/10, 200/20, 200/40, 200/80 and 200/160 mg/L, respectively. (Table 5-16, Fig. 5-33). The comparison of every concentration on day 3, 6, 9, 12 showed the lowest lead content at the concentration of 200/160 mg/L. They were 0.00, 2.29, 3.27, 7.95 mg/g on day 3, 6, 9, 12, respectively (Table 5-16, Fig. 5-33).

5.2.5 Morphological changes

L. minor exposed to lead nitrate solution at 50 mg/L combined with humic acid of every concentration did not show any morphological changes (Fig. 5-34). At lead nitrate concentration of 100 mg/L combined with humic acid of every concentration, *L. minor* showed morphological changes similar to those exposed to lead nitrate solution alone at 100 mg/L (Figs. 5-35 to 5-36). At lead nitrate concentration of 200 mg/L combined with humic acid of every concentration, *L. minor* showed chlorotic fronds on day 6 (Figs. 5-37 to 5-39). The colonies were broken up and the loss of buoyancy of frond occurred on day 9 and these were increased on day 12. In comparison, chlorotic fronds, loss of buoyancy, breaking up of colonies were decreased with the increase of humic acid concentration. At the concentration of 200/160 mg/L, most fronds were still normal and floated on the water surface (Fig. 5-39).

Table 5-1. Effect of lead on total chlorophyll content of *L. minor*.

Lead nitrate concentration (mg/L)	Chlorophyll content (mg/g)														
	Day 0			Day 3			Day 6			Day 9			Day 12		
	Chloro phyll a	Chloro phyll b	Total chloro phyll	Chloro phyll a	Chloro phyll b	Total chloro phyll	Chloro phyll a	Chloro phyll b	Total chloro phyll	Chloro phyll a	Chloro phyll b	Total chloro phyll	Chloro phyll a	Chloro phyll b	Total chloro phyll
Control (metal free)	1.32 ±.08	0.87 ±.07	2.20 ±.23	1.43 ±.08	0.34 ±.07	2.77 ±.23	5.94 ±.53	3.48 ±.38	9.46 ±.91	6.97 ±.21	3.28 ±.28	10.33± .21	21.08± 1.39	15.66± 2.95	37.01± 2.49
30	1.32 ±.08	0.87 ±.07	2.20 ±.23	2.48 ±.08	1.37 ±.08	3.87 ±.16	4.63 ±.35	2.39 ±.40	7.08 ±.73	8.04 ±.89	3.62 ±.32	11.74± 1.19	15.48± .34	7.22 ±.46	22.85± .49
50	1.32 ±.08	0.87 ±.07	2.20 ±.23	2.60 ±.15	1.81 ±.30	4.45 ±.20	4.70 ±.38	1.92 ±.17	6.65 ±.55	6.04 ±1.43	2.98 ±.56	9.08 ±2.00	9.50 ±.32	4.48 ±.05	14.44± .36
100	1.32 ±.08	0.87 ±.07	2.20 ±.23	2.79 ±.18	1.64 ±.16	4.46 ±.33	3.49 ±.25	1.56 ±.10	5.09 ±.35	6.09 ±.81	3.32 ±.36	9.48 ±1.16	2.48 ±.56	1.56 ±.51	4.07 ±1.08
200	1.32 ±.08	0.87 ±.07	2.20 ±.23	2.28 ±.11	1.33 ±.09	3.64 ±.20	3.76 ±.05	2.01 ±.22	5.81 ±.20	1.57 ±.53	0.88 ±.38	2.47 ±.91	1.30 ±.01	0.52 ±.01	1.84 ±.01

Table 5-2. Effect of lead on growth rate of *L. minor*.

Lead nitrate concentration (mg/L)	Growth rate (g/g/d)				
	Day 0	Day 3	Day 6	Day 9	Day 12
Control (metal free)	0	0.05 ±.01	0.13 ±.03	0.09 ±.01	0.14 ±.01
30	0	0.06 ±.01	0.08 ±.01	0.12 ±.01	0.13 ±.01
50	0	0.03 ±.01	0.06 ±.01	0.10 ±.02	0.13 ±.01
100	0	0.04 ±.01	0.05 ±.01	0.08 ±.02	0.08 ±.01
200	0	0.01 ±.01	0.04 ±.01	0.03 ±.01	-0.02 ±.01

Table 5-3. Effect of lead on multiplication rate of *L. minor*.

Lead nitrate concentration (mg/L)	Multiplication rate				
	Day 0	Day 3	Day 6	Day 9	Day 12
Control (metal free)	0	204.89 ±18.24	237.11 ±3.19	290.11 ±8.50	391.33 ±1.64
30	0	187.67 ±3.76	225.22 ±10.38	334.44 ±1.64	397.56 ±4.59
50	0	189 ±2.65	218.89 ±6.35	289.33 ±9.45	379.31 ±6.90
100	0	208.44 ±1.50	104.22 ±11.57	80.28 ±.81	72.89 ±2.54
200	0	41.68 ±1.20	85.17 ±1.32	64.25 ±4.17	42.72 ±1.90

Table 5-4. Lead uptake by *L. minor* exposed to lead nitrate solution.

Lead nitrate concentration (mg/L)	Lead content (mg/g/)				
	Day 0	Day 3	Day 6	Day 9	Day 12
Control (metal free)	0.00 ±.01	0.00 ±.01	0.02 ±.02	0.03 ±.02	0.04 ±.03
30	0.00 ±.01	1.03 ±.39	1.31 ±.69	1.24 ±.46	2.21 ±.71
50	0.00 ±.01	1.32 ±.23	2.37 ±.74	1.2 ±.36	4.28 ±2.40
100	0.00 ±.01	2.19 ±.18	7.19 ±1.46	4.67 ±.98	1.82 ±.57
200	0.00 ±.01	10.86 ±2.10	22.10 ±3.49	17.25 ±7.53	13.49 ±5.61

Table 5-5. Influence of humic acid on the toxic effect lead (50 mg/L) on chlorophyll content of *L. minor*.

Concentration of lead nitrate/humic acid (mg/L)	Chlorophyll content (mg/g)														
	Day 0			Day 3			Day 6			Day 9			Day 12		
	Chloro phyll a	Chloro phyll b	Total chloro phyll	Chloro phyll a	Chloro phyll b	Total chloro phyll	Chloro phyll a	Chlor ophyll b	Total chloro phyll	Chloro phyll a	Chloro phyll b	Total chloro phyll	Chloro phyll a	Chloro phyll b	Total chloro phyll
50/0	1.32 ±.08	0.87 ±.07	2.20 ±.23	1.54 ±.13	0.88 ±.16	2.45 ± 28	1.13 ±.11	0.68 ± 12	1.82 ±.23	6.04 ±1.43	2.98 ±.56	9.08 ±2.00	12.84 ±1.07	5.93 ±1.91	18.90 ±1.91
50/10	1.32 ±.08	0.87 ±.07	2.20 ±.23	1.32 ±.10	0.87 ±.15	2.20 ±.25	1.90 ±.22	1.03 ± 12	2.95 ±.34	17.27 ±1.33	5.14 ±.82	22.55 ±1.27	29.76 ±2.11	11.03 ±1.47	29.76 ±2.11
50/20	1.32 ±.08	0.87 ±.07	2.20 ±.23	1.41 ±.11	0.84 ±.09	2.27 ±.20	1.86 ±.05	1.07 ±.21	2 96 ±.26	16.55 ±1 89	6.97 ±1.36	23.67 ±3.27	19.28 ±1.00	13.22 ±1.43	32.74 ±.74
50/40	1.32 ±.08	0.87 ±.07	2.20 ±.23	1.33 ±.35	0.56 ±.14	1.91 ±.48	1.60 ± 11	1.00 ± 07	2 61 ± 17	17.10 ±1.08	5.96 ±.48	23.20 ±1.54	23.06 ±.10	28.58 ±1.16	52.08 ±1.17
50/80	1.32 ±.08	0.87 ±.07	2.20 ±.23	2.98 ±.28	1.50 ±.13	4.51 ±.40	1.04 ±.36	0.54 ±.23	1 59 ±.60	13.47 ±1.25	4.92 ±.58	18.50 ±1.72	24.17 ±1.48	12.98 ±.50	37.40 ±1.00
50/160	1.32 ±.08	0.87 ±.07	2.20 ±.23	6.97 ±1.24	2.59 ±.26	9.62 ±1.02	5.25 ± 65	2.80 ±.70	8.10 ±1.36	12.62 ±5.49	6.13 ±3.17	18.88 ±8.69	21.70 ±.87	11.11 ±.47	33.04 ±1.30

Table 5-6. Influence of humic acid on the toxic effect of lead (100 mg/L) on chlorophyll content of *L. minor*.

Concentration of lead nitrate/humic acid (mg/L)	Chlorophyll content (mg/g)														
	Day 0			Day 3			Day 6			Day 9			Day 12		
	Chloro phyll a	Chloro phyll b	Total chloro phyll	Chloro phyll a	Chloro phyll b	Total chloro phyll	Chloro phyll a	Chloro phyll b	Total chloro phyll	Chloro phyll a	Chloro phyll b	Total chloro phyll	Chloro phyll a	Chloro phyll b	Total chloro phyll
100/ 0	1.32 ±.08	0.87 ±.07	2.2 ±.23	3.75 ±1.06	2.38 ±.84	6.17 ±1.79	6.41 ±.68	3.14 ±.46	9.72 ±.98	4.42 ±.49	1.53 ±.13	5.98 ±.45	2.48 ±.56	1.56 ±.51	4.07 ±1.08
100/10	1.32 ±.08	0.87 ±.07	2.2 ±.23	5.56 ± 86	3.8 ±.98	9.5 ±1.82	6.42 ±.36	3.24 ±.20	9.73 ± 56	5.9 ±.61	1.48 ±.22	7.42 ±.8	8.72 ±.46	3.93 ±.28	12.73 ±.74
100/20	1.32 ±.08	0.87 ±.07	2.2 ±.23	9.28 ±.42	6.47 ±.64	15.87 ±.58	6.48 ±.87	3.36 ±.63	9.90 ±1.51	6.28 ±.86	2.44 ±.62	8.77 ±1.45	6.84 ±.82	3.33 ±.49	10.28 ±1.29
100/40	1.32 ±.08	0.87 ±.07	2.2 ±.23	8.91 ±3.69	5.66 ±2.62	13.96 ±6.63	6.58 ±.23	3.8 ±.40	10.46 ± 62	6.78 ±.72	2.65 ±.93	9.49 ±1.64	7.46 ±1.83	3.55 ±.89	11.08 ±2.74
100/80	1.32 ±.08	0.87 ±.07	2.2 ±.23	8.65 ±.46	4.81 ±.21	13.55 ±.54	7.1 ± 68	5 27 ±1.11	12.47 ±1.63	5.95 ±3.00	3 27 ±1.44	9.28 ±4.46	7.36 ±.3	2.04 ±1.00	9.46 ±1.14
100/160	1.32 ±.08	0 87 ±.07	2.2 ±.23	8.14 ±.56	1.89 ±.14	10.09 ±.40	12 33 ± 69	5.36 ±1 82	17.81 ±2 54	10.39 ± 05	4.25 ±.89	14.74 ±2.78	7.29 ±.27	2.97 ±4.00	10.33 ±.43

Table 5-7. Influence of humic acid on the toxic effect of lead (200 mg/L) on chlorophyll content of *L. minor*.

Concentration of lead nitrate/humic acid (mg/L)	Chlorophyll content (mg/g)														
	Day 0			Day 3			Day 6			Day 9			Day 12		
	Chloro phyll a	Chloro phyll b	Total chloro phyll	Chloro phyll a	Chloro phyll b	Total chloro phyll	Chloro phyll a	Chloro phyll b	Total chloro phyll	Chloro phyll a	Chloro phyll b	Total chloro phyll	Chloro phyll a	Chloro phyll b	Total chloro phyll
200/0	1.32 ±.08	0.87 ±.07	2.20 ±.23	2.75 ±.12	0.50 ±.02	3.28 ±.11	2.46 ± 09	1.40 ± 13	3.88 ±.22	1.22 ±.12	0.62 ±.10	1.85 ±.22	0.76 ±.23	0.37 ±.12	1.14 ±3.5
200/10	1.32 ±.08	0.87 ±.07	2.20 ±.23	2.95 ±.19	0.67 ±.11	3.63 ±.08	2.5 ±.07	1.45 ±.25	3.98 ±.31	1.53 ±.21	0.71 ±.14	2.25 ±.4	1.66 ±.19	0.83 ±.12	2.51 ±.31
200/20	1.32 ±.08	0.87 ±.07	2.20 ±.23	4.42 ±.58	1.53 ±.25	5.90 ±.5	2.35 ± 61	1 77 ±.42	4.15 ±.42	1.67 ±.47	0.68 ±.17	2.36 ±.65	1.06 ±.08	0.51 ±.05	1.58 ±.13
200/40	1.32 ±.08	0.87 ±.07	2.20 ±.23	3.10 ±.71	0.81 ±.22	3.93 ±.93	2.73 ±.10	1 05 ± 04	3.80 ± 13	2.80 ±.65	1 15 ±.19	3.97 ±.84	0.96 ± 07	0.64 ±.13	1.61 ±.20
200/80	1.32 ±.08	0.87 ±.07	2.20 ±.23	2.63 ±.08	0.60 ±.01	3.25 ±.08	3.46 ±1 24	1 10 ±.56	4 61 ± 70	2 62 ±1.16	1.06 ±.36	3.70 ±1.53	8.34 ±3.77	3.7 ±.58	12.13 ±5.38
200/160	1.32 ±.08	0.87 ±.07	2.20 ±.23	5.38 ±1.15	2.64 ±.47	8.07 ±1.6	9 44 ±.96	3 90 ±.41	13.43 ±1.38	5.37 ±1.13	2.68 ±.56	8.11 ±1.7	9 45 ±.40	4.05 ±.14	13.59 ±.55

Table 5-8. Influence of humic acid on the toxic effect of lead (50 mg/L) on growth rate of *L. minor*.

Concentration of lead nitrate/ humic acid (mg/L)	Growth rate (g/g/d)				
	Day 0	Day 3	Day 6	Day 9	Day 12
50/0	0	0.03±.01	0.06±.01	0.12±.02	0.12±.02
50/10	0	0.11±.00	0.17±.01	0.20±.01	0.18±.00
50/20	0	0.15±.01	0.18±.01	0.19±.01	0.21±.01
50/40	0	0.15±.02	0.12±.02	0.2±.00	0.2±.00
50/80	0	0.24±.01	0.11±.04	0.14±.01	0.13±.00
50/160	0	0.13±.01	0.23±.01	0.22±.01	0.18±.01

Table 5-9. Influence of humic acid on the toxic effect of lead (100 mg/L) on growth rate of *L. minor*.

Concentration of lead nitrate/ humic acid (mg/L)	Growth rate (g/g/d)				
	Day 0	Day 3	Day 6	Day 9	Day 12
100/0	0	0.05±.01	0.09±.03	0.10±.01	0.11±.01
100/10	0	0.11±.02	0.12±.01	0.10±.01	0.10±.01
100/20	0	0.13±.02	0.13±.01	0.13±.01	0.08±.01
100/40	0	0.13±.01	0.12±.01	0.09±.01	0.08±.01
100/80	0	0.15±.04	0.06±.02	0.04±.01	0.09±.01
100/160	0	0.16±.01	0.15±.01	0.13±.02	0.08±.04

Table 5-10. Influence of humic acid on the toxic effect of lead (200 mg/L) on growth rate of *L. minor*.

Concentration of lead nitrate/ humic acid (mg/L)	Growth rate (g/g/d)				
	Day 0	Day 3	Day 6	Day 9	Day 12
200/0	0	0.04 ±.02	-0.01 ±.03	-0.01 ±.01	-0.02 ±.01
200/10	0	0.03 ±.01	0.04 ±.01	0.00 ±.01	0.02 ±.01
200/20	0	0.04 ±.03	0.06 ±.01	0.01 ±.01	0.00 ±.03
200/40	0	-0.07 ±.07	0.02 ±.02	0.01 ±.02	0.02 ±.01
200/80	0	0.06 ±.04	0.02 ±.01	0.02 ±.02	0.02 ±.01
200/160	0	-0.02 ±.01	0.14 ±.02	0.13 ±.03	0.07 ±.01

Table 5-11. Influence of humic acid on the toxic effect of lead (50 mg/L) on multiplication rate of *L. minor*.

Concentration of lead nitrate/ humic acid (mg/L)	Multiplication rate				
	Day 0	Day 3	Day 6	Day 9	Day 12
50/0	0	197.7 ±11.41	206.78 ±6.76	284.44 ±20.17	326.11 ±20.96
50/10	0	189.67 ±2.36	269.33 ±3.93	361.22 ±9.17	405.22 ±.01
50/20	0	272.56 ±76.73	275 ±9.62	304.04 ±45.43	443 ±7.84
50/40	0	194.11 ±1.49	244.67 ±18.05	354.11 ±3.97	417.22 ±6.28
50/80	0	205.73 ±3.42	230.22 ±8.54	301.89 ±4.49	337.22 ±2.08
50/160	0	199.78 ±.89	280.33 ±3.84	349.44 ±5.97	396.18 ±9.98

Table 5-12. Influence of humic acid on the toxic effect of lead (100 mg/L) on multiplication rate of *L. minor*.

Concentration of lead nitrate/ humic acid (mg/L)	Multiplication rate				
	Day 0	Day 3	Day 6	Day 9	Day 12
100/0	0	208.44 ±1.56	104.22 ±11.57	80.48 ±.81	72.89 ±2.54
100/10	0	199.56 ±6.76	115.17 ±3.71	84.81 ±2.91	71.08 ±.73
100/20	0	196.67 ±1.67	114.67 ±2.40	90.96 ±3.25	69.81 ±1.14
100/40	0	195.78 ±1.44	111.17 ±4.87	81.26 ±3.87	68.11 ±2.48
100/80	0	193.89 ±2.0	98.67 ±2.0	63.93 ±1.53	64.08 ±.12
100/160	0	191.11 ±1.18	109.56 ±.72	90.56 ±3.46	75.31 ±3.19

Table 5-13. Influence of humic acid on the toxic effect of lead (200 mg/L) on multiplication rate of *L. minor*.

Concentration of lead nitrate/ humic acid (mg/L)	Multiplication rate				
	Day 0	Day 3	Day 6	Day 9	Day 12
200/0	0	41.08 ±1.2	85.17 ±1.32	64.52 ±4.17	42.72 ±1.90
200/10	0	37.22 ±2.57	101.14 ±10.41	75.58 ±.72	60.03 ±13.87
200/20	0	41.97 ±.77	96.78 ±2.06	78.41 ±.41	54.69 ±3.78
200/40	0	36.61 ±1.27	96.17 ±1.69	78.26 ±1.64	56.14 ±5.75
200/80	0	40.44 ±1.13	90.00 ±3.62	74.54 ±3.06	51.69 ±1.36
200/160	0	58.22 ±12.24	115.44 ±8.88	91.81 ±4.84	71.44 ±1.97

Table 5-14. Influence of humic acid on lead uptake by *L. minor* exposed to lead nitrate concentration of 50 mg/L.

Concentration of lead nitrate/ humic acid (mg/L)	Lead content (mg/g/)				
	Day 0	Day 3	Day 6	Day 9	Day 12
50/0	0.00±.01	1.32±.23	2.34±.47	1.25±.36	0.63±.20
50/10	0.00±.01	1.17±.15	1.43±.27	1.25±.36	0.45±.05
50/20	0.00±.01	0.44±.22	1.57±.35	1.88±.70	0.22±.04
50/40	0.00±.01	0.68±.07	1.8±.18	1.28±.15	0.44±.21
50/80	0.00±.01	0.71±.14	1.19±.04	0.73±.10	0.31±.13
50/160	0.00±.01	0.81±.08	0.44±.05	0.58±.08	0.97±.23

Table 5-15. Influence of humic acid on lead uptake by *L. minor* exposed to lead nitrate concentration of 100 mg/L.

Concentration of lead nitrate/ humic acid (mg/L)	Lead content (mg/g)				
	Day 0	Day 3	Day 6	Day 9	Day 12
100/0	0.00±.1	2.19±.18	7.19±1.46	4.67±.98	1.82±.57
100/10	0.00±.1	1.8±.29	4.57±.57	3.50±.97	2.28±.07
100/20	0.00±.1	1.76±.26	3.14±.14	2.78±.29	2.07±.20
100/40	0.00±.1	1.47±.26	2.57±.12	4.08±1.51	3.44±.59
100/80	0.00±.1	1.85±.47	4.75±1.1	4.92±.18	3.02±.62
100/160	0.00±.1	1.38±.3	1.48±.43	1.64±.66	1.91±.13

Table 5-16. Influence of humic acid on lead uptake by *L. minor* exposed to lead nitrate concentration of 200 mg/L.

Concentration of lead nitrate/ humic acid (mg/L)	Lead content (mg/g)				
	Day 0	Day 3	Day 6	Day 9	Day 12
200/0	0.00 ±.01	10.86 ±2.10	22.10 ±3.94	17.52 ±7.53	13.49 ±5.61
200/10	0.00 ±.01	14.07 ±.07	21.54 ±.96	24.11 ±2.1	25.29 ±6.65
200/20	0.00 ±.01	11.33 ±1.73	26.94 ±5.99	14.73 ±.29	43.52 ±.20
200/40	0.00 ±.01	10.76 ±1.8	16.96 ±.88	9.6 ±1.46	36.01 ±5.75
200/80	0.00 ±.01	11.94 ±1.33	15.75 ±1.98	10.88 ±3.62	23.18 ±3.16
200/160	0.00 ±.01	-0.02 ±.14	2.29 ±.33	3.27 ±.59	7.95 ±4.43

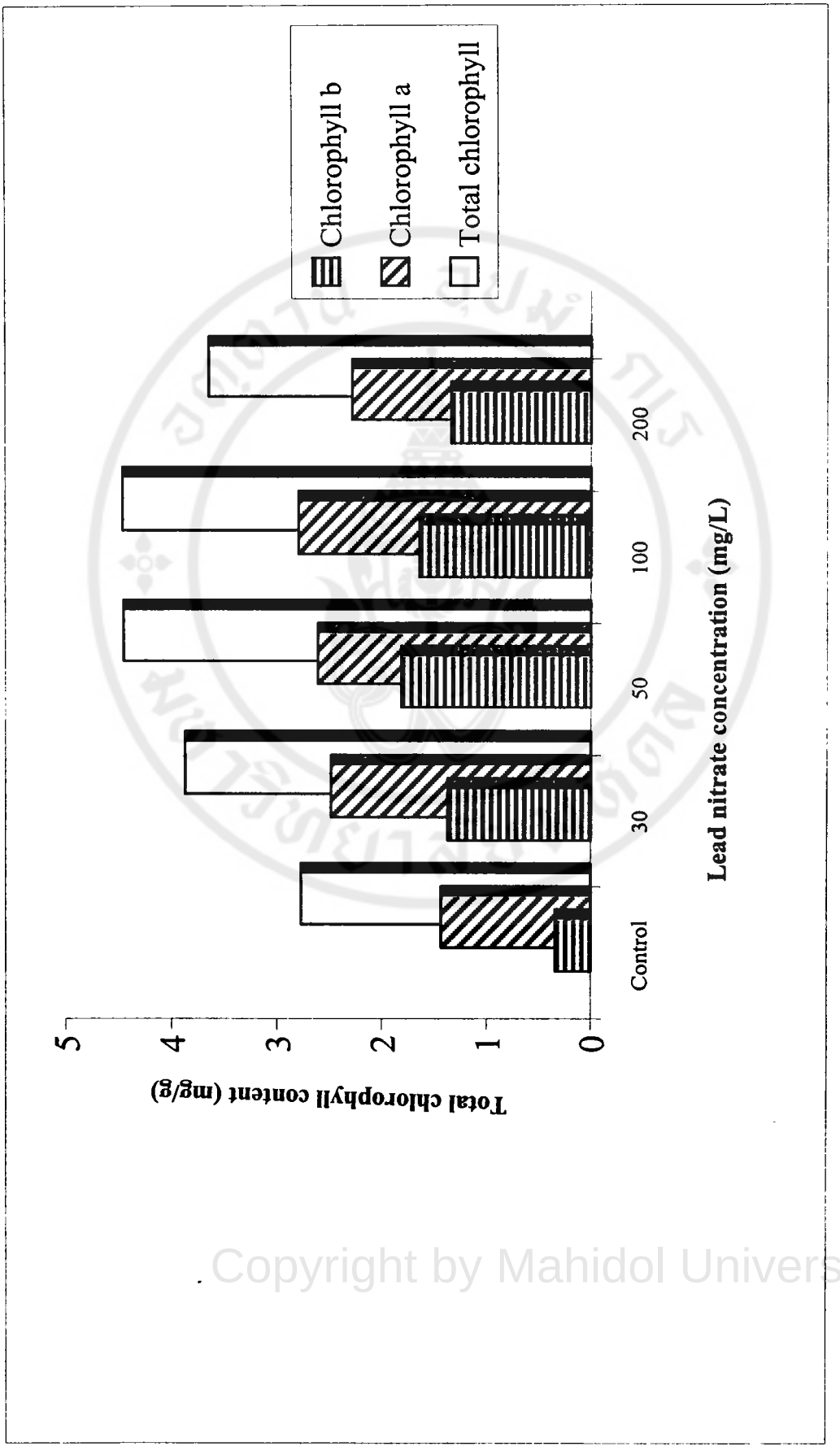


Figure 5-1. Effect of lead on total chlorophyll content of *L. minor* on day 3.

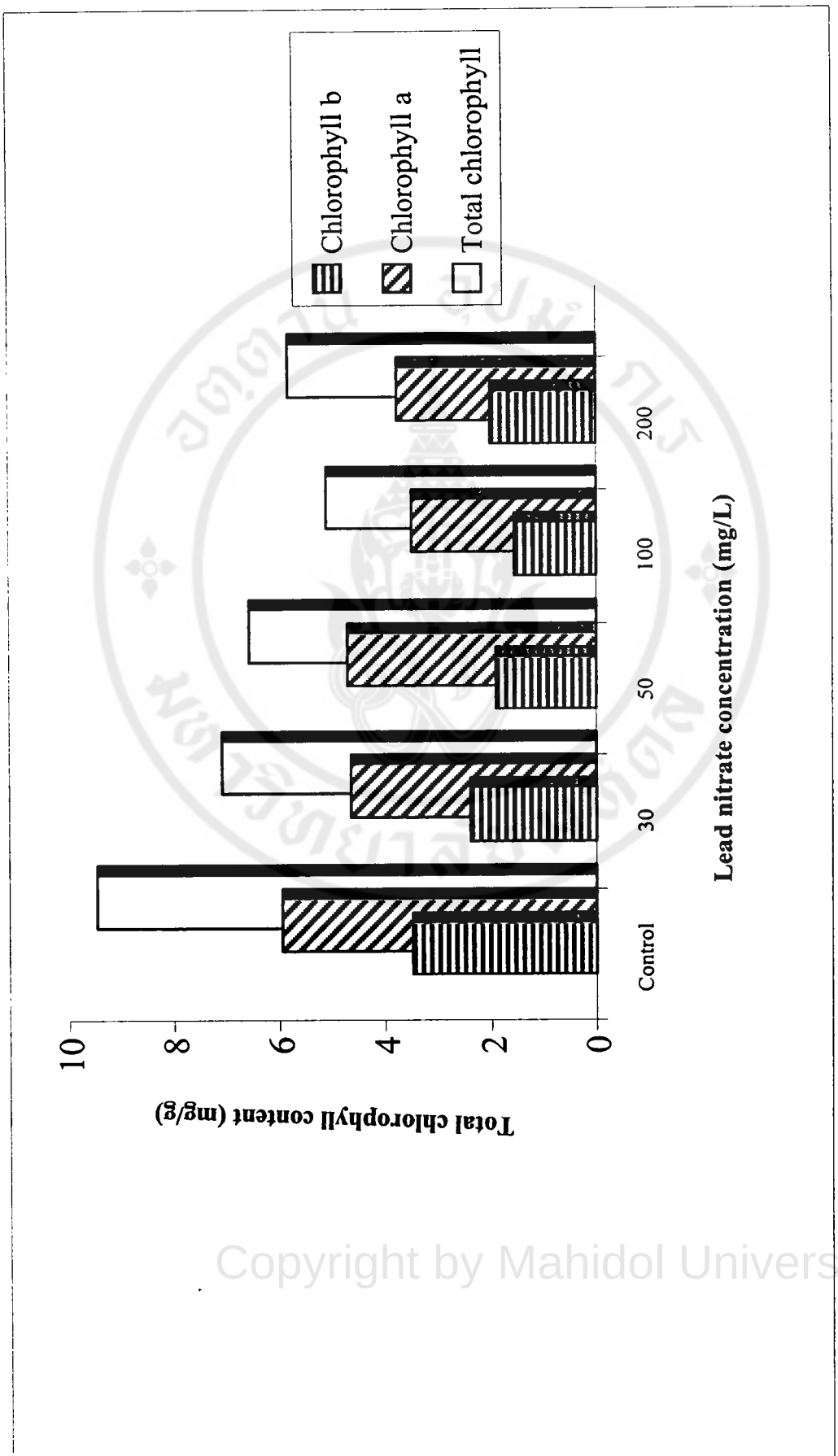


Figure 5-2. Effect of lead on total chlorophyll content of *L. minor* on day 6.

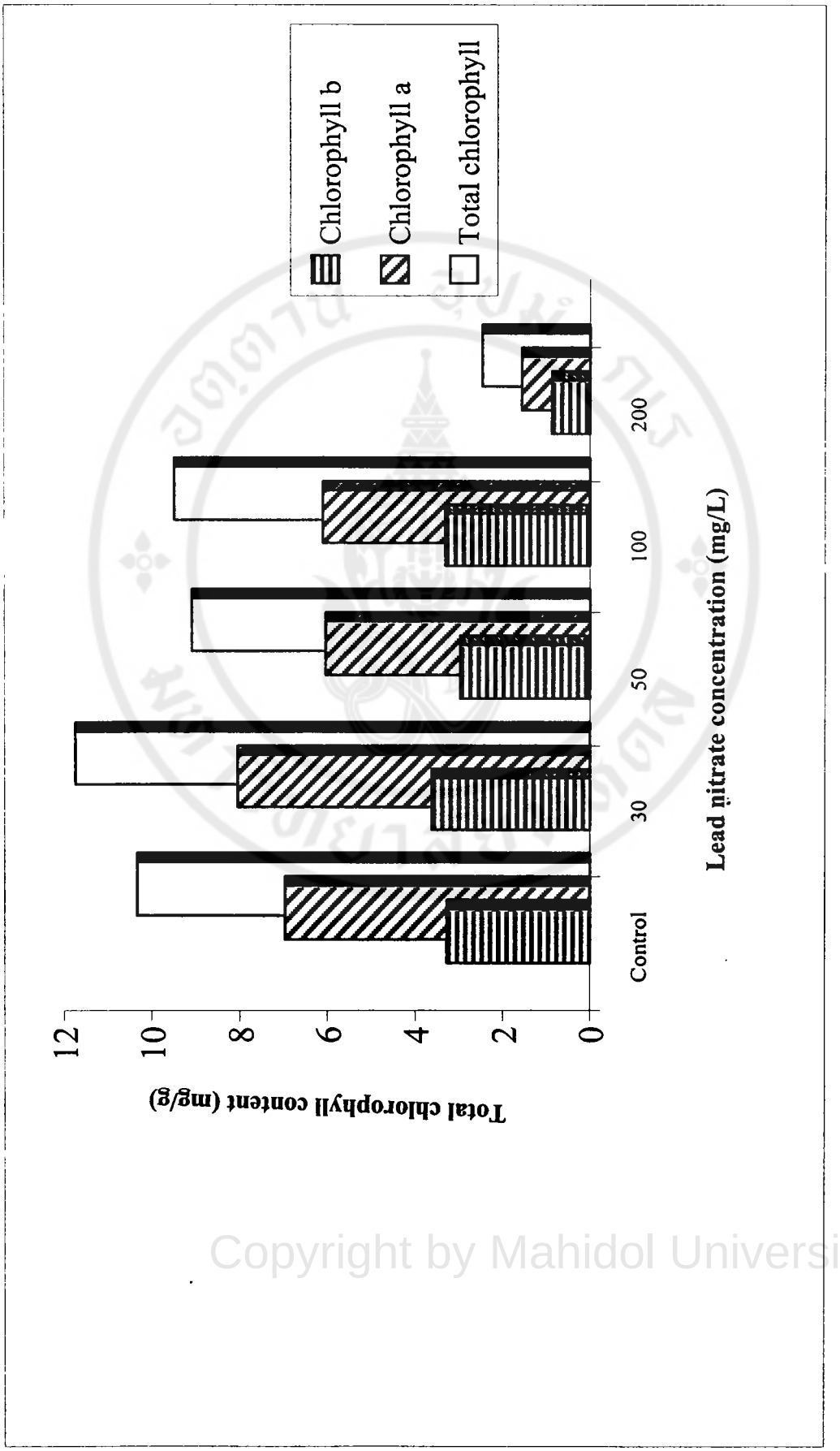


Figure 5-3. Effect of lead on total chlorophyll content of *L. minor* on day 9.

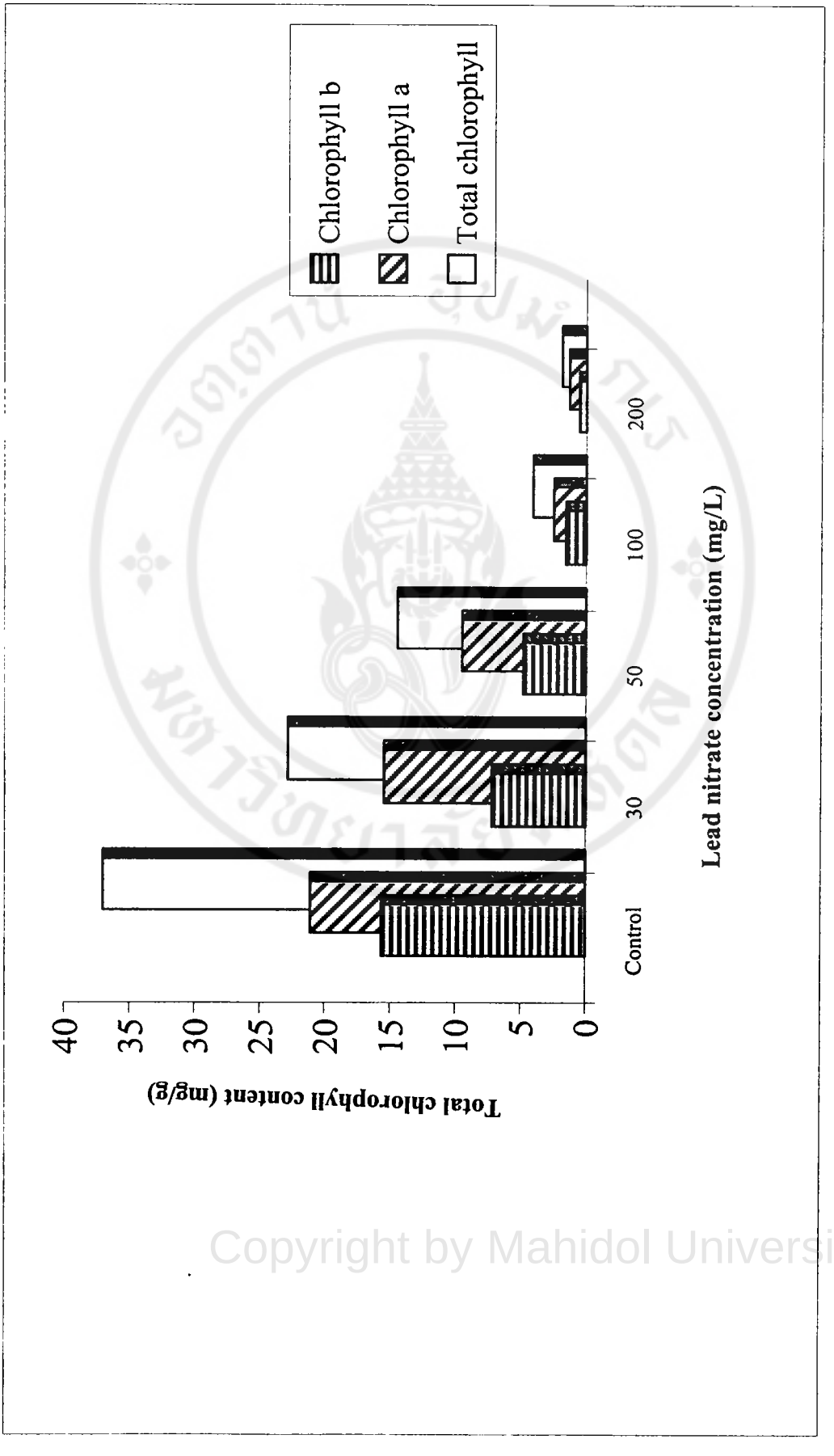


Figure 5-4. Effect of lead on total chlorophyll content of *L. minor* on day 12.

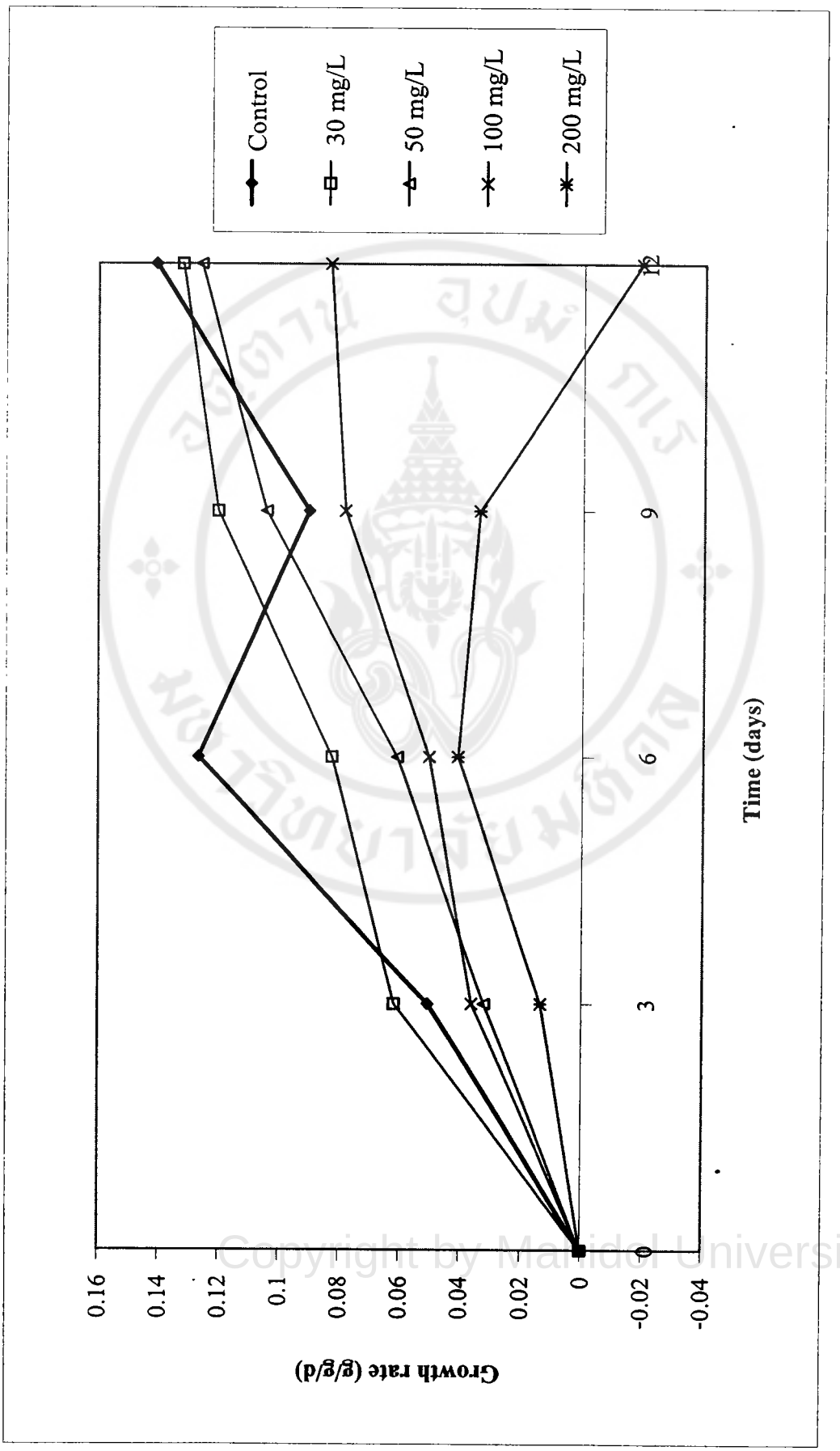


Figure 5-5. Effect of lead on the growth rate of *L. minor*.

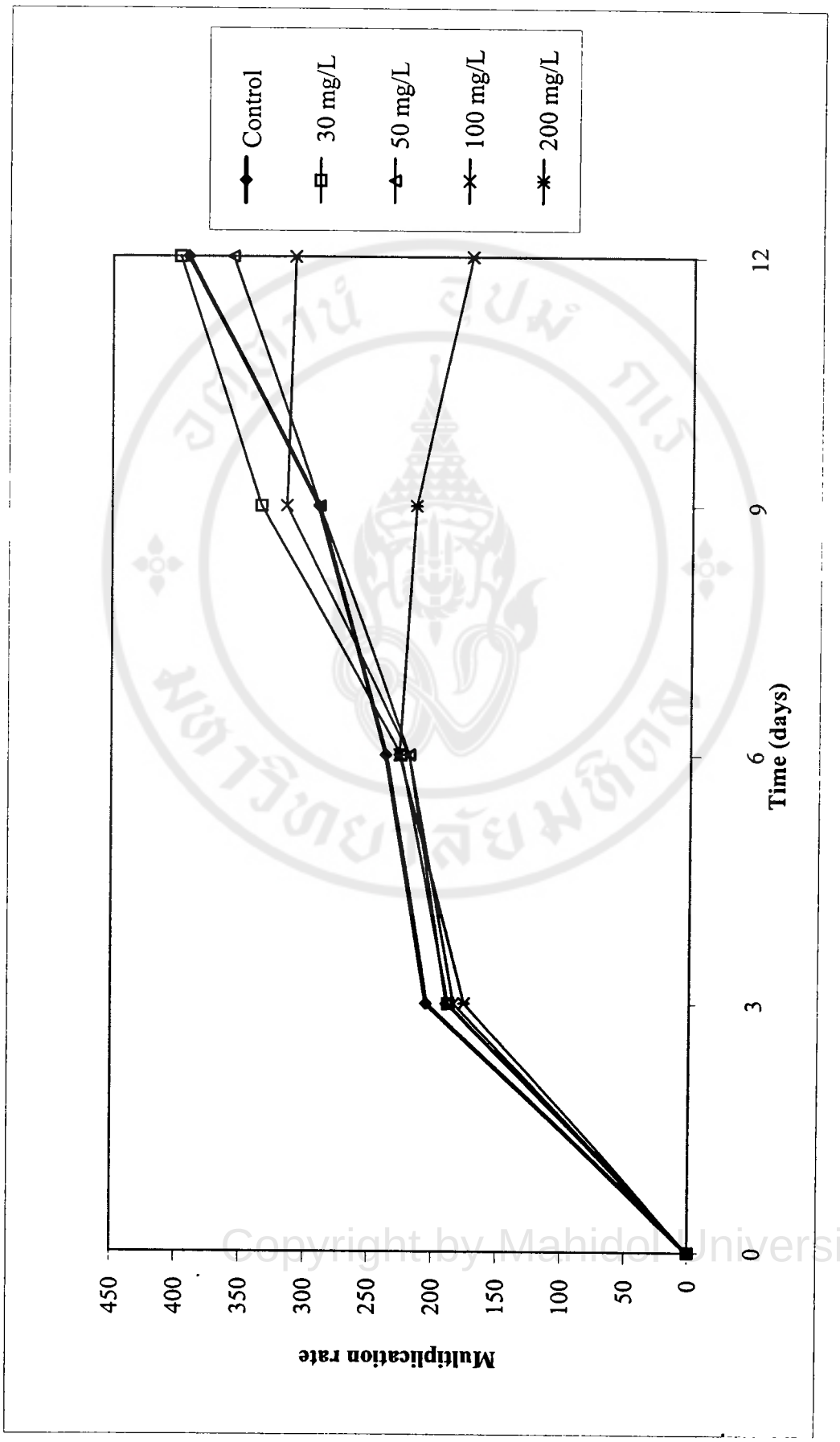


Figure 5-6. Effect of lead on the multiplication rate rate of *L. minor*.

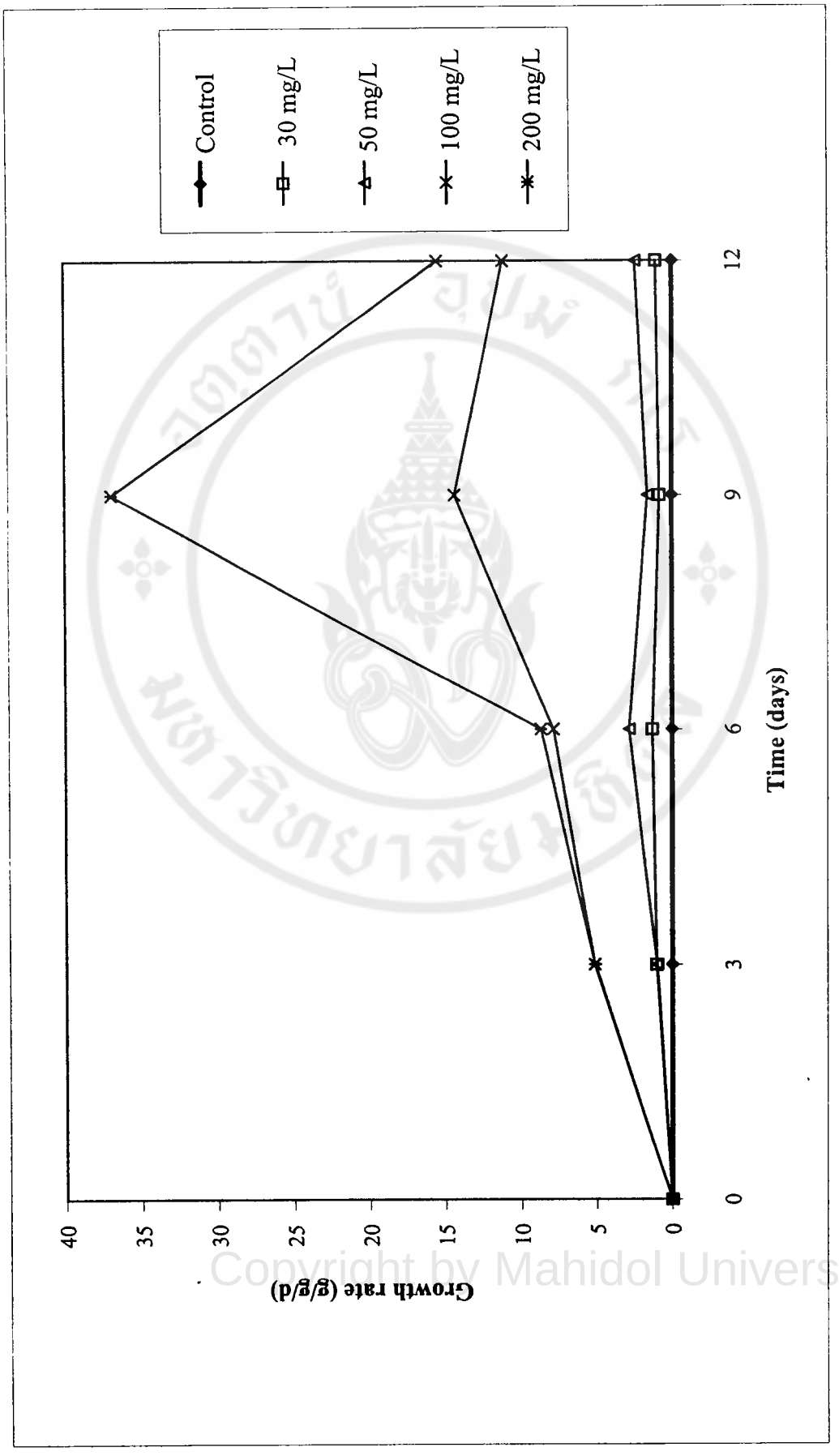


Figure 5-7. Lead uptake by *L.minor* exposed to lead nitrate solution.

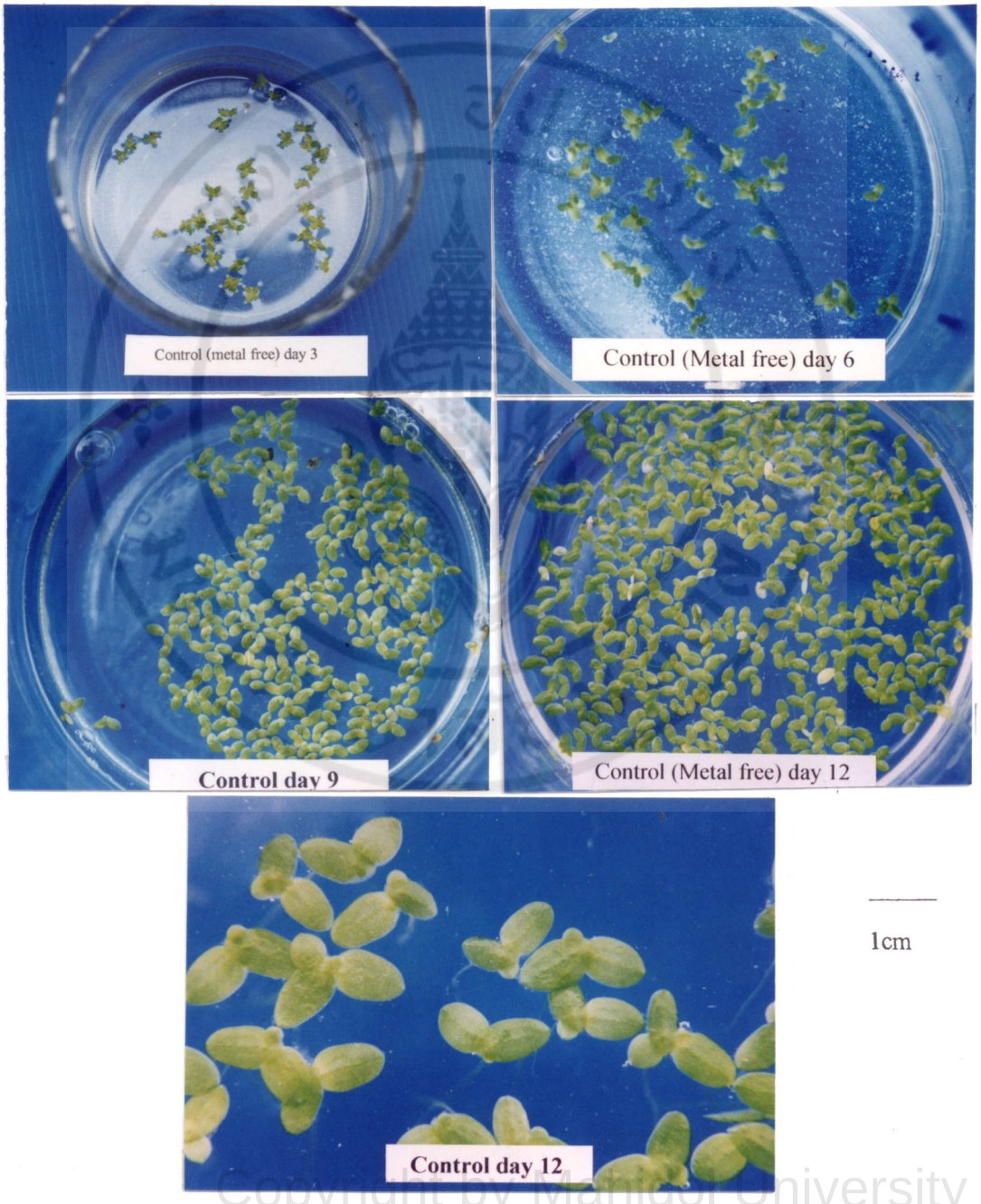


Figure 5-8. Multiplication of *L. minor* incubated in plant nutrients for 3, 6, 9 and 12 days

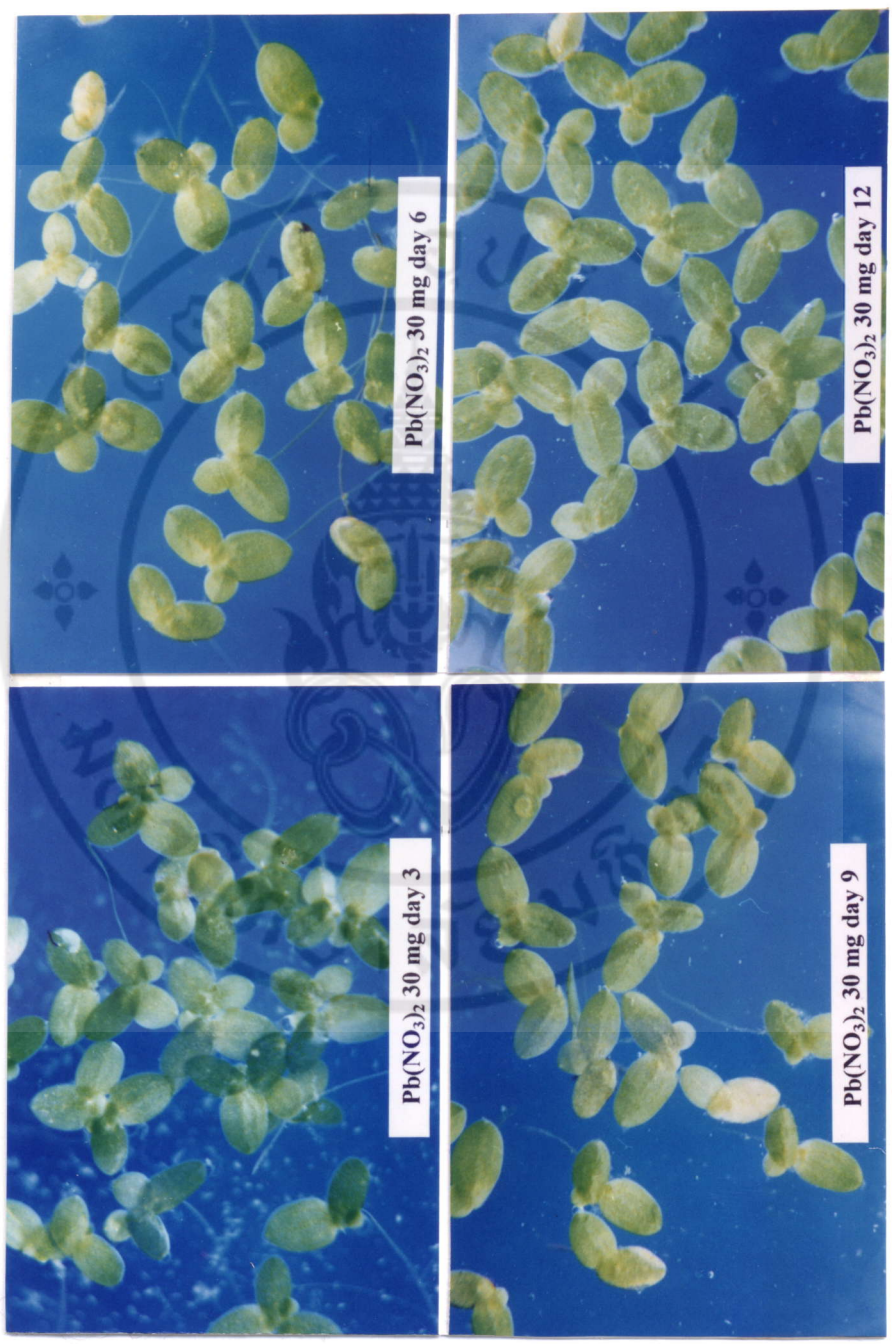


Figure 5-9. Morphological changes of *L. minor* treated with lead nitrate (30 mg/L) for 3, 6, 9, and 12 days.

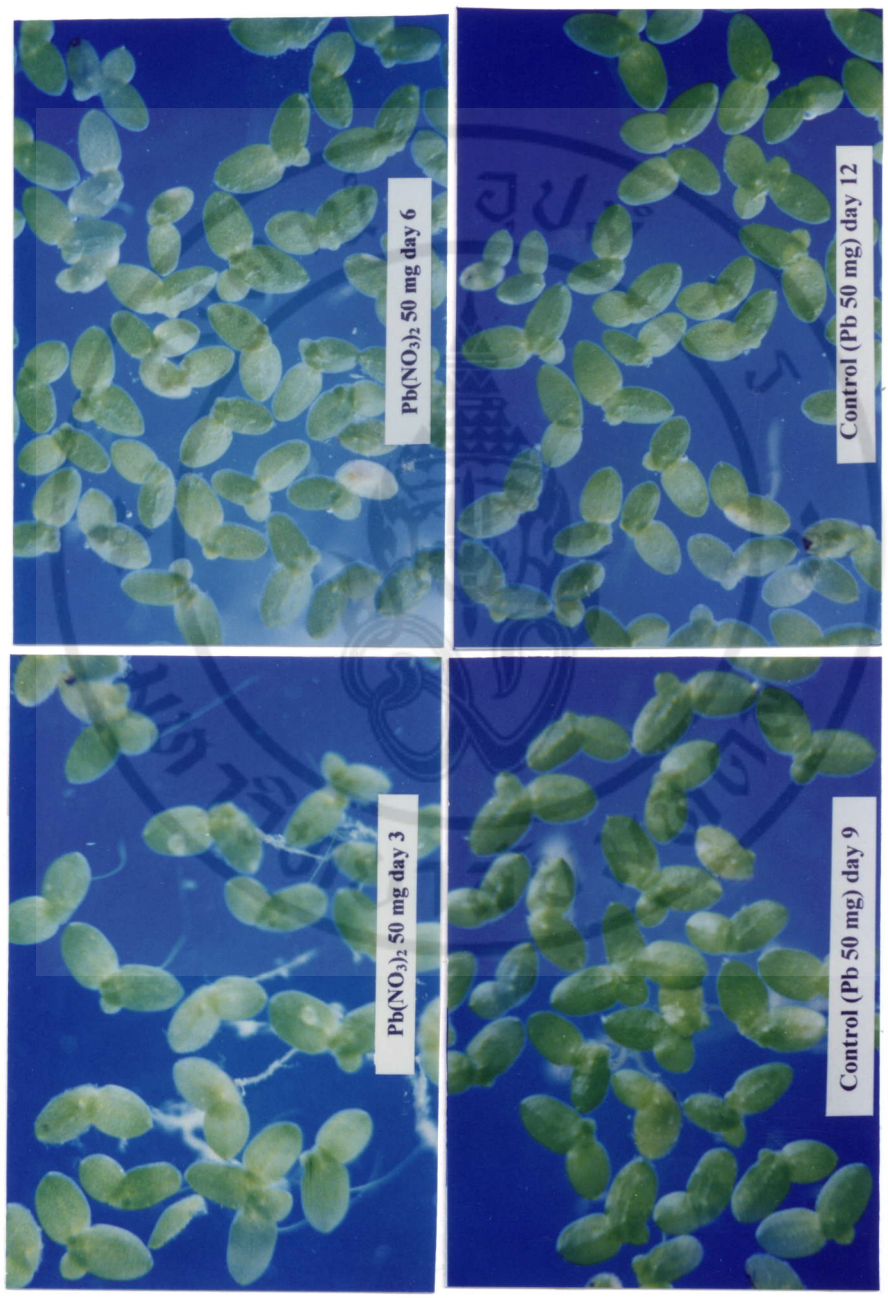


Figure 5-10. Morphological changes of *L. minor* treated with lead nitrate (50 mg/L) for 3, 6, 9, and 12 days.

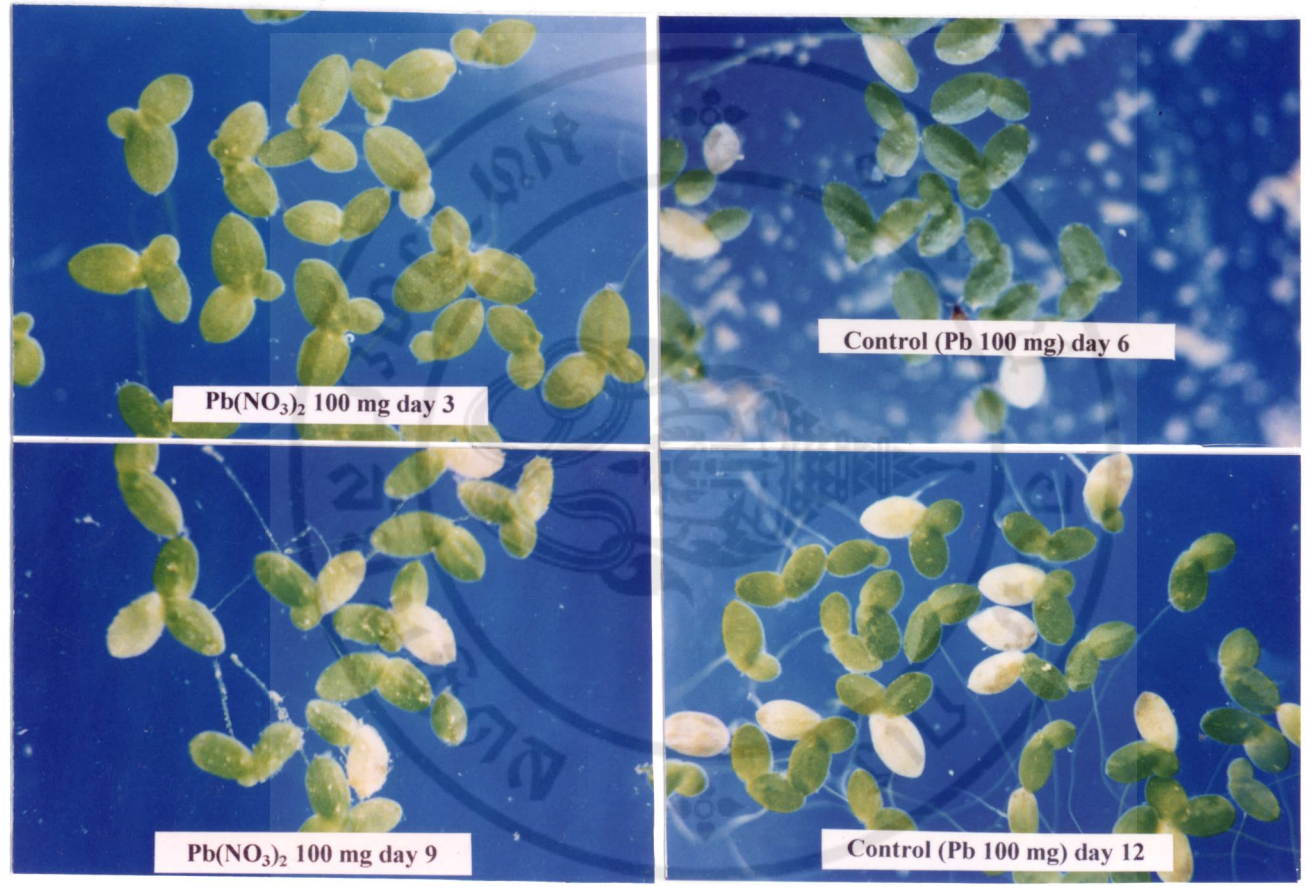


Figure 5-11. Morphological changes of *L. minor* treated with lead nitrate (100 mg/L) for 3, 6, 9, and 12 days.

Figure 5-12. Morphological changes of *L. minor* treated with lead nitrate (200 mg/L) for 3, 6, 9, and 12 days.

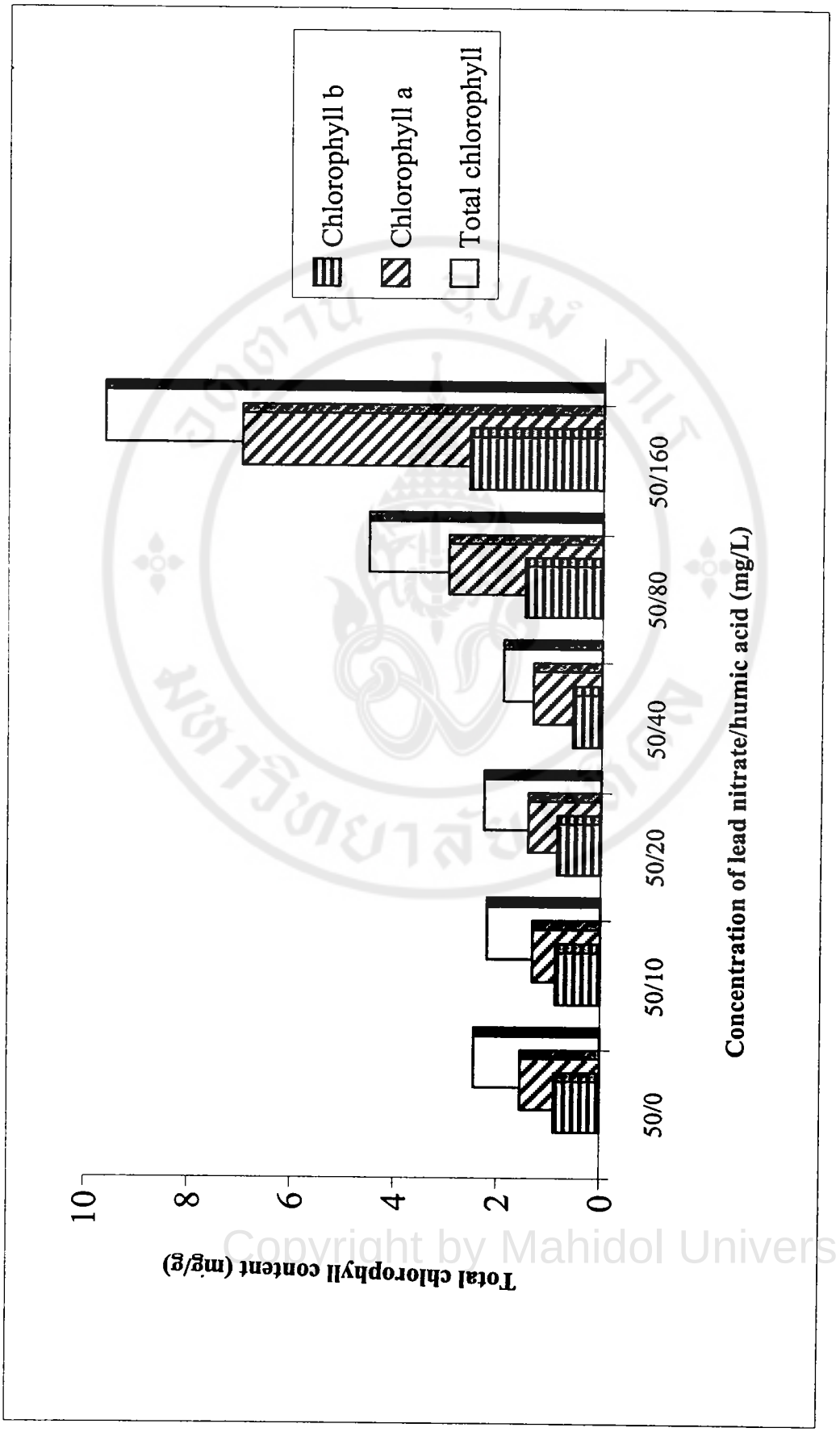


Figure 5-13. Influence of humic acid on the toxic effect of lead (50 mg/L) on total chlorophyll content of *L. minor* on day 3.

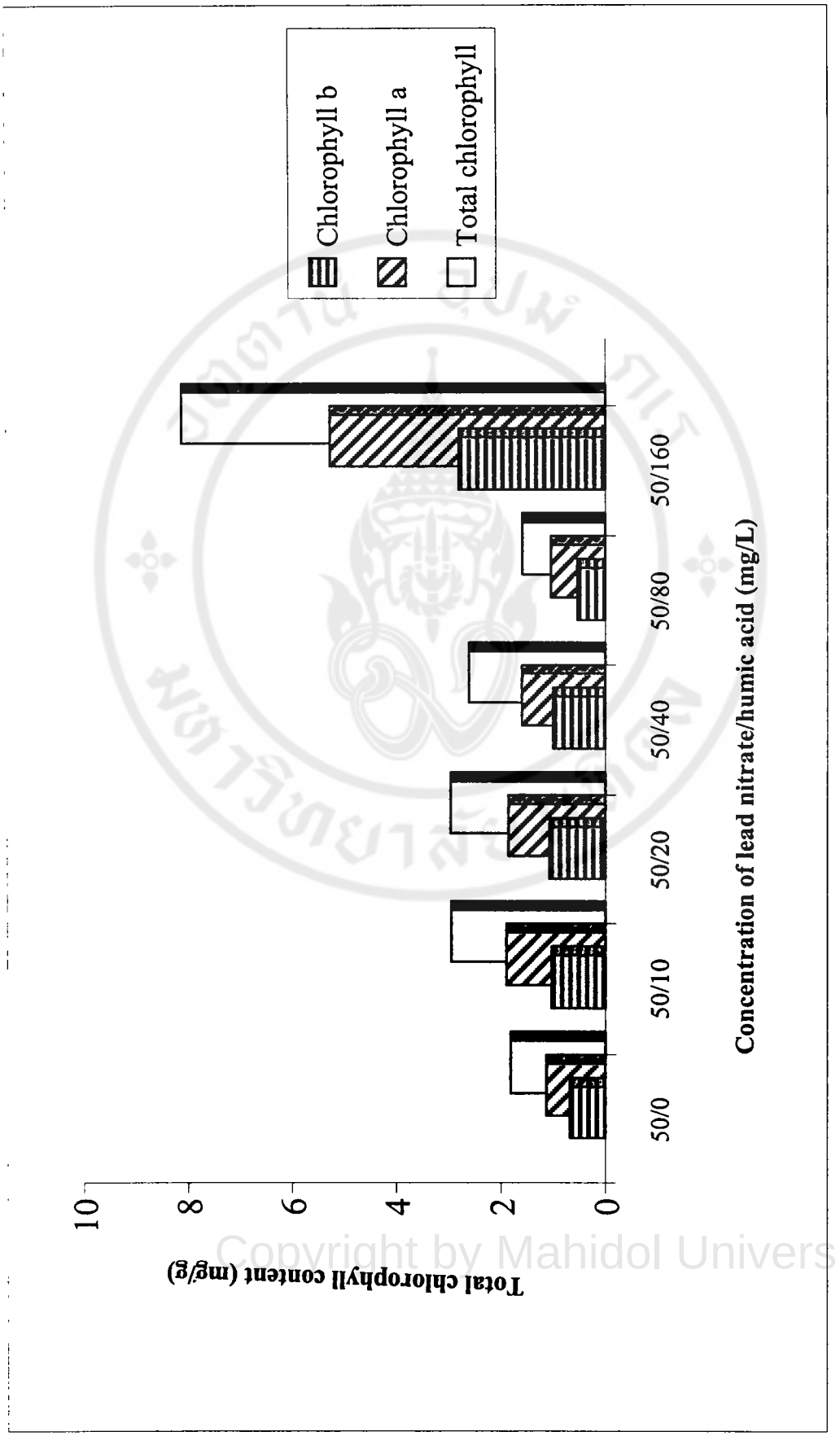


Figure 5-14. Influence of humic acid on the toxic effect of lead (50 mg/L) on total chlorophyll content of *L. minor* on day 6.

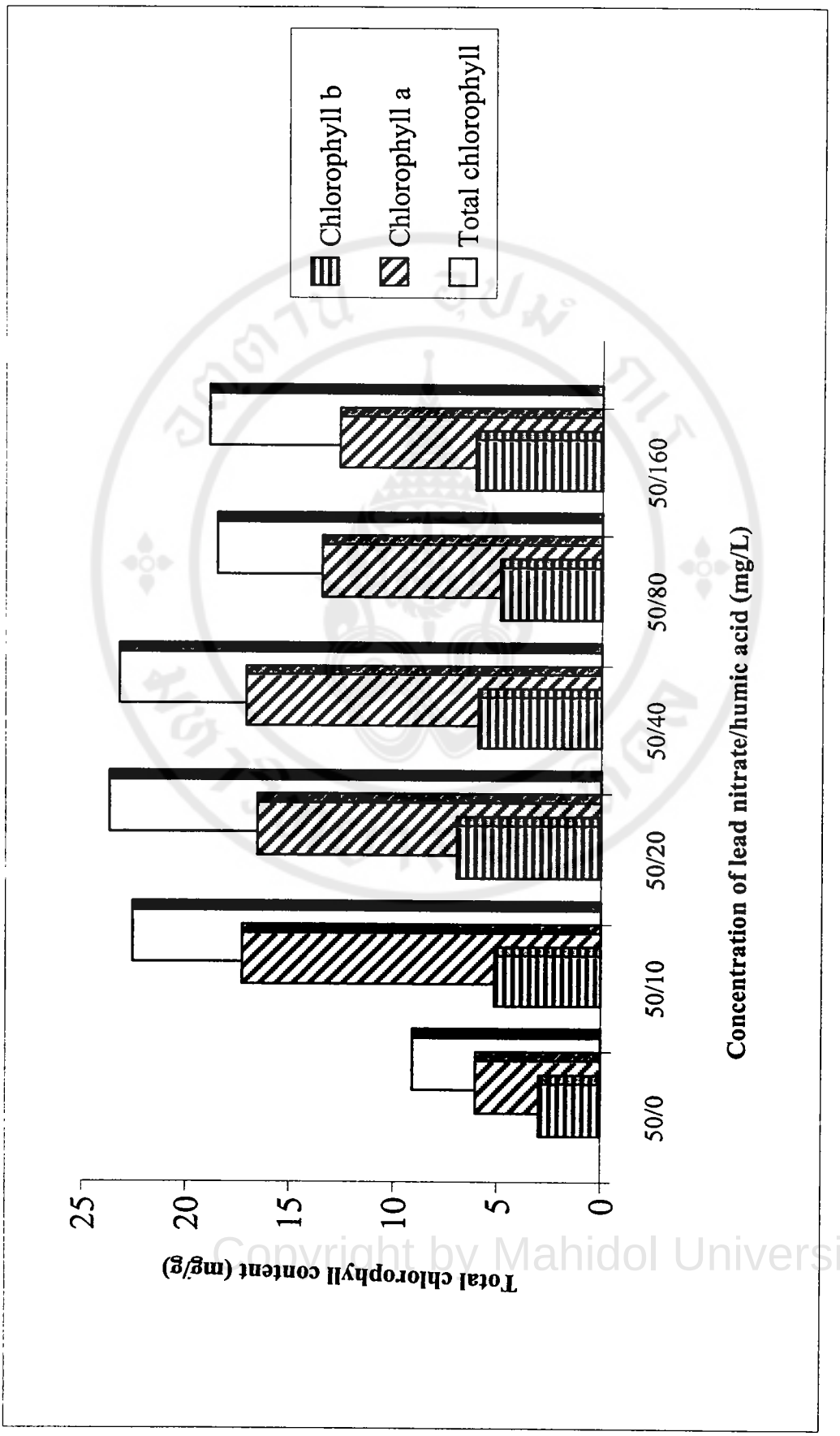


Figure 5-15. Influence of humic acid on the toxic effect of lead (50 mg/L) on total chlorophyll content of *L. minor* on day 9.

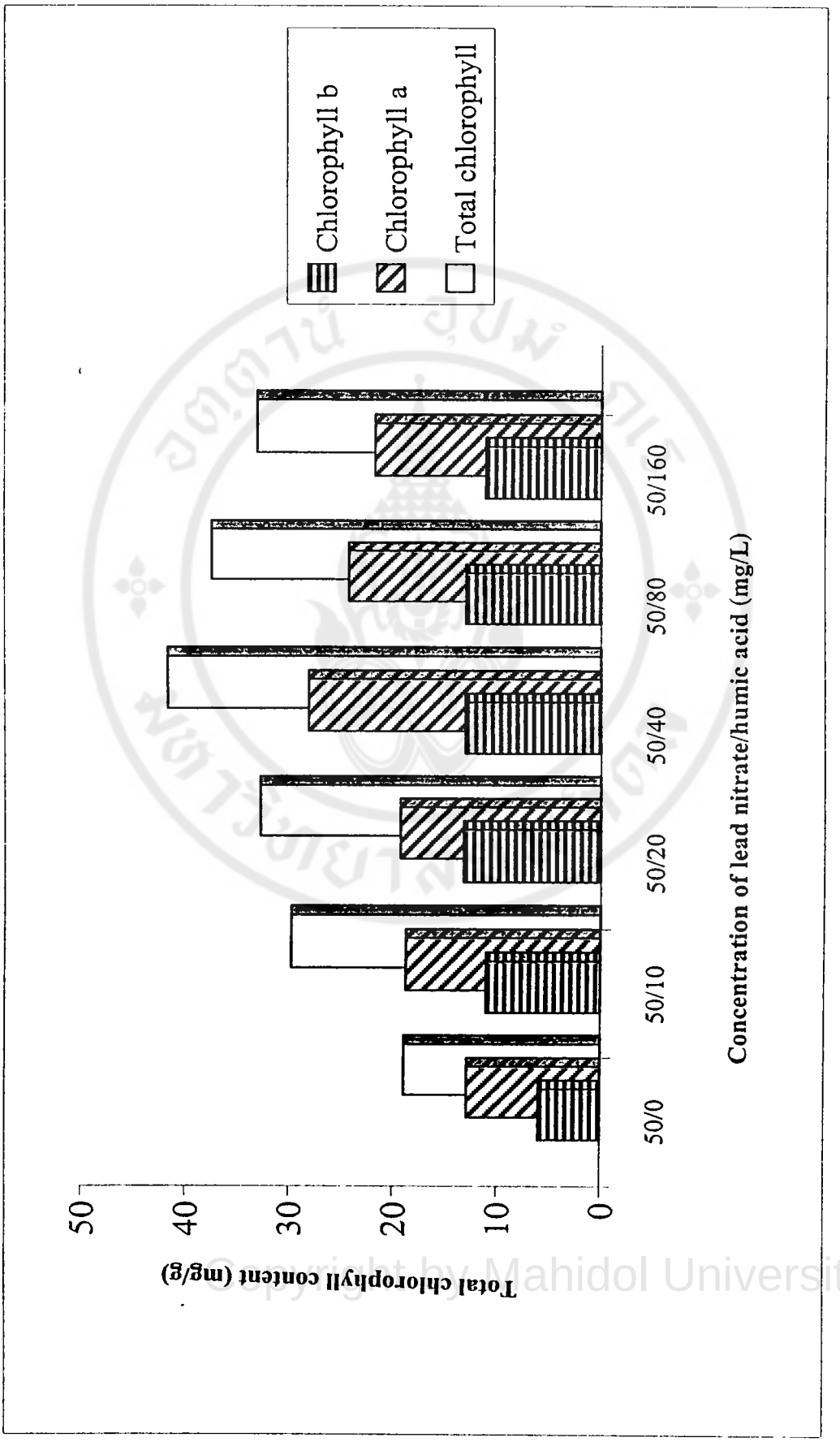


Figure 5-16. Influence of humic acid on the toxic effect of lead (50 mg/L) on total chlorophyll content of *L. minor* on day 12.

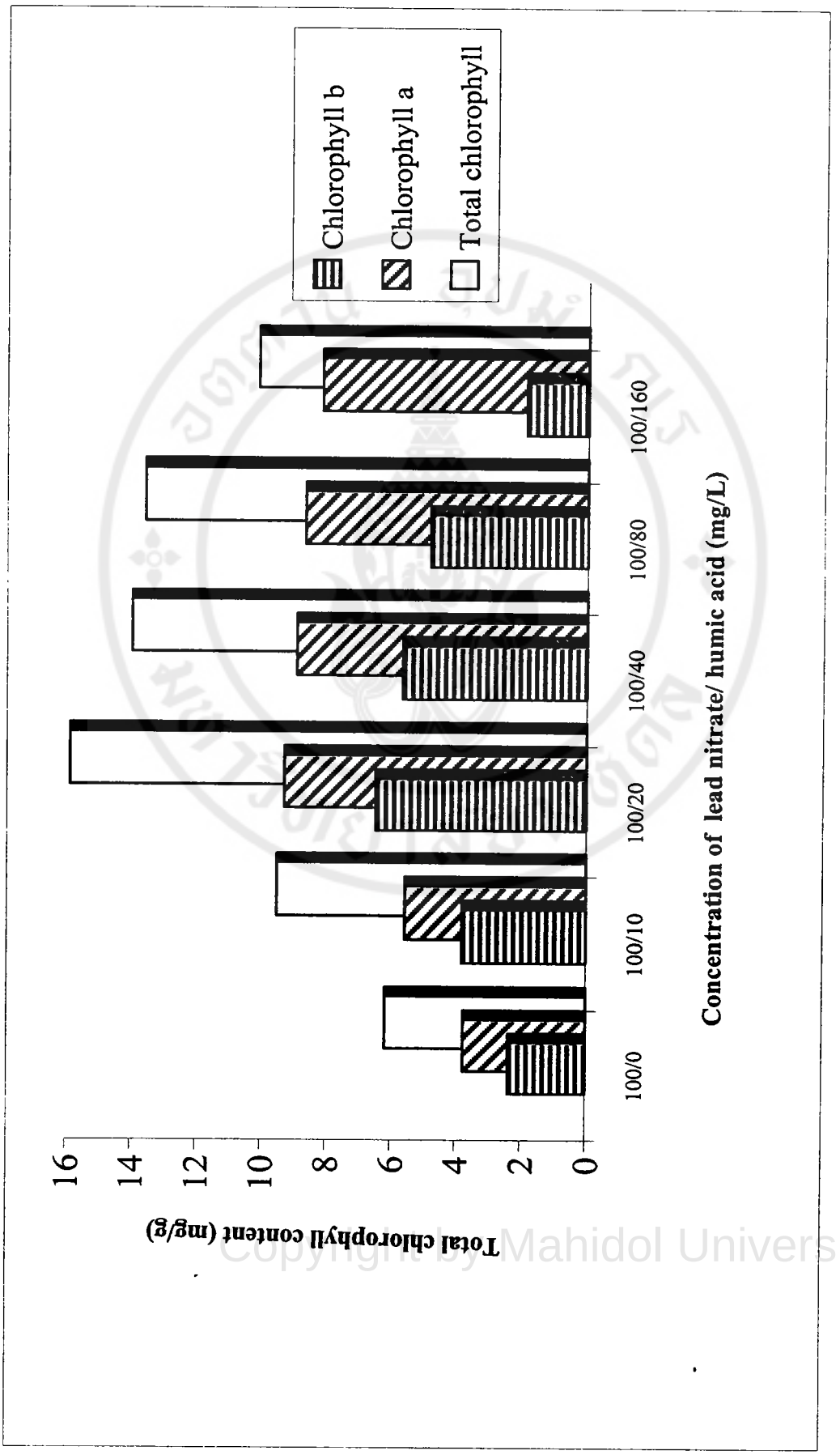


Figure 5-17. Influence of humic acid on the toxic effect of lead (100 mg/L) on total chlorophyll content of *L. minor* on day 3.

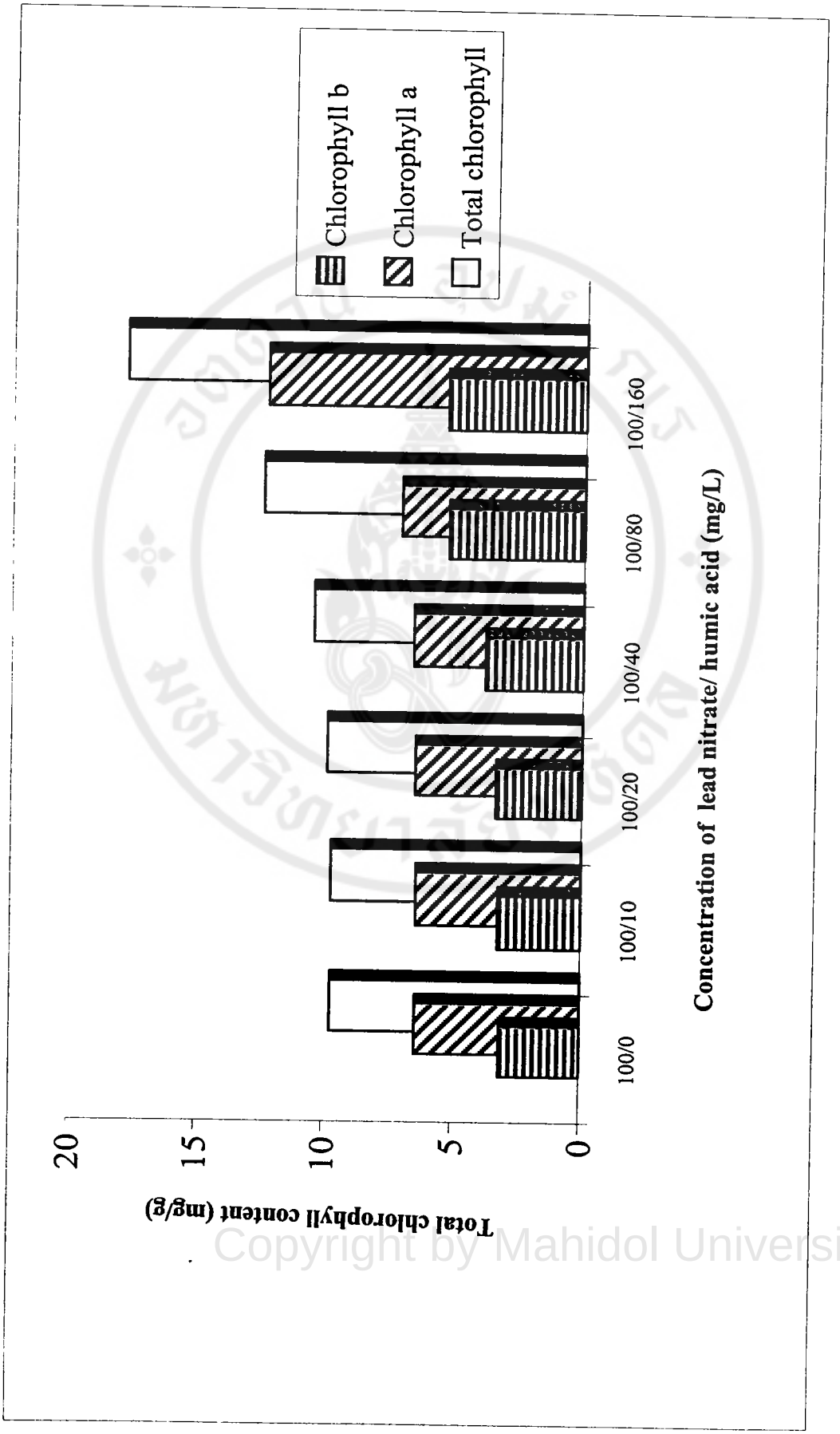


Figure 5-18. Influence of humic acid on the toxic effect of lead (100 mg/L) on total chlorophyll content of *L. minor* on day 6.

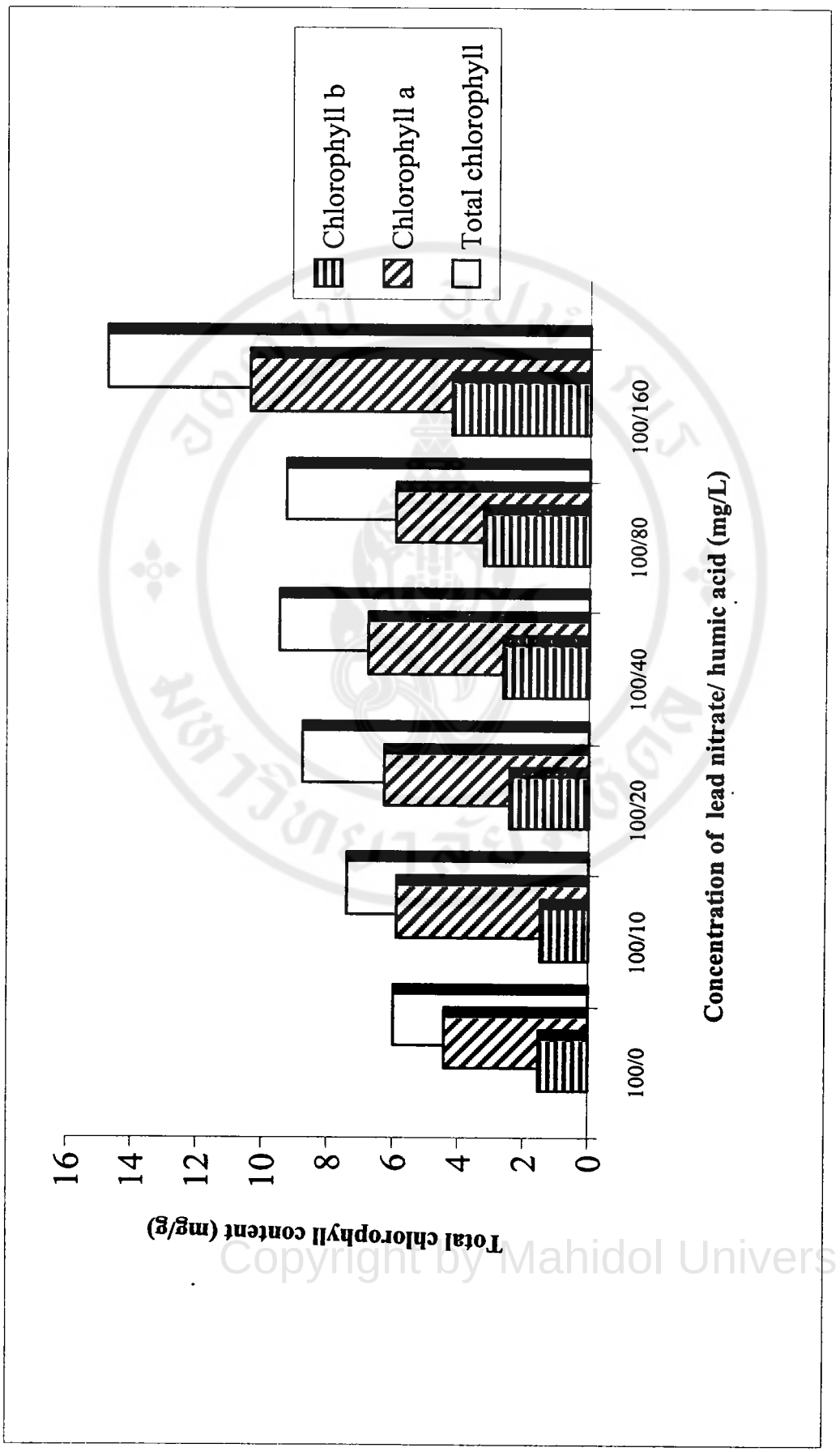


Figure 5-19. Influence of humic acid on the toxic effect of lead (100 mg/L) on total chlorophyll content of *L. minor* on day 9.

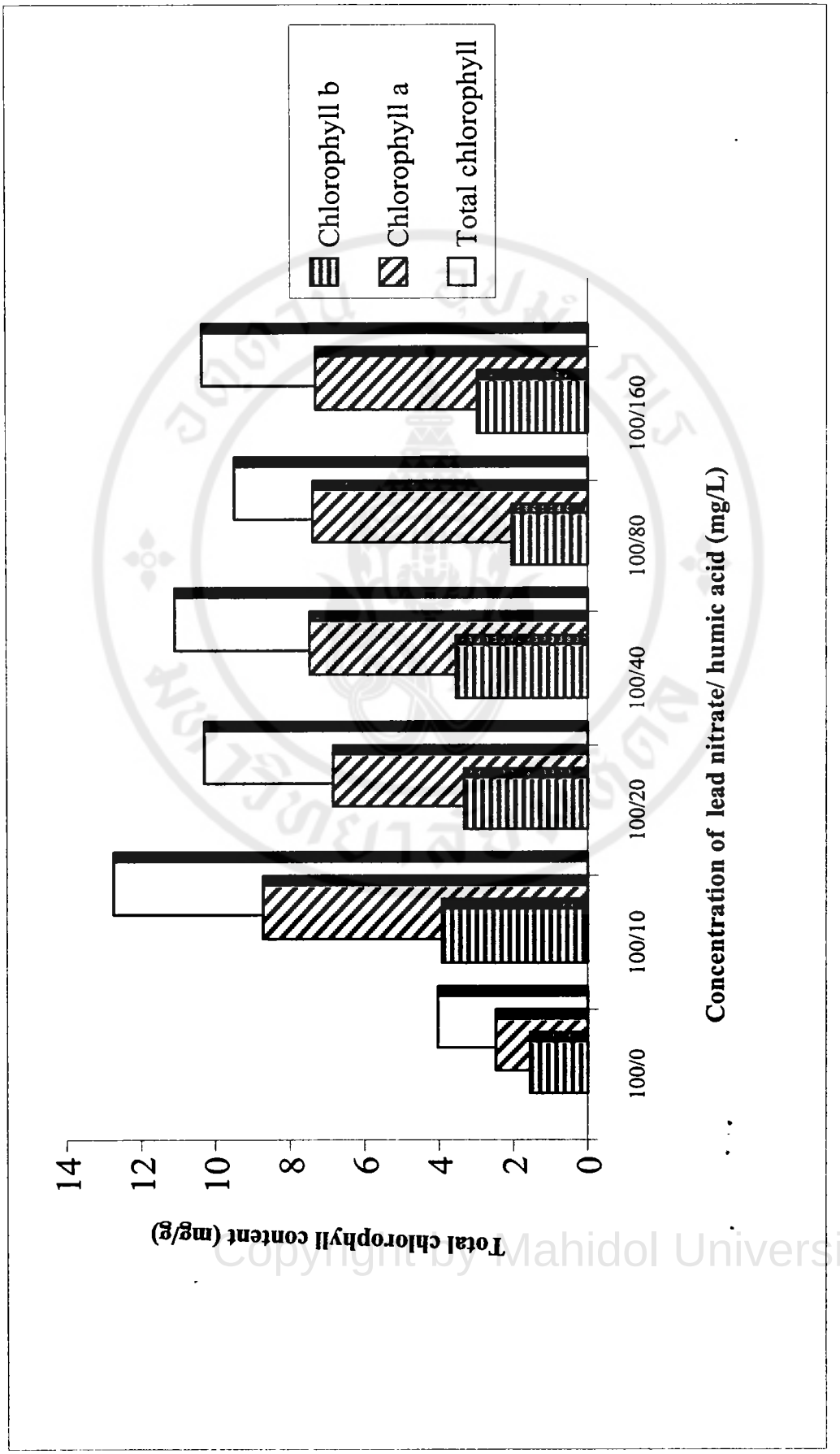


Figure 5-20. Influence of humic acid on the toxic effect of lead (100 mg/L) on total chlorophyll content of *L. minor* on day 12.

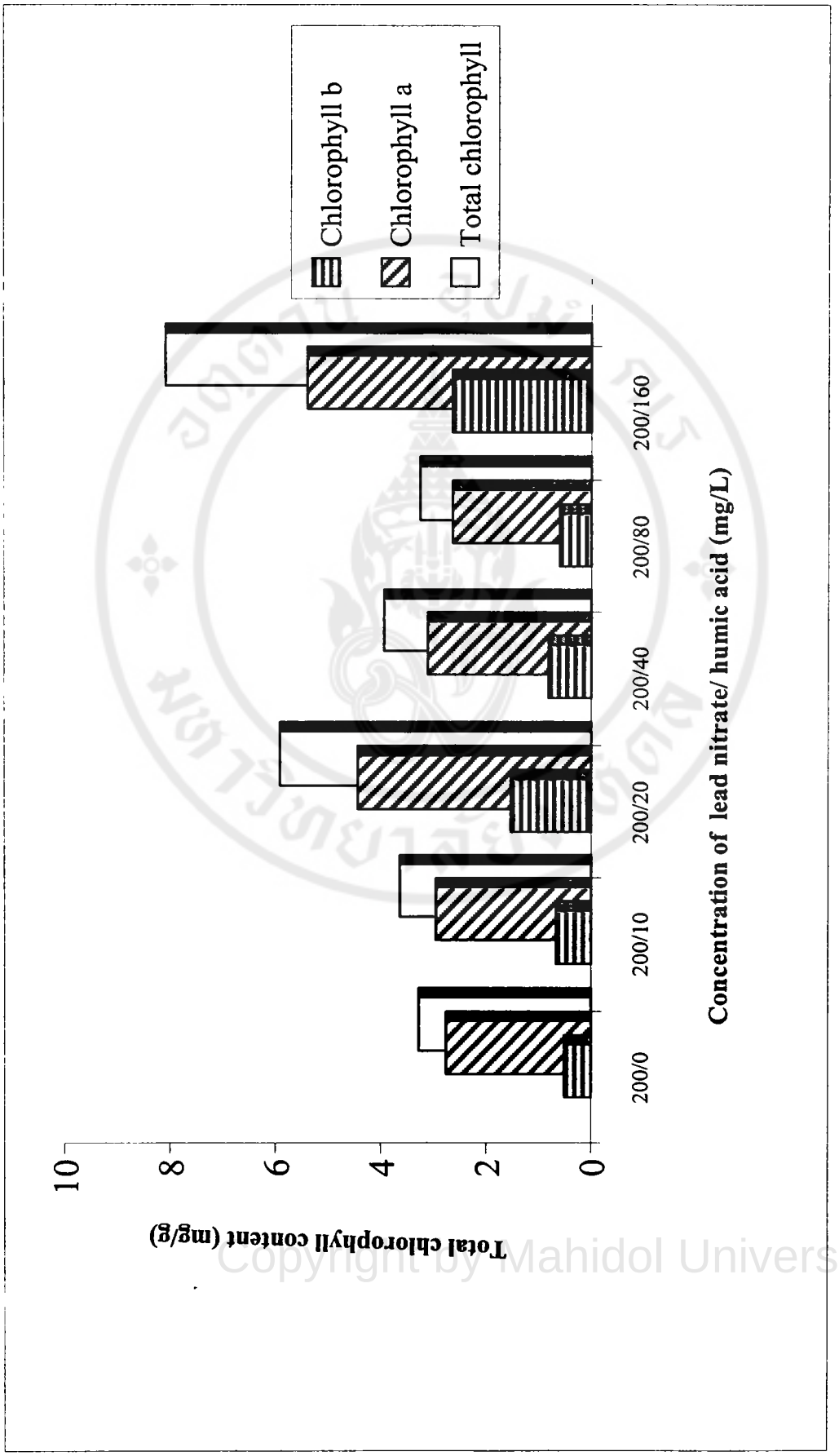


Figure 5-21. Influence of humic acid on the toxic effect of lead (200 mg/L) on total chlorophyll content of *L. minor* on day 3.

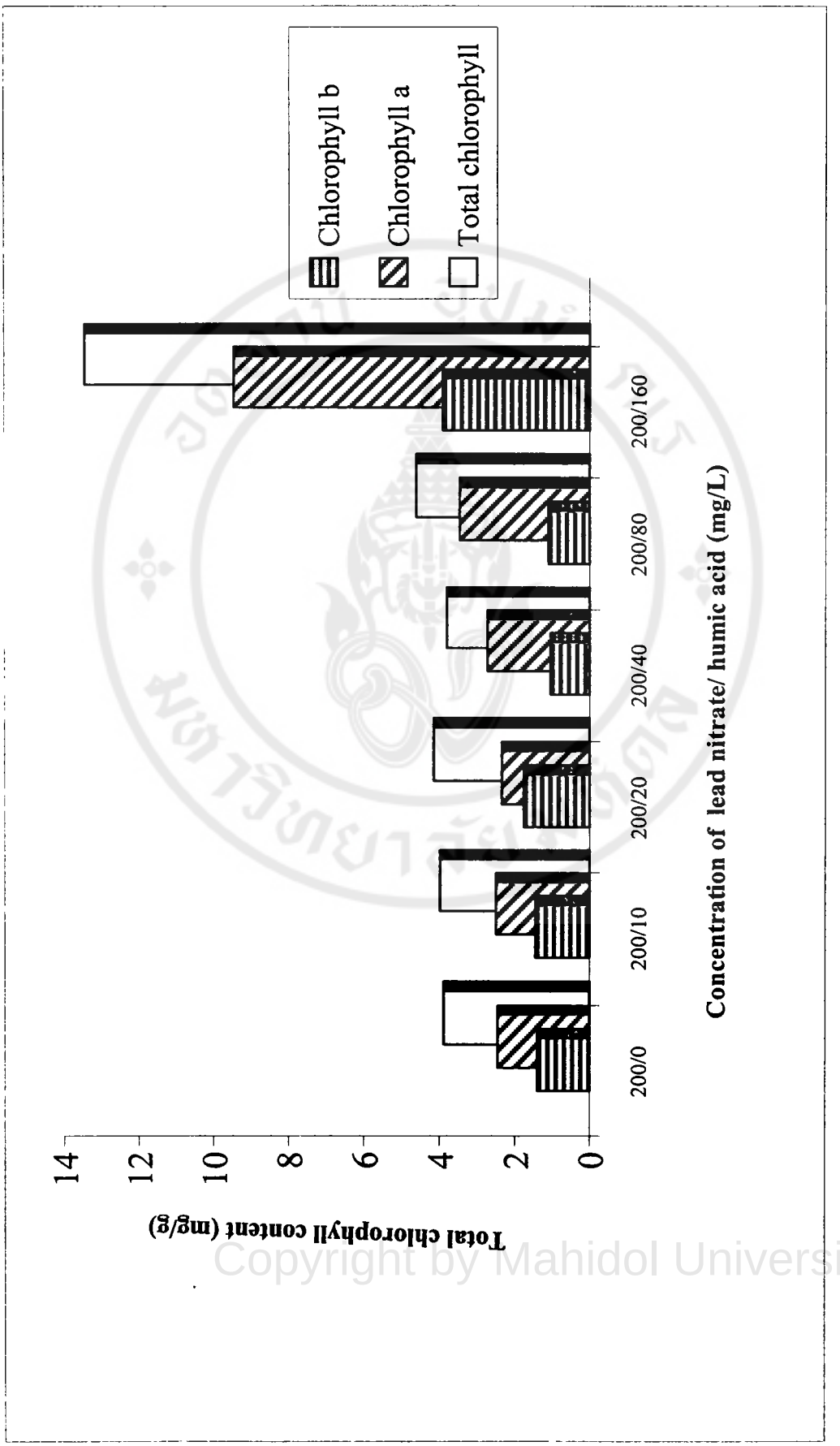


Figure 5-22. Influence of humic acid on the toxic effect of lead (200 mg/L) on total chlorophyll content of *L. minor* on day 6.

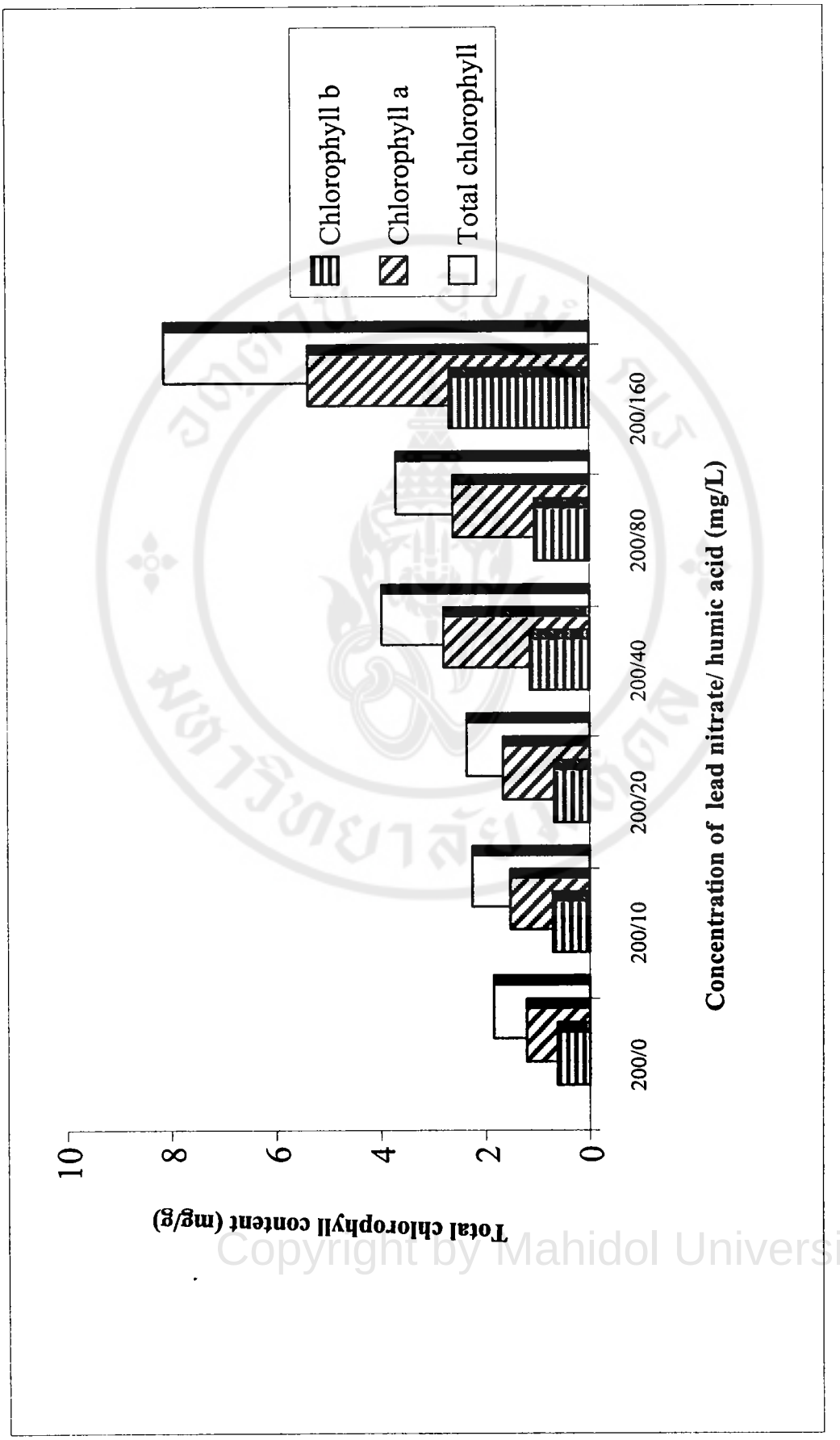


Figure 5-23. Influence of humic acid on the toxic effect of lead (200 mg/L) on total chlorophyll content of *L. minor* on day 9.

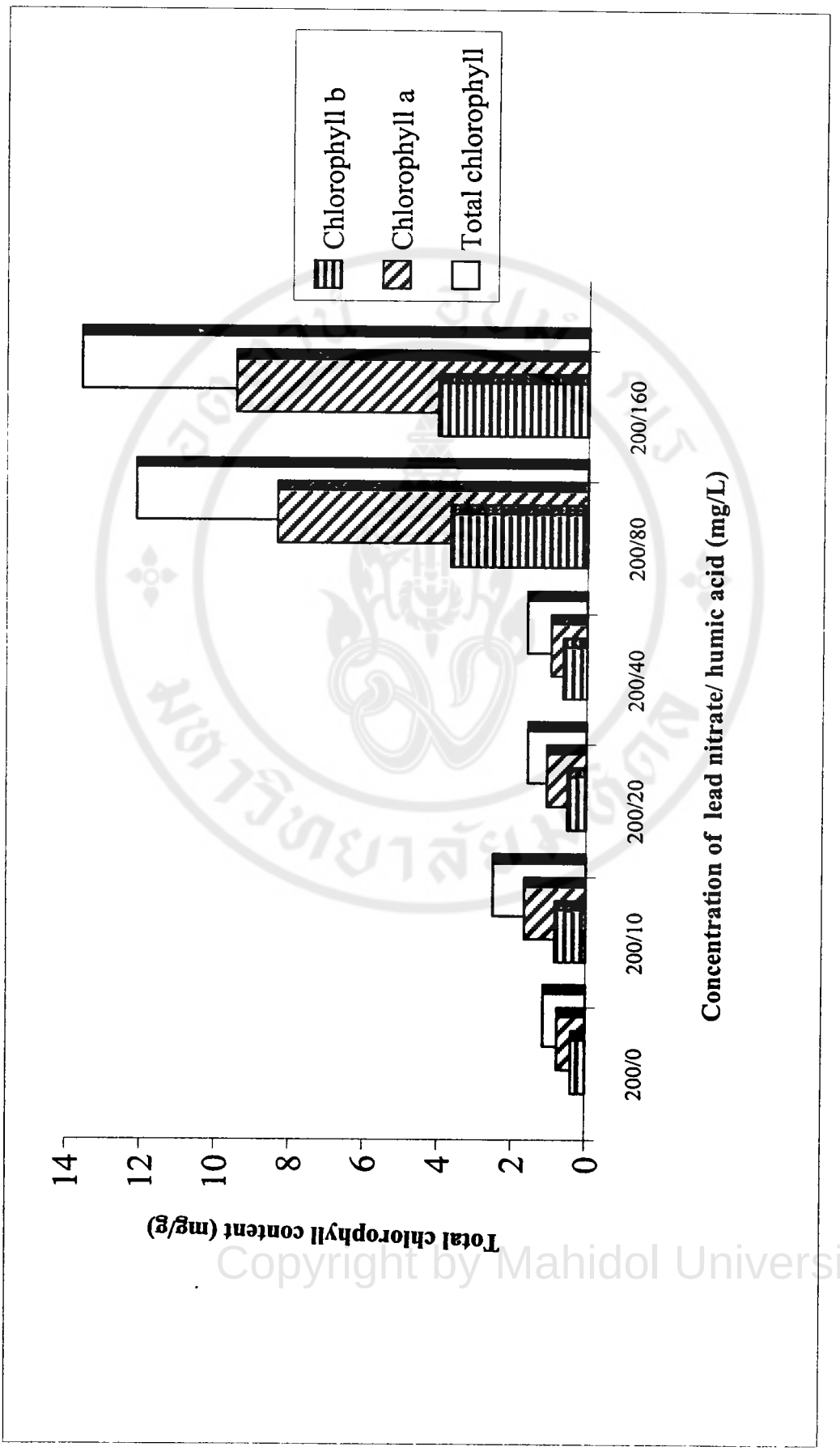


Figure 5-24. Influence of humic acid on the toxic effect of lead (200 mg/L) on total chlorophyll content of *L. minor* on day 12.

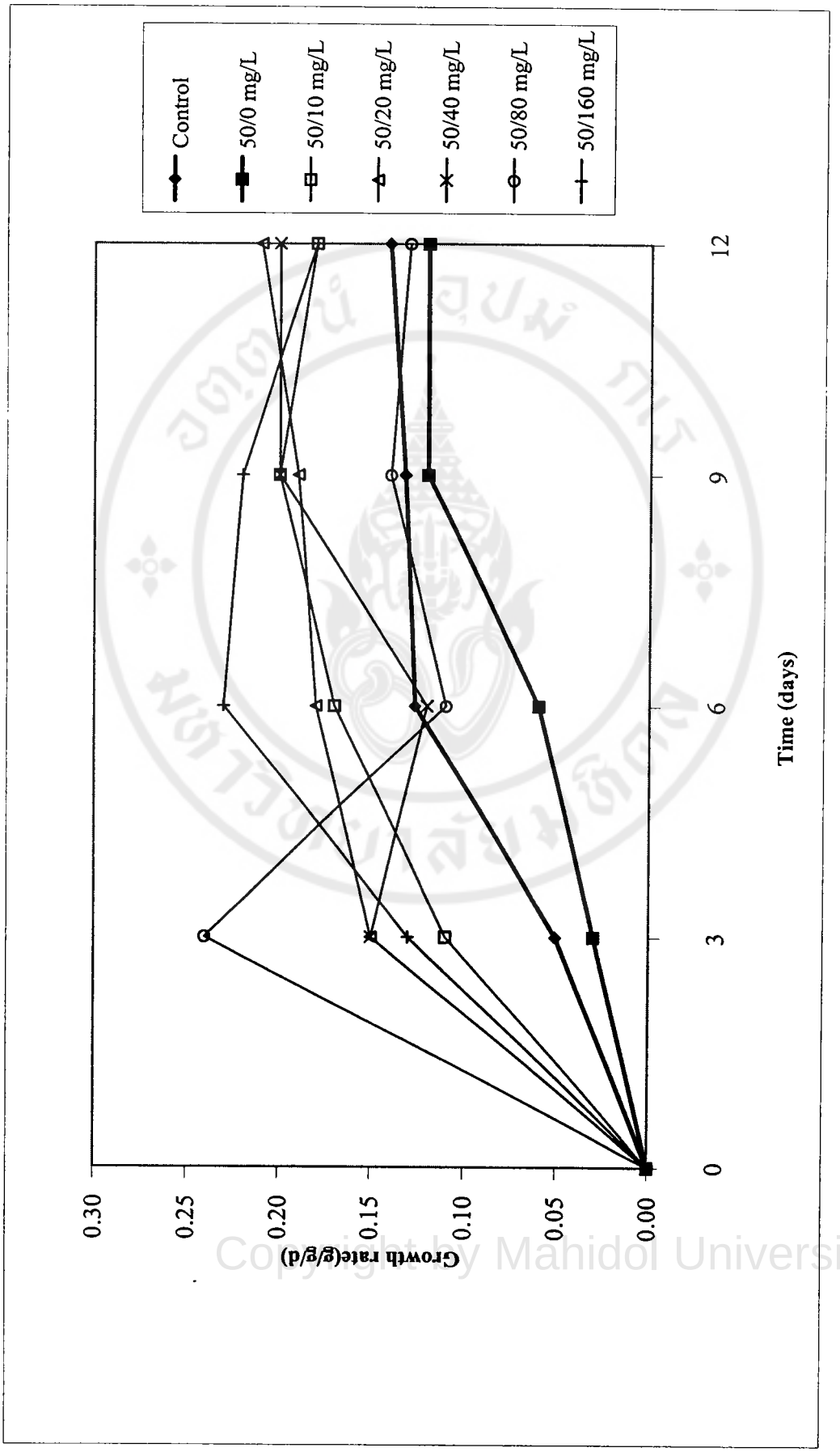


Figure 5-25. Influence of humic acid on the toxic effect of lead (50 mg/L) on growth rate of *L. minor*.

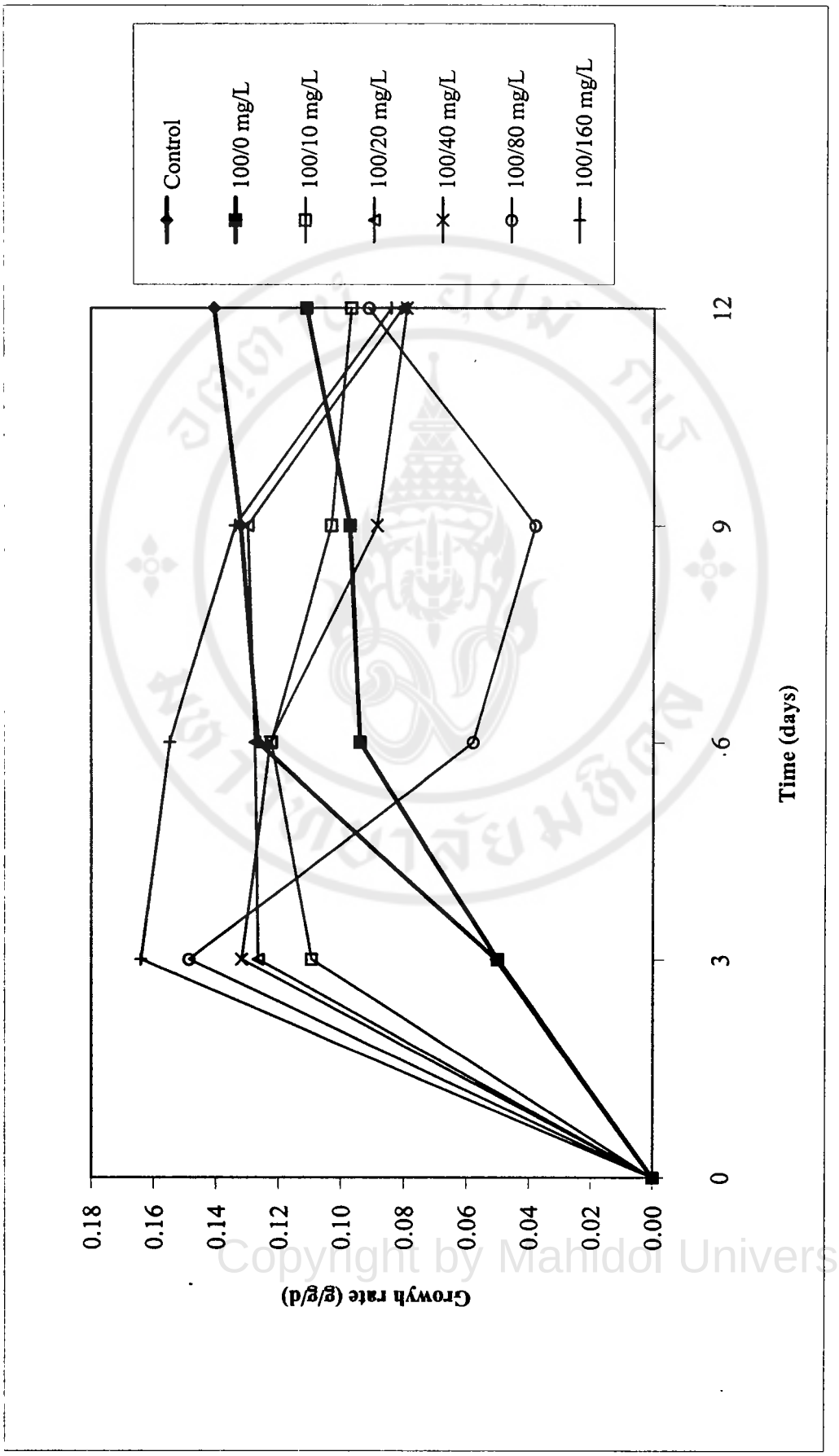


Figure 5-26. Influence of humic acid on the toxic of lead (100 mg/L) on growth rate of *L. minor*.

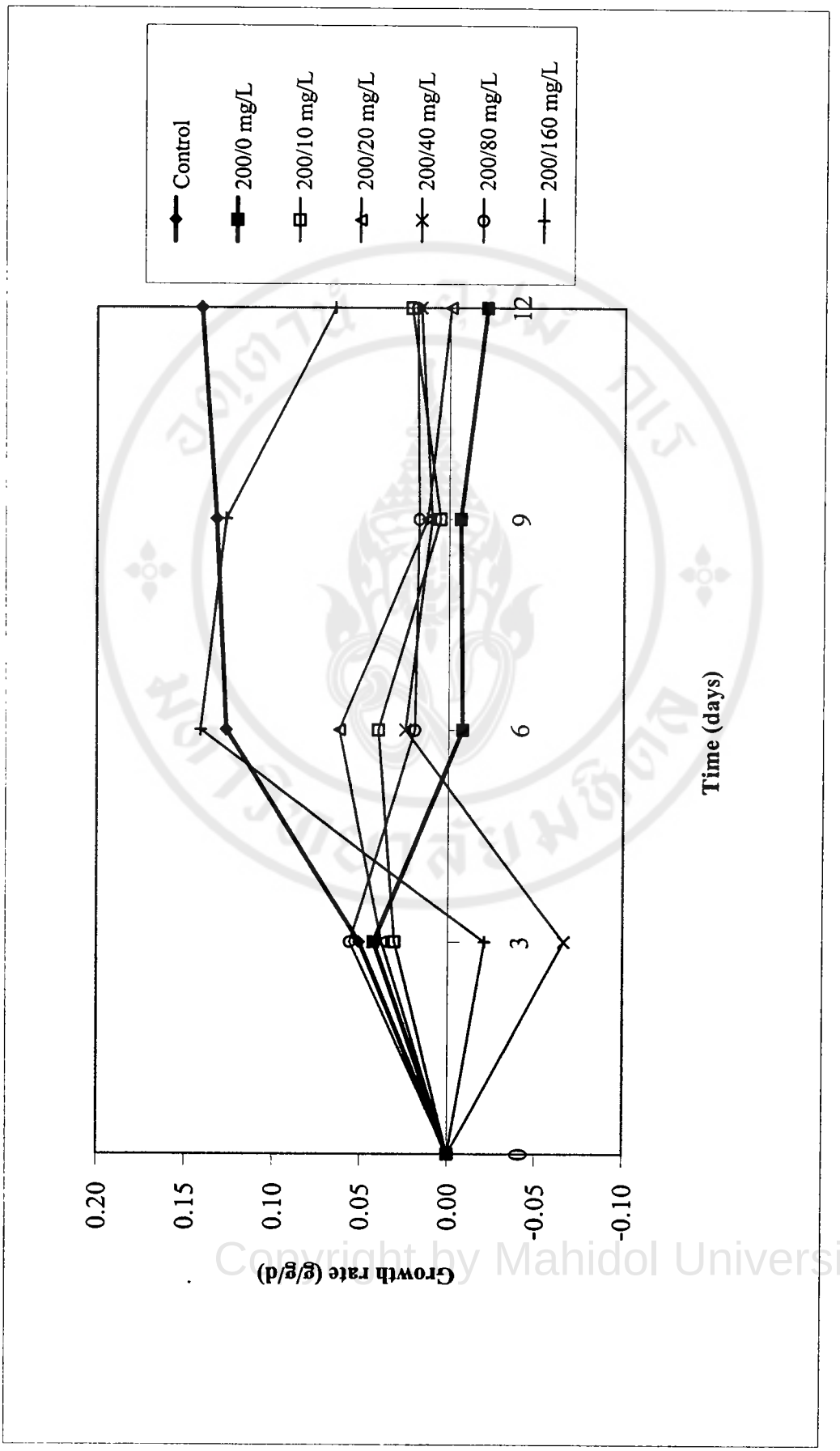


Figure 5-27. Influence of humic acid on the toxic of lead (200 mg/L) on growth rate of *L. minor*.

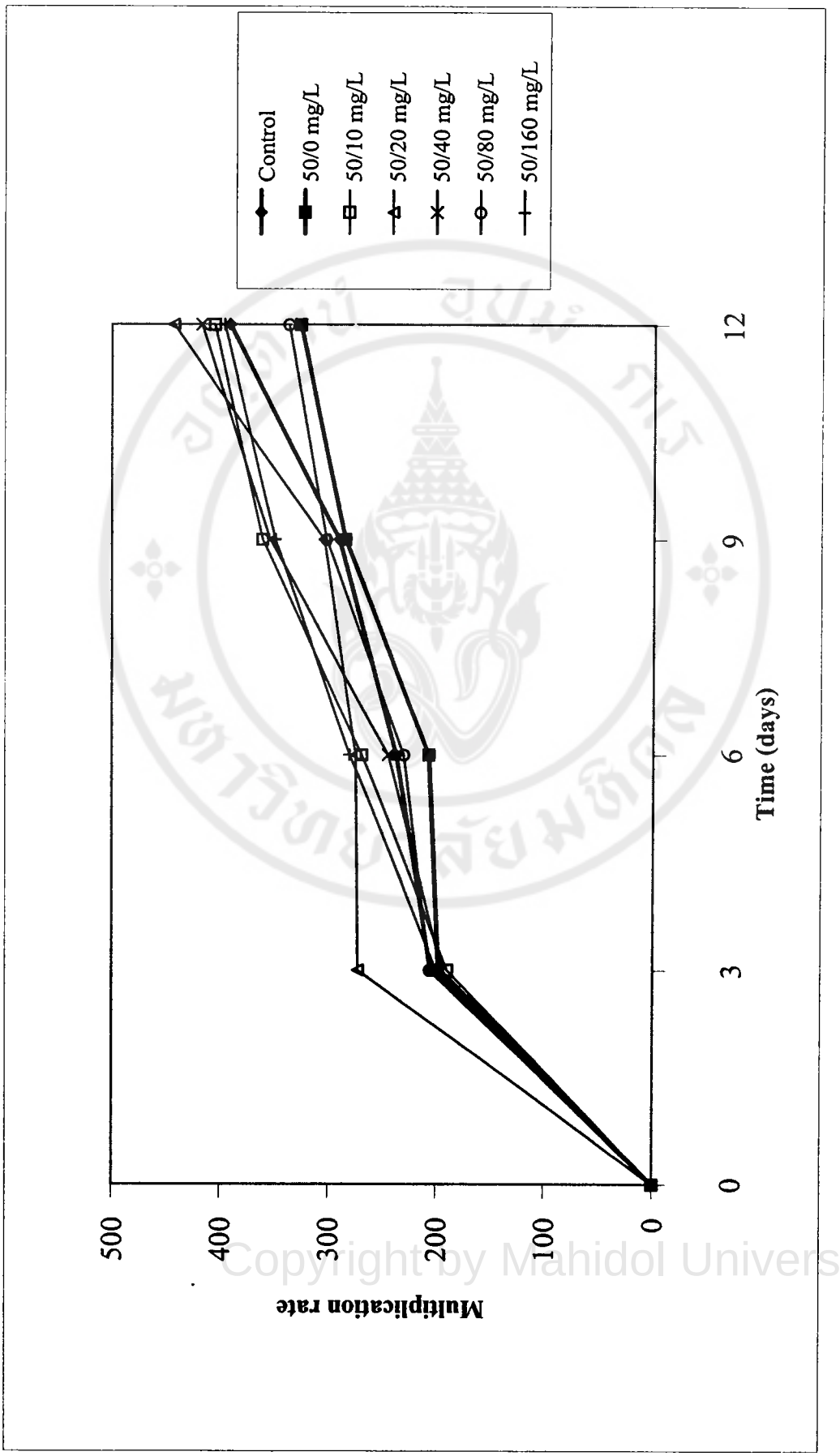


Figure 5-28. Influence of humic acid on toxic effect of lead (50 mg/L) on multiplication rate of *L. minor*.

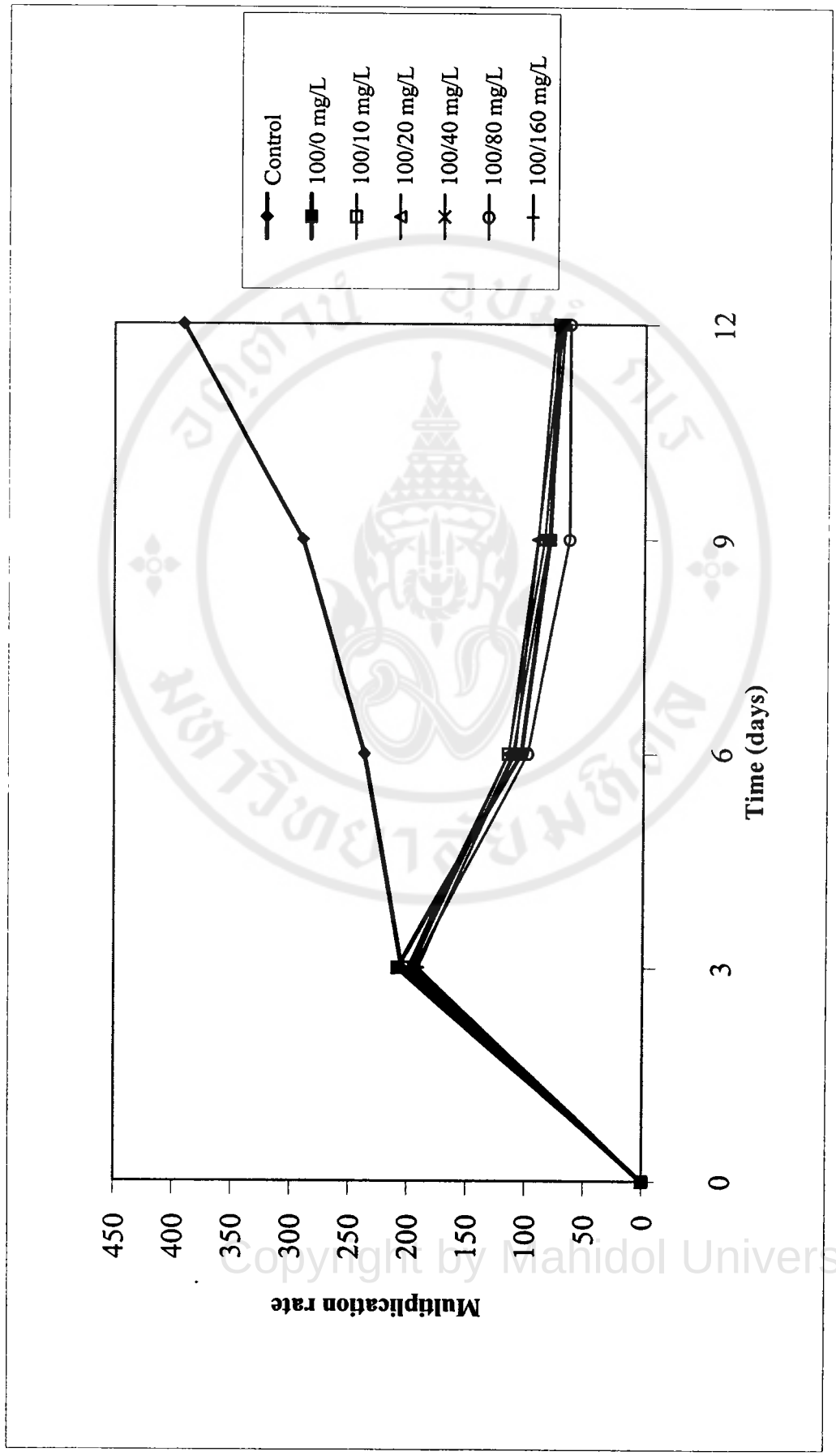


Figure 5-29. Influence of humic acid on the toxic effect of lead (100 mg/L) on multiplication rate of *L. minor*.

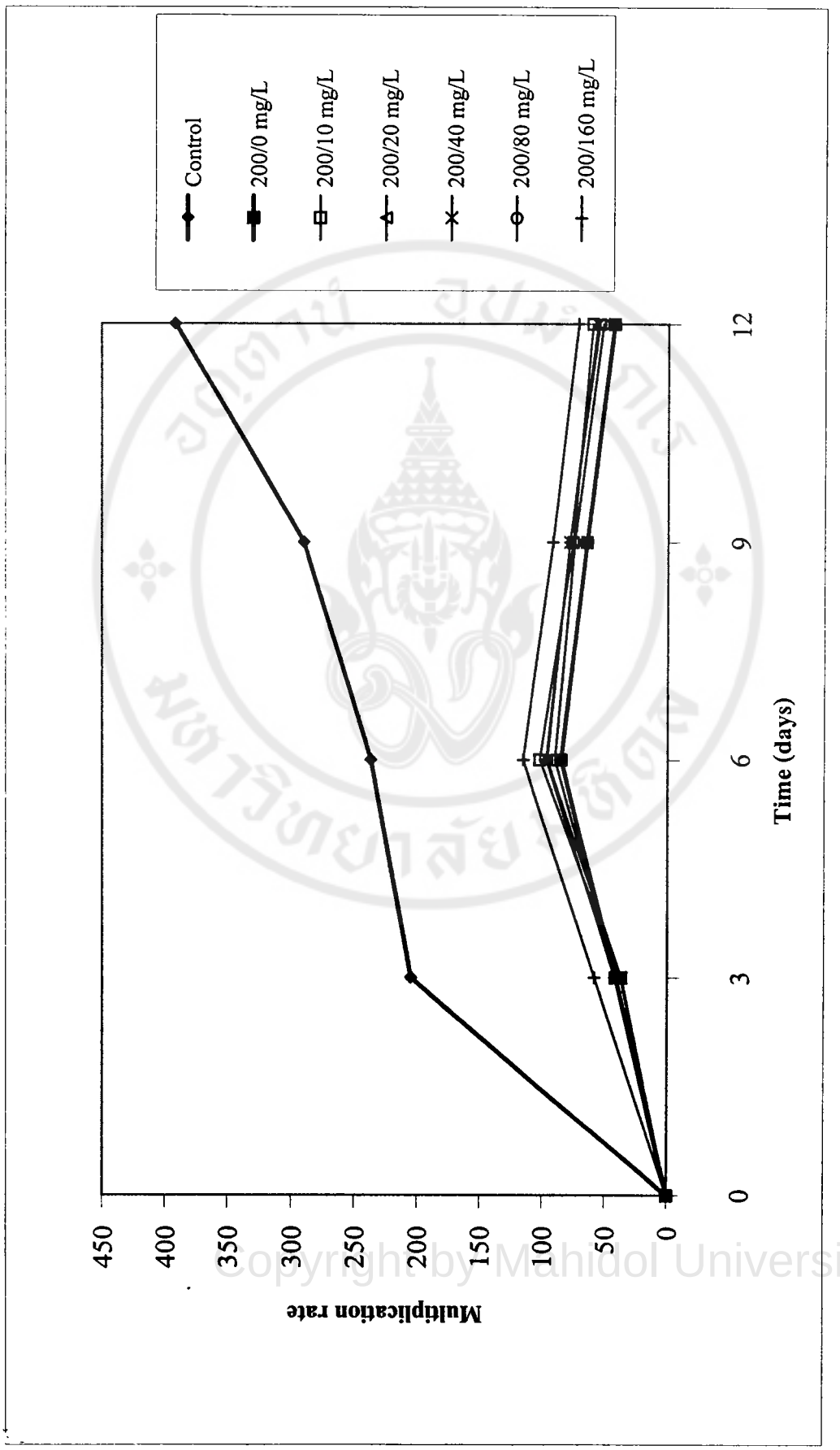


Figure 5-30. Influence of humic acid on the toxic effect of lead (200 mg/L) on multiplication rate of *L. minor*.

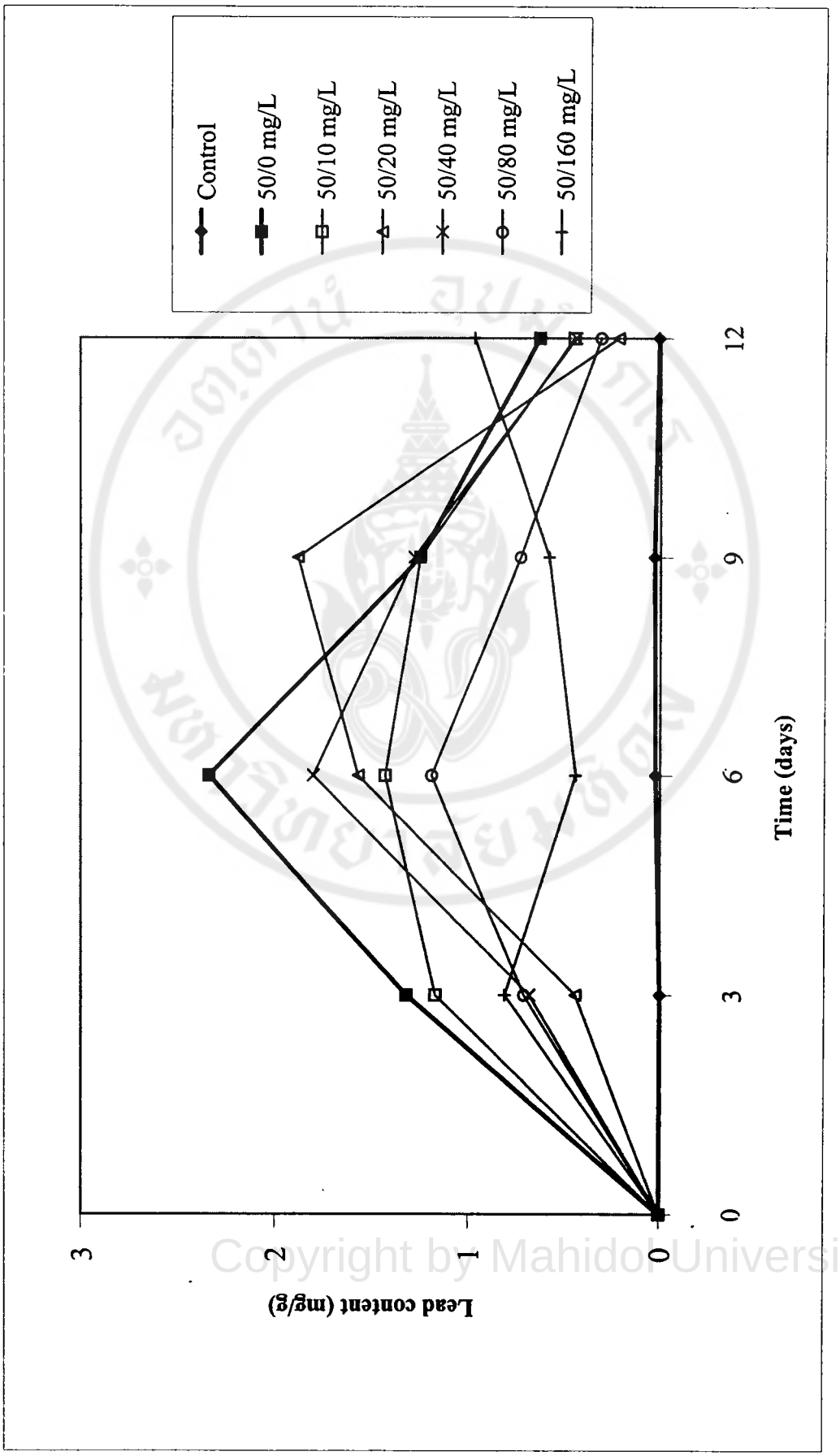


Figure 5-31. Influence of humic acid on lead uptake by *L. minor* exposed to lead nitrate concentration of 50 mg/L.

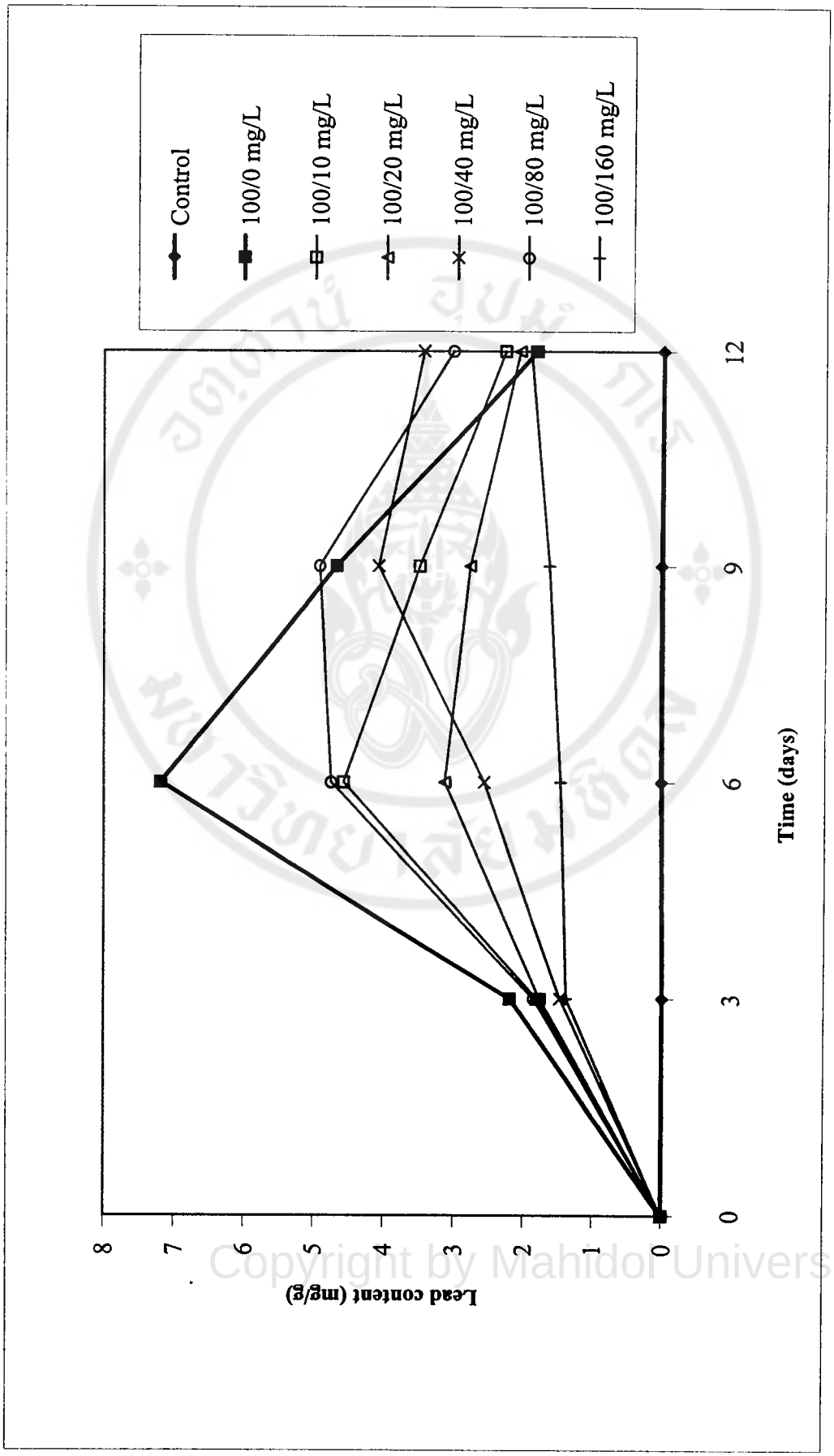


Figure 5-32. Influence of humic acid on lead uptake by *L. minor* exposed to lead nitrate concentration of 100 mg/L.

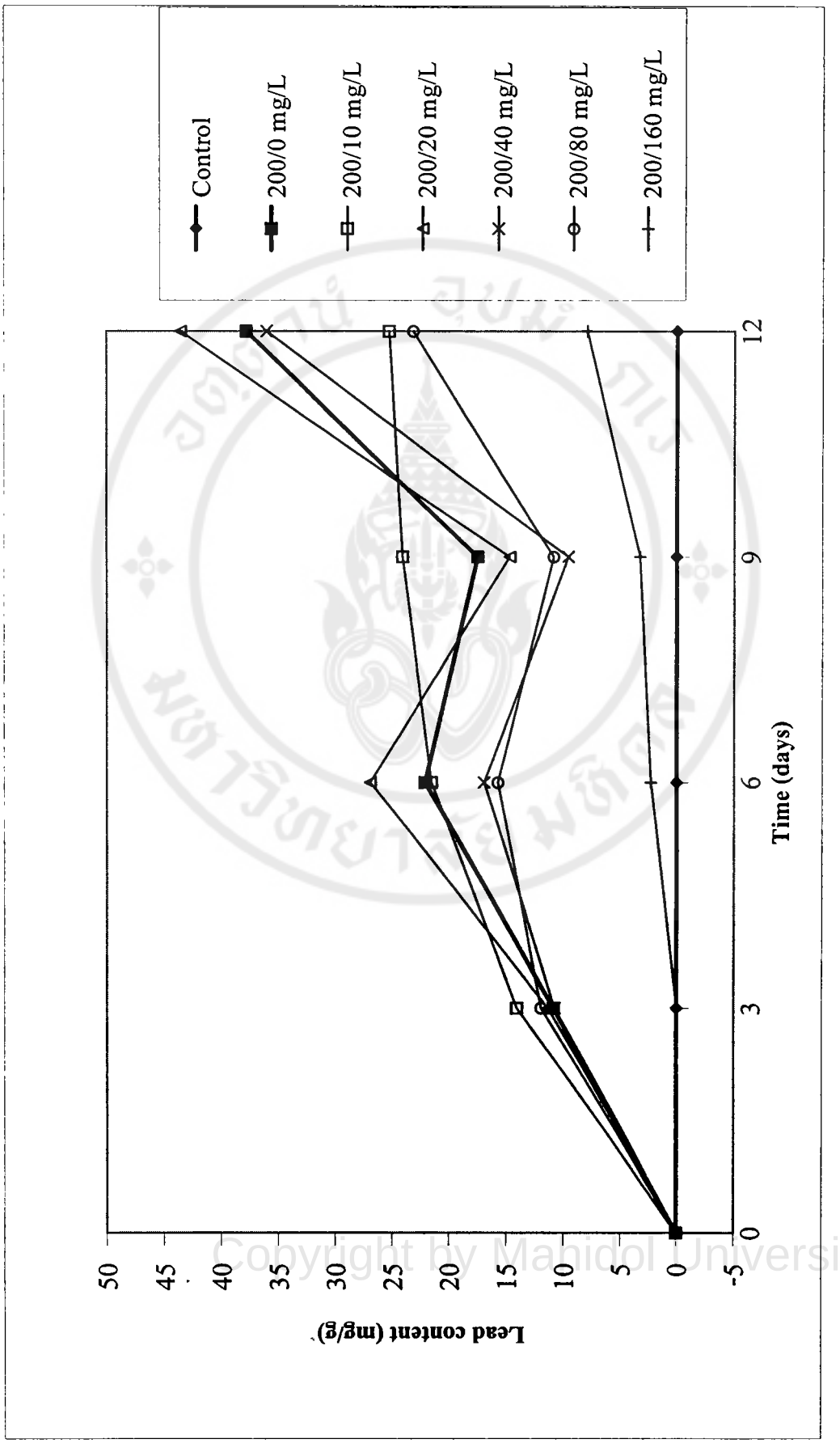


Figure 5-33. Influence of humic acid on lead uptake by *L. minor* exposed to lead nitrate concentration of 200 mg/L.

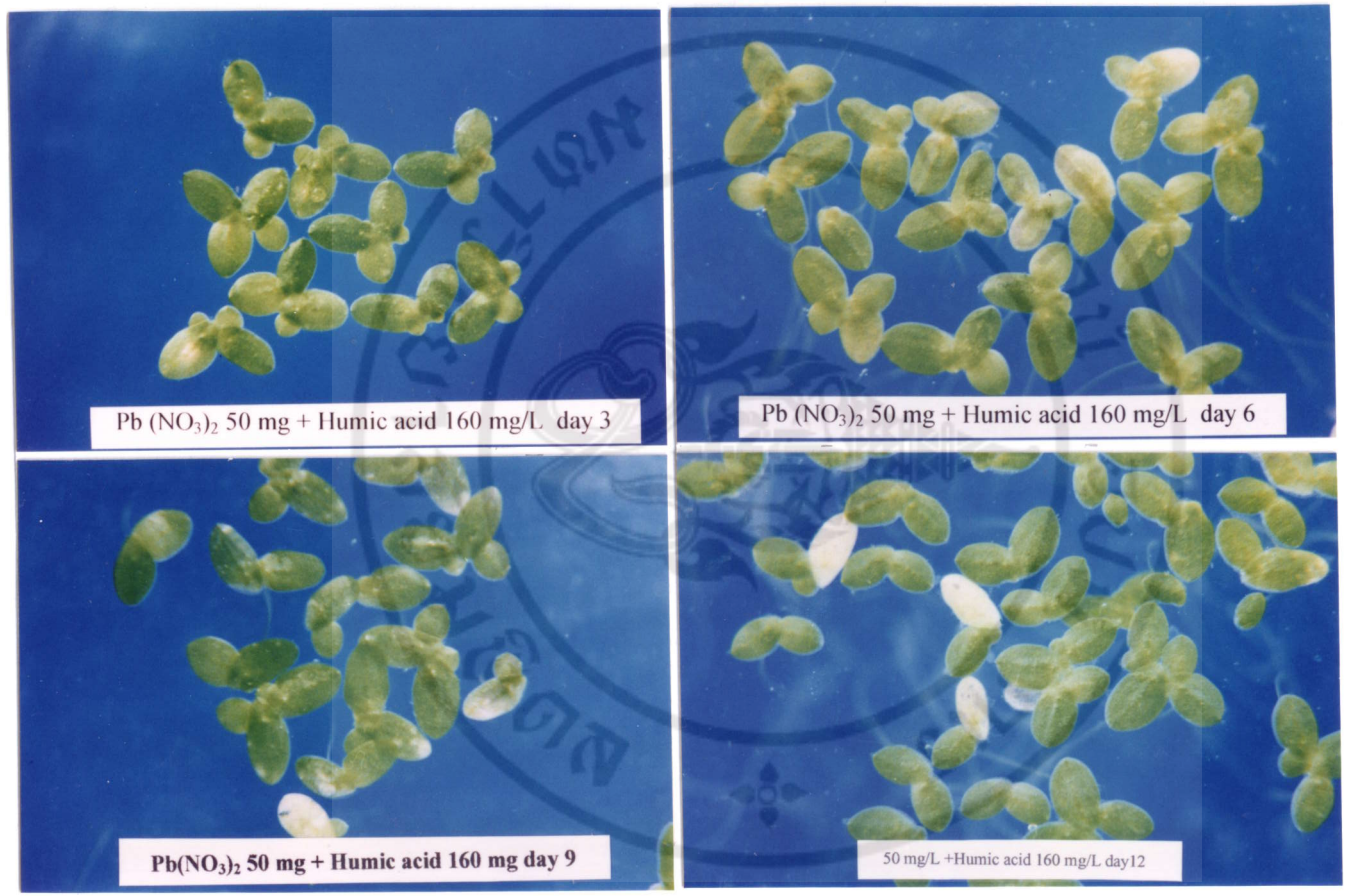


Figure 5-34. Morphological changes of *L. minor* treated with lead nitrate and humic acid (50/160 mg/L) for 3, 6, 9, and 12 days.

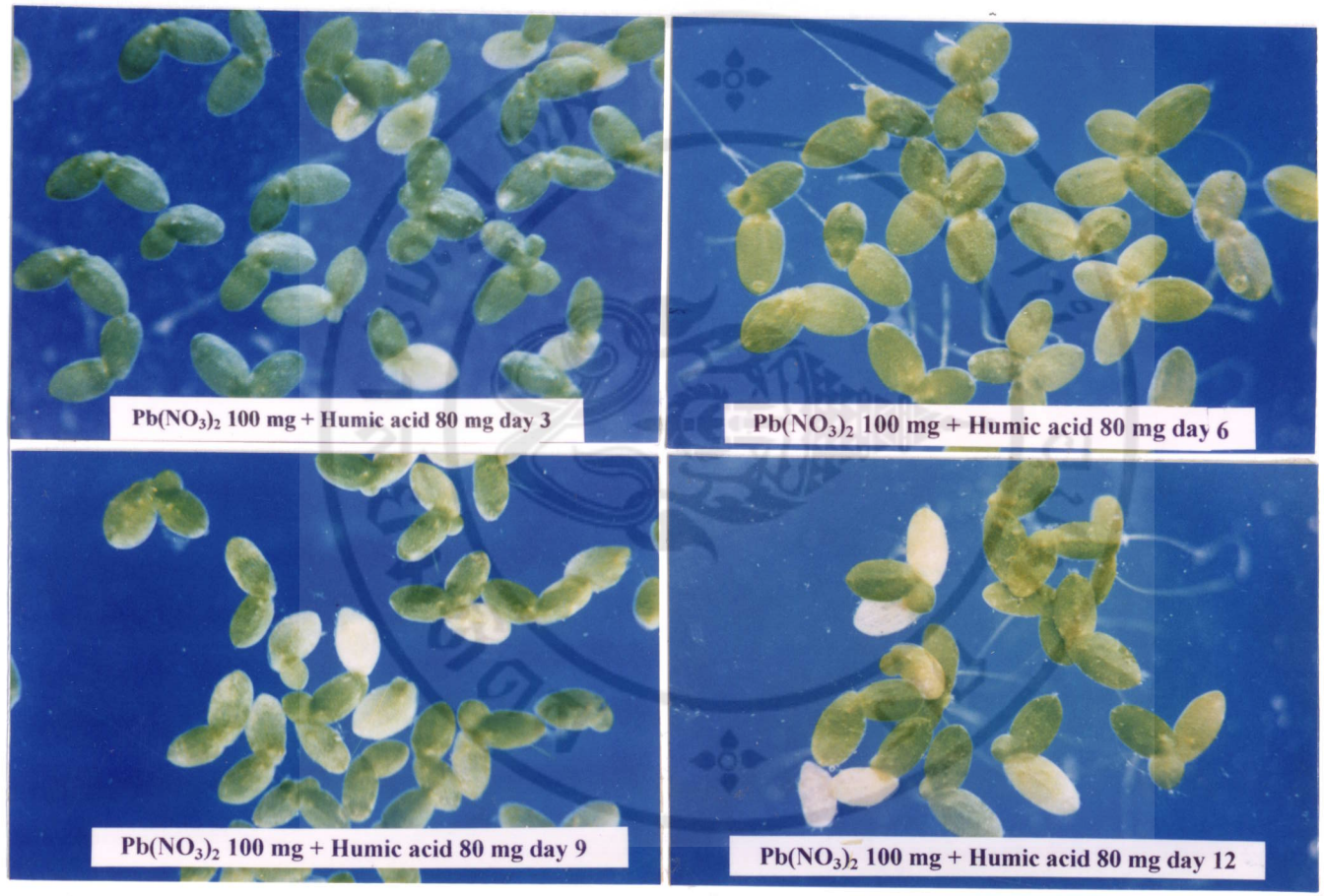


Figure 5-35. Morphological changes of *L. minor* treated with lead nitrate and humic acid (100/80 mg/L) for 3, 6, 9, and 12 days.

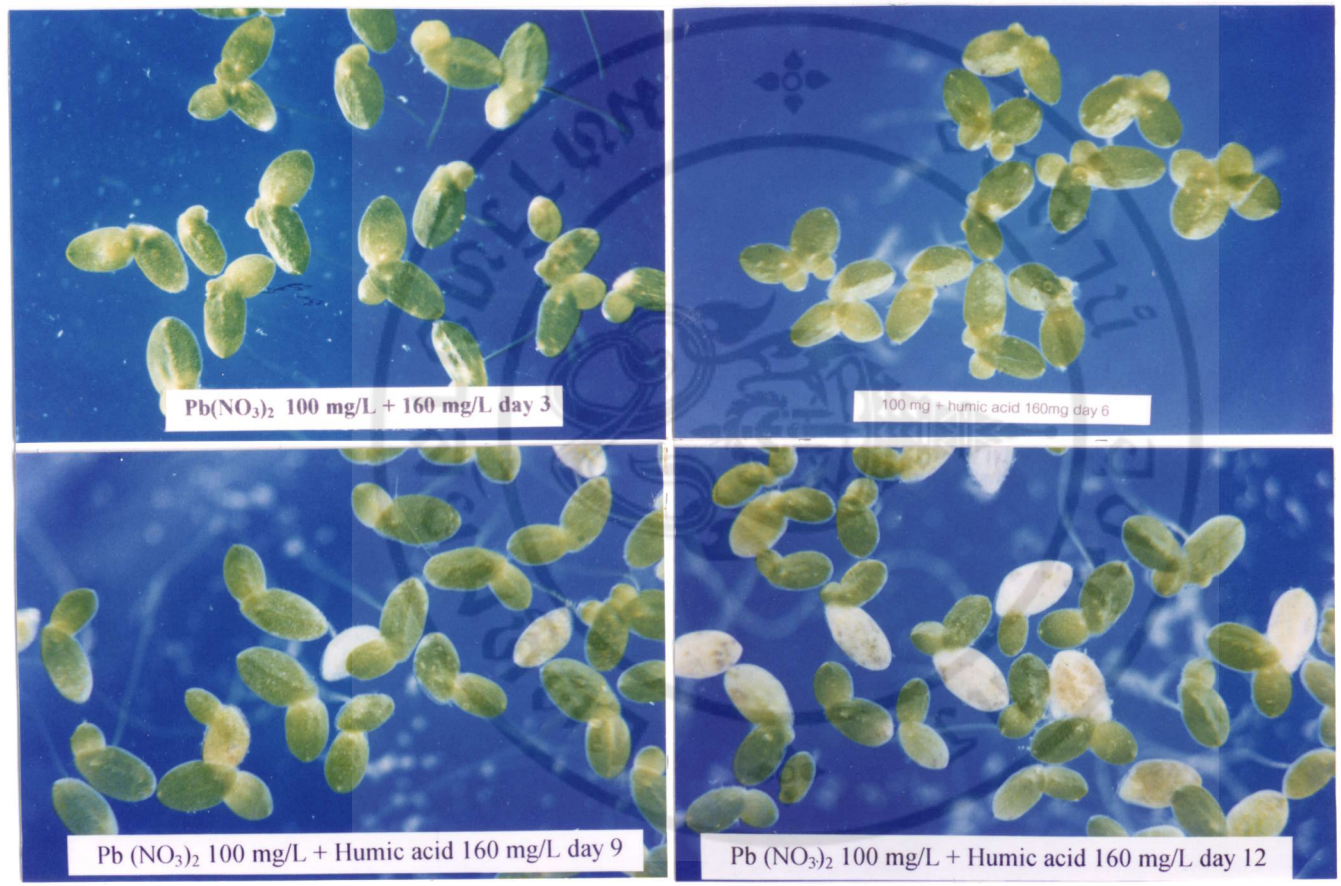


Figure 5-36. Morphological changes of *L. minor* treated with lead nitrate and humic acid (100/160 mg/L) for 3, 6, 9, and 12 days.

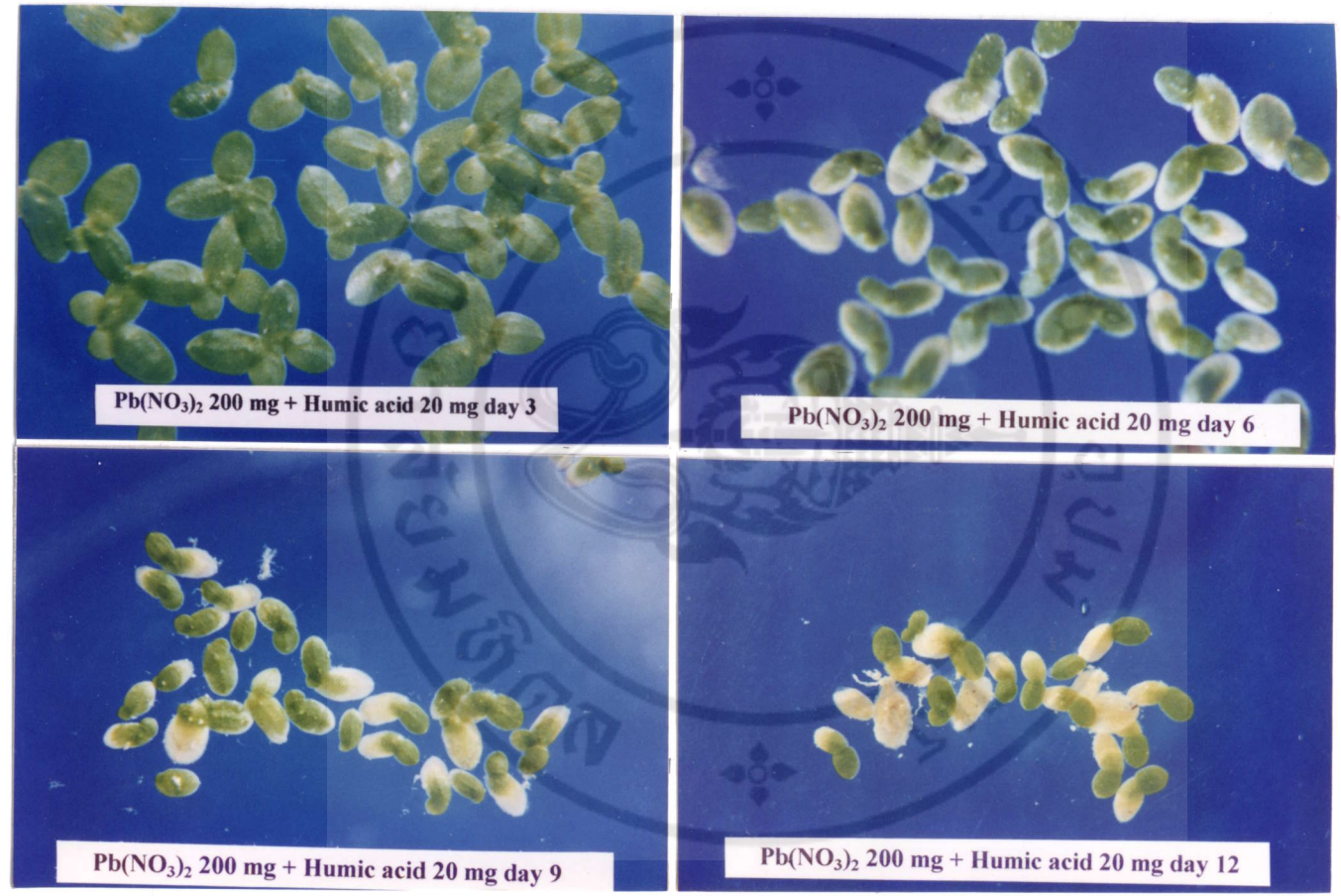


Figure 5-37. Morphological changes of *L. minor* treated with lead nitrate and humic acid (200/20 mg/L) for 3, 6, 9, and 12 days.

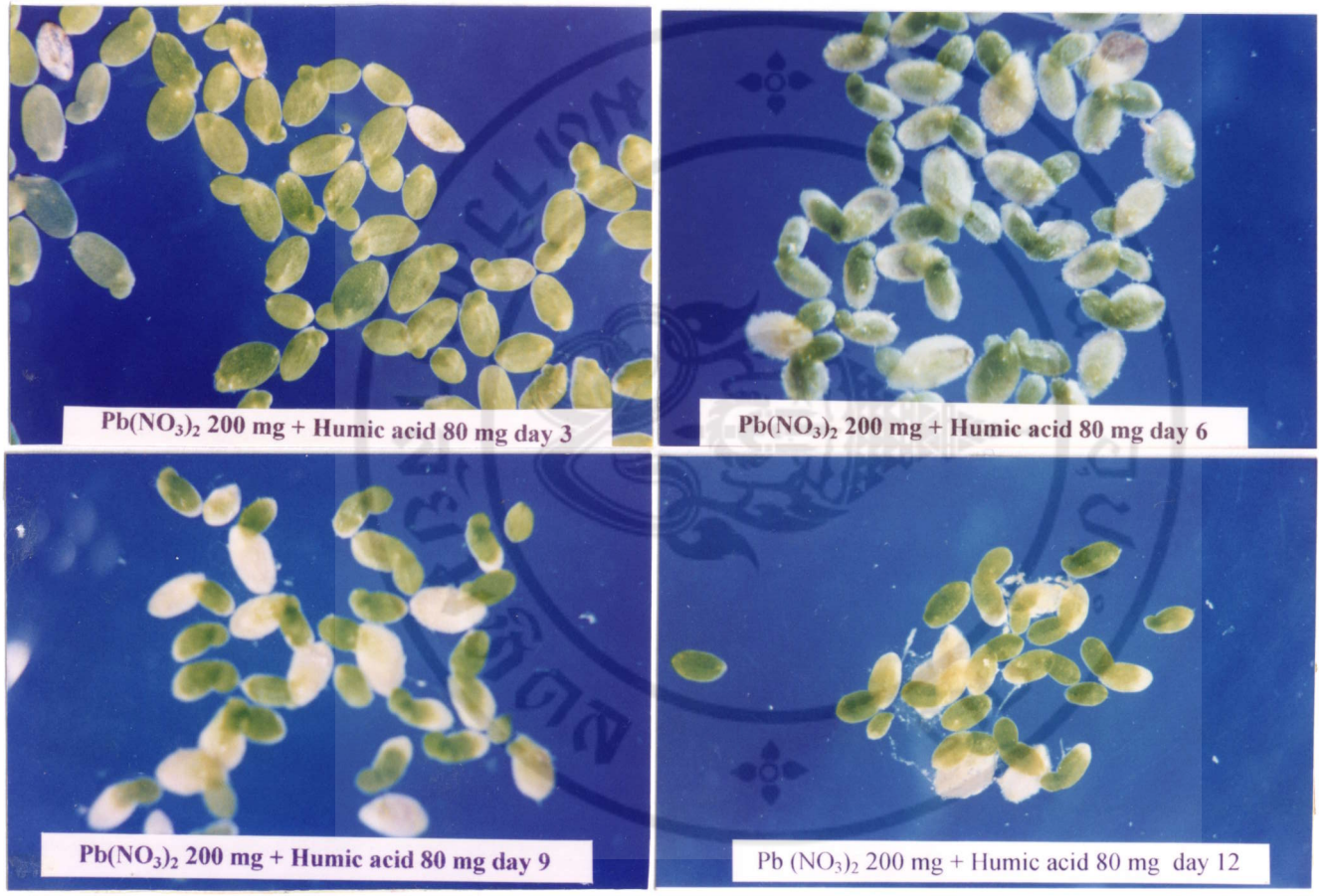


Figure 5-38. Morphological changes of *L. minor* treated with lead nitrate and humic acid (200/80 mg/L) for 3, 6, 9, and 12 days.

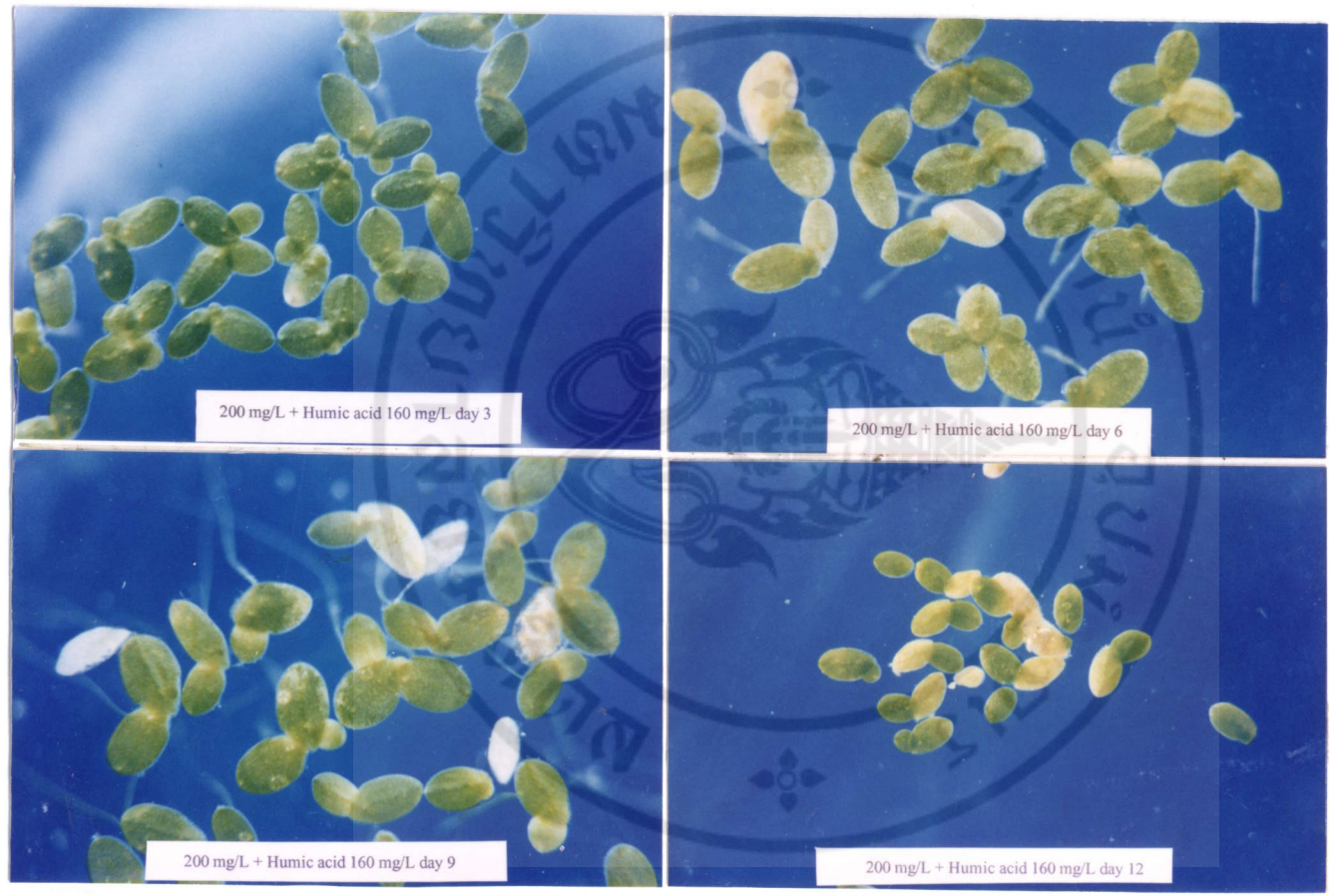


Figure 5-39. Morphological changes of *L. minor* treated with lead nitrate and humic acid (200/160 mg/L) for 3, 6, 9, and 12 days.

CHAPTER VI

DISCUSSION

The present investigations have shown that high concentrations of lead (100 – 200 mg/L) have significant effects on total chlorophyll content, growth rate, multiplication rate, lead uptake and morphological changes of *Lemna minor*. These were decreased when treated with 100 – 200 mg/L of lead nitrate solution within 6 – 12 days. The effects of lead on total chlorophyll contents were also studied by Miranda and Hangovan (39). They found that the accumulation of lead in *Lemna gibba* could reduce the total chlorophyll contents. Lead may inhibit the final reduction step of chlorophyll formation by inhibiting the activity of protochlorophyllide reductase. De fillipis and Pallaghy (46) studied the green alga, *Chlorella vulgaris* grown in the media containing sublethal mercury concentration. They found a decrease of chlorophyll content and enhancement of protochlorophyllide levels. Lead may inhibit the activities of enzymes that has an important role in catalyzing the conversion of δ - ALA into porphobilinogen in the synthesis of chlorophyll. The two different possibilities proposed for the interaction of lead with enzyme were binding of lead to a functional group (lead – SH interaction) and induction of metal deficiency in metalloenzyme and substitution or competition of toxic metal for the essential metal in the enzyme complex *L.minor* that were exposed to high concentrations of lead had a decreased in growth and multiplication rate after 6 – 12 days of exposure.

Miranda and Hangovan(39) studied the lead influence on specific growth rate of *L. gibba*. They found that high lead concentration (200 – 500 mg/L) in the media inhibited significantly the specific growth rate of *L. gibba* under continuous and discontinuous illumination. Shay and Huebert (7) studied the response of *Lemna trisuca* to cadmium. They found that cadmiun (0.64 μg) reduced the multiplication rate of *L. trisulca* after a two – day exposure. Wang (5) studied the phytotoxicity of heavy metals on *L.minor* and showed that copper suppressed both multiplication and frond growth while cadmium suppressed only frond multiplication but did not affect frond growth.

Plants that are exposed to high concentration of lead have lower growth rate and multiplication rate. This might be due to the fact that lead inhibits some process in photosynthesis especially the activity of the enzyme Rubis Co (ribulose 1,5 – diphosphate carboxylase) in Calvin's cycle by interacting with two SH – groups of cysteine which are located in Rubis Co active center (28). These SH – groups are essential for the enzyme activity. The modification in SH – groups results in the loss of enzyme activity. The energy produced by photosynthesis is therefore not enough for normal growth and multiplication of plants (28). Furthermore, lead might induce the activity of enzyme peroxidase that is involved in the degradation of indoleacetic acid (IAA), the hormone which stimulates plant growth and multiplication (47). The induction of peroxidase activity correlates with the lead level in plant tissue.

In addition, lead may inhibit the electron transport and photophosphorylation in photosynthesis . Miller *et al.* (48) isolated the chloroplasts of spinach and tomato treated with lead in order to examine the possible effects of lead on photosystems I and II (PS I, PSII). They found that PS I was not affected by lead, but the primary site of inhibition

was the oxidizing site of PS II, between the primary electron donor of PS II and the site of water oxidation. Furthermore, lead may induce stomatal closure that can be indirectly responsible for a decrease of CO_2 fixation in photosynthesis.

It was shown in the present study that lead uptake by *L. minor* was increased with the increased concentration of lead nitrate. Lead is generally considered to be a non – essential metal to plant. Twenty – five percent of lead in freshwater is in solution form which brings about the toxicity and 75 % is in suspension form (10). The pH regulates the availability of lead owing to its hydrolytic properties (49). Lead may enter the plants through the stomata of leaves and through the roots (46). The stomatal opening ranges from 5 – 30 μm, hence submicron particles containing lead could easily be taken up. The uptake of lead through the root is mainly through transpirational mass flow when plants absorb water and minerals.

The most ardent signs of lead toxicity in *L. minor* were a reduction of leaf development, chlorosis (loss of pigments) and necrosis (localized dead tissue). The present study on morphological changes of *L. minor* treated with lead nitrate showed that the older leaves were more damaged than the younger ones. This might be due to the fact that the older leaves could uptake much more lead than the younger ones because they had more numbers of stomata. In addition, they also had numerous roots which could uptake much more lead directly whereas the younger ones had a few roots or none. Furthermore, lead may damage the membranes of the cells by altering their permeability leading to a leakage of ions such as potassium and other solutes (32). Wang(29), in his review of toxicity test, described that duckweeds could exhibit many symptoms when they were under stress including chlorosis, necrosis, colony breakup, root destruction and loss of buoyancy and gibbosity (swelling). Miranda and

Hangovan (39) studied on the uptake of lead by *L. gibba*. They found that lead reduced the frond size and chlorosis was observed at the concentration of 500 mg/L.

It was shown in the present study that the administration of high concentration of humic acid (160 mg/L) could decrease the lead uptake by *L. minor* and bring about the increases in total chlorphyll content, growth rate and multiplication rate when compared with those of *L. minor* treated with lead nitrate alone. It has been suggested and clearly demonstrated experimentally that natural organic matter, particularly humic substances, under certain conditions could initiate an abiotic reduction of heavy metals such as mercury, cadmiun and lead (50). The reducing effect of natural organic could be less important in soils and sediments, where microbial processes are likely to dominate. However, in aquatic system, where the concentrations of dissolved humic substances generally are in the range of 1 – 10 mg/L, the abiotic redox processes might be of more relative importance (50).

The complexation of humic substances and metal ions is extremely important in affecting the behavior and mobility of metals in the environment. Their ability to form complexes with metal ions can be attributed to their high content of oxygen – containing functional groups with different affinity. The major complexing sites are carboxyl and phenolic groups. If two or more organic functional groups(*e.g.* carboxylate) coordinate the metal ion, forming an internal ring structure, chelation, which is a form of complexation occurs (51). It has been reported that the total binding capacity of humic acid for metal is 200 – 1,600 μmol/g(52). Approximately one - third of the total are cation exchange sites and the remainder are complexing sites.

The possible mechanism of the complex reaction of humic acid and lead ions may be caused by the dissociation of proton from the carboxyl group of humic acid

which starts at pH 6, then it becomes electro – negatively charged and these changes increase with increasing pH (50). In this study, the addition of humic acid to the exposure water containing lead nitrate resulted in a decrease of pH. The solution became a weak base with its pH not more than or equal to 9.

Presumably, there was a dissociation of proton from the carboxyl group and the humic acid could form complexes with lead ions resulting in precipitation which decreased the free lead ions available for the plants, *L. minor*. This mechanism may be responsible for the role of humic acid in decreasing the lead uptake and thereby decreases the toxicity of lead in *L. minor*.

The results of the present study suggested that *L. minor* can be used as a bioindicator due to its capability of accumulating metal in measurable amount, its availability throughout the year with relative ease of collection, its readily availability in terms of quantity and distribution so that unbiased sampling is possible and the cost of its collection and analysis can be acceptable. Moreover, it can be used in tertiary treatment plants to remove heavy metals like lead efficiently. The degree of uptake is affected by the amount of organic matter due to conplexes of humic acid with heavy metals which cause the metals bounded with organic matter to lose their usual characteristics. Humic acid could detoxify lead in plants temporarily. The metal ions might become toxic again depending on environmental factors such as pH and temperature. This type of monitoring is best referred to as ecological monitoring and consists of the surveillance of plants and animals and of the whole system in order to detect changes in environmental quality. But humic acid content should be considered as the important factor related to the heavy metal pollution assessment.

CHAPTER VII

CONCLUSION

The effects of lead and humic acid on total chlorophyll content, growth rate, multiplication rate and morphology of *Lemna minor* were studied. The results were concluded as follows;

1. Effects of lead on *L. minor*

Total chlorophyll content. In the control, 30 and 50 mg/L of lead nitrate, the total chlorophyll content was continuosly increased from day 3 to day 12. But it was significantly decreased on day 9 at the concentration of 100 mg/L and on day 6 at the concentration of 200 mg/L.

Growth rate. In the control, 30, 50 mg/L of lead nitrate, the growth rates were significantly increased from day 3 to day 12. But at lead concentrations of 100 and 200 mg/L, growth rates decreased from day 6 to day 12. The lowest growth rate was found at lead concentration of 200 mg/L and the highest was found in the control.

Multiplication rate. In the control, 30 and 50 mg/L of lead nitrate, the multiplication rates continuously increased from day 3 to day 12. But at lead concentrations of 100 and 200 mg/L, multiplication rates significantly decreased from day 6 to day 12. The lowest multiplication rate was found at lead concentration of 200 mg/L on day 12 and the highest multiplication rate was found in the control.

Lead uptake. The lead content in *L. minor* was increased with the increase of lead concentration. At lead concentration of 30 mg/L, the lead content was increased to the maximum level on day 12. At lead concentrations of 50, 100 and 200 mg/L, the lead contents were increased to the maximum level on day 6. In the control, the lead conten was nearly zero.

Morphological changes. In the control, the number of fronds was double every three days. At lead concentrations of 30 and 50 mg/L, *L. minor* did not show any morphological changes. Chlorotic fronds, loss of buoyancy, breaking up of colonies were found at lead concentrations of 100 mg/L on day 9 and 200 mg/L on day 6.

2. Combined effects of lead and humic acid on *L.minor*

Total chlorophyll content. At 50 mg/L of lead nitrate, the total chlorophyll contents at every concentration of humic acid (10 < 20, 40, 80, and 160 mg/L) were increased to the maximum level on day 12.

At lead concentration of 100 mg/L, the total chlorophyll contents in every concentration of humic acid were decreased from day 3 to day 12. The highest total chlorophyll content was found at the concentration of 100/160 mg/L on day 6. The lowest total chlorophyll content was found in plants exposed to lead nitrate 100 mg/L on day 12.

At lead concentration of 200 mg/L, the total chlorophyll contents at humic acid at concentrations of 10, 20 and 40 mg/L were decreased from day 3 to day 12. But at humic acid concentrations of 80 and 160 mg/L, the total chlorophyll contents were increased from day 3 to day 12.

Growth rate. At lead concentrationof 50 mg/L, the growth rates at every concentration of humic acid increased from day 3 to day 12. The highest growth rate was found a humic acid concentrationof 160 mg/L on day 6 and 9.

At lead concentration of 100 mg/L, the growth rates at every concentration of humic acid increased. The highest growth rate was found at humic acid concentration of 200 mg/L. The growth rates at every concentration of humic acid decreased except at the concentration of 160 mg/L whose growth rate was increased on day 6.

Multiplication rate. At lead concentration of 50 mg/L, the multiplication rates at every concentration of humic acid were increased.

At lead concentrations of 100 and 200 mg/L, the multiplication rates decreased. The highest multiplication rate was found at humic acid concentration of 160 mg/L on day 6, 9 and 12.

Lead uptake. At lead concentration of 50 mg/L, the lead contents were increased to the maximum level on day 6 at every concentration of humic acid. The lowest content was found at humic acid concentration of 160 mg/L.

At lead concentration of 100 mg/L, in 0, 10, 20 and 40 mg/L of humic acid, the lead contents were increased on day 6 and then decreased on day 12. The highest lead content was found in plants exposed to 100 mg/L of lead nitrate alone on day 6. The lowest lead content was found at humic acid concentration of 160 mg/L on day 6, 9 and 12.

At lead concentration of 200 mg/L, the lead contents in 10, 20, 40, 80 and 160 mg/L of humic acid were increased from day 3 to day 12. The lowest lead content was found at humic acid concentration of 160 mg/L from day 3 to day 12.

Morphological changes. At lead concentration of 50 mg/L, *L. minor* did not show any morphological changes in every concentration of humic acid.

At lead concentration of 100 mg/L, the morphological changes of *L. minor* in every concentration of humic acid were similar to those exposte to 100 mg/L of lead alone.

At lead concentration of 200 mg/L and lead combined with every concentration of humic acid, chlorotic fronds were found on day 6 ; breaking up of colonies and loss of buoyancy were found on days 9 and 12. But at humic acid concentration of 160 mg/L, all of these symptoms were decreased.

References

1. Moore JW, Ramamoorthy S. Heavy metals in natural water: applied monitoring and impact assessment. New York: Springer-Verlag Inc., 1984.
2. Cooper WC. Cancer mortality patterns in the lead industry. Annuals of the New York Academy of Sciences.1976; 271:250-9
3. Clifford RL, Stefanie RL. Biomonitoring Water. Environ. Research.1997;96 (4).900-6 Yeo AR. Solute transport in plants. 1st ed. Great Britain: Blackie Academics Profressional, 1992.
4. Riemer DN. Introduction to freshwater vegetation.USA : AVI Publishing Company Inc,1984.
5. Wang W, Williams JM. The use of phytotoxicity tests on common duckweed, cabbage, and millet for determining effluent toxicity. Environ. Monit. Assess. 1990 ; 14 : 59 – 69.
6. Mohan BS, Hesetti BB. Potential phytotoxicity of lead and cadmium to *Lemna minor* grown in sewage stabilization pond. Environ. Pollut.1997 ; 98 : 233-38.
7. Huebert D, Shay JM. The response of *Lemna trisulca* L. to cadmium. Environ. Pollut. 1993 ; 80 : 847 – 53.
8. Lewis MA. Use of freshwater plants for phytotoxicity testing : a review. Envion. Pollut. 1995 ; 57 : 319 – 36.
9. Newman MC, Mc Intosh AW. Metal ecotoxicology : concepts & application USA : Lewis Publishers, 1991.

10. Moore JW. Inorganic contamination of surface water: research and monitoring priorities. New York : Springer-Verlag Inc, 1990.

11. Eaton TG, Parrish PR, Hendricks AC. Aquatic toxicology. Baltimore : American Society for Testing and Materials, 1980.

12. Wilson EL, Burger PE, Dowdle ED. Biochem Environ. J. 1972 ; 29 : 563 – 71.

13. Krenkel DA. Heavy metals in the aquatic environment: An international conference. 1st ed. Great Britain : Pergammon Press Ltd, 1975.

14. Wang W. Literature review on higher plants for toxicity testing. Water Air and Soil Pollut. 1991 ; 59 : 381 – 400.

15. Corn M. Handbook of hazardous materials. USA : Academic Press Inc.,1993.

16. Stoops G, Eswarem H. Soil micromorphology. New York : Van Nostrand Reinhold Company, 1986.

17. Thomas JD. The role of dissolved organic matter, particularly free amino acids and humic substances in freshwater ecosystems. Freshwater Bio. 1997 ; 38 : 1-36.

18. Narine DR, Guy RD. Binding of diquat and paraquat to humic acid aquatic environments. Soil Sci. 1982;133 (6):356-63.

19. Brady NC, Weil RR. The nature and properties of soils. 11th ed. USA : Prentice – Hall International Inc, 1996.

20. Orlov DS. Soil chemistry. USA : Balkema Publishers, 1992.

21. Larson RA, Wabber EJ. Reaction mechanisms in environmental organic chemistry. USA : CRC Press Inc, 1994.

22. Bertion DJ, Albro PW, Haas JR. Enzymatic hydrolysis of carbohydrates in aquatic fulvic acid. Environ. Sci. Technol. 1987 ; 21 : 859 – 63.

23. Clapp CE, Jr. High molecular weight water soluble muck : isolation and determination of constituent sugar of a borax complex –forming polysaccharide employing electrophoretic techniques. Diss. Abstr. 1957;17 : 963 – 64.

24. Tan KH, Clack FE. Polysaccharide constituents in fulvic and humic acids extracted from soil. Geodurma 1965 ; 2 : 245 – 55.

25. Wilson MA, Goh KM, Collin PJ, Greenfield LG. Origin of humus variation. Org Geochem. 1986 ; 9 : 255 – 31.

26. Mierle G, Ingroum R. The role of humic substances in the mobilization of mercury from watersheds. Water Air and Soil Pollut. 1991 ; 56 : 349 – 57.

27. Fasett CN. A manual of aquatic plant. 1 st ed. New York and London: Mc Graw-Hill book Company Inc, 1940.

28. Landis WG, Hoya MI. Introduction to environmental ecotoxicology. USA: Lewis Publishers, 1995.

29. Wang W. Toxicity test of aquatic plants by using common duckweed. Environ. Pollut. (Ser. B) 1986 b;11:1-14.

30. Journey W, Spira W, Skillicorn P. Duckweed aquaculture. 1 st ed. USA : The World Bank, Washington D.C.,1993.

31. Rejmankova E. Comparison of *Lemna gibba* and *Lemna minor* from the production of ecological viewpoint. Aquat. Bot. 1975 ; 1 : 423 – 27.

32. Farago ME. Plants and chemicals elements : biochemistry, uptake, tolerance and toxicity. New York : VCH Publisher,1994.

33. Hillman WJ. The Lemnaceae or duckweeds : A review of the description and experimental literature. Bot. Rev. 1961 ; 27 : 221 – 87.

34. Jones TW, Winchill L. Uptake and photosynthetic inhibition by atrazine and its degradation products on four species of submerged vascular plant. J. Environ. Qual. 1984 ; 13 : 234 – 7.

35. Staves RR, Knaus RM. Chromium removal from water by three species of duckweeds. Aquat. Bot. 1985 ; 23 : 261 – 73.

36. Rodgers JH, Chery DS, Guthrie RK. Cycling of elements in duckweed *(Lemna perpusilla)* in an ash settling basin and swamp drained system. Water Res.1978 ; 12 : 765 – 70.

37. Wang W. Site specific Ba toxicity to common duckweed, *Lemna minor*. Aquat. Toxicol. 1988 ; 12 : 61 – 7.

38. Glandon RP,Mc Nabb CD, The uptake of boron by *Lemna minor*. Aquat. Bot. 1978 ; 4 : 53 : -64.

39. Miranda MG, Hangovan K. Uptake of lead by *Lemna gibba* L : Influence on specific growth rate and basic biochemical changes. Bull. Environ. Contam. Toxicol. 1995 ; 56 : 1000 – 7.

40. Kwan KAM, Smith S. The effect of thallium on the growth of *Lemna minor* and plant tissue concentrations in relation to both exposure and toxicity. Environ. Pollut. 1988 ; 203 – 18.

41. Mackinney G. Absorption of light by chlorophyll solutions. J. Biol. Chem. 1941 ; 144 : 315 – 23.

42. Porath D, Hepher B, Koton A. Duckweed as an aquatic crop : evaluation of clone for aquaculture. Aquat. Bot. 1979 ; 7: 273 – 8.

43. Landolt E, Kandeler R. Biosystematic investigation in the family of duckweeds (Lemnaceae), the family of Lemnaceae – a monographic study 1987 ; 4(2).

44. Anderson R. Sample pretreatment and separation. Singapore : John Wiley & Sons, 1991.

45. Katz SA, Jennis SW. Regulatory compliance monitoring by atomic absorption spectroscopy. USA : Verlac Chemie International Inc, 1983.

46. De Fillipes LF, Pallaghy CK. Zeitschr. Pflanzenphysiol. 1976 ; 78 : 314 – 22.

47. Hoffman DJ, Ratther BA, Burton GA, Cairns J. Handbook of ecotoxicology. USA : CRC Press Inc, 1995.

48. Miller WE, Peterson SA, Green JC, Callahan CA. Comparative toxicology of laboratory organisms for assessing hazardous waste sites. 1985 ; 14 : 569 – 74.

49. O'Neill P. Environmental chemistry. 2nd ed. Great Britain : ST. Edmundsbury Press, 1993.

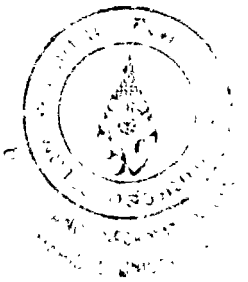
50. Allard B. Arsenic I. Abiotic reduction of mercury by humic substances in aquatic system – an important process for the mercury cycle. Water Air and Soil Pollut. 1991 ; 56 : 457 – 64.

51. Kein TH. Principles of soil chemistry. New York : Marcel Dekken Inc,1993.

52. Merian E. Metals and their compounds in the environment. New York : Verlagsgeselles Chaft Publisher, 1991.

53. Livens FR. Chemical reactions of metals with humic materials. Environ. Pollut. 1991; 70: 183 – 203.

BIOGRAPHY



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