CHAPTER 1

INTRODUCTION

Plant materials provide the main source of minerals to animals and to most of the human ra es. Some of these minerals are essential for the body growth but some are harmful, such as lead, cadmium, arsenic, etc. Fortunately, these toxic substances contaminate vegetable tissues as trace amounts. The main sources of these substances are from soil, water, air, fertilizers and pesticides. The concentration ranges of inorganic elements in natural soil and plants are compared and shown in Table 1.

Lead occurs naturally in the earth's crust in the concentration of about 13 mg/kg (1). The lead content in the acidic soil is generally lower than that in alkaline soil. It is accepted that dominant forms of lead in soil are insoluble and slightly soluble salts adsorbed, multicomponent precipitates such as lead carbonate, lead phosphate and organo-lead complexes, as well as small amounts of lead oxide, lead chloride and lead sulfate(3). The solubility of lead salts is a function of their chemical compositions. Particularly, the formation of chloride and hydroxy salts were found to influence the solubility of the lead salts thus contributing to their mobilization in the environment(4).

The nature of the organic matter in soil also has a considerable influence on the lead content. Some organic matter

Table 1 Ranges of concentrations in soil and plants of inorganic elements which sometimes occur as environmental contaminants (2).

Element	Range of Concentrations	
	Soil(ppm)	Plant(ppm)
Arsenic	0.1-40.0	0.1-5.0
Boron	2-100	30-75
Cadmium	0.1-7.0	0.2-0.8
Copper	2-100	4-15
Fluorine	3-300	2-20
Lead	2-200	0.1-10.0
Manganese	100-4,000	15 - 100
Nickel	10-1,000	1
Zinc	10-300	15-200

is rich in chelating components, and it binds lead, either promoting its movement out or fixing the metal depending on the solubility properties of the complex. Although all of these factors no doubt play a role in determining the lead content of specific soil, the concentration of lead is usually as that found in rocks in the area encountered, with an average of 5.25 mg/kg (5).

Natural water from streams, rivers and lakes generally contains low level of lead. This level is increased in region where lead is used in agriculture or industry, The lead content in ground water was reported in the range of 1 to 60 $\mu g/dm^3$ (6,7), in lakes and rivers was found to be 1-10 $\mu g/dm^3$(8), in sea water off the coast of California was found to be 0.08-0.4 $\mu g/dm^3$(9) and in surface water off Bermuda which was free from continental influence was found to have the average of 0.07 $\mu g/dm^3$ while the central Atlantic water contained the lead content average of 0.7 $\mu g/dm^3$(10).

The atmospheric concentration of lead measured at the sampling points where mainly over remote area of oceans and over Greenland was of the order of 0.0001-0.001 $\mu g/m^3$(11,12,13,14). Studies of heavy metals content especially lead content in plants near major highways were performed by numerous workers(15,16,17,18, 19). Results from these studies showed that the amounts of lead in native plants and crops grown close to highways were influenced by

1) the distance from the highway,

2) the extent of plant surface exposed,

3) the external plant characteristics,

4) the duration of plant exposure,

5) the traffic density, and

6) the direction of prevailing winds.

(Some trees have the capacity to accumulate high concentration of lead.)

Patterson (20) had reported that apart from industrial lead emission 5,000,000 tons of lead were discharged annually in the northern hemisphere of the United States and 250,000 tons(21) from the exhaust of motor vehicles. This distribution of lead was normally 100 m to a maximum of 200 m from the highways, leading to very high lead values, not only in the soil (from 20-300 ppm (22)), but also in the aerial parts (shoots, fruits) of agricultural plants (from 10 - 300 ppm to dry matter (23)).

Fertilizers are the other source of lead provided in plants Lead which was found in fertilizers may come from the process of production and the raw material. Although some workers reported apparent benefits from an addition of low amounts of lead in fertilizers, there is no report that lead is an essential element for plant growth(24). Various growth-inhibiting and promoting effects of lead in plant had been studied. Hewitt(25) showed that among many trace elements; Cr, Mn, Co, Ni, Cu, Zn, Pb, Cd, V and Mo, lead was less active in causing chlorosis in plants grown in sand culture due to excess of lead ions. Baumhardt and Welch(26) studied various plant characteristics of corn as a function of lead in soil and they reported that no effect on emergence of plant height and grain

yielded by lead added. They also notified that no morphological color, maturity or other growth difference during their 2-year study. Dilling (27) suggested that lead salts in concentrations above 0.01 % of lead ion delayed the germination and the growth of seed (cress and mustard); and lead salts in concentrations of about 0.1 to 0.2 % of lead ion inhibited germination of cress and mustard for at least 18 days without destroying the vitality of the seeds, which still germinated on transfer to water culture . Addition of lead chloride at concentrations from $2x10^{-4}$ to $2x10^{-2}$ M reduced the growth of lobloly pine and red maple seedlings in pots of two forest soils(28). Seedings grew better on a sandy loam than on a silt loam, but there was little difference between soil types in influenceing responses to lead(28). It was interesting that an increase of lead in culture media resulted in the decrease of net photosynthesis and transpiration of corn (Zea Mays L. and Glycine max L.) (29). The degree of the effect on photosynthesis and transpiration depended on the amount of lead applied.

Toxicity levels of lead to certain crops established by Barker (30) who studied the depression of the tissue ability (to increase) in fresh water. Lettuce and carrot explants were responsive to lead at 0.5 mg/dm^3. In cauliflower and potato, toxicity of lead became real at concentration somewhere between 0.5 and 5.0 mg/dm^3(30). No discoloration of the tissues or other gross morphological abnormality were observed (30).

With the expansion of agriculture and medicine during the last 100 years, the need for effective insecticides to control pest of crops, animals, and human beings was increased. The earlier insecticides included various inorganic compounds that contained lead, antimony, arsenic,

mercury, selenium, sulfur, thallium, zinc and fluorine as the active ingredients. These compounds, although not very toxic to insects, were very persistent; for instance, sprayed crops sometimes retained sufficient arseniacal residues to be potentially harmful to the consumers and crops were sometimes damaged by residues in soil (31). These chemicals had limited usage and persistent pesticides were not widely used until the discovery of DDT and other organochlorine insecticides during and after the Second World War. However, nowadays there is still some gardeners who use lead compounds such as lead arsenate.

For human being, lead is ingested with the food we eat and the water we drink. Yet, despite its widespread occurrence in food and water, lead has not been found to play any useful metabolic role in life processes. There are three means by which lead can enter the body and produce toxic symptoms: inhalation of dust, fume, vapors, and mists, ingestion through the mount on food, tobacco or on the fingers; and through broken skin or absorption through the skin as tetraethyl lead and other organic compounds.

Minute quantities of lead are found in all foodstuffs. Nevertheless, the normal dietary of lead rarely exceeds 1 mg/day and is

usually much less (32). Monier - Williams (33) had estimated that the daily intake of lead by a normal healthy individual was about 0.4 mg: 0.22 mg being derived from food, 0.10 mg from water and 0.08 mg from inhaled dust. Recently FAO/WHO (34) has proposed a provisional tolerble weekly for man (tentative) as 0.05 mg / kg body - weight. The average daily intake of lead from food and water for an adult is about 200 - 250 µg, this figure being based on both the estimate and the actual metabolic balance (35). Of the lead ingested, adults absorbed 5 - 10 %, confirmed by using ^{204}Pb (36). The usual values of lead for drinking water run about 10 µg/dm^3. The standard consumption of 400 to 500 dm^3 per year would thus contribute 4 or 5 mg of lead per year (37). The studies of Kehoe (35) in human adult volunteers and clinical observation indicated that once excessive lead ingestion was terminated, it took at least twice as long to excrete an excessive body burden as it did to accumulate the burden.

Although metabolic evidence of toxicity usually abates after 6 to 12 months, it may, after protracted ingestion, be several years before blood levels return to normal (32). During this recovery phase most of the residual excess lead is stored in bone. Disorders may also mobilized lead into the soft tissue (32).

In eight normal children aged 3 months to 8.5 years, up to 50% of the intake of lead was absorbed and up to 18 % retained (38). The similar result was also observed in young animals (38). The measurements

of lead in blood provide the best means for following the trends in children with lead intoxication (32).

The absorption of lead is affected by such factors as the presence or absence of food in the gut and the consumption on the diet, although in this respect the influence of dietary milk on absorption remains confusedly. The total body burden of lead is divided among three compartments (39):

1) lead in blood and some soft tissues, this being a rapidly exchangeable pool

2) lead in soft tissue and loosely bound to bone, also rapidly exchangeable and

3) lead tightly bound in the skeleton, forming 60-95 % of the total body burden.

All lead compounds must be considered cumulative poison. Acute plumbism is characterized by distinctive eosinplilic intranuclear inclusion bodies in the liver, kidney, pancreas and brain. In the kidney, these lesions are most common in the proximal tubular epithilicl cells. Functionally, these tubular injury can cause the Falconi syndrome (hypophosphatemia, glycosuria and aminoaciduria) (32). Late lead nephrophathy is characterized mainly by vascular injury resulting in scarred contracted kidneys and renal failure. Albuminuria may also be present. The associated hyperuricemia and resultant gouty manifestations apparently reflect a selective decrease in the clearance of urate.

The metabolic pathway for the biosynthesis of heme is exquistely sensitive to the toxic effects of lead (32). The enzymes definitely inhibited by lead in the pathway for heme formation are sulfhydryl

enzymes. Coproporphyrin and S-amino levulinic acid can be consistently demonstrated in great excess in urine even in the absence of clinical symptoms.

Diagnosis can be established by lead in blood (more than 80 µg/ 100 cm^3 of blood indicates excessive absorption) or lead in urine (more than 150 µg / dm^3 indicates excessive absorption) (40).

Lead intoxication can be cured and the recovery is usually complete. One of the treatments is the intravenous injection of sodium calcium salt of ethylenediamine tetraacetic acid which results in a heavy urinary excretion of lead (41,42). Dimercaprol and Penicillamine also can be used for acute plumbism and cumulative poison (40).

Usually, the most abundant single constituent of vegetable is water which may represent up to about 96% of the total weight. Most of the solid matter of vegetables is made up to carbohydates along with smaller amounts of protein and of fat (43). Included in these groups are the constituents which build up the main structural features of vegetable tissues, the prominent cell walls, the layers of living cytoplasm and, where present, the grain of storage starch. These substances together with water, are called the major constituents. Many other classes of organic compounds and a wide range of mineral elements drawn from the soil, are called the minor constituents, These can have a most important influence on the properties of fruit and vegetable on their colors, flavors and nutritive values and in some cases on their textures.

Lead occurs naturally in all plants, as well as in soil,

air and water. Extremely variable concentrations of lead in plants had been reported but nevertheless, certain generalization were made. Watanabe, et al. (44) compared the atomic absorption spectrophotometric, the alternating current polarographic and oscillopolarographic techniques for determining lead in crop. Laaksovirta, et al. (45) described an isotope-excited x-ray fluorescence analysis to determine lead from lichen and pine bark near highways in the Southern Finland. Heavy metal ions uptake by lower plants such as lichens was studied by Puckett, K.J. et al. (46). Dedolph, et al. (47) studied theimportance of air, water and soil as sources of lead in perennial ryegrass and radish. Two reports suggested that the lead content of plant parts decreases in order: roots, stems, leaves and seeds (48, 49). Warren and Delavault (50) concluded that the normal concentration of lead in leaves and twigs of woody plants was 2.5 mg / kg on a dry weight. Ward, et al. (51) determined lead in soil and vegetable along a New Zealand State Highway with low traffic volume by atomic absorption spectrophotometer, lead levels of 250 - 1100 µg/g were reported in the ash of plants (51).

For vegetables and cereals, Warren and Delavault (50) estimated normal concentrations of lead to be 0.1 - 1.0 mg/kg dry weight. Mitchell (52) found that the concentration of lead in pasture grasses was 1.0 mg/kg dry weight. These figures should be multiplied by a factor of 20 to convert the concentration on a dry weight basis to an ash weight basis.

However, food consumption is a significant source of lead ingestion. The content in various vegetable in many countries

are shown in Table 2. For Thailand there is no report about lead content in vegetables. This made the author enthusiatic to determine lead contents in various vegetables. The purpose of the current study is to determine quantitatively the content of lead in each vegetable species and to investigate the effects of the environment on the content of lead in vegetable species.

The anodic stripping voltammetric technique was selected for this study since there is a trace amount of lead in each vegetable species and this technique has been proved to be powerful for the trace analysis of certain metal ions of environmental concern (57,58).

The fundamental of an anodic stripping voltammetric measurement involves two discrete steps. The deposition step, the analytical species is firstly reduced (electrodeposited or plated) onto or into the working electrode ; and is secondly oxidized (stripped or electrolyzed) back into the electrolyte solution which is known as stripping step. The deposition step can be carried out in two ways. In the stoichiometric procedure the ion of interest is removed completely from a stirred solution at a constant cathode potential. In the nonstoichiometric procedure only a fraction of the ion is deposited ; here, not only the electrode potential must be controlled, but care must also be taken to reproduce the electrode size, the length

Table 2 Lead content in vegetable food

Sample	Lead concentration (ppm)	Country	Method	References
Canned vegetable	0.1	Spain	Atomic absorption spectrophotometer	53
Canned cucumber pickles	1.10-8.71 (4.20)*			
Cucumber in bottled	0.86-1.40 (1.10)*			
Cabbages	0.01-0.51	England	Atomic absorption spectrophotometer	54
Potatoes	0.01-0.14			
Onions	0.01-0.38			
Leeks	0.04-0.07			
Carrots	0.01-0.04			
Swedes	0.01-0.05			
Brussels sprouts	0.01-0.24			
Watercress	0.01-0.33			
Frozen vegetables	0.01-0.10			

Table 2 (continuted)

Sample	Lead concentration (ppm)	Country	Method	References
Cucumber	0.01-0.03	England	Atomic	54
Celery	0.01-0.02		absorption	
Mushrooms	0.03-0.04		spectrophotometer	
Dried herbs	0.10-3.85			
Miscellaneous	0.01-1.50			
Horticultural product (Lettuce, Radish, Beet root)				55
	0.82-2.9	Italy	Spectrophotometer	
Lettuce	185 (max)+	The German Democratic Republic		56

* average result is shown in blanket

+ near lead smelter

of deposition, and the stirring rate for both the sample and the standard solutions employed for calibration. The nonstoichiometric procedure has the advantage of speed, since the electrolysis step needs only a few minutes.

Mercury electrode of various forms have been most widely used for electrolysis, although platinum or other inert metals can also be employed. Generally, it is desirable to minimize the volume of the mercury since this enhances the concentration of the deposited species. For the nonstoichiometric approach several methods have been devised to produce a microelectrode of reproducible dimension and one of these employs the hanging mercury drop electrode which is also used throughout this study. This electrode involves the formation of amalgams of some elements which are soluble in mercury. The sensitivity of the stripping analysis depends on how concentrated an amalgam can be formed during the deposition step. Relatively high amalgam concentrations can be obtained in the limited volume of the hanging mercury drop electrode in reasonable deposit times. The amalgam concentrations can be obtained by considering the diffusion processes with in the hanging mercury drop electrodes and calculated from the approximation equation (59)

$$C_{amal} = 3i(t)_{dep} t / 4\pi F r_o^3 \qquad (1)$$

where C_{amal} = the concentration of the metal in the amalgam , molar

$i(t)_{dep}$ = the deposition current , μa

f = the deposit time , minute

n = the number of electrons involved in the electrode reaction

F = the Faraday value

r_o = the radius of the mercury electrode, cm

A typical value of $i(t)_{dep}$ for a 10^{-5}M solution is the order 0.5 μa and a 5 minute electrolysis would produce an amalgam approximately 5×10^{-3}M, a concentration increase (over the original solution) of about 500. A similar 500 fold increase in the sensitivity of the analysis could be expected during the subsequent stripping analysis.

Deposition currents cannot be measured accurately for solutions more dilute than about 10^{-5} M. However, measurements at higher concentration can be used to calibrate the mass - transfer process, and $i(t)_{dep}$ for the lower concentrations

can be obtained from a linear extrapolation. Thus, above equation can be used to predict the stripping analysis for a particular cell - electrode- stirring arrangement and to help selection of the proper deposition time.

For a single metal ion species (M^{nt}, being reduced at electrode surface, it can be shown that the current flow (the deposition current at time t) is reasonably approximate by the Levich equation (57) :

$$i(t)_{dep} = 0.62\ n\ F\ A\ D^{2/3}\ W^{1/2}\ \mu^{1/6} C(t) \qquad (2)$$

where $i(t)_{dep}$ = limiting current of deposition time t seconds, ma

n = number of electrons transfer

F = the Faraday constant, 96494 coulombs

A = the electrode surface area, cm^2

D = diffusion coefficient, $cm^2\ sec^{-1}$

W = the rate of electrode rotation or solution stirring ($w = 2\pi N$ and N = revolution per second)

μ = the kinematic viscosity of the solution, $cm^2\ sec^{-1}$

C(t) = the ion concentration of deposition time t seconds, mole cm^{-3}.

After the deposition step, the resulting amalgam must be analyzed. This can be accomplised by almost any of the electroanalytical methods, such as square-wave polarography, chronopotentiometry, oscillographic polarography and voltammetry with linearly varying potential. However, most of the important analytical applications of the method have used the technique of voltammetry with linearly varying potential for analysis of the amalgams (60,61)

Thus, the process of an anodic stripping analysis is as followed. In a nonstoichiometric deposition, the Hg drop is formed, stirring is begun, and a potential that is a few tenth of a volt more negative than the half-wave potential for the ion of interest is applied across the calomel - the hanging Hg drop system. Deposition is allowed to occur for a carefully measured period. After completion of the carefully time deposition, the stirring is stopped for perhaps 30 seconds. The voltage is then decreased at a fixed rate from its original cathodic value toward the anodic, and the resulting anodic current is recorded as a function of the applied voltage. A peak appears in the current - potential curve with its potential, a qualitative indication of the identity of the metal ion ; and the peak height, a quantitative measure of its concentration in the solution. The total current (i_t) flowing through the system is

$$i_t = i_f + i_c + i_b \qquad (3)$$

where i_f = the faradaic current, due to oxidation of the species being analyzed and is equal to i_p at the peak potential.

i_c = charging current, due to the changing of the double layer at the electrode solution interface

i_b = background current, due to oxidation of impurities or decomposition of electrolyte.

Together, i_c and i_p make up the residual current or electrochemical noise in the system. The stripping current due to oxidation of each analyte is proportional to the concentration of the analyte on or in the electrode and, thus, in the analytical solution. The oxidation potential have the same qualitative meaning as their half wave curves may be used to strip the deposited analyte from the electrode and obtain the quantitative parameter, the stripping peak current, (i_p).

For a hanging mercury drop electrode , the stripping peak current is given by (62)

$$i_p = 2.72 \times 10^5 n^{3/2} A D^{1/2} C_E \nu \qquad (4)$$

where i_p = the stripping peak current, amperes

n = number of electrons transfer

A = the electrode surface area, cm^2

D = diffusion coefficient, $cm^2\ sec^{-1}$

C_E = the bulk concentration, mole cm^{-3}

ν = the ramp rate for the potential scan, volt sec^{-1}

Quantitative analysis is made by measuring the total peak current, i_p, at the peak potential. The concentration of ion analyzed is then determined by a standard addition method or the previously prepared calibration curve.

For standard addition method the concentration of the test species is calculated by (63)

$$\frac{i'_p}{i_p} = \frac{C_a}{C_x} \qquad (5)$$

and

$$C_a = \frac{C_x V + C_s v}{V + v} \qquad (6)$$

where i'_p = total diffusion current of the known and unknown solution

i_p = the diffusion current of the unknown solution

C_a = the concentration of species determined after the addition of the standard solution of concentration C_s

C_x = the unknown concentration of the ion species determined

V = the volume of the unknown solution used.

v = the volume of the standard solution added

C_s = the concentration of the standard solution.

The basic instrument required for ASV includes a three electrode potentiostat and voltage ramp generator, current measuring circuit (a cell with working, reference, and counter electrode), and a recorder or other readout device. Instrument designed for dc, ac, or pulse polarographic measurement is generally quite adequate for stripping application.

Supporting electrolyte concentrations of 0.05 - 0.5M are typical (57). Almost any indifferent electrolyte can be used, but the choice often improves the reproducibility and resolution of the stripping peaks.

If no supporting electrolyte is present in the solution, there would be another force action on the reducible ion. This could result from the gradient of electrical potential between the two electrodes of the cell. This current is migration current. Thus, the current due to the reduction of metal ion is greater than the diffusion current (64).

In this thesis, conditions for trace determination of lead in vegetables by anodic stripping voltammetry are reported. The decomposition of vegetables and the comparison of lead content in various vegetable species are presented. In addition, the influence of the environment on lead content in vegetables are illustrated.