# CHAPTER I

# INTRODUCTION

Plants provide the main source of minerals to man and other terrestrial animals. Plants are supplied with nitrogen and mineral elements from the soil. Essential mineral elements can be classified into the major elements, calcium, magnesium, potassium, sodium, phosphorus, sulfur and chlorine ; and essential trace elements (sometimes described as micronutrients) such as copper, manganese, iron, zinc, molybdenum, cobalt, iodine and boron. All of these elements are required for the normal growth and maintenance of health in plants and animals. In addition to the essential trace elements, plants are supplied by the other non-essential trace elements such as lead and cadmium that are not at present known to be required either by animals or plants. The main sources of these toxic elements 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.

## 1.1 Lead

Lead is a natural widespread constituent of the earth's crust. Lead in soil ranges from 2 to 200 μg/g and averages 16 μg/g [2]. However, lead compounds are generally insoluble and so poorly transferred from one medium to another. As a result, the concentration in natural bodies of water is extremely low between 0.001 and 0.01 $\mu g/cm^3$. The chemical form and reactions of lead in soils are still very imperfectly understood, but it is known that only a very small

Table 1 Ranges of concentrations of inorganic elements in soil and plants[1]

| Element | Range of Concentrations | |
|---|---|---|
| | Soil (μg/g) | Plant (μg/g) |
| Arsenic | 0.1-40.0 | 0.1-5.0 |
| 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 |

proportion of the total amount in a soil is present in solution. While a plant is growing, its roots remove ions from the soil solution and these are replenished from the solid phase. The natural lead content of surface waters is of the order of 5 $\mu g/dm^3$ [3]. A survey of such waters in the United States gave a range from 0 to 55 $\mu g/dm^3$ [4]. The limit of safety given by the World Health Organization is 50 $\mu g\ Pb/dm^3$ [5]. Most drinking water supplies contain less than 10 $\mu g/dm^3$ but many domestic water supplies can exceed this limit where the water is soft and comes from lead-lined tanks and water pipes[4].

The natural level of lead in air, if there is no contribution from man-made pollution, had been estimated by Patterson[6] to be 0.0005 $\mu g/m^3$. Actual atmospheric Pb levels ranging from 0.4 to 7.6 $\mu g/m^3$ in different cities, at sites with varying motor traffic densities were cited by Hicks[7] in 1972. Most of this lead came from the exhaust fumes of cars burning petrol containing lead alkyl additives. Lead is normally present in soils to the extent of 10 to 100 mg/kg, with more in the surface soil layer than in the lower horizons[3].

Studies of lead content in plants near highways or lead mines were performed by numerous workers[8,9,10], the evidence showed that the lead content decreased with the increasing distance and the outer leaves showed more lead than the inner leaves.

Published values for the lead content of individual food items are so variable that it is difficult to provide a meaningful classification into high, medium, and low groups. Thus Warren and Delavault[11] obtained the following values for English and Canadian grown vegetables from a range of locations : lettuce, 0.3-56 (mean 12); cabbage, 0.2-2.3

(mean 1); potato, 0.2-7.6 (mean 1.6); carrot, 0.2-11(mean 4); and bean, 1-12 (mean 4) µg/g on dry basis. Garcia et al.[12] observed a smaller range of 0.20-0.34 (mean 0.27) µg/g on dry basis for 11 samples of whole kernel dent corn. The levels of lead in wheat, flour, and bread (North American grown) had been reported as follows : common hard wheat, 0.50 ± 0.22; common soft wheat, 1.00 ± 0.61 ; flour, baker's patent, 0.92 ± 0.43; flour, soft patent, 1.02 ± 0.59; and bread, white, 0.41 ± 0.29 µg/g on dry basis. On the basis of these figures lead is not concentrated in the germ and bran and lost in the milling of flour, as with other minerals.

Inorganic compounds, such as the arsenates of copper and lead were formerly used on a large scale as insecticides or herbicides and Bowen[13] stated that until recent years about $3 \times 10^7$ kg was applied to crops annually.

Fertilizers are the other sources of lead provided in plants. Lead which was found in fertilizers came from the process of production and the raw material. Comprehensive information on the trace element content of fertilizers was published by Swaine[14].

No obvious deleterious effects on the growth of plants have been observed on adding soluble lead up to a level of 400 µg/g soil. This is a heavy addition, since uncontaminated soil normally contains around 1 µg/g available lead[4]. Lead, therefore, does not appear to be phytotoxic, even at levels in the heavy contaminated soil. However, there was some evidence based on experimental work with maize and soybeans, that both photosynthesis and transpiration were inhibited by the presence of high lead levels in plants[15]. Motto et al.[8] and

Rolfe[16] reported that most of the lead taken up by plants seemed to accumulate in the root system, and appreciable amounts were only translocated to leaves at relatively high soil lead levels.

The total body burden of lead is divided from food, water and inspired air. In normal adult man ranges from 90 to 400 mg[17,18]. Lead concentrations increase at high-Pb intakes in all tissues, except the muscles, and especially in the bones, liver, kidney, and hair.

The symptoms and pathology of acute lead poisoning have been well documented by M.R. Moore et al.[19] The ingestion, in fairly large quantities, of lead salts such as lead acetate, produces immediate severe illness with burning pain in the mouth, throat and stomach. This is followed by severe colickly abdominal pain and constipation or diarrhoea, often with the passage of blood, because of severe irritation of the gut lining and, presumably, spasm of its muscle wall. In severe poisoning there is then failure of the heart and circulation, the kidneys and the liver, the patient finally lapsing into coma and death.

Chronic lead poisoning is characterized particularly by neurological defects, renal tabular dysfunction, and anemia. Damage to the central nervous system, causing lead encephalopathy and neuropathy, is a marked and common feature, especially in children with their low lead tolerance.

## 1.2 Cadmium

Cadmium is a toxic element and it is also widely distributed in the environment by man's activity. Cadmium is also geochemically

associated with zinc and is found as an impurity (up to 3 percent) in zinc ores such as zincblende and sphalerite (ZnS) or calamine ($ZnCO_3$). Cadmium is found in the rather rase mineral, greenockite (CdS). In uncontaminated soils the cadmium/zinc ratio is usually in the range 1:100 to 1:1,000[20]. Electroplating, iron alloys, pigments, alloys, batteries and plastic additives also utilize cadmium in their processes. Cadmium is also a contaminant in phosphate fertilizers and sewage sludges. There are only small amounts of cadmium in air,water and food. The normal level of cadmium in the air is approximately 0.001 $\mu g/m^3$ which would lead to a maximum inhaled amount of 0.02 µg/person/day[4]. The amount inhaled from the air in most circumstances is insignificant compared with that ingested with the food, with the exception of heavy smokers who could have an intake of 5 µg/day or more from this source alone[20]. Most municipal waters contain less than 1-3 $\mu g/dm^3$ which is well below the upper limit for drinking water of 10 $\mu g/dm^3$ set by the World Health Organization[21]. Food is thus normally the major source of cadmium to animals and nonsmoking humans.

Both airborne and waterborne cadmium can cause increased concentrations of cadmium in soil. In areas not known to be polluted, the cadmium concentration in soil was reported to be less than 1 $\mu g/g$[20]. The other sources which soil can be contaminated with cadmium are sewage sludges used as fertilizer.

The relationship between cadmium uptake by a number of plant species grown in the solution culture and the cadmium concentration in the solution was studied by Page et al.[22] and John[23]. They found

that the tolerance of different species varied to cadmium in the solution in the range 0.1 to 10.0 $\mu g/cm^3$ and the uptake was related to pH, i.e., increased soil acidity resulted in higher cadmium concentrations in plants.

Cadmium is biologically inessential and highly toxic to virtually every system in the animal body, whether ingested, injected, or inhaled. Histological changes have been observed in the kidneys, liver, gastrointestinal tract, heart, testes, pancreas, bones and blood vessels[24]. The most striking morphological change produced by this long-term ingestion of toxic cadmium levels was interlobar hepatic and interstitial renal fibrosis.

For man a "provisional tolerable weekly intake" of 400-500 µg Cd per person had been proposed by the World Health Organization[25]. This approximated 1 µg/kg body weight for most individuals or 55-70 µg Cd/day.

A disease which specifically associated with cadmium poisoning became known as Itai-Itai disease (Ouch-Ouch disease). This disease manifested in renal and gastrointestinal lesions and osteomalacia resulted from the industrial contamination of the food and water supply. Furthermore, it occured mostly in postmenopausal, multiparous women consuming poor diets low in protein and calcium.

1.3 Literature survey of lead and cadmium analyses in food and vegetable samples.

Modern methods of lead and cadmium analyses usually associate with the destruction of organic matter and the conversion of organic

form into the inorganic form of the elements. Both dry ashing and wet digestion procedures have been used. In dry ashing process, sample is ashed in muffle furnace. Many modifications were made by adding some chemicals as ashing aids, for example, magnesium carbonate, calcium nitrate, calcium acetate, calcium hydroxide, magnesium acetate and magnesium nitrate[26,27]. In wet oxidation, such acid systems as nitric-sulphuric acid[28,29], nitric-perchloric acid[30,31], sulphuric acid-hydrogen peroxide[32,33], sulphuric-nitric acid-hydrogen peroxide[34,35], sulphuric-nitric-perchloric acid[36,37], were used.

The determination of lead and cadmium in food and plant material samples have been studied by many workers. They concluded that atomic absorption spectrophotometry[38-43] and atomic emission spectrophotometry[44,45] are commonly used for determining both lead and cadmium in samples. Though these instrumental methods of analysis are rapidly feasible and sensitive, the simultaneous multielement analysis is difficult, requiring more complex equipment (multiple or multielement lamps, multiwavelength monochromator arrangements, etc.).

X-ray fluorescence method is limited by matrix effects and cost factors. It was applied to analysis of lead in leaves[46] and lead-cadmium[47] in vegetable samples.

The determination of cadmium by neutron and photon activation analyses in food and vegetable samples have been compared with atomic absorption[48], the results were agreeable but the accuracy of the former methods were assesed with standard reference materials and the nature of the samples.

Colorimetric technique, from literature survey is less frequently. used in trace analysis of lead and cadmium in food and vegetable samples[49].

Various electrochemical techniques have been investigated for the determination of lead and cadmium at trace levels such as polarography[28,34], alternating current polarography,[50] square-wave polarography[51], pulse polarography[52] and oscilloscopic polarography[53]. All polarographic methods consume a lot of mercury for working electrode. From information in the literature, it appeared that differential pulse anodic stripping voltammetry (higher sensitivity than anodic stripping voltammetry) could be used for simultaneous determination of lead and cadmium in vegetable samples since there are trace amounts of lead and cadmium in each vegetable species and this technique has been proved to be powerful for the trace analysis of certain metal ions of environmental concern[54,55].

Comparison of some analytical methods for the determination of lead and cadmium are presented in Tables 2 and 3[27].

In Thailand, there are a few reports about lead and cadmium contents in vegetables[56,57]. Now, lead and cadmium are known as a highly toxic cummulative poison in man and animals. This made the author enthusiatic to determine the contents of lead and cadmium in various vegetables. The purpose of the current study is to search for the optimum condition for a simultaneous analysis of lead and cadmium in vegetable species by differenial pulse anodic stripping voltammetry and to compare the contents of lead and cadmium in various parts of vegetable species.

Table 2 Comparison of some analytical methods for lead (27)

| Technique | Linear range | Limit of detection | Sample size | Recovery, % | Standard deviation | Substrate |
|---|---|---|---|---|---|---|
| Spectrophotometry: | | | | | | |
| Dithizone | 0–25 μg | 1 μg | | >95 | ± 1 μg | Water |
| | 0–40 μg per 10 ml | | Containing <40 μg Pb | 90–115 | | Tobacco |
| | 1–40 μg | 1 μg | up to 1 400 g | | 5–8% at 1 μg | Apples, biological materials |
| | 1–10 mg kg$^{-1}$ | 0.1 mg | 100 g | >90 | 5% | Canned foods |
| | 0.01–0.1 mg l$^{-1}$ | 1 μg | 100 ml | ~95 | | Urine, faeces, foods |
| Atomic-absorption spectrophotometry: | | | | | | |
| Direct aspiration | 0.1–20 mg kg$^{-1}$ | 0.1 mg kg$^{-1}$ | | | 0.04 at 0.5–20 mg kg$^{-1}$ | Beverages |
| After digestion | | | 10 g dry mass | 91–110 | 5% | Apples |
| | 1–25 μg ml$^{-1}$ | | <10 g | 100 ± 3 | 1–10% | Foods |
| | 0–300 μg kg$^{-1}$ | 40 μg kg$^{-1}$ | 50 μl | | 4–11% | Blood |
| | 0–40 μg | 0.02 mg kg$^{-1}$ | 10 g | 98 | 0.02 mg kg$^{-1}$ in range 0.2–1.0 mg kg$^{-1}$ | Foods |
| With electrothermal atomisation | Up to 2 ng | | 1 ml | 99 | 10–15% | Evaporated milk |
| | 0.22 μg ml$^{-1}$ | | 5–50 l | | 7–18% | Evaporated milk |
| After chelation | 0–2.5 mg kg$^{-1}$ | | 100 g | 96–145 | ~10% | Fruit, vegetables |
| | 1–11 mg kg$^{-1}$ | | 25 g | 100.7 | 0.41 mg kg$^{-1}$ | Fish |
| Polarography | 0–1 μg | 0.1 μg per 20 ml | 1 ml | | | Blood |
| | 1–11 mg kg$^{-1}$ | | 25 g | 97.7 | 0.32 mg kg$^{-1}$ | Fish |
| | 2–40 μg per 2 ml | 1 μg ml$^{-1}$ | 5–20 g | ~95 | | Biological material |
| Anodic-stripping voltammetry | | 0.1 mg kg$^{-1}$ | 2 g | 96 | 13% | Foods |
| | | | 25 ml | | 2% | Sea water |
| | 10–30 μg l$^{-1}$ | 0.7 μg l$^{-1}$ | 50 ml | 90–110 | 10% at 11 μg l$^{-1}$ | Water |
| | 1–400 ng | | 5 ml | 79–95 | | Sewage |
| Spectrography | | 1 mg kg$^{-1}$ | 50 mg | 93–99 | 12–13% | Soil, biological specimens |
| | 1–20 mg kg$^{-1}$ | 1 μg ml$^{-1}$ | 1 g | >90 | ±6% | Tinned meat, soup, cheese |
| | 0.01–0.2 mg l$^{-1}$ | 0.01 mg l$^{-1}$ | 100 ml | ~90 | ±10% | Urine |

Table 3 Comparison of some analytical methods for cadmium (27)

| Technique | Linear range | Limit of detection | Sample size | Recovery, % | Standard deviation | Substrate |
|---|---|---|---|---|---|---|
| Spectrophotometry | Up to 50 $\mu$g | | 1 g | 65–105 at 2 mg kg$^{-1}$ | | Fruit juice, plastics |
| | Up to 50 $\mu$g | 0.01 $\mu$g | 50–100 ml 5–20 g | ~95 | ±1 $\mu$g below 10$\mu$g | Biological material, urine, faeces, blood |
| | Up to 100 $\mu$g l$^{-1}$ | 0.05 $\mu$g | 50–100 ml | >90 | | Urine, water |
| Atomic-absorption spectrophotometry | Up to 1.0 mg kg$^{-1}$ | 0.06 mg kg$^{-1}$ | | 98–117 | 1.8–10.5% at 5.0–0.02 mg l$^{-1}$ | Vegetables, milk, eggs |
| | 0–0.2 mg kg$^{-1}$ | 0.01 mg kg$^{-1}$ | 100 g | 85–103 at 0.01 mg kg$^{-1}$ | 1.3–13.0% at 0.01 mg kg$^{-1}$ | Vegetables, fruit |
| | | | ±0.5 g ash | 95.9 at 10 $\mu$g | ±1.5% | Total-diet samples |
| | 10–100 $\mu$g | 0.05 $\mu$g ml$^{-1}$ | 0.01–6 g | 98–108 | 4.8% | Animal tissues |
| | | 0.01 mg kg$^{-1}$ | 25 g | 94.8 ± 3.0 at 0.10 mg kg$^{-1}$ | | Eggs |
| | 0.05–2.0 mg kg$^{-1}$ | | 50 g | >85 | | 12 food commodities |
| | 1–4 mg l$^{-1}$ | | 1 g | 75–105 | | Plastics, fruit drinks |
| | 0–2$\frac{1}{2}$$\mu$g | 0.002 mg kg$^{-1}$ | 10 g | 90 | | Baby foods |
| Atomic-absorption spectrophotometry with electrothermal atomisation | Non-linear Up to 0.3 $\mu$g ml$^{-1}$ | 2 × 10$^{-12}$ g | 1–100 mg 1 g | 85–96 | ±5% | Fish tissues Bread, potatoes, rice, eggs, spinach |
| Polarography | 1–10 $\mu$g ml$^{-1}$ | | 1 g | 95–100 | | Plastics, fruit drinks |
| | 1.5–500 $\mu$g | 1–3 $\mu$g | 3 ml | ~100 | | Biological material |
| Anodic-stripping voltammetry | 0.05–0.50 $\mu$g ml$^{-1}$ | 0.01 mg kg$^{-1}$ | 2 g 50 g | 90–100 80–110 | ±10% | Foods Total-diet samples |
| Spectrography | 5–200 $\mu$g in 1 ml | 0.4 $\mu$g | 50–100 ml 5–20 g | ~95 | | Biological material |