Chapter I

Introduction
1. INTRODUCTION:

The wealth of India is stored in the enormous natural flora which has been gifted to her endowed with a wide diversity of agro climatic conditions. It has all types of climatic conditions varying from temperate in the Himalayas, tropical in south India, dry in central India, humid wet in Assam and Kerala. These conditions are favourable for the growth of a variety of medicinal and aromatic plants. So India is considered as the Botanical garden of the World. There are about 19,000 species of flowering plants, 11,350 non-vascular plants and 14,500 fungi in India. (Jain, 2000). India has more than 2,200 species of medicinal and essential oil containing plants. Western Ghat is considered as one of the ecological hotspot of the World, which harbors a good number of medicinal plants. North Eastern states are also rich in medicinal plants. Assam is reported to contain about 3,000 medicinal plants. Experts feel that the drugs of natural origin with their wide range of action shall play an important role in health care particularly in the rural areas of India. There are more than 4,22,000 flowering plants reported from the world and more than 50,000 are used in medicine. In India there are more than 13 % of the flowering plants used as medicine (Sanjay et al., 2006). There are more than fifty million people living in and around forest areas in India who depend on forest for survival.

About 1 to 10 % of the plants are used as food by man and other animals. ‘Rigveda’ is the oldest book containing the information regarding the medicinal plants. Hippocrates mentioned 300 to 400 medicinal plants. ‘De Materia Medica’ was written by Dioscoriodes in the first century A.D., in which 2,000 plant species are mentioned, which became the prototype for the modern pharmacopeias. The
Bible offers the description of approximately 30 healing plants. Indeed, Frankincense and Myrrh probably enjoyed their status of great worth due to their medicinal properties. Since they have antiseptic properties, they were even employed as mouth washes.

During the dark ages, the Arab World continued to excavate their own older works and to build upon them. In Asia also pharmacopoeia was compiled. The west saw a revival of ancient medicine during Renaissance years which was built on medicinal plant.

Among the Europeans of the new World, the botanicals were used as alternative to toxic mainstream medicinal practices of the day. In 1887, Homeopathic Pharmacopoeia of the United states was compiled by the alternative practitioners (Cowan, 1999).

There are more than 2,000 registered drug manufacturers in the country. Himalaya Drug house (Bangalore), Vicco (Bombay) and Dabur (New Delhi) are some of the companies producing ayurvedic drugs in India. Central Council of Research on Ayurveda and Siddha takes care of the survey and research in the areas of Pharmacognosy, Pharmacology and Clinical trials.

The history of herbal medicines is as old as human civilization. Evidences say that Neandrathals who lived 60,000 years back used holly hock tree, which are widely used even today in the ethnomedicine. The documents reveal that the plants
were used medicinally in China, India, Egypt and Greece. In 19\textsuperscript{th} and 20\textsuperscript{th} century most of the medicinal plants were used in the form of crude extracts. In China medicinal plants have been in use since 5000 B.C. It contains 365 drugs. The great herbal or Chinese Material Medica ‘Pan Tsao’ was written in 2735 B.C., which includes plants, metallic preparations and animal products. The first written account of medical experiences from Egypt is ‘Papyrus ebrs’, which contains more than 800 formulae and 700 different drugs. The drugs such as \textit{Acacia}, Caster oil and fennel are mentioned along with references to compounds as iron oxide, sodium chloride, sodium carbonate and sulphur.

India had an extensive pharmacopoeia thousands of years ago. The rich heritage of herbal medical culture of China is still very much alive, with some of the written records dating from the beginning of the Christian era. The traditional plant therapeutic knowledge of Africa and South America, along with that of China and India, has given several new drugs to modern medicine. The knowledge of the medicinal plants has been accumulated in the course of many centuries based on different medicinal systems such as Ayurveda, Unani and Sidda. In India it is reported that traditional healers use 2,500 plant species and 100 species of plants serve a regular sources of medicine.

Indians also worked meticulously to examine and classify the herbs, which they came across into groups called ‘Gunas’. Charaka made 50 groups of 10 herbs each of which according to him would suffice ordinary physician’s needs. Similarly Sushrutha arranged 760 herbs in seven distinct sets based on some of their common
properties. A large portion of the Indian population even today depends on Indian
system of medicine ‘Ayurveda’ an ancient science of life. ‘Ayurveda’ is an Indian
system of medicine, based on solid foundations that are fully sustained by long
experimentation and philosophical propositions, dating back to about 1000 BC.

The Ayurvedic system traces it’s roots to the Himalayan mountains of India
over five thousand years ago. According to legend, a conference was held in a
Himalayan cave in which great sages of India met to discuss their knowledge of
their healing arts. These scholars and teachers possessed traditional knowledge
about the medicinal plants of India that had been handed down orally by the tribes
of the Indian forests since the beginning of the history. At this conference the
information was combined into one body called ‘Ayurveda’ meaning ‘Ayur’ or
‘life’ and ‘veda’ or knowledge in Sanskrit.

After this conference the Ayurvedic knowledge was passed orally from
teachers to students for over a thousand years continuously growing as each
Ayurvedic physician added his insights and experiences. The great saint Athreya
preached Ayurveda in Kashi in Vedas period. It was finally written down in the
first century A.D. by the Ayurvedic Physician Charaka in ‘Charaka Samhita’,
Sushrutha in ‘Sushrutha Samhita’ and Vaagbhata in ‘Astanga Hrudaya Samhita’.
There are about 700 herbal drugs mentioned in these treatises.

Ayurvedic medicine probably predates any other healing tradition in
existence today even Chinese medicine. Even before the Ayurvedic conference,
knowledge of the medicinal plants of India had spread to other continents. Knowledge of Ayurvedic medicine has unfortunately been confined to India and the West is largely ignorant of it. Even in India, this traditional medical practice has lost a lot of importance in the urban situations. One of the main reasons for this is that much of the early and core medical literature on Ayurveda is in Sanskrit, the ancient language which ceases to be a day to day language in this century except in extremely small groups of the vast Indian population. Even today a considerable bulk of Ayurvedic knowledge is in the form of ancient palm leaf manuscripts hidden in remote libraries and private collections, and as treasured personal knowledge of a few individuals. The Ayurvedic system grew vigorously till 1300 BC, and the beginning of that century, however, marked the end of a glorious year of growth and standardization. In the medieval times, by a curious combination of circumstances, the rasayana practices reappeared, and the drugs and remedies evolved in these systems got incorporated into Ayurveda.

The Unani Tibb system of medicine is traced to the system of Greek medicine developed during the Arab civilization, is also called the Greco-Arab system. The Unani is prevalent in India, Pakistan and Bangla Desh. The Hamdard Foundation in Karachi has done commendable service to the cause of the Unani system of medicine, more importantly through a series of publications on the subject. The theoretical frame work of Unani is based on the teachings of Hippocrates.
The earliest recorded history of traditional Chinese medicine was in 1800 B.C., the beginning of the Shang dynasty (Pei, 1985) and hence it was a development for far longer than this date.

The homeopathic Materia Medica is largely plant based as even the 12 tissue remedies, which are inorganic salts, are expected to be extracted from plants (Jamil et al., 1997). Medicinal plants provide largely safe sources of primary health care to a majority of the population in India. Poor and marginalized people, who are unable to financially or logistically access formal health care systems, are especially dependent on herbal medicines. World Health Organization recommends and promotes herbal medicines in its National Health Care programmes because the price of such drugs are with in the reach of common man. In 1977, the 30th World assembly of the World Health Organization formed to deal with matters of health, adopted a far reaching resolution urging the governments of the member countries, to give adequate importance to the utilization of their traditional systems of medicine, with appropriate regulations to suit their national health needs. Traditional medicine is defined as ‘the therapeutic practices that have been in existence, often for hundreds of years before the development and spread of modern medicine and are still in use today (WHO, 1991).

According to the World Health organization (WHO) report 80 percent of the Worlds people depend on traditional medicine for their health care needs today. The indigenous plant material has acquired an important position in the modern approach of pest control as they are comparatively safe due to their rapid
biodegradable nature. It was reported about 21,000 plants are of medicinal use. A large number of very specific pharmaceutical products, many of them synthetic, are available to treat almost every disease. Chinese anti-malarial drug artemisin is extracted from *Artemisia annua* and quinine from *Cinchona*. Morphine was isolated by Sertuerner in 1806. The Ephidrine was isolated from *Ephedra* spp. of China in 1887.

Phytochemical studies on medicinal plants are required for the following reasons:

a) Phyto-chemical information on a species of medicinal plant is the essential basis for a fine chemical analysis, to be followed by *in vitro* and clinical studies.

b) Almost every species of medicinal plants contains more than one active compound and it is necessary to know this composition before other studies are undertaken.

c) Phytochemical studies help in discovering alternative sources of therapeutic chemicals of importance. For example, camptothecin, the anticancer drug was originally discovered in the Chinese species *Camptotheca acuminata* (Nyssaceae), for which the later discovered Indian alternative is *Nothapodytes foetida* (Icacinaceae).

With such a vast area of research on medicinal plants, one is compelled to exercise a choice of the type of research work to be undertaken, based on interests and facilities available. The crude drugs are divided into different groups according to the chemical nature of their most important constituent. Since the pharmacological activity and therapeutic significance of crude drugs are based on
the nature of their chemical constituent, it would appear that chemical classification of crude drug is the preferred method of study. The examples of the drugs under chemical classification are glycosides, alkaloids, tannins, volatile oils, lipids, carbohydrates, resins, vitamins and hormones, proteins and enzymes. Most of the plant products are phenols or their oxygenated derivatives (secondary metabolites). Majority of them are insect repellants and terpenoids bring odour, quinines and tannins are responsible for plant pigments.

For the determination of total metal ion concentrations 'Atomic Absorption Spectrophotometer' (AAS) has been used. For the separation of extracts chromatographic techniques have been followed.

The flowchart for the extraction of chemicals in medicinal / aromatic plants is shown below.

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EXTRACTION PROCESS

INFUSION DECOCTION MACERATION PERCOLATION DIGESTION

FRESH CONCENTRATED
INFUSION INFUSION

SIMPLE MACERATION MULTIPLE SIMPLE PERCOLATION
CONTINUOUS WITH MACERATION PERCOLATION PROCESS FOR
MACERATION ADJUSTMENT CONCENTRATION

DOUBLE MACERATION TRIPPLE MACERATION REVERSE PERCOLATION PROCESS MODIFIED PERCOLATION PROCESS
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1.1. Phenolics and polyphenols:

Cinnamic acid and caffeic acids are phenyl propane derived compounds, which are in a oxidation state. Herbs like Tarragon and thyme contain caffeic acid active against bacteria, fungi and viruses. Catechol and pyrogallol (hydroxylated phenols) antibacterial (2-OH groups), (3-OH groups).

Increased hydroxylation increases the toxicity against microbes. These phenols may inhibit enzymes through sulphha hydryl groups or through nonspecific proteins. Phenolic compounds possessing C3 side chain at a lower oxidation level and with out oxygen are essential oils. Eg: Eugenol is found in clove oil and it is antifungal and antibacterial.

1.2. Quinones:

These have two ketone rings and are highly reactive. These are brown and cause colouring reactions in cut fruits. These are also intermediate in the synthesis of melanin. It is also present in henna. Anthraquinone from Cassia italica is against Bacillus anthracis, Corynebacterium pseudodiphterricum and Pseudomonas aeruginosa (Kazmi et al., 1994). Pseudomonas psuedomalliae is killed by hypericin (anthraquinone) from Hypericum perforatum (Duke, 1985).

1.3. Flavones, flavonoids and flavonols:

Flavones are phenolic structures with single carbonyl groups. 3-hydroxy-phenolics give rise to flavonol. Flavonoids give rise to hydroxylated phenolics
occurring as C₆-C₃ unit linked to an aromatic ring. They are produced during microbial infection and act on cell wall or membranes. Catechin is present in Oolong tea is an antimicrobial substance which act on Vibrio cholerae, Streptococcus mutans, Shigella.

1.4. Tannins:

These are polymeric phenolic substances capable of tanning leather and precipitating gelatin. Tannins are produced as a result of condensation of flavon derivatives or polymerization of quinine derivatives produce tannins. Tannins reduced insect growth and disrupt digestive activity in ruminal animals.

1.5. Coumaarins:

These are the phenolic substances made up of a benzene ring and α-pyrone rings. They are antithrombic, anti-inflammatory and vasodialatory, warfarine is used as rodenticide and as an anticoagulant.

Indole compounds related to the metabolism of tryptophan, constitute an extensive family and are found in bacteria, plants and animals. Indolic compounds possess significant and complex physiological roles and especially indole alkaloids have historically constituted a class of major importance in the development of new plant derived drugs. The indole alkaloid alstonine has been identified as the major component of a plant based remedy, used in Nigeria to treat mental illnesses by traditional psychiatrists (Eby Abraham, Ayurvedic medicinal plants. com). The
indole in green tea flavour is the rare phytochemicals that show activity against *Pseudomonas aeruginosa* and *Escherichia coli* (Aya Kubo *et al*., 1996).

1.6. Alkaloids:

The term alkaloid means alkali-like, are the compounds of plant origin having one or more basic nitrogen atoms in heterocyclic ring systems, which induce pronounced physiological activity in animal and in man. Alkaloids are present in dicotyledon plant families occurring in seeds, leaves, bark or root of the plant. A set of alkaloids may be present in each part. They occur as salts of acetic acid, oxalic acid, lactic acid, malic acid, tartaric acid or citric acid.

The alkaloids are extracted by macerating the plant material. If the material is rich in fat (seeds), it is first extracted with ligroin or petroleum ether for their removal. The plant residue is then extracted with methanol and cellulosic material separated by filtration. The filtrate is evaporated to give the crude plant extract. This is then dissolved in dilute acid and extracted with ether. The acid solution of the alkaloid salts is then basified and extracted with ether. Evaporation of ether solution gives a solid mixture of crude alkaloids. It is then subjected to fractional crystallization for separation into individual pure alkaloids. In modern practice the isolation is effected by column chromatography, gas chromatography and by counter current distribution.

Acridone alkaloids are isolated from the plants of family Rutaceae. These show antimicrobial activities. Acronycine has been tested for antitumour properties.
The derivatives of acrycine is effective against *Trichomonas vaginalis*. 30 different acridone alkaloids was evaluated for their antiplasmodial activities in a rodent model (Sherry Queener *et al.*, 1991).

1.7. Terpenes:

Many of the fragrant components of plants are volatile with steam and may be isolated by steam distillation, solvent extraction or other treatment of the plant. These components are called the essential oils. These usually consists of a mixture of hydrocarbons containing 10, 15, 20, 30 or 40 carbon atoms or their oxygenated derivatives. The individual components of essential oils are called the terpenes or terpenoids.

They are isolated from essential oils present in plants. The essential oils are extracted from the plants by steam distillation, digestion with solvents, expression or adsorption in purified fats. Steam distillation is most widely used. The plant tissue is macerated and then steam distilled. If a particular terpene is decomposed under these conditions, it may be removed by extraction with light petrol and the solvent is distilled under reduced pressure. The essential oil contains several terpenes, which are separated by fractional distillation or chromatography.

1.8. Uses of heavy metals in Pharmacology:

Although heavy metals are present in food in very minute quantities the very human existence is due to their role in body metabolism. It has been established
that whatever is taken as food might cause metabolic disturbance if it does not contain the permissible upper and lower limits of heavy metals. Thus both deficiency and excess of essential micronutrients (e.g., iron, zinc, chromium etc.) may produce undesirable effects (Van et al., 1989; Konofal et al., 2004; Koqak et al., 2005). Effect of toxic metals (cadmium, chromium, lead, nickel etc.) on human health and their interactions with essential heavy metals (trace elements) may produce serious consequences (Abdulla and Chmielnieka, 1990; Tokusoglu et al., 2004).

Heavy metal salts are used exclusively as antiseptics. They are capable of destroying the microorganisms in very low concentrations which is called an oligodynamic action. The antibacterial action may be due to the precipitation of bacterial proteins. The heavy metals are used in the traditional formulations. The anticancer molecule cisplatin contains platinum. The ayurvedic preparation ‘Kajjali’ contains mercury and sulphur.

Copper is essential metallo element, like essential amino acids, fatty acids, vitamins required for normal metabolic process. It occurs as complexes in the tissues and biological systems. Copper is absorbed at acidic pH in the stomach and is transported by blood as ternary complexes. It is utilized in the form of copper dependent proteins or enzymes and stored as copper thionein in the tissues.

Quinilince are the fused benzopyridines which have proven antimalarial activity. Copper complexes of substituted quinilines have been reported to possess pharmacological activities (Sridhara Ranganath Pai, 2002).
Copper complexes are reported to have wound healing activity in man. Copper complexes also have anticonvulsant activities, antimutagenic activities, anticancer activities, various copper complexes have been reported to inhibit bacterial fungal, Yeast, algal, viral and mycoplasmal growth. Copper sulphate acts as an emetic, which evokes vomiting by irritation to the gastrointestinal tract. It also has an astringent action.

The approximate daily dietary requirement of Iron is 8 to 13 mg for children, 15 to 20 mg for menstruating women and 10 to 15 mg for men. The foetus accumulates 200 to 400 mg of iron, mainly in the last trimester, further iron is lost during child birth and during lactation. Pregnant and lactating women required 20 to 25 mg of iron daily in food. Women loose approximately about 25mg of iron every month during menstruation.

Lanthanum is used in the treatment of osteoporesis and the deficiency of lanthanum causes chronic fatigue syndrome (Kim et al., 1999).

Significant amount of zinc at concentration around 10μm are present in mammalian brain either protein bound or chelatable form (Fredrickson, 1989, Colin Wright, 1993 and Zerdahelyi, 1984).

Zinc permanganate has greater astringent activity compared to the potassium salt. So it is used for rural irrigation. They are corrosive and have weak antiseptic
properties. Zinc sulphate is used against ulcers, conjunctivitis and chronic inflammation of the cornea. Zinc carbonate (dalmine) is used against skin disorders like eczema, urticaria etc.

Organic arsenicals are used in the chemotherapy or Tripanosomiasis, amoebiasis and trichomoniasis. Earlier organic arsenicals were used against syphilis. Arsenic is stored in the liver, gut, spleen, kidney and lung, small amounts are also present in the brain and skeletal muscles. It also accumulates in bone and hair.

Lead acts as an astringent because of the formation of lead proteinate. Mercury is used as antiseptic and preservative spermicides and diuretics. 1% solution of Cadmium sulphide is used in treating seborrhoeic dermatitis and dandruff.

In traditional Chinese medicine, mercury is part of some preparations under the terminology of ‘cinnabaris’ (mercury sulfide), ‘calomel’ (mercury chloride) or ‘hydrargyri oxydum rubrum’ (mercury oxide). Such products are used for a variety of indications including, for example, as a tranquilliser, an anti-epileptic, for ulcers or to treat insomnia (Koh and Woo, 2000). Lead is used as ‘Mi Tuo Seng’ (Lithargyrum) (Thatte et al., 1993) and arsenic as ‘Xiong Huang’ (Realgar) The Chinese Pharmacopoeia Commission of the Ministry of Public Health (1995) in the manufacture of several TCMs. Strictly speaking, these constituents are thus not contaminants but ingredients deliberately included for a specific curative purpose.
In the present study six indigenous medicinal plants *Adhatoda vasica*, *Alstonia scholaris*, *Mimosa pudica*, *Tabernaemontana coronariae*, *Asperagus racemosus* and *Leucas aspera* were studied for their bioactivity and the presence of metal ions.

**Adhatoda vasica:**

Distribution: The plant is an evergreen branched shrub with large flower, white with red or yellow at throat. It is found in Indomalaysia region.

Uses: The fresh or dried leaves are used in curing bronchial troubles. Leaf juice used in diarrhea, dysentery and glandular tumours. Powdered leaves used in skin infections. In Ayurveda the root, leaf, and flower are used in treating bronchial asthma, hemorrhage, wasting diseases, fever, delirium, leprosy, piles and vomiting.

**Alstonia scholaris:**

Morphological characteristics: The plant is a tall straight tree with rough grayish-white bark. Leaves 4 to 7, in a whorl. Flowers greenish yellow, in compact umbellate cymes, fragrant. Follicle in clusters, cylindric. The bark is bitter tonic, febrifuge, anthelmintic and galactogogue. Used in the liquid extract form for chronic diarrhea, asthma, cardiac troubles. Leaves used in beri-beri, dropsy and congested liver. Latex applied to sores, ulcers, tumours and rheumatic swellings.

Uses: In Ayurveda bark is used in the treatment of fever, leprosy, malarial fever, worm infestation, wounds, cough, pain etc.
**Mimosa pudica:**

Distribution: The plant is native to Brazil.

Morphological characteristics: This evergreen sub shrub is annual. It is grown for its curiosity value of the fern like leaves close up and droop when touched, usually re-opening within minutes. It has prickly stems and small, fluffy, ball shaped pink flowers in summer. It grows to a height of 5ft and spreads around 3 ft a perennial plant, it grows to a height of 0.5 m with a spread of 0.3 m. In some areas this plant is becoming a noxious weed. The stem is erect, slender and branching. The leaves are bipinnate, fern like and pale green closing when disturbed. The flowers are pale lilac pink, occurring in globose heads and appearing in summer. Indigeonous to northern hemisphere, it is adaptable to most soils in an open, sunny position, and is drought and frost tender. Due to its ability to fix nitrogen from the air it does well on poor soil.

**Tabernaemontana coronaria:**

The plant is an evergreen, glabrous, shrub attaining a height of 6 to 8 feet. It is mostly cultivated in gardens on account of the beautiful white flowers it bears.

Distribution: In the wild state it is met with in the forests adjoining the Western Ghats, and the Bellary and Vishakapatnam districts of the Madras presidency.

Uses: The roots are acrid, bitter with a flavor astringent to the bowels, alexipharmic, digestible useful in ‘Kapha’ biliousness and diseases of the blood. The root is a tonic to the brain, liver and spleen, it removes bad humours, useful in
paralysis, weakness of the limbs, strangury, lessens pain in the limbs and joints, cures scorpion sting, epilepsy.

Pillay (1938) reported that the roots of the plant contain fatty acids, phytosterols and some easily oxidisable amorphous bases. Tabernaemontanine, a colour less crystalline alkaloid, coronarine a yellow crystalline alkaloid have been isolated from the alkaloids soluble in light petroleum. The bark has fatty matter, cerotic and oleic acids, resin acids, a resin alcohol (crystalline substance) and caoutchouc, resins and sugars.

*Asparagus racemosus:*

Distribution: This plant is distributed through out the tropical Asia, Africa and Australia. In India it is found in the tropical parts and from Himalaya to the altitude of 1300 to 1400 m.

Morphological characteristics: The stem of this plant is thorny. The leaves are linear, green and needle like. It bears tufts of cladodes axillary to the stem.

Uses: Shatavari roots are used as galactogogue tonic and diruretic. Shatavarin I is reported to possess antioxitocic property. Roots are largely used for medicinal oil recommended in the treatment of rheumatism and nervine disorders. It is used in Ayurveda for abortion and safe delivery justified by uterine blocking activity.
**Leucas aspera:**

Distribution: The plant is a herb growing as a weed on wastelands and road sides all over India.

Morphological characteristics: The plant is an erect or diffusely branched annual herb. The leaves are linear or oblong, blunt tipped, the margin being scalloped.

Uses: The methanol extract of flowers and its fractions contain alkaloidal residue and the expressed flower juice shows antibacterial activity. The plant is used as an insecticide and indicated in traditional medicine for cold, cough, painful swellings and chronic skin eruptions.

A good number of medicinal plants are found in the Western Ghats, one of the ecologically fragile area. Particularly in the foothills of Charmady of Western Ghats. A perusal of available literature has indicated that there are no much scientific information in respect of the chemicals present in the medicinal plants and a large number of plants are in the verge of extinction due to the over exploitation.

Thus with this background it was proposed to explore a scientific work pertaining to the extraction of chemicals with special reference to metal ions in medicinal plants.
Objectives of the work:

1) Collection and extraction of the bioactive compounds from *Adhatoda vasica*, *Alstonia scholaris*, *Leucas aspera*, *Tabernaemontana coronariae*, *Asparagus racemosus* and *Mimosa pudica*.

2) To conduct preliminary phytochemical studies of different extracts of the leaf.

3) To isolate and characterize the bioactive compounds present in *Alstonia scholaris* and *Adhatoda vasica*.

4) To study the antibacterial activity of different extracts.

5) To screen the analgesic activity of *Alstonia scholaris*.

6) To screen the hepatoprotective activity of *Alstonia scholaris*.

9) To study the wound healing activity of the *Alstonia scholaris* extract.

8) To analyze the metal ions like iron, copper, nickel, chromium, zinc etc. in plant residues.