Man's environment is under constant threat from his own several deeds. The rapid developments in industrial revolution, blue revolution, white revolution and agricultural activities to provide food and other basic amenities to ever exploding population, have further vitiated the environment. Over exploitation of natural resources, uncontrolled mechanization, deforestation, extensive use of chemical fertilizers, rapid urbanization and pesticides in green revolution have brought about several changes in various components of the environment.

Metals are intrinsic components of earth's crust. These metals are distributed in various components of ecosystem through the biogeochemical cycles. During the process of evolution, the organisms have adapted themselves to these natural levels of metals. The natural levels of these elements are usually harmless to the organisms including man. The human activities involving the use of metals are ever increasing with civilization. The use of metals and their compounds in almost every sector of economy such as agriculture, housing, industry, energy, transport, communication, etc. is increasing. With increase of human activities involving metals, various organisms in the ecosystem including human beings are being exposed to elevated concentrations of heavy metals, which are causing potential toxicity to these organisms.
Essential elements present in the environment are always beneficial as well as detrimental to the living systems, which include human beings, animals, flora and fauna. Incessant ingestion of diets or ceaseless exposure of total environment that are deficient, imbalance or excessively high in a particular metal, induce changes in the functioning forms, activities or concentrations of that metal in the tissues and fluids of the body.

Most of the metallic substances have direct or indirect influence on the human organisms and may cause serious mortality. Substances such as polycyclic aromatics, pesticides, radioactive substances, metals including trace metals involve directly in endangering human life. The metals are not usually eliminated from the aquatic ecosystem by any natural process in contrast to most organic pollutants, and secondly, most metal pollutants are enriched in organic substances and mineral environment.

Toxic elements such as mercury, cadmium, arsenic, copper, and many other metallic species V⁺², Pb⁺², Ni⁺², Se⁺² tend to accumulate in bottom sediments from which they may be released by various processes of remobilization. When they change their form they can move up the biological chain through different components of environment, thereby reaching human beings, causing either somatic or genetic effect.

1.1 Sources of metal pollution

Elevated levels of heavy metals in the environment can be attributed to both natural and anthropogenic sources. The important natural sources are geological weathering, the dried water droplets from oceans, dust particles from volcanic activities, forest fires, and biodegradation of dead animals and plants from natural calamities.
like tsunami, cyclones, haricans and floods. Important anthropogenic sources of metals in the environment are geological weathering, mining activities, industrial effluents, domestic waste waters, agricultural activities, special sources and sewage irrigated soil profiles.

1.1.1 Geological weathering

Geological weathering is an important source of baseline or background levels. It is to be expected that in areas characterized by metal-bearing formations, these metals will also occur at elevated levels in the water and bottom sediments of the particular area. Obviously, mineralized zones, when economically viable, are exploited to retrieve and process the ore. This in turn leads to disposal of tailings, possibly smelting operations and discharge of effluents that may result to distinguish between natural geological weathering and metal enrichment attributed by human activities. There is evidence that the high mercury content in rocks encountered in the catchments of the La Grande River, Canada, may be responsible for high mercury levels in organisms.

1.1.2 Mining activities

Many mineral ores contain variable quantities of different heavy metals that tend to become free in the environment by ore processing and other mining activities. There are several instances of pollution problems from mines, especially, the waste rocks left at the sites of mining after the mining is over. These rocks are liable to greater physical or chemical weathering and oxidation that release most of the metals in native state. In certain cases, biological weathering plays an important role in the release of metals. Large quantities of iron pyrites (FeS₂), which are left at the sites of coal mining that spread on several
acres of land are known as "gob pile". A single gob pile may cover up to 100 acres of plant area. Acidophilic bacteria in the *Thiobacillus* and *Ferrobacillus* group oxidise iron pyrites by the following reactions

\[
\begin{align*}
\text{Fe}^{2+} & \rightarrow \text{Fe}^{3+} + e^- \\
\text{Fe}^{3+} + 3 \text{OH}^- & \rightarrow \text{Fe(OH)}_3 \\
2\text{S}^{2-} + 3\text{O}_2 + 2\text{H}_2\text{O} & \rightarrow 2\text{SO}_4^{2-} + 4\text{H}^+ + 4e^- 
\end{align*}
\]

The acid concentration from the mining drainage may reach to pH 2-3 or even lower in many areas. The slimes dunes, resulting from exploitation of gold, thorium and uranium bearing ores, are an impressive source of metal supply. It is reported that the very first instance of pollution by mines was caused by the dispersal of toxic metals from Pb, Zn, Hg and As mines in Mid-Wales².

### 1.1.3 Industrial effluents

An industrial activity causes the heavy metal enrichment in aquatic environment. A number of heavy metals are employed by various industries and considerable portion of them finds way into the effluents. Dean *et al.*³ described that various metals are discharged as effluents from major industries like Alkali/Chlorine Batteries, electroplating etc., which are shown in Table 1.1. On the other hand, in several industries only one specific heavy metal is involved. For eg., the tanning industry involves the use of chromium only. Soil contamination by chromium results from land disposal of sludges occurring as by-products at ferro-chrome and chrome-steel production. A few metals may also be disposed of as by-products, while, some other are released during industrial combustion of coal or other minerals. For example the mercury cells employed in caustic soda production is the main source of mercury in the effluent. Industrial sludge contributed by treatment plants is important source of water pollution caused by metals. Electro chemical methods
normally employed in the metal finishing and alloy industries are also partly responsible for contamination of water by toxic metals. The chloral alkali industries release large amounts of mercury as liquid effluent that contaminates the water waste.

Table 1.1
Major trace elements in effluents from various industries

<table>
<thead>
<tr>
<th>Type of Industry</th>
<th>Trace elements in effluents</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cd</td>
</tr>
<tr>
<td>Alkali/Chlorine</td>
<td>*</td>
</tr>
<tr>
<td>Batteries</td>
<td>*</td>
</tr>
<tr>
<td>Electroplating</td>
<td>*</td>
</tr>
<tr>
<td>Fertilizers</td>
<td>*</td>
</tr>
<tr>
<td>Motor vehicles, air craft plating</td>
<td>*</td>
</tr>
<tr>
<td>Pulp and paper mills</td>
<td>*</td>
</tr>
<tr>
<td>Petrochemicals</td>
<td>*</td>
</tr>
<tr>
<td>Pharmaceuticals</td>
<td>*</td>
</tr>
<tr>
<td>Petroleum refining</td>
<td>*</td>
</tr>
<tr>
<td>Steam generation power plants</td>
<td>*</td>
</tr>
<tr>
<td>Textiles</td>
<td></td>
</tr>
</tbody>
</table>

1.1.4 Domestic effluent and urban storm water run-off

Large quantities of metals are present in domestic sewage. The use of shampoos and detergents is responsible for addition of Fe, Mn,
Ni, Zn, Cr, Co, Se and As to the waste waters. A large number of heavy metals may also be contributed by the corrosion of metal pipes. Sewage sludge discharged into waters is also a significant leaching source of Cu, Ag, Cd, Zn and Cr. Urban storm water run-off poses a serious threat of heavy metal contamination. Urban run-off during torrential rain season is rich in certain heavy metals like Cu, Cr, Zn and Pb. However, their relative quantities depend upon the various factors like modernization of road traffic conditions, land use and urban planning. Lead released from auto engines enter into the atmosphere, get deposited on the surfaces and finds its way in the run-off. A statistical summary reveals that urban storm water run-off has been recognized as major and important source of metal pollution of surface water.

1.1.5 Agriculture activities

Agricultural soils are potential sources of heavy metals as a result of the indiscriminate use of various types of pesticides and natural and synthetic fertilizers, and the presence of decaying plant and animal residues. The waste water from irrigation and agricultural run-off further increases the quantity of various metals in the agricultural soils.

1.1.6 Special sources

Upon attempting to leachate the source of metal input of receiving water bodies, a distinction is often made between defused point and non point sources. Such sources of 'mixed' origin will simply be treated as 'special' sources. Essentially, rural areas are regarded as non point sources, since the metal supply originates from vast regional areas. Oil-drilling operations require chemicals that contain various trace elements and thus present a potential pollution problem.
However, this is a localized source of contamination, which is confined to the various chemical forms of trace elements and their location in sediments. In oil-field waters, the concentration of thallium is likely to be extremely high and toxic effects may occur when they are discharged into rivers, lakes and ground water.

1.1.7 Sewage irrigated soil

The elevated heavy metal concentration in the soil is due to long term addition of sewage sludge and pedogenic process. Higher retention of heavy metals is observed in the upper horizon when compared to lower horizon. Sewage-sludge deposition is dominant process in the long-term retention of metals in the soil eco system as reported by Dinesh Mani and Chitranjan Kumar.

1.2 Metals and organic life

It has been known for several decades that the presence of certain elements exert a positive or negative influence on plants, animals and human beings. A greater attention has been drawn with regard to the specific role and significance of trace elements. Generally, the metal is rather loosely employed in current literature to designate the elements, which occur in small concentrations in natural or biological systems. The growing public concern over the deteriorating quality of the environment has led to generalized usage when referring to the trace elements.

1.3 Metals essential to human life

Trace amount of a metal whose concentration is less than 0.01% of the mass of the organism is essential to an organism to grow
or complete its life-cycle. But it becomes toxic when concentrations exceed the desired level of nutritional status by 40-200 folds.

Most of the naturally occurring metals found in the periodical table, are present in human body. But fewer than half of them play a vital role in biological activity. The essential trace elements involved in metallic-enzymes and enzymes have many activities, for example, zinc or molybdenum containing enzymes.

Metals in certain quantities exert immense influence on human body. Hence there is a dire need to monitor the concentrations of metals in human beings and also in other environments. Moreover, estimation of a particular metal health hazard in relation to human beings is essential. Samples of blood, serum, faces, saliva, sweat, hair, nails etc., are used for metal analysis. Levels and distribution of metals in human tissues and fluids are to be studied, since they are the sources of metals. The quantity of metals required for human beings, their functions and hazards of their deficiencies is reported in some studies.

The enhanced stability of DNA is caused by a number of metals acting on RNA. Copper and iron can act as respiratory carriers in human beings. They also function as an integral part of a vitamin, for example, cobalt in Vitamin B₁₂ or hormone. Zinc and chromium can lead in the production and function of insulin and hormones. Several other metals have functions in the mechanism of immune system. Trace elements are involved in the constitution of a large number of enzymes and vitamins, and consequently play the key role of regulators and act as catalysts in various metabolic mechanisms. Zinc plays a crucial role in the development of normal cellular immunity. It is also required for cell replication. Ingestion of lead is harmful to
central nervous system (CNS), gastrointestinal track and kidneys, nerves in brain etc.

There are many reasons for the necessity of online monitoring of the concentrations of the metals in human beings at trace level or ultra trace level. The most obvious is to estimate the health hazard of a particular metal, relating the concentration of the metal in humans to environmental and occupational levels, and is crucial in order to determine areas of health risks. It is, therefore, useful to have reference values of the metal in human beings, as a base line to estimate the significance of swelled levels. Knowledge of both body levels of the metals and human intake of the metals provides data for estimating dose/response relationship. This information is valuable in monitoring health effects and their relation to people's intake.

1.4 Health Hazards due to metals

Trace metals and health hazards are interrelated to each other. Metals either excessive or deficient lead to health hazards of plants, animals and human beings. Each metal has its own effect of toxicity to human beings, animals and plant systems.

Selenium: The essential trace element selenium has recently attracted attention because of its potentialities in the maintenance of human health. Selenium forms part of the active site of the peroxide-destroying enzyme glutathione peroxidase, and it also has other functions, in biotransformation, immune response and detoxification, etc. Although it is toxic in large doses, selenium is an essential micronutrient in all known forms of life. It is a component of the unusual amino acids selenocysteine and selenomethione. In humans, selenium is a trace element nutrient which functions as co-factor for reduction of antioxidant enzymes viz., glutathione peroxidase and
thioredoxin reductase. Selenium also plays a vital role in the functioning of the thyroid gland by participating as a cofactor for thyroid hormone deiodinases. Dietary selenium comes from cereals, meat, fish, and eggs. The recommended dietary allowance for adults is 55 micrograms per day. The environmental chemistry of selenium is shown in Figure 1.1.

Figure 1.1 Environmental chemistry of selenium

**Cobalt:** Cobalt is widely distributed in the animal kingdom. It is present relatively in abundant concentrations (0.10 – 0.25 mg / kg) in the liver, kidney, bone, spleen, pancreas and other glandular species. Cobalt is an essential nutrient for man in the form of vitamin B₁₂. However, the interactions between cobalt and iodine observed in rats¹³,¹⁴ and workers¹⁵ in the USSR, revealed that the cobalt concentration in the soils, foods and waters is inversely correlated
with the incidence of goiter in man and farm animals. The ADI for cobalt is said to be 0.25 mg, but ingestion of higher doses can adversely affect the hemoglobin content of the patient's blood and produce hyperlipemia and polycythemia. Cobalt is used mainly in the steel industry and poisoning is likely to be occupational.

**Chromium:** Chromium exists in both +3 and +6 predominant states. It is recognized as an essential trace element for rats as early as in 1955 and for humans in 1975. Chromium plays a significant role for the maintenance of normal glucose and amino acid metabolism, in the form of 'Glucose Tolerance Factor'. In the human body, chromium probably competes with iron for transferring binding sites. These sites play a predominant role in controlling diabetes mellitus. The physiological role of this element is a co-factor for insulin at the insulin responsive cell membrane. Symptoms of Cr deficiency are Impaired Glucose Tolerance, growth retardation, corneal opacity, skin disorder and necrotic liver degeneration.

**Nickel:** It is an essential trace element. It stabilizes the coiled ribosomes and occurrence in bacterial hydrogenase and urease enzymes shows that Ni is very essential to activity. It is highly carcinogenic and high levels of Ni induce the reduction of N₂ retention and impaired growth. The deficiency of Ni in animals results in impaired growth and an increased fetal death rate.

Many but not all hydrogenases contain nickel in addition to iron-sulfur clusters. Nickel centres are common elements in those hydrogenases whose function is to oxidise rather than evolve hydrogen. The nickel centre appears to undergo changes in oxidation state, and evidence is present that the nickel centre might be the active site of these enzymes. A nickel-tetrapyrrole coenzyme, Co-P430,
is present in the methyl Co- M reductase and in methanogenic bacteria.

**Copper:** Copper is found in sulphide deposits along with Pb, Cd and Zn and is also considered as an important chalcophile element. It is present in zinc concentrates, smelters and water in small quantities. Copper is found in enzymes capable of carrying oxygen. It is an essential nutrient not only for animals but also for plants and lower forms of organisms. Copper has many uses in industrial and household appliances. Abundant absorption of Cu in man results in "Wilson's disease", in which excess Cu is deposited in the brain, liver, skin, pancreas and myocardium. Copper salts are used for controlling biological growths in reservoirs, water distribution pipes and catalyzing the oxidation of Mn. Measurable amounts of Cu may enter into the water in a localized pipe system due to corrosion of Cu containing alloys in pipe fittings. Symptoms of Cu deficiency are loss of weight, microcytic hypochromic anemia, disturbances in ossification and impairment of collagen formation. Toxic symptoms are hemolytic crisis, jaundice and neurological disorders.

**Cadmium:** Cadmium is recognized as one of the most toxic elements in the environment and is widely distributed on the earth's surface. Industrial discharges and deterioration of galvanized pipes are responsible for the occurrence of Cd pollution in the environment. Its availability in the environment, rapid uptake and accumulation by food chain and crops contribute to its potential environmental hazards. It is present in various types of rocks and soils, in water as well as in coal and petroleum. It is not found in pure state, but found as sulfide in Zn ores and in minor amounts in Pb and Cu ores. Cadmium is highly toxic and mainly responsible for several cases of poisoning through food cycle. Chronic Cd poisoning produces proteinuria and causes the formation of kidney stone. High Cd/Zn ratio in the kidney of a man is greatly responsible for hypertension.
The disease "Itai-itai" has been recognized in Japan, specifically associated with Cd poisoning and is related to contamination from mining complex. High concentration of Cd acts as enzyme inhibitor e.g. ATP, carboxy peptidase, carboxy anhydrase and causes renal failure.

**Iron:** Iron is the most abundant transition element and well-known element in biological system. Majority of iron is bound to proteins such as hemoglobin, myoglobin, non-haem compounds such as ferritin, transferrin and haemosiderin, as well as number of enzymes that are mainly taking part in respiration and oxygen transport. The symptoms of iron deficiency are paleness of the skin, conjuctivitis, hypochromic microcytic anemia and reduced concentrations of haemoglobin in blood and myoglobin. In the muscle, the toxicity of iron is inhibited in the cellular type defense of the organization by blocking the reticulo-endothelian system.

**Zinc:** Zinc is an essential trace metal of immense importance. Zn concentration is relatively high in the liver, kidney, pancreas, testis, adrenals, prostate, skin and the tapetum lucidum of the eyes. The main physiological action of Zn is to act as an enzyme activator, e.g. Carbonic anhydrase, alkaline phosphates, pancreatic and brain carboxypeptidases. The biosynthesis of ACTH, insulin and testosterone are influenced by Zn. Zn deficiency causes growth retardation, loss of appetite, bone deformities, alopecia, delayed closure of the epiphyses, hyper and parakeratosis, disturbed wound healing, impaired fertility of both males and females and behavioral abnormalities. Impaired RNA, DNA, protein and lipid synthesis and decreased stability of cell membranes may also be observed. The best indication of the deficiency state is the Zn concentration in plasma, hair and rib. The phytate present in the flour binds Zn, particularly in the presence of Ca and reduces the biological availability of Zn in
these diets, with the result, the requirements for the element are not satisfied\textsuperscript{20}.

1.5 Metals in different components

1.5.1 Metals in water and sediments

Metal and its compounds in the hydrosphere are in three main phases namely dissolved substance, particulate material suspended in the water and sediment deposits. In addition to the main water types, other classes, which are important, include drinking or potable water, domestic waste water, industrial sewage and storm water. Among these, industrial water and domestic waste and sewage water are very significant sources of metal pollution in the hydrosphere. The principal carriers of the metals in the hydrosphere are sediments\textsuperscript{21}. Sediment particles are made up of materials derived from rocks, soil, biological and anthropogenic inputs. It consists of a wide range of particle size, including gravel, sand, silt and clay.

In estuaries, the movement and deposition of sediments depend on the type of mixing process in the estuary. When a salt wedge is present, most of the solids are carried out to sea as a dilute saline layer. For a well-mixed estuary, the sediments move towards the estuary and then get deposited. For a partially mixed estuary, the sediments are deposited near the shore. Estuaries are sinks for river and marine sediments and they act as sheltered environments compared to ocean. A major contributor of high levels of selenium, copper, lead, mercury, nickel, arsenic, zinc and cadmium in river water is the proximity of mining and mineralized areas\textsuperscript{22-24}. The water is often acidic in such a location and contributes to increased solubility of metal species. The levels of metals in rivers are often increasing by the above activity.
1.5.2 Metals in soil

Soil may be described as a composition of mixture of inorganic and organic materials, ranging from small particles to colloids, containing both dead and living materials and water and air masses in variable proportions. The earth's crust, a part of the lithosphere, is not as easily described as the atmosphere and hydrosphere, because of its heterogeneity. The lithosphere is more directly in contact with plants, animals, human beings, and this is the part that human beings depend upon for most of their food. Sedimentary and igneous rocks alter under high pressure and temperature to produce metamorphic rocks and result in numerous new silicate minerals. Many of the minerals in rocks weather more rapidly than others. As a result, the minerals in rocks are weathered and transported to form soils. The extent of weathering and minerals present determine the quality and type of the soil formed and its subsequent properties are developed. Contamination of a soil and the parent material could alter the rate of weathering of the parent rocks, and thereby increase acidity. The important chemical weathering processes are dissolution, hydration, hydrolysis, oxidation, reduction and carbonation\textsuperscript{25}. The solubility of many minerals is also dependent on pH, for example, increase in the pH of water increases the solubility of SiO\textsubscript{2}.

The implantation of metals into plants is mainly achieved by uptake from the available nutrients in soil through the roots. The uptake may also occur from deposits of the heavy metals on the leaves from soil, aerosol or haze particulates\textsuperscript{26-29}. Many factors such as temperature, soil pH, soil aeration and nutrient fertility influence the uptake of metals in the growing environments. Competition between the plant species, their size, and the root system, the availability of the metals in the soil or foliar deposits, the type of leaves, soil moisture and the plant energy are the major suppliers of metals to the roots and leaves.
1.5.3 Metals in plant materials

While considering the metals in plants, the extra dimension compared with the elements in air, water and soil, is the wide range of species. Therefore, it is not straightforward to generalize over concentrations and effects. Metals have been considered essential to plant survival\textsuperscript{30-32}. Metals play a pivotal role in facilitating the optimal growth of the plant in general while some metals play a crucial role in the formation of active constituents, which are responsible for their curative properties. Moreover, plants and their parts like root, stem, bark, leaves, fruits and seed are sometimes employed in urinary tract diseases and cardiovascular diseases.

Because of the importance of metals in various metabolic processes of humans and plants, and also considering the curative properties\textsuperscript{33}, it is imperative to know the concentration of these metals in the leaves, roots and fruits by using appropriate techniques.

1.5.4 Metals in cereals and pulses

The cereals and pulses are some of the main dietary items for supplying metals and nutrients along with some other dietary items. Previous studies are confined to limited number of metals\textsuperscript{34-35}. Metals play a very important role in biological systems especially with respect to the nutritional and health related values. For nutritional evaluations, it is necessary to know the mineral content of food items. They constitute less than 5\% of the material apart from the fiber, phytate, fat and saccharides. Wheat and paddy are the basic cereal food for the human beings. Other cereals like Bajra, Ragi and Jowar are also cultivated and consumed in certain parts of the world. The pulses such as Bengal gram, Red gram and Moong also form part and parcel of the diet. World over, the percentage of calories due to grain
products is more than 80 per cent\textsuperscript{36}. Ragi has been given more importance as diet for controlling diabetes\textsuperscript{37}. Though the nutritional content of these grains differs from one strain to another, a comparison is considered worthwhile for dietary evaluation. Copper deficiency leads to stunted cereals and copper in excess limits results in decreased yield.

1.5.5 Metals in blood, urine and liver

Metals play a pivotal role in liver and blood related factors like infection, hormones, pregnancy etc. Iron, lead, copper, zinc, cadmium, magnesium, manganese, nickel, selenium, vanadium and chromium are important metals present in the liver and blood. The metals are measured in liver and blood to evaluate their status and dietary habits in relation to the status of urine excretion. The level of metals in liver, blood and urine excretion indicates nutritional status.

Zinc occurs relatively at higher concentrations in liver, kidney, pancreas, testis, and adrenals prostate glands. It plays a cofactor of several enzymes\textsuperscript{39} and involves in the immune function\textsuperscript{40}. Zinc and copper are deemed to be essential trace elements in man and are important for medicinal diagnosis\textsuperscript{41}. Selenium is also immensely important due to its physiological significance and toxicity. Lead is a toxic metal dangerous to most of the human body organs and interferes with the body's metabolism and cellular functions. It produces damaging effects in the haematopoetica haematic, renal, reproductive and gastrointestinal systems. Vanadium is an important metal in biological functions of human beings.
1.6 Importance of the topic

The role of metals in the metabolism, maintenance of biological system, steel preparation and pharmaceutical preparation has been recognized for many decades. Pollution due to toxic metals is considered to be of immense importance due to the non-biodegradable nature and long biological half lives. It is also known that their excess or deficiency may result in serious disorders not only to the health of human body but also to the metabolic activities of plants and animals. Exposure to very low concentrations of metals like lead, selenium, copper, cobalt, zinc, nickel and cadmium may produce adverse effects on health and behavior of population. Although metals such as copper, nickel, selenium, cobalt, chromium, cadmium and zinc are essential for human beings, chronic metabolic disturbances may result from excess or deficiency of these metals. Therefore, the knowledge on the role of metals and their presence in biological systems has been the principal target of many a research study in the past and is still continuing. Hence, the continuous research on the study of metals of biological samples, pharmaceutical preparation and steel preparation is conducted to fulfill the lacunae if any, existing in the earlier studies. Hence the author has taken up research in this field and selected the topic "Determination of trace metals (Se, Cr, Co, Cu, Ni, Cd and Zn) in various environmental samples".

1.7 Need for selective analytical techniques

Analysis of environmental samples is one of the most challenging tasks. This is because the environmental problems are complex and the requirements are very stringent. The matrix is quite complex in many cases and the concentration levels of the analytes are quite low and hence very sensitive and element selective analytical methods are needed. There is also an interest on speciation of metal
ions. This is because of the varying toxicities of metal ions in their different oxidation states or because of the formation of organically bound complexes, which are lipid soluble. This has led to a variety of procedures for the separation of the species according to their charge or on the basis of their varying affinities to adsorbents.

1.8 Techniques generally used for the analysis

Reliable and sensitive analytical methods find a significant role in determining and combating the environmental impact of metal pollution. It is realized that no single available analytical technique can be employed for the analysis of the toxic effects of trace metals. Some of the requirements for an analytical method to be acceptable are 1) sensitivity, specificity and accuracy, 2) rapidity of analysis and ease of operation, 3) acceptable automation, 4) low cost of equipment and 5) reliability of statistical validation of results on par with literature methods.

A large number of analytical methods are available for metal determination in various environmental samples. Some of the important techniques are 1) Spectrophotometry, 2) Polarography, 3) Atomic Absorption Spectrometry, 4) Inductively Coupled Plasma - Atomic Emission Spectrometry and 5) Inductively Coupled Plasma - Mass Spectrometry.

1.8.1 Spectrophotometry

It is an instrumental method of analysis widely employed because of its high precision, sensitivity and rapidity. Spectrophotometric method envisages both essential and toxic metals to be determined over a wide range of concentrations in any material of environmental importance.
The absorption spectrophotometry is based on Beer's law, where the absorption of light in a material is proportional to the number of molecules of the absorbing substance in the light path. The measurement of absorbance or transmittance in ultraviolet, visible and IR region of electromagnetic spectrum forms the basis for the qualitative and quantitative analysis of chemical species of particular metal ion present in the sample.

The most advantage of spectrophotometric method is that the radiation absorbed is the characteristic of the material offering simplicity, specificity and sensitivity to the measurement within the limits of Beer's law. It is one of the most useful tools for the elucidation of the composition of the complex ions in solution and for the determination of formation constants.

The methods used in colorimetric determination of Cadmium\textsuperscript{42,43}, Lead\textsuperscript{44-47}, Silver\textsuperscript{48} and Zinc\textsuperscript{49,50} with dithiozone, Chromium with diphenylcarbazide\textsuperscript{51,52} and Iron with phenanthroline\textsuperscript{53,54} in water samples are reported in literature 1-(2-pyridylazo)-2-naphthol (PAN) reagent was employed for the determination of Mn, Zn, Cd, Cu, Co, Ni, In, U, Ga, Hg, Fe and Pd using extractive spectrophotometry in water samples in ppm range\textsuperscript{55-57}.

Jinsook Yun and Choi\textsuperscript{58} studied iron, cobalt, nickel and copper using 1-nitroso-2-naphthol by Micellar Colorimetry. Kubilay et al\textsuperscript{59} developed a combined spectrophotometric - AAS method used for the analysis of trace metal, EDTA and metal-EDTA mixture solutions in adsorption modeling. Ravanasiddappa and Kiran Kumar\textsuperscript{60} reported a facile spectrophotometric method for the determination of selenium using variamine blue at $\lambda_{max}$ 546 nm in Beer's law range of 0.2-2.0 ppm in real samples of water, soil, plant materials, human hair and
synthetic samples of cosmetic and pharmaceutical preparations. Afkhami and Bahram reported H-point standard method for the selective simultaneous determination of nickel and copper using 1-(2-pyridylazo)-2-naphthol (PAN) chromogenic reagent with micellar medium in various environmental samples. Nakano et al described the flow injection spectrophotometric method for determination of vanadium by catalysis of the bromate oxidation of N,N'-bis(2-hydroxy-3-sulfopropyl)-tolidine with Beer's law range 0.01-3.0 ng mL⁻¹. Eskandari et al reported the First- and Second-Derivative Spectrophotometry for simultaneous determination of copper and cobalt using 1-(2-Pyridylazo)-2-naphthol in Tween 80 Micellar Solutions at wave length of 555 and 581 nm for cobalt and copper. Suresh Kumar et al developed an oxidative coupling method for the determination of vanadium in various environmental samples with detection limit of sub ppm using 4-aminoantipyrine with NEDA.

1.8.2 Polarography

Electrochemical analysis is one of the important techniques utilized for the determination of metals. A variety of electrochemical methods have been established based on the versatile electrochemical properties of these metals and their compounds.

Heyrovsky and Shikata studied an apparatus, which increased the applied voltage at a steady rate and simultaneously recorded photographically the current-voltage programme. The proportionality between potential and current constitutes the basis of Polarography.

In a Polarography experiment, a potential difference E is applied across the cell consisting of the dropping mercury electrode and a non-polarizable interface (e.g. a calomel electrode). In response to this potential difference, a current density i' flows across the drop-solution
interface. As each drop grows and falls, the surface area of the drop also grows, and then becomes effectively zero when the drop falls. Thus, the instantaneous current shows fluctuations, but the mean current is a unique function of the potential difference across the drop-solution interface, and therefore of that across the cell.

The essential part of the polarographic set up is a dropping mercury electrode consisting of a glass capillary tube, out of which mercury converges at the rate of a drop for every few seconds. The solution is added to a substance, which is termed as indifferent electrolyte, because its ions do not participate in the charge transfer reaction at the drop-solution interface.

Ladanyi and Topuzovski et al have developed the polarographic method for the determination of trace metals in water. Piperazine was used as a reagent for the polarographic determination of Tl, Pb, Cr and Te. Polarographic study of the extraction of Mn, Co, Fe, Ni and Cd ions by potassium ethyl xanthate in the presence of 2,2'-dipyridyl at pH 5-7 was explained by Ulakhovich et al. Puri et al reported a method for the polarographic determination of E-caprolactum complexes of uranium and tellurium. Inam and Somer have determined the concentrations of selenium and lead in whole blood samples by Differential Pulse Polarography.

1.8.3 Atomic Absorption Spectrometry (AAS)

Atomic absorption spectrometry is a well known analytical method for the determination of trace elements in environmental samples. The method is based on the principle of absorption of radiation by ground state atoms. If light is passed through a flame or furnace containing atoms in the ground state, the corresponding wavelengths will be absorbed and the extent of absorption will be
proportional to the number of ground state atoms present in the flame.

Atomic absorption methods are presently considered to be the workhorse of analytical laboratory. Their main advantages are their high specificity and sensitivity and freedom from inter-elemental interference. This is due to the nature of the absorption line, which is very sharp as there are no associated vibrational or rotational levels. The sensitivity is high for transition metal ions but is relatively poor for refractory oxide forming metals. Flame AAS method has the advantage that the technique is applicable to the analysis of a large number of metal ions at ppm levels. The use of hollow cathode lamps has contributed to the specificity of the method. The use of alternate lines helps in extending the concentration levels of analysis. AAS is a very versatile analytical tool and various types of samples can be analyzed by this technique as shown in Table 1.2. Flame AAS is of advantage for regular analysis of solution samples at ppm levels. The use of matrix matched standards helps in the minimization of errors due to the variation in the nature of samples and standards. The precision obtainable is about 2 percent in the case of the flame AAS. The interferences in the techniques arise mainly due to the chemical interferences like those due to the formation of refractory oxides, sulphates or phosphates in the flame. Some of these effects can be minimized by the use of releasing agents like lanthanum or complexing agents like EDTA, which help in the conversion of a complex into a form, which decomposes and dissociates easily in the flame.

The advantages of the method are specificity, ease of operation, applicability to a large number of elements, wide dynamic range and low detection limits (especially with OFAAS). However, the instrument AAS is inherently designed for single element operation. Hence, simultaneous multielemental analysis is a limitation in AAS.
Lamathe\textsuperscript{72} discussed the extraction of Cu, Pb, Ni, Zn, Cd and Co from sea water with Chelex-100 prior to the determination using AAS. Fernandez and Manning\textsuperscript{73} reported the analysis of trace metals As, Al, Cd, Co, Cr, Fe, Mn, Pb, Sn and Zn in natural water bodies. They obtained better sensitivity for Cd at 1-3 pg and the least sensitivity for Sn at 1500 pg. Nakamoto et al\textsuperscript{74} described atomic absorption spectrometric method using graphite furnace coated with tungsten for the determination of vanadium in heavy oils. Saavedra et al\textsuperscript{75} described the determination of vanadium by using electro thermal atomic absorption spectrometry without chemical modifiers in muscle sample. Minami et al\textsuperscript{76} developed the method for the determination of cobalt and nickel by using graphite furnace atomic absorption spectrometry after co-precipitation with scandium hydroxide at pH 8.0-10.5 in river water sample. The electro thermal atomic absorption spectrometric technique for the analysis of nickel in serum and urine samples was reported by Todorovska et al\textsuperscript{77}.  

<table>
<thead>
<tr>
<th>Area</th>
<th>Elements</th>
</tr>
</thead>
<tbody>
<tr>
<td>Animal Tissue</td>
<td>Na, K, Ca, Mg, Fe, Cu, Zn</td>
</tr>
<tr>
<td>Petroleum products</td>
<td>V, Ni, Pb, Ca, Na, K</td>
</tr>
<tr>
<td>Lubricating oils</td>
<td>Zn, Ca, Ba, Pb</td>
</tr>
<tr>
<td>Metals and Alloys</td>
<td>Many elements</td>
</tr>
<tr>
<td>Ores and Minerals</td>
<td>Many elements</td>
</tr>
<tr>
<td>Soils</td>
<td>Many elements</td>
</tr>
<tr>
<td>Wines and Organic compounds</td>
<td>K, Mg, Ca, Na, Fe, Mn, Zn, Cu</td>
</tr>
<tr>
<td>Pharmaceutical products</td>
<td>Ca, Mg, Fe, Na, K, Li, Ag, Cu, Zn, Bi</td>
</tr>
<tr>
<td>Glass and Ceramics</td>
<td>Hg, As</td>
</tr>
<tr>
<td>Water and Effluent analysis</td>
<td>Many elements</td>
</tr>
<tr>
<td>Gunshot residue</td>
<td>Many metals</td>
</tr>
</tbody>
</table>

\textsuperscript{72} Lamathe, \textsuperscript{73} Fernandez and Manning, \textsuperscript{74} Nakamoto et al, \textsuperscript{75} Saavedra et al, \textsuperscript{76} Minami et al, \textsuperscript{77} Todorovska et al.
Uzun et al\textsuperscript{78} reported the pre-concentration and separation method with Amberlite XAD-4 resin for the determination of Cu, Fe, Pb, Ni, Cd and Bi at trace levels in waste water samples by flame atomic absorption spectrometry. Pankaj Kumar and Ajai Kumar\textsuperscript{79} described the application of thio salicylic acid – immobilized Amberlite XAD-2 to metal sorption behavior for the determination of heavy metal ions employing flame atomic absorption spectroscopy in river water. Mohammad\textsuperscript{80} reported the use of atomic absorption spectrometric determination of trace zinc in alloys and biological samples after preconcentration with [1-(2-pyridylazo)2-naphthol] as microcrystalline naphthalene. Lars and Joakim\textsuperscript{81} carried out determination of Pb, Cd, Zn, Cu, and Fe in food samples using AAS after microwave digestion. Orhan\textsuperscript{82} simultaneously studied the determination of Cadmium, Chromium, Copper and Lead in sediments and soil samples by Electro thermal Atomic Absorption Spectrometry Using Zr, Ir, ethylene diamine tetra acetic acid (EDTA), Zr $^+$ EDTA, Ir $^+$ EDTA, Zr$^+$, Ir and Zr$^+$, Ir $^+$ EDTA as chemical modifiers.

1.8.4 Neutron Activation Analysis (NAA)

Neutron activation analysis as developed by Havesy and Levi in 1936 has become one of the most important analytical tools for the determination of trace elements in a wide variety of complex matrices. Activation analysis is based on the formation of radioactive nuclides as a result of bombardment with nuclear particles to the isotope(s) of the trace element of interest in the test sample. The isotopes in the material are converted into isotopes of the same or different elements. Elements when irradiated with thermal neutrons give rise to radioactive species.

Identification of the element can be done by measurement of the half-life of the energy of the beta particles or by analyzing the gamma
ray spectrum. Neutron activation analysis is a very sensitive analytical technique for the determination of trace elements in the concentration range of $10^{-3}$ to $10^{-7}$ µg/g. The main advantage of neutron activation analysis is its freedom from reagent contamination. The technique is highly sensitive with multi-element capability and is quite specific for different elements.

Blanchard et al\textsuperscript{83} have analyzed 18 trace elements in drinking water by NAA. Nagatsuka and Tanizuki\textsuperscript{84} have estimated Hg and Cu in water by NAA using pre-concentration on emission spectroscopic carbon powder. Lieser et al\textsuperscript{85} have showed the applicability of NAA as a routine method for analysis of trace metals in water. Imai et al\textsuperscript{86} have reported the determination of 19 trace elements in water using NAA. Elemental concentrations of different edible and medicinally valuable leaves along with standard reference materials (SRM) were determined by using NAA\textsuperscript{87}.

NAA is widely employed to measure the various elements like As, Se, Br, V and Cd\textsuperscript{88}. Prompt gamma ray NAA (PGNAA) uses a neutron beam and is an on-line measurement technique, used to measure Cd, Hg and other toxic elements from oil spill and other related matrices\textsuperscript{89}.

1.8.5 Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-AES)

Inductively coupled plasma atomic emission spectrometry is a modern promising analytical technique for the fast multielemental analysis. The method is based on measurement of electromagnetic radiation consequent to the excitation of an atom by plasma. Plasma is a conducting mixture containing significant concentration of cations and electrons. The flame for this instrument is incandescent plasma.
of argon heated inductively by radio-frequency energy. Temperature up to 10000 K is attained which is twice that obtained in conventional flames. The high temperature of plasma and the absence of oxygen are the major advantages of ICP-AES, which keep the interfering chemical reactions to the minimum. The main advantages of ICP-AES are (1) wide linear dynamic range (2) minimum interference by elements, and (3) fast multielemental analysis.

Simultaneous multi element analysis for trace metals in seawater was carried out by ICP-AES after batch pre-concentration on a chelating resin by Cheng et al. In this method pre-concentration factor of 100 was achieved and Al, V, Ti, Mn, Fe, Co, Ni, Zn, Y, Mo, Cd and Pb were estimated with good precision and accuracy. Sugiyama et al. reported the simultaneous pre-concentration of 30 elements in natural water using dithiocarbamate extraction into 2-ethyl hexyl acetate and the determination was carried out by ICP-AES. Simultaneous multi element determination of trace metals in seawater by ICP-AES after co-precipitating with Ga has been reported by Akagi et al. In this method a concentration factor of 7200 could be obtained and the detection limits for Al, Co, Cr, Fe, Pb, Mn La, Ni, Tl, V, Zn and Y were in the range of few ng L⁻¹ to 150 ng L⁻¹. Da-Hai Sun et al. developed a method by comparison of five digestion procedures for the determination of 13 elements in food samples by ICP-AES. In this method, the wet digestion with HNO₃, HClO₄ is the simplest and the most effective procedure for the selected elements except Al and B. Liang et al. evaluated a new method using the micro column packed with nm size titanium dioxide as solid phase extraction for the simultaneous online pre-concentration of trace amounts of Cu, Mn, Cr and Ni prior to their measurements by inductively coupled plasma atomic emission spectrometry in biological samples and lake water with satisfactory results. Grotti et al. reported inductively coupled plasma optical emission spectrometric determination of trace elements in sediments after sequential selective
extraction absorbed analytical signals of major elements affected by the reagents. Sumida et al\textsuperscript{196} described the on-line pre-concentration method using dual mini columns for the speciation of Cr (III) and Cr (IV) and its application to water samples was studied by using inductively coupled plasma atomic emission spectrometry. Zoltan et al\textsuperscript{197} developed a new nebulised system for simultaneous determination of Sb, Sn (hydride generation), vanadium and Zn in spiked water samples and recovery experiments were performed on the NIST 1643 trace metals in water standard reference material with good accuracy.

1.8.6 Inductively Coupled Plasma-Mass Spectrometry (ICP-MS)

Inductively coupled plasma - mass spectrometry (ICP-MS) is a versatile, rapid and precise analytical technique that provides a high quality multielement and isotopic analysis package. The versatility of the ICP-MS technique makes it a multi-disciplinary analytical tool, it can be used in geological and environmental sciences, nuclear and semi-conductor industries, materials science, medicine, agriculture, food and biological sciences. The detection limit for most elements is the sub-parts per billion (ppb) ranges. For some elements it may lie in the sub parts per trillion range (ppt). Ultrasonic nebulizer is used for ultra trace element analysis. The accessories enhance the versatility of the ICP-MS in terms of handling samples of various types, concentrations, chemical matrix, mineralogy and structure. The sample is introduced into radio frequency induced plasma in the form of a solution, vapour or solid. The temperature of the plasma may reach up to 6000 K at the center and 8000 K at the periphery of the plasma. The high thermal energy and electron-rich environment of the ICP results in the conversion of most atoms into ions. A quadruple mass spectrometer permits the detection of ions at each mass in rapid sequence allowing signals of individual isotopes of an element to be scanned.
Determination of lanthanides, some heavy and toxic elements in plant certified reference materials using ICP-MS was done by Ivanova. In this method, the determination of Be, lanthanides, Th and U and other elements at ng g⁻¹ in plants were carried. Bettinelli et al. evaluated an inductively coupled plasma optical emission spectrometry for the determination of heavy metals in soils and sediments by microwave-assisted digestion. Ilia discussed the ICP-MS method for the determination of 60 trace elements in whole blood by sector field with detection limits of a few pg mL⁻¹. Performance optimization of commercially available iminodiacetate resin was done, for the determination of Mn, Ni, Cu, Cd and Pb by on-line pre-concentration by ICP-MS in sea water. Michael et al. developed the microwave digestion methods for the determination of trace elements in brain and liver samples by ICP-MS using digestion mixture of 1.5 mL HNO₃/1 mL H₂O₂. An analytical method was reported using double focusing sector field ICP-MS for multi elemental characterization of human hair and nails. Hsien and Shiuh reported the determination of cadmium, mercury and lead in coal fly ash by slurry sampling using electro thermal vaporization inductively coupled plasma mass spectrometry with Thioacetamide (TAC) as a modifier with detection limit in the range of 24-58, 6-28 and 108-110 ng/g respectively. Martinez et al. developed a new method for the simultaneous chromatography separation and determination of arsenic, selenium and chromium (VI) species by inductively coupled plasma mass spectrometry with good accuracy and repeatability in several environmental surface water samples. Salomon et al. reported a plasma source mass spectrometry as a visible analytical technique for the determination of trace elements in routine practical aspects of importance of environmental matrices. Badal Kumar et al. described the speciation of arsenic in human nail and hair which is collected from arsenic affected area by High Performance Liquid Chromatography coupled with Inductively Coupled Argon Plasma Mass Spectrometry.
The detection limits for a few elements with different techniques are shown in the Table 1.3. The capabilities of different analytical techniques available for determining the environmental samples in terms of accuracy, sensitivity, selectivity, distribution, multi-elemental, sample size, sample type, routine, time interval, accessibility are shown in Table 1.4.

Table 1.3
Detection limits for some elements in µg

<table>
<thead>
<tr>
<th>Element</th>
<th>INAA</th>
<th>XRF</th>
<th>PIXE</th>
<th>ICP-ES</th>
<th>AAS</th>
<th>ICP-MS</th>
</tr>
</thead>
<tbody>
<tr>
<td>As</td>
<td>0.03</td>
<td>4</td>
<td>0.4</td>
<td>3.5</td>
<td>0.2</td>
<td>0.04</td>
</tr>
<tr>
<td>Cd</td>
<td>0.6</td>
<td>6</td>
<td>10</td>
<td>1.7</td>
<td>0.003</td>
<td>0.06</td>
</tr>
<tr>
<td>Cr</td>
<td>0.03</td>
<td>16</td>
<td>0.8</td>
<td>4</td>
<td>0.01</td>
<td>0.06</td>
</tr>
<tr>
<td>Cu</td>
<td>0.03</td>
<td>6</td>
<td>0.3</td>
<td>3.5</td>
<td>0.02</td>
<td>0.32</td>
</tr>
<tr>
<td>Fe</td>
<td>6</td>
<td>12</td>
<td>0.5</td>
<td>0.5</td>
<td>0.02</td>
<td>ND</td>
</tr>
<tr>
<td>Hg</td>
<td>0.03</td>
<td>7</td>
<td>1</td>
<td>17</td>
<td>2</td>
<td>0.02</td>
</tr>
<tr>
<td>In</td>
<td>0.0006</td>
<td>ND</td>
<td>14</td>
<td>40</td>
<td>ND</td>
<td>0.07</td>
</tr>
<tr>
<td>Mn</td>
<td>0.001</td>
<td>12</td>
<td>6.6</td>
<td>0.95</td>
<td>0.01</td>
<td>0.1</td>
</tr>
<tr>
<td>Mo</td>
<td>0.3</td>
<td>5</td>
<td>1.9</td>
<td>5.5</td>
<td>0.02</td>
<td>0.04</td>
</tr>
<tr>
<td>Ni</td>
<td>3</td>
<td>5</td>
<td>0.4</td>
<td>6.5</td>
<td>0.2</td>
<td>0.1</td>
</tr>
<tr>
<td>Pb</td>
<td>0.25</td>
<td>4</td>
<td>0.31</td>
<td>5.2</td>
<td>0.32</td>
<td>0.17</td>
</tr>
<tr>
<td>Sb</td>
<td>0.001</td>
<td>8</td>
<td>14</td>
<td>20</td>
<td>0.1</td>
<td>0.05</td>
</tr>
<tr>
<td>Sc</td>
<td>0.03</td>
<td>2</td>
<td>0.4</td>
<td>50</td>
<td>0.5</td>
<td>0.79</td>
</tr>
<tr>
<td>Se</td>
<td>0.02</td>
<td>0.9</td>
<td>0.35</td>
<td>17</td>
<td>0.15</td>
<td>0.05</td>
</tr>
<tr>
<td>Sn</td>
<td>1</td>
<td>8</td>
<td>16</td>
<td>17</td>
<td>0.1</td>
<td>0.06</td>
</tr>
<tr>
<td>V</td>
<td>0.03</td>
<td>20</td>
<td>1.3</td>
<td>3.5</td>
<td>0.2</td>
<td>0.03</td>
</tr>
<tr>
<td>Zn</td>
<td>0.3</td>
<td>5</td>
<td>0.3</td>
<td>1.2</td>
<td>0.001</td>
<td>0.21</td>
</tr>
</tbody>
</table>

N.D: Not Detected
<table>
<thead>
<tr>
<th>Method</th>
<th>Sample size</th>
<th>Sample type</th>
<th>Accuracy</th>
<th>Sensitivity</th>
<th>Distribution</th>
<th>Multi-elemental</th>
<th>Routine</th>
<th>time interval</th>
<th>Accessibility</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spectrophotometry</td>
<td>mg/L</td>
<td>L</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>3</td>
<td>1</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Derivative Spectrophotometry</td>
<td>ng/L</td>
<td>S</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Atomic absorption spectrometry</td>
<td>mg-ng/L</td>
<td>L</td>
<td>3</td>
<td>1</td>
<td>3</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>ICP-AES</td>
<td>mg-ng/L</td>
<td>L</td>
<td>2</td>
<td>1</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>ICP-MS</td>
<td>ng-pg/L</td>
<td>L</td>
<td>2</td>
<td>1</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>NAA</td>
<td>Ng-pg/Kg</td>
<td>S/L</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>3</td>
<td>3</td>
</tr>
</tbody>
</table>

S = Solid
L= Aqueous solution
1=Good, 2=Average, 3=Not so good
1.9. Preconcentration and separation techniques used in trace metal analysis

Environmental pollution monitoring requires determination of toxic heavy metals in trace levels. Analytical techniques such as spectrophotometry, voltammetry, atomic absorption spectrometry, atomic emission spectrometry are not sensitive enough for the determination of trace metals at very low concentrations. These techniques often require suitable preconcentration methods because the concentration of some metals in environmental samples will be below the detection limit of these techniques. The need for preconcentrating the environmental samples frequently arises for improving sensitivity and to separate interfering species for improving the accuracy. Co-precipitation, solvent extraction, ion-exchange, solid phase extraction and cloud point extraction are some of the preconcentration methods used for the trace metal analysis in environmental samples. These preconcentration methods provide low detection limits and also help to avoid matrix interferences in the analysis of real samples.

1.9.1 Co-precipitation

This is one of the classical methods of separation used in trace metal analysis. In many instances, a precipitate is usually accompanied by other ions present in the solution, which by themselves, would not precipitate. This phenomenon is called as co-precipitation. According to Kolthoff and Sandell\textsuperscript{110} co-precipitation is "the incorporation of impurities into precipitate by substances which under experimental conditions are usually soluble in the liquid phase". The mechanism of co-precipitation is generally considered to include adsorption, occlusion and mixed-crystal formation. Co-
precipitation is favoured when the precipitation process attains equilibrium very slowly. Co-precipitation is widely used in the analysis of fresh water, seawater, tap water and wastewater.

Manganese dioxide\textsuperscript{111-113} has been widely used as collector for precipitating and separating traces of antimony, bismuth and tin in the presence of lead. The traces of As, Sb, Sn, Cd, Pb and other metals are successfully deposited by molybdenum sulphide from water samples\textsuperscript{114}. Boyle and Edmond\textsuperscript{115} developed co-precipitation method for preconcentration of trace metals in sea water using cobalt pyrrolidine dithiocarbamate. Smith et al\textsuperscript{116} reported a preconcentration method using Na-dibenzyl DTC as precipitating agent. Himeno et al\textsuperscript{117} determined heavy metals in waste water after co-precipitation with lanthanum hydroxide. Zucheng and Schramm\textsuperscript{118} employed co-precipitation method for preconcentration of metals in natural waters using Co-DTC. Vircavs et al\textsuperscript{119} reported co-precipitation of twenty one elements with 5,8-polyquinolyl polysulphide. Atanasova et al\textsuperscript{120} developed preconcentration method for the determination of Se, Cu, Pb, Zn, Fe, Co, Ni, Mn, Cr and Cd in water samples after co-precipitation with sodium diethyldithiocarbamate.

1.9.2 Solvent Extraction

Solvent extraction is a process occurring in a two-phase ternary system. It involves distribution of solute in certain rate between two immiscible solvents, one of which is usually aqueous and the other is an organic solvent. This means that concentration of the solute in two phases is mutually related according to the distribution law proposed by Berthelot and Jungfleisch\textsuperscript{121} and developed by Nernst\textsuperscript{122}. This states that a solute is distributed between immiscible solvents in such a way that at equilibrium the ratio of the concentrations of the solute in the two phases is constant at a given temperature, provided that
the solute has the same molecular weight in each phase. It is expressed by the following formula.

\[ K = \frac{C_o}{C_w} \]

Where \( K \) = distribution constant

\( C_o \) = concentration of the solute in the organic phase

\( C_w \) = concentration of the solute in the aqueous phase

Based on observation of Morrison and Freiser\textsuperscript{123}, thermodynamic distribution law states that the equilibrium is reached at a constant temperature and pressure, when the chemical potentials (particular molar Gibbs free energy) of the solute in the two phases are equal.

The distribution constant is calculated as

\[ K = \frac{r_w}{r_o} \exp \left[ -\varphi_o - \varphi_w / RT \right] \]

Where \( \varphi_o \) and \( \varphi_w \) are the chemical potentials of the solute (equal to unity in the organic and aqueous solutions, respectively) and \( r_o \) and \( r_w \) are the corresponding molar activity coefficients in the organic and aqueous phases. The \( \varphi_o \) and \( \varphi_w \) are assumed to be constant, when the pressure of the solute does not significantly affect the mutual solubilities of the organic and aqueous phases for a given system, then the formula takes the form.

\[ K = k \cdot \frac{r_w}{r_o} \text{ where } k \text{ is a constant} \]

Important applications of solvent extraction with reversible chemical reaction are hydrometallurgy and analytical chemistry. It is
necessary to neutralize the ionic charge prior to extraction of cations and anions from an aqueous phase to an organic phase because it is energetically unfavourable to the extraction. The neutralization is achieved either by forming organic soluble neutral complexes in the aqueous phase between cations and anions or by direct reaction between ionic species soluble in the organic phases. The involvement of chelating agents for the extraction of metal ion from a solvent phase became an important and useful step in solvent extraction.

Extractions with dithiocarbamates for preconcentration of metals have been studied by Brooks et al\textsuperscript{124}, Kremling and Peterson\textsuperscript{125}, Jan and Young\textsuperscript{126} and Babu and Naidu\textsuperscript{127}. Tao et al\textsuperscript{128} developed the solvent extraction method for the preconcentration of Cd, Cr, Fe, Mn, Mo, Ni, Pb, U and Zn in river and sea water. Preconcentration of traces of chromium using solvent extraction prior to the determination using 1,5-diphenyl carbazide was reported by Mann and White\textsuperscript{129}. Kammori and Ono\textsuperscript{130}, Kolthoff and Sandell\textsuperscript{131}, Irving et al\textsuperscript{132}, Babko and Pilipenko\textsuperscript{133} reported notable work on dithiozonates and their extraction for the quantitative determination of trace metals in different samples. Balaji et al\textsuperscript{134} used the potassium propyl xanthate for extraction and determination of Cu and Cd in waste water and plant materials. Bengtsson and Johansson\textsuperscript{135} developed two-stage solvent extraction method for the preconcentration of Cd, Cu, Pb, Co and Mn in water samples. Preconcentration of cadmium in water by solvent extraction was reported by Tanigawa et al\textsuperscript{136}. Chen and He\textsuperscript{137} reported solvent extraction method for the preconcentration of Cu, Zn, Cd and Pb in environmental samples. Simultaneous ion–pair solvent extraction method for the determination of twelve elements in natural water samples was reported by Zhang et al\textsuperscript{138}. 

35
1.9.3 Ion Exchange

Ion exchangers are insoluble solid materials, which contain exchangeable cations or anions. These ions can be exchanged for a stoichiometrically equivalent amount of other ions initially present in an electrolyte solution when an ion exchanger is brought into contact with it. Substances containing exchangeable cations are called cation-exchangers and those containing exchangeable anions are called anion-exchangers. Materials capable of both cation and anion exchange are called amphoteric ion-exchangers. On the basis of the nature of the framework material, two main classes of ion exchangers can be distinguished, inorganic and organic. Clays and Zeolites are some of the inorganic ion exchangers; these ion exchangers have special use in radiochemical separations. Molecular sieves, hydrous metal oxides, heteropoly acids are some of the important synthetic organic cation exchangers. Semi synthetic ion exchangers made from sulphonated coal have found little use for analytical purposes because of their low ion exchange capacity, limited mechanical and chemical stability and tendency to peptize. In inorganic analysis, only synthetic ion exchange resins have found wide spread application.

Synthetic organic cation exchangers are cross linked polyelectrolytes which consist of a three-dimensional network of hydrocarbon chains carrying ionic groups such as sulphonate -SO₃⁻, carboxylate-COO⁻, phenolate-O⁻, phosphonate-PO₃⁻, phosphinate-HPO₂⁻. The negative charges of the functional groups are compensated by the positive charges of a stoichiometrically equivalent number of mobile cations known as the counter ions. Within the matrix, counter ions can be exchanged for other ions taken up from the external solution in contact with the ion exchanger. Ion exchange preconcentration of trace metals is usually performed as column or dynamic process, though occasionally static (batch) process may be used. The static method is justified only when the trace metals
to be separated have a very high affinity for the ion exchanger. Recently ion exchange membranes have been used in static\textsuperscript{141,142} and in column process\textsuperscript{142,143} for preconcentration of trace metals. Frache et al\textsuperscript{144} reported on systematic analysis of major, minor and trace elements by ion exchange.

Riley and Taylor\textsuperscript{145} reported Chelex-100 cation exchange method for the preconcentration of trace metals. Orpwood\textsuperscript{146} reported the use of chelating ion exchange resins for the determination of trace metals in drinking water. Potter and Moresby\textsuperscript{147} developed the method for determining copper and iron in distilled water after a 100–1000 fold preconcentration on a sulphonlic acid cation exchanger in $H^+$ form, the ions were estimated spectrophotometrically in the eluate. Preconcentration of trace metals as chloride, bromide, nitrate or thiocyanate complexes on strong base ion exchangers together with selective elution were used for the determination of cobalt\textsuperscript{148} and lead\textsuperscript{149} at ng mL$^{-1}$ levels in natural waters and determined by spectrophotometry or atomic absorption spectrometry. Yoshimura and Ohashi\textsuperscript{150} and Ohzeki et al\textsuperscript{151} improved the sensitivity by using an ion exchange resin and applied 1,5′-diphenyl carbazide for the determination of total chromium in natural waters. Biechler\textsuperscript{152} developed similar method for the determination of Cu, Pb, Zn, Cd, Ni and Fe in industrial waters. Taylor et al\textsuperscript{153} and Sung et al\textsuperscript{154,155} used Muromac A-1 chelating resin for on-line preconcentration of trace metals in waters. Ogura and Oguma\textsuperscript{156} determined molybdenum and vanadium in seawater by ion exchange preconcentration method. Du-Plessis et al\textsuperscript{157} described simultaneous determination of Ba, Ca, Cr, Cu, Fe, Mn and Mo with an on-line ion exchange preconcentration method.
1.9.4 Solid Phase Extraction

Solid-phase extraction is a versatile preconcentration method for separation and determination of trace metal ions. This process, often termed as liquid-solid chromatography, is based on interaction between the solute and fixed active sites on a finely divided solid adsorbent used as the stationary phase. The adsorbent used in this process, has generally a high surface area. Column solid-phase extraction has several advantages over liquid-liquid extraction. The possibility of combination with AAS as well as ICP-AES, allows preconcentration from a large sample volume, establishing high preconcentration factor, simple storage and transport of pretreated sample. The incorporation of column solid-phase extraction in the flow injection systems coupled on-line to atomic spectrometers offers some interesting improvements. The major improvements are, in general, the possibility of working in a closed system with a significant reduction of airborne concentration and fairly high sampling frequency, which justifies current trends towards frequent use of this preconcentration technique.

Alexandrova and Arpadjan\textsuperscript{158} reported column solid-phase extraction using solid ammonium hexamethylenedithiocarbamate (HMDC) mixed with polyurethane foam for the preconcentration of trace amounts of Cd, Cu, Co, Cr, Hg, Fe, Mn, Ni and Pb from analytical reagent grade sodium salts prior to their determination by AAS or ICP-AES. Sorption behaviour of the lanthanum(II), neodymium(II), terbium(III), thorium(IV) and uranium(VI) on Amberlite XAD-4 resin functionalized with bicine ligands was reported by Dev et al\textsuperscript{159}. Karthikeyan et al\textsuperscript{160} described a rapid and sensitive sorbent extraction hydride generation flow injection atomic absorption spectrometric method for the determination of As(III) and As(V) based upon on-line preconcentration on a micro-column packed with activated alumina. In this procedure, the arsenuous are complexed
with quinoline-8-ol-5-sulphonic acid from neutral solutions in the flow injection system and adsorbed on the column. Ibrahim et al\textsuperscript{161} reported adsorption behaviour of some trace metals by activated carbon treated with pyrocatecho1 violet complexes in water samples.

Jain et al\textsuperscript{162} used the Amberlite XAD-2 functionalized o-vanillin-thio-semicarbazone chelating resin for the preconcentration of Cu, Zn and Pb. Saxena and Singh\textsuperscript{163} developed the preconcentration and determination of metal ions in well water samples by solid-phase extraction using pyrocatecho1 violet immobilized Amberlite XAD-2. Electrothermal atomic absorption spectrometric determination of Pb, Cd, Cu and Zn in high salt content samples after simultaneous separation on polyethylene powder impregnated with 1-(2-pyridylazo)-2-naphthol was reported by Bohrer et al\textsuperscript{164}. Kumar et al\textsuperscript{165} reported the preconcentration procedure for the enrichment of trace metals using XAD-2 functionalized Tiron. Determination of trace metal ions in sea water samples by atomic absorption spectrometry after separation/preconcentration with calmagite on Amberlite XAD-1180 was developed by Soylak et al\textsuperscript{166}. Uzun et al\textsuperscript{167} described the preconcentration and determination of trace metals in waste water samples by solid-phase extraction using Amberlite XAD-4 resin. Fillik\textsuperscript{168} developed the preconcentration and speciation of Cr(III) in water by using 5-palmitoyl-8-hydroxyquinoline immobilized on a non polar adsorbent. Narin and Soylak\textsuperscript{169} used 1-(2-pyridylazo)-2-naphthol impregnated Ambersorb 563 resin for the solid-phase extraction for traces of heavy metal ions from natural water samples. Column solid-phase extraction of copper, iron and zinc ions at trace levels in environmental samples on Amberlite XAD-7 was developed by Tuzen et al\textsuperscript{170}. Tunceli and Turker\textsuperscript{171} reported the solid-phase extraction and spectrophotometric determination of Mo (VI) in soil and plant samples using Amberlite XAD-7 resin. Simultaneous preconcentration of Cu, Fe and Pb as methylthymol blue complexes on
naphthalene adsorbent and AAS determination was carried out by Pourreza and Hoveizavi.

1.9.5 Cloud Point Extraction

Separation and preconcentration based on cloud point extraction (CPE) is becoming an important and practical application of surfactants in analytical chemistry. The technique is based on the property of most non-ionic surfactants in aqueous solutions to form micelles and become turbid when heated to a temperature known as the cloud point temperature. Above the cloud point, the micellar solution separates into a small volume of surfactant-rich phase and a diluted aqueous phase, in which the surfactant concentration is close to the critical micellar concentration (CMC); Any analyte solubilized in the hydrophobic core of the micelles will separate and become concentrated in the small volume of the surfactant-rich phase. The use of preconcentration steps based on phase separation by cloud point extraction offers a convenient alternative to more conventional extraction systems. The small volume of the surfactant-rich phase obtained with this methodology permits the design of extraction schemes that are simple, cheap, highly efficient, fast, and of lower toxicity to the environment than extractions that use organic solvents. CPE also provides results comparable to those obtained with other separation techniques. Accordingly, any species that interacts with the micellar system, either directly (generally hydrophobic organic compounds) or after a prerequisite derivatization reaction (e.g. metal ions after reaction with a suitable hydrophobic ligand) may be extracted from the initial solution and preconcentrated.

The successful use of cloud point methodology to preconcentrate trace amounts of Co and Ni as a prior step to their determination by flame atomic absorption spectrometry was reported by
Manzoori et al\textsuperscript{173}. Fan\textsuperscript{174} reported a sensitive and simple method for flame atomic absorption spectrometry determination of antimony species after separation by cloud point extraction. A new method for the determination of trace cadmium as 1-phenyl-3-methyl-4-benzoyl-5-pyrazolone complex in water samples by flame atomic absorption spectrometry after cloud point extraction was proposed by Liang et al\textsuperscript{175}. Yuan et al\textsuperscript{176} developed a method based on cloud point extraction and graphite furnace atomic absorption spectrometry was developed for the analysis of trace tin in the water samples using 8-hydroxyquinoline as a chelating reagent and Triton X-114 was added as surfactant. Cloud point extraction was applied as a preconcentration step for electrothermal atomic absorption spectrometry determination of arsenic species in aqueous solutions by Tang et al\textsuperscript{177}. Chen et al\textsuperscript{178} has used cloud point extraction for the preconcentration of lead, after the formation of a complex with 2-(5-bromo-2-pyridylazo)-5-(diethylamino)-phenol and later analysis was done by graphite furnace atomic absorption spectrometry using Triton X-114 as surfactant. Zhu et al\textsuperscript{179} developed a new method based on cloud point extraction separation and graphite furnace atomic absorption spectrometry detection was proposed for the determination of inorganic tin species. Puff-by-Puff on-line analysis of trace metals in mainstream cigarette smoke has been developed by introduction of an aliquot of smoke aerosol directly into an inductively coupled plasma mass spectrometer by Chang et al\textsuperscript{180}.

2.0 Scope of the work

The anthropogenic input of metals into various environmental samples have attracted a great attention in present days. The input of metals in excess, have resulted the modification of physical, chemical, biological and morphological conditions of the earth biosphere. Apart from organic and inorganic, metallic contamination is also alarming in the sense that many of them are present in the trace or even in ultra...
trace quantities, which have adverse effects on biotic and abiotic system of environment. In view of the detrimental effects of trace metals, it is imperative to identify and determine their presence accurately and rapidly. For the determination of metals in various environmental samples viz., soil, water, leaves, cereals, human blood and hair, several following procedures are adopted.

There are several methods already reported and in use for the determination of trace metals in environmental, biological and pharmaceutical samples which have been discussed in detail so far. Almost all the methods are sensitive, but majority of them need elaborate clean up mechanisms, costly solvents, and sophisticated equipment with skilled personnel to operate.

Hence there is a need to develop simple analytical methods in the laboratory as additions to the existing literature methods.

Economical reagents could be synthesized at laboratory conditions for online monitoring of trace metals in environmental samples.

The developed methods could be tested for statistical validation and compared with reported methods.

The columns could be modified in the laboratory conditions for having good efficiency in terms of performance for monitoring trace metals at low concentrations.

Solid phase extraction method has many advantages in the separation and determination of trace metal ions providing high preconcentration factor.
The cloud point extraction method has distinct advantages, especially for surface water analysis, where the Flame Atomic Spectroscopy is not much suitable.

Solid phase extraction and cloud point extraction techniques have been chosen by the author for preconcentration of trace metals by employing new novel reagents. The developed procedures used along with spectrophotometry and flame atomic absorption spectrometry are found to be simple, sensitive, reliable, accurate and rapid in the determination of trace metals in environmental samples.
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