CHAPTER I

INTRODUCTION

Plants require a wide range of nutrients if they are to grow and produce yields to their maximum capability. Plants can be healthy or sickly on a set of nutrient elements, the requirement of which differ with species. The nonavailability of the nutrients leads to a series of events. Ecologically speaking, plants fail to give their maximum yield, and gradually a stage is reached when they do not grow at all and are replaced by other species which demand a lower level of nutrition or which utilise the available nutrients in a better way. In general terms however, in the event of nonavailability, plants first show the nutritional deficiency by symptoms which are specific for that nutrient; the symptoms are manifested generally in their leaves. Mineral nutrition of plants is a very important discipline and deals with metabolic and biochemical functions of the chemical elements linked with other aspects of plant physiology and biochemistry.

The importance of micronutrients in affecting plant growth is well recognized all over the world. In India, micronutrient deficiencies have been reported from many states (90, 156), particularly from Maharashtra, Gujarat and Bihar. With the
present need for increasing agricultural production, the use of balanced fertilizers including that of micronutrients become inevitable. However, there are situations, where plants do not respond to the supply of these minerals through the soil. Under these circumstances, foliar spray is an effective means of remedy. Further, it is important to note that it is possible to change the plant species to fit the soil in lieu of changing the soil to suit the plant, although the soil can be modified to some extent. From this point of view, it is essential to understand the manner in which the nutrient elements are abstracted by the plant roots. There are other aspects like absorption of nutrients by the foliage from air or from spray liquids. The need to understand these processes has assumed greater importance now than several decades before when human pressure on land for their food was only a fraction of what is today.

It is well known that the mineral nutrient requirement of all plants are not the same and it depends on a number of factors, perhaps, the most important being the absorption capacity of the roots. The function of the roots is dual. What is absorbed is to be delivered to the xylem for further transport to shoot. Furthermore, although generalization on nutrient requirement are often justified, nutritionists and plant physiologists have much to gain from recognizing the fact that variations occur in the mineral requirement of organisms throughout the plant kingdom.
The thesis embodies the results of a number of experiments carried out to obtain answers to several questions relating to our knowledge and understanding of the phenomena of absorption, transport and utilization of zinc and Molybdenum. A number of crop plants served as test objects.

**LITERATURE REVIEW**

**General considerations**

**Importance of Zn and Mo**

Zinc and Molybdenum are well known as important nutrient elements that are required by, and often deficient in agricultural crops. The areas where Zn and Mo deficiencies are noted are shown in Figures 1 and 2 (89). All deficiencies interfere with growth but a deficiency of Zn does so with such dramatic impact that terms like 'little leaf' and 'rosetting' has been applied as specific symptoms. 'Rosetting' refers to the failure of internode to elongate, causing the leaves of several nodes to get together in a plane, i.e., rosette-fashion. However, the role of molybdenum in the enzyme systems of symbiotic nitrogen fixation, suggests that plants relying on symbiotically fixed nitrogen, when subjected to Mo deficiency would in effect become nitrogen deficient, and they do. The same consideration applies to plants where nitrogen source is nitrate.
Functions of Zn and Mo in plants:

In general, micronutrients act as catalysts in the activation of enzyme systems. Following are the functions of Zn in higher plants. 1. It has been reported that Zn activates carbonic anhydrase and the enzyme has been shown to be localized in the chloroplasts (70). 2. In the last few years other Zn metalloenzyme such as a number of dehydrogenases proteinases and peptidases have also been recognized (162). 3. According to Price et al. (134) Zn is closely involved in the N-metabolism of the plant. 4. Zn is also required in the synthesis of tryptophan which is a precursor of indole acetic acid. The formation of IAA is indirectly influenced by Zn (159). 5. Jyung et al. (75) reported that Zn has an important role in starch metabolism.

Mo has long been known to be required for the normal assimilation of N in plants. Of the four enzymes found to contain Mo-aldehyde oxidase, xanthine oxidase, nitrogenase and nitrate reductase - only the latter two are found in plants. The Mo in all four enzymes appears to have similar catalytic functions (122). The increased Mo requirement of most plants grown on NO$_3$-N can almost completely be accounted for by the Mo in nitrate reductase (52). The other major molybdoprotein of plants, nitrogenase fixes N$_2$ as NH$_3$, which is then assimilated by the plant (93). It is composed of at least two proteins, both of which have an Fe requirement and one of which has a Mo requirement (30, 163). There is
some evidence that Mo is required by hydrogenases (91). The fact that P and ascorbic acid metabolism are altered in Mo deficiency may also implicate the metal with these processes.

**Absorption and translocation:**

Although the soil contains the nutrients, the plant root should have the ability to absorb the same from the soil solution. The following are the well known theories which attempt to explain mineral absorption by plant roots. Earlier studies by Osterhaut (128) reveals that the mechanisms of cation absorption is dependent on the acidic substances. According to him, the base is combined with the acidic substance while entering the cell and the following equation indicates how the base enters the cell.

\[ \text{KOH} + \text{HX} \rightarrow \text{KX} + \text{H}_2\text{O} \]

The natural KX complex decomposes at the inner surface of the cell where the sap is more acidic than the external medium. However, he has not mentioned about the anion absorption. In 1947, Jacobson and Overstreet (71) found that general active mechanisms for both anions and cations are by the acidic and basic carriers respectively. The ions combine with some unknown carrier molecules in the cell membrane and get released at the inner side of the membrane. In 1933, Lundegårdh and Burström (104) found the existence of quantitative correlation between anion absorption and a particular
component of respiration which is stimulated by salt. They called this as 'anion' or 'salt' respiration. Accordingly Lundegårdh hypothesis illustrates the existence of oxidation-reduction potential gradient across the functional membrane, where the cytochrome molecules have the tendency to oxidize and reduce at the outer and inner surfaces respectively. Hence absorption of anions are dependent upon the oxidation and reduction of cytochrome molecules.

However, the recent hypothesis of ion uptake are the membrane theory, binding theory and the carrier theory. According to membrane theory, the membranes, the plasmalemma and the tonoplast, constitute the chief barriers. Ion uptake when passive is governed by laws of diffusion provided by electrochemical principles. By passive diffusion, a substance crosses a membrane as a result of random molecular motion. The transported solute is thought not to interact with any molecular species in the membrane (76). When transport is active, metabolic energy is required; movement is against the electrochemical energy gradient and the sites responsible for the transfer are considered to be a part of membrane structure (34, 35, 36). The entire cytoplasm of the cell is covered with plasmalemma functioning as a barrier between the internal and external solution of the cell differing in their composition. So if this membrane below the cell wall is not present, the cell will swell and burst due to high salt component of the external environment (34, 35, 36, 68, 107).
Binding theory includes the association-induction hypothesis of Ling (97) and other similar attempts to explain accumulation, retention and ion selectivity by electrostatic attraction, adsorption processes and/or Donnan systems (65, 98). Primarily this appears to be directed towards the animal cells and other non-vacuolated cells like bacteria and yeast. But it is not true for the higher plants (65), because it is essential that ions accumulated in the protoplast be in some bound state i.e., have in the bulk phase, an electrochemical activity equal to, or lower than that of external medium.

According to carrier theory (40, 42, 43, 94), ions combine with a specific organic substance at one surface, are carried across the membrane and are released on the other side. Following scheme represents the carrier theory:

\[
X_{\text{outside}} + S_0 \xrightarrow{k_1 \text{ or } k^{-1}} XS + \xleftarrow{k_2 \text{ or } k^{-2}} X_{\text{inside}} + S_1
\]

where \(X\) represents the carrier molecule; \(S_0\) and \(S_1\) are substrate ion concentration outside and inside respectively; and \(k\) subscripts are reaction velocity constants. The back reaction \(k^{-2}\) is deemed to be negligible.

The transport of ion takes place either actively or passively. By active transport the solute is accumulated against an electrochemical or osmotic gradient. It is generally believed
that this mechanism requires metabolic energy on the part of the cell as well as a specific membrane carrier molecule. This classic model postulates that the penetrating species combines with a carrier and that the carrier or the carrier-substrate complex is then subjected to modification in the membrane. The carrier substrate complex formed on the outside surface of the membrane crosses the membrane and is modified on the inside surface in such a way that the carrier has a lowered affinity for the substrate. The substrate then is released into the interior of the cell and the carrier is free to cross back the membrane where the cycle is repeated. This active transport is against a concentration gradient and is affected by temperature, pH and metabolic inhibitors.

In 1952, Epstein and Hagen (46) employed enzyme kinetics for ion transport studies. Entry of ions is similar to the enzymatic reactions characterized by Michaelis-Menten equation:

\[
v = \frac{S_0 \cdot V_{\text{max}}}{S_0 + K_m}
\]

where \( v \) is the velocity (rate of ion uptake here); \( S_0 \) is the concentration of the substrate (ion), \( V_{\text{max}} \) is the maximum velocity occurring when the enzyme or the carrier is saturated and \( K_m \) is the Michaelis constant. When \( v = \frac{1}{2} V_{\text{max}} \), \( K_m = S_0 \). Experimentally these values are obtained by measuring rates of tracer uptake of ions over a range of external concentrations. Carrier-kinetics studies show that the absorption of an ion
reaches a saturation level according to the external ion concentration. There are only limited carrier sites where the ion can bind. Once the constituent is saturated, \( V_{\text{max}} \), the maximum rate of uptake will reach. So there will be no enhancement of the movement inside the cell. \( 1/V \) and \( 1/S \) are the reciprocals of uptake rate and substrate concentration. Plotting these two reciprocals (the Lineweaver-Burk plot) gives a straight line. When an ion competes with another for the same site in the carrier, the two lines intersect each other on the axis. But it is not so when the particular ion is not competing at the same site. Multiple sites for inward transport are shown by Epstein and Rains (49) in barley roots.

The rate of absorption differs due to various concentrations, present in the growth medium around the root tissue. According to Epstein and others (50), the uptake process is composed of two mechanisms. There are many differences between them. Mechanism 1 is operating at the lower concentration ranges (upto 0.5 mM) and has high affinity and selectivity for ions. But the mechanism 2 is in higher ion ranges (1 - 50 mM), and has low affinity and this range is relevant to the macronutrients only. Since the amount of micronutrients required by plants are very small, low concentrations of these elements only can be used and hence mechanism 1 is only relevant for such micronutrient uptake studies. Further, mechanism 1 follows the simple Michaelis-Menten kinetics and is not affected by the presence of other anions (49).
There is evidence that both the mechanisms are located on the plasmalemma (167) which is further substantiated by Kannan (78) who observed a typical dual pattern in rubidium absorption by the unicellular green alga, Chlorella pyrenoidosa.

There is another theory by Nissen (123) which shows that the rate of absorption is multiphasic nature. Nissen's theory postulates that there are no separate mechanisms. The inflections of dual mechanisms represent different phases of one transport process only. The phase is determined by external concentration and they follow the Michaelis-Menten kinetics. However, the uptake process is the same and only plotting the values is different.

Translocation:

Higher plants can be considered as having two sites for nutrients. One is the production site and another is the consumption site. The main components which are translocated are water, inorganic ions and organic compounds. Through the xylem elements, water and minerals are taken up from the root medium. Metabolic energy is probably directly involved in both root uptake and release of nutrients into the root xylem and may be involved also in their release from the xylem into surrounding leaf cells. So xylem transport depends on both transpiration pull and on root pressure (116).
However, discussions of the mechanisms of phloem transport are still in a controversial stage. Only little information has been published on micronutrient movement in phloem. The possible mechanism of phloem transport has been discussed by Geiger (55). In most stages of plant growth, it is possible to expect the quantities of micronutrients translocated out of leaves in the phloem to be much less than quantities that ascend the xylem.

Uptake by leaf cells:

Basically the process of nutrient uptake by leaf cells is the same as that of absorption by plant root cells, the main step in the process being the transport through the biological membrane, the plasmalemma. The uptake rate is influenced by the physiological status of the leaf. Elements like Fe, Mn, Zn and Cu are frequently fixed by soil particles and hence scarcely available to the plant roots. So foliar applications in the form of inorganic salts or chelate are useful, where the nutrient uptake from the soil is restricted (160).

Redistribution:

In higher plants, the main transport of the nutrients are through the phloem and xylem and however, they are not directly linked to one another. Redistribution or retranslocation of
micronutrients take place in plants during growth and maturation (1, 56, 102). However, very little is known about the redistribution of micronutrients. Carolus (32) discussed interesting concepts of nutrient conservation and gave data for macro and micronutrient redistribution in asparagus, onion and rhubarb during senescence.

**Ion interactions:**

Ion interactions occur both in the soil and within plant and interactions occur between the micronutrients and macronutrients. It is very important to understand the ionic interactions because they modify the nutrition of the plants. A significant reason for studying and evaluating micronutrient interactions is to improve agronomic practices that can exert a control over the concentrations of trace elements in plants (3). Ion interaction may be defined in two ways. One relates to the influence of an element upon another and the other in relation to plant growth through the differential response to one element in combination with varying levels of a second element applied simultaneously; that is the two elements combine to produce an added effect not due to one of them alone (or a negative effect). However, the type of interaction may vary due to many factors. Loneragan (100) classified terms such as nutrient requirement, critical concentration, and functional requirement. These concepts are especially pertinent in relation to micronutrient interaction.
Chelation:

It has been shown during the last several years that the formation of chelates is important in absorption, transport and metabolic activity of many of the cationic essential trace elements. Chelation is a well known mechanism of chemical combination between certain organic substances and bi- or trivalent cations in which the ions are held partly by co-ordinate bonds in undissociated complexes. The best known synthetic chelating agents are EDTA (Ethylene diamine tetraacetic acid) and EDDHA (Ethylene diamine di(O-hydroxyphenylacetic acid). Normally heavy metals often occur in a chelated form in the plant and organic acids are good chelators. Chelate with a cation, forms very stable complex which is highly soluble in water and also withstands changes in pH. So use of chelates to overcome deficiencies has largely been resorted to, in soil application.

Response of plant genotypes:

Differences in yields of varieties are well documented and well known; varieties grown under the same conditions but having different yields must clearly differ in their efficiencies in dry matter production which is referred to by Vose (164) as "differential yield response". He also noted that such varieties may differ markedly in "differential nutrient uptake", as shown by the concentration and total amounts of elements in the shoots and
roots. It is important to explore varieties for possible differences in their capacity to absorb and or to utilise nutrients under different fertility levels and it is also important to give more emphasis to plant-soil interaction and the breeding or selecting of plants to fit problem soils. The Zn level in the kernels of 31 inbred corn lines was reported by Massey and Loeffel (113) to be genetically controlled. Also nutritional Zn efficiency was reported by Halim et al. (59) to be genetically controlled in several inbreds and single crosses of corn. Response to the application of Zn differs according to species. In general, Kharif crops, especially maize, paddy and sorghum respond to Zn than Rabi crops like wheat and gram (156). Relatively little is known about the fundamental physiological ways in which plants differ in nutrient requirement or uptake (105, 117). Understanding the response of genotypes will also enable crop breeding program to introduce more stress-tolerant cultivars.

Objectives of the study:

Deficiencies of micronutrients have been studied by several workers in order to correct the symptoms. However, the role of metabolism in uptake and translocation of micronutrients needs to be confirmed. Furthermore, the evidence is reasonably good for Mn and Fe, it is quite controversial for Cu and Zn and almost nonexistent for B and Mo. Bowen (14) reported that Zn and
Cu absorption by sugarcane leaf tissue were characteristic of an active process. Tiffin (158) and Ambler *et al.* (8) reported Zn concentrations in xylem exudate from decapitated tomato and soybean plants well above the ambient concentration indicating that absorption is through the metabolic process. Schmid *et al.* (145) showed that Ca strongly inhibited the absorption of Zn by barley roots, whereas Mn had no effect. Increasing Ca concentration was shown to reduce Zn absorption by isolated tobacco leaf cells (77).

Direct evidence to support or refute active transport of Mo in plants is lacking. Stout *et al.* (154) present data where the uptake of Mo by tomato plant was severely depressed by increasing the pH. The enhanced Mo uptake was most pronounced in the tops, and the effect appeared to be specific for phosphate. These kinds of interactions between $\text{SO}_4^-$ and Mo or $\text{PO}_4^-$ and Mo are typical of those seen for ions where active transport can be verified (41).

The thesis explains the absorption and transport mechanisms and the kinetics of Zn and Mo in sorghum, maize, tea and bean seedlings. The interactions with different ions were also investigated and described. The differential response of plant genotypes were discussed by using different cultivars of sorghum, groundnut and cotton during the Zn deficiency stress conditions. Further, utilization of Zn or Mo in plants were discussed by investigating chlorophyll synthesis and nitrate reductase activity.