Chapter-VI

6.1 Introduction:

It is now established that zinc is required to all forms of life, and zinc-deficiency causes a large number of disorders. It is the second most abundant element in our body, following iron. In a healthy adult human body, the amounts of three most abundant metals are: Fe (5-7 g), Zn (2-3 g), Cu (0.2-0.3 g)[1].

Zn (3d^{10}) complexes are colourless and diamagnetic. That is why to study the function of Zn^{2+} in biological systems is relatively difficult compared to iron and copper which often form coloured and magnetically active complexes.

Carbonic anhydrase was the first recognised Zn(II)-containing enzyme. It was established in 1939. In 1955, carboxypeptidase was established as the second Zn(II)-containing enzyme. With the application of different modern techniques, the biochemistry of Zn is now well understood. Now, a large number of Zn-dependent enzymes have been established to function in biological systems. Presently, more than three hundred Zn-dependent enzymes are known. They can catalyse different types of biological reactions. They are of several types: peptidase, aldolase, carboxypeptidase, protease, phosphatase, alcohol dehydrogenase, transphosphorylase, transcarbamylase, DNA-and RNA-polymerase. Some representative examples are given in Table 6.1 [2-5].

The peptidases are forming the main class of Zn-dependent enzymes. Carboxypeptidase A, thermolysin, snake venom proteases, etc. in this class are now structurally well established. Reprolysin are the most poisonous agents of snake venoms [5-8]. These Zn-containing enzymes (e.g. adamalysin, atrolysin C, etc.) are responsible...
for the hydrolysis of extracellular matrix. The proteolytic activity and hemorrhage activity of the reprolisis depend on the presence of Zn²⁺ and these enzymes can be inhibited by the chelating ligands which can inhibit the Zn(II)-activity.

In the different types of Zn-dependent hydrolase enzymes, Zn(II) mainly activates the substrate. In carbonic anhydrase, OH⁻ makes a nucleophilic attack on CO₂, but it is not a hydrolysis reaction. Alcohol dehydrogenase (90 kDa) catalyses the reversible transfer of H⁻ (hydride ion) from alcohol to NAD⁺. Thus it is a redox enzyme[9]. Here, Zn(II) facilitates the nucleophilic attack by H⁻ at the electron deficient carbon center. In Cu-Zn superoxide dismutase (a redox enzyme), Zn(II) offers a structural role.

In the activity of nucleic acid polymerases, Zn(II) plays mainly a structural role. A similar role is played by Zn(II), in forming the peptide into the multiple domains (known as zinc-fingers) where Zn(II) is coordinated by cysteine and histidine residues. The zinc-finger proteins regulate the transcription of DNA to RNA and different gene activity. This DNA-binding metalloprotein consists of several repeated peptide domains. Each domain bears a Zn-binding site. The nucleic acid binding transcription factor TFIIID (found in Xenopus laevis, an African clawed frog) contains 9 Zn-fingers (i.e. 9 domains). It regulates the transcription of 5S ribosomal RNA gene. In TFIIID, Zn²⁺ is tetrahedrally coordinated by two histidine and two cysteine residues. Coordination with Zn²⁺ determines the folding and three dimensional structure of the protein—which looks like a series of spiral loops or fingers. These ‘fingers’ help to recognise the complementary structure of the nucleic acid polymer. Thus, the Zn-fingers act as nucleic acid regulatory proteins[10-11]. The regulatory factor TFIIID possesses 9 fingers, but in order species, such regulatory proteins may contain 2-40 fingers to interact with the nucleic acid strands in different possible
ways. The yeast transcription factor GAL 4 (a transcription factor involved in galactose utilisation) possesses a cluster of two Zn-sites coordinated by cysteine residues (out of 6 cysteine residues, 2 are bridging) and they share a common tetrahedral edge. It has now been established that the regulatory Zn-finger structural domain represents a ubiquitous structural motif for the DNA-binding proteins in eukaryotic cells. Nature’s selection of Zn in this nucleic acid binding protein is quite unique. Zn(II) is inert in terms of redox activity which could damage the DNA but in the case of Fe(III) and Cu(II) and other softer and heavier metal ions would bind DNA bases preferentially to destruct the helical structure[12-13].

Several Zn-dependent DNA and RNA polymerases are now established and Zn(II) may play some roles to stabilise the structure of these genetic molecules. Zn-antagonists such as Cd, Pb can induce disturbance in genetic expression at the molecular level. Male fertility depends on Zn-content in seminal fluid. The birth weight and head circumference are also found to depend on placental content of Zn [14-16]. In fact, pregnant mothers require a higher dose of Zn. Thus there are several evidences to support the fact that Zn is a growth factor. Some types of schizophrenia (mental disease) arise due to low levels of Zn. In such cases, administration of ZnSO₄.7H₂O can improve the situation.

Nutritional Zn-deficiency is quite common in UK and other countries. Zn-deficiency in diets is due to the following causes:

(i) Use of phosphate fertilisers converts Zn⁺⁺ to insoluble Zn₃(PO₄)₂ which renders Zn less readily available to the growing plants.

(ii) Decreased organic contents in the agricultural soils reduces the Zn-uptake capacity of plants. Natural organic
chelating agents can solubilise the different metal ions including Zn(II) into suitable forms for plant uptake. In UK, the organic content of common agricultural land has decreased to 2-4% in comparison with 10-20% for typical virgin grassland.

(iii) Due to the lack of recycling Zn to soils.

Much of the zinc in animals and plants is believed to offer only the structure forming properties towards many proteins.

Much of the zinc in animals and plants is believed to offer only the structure forming properties towards many proteins.

Zinc and copper are generally stored in metallothioneins which are rich in cystein residues. Zinc-thionein is the zinc-storage protein and copper is stored in copper-thionein. These metals are transported loosely associated with the peptides or proteins.

Nature has utilised Zn(II) in different enzymes (more than three hundred enzymes) and many of them participate in hydrolytic reactions. The different properties of Zn(II) in coordination compounds justify this Nature’s unique selection. In fact, in this regard, Zn(II) is a better selection than the other available metal centers like Mg(II), Cu(II), Fe(II)/Fe(III). These special properties are discussed below:

(i) **Zn(II) - a typical Lewis acid**: The Lewis acidity of M²⁺ is determined by its electron affinity (which is equal to the sum of first and second ionisation potentials). These values are: Mg²⁺ (22.6 eV) < Zn²⁺ (27.4 eV) < Cu²⁺ (28.0 eV). It indicates that Zn²⁺ is a better Lewis acid than Mg²⁺, although Cu²⁺ is a better Lewis acid than Zn²⁺. But nature has selected Zn²⁺ rather than Cu²⁺ on another ground.
The 2+ oxidation state of Zn is a very stable and not have any other stable oxidation state.

Zn(II) (d^{10} system) does not have any crystal field stabilisation energy (cfse). It can form complexes with variable coordination number (i.e. fluxional property) like 4, 5 and 6. Coordination number is determined by the relative magnitudes of electrostatic interaction and steric repulsion. Binding of a substrate may increase the coordination number by one unit or the substrate may simply replace a bound water molecule. Thus Zn(II) can act as a good Lewis acid specially in the complexes with lower coordination number. Ligand by the carbonyl oxygen of the peptide linkage or ester group polarises the C=O bond to facilitate the nucleophilic attack at the C-centre. Thus this activation arises due to the Lewis acid character of Zn(II).

(ii) Change of nucleophilicity of Zn(II)-bound H_2O molecule:
The pK_a value of Zn(H_2O)_6^{2+} is about 9.0. Depending on the nature of the ligating sites already present in the coordination sphere, the pK_a value of the bound water molecule may vary. If the positive charge on complex increases and coordination number decreases, the pK_a value decreases. Depending on the situation the pK_a value of bound water molecule may vary in the range 6-9. If the pK_a value decreases to the range 6-7, then it remains in an equilibrium with its conjugate base (CB) in physiological pH range.

\[
(L)\text{Zn(II)} - \text{OH}_2 \rightleftharpoons (L)\text{Zn(II)} - \text{OH (CB)} + \text{H}^+
\]

The metal bound OH group in conjugate base (CB) is a
better nucleophile than the free or metal bound $H_2O$ molecule. Thus the CB form of the enzyme can provide a better nucleophile to attack the substrate. In fact, very often, the hydrolytic cleavage occurs through a nucleophilic attack on the substrate by the metal bound $H_2O$ or $OH$ group.

\[
\text{Zn}^{II} - \text{H} \rightarrow \text{substrate} \\
\]

(iii) **Lability of Zn(II) :** Zn(II) is a labile centre. It is a characteristic feature of $d^{10}$ system. Consequently, the replacement of a water molecule from the Zn(II) coordination sphere by a substrate can occur rapidly. Similarly, the ligand dissociation is also kinetically favoured.

(iv) **Stable oxidation state for Zn :** The 2+ oxidation state is the only stable state for Zn. Hence, for the Zn-metalloenzymes there is no risk to initiate the redox reaction. In fact, nature has not selected Zn for redox activity. The alcohol dehydrogenase (a Zn-containing metalloenzyme) catalyses the redox reaction (through $H^+$ transfer), but here the oxidation state of Zn does not change.

In summary, the properties like good Lewis acidity of Zn(II) specially in complexes with lower coordination number; kinetic lability of Zn(II); rapid interconversion base to provide a better nucleophile; only one stable oxidation state (i.e. 2+) of Zn; etc. make Zn(II) suitable for different biological catalysts.

Zn(II) is a borderline acid and it shows affinity for the combined
N or O donor sites. It can also bind with S sites. Thus in the proteins, the binding sites are provided by histidine (N), glutamate or aspartate (carboxylate O) and cysteine (S) residues. The dominating ligands at the active site of a Zn(II)-containing enzyme are imidazole N-atoms of histidine and the active site is very often occupied by a $H_2O$ molecule or OH group. Thus for the catalytic activity, in general, there is a vacant site (which may be occupied by $H_2O$ or OH group) around the Zn-centre in the resting condition of the enzyme. On the other hand, for structural use, the coordination sites around the tetrahedral Zn-centre are saturated. In such cases, the binding sites are generally occupied by the cysteine residues and the Zn-centre does not show any tendency to expand its coordination number. In fact, the presence of two or more cysteine residues in the coordination sphere of Zn(II) indicates the structural use of the Zn-centre.

Zn is the second most abundant essential trace element (next to Fe). A healthy adult contains $\sim 2$ g Zn present in several enzymes and proteins and an intake of 15 to 20 mg (only half of which is absorbed) is required daily. Zn-deficiency leads to growth retardation, dwarfism, inhibition of sexual maturation, loss of body hair, poor appetite and skin lesions. Zn is important in wound healing as Zn is required in protein and collagen synthesis and in cell replication. Zn-deficiency in pregnant women causes a gross congenital malformation in the offspring. About 30% of total Zn in adults exists in skin and bones which are also likely to be affected in Zn-deficiency. Zn-deficiency seriously affects the function of alkaline phosphatase, carboxypeptidase, thymidine kinase and DNA-polymerase. Zn-deficiency arises in some regions of UK (where the agricultural soil contains a lesser amount of Zn), in certain zones of Middle-East (where the chelating agents like phytates and organic phosphates present in diet chelate Zn(II) and make it inaccessible). Use of phosphate fertilizer produces insoluble Zn-phosphate from which Zn cannot be taken by
the growing plants[17-20].

In the technologically advanced societies, Zn-supplementation in food balances the Zn-deficiency. In Zn-deficiency, ZnSO₄ capsule is clinically recommended.

*Alternaria brassicae* is a plant pathogenic fungus which causes the disease *Alternaria* leaf spot in most crucifer plants and affected the plants at all growth stages. The infection of seedling stems may result in damping-off or stunted plants. Typical lesions begin as small yellow areas that enlarge to about 1.5 cm in diameter and are dark colored spots with concentric rings. A brown discoloration of cauliflower and broccoli heads is caused by infections with this pathogen. The pathogen causes elliptical necrotic spots on seed stalks and seedpods. Pod lesions may extend in to the pod interior and attack the seed causing them to shrivel[21-22].

*Aspergillus niger* is a fungus which is one of the most common species of the genus *Aspergillus*. It causes a disease called black mold on certain fruits and vegetables such as grapes, onions and peanuts and is a common contaminant of food. It is ubiquitous in soil and is commonly reported from indoor environments, where its black colonies can be confused with those of *Stachybotrys* (species of which have also been called “black mold”)[23, 24].

* A. niger causes a serious lung disease aspergillosis in humans when large amounts of spores are inhaled[25, 26]. Aspergillosis is particularly frequent among horticultural workers who inhale peat dust, which can be rich in *Aspergillus* spores. Less commonly, it has been found on the walls of ancient Egyptian tombs and can be inhaled when the area is disturbed. In humans, the major forms of disease are:

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1. Allergic aspergillosis (affects asthma, cystic fibrosis and sinusitis patients).

2. Acute invasive aspergillosis (risk increases if patient has weakened immunity such as some AIDS patients and those undergoing chemotherapy).

3. Disseminated invasive aspergillosis (widespread through body).

*A. niger* is also one of the most common causes of otomycosis (fungal ear infections), which can cause pain, temporary hearing loss and in severe cases, damage to the ear canal and tympanic membrane. *Fusarium oxysporum*, also referred to as Panama Disease or Agent Green, is a fungus that causes Fusarium wilt disease in more than a hundred species of plants. It does so by colonising the water-conducting vessels (xylem) of the plants. As a result of this blockage and breakdown of xylem, symptoms appear in plant such as leaf wilting, yellowing and eventually plant death[27]. *Fusarium oxysporum* has been reported in skin and nail infections[28], in subcutaneous disease[29], in a neutropenic child managed with granulocyte colony-stimulating factor[30], in a disseminated infection in hemophagocytic lymphohistiocytosis[31-33] and in a fatal case involving a cross reaction with a *pan-Candida* genus probe.

The Schiff's bases complexes have been widely investigated because of increasing recognition in biological systems[34-43]. Many investigations indicate that the environment around the metal ion and the conformational flexibility of the ligands are the most important factors because they allow metalloproteins to carry out a special biological function[44]. Further, the interest of studying Schiff's bases and their complexes is due to their significant applications as antifungal, antibacterial, anticancer, antineoplastic, antimalarial,
antiviral, antiflammatory, analgesic, antioxidant, antitumor, antiamoebic, herbicides, insecticides, rotenticides and plant growth regulators[45-60]. Many drugs modify their pharmacological and toxicological properties in the form of metallic complexes. Schiff’s bases with hydrazone-based functional groups have also been found wide applications as pharmaceuticals in the treatment of several diseases such as tuberculosis, leprosy and mental disorders[61]. Several Schiff’s bases have demonstrated inhibitory activity against transplanted rodent neoplasms[62], spontaneous lymphomas of dogs[63] and DNA viruses of the Herpes family[64]. The activity of these compounds is apparently due to inhibition of the biosynthesis of DNA with the metabolic lesion occurring at the level of reduction of ribonucleotides to deoxyribonucleotides by the enzyme ribonucleoside diphosphate reductase[65, 66].

M.T.H. Tafarlder et al.[67] synthesized several new complexes of a tridentate ONS Schiff’s base derived from the condensation of S-benzylthiocarbazate with salicylaldehyde. The Schiff’s base (HONS) forms mono-ligand complexes : [M(ONS)X], [Zn\textsuperscript{II}, Zr\textsuperscript{IV}, or U\textsuperscript{IV} with X= H\textsubscript{2}O, Cl]. The Schiff’s base and its complexes have been screened for their antifungal and antibacterial activities against fungi Candida albicans and Aspergillus ochraceous, and bacteria Bacillus cereus and Pseudomonas aeruginosa.

The neutral tetradentate chelate complexes Zn(II) and VO(II) have been prepared in EtOH using Schiff’s bases derived from acetocetanilido-4-aminantipyrine and 2-aminophenol/2-aminothiophenol by N. Raman et al.[68]. The in vitro antimicrobial activity of the investigated compounds was tested against the microorganisms such as Salmonella typhi, Staphylococcus aureus, Klebsiella pneumoniae, Bacillus subtilis, Shigella flexneri, Pseudomonas aeruginosa, Aspergillus niger and Rhizoctonia bataiola.
Most of the metal chelates have higher antimicrobial activity than the free ligands.

New neutral Schiff’s base complexes Zn(II) derived from 4-aminonitroprusside and N-(1-piperidinobenzy) acetamide (Mannich base) have also been synthesized by N. Raman et al. [69]. The antimicrobial activity of the ligand and its complexes has been extensively studied on microorganisms such as *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli* and *Pseudomonas aeruginosa* and the fungi *Aspergillus niger* and *Rhizoctonia bataica* by well-diffusion technique using DMF as the solvent. It has been found that all the complexes have higher activity than the free ligand and standard.

The synthesis and characterization of some transition metal cis- 3, 7-dimethyl-2,6-octadienemicarbazone (CDOSC) complexes have been reported by R. Sharma et al. [70]. The ligand CDOSC yields: [ML₂Cl₂] and [ML₃Cl]Cl type complexes, where M=Zn²⁺, Cd²⁺ and Hg²⁺, L=CDOSC. All the newly synthesized metal complexes as well as the ligand were screened for their antibacterial activity. All the complexes exhibit strong inhibitory action against Gram (+) bacteria *Staphylococcus aureus* and Gram(-) bacteria *Escherichia coli*. The antibacterial activities of the complexes are stronger than those of the ligand CDOSC itself.

K.S. Abou-Melha et al. [71] synthesized a Schiff’s base bis-[4-hydroxycoumarin-3-yl]-N, N-thiocarbohydroxide (H₂L) by the reaction of 4-hydroxycoumarine-3-carbaldehyde with thiocarbohydrazide in 2:1 molar ratio and its binuclear complexes with Zn(II), Fe(III) and Cr(III) ions. The Schiff’s base and its complexes were screened for their antifungal and antibacterial activities against different species of pathogenic fungi (*Candida albicans* and *Fusarium solani*) and bacteria (*Escherichia coli* and *Staphylococcus aureus*) and their biopotancy have been discussed.
The transition metal carboxylates i.e. 3-[(2,4,6-trichloroanilino) carbonyl] prop-2-enoic acid and 3-[(4-bromoanilino) carbonyl] prop-2-enoic acid have been synthesized by S. Shahzadi et al.[72]. The transition metal complexes were tested in vitro against a number of microorganisms such as *Escherichia coli*, *Bacillus subtilis*, *Shigella flexenari*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Trichophyton longifusus*, *Candida albicans*, *Aspergillus flavus*, *Microsorum canis*, *Fusarium solani* and *Candida glaberata* to assess their biocidal properties.

B.T. Thaker et al.[73] synthesized the novel mononuclear mixed-ligand oxovanadium(VI) complex [VO(PMEP)(Bipy)]ClO₄ by the condensation of VOSO₄·5H₂O with ligands 1-phenyl-3-methyl-4-formyl-2-pyrazoline-5-one (PMFP) and 2,2'-bipyridyl (Bipy). The corresponding Schiff’s bases were prepared by the condensation of [VO(PMFP)(Bipy)]ClO₄ with ethylenediamine, ethanolamine and glycine. The antibacterial activities of PMFP and its Schiff’s base complexes of oxovanadium(VI) were estimated against *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*.

6.2 Synthesis of Zn(II) Complexes:

This chapter deals with the synthesis, characterization and antifungal activity of Zn(II) complexes with semicarbazone of ligands L¹, L², L³ and L⁴. These complexes were characterized on the basis of elemental analyses, magnetic moment and IR studies.

6.2.1 Synthesis of Zn(II) complexes with L¹, L² and L⁴ ligands:

The complexes were prepared by the following method.

A hot ethanolic solution of metal salt (0.05 mol) was mixed with hot ethanolic solution of the corresponding ligand (0.1 mol). The contents were refluxed for about two hours. On cooling the contents,
cream coloured complex was separated out. The precipitates were purified and recrystallized in cold ethanol. The complex was filtered washed with 50% ethanol and dried in a vacuum in desicator over P_4O_{10}. The purity was checked by TLC (M.P. 199, Yield 67%).

6.2.2 Synthesis of Zn(II) complexes with Ligand L^3 ligand:

The complex was prepared by the following method.

A hot ethanolic solution of metal salt (0.05 mol) was mixed with hot ethanolic solution of the corresponding ligand (0.05 mol). The contents were refluxed for about two hours. On cooling the contents cream coloured complex was separated out. The precipitates were purified and recrystallized in cold ethanol. The complex was filtered washed with 50% ethanol and dried in a vacuum desicator over P_4O_{10}. The purity was checked by TLC (M.P. 199, Yield 67%).

6.3 Characterization of Zn(II) Complexes: All the complexes were characterized by Elemental analysis, IR Spectral study and Electronic spectra study.

6.3.1 Elemental Analysis:

The Results are shown in Table 6.2.

6.3.2 IR Spectral of the Complexes:

The IR spectra of complexes with ligands L^1, L^2 and L^3 show absorption bands in the region of 1250-1285 cm\(^{-1}\) \((\nu_g)\) \([\nu_s(\text{NO}_3)]\), 1035-1056 cm\(^{-1}\) \((\nu_p)\), \([\nu_s(\text{NO}_3)]\) and 8079-9005 cm\(^{-1}\) \((\nu_j)\) \(v(\text{NO})\). This indicates that the nitrate group coordinates to central metal as unidentate. The position of band of 1115 cm\(^{-1}\) indicates that nitrate group is uncoordinated.

\(^{(146)}\)
IR spectral bands of acetato complexes with ligands L¹, L², L³ show bands in the region 1123-1175 cm⁻¹ (υ₃), 1097-1079 cm⁻¹ (υ₁), [υₓ(CH₂COO⁻)] and 815-870 cm⁻¹ [υᵧ CH₂COO] corresponding to monodentate nature. On the other hand with ligand L⁴ shows IR bands corresponding to uncoordinated acetate group.

IR spectra of chloro complexes with ligands L¹, L² and L⁴ show absorption bands in the region 245-285 cm⁻¹, [υ (M-Cl)]. It indicates that chloro group coordinates with metal ion. On the other hand in case of ligand L³. There was not band appear in the region 245-285 cm⁻¹ with corresponding metal chloride. It indicates that chloride ion is present outside the coordination sphere.

6.3.3 Electronic spectral study:

Semicarbazone complexes of the ions (NO₃⁻, Cl⁻, CH₂COO⁻) are non electrolyte. Thus the semicarbazone complexes may be formulated as [Zn LₓXᵧ] and [Zn (Lₓ)X]X.

Electronic spectra of the semicarbazone complexes display bands in the range of 36225-38280cm⁻¹. These bands compounds to metal-ligand charge transfer. The results are shown in Table 6.3.

6.4 Results and Discussion:

On the basis of the elemental analysis (Table 6.2) the complexes have the composition Zn(L₁ X₁) (where X=Cl⁻, CH₂COO⁻ and NO₃⁻). Molar conductance (Table 6.2) of the complexes was determined in DMSO and indicates the following nature.

Since Zn(II) has d¹⁰ electronic configuration so all the complexes are diamagnetic in nature.
<table>
<thead>
<tr>
<th>Ligands</th>
<th>Metal Salts</th>
<th>Nature of complex</th>
<th>Formula of the complexes</th>
</tr>
</thead>
</table>
| L₁, L₂, L₄ | ZnCl₂, H₂O  
  Zn (CH₃COO)₂, 2H₂O  
  Zn(NO₃)₂, 6H₂O | 1:2 electrolyte | [Zn (L)₂] X₂ |
| L₃     | ZnCl₂, H₂O  
  Zn (CH₃COO)₂, 2H₂O  
  Zn(NO₃)₂, 6H₂O | 1:1 electrolyte | [Zn (L)X] |

### 6.5 Proposed structure of the complexes:

On the basis of elemental analysis, magnetic moment, IR, studies has been assigned for the complexes with ligand L₁, L₂, L₃ and L₄ as follows:
Zinc(II) complexes of acetoacetate ester semicarbazone

Zinc(II) complexes of isopropyl ester semicarbazone
Zinc(II) complexes of Methyl ester of 4-hydroxy-6-methyl-2-oxo-Pyran-3-carboxylic acid semicarbazone with ligand $L^1$, $L^2$ and $L^3$.

$X=\text{Cl, NO}_3, \text{CH}_3\text{COO}^-$

Zinc(II) complexes of Methyl ester of 4-hydroxy-6-methyl-2-oxo-Pyran-3-carboxylic acid semicarbazone with ligand $L^5$.
Zinc(II) complexes of 4-Formyl methyl salicylate semicarbazone
6.6 Antifungal Activities:

Fungi are the plant-like organisms that lack chlorophyll. Since they do not have chlorophyll, fungi must absorb food from others. Since they do not use light to make food, fungi can live in damp and dark places. Fungi are supposed to “eat” thing when they are dead but sometimes they start eating when the organism is still alive. Many fungi are good and useful e.g. edible mushrooms, while some are pathogenic to plants and human beings. There are over 100,000 species of fungi. Medical mycologists study drugs to cure fungal infections, whereas agricultural and research mycologists study the industrial use of fungi.

Antifungal activity of newly synthesized Zn(II) complexes [74-78] was determined based on the growth inhibition rates of the mycelia of Aspergillus niger and Candida albicans strains grown in potato dextrose broth medium (PDB). Under aseptic conditions 1 ml of spore suspension (5 x 10^6 cfuml) of the fungi being tested was added and 50 ml of PDB medium in an erlenmeyer flask. Appropriate volumes of tested metal complexes was added to produce concentrations ranging from 10-100 M gm ml^-1. The flasks were indicated at 27±1°C in the dark for five day, at which time the mycelia were collected on filter paper. The filter paper was deriv to a constant weight and the level of inhibition relative to the controlled flask was calculated from the following formula:

\[
\frac{W - w}{C} \times 100
\]

\( W = \) Weight of mycelia from the test flasks

\( w = \) Weight of mycelia from the control flasks

(152)
The antifungal inhibition of the Zn (II) complexes given in Table 6.4 to 6.7.

The ligands and their Zn(II) complexes complexes were screened for determining antifungal in vitro against pathogenic fungi results have been compared with the conventional fungicide Captan. The data of the antifungal activities of ligand and complexes are given in Table 6.1. The data reveal that the complexes have the higher activities than the free ligand. This enhancement of the activity of ligand on complexation can be explained by Overtone’s Concept and Chelation Theory[78-82], according to which chelation reduces the polarity of the metal atom mainly because of the partial sharing of its positive charge with donor groups and possible π-electron delocalization over the whole ring through dπ-dπ or dπ-pπ interactions of the orbitals of the ligand and metal ion. This results with increasing of the lipophilic character of the complex and favour the permeation of the complex through the lipid layer of cell membrane and blocking the metal binding sites in the enzymes of microorganisms. Thus, the complex disturbs the respiration process of cell and as a result the synthesis of proteins is blocked in the cell of microorganism.

Further, the degradative enzymes produced by the microorganism are important in host infection. The enzyme production is here intended to mean both, synthesis of the enzymes by the microorganisms and activity of the enzyme in the medium after it is produced. Since the metal complexes inhibit the growth of microorganism, it is assumed that the production of enzyme is being affected and hence the microorganism is unable to utilize the food for itself or the intake of nutrients in suitable forms. Consequently the growth of microorganism is arrested, while higher concentration proves fatal. The higher concentration destroys the enzyme mechanism by blocking any of the metabolism pathway and due to the lack of

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availability of proper food, the organism dies.

The results of antipathogenic activities as enlisted in Tables 6.3-6.6 indicate that the metal chelates are more active than the metal free ligands.
### Table 6.1: Some representative Zn-dependent enzymes

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Metal</th>
<th>Reaction catalysed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carboxypeptidase</td>
<td>Zn(^{2+})</td>
<td>Hydrolysis of C-terminal peptide linkage</td>
</tr>
<tr>
<td>Leucine aminopeptidase</td>
<td>Zn(^{2+})</td>
<td>Hydrolysis of leucine N-terminal peptide linkage</td>
</tr>
<tr>
<td>Neutral protease</td>
<td>Zn(^{2+}), Ca(^{2+})</td>
<td>Hydrolysis of peptides</td>
</tr>
<tr>
<td>Dipeptidase</td>
<td>Zn(^{2+})</td>
<td>Hydrolysis of dipeptides</td>
</tr>
<tr>
<td>Thermolysin</td>
<td>Zn(^{2+}), Ca(^{2+})</td>
<td>Hydrolysis of peptides</td>
</tr>
<tr>
<td>Phospholipase C</td>
<td>Zn(^{2+})</td>
<td>Hydrolysis of phospholipids</td>
</tr>
<tr>
<td>β-Lactamase II</td>
<td>Zn(^{2+})</td>
<td>Hydrolysis of β-lactam ring</td>
</tr>
<tr>
<td>α-Amylase</td>
<td>Zn(^{2+}), Ca(^{2+})</td>
<td>Hydrolysis of glucosides</td>
</tr>
<tr>
<td>Phosphatases</td>
<td>Zn(^{2+}), Mg(^{2+})</td>
<td>Hydrolysis of phosphate esters</td>
</tr>
<tr>
<td>Purple acid phosphatase (PAP) [in bean]</td>
<td>Fe(^{3+}), Zn(^{2+})</td>
<td>Hydrolysis of phosphate ester</td>
</tr>
<tr>
<td>Carbonic anhydrase</td>
<td>Zn(^{2+})</td>
<td>Hydration of CO(_2) and dehydration of H(_2)CO(_3)</td>
</tr>
<tr>
<td>DNA-polymerase</td>
<td>Zn(^{2+}), Mg(^{2+}), Mn(^{2+})</td>
<td>Polymerisation of DNA with the formation of phosphate ester</td>
</tr>
<tr>
<td>Alcohol dehydrogenase</td>
<td>Zn(^{2+})</td>
<td>Hydride transfer from alcohol to NAD(^{+})</td>
</tr>
<tr>
<td>Adenosine deaminase</td>
<td>Zn(^{2+})</td>
<td>Hydrolysis of adenosine</td>
</tr>
<tr>
<td>Cytidine deaminase</td>
<td>Zn(^{2+})</td>
<td>Hydrolysis of cytidine</td>
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### Table 6.2: Physical Parameters of Zn(II) Complexes

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Complex</th>
<th>Colour</th>
<th>Yield (%)</th>
<th>M. Point (°C)</th>
<th>Molecular Weight</th>
<th>Molar conductance</th>
<th>Elemental Analysis found (Calculated)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>[Zn(L)2 Cl2]</td>
<td>White</td>
<td>65</td>
<td>190</td>
<td>509.39</td>
<td>16</td>
<td>32.58 (32.98) 5.00 (5.10) 16.30 (16.55) 18.54 (18.84) 12.56 (12.83)</td>
</tr>
<tr>
<td></td>
<td>Zn C12H10N4O4Cl2</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>2.</td>
<td>[Zn(L)2 (CH3COO)2]</td>
<td>White</td>
<td>60</td>
<td>195</td>
<td>561.39</td>
<td>15</td>
<td>29.85 (29.92) 4.25 (4.27) 19.59 (19.95) 34.10 (34.20) 11.24 (11.53)</td>
</tr>
<tr>
<td></td>
<td>Zn C12H10N4O12</td>
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<tr>
<td>3.</td>
<td>[Zn(L)2 NO3]</td>
<td>White</td>
<td>70</td>
<td>198</td>
<td>557.39</td>
<td>19</td>
<td>38.56 (38.75) 5.57 (5.74) 15.00 (15.07) 28.56 (28.70) 11.53 (11.73)</td>
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<tr>
<td></td>
<td>Zn C12H10N4O10</td>
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<tr>
<td>4.</td>
<td>[Zn(L)2 Cl2]</td>
<td>White</td>
<td>68</td>
<td>215</td>
<td>454.39</td>
<td>18</td>
<td>31.59 (31.69) 5.70 (5.72) 18.37 (18.48) 14.00 (14.08) 14.00 (14.39)</td>
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<tr>
<td></td>
<td>Zn C12H10N4O4Cl2</td>
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<tr>
<td>5.</td>
<td>[Zn(L)2 (CH3COO)2]</td>
<td>White</td>
<td>65</td>
<td>199</td>
<td>501.39</td>
<td>20</td>
<td>38.18 (38.29) 6.30 (6.38) 16.59 (16.75) 25.50 (25.53) 13.00 (14.04)</td>
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<td></td>
<td>Zn C16H12N4O8</td>
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<tr>
<td>6.</td>
<td>[Zn(L)2 (NO3)2]</td>
<td>White</td>
<td>70</td>
<td>202</td>
<td>507.39</td>
<td>19</td>
<td>28.30 (28.38) 5.00 (5.12) 22.00 (22.07) 31.52 (31.53) 12.68 (12.88)</td>
</tr>
<tr>
<td></td>
<td>Zn C12H10N4O10</td>
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<td>7.</td>
<td>[Zn(L)2] Cl2</td>
<td>White</td>
<td>72</td>
<td>220</td>
<td>559.54</td>
<td>96</td>
<td>34.61 (34.81) 3.80 (3.86) 15.10 (15.15) 25.70 (25.79) 10.50 (10.54)</td>
</tr>
<tr>
<td></td>
<td>Zn C12H10N4O4Cl2</td>
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<tr>
<td>8.</td>
<td>[Zn(L)2] (CH3COO)2</td>
<td>White</td>
<td>75</td>
<td>200</td>
<td>620.39</td>
<td>87</td>
<td>39.45 (39.55) 4.40 (4.49) 12.50 (12.58) 33.46 (33.56) 9.59 (9.79)</td>
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<td>Zn C16H12N4O16</td>
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<tr>
<td>9.</td>
<td>[Zn(L)2] (NO3)2</td>
<td>White</td>
<td>75</td>
<td>210</td>
<td>667.39</td>
<td>90</td>
<td>32.07 (32.00) 3.46 (3.56) 16.53 (16.63) 38.00 (38.01) 9.61 (9.71)</td>
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<tr>
<td></td>
<td>Zn C16H10N4O14</td>
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<tr>
<td>10.</td>
<td>[Zn(L)2] Cl2</td>
<td>White</td>
<td>68</td>
<td>215</td>
<td>610.39</td>
<td>12</td>
<td>39.21 (39.31) 3.50 (3.60) 13.66 (13.76) 20.67 (20.97) 10.61 (10.71)</td>
</tr>
<tr>
<td></td>
<td>Zn C20H12N4O4Cl2</td>
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<tr>
<td>11.</td>
<td>[Zn(L)2 (CH3COO)2]</td>
<td>White</td>
<td>70</td>
<td>210</td>
<td>657.39</td>
<td>15</td>
<td>43.60 (43.76) 4.20 (4.25) 12.76 (12.77) 29.10 (29.29) 9.84 (9.94)</td>
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<td>Zn C20H12N4O14</td>
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<tr>
<td>12.</td>
<td>[Zn(L)2] (NO3)2</td>
<td>White</td>
<td>72</td>
<td>210</td>
<td>663.39</td>
<td>16</td>
<td>36.00 (36.17) 3.21 (3.31) 16.78 (16.88) 33.66 (33.76) 9.75 (9.85)</td>
</tr>
<tr>
<td></td>
<td>Zn C24H14N6O12</td>
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<tr>
<td>Sr. No.</td>
<td>Complex</td>
<td>$\lambda_{max}$ cm$^{-1}$</td>
<td>Assignment</td>
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<td>-------------</td>
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</tr>
</tbody>
</table>
| 1.     | [Zn (L)$_2$ Cl$_2$]  
ZnC$_{14}$H$_{28}$N$_6$O$_{4}$S$_2$Cl$_2$ | 34000 37000 | Charge Transfer |
| 2.     | [Zn (L)$_2$ CH$_3$COO)$_2$]  
ZnC$_{14}$H$_{28}$N$_6$O$_{10}$S$_2$ | 34050 36995 | – |
| 3.     | [Zn (L)$_2$ NO$_3$$_2$]  
ZnC$_{14}$H$_{28}$N$_6$O$_{10}$S$_2$ | 33900 36890 | – |
| 4.     | [Zn (L)$_2$ Cl$_2$]  
ZnC$_{14}$H$_{28}$N$_6$O$_{10}$S$_2$Cl$_2$ | 33995 36800 | – |
| 5.     | [Zn (L)$_2$ (CH$_3$COO)$_2$]  
ZnC$_{14}$H$_{28}$N$_6$O$_{10}$S$_2$ | 34155 36900 | – |
| 6.     | [Zn (L)$_2$ NO$_3$$_2$]  
ZnC$_{14}$H$_{28}$N$_6$O$_{10}$S$_2$ | 34059 35925 | – |
| 7.     | [Zn (L)$_2$ Cl$_2$]  
ZnC$_{14}$H$_{28}$N$_6$O$_{4}$S$_2$Cl$_2$ | 33889 35090 | – |
| 8.     | [Zn (L)$_2$ (CH$_3$COO)$_2$]  
ZnC$_{22}$H$_{34}$N$_6$O$_{12}$S$_2$ | 33790 36805 | – |
| 9.     | [Zn (L)$_2$ NO$_3$$_2$]  
ZnC$_{14}$H$_{28}$N$_6$O$_{10}$S$_2$ | 34005 36925 | – |
| 10.    | [Zn (L)$_2$ Cl$_2$]  
ZnC$_{20}$H$_{32}$N$_6$O$_{6}$S$_2$Cl$_2$ | 34785 36785 | – |
| 11.    | [Zn (L)$_2$ CH$_3$COO)$_2$]  
ZnC$_{24}$H$_{36}$N$_6$O$_{16}$S$_2$ | 35000 36725 | – |
| 12.    | [Zn (L)$_2$ NO$_3$$_2$]  
ZnC$_{20}$H$_{32}$N$_6$O$_{12}$S$_2$ | 34995 35600 | – |
### Table 6.4: Antifungal activity of Ligand L¹ and its complexes

<table>
<thead>
<tr>
<th>Compound</th>
<th>Fungal inhibition (% conc. in µg/ml⁻¹)</th>
<th></th>
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<th></th>
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<tbody>
<tr>
<td></td>
<td>A. brassicae</td>
<td>A. niger</td>
<td>F. oxysporum</td>
<td>A. brassicae</td>
<td>A. niger</td>
<td>F. oxysporum</td>
<td>A. brassicae</td>
</tr>
<tr>
<td>Ligand (L₁)</td>
<td>20</td>
<td>31</td>
<td>46</td>
<td>22</td>
<td>31</td>
<td>39</td>
<td>20</td>
</tr>
<tr>
<td>[Zn (L₁)₂Cl₂]NO₃</td>
<td>25</td>
<td>36</td>
<td>48</td>
<td>24</td>
<td>36</td>
<td>44</td>
<td>25</td>
</tr>
<tr>
<td>[Zn (L₁)₂NO₃]</td>
<td>21</td>
<td>32</td>
<td>49</td>
<td>23</td>
<td>32</td>
<td>39</td>
<td>23</td>
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<tr>
<td>[Zn (L₁)₂CH₃COO₂]</td>
<td>23</td>
<td>31</td>
<td>44</td>
<td>28</td>
<td>31</td>
<td>44</td>
<td>23</td>
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</tbody>
</table>

### Table 6.5: Antifungal activity of Ligand L² and its complexes

<table>
<thead>
<tr>
<th>Compound</th>
<th>Fungal inhibition (% conc. in µg/ml⁻¹)</th>
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<th></th>
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</thead>
<tbody>
<tr>
<td></td>
<td>A. brassicae</td>
<td>A. niger</td>
<td>F. oxysporum</td>
<td>A. brassicae</td>
<td>A. niger</td>
<td>F. oxysporum</td>
<td>A. brassicae</td>
</tr>
<tr>
<td>Ligand (L₂)</td>
<td>31</td>
<td>40</td>
<td>59</td>
<td>34</td>
<td>50</td>
<td>59</td>
<td>34</td>
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<tr>
<td>[Zn (L₂)₂Cl₂]</td>
<td>40</td>
<td>58</td>
<td>64</td>
<td>45</td>
<td>57</td>
<td>67</td>
<td>46</td>
</tr>
<tr>
<td>[Zn (L₂)₂(NO₃)₃]</td>
<td>43</td>
<td>60</td>
<td>63</td>
<td>46</td>
<td>60</td>
<td>65</td>
<td>41</td>
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<tr>
<td>[Zn(L₂)(CH₃COO)₂]</td>
<td>35</td>
<td>54</td>
<td>57</td>
<td>35</td>
<td>46</td>
<td>61</td>
<td>41</td>
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</table>

### Table 6.6: Antifungal activity of Ligand L³ and its complexes

<table>
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<th>Compound</th>
<th>Fungal inhibition (% conc. in µg/ml⁻¹)</th>
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<th></th>
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<th></th>
<th></th>
<th></th>
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</thead>
<tbody>
<tr>
<td></td>
<td>A. brassicae</td>
<td>A. niger</td>
<td>F. oxysporum</td>
<td>A. brassicae</td>
<td>A. niger</td>
<td>F. oxysporum</td>
<td>A. brassicae</td>
</tr>
<tr>
<td>Ligand (L₃)</td>
<td>30</td>
<td>39</td>
<td>57</td>
<td>32</td>
<td>44</td>
<td>63</td>
<td>30</td>
</tr>
<tr>
<td>[Zn (L₃)₂Cl]Cl</td>
<td>34</td>
<td>46</td>
<td>59</td>
<td>36</td>
<td>47</td>
<td>65</td>
<td>34</td>
</tr>
<tr>
<td>[Zn (L₃)₂NO₃] NO₃</td>
<td>35</td>
<td>49</td>
<td>61</td>
<td>40</td>
<td>48</td>
<td>66</td>
<td>33</td>
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<tr>
<td>[Zn(L₃)(CH₃COO)₂]</td>
<td>38</td>
<td>44</td>
<td>55</td>
<td>33</td>
<td>45</td>
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</table>
Table 6.7: Antifungal activity of Ligand L₄ and its complexes

<table>
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<th>Compound</th>
<th>Fungal inhibition (%) (conc. in µg/ml⁻¹)</th>
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<th>A. niger</th>
<th>F. oxysporum</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>100</td>
<td>200</td>
<td>300</td>
<td>100</td>
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<td>Ligand (L₄)</td>
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<td>29</td>
<td>44</td>
<td>22</td>
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<tr>
<td>[Zn (L₄)Cl₂]</td>
<td>35</td>
<td>44</td>
<td>51</td>
<td>38</td>
</tr>
<tr>
<td>[Zn (L₄)₂(NO₃)₂]</td>
<td>34</td>
<td>43</td>
<td>49</td>
<td>36</td>
</tr>
<tr>
<td>[Zn (L₄)₂(CH₃COO)₂]</td>
<td>28</td>
<td>35</td>
<td>48</td>
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REFERENCES


