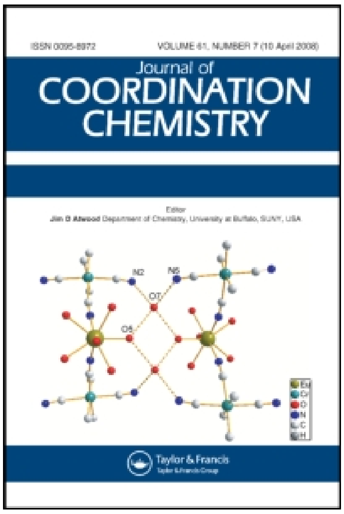

Journal of Coordination Chemistry
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713455674

Synthesis, characterization, spectral studies and antifungal activity of Mn(II), Fe(II), Co(II), Ni(II), Cu(II) and Zn(II) complexes with 3,3'-bis[N,N,di(carboxymethyl)-aminomethyl]-<i>o</i>-cresol sulphonphthalein

Gajanan Pandey[a]; Krishan K. Narang[b]
[a] Department of Applied Chemistry, Madhav Institute of Technology & Science, Gwalior, India [b] Department of Applied Chemistry, Institute of Technology, Banaras Hindu University, Varanasi, India

To cite this Article Pandey, Gajanan and Narang, Krishan K.(2006) 'Synthesis, characterization, spectral studies and antifungal activity of Mn(II), Fe(II), Co(II), Ni(II), Cu(II) and Zn(II) complexes with 3,3'-bis[N,N,di(carboxymethyl)-aminomethyl]-<i>o</i>-cresol sulphonphthalein', Journal of Coordination Chemistry, 59: 13, 1495 — 1507

To link to this Article: DOI: 10.1080/00958970600561332

URL: http://dx.doi.org/10.1080/00958970600561332

Journal of Coordination Chemistry
Vol. 59, No. 13, 10 September 2006, 1495–1507

Taylor & Francis
Taylor & Francis Group

Synthesis, characterization, spectral studies and antifungal activity of Mn(II), Fe(II), Co(II), Ni(II), Cu(II) and Zn(II) complexes with 3,3′-bis[N,N,di(carboxymethyl)-aminomethyl]-*o*-cresol sulphonphthalein

GAJANAN PANDEY*† and KRISHAN K. NARANG‡

†Department of Applied Chemistry, Madhav Institute of Technology & Science,
Gwalior, India
‡Department of Applied Chemistry, Institute of Technology,
Banaras Hindu University, Varanasi, India

(*Received 19 November 2005*)

Mn(II), Fe(II), Co(II), Ni(II), Cu(II) and Zn(II) complexes of the type $Na_4[ML(H_2O)_2]$ of the ligand, 3,3′-bis[N,N-di(carboxymethyl)-aminomethyl]-*o*-cresol sulphonphthalein (Xylenol Orange, Na_4H_2L), have been synthesized and characterized by different physico-chemical (elemental analyses, solubility, electrolytic conductances, magnetic susceptibility measurements) and spectral (u.v.-vis, i.r., e.s.r., and powder X-ray diffraction) techniques for their structure determination. The data suggest 1 : 1 (M : L) compositions and octahedral geometries around M(II) except for Cu(II). Antifungal activity of the complexes measured against ten fungi show significant activity against *Alternaria brassicicola*, *Alternaria solanai*, *Cercospora species* and *Helminthosporium oryzae* and moderate antifungal activity against *Curvularia species*, *Curvularia lunata*, *Curvularia penniseti*, *Colletotrichum capsici*, *Aspergillus niger*, *Aspergillus flavus Erysiphae pisi* and *Fusarium udum* fungi.

Keywords: Metal complex; Antifungal activity; Xylend orange; Ligand

1. Introduction

A number of studies using 3,3′-bis[N,N-di(carboxymethyl)-aminomethyl]-*o*-cresol sulphonphthalein (Xylenol Orange, Na_4H_2L) in spectrophotometric and complexometric determination of metal ions [1–3], chromatographic separation of fatty acids [4], electrochemical studies of complexation behaviour with metal ions [5, 6] and as a metallochromic indicator in EDTA titrations [7] are available. Complexation of xylenol orange occurs through protonated, deprotonated and protonated hydroxo complexes with a number of metal ions, in different metal-ligand ratios under various pH conditions [8, 9], but virtually no effort has been made to obtain solid complexes.

---

*Corresponding author. Email: pandeygajanan@rediffmail.com

Journal of Coordination Chemistry
ISSN 0095-8972 print/ISSN 1029-0389 online © 2006 Taylor & Francis
DOI: 10.1080/00958970600561332

Medicinal potency of coordination compounds has been established by their antitumour [10], antiviral [11], antimalerial [12] and antifungal activity [13]. Transition metal complexes with dyes e.g. copper(II) with methyl orange, show pronounced antifungal and antibacterial activity [14]. The characteristic property has been related to the ability to metal ions to form complexes with ligands containing nitrogen, oxygen and sulfur donor atoms [15–17]. Further, it has been demonstrated that chelation is important for antifungal and antibacterial activity [18].

To study the effect of complexation of sulphonpthalein derivatives on bioactivity and to understand structure-bioactivity relationships, a few 1 : 1 metal-ligand complexes of the type $Na_4[ML(H_2O)_2]$ have been prepared, characterized and their antifungal activity has been measured against ten fungi.

2. Experimental

2.1. *Materials*

Metal salts and the ligand, 3,3′-bis[N,N-di(carboxymethyl)-aminomethyl]-*o*-cresol sulphonphthalein(Na_4H_2L) were of BDH AR or equivalent grades. The solvents (EtOH, MeOH, dioxane, etc.) were purified by distillation. The ligand, Na_4H_2L was purified by the literature procedure [19].

Fungi were isolated on PDA (250 g peeled potato, 20 g dextrose, 15 g agar powder and 1 L distilled H_2O) medium from their respective hosts (table 5) collected from the experimental farm of Banaras Hindu University. The cultures were purified by single spore isolation on PDA slants.

2.2. *Synthesis of complexes*

For the purpose of bioactivity studies, water soluble complexes were prepared by mixing aqueous solutions (0.01 M) of each metal [manganese(II), iron(II), cobalt(II), nickel(II), copper(II) & zinc(II)] salts and the ligand, 3,3′-bis[N,N-di(carboxymethyl)-aminomethyl]-*o*-cresol sulphonphthalein (Na_4H_2L) (0.01 M), in 1 : 1 ratio. The pH of the mixtures was raised to ~11 by addition of NaOH solution and the mixtures were refluxed on a water bath for 2 h. After cooling the solution, Me_2CO (ca $50\,cm^3$) in $100\,cm^3$ aqueous solution was added until shining microcrystals were formed. The metal(II) complexes with Na_4H_2L were analyzed for M, C, H & N & S content. Their physico-chemical and elemental data are presented in table 1.

2.3. *Analyses and physical measurements*

The metal content was determined by atomic absorption spectroscopy using a SOLAAR 32AA instrument. Carbon, hydrogen, nitrogen and sulfur analyses were carried out in a Perkin-Elmer CHN Elemental Analyser (Model 2700). Combustion temperature was at 920°C and reduction temperature was at 640°C.

Magnetic susceptibility measurements were carried out at room temperature on a Faraday type balance (Cahn-electronic balance 75570) using $Hg[Co(NSC)_4]$ as a standard. Experimental magnetic susceptibilities (χ_M) were corrected for diamagnetism

Table 1. Analytical data, magnetic moment and physical properties of complexes of Na_4H_2L with Mn(II), Fe(II), Co(II), Ni(II), Cu(II) and Zn(II).

Complexes Molecular wt.	Colour/ yield (%)	M.p./d.p.°C	Found (Calcd)%						Effective magnetic moment (BM)	Λ_m, Ω^{-1}, cm^2 mol^{-1} (water)
			Na	M	C	H	N	S		
$Na_4[MnL(H_2O)_2]$ 848.9	Deep brown/81.3	>300	10.8 (10.8)	6.45 (6.5)	43.8 (43.8)	3.5 (3.5)	3.9 (3.3)	3.75 (3.8)	4.1	411
$Na_4[FeL(H_2O)_2]$ 849.8	Navy blue/79.8	>300	10.8 (10.8)	6.55 (6.6)	43.8 (43.8)	3.5 (3.5)	3.25 (3.5)	3.75 (3.8)	6.1	409
$Na_4[CoL(H_2O)_2]$ 852.9	Reddish brown/82.5	>300	10.8 (10.8)	6.9 (6.9)	43.6 (43.6)	3.5 (3.5)	3.25 (3.3)	3.7 (3.75)	5.2	409
$Na_4[NiL(H_2O)_2]$ 852.6	Crimson red/83.3	>300	10.8 (10.8)	6.9 (6.9)	43.6 (43.6)	3.5 (3.5)	3.3 (3.3)	3.7 (3.75)	2.9	410
$Na_4[CuL(H_2O)_2]$ 857.5	Brown/83.6	>300	10.7 (10.7)	7.4 (7.4)	43.3 (43.4)	3.5 (3.5)	3.3 (3.3)	3.7 (3.7)	2.1	408
$Na_4[ZnL(H_2O)_2]$ 859.3	Purple/81.6	>300	10.7 (10.7)	7.6 (7.6)	43.3 (43.3)	3.5 (3.5)	3.2 (3.3)	3.7 (3.7)	–	410

L = $C_{31}H_{26}O_{13}N_2S$; Color of Na_4H_2L is violet; All the complexes are water soluble and decompose above 300°C.

using the procedure of Figgis and Lewis [20]. Electronic spectra (1500–200 nm) of the ligand and complexes were recorded on a CARY-2390 UV-Visible spectrophotometer in twice-distilled water. I.r. spectra in the 4000–400 cm^{-1} range were recorded on a JASCO FT/IR-5300 spectrophotometer in nujol mulls. E.s.r. spectra of a copper(II) complex of NaH_2L were recorded on a Varian-E-11 X-band spectrometer at liquid nitrogen temperature and room temperature in the solid state using DPPH (1,1-diphenyl-2-picrylhydrazyl) as a $<g>$ marker. The field setup was 3200 G and the scan range was 2000 G. Powder X-ray diffraction data were analyzed by Ito's method [21]. Molar conductances of the water-soluble complexes were measured at room temperature on a WTW conductivity bridge fitted with a Philips magic eye.

2.4. *Antifungal test*

Aqueous solutions of metal salts $MnCl_2 \cdot 4H_2O$, $FeSO_4 \cdot 7H_2O$, $CoSO_4 \cdot 7H_2O$, $NiSO_4 \cdot 7H_2O$, $CuSO_4 \cdot 5H_2O$, and $ZnSO_4 \cdot 7H_2O$ were prepared by dissolving the required amounts in twice distilled water to concentrations of 1000 ppm. Aqueous solutions of Na_4H_2L were prepared by dissolving the required amount in twice-distilled water to 1000 ppm.

Antifungal activity of the complexes was measured against the fungi *Alternaria brassicicola*, *Alternaria brassicae*, *Alternaria solanae*, *Alternaria triticina*, *Curvularia species*, *Curvularia lunata*, *Curvularia penniseti*, *Helminthosporium oryzae*, *Colletotrichum capsici*, *Aspergillus niger*, *Aspergillus flavus*, *Fusarium udum*, *Cercospora species* and *Erysiphae pisi*, by spore germination [22]. For comparison, antifungal activity of the solutions of metal salts and the ligand were also measured on the same fungi.

A drop of a solution was placed on a grease-free glass slide and 200–300 spores of the test fungi were placed with the help of a sterilized inoculation needle on the solution. The slides were then placed in a moist chamber and incubated for 24 h at $25 \pm 2°C$. After 24 h the spores were fixed and stained with lectophenol cotton blue and spore germination was observed under a light microscope. A similar number of each fungus was mixed in sterilized distilled water, which served as control. For measurement of inhibition, the percentage germination was subtracted from one hundred to get the percentage inhibition. All the experiments were conducted in triplicate. The data were subjected to students '*t*' test for statistical significance.

2.5. *Statistical analysis*

The data recorded for different concentration of solution were subjected to the statistical analysis. The analysis of variance (ANOVA) was carried out separately for each fungus against all the compounds at various concentrations according to the procedure of Randomized Block Design Analysis [23].

3. Results and discussion

Na_4H_2L (figure 1) has several bonding sites but the two acidic chelating groups on the side of its large sulphonphthalein nucleus may behave like branched donating sites

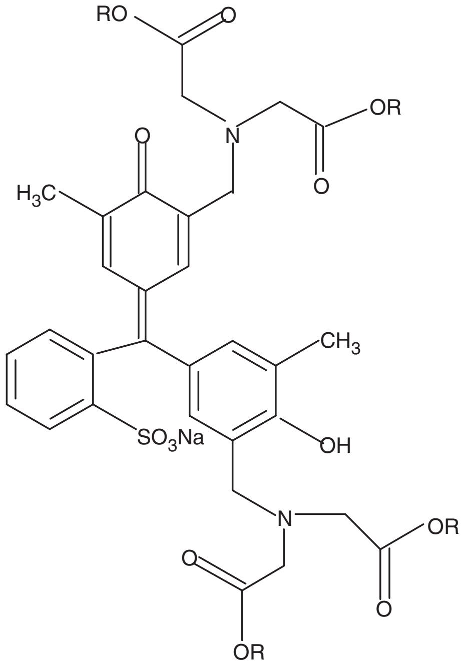

Figure 1. Na_4H_2L.

enhancing the possibility of mononuclear or binuclear complex formation with metal ions [24]. In binuclear complex formation, both sides of the sulphonphthalein nucleus are chelated by PhO^-, N, and two COO^- electron pairs [25]. Empirical formulae derived from analytical data (C, H, N, S and metal contents) suggest formation of 1 : 1 (M : L) complexes. The assumption of 4 : 1 complex electrolyte can be confirmed by high molar conductance values (greater than $400\,\Omega^{-1}\,cm^2\,mol^{-1}$) observed in 1 mM aqueous solution for each complexes [26], though the conductivity values are slightly lower than the value for uni-tetravalent electrolyte [27]. The complex anion with 4^- charge is voluminous and poorly mobile, considerably reducing its contribution to the molar conductivity [28].

Depending on pH, the chelation process indicates stepwise formation of stable complex species viz. MH_2L^{2-}, MHL^{3-} and ML^{4-} in solution [18].

$$M^{2+} + H_2L^{4-} \rightleftarrows MH_2L^{2-} \qquad \text{(i)}$$

$$M^{2+} + HL^{5-} \rightleftarrows MHL^{3-} \qquad \text{(ii)}$$

$$M^{2+} + H_2L^{4-} \rightleftarrows ML^{4-} + 2H^+ \qquad \text{(iii)}$$

At high pH ~ 9–11, deprotonated ML^{4-} metal complex species predominate with different metal ions i.e. manganese(II), iron(II), cobalt(II), nickel(II), copper(II) and zinc(II), according to the reaction.

$$M^{2+} + H_2L^{4-} \rightarrow ML^{4-} + 2H^+$$

Table 2. Tentative assignment#of significant peaks (cm^{-1}) in the IR spectra of Mn(II), Fe(II), Co(II), Ni(II), Cu(II) and Zn(II) complexes with Na_4H_2L.

Name of compound/peaks	ν(OH)/H_2O	ν (C=O)	ν (COO^-) asym	ν (COO^-) sym	ν (S–O)	ν (M–N)
Na_4H_2L	3427	1700	1630	1551	1018	–
$Na_4[MnL(H_2O)_2]$	3452	1643	1624	1598	1018	497
$Na_4[FeL(H_2O)_2]$	3453	1637	1627	1590	1018	451
$Na_4[CoL(H_2O)_2]$	3462	1636	1626	1582	1020	414
$Na_4[NiL(H_2O)_2]$	3418	1638	1628	1598	1018	410
$Na_4[CuL(H_2O)_2]$	3420	1631	1628	1590	1019	408
$Na_4[ZnL(H_2O)_2]$	3418	1635	1589	1594	1018	453

$L = C_{31}H_{26}O_{13}N_2S$.

3.1. *IR spectra*

A strong band at about 3427 cm^{-1} in the IR spectra of Na_4H_2L is observed due to phenolic OH group (table 2). This band should disappear in the spectra of metal complexes indicating deprotonation of phenolic oxygen [29] but overlaps with the bands between 3420–3462 cm^{-1}, for coordinated water. The coordinated water is confirmed by bands between 700–900 cm^{-1} [30]. Antisymmetric and symmetric vibrations of COO^- have been observed between 1589–1630 and 1551–1598 cm^{-1}, respectively showing bidentate chelation of carboxyl groups [31]. The band at ca 1018 cm^{-1} due to SO_3^- remains unaffected.

3.2. *Electronic spectra*

Two bands in $Na_4[CoL(H_2O)_2]$ at 276 and 480 nm are of ligand origin and the band at 580 nm may be due to L → M charge transfer. Weak bands at 586 nm, 698 nm and at 1192 nm correspond to spin allowed d–d transitions i.e. $^4T_{1g}(F) \rightarrow {}^4A_{2g}(F)$, $^4T_{1g}(F) \rightarrow {}^4T_{1g}(P)$ and $^4T_{1g}(F) \rightarrow {}^4T_{2g}(F)$ for Co(II). These are suggestive of octahedral geometry around Co(II) [32]. Similarly, in $Na_4[NiL(H_2O)_2]$ bands at 584 nm, 698 nm and 1180 nm due to $^3A_{2g}(F) \rightarrow {}^3T_{1g}(P)$, $^3A_{2g}(F) \rightarrow {}^3T_{1g}(F)$ and $^3A_{2g}(F) \rightarrow {}^3T_{2g}(F)$ transitions, respectively, indicate octahedral geometry [33]. A broad band centered at ~700 nm in $Na_4[CuL(H_2O)_2]$ is assigned to $^2E_g \rightarrow {}^2T_{2g}$ transition. In case of Mn(II), Fe(II) and Zn(II) complexes the electronic spectra show only intense bands with high ε_{max} values (table 3) due to L–L or L–M (CT) transitions.

3.3. *Magnetic moments*

The observed μ_{eff} value of manganese(II) complex (4.07 BM) is less than the spin only value 5.9 BM, probably due to weak Mn–Mn interaction via Mn–O–Mn or stacking. μ_{eff} of iron(II) (6.06 BM) is slightly higher than the expected value for high spin d^6 probably due to some ferromagnetic exchange interaction [34]. For cobalt(II) complex, (μ_{eff} 5.17 BM) and nickel(II) complex (μ_{eff} 2.92 BM), the observed values of magnetic moments are within the ranges (4.9–5.2 BM) and (2.83–3.5 BM) reported [35]. For the copper(II) complex μ_{eff} value (2.1 BM) corresponds to one unpaired electron.

Table 3. Tentative assignment of significant peaks in the electronic spectra of Mn(II), Fe(II), Co(II), Ni(II), Cu(II) and Zn(II) complexes with Na_4H_2L.

Complexes	λ_{max} (nm)	$E_{max} \times 10^3$ ($dm^3 mol^{-1} cm^{-1}$)	Assignment
$Na_4[MnL(H_2O)_2]$	244	11.05	Ligand origin
	476	8.94	
	572	11.21	LMCT
$Na_4[FeL(H_2O)_2]$	274	6.99	Ligand origin
	480	5.69	
	572	10.41	LMCT
	702		$^5T_2g \rightarrow {}^5Eg$
$Na_4[CoL(H_2O)_2]$	276	8.16	Ligand origin
	480	10.78	
	580	14.37	LMCT
	586		^4T_1g (F) $\rightarrow {}^4A_2g$(F)
	698		^4T_1g (F) $\rightarrow {}^4T_1g$(P)
	1192		^4T_1g (F) $\rightarrow {}^4T_2g$(F)
$Na_4[NiL(H_2O)_2]$	274	7.51	Ligand origin
	468	9.96	
	578	9.47	LMCT
	584		^3A_2g(F) $\rightarrow {}^3T_1g$(P)
	698		^3A_2g(F) $\rightarrow {}^3T_1g$(F)
	1180		^3A_2g(F) $\rightarrow {}^3T_2g$(F)
$Na_4[CuL(H_2O)_2]$	240	10.84	Ligand origin
	476	8.70	
	578	11.17	LMCT
	700		$^2Eg \rightarrow {}^2T_2g$
$Na_4[ZnL(H_2O)_2]$	274	8.06	Ligand origin
	460	10.37	
	580	15.47	LMCT

$L = C_{31}H_{26}O_{13}N_2S$.

On the basis of analytical data and spectral evidences, a general structure of 1 : 1 (M : L) complexes has been proposed in figure 2.

3.4. *ESR spectra*

ESR spectra of $Na_4[CuL(H_2O)_2]$ have been recorded at room temperature and liquid nitrogen temperature. The bonding parameters computed from the spectra are included in table 4. The spectra were analyzed by the method reported elsewhere [36].

The anisotropy is not clearly visible at room temperature but can be seen in the liquid nitrogen temperature spectral pattern, though the resolution is very low. Four bands in the spectrum indicate a single copper is present in $Na_4[CuL(H_2O)_2]$. Four bands, each due to $g_\parallel$, $g_\perp$, $A_\parallel$ and $A_\perp$, were identified and g_{av}, A_{av} and G were calculated using the expressions.

$$g_{av} = \frac{1}{3}(g_{||} + 2g_\perp), \quad A_{av} = \frac{1}{3}(A_{||} + 2A_\perp), \quad G = \frac{g_{||} - 2}{g_\perp - 2}$$

The closeness of the g value to 2.0023 (the free electron value) suggests little spin-orbit coupling. Also the G value, presented in table 4, indicates that there is no interaction between copper centers [37].

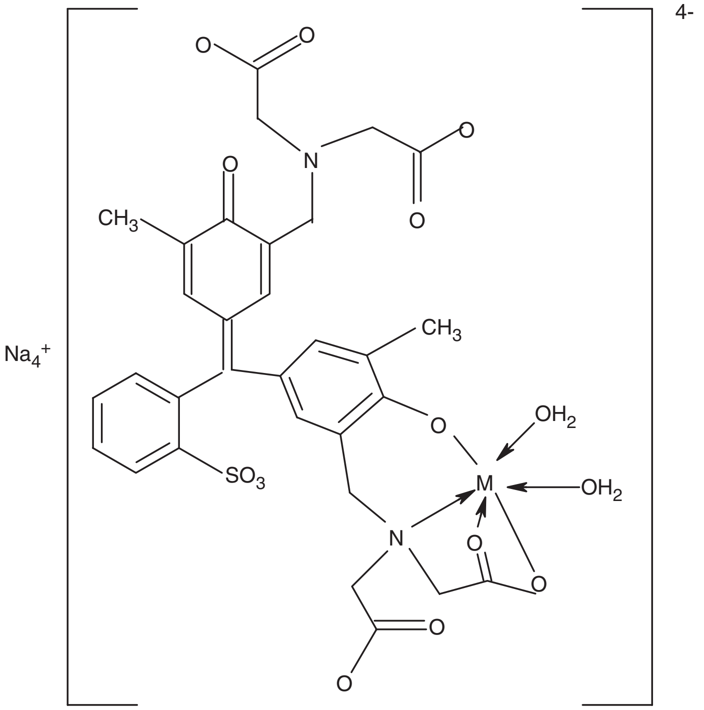

Figure 2. Proposed structures of manganese(II), iron(II), cobalt(II), nickel(II), copper(II) & zinc(II) complexes with Na_4H_2L.

Table 4. ESR parameters of Cu(II) complex with Na_4H_2L.

	$g_{\parallel}$	$g_{\perp}$	g_{av}	G	$A_{\parallel} \times 10^{-4}\,cm^{-1}$	$A_{\perp} \times 10^{-4}\,cm{-}1$	$A_{av} \times 10^{-4}\,cm^{-1}$
Room temp.	2.1516	2.0504	2.0841	3.0079	175.82	57.45	96.91
Liquid N_2 temp.	2.1629	2.0553	2.0912	2.9457	181.79	62.38	102.18

The temperature dependence of the spectrum is reminiscent of the behavior of octahedral complexes with a tetragonal Jahn-Teller distortion. At high temperature, the system resonates among three equivalent tetragonal distortions along the three four-fold axes of the octahedron, giving rise to an isotropic g value [38]. At lower temperature, the complex is frozen into one of the distortions and an anisotropic 'g' value is observed. The e.s.r. parameters follow the trend $g_{\parallel} > g_{\perp} > g_e$ (2.0023) indicating that the unpaired electron is present in the d_{x2-y2} orbital of the copper(II) in octahedral geometry for $Na_4[CuL(H_2O)_2]$ [39].

3.5. *X-ray diffraction*

Powder X-ray diffraction of iron(II), nickel(II) and copper(II) complexes were recorded and the lattice parameters were calculated by Ito's method. The lattice parameters for $Na_4[FeL(H_2O)_2]$ were found to be: $a = 20.671\,\text{Å}$, $b = 15.127\,\text{Å}$, $c = 11.430\,\text{Å}$,

$\alpha = 108.77$, $\beta = 94.90$, $\gamma = 128.08$ and cell volume $V = 2462.395$ Å^3; for $Na_4[NiL(H_2O)_2]$, $a = 16.775$ Å, $b = 12.595$ Å, $c = 11.730$ Å, $\alpha = 93.97$, $\beta = 121.45$, $\gamma = 105.01$ and $V = 1978.509$ Å^3; and for $Na_4[CuL(H_2O)_2]$ $a = 27.541$ Å, $b = 22.481$ Å, $c = 5.441$ Å, $\alpha = 91.91$, $\beta = 92.38$, $\gamma = 148.98$ and $V = 1718.828$ Å^3. These lattice parameters suggest that all the three systems are triclinic [21].

3.6. *Antifungal activity*

In order to understand the effect of chemicals on microorganisms like bacteria, fungi, virus, cancer etc., one has understand cellular structure and composition. A living cell is a self-contained, self-assembling, self-adjusting, self-perpetuating, isothermal system of organic molecules that extracts free energy and raw materials from its environment [40]. Cell walls have pores such that hydrated metal ions are unable to pass through while organic chemicals (lipo-soluble) pass through H-bonds exchange interactions with cell wall chemicals. Metal complexes of large organic molecules like the one under study here, surrounded by an organic moiety, are acceptable to the cell walls. Thus the metal ions go inside the cell, release the metal ion and cause the resultant toxic effect. Structure of the metal complex is equally important if it gives matching cone and socket or key-lock type structural attachment with the cell structure.

A number of studies on antibacterial, antifungal etc. activities of metal complexes with various ligands [41–44] report the toxic effects but the mechanisms of interaction are still not clear.

Antifungal activity has been studied in this paper by studying the effect of metal ions, ligand (xylenol orange, Na_4H_2L) and complex, $Na_4[CuL(H_2O)_2]$ on various fungi in molar ratio (in this case it is assumed that one or more ions will interact with one fungus cell) and the effect of certain maximum inhibitory concentration (1000 ppm) of the ligand and the metal complexes on various fungi.

Individually copper sulphate (1 mmol) and Na_4H_2L (1 mmol) were less effective than $Na_4[CuL(H_2O)_2]$ (0.6 mmol) in inhibiting spore germination of *Alternaria Solanae* where inhibition was observed to be 74, 64 and 98% respectively. Similarly, on *Cercospora Species* the inhibitory effect of $Na_4[CuL(H_2O)_2]$ was more (99%) than individual copper sulphate (78%) or Na_4H_2L (47%). On the remaining fungi the inhibitory effect of $Na_4[CuL(H_2O)_2]$ was more than Na_4H_2L, indicating the effect of complexation (table 5). This effect may be attributed to slow release of Cu(II) ions into the culture medium thereby causing inhibition of fungi [45].

The effect of complexation can also be seen in the data collected in table 6. Na_4H_2L was considerably less effective on *Alternaria brassicicola* where the inhibition was 38% but its manganese(II), iron(II) and cobalt(II) complexes were very effective showing 100, 99.5 and 96% inhibition respectively at 1000 ppm concentration (table 6). In case of *Helminthosporium oryzae* similar trends were obtained; in comparison to the ligand (25%), manganese(II), cobalt(II), nickel(II), copper(II) and zinc(II) complexes were more effective showing 97.8, 91.5, 81.3, 82.4 and 95.2% inhibition at 1000 ppm.

The metal complexes in general are more toxic to fungi than the parent ligand, probably due to chelate formation of the ligand with metal ions. In certain cases, the complexes in solution (metal salts + ligands) are more effective i.e., show more fungi-toxicity than individual metal salts or the ligand, indicating a good synergetic effect or the effect of complexation in solution [18].

Table 5. Effect of $CuSO_4$, Na_4H_2L and $Na_4[CuL(H_2O)_2]$ on spore germination of some fungi (% inhibition).

Fungus/Treatment	Host	Control	A	A_1	A_2	A_3	B	B_1	B_2	B_3	1	2	3	4
Alternaria brassicicola	*Brassica campestris*	0.83	96.42	91.15	81.19	62.22	87.65	81.21	75.24	64.67	91.15	82.31	69.57	57.64
Alternaria brassicae	*Brassica campestris* var. capitiata	3.41	99.03	93.90	90.16	86.45	34.65	29.21	26.24	19.67	79.35	77.00	59.82	54.83
Alternaria solanae	*Solenum tuberosum*	3.00	74.29	71.00	69.50	63.20	64.50	54.22	50.00	44.00	98.10	96.32	91.06	88.64
Cercospora species	*Blumea species*	31.79	78.12	74.91	73.90	71.96	47.04	46.10	43.14	39.79	99.83	99.12	98.04	96.77
Helminothosporium orazae	*Oryza sativa*	50.37	99.68	97.94	94.52	93.17	16.50	15.60	8.85	7.11	65.46	64.52	62.68	61.52
Curvularia penniseti	*Pennisetim pyphosias*	32.64	98.37	97.04	95.46	95.00	95.34	92.29	87.07	82.57	96.57	95.82	64.83	92.14
Curvularia lunata	*Oryza sativa*	11.79	91.69	86.51	85.34	79.37	97.88	95.93	92.32	87.74	71.67	79.16	87.85	88.75
Aspergillus nigger	*Air born saprophyte*	30.83	95.50	89.67	85.67	80.74	90.17	88.16	81.32	74.68	92.43	89.33	86.11	84.80
Aspergillus flavus	*Air born saprophyte*	19.33	91.09	77.74	69.01	57.84	94.56	79.32	72.33	59.94	93.51	98.03	79.19	73.94
Erysiphae pisi	*Pisum sativum*	70.81	94.43	92.43	90.00	88.09	94.07	91.47	89.83	96.84	96.90	92.95	91.85	87.76

Strength of the solutions:
$A = 1$ mmol $B = 1$ mmol $1 = 0.6$ mmol
$A_1 = 0.5$ mmol $B_1 = 0.5$ mmol $2 = 0.3$ mmol
$A_2 = 0.25$ mmol $B_2 = 0.25$ mmol $3 = 0.15$ mmol
$A_3 = 0.125$ mmol $B_3 = 0.125$ mmol $4 = 0.075$ mmol
A, A_1, A_2, A_3 = Copper sulphate; B, B_1, B_2, B_3 = Na_4H_2L (Xylenol Orange); 1, 2, 3, 4 = $Na_2[CuL(H_2O)_2]$.

Table 6. Effect of Na_4H_2L and $Na_4[ML(H_2O)_2]$ on spore germination of different fungi (% inhibition).

			Na_4H_2L		$Na_4[MnL(H_2O)_2]$		$Na_4[FeL(H_2O)_2]$		$Na_4[CoL(H_2O)_2]$		$Na_4[NiL(H_2O)_2]$		$Na_4[CuL(H_2O)_2]$		$Na_4[ZnL(H_2O)_2]$	
Fungus/Treatment	Host	Control	1	2	1	2	1	2	1	2	1	2	1	2	1	2
Alternaria brassicae	*B. Campestris* Var. capitiata	7.34	98.5	85.7	81.0	8.9	45.0	21.7	99.67	89.1	98.8	86.7	77.6	71.3	100.0	97.1
Alternaria brassicicola	*B. Campestris*	1.67	38.5	10.7	100.0	95.5	99.5	89.9	96.00	95.5	26.0	1.9	89.5	83.7	15.0	2.3
Alternaria solanae	*Solenum tuberosum*	10.0	72.0	47.4	98.2	87.2	97.6	71.5	91.4	60.2	99.3	88.3	92.7	92.2	97.1	86.3
Curvularia lunata	*O. sativa*	32.18	99.6	90.2	98.6	79.7	100.0	95.9	95.5	73.5	98.0	71.3	76.6	72.4	91.4	76.4
Helminthosporium orazae	*Oxyza sativa*	1.17	25.5	10.0	97.8	4.4	19.0	6.7	91.5	77.6	81.3	55.5	82.4	78.9	95.2	88.1
Curvularia species	*Brassica Campestris*	3.67	81.6	65.4	100.0	94.3	99.3	88.4	79.8	61.3	100.0	97.1	91.4	78.4	76.5	60.2
Colletotrichum capsici	*Capsicum annum*	36.43	92.5	78.5	37.5	85.3	83.3	51.5	63.3	48.8	89.9	70.2	98.5	88.5	76.6	61.1
Aspergillus nigger	*Saprophyte*	33.67	99.4	92.17	81.7	55.4	56.8	39.4	99.6	92.3	90.3	72.4	100.0	95.6	98.3	85.1
Aspergillus flavus	*Saprothyte*	19.67	100.0	95.67	99.5	86.7	87.3	71.1	99.1	92.1	92.5	76.54	98.7	91.2	67.8	56.8
Fusarium udum	*Cajanus cajan*	1.37	96.7	81.1	99.0	85.6	90.1	71.5	99.6	85.5	31.8	7.8	98.9	88.7	73.5	59.7

Strength of solutions (in ppm): 1. 1000, 2. 500.

For practical utility of these compounds, the inhibiting capacity of the complexes was compared with commercially available dithane-M-45 (a broad spectrum fungicide) which is used for complete inhibition of spore germination of several of the fungi using 2500 ppm under field condition. We observe that $Na_4[MnL(H_2O)_2]$ against *Alternaria brassicicola* and *Cercospora species*, $Na_4[ZnL(H_2O)_2]$ against *Alternaria brassicae*, Na_4 $[FeL(H_2O)_2]$ against *Curvularia lunata*, $Na_4[MnL(H_2O)_2]$ against *Curvularia Species*, $Na_4[NiL(H_2O)_2]$ against *Curvularia species* and $Na_4[CuL(H_2O)_2]$ against *Aspergillus niger*, were more effective showing 100% inhibition at 1000 ppm (others show nearly 100% inhibition at 1000 ppm) than the commercial fungicide dithane-M-45 which shows 100% inhibition at 2500 ppm on these fungi. This observation opens up a new field of research where metal complexes of organic molecules can be better fungicides than the commercial products.

Acknowledgements

Mr. Gajanan Pandey is grateful to CSIR New Delhi for financial support as a SRF. The authors also thank Prof. U. P. Singh, Department of Mycology and Plant Pthology, Institute of Agriculture Science, Banaras Hindu University, Varanasi for antifungal activity measurements.

References

[1] V.N. Tikhonov. *Zh. Anal. Khim.*, **41**, 1353 (1986).
[2] A. Ishida, E. Kaneko, T. Yotsuyangi. *Chem. Lett.*, **4**, 351 (1999).
[3] C. Gay, J. Collins, J.M. Gebicki. *Anal. Biochem.*, **273**, 143 (1999).
[4] K. Sugino. *Biosci. Biotechnol. Biochem.*, **63**, 773 (1999).
[5] K. Ogura, S. Kurakami, K. Seneo. *J. Inorg. Nucl. Chem.*, **43**, 1243 (1981).
[6] Refat A.-Hamid, Hussein M. El-Sagher, Mostafa K. Rabin. *Can. J. Chem.*, **75**, 162 (1997).
[7] M.A.H. Hafez. *Talanta*, **39**, 1189 (1992).
[8] S. Murakami, K. Ogura, T. Yoshino. *Bull. Chem. Soc. Jpn*, **58**, 2228 (1980).
[9] H.P. Srivastava, D. Tiwari. *Ind. J. Chem.*, **34A**, 550 (1995).
[10] W.E. Antholine, J.M. Knight, D.H. Petering. *Inorg. Chem.*, **16**, 569 (1977).
[11] D.H. Jones, R. Slack, S. Squires, K.R.H. Wooldrige. *J. Med. Chem.*, **8**, 676 (1965).
[12] D.L. Klayman, J.P. Sconill, J.F. Bafosevich, J. Bruce. *J. Med. Chem.*, **26**, 35 (1983).
[13] B.K. Sarma, J.S. Srivastava, B. Prithiviraj, U.P. Singh, S.N. Pandey. *Folia Microbiologica*, **43**, 393 (1998).
[14] J.D. Leach, R.H. Garber, W.H. Lange. *Plant Disease Reptr.*, **Suppl. 259**, 213 (1959).
[15] K.C. Agrawal, A.O. Santoelli. *Prog. Med. Chem.*, **15**, 349 (1978).
[16] A. Balasubrahmanyan, V.K. Baranwal, M.L. Lodah, A. Varma, H.C. Kapoor. *Plant Science (Limerick)*, **154**, 13 (2000).
[17] D. Peciulyte and A. Lugauskas, *Proceedings of the International Conference: Development of Environmentally friendly plant protection in Baltic Region*, edited by L. Metspalu and S. Mitt, Tartu, Estonic (2000).
[18] M.J.M. Campbell. *Coord. Chem. Rev.*, **15**, 2799 (1975).
[19] (a). H. Sato, Y. Yokoyama, K. Momoki. *Talanta*, **94**, 217 (1977); (b) D.C. Olson, D.W. Margerum. *Analyst. Chem.*, **34**, 1296 (1962).
[20] B.N. Figgis, J. Lewis, in *Modern Coordination Chemistry*, edited by J. Lewis and R.C. Willkins, p. 403, New York, Intersciences (1964).
[21] L.G. Azaroff, M.J. Buerger, *The Powder Method in X-ray Crystallograph*, pp. 106–123, McGraw-Hill Book Company, New York (1958).
[22] U.P. Singh, V.N. Pandey, K.G. Wagnar, K.P. Singh. *Can. J. Botany*, **68**, 1354 (1990).
[23] V.G. Panse, P.V. Sukhatme, in *Statistical Methods for Agricultural Workers*, edited by C.G. Raghava Kurup, pp. 152–157, ICAR, New Delhi (1967).

[24] A.K. Agarwal, V.M. Bucha. *Ind. J. Chem.*, **24A**, 163 (1985).
[25] H. Mizuguchi, T. Yotsuyangi. *Anal. Sci.*, **17**, 1687 (2001).
[26] W.J. Geary. *Coord. Chem. Rev.*, **7**, 81 (1971).
[27] S. Glasstone, *An Introduction to Electrochemistry*, p. 71, Litton Educational Publishing, Inc., New York (1999).
[28] Z.M. Miodragovich, G. Vuckovic, V.M. Leovac. *J. Serb. Chem. Soc.*, **66**, 597 (2001).
[29] K. Nakamoto, in *Advance in Chemistry of Coordination Compounds*, edited by S. Krishner, p. 437, McMillen, New York (1961).
[30] V. Stefov, V.M. Petrusevski, V. Soprajanov. *J. Mol. Stru.*, **293**, 97 (1993).
[31] K. Nakamoto, *Infrared and Raman Spectra of Inorganic and Coordination Compounds, Part B*, p. 60, John Willey & Sons, Inc. (1997).
[32] B.N. Figgis, *Introduction to Ligand Fields*, p. 220, Wilay Eastern Ltd. (1976).
[33] M.N. Chary, B.A. Sastry, G. Ponticellis, J.S. Shannon. *Aust. J. Chem.*, **18**, 673 (1963).
[34] V.V. Ramanujan, B. Shivshankar. *J. Ind. Chem. Soc.*, **62**, 734 (1985).
[35] M.A. Ali, R. Bose. *J. Inorg. Nucl. Chem.*, **39**, 265 (1977).
[36] H.R. Germasan, J.D. Swalen. *J. Chem. Phys.*, **56**, 3221 (1962).
[37] B.J. Hathway, D.E. Billing. *Coord. Chem. Rev.*, **6**, 143 (1970).
[38] B.H.C. Allen Jr, G.F. Kokostzka, R.G. Inskeep. *J. Am. Chem. Soc.*, **86**, 1023 (1964).
[39] M. Fujwara, H. Wakita, T. Masushita. *Polyhedron*, **10**, 1773 (1991).
[40] D.L. Nelson, M.M. Cox, *Lehninger Principles of Biochemistry*, 3rd edn, p. 18, Worth Publisher, New York (2000).
[41] S. Bellu, E. Hurre, M. Trape, C. Trosserer, G. Molina, C. Drogo, P.A.M. Williams, A.M. Aria, J.C.M. Acevedo, S. Zacchino, M. Sortino, D. Campagnoli, M. Razzotti. *Polyhedron*, **24**, 501 (2005).
[42] M.T.H. Tarafder, K.-B. Chew, K.A. Crouse, A.M. Ali, B.M. Yamin, H.-K. Fun. *Polyhedron*, **21**, 2683 (2002).
[43] S.M.M.H. Majumdar, M.A. Ali, F.E. Smith, A.U. Mridha. *Polyhedron*, **21**, 2183 (1988).
[44] A. Balasubrahmanyan, V.K. Baranwal, A. Varma, H.C. Kapoor. *Plant Science (Limerick)*, **154**, 13 (2000).
[45] G. Cik, A. Bujdakova, F. Sersen. *Chemosphere*, **44**, 313 (2001).