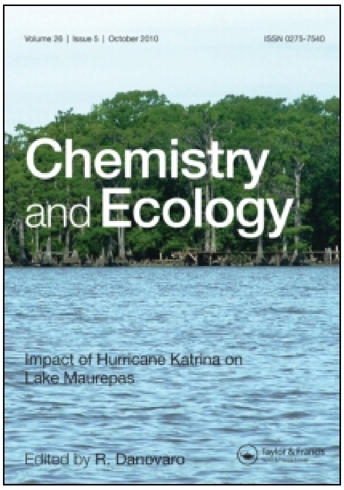

**Chemistry and Ecology**
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713455114

# Formation of Soluble Complexes of Metals with Humic Acid and Its Environmental Significance

Ashok Kumar Pandey[a]; Shri Dhar Pandey[a]; Virendra Misra[a]; P. N. Viswanathan[a]
[a] Ecotoxicology Section, Industrial Toxicology Research Centre, Lucknow, India

**To cite this Article** Pandey, Ashok Kumar , Pandey, Shri Dhar , Misra, Virendra and Viswanathan, P. N.(1999) 'Formation of Soluble Complexes of Metals with Humic Acid and Its Environmental Significance', Chemistry and Ecology, 16: 4, 269 — 282

**To link to this Article: DOI:** 10.1080/02757549908037652

**URL:** http://dx.doi.org/10.1080/02757549908037652

*Chemistry and Ecology*, 1999, Vol. 16, pp. 269–282
Reprints available directly from the publisher
Photocopying permitted by license only

Published by license under
the Gordon and Breach Science
Publishers imprint.
Printed in Malaysia.

# FORMATION OF SOLUBLE COMPLEXES OF METALS WITH HUMIC ACID AND ITS ENVIRONMENTAL SIGNIFICANCE

ASHOK KUMAR PANDEY, SHRI DHAR PANDEY,
VIRENDRA MISRA* and P. N. VISWANATHAN

*Ecotoxicology Section, Industrial Toxicology Research Centre,*
*Post Box No. 80, M.G. Marg, Lucknow-226 001, India*

*(Received 12 January 1999; In final form 22 March 1999)*

In order to investigate the role of functional groups present in humic acid(s) (HA) during complex formation, water soluble complexes between HA isolated from soil and metals (Pb, Ca, As, Ni, Cr, Co, Cu, Cd, Fe, Mn, Mg and Zn) were prepared and subjected to infrared (IR) spectroscopy. The IR data revealed the involvement of the —OH group of humic acid in complex formation with all metals except copper and arsenic, whereas the —COOH group of HA was found to be the preferred site of binding for all the metals. The significance of findings in relation to detoxification of environmental pollutants is discussed in this paper.

*Keywords:* Complex formation; humic acid; infrared (IR) spectroscopy; bioavailability; detoxification

## INTRODUCTION

In water bodies polluted by heavy metals, insoluble species settle out and get bonded to various compartments of the sediment (Nriagu and Pacyna, 1988). Sediment acts as an intermediate sink for most of the trace metals which may re-enter the water column by various physico-chemical and biological processes. Humic substances are found in natural surface waters of sediment and soils, in both soluble and

---

*Corresponding author. e-mail: intox@itrc.sirnetd.eruet.in insoluble forms. They act as a ligand and play a crucial role in the speciation, transport and deposition of a variety of compounds ranging from metal ions to lipophilic compounds (MacCarthy, 1989). Humic substances are considered as natural polyelectrolytic organic compounds of complex structure involving a proportion of more or less condensed aromatic rings with a large number of attached —OH and —COOH groups (Stevenson, 1982). They are mixtures of cross-linked polymers of different molecular weight and charge-densities, their solubility probably being related to these factors (Stevenson, 1985; Wershaw *et al.*, 1986).

Complexation is an important aspect of environmental analytical chemistry for understanding the fate of environmental pollutants. The dynamic nature of humic substances in ecosystems and the distribution and total mass of pollutants in the ecosystem could be governed *inter alia* by pollutant-humus interaction. Humic acid can act as a reducing agent reducing chromium (VI) to chromium (III), altering bioavailability and toxicity of metals (Sparks, 1995). The results of Warwick *et al.* (1997) indicated that nickel-humic acid binding decreases with increasing temperature. Also at higher temperature some coagulation of the colloidal nickel humate occurs. Due to its enormous chelation capacity humic acid is capable of detoxifying lakes that are affected by metal pollution (Tan, 1994). The role of humic acid in the sequestration of metals using fluorescence quenching was reported by Misra *et al.* (1996). As an extension of their studies complexes of various metals with humic acid, isolated from soil, were prepared and characterized by IR spectroscopy, to investigate the involvement of functional groups of humic acid in the binding of metals.

## MATERIALS AND METHODS

Humic acid isolated from local soil was used in all the experimental studies (the percentage of carbon, hydrogen and nitrogen of humic acid isolated from natural soil was found similar to Aldrich humic acid). Other chemicals used in the study were from E. Merck and Qualigens AnalaR grade.

## Isolation of Humic Acid from Soil and Elemental Analysis

The method of Haworth (1971) was employed for the extraction of humic acid from the natural soil. Two hundred grams of air dried soil, rich in humus, collected locally, was defatted with ethanol-benzene mixture (1:1). It was then shaken with 0.2 N sodium hydroxide (12 volumes), centrifuged and the pH of the clear supernatant adjusted to 1.0 with 2 N hydrochloric acid. A gelatinous precipitate was obtained which was filtered on a sintered glass funnel and dried at 90°C, with the recovery of about 1.2% HA. Humic acid samples from soil and Aldrich were analysed for elemental analysis on a Carlo-Erba EA 1108 elemental analyser (Tab. I)

## Preparation of Humic Acid Solution

Humic acid solutions were prepared by dissolving Aldrich humic acid/humic acid isolated from soil in deionized water. Fresh solutions were prepared prior to each experiment and stored in the dark at 4°C. The pH of the solution was 6.85.

## Preparation of Soluble Complexes

For soluble complex formation, equimolar concentrations of metal salts ($CaCl_2 \cdot 2H_2O$, $CrCl_3 \cdot 6H_2O$, $MnCl_2 \cdot 4H_2O$, $FeCl_3$ (anhydrous),

TABLE I Elemental analysis of humic acid isolated from soil and procured from commercial sources (Aldrich)

| *Element* | *HA (Aldrich)* | *HA (isolated from soil)* |
|---|---|---|
| C % | 44.67 | 43.88 |
| H % | 5.87 | 5.74 |
| N % | 4.88 | 5.17 |
| O % | 43.9 | 44.6 |
| Total acidity (mol/kg) | 12.3 | 12.5 |
| COOH (mol/kg) | 4.1 | 4.2 |
| Phenolic OH (mol/kg) | 8.2 | 8.3 |
| Ash (%) | 0.58 | 0.64 |
| C/N Ratio | 9.1 | 8.4 |
| C/H Ratio | 7.6 | 7.7 |
| O/H Ratio | 7.4 | 7.8 |

$CoCl_2 \cdot 6H_2O$, $NiSO_4 \cdot 7H_2O$, $CuCl_2 \cdot 2H_2O$, $ZnSO_4 \cdot 7H_2O$, $(CH_3COO)_2Pb \cdot 3H_2O$, $Na_2HAsO_4 \cdot 7H_2O$, $CdCl_2 \cdot H_2O$, $MgCl_2 \cdot 6H_2O$) and Aldrich humic acid were prepared in deionized water. The pH of the salt solutions ranged from 6.28 to 7.54. Both were mixed according to the method of Job as cited in Rossotti and Rossotti (1961). The final pH of the metal ion/humic acid mixture ranged from 6.10 to 6.56.

The mixture was incubated for 15 days in the dark with occasional shaking. After 15 days the complex mixture was centrifuged at 10,000 rpm to get the soluble complexes.

### Spectroscopic Studies

The soluble complexes prepared above were subjected to IR spectral study using Perkin Elmer-881 IR Spectrophotometer either as KBr pellet, or neat, taking care to avoid moisture.

## RESULTS

Humic acid showed IR peaks at 3808, 3468, 2936, 2336, 1626 and 1400 $cm^{-1}$ (Fig. 1). The peak at 3808 $cm^{-1}$ of humic acid is due to the free —OH group present in humic acid. The shifting of a peak on treatment with arsenic at 3660 and 3592 $cm^{-1}$ (Fig. 2), with copper at 3650, 3586 and 3510 $cm^{-1}$ (Fig. 3), with cadmium at 3492 $cm^{-1}$ (Fig. 4), with manganese at 3438 $cm^{-1}$ (Fig. 5) and with nickel at 3438 $cm^{-1}$ (Fig. 6) indicated the binding of free —OH group of humic acid with these metals. The band at 3468 $cm^{-1}$ of humic acid showed —OH stretching vibration of phenolic —OH (contribution from aliphatic OH). The —NH stretching band also contributes to absorption in this region. Shifting of a band on treatment with iron at 3422 $cm^{-1}$ (Fig. 7), chromium at 3418 $cm^{-1}$ (Fig. 8), lead at 3410 $cm^{-1}$ (Fig. 9), magnesium and zinc at 3404 $cm^{-1}$ (Figs. 10 and 11), calcium at 3378 $cm^{-1}$ (Fig. 12) and cobalt at 3324 $cm^{-1}$ (Fig. 13), indicated the binding of these metals with this group of the humic acid molecule. Copper and arsenic were also found to show similar type of binding. The 2936 $cm^{-1}$ band observed indicates the characteristic of aromatic —CH stretching vibration. This suggests that the aromatic ring of humic acid is not completely substituted. The band centered at

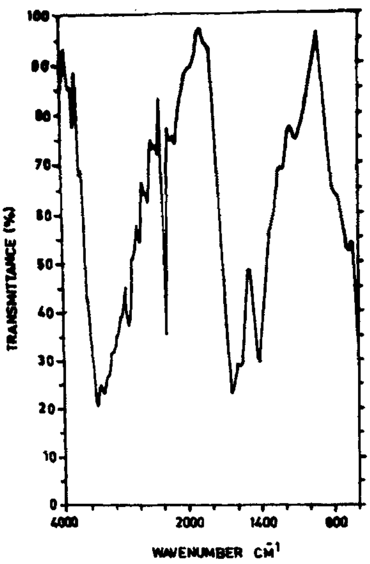

FIGURE 1 Infrared spectra of humic acid.

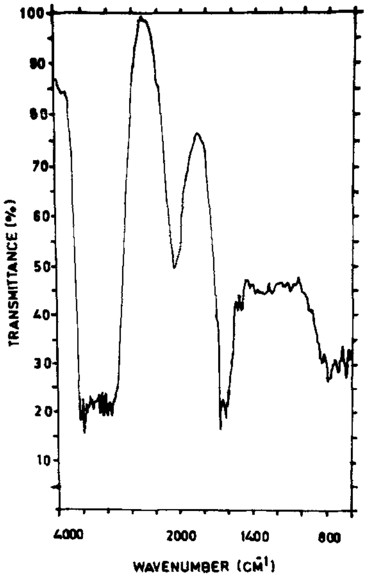

FIGURE 2 Infrared spectra of humic acid–As complex.

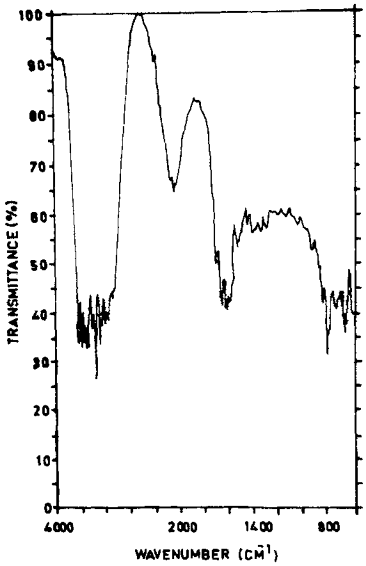

FIGURE 3 Infrared spectra of humic acid–Cu complex.

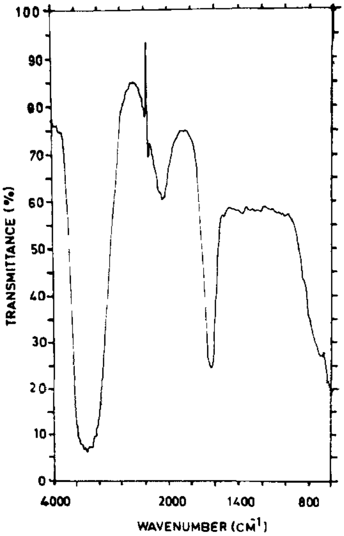

FIGURE 4 Infrared spectra of humic acid–Cd complex.

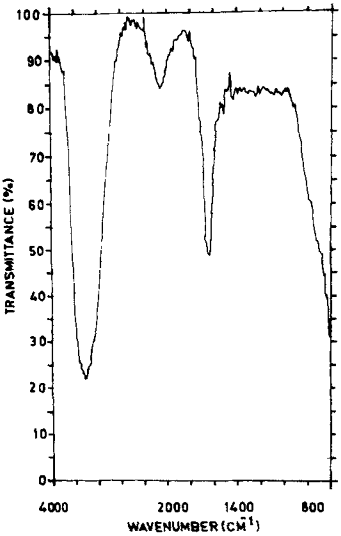

FIGURE 5 Infrared spectra of humic acid–Mn complex.

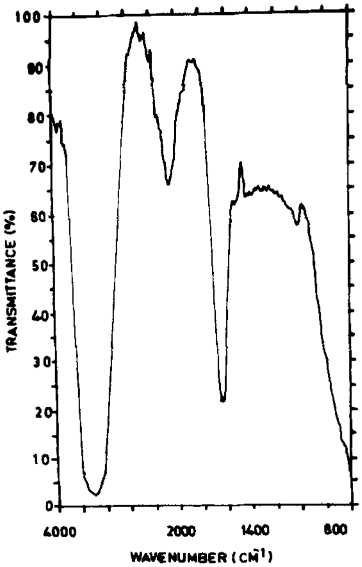

FIGURE 6 Infrared spectra of humic acid–Ni complex.

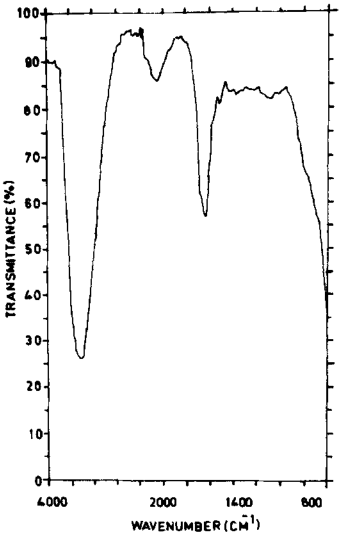

FIGURE 7 Infrared spectra of humic acid–Fe complex.

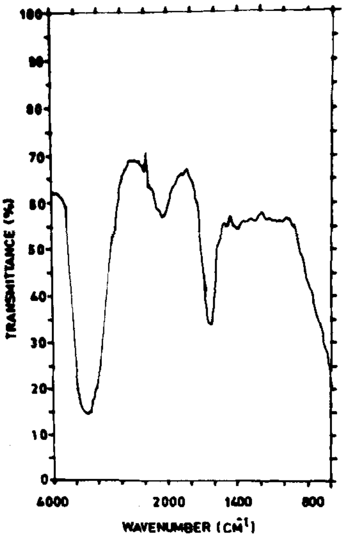

FIGURE 8 Infrared spectra of humic acid–Cr complex.

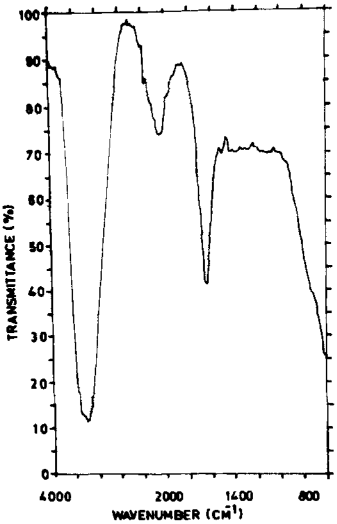

FIGURE 9 Infrared spectra of humic acid–Pb complex.

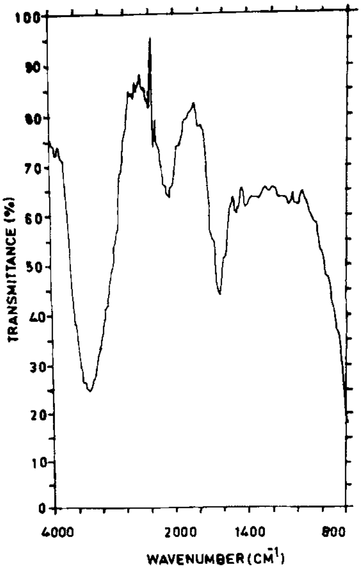

FIGURE 10 Infrared spectra of humic acid–Mg complex.

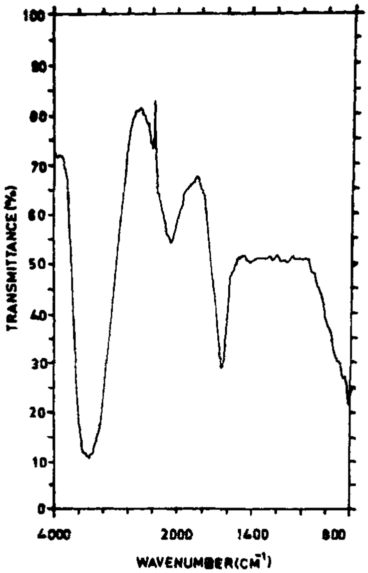

FIGURE 11 Infrared spectra of humic acid – Zn complex.

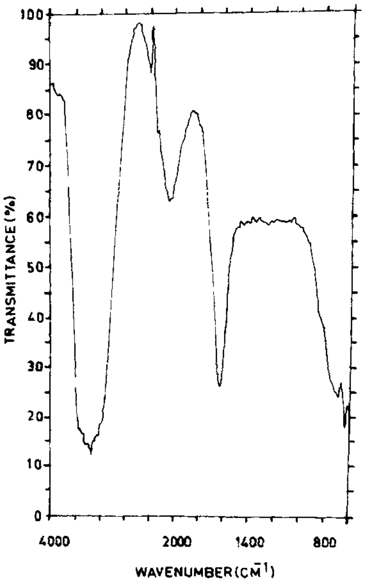

FIGURE 12 Infrared spectra of humic acid – Ca complex.

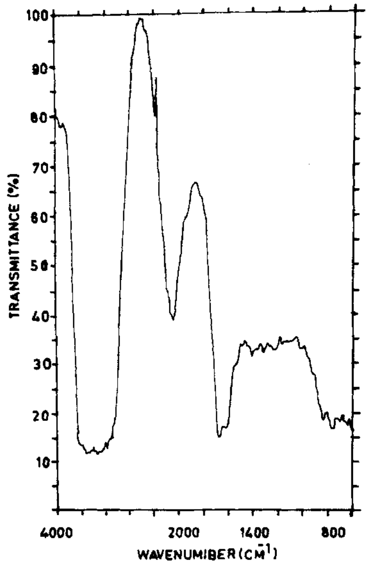

FIGURE 13 Infrared spectra of humic acid–Co complex.

about 2336 cm$^{-1}$ observed is due to —OH stretching vibration of H-bonded —COOH and —C≡C— stretching of acetylenic compounds. Shifting of this band to different frequencies reveals binding of this group with all the metals. The sharp band at 1626 cm$^{-1}$ and 1400 cm$^{-1}$ in case of humic acid is due to C=O stretching vibration, mainly of —COOH. In this case the carboxylate anion has an asymmetrical stretching vibration at 1626 cm$^{-1}$ and symmetrical stretching vibration at 1400 cm$^{-1}$. The position of this band suggests that the H-bonding of —COOH is not similar to H-bonding of —OH groups. Shifting of the band to lower or higher side of frequency is due to the binding of above metals with this group. Such type of binding was noticed in case of copper and arsenic.

## DISCUSSION

The present spectral data indicate the complex formation of metals with humic acid. Humic acids, having large molecular masses and various functional groups, are complex structures undergoing diverse mutual interactions in different degrees, depending also in the environment (for instance pH or concentration of different cations). Humic acid is an important ligand for most heavy metals in soils, waters and sediments (Livens, 1991). It also influences metal transport through soil layers and control the uptake by plants *via* root systems (Giusquiani *et al.*, 1992; Evans, 1989). Misra *et al.* (1999) have studied the influence of humic acid on the bioavailability of gamma —HCH in aquatic macrophyte to understand the behaviour of gamma —HCH in the fresh water ecosystems using sediment-water-biota compartments on different photoperiods. Humic acid is also reported to affect the bioavailability of several xenobiotics to *Daphnia magna* (Steinberg *et al.*, 1993). The active functional groups of humic acid form complexes with paramagnetic metal cations (Schnitzer and Khan, 1972; Mikkii *et al.*, 1997). Humic acid forms charge transfer complexes with copper (II) (Boyd *et al.*, 1981). Senesi *et al.* (1986) have found that soil humic acid has two different classes of binding sites for copper (II) and iron (III). Stable and soluble complexes (mainly as hydroxo-complexes) of iron (III) and copper (II) under natural environmental conditions, with humic substances and leaf litter extracts have been reported (Buffle *et al.*, 1980; Senesi *et al.*, 1989). In this study, all the metals investigated were strongly complexed at the —COOH group of humic acid suggesting —COOH group is the preferred site for binding. However, the involvement of —OH group of humic acid in the complex formation was also noticed with all the metals with the exception of copper and arsenic, thereby limiting its availability for plant or animal uptake.

The shifting of peaks towards higher frequencies in IR spectra could be due to the reduction in hydrogen bonding between functional group of humic acid and metals whereas shifting towards lower frequencies could be the result of strong hydrogen bonding. However, there are three possibilities of binding of humic acid with metals:

(a) by chelation between carboxyl and phenolic hydroxyl;
(b) by chelation between two carboxyl groups; and
(c) by complexation with a carboxyl group.

Manahan (1994) reported that these stable, soluble complexes can diminish the removal effectiveness of heavy metals from leachate waters of hazardous waste sites when precipitation techniques are employed as the remediation technique in water treatment scenarios. On the other hand, the binding of heavy metals to humic acid can enhance biological treatment of inorganic contaminants by reducing toxicity of heavy metals to the microbes.

## CONCLUSIONS

Formation of stable and soluble complexes as a result of interaction of all the above metals with the functional groups (—COOH or —OH) present in humic acid may play a significant role in environmental detoxification. Such studies can also provide an insight into element distribution patterns, identify metal bioavailability and toxicity in ecosystems and explain transformation and mobility of metal species into sediment, water and biota compartments.

### *Acknowledgements*

Thanks are due to Dr. P. K. Seth, FNA, Director, Industrial Toxicology Research Centre, Lucknow for his keen interest in this work. The excellent word processing by Mr. Pramod Kumar Srivastava is also acknowledged. This is ITRC Publication No. 2023.

### *References*

Boyd, S. A., Sommers, L. E., Nelson, D. W. and West, D. X. (1981) The mechanism of Cu(II) binding of humic acid an electron spin resonance study of a Cu(II) humic acid complex and some adducts with nitrogen donors. *Soil Sci. Soc. Amer. J.*, **45**, 445–448.

Buffle, J., Deladoey, P., Greter, F. L. and Haerdi, W. (1980) Study of the complex formation of Cu(II) by humic acid fulvic substances. *Anal. Chim. Acta*, **116**, 255–274.

Evans, L. J. (1989) Chemistry of metal retention by soils. *Environ. Sci. Technol.*, **23**, 1046–1056.

Giusquiani, P. L., Giglioth, G. and Businelli, D. (1992) Heavy metals in the environment. *J. Environ. Qual.*, **21**, 330–335.

Haworth, R. D. (1971) The chemical nature of humic acid. *Soil Science*, **111**, 71–79.

Livens, F. R. (1991) Chemical reactions of metals with humic material. *Environ. Pollut.*, **70**, 183–208.

MacCarthy, P. (1989) Aquatic humic substances and their influence in the fate and treatment of pollutants. In: *American Chemical Society*, Eds. Suffet, I. H. and MacCarthy, P., Washington, DC. pp. XVII–XXX.

Manahan, S. E. (1994) Complexation of humic substances. In: *Environmental Chemistry* 6th Ed. Lewis Publishers, CRC Press, Inc., Boca Raton, USA, pp. 80–83.

Miikki, V., Senesi, N. and Honninen, K. (1997) Characterization of humic material formed by composting of domestic and industrial biowastes. *Chemosphere*, **34**, 1639–1651.

Misra, V., Pandey, S. D. and Viswanathan, P. N. (1996) Environmental significance of humic acid in the sequestration of metals. *Chemistry and Ecology*, **13**, 103–112.

Misra, V., Pandey, S. D. and Viswanathan, P. N. (1999) Effect of humic acid on the bioavailability of gamma-hexachlorocyclohexane in *Marsilea minuta* (L). *Environ. Monit. Assessment.*

Nriagu, J. O. and Pacyna, J. M. (1988) Quantitative assessment of world wide contamination of air, water and soils by trace metals. *Nature*, **333**, 134.

Rossotti, F. J. C. and Rossotti, H. (1961) *The determination of stability constants*, McGraw-Hill Book Co., pp. 38–57.

Schnitzer, M. and Khan, S. U. (1972) *Humic Substance in the Environment*. Marcel Dekker, New York, p. 319.

Senesi, N., Sposito, G. and Martin, J. P. (1986) Cu(II) and Fe(III) complexation by soil humic acids: an IR and ESR study. *Sci. Total Environ.*, **55**, 351–362.

Senesi, N., Sposito, G. and Bradford, B. R. (1989) Iron, copper and manganese complexation by forest leaf litter. *For. Sci.*, **35**, 1040–1057.

Sparks, D. L. (1995) Chemistry of soil organic matter. In: *Environmental Soil Chemistry*, Academic Press, Inc. N. York, pp. 53–80.

Steinberg, C. E. W., Xu, Y., Lee, S. K., Freitag, D. and Kettmp, A. (1993) Effect of dissolved humic material (DHM) on bioavailability of some organic xenobiotics to *Daphnia magna. Chem. Speciation Bioavailability*, **5**, 1–9.

Stevenson, F. J. (1982) *Humus Chemistry-Genesis, Composition, Reaction*. Wiley & Sons, New York, pp. 2–53.

Stevenson, F. J. (1985) Geochemistry of humic substances, In: *Humic Substances in Soil, Sediment and Water*, Eds. Aiken, G. R., McKnight, D. M., Wershaw, R. L. and McCarthy, P., Wiley & Sons., New York, pp. 13–52.

Tan, K. H. (1994) *Environmental Soil Science*, Marcel Dekker, Inc., New York, pp. 159–177.

Warwick, P., Kall, A., Jhu, J., Dimmock, P. W., Robbins, R., Carlsen, L. and Larsen, P. (1997) Effect of temperature on the nickel humic acid equilibrium reaction. *Chemosphere*, **35**, 2471–2477.

Wershaw, R. L., Thorn, K. A., Dinckney, D. J., MacCarthy, P., Rice, J. A. and Hemond, H. F. (1986) Application of membrane model to the secondary structure of humic material in peat. In: *Peat and Water*, Ed. Fuchsman, C. H., Elsevier Applied Science Publishers, pp. 133–157.