Asian Journal of Chemistry Vol. 17, No. 3 (2005), 1363–1370

REVIEW

Resistance Mechanism of Plants against Cadmium

RIFFAT JOHN, KASTURI GADGIL* and SATYAWATI SHARMA†
Centre for Energy Studies, Indian Institute of Technology,
Hauz Khas, New Delhi-110 016, India
E-mail: kgadgil@hotmail.com

Cadmium in soils is known to originate from geogenic (natural) and anthropogenic (industrial) sources. Cadmium in soils is known to be more mobile and readily absorbed and incorporated into plant tissues compared with lead and mercury. Due to inherent genetic and physiological characteristics plants have long been known to accumulate cadmium from soils. Some plants known as hyper-accumulators are able to tolerate high levels of elements in the root and shoot cells. The ability to both tolerate elevated levels of heavy metals and to accumulate them to unusually high concentrations is the result of various biological mechanisms which plants have developed during evolution. Phytoremediation is the use of specially selected and engineered metal accumulating plants for environmental clean-up.

Key Words: Cadmium, Resistance mechanisms, Plants.

INTRODUCTION

Cadmium is one of the components of the earth's crust and present everywhere in the environment. The natural occurrence of cadmium in the environment results mainly from gradual phenomena such as rock erosion and abrasion that estimates for 15,000 mt per annum[1]. Naturally existing concentration of cadmium in atmosphere is 0.1–0.5 ng/m^3, in earth's crust is 0.1–0.5 μg/g but much higher levels may accumulate in sedimentary rocks and marine phosphates. The widespread use of cadmium is based on its unique physical and chemical properties. It is highly resistant to chemicals, high temperature and ultraviolet light[2]. Cadmium is widely used in special alloys, pigments coatings, stabilizers and above all (almost 70% of its use) in Ni-Cd batteries[3]. It can enter air from the burning of coal, household waste and metal mining as well as refining-process which may increase the level of cadmium in the soil varying from 100–600 mg/kg dry wt.[4, 5] or more[6]. The general trend of metal enrichment appears to be urban > rural > remote location.

---

†Center for Rural Development and Technology, Indian Institute of Technology, Hauz Khas, New Delhi-110 016, India.

Some countries have set tolerance limits on heavy metal additions to soil because their long-term effects are unknown. These limits are usually set for plough layer of soil where most of the root activity occurs. The value of potentially toxic elements (PTE) proposed by Council of European Economic Committee[7] for cadmium concentration in soil is 1.0–3.0 mg/kg of dry soil and maximum annual addition of total cadmium to soils[8] is 150 g ha^{-1}.

Cadmium appears to be absorbed passively[9] and translocated freely. The uptake of cadmium from soil to above ground parts depends upon many factors including (a) the position of the total cadmium that is available to the plant root system and (b) the pH. Optimum cadmium mobility is achieved[10, 11] at pH 4.5–5.5. The absorption of cadmium almost doubled for each increase of 0.5 units[12] in the pH from 4–7.

Cadmium accumulation in plants: Most of the cadmium that enters the plant system accumulates in the roots and only a small portion is translocated to stem, leaves, pods and seeds[13]. Accumulation of Cd is directly proportional to the concentration of cadmium supplied to the growing plant as well as the phonological stage at which cadmium is supplied. However, the actual accumulations are influenced by the plant species and soil properties[14, 15]. Vassilev *et al.*[16] observed 10 times higher Cd accumulation in roots than over ground parts. Cadmium retention in the roots might be due to the cross linking of Cd to carboxyl groups of cell wall proteins[17] and/or an interaction with the thiol groups of soluble proteins and non-proteins thiol operating as a tolerating mechanism in root cells[18]. There are some plants (e.g., *Alyssum spp., T. carrulescens*) that accumulate maximum cadmium in their biomass than the surrounding environmental concentrations in which they grow. These are called hyper-accumulators[19, 20].

Mechanism of tolerance: All organisms possess mechanisms that regulate metal ion accumulation and, thus, avoid their toxicity[21]. The mechanisms that provide tolerance in plants are largely unknown[22]. Since plants generally have no choice about where to germinate and grow, they must develop some specific physiology to collectively enable them to adapt to unfavourable environmental conditions to survive[23, 24].

Binding of metal to cell wall

In nature, the root system of plants acts usually as the first barrier to heavy metals in the soil. In spite of differential mobility of metals in plants, root system accumulates them to a significantly higher extent[16]. At the first barrier, cadmium can be immobilized by means of cell wall[25, 26] and extra-cellular carbohydrates like mucilage and callose[27, 28]. In some cases, Cd ions seem to be mostly bound by pectic sites and hystidyl groups of cell wall[29]. The tolerance imparted by these mechanisms is primarily dependent on the concentrations of cadmium supplied[30].

Immobilization of metal through phytochelatin

Once cadmium has entered the cytosol, a system strictly related to sulphur metabolism is promptly activated, finally resulting in the production of important complexing agents termed as phytochelatins[31–34]. They form a family of structures with increasing repetition of the $(\gamma\text{-Glu-Cys})_n\text{-Gly}$ where n has been reported to be as high as 11, but is generally in the range of 2–5. Phytochelatins form various complexes with cadmium (with molecular mass of about 2500 or 3600), due to the presence of thiol groups of Cys, which chelate cadmium, and as a result prevent it from circulating as a free Cd^{2+} inside the cytosol[35]. The production of phytochelatins is a widespread mechanism of cadmium detoxification in higher plants[33, 36, 37].

Metallothioneins

It has been documented that in animals and certain fungi metal uptake induces the production of small cysteine-rich proteins known as metallothioneins (MTs). Metallothioneins are low-molecular-weight, cysteine-rich proteins that have high affinity for binding metal cations[38], such as Cd, Cu and Zn. In plants metallothioneins have been identified only in wheat[39] and *Arabidopsis.* These are small gene coded cys rich polypeptides, generally lacking aromatic amino acids[40] and have a high metal content in coordination of metal thiolate clusters. Their molecular weight varies[41] from 8–14 K Da and are thought to be aggregates of phytochelatins. MTs from many species, although differing in some respects, are remarkably similar in structure and retain a high degree of homology at the protein level[42] implying important biological functions. A critical and essential biological function for MTs, individually or collectively, has yet to be identified. However, a number of roles associated with cellular and tissue stress has been attributed to MT. These roles include the detoxification of heavy metals, homeostatic regulation of essential metals and protection of tissues against various forms of oxidative injury[43]. MTs behave similarly as PCs and often metal complexation duties shared between MTs and PCs as seen in *Datura* and *Zea mays*[44].

Vacuolar compartmentalization

A very significant role in detoxification and tolerance is played by vacuolar compartmentalization. It prevents free circulation of Cd ions in the cytosol and forces them into a limited area[30]. Cadmium, free and complexed, is sequestered in vacuole of root cells in most species. It is actively transported from cytosol into vacuole across the tonoplast *via* an H^+/Cd^+ antiport or an ATP-dependent phytochelatin transporter[45, 46]. Cd-Pc complexes as well as apo-chelatin are transported against the concentration gradient across the tonoplast by means of specific carriers. They accumulate inside tonoplast vesicle up to 38 times more than external solution[47]. In the vacuole because of acidic pH, these complexes dissociate and cadmium can be complexed by vacuolar organic acids like citrate, oxalate and malate[48] and possibly by amino acids. Apo-phytochelatins may be degraded by vacuolar hydrolysis and/or return to the cytosol, where they can continue to carry out their shuttle role.

Stress proteins

Plants when subjected to cadmium show the induction of several classes of heat shock proteins (hsps) (hsp 100, hsp 90, hsp 70, hsp 60) or stress proteins or hsp cognates[49]. It has been demonstrated that the DNA of CD stressed cells produces specific mRNA transcripts, which regulate the synthesis of stress proteins[50, 51]. In several species, Cadmium exposure induces the synthesis of stress proteins with molecular mass ranging[52, 53] from 10,000-70,000 Da. Rivetta *et al.*[54] demonstrated that cadmium binds to calmodulin and competes with calcium in this binding.

Stress ethylene and stress triterpenes

Cadmium has shown to stimulate ethylene biosynthesis through methione-S-adenosyl methionine, 1-aminocyclopropane-1-carboxylic acid ethylene (MSEA) pathway[55]. Production of stress ethylene increased the activity of guaiacol peroxidases and accumulation of soluble and insoluble phenolics[56] and it was hypothesized that this increase in ethylene production could be contributed to cadmium sequestration, which diminished the cadmium stress.

Phytoremediation

The use of specially selected and engineered metal accumulating plants for environmental clean up is an emerging technology called phytoremediation[57–60].

Phytoremediation of metal contaminated sites offers a low cost method for soil remediation and some extracted metals may be recycled for value. Because cost of growing a crop is minimal compared to those of soil removal and replacement, so the use of plants to remediate hazardous soils is seen as having great promise. Other recent reviews on many aspects of soil phytoremediation are available[57, 61–64]. Phytoremediation is the use of plants to make soil contaminants non-toxic and is also often referred to as bioremediation, botanical bioremediation or Green Remediation. The idea of using rare plants which hyperaccumulate metals to selectively remove and recycle excessive soil metals was introduced by Chaney *et al.*[65] and has increasingly been examined as a potential practice and a more cost-effective technology than soil replacement, solidification or washing strategies presently used[62, 66].

Strategies of Phytoremediation

Rhizosphere biodegradation: In this process, the plant releases natural substances through roots that supply nutrients to microorganisms in the soil. The microorganisms enhance degradation[62].

Phytostabilization: In phytostabilization, chemical compounds released by the plants immobilize contaminants rather than degrading them[60].

Phytoextraction: Plant roots absorb the contaminants along with other nutrients and water. The contaminant mass is not destroyed but ends up in the shoots and leaves. This method is used primarily for wastes containing metals[67].

Rhizofiltration: It is similar to phytoaccumulation, but the plants used for cleanup are raised in greenhouses with their roots in water. As the roots become saturated with contaminant, they are harvested and disposed[68].

Phytovolatilization: Here plants take up water containing organic contaminants and release the contaminants into the air through their leaves.

Phytodegradation: Phytodegradation involves metabolization and destruction of contaminants within the plant tissues.

Plant assisted bioremediation in which plant roots in conjunction with their rhizophoric microorganisms are used to remediate soils contaminated with organics.

The best hyperaccumulators[62, 70] have (a) high accumulation rate, even at low environmental concentration of the contaminant; (b) ability to accumulate very high levels of contaminants; (c) ability to accumulate several metals; (d) fast growth; (e) High biomass production and (f) Resistance to diseases and pests.

Genetic engineering for phytoremediation

Improvement of plants by genetic engineering, *i.e.*, by modifying characteristics like metal uptake, transport and accumulation as well as tolerance, opens up new possibilities for phytoremediation[22]. This also yields insights into the molecular regulation and control of plant heavy metal and micronutrient accumulation and homeostasis, as well as provides information that will contribute for the improvement of phytoremediation. Enhancing rhizophoric detoxification by enlarging root mass may be effected by using *Agrobacterium tumefaciens* and *Agrobacterium rhizogenes* which can mediate DNA transfer from bacterium to plant cells[71].

It has been found that cadmium tolerance and accumulation is enhanced by overexpression of gamma-glutamylecysteine synthetase[72]. Indian mustard (*Brassica junceae*) was genetically engineered to over-express the *Escherichia coli* gshI gene encoding gamma-glutamylcysteine synthetase (γ-ECS). γ-ECS transgenic seedlings showed increased tolerance to cadmium and higher concentration of PCs, gamma-GluCys, glutathione and total non-protein thiols compared with wild type seedlings. In spite of their higher tissue cadmium concentration, γ-ECS plants grew better in the presence of cadmium than wild type. Thus, over-expression of γ-ECS appears to be a promising strategy for the production of plants with superior heavy metal phytoremediation capacity. Metal chelating characteristics of synthetically prepared phytochelatin analog peptide $(\text{Glu-Cys})_2\text{Gly}$ to determine if a gene encoding such a peptide might be useful in phytoremediation were studied by Bae *et al.*[73] Studies with Cd(II), Hg(II) and Pb(II) show that synthetic $(\text{Glu-Cys})_2\text{Gly}$ peptides exhibits metal-chelating properties similar to the phytochelatin $(\gamma\text{-Glu-Cys})_2\text{Gly}$. GSH-bound metals were also shown to be quantitatively transferred to $(\text{Glu-Cys})_2\text{Gly}$. The Cd(II)-form of the synthetic $(\text{Glu-Cys})_2\text{Gly}$ peptide-like PCs was able to form stable complexes with sulfide. The spectroscopic properties of $(\text{Glu-Cys})_2\text{Gly}$-coated complexes of CdS were comparable to those exhibited by $(\gamma\text{-Glu-Cys})_2\text{Gly}$-coated CdS particles. Both $(\gamma\text{-Glu-Cys})_2\text{Gly}$ and $(\text{Glu-Cys})_2\text{Gly}$ exhibited a Cd-binding stoichiometry of 0.5 Cd per peptide molecule. UV-Visible, HPLC and mass spectral analyses indicated that one Hg(II) ion was chelated by each molecule of $(\gamma\text{-Glu-Cys})_2\text{Gly}$ or $(\text{Glu-Cys})_2\text{Gly}$. Each molecule of $(\gamma\text{-Glu-Cys})_2$ or $(\text{Glu-Cys})_2\text{Gly}$ bound to one atom of Pb(II)[73].

Conclusion

Phytoremediation of metal polluted soils to be successful, a strategy should be considered that combines rapid screening of plant species possessing ability to tolerate and accumulate heavy metals with agronomic practices that enhance shoot biomass production and/or increase metal bioavailability in the rhizophore[74]. However, biology alone cannot make phytoremediation work. The highly integrated nature of phytoremediation requires synergy with many other disciplines.

VARIOUS SPECIES OF PHYTOREMEDIATORS

Plant	Cd supplied	Root	Shoot	Reference
Maize	15	10	76	75
Pea	15	10	64	75
Cucumber	2.5	1.58	8.68	76
Barley	45	17	196	16
Thlapsi	500	2700	—	5
Tobaccoo	18	120	—	77
Silene	5.5	19	—	65
Radish	40	13	3	75
Cowpea	2	1.1	7.9	78

REFERENCES

1. World Health Organization (WHO), Environmental Health Criteria 134: Cadmium International Programme on Chemical Safety (IPCS), Monograph (1992).
2. H. Morrow and J. Keatings, Overview paper for OECD workshop on the effective collection and recycling of nickel-cadmium batteries, Lyon, France (September 23–25, 1997).
3. H. Morrow, Questioning the need to develop alternatives for cadmium coatings, Proceedings of 2nd Annual Cadmium Alternative Conference, National Defense Center for Environmental Excellence, Johnstown, Pennsylvania, USA (May 13–15, 1996).
4. W. Ernest and H.J.M. Nielson, *Environ. Pollu.*, **107,** 329 (2000).
5. E. Lombi, F.J. Zhao, S.J. Dunham and S.P. McGrath, *New Phytol.*, **145,** 11 (2000).
6. R.B. Meaghler, *Curr. Opi. Plant. Biol.*, **3,** 153 (2000).
7. S.R. Smith, CAB International, Willingford, UK, p. 382 (1996).
8. M. Palanaippan, K. Shannumugam and S. Ponnusamy, Soil degradation due to heavy metal accumulation under long-term fertilization, The 17th World Congress of Soil Science (WCSS), Bangkok (Thailand) (2002).
9. J.M. Cutler and D.W. Raini, *Plant Physiol.*, **54,** 51 (1974).
10. E.J. Jonner and C. Leyval, *New Phytol.*, **435,** 352 (1997).
11. F.T. Bingham, A.L. Page and J.E. Strong, *Soil Sci.*, **130,** 32 (1980).
12. M. Anderson, *Water, Air and Soil Pollut.*, **39,** 439 (1988).
13. A.P. Vitoria, P.J. Lea and R.A. Azevedo, *Phytochem.*, **57,** 701 (2001).
14. E. Fediuc and L. Erdei, *Radiol. Oncol.*, **36,** 156 (2002).
15. F. Jemal, L. Didierjean, R. Ghirir, M.H. Gorbal and G. Burkard, *Plant Sci.*, **137,** 143 (1998).
16. A. Vassilev, T. Tsonev and I. Yordanov, *Environ. Pollut.*, **103,** 287 (1998).

17. J. Barcelo and C. Poschenriender, *J. Plant Nutr.,* **13,** 1 (1990).
18. A. Chaoui, S. Mazhaudi, M.H. Ghorbal and E.E. Ferjoni, *Plant Sci.,* **126,** 1 (1997).
19. U. Kramer, J.D. Otter-Howells, J.M. Charnock, A.J.M. Baker and J.A.C. Smith, *Nature,* **379,** 635 (1996).
20. Y.M. Li, R.L. Chaney, J.S. Angles, K.Y. Chen, B.A. Kerschner and A.J.M. Baker, *Agron. Abstr.,* p. 27 (1996).
21. G.R. Ybarra and R. Webb, *J. Hazar. Subs. Res.,* **2,** 1.1 (1999).
22. S. Karenlampi, H. Schat, J. Vangronsveld, J.A.C. Verkleij, D. Leila, M. Mergeay and A.I. Tervahauta, *Environ. Pollu.,* **17,** 225 (2000).
23. C.S. Cobbett, *Plant Physiol.,* **123,** 825 (2000).
24. A. Brune, W. Urabacch and K.J. Dietz, *J. Exp. Bot.,* **45,** 1189 (1994b).
25. H. Nishizono, K. Kubata, S. Suzuki and F. Ishii, *Plant Cell Physiol.,* **30,** 595 (1989).
26. E. Who, Effects of heavy metals in plants at the cellular and organismic level, in: G. Schuurman and B. Marker (Eds.), Ecotoxicology, John Wiley & Sons Inc. and Speckturm Akademischer Veriog (1998).
27. G.J. Wagner, *Adv. Agron.,* **51,** 173 (1993).
28. J.A.C. Verkleij and H. Schat, Mechanism of metal tolerance in higher plants, in: A.J. Shaw (Eds.), Heavy metal tolerance in plants: Evolutionary aspects, CRC Florida Press Inc., Boca Raton, pp. 179–194 (1990).
29. L. Leita, M. DeNobili, S. Cesco and C. Mondini, *J. Plant Nutri.,* **19,** 527 (1996).
30. L. Sanita di Toppi and R. Gabbrielli, *Environ. Exp. Bot.,* **41,** 105 (1999).
31. H. Kubota, K. Sato, T. Yamod and T. Maitani, *Phytochem.,* **53,** 239 (2000).
32. E. Grill, *Mol. Bio. Chem.,* 283 (1989).
33. W. Grekeler, E. Grill, E.L. Winnacker and M.H. Zenk, *Naturforsch.,* **44C,** 361 (1989).
34. E. Grill, S. Loffelr, E.L. Winnacker and M.H. Zenk, *Proc. Natl. Acad. Sci. (USA),* **86,** 6838 (1989).
35. E. Grill, M.H. Zenk and E.L. Winnacker, *Naturwissenscchaften,* **72,** 432 (1985).
36. J.L. Hall, *J. Exp. Bot.,* **53,** 1 (2002).
37. J.A.C. Verkleij, P. Koevoets, J. van't Reit, R. Baker, Y. Mijdam and E. Who, *Plant Cell Environ.,* **13,** 913 (1990).
38. C. Cobbett and P. Goldsbrough, *Annu. Rev. Plant Physiol. Plant Mol. Biol.,* **53,** 159 (2002).
39. B. Lane, R. Kajoika and T. Kennedy, *Biochem. Cell. Biol.,* **65,** 1001 (1987).
40. J.H.R. Kagi, *Methods Enzymol.,* **205,** 613 (1991).
41. N.T. Robinson, A.M. Tommey, C. Luske and P.J. Jackson, *Biochem. J.,* **295,** 1 (1993).
42. A.T. Miles, G.M. Hawksworth, J.H. Beattie and V. Rodilla, *Crit. Rev. Biochem. Mol. Biol.,* **35,** 35 (2000).
43. J. Koropatnick and R.K. Zalups, Toxic and essential metals in the cellular response to signals, Molecular Biology and Toxicology of Metals, Taylor and Francis, London, UK, pp. 551–576 (2000).
44. I.F. Rivari, H. Koyama and S. Suzuki, *Jap. J. Health Huma. Ecol.,* **56,** 168 (1990).
45. G.E. Gries and G.J. Wagner, *Planta,* **204,** 390 (1998).
46. D.E. Salt and R.J. Wagner, *J. Biol. Chem.,* **268,** 12297 (1993).
47. D.E. Salt and W.E. Rauser, *Plant Physiol.,* **107,** 11293 (1995).
48. R.M. Krutz, B.P. Evangelou and G.L. Wagner, *Plant Physiol.,* **91,** 780 (1989).
49. E. Vierling, *Ann. Rev. Plant Physiol. Plant Mol. Biol.,* **42,** 579 (1991).
50. L. Edelman, E. Czarnecka and J.L. Key, *Plant Physiol.,* **86,** 1048 (1988).
51. E. Czarnecka, L. Edelman, F. Schoff and J.L. Key, *Plant Mol. Bio.,* **3,** 45 (1984).
52. S.I. Fenick, V.G. Solodushko, T.B. Trofimyak and Y.B. Blume, *J. Exp. Bot.,* **48,** 99 (1997).

53. M.N.V. Prasad, Trace Metals Plant Ecophysiology, Wiley, New York, pp. 207–249 (1997).
54. N. Rivetta, N. Negrini and M. Cocucci, *Plant Cell Environ.*, **20,** 600 (1997).
55. D.O. Adam and S.F. Yang, *Proc. Natl. Acad. Sci. (USA),* **76,** 170 (1997).
56. J. Fuherer, *Plant Cell Environ.*, **5,** 263 (1982b).
57. S.D. Cunningham, W.R. Berti and J.W. Haung, *Trend Biotechnol.,* **13,** 393 (1995).
58. B.D. Ensley, I. Raskin and D.E. Salt, Phytoremediation application for removing heavy metals contamination from soil and water, in: G.S. Sayler, J. Sanseverino and K.L. Danis (Eds.), Biotechnology in the Sustainable Environment, Plenum Pres, New York, pp. 59–64 (1997).
59. R.R. Brooks, M.F. Chambers, L.J. Nicks and B.H. Robibson, *Trends Plant Sci.,* **3,** 359 (1998).
60. D.E. Salt, R.D. Smith and I. Raskin, *Ann. Rev. Plant Physiol. Plant Mol. Biol.,* **49,** 643 (1998).
61. I. Raskin, P.B.A.N. Kumar, S. Dushenkov and D.E. Salt. *Curr. Opin. Biotech.,* **5,** 285 (1994).
62. D.E. Salt, M. Blaylock, N. Kumar, S. Dushenkov, B. Ensley, I. Chet and I. Raskin, *Biotechnol.,* **13,** 468 (1995).
63. I. Raskin, *Proc. Natl. Acad. Sci. (USA),* **93,** 3164 (1996).
64. A.S. Moffat, *Science,* **285,** 369 (1999).
65. S.L. Brown, R.L. Chaney, J.S. Angle and J.M. Baker, *Environ. Sci. Tech.,* **29,** 1581 (1995).
66. S.D. Cunningham and D.W. Ow, *Plant Physiol.,* **110,** 715 (1996).
67. ———, *Environ. Sci. Tech.,* **29,** 1232 (1995a).
68. V. Dushenkov, P.B.A.N. Kumar, H. Motto and I. Raskin, *Environ. Sci. Tech.,* **29,** 1239 (1995).
69. B.T. Walton and T.A. Anderson, *Curr. Opin. Biotech.,* **3,** 267 (1992).
70. L. Marchiol, S. Assolari, P. Sacco and G. Zerbi, *Environ. Pollut.,* **132,** 21 (2004).
71. A.M. Stomp, K.H. Hann, S. Wilbert, M.P. Gordon and S.D. Cunningham, *Annals New York Acad. Sci.,* **721,** 481 (1994).
72. Y.L. Zhu, E.A. Pilon-Smith, A.S. Tarun, S.U. Weber, L. Jouanin and N. Terry, *Plant Physiol.,* **121,** 1169 (1999).
73. W. Bae and R.K. Mehra, *J. Inorg. Biochem.,* **68,** 201 (1997).
74. S.D. Ebbs and L.V. Kochian, *J. Environ. Qual.,* **24,** 126 (1997).
75. E. Lozano-Rodriguez, L.E. Hernandez, P. Bonay and R.O. Carpena-Ruiz, *J. Exp. Bot.,* **48,** 123 (1997).
76. F.M.G. Tack, J. Esteban-Mozo and M.G. Verloo, *Commun. Soil. Sci. Plant Anal.,* **29,** 3015 (1998).
77. M.J. Mench, V.L. Didier, M. Loffler, A. Gomez and P. Masson, *J. Environ. Qual.,* **23,** 58 (1994).
78. B.S. Panwar, J.P. Singh and R.D. Laura, *Water, Air and Soil Pollut.,* **112,** 163 (1999).

(*Received*: 2 April 2004; *Accepted:* 11 February 2005) AJC-4108