UKaaZ

Annals of Phytomedicine 2(2): 110-113, 2013

ANNALS OF PHYTOMEDICINE
An International Journal

ISSN 2278-9839

Heavy metals contamination in ayurvedic plant drug of *Withania somnifera* L. Dunal and their remediation

Ch. Saidulu, C. Venkateshwar and S. Gangadhar Rao

Department of Botany, University College of Science, Osmania University, Hyderabad-500007, Andhra Pradesh, India

Received September 15, 2013: Revised October 30, 2013: Accepted November 15, 2013: Published online December 30, 2013

Abstract

Withania somnifera L. Dunal is one of the most important ayurvedic medicinal plants which was investigated to know how heavy metal content distributes into the plant parts and their remediation. Plants were grown in pot culture experiments with three treatments in black soil. Treatment No. I. A control without any addition to the soil, Treatment No. II. Cadmium 10ppm, Chromium 20ppm, Nickel 16ppm were introduced into the soil, Treatment No. III. one % of Calcium hydroxide was also added along with heavy metals to the soil, and were grown up to the productivity levels. The amount of heavy metals distribution in different plant parts of *Withania somnifera* was analyzed with AAS (atomic absorption spectroscopy) after acid digestion. Heavy metals were more in plants, grown only in heavy metal treated soils (Treatment No. II) when compared to 1% Calcium hydroxide treated soil (Treatment No. III). The Ni, Cd, Cr, Cu, Co, Zn were present in higher concentration in the roots, leaves, stem and seeds except in the plants treated with 1% Ca $(OH)_2$ and heavy metals. In addition, the plants grown in 1% Calcium hydroxide treated, reversed the growth suppression of heavy metal toxicity in plants as evidenced by reduced metal contents in plants, grown with remediation. This study is a boon for cropping pattern of growing *Withania somnifera* L. Dunal even in polluted areas with 1% Calcium hydroxide treatment, results in genuine pure ayurvedic plant parts which can be used in medicine.

Key words: *Withania somnifera*, atomic absorption spectroscopy, remediation,

Introduction

Withania somnifera L. Dunal is an important medicinal plant, belongs to the family Solanaceae, commonly known as ashwagandha or winter cherry (Vernaculars: Sanskrit: ashwagandha; Telugu: Panneru; Trade name: Ashwagandha), is used in more than 100 formulations of Ayurveda, Unani and Siddha and is therapeutically equivalent to ginseng (Sangwan *et al.*, 2004). The genus *Withania* is systematically placed into the division Magnoliophyta, class Magnoliopsida, order Solanales and family Solanaceae (Heiser and Smith, 1953). It is well known for its rejuvenating properties, and hence called "Indian Ginseng" (Singh and Kumar, 1998). The heavy metal contamination in the environment is a major problem for human health and environmental quality (Chen *et al*, 1996; Ma and Rao, 1997). Most of the heavy metals are present in soil because of their immobility, eventhough their phytoavailability has been increased in recent times due to agricultural soil contamination, leading to damage of foliage, growth and crop productivity (Adriano, 2001). The effect of ionic strength and pH variation on the absorption of Cd, Cr and Ni metals is variable for different soils (Naidu *et al.*, 1994). Several crops grown in contaminated soils, showed bioaccumulation and also hyper accumulation (Alloway, 1990). Heavy metal content in polluted soils is about Cd-10ppm, Cr- 20ppm, and Ni-16ppm, therefore, the same concentrations are maintained in the present study.

Author for correspondence: Professor C. Venkateshwar
Department of Botany, University college of Science, Osmania University, Hyderabad-500007,A.P., India.
E-mail: cvlaxman2004@yahoo.com
Tel.: +91-9440487742

Soil remediation techniques have been conducted by Chen *et al.* (1994, 2000). Wang and Bai (1994), Chen and Lee (1997), in which some chemical treatments were applied like dolomite, phosphates or organic matter residues into the polluted soils to reduce the soluble nature of heavy metals in soils, *i.e.*, by precipitation, adsorption or complexion of molecules (Impens *et al.*, 1991; Mench *et al.*, 1994 ; Chen and Lee, 1997). Further, application of Calcium carbonate has significantly reduced the solubility of heavy metals in contaminated soils (Mc Bride and Blasiak, 1979; Sommers and Lindsay, 1979; Mc Bride, 1980; Chen and Lee, 1997; Liu *et al.*, 1998). Even hydrous iron oxides or manganese oxides mixed into contaminated soils, could reduce the concentration of soluble Cd or Pb in soils (Mc Kenzie, 1980; Tiller *et al.*, 1984; Khattak and Page, 1992; Mench *et al.*, 1994; Chen and Lee, 1997), but these hydrous metal oxides lastly dissolve in water. Therefore, presently Calcium hydroxide is used for the remediation of heavy metals in soil which precipitates the heavy metals. Pot experiment was conducted to know (i) the ability of Calcium hydroxide remediation in contaminated soils. (ii) To evaluate the effectiveness of most toxic heavy metals in presence of Calcium hydroxide and their accumulation in the plant parts of medicinally important crude drugs of plant origin.

Material and Methods

Soil preparation

Semiblack sandy loam soil was taken from the Botanical garden, Department of Botany, Osmania University, Hyderabad, India. Freshly collected soil was passed through a 2mm sieve and air dried for one week. The clay content was 15.4%, total carbon was 3.5% and the pH was 6.5. 900kgs of air dried soil was mixed well and from that 300kgs were separated as control soil. 45gms of Cadmium sulphate ($CdSO_4$. $4H_2O$) was weighed so as to obtain 10ppm of Cd metal alone. 92.3gms of Chromium nitrate ($Cr(NO_3)_3$. $9H_2O$) was weighed so as to obtain 20ppm of Cr metal and 33.6gms of Nickel chloride ($NiCl_2$. $6H_2O$) was weighed so as to obtain 16ppm of Ni. All the three heavy metals were dissolved in 150 liters of distilled water and sprayed on the 600kgs of dry soil by mixing thoroughly to obtain mud slurry. Which was again air dried. 300kgs of this soil is separated, powdered and the appropriate amount of 1.5kg of $Ca(OH)_2$ mixed with 300kgs of heavy metals treated soil as a remedial measure, filled into clay pots and then divided into three treatments. Treatment No. I Control without any addition of either the heavy metals or Calcium hydroxide to the soil. Treatment No. II Cadmium 10ppm, Chromium 20ppm, Nickel 16ppm were introduced into the soil. Treatment No. III One % of Calcium hydroxide was also added along with heavy metals to soil. These treatments were replicated three times.

Plant material source

Withania somnifera seeds were procured from the CIMAP, Hyderabad. The seeds were sown in earthen pots in Green house of Botanical Garden, Department of Botany, Osmania University, Hyderabad.

Acid digestion of plant samples

The method followed for the digestion of plant tissue was by dry acid digestion in triple acid ($HCLO_4$: HCL: HNO_3) Allen *et al.* (1976). Weighed sample placed in digestion vessel in acid ratio of (5:2:6), is added and heated at 80°C for several hours and samples were dried out completely. After digestion, samples were diluted to specific volume and analyzed directly by using Atomic Absorption Spectrophotometer (Model: Perkin Elmer Analyst 100).

Results and Discussion

Nickel

It was observed that the plants grown in control soil, the concentration of nickel in root was 0.884± 0.037mg/kg, in leaf 0.867±0.006 mg/kg, in stem 0.87±0.006mg/kg and in seed 0.915±0.032mg/kg. Plants grown in heavy metal treated soil, the Ni concentration in root was 1.62±0.013mg/kg, in leaf 1.361±0.008mg/kg, in stem 1.35±0.001mg/kg and in seed 1.348±0.013mg/kg, and in plants grown in soil with heavy metal treatment + 1% $Ca(OH)_2$ as barrier and the Ni concentration in root was 0.93±0.014mg/kg, in leaf 0.902±0.032mg/kg, in stem 1.21±0.032mg/kg and in seed 1.261±0.002mg/kg. In 1984, FAO/WHO set permissible limit for Ni is 1.683ppm. Thus, in *Withania somnifera* concentrations of nickel in root, leaf, stem and seed are within the permissible limit. Hence, it is recommended that application of 1% Ca $(OH)_2$ is a good barrier to stop heavy metal entry into *Withania somnifera* (Table 1).

Cadmium

The concentration of cadmium in root was 0.01±0.002mg/kg, in leaf 0.008±0.002mg/kg, in stem 0.008±0.001mg/kg and in seed 0.014±0.009mg/kg when plants grown in control soil. Plants grown in heavy metal treated soil, the Cd concentration in root was 0.028±0.001mg/kg, in leaf 0.014±0.001mg/kg, in stem 0.016±0.002mg/kg and in seed 0.006±0.001mg/kg and in plants grown with heavy metal treated +1% Ca $(OH)_2$ as barrier soil, Cd concentration in root was 0.024±0.0006mg/kg, in leaf 0.009±0.001mg/kg, in stem 0.008±0.0006mg/kg and in seed 0.025±0.0007mg/kg. The permissible limit set by WHO is 0.2 to 0.81ppm. Thus, in *Withania somnifera* concentrations of Cadmium in root, leaf, stem and seed are within the permissible limit. Hence, it is recommended that application of 1% Ca $(OH)_2$ is a good barrier to stop heavy metal entry into *Withania somnifera* (Table 1).

Table 1: Heavy metal contents of *Withania somnifera* (mg/kg of dry weight)

Parameters	Heavy metals	Treatment No: I (Control soil)	Treatment No: II (Soil + Heavy metal)	Treatment No: III (Soil + Heavy metal + 1% Ca $(OH)_2$)
		Mean ± S.D	Mean ± S.D	Mean ± S.D
Root	Nickel	0.884 ± 0.037	1.628 ± 0.013	0.93 ± 0.014
	Cadmium	0.01 ± 0.002	0.028 ± 0.001	0.024 ± 0.0006
	Chromium	0.452 ± 0.014	0.77 ± 0.072	0.611 ± 0.008
	Copper	0.145 ± 0.003	0.336 ± 0.002	0.27 ± 0.004
Leaf	Nickel	0.867 ± 0.006	1.361 ± 0.008	0.902 ± 0.032
	Cadmium	0.008 ± 0.001	0.014 ± 0.001	0.009 ± 0.002
	Chromium	0.393 ± 0.016	0.925 ± 0.016	0.759 ± 0.026
	Copper	0.146 ± 0.001	0.174 ± 0.004	0.133 ± 0.005
Stem	Nickel	0.87 ± 0.006	1.355 ± 0.001	1.211 ± 0.032
	Cadmium	0.008 ± 0.001	0.016 ± 0.002	0.008 ± 0.0006
	Chromium	0.519 ± 0.041	0.91 ± 0.018	0.669 ± 0.039
	Copper	0.129 ± 0.003	0.166 ± 0.002	0.127 ± 0.004
Seed	Nickel	0.915 ± 0.032	1.348 ± 0.013	1.261 ± 0.002
	Cadmium	0.014 ± 0.009	0.025 ± 0.007	0.006 ± 0.0010
	Chromium	0.562 ± 0.020	1.072 ± 0.029	0.718 ± 0.018
	Copper	0.159 ± 0.001	0.167 ± 0.005	0.133 ± 0.003

Chromium

The concentration of chromium in root was 0.452±0.014mg/kg, in leaf 0.393±0.016 mg/kg, in stem 0.519±0.041mg/kg and in seed 0.562±0.02mg/kg when plants grown in control soil. Plants grown in heavy metal treated soil, the Cr concentration in root was 0.77±0.072mg/kg, in leaf 0.92±0.016mg/kg, in stem 0.91±0.018mg/kg and in seed 1.072±0.02mg/kg and in plants grown with heavy metal treated +1% Ca $(OH)_2$ as barrier soil, Chromium concentration in root was 0.61±0.008mg/kg, in leaf 0.759±0.02mg/kg, in stem 0.669±0.039mg/kg and in seed 0.718±0.018mg/kg. For medicinal plants, the WHO (2005) limits for chromium have not yet been established. However, permissible limits for chromium set by Canada were 2ppm in raw medicinal plant material and 0.02mg/day in finished herbal products WHO. Comparison of metal levels in the medicinal plants investigated with those proposed by FAO/WHO showed that the herbs have chromium concentrations equivalent to the limits permissible in edible plants. Thus, in *Withania somnifera* concentrations of chromium in root, leaf, stem and seed are within the permissible limit. Hence, it is recommended that application1 of % Ca $(OH)_2$ is a good barrier to stop heavy metal entry into *Withania somnifera* (Table 1).

Copper

The concentration of copper in root is 0.145± 0.0031mg/kg, in leaf 0.146±0.0013mg/kg, in stem 0.129±0.003mg/kg and in seed 0.159±0.001mg/kg when plants grown in control soil. Plants grown in heavy metal treated soil, the Cu concentration in root was 0.33±0.002mg/kg, in leaf 0.174±0.004mg/kg, in stem 0.166±0.002mg/kg and in seed 0.167±0.005mg/kg and in plants grown with heavy metal treated +1% Ca $(OH)_2$ as barrier soil, Copper concentration in root was 0.27±0.004mg/kg, in leaf 0.133±0.005mg/kg, in stem 0.127±0.004mg/kg and in seed 0.133±0.003mg/kg. In 1984, FAO/WHO set permissible limit for Cu is 3.00ppm. Thus, in *Withania somnifera* concentrations of Copper in root, leaf, stem and seed are within the permissible limit. Hence, it is recommended that application of 1% Ca $(OH)_2$ is good barrier to stop heavy metal entry into *Withania somnifera* (Table 1).

Zinc

The concentration of Zinc is not detected in the root, leaf, stem and seeds of *Withania somnifera*, grown in different exposures. It was reported by Jabeen *et al.* (2010) that the permissible limit set by FAO/WHO in edible plants was 27.4ppm. Thus, in *Withania somnifera* concentrations of zinc in root, leaf, stem and seed are within the permissible limit (Table 1).

Cobalt

The concentration of cobalt is not detected in the root, leaf, stem and seeds of *Withania somnifera*, grown in different exposures. There are no regulatory limits by WHO/FAO for cobalt content in herbal plants and preparations. It was reported by Jabeen *et al.* (2010) that the study carried out in seven herbs in Turkey, determined cobalt concentration ranged between 0.14ppm to 0.48ppm. Thus, in *Withania somnifera* concentrations of cobalt in root, leaf, stem and seed are within the permissible limit. (Table 1).

Conclusion

The results suggest that medicinal plants used for human consumption or for preparation of herbal products and standardized extracts should be collected from an unpolluted natural habitat. Outcome of the present work: Heavy metal content in the plants, growing in polluted areas can be reduced when the soil is treated with of 1% $Ca(OH)_2$. By this method, we can achieve heavy metal free plants and plant drugs like *Withania somnifera.*

Acknowledgment

We acknowledge the support given by DST-PURSE, Research Project Ltr No. A-46/BOT//DST- PURSE/2011, New Delhi, India. We are very thankful to Professor B. Sashidhar Rao, Co-ordinator, OU-DST PURSE Programme, Department of Biochemistry, Osmania University, Hyderabad for his support.

Conflict of interest

We declare that we have no conflict of interest.

References

Adriano, D.C., (2001). Trace elements in terrestrial environments: Biogeochemistry, bioavailability and risks of metals. Springer Verlag, New York, pp:866.

Allen, S.E.; Grimshaw, H.M.; Parkinson, J.A.; Quarmby, C. and Roberts, J.D. (1976). Chemical analysis ***In***: Champman, S B (ed),Methods in plant Ecology, Black Well Scientific Publication, Oxford, pp:424-426.

Alloway, B.J. (1990). Cadmium in heavy metals in soils. Blackie, Glasgow, pp:321.

Chen, M.; Chau, L. and Ching, K. (2000). Oxidative stress in copper treated rice roots. Bot. Bull. Acad. Sin., **41**:99-103.

Chen, I.C.K.; Coffey, J.T. and Mudge, T.N. (1996). Analysis of branch prediction *via* data compression. Cambridge, Massachusetts. ***In***: ASPLOS **7**:128-137.

Chen, W.; Mahlke, S.; Warter, N.; Anik, S. and Hwu, W. (1994). Profile assisted instruction scheduling. Int. J. of Parallel Programming, **22**(2):151-181.

Chen, Z.S. and Lee, D.Y. (1997). Evalution of remediation techniques on two Cadmium polluted soil in Taiwan. ***In***:Iskandar, A.,A Adrinano, D.C (Eds), Remediation of soil contaminated with metals. Book Chapter of special Volume of Second International Conference on the Biogeochemistry of Trace Elements.(5-10 September, 1993, Taipei, Taiwan). Science Review, London, pp:209-223.

FAO/WHO (1984). Contaminants in *codex alimentarius*, Vol. XVII, Edition 1. FAO/WHO, Codex Alimentarius Commision, Rome.

Heiser, C.B. and Smith P.C. (1953).The cultivated capsicum. Econ. Bot., **1**:214-227.

Impens, R.; Fagot, J. and Avrril, C. (1991). Gestion des sals Contamines par les Metaux Lourds. Association Francaise Inter. Professionnelle du Cadimum, Paris, France.

Jabeen, S.M.; Shah, T.; Khan, S. and Hayat, M.Q. (2010). Determination of major and trace elements in ten important folk therapeutic plants of Haripur basin, Pakistan. Journal of Medicinal Plants Research, **4**(7):559-566.

Khattak, R.A. and Page, A.L. (1992). Mechanism of manganese adsorption on soil constituents. ***In***: Biogeochemistry of Trace Metals. Lewis Publ., Boca Raton, FL. pp:383-400.

Liu. J.C.; Looi, K.S.; Chen, Z.S. and Lee, D.Y. (1998).The effects of composts and lead by vegetables grown in polluted soil. J. Chinese Inst. Environ. Engrg., **8**:53-60.

Ma, L.Q and Rao, G.N. (1997).Chemical fractionation of cadmium, copper, nickel and zinc in contaminated soils. J. Environ. Qual., **26**: 256-264.

Mc Bride, M. B. and Blasiak, J. J. (1979). Zinc and copper solubility as a function of pH in an acidic soil. Soil Sci. Soc. Am. J., **43**:866-870.

Mc kenzie, R.M. (1980). The adsorption of lead and other heavy metals on oxides of manganese and iron. Aust. J. Soil Sci., **18**:61-73.

Mench, M.; Didier, V.; Loffler, M.; Gomez, A. and Masson P. (1994). Mimicked *in-situ* remediation study of metal contaminated soils with emphasis on Cd and Pb. J. Environ. Qual., **23**:58-63.

Naidu, R.; Bolan, N.S.; Kookana, R.S. and Tiller, K.G. (1994). Ionic strength and pH effects on the absorption of cadmium and the surface charge of soils.European J.Soil Sci., **45**:419-429.

Sangwan, R.S.; Chaurasiya, N.D.; Misra, L.N.; Lal, P.; Uniyal, G.C.; Sharma, R.; Sangwan, N.S.; Suri, K.A.; Qazi, G.N. and Tuli, R. (2004). Phytochemical variability in commercial herbal products and preparations.

Singh, S. and Kumar S. (1998). *Withania somnifera* - The Indian Ginseng Aswagandha. Central Institute of Medicinal and Aromatic Plants.

Sommers, L.E. and Lindsay, W.L. (1979). Effect of pH and redox on predicted heavy metal chelate equilibria in soil.Soil Sci. Soc. Am. J., **43**:39-47.

Tiller, K. G.; Gerth, J. and Bruemmer, G.W. (1984).The relative affinities of Cd, Ni, and Zn for different clay fraction and goethite.Geoderma., **34**:17-35.

Wang, L. and Bai, J. (1994).The pollution investigation and prevention strategies in vegetables at Shenyan. Angro-environmental protection **13**(2):84-88(In Chinese with English abstract).

World Health Organization (WHO) (2005). Quality control methods for medicinal plant materials. Revised, Geneva.

World Health Organization (1998). Quality control methods for medicinal plant materials. Published by WHO, Geneva.