

Full Length Research Paper

Comparative elemental analysis of *Solanum macrocarpum* (L.) and soil sample from Alau, Borno State, Nigeria

O.A Sodipo^{*1}, F.I Abdulrahman² and J.C Akan²

¹Department of Clinical Pharmacology and Therapeutics, College of Medical Sciences

²Department of Chemistry, Faculty of Science, University of Maiduguri, P.M.B. 1069, Maiduguri, Nigeria

Accepted 26 March, 2012

The fresh fruit of *Solanum macrocarpum* (Solanaceae) and the soil in which the plant was grown were obtained from Alau in Konduga Local Government, Borno state. The concentration of elements which included Fe, Mg, Zn, Cu, Mn, As, Cr, Cd, Se and Pb were determined using Atomic Absorption Spectrometer (AAS). Flame Emission Spectrophotometer (FS), Gallenkamp (FGA 330), was used to determine Na, K and Ca while S and P was determined using UV/V spectrophotometer. The results obtained from this analysis revealed that S, Na and K were in high concentrations; Fe, Zn, Mg, Cu and P in moderate concentrations while Pb, Cd and Se were not detected in the fruit. In the soil sample however, Fe, Na and K were in high concentrations, S, Zn, Mg, Ca and P in moderate concentrations, while Pb, Cd and Se were not detected. The fruit contained much higher elemental concentrations than the soil generally except in the soil where the concentrations of Fe, P, Cr and As were higher, but the concentration of Mn was the same in both the soil and the fruit. Generally, the concentration of trace elements, Zn, Cu, Fe, Mn, Cr and heavy metals like in fruit are within safety limits as reported by WHO. However, the concentration of some essential elements (K, Na and S) is much higher than reported levels. Thus, the values of these elements in the fruit could probably be due to the topography, soil-water-plant exchange complex and evapo-transpiration of the environment. Also, the values of the elements show that the use of this fruit would not pose a health risk.

Keywords: Elemental Content, *Solanum macrocarpum* Linn; soil, fruit, Borno State.

INTRODUCTION

Solanum macrocarpum Linn. otherwise called garden egg or *Solanum macrocarpum* L. sensu stricto or *Solanum daysphyllum* Schumacher and Thon belongs to the family Solanaceae. It is extensively cultivated in the North East Arid Zone of Nigeria, Sierra Leone, Kenya and Uganda (Grubben and Denton, 2004). Traditional medical uses of the plant include the young fruits and flowers as laxatives, for cleaning the teeth and in the treatment of cardiac diseases and hyperlipidaemia in Nigeria. The heated leaves are chewed to treat throat troubles in Sierra Leone. The juice of boiled roots is drunk in Kenya to get rid of hook worm while crushed leaves are taken to treat stomach troubles (Grubben and Denton, 2004). The

young leaves and young fruits are cooked and consumed as a vegetable. The fruit is a depressed glabrous berry, 2 – 6 cm x 3 – 10 cm, green, ivory or purplish – white with dark stripes when young, yellow to brownish when ripe, partly covered by the enlarged calyx lobes; fruit stalk is erect or decurved, 1 – 4 cm long (Grubben and Denton, 2004), in contrast to that of *S.melongena* whose fruit is a large, egg – shaped berry, varying in colour from dark purple to red, yellow or white (ANON, 2007; Sodipo, 2009).

Solanum macrocarpum Linn aqueous fruit extract has been reported to exhibit laxative and hypotensive effects (Sodipo *et al.*, 2008). The aqueous fruit extract has also been shown to possess lipid lowering activities (Sodipo *et al.*, 2009c; 2011a; 2012a) and at the same time has renal and hepatoprotective effects (Sodipo *et al.*, 2009a,b; 2012c). The aqueous fruit extract also demonstrated antianaemic properties (Sodipo *et al.*, 2009d; 2011b;

*Corresponding author E-mail:
sodipoofunke@yahoo.com

2012b).

Mineral elements (most of which are present in medicinal plants) serve not only as sources of nutrition to plants and animals, but also play other functions fundamental to the environment. Research has shown that inorganic elements are essential in nutrition and play important roles as structural components in cellular processes (Hakeem, 1987). Also, there is a growing recognition of the adverse effect of the cumulative exposure to heavy metals in small quantity and essential elements in large concentrations (WHO, 1973, U.S. Food and Drug Administration 1999; Abdulrahman, 2004; Sodipo, 2010; Akan *et al* 2010a,b). These adverse effects of elements are of major concern. Plants are sensitive to environmental conductors and their elemental contents respond to changes in the condition of the environment (Vtorova, 1987; Kabata and Pendias, 1984). The uptake of trace elements by plants depends on the reserves of the nutrients in the soil and its availability (Koke *et al.*, 1984). Furthermore, the soil pH, soil organic matter content and plant genotype have a marked effect on nutrient availability (Kloke *et al.*, 1984). In view of the various uses of this plant, there is therefore a need to investigate the elemental contents of the fruit and the soil from which the plant was obtained in order to ascertain the levels of the elements in the fruit so as to avoid toxic concentrations and also to know if the concentration in the fruit are due to the level in the soil and its availability or otherwise.

EXPERIMENTAL

Sample collection and identification

The plant material and the soil sample used in this study were obtained from Alau in Konduga Local Government Area, Borno State between October and November, 2007. The plant was identified and authenticated by Prof S. S. Sanusi of Biological Sciences Department, University of Maiduguri, Maiduguri, Nigeria. Specimen voucher (No. 548A) was deposited at the Research Laboratory of the Department of Chemistry.

Sample Preparation

The air-dried powdered fruit (5g) was placed in an evaporating dish in an oven at 80°C and dried to a constant weight. The sample was placed in a weighed porcelain crucible and ashed at 500°C in a hot spot furnace for 3h. The ashed material was then digested and prepared for determination of elements. The cooled, ashed sample (0.5g) was digested by heating for 3h with a mixture of 10ml each of c.HNO₃, HCl and HClO₄ in a 500ml flask.

This material (aliquot) together with the residue was

mixed with 100 ml 2M HNO₃ and 30ml distilled water in 100 ml volumetric flask. The volume was made up to the mark with distilled water (Radojevic and Bashkin, 1999). Blank sample (using the same procedure but omitting the plant material) and standard for the various elements were similarly prepared.

Some air-dried soil sample was ground and sieved (<2mm). 1.0 g was then weighed and placed in a 100 ml tall-form beaker. 30 ml 1:1 HNO₃ acid (10 ml H₂O and 10 ml. c. HNO₃) was added and boiled gently on a hot plate until the volume reduced to approximately 5 ml while stirring. A further 10 ml of 1:1 HNO₃ acid was added and the procedure was repeated. It was cooled and the extract was filtered through a Whatman No. 541 filter paper into a 100 ml volumetric flask. The volume was made up to the mark with distilled water (Radojevic and Bashkin, 1999). Blank sample using the same procedure but omitting the soil) and standard dilutions for the various elements were similarly prepared.

All samples were stored in plastic containers in a refrigerator maintained at 4°C prior to analysis

Elemental analysis

The resulting solutions from the perchloric acid and nitric acid digestion for the fruit and soil respectively were used for elemental analysis using Atomic Absorption Spectrometry (AAS) with SP/Cr Unicam Model Solar 32 Data station V7.10 System at the appropriate wavelength, temperature and lamp current for each element under study for the determination of Fe, Mg, Zn, Ca, Cu, Mn, As, Cr, Cd, Se and Pb, Flame Emission spectrophotometry (FES), Gallenkamp (FGA 330) for determination of Na, K and Ca while S and P were determined using uv/v spectrophotometry (Ogugbuaja, 2000).

Statistical Analysis

The results of the elemental analysis were expressed as the mean value ± standard deviation (S.D). The result obtained was subjected to analysis of variance (ANOVA) using SPSS/PC 4 package and differences between means were compared using Ozdamar's (1991) multiple range test.

RESULTS

Tables 1 and 2 show the mean concentration of elements in the fruit of *Solanum macrocarpum* and the soil on which the plant was grown. From the result of the study, the elemental concentration ranged between 13.02 ± 1.44µg/g to 12,536.00±85.60µg/g) in the fruit (Table 1).

Table 1: Elemental concentration of the fruit of *Solanum macrocarpum*

S/N	Elements	Concentration ($\mu\text{g/g}$)
1	Potassium (K)	4,234 \pm 51.56
2	Sodium (Na)	6,238.50 \pm 215.08
3	Calcium (Ca)	254.31 \pm 9.87
4	Magnesium (Mg)	543.75 \pm 30.86
5	Lead (Pb)	ND
6	Zinc (Zn)	505.71 \pm 5.21
7	Copper (Cu)	151.75 \pm 2.69
8	Cadmium (Cd)	ND
9	Iron (Fe)	552.45 \pm 7.38
10	Manganese (Mn)	80.00 \pm 2.83
11	Arsenic (As)	13.02 \pm 1.41
12	Sulphur (S)	12,536.00 \pm 85.60
13	Phosphorus (P)	63.00 \pm 4.24
14	Selenium (Se)	ND
15	Chromium (Cr)	33.00 \pm 4.24

ND = Not detected

The values given in the table above are means of replicate values (n=25)

Table 2: Elemental Concentration of the Soil from which *Solanum macrocarpum* fruit was grown

S/N	Element	Concentration ($\mu\text{g/g}$)
1	Potassium (K)	1,435 \pm 24.60
2	Sodium (Na)	1,224 \pm 11.23
3	Calcium (Ca)	123.40 \pm 2.65
4	Magnesium (Mg)	469.17 \pm 5.24
5	Lead (Pb)	ND
6	Zinc (Zn)	84.06 \pm 2.81
7	Copper (Cu)	10.21 \pm 1.23
8	Cadmium (Cd)	ND
9	Iron (Fe)	1,780.40 \pm 8.60
10	Manganese (Mn)	80.00 \pm 1.53
11	Arsenic (As)	15.01 \pm 2.63
12	Sulphur (S)	853.20 \pm 30.82
13	Phosphorus (P)	80.00 \pm 5.42
14	Selenium (Se)	ND
15	Chromium (Cr)	51.00 \pm 1.53

ND = Not detected

The values given in the table above are means of replicate values (n=25)

and 10.21 \pm 1.23 $\mu\text{g/g}$ to 1,780.40 \pm 8.60 $\mu\text{g/g}$ in the soil (Table 2). From the results of the study, the elemental concentration in the fruit sample showed high concentration of sulphur, S (12,536.00 \pm 85.60 $\mu\text{g/g}$), Sodium, Na (6,238.50 \pm 215.08 $\mu\text{g/g}$), potassium, K (4,234 \pm 51.56 $\mu\text{g/g}$), whilst arsenic, As (13.02 \pm 1.44 $\mu\text{g/g}$) and chromium, Cr (33.00 \pm 4.24 $\mu\text{g/g}$) occurred in low quantities. The following elements occurred in moderate concentrations, iron, Fe (552.45 \pm 7.38 $\mu\text{g/g}$), magnesium, Mg (543.71 \pm 30.86 $\mu\text{g/g}$), Calcium, Ca (254.30 \pm 9.87 $\mu\text{g/g}$) copper Cu (151.75 \pm 2.69 $\mu\text{g/g}$), manganese, Mn (80.00 \pm 2.83 $\mu\text{g/g}$), zinc, Zn (505.71 \pm 5.21 $\mu\text{g/g}$) and

phosphorus, P (63.00 \pm 4.24 $\mu\text{g/g}$). In the soil sample however, Fe, Na and K were in high concentrations (1,780.40 \pm 8.60 $\mu\text{g/g}$, 1,224.00 \pm 11.23 $\mu\text{g/g}$ and 1,435.00 \pm 24.60 $\mu\text{g/g}$) respectively S, Zn, Mg, Ca and P in moderate concentrations (853.20 \pm 30.82 $\mu\text{g/g}$, 84.04 \pm 2.81 $\mu\text{g/g}$, 469.17 \pm 5.24 $\mu\text{g/g}$, 123.40 \pm 2.60 $\mu\text{g/g}$, 80.00 \pm 1.56 $\mu\text{g/g}$ respectively) while As and Cr occurred in low quantities (15.01 \pm 2.63 $\mu\text{g/g}$ and 51.00 \pm 1.53 $\mu\text{g/g}$ respectively). In both the fruit and soil, lead, Pb, cadmium, Cd and selenium, Se were not detected with the method adopted for the analysis. In the fruit, S showed the highest concentration whilst in the soil; it was

Fe that showed the highest concentration. In both the fruit and soil, As recorded the lowest concentration. The concentration of Mn was the same ($80.00 \pm 2.83 \mu\text{g/g}$) in both the fruit and soil.

Results of analysis of variance (ANOVA) showed that variation between the elements in the fruit and soil were statistically significant ($P < 0.05$).

DISCUSSION

The concentration of the essential elements (Zn, Cu, Fe, Mn), and the non-essential elements (Cr and As) detected in the fruit were within safety limits that has been reported by Alloway (1995); WHO (1996); However, the concentration of some essential elements (K, Na and S) is much higher than reported or within acceptable levels. The high concentration of K, Na and S may be due to deposition of particulate matter on the leaves of the plant (Reuben *et al.*, 2008), as the values of these macro nutrients were lower in the soil. Thus, the concentration of these elements is not due to root uptake from the soil. The moderately high concentration of Fe in the fruit may be due to the uptake from the

soil as the concentration of Fe in the soil was very high ($1,780.40 \pm 8.60 \mu\text{g/g}$) as compared to that in the fruit ($552.45 \pm 7.38 \mu\text{g/g}$). Thus the intensity of extent of the uptake therefore influences the actual contents of an element in the plant. Presence of Fe in high concentration in plant poses serious pollution and health problem. Toxicity of Fe in human leads to vomiting, cardiovascular collapse and diarrhoea (Turnland, 1988). Since the Fe content in the fruit is moderate, the fruit is probably not toxic.

Ca in the fruit and soil are moderate $254.31 \pm 9.87 \mu\text{g/g}$ and $123.40 \pm 2.65 \mu\text{g/g}$ respectively. The lower concentration in the soil implies that the Fe content in the fruit is not due to the soil-water complex or evapotranspiration, but it is probably due to the deposition on the leaves. Ca is used in bone formation and usually occurs in the body with flourine in the ratio 2:1 (Aliu, 2007).

Pb, Cd and Se were not detected in both the soil and the fruit. They are toxic metals which can accumulate in the human tissues when they are not metabolized by the body for absorption and utilization (Ewers and Shlipkoter 1991, Health Concerns, 2003; Amartey *et al.*, 2011). Thus, the fruit is safe for human consumption and the soil has probably not been contaminated with these heavy metals. The danger of these toxic metals on the human body is enhanced when there is low intake of the essential mineral nutrients for their absorption (Wilson, 2008). For instance, Cd causes kidney damage and bone degradation because it affects calcium metabolism (Waalkes, 1991; Amartey *et al.*, 2011). The low concentration of As and Cr may be an indication of degree of pollution in the area where the fruit was

obtained or poor absorption of these elements by the plant root (Abdurahman, 2004). Borno State is not an industrial area, hence it is expected that the production and disposal of these toxic metals will be minimal as to contribute to environmental pollution. This must have contributed to the low accumulation of these toxic metals in the plant (Abdurahman, 2004). The low concentration of the toxic elements may also be due to the low deposits of these elements in the soil, since the concentration of elements in the plant is a reflection of the concentration in the soil (Abdurahman, 2004).

The Fe which is present in the fruit in moderate concentration is within safety limits as reported by (Alloway, 1998; WHO, 1998), is important in haemoglobin production and is used in the treatment of iron deficiency anaemia (Lawrence *et al.*, 1997). The moderate level of Fe in the fruit of the plant is probably an indication of the concentration of Fe in the soil ($1,780.40 \pm 8.60 \mu\text{g/g}$). Also it may be related to the high Packed Cell Volume (PCV), Red Blood Cell (RBC) and Haemoglobin (Hb) recorded when graded doses of the fruit extract were administered to hypercholesterolaemic rats (Sodipo *et al.*, 2009a).

It has been reported by Kloke *et al.*, (1984) that Cd, T and Zn have the highest soil to plant transfer coefficient, in part because of their relatively poor sorption in the soil, while elements such as Cu, Ca, Cr, and Pb have low transfer coefficient and are strictly bound in the soil structure.

Cu is an essential substance to human life, however in high concentration it can be highly toxic to fish (Grosell *et al.*, 1997) and causes anaemia, liver and kidney damage, stomach and intestinal irritation (Turnland, 1988). From the study, concentration of Cu in the soil was small, ($10.21 \pm 1.23 \mu\text{g/g}$) whilst that in the fruit was moderate $151.75 \pm 2.69 \mu\text{g/g}$. The moderately high concentration of the Cu in the fruit was therefore not due to the uptake from the soil and the fruit is probably not toxic.

CONCLUSION

The implication of the low concentration of some heavy metals and the non-detection of others in both the fruit and soil is that heavy metal intoxication following the administration of either the whole fruit or its extract to man and animal is safe and that the degree of pollution in the area where the fruit was obtained was low or there was poor sorption of these elements by the plant root.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge the technical assistance of Mr. Fine Akawo and the National Agency for Food and Drug Administration and Control (NAFDAC, Maiduguri office for elemental analysis determination.

REFERENCES

- Abdulrahman FI (2004). Studies on the chemical contents and pharmacological activities of the root-bark extract of *Vitex doniana* (Black Pum). PhD. Thesis University of Maiduguri, Maiduguri Nigeria pp 166.
- Akan JC, Abdulrahman FI, Sodipo OA, Chiroma YA (2010b). Distribution of heavy metals of the liver, kidney and meat of beef, mutton, caprine and chicken from Kasuwan Shanu market in Maiduguri Metropolis, Borno state, Nigeria. *Res J. Appl. Sci. Engin. Tech* 2 (8): 743-748
- Akan JC, Abdulrahman FI, Sodipo OA, Ochanya AE, Askira YK (2010a). Heavy metals in sediments from river Ngada, Maiduguri Metropolis, Borno state, Niger. *J. Environ. Chem. Ecotoxicol.* 2 (9): 131-140
- Aliu YO (2007). *Veterinary Pharmacology 1st ed.* Tamaza Pub. Com. Ltd. Zaria. Nigeria pp 282 – 341.
- Alloway BJ (1998). *Heavy Metals in Soils.* 2nd ed. Blaker, London p. 91
- Amartey EO, Asumadu-Sakyi AB, Adjei CA, Quashie TK, Duodu GO, Bentil NO (2011). Determination of heavy metals concentration in hair pomades on the Ghanaian markets using atomic absorption spectrometry technique. *Bri. J. Pharmacol. Toxicol.* 2(1): 192 – 198.
- ANON (2007). Solanaceae. http://www.medicalglossary.org/solanacea_solanum_definition.html. Access date:- 26/5/2007
- Ewers U, Schlipkoter HW (1991) Lead In: *Metals and Their Components in the Environment. Occurrence, Analysis and Biological Relevance* (Ed. E. Merian) Wiley – VCH Weinheim Pub. pp1,438
- Grosell MH, Hogstrand C, Wood CM (1997). Copper uptake and turnover in both copper acclimated and non-acclimated rainbow trout (*Oncorhynchus mykiss*). *Aquat Toxicol.* 38: 257 – 276.
- Grubben GJH, Denton OA (2004). PROTA 2. *Plant Resources of Tropical Africa 2 Vegetables* Ponen nad Looijen hy, Wagening en, Netherlands, pp 667
- Hakeem AH (1987). *Medical Elementology: A Bibliography of Work Done in India, Pakistan and Bangladesh.* Institute of Medicine and Medical Research. Hamdard Nagas, New Delhi India. P 38.
- Health Concerns (2003). Heavy Metal Toxicity. Life Extension Foundation <http://www.bewholewell.com/what-you-should-know-about-heavy-metals>. Access date: 25/05/2011.
- Kabata PA, Pendias H (1984). *Trace Elements in Soils and Plants* CRS Press. Boca Raton.
- Kloke A, Sauerback DR, Vetter H (1984). *Changing Metal Cycles and Human Health.* Springer – Verlag, Berlin.
- Lawrence DR, Bennet PN, Brown MJ (1997). *Clinical Pharmacology.* 8th ed. Churchill Livingstone, New York, USA pp. 478 – 483.
- Ogugbuaja VO (2000). *Absorption / Emission Spectroscopy: An Instrumental Methodology in Analytical Chemistry.* 1 – 189.
- Ozdamar K (1991). *Biostatistics with SPSS* Kan Press, Eskisehir.
- Radojevic M, Bashkin V (1999). *Practical Environmental Analysis.* The Royal Society of Chemistry. Thomas Goraham House, Science Park, Milton Rd, Cambridge, U.K. pp. 391 – 396.
- Reuben KD, Akan JC, Abdulrahman FI, Sodipo OA (2008). Elemental content in plant sample of *Croton zambesicus* from Mubi, Adamawa State, Nigeria. *Continental J. Appl. Sci.* 3: 46 – 50.
- Sodipo OA (2009). Studies on chemical components and some pharmacological activities of *Solanum macrocarpum* Linn. fruit (Garden egg). Ph D Thesis, University of Maiduguri, Nigeria. pp 387.
- Sodipo OA (2010). Heavy metal toxicity and human health. In; *Religion and Environment* (ed. Yahya O. Imam). Book Wright Nig Pub. Ibadan, Nigeria. A Publication of ASIRS (Association for the Study of the Interplay Between Religion and Science) pp. 229-243.
- Sodipo OA, Abdulrahman F, Sandabe U (2012b). Effects of the aqueous fruit extract *Solanum macrocarpum* Linn on haematological parameters of chronic triton-induced hyperlipidemic rats. *J. Phys. Pharm. Adv.* 2 (2): 122-132
- Sodipo OA, Abdulrahman FI, Sandabe UK (2012a). Total lipid profile, faecal cholesterol, very low density lipoprotein cholesterol (VLDL-C), atherogenic index (A.I) and percent atherosclerosis with aqueous fruit extract of *Solanum macrocarpum* in chronic triton-induced hyperlipidemic albino rats. *Current Res J. Biol. Sci* 4(2):26-34.
- Sodipo OA, Abdulrahman FI, Sandabe UK (2012c). Biochemical kidney function with aqueous fruit extract of *Solanum macrocarpum* Linn. albino rats chronically administered triton-X to induce hyperlipidemia *J.Med.Medical Sci.* 3 (2): 93-98
- Sodipo OA, Abdulrahman FI, Sandabe UK, Akinniyi (2008). Effect of aqueous fruit extract of *Solanum macrocarpum* on cat blood pressure and rat gastrointestinal tract. *J. Pharm. Biores.* 5 (2): 52-59
- Sodipo OA, Abdulrahman FI, Sandabe UK, Akinniyi JA (2009a). Effects of the aqueous fruit extract of *Solanum macrocarpum*. Linn on some haematological indices in albino rats fed with cholesterol-rich diet. *Sahel J. Vet. Sci.* 8(2): 5 – 12.
- Sodipo OA, Abdulrahman FI, Sandabe UK, Akinniyi JA (2009b). Effect of *Solanum macrocarpum* on biochemical, liver function in diet-induced hypercholesterolaemic rats. *Nig. Vet. J* 30 (1): 1-8
- Sodipo OA, Abdulrahman FI, Sandabe UK, Akinniyi JA (2009c). Total lipid profile with aqueous fruit extract of *Solanum macrocarpum* Linn. in hypercholesterolaemic albino rats. *J. Pharm. Biores.* 6 (1): 10-15
- Sodipo OA, Abdulrahman FI, Sandabe UK, Akinniyi JA (2009d). Effects of the aqueous fruit extract of *Solanum macrocarpum* Linn. on some haematological indices in albino rats fed with cholesterol-rich diet. *Sahel J. Vet. Sci.* 8 (2): 5-12
- Sodipo OA, Abdulrahman FI, Sandabe UK, Akinniyi JA (2011a). Total lipid profile and faecal cholesterol with aqueous food extract of *Solanum macrocarpum* in triton-induced hyperlipidemic albino rats. *J. Medicinal Plants Res.* 5 (6): 3833-3856
- Sodipo OA, Abdulrahman FI, Sandabe UK, Akinniyi JA (2011b). Effects of the aqueous fruit extract of *Solanum macrocarpum* Linn. on the hematological parameters of triton-induced hyperlipidemic rats. *Afr. J Pharm. Pharmacol* 5 (5): 632-639
- Turnland JR (1988). Copper nutrition, bioavailability and influence of dietary factors. *J. Am. Dietetic Assoc.* 1: 303 – 308.
- US Food and Drug Administration (1999). Centre for Food Safety and Applied Nutrition. *Economic Characterization of Dietary Supplement Industry Final Report.* pp. 1 – 113.
- Vtorova VN (1987). Substantiation of methods and objects of observations over chemical composition of plants during monitoring of forest ecosystem. Information Bulletin on Problem III of the Council of Mutual Economic Help. Nos. 1–2 Ecological Cooperation. Bratislava.
- Waalkes MP (1991). Cadmium and human health. *Health Environ. Digest.* 5(4): 1 – 3.
- WHO (1973). *Environmental Health Criteria 3: Principles and Methods for Evaluating the Toxicity of Chemicals.* Part I, Geneva.
- WHO (1996) Guidelines for Elemental Concentration. Trace Elements in Health and Human Nutrition pp. 50 – 228.
- Wilson I (2008). The dangers of toxic metals <http://www.dreddyclinic.com/dangers-of-toxic-metals.htm>. Access date: 25/05/2011.