

***Euphorbia granulata* Forssk as a Source of Mineral Supplement**

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Abstract: Medicinal plants serve dual function of providing the therapeutic agents and supplying the mineral nutrients. In the present study *Euphorbia granulata* Forssk was evaluated for its role in supplementing the essential mineral nutrients to the patients. Macro-minerals (K, S, Ca, P, Mg and N) and micro-minerals (Fe, Mn, Zn, Cu, Mo, Na, Cl and Se) were analyzed. The Fe contents were 801 ppm while the suggested RDA is 8 mg/day. Similar trends of good quantities of mineral nutrients were recorded for Mn (4883 ppm), Zn (47.1 ppm), Mo (1.9 ppm) and Se (34.46 ppm). Cu and Cl were not detected in whole plant. Na, K, Ca and P were also found above the recommended dietary allowances. The results support the beneficial use of the plant as a nutritional supplement.

Key words: Minerals • Nutrients • *Euphorbia granulata* • Recommended Dietary allowances • Permissible limits and Edible plants

INTRODUCTION

For proper physiological and biochemical functioning both plant and animals need mineral nutrients. The root cause of many health problems in man are either deficiency or excess of minerals [1]. Plant is one of the sources of mineral for man and animals. Researchers are trying to find out the mineral composition of medicinal plant and correlate for treating the diseases. Elemental composition [2] of lemon grass collected from three different areas and reported that sodium, potassium, calcium, magnesium and silicon were present in high quantity (2.5, 53.4, 26.2, 2 and 10% respectively). The remaining of elements were less than 2% in all the analyzed samples. It was reported [3] that stem bark of *Harungana madagascariensis* had Cl, Ca, Mn, K and Sr between 10.5 to 774.3 mg/g while heavy metals were 1.5 to 7.2 mg/g. The presence of 14 elements in five medicinal plants [4]. *Silybum marianum*, *Fagonia indica*, *Argemone Mexicana*, *Solanum suratense* and *Cnicus benedictus* were found with higher concentration of Fe, Mg, Na and K. Calcium, Co, Mn and heavy metals (Ni, Li, Cr, Cu, Pb, Cd and Zn) were present in low concentration. *Withania somnifera* an important medicinal plant was collected from two important geographical regions and was studied for

its elemental quantities. Sample from one region contain high levels of N, P, Mg, Zn, Cu and Mn while samples from other region were rich in Na, K and Ca. Iron was present satisfactorily in both samples. This shows that habitat have impacts on nutritional status and medicinal value of a medicinal plant [5].

MATERIALS AND METHODS

Protocol used by [6] with minor modification was followed for determining the micro and macro-minerals. From standard stock solution of each element, working solutions were prepared. The samples were subjected to atomic absorption (Perkin-Elmer 1100 B, USA) spectroscopy and the reading noted. A calibration curve was prepared for each sample by plotting absorbance versus concentration. The absorbance for each test element was compared with standard working solution by putting in the calibration curve. The concentrations of test samples were determined from this each curve.

Statistical Analysis: The present data is the results of three repeats for all nutrients. Graphpad prism 5 software was used to processing the data for mean and \pm standard error of mean (\pm SEM).

RESULTS AND DISCUSSION

The mineral contents in soil are presented in Table 2. The quantity of different mineral in whole plant and its parts are summarized in Table 1. In soil the high to low concentration order was S > Ca > P > Fe > K > Na > Mg > Cl > Mn > Zn > Cu > Mo > Se > N. In whole plant these 14 elements were present as S > K > Mg > Na > Ca > P > Fe > Mn > Zn > Se > N > Mo > Cu = Cl (Table 1). The differences in the two orders of the elements suggest that plants are highly selective in absorbing minerals from soils. All the elements in the soil were found within the permissible limits [7].

Mineral may be either requiring in small (micro-minerals) or large amount (macro-minerals). There is no precise definition for trace or micro-mineral yet these are the elements that constitutes <0.01% of the total body weight [8]. Optimum level of mineral (both macro and micro) nutrient is essential for good health. Deviation from optimum limits growth of plants and produces physiological problems in man. Various elements play different roles in plants metabolism.

Iron is an important trace element. It is part of hemoglobin and plays role in the metabolism of lipids, carbohydrates and protein. Its deficiency can lead to anemia [9, 10, 5]. Higher concentration was noted in

Table 1: Distribution of minerals elements in plant and plant parts of *Euphorbia granulata*

| Mineral | Mineral composition (ppm) | | | | |
|---------|---------------------------|--------------|----------------|---------------|---------------|
| | Whole plant | Roots | Stem | Leaves | Seeds |
| Fe | 803.3 ± 4.4 | 416 ± 2.96 | 682 ± 2.33 | 919 ± 2.08 | 105 ± 0.75 |
| Mn | 65.1 ± 0.38 | 59.2 ± 0.11 | 64.43 ± 0.23 | 68.9 ± 0.8 | 18.53 ± 0.088 |
| Zn | 47.1 ± 0.09 | 52.7 ± 0.12 | 45.0 ± 0.07 | 40.0 ± 0.12 | 19.45 ± 0.058 |
| Cu | BDL | 1.9 ± 0.02 | BDL | 0.9 ± 0.02 | 0.66 ± 0.017 |
| Mo | 1.9 ± 0.03 | 2.3 ± 0.03 | 1.5 ± 0.29 | 2.1 ± 0.04 | BDL |
| Ca | 3007 ± 14.5 | 2167 ± 12.01 | 3223 ± 20.27 | 4130 ± 15.27 | 3164 ± 7.44 |
| P | 1638 ± 6.0 | 1378 ± 2.08 | 1605 ± 7.63 | 1998 ± 6.0 | 1942 ± 4.41 |
| Mg | 4883 ± 20.27 | 2273 ± 6.66 | 4970 ± 5.77 | 5265 ± 7.63 | 2198 ± 6.0 |
| S | 31183 ± 60.09 | 36157 ± 34.8 | 26500 ± 76.37 | 35633 ± 60.09 | 21070 ± 17.32 |
| N | 2.80 ± 0.02 | 2.75 ± 0.009 | 2.52 ± 0.006 | 3.15 ± 0.01 | 3.58 ± 0.012 |
| K | 12533 ± 109.3 | 2571 ± 10.9 | 12800 ± 15.275 | 9675 ± 7.638 | 1332 ± 4.41 |
| Na | 3498 ± 19.22 | 4261 ± 4.41 | 2885 ± 7.63 | 882 ± 7.26 | 470 ± 5.23 |
| Cl | BDL | 1.98 ± 0.06 | BDL | BDL | BDL |
| Se | 34.46 ± 0.08 | 89.8 ± 0.04 | 23.57 ± 0.06 | 27.81 ± 0.038 | 2.78 ± 0.043 |

Values are the mean of 3 replicates with ±SEM.

Table 2: Mineral composition of representative soil sample where EG grown and permissible limits for these elements in soil

| Mineral | Concentration in soil with ±SEM | Permissible concentration for soil | Reference source |
|---------|---------------------------------|------------------------------------|----------------------------------|
| Fe | 23233 ± 504.4 | 129000ppm | [7] Ramamurthy and Kannan (2009) |
| Mn | 516 ± 12.01 | 1000ppm | [7] Ramamurthy and Kannan (2009) |
| Zn | 226 ± 8.8 | 300ppm | [7] Ramamurthy and Kannan (2009) |
| Cu | 2.7 ± 0.06 | 100ppm | [7] Ramamurthy and Kannan (2009) |
| Mo | 1.5 ± 0.019 | 4ppm | [17] Chesworth, (2008) |
| Ca | 33200 ± 86.8 | 52000ppm | [7] Ramamurthy and Kannan (2009) |
| P | 27116 ± 116.66 | Not specified | - |
| Mg | 5433 ± 60.09 | 9000ppm | [7] Ramamurthy and Kannan (2009) |
| S | 45633 ± 88.19 | Not specified | - |
| N | Not done | Not specified | - |
| K | 23150 ± 86.60 | 37000ppm | [7] Ramamurthy and Kannan (2009) |
| Na | 19050 ± 86.60 | 25000ppm | [7] Ramamurthy and Kannan (2009) |
| Cl | 1596 ± 8.8 | Not specified | - |
| Se | 1.4 ± 0.05 | 2ppm | [7] Ramamurthy and Kannan (2009) |

Values are the mean of 3 replicates with ±SEM.

Table 3: Recommended Dietary allowances (RDA) and permissible limits (PL) for mineral elements

| Mineral | *RDA (mg / day) | Reference and authority | PL in edible plants and authority | Reference source |
|---------|------------------|---|-----------------------------------|----------------------------------|
| Fe | 8.0 | Gropper <i>et al.</i> (2009); NAS, (2005) | 20ppm (FAO / WHO) [20] | [11] Mtunzi <i>et al.</i> (2012) |
| Mn | 2.3 | Gropper <i>et al.</i> (2009); NAS, (2005) | 2ppm (FAO / WHO) [20] | [11] Mtunzi <i>et al.</i> (2012) |
| Zn | 11.0 | Gropper <i>et al.</i> (2009); NAS, (2005) | 27.4ppm (FAO / WHO) [20] | [18] Jabeen <i>et al.</i> (2010) |
| Cu | 0.9 | Gropper <i>et al.</i> (2009); NAS, (2005) | 3ppm (FAO / WHO) [20] | [11] Mtunzi <i>et al.</i> (2012) |
| Mo | 0.045 | Gropper <i>et al.</i> (2009); NAS, (2005) | 0.02ppm (FAO / WHO) [20] | [18] Jabeen <i>et al.</i> (2010) |
| Ca | 1000 | Gropper <i>et al.</i> (2009); NAS, (2005) | Not specified | [19] Duruibe, (2010) |
| P | 700 | Gropper <i>et al.</i> (2009); NAS, (2005) | Not specified | - |
| Mg | 400-420 | Gropper <i>et al.</i> (2009); NAS, (2005) | Not specified | - |
| S | Not specified | Dietary mineral | Not specified | - |
| N | 56000 (proteins) | Gropper <i>et al.</i> (2009); NAS, (2005) | Not specified | - |
| K | 4700 | Gropper <i>et al.</i> (2009); NAS, (2005) | Not specified | - |
| Na | 1300-1500 | Gropper <i>et al.</i> (2009); NAS, (2005) | Not specified | - |
| Cl | 2300 | Gropper <i>et al.</i> (2009); NAS, (2005) | Not specified | - |
| Se | 0.055 | Gropper <i>et al.</i> (2009); NAS, (2005) | Not specified | - |

*RDA= Recommended dietary allowances.

leaves (919 ± 2.08 ppm) while seeds had low (105 ± 0.75 ppm) concentration. The high concentration in leaves suggest the high metabolic activities of leaves. As seeds remain dormant for long time they require small amount of iron. In whole plant iron concentration was 803.3 ± 4.4 ppm. The permissible limit (Table 3) of iron for edible plants is 20 ppm [11]. This shows a concentration for high than permissible limit but *Euphorbia granulata* is a medicinal plant and is used in small amount. Iron is a micro-mineral and is needed for good health [8]. For a healthy male of age between 19 to 50 years the suggested RDA is 8 mg/day (Table 3). As iron is part of hemoglobin and many metabolic enzymes this suggests that *Euphorbia granulata* might be a good source of Iron.

Table 1 shows high manganese accumulation (68.9 ± 0.8 ppm) in leaves. There were little variations in roots, stem and leaves. However seeds contained 4 times less manganese (18.53 ± 0.088 ppm) than the leaves. Manganese, a micro-mineral, is needed for proper functioning of enzymes like transferases, hydrolases, oxidoreductases, ligases and lyases [8]. It is important for the development of bones, fats and carbohydrates metabolism. Mg deficiency causes myocardial infarction, formation of defective cartilages in infants, immunodeficiency and rheumatoid arthritis [10, 11, 9]. For ingestion the permissible range is only 2 ppm for edible plants (Tables 1 and 3). Although Mn is very high but the amount of plant usually ingested is small that will not be harmful. Furthermore, the element is essential to be taken in diet. The RDA for Mn is 2.3 mg (Table 3). The concentration found in *Euphorbia granulata* and the daily need of the body suggests that the use of plant for medicinal purpose can be a good source of this micro-mineral.

Zinc is needed for normal insulin secretion, wound healing, normal growth and development, brain health and behavioral development [9]. The distribution order of Zn in plant parts was as; roots > whole plant > stem > leaves > seeds (Table 1). High amount in roots indicates the high retention of Zn. The permissible limit for edible plants is 27.4 ppm, while the Zn found in whole plant was 47.1 ± 0.09 ppm (Table 1 and 3). Roots contained 52.7 ± 0.12 ppm while seeds 19.45 ± 0.058 ppm. The present concentration in the whole plant is almost double of the permissible limit. Based on the need of the body for Zn the suggested RDA is 11.0 mg (Table 3). *Euphorbia granulata* as a medicinal plant will help providing the Zn in sufficient amount to fulfill the need of patient.

Copper is also an essential micro-mineral but may be toxic if taken in large amount. The permissible amount to be present in edible plants is 3ppm (Tables 1 and 3). In the present study copper was not detected in whole plant. However, roots were noted with 1.9 ± 0.02 ppm and seeds with 0.66 ± 0.017 ppm (Table 1). This small concentration reduces the chances of copper toxicity with consumption of plant. Furthermore, ceruloplasmin, superoxide dismutase, cytochrome c oxidase, amine oxidases, lysyl oxidase and many more enzymes need copper as a cofactor [8]. Copper is required for absorption and incorporation of iron in to hemoglobin, RBCs formation, mitochondrial activities and RNAs the deficiency leads to anemia, fragility of blood vessels and bone cortices [10, 5]. Sufficient amount of the copper should be present in the diet to fulfill the daily requirements. The suggested RDA is 0.9 mg (Table 3). The amount found in *Euphorbia granulata* suggests that this plant might not be a good source of copper. However, the amount found in roots and seeds will contribute to the overall need of the body.

Molybdenum is essential part of body enzymes like xanthine oxidase, nitrate reductase and sulfite oxidase (essential enzymes for electron transport). Molybdenum within permissible limits help in copper utilization but high intake produces copper deficiency which leads to microcytic anemia [12, 9]. To meet the need of the body metabolic activities the suggested RDA is 45µg for molybdenum (Table 3). The element found in this plant was in order of, roots > leaves > whole plant > stem > seeds (Table 1). Mo concentration in whole plant was 1.9 ± 0.03 ppm, while permissible concentration in edible plants is 0.02 ppm (Tables 1 and 3). Higher concentration was noted for roots (2.3 ± 0.03 ppm) while it was not detected in seeds. The high concentration noted for Mo in plant and plant parts apparently looks high but the plant is not an edible one and used in small amount. The overall amount found in plant suggests that its ingestion will help in providing molybdenum to the daily need of consumer.

Selenium is a micro-mineral that plays important role in preventing liver cell necrosis. It is a prosthetic group for the enzyme glutathione peroxidase which is involved in reducing hydrogen peroxide to water and hence helpful anti oxidant role [12, 9]. The concentration in whole plant was 34.46 ± 0.08 ppm, while major portion of this is located in roots, was 89.8 ± 0.04 ppm. Seeds contained only 2.78 ± 0.043 ppm. Selenium is needed in small amount (RDA 55 µg/day). The recorded concentration suggests that it will benefit the health of consuming patient.

Macro-minerals are elements that at least each of them constitute 0.01% of the total body weight [9]. Calcium, a macro-mineral, is vital for bones and teeth health. It is needed for cardiac muscles and nerve impulses, blood and milk clotting [5, 13, 11]. For a person having an age between 19 and 50 years the suggested adequate intake (AI) is 1000 mg [9]. This plant was found with good quantity of calcium. Higher amount was found in leaves compared to concentration found in whole plant and the rest of the plant parts. High amount of calcium is necessary for high metabolic activity in meristematic regions of plants because calcium is essential for cell division [5]. Calcium concentration in whole plant was 3007 ± 14.5 ppm. The high concentration noted for Ca in plant and plant parts may be good for the consumer health. Phosphorus is also an essential part of bones, phospholipids (an essential cell membrane component), constitute part of ATP (an energy rich molecules) and nucleic acids [10, 14]. The results (Table 1) revealed high phosphorus contents in leaves than in other parts. Cells multiplication and growing cells need energy as ATP molecules and for nucleic acids synthesis. This

necessitates the availability of phosphorus. Therefore good quantity of phosphorus is necessary for growing parts of the plants. Phosphorus concentration in whole plant was 1638 ± 6.0 ppm, while in roots it was 1378 ± 2.08 ppm. Almost equal quantities were found in leaves and seeds (1998 ± 6.0 and 1942 ± 4.41 respectively). High amount in leaves is required for active metabolism while seeds need more P at the time of germination. Based on the high needs of phosphorus (macro-mineral) the body metabolic activities high RDA (700 mg/day) is suggested by NSA [15]. As macro-nutrients are required in large amount permissible concentration in edible plants is not specified (Table 1 and 3).

Almost 55 to 60% of total magnesium is associated with bones while the rest is found in muscles and extracellular fluids. It is needed for enzymes of respiration (glycolysis and TCA) especially ATP or ADP and associated enzymes [9]. Deficiency can lead to muscle irritability and convulsion [14, 5]. For adult male the RDA is 400-420 mg (Table 3). The leaves were rich in Mg that can be an efficient source (Table 1). Mg concentration in whole plant was 4883 ± 20.27 , while it was 5265 ± 7.63 ppm in leaves. The least Mg quantity in seeds (2198 ± 6.0 ppm) indicates the reduced metabolic activities of seeds. As Mg is required in large amount the permissible concentration in edible plants is not specified (Tables 1 and 3).

Sulfur is part of acetyl Co-A, amino acids, vitamins and lipids like mucic acid and chondroitin [12, 9]. Distribution in plant parts was as; roots > leaves > whole plant > stem > seeds (Table 1). High level in roots (35633 ± 60.09 ppm) might be the retention by the roots exudates. Sulfur concentration in whole plant was 31183 ± 60.09 ppm while seeds contained least amount of 21070 ± 17.32 ppm. Both permissible concentrations in edible plants and RDA for human are not specified (Tables 1 and 3). The high S concentration observed is good that reflects sulfur containing amino acids, lipid and vitamins which are in sufficient amount and is good for the patient health.

Nitrogen is an essential element and parts of protein, porphyrins, lipid, purines, pyrimidine and vitamins. The nitrogen contents of a plant reflect the protein contents in the plant which is already discussed in protein portion. For adult male the required RDA is 56.0 gm proteins/ay (Table 3).

Potassium is the major extracellular cation widely present in body fluids and tissues. It is involved in muscular activities, acid base balance and neuromuscular activities. It is part of certain metabolic enzymes like pyruvate kinase and has important role in cardiac function [12, 9, 5, 16]. Potassium was found distributed in different

parts as; stem > whole plant > leaves > roots > seeds (Table 1). Similar results for potassium distribution in plant parts were also reported by [10]. Potassium concentration in whole plant was 12533 ± 109.3 ppm. Highest concentration was noted for stem (12800 ± 15.275) and the lowest for seeds (1332 ± 4.41). High concentration in the plant may help restoring the electrolyte balance in patient of diarrhea. The high RDA for potassium (4700 mg) indicates the need of a continuous supply. While taking plant by a person, some parts of the needed potassium will be obtained from this plant.

Sodium is found in extracellular body fluids. Its major role is maintaining electrolyte balance [12, 9, 5]. Sodium is also needed in high amount by the human body (RDA 1300-1500 mg daily). There was 19050 ± 86.60 ppm in whole plant (Table 1). The major portion of which is distributed into the roots (4261 ± 4.41 ppm). Only 470 ± 5.23 ppm was recorded for seeds. This shows that plant roots contained 9 times more sodium than the seeds. As roots are in direct contact with soil and soil is the only source of electrolytes for plants the large proportion in roots might accumulation due to this direct contact with soil. Plant most often contain variable amount of sodium but the daily requirements of human are most often met from table salt.

Chlorine is important for production of HCl in stomach and shifting chloride during respiration [12, 9]. Chloride was detected in roots only (1.98 ± 0.06). Therefore, this plant was not good source of chlorine while the human need it in large amount (RDA 2300 mg/day) to fulfill the requirements. Like sodium the human get the additional chloride from table salts (NaCl).

CONCLUSIONS

Most of the essential mineral elements are required in minute amount (Fe, Mn, Zn, Se and Mo etc); and the plant has the potential to supply these elements at therapeutic dose. Analysis of *Euphorbia granulata* revealed that only copper and Chlorides were present in small amount while the rest of micro and macro minerals are present in higher concentrations. It is suggested that the plant could be a good source of these minerals.

REFERENCES

1. Saeed, M., H. Khan, M.A. Khan, F. Khan, S.A. Khan and N. Muhammad, 2010. Quantification of various metals and cytotoxic profile of aerial parts of *polygonatum verticillatum*. Pak. J. Bot., 42(6): 3995-4002.
2. Aftab, K., M.D. Ali, P. Aijaz, N. Beena, H.J. Gulzar, K. Sheikh, Q. Sofia and S.T. Abbas, 2011. Determination of different trace and essential element in lemon grass samples by x-ray fluorescence spectroscopy technique. Int. Food Res. J., 18: 265-270.
3. Iwalewa, E.O., N.O. Omisore, O.M. Daniyan, C.O. Adewunmi, B.J. Taiwo, O.A. Fatokun and I.O. Oluborode, 2009. Elemental compositions and anti-anemic property of Harungana madagascariensis stem bark. Bangladesh J. Pharmacol., 4: 115-121.
4. Zafar, M., M.A. Khan, M. Ahmad, G. Jan, S. Sultana, K. Ullah, S.K. Marwat, F. Ahmad, A. Jabeen, A. Nazir, A.M. Abbasi, Z. Rehman and Z. Ullah, 2010. Elemental analysis of some medicinal plants used in traditional medicine by atomic absorption spectrophotometer (AAS). J. Med. Plants Res., 4(19): 1987-1990.
5. Krishnamurthy, S.R. and P. Sarala, 2010. Proximate nutritive values and mineral components of *Withania somnifera* (Linn.) Dunal. E-Jour. Chem., 7(3): 985-996.
6. Aslam, M., F. Anwar, R. Nadeem, T. Rashid, T.G. Kazi and M. Nadeem, 2005. Mineral Composition of *Moringa oleifera* leaves and pods from different regions of Punjab, Pakistan. Asian J. Plant Sci., 4(4): 417-421.
7. Ramamurthy, N. and S. Kannan, 2009. Sem-eds analysis of soil and plant (*Calotropis gigantea* Linn) collected from an industrial village, cuddalore dt, tamil nadu, India. Rom. J. Biophys., 19(3): 219-226.
8. Gropper, S.S., J.L. Smith and J.L. Groff, 2009. Advanced nutrition and human metabolism. 5th ed. Wadsworth, Cengage Learning, Belmont, USA.
9. Khan, S.A., L. Khan, I. Hussain, K.B. Marwat and N. Akhtar, 2008. Profile of heavy metals in selected medicinal plants. Pak. J. Weed Sci. Res., 14(1-2): 101-110.
10. Shad, A.A., H. Shah, F.K. Khattak, N.G. Dar and J. Bakht, 2002. Proximate and mineral constituents of medicinal herb *Fagonia Arabica*. Asian J. Plant Sci., 1(6): 710-711.
11. Mtunzi, F., E. Muleya, J. Modise, A. Sipamla and E. Dikio, 2012. Heavy metals content of some medicinal plants from Kwazulu-Natal, South Africa. Pak. J. Nutr., 11(9): 757-761.
12. Erdman, J.W., I.A. Macdonald and S.H. Zeisel, 2012. Present Knowledge in Nutrition, 10th edi. Wiley Blackwell, A John Wiley and Sons, Ltd., Publication, Iowa USA.

13. Hussain, I., R. Ullah, M. Khurram, N. Ullah, A. Baseer, F.A. Khan, N. Khan, M.U.R. Khattak, M. Zahoor and J. Khan, 2011. Heavy metals and inorganic constituents in medicinal plants of selected Districts of Khyber Pakhtoonkhwa, Pakistan. *African J. Biotech.*, 10(42): 8517-8522.
14. Yusuf, A.A., B.M. Mofio and A.B. Ahmed, 2007. Proximate and mineral composition of *Tamarindus indica* Linn 1753 seeds. *Sci. World J.*, 2(1): 1-4.
15. NAS (National Academies of Sciences), 2005. Dietary Reference Intakes series. National Academies Press USA.
16. Kawo, A.H., B.A. Abdullahi, Z.A. Gaiya, A. Halilu, M. Dabai and M.A. Dakare, 2009. Preliminary phytochemical screening, proximate and elemental composition of *Moringa oleifera* lam seed powder. *Bajopas*, 2(1): 96-100.
17. Chesworth, W., 2008. *Encyclopedia of Soil Science*. Springer, The Netherlands.
18. Jabeen, S., M.T. Shah, S. Khan and M.Q. Hayat, 2010. Determination of major and trace elements in ten important folk therapeutic plants of Haripur basin, Pakistan. *J. Med. Plants Res.*, 4(7): 559-566.
19. Duruibe, J.O., M.O.C. Ogwuegbu and J.N. Ekwurugwu, 2007. Heavy metal pollution and human biotoxic effects. *Int. J. Phys. Sci.*, 2(5): 112-118.
20. FAO/WHO, 1984. Contaminants. In *Codex Alimentarius*, Vol. XVII, Edition 1. FAO/WHO. Codex Alimentarius Commission, Rome.