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Study of Some Heavy Metals Contamination in Two *Tinospora* (ဆင်တုံးမန္ဓယ်) Species

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Medicinal plants are the most important source of life saving drugs for the majority of the world population. World Health Organization (WHO) estimates that more than 80% of people in developing countries depend on traditional medicine for their primary health needs. Heavy metals are metallic elements with high atomic number and poisonous to living organisms. Plants may absorb heavy metals from soil, water or air. The purpose of present study was to determine the heavy metals contamination in *Tinospora cordifolia* (ဆင်တုံးမန္ဓယ်ပြောင်ချော) and *Tinospora crispa* (ဆင်တုံးမန္ဓယ်ဆူးပေါက်), which are reputed to have antidiabetic, antiinflammatory, antiarthritic, antispasmodic and antiallergic activities. The atomic absorption spectrophotometer (AAS) was used for determination of heavy metals (Cd, Cr, Cu, Fe, Pb and Zn) in two *Tinospora* species and their surrounding soils from Mandalay, Pyin Oo Lwin and Shwe Bo. These two *Tinospora* species contained metals (Cd, Cr, Cu, Pb and Zn), which were within permissible limits except Fe content more than (20 ppm), set by WHO, 2005. All soils contained metals which were within permissible limits. These findings obtained from present study will be helpful for herbal medicine users, local practitioners and pharmaceutical industries using these two *Tinospora* species for different types of ailments. In conclusion, monitoring such medicinal plants for heavy metals is important in protecting the public from adverse and hazardous effects of heavy metals.

Key words: Heavy metals, *Tinospora* species, AAS

INTRODUCTION

Medicinal plants are important source of life saving drugs for the majority of the world population. There is a common concept among people that herbal medicines have no side effects and that "being natural in origin, herbs are safe".[1] World Health Organization estimates that more than 80% of people in developing countries depend on traditional medicine for their primary health needs.[2]

Heavy metals are metallic elements with high atomic number and poisonous to living organisms.[3] Heavy metals are naturally present in the environment.[4] Plants may absorb heavy metals from soil, water or air.[5] Heavy metal contents in medicinal plants depend on climatic factors, plant species, air pollution and other environmental factors. There are some studies from different areas on the accumulation of heavy metals in certain medicinal plants.[1] *Tinospora* species are one of such plants which is widely used in indigenous system of medicine.[6]

The notable medicinal properties of *Tinospora cordifolia* (ဆင်တုံးမန္ဓယ်ပြောင်ချော) are antidiabetic, antiperiodic, antispasmodic, antiinflammatory, antiosteoporetic, anti-arthritic, antioxidant, antiallergic, antistress, antileprotic, antimalarial, hepatoprotective, immunomodulatory, antitumor and anti-neoplastic activities.[7]

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Tinospora crispa (ဆင်တုံးမန္ဓယ်ဆူးပေါက်) is used for the treatment of flatulence, indigestion and diarrhea and as tonic, anti-spasmodic, antiinflammatory, antiarthritic, antiallergic and antidiabetic.[8] *Tinospora crispa* possesses more therapeutic action than *Tinospora cordifolia.* However, in Myanmar, many traditional practitioners used *Tinospora cordifolia,* because it is readily available.

The aim of this study was to determine the heavy metals contamination of two *Tinospora* species (*Tinospora cordifolia* and *Tinospora crispa*) and their surrounding soil samples from Mandalay, Pyin Oo Lwin and Shwe Bo. Pyin Oo Lwin has many rock mining sites. Shwe Bo is agricultural area and also near the production of glaze earthen jar. Mandalay area is comparing for above two places. Thus, it supports the idea that every medicinal plant must be tested for contamination of heavy metals before dealing out it for further their use in medications. This study aimed to find out the evidence of heavy metals contamination in two *Tinospora* species.

MATERIALS AND METHODS

Reagents and chemicals

Analytical grade (Merck) cadmium (Cd), chromium (Cr), copper (Cu), iron (Fe), lead (Pb), zine (Zn) standard, 70% nitric acid (HNO_3), 69% hydrochloric acid (HCl), air-acetylene gas and double deionized water (DDW) were used.

Instruments and apparatus (glassware)

Atomic Absorption Spectrophotometer (AAS), AA 6650 with auto sampler for flame ASC 6100, Shimadzu Japan, muffle furnace (LEF 1035), oven, soil pH meter, analytical balance, vortex, heating magnetic stirrer, volumetric flask, measuring cylinder, beaker, pipette, crucible and desiccators were used.

Plant authenticity

Sample plants were identified and confirmed for its specific botanical name by competent taxonomists from Department of Botany, University of Mandalay.

Sample collection

Two *Tinospora* species were collected from Mandalay (Mdy), Pyin Oo Lwin (POL) and Shwe Bo (SB). Soil samples were collected from surrounding plants at about 8-10 cm depth of the soil. Both plant and soil samples were placed in sampling bags. Water samples were collected at depth of 20 cm from the water surface directly into one-liter polyethylene bottle.

*Plant samples ana*lysis

The plant samples were thoroughly washed with tap water and rinsed with distilled water to remove dust and other particles. The cleaned plant samples were dried in shade at room temperature. The dried samples were crushed, powdered and homogenized, using mortar and pestle. Then, they were dried in oven at 60°C to get constant weight and ground to powder for analysis of heavy metals.

Digestion of plant samples

Specified weight (2.5 g) of crushed and powdered portion from each plant of two *Tinospora* species was taken into crucible for heating in oven at 110°C for 2 hours to remove moisture. Then, they were placed in furnace. The furnace temperature was gradually increased from room temperature to 550°C within 30 minutes. It was maintained for about 4 hours until white or grey ash residue were obtained. The content of crucible were cooled in desiccators and weighed. Then, 5 ml of 6 M HNO_3 were added to ash samples of each plant to dissolve and digest the contents. The solution was filtered by Whatman 70 mm filter papers, transferred to 50 ml volumetric flasks and diluted to the mark with DDW.[9]

Digestion of soil samples

Soil samples were dried in an oven at 110°C for 2 hours until they were brittle and crisp. One gram of dried soil sample from each site was placed separately in 250 ml Pyrex beakers and then digested

with 12 ml of HNO_3-HCl mixture (1:3 v/v) to near dryness in an oven at 110°C for 3 hours and cooled. And then, 20 ml of 2% HNO_3 was added into each beaker and heated on a hot plate to boil for 10 minutes and cooled. The digested samples were filtered into 100 ml volumetric flask using Whatmann 70 mm filter paper and diluted to the mark with DDW.[10] A blank control was carried out in the same way by using solvent alone.

Standard solutions of each metal were separately prepared from their respective concentration of 1000 mg/ml stock solutions, from which further serial dilutions were made to cover the optimum absorbance range for standard calibration curve. Reagent blank determination was used to correct the instrument readings. Sample run was conducted in triplicates.[11]

Physico-chemical analysis of water and soil samples

Physico-chemical properties like electrical conductivity (EC), total dissolved solids (TDS) and salinity of the water and soil samples were measured by using Pocket Pro™ Tester, HACH, China. pH was also recorded by using Waterproof Handheld (H160), HACH, China, portable pH meter. Prior to estimation of soil pH, the instrument was calibrated with buffer solutions of pH 4, 7 and 10. Twenty grams of air dried soil were mixed with 20 ml DDW to make 1:1 slurry. The slurry was agitated thoroughly for 30 minutes to stabilize the pH and then electrode was immersed to take the reading.

Ten grams of dry crushed soil sample (<0.2 mm) of each type were mixed with 50 ml of deionized water in a bottle to make 1:5 ratio (w/v) slurry and the mixtures were shaken thoroughly for complete dissolution of soluble salts. The soil was allowed to settle down and then conductivity cell was inserted to take the readings. Then, filtrates of soil-water slurry 1:5 ratio (w/v) were used for analysis of TDS and salinity.[12]

Phytochemical test for types of compounds

Phytochemical compounds present in two *Tinospora* species were tested according to phytochemical methods.[13]

Statistical analysis

Data were analyzed by using Microsoft Excel v. 2007. Results were calculated on dry weight basis (µg/g) and presented as mean±SE.

RESULTS AND DISCUSSIONS

Plant authenticity

Morphology, taxonomy and anatomy of the plants were observed to agree with following botanical names.[14]

Tinospora cordifolia (Willd.) Hook. F & Thoms (ဆင်တုံးမန္နယ်ပြောင်ချော)

Tinospora crispa (L) Hook. F & Thoms (ဆင်တုံးမန္နယ်ဆူးပေါက်)

Tinospora cordifolia (Willd.) Hook. F & Thoms is deciduous lianas belonging to the family Menispermaceae. The stems are cylinder, smooth, with aerial roots, glabrous. Leaves are simple, alternate, exstipulate. Inflorescences axillary raceme of staminate flowers, the pistillate ones solitary in raceme. Flowers are yellowish green, 5.0-7.0 mm in diameter at anthesis, unisexual actinomorphic trimerous, hypogynous. Flowering period is February to May. Fruits are drupes, ovoid, indehiscent, red when ripe. Seeds are with endosperm, oblong- ellipsoid and glabrous.

Tinospora crispa (L) Hook. F & Thoms is perennial, dioecious and climbing vines belonging to the family Menispermaceae. Stems and branches are tuberculate with aerial roots. Leaves are simple, alternate and exstipulate. Inflorescences raceme or

paniculate fascicles at the leafless nodes, often pendulous. Flowers are green or greenish yellow, 5.0-7.0 mm in diameter at anthesis, unisexual actinomorphic trimerous, hypogynous. Flowering period is January to March. Fruits are drupaceous, druplets 1-3, subgloboid, indehiscent, with one pyrene, orange when ripe. Seeds are with endosperm usually ruminate, ventrally grooved or curved. In all studied soils samples, 'Cd' and 'Cu' were not detected. The findings of this study indicated that the presence of heavy metals analyzed at three different soil samples were within permissible limit set by FAO/WHO, 2001[16] (Table 1).

Table 1. Content of heavy metals (ppm) in soil samples from different places

Metal	Mdy	POL	SB	Ref: value[#]
Cd	ND	ND	ND	≤3
Cr	20.97±0.31	14.20±0.29	15.09±0.33	≤100
Cu	ND	ND	ND	≤100
Fe	24561±6.04	2479.14±4.67	2329.07±6.57	≤50000
Pb	ND	ND	25.57±0.01	≤100
Zn	46.14±0.59	49.31±0.67	47.78±0.55	≤300

Cd=Cadmium, Cr=Chromium, Cu=Copper, Fe=Iron Pb=Lead, Zn=Zine, ND=Not detected, Mdy=Mandalay, POL=Pyin Oo Lwin, SB=Shwe Bo, #=FAO/WHO, 2001[16]

Table 2. Level of heavy metals (ppm) in plant samples from different places

Metal	*Tinospora cordifolia* (ဆင်တုံးမန္နယ်ပြောင်ချော)			*Tinospora crispa* (ဆင်တုံးမန္နယ်ဆူးပေါက်)			Ref: value[#]
	Mdy	POL	SB	Mdy	POL	SB	
Cd	0.08 ±0.002	ND	0.14 ±0.01	0.25 ±0.02	0.25 ±0.01	0.3 ±0.00	≤0.3
Cr	2 ±0.1	1.87 ±0.1	0.95 ±0.07	1.7 ±0.04	1.75 ±0.04	1.2 ±0.04	≤2
Cu	4.64 ±0.09	8.69 ±0.22	4.03 ±0.14	3.69 ±0.12	5.85 ±0.19	3.55 ±0.12	≤20
Fe	**57.32 ±0.4**	**29.66 ±0.35**	**27.67 ±0.43**	**52.03 ±0.77**	**22.22 ±0.45**	**46.6 ±0.17**	**≤20**
Pb	3.47 ±0.25	5.19 ±0.17	2.85 ±0.15	4.59 ±0.19	3.45 ±0.08	5.31 ±0.24	≤10
Zn	17.04 ±0.33	39.48 ±0.51	12.65 ±0.27	11.71 ±0.22	20.06 ±0.38	27.86 ±0.52	≤50

Cd=Cadmium, Cr=Chromium, Cu=Copper, Fe=Iron Pb=Lead, Zn=Zine, ND=Not detected, #=WHO, 2005[15] Mdy=Mandalay, POL=Pyin Oo Lwin, SB=Shwe Bo

'Cd', 'Cr', 'Cu', 'Pb' and 'Zn' content in two *Tinospora* species were also within maximum permissible limit (MPL) set by WHO, 2005 (Table 2). In Shwe Bo, upper MPL of 'Cd' may be due to near the production of glaze earthen jar and fertilizer used paddy field area. Upper MPL of 'Cr' may be due to the plant sample collected from Mandalay area is near fertilizer used agricultural area. And then, chromium occurs naturally in rocks, soils and gases. However, the content of 'Fe' ranged between 22.22±0.45 ppm in *Tinospora crispa* at Pyin Oo Lwin to 57.32±0.4 ppm in *Tinospora cordifolia* at Mandalay. These values exceed MPL, 20 ppm.[15] High amount of Fe in plants may also be due to the foliar absorption from the surroundings air.[19] And also, due to the dam water drained from the hilly areas which have also been exposed to mining work. In mining sites, dust laden metals spread on every surface in the area due to blasting of the rocks during mining with concentration on soil, water and plants depending on distance from the mine and the form in which it is transported.[20]

The increasing the quantity of dust around mining operations degrades air quality in the immediate area, has an adverse impact on vegetative life.[21] However, Khin Phyu Phyu, *et al*. (2014) reported that all plants in her study contain high amount of 'Fe' ranged between 76.78-356.05 ppm, also more than MPL, 20 ppm.[22] And Then, 'Fe' concentration in this study was comparable to the study reported by Jabeen, *et al*. (2010), the range of 'Fe' in selective medicinal herbs of Egypt in the study carried out was between 261 to 1239 ppm.[23] The measurement of pH shows the acidity and basicity or alkalinity.[24] Most minerals and nutrients are more soluble or available in acid than in neutral or slightly alkaline soils. Soil pH varied from slightly acid to neutral (5.5-6.0). This condition is favorable to nutrient uptake by plants.[25] The type of soil controlled by pH value at 6.0-8.2 pH will bacteria predominate.[24] In the present study, all soil pH ranged between (7.16-7.99). Thus, study soil samples are slightly alkaline and acceptable for plants growth (Table 3).

Table 3. Physico-chemical parameters for soil samples from different places

Location	Conductivity (μS/cm)		TDS (mg/l)		Salinity (g/l)		pH		Temperature (°C)	
	Mean±SE	Range	Mean±SE	Range	Mean±SE	Range	Mean±SE	Range	Mean±SE	Range
Mdy	124.73±10.56	115.3-145.8	106.07±8.99	96.3-124	0.08±0.01	0.07-0.09	7.99±0.03	7.92-8.03	23.63±0.07	23.5-23.7
POL	142.73±12.75	119.5-163.4	121.33±10.73	102-139	0.09±0.01	0.07-0.10	7.80±0.01	7.79-7.82	23.27±0.03	23.2-23.3
SB	105.13±4.08	97-109.7	89.5±3.51	82.5-92.7	0.07±0.03	0.06-0.07	7.16±0.03	7.10-7.19	23.4±0.06	23.3-23.5

Mdy=Mandalay, POL=Pyin Oo Lwin, SB=Shwe Bo

Table 4. Physico-chemical parameters for water samples from different places

Location	Conductivity (μS/cm)		TDS (mg/l)		Salinity (g/l)		pH		Temperature (°C)	
	Mean±SE	Range	Mean±SE	Range	Mean±SE	Range	Mean±SE	Range	Mean±SE	Range
Mdy	355±15.64	367-400	318±2.08	314-321	0.01±0	0.01-0.01	8.44±0.02	8.39-8.46	23.83±0.03	23.8-23.9
POL	195.33±2.19	191-198	165.67±1.86	162-168	0.12±0.003	0.11-0.12	7.27±0.01	7.25-7.28	23.47±0.03	23.4-23.5
SB	154.33±10.85	144-176	130.67±9.18	121-149	0.09±0.003	0.09-0.1	6.88±0.06	6.76-6.98	23.87±0.03	23.83-23.90
Ref. value	1500[17]		500[17]		Non saline <1, Slightly saline 1-3, Moderately saline 3-10, Very saline >10[18]		6.5 -8.5[17]		25[#]	

Mdy=Mandalay, POL=Pyin Oo Lwin, SB=Shwe Bo, #=(WHO, 1996)

The measurement of electrical conductivity (EC) is to measure the current that gives a clear idea of soluble salt present in the soil.[24] Conductivity will vary with water source: ground water, water drained from agricultural fields, municipal waste water and rainfall.[26] Soil with EC below 400 μS/cm are considered marginally or non-saline, while soils above 800 μS/cm are considered severely saline.[12] In the present study, soil EC ranged between 154-355 μS/cm. Thus, study soil samples are non-saline and consider to be acceptable.

Salinity is a measure of the amount of salts in the water.[26] It is widely accepted that the salinity of soil water is equal to approximately three times the salinity of irrigation water, assuming relatively little leaching is occurring.[27] In the present study, soil salinity ranged between 0.01-0.12 g/l. In the present study, soil TDS ranged between 130.67-318 mg/ml. If pH and TDS are taken into consideration, it is clear that high pH soil lacks in several ions, it may be due to lack of microbial activity in the soil.[12]

Total dissolved solids are the concentration of a solution as the total weight of dissolved solid which expresses the degree of salinity in a medium. Soil salinization is the increase in the concentration of total dissolved salts in the soil and water; it leads to loss of habitat and reduction of biodiversity.[28]

Thus, study soil samples are non-saline. It is documented that, consumption of water with high concentrations of total dissolved solids caused disorders of alimentary canal, respiratory system, nervous system, coronary system besides, causing miscarriage and cancer.[29] In the present study, soil temperature ranged between 23.47-23.87°C. The temperature of soil greatly affects the physical, biological and chemical processes occurring in soils. Temperature plays a very important role in soil characteristics and seed germination.[26] In this study, all physico-chemical parameters including pH, conductivity, salinity, TDS and temperature of the soil and water samples were considered to be acceptable (Table 4).

According to phytochemical test, two *Tinospora* species contained alkaloids, α-amino acid, carbohydrate, glycosides, protein, reducing sugar, saponins, starch, steroids, tannins and tri-terpene (Table 5). Alkaloids possess a good analgesic, anti-inflammatory, anticancer and antioxidant activity.[30] Glycosides shows anticancer, purgative, and treatment of skin diseases.[31] Saponins shows hypolipidemic and anti-cancer activity.[31]

Table 5. Phytochemical constituents of plant samples from different places

Phytochemical	*Tinospora cordifolia*			*Tinospora crispa*		
	Mdy	POL	SB	Mdy	POL	SB
Alkaloids	+	+	+	++	++	+
α-amino acid	+	+	+	+	+	+
Carbohydrate	+	+	+	+	+	+
Flavonoids	-	-	-	-	-	-
Glycosides	+	+	+	+	++	+
Phenols	-	-	-	++	+	+
Protein	+	+	+	+	+	+
Reducing sugar	+	++	++	+	++	++
Saponins	++	++	++	++	++	++
Starch	+	+	+	+	+	+
Steroids	+	+	+	+	+	+
Tannins	+	+	+	+	+	+
Tri-terpene	+	+	+	+	+	+

Mdy=Mandalay, POL=Pyin Oo Lwin, SB=Shwe Bo
(+)=Presence, (-)=Absence

Steroid has specific and powerful action mainly on the cardiac muscle and promote nitrogen retention in osteoporosis.[31] Tannins are also known antimicrobial agents. Tannins have antiirritant, antisecretolytic, antiphlogistic, antimicrobial and anti-parasitic effects.[32] Terpenoids acts as anti-bacterial, antiinflammatory and anti-neoplastic.[30, 31] These two species have quite a number of chemical constituents which may be responsible for many pharmacological activities and in accordance with the medicinal usage of literature review .

Conclusions

The present study showed that the amount of heavy metals are different in the same medicinal plant taken from diffeent locations. Although the levels of all metals except 'Fe' analyzed in two *Tinospora* species are below the permissible level set by WHO/FAO, 2001, this study further confirms the increased danger of environmental pollution. Thus, it supports the idea that, every medicinal plant must be tested for contamination of heavy metals before dealing out it for further use in medications. These findings obtained from current study would go a long way in providing baseline data which will give a variety of useful information and methodology towards achieving safety and quality of plants. Then, it will be helpful for herbal medicine users, local practitioners and pharmaceutical industries, using these plants for different types of ailments. In conclusion, monitoring such medicinal plants for heavy metals is applicable for references and importance in protecting the public from adverse and hazardous effects of heavy metals.

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REFERENCES

1. Rahimi M, Farhadi R & Salehibalashahri M. Effects of heavy metals on the medicinal plant. *International Journal of Agronomy and Plant Production* 2012; 3(4): 154-158.
2. Lawal AO, Batagarawa SM, Oyeyinka OD & Lawal MO. Estimation of heavy metals in neem tree leaves along Katsina-Dutsinma-Funtua Highway in Katsina State of Nigeria. *Journal of Applied Sciences and Environmental Management* 2011; 15(2): 327-330.
3. Uwah EI & Ogugbuaja VO. Investigation of some heavy metals in *citrullus vulgaris, cucumis sativus* and soils obtained from gardens being irrigated with wastewater in Maiduguri, Nigeria. *Global Research Journal of Agriculture Biological Sciences* 2012; 3(5): 373-380.
4. Khattak MI & Khattak MI. Study of heavy trace metals in some medicinal-herbal plants of Pakistan. *Pakistan Journal of Boanyt* 2011; 43(4): 2003-2009.
5. Ullah R, Khader JA, Hussain I, *et al*. Investigation of macro and micro-nutrients in selected medicinal plants. *African Journal of Pharmacy and Pharmacology* 2012; 6(25): 1829-1832.
6. Sivakumar V & Rajan MSD. Standardization and characterization of *Tinospora cordifolia* (Willd) Miers ex Hook. F. & Thoms. Plant stem extract I different solvent fractions. *Asian Journal of Biochemical and Pharmaceutical Research* 2011; 4(1): 105-110.

7. Singh SS, Pandey SC, Srivastava S, Gupta VS, Patro B & Ghosh AC. Chemistry and medicinal properties of *Tinospora Cordifolia* (Guduchi). *Indian Journal of Pharmacology* 2003; 35: 83-91.
8. Hipol RLB, Cariaga MFNM & Hipol RM. Anti-inflammatory activities of the aqueous extract of the stem of *Tinospora crispa* (family Menispermaceae). *Journal of Nature Studies* 2012; 11(1 & 2): 88-95.
9. Niaz A, Ullah N, *et al*. Pollution based study of heavy metals in some selected medicinal plants by dry digestion method. *International Journal of Pharma Sciences and Research* 2013; 4(2): 17-24.
10. Chen M & Ma LQ. Comparison of three aqua regia digestion methods for twenty florida soils. *Soil Science Society America Journal* 2001; 65: 491-499.
11. Musa U, Hati SS & Mustapha A. Levels of Fe and Zn in staple cereals micronutrient deficiency implication in Rual Northeast Nigeria. *Food and Public Health* 2012; 2(2): 28-33.
12. Zaiad GM. Physico-chemical analysis of soils in Al-Khums city, Libya. *Journal of Applied Sciences Research* 2010; 6(8): 1040-1044.
13. Horborne JB; A guide to modern techniques of plant analysis; Phytochemical methods, 2nd ed., London, New York, Chapman and Hall, 1984.
14. Nwe Nwe Yi. Taxonomic study on magnoliophyta (Angiospermae) of Loikaw Township. [PhD *thesis*]. University of Medicine: Mandalay; 2007.
15. Quality control methods for medicinal plant materials. Revised, Geneva, WHO, 2005.
16. Codex alimentarius commission. Food additives and contaminants. Joint FAO/WHO Food Standards Program; ALINORM01/12A: 1-289, 2001.
17. Venkateswaran S, Karuppannan S & Shankar K. Ground water quality in pambar Sub-Basin, Tamil Nadu, India Using Gis. *International Journal of Recent Scientific Research* 2012; 3(10): 782-787.
18. Rabinove CJ, Langford RH & Brookhart JW. Saline water resources of North Dakota. US Geol. Sur. Water Supply Paper 1958; 1428: 72.
19. Khan SA, Khan L, Hussain I, Marwat KB & Akhtar N. Profile of heavy metals in selected medicinal plant. *Pakisatan Journal of Weekly Science Research* 2008; 14(1-2): 101-110.
20. Amune M, Christiana O & Samuel K. Impact of mining and agriculture on heavy metal levels in environmental samples in Okehi Local Government Area of Kogi State. *International Journal of Pure and Applied Sciences and Technology* 2012; 12(2): 66-77.
21. Environmental impact of the coal industry. From Wikipedia, the free encyclopedia. (Retrieved on 9.3.14 printed).
22. Khin Phyu Phyu, *et al*. Comparative study of heavy metals in selected medicinal plants and soils from different sites Mandalay Region. *Myanmar Health Sciences Research Journal* 2014; 26(2): 141-143.
23. Maobe MAG, Gatebe E, Gitu L & Rotich H. Profile of heavy metals in selected medicinal plants used for the treatment of diabetes, malaria and pneumonia in Kisii Region, Southwest Kenya. *Global Journal of Pharmacology* 2012; 6(3): 245-251.
24. Rai S, Chopra AK, Pathak C, Sharma DK, Sharma R & Gupta PM. Comparative study of some physicochemical parameters of soil irrigated with sewage water and canal water of Dehradun city, India. *Archives of Applied Science Research* 2011; 3(2): 318-325.
25. Ololade IA. A study on effects of soil physico-chemical properties on cocoa production in Ondo State. *Modern Applied Science* 2010; 4(5): 35-43.
26. Electrical Conductivity/Salinity Fact Sheet 3.1.3.0. (Retrieved on 13.11.14 printed).
27. Warrence NJ, Bauder JW & Pearson KE. Basics of salinity and sodicity effects on soil physical properties. Department of Land Resources and Environmental Sciences, Montanta State University-Bozeman; 2002.
28. Kuzhali SS, Manikandan N & Kumuthaka-lavalli R. Physico-chemical and biological parameters of paper industry effluent. *Journal of Naturnal Product and Plant Resourse* 2012; 2(3): 445-448.`
29. Olusegun AJ & Samuel AA. Assessment of soil sediments and salinity status of wetland landscape as affected by climate change in South-western Nigeria. *Standard Global Journal of Agricultural Sciences* 2014; 1(1): 001-009.
30. Nasreen S, Radha R, Jayashree N, Selvara B & Rajendran A. Assessment of quality of *Tinospora cordifolia* (Willd.) Miers. (menisper-maceae): Pharmacognostical and phyto-physicochemical profile. *Pharmacie Globale: International Journal of Comprehensive Pharmacy* 2010; 5(3): 1-4.
31. Doughari JH. Phytochemicals: Extraction methods, basic structures and mode of action as potential chemotherapeutic agents. Phyto-chemicals: A global perspective of their role in nutrition and health.
32. Najafi S, Nejadv BS, Deokule SS & Jasem Estakhr J. Phytochemical screening of *Bidaria khandalense* (Sant.) *Loranthus capitellatus* Wall. *Viscum articulatum* Burm.f. and *Vitex negundo* Linn. *RJPBCS*, 2010; 1(3): 388-393.