Study of some heavy metals contamination in two *Tinospora* (ဆင်တုံးမန္ဓယ်) species

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Background Information

- Medicinal plants are the most important source of life saving drugs for the majority of the world population [1]
- common concept among people that herbal medicines have no side effects and that “being natural in origin, herbs are safe [2]
- more than 80% of people in developing countries depend on traditional medicine for their primary health needs[3]
- Heavy metals (HM) are metallic elements with high atomic number and poisonous to living organisms [4]
- contents in medicinal plants depend on climatic factors, plant species, air pollution and other environmental factors
- some researchers reported from different areas which were conducted to search of the accumulation certain medicinal plants [1]
- *Tinospora* species are one such plant which is widely used in indigenous system of medicine [6]

Medicinal plants under investigation; name, parts used in the studied and their medicinal properties

Plant species	Myanmar name	Part used	Medicinal properties	Collection Place
Tinospora cordifolia (Willd.) Hook. F & Thoms	(ဆင်တုံးမန္ဓယ် ပြောင်ချော) STM(PC)	stem	antidiabetic, antiperiodic, antispasmodic, antiinflammatory, antiosteoporetic, antiarthritic, antioxidant, antiallergic, antistress, antileprotic, antimalarial, hepatoprotective, immunomodulatory, antitumor and antineoplastic activities (7)	Mandalay Pyin Oo Lwin Shwe Bo
Tinospora crispa (L) Hook. F & Thoms	(ဆင်တုံးမန္ဓယ် ဆူးပေါက်) STM(PC)	stem	treatment of flatulence, indigestion and diarrhea, tonic, antispasmodic, anti-inflammatory, antiarthritic, antiallergenic, and antidiabetic(8)	

General objective

To find out the evidence of heavy metals contamination in two *Tinospora* species

Specific objectives

- To measure the amount of heavy metals: cadmium (Cd), chromium (Cr), Copper (Cu), iron (Fe), lead (Pb) and zinc (Zn) in two *Tinospora* species
- To compare the metal content in their surrounding soils and two *Tinospora* species from three different places
- To analyze physico-chemical properties of their surrounding soils and water from three different places
- To find out phytochemical constituents of two *Tinospora* species

Materials and Methods

Materials

Reagents and Chemicals

- Analytical grade reagents (Merck),

  Cd, Cr, Cu, Fe, Pb and Zn standard

- 70% HNO_3 & 69% HCl

- Double de-ionized water (DDW)

Instruments & Apparatus

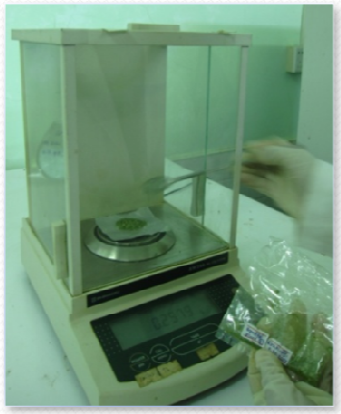
Atomic absorption spectrophotometer AA 6650

Muffle furnace

pH meter

Pocket Pro ™ Tester

Analytical balance



Oven

Vortex mixture

Heating magnetic stirrer

Volumetric flask

Methods

Plant authenticity

- **Identified and confirmed by competent taxonomists Department of Botany, University of Mandalay**

Plant samples collection

- Two *Tinospora* species
  Mandalay (Mdy), Pyin Oo Lwn (POL) and Shwe Bo (SB)

- Pyin Oo Lwin - many rock mining sites
- Shwe Bo - near the production of glaze earthen jar and agricultural area
- Mandalay area - compare for above two places

- That's why we choose these study places

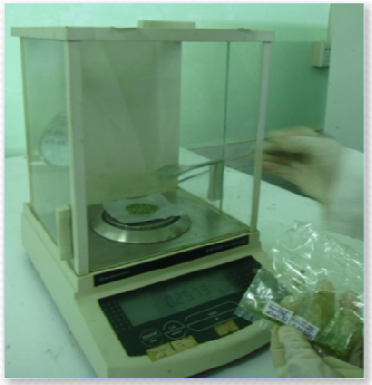
Soil & Water samples collection

- Soil samples were collected from surrounding plants at about 8-10 cm depth of the soil
- Water samples were collected at depth of 20cm from water surface directly into one liter polyethylene bottle

Plant samples analysis

- thoroughly washed with tap water and rinsed with distilled water to remove the dust and particles
- dried in shade at room temperature
- crushed, powdered and homogenized, using mortar &pestle
- dried in oven at 60ºC to constant weight, ready for analysis of heavy metals

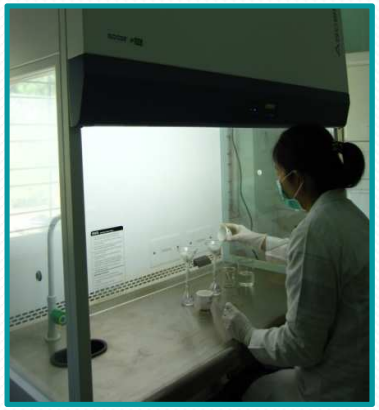
Digestion of plant samples (9)



Plant powder (2.5) g

Heating 110 °C, 2hrs, in an oven to remove moisture

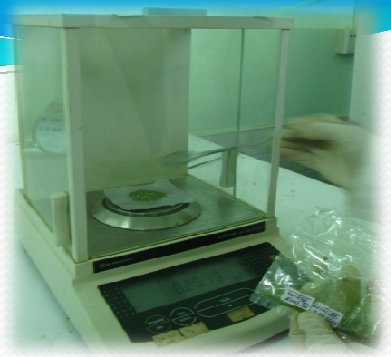
furnace, at 550°C, 4hrs, to obtain grey ash, & cool



5 ml of 6M HNO_3, to dissolve & digest, filter

Made up with DDW (50ml Volumetric flask)

Digestion of soil samples (10)



Dried soil (1) g

Digest with 12ml HNO_3-HCl (1:3 v/v)

Dryness in an oven at 110°C for 3 hr, cooled

20 ml 2% HNO_3 boil 10 mins, cooled & filtered

Made up with double de-ionized water (100ml Volumetric flask)

- **Blank control was carried out in the same way of sample preparation using solvent alone**
- **Standard solutions of each metal was separately prepared from their respective concentration of 1000mg/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 runs was conducted in triplicates [11]**

Instrumental condition for analysis

Parameter	Cd	Cr	Cu	Fe	Pb	Zn
Wavelength (nm)	228.8	357.9	324.8	248.3	283.3	213.3
Slit width (nm)	0.5	0.5	0.5	0.2	0.5	0.5
Lighting mode	BGC-D_2	BGC-D_2	BGC-D_2	BGC-D_2	BGC-D_2	BGC-D_2
Flame type	Air/ C_2H_2	Air/ C_2H_2	Air/ C_2H_2	Air/ C_2H_2	Air/ C_2H_2	Air/ C_2H_2
Current	8mA	10mA	6mA	12mA	10mA	8mA
AAS Technique	Flame	Flame	Flame	Flame	Flame	Flame

BGC-D_2 – Background Correction Deuterium Lamp

Physico-chemical analysis of soil & water samples (12)

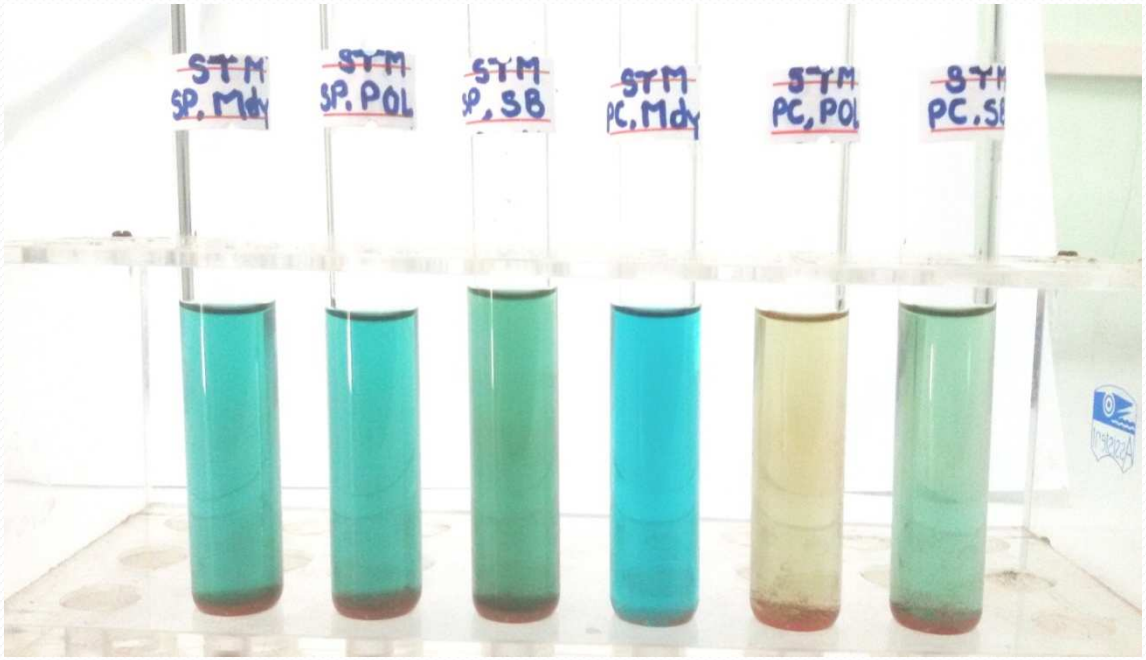
- instrument was calibrated with buffer solutions of pH 4, 7 and 10
- Filtrates of soil-water slurry (1:1 w/v) – pH
- Waterproof Handheld (H160), HACH, China, portable pH meter

- **Filtrates of soil-water slurry (1:5 w/v)**
  Electrical Conductivity (EC), Total dissolved solids (TDS) & Salinity

- **Pocket Pro ™ Tester**
  HACH, China

Phytochemical test for types of compounds [13]

- **Harborne J.B (1984) & WHO (1998) Phytochemical Methods**



Statistical analysis

- Microsoft Excel v. 2007
- Results were presented as mean ± SE

Results

Plant Authenticity

Family - Menispermaceae
Stem - cylinder, smooth, with aerial roots, glabrous.
Leaves - simple, alternate, exstipulate
Inflorescences axillary raceme of staminate flowers, the pistillate ones solitary or in raceme
Flowers - yellowish green, 5.0-7.0 mm in diameter at anthesis, unisexual actinomorphic trimerous, hypogynous, February to May
Fruits - drupes ovoid, indehiscent, red when ripe
Seeds - with endosperm, oblong- ellipsoid and glabrous [14]

Tinospora cordifolia
(Willd.) Hook. F &Thoms
(ဆင်တုံးမန္ဓယ်ပြောင်ချော)
STM(PC)

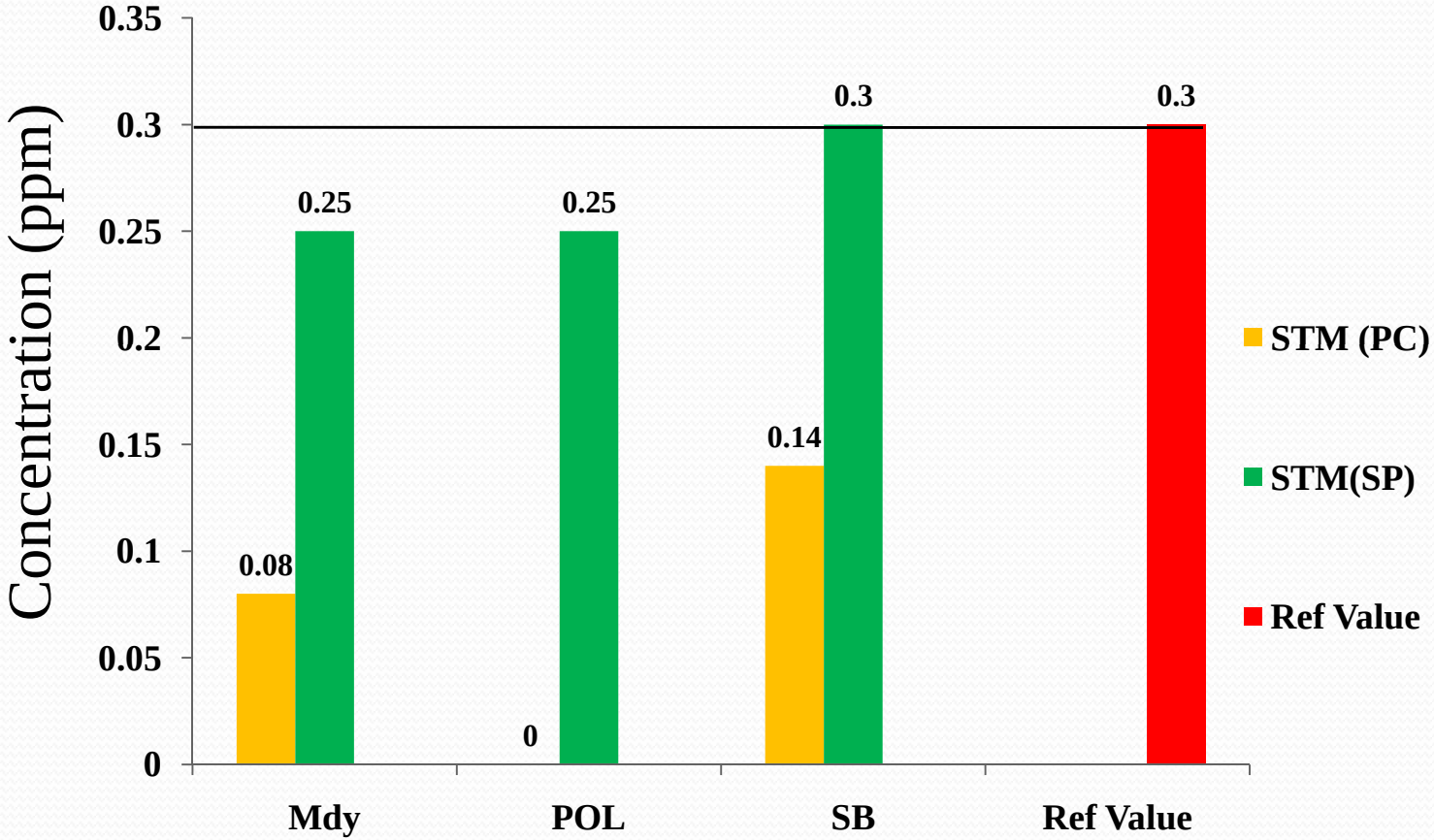
Tinospora crispa (L) Hook. F& Thoms
(ဆင်တုံးမန္ဓယ်ဆူးပေါက်)
STM(SP)

Family - Menispermaceae
Stems and branches - tuberculate with aerial roots
Leaves - simple, alternate and exstipulate
Inflorescences raceme or paniculate fascicles at the leafless nodes, often pendulous
Flowers - green or greenish yellow, 5.0-7.0 mm in diameter at anthesis, unisexual actinomorphic trimerous, hypogynous. January to March
Fruits - drupaceous, druplets, 1-3, subgloboid indehiscent, with one pyrene, orange when ripe
Seeds - with endosperm usually ruminate, ventrally grooved or curved (14)

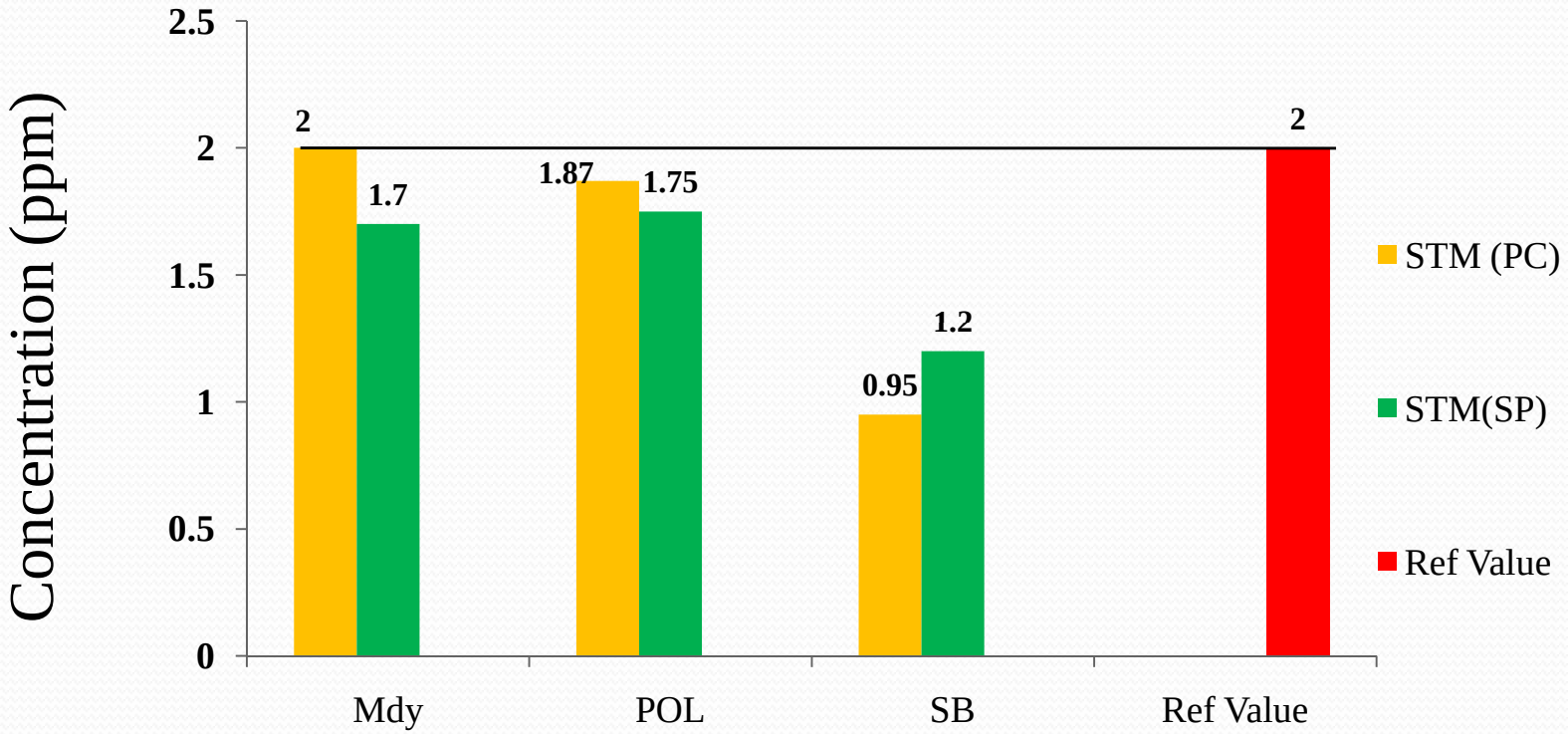
Table 1. Level of heavy metals (ppm) in two *Tinospora* species from three different places

Metal	*Tinospora cordifolia* ဆင်တုံးမန္ဓယ်ပြောင်ချော			*Tinospora crispa* ဆင်တုံးမန္ဓယ်ဆူးပေါက်			Ref: value[15] (WHO, 2005)
	Mdy	POL	SB	Mdy	POL	SB	
Cd	0.08 ±0.002	ND	0.14 ±0.01	0.25 ±0.02	0.25 ±0.01	0.30 ±0.00	≤ 0.3
Cr	2.00 ± 0.1	1.87 ±0.1	0.95 ±0.07	1.70 ±0.04	1.75 ±0.04	1.2 ±0.04	≤ 2
Cu	3.64 ±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**	**46.60 ±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

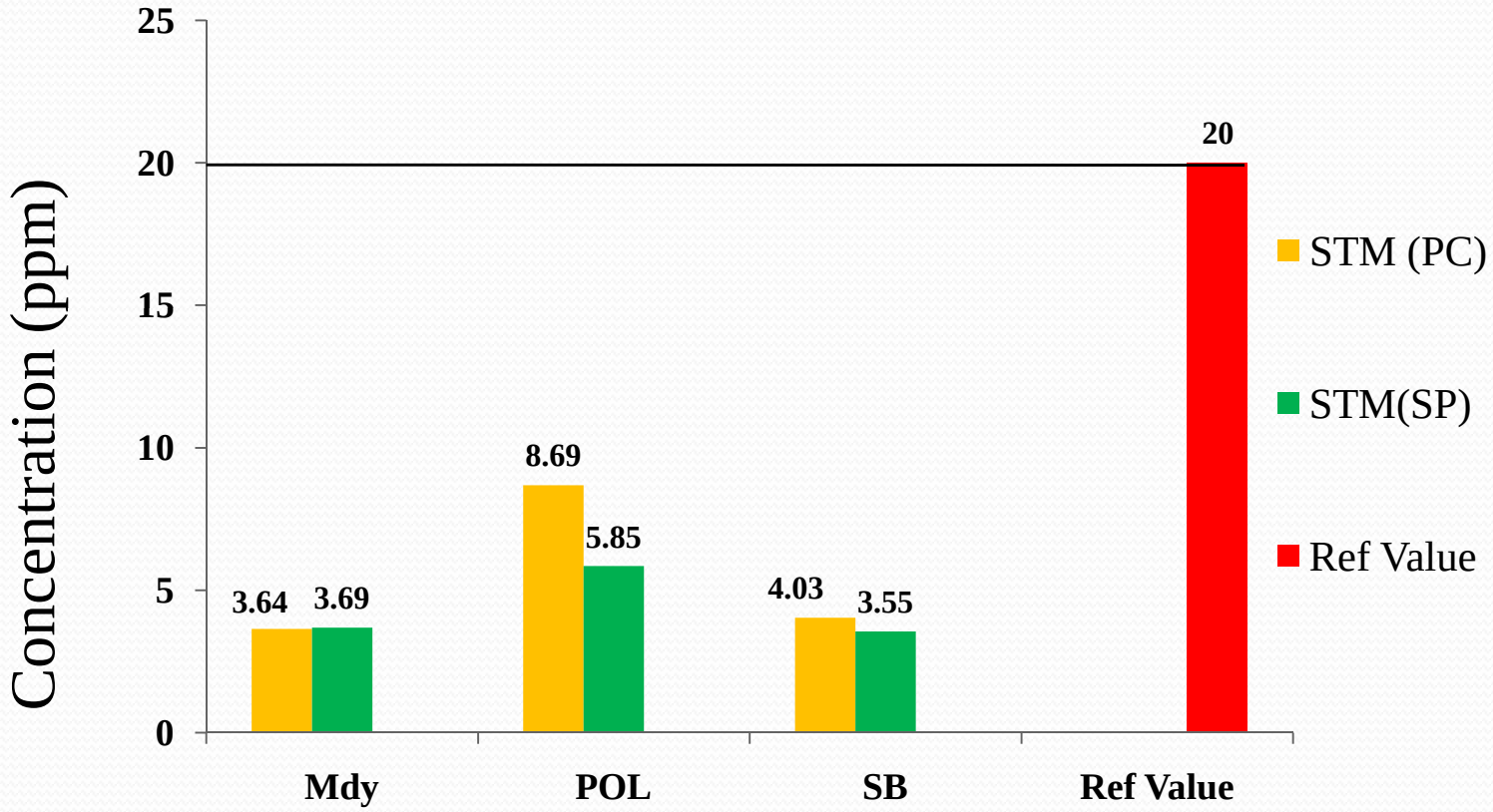
Concentration of Cd (ppm) in two *Tinospora* species



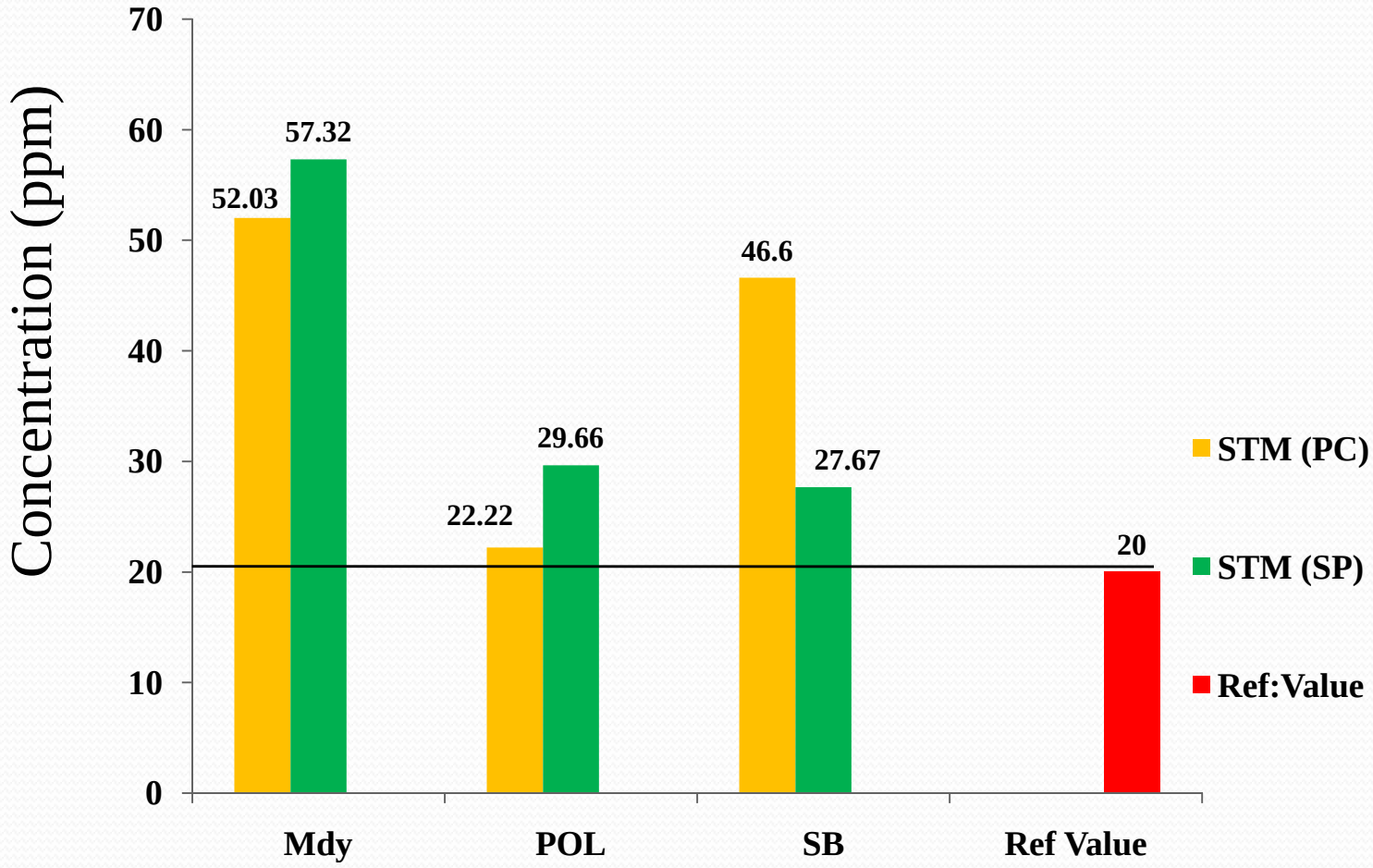
Concentration of Cr (ppm) in two *Tinospora* species



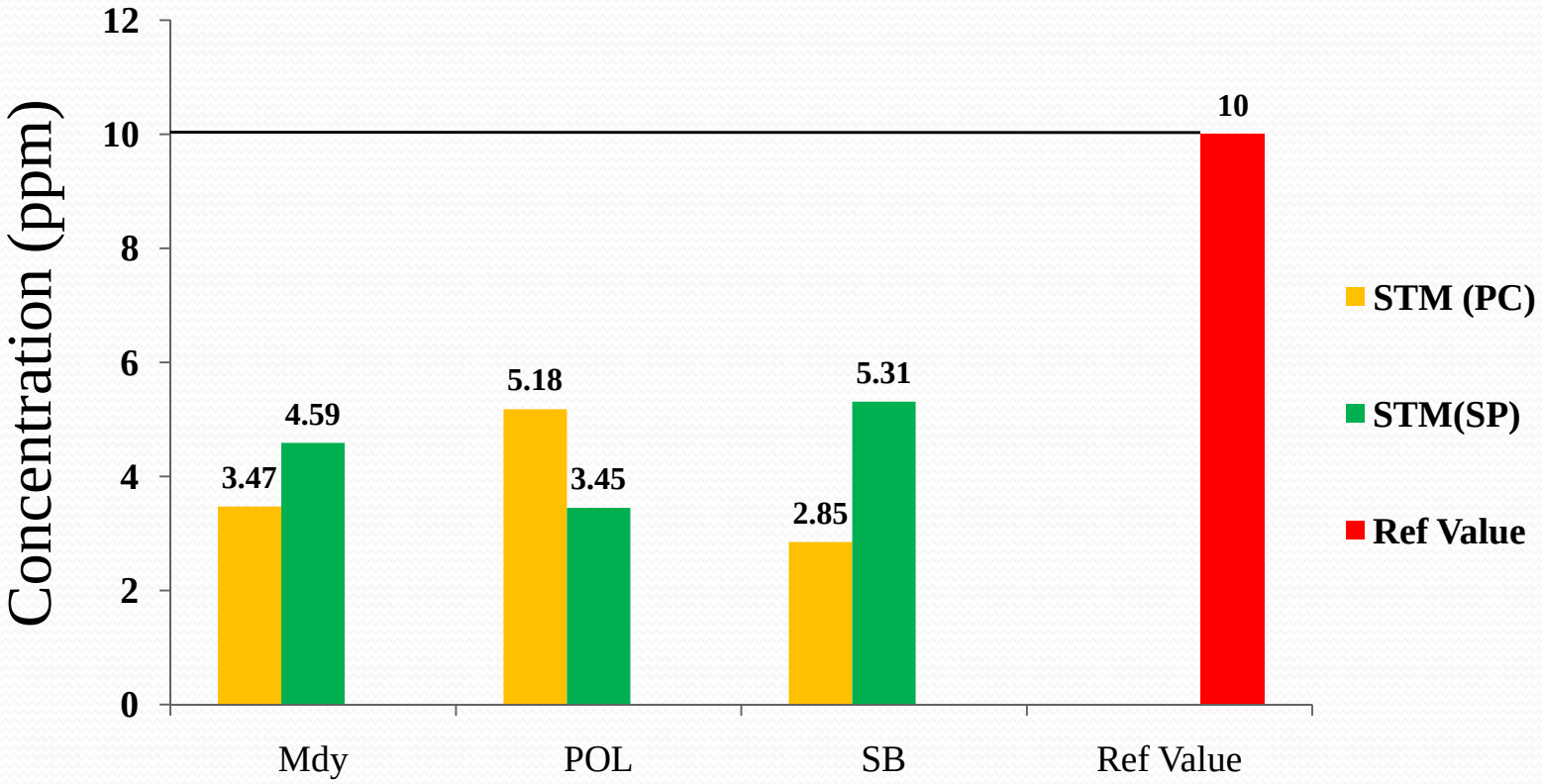
Concentration of Cu (ppm) in two *Tinospora* species



Concentration of Fe (ppm) in two *Tinospora* species



Concentration of Pb (ppm) in two *Tinospora* species



Concentration of Zn (ppm) in two *Tinospora* species

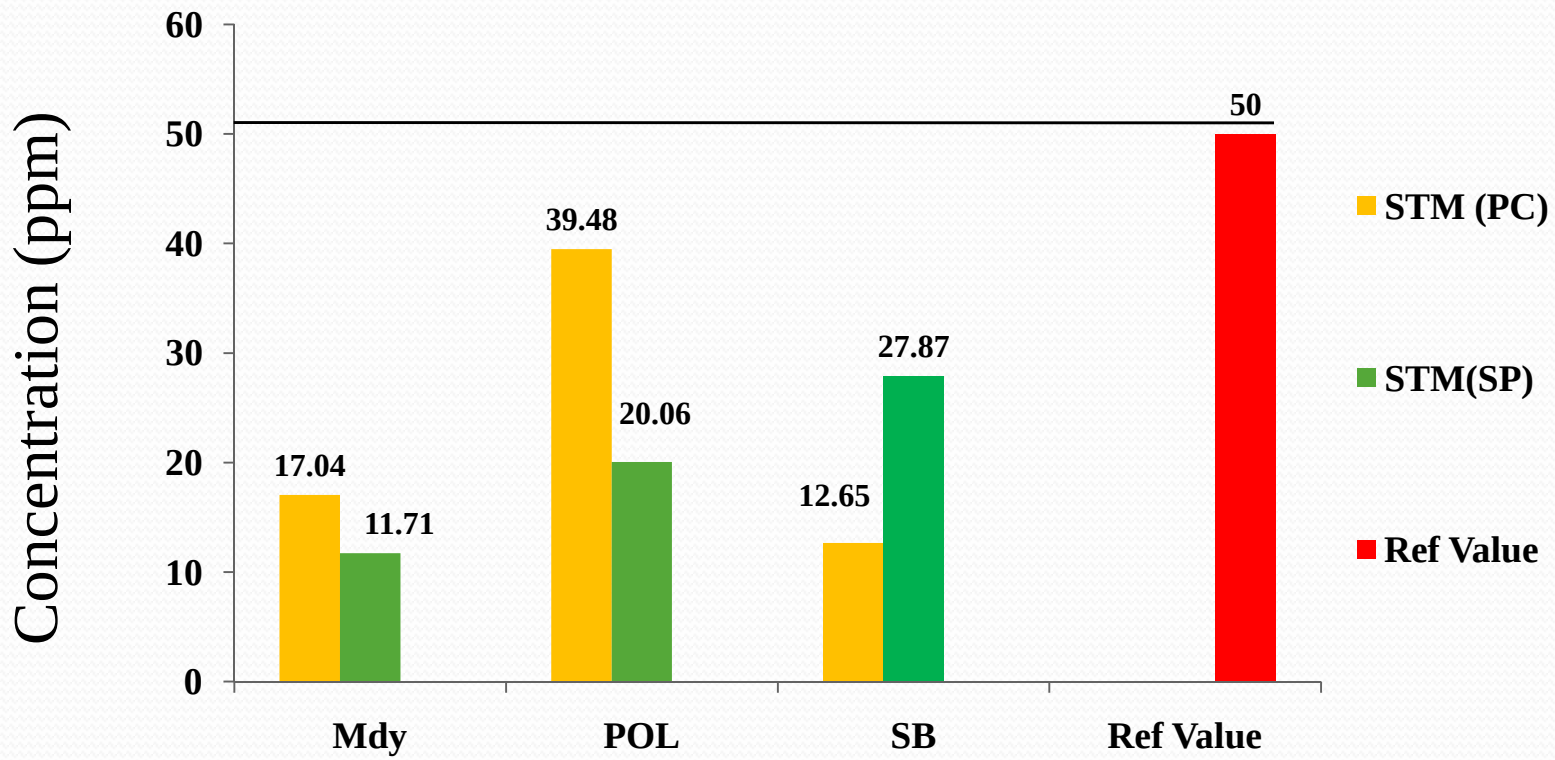


Table 2. Content of heavy metals (ppm) in soil samples from three different places

Metal	Mdy	POL	SB	Reference value, FAO/WHO, 2001[16]
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	2456.1 ± 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

Table 3. Content of some physico- chemical parameters for soil samples from three different places

Sampling 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	234 ±0.06	23.3-23.5

Table 4. Content of some physico- chemical parameters for water samples from three different places

Sampling 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	355.00 ±15.64	367-400	318 ±2.08	314-321	0.01 ±0.00	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	24.2-24.4
Ref: Value	1500 (WHO,2008)		500 (WHO,2008)		Non Saline <1 Slightly Saline 1-3 Moderately Saline 3-10 Very Saline >10 Rabinove,1958(18)		6 -9 (WHO, 2006) (19) 6.5 -8.5(WHO, 2008) (17)		25 (WHO, 1996)	

Table5. Phytochemical constituents of two *Tinospora* species from three different places

Sr. No.	Phytochemical	*Tinospora cordifolia*			*Tinospora crispa*		
		Mdy	POL	SB	Mdy	POL	SB
1.	Alkaloids	+	+	+	++	++	+
2.	α amino acid	+	+	+	+	+	+
3.	Carbohydrate	+	+	+	+	+	+
4.	Flavonoids	-	-	-	-	-	-
5.	Glycosides	+	+	+	+	++	+
6.	Phenols	-	-	-	++	+	+
7.	Protein	+	+	+	+	+	+
8.	Reducing sugar	+	++	++	+	++	++
9.	Sapponins	++	++	++	++	++	++
10.	Starch	+	+	+	+	+	+
11.	Steroids	+	+	+	+	+	+
12.	Tannins	+	+	+	+	+	+
13.	Tri-terpene	+	+	+	+	+	+

(+) = presence (-) = absence

Discussion

- All studied soils samples, ‘Cd’ and ‘Cu’ were not detected in three different places
- the presence of the heavy metals analyzed at three different soil samples were within permissible limit set by FAO/WHO, 2001[16]
- the content of ‘Cd’, ‘Cr’, ‘Cu’, ‘Pb’ and ‘Zn’ in two *Tinospora* species were within maximum permissible limit (MPL) set by WHO, 2005
- upper MPL of ‘Cd’ and ‘Cr’ may be due to the area is rocky, near the production of glaze earthen jar & fertilizer used agricultural area
- chromium occur naturally rocks, soils and gases
- the content of ‘Fe’ ranged between 22.22 ± 0.45 ppm in *Tinospora crispa* at POL to 57.32 ±0.4 ppm in *Tinospora cordifolia* at Mdy were exceed MPL, 20 ppm[15]

- high amount of Fe in plants may also be due to the foliar absorption from the surroundings air [20]
- 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[21]
- mining operations degrades air quality in the immediate area, has an adverse impact on vegetative life [22]
- Khin Phyu Phyu *et al*. (Jan, 2014) reported that all plants contain in her study, 'Fe' ranged between 76.78 - 356.05 ppm [23]
- 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 [24]

- pH shows the acidity and basicity or alkalinity
- 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) favorable to nutrient uptake by plants
- pH value at 6.0-8.2 pH will bacteria predominate[25, 26]
- Electrical conductivity (EC) is for measure the current that gives a clear idea of soluble salt present in the soil
- Conductivity will vary with water source: ground water, water drained from agricultural fields, municipal waste water & rainfall
- Soil with EC below 400 µS/cm non-saline,
- above 800 µS/cm are considered severely saline[12, 25, 27]
- Salinity is a measure of the amount of salts in the water
- Salinity of soil water is equal to approximately three times the salinity of irrigation water

- pH and TDS are taken into consideration, high pH soil lacks in several ions, it may be due to lack of microbial activity in the soil [12, 27, 28]
- TDS 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 TDS in the soil and water; it leads loss of habitat and reduction of biodiversity
- Consumption of water with high concentrations of TDS cause disorders of alimentary canal, respiratory system, nervous system, coronary system besides, causing miscarriage and cancer [17, 29]
- Temperature plays a very important role in soil characteristics and seed germination [25]
- All physico-chemical parameters including pH, conductivity, TDS, salinity and temperature of the soil and water samples are considered to be good

- two *Tinospora* species contained alkaloids, α amino acid, carbohydrate, glycosides, protein, reducing sugar, saponins, starch, steroids, tannins and tri-terpene
- Alkaloids - analgesic, antiinflammatory, anticancer & antioxidant
- Glycosides - anticancer, purgative & treatment of skin diseases
- Alkaloids, flavonoids & tannins - phenolic compounds with antioxidant properties
- Glycosides, flavonoids, tannins - hypoglycemic activities
- Saponins - hypolipidemic and anticancer activity
- Steroid - specific and powerful action mainly on the cardiac muscle and promote nitrogen retention in osteoporosis
- Tannins - antimicrobial agents
- Terpenoids - antibacterial, antiinflammatory and antineoplastic activities [30,31,32,33,34]
- chemical constituents which may be responsible for many pharmacological activities and accordance with the medicinal usage of literature review

Conclusion

- all metals except ‘Fe’ analyzed in two *Tinospora* species are below the permissible level set by WHO/FAO, 2001
- every medicinal plant must be tested for contamination of heavy metals before dealing out it for further their use in medications
- providing baseline data, will give a variety of useful information and methodology towards achieving safety and quality of plants
- helpful for herbal medicine users, local practitioners and pharmaceutical industries, using these plants for different types of ailments
- monitoring such medicinal plants for heavy metals is applicable for references and supreme importance in protecting the public from adverse and hazardous effects of heavy metals

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Thank you very much for your kind & patient attention