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Research Article

The Heavy Metal Analysis of Three *Tinospora* Species Frequently used in Traditional Medicine Preparation Sampled from Different Environmentally Diverse Locations

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ABSTRACT

Herbal drugs have been used as remedies for the treatment of a large number of human ailments since ancient times as part of the traditional medicine system. Significant groups of the indigenous systems of medicine are based on herbal drugs. Of these herbal drugs, *Tinospora cordifolia* plays a vital role in treating various diseases and disorders. But nowadays, these plants are heavily contaminated with heavy metals. Heavy metal contamination in herbal medicines is a global threat to humans, especially at levels above known threshold concentrations. This investigation aimed to evaluate the concentrations of four heavy metals: cadmium (Cd), lead (Pb), arsenic (As) and mercury (Hg) in three *Tinospora* species: *T. cordifolia*, *T. crispa* and *T. malabarica* using inductively coupled plasma - optical emission spectrometry (ICP-OES). Samples were collected from environmentally diverse locations. Our investigation made it clear that *T. crispa* had the highest quantity of heavy metals, followed by *T. malabarica* and *T. cordifolia*. In most samples, mercury and arsenic were above the detection limit recommended by WHO. Warty tubercle samples of *T.* species had significantly higher amounts of heavy metal when compared to the rest of the parts tested. In addition to this, the samples collected from industry/urban areas recorded a considerable amount of heavy metals. These results suggest that *T. cordifolia* for formulation should be properly collected from wild/rural areas in order to avoid the accumulation of harmful heavy metals. Furthermore, it was suggested that the cultivation of medicinal plants should be curtailed near environmentally polluted areas, especially industrial areas, to avoid health hazards. Usually, *T. malabarica* and *T. crispa* will substitute or adulterate for *T. cordifolia.* So, identifying *T. cordifolia* is very important before going for ayurvedic formulations. The present study's findings also highlight the need for relentless monitoring of heavy metal residues to ensure the quality and safety of finished herbal products.

Introduction

Tinospora species are renown ayurvedic medicinal plants distributed throughout the tropical and subtropical regions of the Indian subcontinent and Southeast Asia. The three main *Tinospora* species are *T. cordifolia* (TCF), *T. crispa* (TCP) and *T. malabarica* (TCM). Out of these three species, *T. cordifolia*, also known as guduchi/giloy is widely used in various traditional ayurvedic formulations.[1] The estimated annual consumption of *T. cordifolia* in the Indian system of medicines is roughly about 1,000 tonnes.[1] Guduchi is a potent ayurvedic medicine quoted in several traditional texts to treat various alignments such as fever, diabetes, skin problems, jaundice, antiperiodic, antispasmodic, antiinflammatory, antiarthritic, antioxidant, antiallergic, antistress, antileprotic, antimalarial, hepatoprotective, immunomodulatory and antineoplastic activities.[2] In this context, guduchi is considered a nectar plant, and its detoxifying, revitalizing, and immune-boosting properties have garnered it the name amrita in Sanskrit.[3] In modern medicine, the plant

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has been extensively evaluated and studied, and the drug is now being used to combat the detrimental effects of chemotherapy. So, *T. cordifolia* is the most important medicinal herb considered by ancient rishis in vedic times, having a great potential (medicinal attributes) for curing a variety of ailments. So, there is no question regarding the efficacy of this plant in managing various ailments. Nowadays, this plant is widely contaminated, especially with heavy metals. The occurrence of a significant quantity of heavy metals in *T. cordifolia* is well reported in the literature.[4-8]

The leaves of *T. cordifolia* are simple, alternate, exstipulate, cordate (heart-shaped), with long petioles up to 15 cm, roundish and pulvinate. Both at the base and apex, with the basal one being longer and partially and halfway round.[4,9] Stems are somewhat succulent, with long, filiform fleshy aerial roots arising from the branches. Flowers are yellow or greenish-yellow on axillary and terminal racemes, unisexual, small on separate plants and appearing when the plant is leafless. Fruits are aggregates of 1-3 ovoid smooth druplete on a thick stalk with sub-terminal style scars, scarlet or orange-red in color.[9] Seeds are white, bean shaped, and curved. The bark is creamy-white to grey-brown, warty, and deeply left spirally, the space in between being spotted with prominent rosette-like lenticels.[10] The different parts of *T. cordifolia* are shown in Fig. 1. A variety of phytoconstituents have been isolated from *T. cordifolia*. They belong to different classes such as alkaloids, diterpenoid lactones, glycosides, sesquiterpenoids, aliphatic compounds, phenolics, polysaccharides, steroids like tinosporine, tinosporide, tinosporaside, cordifolide, cordifol, heptacosanol, clerodane furano diterpene, diterpenoid furanolactone tinosporidine, columbin and β-sitosterol.[11] Whereas *T. malabarica/sinensis* deviates from *T. cordifolia* morphologically by its large tomentose leaves, uniseriate multicellular type of trichome present on young stems and on both surfaces of the leaves, watery latex and also by its prominent scattered oval-shaped lenticels. The major phytochemicals present in *T. sinensis* are trans-syringing, 3'-dymethyl-phillyrin, sesquiterpene glycoside, vanillin, daucosterol, I- sitisterol, 20β-hydroxyecdysone, tinosporaside, cordicoside, columbin etc.[12]

T. crispa is an herbaceous vine that extensively grows in tropical and subtropical regions of Southeast Asia. The old stems of *T. crispa* are fleshy, with numerous prominent blunt tubercles, whereas younger stems are slightly succulent, thin epidermis, membranous, brownish, and glabrous. The leaves are large, heart-shaped 6–12 cm long and 7–12 cm wide. Petioles are glabrous and 5–15 cm long. Leaf-blade is slightly fleshy, both surfaces glabrous and very delicate when dried (Fig. 1). *T. crispa* contains two or three small yellow or greenish yellow color flowers that are fascicled. The major phytochemicals present in the *T. crispa* are diosmetin, genkwanin, luteolin 4-methyl ether 7-glucoside, genkwanin 7-glucoside, cycloeucalenol, cycloeucalenone, 2-O-lactoylborapetoside B, 6-O-lactoylborapetoside B, borapetoside, rumphioside C, rumphioside F, rumphioside, columbin, tinocrisposide A, tinocrisposide B, tinocrisposide C, and tinocrisposide D.[13] But the traditional Indian system of medicine will solely use *T. cordifolia* for preparing various formulations. But

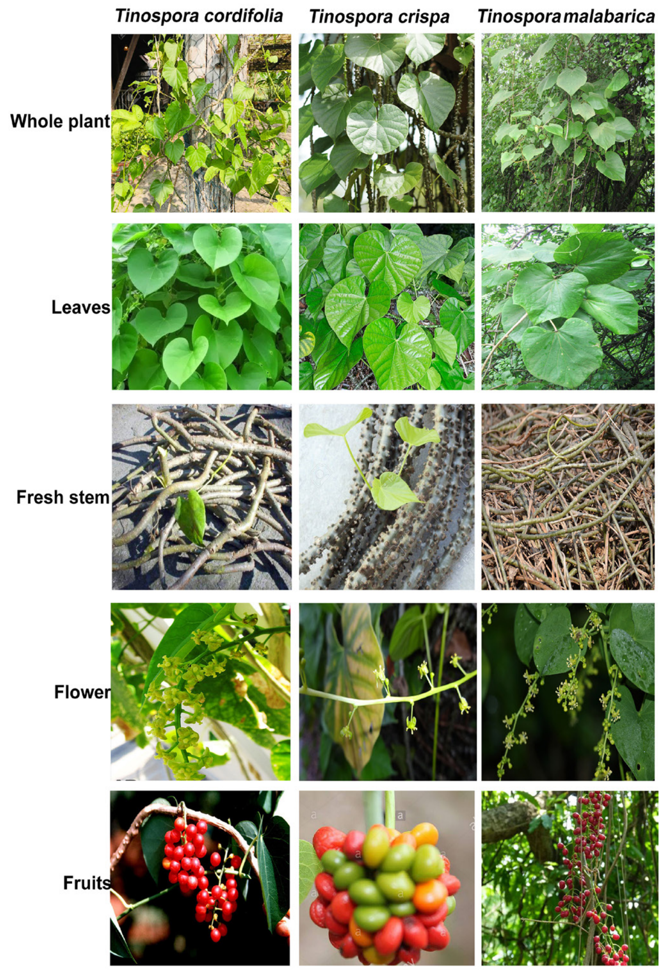

Fig 1: Anatomical difference of three *Tinospora* species.

Table 1: List of the *Tinospora* species collected various locations

Tinospora species	*Number of samples collected for analysis*				
	Wild/rural area	*Industrial/urban area*	*Market samples*	*Physic garden*	*Total*
T. cordifolia	16	10	10	7	43
T. malabarica	10	8	6	5	29
T. crispa	18	14	8	4	44

T. crispa has been used in conventional medicine to treat numerous pathologies in Malaysia, Indonesia, Thailand, and the Philippines.[13] Even though the pharmacognostic properties of *T. cordifolia*, *T. malabarica* and *T. crispa* are different, the people who collect *T. cordifolia* for preparing Ayurvedic formulations may mistakenly gather *T. malabarica* and *T. crispa*. So, *T. malabarica* and *T. crispa* will act as a substitution or adulteration for *T. cordifolia.*

Due to their rising popularity and global market expansion, the safety and efficacy of herbal products have become a foremost public health concern.[14] Due to a lack of regulation and a lack of control over distribution channels (including Internet sales), unpleasant reactions from herbal medicines of poor quality may occur. Adulteration or substitution of herbals with similar species or misidentification with toxic plant species and use of products severally contaminated with potentially hazardous components like heavy metals, mycotoxins, especially aflatoxin, and agrochemical residues are among the most common causes.[15,16] Many pollutants, such as radionuclides and metals, are found naturally in the ground and in the environment. Some are caused by previous or current usage of substances that damage the environment and, as a result, medicinal plants, such as industrial emissions or chemical residues. Even if an herb is organically grown, pollutants from environmental sources may be present due to their excessive usage and disposal.[17] Harmful pollutants can also come from the growing circumstances of medicinal plants, post-harvest treatment of herbal material (e.g. fumigants), and the final product manufacturing stages (e.g. organic solvent residues).[17]

Heavy metals play a significant role in the issues mentioned above affecting herbal raw materials. Numerous harmful heavy metals have contaminated our land, water, and air due to various human activities, particularly urbanization and industrialization.[18] Heavy metals accumulate in natural settings due to multiple human activities such as industry, metallurgy, mining, chemical fertilizers containing heavy metals, and traffic activities. Heavy metals released into the air, soil, and environment can be absorbed by plants through their roots and leaves, resulting in plant metabolism malfunction and a serious health threat.[19] Heavy metal contamination of medicinal plants and by-products occurs during cultivation due to pollution of the water, soil, and air and product processing such as drying, storage, shipping, and maintenance. When these heavy metals come into contact with human beings, they pose serious health risks. So, it is necessary to thoroughly pre-process the raw materials before using them for the ayurvedic formulations. Unfortunately, most manufacturers produce, sell, and market herbal preparations without any evidence-based scientific study regarding their safety and efficacy. Normally, ordinary people will collect the medical herbs collected from various locations, especially from the roadside and consume them without any processing, leading to various health hazards due to the presence of the above-mentioned contaminations, especially heavy metals. In the case of *Tinospora* species, the chance of substitution and adulteration is greater as they are readily available around us. Under certain conditions, these herbal drugs have been found to hurt human life in place of healing. Reports from patients experiencing negative health consequences caused by the use of herbal medicines are on the rise. One of the major causes of adverse effects is directly linked to the poor quality of herbal drugs.[20] So, regulatory authorities and pharmaceutical companies should be more vigilant about the quality of the raw materials.

The basis of any pharmaceutical industry is efficacy and safety, followed by the quality of the raw materials used for the formulations. As said in the above paragraphs, the efficacy of *Tinospora* species was established several hundred years ago. But nowadays, the actual problem faced by the pharmaceutical industry is the quality of raw materials collected from various locations. Moreover, if we compromise on the quality of raw materials, it will affect the final product. The present study mainly concentrated on the safety issues that arise if an industry compromises the quality of raw materials. Heavy metal contamination in Ayurvedic plants, particularly *Tinospora* species, is a significant obstacle to pharmaceutical firms maintaining quality assurance and quality control, as well as delivering high-quality final products to consumers. In the end, it has turned into a serious health-related social problem that has resulted in a variety of illnesses and dangerous diseases such as kidney damage, liver damage, and cancer. As a result, heavy metal levels in raw materials used for herbal formulations must be scientifically assessed.

Any pharmaceutical dealing with medicinal preparations should follow a major thumb rule which includes four pillars, i.e., safety, efficacy, quality and stability. Out of the four pillars, the three pillars except stability mainly concentrate on the selection of crude drugs and formulations. In the case of herbal drug formulation, adulteration, use of spurious drugs and contamination will spoil the three principles associated with the herbal industry. Hence, these principles play a pivotal role in the herbal pharmaceutical industry. For these reasons, the present research study plays an important role in substantiating whether the herbal raw material selected by the industry fulfils the three major principles or not. In addition to this, if we select a wrong medical plant for drug preparation, it will affect the overall quality and efficacy of the final formulation. Therefore, the present work highlighted the importance of selecting accurate raw materials for preparing the herbal formulations. *T. cordifolia* is one of the major medicinal plants widely used for the preparation of several medicines by the herbal pharmaceutical industry. But nowadays, these plants are heavily contaminated especially with heavy metals due to various causes. Thus, by implementing strict

Table 2: ICP OES instrumental parameters.

Parameters	*Conditions*
Radiofrequency power	1.20 kW
Pump flow	12 rpm
Plasma argon flow	12 L/min
Auxiliary argon flow	1 L/min
Nebulizer gas flow	0.75 L/min
Plasma view	Axial
Analytes	Wave length
Pb	220.353
Cd	226.502
As	188.980
Hg	194.164
Reading/Replicates	3

Table 3: Concentration of heavy metals in the various part of *Tinospora* species

Plant part	*Mean heavy metal concentration in the samples (ppm)*			
	T. cordifolia			
	As	*Cd*	*Pb*	*Hg*
Warty tubercle	3.24 ± 1.63	0.12 ± 0.06	0.18 ± 0.17	3.88 ± 2.68
Whole stem	1.62 ± 0.79	0.06 ± 0.03	0.19 ± 0.18	1.99 ± 1.77
Leaf	1.71 ± 1.13	0.08 ± 0.05	0.17 ± 0.17	1.92 ± 1.37
Cambium	1.51 ± 0.98	0.06 ± 0.04	0.13 ± 0.18	1.52 ± 1.05
	T. malabarica			
	As	*Cd*	*Pb*	*Hg*
Warty tubercle	2.74 ± 0.84	0.13 ± 0.08	0.24 ± 0.61	2.55 ± 0.76
Whole stem	2.05 ± 0.62	0.11 ± 0.06	0.16 ± 0,09	2.06 ± 0.60
Leaf	2.65 ± 1.45	0.24 ± 0.36	0.25 ± 0.23	2.23 ± 1.95
Cambium	1.12 ± 0.67	0.11 ± 0.08	0.27 ± 0.33	1.32 ± 0.64
	T. crispa			
	As	*Cd*	*Pb*	*Hg*
Warty tubercle	8.23 ± 2.05	0.29 ± 0.64	0.12 ± 0.07	6.69 ± 2.05
Whole stem	5.00 ± 1.61	0.07 ± 0.05	0.16 ± 0.07	4.08 ± 1.31
Leaf	2.86 ± 0.69	0.07 ± 0.05	0.12 ± 0.09	2.54 ± 0.57
Cambium	2.04 ± 0.64	0.09 ± 0.08	0.16 ± 0.09	1.60 ± 0.66

quality control and changing the pre-processing before formulation, we can easily slow the above-mentioned problems associated with *T. cordifolia*.

Materials and Methods

Chemicals and Reagents

All chemicals and reagents used were of analytical-reagent grade with no further purification. Nitric acid (HNO_3) and hydrogen peroxide (H_2O_2) were purchased from Merck (Darmstadt, Germany) were used for sample digestion. Ultrapure water was used for solution preparation and dilution. The heavy metal standard solutions used for the calibration were prepared by the step dilution of certified stock solutions of 1000 mg/L of each heavy metal (Pb, Cd, Hg and As) purchased from Merck (Darmstadt, Germany). To avoid sample contamination, all glassware, plastic ware, and digestion vessels were washed with Extran® detergent, soaked in 10% (v/v) HNO_3 for a minimum of 24 hours, washed several times with tap water, rinsed thoroughly with ultrapure water, dried and stored in closed polypropylene containers until use.

Table 4: Concentration of heavy metals in the various part of *T. cordifolia* collected from different locations

Plant part	*Mean heavy metal concentration in the samples (ppm)*			
	Wild/rural area			
	As	*Cd*	*Pb*	*Hg*
Warty tubercle	3.48 ± 1.2	0.12 ± 0.12	0.12 ± 0.12	2.77 ± 0.86
Whole stem	1.57 ± 0.84	0.06 ± 0.03	0.20 ± 0.22	2.57 ± 1.72
Leaf	1.43 ± 0.81	0.07 ± 0.03	0.09 ± 0.06	1.86 ± 1.45
Cambium	0.86 ± 0.37	0.06 ± 0.03	0.10 ± 0.06	0.90 ± 0.40
	Industrial/urban area			
	As	*Cd*	*Pb*	*Hg*
Warty tubercle	3.57 ± 1.2	0.13 ± 0.13	0.36 ± 0.35	7.56 ± 2.92
Whole stem	2.13 ± 0.87	0.07 ± 0.03	0.22 ± 0.13	2.43 ± 0.89
Leaf	2.56 ± 1.41	0.08 ± 0.04	0.28 ± 0.26	2.29 ± 1.93
Cambium	1.98 ± 0.80	0.07 ± 0.04	0.23 ± 0.29	1.99 ± 1.62
	Market samples			
	As	*Cd*	*Pb*	*Hg*
Warty tubercle	3.07 ± 1.45	0.10 ± 0.04	0.13 ± 0.08	3.20 ± 0.98
Whole stem	1.39 ± 0.46	0.05 ± 0.01	0.20 ± 0.00	0.66 ± 0.67
Leaf	1.73 ± 1.05	0.14 ± 0.07	0.18 ± 0.09	1.90 ± 0.78
Cambium	2.70 ± 0.85	0.04 ± 0.00	0.06 ± 0.03	2.02 ± 0.75
	Physic garden			
	As	*Cd*	*Pb*	*Hg*
Warty tubercle	2.08 ± 0.86	0.05 ± 0.00	0.06 ± 0.01	1.56 ± 1.06
Whole stem	1.13 ± 0.30	0.04 ± 0.01	0.07 ± 0.02	1.53 ± 0.17
Leaf	0.83 ± 0.38	0.04 ± 0.01	0.12 ± 0.09	1.44 ± 0.47
Cambium	0.73 ± 0.39	0.04 ± 0.01	0.05 ± 0.02	1.64 ± 0.78

Study Area and Sample Collection

A total of 110 samples of three *Tinospora* species were collected from different area during the period of 2019-2021. The sample were collected from wild/rural area, industrialised/urban area, from different herbal medicine outlets and also from Physic Garden. The collected *Tinospora* species were authenticated by the experts in identifying the medical plants. Voucher specimens were partly deposited at herbarium of Dravyaguna Department, Pankajakasthuri Ayurvedic Medical college and PG centre, Killy, Kattakada, Trivandrum, Kerala, India. Table 1 shows a list of the *Tinospora* species collected and the number of samples analysed along with different locations.

Processing of Sample

The samples were washed with Milli-Q water and dried at 40–60˚C for 5–6 hours. After drying, the samples were stored in an air tight polyethylene container.

Microwave Acid Digestion of the Sample

In a microwave oven (UniClever BM—1z, Plazmatronika, Poland), the dried sample (0.5 g) was digested with 6 cm^3 of concentrated HNO_3 and 2 cm^3 of concentrated HCl in closed polytetrafluoroethylene (PTFE) vessels. A three-step protocol (described further below) was used. After digestion, the solution with a solid phase was transferred to a 100 cm^3 volumetric flask, filled to capacity with type I (ISO 3696) deionized water with a resistivity greater than 10 MΩ·cm, and filtered through a filter paper (pore size 8 µm, medium porosity) to a clean polyethylene bottle.

Microwaves operation parameters as applied in method

	heating time [min]	pressure [atm]	power [%]
Step 1	5	17–20	60
Step 2	10	24–27	80
Step 3	10	27–30	100

Elemental Analysis by Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES) Technique

A clear solution was obtained after microwave-assisted digestion with a mixture of nitric acid and hydrogen peroxide, and the analytes were determined using ICP OES (Agilent ICP OES–5110, Santa Clara, California, United States). Table 2 shows the instrumental conditions maintained for the analysis of Cd, Pb, AS and Hg.

Statistics

Statistical analysis SPSS statistical programme was used for statistical analysis. Average, SD, minimum, and maximum values, which were read for three times, have been calculated.

RESULTS

In this study, arsenic and mercury were present in the three *Tinospora* species examined. Lead and chromium were also detected in samples but was below the detection limit (0.001). In most of the samples, arsenic and mercury concentrations were beyond the permissible limits defined by WHO. Out of three *Tinospora* species tested, a significantly higher concentration of heavy metals was detected in *T. crispa*, followed by *T. malabarica*. But *T. cordifolia* recorded lesser quantity of heavy metals when compared to the rest of the species tested (Fig. 2 and Table 3).

In the plant tissues, warty epidermis recorded a very high concentration of heavy metals, followed by whole stems (Fig. 3 and Tables 4-6). Surprisingly, the cambium recorded a significantly lower concentration of all heavy metals followed by leaf.

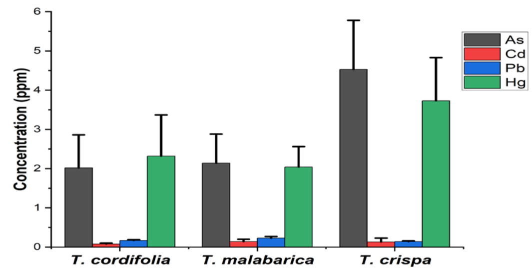

Fig 2: Concentration of heavy metals in the *Tinospora* species

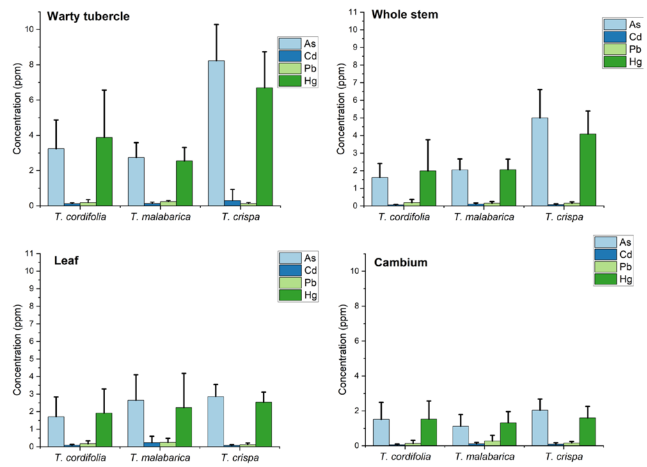

Fig 3: Concentration of heavy metals in the various part of *Tinospora* species. The analysis clearly indicated that warty tubercle recorded highest concentration of heavy metal.

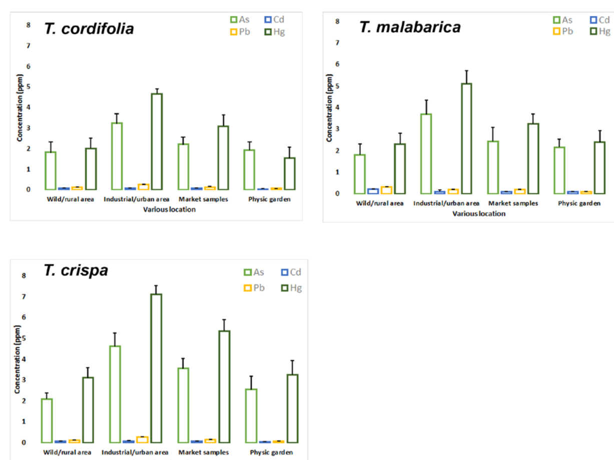

Fig. 4: Concentration of heavy metals in *Tinospora* species collected from different locations.

A significant difference in heavy metal concentration was observed among the plants in the four tissue samples collected from diverse geographical locations (Tables 4-6). Samples collected from industrial areas recorded a significantly higher concentration of heavy metals, especially mercury and arsenic, followed by samples

Table 5: Concentration of heavy metals in the various part of *T. malabarica* collected from different locations

Plant part	*Mean heavy metal concentration in the samples (ppm)*			
	Wild/rural area			
	As	*Cd*	*Pb*	*Hg*
Warty tubercle	2.59 ± 0.69	0.06 ± 0.04	0.32 ± 0.06	2.17 ± 0.68
Whole stem	1.88 ± 0.54	0.09 ± 0.02	0.22 ± 0.07	1.75 ± 0.40
Leaf	2.07 ± 0.99	0.64 ± 0.91	0.39 ± 0.30	1.38 ± 1.46
Cambium	0.70 ± 0.12	0.08 ± 0.02	0.32 ± 0.30	1.02 ± 0.90
	Industrial area			
	As	*Cd*	*Pb*	*Hg*
Warty tubercle	3.22 ± 0.69	0.11 ± 0.06	0.30 ± 0.23	3.08 ± 0.83
Whole stem	2.23 ± 0.77	0.08 ± 0.03	0.12 ± 0.10	2.21 ± 0.63
Leaf	2.93 ± 1.03	0.15 ± 0.09	0.16 ± 0.12	3.48 ± 2.68
Cambium	1.37 ± 0.62	0.09 ± 0.08	0.18 ± 0.08	1.38 ± 0.66
	Market samples			
	As	*Cd*	*Pb*	*Hg*
Warty tubercle	2.61 ± 1.2	0.19 ± 0.08	0.16 ± 0.07	2.33 ± 0.61
Whole stem	2.11 ± 0.70	0.17 ± 0.09	0.12 ± 0.05	2.07 ± 0.66
Leaf	3.26 ± 2.60	0.30 ± 0.13	0.20 ± 0.09	1.43 ± 1.42
Cambium	1.07 ± 0.71	0.17 ± 0.10	0.46 ± 0.65	1.52 ± 0.29
	Physic garden			
	As	*Cd*	*Pb*	*Hg*
Warty tubercle	2.36 ± 0.77	0.15 ± 0.07	0.09 ± 0.04	2.78 ± 0.54
Whole stem	2.03 ± 0.42	0.08 ± 0.03	0.09 ± 0.03	2.58 ± 0.57
Leaf	2.62 ± 0.41	0.06 ± 0.04	0.10 ± 0.04	3.17 ± 0.32
Cambium	1.63 ± 0.39	0.09 ± 0.04	0.09 ± 0.06	1.10 ± 0.21

Table 6: Concentration of heavy metals in the various part of *T. crispa* collected from different locations

Plant part	*Mean heavy metal concentration in the samples (ppm)*			
	Wild/rural area			
	As	*Cd*	*Pb*	*Hg*
Warty tubercle	7.34 ± 1.95	0.19 ± 0.19	0.14 ± 0.09	5.76 ± 2.24
Whole stem	5.15 ± 1.14	0.08 ± 0.07	0.14 ± 0.07	4.02 ± 1.07
Leaf	2.83 ± 0.57	0.08 ± 0.05	0.11 ± 0.10	2.38 ± 0.39
Cambium	1.79 ± 0.58	0.11 ± 0.10	0.15 ± 0.08	1.43 ± 0.81
	Industrial area			
	As	*Cd*	*Pb*	*Hg*
Warty tubercle	9.51 ± 1.52	0.51 ± 1.10	0.09 ± 0.06	7.80 ± 1.62
Whole stem	5.32 ± 0.92	0.08 ± 0.04	0.00 ± 0.00	4.44 ± 1.65
Leaf	3.15 ± 0.81	0.08 ± 0.06	0.08 ± 0.03	2.89 ± 0.80
Cambium	2.34 ± 0.77	0.08 ± 0.07	0.13 ± 0.14	1.88 ± 0.62
	Market samples			
	As	*Cd*	*Pb*	*Hg*
Warty tubercle	7.28 ± 1.94	0.03 ± 0.00	0.00 ± 0.00	7.13 ± 1.81
Whole stem	4.65 ± 1.56	0.05 ± 0.01	0.00 ± 0.00	3.85 ± 1.37
Leaf	2.56 ± 0.75	0.06 ± 0.02	0.03 ± 0.00	2.30 ± 0.17
Cambium	1.99 ± 0.47	0.05 ± 0.01	0.00 ± 0.00	1.41 ± 0.27
	Physic garden			
	As	*Cd*	*Pb*	*Hg*
Warty tubercle	9.61 ± 1.47	0.00 ± 0.00	0.00 ± 0.00	6.19 ± 0.80
Whole stem	3.96 ± 0.46	0.05 ± 0.02	0.22 ± 0.02	3.50 ± 0.86
Leaf	2.57 ± 0.44	0.05 ± 0.04	0.27 ± 0.08	2.50 ± 0.38
Cambium	2.22 ± 0.16	0.07 ± 0.02	0.19 ± 0.10	1.78 ± 0.17

collected from the market (Fig. 4). Here also *T. crispa* collected from industrial area recorded significant concentration of heavy metals. Unfortunately, the concentration of mercury was recorded in a higher quantity in the tested samples. Samples collected from the wild/rural area recorded lower quantity of heavy metals.

Discussion

Because of the rapid development of industries and motorization, as well as the widespread use of pesticides and fertilisers, the atmosphere and soil are constantly polluted with chemicals and heavy metals. These pollutants and heavy metals are then deposited on the plants that grow in polluted areas, where they enter the human food chain *via* plant parts and/or extracts.[21] Heavy metal contamination of medicinal herbal products occurs during cultivation, cross-contamination during processing or their deliberate introduction as therapeutic ingredients. Cultivation in soils containing high concentrations of heavy metals is one mechanism by which heavy metal contamination of herbal products has been documented.[22]

Medicinal plants have been cited as a potential source of heavy metal toxicity to both humans and animals.[22] Lead, mercury, arsenic, and cadmium are the most common heavy metals implicated in human toxicity, though aluminium and cobalt may also be toxic. As a result, the World Health Organization recommends that medicinal plants, which are the raw materials for the majority of herbal remedies, be tested for heavy metals. The majority of people who live in areas where these plants grow, however, harvest them locally for personal or family use without checking for heavy metal accumulation. The general belief that medicinal plants are safe and free of heavy metal toxicity may be incorrect. Hence, in the present study, we investigated the presence of heavy metals in the various parts of the three *Tinospora* species collected from various locations and the results are presented in Table 3.

In the present study, we studied the presence of four heavy metals in the three major *Tinospora* species used in Ayurveda and other systems of medicine. The samples were collected from various locations, including wild, rural, urban, etc. Our results clearly pointed out that heavy metals were present in significant quality in the *Tinospora* species tested.

Lead and its complexes are toxic to many tissues in the body, including the heart, blood, generative system, digestive tract, kidneys, and nerves. Lethal doses cause severe illness, resulting in long-term difficulties with cerebral issues. Lead toxicity causes stomach pain, nuisance, anaemia, peripheral neuropathy and irritability. In our study, the concentration of lead in the *Tinospora* species contained lower concentrations than the permissible quantity. Cadmium *is* a non-essential hazardous heavy metal that causes serious effects on the kidney, liver, vascular and immune system. Prolonged exposure to high-doses of Cd results in the Itai-Itai disease that is characterized by severe renal tubular dysfunction, ostomalacia and osteoporosis.[23] Surprisingly, the cadmium concentration in the *Tinospora* species investigated was also the level recommended by the WHO.

Arsenic is a highly toxic non-essential metalloid that significantly affects human health, leading to gastro-intestinal and hepatic disorders, hypotension, poly-neuropathy, loss of sight and carcinogenic effects.[24] Plants are exposed to As mainly from anthropogenic activities such as mining and smelting, the use of arsenic-based pesticides in agriculture and of arsenic-contaminated groundwater for irrigation purposes.[25] Herein, we have tested the presence of arsenic in the *Tinospora* species collected from various locations and the results clearly point out the presence of a significant quality of arsenic in the sample. A significantly higher amount of arsenic was recorded in *T. crispa*. Mercury is a highly toxic non-essential metal that is considered to be a dangerous xenobiotic in living cells. The consequences of Hg toxicity, mainly as organic mercury compounds, include central nervous system damage, alteration of motor function, neuro-behavioral, neuro-developmental, immunological effects and cardiovascular and kidney injuries. Exposure of plants to Hg derives from natural sources (volcanic eruptions, emissions from the ocean) and anthropogenic activities (burning of fossil fuels, production of cement, mining, smelting, production of ferrous and non-ferrous metals).[26] In this investigation, Hg levels were above the approved limit recommended by the WHO.

Plants growing along the roadside may be subjected to high levels of metal pollution, particularly vehicle emissions and trace amounts of metal in the air. It has been widely reported that the accumulation of toxic industrial effluents in the soil is steadily increasing due to rapid urbanisation and widespread pollution of the environment. Among these toxic substances, heavy metals, which are ubiquitous in nature, have serious adverse effects on living organisms.[27,28] Plants are sensitive to environmental conditions and accumulate heavy metals in their parts (*via* root uptake, foliar adsorption, and deposition of specific elements in leaves and storage organs), and the intensity of this uptake process alters the plant's overall elemental composition.[29] The uptake, accumulation, and concentration of heavy metals in plants are influenced by a variety of factors, including atmospheric deposition (depending on traffic density, metal mining and smelting operations), soil concentration and bioavailability (*via* the addition of pesticides and sewage sludge), and the nature of the soil where herbs are grown.[21] All these research clearly show that the herbal plants that grow in the industrial/urban areas will have a greater amount of heavy metals due to the absorption of heavy metals from the soil and atmosphere. In our investigation, *Tinospora* species collected from urban/industrial areas recorded a significant quantity of heavy metals compared to other areas. This was also supported by the research reports of several investigators around the globe. Feng *et al.*[30] suggest that heavy metals from traffic emissions may accumulate in roadside plants from the soil. Meanwhile, other researchers reported that heavy metals in the air could be deposited and absorbed by the leafy part of the plant.[31,32] Heavy metals emitted by traffic may accumulate in the soil before being absorbed by plant roots. Because heavy metals are resistant and persist in plants for long periods,[33] they may be transferred to humans through the food chain.[34] Fast urbanisation in developed cities is also responsible for a substantial increase in metal concentration in surrounding areas and plant species thriving in those places, according to Princewill-Ogbonna and Ogbonna.[35] As per AAS-mediated chemical profiling, greater liberation of vehicle pollution in Aba, Nigeria, resulted in a varied concentration of heavy metals in diverse medicinal plants. Similarly, in numerous spice and medicinal plant species collected from Egypt's exportation zones, analytical profiles of Pb, Cd, Cr, Ni, Sn, Zn, Mn, Cu, and Fe revealed quantities above-permitted limits.[36]

The result shows that the maximum and minimum concentrations of metals in soil were observed in the industrial area and the residential area, respectively. The lower concentration of metals in the residential area (i.e. control) is attributed to the absence of industries in that location. The highest concentrations of Hg and As were found in the industrial area sample, and the values obtained were significantly higher than those found in the residential area. The high values of Hg and As at the industrial area are attributed to various industrial activities taking place in the location. An important process in the geochemical cycling of heavy metals is the mobilisation of metals into the atmosphere as a result of anthropogenic activities. This is especially noticeable in urban areas, where a variety of stationary and mobile sources emit large amounts of metals into the atmosphere and soil.[37]

However, the accumulation of heavy metals varied from species to species. In our study, *T. crispa* recorded the highest concentration of heavy metals and the lowest concentration was recorded by *T. cordifolia*. Interestingly, if we analyse the presence of heavy metals in different plant parts, certain parts will record a significant concentration of heavy metals when compared to the rest of the parts. In the case of *Tinospora* species, a significantly higher concentration of heavy metals was recorded at the warty tubercle and a lower concentration was recorded at the cambium (Fig. 3).

As we said earlier, the significant quality of heavy metals was recorded in *T. crispa*. In addition to this, the warty tubercle of the *Tinospora* species recorded a higher quantity of heavy metals when compared to other parts. If we examine the three *Tinospora* species used in the study, *T. crispa* has an enormous number of warty tubercles in the stem compared to *T. cordifolia and T. malabarica.* It is believed that *Tinospora* species will absorb and accumulate heavy metals from the soil and store them in these warty tubercles. The higher concentration of heavy metals will be found in *T. crispa* due to the presence of a large number of warty tubercles. This was also clearly proved in our investigation. So, if we are using *T. crispa* instead of *T. cordifolia* in the pharmaceutical industry, heavy metal poisoning is more likely. Thus, substitution or adulteration of *T. cordifolia* with *T. crispa* is a great peril for the pharmaceutical industry, as the traditional pharmaceutical industry solely depends on *T. cordifolia* for making medicines.

Conclusion

Medicinal plants contain a wide range of active principles used in both herbal and modern medicine. *Tinospora species, especially T. cordifolia* are renown ayurvedic medicinal plants with several medicinal properties beneficial to humans. However, the continuous increase in environmental pollution is leading to the build-up of these pollutants, including heavy metals in the plant parts that eventually enter the human food chain. Thus, medicinal plants for the formulation of herbal remedies should be harvested from pollution free natural habitats. Our findings further indicate that the medicinal plants, used for local or pharmaceutical purposes, should be collected from areas not contaminated with heavy metals. The results suggest that regular and systematic screening of raw medicinal herbs on a quantitative basis is necessary to check the levels of heavy metal pollutants prior to using them for consumption or manufacture of herbal medicinal formulations so that the possible contamination cannot reach the finished herbal products. In addition to this, due to the close anatomical features, *T. malabarica* and *T. crispa* will act as a substitution or adulteration for *T. cordifolia.* So, regular quantity checking is very important to identify the adulterant. Our study clearly pointed out that *T. crispa* has a huge amount of heavy metals compared to *T. cordifolia.* Furthermore, it is very important to remove the warty tubercle before formulation as it contains a large amount of heavy metals when compared to the rest of the parts. Moreover, assessment of heavy metals in medicinal plants will pave the way for excluding extensively polluted environmental sites for collection of raw materials required for herbal drug preparation.

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