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Effect of Heavy Metals in Naturally Occurring Medicinal Plants of India

GOVINDASAMY AGORAMOORTHY, MINNA J. HSU† and PO-CHUEN SHIEH*
Department of Pharmacy, College of Environmental and Health Sciences, Tajen University, Yanpu, Pingtung 907, Taiwan
E-mail: pochuenshea@gmail.com

This paper presents the effect of heavy metal bioconcentration on 23 plant species that occur in natural ecosystems of India. The average concentration of mercury in the coastal plants was 15.3 times higher than that of the inland plants. The average concentration of zinc in the inland plants was significantly higher than coastal medicinal plants. With the exception of one plant (*Eclipta alba*), the concentrations of lead among 22 plant species investigated were higher than the normal range of lead concentration showing signs of environmental contamination. Local people continue to use plants reported in this paper in the traditional medicine to treat various ailments, which may increase the risk of heavy metal toxicity. It is vital to develop long-term environmental monitoring in India's natural ecosystems to evaluate levels of contaminants in organisms so that counter measures can be deployed to decelerate environmental toxicity and to safeguard human health in future.

Key Words: Plant, Ecosystem, Heavy metal, Pollution, Environment, India.

INTRODUCTION

India is one among the richest and highly endangered eco-regions of the world and it harbors diverse species of animals and plants[1,2]. Plants play a major role to give life on the planet by tapping the sun's energy. They serve as food and medicine for many animals including human and they improve the quality of air that we breathe. India's forested areas support plant endemism, which is estimated at 33 % with 140 endemic genera. About 6 % of the global diversity of flowering plants (15,000 species) occurs in India alone[3]. However, threats ranging from habitat destruction to biological invasion and commercial exploitation to industrial pollution continue to threaten the survival of various species of plants across India.

Heavy metals are both toxic and common in natural environments since they occur in soil, surface water and plants. Heavy metals are readily mobilized by human activities that include mining and discarding industrial toxic waste in nature and they pose a potential threat to terrestrial and aquatic organisms[4,5]. However, scientific

†Department of Biological Sciences, National Sun Yat-Sen University, Kaohsiung 80424, Taiwan.

data on the toxic levels of heavy metals found in India's plants in natural ecosystems that occur along the coastal and inland riverine habitats are limited. Mangrove forests occur along the coastal habitat while riverine forests occur along inland areas. Mangrove forest is among the highly endangered ecosystems of the world since the plants require specific conditions to grow, thus restricting their geographic range. Both the mangrove forests and the riverine forests in Asia are diminishing in recent years due to uninterrupted man-made disturbances that range from deforestation to pollution[4].

Heavy metal contamination poses a major threat to ecosystems including water, soil and organisms including human health[5,6]. When the levels of heavy metals exceed in plants and animals, it can induce a variety of acute and chronic effects in wide range of organisms in various ecosystems. In USA for example, heavy metals have caused natural forest to decline[7]. Due to rapid development, coastal and inland habitats of most Asian countries including India are subjected to serious environmental stress and the increasing intensity of domestic sewage, industrial waste, agricultural runoff, heavy metals and other toxic waste materials leading to disastrous environmental consequences[5,8,9]. Therefore, it is important to gather information, hitherto not available, on heavy metal levels in plants that naturally occur along the coastal and inland riverine habitats throughout India.

In this study, we have investigated the bioconcentration of heavy metals in the naturally occurring 11 inland plant species and 12 coastal plant species in Cuddalore District of Tamilnadu State, India. These plant species have been locally used by people in traditional medicine to treat various illnesses for decades. We have analyzed for essential (Cu, Fe, Mg, Mn and Zn) and non-essential (environmentally toxic Hg, Pb and Sn) trace metals.

EXPERIMENTAL

Study area and sample collection: Coastal plants were collected from the Pichavaram forest located between Vellar and Coleroon estuaries (latitude 11°22′ N and 79°45′ E) in Cuddalore District of Tamilnadu, India. Inland plants were collected along the forest areas of Coleroon river. Plants were identified using field guides and species identification confirmed at the Department of Botany, Annamalai University[10]. The climate of the study area is humid with an average rainfall of 1310 mm. Rains occur during the northeast monsoon season (October-December) each year[11]. In order to study the bioconcentration levels of heavy metals, 11 inland plants (*Ipomoea pes-caprae, Acalypha indica, Cassia auriculata, Cissus quandrangularis, Eclipta alba, Hibiscus cannabinus, Lawsonia inermis, Phyllanthus niruri, Sauropus androgynus, Tridax procumbens* and *Vitex negundo*) and 12 coastal plants (*Avicennia officinalis, Bruguiera cylindrica, Ceriops decandra, Rhizophora apiculata, R. mucronata, Aegiceras corniculatum, Excoecaria agallocha, Acanthus ilicifolius, Arthrocnemum indicum, Suaeda monoica, S. maritime* and *Sesuvium portulacastrum*) were collected from dawn to dusk on 26 April 2007. Five leaves each from 4 different plants belonged to the same species were collected contributing to a total of 20 leaves for each species. Collected samples were stored separately in polythene bags and transported to the laboratory for processing the same day.

Sample preparation and laboratory analysis: Plant samples were digested for heavy metal analysis with a 90 ºC mixture of concentrated nitric acid and hydrogen peroxide and made to 25 mL volume[12]. Digested samples were stored in labeled, acid-washed glass vials. Analysis for heavy metals was carried out immediately on the resultant digests using air/acetylene atomic absorption spectroscopy, with the use of prepared standards (run before each batch) to determine sample concentrations. In order to ensure precision of atomic absorption spectroscopy results, three replicates of each sample were run to ensure measured absorbencies were consistent. Metal concentrations were calculated from each replicate absorbance value, which was then used to calculate an average sample metal concentration. Only 8 metals (copper, iron, mercury, magnesium, manganese, lead, tin and zinc) within leaf tissue were of interest in this study since arsenic and cadmium were not detectable in most plants. Elements such as copper, zinc and lead were given emphasis since these metals can be used as bio-indicators of heavy metal exposure. All concentrations were expressed in µg g^{-1} (ppm) on a dry-weight basis using weights obtained from oven-dried specimens. All specimens were run in batches, which included known standards, method blanks and spiked specimens. The absorbance of a blank sample was also conducted to allow background correction. Accepted recoveries ranged from 85 to 105 % and batches with recoveries less than 85 % were rerun.

Bioaccumulation factor and statistical analysis: Two basic approaches were used to quantify the bioaccumulation of environmental pollutants with the assumption that organisms achieve a chemical equilibrium with respect to a particular media or route of exposure[6]. This approach used bioconcentration or bioaccumulation factors (BCFs or BAFs) to estimate chemical residues in plants from measured concentrations in the appropriate reference media. Hence, in present study of coastal and inland plant species, the reference media was soil, water and air. They were considered as reference media to calculate bioconcentration factors. Soil metal concentrations were normalized with metal values of Earth's Upper Continental Crust[13]. The bioconcentration factor (BCF) is defined as BCF = C_{biota}/C_{soil}, where C_{biota} and C_{soil} were the total metal concentrations in taxa and soil, respectively, in µg/g. The same formula was adopted to calculate bioconcentration factor for all the collected samples of plants with the assumption that the distribution of heavy metals in the environment is controlled by a continuous exchange among phases such as air, water, soil/sediment and biota.

Statistical analysis system software was used for data analysis and all means are presented as the values ± 1 standard deviation[14]. The averages of metal concentrations between coastal and inland medicinal plants were tested using non-parametric Wilcoxon rank test. We calculated Spearman rank correlation coefficients among the eight heavy metal elements. In order to know the significant groupings of studied metals and delineate the dominant processes by which metals sourced in the studied plants, the metals data set was subjected to factor analysis extracted using Varimax rotation scheme[14].

RESULTS AND DISCUSSION

The concentration of mercury in 11 plant species (with the exception of *Arthrocnemum indicum* and *Sesuvium portulacastrum*) was within the normal range of plant materials[15]. However, the average concentration of mercury in the coastal plants (0.21 μg g^{-1}) was 15.3 times higher than that of the inland plants (0.01 μg g^{-1} Wilcoxon Rank test $p < 0.001$; Fig. 1). The concentration of magnesium was positively correlated to the concentration of mercury ($p < 0.05$), while mercury was negatively correlated with zinc ($p < 0.001$) (Table-1). The average concentration of zinc in the inland medicinal plants was 335.9 ± 117.2 μg g^{-1}, which is significantly higher than coastal medicinal plants (55.64 ± 36.4 μg g^{-1}, Wilcoxon Rank test $p < 0.001$; Fig. 1). The concentration of cadmium was not detectable with the exception of 4 species (*Eclipta alba*-0.11 μg g^{-1}, *Sauropus androgynus*-0.90 μg g^{-1}, *Arthrocnemum indicum*-0.31 μg g^{-1} and *Sesuvium portulacastrum*-0.19 μg g^{-1}) suggesting less airborne contamination of cadmium in the coastal and inland habitats of the study area.

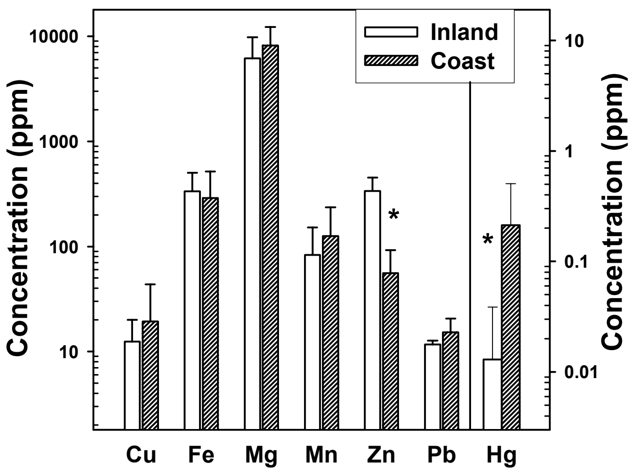

Fig. 1. Metal concentrations in 11 species of inland and 12 species of coastal plants of India, *Significant in Wilcoxon rank test ($p < 0.001$)

The mean bioconcentration factors (BCF) for iron and manganese were low while magnesium, zinc and lead were higher than 1.6. The bioconcentration factor for zinc in coastal plants (1.11 ± 0.73, n = 12) was significantly lower than that in the inland plants (6.72 ± 2.34, n = 11). The bioconcentration factor for lead in coastal plants (1.91 ± 0.66) was higher than that in the inland plants (1.46 ± 0.13) but the difference was not significant. The factor analysis indicated three factors

TABLE-1
SPEARMAN CORRELATION MATRIX FOR HEAVY METALS FOUND IN 23 PLANTS

Metal	Copper	Iron	Magnesium	Manganese	Zinc	Mercury	Lead
Copper	–						
Iron	-0.06	–					
Magnesium	0.28	0.18	–				
Manganese	0.32	-0.30	-0.14	–			
Zinc	-0.28	0.30	-0.27	-0.09	–		
Mercury	0.17	-0.01	0.47†	-0.23	-0.68‡	–	
Lead	0.23	-0.22	-0.22	0.21	-0.24	0.29	–

†$p < 0.05$; ‡$p < 0.001$.

describing 72.3 % of total data variability in 23 different samples of plants from costal and inland ecosystems. The dominant factor accounted for 28.7 % of the total variance that portrays accumulation of essential elements such as magnesium and iron and a non-essential lead confirming the contaminated condition of the studied ecosystems. This was supported by the factor 2 that accounted for 24.4 % of the total variance and it showed accumulation of essential element zinc and toxic elements such as mercury and lead confirming the polluted nature of the studied ecosystems. Factor 3 accounted 19.2 % of total variance that showed significant loading on copper and manganese. The results indicate that the anthropogenic sources of metals pollute the biotic community in the coastal and inland ecosystems of Cuddalore District in south India.

Toxic heavy metals accumulate faster in the coastal and riverine forest habitats due to their close proximities to development areas and metal contamination range from industrial waste to garbage dumps[16]. Metals such as copper, lead and zinc are of greatest eco-toxicological concern even in the less populated coastal habitats of Australia[17]. The zinc concentrations observed in the inland areas of this study are higher than reported in other coastal forest areas of India and elsewhere in Australia and Panama[12,18,19]. The average concentration of mercury in the coastal plants was over 15 times higher than that of the inland plants, which indicates that the atmospheric pollutants through industrial sources from near by towns find their way into the natural coastal ecosystem. Mercury is a severe environmental pollutant due to its toxicity even at low concentrations in biological systems and the coastal habitats are known to be vulnerable to mercury pollution.

Lead accumulates in plants primarily from the atmosphere[20]. With the exception of *Eclipta alba*, the concentrations of lead among the other 22 plant species investigated were higher than the normal range of lead concentration (5.0-0.0 µg g^{-1}) of plant materials showing apparent signs of environmental contamination. Similar concentrations of cadmium were reported in the Indian herbal drugs made from the leaves of *Coleus forskohlii* and *Alpinia galangal* highlighting the potential threat of cadmium in plants that are used in herbal medicines for human consumption[21]. Higher airborne cadmium can be inferred from its concentration between 0.2 and 1.0 µg g^{-1} in plants[20].

The plant species investigated in this study have been used in traditional medicine. For example, plants such as *Bruguiera cylindrica*, *Rhizophora apiculata* and *R. mucronata* are rich in polyphenols[22]. Some plants are used as tea and the tea prepared from the flowers of *Cassia auriculata* has been used to treat diabetic patients in rural areas[23,24]. The ashes of *Arthrocnemum indicum* have been prescribed in the treatment of snakebites and traditional healers use *Sesuvium portulacastrum* for the treatment of kidney problems and fever[25,26]. Leaves of *Acanthus ilicifolius* have been used as an emollient fomentation for treating rheumatism and neuralgia. The polysaccharide extracted from *Rhizophora mucronata* showed *in vitro* anti-HIV activity[27]. *Acalypha indica* has been used as an antidote for poisonous snake bites in rural India[28]. *Cissus quandrangularis* has been used for the treatment of gastritis, bone fractures, skin infections, eye diseases, anemia, asthma, burns and wounds[29]. The plant *Eclipta alba* has been mentioned in the ancient Indian texts as a nervine tonic[30]. *Vitex negundo* is a small tree and extract made from its leaves have been used in India's traditional medicine (Ayurveda) as antiinflammatory, analgesic and antiitching agents, both internally and externally. In different parts of India, *Phyllanthus niruri* has been used as traditional medicine for centuries[31]. Henna, an extract of the plant *Lawsonia inermis* has been used in many cultures as a dye for hair/nails and for decorative body painting[32]. *Tridax procumbens* is a weed found throughout India and it has been used to treat ailments such as jaundice, bronchial catarrh, diarrhea and dysentery[33]. People continue to use these plants from the coastal to inland habitats throughout India to treat various ailments. Thus there is significant health risk related to heavy metal toxicity for those who consume medicines/tea made from plants.

India has a vast coastline that stretches over 7000 km and rivers discharge about 1645 km^3 of freshwater of which, 75 % enters the Bay of Bengal[34]. The Mangrove forest of Pichavaram and the riverine forest of Coleroon in Cuddalore District are located along the Bay of Bengal Coast in south India. This region harbors diverse natural ecosystems that include estuaries, mangroves, lagoons, beach ridges and riverine forests. The district in general has seen enormous growth in the industrial sector in recent years. On the bank of the river Uppanar estuary near the town of Cuddalore, one of the major chemical industrial complexes namely the State Industries Promotion Corporation of Tamil Nadu is located in an area of 520 acres. Industrial wastes from the district are often drained directly into the estuary, rivers and coastal areas that impact flora and fauna. Present results suggest that heavy metal toxicity is becoming increasingly common among coastal and riverine habitats in India. Present findings are similar to heavy metal toxicity reported in the coastal sediments and marine organisms along the coastal areas of south India confirming the ongoing threat to coastal and terrestrial biota[35-37]. Present findings are also similar to heavy metal toxicity reported in protected forest areas of Taiwan[5].

The Millennium Ecosystem Assessment 2005, a comprehensive analysis produced by 1,360 scientists determined recently that the health of the world's ecosystems has been severely damaged. Ecosystems provide essential services to people around the world[38]. Of the 24 ecosystem services examined including provision of freshwater, food, regulation of climate and air quality, the assessment found that 15 (62.5 %) of them are being degraded or used unsustainably. Furthermore, another assessment from the World Conservation Union summarizes a grim statistics on the current status of global plant diversity and 70 % of the plant species assessed, 45 % has been classified as endangered or critically endangered[39]. Therefore it is not only essential to protect India's delicate coastal and riverine forest ecosystems, but also vital to monitor the impact of heavy metals and other contaminants affecting plants that are commonly used by people in traditional medicine.

India is the second most populous country in the world with over 1 billion people. The population is expected to double in the next few decades. Environmental pollution is a serious problem in India and about 70 % of India's ground water resources are contaminated by biological, toxic organic and inorganic pollutants[40]. Heavy metal depositions are associated with a wide range of sources that include small/large industries, battery production, metal products, metal smelting, cable coating, brick kilns, vehicular emissions, diesel generators, sewage, pesticides, fungicides, fertilizers and sewage sludge. India is the second-largest user of mercury in the world (170-190 tons/year) after USA (372 ton/annually). Unfortunately, India does not have strict regulations to control mercury in various products. The Pollution Control Boards managed by the state and central governments in India have identified 1,532 'over polluting' industries across the country drastically influencing natural ecosystems[40]. Most of India's industries do not comply with international environmental pollution emission standards[41]. To make matters worse, millions of kilograms of electronic waste are being generated in developed countries each year of which an estimated 50-80 % is being shipped for recycling in countries such as India, China, Pakistan and Bangladesh usually end up in rivers, waterways and coastal areas[8]. Cheap manpower, inadequate environmental standards and lack of enforcement of environmental laws in Asian countries attract the toxic garbage dumping in the name of recycling[4]. The on-going toxic impact including heavy metals affect various species of fauna and flora in natural environment in India[42].

The recognition of the occurrence, importance and effects of contaminants on food chains and ecosystems must lead to the development of stringent environmental monitoring research in India aimed at directly measuring the levels of contaminants in various organisms through systematic long-term research. If industrial and vehicular pollution of water, soil and air can be reduced in future, it will prevent high concentrations of heavy metals entering natural ecosystems and associated food chain affecting human and environmental health. The aerial deposition of heavy metals can be reduced by setting and enforcing emission standards for heavy metals, systematic monitoring of heavy metals in aerial deposition and by establishing master plans for rural/urban industrial development plan to regulate heavy metals affecting biota including human health across India's unique and delicate ecosystems.

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ERRATUM

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Stability Study on *Boswellia serrata* (Hydro-alcoholic) Extract

NARENDRA KUMAR SAHOO†, SIBAJI SARKAR* and BHUSHAN PATWARDHAN‡
J.J.C. Trust Sanchalit, N.R Vekaria Institute of Pharmacy and Research Center
C.L. College Campus, Bilkha Road, Junagadh-362 001, India
E-mail: sibajisarkar004@gmail.com

The corresponding author for this manuscript is **Dr. Narendra Kumar Sahoo*** and his affiliation is Poona College of Pharmacy, Bharati Vidyapeeth University, Erandwane, Pune-411038, India, E-mail: sahoo23@rediffmail.com