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A Comparative study of heavy metals in *Emblica officinalis, Phyllanthus emblica and Azadirachta indica*

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Abstract

Medicinal plants play vital role in healthcare sector for developing nation and potent source of therapeutic molecules to heal various diseases in the world. The quantity of heavy metals in plants were analyzed to show the potential threat of their effects to the animals and human beings who consume them as such or their derived products. The work is much more beneficial as the actual nutrient content of the medicinal plants in terms of the essential trace elements could also be identified. The concentration levels (mg/l) of the selected trace metals (Ni, Cu, Cr, Zn, Mn, Pb) were estimated in some medicinal plants of the Muzaffarnagar district. The atomic absorption spectrophotometer was employed for the estimation of heavy metals of three different plant species that were collected from different locations in Muzaffarnagar district. Emblica officinalis, Phyllanthus emblica and Azadirachta indica *are selected for the analysis. In this investigation the trace elements in the samples were determined and was established,* Emblica officinalis *has the level of metal in the range of Cu>Cr>Mn>Zn,* Phyllanthus emblica *Cu>Cr>Zn>Mn* and Azadirachta indica *has Cu>Zn>Cr>Mn, Ni and Pb was completely absent in all three plants.*

Keywords: Medicinal plants, heavy metals, Emblica officinalis, Phyllanthus emblica, azadirachta indica, atomic absorption spectrophometer.

Introduction

Heavy metals are natural elements of the earth's crust and they can enter in water and food cycles through a variety of chemical and geochemical process[1]. The living organism requires a number of minerals for their growth and other activities which are obtained from plants, since plants uptake and accumulate minerals from the environment which is necessary for its growth. Plants requires trace amount of heavy metals like Co, Cu, Fe, Mn, Mo, V and Zn found in excessive levels, these metals and other heavy metal such as Cd, Hg and Pb have not known beneficial effect on organism and their accumulation in mammals can cause serious illness[2]. Among the different type of minerals, traces of Cd and Pb has been detected and reported in all plants and foodstuffs.

Medicinal plants have played an essential role from the ancient period as they are used in conventional medicine and also as home remedies. Pollution, environment, soil, atmosphere, harvesting and handling are some of the factors, which play a major role in contamination of medicinal plants by metals and also by microbial growth. Therefore it is necessary to measure and establish the levels of metallic elements in the herbal plants as these elements when consumed at higher levels become toxic. The World Health Organization were also emphasized the quality of plant and its products by using modern techniques and applying suitable standards[3]. In the present study, the concentration of various metallic elements in the medicinal plants *Emblica officinalis, Phyllanthus emblica* L., *Azadirachta indica* A. Juss, were analyzed using atomic absorption spectrophotometry.

Emblica officinalis is commonly known as amla. The fruit of this plant is used in world-wide as a traditional medicine. The fruit of *Emblica officinalis* was used as diuretic, adaptogenic, hepatoprotective, antitumor, hypocholestrolemia, antioxidant, antiulcerogenic, anti-inflammatory, antimicrobial, analgesics and antipyretic agents[4-6]. The fruit of *E. officinalis* has been found to contain tannic compounds emblicanin A and B which found to have significant *invitro* antioxidant activity[7]. *Phyllanthus emblica* L. is a tree of small or moderate size with a greenish-grey bark[8]. *Phyllanthus emblica* L has several class of secondary metabolites used as anti-inflammatory and anti-pyretic agent by the rural population and for treating several disorders such as the scurvy, cancer and heart diseases and anti-neutrophilic and anti-platelet activity[8,9], anti-viral, antimutagenic, anti-allergic, anti-bacterial, gastro-protective and anti-proliferative activities[8-10]. *Azadirachta indica* A. Juss (syn. *Melia azadirachta)* is known as Indian neem (margosa tree) originates from the Indian sub-continent and now grow in dry regions of more than 50 tropical countries around the world[11,12]. Nimbidin, a bitter crude extracted from the oil of seed kernels have been reported to have several biological activities[11]. Azadirachtin isolated from the neem seed has strong antifeedant[11,13] and antimalarial activity. A deoxygedunin compound isolated from the seed oil of the plant has been reported to exhibit moderate antibacterial activity against some strains of human pathogenic bacteria[11].

Heavy metal uptake by the plants is the main pathway of metal transfer from sediments and water to the food web. The metal uptake by the plant is determined by the metal mobility and bioavailability Most of the procedures adopted involve dry ashing or wet digestion using nitric acid alone, or in combination with perchloric acid or hydrogen peroxide. This study adopts the procedure of overnight wet digestion with nitric acid alone for the determination of heavy metals[14]. Plants can accumulate metal in their parts and transfers it from soils into the food chain[15]. This accumulation is one of the most serious environmental concerns because of the potential harmful effects that toxic metals could have on animals and human health. Some metals like Zn, Fe, Cu, Cr and Co are toxic at high concentrations and Pb, Hg and Cd are exclusively toxic[16].

Material and Methods

Sample Collection: The plant leaves of *Emblica officinalis, Phyllanthus emblica, and Azadirachta indica* were collected from Shri Ram College Muzaffarnagar district, Uttar Pardesh, India and transferred in a sterile cloth bag and were transferred to the laboratory.

Material Preparation: The samples were thoroughly washed with deionized water, shade dried at room temperature for 15 days, ground into powder with a mechanical grinder and homogenized. The samples were subsequently stored in separate sample bottles[17].

Treated with conc. HNO_3: 10 ml of Concentrated HNO_3 (extremely pure 65%) was added to 1 g leaf sample and concede to stand overnight at room temperature. The samples were heated at 120°C, after which the temperature was enhanced to 140°C for 4 hrs. The digestion was continued at this temperature until about 1ml of acid remained. After cooling, the suspension was separated in a 50ml volumetric flask and diluted to the mark[20]. Stock standard solutions of Cu, Cr, Mn, Ni, Zn and Pb containing 1000ppm of each metal were prepared by dissolving weighed amounts of suitable anhydrous analytical grade salts in distilled water. Calibration standards of 1ppm, 2ppm and 3ppm of each element were found by proper dilution of the stock solutions. Cu, Cr, Mn, Ni, Zn, Pb contents were measured by using flame atomic absorption spectrometry. The elements were measured under the suitable conditions with air-acetylene flame.

Results and Discussion

The six elements (Cu, Cr, Mn, Ni, Zn, and Pb) were determined in the powdered leaf samples of medicinal plants using atomic absorption spectrophotometer (AAS). Figure 1 show the concentration of individual metals, which were analyzed. This study discovered that all the metals were accumulated to greater or lesser extents by the three plant species studied, except that of nickel and lead.

Copper is one of the indispensable metals which are needed for the normal plant growth and development. Copper is a co-factor for metalloproteins and play a major role in several metabolic pathways. Though it is essential the excess levels of copper in the plant would inhibit its growth and alters certain cellular processes. The release of heavy metals into the environment by mining, smelting, manufacturing, agriculture and waste disposal technologies is a major cause for the excess accumulation of copper in nature as well as in plants[18]. Since the higher concentration of Cu becomes toxic due to its high redox properties it has to be maintained in lower levels in the body[19].

Chromium has a wide use in the industries and considered as a serious environmental pollutant. In plants the toxSicity of chromium is based on its valence state i.e. Cr (III). Cr (III) is less toxic than Cr (VI). Chromium is not absorbed directly by plants rather it gets accumulated by carrier ions like sulfate or iron. Chromium toxicity alters the plant germination, its complete growth by affecting photosynthesis, other metabolic processes and the total dry matter production[20].

Zinc is an essential micronutrient which is involved in many biochemical reactions in the plants. It is required for the optimum crop growth and it is taken in divalent form by the plants[21]. Manganese is also an essential metallic compound that plays a vital role in the photosynthesis, nitrogen metabolism and in the formation of other compounds that are required for the plant metabolism[22]. Figure 1 shows the heavy metal concentration in the different medicinal plants like *Emblica officinalis, Phyllanthus emblica* and *Azadirachta indica.*

In *Emblica officinalis*, nickel and lead metals were not found. The level of copper was found to be high in the case of the leaf sample of *Emblica officinalis* followed by chromium, manganese and zinc. In *Phyllanthus emblica* copper concentration was found in high level when compared to other metals (chromium, zinc and manganese) detected. The neem plant *Azadirachta indica* was already investigated for the presence of certain heavy metals and their concentration was found to be zinc 15.7, copper 1.12, iron 188, manganese 46.5, sodium 138, lead 0.49, potassium 19220, magnesium 5630, calcium 3543, and phosphorous 900 ppm levels[1]. In the present study *Azadirachta indica* showed different concentration of heavy metals like copper 1.655, chromium 0.145, zinc 0.294, manganese 0.348 ppm where the copper concentration was found to be at the maximum level followed by the manganese, zinc and chromium. The heavy metal content in the above plants was not found in large amounts. In this comparative study, the application shows the accumulation capacity of heavy metals in different medicinal plants.

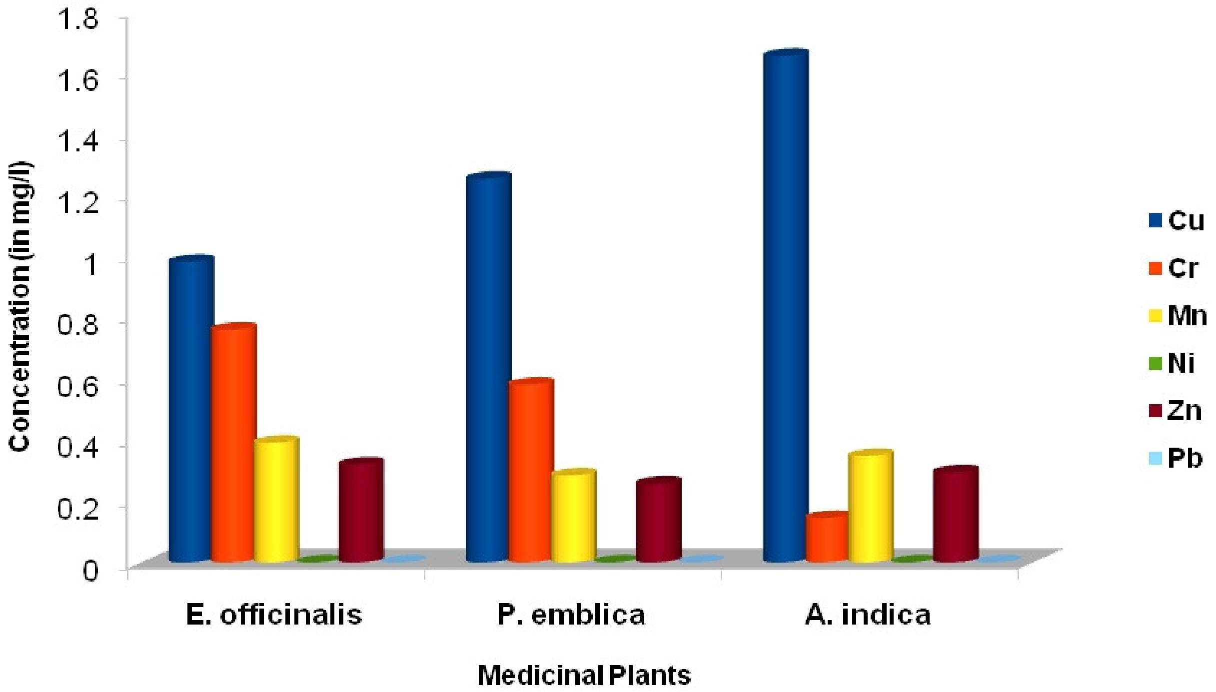

Figure-1
Heavy metal concentration in different medicinal plants (*Emblica officinalis, Phyllanthus emblica and Azadirachta indica*)

All the investigated plants showed the highest level of copper in the leaf parts. The heavy metal content in the plant induces stress to the plant and causes various disease, other stress. That is the main need to control the quality of the products obtained from the medicinal plants has attained a great interest particularly in the field of pharmaceutical industries. Medicinal plants were given the standard values for the presence of heavy metal content by the World Health Organization. These measures help us to be free from metal contaminated plant products.

The source of the metal content in the soil is mainly due to the over usage of the artificial fertilizers, industrial effluent drop out to the agricultural land areas, and other man made activities. Thus, from the soil the heavy metal content were transferred to the plant parts that were consumed by the human and animal species. Hence, the bioaccumulation of the heavy metal content would attain the greater deposition in various areas of plant, animal and human. The standard levels obtained for the four metals such as copper was found to be 4-15 ppm, zinc in the wide range of 15-200ppm, followed by the manganese concentration as 2-685 ppm[3]. The detection level of chromium has a limitation of about 0.12ppm per gram[17].

Comparing the obtained values to the standard limitation values, we can conclude that the copper concentration was found to be less than the detection limit and hence do not possess any harm in the products obtained out of them. Same way, the zinc and manganese concentration of the above plants were also found to be less that the standard limits. But, chromium concentration was found to be higher than that of the standard level in all the three plants out of which *Emblica officinalis* reaches the peak in the graph obtained.

Conclusion

The heavy metal stress in the plant affects the entire biological life cycle due their accumulation in the biological species and further biological magnifications to the higher order levels thereby accumulating as much of metal that causes stress to the environment and the heavy metal concentration in the plant was beneficial only to a certain limit. Heavy metals cause stress to the cells in the living organism and therefore affects the oxidation process called as the oxidative stress in human oxidative stress is involved in the development of cancer, Alzheimer diseases, Parkinson's diseases, myocardial infection etc. In this comparative study shows the higher concentration amount is Cu in *A. indica and low concentration of Cu in E. officinalis,* the high concentration amount of Zn and Mn in *E. officinalis and lower concentration shows in P. emblica, and* the higher concentration amount of Cr in *E. officinalis and lower concentration shows in A. indica.*

The determination of heavy metal content in the plant depends on the factors such as the area of sample collection, procedures of sample collection, preparation of the sample extract by various treated methods, etc. The present research has to be developed in the area of medicinal plants to produce both the therapeutic molecule and for the development of protective mechanism such as the metal absorption information through the field studies has to be done.

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