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Calotropis as Bioindicator Plant for Assessing Trace Elemental Contamination of Environment

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Abstract: This paper is an attempt to study the efficacy of *Calotropis*, a perennial wild plant, to act as a bioindicator of trace elemental pollution at Central East Indian locations. In the study the *Calotropis* has been found to be an efficient indicator of trace element. Corresponding physical change was observed in the wild plant due to the accumulation of trace element. High values of trace element correspond to stunted growth of the plant with small sized leaves and flowers. Concentration of trace element obtained indicated the degree of disturbance when assessed against background values obtained from unpolluted vegetation. Results show that the plant can be used as an effective bioindicator of metal pollution. Its sensitivity towards the metal in environment follows the order of Fe > Ni > Pb > Mg > Zn > Mn > Ca > Cu. Enrichment calculations show high enrichment of Pb and Cu.

Key words: *Calotropis*, enrichment factor.

Introduction

Elements such as Cu, Mo, Zn, Mn, P and S are essential micro-nutrients that the plants require for their proper growth. The essential micronutrients are readily taken up by plants, provided the concentration in the soil substrate does not exceed a certain critical level. This critical level is species dependent. However, there are non-essential elements, which, if dissolved in nutrient solution, enter into a plant's dynamic system by passive uptake (Berry, 1986). The availability of mineral nutrients in the root environment influences the metal content of a plant. With the increase in concentration of an element, the normal functioning of an element is hampered and plants may develop symptoms of toxicity such as dwarfism, chlorosis and necrosis (Berry et al., 1981). In extreme cases (with lethal levels of concentration), a plant may restrict the inflow of toxic metals through its roots or may simply fail to grow due to the death of the root. Some plants may take up metal passively and deposit it in senescent organs such as leaves and needles. The senescent parts are subsequently deposited in such a manner that the normal metabolism process continues unhindered.

Metal concentrations in plants depend not only on the total soil concentration, but also on the chemical speciation of metals in soil and soil solution (Spasito et al., 1984). Sometimes a set of species or the structure and function of an entire biological community may function as a bioindicator. Bioindicators are generally used to detect or demonstrate the existence of a particular environmental state and to monitor changes in that state. In this way they are used as tools to extract abiotic or biotic information from the environment so that this information can be used for making scientifically based management decisions. Any plant species which is native to the area under consideration may act as an effective bioindicator if it gives some recognizable visible and chemical information. Forest and aquatic mosses have been used to monitor metal pollution in rivers (Say et al., 1981; Chakrabortty et al., 2006) while Sphagnum has been used to monitor atmospheric deposition of As

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and Se in Canada (Glosschenko et al., 1988). Lichens are used to monitor air pollution (Ferry et al., 1973, Tony R. Walker, 2008). Since the behaviour of trace elements in an ecosystem is highly complex they are usually studied separately for air, water, soil and biota (Kabata-Pendias, 2001). The capacity of plants to mobilize the toxic elements was studied in various ecosystems (Loko-bauer et al., 1993; Pascoa et al., 1996; Sastre et al., 1999).

In the present paper, a perennial shrub *Calotropis* was studied for its efficacy to act as a bioindicator. *Calotropis* is of the family Asclepeadaceae. It is a treelet with thick colony tomentose leaves when young and frequently glabrescent when fully developed. Different parts of *Calotropis* were used to find the trace elemental levels for studying its capability to sequester the trace elements from the soil and its probable role as a potential bioindicator.

Material and Methods

Study Site

Chhattisgarh state is among the fast developing industrial state of India where the large availability of coal and iron ore has led to establishment of a large number of primary and secondary metallurgical industries and coal-based power plants. Bhilai is the city where one of the largest integrated steel plants is located. The nearby city of Raipur (the capital) also supports large number of secondary metallurgical industries. Most of these industries are causing heavy particulate emission which is likely to cause many direct or indirect impacts on the health of ecosystem. The sampling sites were mostly located along the Mumbai Howrah National Highway passing through Rajnandgaon, Durg, Bhilai, Raipur and Bilaspur districts. Our sampling sites were located within 100-metre distance from the roads. However an effort was made not to take the plants which were located very close to the highway. Sampling locations are shown in Figure 1. Vegetation samples were collected from along the highway passing from Bilaspur to Rajnandgaon by grid sampling method. The samples collected were *Calotropis* (giant milkweed) and other native shrubs of the locality.

Sampling Strategy and Pretreatment

Samples were collected manually for the study from February 2007 to March 2008. Each location was

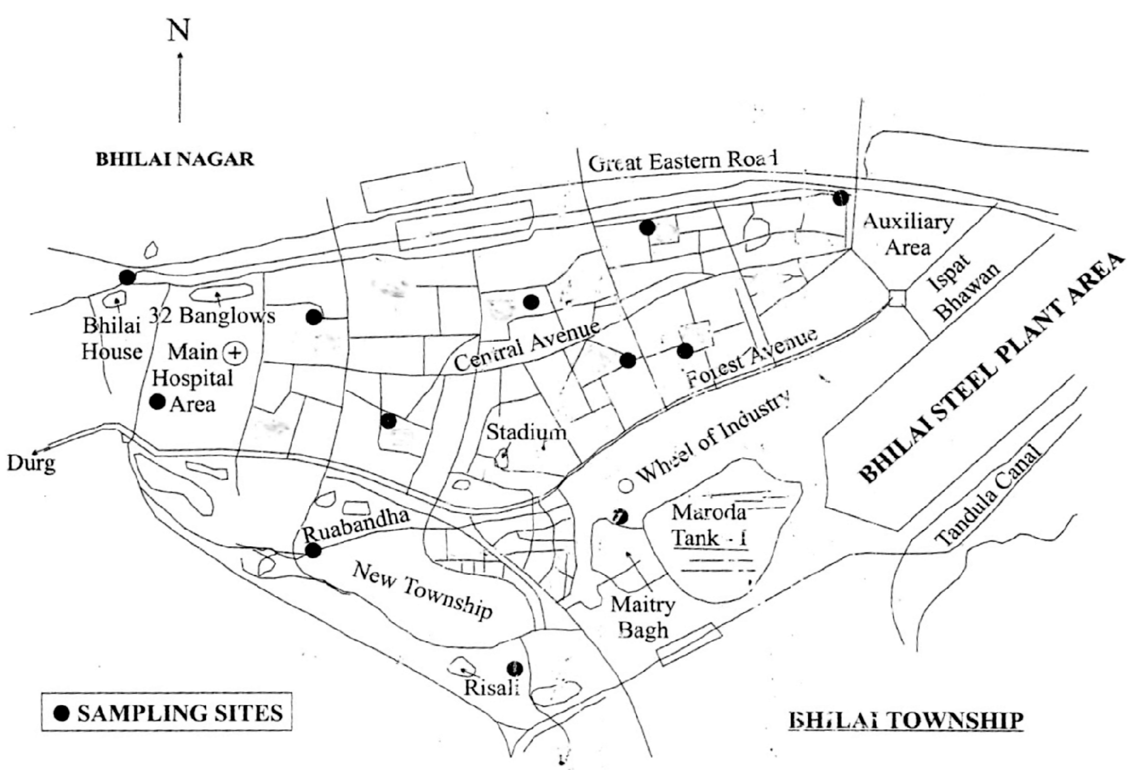

Figure 1: Map of Bhilai township showing sampling locations.

randomly sampled in a 5 m^2 square plot. Samples of leaves, buds, flowers, stem and soil were collected. After collection, the samples were placed in large plastic bags and immediately transported to the laboratory. Samples were rinsed thoroughly with deionised water, shaken to remove most of the water and allowed to air dry for one week. All the samples were dried in an oven at 60°C till dryness. After drying the plant samples were grounded in a ceramic mortar. Dried plant samples were crushed and sieved with 450 μ mesh size and stored in a dry atmosphere before analysis.

Digestion

One gm of dry samples of leaves, buds, flowers and stem were weighed and digested with 5 ml of conc. HNO_3 (Merck) in two installments till dryness, residue was dissolved in 50 ml 1:1 HCl and left overnight. All the digested samples were vacuum filtered by using micro glass filter paper. Finally the volume was made to 100 ml with 1:1 HCl (Merck).

One gm of soil from each sampling site was treated with 12 ml conc. HNO_3 in 100 ml Teflon beakers and heated to dryness, if sample was still evolving brown fumes of NO_X this step was repeated. Residue was treated with 12 ml of HF and 8 ml of $HClO_4$. The HF-$HClO_4$ digestion was used for dissolution. Heating was continued until the evolution of dense white perchloric fumes occurred and the solution reached incipient dryness and then allowed to cool. In this residue 15 ml of conc. HCl was added and left overnight. Then this solution was filtered using micro glass filter paper and volume made up to 50 ml with deionised water (APHA, 1992).

The analysis of all digested samples was carried out by Atomic Absorption Spectrophotometer (Varian AA 240 FS). Quality control and quality assurance protocol consisted of duplicate analysis of samples, use of standard elemental solutions, analysis of procedural and digestion blanks. Metal concentration in ppm was determined for each sample and average was calculated from three replicates. The absorbance of a blank sample was also conducted to allow background correction.

Result and Discussion

Trace Elemental Concentration in Various Body Parts of *Calotropis*

The concentration of various elements in different parts of the plant *Calotropis* was carried out. Based on the results (Table 1) it was found that the flower of *Calotropis* is the part which exhibit maximum concentration of elements. It was interesting to note that the concentration of trace elements like Ni, Pb, Zn and Cu was very high compared to the average levels of those elements in leaves and stem. Percent enrichment of Ni, Pb, Zn and Cu in the flower was 19.166; 10.264; 9.524 and 1.652 respectively compared to the other body parts average.

Selection of *Calotropis* as Biomonitoring Species

Theoretically, biomonitoring species for trace-element air pollution are selected on the basis of specificity (Rühiling, 1994). A suitable biomonitor should be common in the area of interest, available for sampling during all seasons, and it should be tolerant of pollutants at the relevant levels.

Apart from the above, a suitable biomonitor should also show element uptake independent of local conditions, its biological variation should be limited and the accumulated concentration levels must be measurable by routine analytical techniques. It is also desired that the physiological mechanisms for uptake of elements should be known to facilitate interpretation of the results. Further, the elemental concentrations should be a function of the exposure to the elements and the organism should have low background concentrations of these elements. The sampling method and protocol for sample preparation for measurement should be simple and quick in a good biomonitor.

Suitable biomonitors, which meet the requirements, make continuous monitoring and even retrospective monitoring of pollution possible at relatively low cost. When information on time-averaged trace-element concentrations at specific sites in the environment is the aim, the use of such non-mobile monitors is preferred (Chakrabortty et al., 2006).

Table 1: Mean concentration of elements in various parts of *Calotropis* in township area (mg/kg)

	Fe	*Zn*	*Mn*	*Ni*	*Ca*	*Mg*	*Cr*	*Al*	*Pb*	*Cu*
Leaf	1076.25	15.06	141.87	0.7	14256	8588	38.05	BDL	4.1	4.41
Stem	257.33	12.16	42.75	BDL	9478	5268	3.05	BDL	2.33	BDL
Flower	1623	219.39	135.16	134.16	16565	10281	BDL	BDL	330	420

From the above viewpoints, the *Calotropis* was judged for its biomonitoring potential. It was observed that *Calotropis* is a perennial woody plant which can survive in hot dry climate also. This plant possesses well developed root system. The soft hairy leaf and stem structure appears to promote the deposition of dust where large surface to width ratio appears to be promoting the absorption of elements.

This plant appears to be tolerant of pollutants. The results indicate elemental uptake corresponding to the elemental levels in soil and air. Mean concentration of trace metal contents in the flowers of *Calotropis* is presented in Table 1. The range of Fe in the samples is in between 1187.72 mg/kg and 1936.37 mg/kg, Zn 96.018 mg/kg and 241.34 mg/kg, Mn 128.5 mg/kg and 191.2 mg/kg, Ni 0.0346 mg/kg and 134.16 mg/kg, Pb 150 mg/kg and 330 mg/kg, and Cu 67.5 mg/kg and 420 mg/kg. This study has found that *Calotropis* is a metal loving (particularly Fe, Mn, Ni and Zn) plant and it can be used for the phytoremediation of metal contaminated soils. However very high concentration of above metals in the soil was ultimately found to stunt the growth of plant

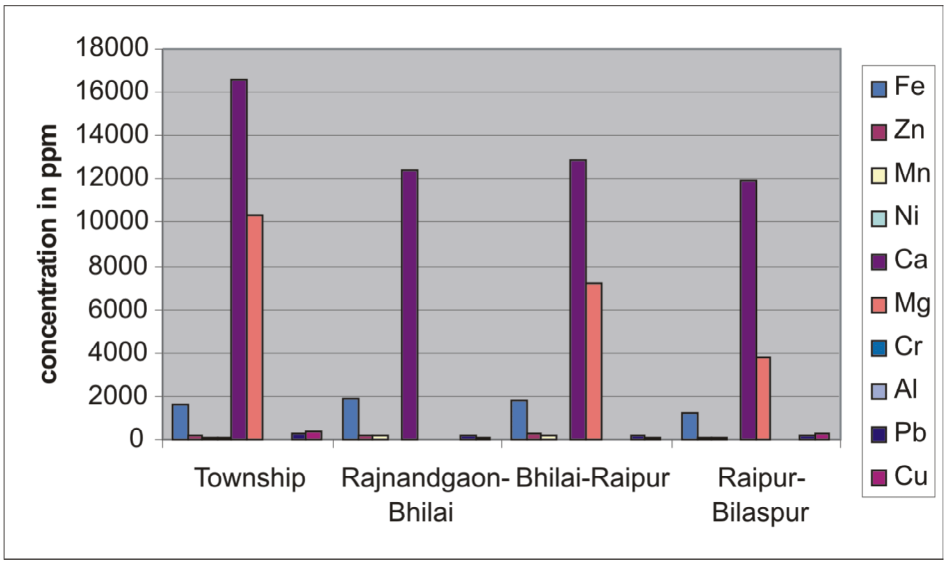

Figure 2: Mean concentration of elements in the flower of *Calotropis* grown at different locations.

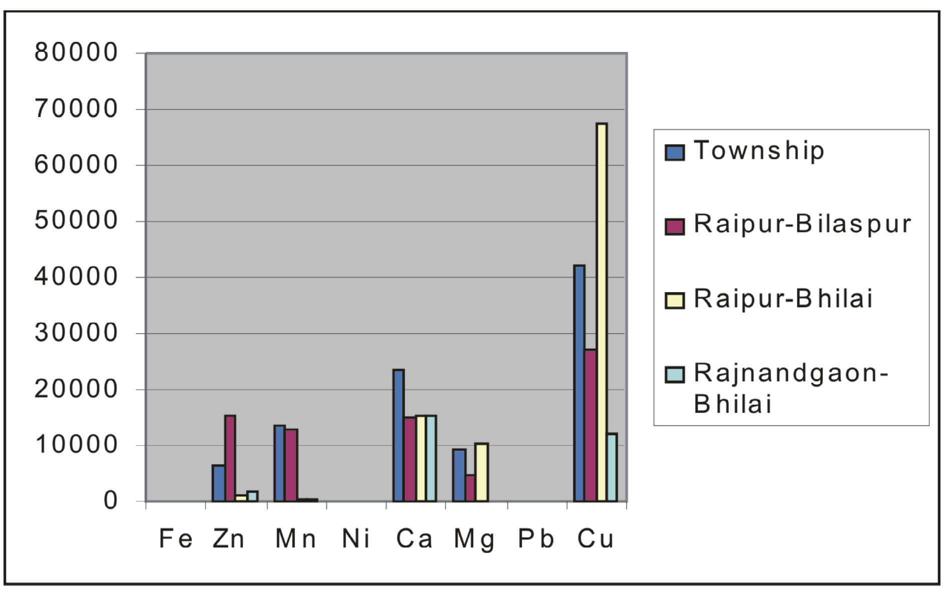

Figure 3: Enrichment factor of elements in the flowering parts.

Table 2: Mean concentration of elements in flower of *Calotropis* grown at different locations (mg/kg)

Location	*Fe*	*Zn*	*Mn*	*Ni*	*Ca*	*Mg*	*Cr*	*Al*	*Pb*	*Cu*
Township	1623	219.39	135.16	134.16	16565	10281	BDL	BDL	330	420
Rajnandgaon-Bhilai	1936.37	164.5	180	0.35	12390	BDL	BDL	BDL	170	121.5
Bhilai-Raipur	1829.6	241.34	191.2	0.66	12857	7160	BDL	BDL	180	67.5
Raipur-Bilaspur	1187.72	96.01	128.5	0.03	11953	3805	BDL	BDL	150	271

Table 3: Enrichment factor of elements in the flowering part of *Calotropis*

Element	*Township*	*Raipur-Bilaspur*	*Raipur-Bhilai*	*Rajnandgaon-Bhilai*
Fe	5.7792	3.4974	5.7107	7.2898
Zn	6268.38	15500.56	950.1574	1731.57
Mn	13516.67	12850.15	285.3731	272.7272
Ni	30.84	2.4539	79.6139	48.0272
Ca	23664.28	14941.25	15305.95	15487.5
Mg	9346.36	4756.25	10228.57	0
Pb	63.4615	47.619	48	70.8333
Cu	42000	27100	67500	12150
Gross enrichment	**94,895.77**	**75,201.78**	**94,403.38**	**29,767.95**

due to which *Calotropis* plant situated in contaminated area were found to have lesser plant height and spars foliage.

It has been reported that the plants which can accumulate about 100 mg/kg of their biomass are preferred candidate for phytoremediation. In our study *Calotropis* has shown to accumulate Fe, Mn, Ni and Zn of 100 mg/kg dry weight. It has also shown to significantly accumulate Pb and Cu.

Elemental Uptake and Enrichment Factor

Results in Table 2 show that the *Calotropis* possesses high metal accumulation capability which lies in the order Fe> Ni >Pb >Mg > Zn > Mn > Ca >Cu. Thus it can be seen that *Calotropis* hyper accumulates Cu in the maximum percentage and thus this plant may fall among the few Cu reported plants (K. Czarnowska et al., 1999; Ahmet Aksoy et al., 2000; Soraya et al., 2005). Mean concentration of elements in the flower of *Calotropis* grown at different locations is shown in Figure 2.

To find the extent of metal accumulation by this bioindicator plant, analysis of soil of the area was also carried out. Table 3 gives the average concentration of metals in soil in the various locations. Based on the above two estimations the enrichment calculations were carried out by the following formula:

Metal enrichment % = (concentration of element in biomass/concentration of that element in soil) × 100

Metal enrichment factor is shown in Figure 3.

Comparison of Trace Metal Level with Other Native Plants

If the bioindicator properties of *Calotropis* taken into consideration show distinctive element accumulation properties which corresponds to the level of metals in the soil and environment. Accordingly the *Calotropis* plants growing in township area and those growing along the Raipur-Bhilai road show highest gross enrichment of the studied elements (Table 2). Incidently the township and Raipur-Bhilai area is having high industrial activity where large number of metallurgical industries are located. The industrialization is less on the way to Raipur to Bilaspur and is minimum in Bhilai to Rajnandgaon region under consideration. We carried out analysis of a significantly large number of samples in the above four regions.

A very interesting feature in the *Calotropis* plant has been noted that it has shown no accumulation of Cr and Al. We are unable to offer any concrete explanation for the phenomenon at this moment. However it is clear that the periodic assessment of the accumulation of trace elements in urban areas in *Calotropis* can be very useful to evaluate the rate of environmental pollution. The *Calotropis* accumulated heavy metals in much higher concentrations. The elemental study obtained from flowers of the shrub was compared with leaves and stem of the shrub (Table 3). Flowers showed maximum efficiency of element accumulation. The results obtained from the calotropis was further compared with other native species like *Acacia nilotica* (Babool) and

Pongamia pinnata (Karanj). *Acacia nilotica* showed significant elemental uptake of Fe 225 mg/kg, Ca 150 mg/kg and Mg 50 mg/kg. The elemental uptake of *Pongamia pinnata* is Fe 749.5 mg/kg, Ca 450 mg/kg, Mg 150 mg/kg, Zn 104 mg/kg and Mn 35.5 mg/kg. It appears that *Calotropis* is more efficient than the other native species in uptake of elements. This attests to the bioaccumulation capacity of the *Calotropis*. Such a plant could also be used as 'active biomonitors' compared to other native species which may best be termed as 'passive biomonitors'.

The *Calotropis* plant possesses two major species that is *Calotropis procera* and *Calotropis gigantea*. However both the species are distinctly identifiable by the colour of their flowers, apart from that the biological variation is limited. The analysis of accumulated trace elemental level can be easily measured by instrument like AAS provided the digestion has been carried out properly. Inter species differences in the metal uptake capacities of the two species was not significant. This plant can also serve as a visual indicator because it was found that higher atmospheric pollution caused stunted growth of the plant, reduction in the leaf and foliage size, reduction in the succulence of the plant and flower body.

As far as the physiological mechanism of uptake of element is concerned it appears that *Calotropis* actively absorb these elements probably from both soil and atmosphere. This active uptake appears as a part of its defense mechanism. The presence of alkloids and tannins appears to be the medium by *Calotropis* which reduces the toxic effects of the accumulated trace elements. Thus *Calotropis* meets the various requirements of a suitable biomonitor. The plant can be used for both continuous monitoring and retrospective monitoring of elemental contamination of environment. The non-mobile and recalcitrant nature of the plant makes it as a very good candidate for biomonitoring.

Conclusion

Results show that atmospheric pollution level is responsible for the different elemental concentration in the *Calotropis* collected from different areas. Hence it can be the candidate plant for the biomonitoring of atmospheric pollution level. This plant can also serve as a visual indicator of pollution as higher atmospheric pollution has resulted the stunted growth, shorter leaves, less succulent and flower body. The periodic assessment of the accumulation of trace elements in *Calotropis* can be useful to evaluate the rate of environmental pollution, particularly in the urban areas.

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