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Full Length Research Paper

Heavy metal contamination in leaves of *Mangifera indica* around a coal fired thermal power plant in India

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The aim of this study is to evaluate the differences in the bioaccumulation of some elements in *Mangifera indica* tree leaves grown around a coal fired Thermal Power Plant (affected areas) and in other areas (control) 25 km away from Thermal Power Plant (TPP). Toxic group of metals (Pb, Cd and Cr) in an average displayed higher levels (28.0, 2.4 and 3.2 ppm for Pb, Cd and Cr respectively) in plants grown in affected sites with distinct temporal and spatial variation in comparison to samples in control areas (0.09 to 2.2 ppm for Pb, 0.01 to 1.1 ppm for Cd and 0.01 to 0.65 ppm for Cr). Such types of variations were not encountered significantly in cases of the essential metals (Cu, Zn, Ni and Fe). Strong positive correlation (P > 0.05) between the pairs of toxic metals in affected sites could be associated with their similar anthropogenic source. This resulted in higher contamination factor (CF > 6) of the toxic metals in plant leaves grown in the periphery of the TPP. The ash contamination from TPP might play an active role for this metal enrichment in plant leaves. The leaves of this plant could be classified as considerable degree of heavy metal pollution during the year and this might affect the productivity of this important commercial plant to a great extent. The winter months might represent the best sampling period for evaluation of the suitability of this plant as bio-indicator of metal pollution.

Key words: Bioaccumulation, degree of contamination, heavy metals, *Mangifera indica*, spatial and temporal variation.

INTRODUCTION

Thermal power plants produce large amount of fly ass with varying sizes of the ass particles. The finer sized components of ash ranging from 0.5 to 200 µm (Baba, 2002) is disposed off through the chimney into the air and the coarse grained fraction of ash collected from the bottom of the boiler (bottom ash) is disposed off in slurry form to the large ponds located in areas near the vast agricultural fields. The unmanaged landfills or ponds or illegal dumping in the territory may serve a potential source of ash contamination through leaching, wind blown or atmospheric deposition on the nearby soil and agricultural plants (Kim and Kim, 1998; Sharma et al., 2000). The ash contamination up to a certain extent may primarily improve the soil quality, as well as the growth and development of the plants due to presence of most essential micro and macronutrients (Elseewi et al., 1980; Chang et al., 1977). However an excessive ash contamination can cause potential damage to both the soil and the prevailing communities, solely due to higher degree of accumulation of elements present in ash (Singh and Yunus, 2000; Niess, 1999). The essential heavy metals play a vital role in many physiological processes in trace amounts; several of these ions are required for growth, metabolism and development (Shi and Sengupta, 1995; Singh and Yunus, 2000). The problem arise when cells are confronted with an elevated level of these vital ions (Adriano et al., 1978) or with non essential ions that lead to wide range of cellular damage through inactivation of bio-molecules by either blocking essential functional groups or by displacement of essential metal ions (Wood, 1974). In addition, fly ash dust under certain condition of humidity sticks to the leaves or fruits and promotes chemical, as well as

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Table 1. Location of sampling areas and nearby anthropogenic source.

Spot No.	Name of the sampling sites	Latitude	Longitude	Nearby anthropogenic source
1.	Rubia	$24^{0}\ 45^{/}\ 42^{//}$ N	87° $54^{/}22^{//}$ E	Illegal ADS
2.	Ballalpur	$24^{0}\ 44^{/}\ 45^{//}$ N	87° $54^{/}\ 08^{//}$ E	Illegal ADS
3.	Malancha	$24^{0}\ 40^{/}\ 28^{//}$ N	87° $55^{/}\ 00^{//}$ E	Presence of AP
4.	Nishindra	$24^{0}\ 47^{/}\ 45^{//}$ N	87° $54^{/}\ 08^{//}$ E	Presence of AP
5.	Ratanpur (control)	$24^{0}\ 40^{/}\ 19^{//}$ N	87° $56^{/}\ 41^{//}$ E	Far away from AP or illegal ADS
6.	Solomile (control)	$24^{0}\ 52^{/}\ 12^{//}$ N	87° $56^{/}\ 41^{//}$ E	Far away from AP or illegal ADS

physical injuries directly (Singh and Yunus, 2000). This affects not only the agriculture of entire region but also the humans by way of transfer of accumulated toxic metals through food crops and vegetables.

In this study area, most village people earn their livelihood through agricultural practices like horticulture, paddy, sugarcane, wheat, jute, turmeric, pulses and oil seeds. About 45% of the net area under cultivation is used for orchard cultivation of which, 70 to 75% is used for *Mangifera indica* plantation and the large fraction of people depend on the production of mango (local name of *M. indica*) fruits during four to five months in a year. The average annual production of mango is around 2000 metric tons per year; most is exported to other countries. These trees can take up trace metals from soil, water or air and retain them for long time. Accordingly, higher plants act as bio-monitors in the assessment of heavy metal contamination by means of their bio-accumulative properties and this concept gained wide acceptance (Sebastiaan et al., 2002). The concentration of different elements in the tree can vary due to large number of factors (Kabata-Pendias and Pendias, 1992). Among them seasonal variability is the most important and depends on the prevailing climatic factors in the area. Besides, certain kinds of contaminants, such as heavy metals occur naturally in the environment and it is important to be able to distinguish between anthropogenic contamination and natural level to enable accurate evaluation of the degree of contamination. Therefore, the use of tree as bio-indicator is the best option available when information about the bioavailability of contaminants is required. In the light of the above facts, some basic knowledge is needed before using any tree as bio-indicator of metal pollution of the study area. In previous study (Sengupta et al., 2010), we documented the degree of trace metal enrichment in agricultural topsoils around the TPP and we also analyzed the pollution levels in surface soils within this area.

Hence, the main objectives of this study were: (1) to monitor the concentration of seven heavy metals (Fe, Cu, Ni, Zn, Pb, Cd and Cr) in the foliar parts of the *M. indica* tree around the periphery of TPP (affected) and in control areas for comparison. (2) To investigate the trend of temporal and spatial variations of metal level, which may help to determine the optimal sampling time as well as space for evaluation of the suitability of this plant as bio-indicator of metal pollution. (3) To quantify the degree of heavy metal contamination in the study area with respect to control areas.

MATERIALS AND METHODS

Physiographic condition of the study area

The largest thermal power plant in eastern India under the umbrella of National Thermal Power Corporation (NTPC) is situated at Farakka West Bengal, India (within the longitude between 87°53'39E and 87°56'41E and latitude 24°40'19N and 24°52'12N) and is in operation since 1982. The physiographic conditions of the sampling locations are presented in Table 1. The climatic condition of the area is mainly dry in nature with maximum and minimum temperature 43 and 8°C respectively, where as the average rainfall is 1400 mm/year. The soil quality of this area is neutral in nature and contains low organic carbon (Sengupta et al., 2010).The seasons here are categorized as pre monsoon (May to June) with higher temperature and thunder storms, monsoon (July to October) with rainfall and post monsoon (November to February) with lower temperature and negligible rainfall.

Collection and processing of the samples

The collections of foliar components of the *M. indica* tree were done during the month of January, February, March, May, September and October in the year 2007. Five trees were chosen and marked for seasonal monitoring from each of the four identified locations situated within 5 km radius of the TPP, near to the ash ponds (AP) or ash dumping sites (ADS). These locations were assumed to be largely affected by the discharge of ash particles and designated as the affected sites. In a similar way two background samples were also collected simultaneously from a distance of more than 25 km and were considered as control sites which are minimally affected by the fly ash disposal. The sampling strategy and number of foliage samples to be collected in a location were according to the procedure as recommended by UN/ECE-EC (1998). In order to optimize sampling consistency, an attempt has always been made to reduce the variability as far as possible. The collection of shaded and infected leaves were avoided and instead only the mature leaves with greater aerial exposure from about 4.5 m height of the tree in a particular location were selected and collected by hand picking. In all locations samplings were done at similar time of the year and from the same plant species and were kept in plastic container. Samples were first thoroughly washed with single distilled water and later with 0.2 M HCl and Millipore water. The leaves were then air dried, chopped and further dried in oven for

24 h at 80°C. The dried samples were ground finally in a mortar and passed through a 500 µm stainless-steel sieve.

Chemical analysis

About 0.5 to 1.0 g of the powder was taken and carefully digested with 5:1 (v/v) mixture of concentrated HNO_3 and $HClO_4$. The digestion was carried out on a hot plate at 80°C in digestion chamber. The solution obtained after digestion was analyzed for Lead (Pb), Cadmium (Cd), Chromium (Cr), Cupper (Cu), Zinc (Zn), Nickel (Ni) and Iron (Fe) by atomic absorption spectrophotometry (AAS, Model: Varian spectrAA 55). A sample blank was prepared following the same sequence of digestion procedure and the element analysis was performed against the blank. AAS settings were those recommended by the manufacturer. A standard curve was run with each analysis. All chemicals used in the study were obtained from Merck and were of analytical grade. Quality control was carried out by parallel analysis of certified reference materials. The results were expressed as ppm of dry weight of leaves.

Contamination factor (CF) has been calculated to obtain the degree of heavy metal accumulation in plants growing in affected areas with respect to the plant growing at unaffected (control) sites:

CF = Metal concentration in plant leaves at affected sites / Metal concentration plant leaves at control sites.

Statistical analysis

The normality of the trace metal distribution was tested for each group by the Kolmogorov-Smirnov test. We did not transform the variables logarithmically for normal distribution. Due to large heterogeneity of variance, we used non-parametric tests: Mann-Whitney test was used to compare means, between the concentration of trace elements in affected leaves and these in control sites, with differences being considered significant at $P < 0.05$; Kruskal-Wallis test was used for analysis of temporal variation. Correlation analysis was carried out using the non-parametric Spearman rank coefficient correlation procedure with minimum significance level of $P < 0.05$ being accepted. All these statistical analysis were carried out using SPSS-11 statistical package for windows.

RESULTS AND DISCUSSION

Distribution of metals in leaves

The average levels of essential metals in leaves were 5.0, 14.3, 35.5 and 8.0 ppm of Fe, Cu, Zn and Ni respectively and the toxic metals of Pb, Cd and Cr were of 28.0, 2.4, and 3.2 ppm (Figure 1). The degree of bio-accumulation of Pb is considerably higher than those of Cd (12 fold) and Cr (9 fold) with respect to control area. However, plants in control areas contained considerably lower amount of these toxic metals ranging from 0.09 to 2.22 ppm for Pb, 0.01 to 1.11 ppm of Cd and 0.01 to 0.65 ppm of Cr with the mean of 2.03, 0.39 and 0.28 ppm respectively. This indicated that, the plants in control area were also not devoid of toxic metal exposure and accumulation but may thus be considered as a natural level. The accumulation of Cu in leaves from the control and in affected sites was almost equivalent in level compared with the range considered as “normal” 6 to 14 ppm (Bowen, 1979). Relatively lower uptake of Cd, Ni and Cr could be due to antagonistic interactions with other elements, which can reduce their uptake from both the root and foliar system (Kabata- Pendias and Pendias, 1992). The concentration of Pb, Cd and Cr reached maximum values in pre monsoon (March to May) period (35, 4.5 and 5.5 ppm for Pb, Cd and Cr respectively). These high values were above the limits of phytotoxicity as reported by Kabata-Pendias and Pendias (1992) for Pb (30 ppm) and Cr (0.2 ppm) for natural concentration in plants (Table 2). Iron accumulation in the leaves was very high and the precise definition of the critical toxicity concentration for Fe is difficult, as the relationship between the proportion of Fe (III), which precipitates preferentially in the apoplast, and the highly toxic Fe^{2+}, which freely circulates in the cytoplasm and cell organelles, is not known (Romheld and Marschnner, 1991). The critical level of Cu and Zn toxicity are around 20 to 30 and 100 to 120 ppm of dry tissues respectively (Vangrosveldt, and Clijster, 1994; and Cakmak, 2000). However, expressed as mean values, all of these metal concentrations were within the critical level of toxicity.

The most variable metal concentrations, according to coefficient of variation (CV) were those of Pb, Cd, Cr and Fe in both analyzed areas. Usually, environmental variability of the metals arises from the acute increase in their concentration relative to background level due to contamination from anthropogenic sources and the plants have the capacity to regulate them through translocation into different organs depending on the extent of contamination. In this study, the variability of the essential metal was found well controlled by this plant in both areas. In contrast the mean values of most of the toxic metals were always higher than their median values indicating skew to the right in the distribution, which is often found in the pollution studies (Villares et al., 2003). However the normality study on the metal distribution highlighted that the higher CVs of the metals were not always associated with normal distribution pattern, as found in case of Cd and Ni in affected areas and Cr in control areas, where high CVs were encountered with normal distribution. This indicates that, environmental variability in a symmetric pattern may lead to normal frequency distribution of the elements. Thus the metals, Pb, Cd, Cr and Fe in leaves were largely associated with anthropogenic sources not only in affected areas but also in control areas. In fact heavy metals occurred naturally in the environment and to find truly pristine area is scarce or even non-existent. Furthermore, some contaminants are easily transported and can be found in areas far from the point of emission and thus the degree of contamination that was observed in control areas has been taken into account as due to natural variability.

Spatial and temporal variation

The distribution pattern of the metals in affected sites

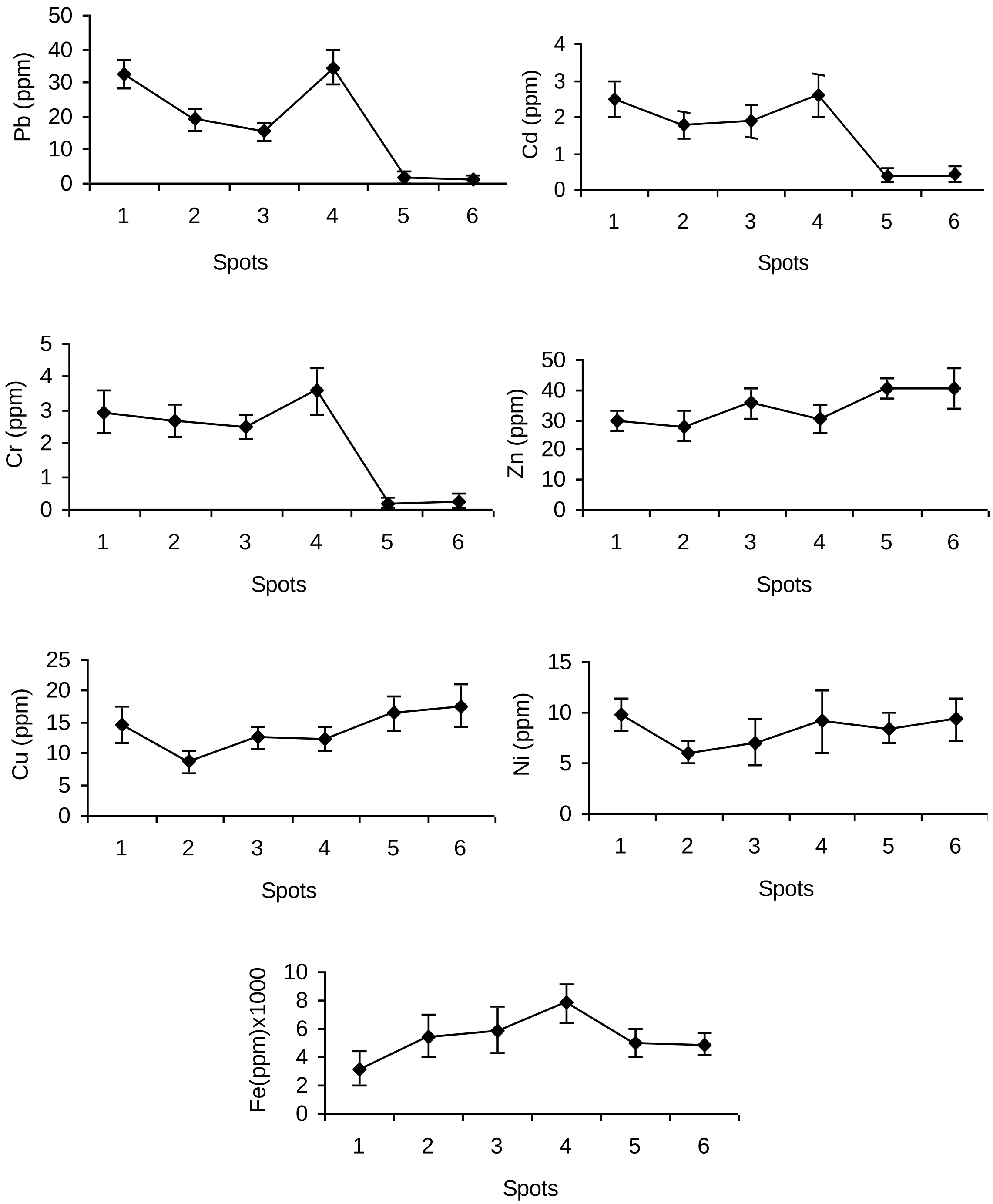

Figure 1. Spatial variation of heavy metals in *M. indica* leaves. This figure reveals the distribution of heavy metal at seven different sites (Spot 1, 2, 3,4,5,6 and 7). The data has been presented as the mean of five different observations with the standard error bar.

comprising of locations 1 to 4 (Figures 1 and 2), was almost similar without any significant differences in levels. The locations, 5 and 6 at control sites also showed close similarity in metal level between these two stations (Figure 1). As a consequence, the metal levels in all locations of affected and control sites were averaged separately. This may represent well the metallic burden of the respective areas. Therefore, the average values in both sites were mutually compared (Mann Whitney U test). It has been shown that, only the level of toxic group of metals were significantly different (Table 3) at 95% confidence level indicating efficient enrichment of them in leaves grown in affected sites.

The results of monthly analysis of the mean level of

Table 2. Some heavy metals with their natural concentration in plants and adequate concentration for normal growth as well as their toxic effects.

Metals	Natural concentration in plants (ppm) Paias (1991)(missing)	Essential or non essential	Concentration for normal growth (M mole/Kg) Hopkins (1999)	Potential toxicity to plants Adriano (1991)
Cd	-	No	-	Toxic
Cr	0.2	No	-	Carcinogenic
Pb	-	No	-	Cumulative Poison
Cu	14	Yes	0.1	Relatively nontoxic
Zn	100	Yes	0.3	Relatively nontoxic
Ni	3	Yes	0.05	Relatively nontoxic
Fe	200	Yes	-	Relatively nontoxic

Table 3. Differences between concentrations of metals in leaves of affected and control sites using Mann-Whitney U test.

	Pb	Cd	Cr	Cu	Zn	Ni	Fe
U values	0.00	1.00	0.00	10.00	11.00	17.00	18.00
Significance	0.002**	0.004**	0.002**	0.240	0.340	0.937	1.000
d. f.	5	5	5	5	5	5	5

**P < 0.01; *P < 0.05.

metals (Figure 2) revealed that the values increased from starting sampling in January with a peak reached in May. The concentration then decreased during September with minimum values being found in October. The variation of metal content in two successive months was sometimes very large, for example, the level of Fe increased by two times between February and March. The pattern of temporal variation was to a large extent similar in all metals at both areas. This similarity in changes of the different elements is also reflected by their strong correlations (Table 5). Nevertheless, in control areas only Cd displayed strong positive correlation between Cr, Zn and Fe. This clearly indicates that most of the elements are originated from a single source in affected sites. Similarly, the degree of variation of the mean levels of monthly samples were not same and significant statistical differences (Kruskal Wallis Test) were observed (Table 4) only for toxic metals (Pb, Cd and Cr) in tissues of affected sites, while on control areas, although the level varied through the months, these variations were not significant at all in any of the metals, highlighting insignificant enrichment of the accumulated metals.

The intensity of seasonal changes of bioaccumulation is reported to relate to the amount of available metals present in the external environment (Greger, 1999). Metals from both natural and pollutant sources have the potential for being assimilated by the plant through foliar or root absorption processes and the bioaccumulation of trace metals in the leaves is the combined result of these uptake processes. The root absorption processes are no doubt a long- term process due to great deal of complication by the mediating effect of soil properties and soil and plant processes (Cataldo and Wildung, 1978; Jones, 1991). This process is characterized by higher degree of relative accumulation in root or lower stem as the most toxic metal are not easily mobile within the plant. Therefore, enriched level of metals in leaves may not be normally expected from this process. Indeed, the distinction between the proportions of elements taken in after airborne deposition and from those translocated via the roots from the soil is difficult to ascertain (Bargagli, 1998). In this study, we have not analyzed the metal levels in lower parts of the tree and therefore we could not exactly highlight that the leaf metal accumulation is originated either from root or foliar absorption. Instead, we shall attempt to give impetus on the easy accessible foliar absorption process on the basis of some important clues. The soil pH in these areas was found alkaline in range, which is known to restrict the availability of metals to the root zone. As a result, higher degree of monthly increment of metal level in leaves may not be quenched by the supply of root uptake processes alone especially during dry period of March and May. Besides these trees are deep rooted and the metals contaminated in topsoil are to face lot of hindrance to reach to the root zone.

On the other hand, the degree of seasonal changes of climatic conditions (Bache et al., 1991; Harrison and Chirgawi, 1989) in the study area could play vital role in atmospheric depositions on leaves and may serve as the potential source of bioaccumulation. Generally, after the

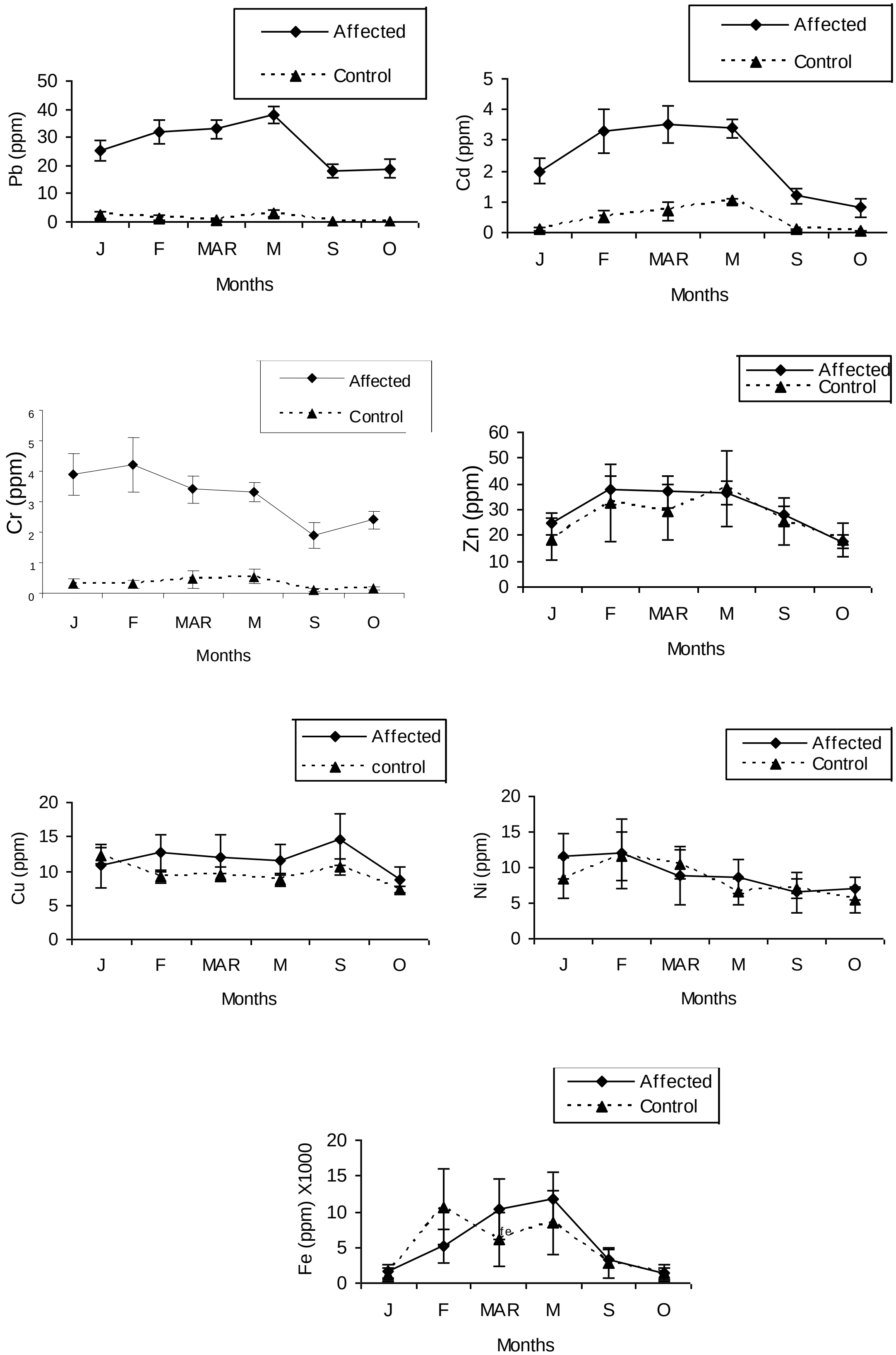

Figure 2. Temporal variation of heavy metals in *M. indica* leaves. The mean value of five different observations has been cited with the standard error. The figure also depicts the degree of heavy metal contamination in the affected areas in the light of control data. J,F, MAR, M, S and O in the X-axis are standing for January, February, March, May, September, October respectively.

Table 4. Kruskal-Wallis test of differences between mean concentration of metals in monthly samples in affected and control sites.

Variable		Pb	Cd	Cr	Cu	Zn	Ni	Fe
Affected sites	Chi-square	10.74*	15.10**	13.94**	4.69	9.46	7.47	16.37**
	d. f.	5	5	5	5	5	5	5
Control sites	Chi-square	5.18	8.77	4.64	8.63	8.27	8.12	8.31
	d. f.	5	5	5	5	5	5	5

**P < 0.01; *P < 0.05.

Table 5. Spearman rank correlation co efficient of the metals in affected areas and control areas.

Affected sites	Pb	Cd	Cr	Cu	Zn	Ni	Fe
Pb	1						
Cd	0.646*	1					
Cr	0.511*	0.445*	1				
Cu	0.027	0.284	-0.327	1			
Zn	0.130	0.521	0.482*	0.243	1		
Ni	0.607*	0.755**	0.370	0.339	0.498*	1	
Fe	0.252	0.498	0.450*	-0.059	0.592*	0.134	1
Control sites							
Pb	1						
Cd	-0.123	1					
Cr	0.470	0.456	1				
Cu	-0.475	0.340	0.091	1			
Zn	-0.036	0.755	0.314	0.497	1		
Ni	-0.374	0.382	0.273	0.214	0.391	1	
Fe	-0.114	0.655*	0.232	0.510	0.864	0.564**	1

**P < 0.01; *P < 0.05.

monsoon (July to October), winter prevails till January and is characterized by lower temperature (8 to 14°C) with nil rainfall. After then, air temperature increases gradually from February and attains maximum values (39°C) in May during summer season and makes complete dryness of the area, even also the surrounding AP and ADS. Besides, this is the time when southern winds start blowing together with occasional storms known locally as Kalbaisakhi (Nor'Wester) accompanied with huge amount of aerial particles in suspensions, what may act as a source of higher degree of foliar deposition on the trees in the region. The deposition remains for a long time until the rain-washes it out during next monsoon, thereby, increasing the retention time of the deposited materials and facilitates the enhanced level of bioaccumulation of the elements. Hence the seasonal variation of metal levels in leaves in this study may be attributed to higher concentration during dry period at favorable condition of foliar deposition and lower in monsoon due to removal of surface deposition by precipitation. Consequently foliar deposition process is likely to be more pronounced during this study.

The toxic groups of metals in leaves of affected areas are well differentiated by their distinct seasonal variation from essential heavy metals and also showed spatial variation in relation to control sites. Consequently the *M. indica* leaves do possess some intrinsic property of showing higher degree of bioaccumulation of toxic metals (Streit and Stum, 1993: Yadav et al., 2005) without affecting the level of other essential metals appreciably. This may happen only when the plants after the metal accumulation from foliar route have the capacity to transfer the nutritional elements towards the lower organs restricting their considerable enrichment in leaves. There are several studies which highlighted that the distribution of essential metal in plants to a large extent is metabolically controlled (Cataldo and Wildung, 1978) and that the metal levels are evenly distributed throughout the all organs of the plant. This resulted in lack of significant seasonal, as well as spatial variation of the essential

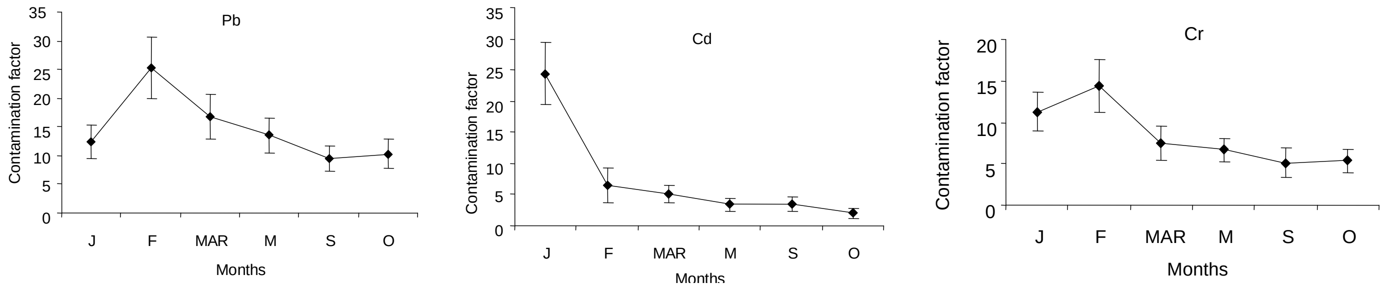

Figure 3. Temporal variation of the degree of contamination of heavy metals in *M. indica* leaves. Pb, Cr and Cd show significant level of contamination in comparison with the control sample. Therefore, the data illustrated the degree of contamination of all those metals with their mean values and standard errors.

metal levels during the present study area in both affected and control sites. On the other hand, the toxic metals due to their negative role in plant physiology may be preferentially partitioned by way of sequestration with organic molecules in the foliar component, which may prevent their mobility and translocation of ions in the plant system. These peculiarities of the toxic metals could be responsible for sharp spatial and temporal variation in bioaccumulation within the study area and due to this, the foliar component of the mango trees may serve as efficient bio-indicator of toxic metals of Pb, Cd and Cr.

Degree of contamination

The level of contamination in mango leaves grown in affected sites is calculated in forms of contamination factor (CF), defined as:

CF = Concentration of metals in plant parts / Concentration of metals in control areas.

The CF reflects the degree of contamination by a particular element in a sample. CF < 1 indicates that, the content of element is of natural origin and there is no contamination. While CF >1 indicates element enrichment. In order to take into consideration the natural variability that exists within the ecosystems and to overcome the possible analytical error, a sample should not be classified as contaminated unless CF > 2. For this, we classified the sampling according to CF, using the following categories of contamination (Hakanson, 1980):

CF < 1 Low contamination, 1 ≤ CF < 3 Moderate contamination, 3 ≤ CF < 6 Considerable contamination, CF ≥ 6 Very high contamination

In the present study, the CF values for the elements that are important in the metabolism, such as Cu, Zn, Ni and Fe were less than 1, indicating that there is less accumulation of these elements in the leaves of the affected sites. However, for elements typically regarded as contaminants such as Pb, Cd and Cr showed higher values revealing considerable enrichment of these metals (Figure 3). CF values also showed a sharp seasonal variation with higher values during January to February and lower values in September to October for all toxic metals, while bioaccumulation of metals showed high peak values during March to May. Hence, higher degree of bioaccumulation does not exactly correspond to the higher CF values. This arises solely due to the facts that, the metal accumulation of leaves in control areas also undergoes seasonal variation with comparatively higher accumulation during March to May, what decreases the ratio (CF) values of the metals in the relevant months. The classification of the degrees of contamination from Figure 3 showed that in an average the toxic metal Pb and Cr all the times of the year showed high degree of pollution as the CF values were always higher than 6 with the intensity more pronounced during winter season (January to February). On the other hand, Cd showed moderate to considerable degree of pollution in other months excepting in January. Accordingly, winter season could be recognized as the most suitable time for the collection of leave samples in order to select the plant as bio-indicator of metal pollution in this area.

Conclusions

The level of metals and the mode of distribution of the studied metals in mango tree grown in the periphery of TPP were not alike. In affected sites the leaves showed considerable accumulation of Pb, Cd and Cr in comparison to control areas. The seasonal changes in levels of metals of these toxic elements followed a similar pattern in affected sites with minimum values being found in monsoon and post monsoon season and maximum values in pre monsoon. This is apparently caused by the foliar deposition of fly ash on leaves during dry period, which were being washed out in pre monsoon period due to rainfall.

The large variability in metal content found in the mango tree leaves in consecutive months must be taken into account when designing bio-monitoring program because different conclusions can be reached when using even slightly different sampling times. These results also demonstrated how complicated it is to compare data from studies carried out at different times of the year. Contamination factors calculated on the basis of control areas showed a distinct seasonal variation with comparatively higher values in January to February and lower values during September to October, but all the seasons CF values were higher indicating higher degree of contamination in leaves of *M. indica* due to anthropogenic stress.

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