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Bioaccumulation of Heavy Metals in Plants near Mining and Non-Mining Areas

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Abstract: Plants take heavy metals from soil through different reactions. In the present study, heavy metals concentration in leaves and soil of few common plants like Bougainvillea glabra, Delonix regia and Azadirachta indica in mining and non-mining area has been determined. Effect of air pollution and metal content in soil on metal uptake by plant leaves has been done. Metals are present at higher concentration level in soil and leaves of mining area as compared to non-mining area. Metals are present well within the normal range in leaves of the three plants of non-mining area. Cr concentration in leaves of plants in mining area has been found above the normal range. Cu and Pb concentration in leaves of Gulmohar and Neem has also been found above the normal range in mining area. But the high concentration of metals did not show any symptoms of phytotoxicity in plants of mining area.

Key words: Phytotoxicity, soil contamination, metal uptake.

Introduction

Heavy metals are natural components of earth crust. The most common heavy metal contaminants are Cd, Cr, Cu, Hg, Pb and Zn. Some of these metals are micronutrient necessary for plant growth such as Zn, Cu, Mn, Ni and Co. However at higher concentration they can lead to poisoning or toxicity while other heavy metals such as Cd, Co, Hg and Pb cannot be biodegraded but can be accumulated in plants (Selim and Amacher, 1996). Bioaccumulation of metals in any plant is a function of the soil concentration, solubility and mobility of the metal within the plants. The high concentration of heavy metals in soil is reflected by higher concentration of metals in plant and consequently in animal and human bodies (Buszewski et al., 2000). Excess concentration of heavy metals in soil increases due to the application of fertilizers, mining activities and sewage sludge that exert toxic effect on plants (Marschner, 1995). Coal mine waste usually contains more than one metal and some of these might occur at toxic concentration (Deo, 1992). In coal mine waste, most of the plants grow well but some species show abnormal growth because of nutrient-deficiency and presence of heavy metals (Deo, 2005). The heavy metals like Cu, Pb, Cd, Cr and Mn are common anthropogenic pollutants that are frequently investigated in studies analyzing the metal content of soil and plant tissue (Kabata-Pendias and Pendias, 1992). Heavy metals effect on plant result in growth inhibition, structural damage, a decline of physiological and biochemical activities. Plants have their own resistance mechanism against toxic effects and for detoxifying heavy metal pollution (Cheng, 2003).

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Amount of Cu available to plant varies widely by soils. Available Cu can vary from 1 to 100 mg/kg in uncontaminated soil (Keane et al., 2001). Cu is one essential micronutrient for plant growth. Normal range of Cu in between 7.53 and 8.44 mg/kg is required by plant (Kabata-Pendias and Pendias, 1992). It is involved in numerous physiological functions as a component of several enzymes, mainly those which participate in electron flow, catalyze redox reaction in mitochondria and chloroplast (Hansch and Mendel, 2009). Cu also plays an important role in photosynthesis-related plastocyanin and membrane structure (Li and Xiong, 2004). Critical range is 25-90 mg/kg of Cu in plant (Kabata-Pendias and Pendias, 1992). Especially excess Cu can cause chlorosis, inhibition of root growth and damage to plasma membrane permeability, leading to ion leakage (Upadhyay and Panda, 2009). Due to high concentration of Cu, the root and shoot-elongation has been poor and the contaminant decreases in root and shoot length (Bouazizi et al., 2010).

Pb is not an essential nutrient for plant and it is one of the hazardous heavy metal pollutants of the environment that originate from various sources like mining and smelting activities, burning of coal etc. (Eick et al., 1999). Normal range of Pb in plant is 0.2-20 mg/kg and 30-300 mg/kg is critical range (Kabata-Pendias and Pendias, 1992). Response of plant to Pb exposure includes decrease in root elongation and biomass, accelerate leaf senescence, inhibition of chlorophyll biosynthesis, inhibition of seed germination, a wide range of adverse effect on growth and metabolism of plant.

Cadmium concentration in field grown crops is usually negatively related to soil pH. The uptake of cadmium in plants increases with increasing pH from 5.0 to 7.0, but its uptake decreases due to the acidification i.e. due to high concentration of hydrogen ion in soil (Hatch et al., 1988). Cadmium in soil readily attaches to clay particles and organic matter, making it less available for uptake by plants. Cadmium adversely affects the absorption and transport of essential elements, disturbs the metabolism and has an impact on growth and reproduction in plants.

Chromium is the 21st most abundant element and it occurs in nature in bound forms that constitute 0.1-0.3 mg/kg of the Earth's crust (Zayed and Terry, 2003). Chromium is bound very strongly by soil particles, especially by soil clays and organic matter. They are most strongly bound in near-neutral soils (pH 6–8) and become more soluble in acidic soils (pH less than 5) and highly alkaline soil (pH more than 9). The background level of chromium in soil is 3-200 mg/kg; above this level chromium is considered to be toxic and the soil is contaminated by high chromium concentration (Keane et al., 2001). It causes leaf chlorosis and necrosis in plants (Cervantes et al., 2001).

Hue et al. (2001) stated that soils with "high" Mn levels became phytotoxic at pH less than 6.0. Manganese is not toxic at background level of 7-3000 mg/kg but it becomes toxic beyond 3000 mg/kg in soil (Keane et al., 2001). Although manganese is an essential plant nutrient, excessive quantities of manganese may be detrimental to plant growth. Manganese with particular higher concentrations shows the symptoms of toxicity such as appearance of brown spots, chlorosis and necrosis of leaves (Demirevska et al., 2004).

The aim of present study is to determine the heavy metals concentration in leaves of few common plants like Bougainvillea *glabra* (Paper flower), *Delonix regia* (Yellow gulmohar) and *Azadirachita indica* (Neem) collected from Jharia coalfields and compared with the plants grown in non-mining area (C.I.M.F.R. campus) and study the effect of environment like air pollution and soil on metal contamination in leaves of three plants.

Methodology

Study Site

The study site Jharia Coalfields is the most important coalfield in India, located in Dhanbad district. It is located within latitude 23° 39' to 23° 48' north and longitude 86° 11' to 86° 27' east. The mining activities in these coalfields started in 1894 and had really intensified in 1925. The climate of the area under study is subtropical which is characterized by hot summer from March to May and well distributed rainfall during south-west monsoon from June to September. The annual rainfall is approximately 1306 mm. The study has been conducted in the month of May, 2012. Sampling has been done from the Kusunda and Dhansar area no. 6 of Jharia coalfields and C.I.M.F.R. campus as a non-mining area.

Selection of Plant

Two plants, *Azadirachta indica* (Neem) and *Delonix regia* (Yellow Gulmohar) have been selected from Dhansar area which is found at a distance of about 150-200 m and 400-450 m respectively from the mining site. Another shrub *Bougainvillea glabra* (Paper Flower) has been selected from Kusunda area which is found at a distance of about 400 m from the mining site. The same plants have also been selected from less polluted non-mining C.I.M.F.R. campus. Above mentioned plants have been chosen because their leaves could be easily digested and these plants are more common and also easily found in mining area.

Soil Sampling

Soil samples have been collected at a depth of 2-10 cm from three different places, near the selected plant. The representative soil sample has been prepared by Quoni-Quartering in the laboratory. Thus obtained soil has been powdered by using mortar and pestle and then left overnight for bench drying in laboratory. After bench drying, sample has been sieved by using 72 BSS sieve. The powdered samples have been in -72 BSS sieve size, kept in Petri dishes for further processing.

Leaf Sampling

Leaves have been collected from the selected plants by removing several leaves from the stem and kept in the plastic container and labelled properly. Then brought to the laboratory and washed with deionized water thoroughly for 3-4 times, followed by distilled water. The samples have been properly dried in sunlight for a whole day followed by the oven drying at 75°C for 48 hours. Then homogenized by mortar and pestle and were sieved with 40 BSS sieve. Powdered samples in –40 BSS sieve size were kept in plastic container for metal analysis.

Analysis of Soil Samples

The analysis of pH, moisture content and loss of ignition in soil samples has been done by using standard method (Arnold et al., 1992). To determine heavy metal content in soil, 2 gm of dried soil from each sample has been taken in a beaker and 25 ml of 6N HNO_3 has been added and kept for overnight. Single acid digestion has been done on a hot plate at 70-80°C for 4-5 hours to extract total soil metal, cooled, filtered and analyzed for determination of metal by Atomic Absorption Spectrometer (Arnold et al., 1992).

Analysis of Leaf Samples

Five gram of each powdered leaf tissue has been ashed in a muffle furnace, initially at 450°C for 30 minutes and then at 850°C for one hour. The resulting ash has been dissolved in 25 ml of 6N HNO_3 and heated at low temperature at about 70°C for whole day on a hot plate to extract total leaf metals. Sample has been cooled and filtered into a 50 ml volumetric flask with distilled water. After dilution, samples have been kept for metal analysis by AAS.

Metal Analysis

Determination of the concentration of Cu, Pb, Cd, Cr and Mn in leaf and soil extracts has been carried out by flame Atomic Absorption Spectroscopy using a 'Thermo Scientific M series' Atomic Absorption Spectrometer. This instrument has been calibrated using standard solution containing known concentration of each metal (Cu 1 to 5 ppm, Cr 2 to 10 ppm, Cd 0.2 to 0.8 ppm, Mn 1 to 4 ppm and Pb 2.5 to 10 ppm). Standard solution has been prepared by diluting 1000 ppm of high purity stock solution (Merck standard solution) of each metal in acidic water of pH <2. These external standard solutions have been used to generate calibration curves for assessing the concentration of each metal in the soil and leaf sample. Analysis has been done by using respective wavelength of Cu at 324.7 nm, Cr at 357.9 nm, Mn at 279.8 nm, Pb at 217 nm and Cd at 228.8 nm. Data quality has been assessed by preparing and analyzing a replica of every soil and leaf samples and by periodically reading the standard solution during analysis.

Results and Discussion

Analysis of Jharia coal mine soil and CIMFR soil as non-mining of three plants that is *Bougainvillea glabra* (Paper Flower), *Delonix regia* (yellow Gulmohar) and *Azadirachita indica* (Neem) for pH, organic carbon and moisture content has been shown in Table 1. The CIMFR soil is slightly acidic than coal mine soil. In mining area some morphological abnormalities in *Delonix regia* (yellow Gulmohar) and *Azadirachita*

Table 1: pH, moisture content and loss on ignition of soil in mining and non-mining areas

Parameters	*Mining area*			*Non-mining area*		
	Paper flower	*Yellow gulmohar*	*Neem*	*Paper flower*	*Yellow gulmohar*	*Neem*
pH	6.77	7.44	7.53	5.68	6.33	6.45
% moisture	1.00	0.02	1.42	1.01	1.49	1.11
% LOI	18.02	39.80	32.73	12.13	17.69	11.13

indica (Neem)—small sized leaves, less leaf area and less in number—have been observed. Less developed and stunted growth of plant in *Bougainvillea glabra* has also been observed. Blackish layer of coal dust on leaf surface of coal mine area plant and black colour of coal mine soil sample has also been observed. This has been due to continuous coal mining activities in the area.

In present study, attempts have been made to compare the five heavy metals content in soil of three selected plants of mining and non-mining area (Table 2) and metal accumulated in corresponding leaves (Table 3). Various interaction of heavy metals between plants and soil has been studied (James and Bartlett, 1984).

The data of figures 1, 2, 3, 4 and 5 show the relationship among five heavy metals Cu, Pb, Cd, Cr and Mn respectively drawn with a view to study the heavy metal content of soil and accumulation in leaves of three selected plants species. Transport and accumulation of heavy metals in different plant species have been reported earlier in other mine spoils (Haritonidis and Malea, 1995).

Copper Concentration in Soil and Leaves

Maximum concentration has been found in mining *Delonix regia* (Yellow Gulmohar) soil whereas minimum concentration in non-mining Neem soil. Soil of all plants of mining area has more concentration of Cu than soil of non-mining area. This may be due to more pollution found near mining area. Normal range of Cu concentration is 1-100 mg/kg in uncontaminated soil, (Keane et al., 2001). From Table 2 and Figure 1, it has been observed that the entire soil sample of mining and non-mining area has less than 100 mg/kg of Cu concentration.

Morphologically less leaf area and less number of leaves has been found in mining area rather than plants of non-mining area. This may be due to high concentration of Cu in mining area than non-mining. The reduction of leaf area due to Cu has also been observed by others (Zheng et al., 2004). Maximum uptake of Cu has been found in leaf of *Delonix regia* of mining area, may be due to presence of maximum concentration of Cu in corresponding soil (Table 3, Figure 1). This was previously reported that high concentration of heavy metals in soil is reflected by higher concentration of metal in plants (Deo, 1992). On comparing the uptake of Cu in all plants, Cu uptake is more in *Bougainvillea glabra* (Paper flower) leaves, may be due to the reason that bougainvillea is shrub. This view has been supported by the previous observations that concentration of Cu was maximum in leafy parts of tree and shrub sample (Deo, 2004). Cu content found in leaves of all plants is not phytotoxic because Cu concentrations are in normal range i.e 7.53-8.44 mg/kg in leaves (Kabata-Pendias and Pendias, 1992). Only leaves of *Delonix regia* (Yellow Gulmohar) of mining area had slightly more concentration of copper.

Lead Concentration in Soil and Leaves

Maximum Pb concentration has been found in mining *Azadirachita indica* (Neem) soil whereas minimum concentration in non-mining bougainvillea soil (Table 2,

Table 2: Heavy metals concentration (mg/kg) in soil of mining and non-mining areas

	Mining area			*Non-mining area*		
Metal analysed	*Paper flower*	*Yellow gulmohar*	*Neem*	*Paper flower*	*Yellow gulmohar*	*Neem*
Cu	30.50	72.00	49.00	14.50	23.00	10.75
Pb	61.74	155.92	229.52	37.45	50.20	65.13
Cd	0.92	1.64	7.33	0.77	1.00	0.81
Cr	51.03	181.06	33.76	20.52	23.18	17.26
Mn	705.43	988.31	621.96	636.84	612.93	566.59

Table 3: Metals concentration (mg/kg) in plant leaves of mining and non-mining areas

	Mining area			*Non-mining area*		
Metal analysed	*Paper flower*	*Yellow gulmohar*	*Neem*	*Paper flower*	*Yellow gulmohar*	*Neem*
Cu	6.50	9.30	7.50	4.50	8.30	6.50
Pb	13.08	15.13	25.09	7.00	8.88	17.24
Cd	0.56	0.57	0.41	0.44	0.59	0.45
Cr	9.50	7.09	8.20	4.97	2.98	2.68
Mn	467.64	307.92	27.14	224.19	136.72	42.943

Figure 2). All soil samples of mining area have greater concentration of Pb than non-mining area. This may be due to the coal mining activities, burning of coal or coal dust (Eick et al., 1999). All the soil of non-mining area and *bougainvillea* soil of mining area have been found as uncontaminated because its concentration is about 10-70 mg/kg which is in uncontamination range of soil (Keane et al., 2001). But in mining area *Delonix regia* (Yellow Gulmohar) and *Azadirachita indica* (Neem) soil has above uncontaminated range, so it has been referred as contaminated soil with respect to Pb. Maximum uptake of Pb has been found in *Azadirachita indica* (Neem) leaf of mining area (Table 3, Figure 2). This may be due to maximum concentration of Pb in soil. This has been supported by previous report that the high concentration of heavy metal in soil is reflected by higher metal in plant (Deo, 1992).

Minimum uptake of Pb has been found in bougainvillea leaf of non-mining area. All the plant leaves of mining area have greater concentration of Pb than mining area. On the basis of morphological observation, less in number and small size of leaf in mining area plant has been observed. It may be due to greater concentration of Pb uptake by mining area plant. Excess Pb concentration interferes with nutrient uptake, influence the net photosynthesis rate and respiration and alternate permeability of cell membrane (Sharma and Dubey, 2005). Pb content found at non-toxic level in leaves of all three plants of non-mining area, is in range 0.2-20 mg/kg which is a normal range (Kabata and Pendias, 1992). Only *Azadirachita indica* (Neem) leaves of mining area has slightly more concentration than normal range, so it may be mildly phytotoxic for plant.

Cadmium Concentration in Soil and Leaves

Maximum cadmium concentration has been found in mining *Azardirachta indica* (Neem) soil whereas minimum concentration has been found in non-mining Bougainvillea plant's soil (Table 2, Figure 3). All soil samples of mining area have greater concentration of cadmium than non-mining area. This may be due to coal mining activity, burning of coal and coal dust accumulation in soil. No Cd contamination has been observed in the soil of non-mining area and of mining area except the soil of *Azardirachta indica*. The range of concentration of cadmium is about 0.05 – 1.5 mg/kg in soil and if the concentration was found to be above this range then it would have been considered as contaminated soil (Keane et al., 2001). Maximum uptake of cadmium has been found in leaves of *Delonix regia* (Yellow Gulmohar) of non-mining area. This may be due to more concentration of cadmium in soil of non-mining area (Table 3, Figure 3). *Delonix regia* leaves of mining area have greater concentration of cadmium than Bougainvillea *glabra* (Paper flower) and *Azardirachta indica* leaves of non-mining area. *Delonix regia* and *Azardirachta indica* (neem) has high concentration of cadmium than *Delonix regia* and *Azardirachta indica* of mining area (Table 3, Figure 3). But Bougainvillea leaves of non-mining area have low concentration than mining area. Cadmium content in leaves of all three plants of non-mining area as well as of mining area has been found to be non-toxic as cadmium's concentration level is in between 0.15 and 2.3 mg/kg concentration (Kabata-Pendias and Pendias, 1992) which is cadmium's concentration range in plant's leaves and above this range only it would have been phytotoxic.

Chromium Concentration in Soil and Leaves

Maximum Cr concentration has been found in mining area *Delonix regia* (Yellow Gulmohar) soil whereas minimum concentration in non-mining *Azardirachta indica* (Neem) soil (Table 2, Figure 4). All soils of mining area have greater concentration of Cr than non-mining. This may be due to the coal mining activities, burning of coal and coal dust (Eick et al., 1999). All the soils of mining as well as non-mining area were found to be uncontaminated because they lie in 3-200 mg/kg range which is background level (Keane et al., 2001). All the leaves of plants of mining area have greater concentration of Cr than in non-mining area and maximum uptake of Cr is found in leaves of *Bougainvillea glabra* (Paper flower) of mining area (Table 3, Figure 4). Cr content found in the plant leaves of non-mining area is not toxic, only a slight variation from normal level has been observed in case of *Bougainvillea glabra* which is 4.97 mg/kg whereas leaves of the corresponding plants of mining area have been found to be mildly phytotoxic; this may be due to high pollution level in mining area through mining activities, coal dust and high vehicle pollution. On the basis of morphological observation, we found less number and small sized leaves in mining area plant than in non-mining area. This may be due to high concentration of Cr in leaves of plants growing in mining area because increasing chromium concentrations in the soil decreased the N, P, K, Ca and Fe contents of shoot system which decrease the growth rate and breakdown of membrane function due to high oxidative potential of Cr(VI) (Zayed and Terry, 2003).

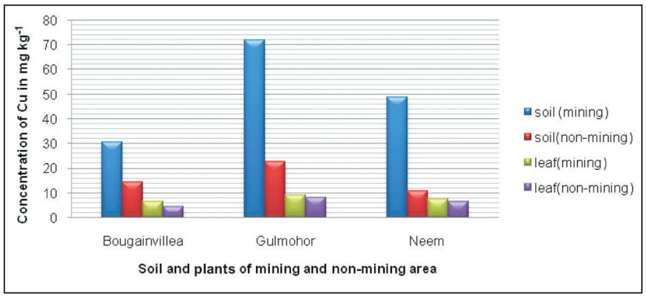

Figure 1: Concentration of Cu in leaves and soil.

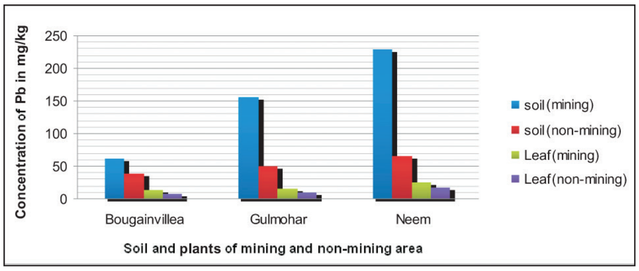

Figure 2: Concentration of Pb in leaves and soil.

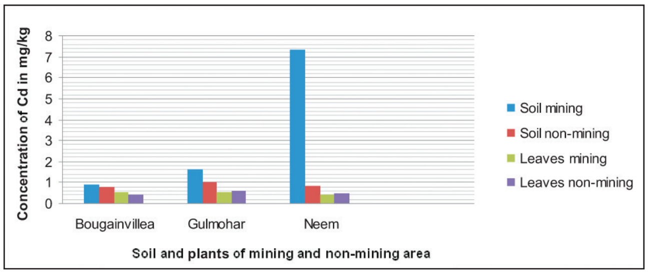

Figure 3: Concentration of Cd in leaves and soil.

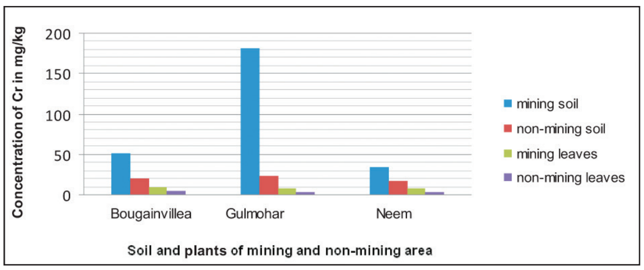

Figure 4: Concentration of Cr in leaves and soil.

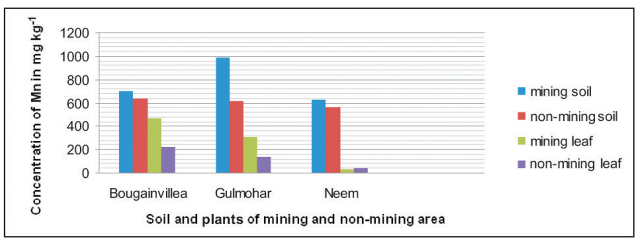

Figure 5: Concentration of Mn in leaves and soil.

Manganese Concentration in Soil and Leaves

All soil samples of mining area have greater concentration of Mn than that of non-mining. This may be due to the coal mining activities, burning of coal and coal dust (Eick et al., 1999). All the soils of mining as well as non-mining area have been found to be uncontaminated because they lie in 7-3000 mg/kg that is background level (Table 2, Figure 5). Maximum Mn concentration has been found in mining area Gulmohar soil whereas minimum concentration in non-mining Neem soil. All the leaves of plants of mining area have greater concentration of Mn than that of non-mining area; only a slight variation from 30-500 mg/kg that is normal level (Keane et al., 2001) is observed in case of Neem of mining area (Table 3, Figure 5). Mn content found in the leaves of both mining and non-mining area is not toxic. Manganese content in leaves of Neem plant is significantly lower as compared to other plants, irrespective of location of the plants, thereby maintaining a gradient between these plants. This may be possible due to high deposition of lead which is responsible for decrease in uptake of manganese (Geeblen et al., 2002).

Conclusion

The present study reveals the concentration of heavy metals (Cu, Pb, Cd, Cr and Mn) in soil and leaves of three plants—*Bougainvillea glabra* (Paper Flower), *Delonix regia* (Yellow Gulmohar) and *Azadirachta indica* (Neem)—of mining and non-mining area. It appears that all metals are present at higher concentration level in soil and leaves of mining area as compared to non-mining area. Concentration of Pb in soil of Gulmohar, Neem and concentration of Cd in soil of Neem has been found above the uncontaminated range in mining area. Concentration of Cd is slightly less in leaves of Gulmohar and Neem of mining area and also less concentration of Mn in leaves of Neem of mining area has been found.

Most of the metals under study are present well within the normal range in leaves of three plants of non-mining and mining area. Except Cr in leaves of all plants, Cu in leaves of Gulmohar and Pb in leaves of Neem, have been found at higher concentration than the normal range in mining area. But the high concentration of these metals did not show any symptoms of phytotoxicity in plants.

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