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Accumulation and translocation of heavy metals by naturally grown plants from fly ash contaminated area

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Abstract

The current research shows that the accumulation of heavy metals in fields contaminated with fly ash from a thermal power plant and its uptake in different parts of naturally grown plants in *Argemone Mexicana, Datura stramonium* and *Solanum Nigrum* Research shows that in the contaminated area, the accumulation level of all the metals (Fe, Pb, Cu, Zn and Cr) in soil and in different parts (stem and leaves) of plant species were found higher than the uncontaminated area. The current research provides us a clue for the selection of best plant accumulator species, which show natural resistance against toxic metals and which are best efficient metal accumulators.

Keywords: Accumulation, fly ash, accumulator plant species, heavy metals, Fe, Pb, Cu, Zn and Cr

Introduction

The productivity of electricity in India is mainly dependent on coal fired thermal power plants. And we are mainly dependent on this, fly ash is a by-product of coal fired thermal power industries, amount around 35-40% of the coal is used by the thermal power plants. In the others words, we can justify that generation of one MW of electricity from coal requires about one acre of land for disposal of fly ash (Sahu *et al*., 1994). In India Fly ash productivity was around 163.56 million tons during 2012-2013 and it is expected to be about 150-170 million tons per annum by the year end of 2020 (MOEF, 2007; Pandey *et al*., 2009) [24, 25]. However, production of fly ash in such a large scale by thermal power industries would poses a serious threat to the environment as a major source of inorganic pollution. The behaviour of many metal pollutants and the release of such metals during storage can have deleterious effects on the environment as well as on human health.

Besides many essential macronutrients (P, K, Ca and S) and micronutrients (Fe, Mn, Ni, Cu, Co, B and Mo), fly ash also contains a number of toxic heavy metals such as Cd, Pb and Se (Rautary *et al*., 2003; Adriano *et al*., 1980) [28, 1]. All the heavy metals found in fly ash are generally toxic in nature. The total land required for ash disposal would be about 82,200 ha by the year 2020 at an estimated 0.6 ha per MW. Sometimes, the concentration of trace metals in fly ash exceeds the levels of these metals found in normal soil (Kalra *et al*., 1996) [15]. Various studies concerning the impact of fly ash on soil or plant productivity have been mostly carried out under laboratory conditions (Garg *et al*., 1996; Karla *et al*., 1997; Mishra and Shukla, 1986; Sikka and Kansal, 1994; Sinha and Gupta, 2005) [11, 16, 23, 32, 13]. Fly ash can be treated as a byproduct rather than waste.

Fly ash particles are generally spherical in shape and range in size from 0.5 µm to 100 µm. They consist mostly of silicon dioxide (SiO_2), which is present into two forms: amorphous, which is rounded and smooth, and crystalline, which is sharp, pointed and hazardous; aluminium oxide (Al_2O_3) and iron oxide (Fe_2O_3). Fly ashes are generally highly heterogeneous, consisting of a mixture of glassy particles with various identifiable crystalline phases such as quartz, mullite, and various iron oxides. (Saxena M, and Prabhakar J, 2000) [31].

There is an inherent tendency of plants to accumulate toxic substances including heavy metals that are subsequently transferred by the food chain from one phase to another phase. Use of contaminated land or water for irrigation of crops mainly accounts for decrease in the overall productivity and which results in contaminated food grains and vegetables which adversely affects human health and sometimes it results in with severe impact. The main advantage associated with study of naturally grown plants is to check their capacity to accumulate metals, and to find out the best accumulator plant species if grown on metal polluted land or irrigated with polluted water. Thus, accumulator plants serve as a good tool for phytoremediation. However, determination of the nature of toxicity, distribution of toxicants and level of accumulation in different plant parts would be essential before selection and cultivation of plants for phytoremediation (Barman and Lal, 1994; Barman and Bhargava, 1997; Barman and Ray, 1999; Barman *et al*., 1999, 2000, 2001) [3, 4, 6, 5, 7, 8]. Many studies have shown that plants can automatically acquire characteristic resistance against toxicants including heavy metals, depending upon the various eco physiological factors in time and space (Gregory and Bradsaw, 1965; Antonovics *et al*., 1967; Porter and Peterson, 1977; Ray *et al.*, 1988) [12, 2, 27, 29]. However, all plants are not equally resistance to all types of pollutants in the environment. It appears that the plant resistance against a particular toxicant is also dependent on the cyto-genetic makeup of the particular species.

The current research relates to the study of levels of metals accumulation in different parts of three plant species, which are growing naturally in fly ash contaminated area. This research is expected to provide us clues for the selection of best accumulator/resistant plant species towards metal found in the fly ash, contaminated area.

Materials and Methods

Study area and samples collection: The study area selected for the research was near by a coal based thermal power plant, NTPC Tanda Ambedkar Nagar district of Uttar Pradesh. The thermal power plant lies between latitude 26°33’00’’N and longitude 82°39’00’’E and is located in a rural area.

Three plant species were collected from the contaminated area near by NTPC during March 2018 and their scientific and common local name are *Datura stramonium* (*Datura*), *Solanum nigrum* (Kali Makoy) and *Argemone Mexicana* (Kateli ka phool). These three plant species, are naturally growing plant species. These three naturally growing plant species was also collected from uncontaminated area (about 20 km away from the NTPC) served as control. All the plant species samples were uprooted at maturity stage and than separated into stem and leave parts for estimation of metal content.

Three plants species were collected randomly from fly ash contaminated area and as well as from the control site. Soil samples were also drawn from contaminated area and control site with an average depth of 20 cm. And fly ash sample were also collected. During plant sampling, it was ensured that different plant samples of each species had the same physiological age, identical size and appearance. The plant samples were first washed with running tap water followed by distilled water to remove extraneous matter. After washing, the plant material was oven dried at 65°C for 24 hr and chopped. The soil samples were taken from both contaminated as well as control sites and were air dried and sieved before analysis.

Metal analysis: One gram of each plant and soil sample (each in triplicate) was digested overnight with a mixture of HNO_3: $HClO_4$ (4:4, v/v). Samples were slowly digested on the hot plate until a clear solution was obtained (Barman *et al.*, 2000: Kisku *et al.*, 2000) [7, 17]. It was then filtered and assayed by AAS (LABINDIA AA 7000) for Fe, Pb, Cu, Zn and Cr. The AAS value of blank (without sample) of each metal was deducted from the sample value for final calculations.

Results and Discussion

Accumulation of metals: The average metal content of contaminated soil was found in the order of Fe (307.98) > Cr (0.26) > Zn (0.23) > Cu (0.05) > Pb (0.04) µg g^{-1} d.w. whereas, in case of control soil it was found almost in the same sequence of Fe (307.98) > Cu (0.05) > Zn (0.00) > Cu (0.00) > Pb (0.00) µg g^{-1} d.w. but in contaminated soil the mean metals levels were significantly ($p<0.01$) higher than the uncontaminated soil.

Among the three plant species, the mean concentration of each metal in stem, in contaminated site the respective metal levels were found in the order and the maximum and minimum concentration (µg g^{-1} d.w.) of metals in the stem part of plant grown in contaminated area was found as Fe > (4.80) > Pb (0.58) > Cu (0.51) > Zn (0.14) > Cr (0.00) in Argemone Mexicana, Fe > (22.25) > Zn (3.15) > Cu (0.86) > Cr (0.07) > Pb (0.00) in Solanum Nigrum, Fe > (18.04) > Zn (2.34) > Cu (1.77) > Cr (0.03) > Pb (0.00) in Datura Stramonium and in uncontaminated area it was found Cu > (0.11) > Zn (0.03) > Pb (0.01) > Fe (0.00) > Cr (0.00), in Argemone Mexicana, Fe > (0.00) > Zn (0.00) > Cu (0.00) > Cr (0.00) > Pb (0.00) in Solanum Nigrum, Fe > (4.17) > Zn (0.22) > Cu (0.00) > Cr (0.00) > Pb (0.00) in *Datura stramonium*. The maximum and minimum values of each metal were found to be comparatively higher in contaminated plant species than in uncontaminated plant species.

In the leave part, in contaminated site the respective metal levels were found in the order and the maximum and minimum concentration (µg g^{-1} d.w.) of metals in the leave part of plant grown in contaminated area was found as Fe > (196.65) > Zn (0.71) > Cu (0.22) > Pb (0.11) > Cr (0.00) in Argemone Mexicana, Fe > (155.63) > Cu (6.07) > Zn (1.47) > Cr (0.00) > Pb (0.00) in Solanum Nigrum, Fe > (91.08) > Cu (2.07) > Zn (0.23) > Cr (0.00) > Pb (0.00) in Datura Stramonium and in uncontaminated area it was found Fe > (48.37) > Zn (0.19) > Cu (0.05) > Pb (0.00) > Cr (0.00), in Argemone Mexicana, Fe > (8.64) > Cu (0.42) > Zn (0.00) > Cr (0.00) > Pb (0.00) in Solanum Nigrum, Fe > (21.42) > Cu (0.35) > Zn (0.00) > Cr (0.00) > Pb (0.00) in *Datura stramonium*. The maximum and minimum values of each metal were found to be comparatively higher in contaminated plant species than in uncontaminated plant species.

The maximum and minimum concentration (µg g^{-1} d.w.) of metals in the leave part of plant grown in contaminated soil was found as 196.65 Fe in Argemone Mexicana and 155.63 in Solanum Nigrum for Fe, 91.08 for Fe in *Datura stramonium* respectively.

The concentrations of five metals in three parts (soil, stem and leaves) of 3 naturally grown plant species at two sites contaminated and uncontaminated were analyzed statistically and summarized. The metal concentrations in contaminated area was found to be higher than the uncontaminated area for all parts (soil, stem and leaves) and showed heterogeneous accumulation.

Table 1: Metal Accumulation in Soil and in Fly Ash

Metals	Contaminated Site	Control Site	Fly Ash
Fe	307.98	307.77	43.44
Pb	0.04	0.00	0.00
Cu	0.05	0.05	2.12
Cr	0.26	0.00	0.05
Cd	0.00	0.00	0.00
Zn	0.23	0.00	0.06

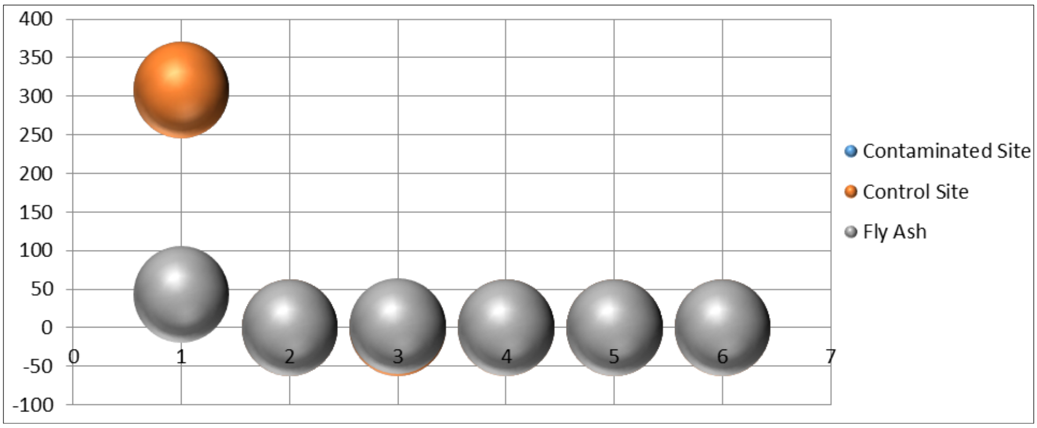

Fig 1: Metal Accumulation in Soil and Fly Ash

Futher, in the samples of naturally grown plants namely *Argemone Mexicana*, *Solanum Nigrum* and Datura Stramonium, and the concentration of each metal in leaves varied over a wide range.

1. Argemone Mexicana: Fe > Zn > Cu > Pb > Cr
2. Solanum Nigrum: Fe > Cu < Zn < Cr > Pb
3. Datura Stramonium: Fe < Cu < Zn < Pb < Cr

And the concentration of each metal in stem varied over a wide range

1. Argemone Mexicana: Fe > Pb > Cu > Zn > Cr
2. Solanum Nigrum: Fe > Zn < Cu > Cr > Pb
3. Datura Stramonium: Fe < Zn < Cu < Cr < Pb

And the concentration of each metal in soil varied over a wide range

Fe < Cr < Zn < Cu < Pb

Table 2: Leaves

Plants	Contaminated Site					Control Site				
	Fe	**Pb**	**Cu**	**Zn**	**Cr**	**Fe**	**Pb**	**Cu**	**Zn**	**Cr**
Argemone Mexicana	196.65	0.11	0.22	0.71	0.00	48.37	0.00	0.05	0.19	0.00
Datura Stramonium	91.08	0.00	2.07	0.23	0.00	21.48	0.00	0.35	0.00	0.00
Solanum Nigrum	155.63	0.00	6.07	1.47	0.00	8.64	0.00	0.42	0.00	0.00

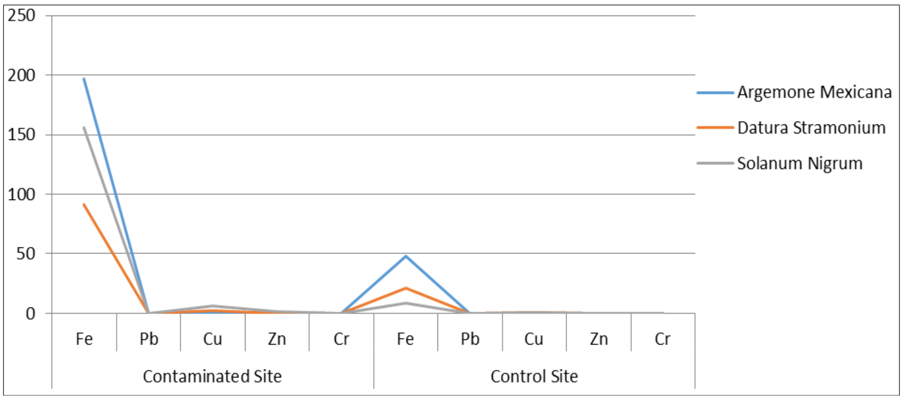

Fig 2: Accumulation of metals in leaves of the naturally grown plant species in contaminated and uncontaminated area

Table 3: Stems

Plants	Contaminated Site					Control Site				
	Fe	**Pb**	**Cu**	**Zn**	**Cr**	**Fe**	**Pb**	**Cu**	**Zn**	**Cr**
Argemone Mexicana	4.80	0.58	0.51	0.14	0.00	0.00	0.01	0.11	0.03	0.00
Datura Stramonium	18.04	0.00	1.77	2.34	0.03	4.17	0.00	0.00	0.22	0.00
Solanum Nigrum	22.25	0.00	0.86	3.15	0.07	0.00	0.00	0.00	0.00	0.00

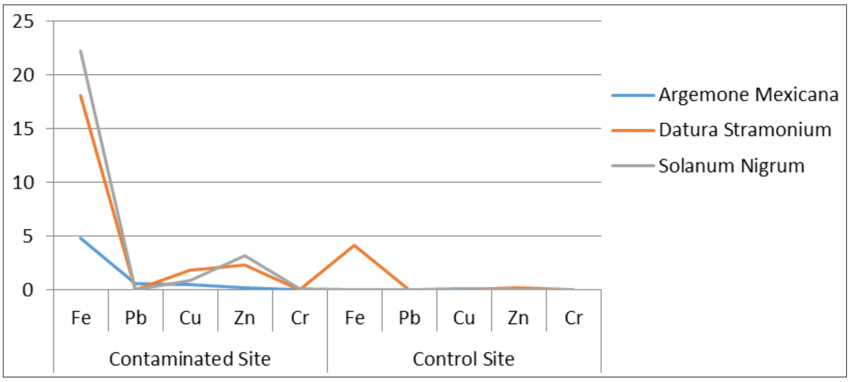

Fig 3: Accumulation of metals in stems of the naturally grown plant species in contaminated and uncontaminated area.

The distribution of metals in soil contaminated with fly ash and thereby increasing the metal accumulation in plants species grown on the contaminated soil (Kisku *et al.,* 2000: Gupta *et al.,* 2008) [17, 13]. Among the five metals estimated, the maximum higher range of Fe was found and followed by soil, stem and in leaves part but in overall, the sequence did not follow any specific pattern.

The accumulation and translocation of metals in some species showed higher bio magnification of metals in contaminated soil whereas the same species showed less accumulation of a particular metal in uncontaminated soil such as the species Solanum Nigrum in leaves and stem, species which is grown in fly ash contaminated soil. Research shows that some metals were also less translocated in the contaminated soil than in the control soil, which indicates that cytogenetic make-up as well as other unknown factors are responsible for different patterns of translocation of metals to the upper part of the plant.

Furthermore, establishing a pattern of translocation of metals from soil to other parts of a plant species can be very useful in biological monitoring of heavy metal contaminated as well as for the selection of best accumulator or tolerant plant species. The metal translocation process in plant species is a critical factor in determining the metal distribution in different plant tissues (Xiong 1998) [35]. A number of factors are involved including anatomical, biochemical and physiological factors (Salt *et al.*, 1995) [30] which contributes to heavy metal accumulation and distribution in upper vegetative parts.

Metals are mobilized and taken up by root cells from soil, bound by cell wall and then transported across the plasma membrane, driven by ATP – depended proton pumps that catalyzes H^+ extrusion across the membrane. Along with cationic nutrients, plant transporters are also involved in shuttling potentially toxic cations across plant membranes (Master *et al.,* 2001; Singh *et al.,* 2003) [33]. The tolerance of plants to increasing levels of toxic elements can result from the exclusion of toxic elements or their metabolic tolerance to specific elements. The major mechanism in tolerant species of plants appears to be compartmentalization of metal ions, *i.e.* sequestration in the vacuolar compartment, which excludes them from cellular sites where processes such as cell division and respiration occur, thus proving to be as effective protective mechanism (Chaney *et al.*, 1997; Hall 2002; Lee *et al.*, 1977) [9, 14, 19]. Higher Fe content in soil, stem and in leaves of a metal accumulator plant species is mainly dependent on at least two factors namely: sequestration and/or translocation. Ni could be transported as a nickel-citrate complex (Lee *et al.*, 1977) [19] or as a nickel-peptide complex or as a nickel-histidine complex (Kramer *et al.*, 1996) [18] to ensure high mobility of Ni within the plant. The Ni tolerant proteins TgMTPIs from the Ni hyper accumulator species *T. goesingense* have been suggested to be responsible for metal ion accumulation in the shoot vacuoles of this plant (Persans *et al.,* 2001) [26]. Similarly, the protein ZAT 1 has been implicated in the vacuolar sequestration of Zn (Zaal *et al.*, 1999) [36]. Memin and Yatazawa (1984) [21] suggested from teir for Mn distribution in plant species of *Acanthopanz sciadophlloides* and *Thea sinensis,* that Mn^{+2} is taken up at plasma membrane and binds with malate in the cytoplasm and this Mn-malate complex is transported through tonoplast membrane to the vacuole where Mn dissociates from malate and complexes with oxalate. Here malate functions as a “transport vehicle” through the cytoplasm and oxalate as the “terminal acceptor” in the vacuole. Several other mechanisms may contribute to heavy metal tolerance depending on the type of metal and plant species (Memon and Yatazawa, 1984; Memon *et al.,* 2001) [21, 22]. Complex with metal binding peptide, metallothioneins and phytochelatins may also serve to alleviate the toxicity of heavy metals in plants. Metallothioneins such as cysteine-rich proteins have high affinity for binding metal cations such as Cd, Cu and Zn (Singh *et al.,* 2003) [33]. Dan *et al.,* (2000) [10] suggested that lignifications of cell wall and formation of metal-lignin complex might be one of the primary mechanisms of Pb tolerance in the roots of scented *Geranium* plant. In the current research, Fe accumulation/translocation is higher in *Argemone Mexicana* and in *Solanum Nigrum.* The uptake and translocation of Fe may also be dependent on the mobility of Fe as well as its competition with other metals within plants.

The research revealed that fly ash contributed a high level of Fe, Cu, Zn, and Cr contaminated soil and subsequent higher accumulation in plants parts. Higher level of metal accumulation in plant parts especially in upper parts of *Argemone Mexicana* and *Solanum Nigrum* showed biomagnification of metals and thus these plants can be considered as best acuumulator plant species.

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