Bio-monitoring of metal deposition in Ranthambhore National Park (Rajasthan), India using *Plagiochasma rupestre* (G. Frost) Stephani

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Abstract: In the present study the level of S, Cu, Cr, Cd, Zn, Pb and Cr was estimated in samples of the common thalloid liverwort *Plagiochasma rupestre* (G. Frost) Stephani, from Ranthambhore National park, Rajasthan (India). High metallic load was observed both in substrate as well as in plant tissue at locations adjacent to higher vehicular load, during winter the metallic content is highest, followed by summer and monsoon season. Elemental concentration in substrate for *Plagiochasma rupestre* was in the order of Zn> S> Pb> Cu> Cd> Cr, while in plant tissue it showed S> Zn> Pb> Cu> Cd> Cr, which is indicative of air borne trace elemental load. Quantitative analysis of these elements in vegetative thalli of *Plagiochasma rupestre* and the substrate showed an increase in metallic content during winter, which reveals the significance of this liverwort as a biological sink of mineral elements present in the soil and air and may provide an important tool in estimation of both aerial pollution and mineral enrichment in soil.
Keywords: Bio-monitoring, Elemental concentration, Liverwort, *Plagiochasma*, Ranthambhore.

1. Introduction

Richards (1932) remarked that "the responses of bryophytes to edaphic factors are certainly sharper and clearer than the flowering plants". Bryophytes are major constituent of hilly forest cover and pioneer in colonization. They play a vital role in carbon assimilation, mineral and moisture enrichment as well as contribute organic matter, nutrients materials and moisture to substratum which they absorb from air. In recent past, there has been a surging curiosity regarding bryophyte responses to environment due to their characteristic thallus composition as they are composed of sheets of tissue, therefore they are in close vicinity with the environment and respond more rapidly to environmental changes than vascular plants, because they get water, nutrients and elements mainly from the atmosphere.

They are very handy tool in biological monitoring where the use of organisms provides information on certain aspect of biosphere like air-borne metal content. They are suitable and inexpensive method to quantify the possible emanation of metals because they are able to mount up elements in extremely high concentration and this provide easy detection of elements present even in very low concentration in the environment. On being trapped metals bind to the cell wall and remain retained for years and can be analyzed in future also. The lack of transportation system, results absorbed minerals to be adequately preserved.

Bryophytes tolerance and well suited and safe for the purpose of bio-monitoring (Giordano et al., 2005) like other bio-monitoring organism they also possess the ability to survive quantity specific essential and non essential elements present in the environment and utilize them in their metabolism, these elements show toxicity at higher concentration by sensitive species, however, excess elements are detoxified by some molecular proteins in tolerant species. The work carried out earlier has been largely associated with musci as compared to hepaticae. Among the hepaticae, Marchantialean forms are considered to be most resistant against all types of adverse conditions as they are also called "resurrection plants" hence can profitably be used for such studies. Several contributions mostly from abroad and elsewhere have been made in this direction (Satake, 1951; Pearson, 1956; Martensson and Berggren, 1951; Schatz, 1955; Canon, 1960; brooks, 1972; James, 1973; Rejment, 1976; Ward et al., 1976; Shacklette and Erdman, 1982; Shacklette, 1984; Smith, 1986; Shaw and Schneider, 1995; Frahm, 1998). But in India these types of studies is restricted to few workers only (Pant and Tewari, 1998; Glime and Saxena, 1991; Alam and Srivastava, 2009; Alam et al, 2011].

2. Materials and Methods

Materials

Considering the need of such studies it was thought desirable to assess the terrestrial liverwort (*Plagiochasma rupestre*) of order Marchantiales as an indicator plant as well as accumulator of mineral elements/ heavy metals from soil and atmosphere.

Characteristics of *Plagiochasma rupestre*:

Thallus green, large, dichotomously branched, apex notched. Midrib somewhat indistinct. Ventral surface covered with numerous rhizoids and prominent ventral scales in single row on each side of midrib. Thallus internally well differentiated into upper assimilatory zone having air chambers with assimilatory filaments, storage zone present up to the margins.

Studied Area:

The Ranthambhore Tiger Reserve is the single biggest stretch of dry deciduous *Anogeissus pendula* forest left unbroken in India. In Ranthambore the biodiversity is made even richer by the invasion of the Vindhyan hill system. The terrain of Ranthambhore tiger reserve is mostly craggy and hilly and is intimately related to the Great boundary fault. The highest point of this is Gazella peak, 507 m above sea level. The lowest altitude of this tract is 244 m above sea level at Bodal. Streams flowing in northern tract form the catchment of the river Banas and streams flowing in southern tract drain directly in the river Chambal. Its cardinal points are 25° 54' to 26° 12' N Lat. and 76° 23' to 76° 39' E Long., annual average rainfall 800m, temperature range 4°C (minimum) - 47°C (maximum). The annual mean humidity is 72%, which reaches to its maximum (99%) during monsoon. The sand stone beds of these hills are flat-topped and form extensive table lands known as "Dangs". These dangs rise abruptly from flat ground and have sandstone ridges running continuously along their edges. At places, small and short-lived streams have eroded deep, long and narrow gorges that are locally known as "Khohs" (Alam et al., 2011).

Methods

The materials used for the present study include (i). The vegetative thalli of *Plagiochasma rupestre* and, (ii). The soil samples of substrata from different sites on which they grow. Vegetative thalli of *Plagiochasma rupestre* and soil samples from the substrate from 2 major regions (peripheral zone and undisturbed zone) of Ranthambhore National Park were collected between 15.05.2012 to 05.02.2013. The quantitative analysis of S, Fe and heavy metal content (S, Cu, Cd, Zn, Pb and Cr) was carried out in both the plant thalli and soil samples from different localities. The soil samples were also analyzed for its physical and chemical properties to find out the similarities and differences in soil properties of different regions. The presence of mineral

elements such as S, Cu, Cr, Cd, Zn, and Pb in soil as well as in the plant tissue was estimated to see the extent of accumulation of soil elements in plant material.
The plant material was collected along with soil samples from different sites and were individually sealed in polythene bags and brought to laboratory. The plants were examined under microscope and identified for their taxonomic status. The substrate matter attached to the plant material was removed by high pressure washing with tap water and then by repeated washing with distilled water and finally immersed in double distilled water for 2 hours. The meticulously washed plant samples employed for chemical analysis were dried at 70^{o} C for 48 hours in labeled bamboo paper bags. The oven dried plant material was used for analysis of macronutrient (i.e., S) after wet digestion employing Piper's method (Piper, 1942). Further estimations were done according to the methods employed by various workers as tissue Sulphur (Chesnin and Yein, 1951), and tissue Copper, Cobalt and lead were analyzed by DTPA extraction method (Lindsay and Norvell, 1978) using Atomic Absorption Spectrophotometer (AAS). The pH of soil was measured using digital pH meter.

3. Observations

3.1 Estimation of pollutants
Sulphur (S):
High influx of tourist, the consumption of fuels can be the reason for high increase in S content throughout the year near densely populated areas (Thőni et al., 1996). The result is clarifying the statement that at tourist places there will always be escalating drift observed for vehicle pollution; consequently increase in the metals, associated with the vehicular pollution (Poikolainen et al., 2004; Alam and Srivastava, 2009) as it appear as many fold increase in its value during winter could be due to enhanced traffic in winter seasons The overall maximum percent (%) S load in location of high traffic movement at their maximum value (Table 1, 2; Fig. 1a-1c; Fig.2a-2c).
Moreover, the tourist influx during winter in this tourist spot find itself choked with tourist traffic in and indeed they are more crowed in each season except monsoon Saxena et al., 2010). The deposition values are positively related to the traffic (and population) density. This reflects their high value in plants growing at petrol pumps or along the road (near taxi stands) in Ranthambhore compared to other catchment areas.
Comparison of metal S concentration in different seasons exhibited its lowest concentration in rainy season could be attributed to decrease of tourists in the rainy season and secondly pollutant leaching and increase in growth and biomass occurs more rapidly as a result it reduces the sulphur percentage in leafs in proportion to biomass (Fernández et al., 2009).
Lead (Pb):
Excessive fuel consumption could be the reason for its high value in all seasons near proximity to the town, along the roads or near these hill stations' taxi stand which intensified during winter, a tourist season (Halleraker et al., 1998). The result is explanatory the statement that at tourist places there will always be increasing trend observed for vehicle pollution; consequently increase in the metals, associated with the vehicular pollution (Poikolainen et al., 2004). Present experimental data further revealed that many fold increase in its value during winter could be due to enhanced traffic in winter seasons (Table 1). The overall maximum percent (%) metal Pb load in both locations stands with their maximum value (Table 1, 2; Fig. 1a-1c; Fig.21a-2c). A decrease in metal lead load during monsoon could be due to the atmospheric metal load is washed during heavy rains ((Table 1, 2; Fig. 1a-1c; Fig.2a-2c). Combustion of leaded, low-leaded and unleaded gasoline continues to be the major source of atmospheric Pb emissions (Bate, 1992). A decrease in metal lead load during monsoon could be due to the atmospheric metal load is washed during heavy rains.
Since high-octane petrol (with 0.45 g lead/ l) is still used in the majority of the cars, the deposition values are positively related to the traffic (and population) density. This reflects their high value in

plants growing at petrol pumps or along the road (near taxi stands) in as compared to other catchments areas.

Like Sulphur comparison of metal Pb concentration in different seasons revealed its lowest concentration in monsoon season due to decrease of tourists in the rainy season and leaching increase in growth and biomass occurs more rapidly as a result it reduces the metal percentage in thalli in proportion to biomass.

Zinc (Zn):

Maximum percent increase of metal Zn was observed at the same locations similar to Pb. A seasonal trend for metal Zn justifies that winter had high value of Zn followed by summer and rain (Table 1, 2; Fig. 1a-1c; Fig.2a-2c). Heavy rain fall could be one another way to explain the decrease in Zn load as most of the metal Zn was surface adsorbed. The enrichment ratios for Zn in thalli were not constant throughout the year; therefore, interpretation of enrichment of Zn is complicated. Here, the role of traffic cannot be over ruled to increase Zn content of the air as it is the part of automobile stratum (Makholm and Miladenoff, 2005).

Copper (Cu):

In comparison to Pb and Zn, a moderate deposition rate of metal copper was observed from same catchment areas. Metal Cu was high in populated as well as rural areas. Maximum value of copper was in peripheral zone during all the three seasons (Table 1, 2; Fig. 1a-1c; Fig.2a-2c). Therefore, present finding is further supported by the presence of abundant Cu absorbing bryophytes in these areas.. A decrease in Cu in summer with respect to winter could be explained by considering that dry deposition increases on moving from humid to arid climates (Couto et al., 2004).

Concentration and distribution pattern of both metal Zn and Cu in *Plagiochasma rupestre* were quite similar and both metals were high in rural transplants located in vicinity of national park. Cu pollution may also originate from domestic waste disposal. The use of $CuSO_4$ mixed kerosene oil could also one of the facts of increase of Cu concentration in domestic areas.

Cadmium (Cd):

Cadmium metal is very easily leached out from surface and the similar results were observed in monsoon season in *Plagiochasma rupestre.* All catchment's area shows significant decrease in metal Cd value in rainy season with respect to summer and winter (Table 1, 2; Fig. 1a-1c; Fig.2a-2c). Easily leaching property could be the reason for their low value even in the forest areas (Bate, 1992). There is a frequent use of breaks in the hilly region compare to the land areas. This could be the reason for high value of Cd in peripheral zone during winter. An increase in the Cd on such places could be from abrasion of clutch, breaks of the vehicles. Higher concentration in agricultural land might be due to the use of phosphate fertilizers (Otvos et al., 2003). Perhaps Cd is also present in the petrol as mining impurities. Service shops related with metals are the other factors for this increase.

Chromium (Cr):

Chromium resembles iron in its chemical distinctiveness and is often found with iron in ores. It is enormously important in the manufacture of steel alloys, and is used in catalysts, electroplating, pigment manufacture, leather tanning and some wood preservatives. Chromium can occur as a byproduct of the combustion of coal (Rühling et al., 1985). It is also used to prevent corrosion in power plant cooling towers and can be released into the atmosphere in steam released from such towers. Chromium can be enhanced near urban/industrial areas, with levels of 25 to 130 ppm. Levels of about 10 ppm in bryophytes were considered enhanced (Gydesen et al., 1980)

3.2 Results:

Percent metal load and pollution index value:

The pollution index value at peripheral zone and undisturbed zone of Ranthambhore National Park was +1.212453 and +0.877541 respectively.

The PI value was highly positive at the areas near by taxi stand and at petrol pumps. This was further supported by percent metal loading in respective locations during these years. The maximum positive values were measured in proximity to the city area. Negligible value at undisturbed zone reveals that it is relatively cleaner site (pollution free). The effect of intensity of traffic compared to the influence of other factors like farming on sampling points are much higher as the spots were nearer to the roads (except control site). Besides this, contamination was high and reduces with distance (Alam and Sharma, 2012).

Table 1: Metals concentration (µg/g dry weight.) ± SE in seasonally exposed samples of *Plagiochasma rupestre* at two different localities of Ranthambhore National park. Significance differ from control (a) p ≤ 0.05 and (b) p ≤ 0.01 significance level

Study sites	Winter (Fig. 1a)					
	Cu	Cr	Zn	Pb	S	Co
Peripheral (Disturbed Zone)	18.15±0.98	5.17±0.74	27.90±0.94	21.56±1.02	20.90±0.72	6.10±0.72
Central (Undisturbed Zone)	12.10±0.87	1.47±0.16	17.36±0.99	10.36±0.81	08.20±0.82	02.20±0.77
	Summer (Fig. 1b)					
Peripheral (Disturbed Zone)	26.25±0.78	4.82±0.52	29.71±0.42	25.86±0.92	23.70±0.32	06.70±0.12
Central (Undisturbed Zone)	17.32±0.62	2.27±0.14	19.26±0.32	11.46±0.43	10.60±0.72	03.05±0.64
	Monsoon (Fig. 1c)					
Peripheral (Disturbed Zone)	11.58±0.72	2.35±0.24	22.57±0.17	15.34±0.81	19.70±0.32	02.80±0.23
Central (Undisturbed Zone)	5.26±0.72	0.77±0.15	8.08±0.72	7.23±0.72	5.10±0.32	0 1.27 ±0.72

Table 2: Metals concentration (µg/g dry weight.) ± SE in soil samples at *Plagiochasma rupestre* at two different localities of Ranthambhore National park. Significance differ from control (a) p ≤ 0.05 and (b) p ≤ 0.01 significance level

Study sites	Winter (Fig. 2a)					
	Cu	Cr	Zn	Pb	S	Co
Peripheral (Disturbed Zone)	240.12±0.12	41.22±0.15	60.11±0.14	79.17±0.12	14.82±0.32	11.10±0.24
Central (Undisturbed Zone)	69.18±0.24	25.3 7±0.22	33.26±0.12	27.15±0.82	04.24±0.12	04.12±0.15
	Summer (Fig. 2b)					
Peripheral (Disturbed Zone)	178.16±0.32	34.14±0.62	52.11±0.15	74.34±0.24	13.89±0.12	11.87±0.32
Central (Undisturbed Zone)	59.18±0.24	19.77±0.22	24.36±0.12	23.15±0.82	04.40±0.12	03.92±0.15
	Monsoon (Fig. 2c)					
Peripheral (Disturbed Zone)	158.11±0.12	31.34±0.12	34.26±0.12	62.34±0.12	12.19±0.32	10.77±0.12
Central (Undisturbed Zone)	32.12±0.82	15.27±0.28	18.46±0.42	18.72±0.24	03.42±0.32	02.98±0.24

4. Discussion and Conclusion

The bryophytes in general are not only efficient absorbers of heavy metals but also useful indicators of aerial fallout of heavy metals usually much more than the vascular plants (Richards, 1949; Brüning and Kreeb, 1993). These studies have proven that the chemical analysis of carpet forming bryophytes is the rapid and comparatively economical tool for surveying heavy metal deposition in terrestrial ecosystem and in surrounding ambient air. The monitoring by bryophytes can be done even in any remote area where no expensive technical equipment can be transported for estimation. Collection and sampling is an easy task in case of bryophyte and can be done by any one suspecting that air has been contaminated. Their habitat diversity, structural simplicity, totipotency and rapid multiplication rate, make them an ideal organism for studies related to pollution and mineral enrichment besides phyto-sociological and eco-physiological aspects.

The mineral elements (S, Pb, Zn, Co, Cr and Cu) estimated in thallus tissue of *Plagiochasma rupestre* are not only essential elements but also contribute as an important component of the plant organization by playing major role in their metabolic activities. Heavy metals such as Cu, Co, and Cr have also been estimated to find out their extent of accumulation in the thalli from soil as well as aerial fall out. The sites of undisturbed zone have been considered as control as the conditions in this region is almost ideal for the growth of *Plagiochasma rupestre* because of least population and least atmospheric pollution as compared to the sites in peripheral zone. Likewise, winter season is more affected with metals' pollution due to heavy influx of tourists to the national park from country and abroad.

On the basis of present study it has been revealed that *Plagiochasma rupestre* may be considered as accumulator of certain elements, whose concentration in plant tissue is generally related to the element content in the supporting substrate (soil). However, this relationship tends to vary in case of certain elements such as S due to aerial fall out in polluted areas. The tissue chemical content of the same species also varies according to its changed climatic conditions, but the basic level of requirement of a particular element is almost the same. The variation caused in the tissue chemical content may be due to the effect of pollutants (Table 1, 2; Fig. 1a-1c; Fig.2a-2c).

The Bryo-vegetation is an exploratory "tool" and should be widely accepted as readily as have rocks, soils, water and stream sediments. It should not be taken as a "nuisance" and must not be mercilessly bulldozed out because they accumulate pollutants and make the surrounding atmosphere free from the pollution (Brooks, 1972) and is evidently supported in the present investigation. The *Plagiochasma rupestre* thalli accumulate S to a large extent besides serving as sink for heavy metals like Co, Cu, Pb, Zn and Cr and may act as soil purifier. Further investigations employing other species of bryophytes may profitably be used in monitoring environmental pollution as well as indicators of mineral enrichment of soil.

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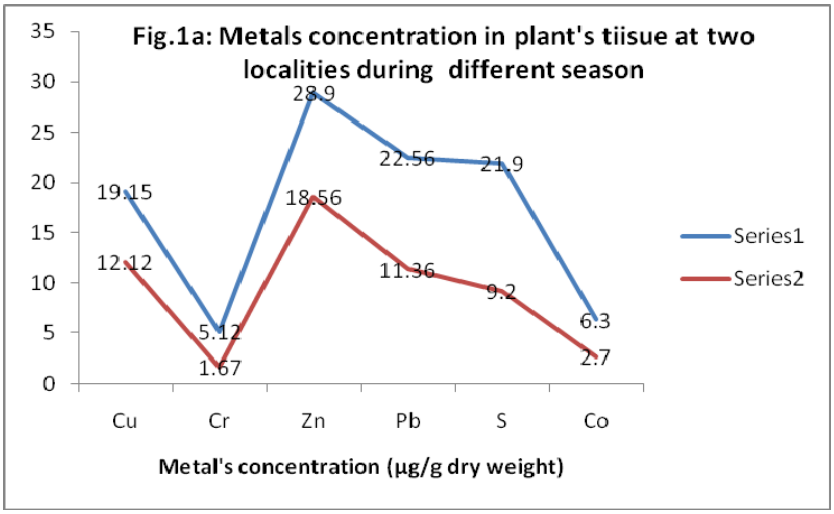
Fig.1a: Metals concentration in plant's tiisue at two localities during different season
35
30
25
20
15
10
5
0
19.15
12.12
5.12
1.67
28.9
18.56
22.56
11.36
21.9
9.2
6.3
2.7
Cu
Cr
Zn
Pb
S
Co
Metal's concentration (µg/g dry weight)
Series1
Series2

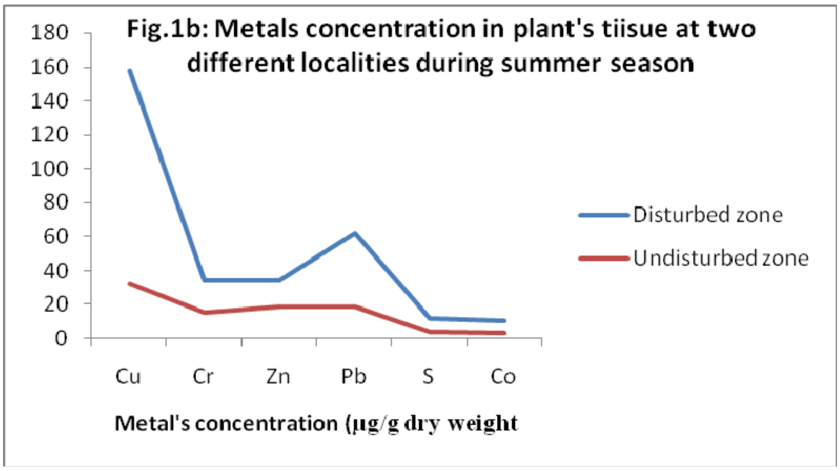
Fig.1b: Metals concentration in plant's tiisue at two different localities during summer season
180
160
140
120
100
80
60
40
20
0
Cu
Cr
Zn
Pb
S
Co
Metal's concentration (µg/g dry weight
Disturbed zone
Undisturbed zone

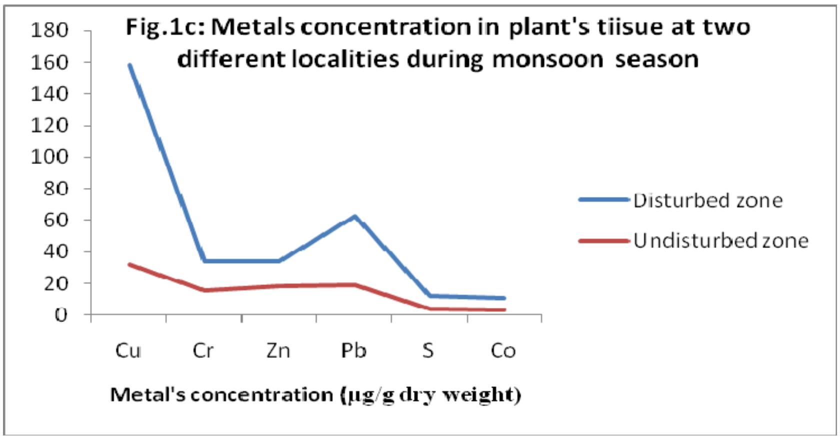
Fig.1c: Metals concentration in plant's tiisue at two different localities during monsoon season
180
160
140
120
100
80
60
40
20
0
Cu
Cr
Zn
Pb
S
Co
Metal's concentration (µg/g dry weight)
Disturbed zone
Undisturbed zone

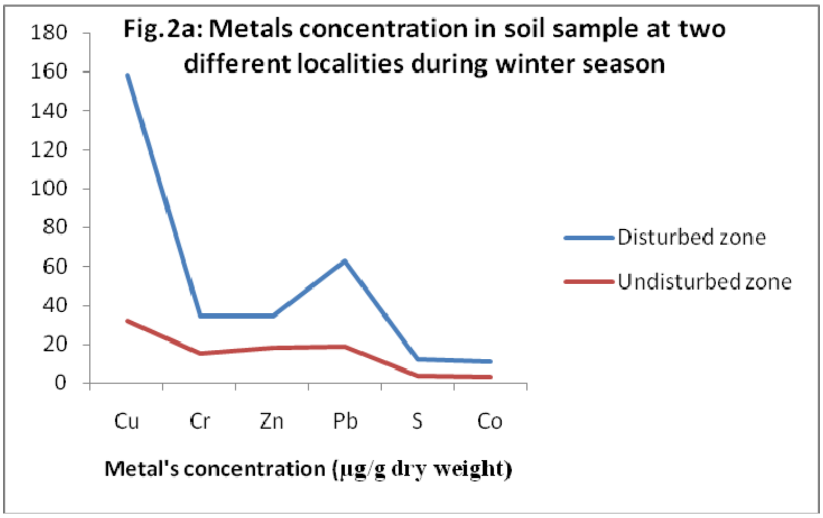
Fig.2a: Metals concentration in soil sample at two different localities during winter season
180
160
140
120
100
80
60
40
20
0
Cu
Cr
Zn
Pb
S
Co
Metal's concentration (µg/g dry weight)
Disturbed zone
Undisturbed zone

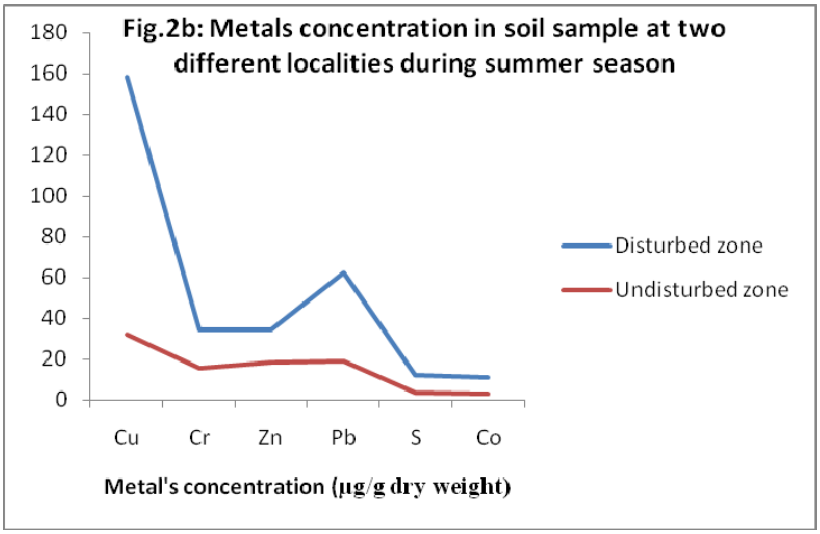
Fig.2b: Metals concentration in soil sample at two different localities during summer season
180
160
140
120
100
80
60
40
20
0
Cu
Cr
Zn
Pb
S
Co
Metal's concentration (µg/g dry weight)
Disturbed zone
Undisturbed zone

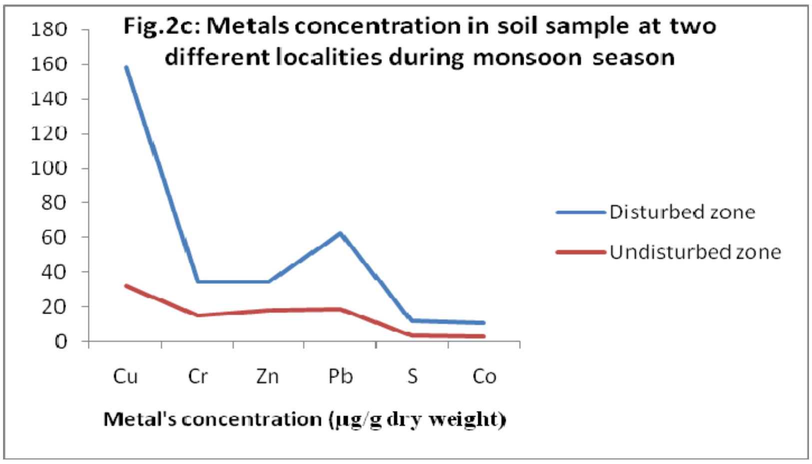
Fig.2c: Metals concentration in soil sample at two different localities during monsoon season
180
160
140
120
100
80
60
40
20
0
Cu
Cr
Zn
Pb
S
Co
Metal's concentration (µg/g dry weight)
Disturbed zone
Undisturbed zone