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HIGH THROUGHPUT ANALYSIS OF TEMPORAL AND SPATIAL EFFECTS ON BIO ACCUMULATION OF PARTICULATE AIR POLLUTANTS OF METALS AND METALLIC OXIDES IN A BIOINDICATOR

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ABSTRACT

Particulate pollution by metals and metal oxides cause environmental and health hazards in different levels depending upon the spatial location and seasonal temporal variations. This paper aims to determine the level of bioaccumulation of particulate pollutants and its variation due to environmental location and seasonal impacts in a well-known versatile tropical waste land plant and bioindicator, *Ricinus communis*. The bioaccumulation across 3 sites namely residential, traffic prone and industrially polluted sites were chosen over two seasons namely dry and wet. The elemental compositional analysis was carried out using X-Ray fluorescence technology and micrographic images (SEM) were used to examine and compare the bio-accumulated particulate metallic pollutants in the aerial parts of the bio-indicator plant. The bioaccumulation of Pb was high in wet season than in dry season across all sites irrespective of the location, but the level of Pb accumulation was varying between sites due to influence of seasonal effects caused by monsoon indicating more accumulation in residential site due to industrial leach and percolation. Particulate accumulation of Ca and Fe and its oxides were persistently high both in dry and wet seasons in traffic prone site than the other 2 sites. Similarly, S, Si and their oxides were persistent across 2 seasons but, their levels were influenced by temporal factor of seasonal characters such as rain and arid climatic conditions, scoring high for wet season. The bio- accumulation of alkali earth metals, Na and Cl were influenced both by spatial and temporal factors such as anthropogenic activities and monsoon effects, accumulating more in industrial site and in wet season. This paper clearly demarcates the influence of spatial and temporal factors on 19 metallic and non metallic particulate matters and their oxides.

Keywords: bioaccumulation, particulate matter, spatial effect, temporal effect, bio-indicator.

INTRODUCTION

Plants are bio-indicators of environmental pollution. They accumulate heavy metals from environment Buszewski B. *et al.* [6]. Plants naturally acquire pollutants from their local environment as a part of their phyto-accumulation and variation in chemical composition profile indicating the degree of disturbances from pollutants Tangahu B.V. *et al.* [22]. The most common sources of environmental pollution are mining, vehicular exhaust, tannery and industrial activities, etc., plants can absorb metals and metal oxides Porebska G. *et al.* [18]. Of many bio-indicating plants, Castor (*Ricinus communis)* of Euphorbiaceae family is commonly available in wastelands. It is mainly cultivated for oil production. Earlier used as good lubricant, several extracts of Castor is used in medicine due to the presence of anti-microbial, analgesic, antihistamine and anti-inflammatory properties and it is used as insecticide. Raw castor bean are toxic due to presence of Ricin, about 4-8 seeds are considered as lethal dosage in adults (Worbs, S. *et al.* [24]).

Health hazard studies reveal that the spatially varying factors such as land use patterns and industrial exposures modified the effect of air pollution. (Lianfa Li, *et al.*[11]). Chen *et al.* [8] has reported seasonal variational pattern on particulate air pollution. Such references outline a fundamental correlation of the particulate pollution level fluctuations with space and time.

This paper aims to analyse the fluctuations in phyto accumulation of metallic and non-metallic particulate matters in the above mentioned bio-indicator with reference to space and time using precise high throughput analytical techniques such as XRF and SEM.

XRF (X-Ray Fluorescence spectroscopy) is a non-destructive analytical tool used for quantitative analysis of majority of trace elements. Reasons for using XRF are low cost of sample preparation, easy to use spectrometer, accuracy and precision of results. These make XRF as a most widely used analytical tool. (Carvalho, M.L. *et al.* [7]; Rizescu C.Z, *et al.* [20]).

Scanning Electron Microscope (SEM) which produces scanned images with the focused beam of electrons can be used to determine aerodynamic diameters of metallic and non-metallic particulate pollutants (Luis *et al.* [13])

MATERIALS AND METHODS

Study sites -for spatio- temporal effect s

Three sites were chosen keeping the criteria of pollution diffusion as the basis for spatially segregating the sites to study the effect of spatial variations on bioaccumulation of particulate pollutants of metals and non-metals. The study was carried out in the area which

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falls as district boundary between Vellore and Thiruvallur district, Tamil Nadu, South India. Ranipet, an urban town which is designated as state industries promotion corporation of Tamil Nadu (SIPCOT) area where number of medium and large scale leather industries for making leather articles such as shoes and garments for export are located happens to be the best choice of area to study about the extent of influence of industrial particulate pollutants including heavy metals such as lead and their extent of bio-accumulation in plants. Effect of industrial pollution in plants has been carried out by Nouri J. [17] and Ramamurthy N. [19]. Second site of plant samples collection was carried out from traffic prone area about 1.5 km from the centre of the town- Sholinghur, Vellore district. A similar study has been done in traffic prone area by Naveed N.H. *et al*. [15]. Thirdly, the authentic reference samples (ARS) collection site was carried out from rural residential area of Veeranathur village in Thiruvallur district, Tamil Nadu which is located completely in isolation from pollution diffusion and hence it could be tagged as clean residential zone.

Temporal effects on phyto-accumulation was observed based on pollution levels in two seasons of 2016, namely dry (Feb-Aug) and wet (Sep-Jan) in Thiruvallur District, Tamil Nadu. The samples were collected in triplicates at the end of dry and wet seasons in order to assess the cumulative pollutant deposition in the aerial parts of the bio-indicator.

Plant sample collection

The plant sample- Castor *Ricinus communis* which was collected from a clean zone had been included as standard for comparison with the plant samples collected from polluted sites-Ranipet (industrial pollution) and Sholinghur (transportation pollution).About 20-25 leaves were collected from various positions in the plant namely top, middle and basal portions of five plants randomly chosen from above sites of each location at the end of two seasons. In case of plant sample collected from polluted areas, the sites included leather industry polluted sites at Ranipet and traffic prone district road near Brakes India limited, Sholingur,Vellore district, Tamilnadu 9site2 as mentioned above).

Sample preparation and analysis

The leaves were washed with clean water to remove the dust, soil present on the surface. Then the cleaned leaves samples were shade dried to become brittle. Dried samples were finely powdered by using mixer grinder. Each of the powdered samples collected from above mentioned sites were analysed in triplicates to reduce the risk of analytical error using X-ray fluorescence spectrometer and SEM. In case of XRF analysis, the powdered samples were placed on top of boric acid taken in an alumina cup and then pelletized using a 25 ton hydraulic press to obtain thin disc of 34mm diameter. The elemental composition of samples were determined using XRF (S8 Tiger, Bruker AXS, Germany) using a 4 kW Rhodium anode X-Ray tube. Analytical precision for sample replicates was always better than 90%.

The spectrometer used in this work is based on a three axial geometry which reduces the background by polarization of the radiation, as pointed out by Standzenieks *et al*. [21]. The primary beam from the X-ray tube impinges on a secondary target, which emits almost monochromatic X-ray radiation. This monochromatic beam is then used to excite characteristic radiation from the atoms of the sample.

The micrographic analysis was carried out using Field Emission Scanning Electron Microscope model JSM 6701F, JEOL, Japan. 100% dried samples were coated with gold nano particles and were scanned using an electron beam (0.3kv - 10kv) and the images were magnified to 6 lakhs in 25X.

RESULTS

The Table (Tables 1, 2, 3 & 4) below shows the elemental concentration obtained using XRF for the samples in two seasons from three different sites. The analyses were done for metal elements namely Ca, K, Mg, Fe, Al, Na, Mn, Ru, Sr, Mo, Ti, Pb, Cu, and Zn. Similarly for the nonmetals, namely O, Cl, S, P and Br, elemental concentrations were estimated. In addition, elemental concentrations for following metal oxides namely CaO, K_2O, MgO, Fe_2O_3, Al_2O_3, Na_2O, MnO, MoO_3, TiO_2,SrO, PbO, CuO, ZnO and nonmetals SO_3,SiO_2, P_2O_5 were also estimated. (Yagi S. [25] and Cheng ma *et al*. [9]).

Spatial effects on bioaccumulation of metallic and non-metallic particulate pollutants for dry season

Table-1. Mean concentration of different metals and nonmetals in plant leaf samples of *Ricinus communis* collected from each site during dry season.

Estimated concentration (%)	Site 1	Site 2	Site 3
Ca	60.65%	64.63%	57.86%
K	16.31%	14.65%	20.47%
Cl	8.05%	4.62%	5.27%
S	6.42%	5.32%	5.65%
Mg	2.64%	1.60%	2.14%
Si	2.21%	3.77%	3.35%
P	1.57%	1.20%	1.47%

Fe	0.91%	1.69%	1.32%
Al	0.67%	1.42%	1.11%
Na	0.25%	0.37%	0.34%
Mn	0.08%	0.25%	0.09%
Ru	0.06%	-	0.04%
Sr	0.06%	0.05%	0.03%
Mo	0.05%	-	0.04%
Ti	0.04%	0.16%	0.14%
Br	0.03%	-	-
Pb	-	0.12%	0.16%
Cu	-	0.08%	0.44%
Zn	-	0.07%	0.06%

Table-2. Mean concentration of different metallic and nonmetal oxides in plant leaf samples of R*icinus communis* collected from each site during dry season.

Estimated concentration (%)	Site 1	Site 2	Site 3
CaO	40.25%	44.42%	7.10%
K_2O	19.64%	17.65%	6.40%
SO_3	16.03%	13.28%	4.85%
SiO_2	4.72%	8.06%	1.79%
Cl	8.05%	4.62%	1.75%
P_2O_5	3.61%	2.74%	0.95%
MgO	4.39%	2.65%	0.58%
Fe_2O_3	1.31%	2.42%	0.45%
Al_2O_3	1.26%	2.69%	0.41%
CuO	-	0.11%	0.16%
PbO	-	0.12%	0.15%
MoO_3	0.07%	-	0.06%
Na_2O	0.34%	0.50%	0.06%
TiO_2	0.07%	0.27%	0.04%
ZnO	-	0.08%	0.02%
SrO	0.07%	0.06%	0.02%
MnO	0.10%	0.33%	0.02%
RuO	0.06%	-	-
BrO	0.03%	-	-

Site1- Rural resident area; Site 2 - Area polluted by Transportation; Site3- Industrially polluted area.

The above tables 1& 2of relative comparison of metals, nonmetals and their oxides in the above mentioned sites illustrated that site 1(Rural residential site) recorded high concentrations of Cl, S, Mg, P, Ru, Se, Mo and Br; site 2 (Traffic prone site) measured high amounts of Ca, Si, Fe, Al, Na, Mn, Ti and Zn; site 3 (Industrially polluted site) was found to have high levels of K, Cu and Pb. Similarly for metal oxides site 1 had high K_2O, SO_3, oxides of Cl, Br & Ru, MgO, P_2O_5, MoO_3 and SrO; site 2 recorded high SiO_2, Fe_2O_3, Al_2O_3, Na_2O, TiO_2 and ZnO. High concentrations of CuO and PbO were recorded in site3 (industrially polluted site).

The analysis showed that site 1 did not show evidence for the presence of Pb, Cu, Zn, PbO, CuO and ZnO; and site 2 did not show evidence for the presence of Ru, Mo, Br, RuO, BrO and MoO_3; site 3 did not show evidence for the presence of oxides concentrations of Ru and Br. It was observed that presence of Bromine was observed only in site1.

Metals and non metals

Among the nonmetals, S, Mg showed equivalent levels of concentrations in the sample collected from rural, industrially polluted and traffic prone sites as mentioned above. This is attributed to the reason that presence of these elements is distributive in nature in the same range of concentrations across all environments whereas, the

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concentration of Phosphorus is recorded in equal range among the rural and industrially polluted sites. The Phosphorus accumulation in rural area is attributed to P fertilizer addition in those areas. Moreover as leather industries are available in the second site, the effluent is rich in P and hence the uptake by plants had recorded high concentration of P. The presence of nonmetal Br is recorded only in rural site implicating the uptake of Br dissolved in ground water by the castor plants grown in that area. Cl concentration is higher than other two sites which may be due to salinity of underground water of rural area which led to accumulation of Cl in the aerial parts of the plant due to root uptake.

In case of metals, the concentration of Pb, Cu and Zn were high in the samples collected and analysed from the industrially polluted site. According to Aragon Pina *et al.* [2, 3, 4], the industrial and mining wastes contribute to the high concentrations of heavy metals including Lead and Arsenic in addition to Calcium sulphate and Chlorides. The concentrations of Na and Al showed remarkably high levels in traffic prone site, whereas, the concentration of Chlorine was distinctively high in the plant samples analysed from rural site. The combustion of agricultural residues generated the accumulation of non-metals such as KCl (Niemi J.V. *et al.* [16]) which could have caused high accumulation of Cl.In case of metals such as Mn and Sr, the concentrations are on par with the range of concentrations recorded in all 3 sites. Notably no trace of Ru and Mo were recorded in traffic prone area and no traces of RuO, BrO and Mo were reported from industrially polluted sites. In partial agreement with the present result, the XRF estimation of most abundant elements by Lough *et al.* [12] indicated the presence of Ca, Na, Mg, S, Al, and K in the road dust. The less abundant elements such as Pb, Ar, Cr, Cd, Ni, Mn, Cu and Zn were also observed in the same study on road dusts.

The concentration of Si (metalloid) was reported to be high in traffic prone area followed by rural area indicating presence of air suspended Si particles in the air owing to traffic and agricultural operations such as ploughing.

Metallic and non metallic oxides

Table-2 had clearly depicted that oxide concentrations for all non metals except for Na_2O, CaO was high for residential site sample compared to traffic prone and industrially polluted sites. Whereas, the percentage of accumulation of metallic oxides in the samples of traffic prone site recorded high values except for MgO than the residential and industrially polluted sites.

It was also obvious from Table 2, that the concentrations of the metals and nonmetallic oxides in the samples collected from industrially polluted area, remarkably was low except CuO, but still the results showed presence of oxides of heavy metals such as PbO. The bioaccumulation of heavy metals could be attributed to the presence of leather processing factory in the location. A similar result for higher concentrations of heavy metal oxides had been observed in the samples of industrially (cement factory) polluted area (Elena D. *et al.* [10]).

Spatial effects on bioaccumulation of metallic and non-metallic particulate pollutants for wet season:

Table-3. Mean concentration of different metals and nonmetals in plant leaf samples of R*icinus communis* collected from each site during wet season.

Estimated concentration (%)	Site 1	Site 2	Site 3
Ca	25.95%	57.09%	53.74%
K	55.28%	24.79%	26.29%
Cl	7.28%	7.61%	9.38%
S	4.39%	3.86%	3.49%
Mg	2.50%	2.04%	2.26%
Si	0.63%	1.26%	1.56%
P	2.37%	1.78%	2.13%
Fe	0.39%	0.68%	0.56%
Al	0.16%	0.41%	0.54%
Na	-	0.10%	0.18%
Mn	0.13%	0.06%	0.06%
Ru	-	0.05%	-
Sr	0.04%	0.04%	0.05%
Mo	0.06%	-	-
Ti	-	-	0.06%
Br	-	-	-

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Pb	0.58%	0.29%	0.29%
Cu	0.14%	0.03%	0.03%
Zn	0.11%	0.06%	0.06%

Table-4. Mean concentration of different metallic and nonmetal oxides in plant leaf samples of R*icinus communis* collected from each site during wet season.

Estimated concentration (%)	Site 1	Site 2	Site 3
CaO	36.31%	40.21%	35.52%
K_2O	32.44%	29.86%	31.67%
SO_3	10.95%	9.64%	8.72%
Cl	7.28%	7.61%	9.38%
P_2O_5	5.43%	4.07%	4.89%
MgO	4.15%	3.39%	3.75%
SiO_2	1.34%	2.69%	3.33%
PbO	0.63%	0.30%	0.30%
Fe_2O_3	0.56%	0.98%	0.80%
Al_2O_3	0.31%	0.77%	1.03%
CuO	0.17%	0.05%	0.04%
MnO	0.16%	0.09%	0.08%
ZnO	0.13%	0.08%	0.08%
MoO_3	0.09%	-	-
SrO	0.04%	0.05%	0.06%
Na_2O	-	0.14%	0.25%
RuO	-	0.05%	-
TiO_2	-	-	0.11%

Site1- Rural resident area; Site2 - Area polluted by Transportation; Site3- Industrially polluted area.

The above Tables 3 & 4 of relative comparison of metals nonmetals and their oxides between 3 sites illustrated that site 1(Rural residential site) recorded high concentrations of K, S, Mg, P, Mn, Pb, Cu and Zn; site 2 (Traffic prone site) measured high amounts of Ca and Fe; site 3 (Industrially polluted site) was found to have high levels of Cl, Si, Al, Na and Sr. Mo presence was noticed only in site 1. Whereas, Ru was found to be present only in site 2; Ti was present only in site 3.

Similarly for metal oxides site 1 had high K_2O, SO_3, MgO, P_2O_5, Pbo, MoO_3, CuO, MnO and ZnO; site 2 recorded high CaO, Fe_2O_3. High concentrations of oxides of Cl, SiO_2, Al_2O_3, SrO, and Na_2O were recorded in site3 (industrially polluted site).

The analysis showed that site 1 did not show evidence for the presence of Na, Ru, Ti, Br, Na_2O, RuO_2,TiO_2; and site 2 did not show evidence for the presence of Mo,Ti,Br,$TiO_2$and MoO_3; site 3 did not show evidence for the presence of oxides concentrations of Ru ,Mo and Br.

It was observed that $MoO_3$was present only in site1.RuO_2 was recorded only in site2, and TiO_2 was observed only in site 3.

Metals and nonmetals

The presence of all alkali earth metals except sodium were higher in residential site during wet season compared to dry season due to surface run off, leaching and percolation during precipitation. Pb was notably high in site1 but it was appreciably present in all 3 sites including residential, traffic prone and industrially polluted sites. According to Weerasuriya *et al*. [23], the lead contamination in residential area was due to industrial pollution in the city canal of Colombo, Srilanka which highlights the possibility of industrial leachates in the surface and underground water of residential locations during North east monsoon season in the study site. Sodium and Chlorine was observed high in industrially polluted area as sodium chloride is heavily used for tanning and leather treatment. According to Boopathy *et al.* [5], the solid wastes of leather industries are laden with sodium chloride as it is used for preservation of hides. As a leather industry is located in the present industrial study site, the above reason could logically fit into the reason for high levels of sodium and Chlorine recorded in the industrial site.

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Metallic and non metallic oxides

As heavy metal (Pb) contamination was high in the residential site, its corresponding oxide level was also recorded to be high in site1 (residential site). Oxides of Potassium, Phosphorous, Magnesium and Sulphur had been noticeably high in the rural residential area; these are main operative components of inputs as fertilizers for crop production. Although Iron particles had been recorded in all sites of present study, Iron as element and as oxide was found to be comparatively higher in traffic prone site owing to the metal body vehicle erosion and deposition as suspended particulate matter in the atmosphere. The study of Mafuyai *et al* [14] has determined that the reason for presence of Cu, Pb, Ni, Zn, Fe, Cd, Mn and Cr in the road dusts were due to human activities, emissions from vehicles and lithogenic occurrences of the metals owing to construction of roads. In perfect alliance with Na, and Cl bioaccumulation in industrially polluted sites as mentioned above, the oxides of sodium and chlorine was also observed to be higher in the present industrial study site where a leather industry is located.

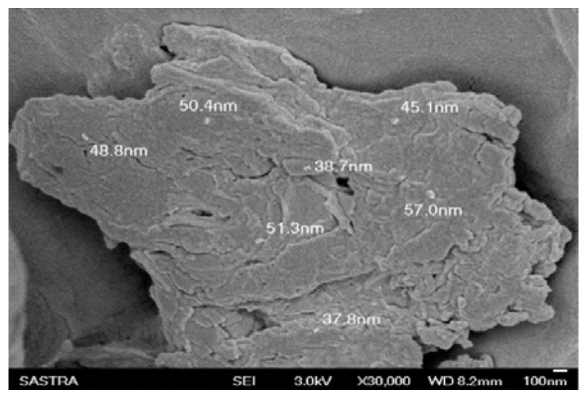
DISCUSSIONS

Critical comparison between wet season and dry season across residential, traffic prone and industrially polluted sites

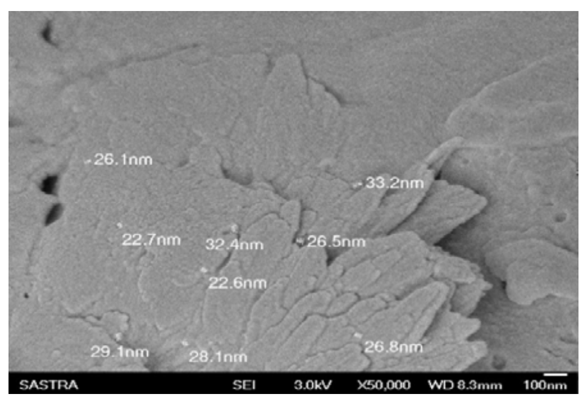
In both seasons, Ca and Fe phyto accumulation was recorded to be higher in traffic prone site owing to the reason that anthropogenic activity based pollution by vehicular emissions and construction of roads are ever present in any traffic prone site through the year. The level of Pb fluctuated from nil to high in rural residential site owing to high amount of bioaccumulation of leachates by the bio-indicator during wet season. Within each season, the prevalence of metal, non metals and the level of their respective oxides corroborated well with their elemental amounts of bioaccumulation (e.g.) Cl, Na, Fe, Pb. Moreover, Cu, Zn were available both in their elemental and oxides form in residential sites only in wet season and not in dry season attributing the reason of surface run off and percolation during precipitation. In addition, the suspended particulate matters from air might form deposits of Sulphur oxides and acid rains during dry and wet seasons respectively as indicated by the levels of high S, SO_3 in 2 seasons across 3 sites. Al-Jahdali and Bin Bisher [1] have claimed that SO_2 which is trapped in the lower part of the atmosphere gets accumulated on surface soil due to rainfall and the hot climate increases the solubility of SO_2 in the air. The elemental and oxide forms of Si were high in traffic prone site during dry season, whereas in wet season, the bioaccumulation of SiO_2 was recorded high in industrially polluted site. This may be attributed to the reason of high rate of air suspension during dry season at traffic prone area and its deposition in soil during wet season and its increased uptake due to rapid vegetative growth of the bio-indicator (Castor) during monsoon and its increased phyto-accumulation from soil in the outlets of industrial wastes.

Micrographic images of metallic and metallic oxide particulate pollutants

The overall micrographic images indicated the deposits of metallic and metallic oxide pollutants ranging from size of 22.6nm to 548nm. It was remarkable to note that the size of particulate pollutant deposits were in the high range of 226nm to 548nm which attributes to the presence of metallic oxides especially heavy metal oxides such as PbO which corroborates well with the XRF results as enlisted in Tables 2 & 4. The size of micrographic image particulate matters accumulated in the traffic prone area is comparatively more in the wet season than in the dry season implicating formation of more oxides and hydroxides of metals in wet season. The size of particulate pollutants in wet season is significantly higher than in the dry season of rural residential site owing to the reason of increased oxides due to agricultural operations such as ploughing, dusting of fertilizers, spraying of pesticides and combustion of agricultural wastes during wet season.



(a)



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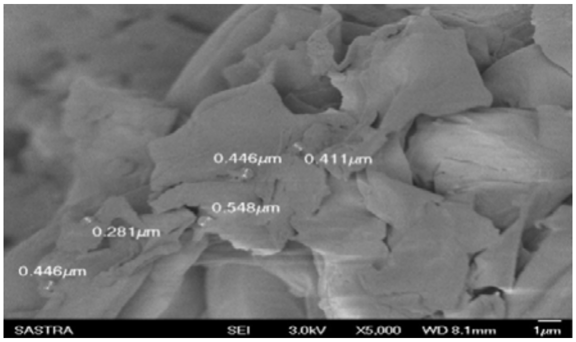
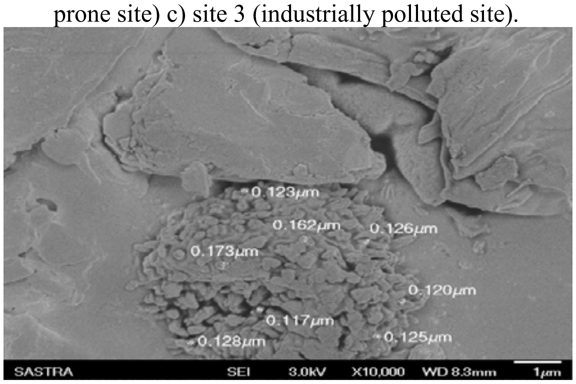


(c)

Figure-1. Micrographic images of samples from dry season at a) site 1(rural residential site) b) site 2 (traffic prone site) c) site 3 (industrially polluted site).



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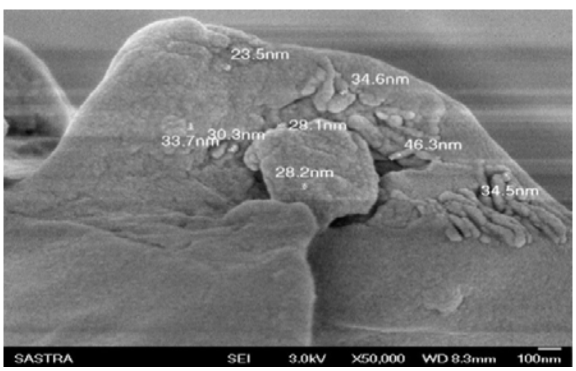

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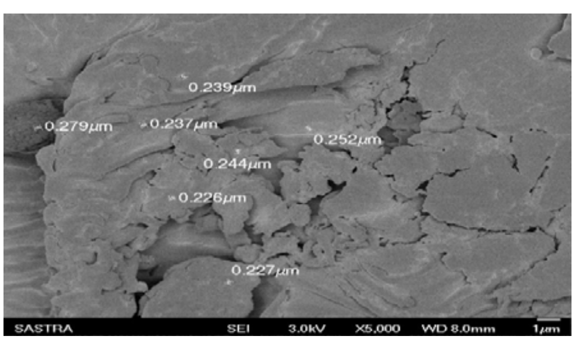

(f)

Figure-2. Micrographic images of samples from wet season at d) site 1(rural residential site) e) site 2 (traffic prone site) f) site 3 (industrially polluted site).

CONCLUSIONS

The high throughput techniques employed in the present study were precise enough to determine the spatio-temporal variations induced fluctuations in the particulate pollutants of metals and metallic oxides of the bioindicator- *Ricinus communis.* This plant serves as the best bioindicator with varied deposits of particulate pollutants in multi locations and bivariate seasons. The results clearly demarcate the phyto accumulation of metal and metallic oxide pollutants differentially scoring high for residential, traffic prone area and industrially polluted site for both dry and wet seasons. In addition a distinctive accumulation in particle size was noted between wet and dry seasons of rural residential sites. The castor plants could serve as a good pollution bioindicator. Thus, this effort has rendered to clearly understand that there is a definite spatial and temporal impact both in the concentration as well as in the size of particulate pollutants of metals and metallic oxides bioaccumulated depending upon the location and season. This could be used as a best parameter in estimating extent of air pollution for epidemiological studies. Moreover, castor can be used both as a bio-indicator and as a detoxifier appreciating its capability of phyto-accumulation of metallic and non metallic elements and oxides.

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