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Azadirachta indica as indicator for heavy metals pollution

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ABSTRACT

In this study, the *Azadirachta Indica* tree was evaluated as the biomonitors of heavy metals such as Fe, Mn, Zn and Cu contaminated in Madurai City. The soil samples at depth (0-20cm) and Azadirachta Indica leaves were taken from different sampling sites namely Kalavasal (S1), Palaganatham (S2), Periyar (S3), Simmakal (S4), Goripalayam (S5) and Mattuthavani (S6). Then, the concentrations of Fe, Mn, Zn and Cu were measured using Flame Atomic Absorption Spectrophotometer Perkin Elmer Model 1100. The result of this study showed that the concentration of Fe, Mn, Zn and Cu varied between 11.96µg/m^3-21.6 µg/m^3, 5.36 µg/m^3 - 16.25 µg/m^3, 4.38 µg/m^3 - 7.22 µg/m^3 and 1.58 µg/m^3 - 5.03 µg/m^3respectively at depth 0-20cm. The concentration of heavy metals such as Fe, Mn, Zn and Cu in *Azadirachta Indica* ranged between 206.92 µg/m^3- 1661.3 µg/m^3, 45.0 µg/m^3 – 273.75 µg/m^3, 112.67 µg/m^3 – 345.67 µg/m^3 and 15.0 µg/m^3 - 23.25 µg/m^3 respectively. According to these results the concentration of heavy metal Fe was found to be high in all the sampling sites at depth of soil 0-20cm. The mobility ratio value for heavy metals in *Azadirachta Indica* was found to be greater than one in all the sampling sites, thus *Azadirachta Indica* is said to be heavy metal accumulator. The variation in heavy metal concentrations is due to changes in traffic density and anthropogenic activities. It is concluded that *Azadirachta Indica* can be applied to monitor polluted sites.

1.Introduction

One of the most serious environmental problems is the accumulation of heavy metal in plants, and hence in the food chain, as a result of the pollution of soils and water with these substances. The pollutants in the urban atmosphere are discharged from many sources the major contributors are traffic and industrial establishments. As such, an increased concern all over the world has been observed lately over metal pollution. A lot of work has been done all over the world related to the heavy metal bio monitoring features of plants; Seaward and Mashhour (1991), Ozturk and Turkan (!993), Sawidis et al; (1995), Aksoy and Ozturk (1996;1997). Increasing of anthropogenic activities leads to the emission of various pollutants into the environment and different types of hazardous substances are consequently appeared into the atmosphere (Onder and Dursun, 2006; Kho et al; 2007, Sarala et al; 2012). The use of plant tissues in sampling has long been shown to be an effective indicator of atmospheric pollution (Goodman and Roberts, 1971). Vegetation is a proper indicator to assess the impact of a pollution source on the vicinity which is due to high metal accumulation of plants (Onder and Dursun, 2006). Heavy metals cause serious environmental risks and therefore, its effect has been examined extensively (Abdel-Ghani et at; 2007). Uptake of elements into plants can happen via different ways. The elements can be taken up via roots from soil and transported to the leaves; also they may be taken up from the air, or by precipitation directly via the leaves. According to Wittig (1993), the basic criteria for the selection of a species as a bio monitor, it should be represented in large numbers all over the monitoring area, have a wide geographical range, should be able to differentiate between air borne and soil borne heavy metals, be easy to sample and there should be no identification problems. *Azadirachta Indica* has been widely found all over the Madurai region as medicinal plants and shade bearing characteristics. It was selected for bio monitoring studies because it fulfills all the basic criteria given by Witting (1993). The aim of this study was to investigate the metal pollution levels in the Madurai region at different sampling sites. The heavy traffic load are the main cause of Pollution in this area in particular heavy metal pollution.

2. Materials and Methods

2.1 Study area

Madurai is the city of southern region, of Tamil Nadu which has an elevation of around above sea level, at latitude 9.933 and longitude 78.1167. The city suffered from high traffic density caused by vehicles. The average number of vehicles movement per hour in the study area are 460, 540, 645,745, 777, 954, and 975 respectively. The selected sampling plant species such as *Azadirachta Indica* covers the majoring of urban trees in Madurai city due to its ever greenness.

The selected sampling sites for the study are given in Table 1.

Table 1 Six Sites were selected around Madurai city for data collection

Sampling Sites	Name of the site
S1	Kalavasal
S2	Palaganatham
S3	Periyar
S4	Simmakal
S5	Goripalayam
S6	Mattuthavani

2.2 Sampling and analysis

The soil samples and plant leaves were collected in six different locations during the month of July and August 2012. Twelve samples of soil at surface level (0-20cm) and plant

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leaves were selected from each sampling sites covering the traffic area. The soil samples and plant leaves were separately collected into a clean cellulose bags and brought to the laboratory on the same day. The collected soil samples were air-dried and sieved into coarse and fine fractions. Well mixed samples of 2g each were taken in 250ml glass beakers and digested with 8 ml of aqua regia on a sand bath for 2 hours. After evaporation to near dryness, the samples were dissolved with 10 ml of 2% nitric acid, filtered and then diluted to 50 ml with distilled water. Fe, Mn, Zn and Cu heavy metal concentrations of each fraction were analyzed by Atomic Absorption Spectrophotometry (Perkin Elmer Model 1100). Quality assurance was guaranteed through double determinations and use of blanks for correction of background and other sources of error. The *Azadirachta Indica* leaves were carefully washed three times with distilled water to remove adhering particle (Babaoglu, et al; 2004). All samples were weighed and then dried in an oven at 70c for 48hr. The samples (1g) of finely ground samples were digested with concentrated HNO_3 in a microwave system. Heavy metal concentrations were measured by the flame atomic absorption spectrophotometer Perkin Elmer AAS analysis 300 model, with three replicates.

Fig 1. Azadirachta Indica leaves

2.3 Analytical methods

For the limited number of samples and locations, exploratory data methods were used to study and present the data. The use of box plots median minimizes the problems related both to the fact that different number samples from different catchments were used together with the skew stations and some outliers that play an important role in the data set. Mobility ratio that expresses the ratio of metal concentration in plants to its concentration in soil.

$MR = M_{plant} / M_{soil}$

3. Results and Discussion

3.1 Heavy metal concentration in soil

The heavy metal concentration in soil sample at depth 0-20cm and in *Azadirachta Indica* are shown in table 2

The heavy metal concentration in the soil (0-20cm depth)at the sampling site 1 was found to be in the order of Fe (14.7 $\mu g/m^3$) > Mn (10.1 $\mu g/m^3$) > Zn (4.4 $\mu g/m^3$) > Cu (1.9 $\mu g/m^3$). At sampling site 2 the metal concentration in the soil at depth 0-20cm was in the order of Fe (14.4 $\mu g/m^3$) > Zn (7.2 $\mu g/m^3$) > Mn (5.4 $\mu g/m^3$) > Cu (5.0 $\mu g/m^3$). It has been found that the concentration of heavy metals in the soil (0-20cm depth)at the sampling site 3 was in the following order of Fe (18.1 $\mu g/m^3$) > Mn (8.9 $\mu g/m^3$) > Zn (7.1 $\mu g/m^3$) > Cu (2.2 $\mu g/m^3$). The heavy metal concentration at sampling site 4 in the soil (0-20cm depth)was in the order of Fe (21.6 $\mu g/m^3$) > Mn (6.7 $\mu g/m^3$) > Zn (6.1 $\mu g/m^3$) > Cu (2.4 $\mu g/m^3$). At sampling site 5 in the soil (0-20cm depth) , the heavy metal concentration were found to be in the order of Mn (16.3 $\mu g/m^3$) > Fe (11.9 $\mu g/m^3$) > Zn (5.9 $\mu g/m^3$) > Cu (2.3 $\mu g/m^3$). The heavy metal concentration in the soil (0-20cm depth) at sampling site 6 was in the order of Fe (15.7 $\mu g/m^3$) > Mn (9.1 $\mu g/m^3$) > Zn (4.9 $\mu g/m^3$) > Cu (1.6 $\mu g/m^3$). On comparing, the heavy metal concentration in the surface soil iron was found to be maximum in all five sampling site except (S5) { Fe [14.7 $\mu g/m^3$ (S1)], [14.4 $\mu g/m^3$ (S2)], [18.1 $\mu g/m^3$ (S3)], [22.4 $\mu g/m^3$ (S4)]and [15.7 $\mu g/m^3$ (S6)]}. The concentration of Mn [16.3 $\mu g/m^3$] was maximum in the sampling site (S5). The reason for the maximum concentration of Fe is due to the automobiles cause iron contribution to the environment from urbanization. The auto body rust and engine parts are releasing iron to the environment. Manganese could also come from metallurgical industries and is a component of antiknock compounds. The heavy metal concentration in plant leaves at the sampling site 1 was found to be in the order of Fe (485$\mu g/m^3$) > Zn (346 $\mu g/m^3$) > Mn (56 $\mu g/m^3$) > Cu (22 $\mu g/m^3$). At sampling site 2, the metal concentration was in the order of Fe (673 $\mu g/m^3$) > Zn (233 $\mu g/m^3$) > Mn (45 $\mu g/m^3$) > Cu (15.0 $\mu g/m^3$). It has been found that the concentration of heavy metals in plant leaves at the sampling site 3 was in the following order of Fe (768 $\mu g/m^3$) > Zn (114 $\mu g/m^3$) > Mn (45.0 $\mu g/m^3$) > Cu (20 $\mu g/m^3$) . The heavy metal concentration at sampling site 4 was in the order of Fe (1661 $\mu g/m^3$ > Zn (113 $\mu g/m^3$) > Mn (51 $\mu g/m^3$) > Cu (26 $\mu g/m^3$). The results implies that at sampling site 5 the heavy metal concentration were found to be in the order of Fe (451 $\mu g/m^3$) > Zn (149 $\mu g/m^3$) > Mn (52 $\mu g/m^3$) > Cu (20 $\mu g/m^3$). The heavy metal concentration at sampling site 6 was in the order of Mn (274 $\mu g/m^3$) > Fe (207 $\mu g/m^3$) > Zn (180.0 $\mu g/m^3$) > Cu (17.0 $\mu g/m^3$) On comparing, the heavy metal concentration in plant leaves iron was found to be maximum in all five sampling site except (S6) { Fe [485 $\mu g/m^3$ (S1)], [673.0 $\mu g/m^3$ (S2)], [768.0 $\mu g/m^3$ (S3)], [1661 $\mu g/m^3$ (S4)]and [451.0 $\mu g/m^3$ (S5)]}. The concentration of Mn [274 $\mu g/m^3$ (S6)] was maximum in site S6. Since the concentration of Fe found in all the sampling stations were high at depth of soil (0-20cm) , the content of heavy metals in *Azadirachta Indica* was found to be high.

3.2 Mobility Ratio

The mobility ratio values at soil depth (0-20cm) for *Azadirachta Indica* at different sampling sites are given in table 3.

Table 3 Mobility ratio values at soil depth (0-20 cm) for *Azadirachta Indica*

Sampling site	Mobility ratio			
	Fe	Mn	Zn	Cu
Site 1	0.33	5.56	78 .99	11.70
Site 2	46.70	8.39	32.27	2.98
Site 3	42.45	5.03	15.97	9.30
Site 4	76. 89	7.62	18 .40	10.66
Site 5	37.71	3.20	25.34	8.81
Site 6	13.18	29.98	36.36	10.76

The ratio between plant and soil concentration (MR) is an index of element soil-plant transfer that may favor the understanding of the plant uptake characteristics (Chamberlain, 1983). MR > 1 indicates that the plant enrich these elements, a ratio at around 1 indicates a rather in different behavior of the plant towards these elements (indicator, Baker, 1981) and a ratio clearly <1 shows that the plant exclude these elements from uptake (excluder, Baker 1981). Table 4 displays the mean MR values for soil and leaves. The results revealed that the plants are enriched with heavy metals such as Mn, Zn and Cu in all the sampling sites (MR>1). But the mobility ratio value was less than one for Fe in sampling site 1 (Kalavasal), which infers that the *Azadirachta Indica* leaves exclude this metal uptake. Since MR > 1 for Fe, Mn, Zn and Cu heavy metals indicating *Azadirachta Indica* acted as heavy metal accumulator.

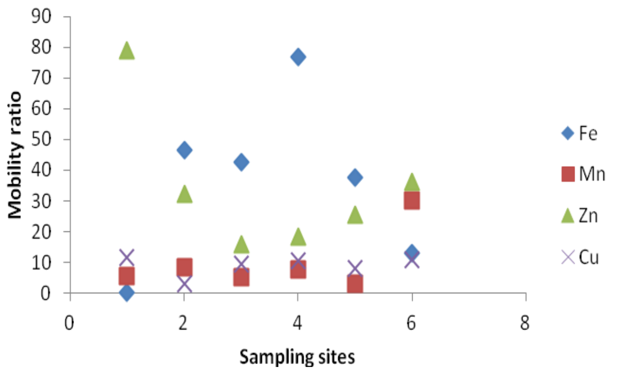
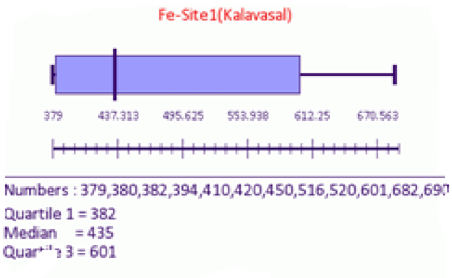
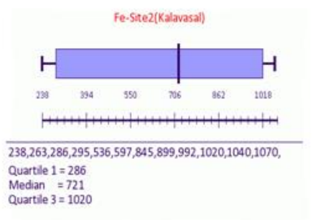
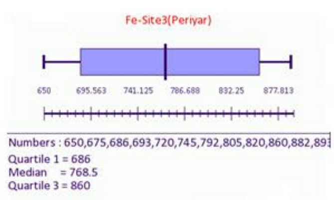


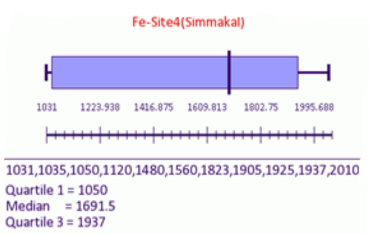
Fig 2. Mobility ratio soil depth 0-20cm for *Azadirachta Indica*

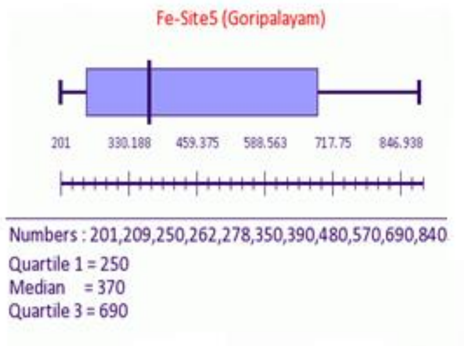
Analytical results for heavy metals such as Fe, Mn, Zn and Cu in all sampling sites are given in fig 4, 5 and 6 as box plots with metals sorted by increased median value.











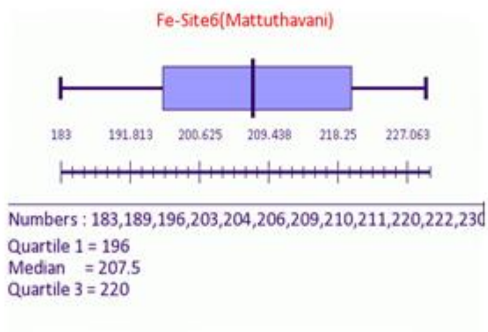
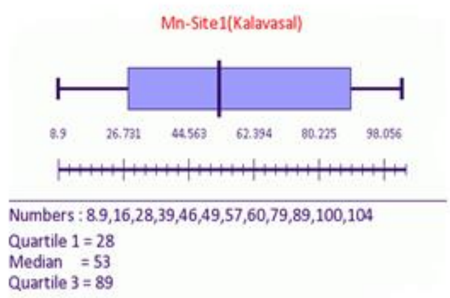
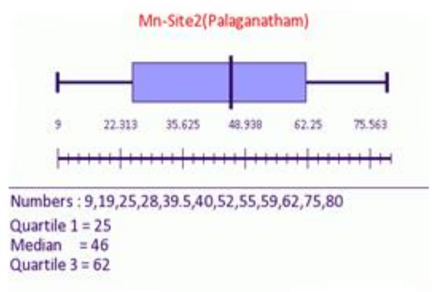
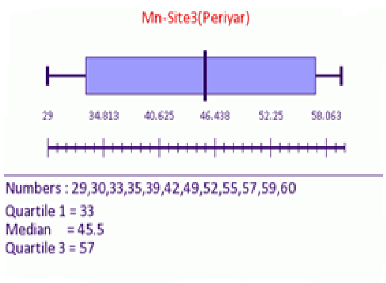


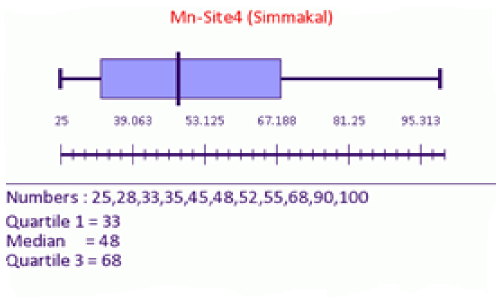
Fig 3. Box plot for heavy metal -Fe

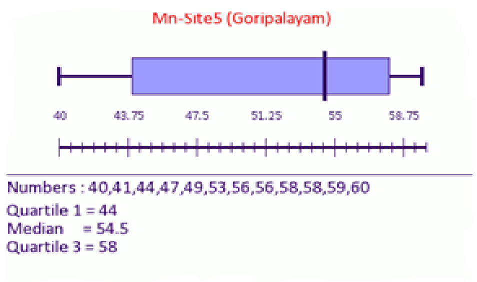
From the fig 3, it has been revealed that the concentration of heavy metal Fe at all sampling sites ranged between 207.5 μg/m^3 to 1691.5 μg/m^3. On comparing the concentration of metal Fe, at sampling site 4 (Simmakal) was found to be maximum. Since the heavy metal Fe is a typical soil constituents.











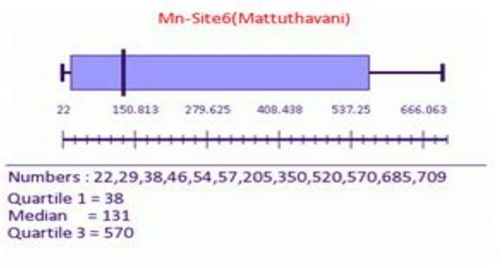
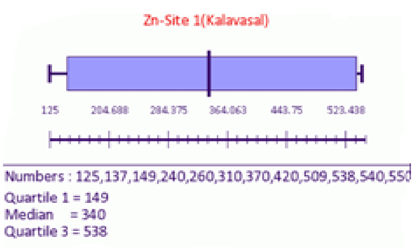
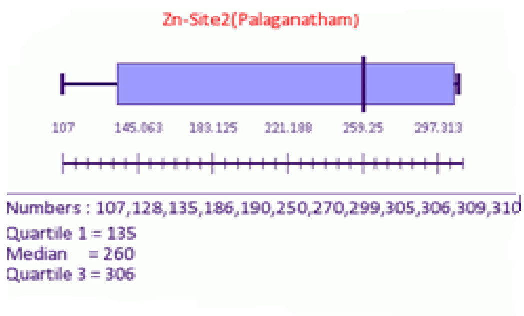
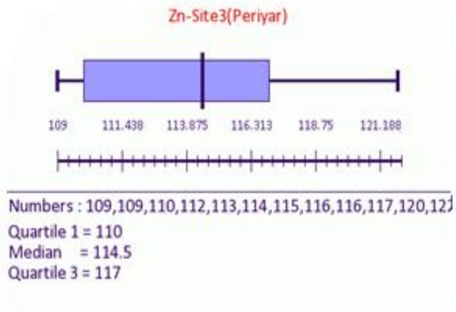


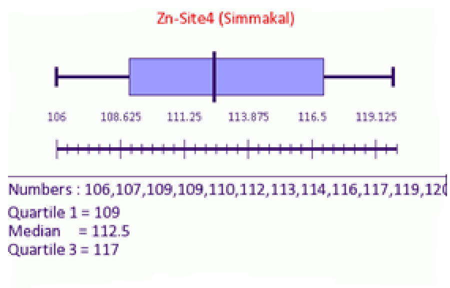
Fig 4. Box plot for heavy metal – Mn

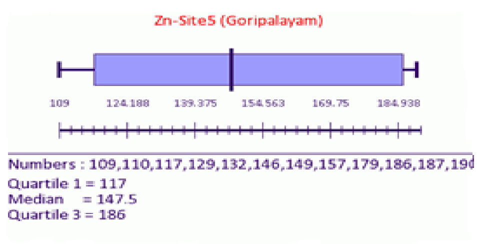
The fig 4 implies that the concentration of heavy metal Mn at all sampling sites ranged between 45.0 µg/m^3 to 276.92 µg/m^3. It was observed that the concentration of Mn was maximum at sampling site 6(Mattuthavani). The major source of Mn is metallurgical industries and is a component of anti-knock compounds (Querol et al; 2002).











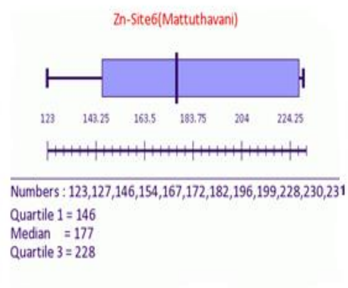
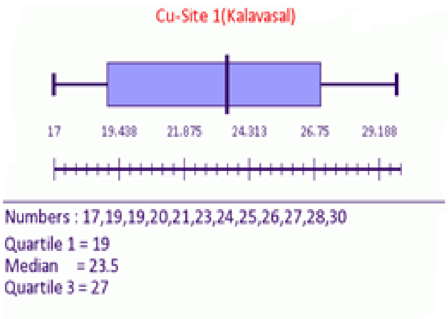
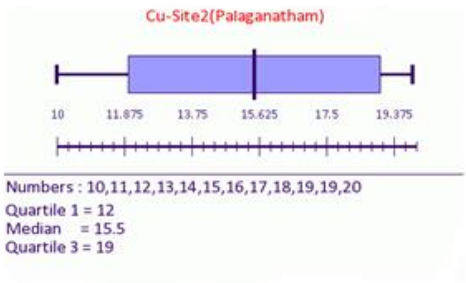
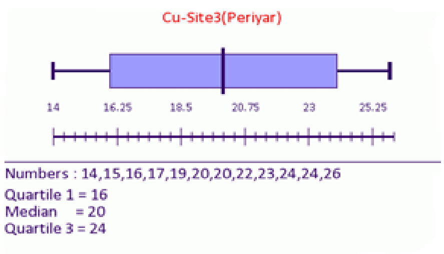


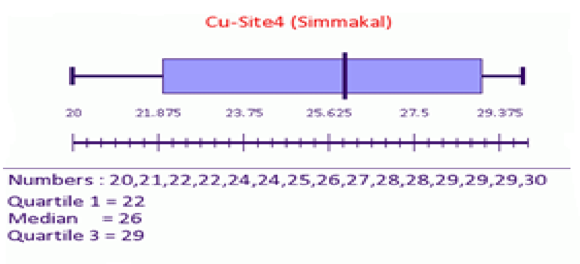
Fig 5. Box plot for heavy metal – Zn

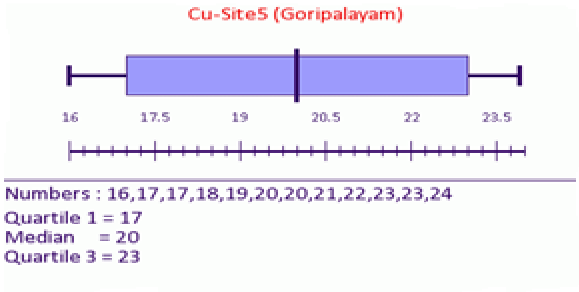
From the fig 5, it has been revealed that the concentration of Zn at all sampling sites was ranged between 112.67 µg/m^3 to 345.67 µg/m^3 . The concentration of heavy metal Zn was found to be maximum at sampling site 1 (kalavasal) on comparing it with other sampling sites. Zinc is ubiquitous in the environment. It is a component of tires, which is released as they wear (Doss et al; 1995). Zn is a microelement essential in all organisms and plays an important role in biosynthesis of enzymes. Zn is not considered to be highly phytotoxic and toxicity limit for Zn (300-400 mg kg^{-1}) depends on the plant species, as well as on the growth stage (Kabata –Pendias and Pendias 2001). According to Padmavathiamma and Li (2007), normal concentrations of Zn in plants are in the range of 10-150 mg kg^{-1}.











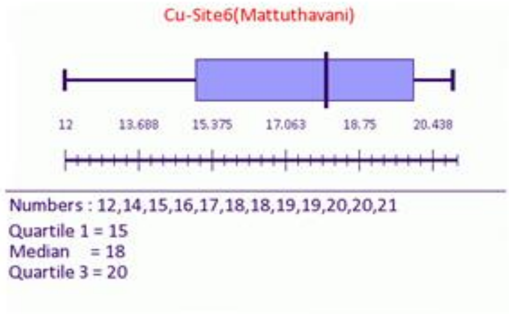


Fig 6. Box plot for heavy metal- Cu

The fig 6 revealed that the concentration of heavy metal Cu was ranged between 17.42 µg/m^3 to 25.75 µg/m^3. On comparing, it has been inferred that metal Cu was found to be maximum at sampling site 4 (Simmakal). A certain content of Cu in plants is essential for their health. The normal concentrations of Cu in plant shoots range from 2-20mg kg^{-1} dry weight, and the levels of 30mg kg^{-1} are phytotoxic (Kabata-Pendias and Pendias 2001). According to Padmavathiamma and Li (2007) concentrations of Cu in the range of < 1-5mgkg^{-1} are deficient, from 3-30mg kg^{-1} are normal, and phytotoxic concentrations in plants are in the range of 20-200mg kg^{-1}. Under natural and anthropogenic conditions the majority of plant species can accumulate much more Cu (Kabata-Pendias and Pendias, 2001).

The data presented in the table 5 revealed the average concentration of heavy metals such as Fe, Mn, Zn and Cu at different sampling sites namely Kalavasal (S1), Palaganatham (S2), Periyar (S3), Simmakal (S4), Goripalayam (S5) and Mattuthavani (S6) at different depth 0-20cm and 20-40cm. It has been found that the concentration of heavy metal Mn was within the normal range (20-10,000 µg/gm) at different soil depth 0-20cm and 20-40cm. The major source identified may be due to cumulative effect of coal burning and fugitive emissions. The results revealed that in all the sampling sites Zn concentrations was within the recommended range (1-100µg/gm) value. Hence, despite the close proximity of cultivated land to high-traffic areas, roadside soil was not significantly contaminated by Zn from motor vehicles. Similarly, the concentration of heavy metal Cu was within the normal range (2-250 µg/gm) at different soil depth 0-20cm and 20-40cm. The probable source of Cu is due to the combustion petrol products which contain Pb and Cu (J.T. Nyangababo, et al; 1986), Cu is also derived from engine wear (M.S. Akhter, et al; 1993).

Table 2 Concentration of heavy metals in road side soil and plant leaves (*Azadirachta Indica*) (μg/m³) at different sampling site

Sampling site	Statistical data	Heavy metals in soil (0-20cm)				Heavy metals in *Azadirachta Indica*			
		Fe	Mn	Zn	Cu	Fe	Mn	Zn	Cu
Site 1	Min	14.20	9.50	4.00	1.40	379.00	9.00	125.00	17.00
	Max	15.10	10.70	4.80	2.40	690.00	104.00	550.0	30.00
	Mean	14.71	10.08	4.38	1.88	485.33	56.33	345.67	23.25
	Median	14.75	10.05	4.35	1.85	435.00	53.00	340.00	23.50
	SD	0.28	0.39	0.23	0.31	116.27	31.49	165.14	4.09
	CV	0.02	0.04	0.05	0.17	9.46	2.59	13.27	0.52
Site 2	Min	13.90	4.70	6.30	4.40	238.00	9.00	107.00	10.00
	Max	14.90	6.10	7.90	5.70	1070.00	80.00	310.00	20.00
	Mean	14.41	5.36	7.22	5.03	673.00	45.00	233.0	15.00
	Median	14.45	5.25	7.35	5.05	721.00	46.00	260.00	20.00
	SD	0.30	0.43	0.52	0.36	339.70	22.31	79.08	3.37
	CV	0.02	0.08	0.07	0.07	27.19	1. 87	6.44	0.42
Site 3	Min	17.60	8.50	6.30	1.60	650.00	29.00	109.00	14.00
	Max	18.60	9.40	7.80	2.70	893.00	60.00	122.00	26.00
	Mean	18.09	8.95	7.14	2.15	768 .42	45.00	114.42	20.00
	Median	18.05	8.95	7.20	2.15	768 .50	45.50	114.50	20.00
	SD	0.30	0.26	0.47	0.35	85.00	11.67	4.12	3.91
	CV	0.02	0.03	0.07	0.17	7.16	1.09	0.95	0.49
Site4	Min	20.70	5.90	5.30	1.90	1031.00	25.00	106.00	20.00
	Max	22.40	7.20	6.90	2.90	2060.00	100.00	120.00	30.00
	Mean	21.60	6.69	6.14	2.44	1661.30	50.92	112.67	25.75
	Median	21.55	6.75	6.20	2.45	1864.00	46.50	112.50	26.50
	SD	0.47	0.36	0.53	0.33	403.21	24.16	4.64	3.36
	CV	0.02	0.06	0.09	0.14	32.35	2.02	0.96	0.54
Site 5	Min	11.40	15.40	5.40	1.70	201.00	40.00	109.00	16.00
	Max	12.60	16.90	6.50	2.90	890.00	60.00	190.00	24.00
	Mean	11.96	16.25	5.88	2.27	490.83	51.75	149.25	20.00
	Median	11.95	16.25	5.85	2.25	370.00	54.50	147.50	20.00
	SD	0.39	0.43	0.33	0.34	244.43	7.26	30.60	2.66
	CV	0.03	0.03	0.06	0.12	19.58	0. 84	2.65	0.43
Site 6	Min	15.00	8.50	4.00	1.00	183.00	22.00	123.00	12.00
	Max	16.30	9.90	5.70	2.20	230.00	709.00	231.00	21.00
	Mean	15.71	9.14	4.95	1.58	206.92	273.75	179.58	17.42
	Median	15.75	9.05	5.05	1.65	207.50	131.00	177.00	18.00
	SD	0.41	0.45	0.51	0.36	13.48	276.92	165.14	4.09
	CV	0.03	0.05	0.11	0.24	1.61	22.14	3.25	0.41

Table 4 Heavy metal concentration in soil sample at surface (0-20cm) with normal range

Heavy metals	Normal Range μg/gm	Critical soil concentration μg/gm	Average concentration in soil samples (0-20cm)					
			S1	S2	S3	S4	S5	S6
Fe	----	-----	14.71	14.41	18.09	21.60	11.96	15.71
Mn	20 - 10,000	1500-3000	10.08	5.36	8.95	6.69	16.25	9.14
Zn	1 - 900	70-400	4.38	7.22	7.14	6.14	5.88	4.95
Cu	2 - 250	60-125	1.88	5.03	2.15	2.44	2.27	1.58

Table 5 Heavy metal concentration in *Azadirachta Indica* with normal range

Heavy metals	Normal Range μg/gm	Average Concentration of heavy metals in *Azadirachta Indica*					
		S1	S2	S3	S4	S5	S6
Fe	---	485.33	673.00	768.42	1661.3	490.83	206.92
Mn	20	56.33	45.00	45.00	50.92	51.75	273.75
Zn	80-100	345.67	233.00	114.42	112.67	149.25	179.58
Cu	2-20	23.25	15.00	20.00	25.75	20.00	17.42

All the heavy metal concentration (Fe, Mn, Zn and Cu) in soil were found to be within the normal range at all the sampling site and also at different depth.

The data presented in the table 6 implies that the concentration of heavy metals such as Fe, Mn, Zn and Cu at different sampling sites in *Azadirachta Indica*, Fe is not considered a toxic heavy metal, because of it function in a number of normal physiological processes in plants (Bailey and Danin, 1981). Manganese plays a significant role in carbon dioxide assimilation and nitrogen metabolism (Katyal and Randhawa, 1983). The minimal level of Mn for normal growth and development of plants is 20µg/m^3 (Jaloud, et al; 1994). The results revealed that the concentration of heavy metals Mn was found more than sufficient for the normal growth and development. Zinc is an essential element in all organisms and considered an important factor in the biosynthesis of enzymes, auxins and some proteins. Plants with symptoms of Zn deficiency experience a retarded elongation of cells. A critical toxic level of Zn in the leaves is about 100 ppm in dry plant matter (Allen et al; 1974; Yilmaz and Zengin, 2003). The high content of Zinc in plant leaves may cause the loss of food production and low levels in plants may cause deformation of leaves (Bucher and Schenk, 2000; Celik et al; 2005; Kashem et al; 2007). A report from the study carried out by Fatoki and Ayodele (1991), showed that high concentration of Zn in the vegetation of roadside was as a result of motor vehicle emission because Zinc additives are often used as lubricants in oils. The report implies that the concentration of Zn exceeded the normal range in all the sampling sites. Copper is minor trace metal, with 70% copper in leaves contained in the chloroplast of land plants (Wilkinson, 1994). It is an important constituent of many enzymes of oxidation-reduction reactions (Celik et al; 2005). Kabata-Pendias and Piotrowska (1984) reported the normal content of Cu in plants ranges to be 2-20 ppm, but in most plants, the normal Cu contents are in a lower range of 4-12 ppm. Results indicated that the highest Cu value was found in sampling sites S1(23.25µg/m^3) and S4 (25.75 µg/m^3) whereas in rest of the sampling sites the concentration of Cu was found to be minimum. It was concluded that the heavy metals concentration in leaves of *Azadirachta Indica* were found to be above the normal range at all the sampling sites.

Conclusion

The results of our study indicate that the concentration of heavy metals such as Fe, Mn, Zn and Cu from the traffic area is an indicative of anthropogenic pollution. It was concluded that with an increase in the amount of heavy metals in soil, their uptake by plants also increased. The mobility ratio analysis data also showed that heavy metals (Fe, Mn, Zn and Cu) are highly trans located from soil to plant leaves in all the sampling sites. According to our study, *Azadiracta Indica* located in the sampling sites are said to be a heavy metal accumulator. High metal concentrations in plants are contained in urban and highway roadsides due to the anthropogenic activities in addition to the traffic density. The heavy metal concentration was maximum in the study area of Maduria city indicates the need for pollution control in the city environment. *Azadirachta Indica* is widely distributed in Madurai city and is used as a road side ornamental and herbal tree. In accordance with the data presented here, Azadirachta Indica possess all the characteristic for its selected as a bio monitor.

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