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RESEARCH ARTICLE

HEAVY METAL MONITORING BY *LIGUSTRUM LUCIDUM, FAM: OLEACEAE* VASCULAR PLANT AS BIO-INDICATOR IN DURRES CITY

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ABSTRACT

The object of this study is to determine and compare level of heavy metal in the leaves of vascular plants. The study was conducted in Durres area, Albania. During March 2013 these leaves were collected with different anthropogenic impact in the city. Concentrations of Cu, Pb, Zn, Mn, Fe, Na, K, Mg, Ca and Hg present in leaves samples have been detected using furnace AAS. To study the level of accumulation of each element in leaves sample was carried out by statistical analysis. The trace metal contents were expressed as arithmetic means and standard deviation. Statistical analysis of the data was carried out using EXCEL and MINITAB-15 Package Programs. The trend of the distribution of each element is: Hg< Pb< Cu< Zn< Mn<Fe< Na< Ca < Mg< K. Correlation analysis ($p<0.05$) was carried out on the data set of heavy metals to describe their behavior and the association. A good correlation were found between Pb-K ; Mn-Ca; Fe- Mg and Cu- Mg, suggested the same origin of pollution. *Ligustrum lucidum* leaves showed the ability to absorb heavy metals. The data were also processed with factor analysis (FA) in order to identify the main source categories of the analysed samples regarding site contamination and elements distribution.

INTRODUCTION

Environment pollution of heavy metals is an universal problem, because heavy metals are indestructible and most of them have toxic effects on living organisms, when permissible concentration levels are exceeded. (Amusan *et al.,* 2003) In urban areas the population is very numerous and the vehicle traffic is relatively high, so the exposure of people to the traffic related concentrations is significant. In the last years, the vehicle traffic increased especially in these areas. As a consequence, although there were implemented new techniques for controlling the emissions the urban air quality was not improved significantly. In cities with many tall buildings, placed on both sides of the street, the pollution level often does not comply with air quality standards. (Popescu *et al.,* 2011) Every year, millions tones of toxic pollutants are emitted in the air, both from natural sources and especially from anthropogenic sources. There are four categories of emission sources: stationary (industrial processes, industrial and domestic combustions); mobile (road and stationary traffic); natural (volcanic eruptions, forest fires); accidental pollutions (discharges, industrial fires). In the most cases, heavy metals pollution is a problem associated with the intense industrialized areas. However, high vehicle traffic was proved to be one of the important heavy metals emissions sources. Zinc, copper and lead are three of the most common heavy metals emitted by vehicle traffic. Also, vehicle traffic is responsible for the emission of some small quantities of other metals, like nickel and cadmium. (United States Office of Air Quality, 2007) Bio-indication methods were more frequently used last year, basically for heavy metals in environment pollution study (Gjorgieva *et al.,* 2011) The high concentration of heavy metals in plants is reflected by high concentration in animal and in human bodies. The abilities of some plants to absorb and accumulate metals make them useful as indicators of environmental pollution. *Ligustrum lucidum, Fam: Oleaceae* is one of the well known plants used as bio-indicator. Municipality of Durres (2015) (Bekteshi *et al.,* 2013). It is geographically widely spread and easily to identify, characterized by a relatively high tolerance to environmental pollutants showing a good correlation between pollution level of the element in the air and the concentration of this element in plant tissues. *Ligustrum lucidum* (Mills *et al.,* 1985) tree grows in full sun or partial shade on various soil types, and is moderately salt-tolerant. It is used as a street tree along boulevards where in these locations the space is limited. (Kabata *et al.,* 1997) This species plant require only pruning to maintain shape and remove dead wood. (Brodribb *et al.,* 2010) The aim of this study is to investigate the level of metal contaminants (Cu, Pb, Zn, Mn, Fe, Na, K, Mg, Ca and Hg) in vascular plants of Durres area, using one vascular plant species that is able to accumulate the heavy metal in the air. The most toxic metals for vascular plants are Zn, Cu, Ni, Cd and Hg.

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(Krolak *et al,* 2003) Toxic metals are biological magnified through the food chain. They infect the environment by affecting soil properties, its fertility, biomass, crop yields and essentially human health. It is a big issue of accumulation of heavy metals in soils as a result of industrial effluents and atmospheric emissions like paper mill, fertilizers, glasses and Mining wastes. (Mudgal *et al.,* 2010) The presence of heavy metals in toxic concentrations can result in the formation of superoxide radicals ($O^{-}2$), in hydrogen peroxide (H2O2), in hydroxyl radicals (OH^{-}), etc, can cause severe damage to bio-molecules like lipids, proteins and nucleic acids. Cu and Zn (Smolders *et al.,* 2002) can increase the activity of various antioxidant enzymes and non-enzymes like glutathione. The level of heavy metals absorbed in plants, depends not only from the physical and chemical composition of the heavy metals in the air. The concentration varies in different parts of plants. In this case exception makes the vascular plant, because it has the ability to tolerate elevated levels of heavy metals and accumulate them in very high concentration. Filipovic – Trajkovic (2012) Certain vascular plants absorb these toxic metals and help to clean up the air from them, being hyper accumulators. (Malaj *et al.,* 2011) These plants have shown to be resistant from heavy metals and are capable to accumulate them into their roots and leaves.

Heavy metals contamination is one of the major kind of environmetal pollution in urbanized cities due to emissions from heating, transport, industry and other human activities. In the past, the main contribution to heavy metals contamination has been due to using (Popescu *et al.,* 2011) anti-detonation agent in fuels. At the end of 1998, the European Parliament and Council with the Directive 98/70/EC prohibited the marketing of leaded petrol within their territory. Since that date, the contribution of heavy metal pollution depend from other anthropogenic sources (i.e., exhausted batteries, cars brake and other industrial wastes).

MATERIALS AND METHODS

Sampling

Durres is one of the largest cities of Albania, located near the Adriatic shore in western Albania. The main source for heavy metal pollution in the city is port activity. (Mills W. B *et al,* 1985) Type of vascular plant that was used for monitoring is : *Ligustrum lucidum*, *Fam: Oleaceae* plant. Samples were collected during the last 10 days of March 2013, at 13 stations in Durres city, at about one week after rainy days.

Fig. 1. *Ligustrum lucidum, Fam: Oleaceae* vascular plant

The different stations, where are taken the samples (with the only species: *Ligustrum lucidum*) are shown in Figure 1:

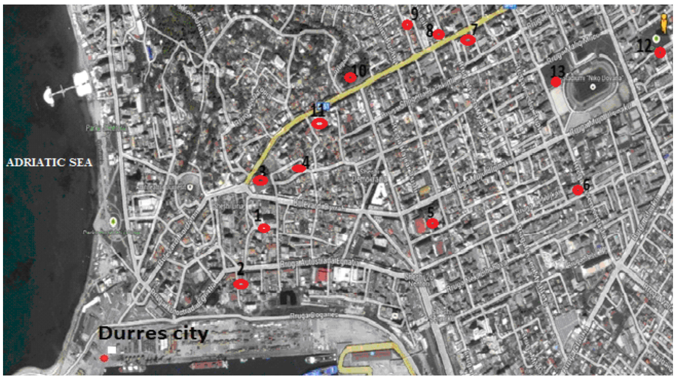

Fig. 2. The map of monitoring stations in Durres City, Albania

Sample digestion

After the transport of the leaves sample in laboratory, leaves samples were dried at ambient air on sheet papers until a constant weight was reached. Then the dried leave samples were homogenized to a fine material. The leaves were crushed by hand, wearing laboratory polyethylene gloves without powder. Dried plant samples were digested with nitric acid (ultra pure, 65%) and deionised water (9:1) in half pressure Teflon tubes. The experiment dishes are cleaned with dilute nitric acid and are washed with distilled water. Elements standard solutions used for calibration curves were prepared by dilution of base standard liquids of 1000 mg / L. 0.5 g dry leaves of each sample were digested with 10 mL of nitric acid HNO3 (9:1). Teflon tubes were closed and then were left at room temperature for 48 h, and then were digested for 3 hours at 80-90 ° C. Subsequently, the temperature was increased to 200° C for 1 hour to realize the full digestion. After cooling, the Teflon tubes were opened and left for evaporation of nitric acid to small volumes. Samples were then diluted with deionised water to a total volume of 50 mL.

The vascular plant digests were analysed for Cu, Zn, Mn, Fe, Na, K, Mg, Ca and Hg using flame AAS Atomic Absorption Spectrophotometry by using air-acetylene flame. Determination of Pb metal was made by using AAS with electro-thermal atomiser with graphite furnace. (Kròlak *et al.,* 2003)

Calibration method

Linear calibration method that find the link between the analytical signal and the measured analyses concentration were used for obtaining the calibration curves of each element. Blank solution of each element was used. Heavy metal concentration in samples were calculated based on their analytical signal (value of absorbance and the relevant equations of calibration curves).

RESULTS AND DISCUSSION

The descriptive statistic analysis was carried out on the data sets as reported in Table 1. The analytical data were subject to the statistical analysis. Statistical analysis of the data was carried out using EXCEL and MINITAB-15 Package Programs. In Table 2 we note the tendency for distribution of the elements listed in an ascending order of accumulation factor.

Being a coastal area affects in Hg level of concentration. The station that represents high value of Hg is located near the crossroad Nici A. and Goga A., which are affected from the presence of the sea. Accumulation (and distribution) of heavy metals with anthropogenic origin in vascular plant may depend

Table 2. Results of statistical data processing (EXCEL, Descriptive Statistic) (mg/kg, DW)

Parameters	Cu	Pb	Zn	Mn	Fe	Na	K	Mg	Ca	Hg
Mean	8.32	1.61	38.43	76.53	213.69	226.92	5737.6	3752.2	3505.7	0.268
Median	9.3	1.42	40.4	57.8	233	106	2406	3744	3288	0.2
Minimum	2.33	0.4	20.4	17.6	118	13	1956	1462	2423	0.07
Maximum	11.63	4.01	63.5	164.7	289	1191	26672	6502	5654	0.55

Table 3. Presentation of the reports Mean/Median and Max/Min

Parameter	Cu	Pb	Zn	Mn	Fe	Na	K	Mg	Ca	Hg
Mean/Med	0.89	1.13	0.95	1.32	0.917	2.14	2.38	1.002	1.01	1.34
Max/Min	4.99	10.02	3.11	9.35	2.44	91.61	13.63	4.45	2.33	7.85

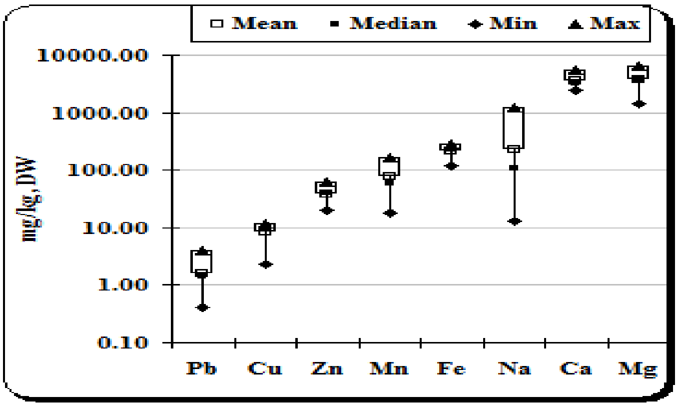

Fig. 3. The Box-plot graphic of the distribution of the elements (log- normal scale)

The trend of the distribution of each element is: Hg< Pb< Cu< Zn< Mn<Fe< Na< Ca < Mg< K. Here we can distinguish more clearly the changes in concentration of different elements in various monitoring points. K result the element with high level of concentration at Goga A. street. (A. Goga street is resulted with a high level concentration of K element) Ca and Mg are the only elements which have the same magnitude of the results, because they have a similar origin while Mg element is an important nutrient for *Ligustrum Lucidum* plants from natural origin.

on wet and dry depositions that convey particles from air to leaves. Resources of Cd, Zn and Ni are originated from oils, pneumatics and old car pieces in general, (Malizia *et al*., 2012) Copper (Cu) source is from the cars and other electric vehicles while Manganese (Mg) element comes from natural sources.

The concentration data that represent very high values of Max/Min values belong a Na, with high level of concentration at Grigor D. Street. This value is connected with anthropogenic origin from traffic emission and natural origin as an important nutrient for *Ligustrum lucidum* plants.

Descriptive statistical help us to explain the behavior of heavy metals

In Table 5 was note that Fe has low variation of the data(C%<23%); Mg, MN, Zn, Cu and Pb have a moderate variation of the data (C%<75%); Na and K have a high variation of the data (C%>75%) because they are an important nutrient elements for the plants. Another important parameter is Skewness. Ca, Pb, Na and K have a positive skewed that is affected strongly by anthropogenic factors. Correlation analysis ($p<0.05$) was carried out on the data set of heavy metals to describe their behavior and the association. The traffic emission was the reason for the high metals level in the samples for all the areas. It is reported from (Amusan *et al.,* 2003) that traffic density influences the increase of the concentration of heavy metals on plants alongside the roads.

Table 4. Correlations between elements Cu, Pb, Zn, Mn, Fe, Na, K, Mg, Ca, Hg. We note a high correlation between Pb-K ; Mn-Ca; Fe- Mg and Cu- Mg. It is related by the high traffic density of all types of automobiles

	Cu	*Pb*	*Zn*	*Mn*	*Fe*	*Na*	*K*	*Mg*	*Ca*	*Hg*
Cu	1.000									
Pb	0.090	1.000								
Zn	0.462	-0.112	1.000							
Mn	0.306	0.406	0.041	1.000						
Fe	0.398	0.147	0.285	0.355	1.000					
Na	-0.124	0.383	0.280	-0.103	0.306	1.000				
K	-0.115	0.792	-0.269	0.133	-0.211	0.007	1.000			
Mg	0.639	-0.004	0.262	0.491	0.683	-0.135	-0.141	1.000		
Ca	0.504	0.480	-0.060	0.699	0.457	0.051	0.111	0.576	1.000	
Hg	-0.157	-0.399	0.230	-0.006	0.255	-0.043	-0.373	0.237	-0.159	1.000

Table 5. Descriptive statistical between elements, Pb, Zn, Mn, Fe, Na, K, Mg, Ca, Hg

Parameters	Cu	Pb	Zn	Mn	Fe	Na	K	Mg	Ca
Mean	8.32	1.61	38.43	76.53	214	227	5738	3752	3506
Standard Error	0.71	0.27	3.60	14.33	14	90	2101	411	243
Median	9.30	1.42	40.40	57.80	233	106	2406	3744	3288
Standard Deviation	2.55	0.98	12.97	51.67	49	326	7574	1482	875
Sample Variance	6.50	0.96	168	2670	2408	106219	57364052	2196542	766215
CV%	31	61	34	68	23	144	132	39	25
Kurtosis	1.15	1.74	-0.12	-1.27	-0.57	6.83	4.90	-0.25	1.87
Skewness	-1.07	1.23	0.37	0.48	-0.38	2.51	2.33	0.18	1.24
Minimum	2.33	0.40	20.4	17.6	118	13	1956	1462	2423
Maximum	11.63	4.01	63.5	164.7	289	1191	26672	6502	5654
Count	13	13	13	13	13	13	13	13	13
Confidence Level (95.0%)	1.54	0.59	7.84	31.2	29.7	197	4577	896	529

It is known from (Smolders *et al.*, 2002), that Zn is used in the production automobile tires, and also that Pb and Mn are metals that come from gasoline used in automobiles. Station 3 is located near Durres port and the high concentration of metals comes from the port activity. Station 13 has a low concentration of metals from the other station, the reason is low density of traffic and because there is no industrial activity, but the most important is that this station has large green surface than the other stations in Durres city.

Therefore to distinguish natural or anthropogenic origin of elements analysed in leaves samples was carried correlation analysis and analysis of elements in groups (Cluster Analysis) elements:

Cluster Analysis of Observations: Cu, Pb, Zn, Mn, Fe, Na, K, Mg, Ca, Hg

Euclidean Distance, Complete Linkage

Step	Number of clusters	Similarity level	Distance level	Clusters joined		New cluster	Number of obs. in new cluster
1	12	98.2183	441.7	5	8	5	2
2	11	96.7997	793.3	6	7	6	2
3	10	96.2933	918.9	5	9	5	3
4	9	95.4898	1118.0	1	10	1	2
5	8	94.3190	1408.3	1	2	1	3
6	7	91.9974	1983.8	1	5	1	6
7	6	90.0245	2472.8	3	4	3	2
8	5	85.5758	3575.7	1	6	1	8
9	4	78.2128	5400.9	1	3	1	10
10	3	74.9071	6220.3	1	11	1	11
11	2	60.3747	9822.8	12	13	12	2
12	1	0.0000	24789.2	1	12	1	13

Final Partition
Number of clusters: 5

	Number of observations	Within cluster sum of squares	Average distance from centroid	Maximum distance from centroid
Cluster1	8	14542791	1232.88	2093.71
Cluster2	2	3057492	1236.42	1236.42
Cluster3	1	0	0.00	0.00
Cluster4	1	0	0.00	0.00
Cluster5	1	0	0.00	0.00

Cluster Centroids

Variable	Cluster1	Cluster2	Cluster3	Cluster4	Cluster5	Grand centroid
Cu	8.43	9.89	5.81	9.3	5.8	8.32
Pb	1.25	1.47	1.62	4.0	2.4	1.61
Zn	39.90	45.50	20.40	25.9	43.1	38.43
Mn	61.39	151.95	29.40	148.0	22.5	76.53
Fe	201.88	267.50	262.00	190.0	176.0	213.69
Na	243.00	39.50	532.00	123.0	272.0	226.92
K	2402.75	2089.50	7313.00	26672.0	17203.0	5737.62
Mg	3326.50	6145.00	3744.00	4065.0	2068.0	3752.23
Ca	3295.50	4471.00	3058.00	4442.0	2769.0	3505.77
Hg	0.29	0.33	0.20	0.2	0.1	0.27

Distances Between Cluster Centroids

	Cluster1	Cluster2	Cluster3	Cluster4	Cluster5
Cluster1	0.0	3078.6	4942.7	24308.0	14863.1
Cluster2	3078.6	0.0	5941.8	24670.6	15748.5
Cluster3	4942.7	5941.8	0.0	19415.9	10038.9
Cluster4	24308.0	24670.6	19415.9	0.0	9822.8
Cluster5	14863.1	15748.5	10038.9	9822.8	0.0

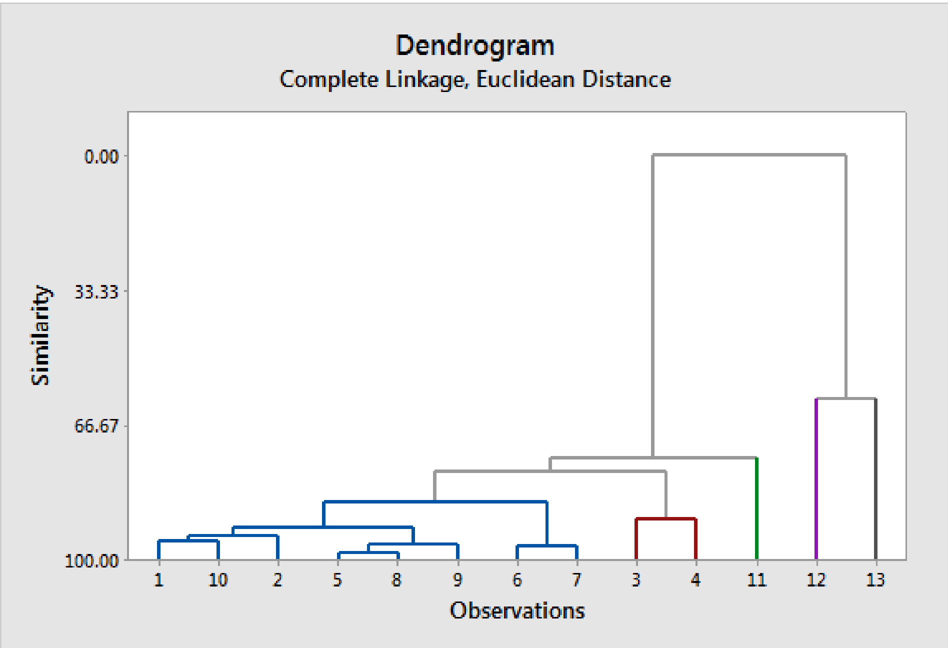

Fig 3. The dendrogram of the distribution of the stations classified on their similarity, Euclidean Distance, Complete Linkage, Amalgamation Steps; Final Partition: Number of clusters: 5

Cluster 1 In this group we have these stations 1, 10, 2, 5, 8, 9, 6, 7, which represent an urban area with high level of heavy metals. These values depend on traffic emission, because they are the main streets and the most used in Durres city.

Cluster 2 In this group we have these stations 3, 4, which are around of Culture Palace, therefore they have similar environmental conditions of heavy metals values. These stations are located near Durres port and high concentration of metals comes from the port activity.

Cluster 3 In this group we have the station 11, part of Goga A. St, which is the longest road in Durres area, so bring to us a high level of air pollution from traffic emission.

Cluster 4 In this group we have the station 12, that belong a Sotiris K. St, which is near “Niko Dovana” stadium. This location is distant from the sea, it is reflected in low concentration of some heavy metals like Mercury (Hg), also influences the fact that there is no industrial activity.

Cluster 5 In this group we have the station 13, belong a Ramazoti A. St, which resulted to have a lower concentration of metals than other stations, the reason is traffic low density as well as it has the largest green surface of all other stations in Durres city.

Factor analysis was carried out for a better interpretation of the results:

Cluster Analysis of Variables: Cu, Pb, Zn, Mn, Fe, Na, K, Mg, Ca, Hg

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Correlation Coefficient Distance, Complete Linkage, Similarity level: 70%
Amalgamation Steps
                                                                    Number
                                                                   of obs.
      Number of  Similarity  Distance  Clusters      New  in new
Step   clusters       level     level   joined   cluster  cluster
   1          9     89.6089   0.20782  2      7        2        2
   2          8     84.9535   0.30093  4      9        4        2
   3          7     84.1357   0.31729  5      8        5        2
   4          6     73.0974   0.53805  1      3        1        2
   5          5     67.7448   0.64510  4      5        4        4
   6          4     50.3485   0.99303  2      6        2        3
   7          3     47.0140   1.05972  1      4        1        6
   8          2     42.0387   1.15923  1     10        1        7
   9          1     30.0643   1.39871  1      2        1       10
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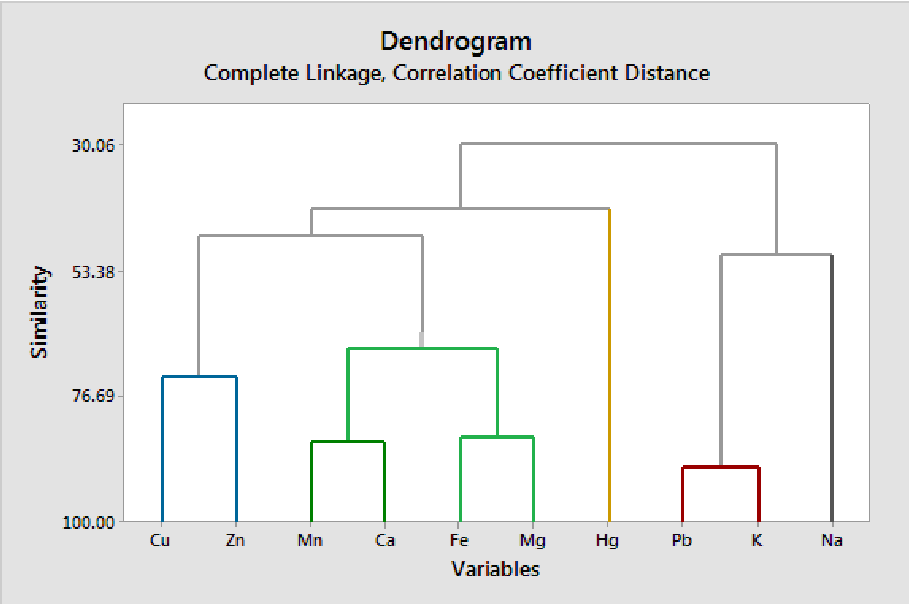

Fig 4. The dendrogram of the distribution of the elements classified on their similarity, Euclidean Distance, Complete Linkage, Amalgamation Steps; Final Partition: Number of clusters: 5; Cluster 1: Cu Zn; Cluster 2: Pb K; Cluster 3: Mn Ca Fe Mg; Cluster 4: Na; Cluster 5: Hg

Cluster 1 Cu, Zn: These associations may be attributed to anthropogenic origin of brake lining and tire wear particle. Traffic density influences the increase of the concentration of heavy metals specially of Zn, that is used in the production automobile tires.

Cluster 2 Pb, K: These associations are connected with anthropogenic input from traffic emission. In this study K element appeared to be in higher than the other elements. The highest value is reached in Goga A. street, where the density of traffic is higher. Pb element acts as marker element for motor vehicle emissions. The presence of these two elements in this group also indicate the origin from the soils of the area that pass from the roots to the leaves.

Cluster 3 Mn, Ca, Fe, Mg: These associations of Mn and Ca are bonded together through the origin of the area soils. Mn is mainly of geochemical origin of wind soil dusts and the feeling of the plants from their roots system. Also Ca element can pass from the roots to the leaves.

Mg and Fe are connected with anthropogenic input by various plants and cars brake. Mg element is an important nutrient for *Ligustrum Lucidum* plants from natural origin. The concentration of Fe element is related by the high traffic density of all types of automobiles.

Cluster 4 Na: Na element is an important nutrient for the vascular plants, in this case for *Ligustrum lucidum,* from natural origin.

Cluster 5 Hg: Hg element is associated with anthropogenic input by burning urban wastes. Also it is related to port activity, in Durres area

For better interpretation PC analysis is done. The results of PC analysis are shown below :

Principal Component Analysis: Cu, Pb, Zn, Mn, Fe, Na, K, Mg, Ca, Hg

Eigenanalysis of the Correlation Matrix

Eigenvalue	3.2516	2.4343	1.4291	1.0437	0.7132	0.5421	0.2820	0.1738	0.1103	0.0198
Proportion	0.325	0.243	0.143	0.104	0.071	0.054	0.028	0.017	0.011	0.002
Cumulative	0.325	0.569	0.711	0.816	0.887	0.941	0.970	0.987	0.998	1.000

Variable	PC1	PC2	PC3	PC4
Cu	**0.402**	0.112	0.099	**0.578**
Pb	0.215	**-0.541**	-0.244	-0.062
Zn	0.180	**0.304**	-0.416	**0.425**
Mn	**0.403**	-0.144	0.216	-0.249
Fe	**0.412**	0.173	-0.244	-0.264
Na	0.057	-0.088	-0.773	-0.112
K	0.011	**-0.546**	-0.010	-0.000
Mg	**0.462**	0.199	0.168	-0.054
Ca	**0.459**	-0.181	0.148	-0.106
Hg	0.011	**0.416**	-0.056	-0.568

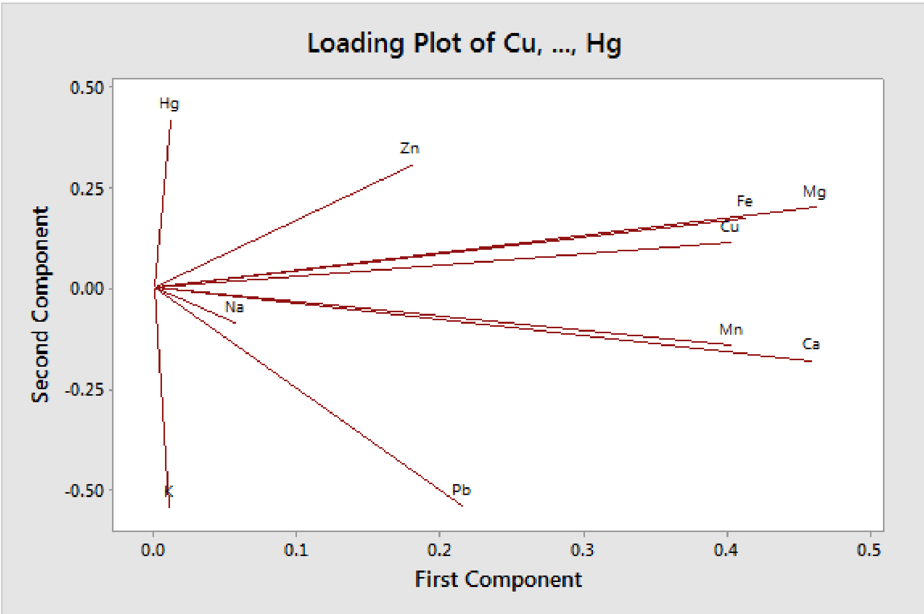

Fig 5 - The dendrogram of Principal Component Analysis of the Correlation Matrix

From the PCA analysis we have selected five principal components (PC's with Eigenvalue >1) that affect differences in trace metals distribution in vascular plants species:

PC 1 is the most important principal component that represents 32.5% of the total variance and is characterized by high loads of Fe, Mg, Ca and Cu. These associations may be attributed to anthropogenic PC of brake lining, tire wear particle, car's brake and other electric vehicles. Mg element is connected with soil dust, because he is an important nutrient for *Ligustrum Lucidum* plants from natural origin.

PC 2 is the second important principal component that represents 24.3% of the total variance and is characterized by high positive loads of Hg and Zn and negative loads of K and Pb. The negative loads of K and Pb came because Pb element acts as marker element for motor vehicle emissions but the presence of K (an important nutrient for *Ligustrum Lucidum* plants) may indicate the origin from the soils of the area that pass from the roots to the leaves. So when the level concentration of Pb increase bring a high dense traffic and the level concentration of K decrease. The opposite happen with Hg and Zn with positive loads. Traffic density influences the increase the concentration of these heavy metals, when Zn is used in the production automobile tires and Hg also is associated with anthropogenic input especially by burning urban wastes.

PC 3 is the weak principal component that represents only 14.3% of the total variance and is characterized by high negative loads of Na and Zn. These happen because traffic emission is the source of Zn (anthropogenic origin), but Na has natural source, because is an important nutrient for the vascular plants. So the increasing of Zn element brings reduced of Na element.

PC 4 is the weakest principal component that represents only 10.4% of the total variance and is characterized by high positive loads of Cu and Pb and negative loads of Hg. This situation is related by the high traffic density of all types of automobiles, that is more higher than the sources of Hg.

Conclusion

The object of this study is to determine and compare level of heavy metal in the leaves of vascular plants. The study was conducted in Durres area, Albania, using *Ligustrum lucidum* as bio-indicator from *Oleaceae* family. The trend of the distribution for each element is: Hg< Pb< Cu< Zn< Mn<Fe< Na< Ca < Mg< K. From this study K result the element with high level of concentration at Goga A. Street. The presence of K may also indicate the origin from the soils of the area that pass from the roots to the leaves.

The station that represent a high value of Hg is located near the crossroad Nici A. and Goga A. streets, which is affected from the presence of the sea. Was observed a high correlation between Pb-K; Mn-Ca; Fe- Mg and Cu- Mg, related by the high traffic density of all types of automobiles. Was found the resources of Ca, Zn and Pb that cause air pollution, are originated from oils, brake lining, tire wear particle and cars brake in general. Cu source is related from cars and other electric vehicles, while Mg element is connected with soil dust, because he is an important nutrient for *Ligustrum Lucidum* plants from natural origin.

The concentration data that represent very high values of Max/Min values belong a Na, with high level of concentration at Grigor D. Street. This value is connected with anthropogenic origin from traffic emission and natural origin as an important nutrient for *Ligustrum lucidum* plants.

After investigation is concluded that *Ligustrum lucidum, Fam : Oleaceae* is able to accumulate heavy metal in the air, in the best way with low economic cost.

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