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Heavy metals accumulation in *Nerium oleander* leaves across urban areas in Setif region, Algeria

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Abstract. *Koucim MA, Belguidoum A, Lograda T, Ramdani M. 2021. Heavy metals accumulation in Nerium oleander leaves across urban areas in Setif region, Algeria. Biodiversitas 22: 3083-3091.* Pollution by Metallic trace elements (MTE) has become one of the most serious environmental problems resulting from human activity. Plants, which are the base of the food chain, can take up MTE from the soil solution; hyper-accumulators can store high levels of heavy metals in their aerial parts at high concentrations. These plants can be used in phytoremediation. This study aimed to investigate the accumulation of MTE in the leaves of *Nerium oleander* to monitor environmental pollution of several areas in the province of Setif, Algeria. The samples of *N. oleander* leaves were collected from 20 urban areas in Setif Province. The concentrations of seven metallic trace elements (Cd, Mn, Pb, Sb, Cu, Bi and Fe) were determined using Flame Atomic Absorption Spectrophotometry (AASF). The results show that the concentrations of heavy metals in the leaves of *N. oleander*, in general, were very high, far exceeding the certified standard ranges. The order of MTE in the leaves was found as follows: Mn> Sb> Bi> Pb> Fe> Cu> Cd. Our findings indicate that although *N. oleander* showed a significant capacity to accumulate MTE, the urban areas of Setif province were highly polluted by heavy metals. The presence of metal ions in the aerial parts of the plant indicates that *N. oleander* is a hyper-accumulator of metals with tolerance to Mn, Sb, and Pb, and can be used as a bio-monitor. This opens up prospects for its application for soil phytoremediation.

Keywords: phytoremediation, bio-indicator, bio-accumulation, *Nerium oleander*, heavy metals, pollution, Algeria

INTRODUCTION

The environmental pollution by heavy metals and their accumulation have increased dramatically in the past decades, raising significant concerns worldwide (Suman et al. 2018; Ashraf et al. 2019). Heavy metals are not biodegradable and are prone to accumulate in living organisms as metallic trace elements (MTE) (Tchounwou et al. 2012). MTE are a group of metallic chemical elements from natural or anthropogenic sources (Farahat and Linderholm 2015; Chen et al. 2016; Hodomihou et al. 2016; Iqbal et al. 2016; Pichtel 2016). Many metallic elements are known for their toxicities (Nordberg et al. 2015). While some metals such as zinc, iron and copper are essential for plants and animals, they can be very toxic to humans (Tchounwou et al. 2012).

The mitigation of MTE toxification risks in humans requires environmental monitoring to help developing appropriate treatments, for example establishing safe disposal of pollutants (Schreck et al. 2012). The implementation of quality monitoring of the contaminated environment is imperative to reduce pollutant levels, particularly in urban areas where metallic pollutants often act together, making it difficult to reveal their effects (Lodenius et al. 2010; Pandey et al. 2014; Gonzalez-Castanedo et al. 2014; Parzych et al. 2016; Chen et al. 2016; Hankey et Marshall 2017; Fioravanti et al. 2018). One method of MTE monitoring in contaminated soils uses chemical and physical technologies, however, this approach is neither environmentally friendly nor cost-effective, especially in large and unproductive urban areas; in addition, it requires huge financial and labor investments (Venkateswarlu et al. 2016; Napoli et al. 2019).

Another monitoring method of MTE pollution is the use of bio accumulators, such as plants, in a diagnostic study. This method is very advantageous since the cost is low and it can be used on a large scale with high efficiency, offering an interesting tool to monitor heavy metal contamination in the air and soils (Pirintsos and Loppi 2008; Stamenkovic et al. 2013; Antonucci et al. 2016; Belguidoum et al. 2020). Several higher plants have been used to assess metal contamination in the air and soils because of their accumulation properties (Al-Khlaifat and Al-Khashman 2007; Berlizov et al. 2007; Di Lonardo et al. 2011; Youssef et al. 2014, Belguidoum et al. 2020; Vannucchi et al. 2020). Studies have shown that the leaves of higher plants adsorb MTE from wet and dry atmospheric deposition and accumulate metals from the soil and the atmosphere (Maher et al. 2008; Barış et al. 2015; Lograda et al. 2016).

Plant leaves are widely used as a bioindicator due to their ability to absorb air pollutants through the stomata, cuticle or indirectly through uptake through the roots after deposition of air pollutants from the soil (Ernst 2003). Urban trees can remove some of the particles from the air because they have a large leaf area, capturing pollutants on the foliage (Tallis et al. 2011; Speak et al. 2012). Besides the role of plants as bio-monitoring agents of polluted environment, a study by Sivakumar et al. (2020) showed that several plants are also very effective for phytoremediation of heavy metals, suggesting that they could be used to decontaminate soil polluted by heavy metals.

Nerium oleander is a xerophytic and perennial evergreen species of the *Apocynaceae* family. It can survive in soils polluted by heavy metals due to its strong exclusionary behavior (Franco et al. 2012, 2013). Showing a good capacity for bioaccumulation of Pb, Cu, Cd, Sb, and Mn (Aksoy and Ozturk 1997; Espinosa and Oliva 2006; Mingorance and Oliva 2006; Mingorance et al. 2007; Santos et al. 2019; Ibrahim and El Afandi 2020a and b), it is used as a biomonitor of heavy metals in Mediterranean areas. In Algeria, this species is widely distributed in urban areas.

The main target of this study is to determine heavy metal concentrations in leaves of *Nerium oleander* planted in the roads of twenty stations in the province of Setif (Algeria) in order to evaluate its ability to remove pollutants from, and thus to be used for monitoring environment quality.

MATERIALS AND METHODS

Study area

Twenty observation stations located in the province of Sétif, Algeria, were selected to sample *Nerium oleander* (Figure 1; Table 1). The samples were collected in urban areas of the Sétif region, Algeria, during March 2020. The climate of the study area is semi-arid, cold in winter, and hot in summer. The average annual rainfall is 322mm and rarely exceeds 500mm and the annual average temperature is 15°C (Gharzouli 2007).

The sources of pollution in the Setif region are mainly from industry, agriculture, a road network which is composed of several types (the East-West Highway, the national roads (RN) (NR5, RN9, RN28, RN75, RN78,), a set of secondary roads and more or less developed tracks), two railway lines and airport.

Selection of the plant

Nerium oleander is a species with very original leaves, persistent, gray, and thick, forming a dense ground cover. It flowers in spring with bright yellow color. This species is native to Algeria and popular in the urban area. The selection of *N. oleander* is based on a previous study on the bioaccumulation of heavy metals in this species (Santos et al. 2019; Belguidoum et al. 2020; Ibrahim and El Afandi 2020a and b). The selected plant is a common and typical shrub and is planted for landscape purposes in Setif. It grows in almost all urban parks and streets, even where urban pollution levels are high. This plant is found in large numbers in the monitoring areas and can be easily identified. Sampling was done randomly, depending on the abundance of the plant species at the site level. The samples were transported to the laboratory in paper bags.

Sample's preparation

Cleaning and grinding

These activities consisted of eliminating any atmospheric deposits. After measuring the fresh weight, a sample of 400g of plants was dried at 80°C for 48h, and then the dry weight was measured and crushed. Since this step is highly critical since it can cause metal contamination or loss, a porcelain mortar was used to avoid contamination. Then, the samples were sieved through a nylon sieve to obtain a fine powder.

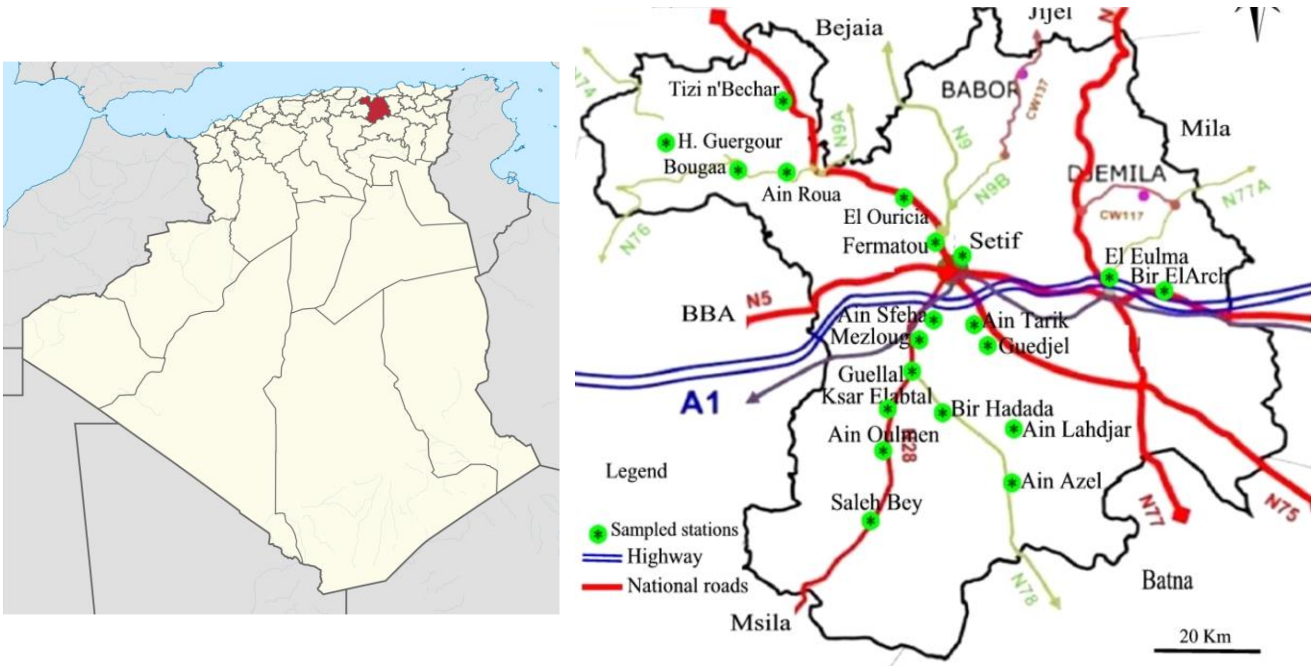

Figure 1. Observation and sampling stations for *Nerium oleander* populations in the province of Sétif, Algeria

Table 1. Geographical coordinates and altitude of the sampling stations in Setif Province, Algeria

Station	Latitude (N)	Longitude (E)	Altitude (m)	Sampling locations
Tizi n'Bechar	36° 43' 55"	5° 32' 91"	881	RN9
H. Guergour	36° 33' 63"	5° 04' 99"	831	RN75
Bougaa	36°33' 51"	5° 09' 69"	950	RN75
Ain Roua	36° 33' 78"	5° 15' 69"	1085	RN75; presence of quarries
El Ouricia	36° 26' 99"	5° 40' 82"	1103	RN9
Fermatou	36° 20' 54"	5° 42' 90"	1110	RN9
Setif	36° 18' 62"	5° 44' 51"	1115	RN5
El Eulma	36° 15' 75"	5° 67' 49"	950	Highway A1
Bir Arch	36° 13' 08"	5° 39' 76"	1105	RN5
Ain Sfiha	36° 17' 34"	5° 39' 76"	1100	RN28
Mezloug	36° 10' 65"	5° 33' 99"	933	RN 28, Intense industry
Guellal	36° 04' 29"	5° 33' 84"	911	RN28, presence of quarries
Ksar El Abtal	35° 97' 65"	5° 30' 73"	897	RN28
Ain Oulmene	35° 94' 01"	5° 30' 22"	950	RN28
Saleh Bey	35° 51' 04"	5° 17' 34"	978	RN28
Bir Hadada	35° 96' 01"	5° 43' 51"	934	RN78, presence of quarries, agriculture
Ain Lahdjar	35° 93' 94"	5° 54' 02"	916	RN78, presence of quarries
Ain Azel	35° 80' 91"	5° 51' 10"	916	RN78, zinc mine
AinTarik	36° 15' 16"	5° 44' 01"	1107	RN75, industrial area
Guidjel	36° 09' 02"	5° 48' 98"	985	RN75, Agriculture

Calcination

The method of Tauzin and Just (Belguidoum et al. 2020) was used. The resulting fine powder (1 to 2g of leaves) was calcined using quartz capsules in a muffle oven with the temperature was gradually increased to 500°C from 2 hours to 4 hours.

Mineralization

The method of Tauzin and Just (Belguidoum et al. 2020) was applied to assess the metal concentration in the sample. It consisted of calcining 1 to 2g of leaves in a muffle furnace at 450°C for four hours. The ashes obtained were mineralized by aquaregia (25% HNO_3 and 75% HCl) then dried on a sand bath until discoloration of the mineral deposit occurred. The residue was dissolved in 10 mL HCl 5%, and then filtered on Whatman paper with 0.45µm of diameter, and completed to 20 mL with HCl 5%. Heavy metals were assayed using Atomic Absorption Spectrophotometer with Flame (AASF) in the laboratory of Valorization of Natural Biological Resources, Setif University.

Analytical procedures for MTE concentrations

The concentration of the following elements (Cd, Mn, Pb, Sb, Cu, Bi and Fe), were determined by Atomic Absorption Spectrophotometry with Flame (AASF). There are no established standards of trace element concentration in ppm. To interpret the results of each element studied, we used standard reference values according to the unit's concentration ranges. The obtained results (in g/L) were transformed into mg/kg using the following relationship:

$$T\ (mg/kg) = C\frac{V}{S}$$

Where;

T: element concentration in mg/kg

C: concentration of the element in mg/l determined by the calibration curve

V: extraction volume in mL

S: sample weight in grams

Statistical analysis

Data were first subjected to Principal Components Analysis (PCA) to examine the relationships among the trace elements and the bioaccumulation by this plant, and the relationships between the presence of these elements and the vehicle circulations. Cluster analysis using unweighted pair group method with arithmetic mean (UPGMA) was carried out on the original variables and on the Manhattan distance matrix to seek hierarchical associations among the elements and stations. The statistical analyses were carried out using STATISTICA 10 software.

RESULTS AND DISCUSSION

The results of the metallic trace elements (MTE) of Cd, Mn, Pb, Sb, Cu, Bi, and Fe, accumulated in the leaves of *Nerium oleander* from 20 stations in Setif Province, Algeria is presented in (Table 2). In general, the concentrations of MTE in the leaves vary and exceed the certified standard values.

Cadmium contents in *Nerium oleander* leaves varied, showing a total absence in seven stations, while the other stations had values exceeding the certified standards. The highest concentration of Cd was recorded in the Bir Hadada station with 99 mg/kg). Similarly, the accumulation of Mn was very high and variable, with an average of 2133.5 ± 1579.86 mg/kg, thus far exceeding the certified standard of 33 mg/kg. The highest level of Mn was also found in the Bir Hadada station with 5980 mg/kg, which is probably due to the proximity of this station to agricultural activities and the presence of twelve quarries, which negatively affects the environmental quality of the region.

A strong accumulation of Pb was recorded with an average of 550.5 ± 280.97 mg/kg, exceeding the certified limits. The stations of Farmatou, Guidjel, and Mezoug had the highest rates of Pb concentration in the leaves of *Nerium oleander*. These stations are located in the vicinity of roads with high traffic and affected by industrial activities that use lead as material sources. In addition, a strong accumulation of iron was noted in the studied stations, particularly in Mezloug station in which this station is located near chemical industries. The Farmatou station has the highest level of Sb with 3210 mg/kg. While the Bougaa station has the lowest rate of this element. The accumulation of elements (Fe, Cu, Bi, and Sb) in the leaves of *N. oleander*, reveal values exceeding the certified standards, with high averages. The rank of metallic trace elements analyzed in this study based on the averages is Mn> Sb> Bi> Pb> Fe> Cu> Cd.

The results reveal a significant variation in MTE among the stations studied and among the concentrations of MTE. The accumulation of Sb and Mn was very high in the stations studied, particularly at Bir Hadada, Farmatou, and Mezloug. These results show that this species is a hyper-accumulation of Sb and Mn.

The MTE concentrations varied across the sampled stations. The Mn element shows the greatest variability in *N. oleander* populations, while the Cd level is the least variable (Figure 2).

UPGMA statistical analysis of the results shows that MTE are separated into two clusters. The first cluster includes the elements of Sb and Mn in which their concentrations were very high in all populations. The second cluster brings together two groups. The elements of Bi and Pb from the first group with high to medium concentrations, while Cd, Cu, and Fe form the second group with more or less low concentrations (Figure 3). This analysis confirms the results of the classification of MTE Mn> Sb> Bi> Pb> Fe> Cu> Cd.

Based on the first three axes from the PCA, the three-dimensional spatial projection of the stations shows the formation of a point cloud with the individualization of three populations (Fermatou, Mesloug, and Bir Hadada) (Figure 4). High rates of MTE characterize these three populations. The remaining populations are a group where the populations were not individualized

Table 2. MTE concentration in *Nerium oleander* leaves (mg/kg) across 20 observation stations in Setif Province, Algeria

Stations	Cd	Mn	Pb	Sb	Cu	Bi	Fe
Tizi n'Béchar	26	910	230	1630	55	850	220
H. Guergour	0	770	530	2880	80	680	200
Bougaa	24	560	270	1250	95	790	210
Ain Roua	60	2510	610	2586	208	760	210
EL Ouricia	30	1750	510	2580	920	780	200
Farmatou	0	4930	1550	3210	590	660	150
Setif	40	1120	500	2620	106	920	170
El Eulma	3	2520	460	2270	105	680	610
Bir Arch	0	2700	670	1450	152	850	90
Ain Sfiha	30	1070	330	1380	98	920	240
Mezloug	0	4900	660	1350	800	800	3070
Guellal	0	1120	470	1940	670	670	370
Kasr El Abtal	3	640	520	2600	850	850	230
Ain Oulmen	5	2790	550	2060	240	1000	23
Saleh Bey	0	920	600	1380	201	650	60
Bir Hadada	99	5980	500	1830	600	850	370
Ain Lahdjar	0	980	450	2350	149	620	150
Ain Azel	62	1060	310	1590	144	850	310
Ain Trik	43	2530	390	2250	360	700	180
Guidjel	39	2910	900	2160	240	890	470
Average	23.2	2133.5	550.5	2068.3	333.15	788.5	376.65
SD	27.67	1579.9	280.97	573.55	289.37	107.37	648.73
RSD	119.26	74.1	51.04	27.73	86.86	13.62	172.24
Min.	0	560	230	1250	55	620	23
Max.	99	5980	1550	3210	920	920	3070
Certified standards	0.6	33	40.9	0.35	7.03	4.12	804

The result of UPGMA analysis shows the heterogeneity of the accumulation of MTE in *N. oleander* across stations and confirms the separation of populations studied into two groups (Figure 5). The first group is represented by the stations of Mezloug, Farmatou and Bir Hadada, characterized by a strong accumulation of MTE in the leaves; particularly Mn and Sb. These stations have high levels of pollution from MTE. These high rates are probably due in large part to the region's significant agricultural activities and some industrial activities. While the second group is divided into two branches. The stations of Bir Arch, Ain Roua, El Eulma, Gudjel, Ain Trik, and Ain Oulmen represent the first sub-branch, with a high accumulation of MTE. The second sub-branch, includes the least polluted stations, still, the MTE accumulation rate is much higher than the standard values. In contrast, the populations of Tizi n'Bechar accumulate low concentrations of MTE, with the lowest Cu concentration compared to the different stations studied (55 mg/kg).

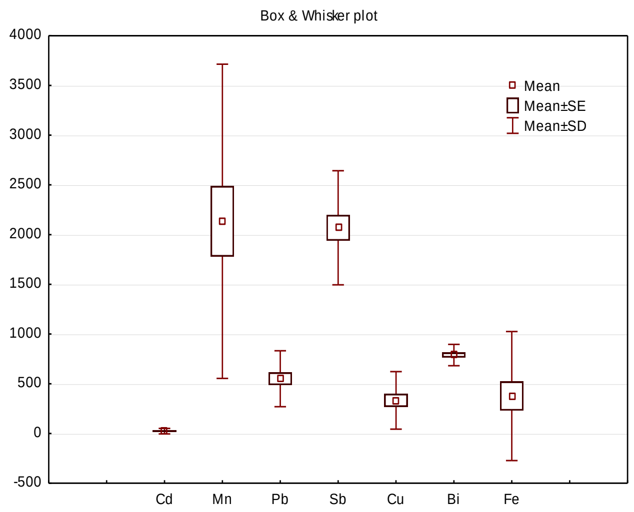

Figure 2. Variability of MTE concentrations in *Nerium oleander* leaves in Setif Province, Algeria

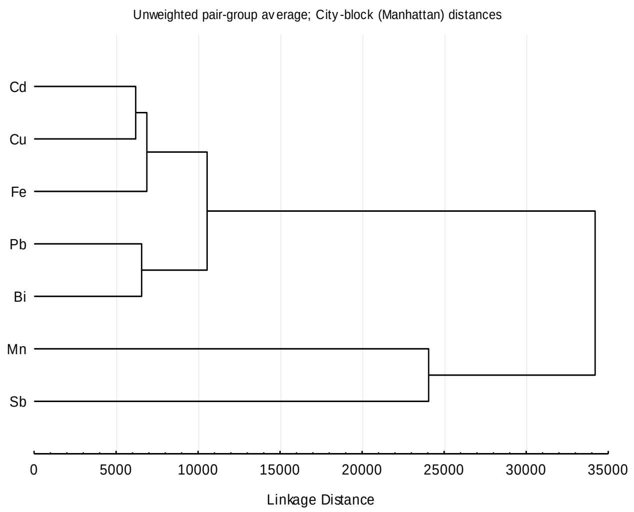

Figure 3. The clustering analysis of MTE accumulations in the leaves of *N. oleander* in Setif Province, Algeria

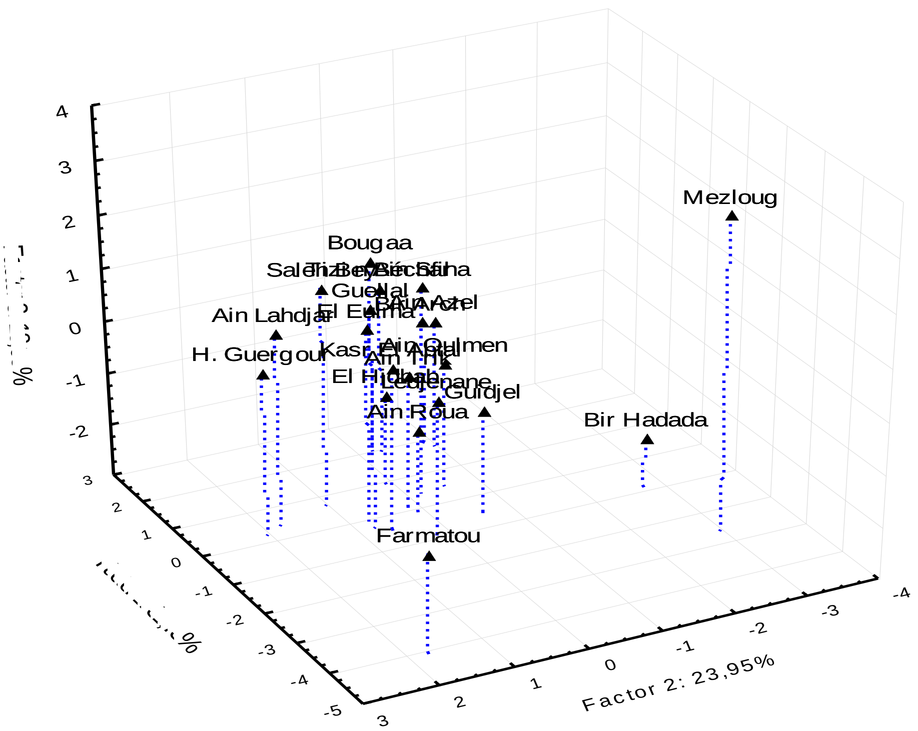

Figure 4. Spatial projection of stations based on the first three axes of the PCA

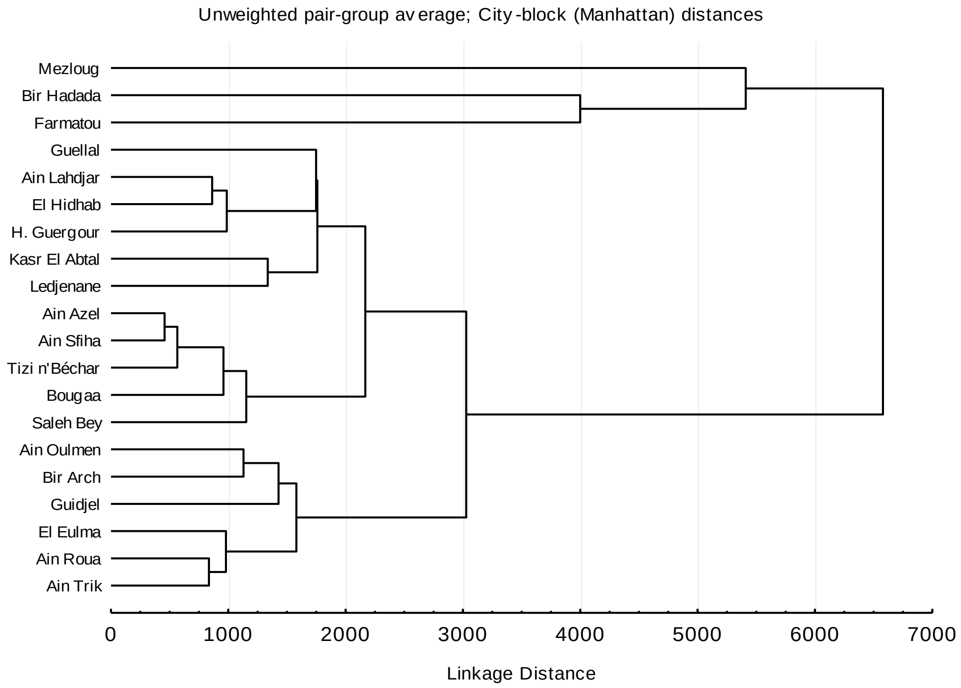

Figure 5. The clustering analysis of twenty *Nerium oleander* populations in Setif Province, Algeria based on MTE concentration

Discussion

Trace metallic elements (MTE) from natural and anthropogenic sources degrade the initial state of urban environment (Aksu 2015; Linderholm 2015; Chen et al. 2016; Hodomihou et al. 2016; Pichtel 2016; Iqbal et al. 2016; Yan et al. 2020) as observed in the study region. The concentration of MTE accumulated by *Nerium oleander* in Setif Province, Nigeria shows the rank in concentration of the elements Mn> Sb> Bi> Pb> Fe> Cu> Cd. Other studies on the concentration of MTE in the leaves of *N. oleander* have revealed similar results (Espinosa and Oliva 2006; Mingorance and Oliva 2006; Mingorance et al. 2007; Santos et al. 2019; Fliou et al. 2020; Ibrahim and El Afandi 2020a).

The evaluation of the levels of MTE accumulation in the leaves of *N. oleander* shows extremely high values. In general, the concentrations of these elements are closely linked to intensive agricultural activities, which is in accordance with other observations (Abbasi et al. 2014; Song et al. 2020; Theo et al. 2020). Similarly, severe emissions from metallurgical industrial activities in the region also contribute to the increase of these elements in the environment (Hu et al. 2014; González-Castanedo et al. 2014; Pollard et al. 2015).

The MTE concentrations in the leaves of *N. oleander* show a strong accumulation in the Bir Hadada station. This accumulation is probably due to the various quarries surrounding the study station, which is located between two mountains, i.e., Djebel Sakreen to the south and Djebel Youssef to the north. Overall, the region has 12 quarries, which might negatively affect the environment (Issah et al. 2010; Midhat et al. 2019; Rimondi et al. 2020). The high rates of MTE accumulation at Bir Hadada is presumably not only linked to a large number of quarries, but also related emissions from intensive agricultural operations which degrade the environment through the release of pesticides and chemicals that generate associated toxic heavy metal particles suspended in the environment (Wei and Yang. 2010; Vázquez et al. 2016; Kirpik et al. 2017; Oumenskou et al. 2018).

This study is consistent with the results of other works, showing the relationship between the presence of MTE in the environment and their bioaccumulation in higher plants (Kabata-Pendias and Pendias 2001; Mohamed et al. 2013; Nguyen-Van et al, 2016; Ibrahim and El Afandi. 2020b). The concentrations of Sb, Mn, Cu and Pb were high at stations located near roads. The high levels of these elements have been positively correlated with the traffic because vehicle exhaust emissions have a significant role in environmental contamination (De Silva et al. 2016; Kirpik et al. 2017; Yang et al. 2020). Studies on the accumulation of MTE by the plants located in the proximity roads showed a close relationship between levels of heavy metals and the volume of traffic (De Silva et al. 2016; Yang et al. 2020; Belguidoum et al. 2021). In Turkey, *Nerium oleander* has been used as a biomonitor tool for heavy metal accumulation in intense human activity and heavy road traffic (Banu-Doganlar et al. 2012).

The Mezloug station reveals a high level of iron due to the transport of airborne particles from the chemical industry area, which contributes significantly to this pollution (Odabasi et al. 2010). The plants accumulate more heavy metals in industrial areas than in agricultural areas (Hanedar 2015). Lograda et al. (2016) and Belguidoum et al. (2020) have reported the same observations.

The concentrations of Cd and Pb far exceed the standard values in the Ain Azel station, which is in accordance with the findings of Lograda et al. (2016) and Belguidoum et al. (2020). The high levels of these elements are probably associated with mining the Kherzet Youcef polymetallic deposit, which constitutes a significant source of environmental contamination (Bellouche 2016; Dan-Badjo et al. 2019). In Morocco mining areas, Duraes et al. (2015) found the presence of high levels of Pb and Cu in the leaves of *N. oleander*. Similar observations have been made in Spain on the oleander plant in extremely acidic mine drainage environments (Franco et al. 2013). Therefore, the establishment of a monitoring and treatment program for soil and polluted water in mining stations would be necessary to preserve human health (Dan-Badjo et al. 2019).

The concentration of MTE accumulated in the *N. oleander* leaves exceeds the certified standards. This condition is related to the biogenic capacity of the plant, which produces chemical chelators favoring the accumulation of these elements (Elloumi et al. 2017; Suba et al. 2018). The evaluation of the kinetics of some MTE in *N. oleander* seedlings by Franco et al. (2013) shows a high level of Mn, Fe, and Cu in *Nerium* seedlings, which confirms our results. The super accumulating plants of MTE can be used for the bioremediation of the environment polluted by these heavy metals (Morel 2002, Ali et al. 2013; Haq et al. 2020; Morel 2021). Therefore, *N. oleander* can be considered as a hyper-accumulator of metals with a high tolerance to Mn, Sb and Pb, thus offering prospects for application to phytoremediation in large polluted urban geographic areas.

In conclusion, the study results provide an assessment of the pollution in Setif region by using the concentration of heavy metals of Cd, Mn, Pb, Sb, Cu, Bi and Fe in *Nerium oleander* leaves. It was shown that the sampled stations were heavily polluted by the presence of high levels of MTE. Overall, the MTE concentrations were higher than the standards with Mn and Sb concentrations greatly exceeded the certified standards. The element of Mn was found to be the highest and Cd was the lowest according to the sequence of Mn> Sb> Bi> Pb> Fe> Cu> Cd. Tizi n'Bechar can be considered as the less polluted site, while Mezloug, Bir Hadada and Fermatou were the most polluted sites with high human activity and vehicular density. The results indicate a good agreement between the concentrations of heavy metals in *N. oleander* and the anthropic impacts, which are notably represented by mining, agricultural activities, chemical industries, and the combustion processes linked to vehicle emissions. The results suggest that *Nerium oleander* can be used as a bio-accumulator of MTE in soil pollution monitoring studies, thus offering prospects for application in phytoremediation. We thus propose the use of *Nerium oleander* in the rehabilitation of the polluted urban landscape to minimize the impact of pollution, and simultaneously enrich the landscape without altering the ecosystem.

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