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Sampling Standardisation of Date Palm (*Phoenix dactylifera* L.) Leaflets as a Biomonitor of Metal Pollutants in Arid Environments

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Sampling standardisation is a basic requirement for obtaining spatially and temporally reproducible comparable results. Date palm, *Phoenix dactylifera* L., leaves showed to be suitable as a biomonitor for atmospheric metal pollution. Thus this work was undertaken to establish a standard sampling technique for the analysis of date palm leaves. The results showed that metal distribution in the date palm is related to the age of leaves. The metal content was investigated as a function of a leaflet's position along the rachis, and also of distribution within an individual leaflet.

INTRODUCTION

Standardisation of a sampling procedure is a way to obtain representative, random samples. It is a basic requirement in order to quantitatively compare data between different sites and time periods. For this in practice a multitude of factors and sampling parameters have to be kept within more or less narrow limits in order to reduce the spatial, temporal, and biological variability.

Sampling procedures for each site, each crop, and specific plant parts in the same stage of growth must be standardised for obtaining compatible results of metal content that could be classified as deficient, sufficient, or excessive or toxic for plants[1].

There are many variables involved in sampling procedures, including sampling time, weather conditions, plant age, leaf (age, size, shape, height from the ground and surface texture). Several studies[2–4] have investigated the effects of such factors on the effectiveness of particular leaves to biomonitor atmospheric heavy metals. Little[5] has shown that the assumption that unwashed and washed samples of leaves have the same original concentrations of metals may be quite erroneous, unless the sampling procedure is carefully controlled. Therefore, to assess metal burdens before and after washing, Little[5] and Little & Martin[6] cut elm leaves into two halves along the main vein; one set was washed and the other set of matching halves remained unwashed for comparison.

Little and Wiffen[7] in their studies of exhaust lead deposition on plant surfaces found that sectioning of whole leaves indeed demonstrated that lead deposition was heaviest at the leaf-tip and around the leaf margins.

The mineral composition of date palm *Phoenix dactylifera* L. foliage has been

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studied by Reuther[8], who found striking changes in some of the mineral constituents and chlorophyll occur in date palm leaves as they develop and grow old. He emphasised the importance of sampling leaves of comparable age when attempting to compare the mineral composition of samples of date palm foliage from different locations or treatments. This requires a precise sampling procedure and an intimate knowledge of the plant. Khalil *et al.*[4] investigated the effect of age on chlorophyll content of date palm leaves, and found that the dry weight of leaves increased with age faster than the increase in chlorophyll content. The mineral composition of leaves of four date plam cultivars in relation to tree age has been undertaken by Gasim *et al.*[2], who concluded that age of leaves rather than age of tree may be of more importance in influencing the mineral content of date palm tree.

Nixon[9] studied the size of date plam leaves in relation to age and found that the size of leaves varies with age. Even before the leaves reach maximum size, the spines have reached their maximum size, and the pinnae have attained their approximate maximum size but not their maximum number.

Of all cultivated plants, the date palm is probably one of the most mechanical or geometrical in its external structure. The rachis, or rib, is long, smooth and rodlike, and its expansion toward the base is along symmetrically moulded surfaces[10]. The leaflets, or pinnae, are in symmetrical ranks from either side of the rib, with each blade folded lengthwise with machine work precision. Due to these morphological features, sampling of the date palm can be precisely defined and easily applied. Therefore, it was the aim of this work to standardise the sampling technique of P. *dactylifera* leaflets to be used as a biomonitor of the atmospheric metal pollution.

EXPERIMENTAL

Sample collection and treatment: Seventeen date palm trees were chosen randomly for this investigation to represent different environments with different pollution levels in Riyadh city, Saudi Arabia. Since it is conceivable that the rapid and vigorous growth of a relatively small palm might prevent the accumulation of food reserves in the trunk[9], young palms with a maximum one metre trunk height were selected in order to reduce age and exposure period variation between tree samples. All samples were collected in two successive days to eliminate the weather variations.

For the study of the relation of age of leaf to its metal content, palm leaves were divided into three main parts according to their location on the tree and therefore their age, *i.e.*, old (A), middle age (B), and young (C) leaves.

To study the metal content of the leaflet related to their position along the rachis, leaflets were collected from basal, median and distal positions (Fig. 1). The distal portion of middle age leaves was shown to be the most suitable portion for sampling. This portion was then further divided into three (Fig. 2), with basal, median and subterminal subsections sampled separately for metal determination.

All collected samples were divided into two subsamples. One was thoroughly washed with running distilled water to remove deposited dust particles, the other remained untreated. Samples were then oven-dried at 80°C to a constant weight, milled in a micro-hammer cutter, then stored in clean, self-sealing, plastic bags with distinctive labels.

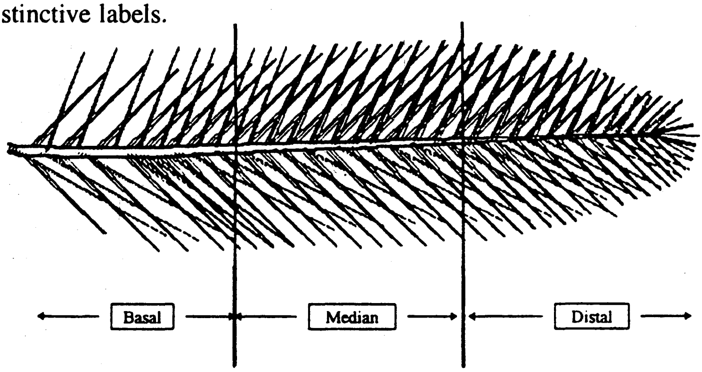

Fig. 1. Date palm (*Phoenix dactylifera* L.) leaf

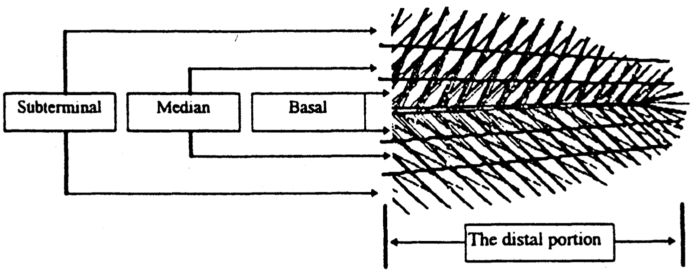

Fig. 2. Leaflets divisions along the distal portion of the date palm (*P. dactylifera* L.) leaf

Extraction: 1 g samples of dry milled plant material were ashed in an electric muffle furnace at 480°C for 24 h. The weighed ash was digested in 10 mL AR concentrated HNO_3, evaporated to near dryness on a hot-plate and made up to volume with 1% HNO_3.

Element concentrations were determined using atomic absorption spectrophotometer (Perkin-Elmer model 1100, micro-computer controlled with integrated CRT screen and keyboard function). The precision of the results was checked by duplicating 20% of the samples chosen randomly. In order to ascertain the accuracy of the method employed, two reference materials were included with every batch (SRM 1547 Peach leaves and CRM 281 Rye grass).

RESULTS AND DISCUSSION

Leaf age in relation to metal content

The data summarised in Table-1 and Fig. 3 were obtained by averaging the mesurements of the subsections represented in Table-2. Thus, the data for each age category represent the averge of the results obtained from the basal, median and distal parts of seventeen trees. The metal content for all trees results showed the same general trends shown by their means presented in Figs. 3 and 4.

In the case of unwashed leaves, the means of Pb, Cr, Ni and Zn showed a gradient of concentrations related to leaf age (Table-1). However, the application of the analysis of variance (ANOVA) test, followed by Duncan's multiple range test, shows significant differences between their means. Although the mean concentrations of Pb, Ni and Zn were greater in old leaves (81.25, 5.60 and 25.16 $\mu g\ g^{-1}$, respectively) than in young leaves (35.15, 3.24 and 21.18 $\mu g\ g^{-1}$, respectively), but only Pb and Ni showed significant differences between old and young leaves. The same conclusion was obtained when comparing the levels of these metals in middle age and young leaves. On the other hand, the mean concentrations of Pb, Ni and Zn in old leaves were higher than their means in the middle age ones, but these were shown to be insignificant differences.

The higher concentrations found in old leaves can be attributed to the closeness of these leaves to the surface soil: reinternment of dust and soil particles is taking place, leading to a rise in metal concentrations in old leaves more than the others. This became obvious from measurements determined from the distal part of the old leaves (Fig. 4), which is the nearest part to the soil due to the curvature of the midrib (rachis) and in some cases actually lies on the ground. Furthermore, car splash, albeit infrequent, can form another source leading to a rise in metal concentrations of the old leaves. In contrast, the young leaves, being highly sheltered and held almost vertically, reduce the amount of dust particulates to be deposited on them.

The means of Pb, Ni and Zn showed a similar concentration gradient in the washed leaves. Zn did not show any significant differences in leaves in relation to the age, but Pb and Ni were significantly higher in old and middle age leaves than in the young ones. These high levels in the old leaves could be due to the cracks in the cuticle and/or to losses of cuticular cover by normal weathering and by mechanical damage[11]. Chromium in the young leaves was less than that in old and middle age leaves but not significantly.

Lithium was easily removed by washing, being significantly high in unwashed median and basal parts of the middle age leaves and the distal part of the young leaves, but did not appear to respond to age differences in the washed leaves.

In contrast to the other metals, copper was significantly higher in young leaves compared with old and middle age leaves, probably due to its uptake from soil by roots as an essential plant element. Therefore, the young leaves could be used to monitor the copper in soil. Relatively high levels of copper were found in basal, median and distal parts of the young leaves (Fig. 4).

Nixon[12, 13] found that flower and fruit production in the date palm are increased by the retention of old leaves. In spite of this, a large number of green leaves, regarded as old, are cut off every year by the municipality of Riyadh city. Thus, the old leaves cannot be found in every time and in all sampling locations compared with the middle age and young leaves. Therefore, due to uncontrolled

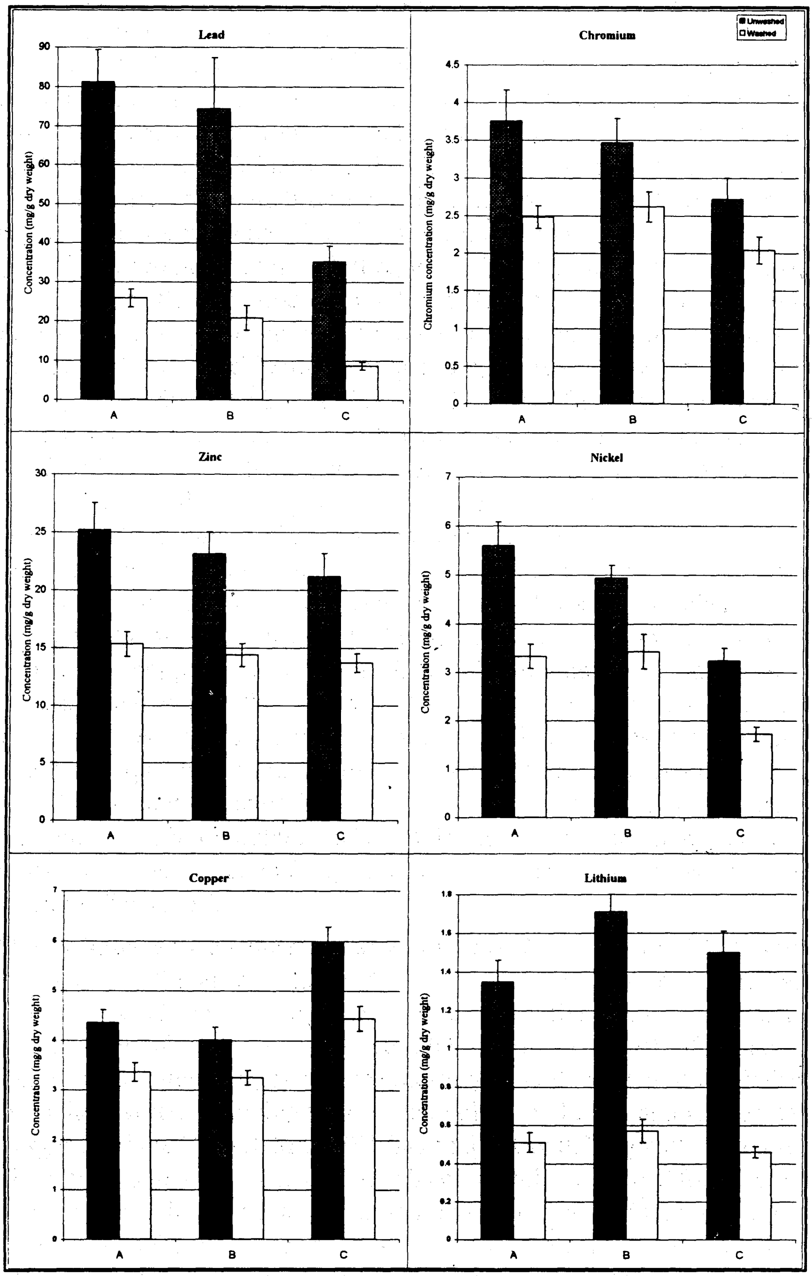

Fig. 3. Metal concentrations (μg/g dry weight) in old (A), middle age (B) and young (C) leaflets of *P. dactylifera* (mean and SE error bars).

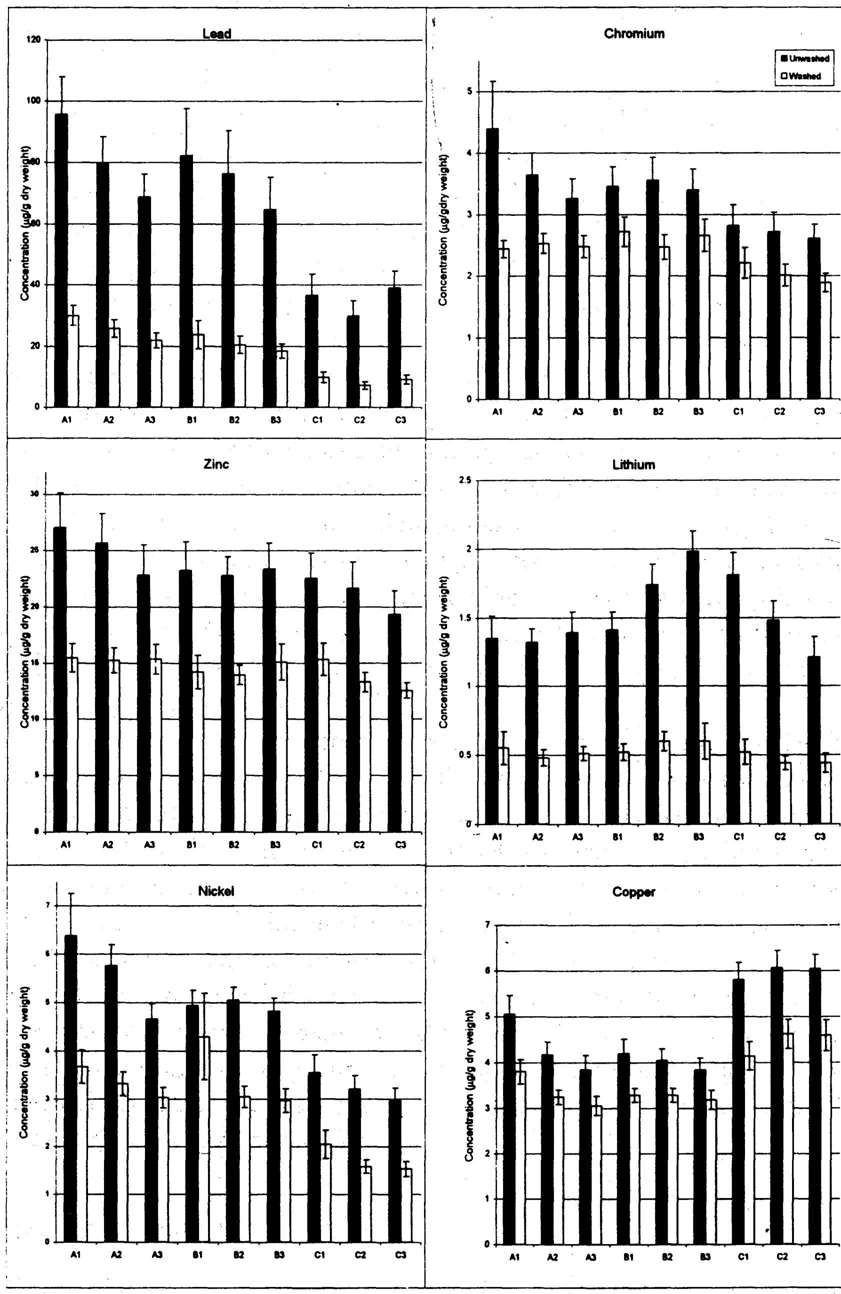

Fig. 4. Metal concentrations (µg/g dry weight) in distal (1), middle (2) and basal (3) parts of old (A), middle age (B) and young (C) leaves of *Phoenix dactylifera* (mean and SE error bars).

TABLE-1

METAL CONCENTRATIONS (μg/g DRY WEIGHT; MEAN ± SE) IN *Phoenix dactylifera* LEAVES OF DIFFERENT AGES.

Element	Old leaves (A)		Middle age leaves (B)		Young leaves (C)	
	Unwashed	Washed	Unwashed	Washed	Unwashed	Washed
Lead	81.25a* ± 8.13	25.81c ± 2.31	74.43b ± 12.88	20.81d ± 3.10	35.15ab ± 4.01	8.69cd ± 0.98
Copper	4.36a ± 0.26	3.36c ± 0.19	4.02b ± 0.26	3.25d ± 0.15	5.98ab ± 0.3	4.45cd ± 0.25
Chromium	3.76 ± 0.41	2.48 ± 0.15	3.47 ± 0.32	2.62 ± 0.2	2.72 ± 0.28	2.04 ± 0.18
Nickel	5.60a ± 0.48	3.33c ± 0.25	4.94b ± 0.26	3.43d ± 0.36	3.24ab ± 0.26	1.72cd ± 0.15
Lithium	1.35 ± 0.11	0.51 ± 0.05	1.71 ± 0.09	0.57 ± 0.06	1.5 ± 0.11	0.46 ± 0.03
Zinc	25.16 ± 2.37	15.33 ± 1.07	23.13 ± 1.91	14.39 ± 1	21.18 ± 1.98	13.71 ± 0.81

*Means, within a row followed by the same letter, differ significantly at 1% level of probability according to Duncan's Multiple Range Test.

TABLE-2

METAL CONCENTRATIONS (μg/g DRY WEIGHT; MEAN ± SE) IN DIFFERENT PARTS OF OLD (A), MIDDLE AGE (B) AND YOUNG (C) LEAVES OF *Phoenix dactylifera* (n = 153)

1. Old leaves (A):

Element	Distal		Median		Basal	
	Unwashed	Washed	Unwashed	Washed	Unwashed	Washed
Lead	95.59 ± 12.29	29.96 ± 3.25	79.51 ± 8.79	25.70 ± 2.85	68.67 ± 7.63	21.78 ± 2.46
Copper	5.06 ± 0.41	3.80 ± 0.27	4.18 ± 0.27	3.24 ± 0.16	3.84 ± 0.32	3.05 ± 0.21
Chromium	4.39 ± 0.77	2.44 ± 0.14	3.65 ± 0.35	2.53 ± 0.16	3.26 ± 0.32	2.48 ± 0.18
Nickel	6.38 ± 0.88	3.67 ± 0.35	5.76 ± 0.43	3.31 ± 0.25	4.66 ± 0.31	3.02 ± 0.21
Lithium	1.35 ± 0.16	0.55 ± 0.12	1.32 ± 0.10	0.48 ± 0.06	1.39 ± 0.15	0.51 ± 0.05
Zinc	27.04 ± 3.08	15.45 ± 1.27	25.66 ± 2.63	15.22 ± 1.13	22.80 ± 2.69	15.33 ± 1.33

2. Middle age leaves (B):

Element	Distal		Median		Basal	
	Unwashed	Washed	Unwashed	Washed	Unwashed	Washed
Lead	82.11 ± 15.31	23.67 ± 4.68	76.45 ± 13.75	20.38 ± 2.82	64.73 ± 10.52	18.38 ± 2.32
Copper	4.19 ± 0.32	3.28 ± 0.15	4.05 ± 0.25	3.28 ± 0.15	3.84 ± 0.26	3.18 ± 0.21
Chromium	3.46 ± 0.32	2.72 ± 0.24	3.56 ± 0.37	2.47 ± 0.20	3.40 ± 0.34	2.66 ± 0.26
Nickel	4.94 ± 0.31	4.29 ± 0.90	5.05 ± 0.27	3.04 ± 0.22	4.82 ± 0.27	2.96 ± 0.24
Lithium	1.41 ± 0.13	0.52 ± 0.06	1.74 ± 0.15	0.60 ± 0.07	1.98 ± 0.15	0.60 ± 0.13
Zinc	23.24 ± 2.53	14.18 ± 1.50	22.78 ± 1.65	13.92 ± 0.86	23.38 ± 2.28	15.07 ± 1.61

3. Young leaves (C):

Element	Distal		Median		Basal	
	Unwashed	Washed	Unwashed	Washed	Unwashed	Washed
Lead	36.58 ± 6.89	9.81 ± 1.75	29.84 ± 4.90	7.16 ± 1.26	39.04 ± 5.37	9.11 ± 1.38
Copper	5.81 ± 0.37	4.14 ± 0.31	6.07 ± 0.37	4.62 ± 0.32	6.05 ± 0.30	4.59 ± 0.34
Chromium	2.82 ± 0.34	2.21 ± 0.25	2.72 ± 0.32	2.01 ± 0.18	2.61 ± 0.23	1.89 ± 0.15
Nickel	3.55 ± 0.37	2.05 ± 0.30	3.20 ± 0.28	1.58 ± 0.14	2.96 ± 0.26	1.53 ± 0.16
Lithium	1.81 ± 0.16	0.52 ± 0.09	1.48 ± 0.14	0.44 ± 0.05	1.21 ± 0.15	0.44 ± 0.07
Zinc	22.55 ± 2.22	15.32 ± 1.44	21.67 ± 2.32	13.27 ± 0.86	19.33 ± 2.10	12.53 ± 0.67

factors such as the closeness to the soil and car splash together with limited availability, in terms of time and space, in sampling locations, old leaves are not a suitable choice to represent the palm tree.

However, although young leaves can be used to monitor copper in soil, but due to their sheltered nature, they cannot be used to directly monitor atmospheric metal pollution. Nevertheless, young leaves may be used to monitor atmospheric metal pollution indirectly after absorption of these pollutants by the roots after being deposited on and then in the soil.

Finally, middle age leaves, 1.5–2.0 metres above ground and with horizontally oriented leaves, have been found to be the most suitable to represent the palm tree for metal pollution analysis. This can be supported by the fact that the metal content in washed and unwashed middle age leaves proved to be closer than other leaves sampled to the mean values of these metals for the tree (Fig. 5).

Metal content of leaflets related to position along the rachis

The metal contents of washed and unwashed basal, median and distal leaflets of the middle age leaves are shown in Table-2 and Fig. 4. The mean concentrations of lead in unwashed distal, median and basal leaflets were 82.11, 76.45 and 64.73 $\mu g\ g^{-1}$ dry weight. Although lead concentrations showed a gradient, the differences between these means were insignificant when applying the ANOVA test. Similarly, a gradient, albeit less significant, was found for lead in washed leaflets for the same parts. This gradient could be explained as follows.

Firstly the basal leaflets contain spines which have a low surface area. Moreover, they are more sheltered than the median leaflets, and thus reduce the possibility of dust deposition into this part compared with the other parts. Secondly, despite the slight difference between the median and distal leaflets in the surface area, the former showed less concentration due to the cover by higher leaves. Finally, the distal leaflets are more exposed to atmospheric pollution, and therefore contain high concentrations. Similar gradients were shown by copper and nickel in unwashed and washed leaflets respectively. Chromium and zinc did not show any trends regarding the leaflets position along the rachis, but lithium levels were significantly higher in basal and median leaflets than in distal leaflets.

From the results obtained above, it was decided to use the distal leaflets of the middle age leaves for further investigation.

Metal variation along the leaflet (Pinna)

The data in Table-3 are represented graphically in Fig. 6 and show the mean metal concentrations in washed and unwashed basal, median and subterminal parts of the leaflets. The unwashed subterminal portions show higher metal concentrations than other parts, possibly due to the accumulation of dust particles at the leaf tips with an absence of surface wax[11]. Application of the ANOVA test did not reveal any significant differences in metal concentrations, except for lithium which was significantly higher in washed and unwashed subterminal

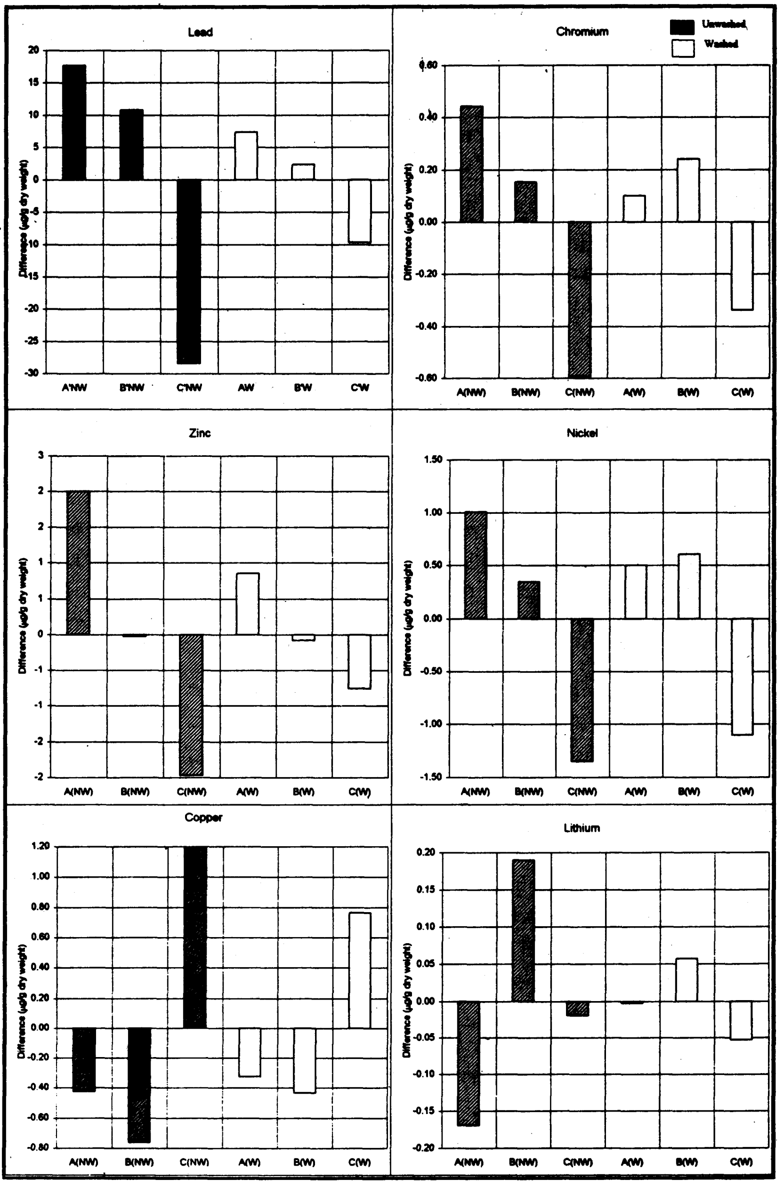

Fig. 5 Metal differences (µg/g dry weight) between old (A), middle age (B) and young leaves (C) and mean value of *Phoenix dactylifera* as a whole.

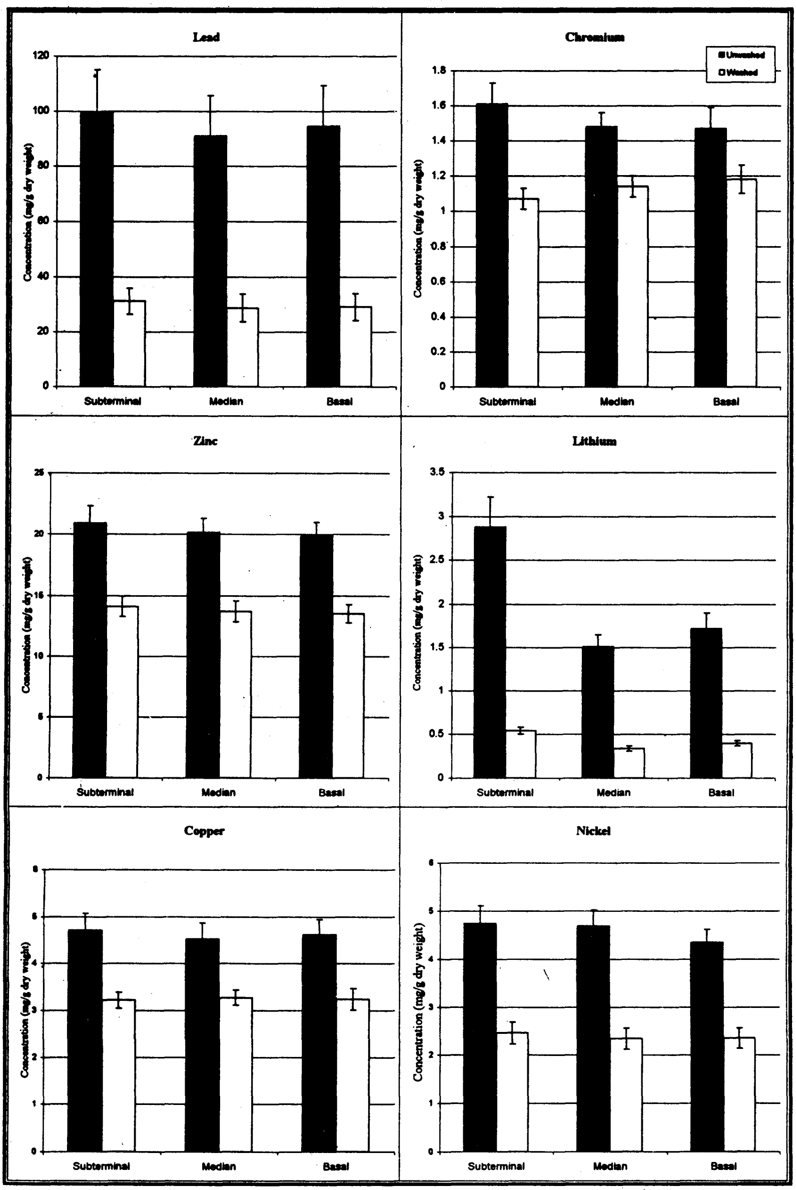

Fig. 6 Metal concentrations (μg/g dry weight) in different parts of *Phoenix dactylifera* pinnae (mean and SE error bars.)

TABLE-3
METAL CONCENTRATIONS (μg/g DRY WEIGHT; MEAN ± SE) IN DIFFERENT PARTS OF *P. diactylifera* PINNAE

Element	Subterminal		Median		Basal	
	Unwashed	Washed	Unwashed	Washed	Unwashed	Washed
Lead	99.65 ± 15.35	31.17 ± 4.70	90.97 ± 14.72	28.70 ± 5.01	94.51 ± 14.87	29.10 ± 4.84
Copper	4.71 ± 0.36	3.22 ± 0.17	4.53 ± 0.34	3.27 ± 0.16	4.62 ± 0.32	3.24 ± 0.23
Chromium	1.61 ± 0.12	1.07 ± 0.06	1.48 ± 0.08	1.14 ± 0.06	1.47 ± 0.12	1.18 ± 0.08
Zinc	20.91 ± 1.41	14.12 ± 0.85	20.16 ± 1.12	13.71 ± 0.87	19.88 ± 1.08	13.54 ± 0.74
Nickel	4.74 ± 0.37	2.46 ± 0.23	4.69 ± 0.33	2.34 ± 0.22	4.35 ± 0.27	2.35 ± 0.21
Lithium	2.88 ± 0.34	0.54 ± 0.04	1.51 ± 0.14	0.34 ± 0.03	1.72 ± 0.18	0.4 ± 0.03

portions than the others. However, for the other metals there is no significant difference between the basal, median and subterminal parts, but to bring the sampling variation as low as possible it was decided to choose only subterminal of distal leaflets of middle age leaves for pollution monitoring in Riyadh city.

Conclusions

From the results obtained, it was found that metal distributions on and in date palm leaves were related to their age and position on the tree, and that the middle age leaves appear to be the most representative of the palm tree.

Variation of metals accumulated in the leaflets along the rachis showed a gradient for some metals but without significant differences, which may depend on the local availability of a particular metal. If metal levels are high enough, they show a distinct gradient in leaflets along the rachis. However, from our results, it would appear that the distal part of middle age leaf was the most suitable for sampling.

Study of the metal variation along distal leaflets did not reveal any significant differneces, except in the case of lithium. Nevertheless, the distal part of these leaflets shows relatively higher concentrations than the other parts. Therefore, it was decided to use this part of the palm tree for a widescale pollution monitoring programme in Riyadh city.

To standardise sampling procedures, the following should be addressed:

(1) Sampling time to be specified (2) Weather conditions chosen to be fully comparable (3) Samples to be taken from the same height above the ground (1.5–2.0 m), from the distal portion of the middle age leaves which are not covered by higher leaves, and from all directions as a mixed sample.

REFERENCES

1. A. Kabata-Pendias and Henryk Pendias, Trace Elements in Soils and Plants, CRC Press, Boca Raton (1984).
2. S.M. Al-Shayeb, M.A. Al-Rajhi and M.R.D. Seaward, *Science of the Total Environment*, **168**, 1 (1995).
3. A.A. Gasim, G.M. El–Hassan and A.A. Khateeb, *Proc. 2nd Symp. on Date Palm in Saudi Arabia*, **2**, 377 (1986).
4. M.M. Khalil, M.M. Aboeinil and A.S. Al-Ghamdi, *Proc. 2nd Symp. on Date Palm in Saudi Arabia*, **2**, 591 (1986).
5. P. Little, *Environmental Pollution* , **5**, 159 (1973).
6. P. Little and M.H. Martin, *Environmental Pollution*, **6**, 1 (1974).
7. P. Little and R.D. Wiffen, *Atmospheric Environment*, **11**, 437 (1977).
8. W. Reuther, *Amer. Soc. Hort. Sci. Proc.*, **51**, 137 (1948).
9. ————, *Amer. Soc. Hort. Sci. Proc.*, **57C**, 179 (1951).
10. S.C. Mason, *USDA Bul.*, **223**, 28 (1915).
11. M.H. Martin and P.J. Coughtrey, Biological Monitoring of Heavy Metal Pollution, Land and Air Applied Science Publishers, London (1982).
12. R.W. Nixon, *Ann. Date Grower's Inst. Ret.*, **20**, 7 (1943).
13. ————, *Ann. Date Grower's Inst. Rept.*, **24**, 23 (1947).

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