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Lead Pollution Levels in Sultanate of Oman and its Effect on Plant Growth and Development

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Abstract
Lead pollution is a major environmental problem where leaded petrol is used. The effect of lead levels on plants (date palm *Phoenix dactylifera* and tomato *Lycpersicon esculentum* Mill.) and the proximity to major roads on lead levels have not been investigated in the Sultanate of Oman. A study is conducted to find out lead levels in an area near a major highway and the effect of high lead levels on plant growth. The results indicate that contamination by lead decreases rapidly with increasing distance from the highway. Atmospheric depositions appeared to be the main contributor to plant contamination by lead. The absolute amount of lead absorbed by plants is positively correlated with the amount of lead in the soil, whereas the percentage uptake showed a negative correlation with lead concentration in the soil. Lead concentrations of 100, 500 and 1000 ppm have significant effects on roots of tomato plants. SOS-PAGE studies revealed the presence of two new low-molecular weight proteins associated with lead stress.

Introduction
Environmental pollution, especially by chemicals, has received a great deal of attention in recent years. Among all chemical pollutants, heavy metals have specific ecological, biological and health significance. Most of them are not essential for life and their depositions in the atmosphere contribute to the contamination of the biosphere components viz. water, soil and vegetation (Kabata-Pendias and Pendias, 1984).

Atmospheric lead from industrial sources contributes significantly to environmental pollution. Most of the lead in the environment comes from human consumption by different means of which fuel combustion from automobiles forms one of the main sources of atmospheric and soil pollution particularly by lead compounds (Fergusson, 1990; Murphy, 1987). Tetraethyl and tetramethyl-lead are added to petrol as additives in order to increase the octane rating of fuel (Fergusson, 1990). Lead forms 21 per cent of the line particles emitted by the automobiles (Seaward and Richardson, 1990). The principal lead products released by the automobiles are lead halides, ammonium lead halides and lead chlorobromide (Ter Haar and Bayard, 1971). Atmospheric pollution by lead is affected by a number of factors including wind velocity, traffic density and car status.

Atmospheric deposition is the most consequential source of lead accumulation in the aerial parts of plants (Pietrzak-Flis and Skowronsk-Smolak, 1995). Most of the lead absorbed by the root is bound to the cell wall and only small amount of it i.e., about 3 per cent, is translocated to other parts of the plant. Lead assimilation by the roots is a passive process, which depends on a number of factors such as lead concentration in the soil, soil pH and the form of lead compounds in the soil (Kabata-Pendias and Pendias, 1984). Plant cells are known to be able to adapt and develop tolerance to toxic levels of heavy metals by synthesizing a group of non-protein metal binding polypeptides, known as phytochelatines. These are involved in the detoxification of the excess metal ions. Failure to synthesize these polypeptides may cause growth inhibition or cell death (Steffens, 1990). Phytochelatines functionally resemble metallothioneins produced by mammals, insects, fungi and invertebrates in response to excess intracellular heavy metals. Production of phytochelatines has been demonstrated for a number of heavy metals including copper, zinc and cadmium (Steffens, 1990). These polypeptides have low molecular weight that varies from 3 to 10 kd and possess unusual structure (y-Glu-Cys)„-Gly, where n = 2-7 which is similar to glutathione (GSH) that possess the structure of y-Glu-Cys-Gly (Steffens, 1990; Steffens *et al*., 1986). The role of these polypeptides is not clear but the similarity between the phytochelatines and GSH suggests that they may carry out the same redox reactions in which GSH is involved, or they may participate in glutathione-S-transf erase reactions used in detoxification (Steffens, 1990).

Sultanate of Oman is marching fast towards the development and industrialization. Population is increasing steadily and its growth is associated with the geometrical increase in the numbers of automobiles. This combination of factors contribute greatly to environmental pollution with heavy metals, it is therefore necessary to investigate and put the levels of these pollutants under control before they cause any major damage to the environment. Lead is on the top of the list of heavy metal pollutants. Few studies have been carried out in Oman e.g., El-Mardi *et al*. (1995) assessed the effect of sewage water on the vegetation in general and date palm in particular. They showed that lead content is higher in plants irrigated with treated sewage water than those irrigated with desalinated or well water.

However, since it is known that lead affects humans, animals and plants, it becomes essential to explore lead levels in the environment since the Sultanate is still using leaded petrol. Date palm (*Phoenix dactylifera* L.) is used as biomonitor in this because it is the most common plant in Oman and it is found particularly on the roadsides and has been used as biomonitor in different studies in the arid environments (Al-Shayeb *et al*., 1995). In addition, there is a need to study the effects of high lead level on plants by examining the symptoms and changes that may occur at the molecular level. For this purpose tomato plants (*Lycopersicon esculentum* Mill.) were grown in soil with different concentrations of lead to assess the threshold level of lead and to determine lead uptake by plants from the soil.

Materials and Methods

The study area was chosen in the Wilayat of Barka of the Batinah coastal plain, which is considered to be the most important area for agronomy in the Sultanate of Oman and contains a number of important urban centers such as Seeb, Barka, Suwaiq, Khaburah, Saham and Sohar.

An area of 330.8x85 m was chosen at Al Naseem Public Park as the study area from which both plant leaves and soil samples were collected. This area is 68.2 m away from the Muscat-Sohar highway, which is a heavy traffic road and 30.88 m away from a service road. The experimental area has date palm plantation in regular rows and was divided into several small blocks each containing one date palm tree. 88 Blocks were used for sample collection from the total of 344 blocks. A small portion of the leaves was randomly selected from the plant, cut and kept in labeled polyethene bags and brought to the laboratory for analysis. For soil samples, each of the small blocks were further segmented into five sectors for randomization. Top surface soil at about 15 cm depth was collected. Quantitative estimation of lead in the leaves was analyzed by using Atomic Absorption Spectrophotometer (Rabinowitz, 1972), while soil lead content was analyzed by using Atomic Absorption Spectrophotometer (Fig. 1).

To study the effect of lead concentrations on plants, tomato plants were used. Seeds were germinated on moist tissue paper in the laboratory. Seedlings were transferred to pots containing soil mixture with different lead concentrations. Plants were provided with 25 ml of Hogland's nutrient solution twice a week. Lead nitrate solution of the desired concentration was prepared and added to the pots. The pots were kept under the shade house. Analyses were carried out after one and two months. Leaf area was measured from all the treatments using Leaf Area Meter. Dry weight of plants was recorded. Three plant samples were chosen randomly from each treatment to measure the lead uptake by the plants. 7.5% Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE) was performed for the analysis of water-soluble proteins by the standard protocol of Laemmli (1970). 3.5 g of the leaves were cut into small pieces and ground in 5 ml of the lyzing buffer and centrifuged at 100 g for 10 minutes at 4°C. Supernatant was centrifuged in again in a Beckman ultracentrifuge at 55000 g for 40 min at 6°C to precipitate the proteins. The pellet was dissolved in 500 µl of lyzing buffer with 12.5 µl of SDS and was stored at -80°C until further use.

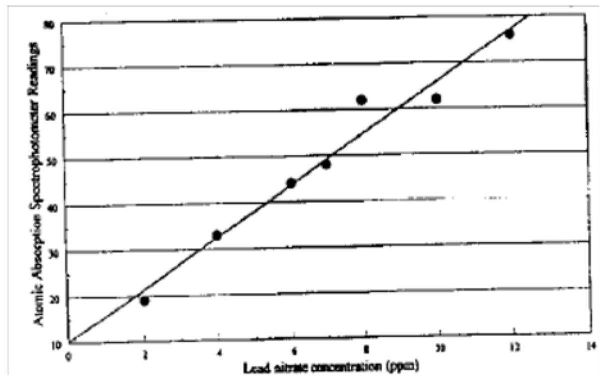

Fig. 1: An example of the standard curve used for lead determination using atomic absorption spectrophotometer.

Results

There was a decrease in the lead concentration in the soil with increasing the distance from the road. In general, lead concentration was relatively high at the beginning of the study area. High lead levels were recorded at the distance of 127.4 m to 171.8 m away from the highway with the maximum 0.14 ppm (µg/g) at the distance of 142.2 m. Then there was a steep decrease from 0.12 ppm at 171.8 m to 0.02 ppm at 186.6 m.

Lead concentrations in plant samples decreased with increased the distance from the highway. Plants at the distance of 127.4 m and up to 186.6 m away from the road showed the highest lead concentration in both the washed and the unwashed plant leaves. Lead concentration of the unwashed leaves increased from 11.25 ppm to a maximum of 12.23 ppm and then decreased to 11.44 ppm. Washed leaves followed similar pattern as the unwashed samples. The maximum lead concentration of 7.99 ppm was recorded at a distance of 142.2 m from the highway (Table 1).

Lead content in the one-month-old tomato plants was positively correlated with the concentration of lead in the soil. Four weeks later the increment in the amount of lead in the plants grown in the soil with high lead concentration was higher than plants grown in low lead concentrations. Lead accumulation in the plants did not increase greatly during the second month. The total amount of lead after the second month in the plants of treatment 500 ppm was 13.9 ppm while in the first month it was 9.3 ppm. The percentage of lead uptake was found to be much higher in the plants grown in the low lead content soil than in high lead soil. For instance, the plants grown in soil with 5 ppm, lead absorbed was 1.3 ppm which is 26 per cent of the given lead, whereas those grown in the soil with 1000 ppm of lead absorbed only 21.0 ppm which is 2.1 per cent of lead present in the soil.

Fig. 2: Effect of lead on the roots of tomato plants
1a = 500 ppm; b = control; c = 1000 ppm of lead

Pronounced effect was found in the root system of the treated plants while the effect was little on the leaves and the stems. The roots of the plants of the 100 ppm treatment were relatively shorter than the control plants. Drastic reduction in the root length was noticed in the 500 ppm treatment (Fig. 2).

Protein analyses using SDS-PAGE from the treated tomato indicate profiles with an extra band in plants grown in the different lead treatments. An additional protein band was noticed in the samples of 50, 100, 500 and 1000 ppm !cad treatment. Using standard protein markers, the molecular weights of these protein bands was found to be 9 and 3 kd respectively (Fig.3).

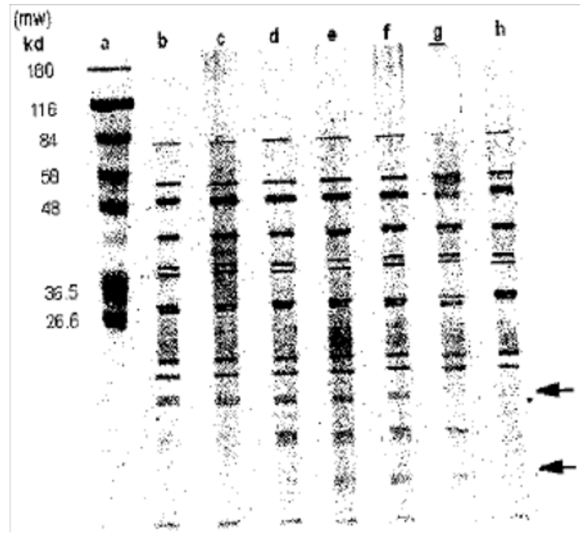

Fig. 3: Analysis of proteins by SDS-PAGE Electrophoresis
a. Standard protein markers; b. 5 ppm; c.10 ppm; d. 50 ppm; e. 100 ppm; f. 500 ppm; g. 1000 ppm; h. Control.

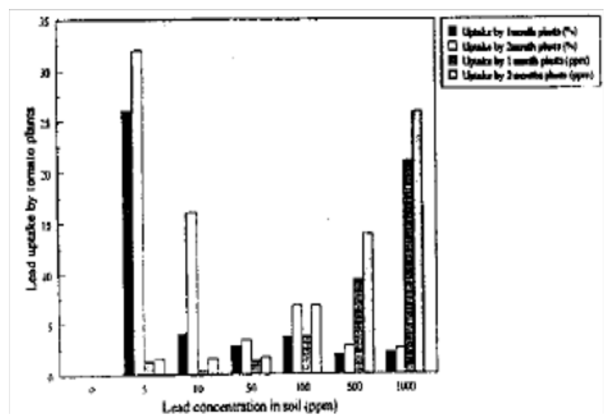

Fig. 4. Lead uptake by tomato plants

Discussion

Lend usually combines with organic matter in the soil forming different compounds (Zimdahl and Skogerboe, 1977). The soil in the study area is sandy and has low organic matter content, this may be a factor in the low lead levels detected. In sandy soils the top layer could be easily blown off by wind, as it is not covered by vegetation, this yfeatly limits or removes the accumulation effects.

Lead levels in the soil of the study area were found to be low (0.0 to 0.14 ppm) compared to data available for other countries (10 to 20 ppm) (Kabata-Pendias and Pendias, 1984). This could be due to the low population and the relatively limited number of vehicles on the streets and also to the fact that lead-containing pesticides are banned in Oman. Lead concentration was relatively or low at the outset of the study area; it increased steadily until it reached its maximum value and then started to decrease gradually until it reached zero towards the end of the study area. This pattern of decrease followed by an increase at the beginning of the area may be due to the presence of a large canopy of trees in front of the area which may act as a barrier, thus causing lead particles travel for a longer distance before they settle down. The decrease in lead showed almost curvilinear pattern with slight fluctuation. These results agree with those reported by Davies (1990). Al-Shayeb *et al*. (1995) stated that it was shown in several studies around the world that the leaves of higher plants

Table 1: Lead concentration (ppm) in soil and leaf samples.

Distance From main road (m)	Mean ± S.D. lead in soil (ppm)	Mean ± S.D. lead in unwashed leaves (ppm)	Mean ± S.D. lead in washed leaves (ppm)
68.2	0.1119 ± 0.1165	8.0339	3.4720
83.0	0.0679 ± 0.0790	12.0959 ± 5.3920	7.3308 ± 3.7145
97.8	0.0963 ± 0.0637	10.2975 ± 2.8360	8.0217 ± 2.8175
112.6	0.0775 ± 0.0704	10.4597 ± 5.4270	6.6122 ± 6.0683
127.4	0.1318 ± 0.1163	11.2525 ± 1.2107	7.7141 ± 0.9317
142.2	0.1391 ± 0.2959	12.0961 ± 2.5465	7.9922 ± 3.4496
157.0	0.1373 ± 0.1645	12.2277 ± 5.9728	7.1261 ± 4.8690
171.8	0.1157 ± 0.0607	11.7829 ± 1.7179	5.2840 ± 7.4727
186.6	0.0197 ± 0.0229	11.4431 ± 0.8020	5.4606 ± 0.7592
201.4	0.0395 ± 0.0102	7.6394 ± 0.5798	1.9995 ± 3.4633
216.2	0.0178 ± 0.0286	10.9429	7.4964
231.0	0.0172 ± 0.0288	8.1560 ± 3.7441	6.9784 ± 2.1693
245.8	0.0388 ± 0.0388	15.3238 ± 3.1728	9.2946 ± 1.7131
260.6	0.0550 ± 0.0453	6.9853 ± 3.5147	5.2307 ± 1.7404
275.4	0.0257 ± 0.0360	5.4977 ± 0.9966	4.1519 ± 1.0268
310.2	0.0337 ± 0.0347	7.3260 ± 4.0786	5.8261 ± 1.7581
325.0	0.0303 ± 0.0404	3.2474 ± 4.5925	3.2332 ± 4.5724
339.8	0.0232 ± 0.0325	5.4807 ± 4.0227	1.6123 ± 3.2247
354.6	0.0446 ± 0.0163	5.9903 = 4.3541	4.4932 ± 5.0636
369.4	0.0227 ± 0.0268	1.7500 ± 2.4748	0
384.2	0.0112 ± 0.0225	3.4966 ± 3.1184	1.9966 ± 3.4583
399.0	0 ± 0	4.9827 ± 4.2923	2.4905 ± 3.1196

can be used as monitors for the accumulation of a number of heavy metals. They indicated that date palm has the ability to retain the deposited metals due to the special morphological features of their leaves and their long life span. At the beginning of the study zone, lead concentration in the unwashed leaves was relatively high. It increased to a maximum, then decreased steadily towards the end of the study area. The presence of the large canopy of trees may be the cause for this pattern. Values of lead levels obtained in this study are relatively low compared to lead levels obtained in date palm trees from Saudi Arabia (Al-Shayeb *et al*., 1995). However, the general pattern of decrease in lead levels with the distance in the rest of the samples indicates clearly that the closeness to the road affects the lead content in the unwashed leaves. It has been shown that the main source of lead in the roadside ecosystem is the leaded petrol (Davies, 1990).

Lead concentration in plants was much higher than that of the soil. This indicates that the soil may not be the only source of the lead accumulation in the plants and that there are other sources that contribute to the lead pollution in the plants. Lead may accumulate on the leaf surface by foliar retention, foliar absorption or by the uptake from soil. These particles could then enter into the leaves through the stomata where lead could be seen as electron dense particles in the substomatal cavity and around the epidermis. Atmospheric depositions have been reported to be the main source of lead accumulation in the plants (Al-Shayeb *et al*., 1995). 84 to 89% of lead aerosols are within the range of 2.5-15 μm in diameter that can settle down on the available surfaces including plant leaves (Al-Shayeb *et at*., 1995). When lead content in plants grown in the field was compared with those grown under a tent, lead concentration in the aerial parts of the field plants was found to be higher than that of the tent plants (Pietrzak-Flis and Skowronska-Smolak, 1995). Lead concentration in the roots of plants gown in the field had similar concentration to those grown under the tent. The high concentration in the shoots of the field plants resulted from the atmospheric depositions. Lead concentration in the washed date palm leaves followed the same pattern as that of unwashed samples. However, in the present study it was found that 14-73 per cent of lead in the unwashed leaves was removed by washing. These results are in agreement with those reported by Kabata-Pendias and Pendias (1984). Similarly, Albasel and Cottenie (1985) stated that lead accumulation in the unwashed plants is up to three fold higher than that of the washed plants. Moreover, Al-Shayeb *et al*. (1995) showed that 26-68 per cent of lead could be removed from the date palm leaves by washing.

The amount of lead absorbed by tomato plants was found to be directly related to the amount of lead in the soil (Fig. 4). These results are in conformity with those reported by Kabata-Pendias and Pendias (1984). Tomato plants absorbed most of the lead during the early stages of growth. It is probable that the plants may absorb a large amount of lead at seedling stage, then only a small amount is assimilated as they grow. It should be noted that plants did not absorb all of the lead in the soil. The percentage uptake of lead by the plants decreased as the lead concentration in the soil increased. These results gather support from the reports of Simon and Ibrahim (1987). It can be notpd that with a low soil concentration of lead, the uptake percentage of the plant increased linearly as lead concentration in soil increased. However, the correlation between the plant's ability to absorb lead and soil lead concentration was significantly less in soil, with high lead concentration that is close to saturation point. This study showed that the plant uptake as a function of substrate concentration increases asymptomatically towards a saturation point, this agrees with the findings by Simon and Ibrahim (1987). Solubility of lead in the soil and its availability to the roots is not the only factor that limits the lead content in aerial parts of the plants, but another internal factor in the plant appears to control the mobility of lead within the plant. Most of the lead is bound at the site of the absorption whether it is in the leaves or the roots, only little amount is translocated to the other parts of the plant (Rabinowitz, 1972). Our findings support this view.

The treated plants showed large leaf area independent of lead concentration in some treatments. Statistical analyses of the leaf area and the dry weight of the tomato plants showed significant differences. The average values were higher than the control plants. Consequently, the dry weight of these plants was higher; the variation among the plants seems to be due to other factors rather than to the effect of lead. In general, the leaves appeared healthy without any visible symptoms. On the other hand, the roots of plants grown in high lead levels i.e., 100, 500 and 1000 ppm were significantly affected (Fig. 2). The roots were considerably short and brown in the 1000 ppm treatment. It is apparent that the effect of lead can be clearly noticed at the absorption site rather than the other parts of the plant. This is in agreement with the findings reported by Kabata-Pendias and Pendias (1984) who illustrated that lead poisoning results in dark green leaves, wilting of older leaves and brown short roots. In this study, the leaves exhibited no phytotoxicity symptoms. This may be due to the young age of the plants, or just the site of absorption is effected. Tung and Temple (1996) suggested a pathway for lead entrance and accumulation in the roots. The most active sites for lead absorption in the root are the walls of the primary epidermal cells at the meristematic zone. Lead accumulates mainly in the root apex, root hair zone, root primordia and the uppermost part of the root tissue. The greatest lead accumulation being at the growing root tip while it decreases as the distance from the tip increases, Lead in the root hair zone can not pass through the casparian strip within the endodermis. Entrance can take place through the non-thickened passage cells. However, the short root system may cause death to the plant, for its inability to absorb enough nutrients to support the growth. Thus, the range of 100 to 500 ppm seems to be the critical range at which lead starts to affect the plants significantly. These results are in accordance with Kabata-Pendias and Pendias (1984).

SDS-PAGE of protein revealed a number of bands in all the plant samples however, in the lead treated plants an extra band with an approximate molecular weight of 9 kd was detected, this band was not detectable in the control plants. An additional band with the molecular weight of 3 kd appeared in the plant sample that received lead concentration of 50, 100, 500 and 1000 ppm. Therefore, it is apparent that even low lead levels affect plants and cause the synthesis of stress proteins, which are probably involved in detoxification of lead. Steffens (1990) demonstrated that with excess heavy metals, plant cells respond by synthesizing low molecular weight polypeptides called phytochelatines. The molecular weight of the phytochelatines, ranges between 3 to 10 kd. It is believed that these polypeptides are metal binding and involved in the detoxification of the heavy metals.

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