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Heavy Metal Content in Leaves of *Ficus retusa* Collected from Contaminated and Uncontaminated Sites in Northern Egypt: Mitigation of Toxicity by Washing Treatments

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Abstract:
Leaves of *Ficus retus*a were collected from three locations; suburban, urban and industrial in Northern Egypt. Concentrations of heavy metals in soils collected from the suburban site were lower than that collected from other sites. Plants washed with 1% soap showed better growth, higher chlorophyll content and higher photosynthetic rates than those remained unwashed or washed with tap water or 0.1N HCl. Our results indicated that washing treatments alleviated the phytotoxic effects of heavy metal pollution on plants.

Keywords: Growth, Heavy metals, Pollution, Photosynthesis, Washing

1.0 Introduction:

To date an unprecedented, rapid change in environmental conditions is observed, which is likely to override the adaptive potential of plants. In most terrestrial ecosystems, there are two sources of these environmental changes which have caused air and soil pollution (Scützandübel & Polle, 2002**).**These sources are the underlying parent material and the atmosphere. The concentrations of heavy metals in soils depend on weathering of the bedrock and on atmospheric inputs of metals. In addition, plants are exposed to natural climatic or edaphic stresses, for example air pollution, chilling, heat, drought, flooding and nutrient imbalance. Some of these factors may fluctuate significantly in intensity and duration.

The heavy metals such as lead (Pb), cadmium (Cd) and zinc (Zn) are very common in areas surrounding non-ferrous smelters and they are readily absorbed by plants (Hassan & Gewifel, 1998: Manworing, 2001). Increased concentrations of these metals causes damage and visible injury in vegetation and trees (Sawidis *et al*., 2000). Metals my inhibit several biological processes such as delay in flowering due to inhibition in hydrolysis of starch and sucrose as well as transportation of sucrose followed by secondary effects of a disturbed nitrogen metabolism. Moreover, heavy metals cause other physiological disorders in plants such as reduction in growth and yield, alteration in Photosynthetic rates and stomatal conductance, disturbance in mineral nutrition and therefore, strongly influence biomass production (Shamsi, *et al*., 2008).

There are considerable data regarding the retention and bioaccumulation of metals from plant leaves and the biological effects caused from their toxicity (Duruibe *et al*., 2007). However, metal uptake by trees has been unclear and also no correlation with heavy metal doses and corresponding effects on tree leaves (Duruibe *et al*., 2007). In contrast, Gue *et al.* (2003) reported a strong correlation between heavy metal toxicity and changes in lipid composition, alteration in antioxidant systems, and the reduction in photosynthetic ic rates due to reduced chlorophyll content plant material for trace elements (Hassan, 1994).. Recently, Agrawal and Sharma (2008) found similar results as they correlated toxicity of metals with reduction in growth and yield. It has been shown that bioavailability is affected by soil properties like pH, organic matter or clay content (Brink *et al*., 2010).

It is important to distinguish between mineral deposited on the leaf surface through atmospheric fallout (dry or wet deposition) and those contained within the leaf tissue obtained from the soil in order to analyse plant material for trace elements (Hassan, 1994), and those contained within the leaf tissue to remove surface contamination (Porter, 1986). There are several techniques present to investigate the plant response to pollutants singly and in combination. The most reliable one is to carry out experiments under natural field conditions along a transact from the source point to a place away from it (upwind) (*e.g* Ali, 1993). Romano and Abate (1995) analysed heavy metals in leaves of ornamental plants grown along urban roads and urban areas before and after washing plant materials with distilled water. They found higher concentrations of metals in samples collected from urban areas than those collected from rural areas. Moreover, washing the leaves caused removal of heavy metals by about 58 to 74% depending on the nature of the metal. The aim of this study was to investigate the effects of heavy metal pollution and washing treatments on growth and physiology of *Ficus retusa* grown in three different locations in Northern Egypt.

2.0 Materials and Methods:

2.1 Study Area and Plant Culture:

Three locations were chosen in Alexandria City to conduct the experiments for two successive years (2004, 2005). The first location was at Al Montazah Botanical Garden, a suburban location, located 17 km to the east of Alexandria City centre. It is surrounded by palm trees and casuarinas (Hassan *et al*., 1995). The second one was located in the Botanical Garden of Faculty of Agriculture, Alexandria University, urban area, near the city centre and very close to a congested traffic road, while the third location was located at a garden of Abou Quir company of fertilizers and Chemicals, industrial location, located 21 km east of Alexandria city centre. Rooted cuttings of *Ficus retusa* with average length of 40 cm were grown in 5 L clay pots filled with loamy clay soils collected fro, the surrounding soils in each site (pH 7.7) for twelve months (Dec. 2003 – Dec. 2004, and then repeated again (12/2004 – 12/2005). There was one plant/pot. NPK was added at a rate of 5 g/pot/month to all plants according to normal agricultural practice in Egypt (El Laithy, 1987; Hassan, 2010) in the three locations. There were 100 pots in each location.

2.2 Washing Treatments:

There were four washing treatments applied to plants in all locations 8 weeks after planting every month throughout the entire course of the experiment. Pot surfaces were covered with polyethylene cover to avoid falling of washing solutions on the soil during spraying. Treatments were as follows: (I) control (without washing); (II) washing with tap water, (III) washing with 0.1 N HCl solution and (IV) washing with 1% soap solution (Nastapon) (using this trade mark does not mean its recommendation). There were 25 pots from each treatment / location.

2.2.1 Non Destructive Harvest

Photosynthetic rates (A) (µmol m^{-2} s^{-1}) and stomatal conductance (g_s) (mmol m^{-2} s^{-1}) were measured 60 days after planting (DAP) using a portable Infra Red Gas Analyser (Li – COR 6200, Lincolin, UK). The measurements were carried out on first foliage leaf every ten days. In case of sever damage of first leaf, measurements were carried out on the second leaf. Onset of flowering time was recorded for all plants in all locations.

2.2.2 Destructive Harvest:

Plants were harvested destructively at the end of the experiment (after 12 month) in both experiments. (26 Dec 2004 and 28 Dec 2005 in the first and second experiment, respectively). Plants were divided into different organs and dried in dry oven at 70 ^{o}C for 72 hrs and then weighed.

2.3 Determination of Chlorophyll Content:

Five oven-dried leaves from each treatment from each site were ground to produce subsamples of 1.0 g. and total chlorophyll was determined according to Garty et al (2001), measurements were carried out on PEKMAN Spectrophotometer 1201

2.4 Determination of Heavy Metal Concentrations:

Ten oven-dried leaves/ treatment/site were pulverized in a mortar under liquid nitrogen. Then 250 mg of this powder were digested in 10 ml of 1 N HNO_3 in 50 ml test tube in a heating block for 10 h at 100 ^{o}C. The concentrations of heavy metals were determined in soil and leaves by inductively coupled atomic emission spectrometry (ICP – AES) by spectroflame ICP (Spectr, Klave, Germany).

2.4 Statistical Analysis:

Two way ANOVA was applied to log-transformed data to evaluate effects of washing treatments and sampling sites on growth and physiology of the plant. (Statgraphics Statistical Package 4, London, UK).

3.0 Results and Discussion:

Visible injury symptoms were very clear on plants (Figure 1). It shows that air pollution caused severe symptoms on leaves collected form an industrial area compared to those collected from uncontaminated area. Egyptian soils characterized by being slightly alkaline pH (7.7) Table (1). Moreover, soils collected from Abou Quir locations had the highest concentrations of heavy metals (31.65, 595.15, 786.88 for Cd, Zn and Pb, respectively, Table 1), while soils collected from Al Montazah showed the lowest concentrations for the same metals. However, the third location (El Shatby) had intermediate concentrations of these heavy metals (table 1).

Figure 1. Visible injury symtoms on plants (The leaf on the right collected from Al Montazah "Suburban area" and the leaf on the left collected from Abou Quir "industrial area").

Table 1: Concentrations of heavy metals (ppm) in soils (+ SD)

Location	Parameter			
	Cd	Zn	Pb	pH
El Montazah	2.43 ±0.3	54.92 ±6.4	55.50 ±7.5	7.7
El Shatby	8.95 ±1.1	150.20 ±8.4	110.35 ±8.9	7.7
Abou Quir	31.65 ±4.2	595.15 ±11.7	786.88 ±18.4	7.7

Heavy metal contents of soils showed that Al-Montazah site (suburban) was the least contaminated, while the other two sites were more polluted with Abou Quir site (Industrial) was the most polluted one and this is in agreement with the previous published data (Hassan, 1999: EEAA, 2007). This indicates that Al-Montazah site could be considered as an uncontaminated site while Abou Quir is the contaminated one. Our results are in agreement with the results of Ke *et al*. (2007), who described the site with low heavy metal contamination as an uncontaminated site, while that near industrial point as a contaminated one. This finding was supported recently with the results of Brink et al. (2010) who found similar results in the Netherlands. In agreement with Brink *et al*. (2010), metal accumulation by plants appears to be highest in contaminated sites (Abou Qir and El Shatby) compared to an uncontaminated one (Al-Montazah) in the present study. Trees appear most efficient in accumulating Pb, Cd and Zn than grasses or shrubs (data not shown) and this is in agreement with the results of many published data (e.g. El Kiey & Ormrod, 1987; Sawidis et al., 2007; Brink et sl., 2010; Zahran, 2011). In contrast to Ke *et al*. (2007), our results indicated that plants grown in the contaminated sites had lower growth rates than those grown in an uncontaminated site. This may be ascribed to differences in soil properties and/or genetic characteristics.

Table 2: Growth parameters of *Fiscus retusa* response to different washing treatments.

Site	Parameter	Treatment Control	H_2o	HCl	Soap
Al Montazah (Suburban)	Height (cm)	48.93[a]	53.63[b]	54.60[b]	56.33[c]
	No. of leaves	87.37[a]	91.50[b]	106.20[c]	154.60[d]
	No. of dead leaves	17.50[c]	14.60[b]	13.20[a]	12.40[a]
	TDW (g)	48.76[a]	55.96[b]	62.34[c]	65.21[c]
	SDW (g)	33.72[a]	39.87[b]	43.79[c]	47.25[d]
	RDW (g)	15.04[a]	16.09[a]	18.55[c]	17.96[b]
	RSR	0.47[d]	0.40[ab]	0.42[bc]	0.38[a]
El Shatby (Urban site)	Height (cm)	46.21[a]	48.21[a]	51.01[b]	50.00[b]
	No. of leaves	51.86[a]	54.12[a]	60.35[b]	72.66[c]
	No. of dead leaves	26.21[b]	24.20[a]	23.70[a]	25.08[b]
	TDW (g)	45.79[a]	47.11[a]	56.32[b]	61.09[b]
	SDW (g)	32.31[a]	37.01[b]	40.25[b]	45.73[c]
	RDW (g)	13.48[a]	10.10[a]	16.07[bc]	15.36[ab]
	RSR	0.42[c]	0.27[a]	0.40[c]	0.33[b]
Abou Quir (Industrial)	Height (cm)	43.20[a]	45.10[ab]	47.32[b]	48.25[bc]
	No. of leaves	49.84[a]	51.16[a]	54.37[b]	56.12[b]
	No. of dead leaves	34.02[d]	29.46[c]	24.62[b]	20.03[a]
	TDW (g)	40.39[a]	44.98[b]	50.08[c]	52.73[c]
	SDW (g)	29.45[a]	31.73[a]	39.79[b]	41.21[b]
	RDW (g)	10.94 [a]	13.25[b]	10.29[a]	11.52[b]
	RSR	0.37[b]	0.42[c]	0.26 [a]	0.28[a]

Each figure is a mean of 40 plants (the data averaged over the two experimental years); TDW = total dry weight; SDW = Shoot dry weight; RDW = root dry weight; RSR = Root: shoot ratio; Figures not followed by the same letter are significantly different from each other at P≤ 0.05.

Plant height was significantly (P≥ 0.05) affected by washing plants with different liquids, location and their interaction (for simplicity, data represented in this paper is the average of results of two successive seasons, 2004 & 2005). The highest length was found in plants grown in Al Montazah (suburban area) (48.93 cm) while the shortest length was recorded in plants grown in Abou Quir (43.20 cm). Moreover, washing plants with 1% soap protected plants better than other treatments, it caused increase in height by 15, 8 and 12% and in root: shoot ratio (RSR) by 19, 21 and 24% in plants grown at Al Montazah, El Shatby and Abou Quir, respectively). Other growth parameters showed the same trend (table 2).Interaction between treatment and location was less than additive (Table 3). The higher growth rates of plants at Al Montazah was significantly correlated (R^2 = -0.261) with good quality and low levels of metal content of soil (data not shown). On the other hand low growth rates were correlated with high levels of heavy metals in the soil at Abou Quir location (R^2 = -0.306).

Table 3: ANOVA showing effects of different washing treatments, locations and their interaction on growth parameters

Parameter	Washing treatment	Site	Washing X site
Height (cm)	*	*	n.s
No. of leaves	*	**	*
No. of dead leaves	**	**	*
TDW (gm)	*	*	n.s
SDW (gm)	*	*	n.s
RDW (g)	*	*	*
RSR	*	**	*

Legends as table 1; * = significant at 0.01 ≥ P ≥ 0.05; ** = significant at 0.001≥ P ≥ 0.01; n.s. = non significant (P≥ 0.05)>

Table 4: Concentrations of Cd (ppm) in leaves of *Fiscus retusa*

Location	Treatment			
	Control	H_2O	HCl	Soap
El Montazah	0.50	0.46	0.38	0.32
El Shatby	1.08	0.99	0.90	0.76
Abou Quir	1.66	1.40	1.11	0.90

LSD $_{0.05}$ for Location = 0.05
LSD $_{0.05}$ for treatment = 0.04
LSD $_{0.05}$ for location * treatment = 0.06

Legends same as mentioned in Table 2.

Table 5: Concentrations of Zn (ppm) in leaves of *Fiscus retusa*

Location	Treatment			
	Control	H_2O	HCl	Soap
El Montazah	65.30	46.52	40.03	38.72
El Shatby	101.13	84.68	67.39	50.01
Abou Quir	127.64	96.48	76.31	49.54

LSD $_{0.05}$ for Location = 6.11
LSD $_{0.05}$ for treatment = 10.72
LSD $_{0.05}$ for location * treatment =6.97

Table 6: Concentrations of Pb (ppm) in leaves of *Fiscus retusa*

Location	Treatment			
	Control	H_2O	HCl	Soap
El Montazah	1.43	1.39	1.00	0.70
El Shatby	7.94	5.55	5.02	4.12
Abou Quir	29.97	20.32	18.64	10.10

LSD $_{0.05}$ for Location = 0.02
LSD $_{0.05}$ for treatment = 5.14
LSD $_{0.05}$ for location * treatment = 6.19

Washing plants with 0.1N HCl and 1% soap caused increase in Photosynthetic rates (A) by 22 and 26% in plants grown at Al Montazah, and by 19 and 21% in plants grown at El Shatby and by 15 and 18% in plants grown at Abou Quir, respectively. However, washing plants with tap water had no significant effect ($P \geq 0.05$) at all locations (Figure 2). Similarly, washing with 1% soap caused increase in Stomatal conductance (g_s) by 6, 17 and 18% in plants grown at Al Montazah, El Shatby and Abou Quir, respectively (Figure 3). Nevertheless, lower photosynthetic rates and stomatal conductance were correlated to high concentrations of heavy metals in the soil (R^2 = 0.211).

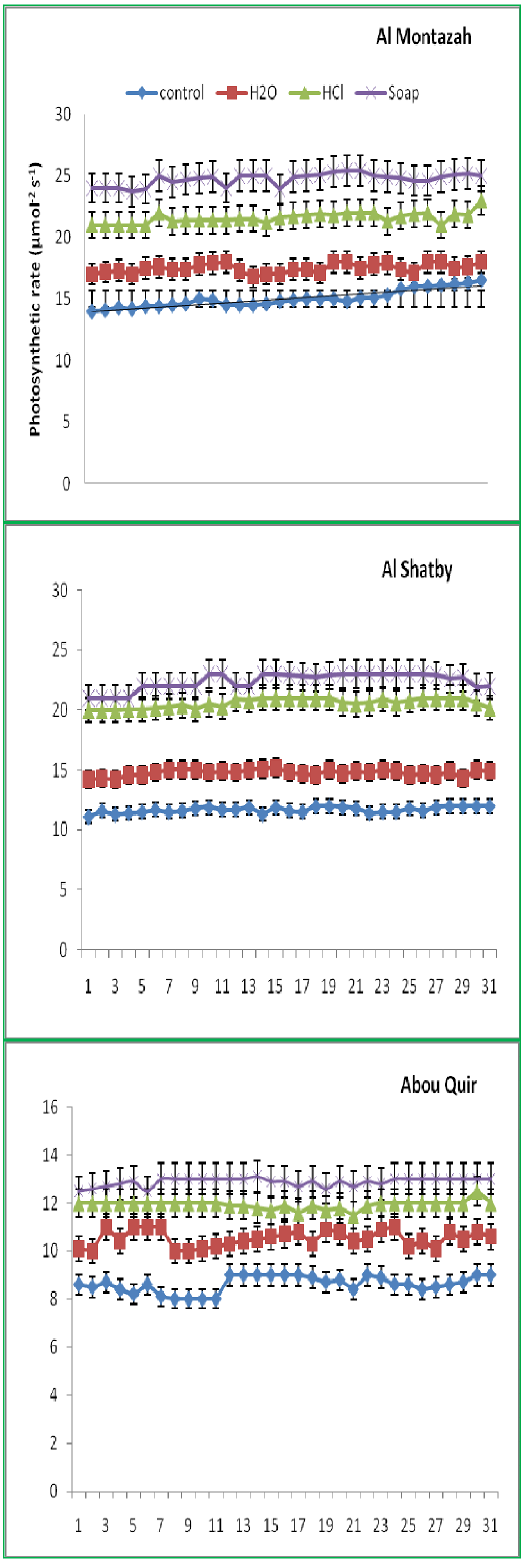

Fig.2: Effect of different washing treatments on Photosynthetic rates (A) of plants grown at different sites. Each figure is a mean of 15 readings ± 1 SE.

Chl *b* was more sensitive to washing treatments than Chl *a*, as it was found to be increased by 85, 1 and 2 fold at Al Montazah, El Shatby and Abou Quir, respectively, while these parameters increased by 62, 50 and 57% at the same locations, respectively, due to washing with 1% soap(Figure 4). Heavy metal contents of leaves showed that their concentrations increased with the increase in the concentration of these metals in the soil: plants grown at Abou Quir had the highest concentrations(1.66, 127.64, 29.97 ppm for Cd, Zn and Pb, respectively), while those grown at Al Montazah had the lowest concentrations (0.50, 65.30, 1.43 ppm for the same elements, respectively), and those grown at El Shatby had intermediate concentrations of these elements (1.08, 101.13, 7.94, respectively) (Tables 4,5,6).The concentrations of metals were significantly correlated to concentrations of these elements in the soil (R^2 = 0.196, R^2 = 0.201, R^2 = 0.276, for Cd, Zn and Pb, respectively).

Washing treatments significantly ($P \leq 0.05$) reduced concentrations of heavy metal contents in the leaves of all plants collected from all locations. Reductions due to washing with 1% soap ranged between 30% for ZN in plants grown at El Shatby to 98% for Pb in plants grown at Al Montazah (Tables 4, 6). Interaction between locations and washing treatments was more than additive. Heavy metals may interfere with essential nutrient uptake and transport and thereby disturb mineral nutrition composition of plants. Consequently, heavy metals may result in phytotoxic effects by influencing metal nutrition metabolism of plants (Monni *et al*., 2000; Ke *et al*., 2007). Such disturbance in metabolism would affect physiology and growth of plants. It is generally regarded that damage to plasmalemma of root cells constituted the first effect of heavy metal toxicity causing a loss of ions and lipid peroxidation (Woolhouse, 1983; De Vos *et al*., 1993). So root growth is more sensitive to heavy metal accumulation than shoot growth (Prajapati & Tripathi, 2008).

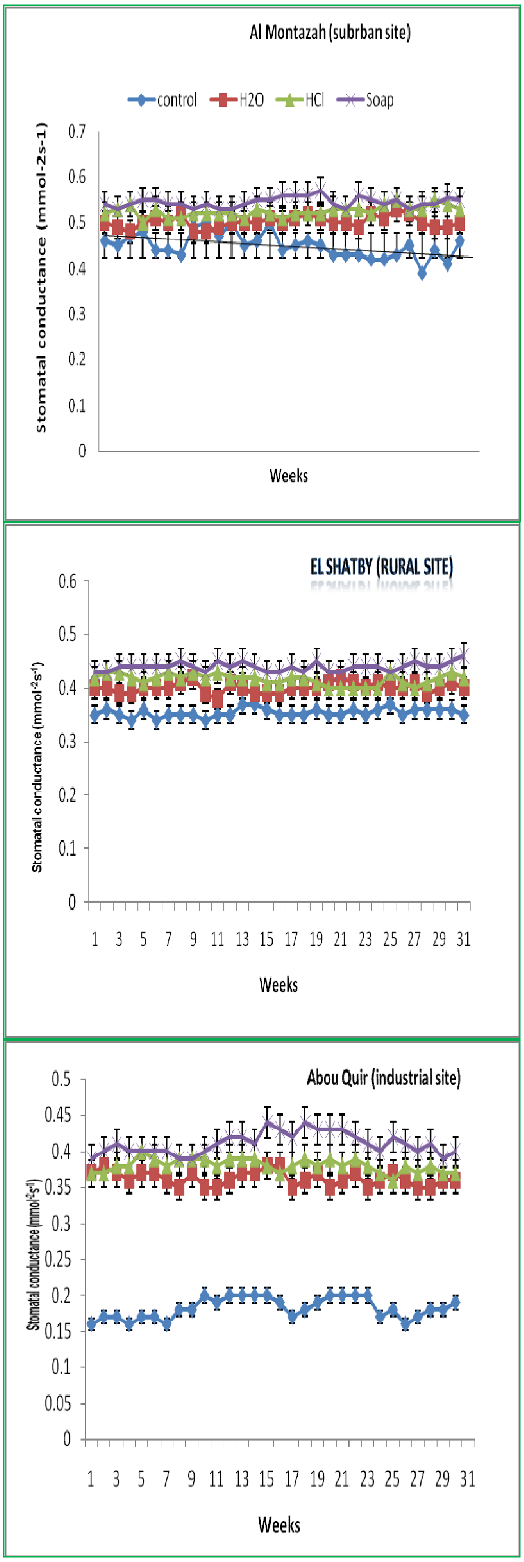

Fig.3: Effect of different washing treatments on and stomatal conductance (g) of plants grown at different sites. Each figure is a mean of 15 readings ± 1SE

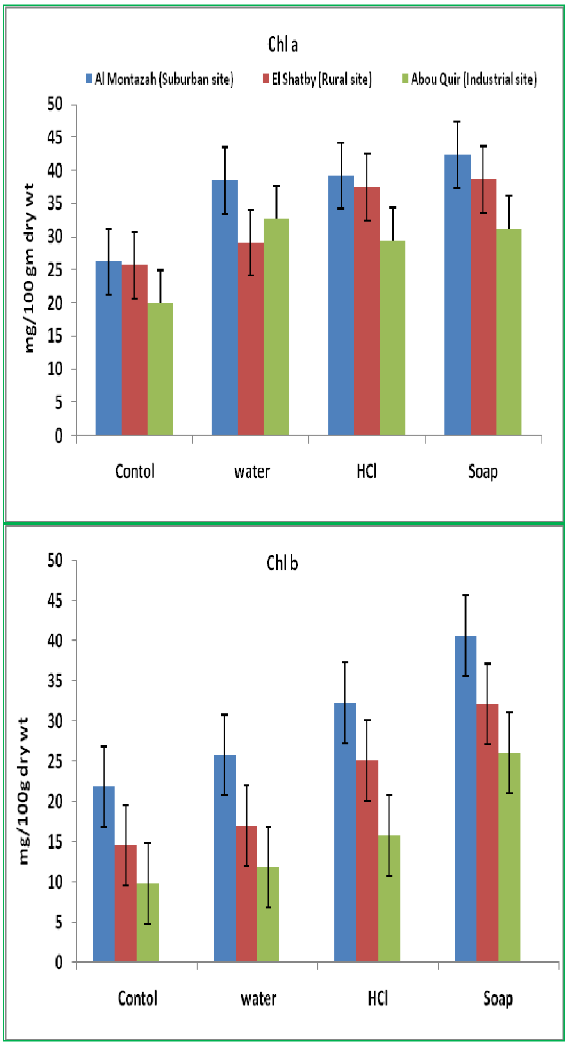

Fig.4: Effect of different washing treatments on Chlorophyll *a* and *b* of plants grown at different sites. Each figure is mean of 15 readings ± 1 SE.

In the present study, it was noticed that root growth was more affected than shoot and this resulted in lower root:shoot ratio (RSR) in plants grown in contaminated sites than those grown in an uncontaminated one. Such alteration in growth was associated with the reduction in chlorophyll content, lower photosynthetic rates and alteration in stomatal conductance, and this is in agreement with the results of (Garty *et al*., 2001; Scützandübel & Polle, 2002; Ke *et al*., 2007; Brink *et al*., 2010). There is need for future investigation to be carried out in other areas and in combination with heavy metal accumulation in relation with physiological changes, nutrient variations, combined stresses etc.

Washing treatments alleviated the phytotoxic effects of heavy metal pollution, plants washed with 1% soap showed better growth, higher chlorophyll content and higher photosynthetic rates than those remained unwashed or washed with tap water or 0.1N HCl and this is in agreement with the results of Wallace *et al*. (1999) *on Macadonia tenifolia.* Recently, Novak et al. (2005) indicated that industrial emission caused similar effects on different woody plants and washing treatments showed wide range of responses. Moreover, reported that washing plants with soap caused the highest growth and photosynthetic rates in plants compared to unwashed or washed with other agents.

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