EVALUATION OF AIR AND SOIL POLLUTION USING THE BIOMARKER *MORINGA OLEIFERA* IN JEDDAH CITY, SAUDI ARABIA

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Abstract. Physiological processes, photosynthesis, carbon and nitrogen metabolism, osmotic adjustment and secondary metabolites are known to be affected by air and soil pollutants. The present study was designed to evaluate the impact of air and soil pollution in Jeddah city, Saudi Arabia, on the physiological processes of *Moringa oleifera*. Four accessions differing in their pollution intensity were selected for this study (S1-S4). Elemental analysis revealed that Pd, Cu, Mn, Cr, Fe and Zn were the main soil pollutants in study accessions and trees growing in S2 have accumulated large quantities of these metals in their leaves. The physiological response to environmental pollution in *M. oleifera* tree was found to be dependent on pollution intensity. The heavily polluted accession showed a different response from the rest of accessions. As an adaptive strategy to environmental stress, this site has shown an increase in some metabolites such as lipids, flavonoids, free amino acids, alkaloids and saponins. However, some metabolites were decreased in response to pollution intensity such as soluble sugars and proteins, ascorbic acid, and terpenoids.
Keywords: *Moringa oleifera, heavy metals, phytotoxicity, physiological response, antioxidant capacity*

Introduction

Moringa (*Moringa oleifera* Lam.) is the most broadly cultivated species within Moringaceae family and is known as a rapidly-growing tree. It is commonly distributed in the sub-Himalayan parts of India, Pakistan, Bangladesh and Afghanistan. It has been introduced and become naturalized in many other parts of the world including Arabian peninsula. *Moringa oleifera* has many common names as the horseradish tree, drumstick tree, benzolive tree, kelor, marango, mlonge, moonga, and many other synonyms (Fahey, 2005). *Moringa oleifera* is widely cultivated for many purposes as efficient food stuff, biofuel production, and other implementations. Traditionally, it is used in therapeutic objectives around the world due to actual manifestations. These advantages have been related to metabolites like phenolics, vitamins and proteins (Health, 2005; Adedapo et al., 2009). *Moringa oleifera* offers a rich and unusual mixture of nutrients in its leaves, pods and seeds, amino acids that possess many therapeutic activities like antioxidant, anti-aging and anti-inflammatory. Moringa is sometimes called “Mother’s Best Friend” and “Miracle Tree” as it has been advised as a complementary to treat malnutrition (Sreelatha and Padma, 2009).

In fact, moringa is said to provide 7 times more vitamin C than oranges, 10 times more vitamin A than carrots, 17 times more calcium than milk, 9 times more protein than yoghurt, 15 times more potassium than bananas and 25 times more iron than spinach (Rockwood et al., 2013). The leaves of *M. oleifera* has been established as a rich source of phenolics and glucosinolates (Amaglo et al., 2010), minerals like iron, calcium, phosphorus and copper, (Saini et al., 2014a), tocopherols (Saini et al., 2014b) and antioxidants (Dillard and German, 2000). It also contains high concentrations of

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ascorbic acid, estrogenic substances and beta-sitosterol, vitamins A, B and C, α-tocopherol, riboflavin, nicotinic acid, pyridoxine, β-carotene, protein, and essential amino acids such as methionine, cystine, tryptophan and lysine (Rajput et al., 2017). These components have pharmacological potentials like antimicrobial, anticancer, antihyperlipidemic, antidiabetic, antiulcer, analgesic, antifertility, anticonvulsant and hepatoprotective (Ganatra et al., 2012).

Global awareness of air pollution has been rising due to its massive danger to public health. World Health Organization (WHO) reported that annual concentration of ambient air pollution was increased worldwide by 8% during the recent five years (Nakao et al., 2017). In regard to plants, unrestrained use of fossil fuels in industries and transport sectors has led to the increase in concentrations of gaseous pollutants such as SO_2, NO_2, etc. (Rai et al., 2011). It is a known fact that 60 % of air pollution in city is caused by automobiles only. On sensitive species of both plants and animals, the effect of these pollutants is observed at acute level. Response of plants towards air is being assessed by the air pollution tolerance index (APTI). Some plant species and varieties are so sensitive that they can be easily used as biological indicators or monitors of specific pollutants. They can further assist the planner in managing the urban cities (Horaginamani and Ravichandran, 2010).

With the extension of the urban expansion of the city of Jeddah, Kingdom of Saudi Arabia (KSA), many human requirements led to the accumulation of various wastes containing hazardous and non-hazardous components. The lack of proper disposal of those wastes cause environmental disasters on all surroundings. Solid waste management studies have shown that the amount of solid household waste produced per individual in industrialized and developed countries ranges from 2 to 2.5 kg/ day. The World Health Organization classified the Arab Gulf countries among the rich countries characterized by the rise of solid waste for individuals, which led to the emergence of the problem of open and closed garbage dump sites. Due to the fact that plants play an effective role in purifying the environment of pollutants, *M. oleifera* was selected for this study. It was noted that its fruits and leaves are palatable by the expatriate workers, despite of it was planted in areas close to garbage dumps and in heavy traffic roads. This study provides an overview on the nutritional value, antioxidant capacity, secondary metabolites, as well as heavy metals exclusion capacity from air and soil by *M. oleifera* in four differentially polluted accessions in Jeddah city, KSA.

Materials and methods

Green leaves of *M. oleifera* tree were collected during the summer season of 2017 from four different accessions in Jeddah city, KSA. The first accession (S1) was a double public road passing by cars (21.33.32 N; 39.10.33 E), the second accession (S2) was a polluted area by flaming fires, factories wastes and random waste disposal (21.21.16 N; 39.13.09 E). The third accession (S3) was a narrow bystreet with rare traffic (21.33.29 N; 39.10.32 E) and the fourth accession (S4) was a secondary street with average traffic (21.33.49 N; 39.10.44 E). The map of different experimental sites is provided in *Photo 1*. The mature and fully expanded leaves were collected from the top branches of the trees in the study sites. A total of 250 leaves were collected from 5 trees at each experimental site. Leaf samples were washed with tap water several times and once with distilled water, placed in an air-forced oven at 60 °C for five days. Dried samples were grinded into fine powder by an electrical mixer, sieved through 0.2 mm

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sieve and stored in paper bags for analysis. Soil samples were collected at the time of leaves collections from the study sites. Soil samples were collected from the surface soil layers with a depth of about 0 to 20 cm at the tree rhizosphere. Soil samples were replicated three times per each experimental site. The collected soil samples air dried and sieved for physical and chemical characterization.

Photo 1. *A satellite image of the four experimental sites*

Mineral analysis of plant and soil samples

Plant and soil samples were digested with a mixture of 69% HNO_3 and 30% H_2O_2 (5:2 v/v). The mineral content concentrations in digested solutions were determined using inductively coupled plasma-optical emission spectroscopy (Polyscan 61E, Thermo Jarrell-Ash Corp., Franklin, MA, USA).

Estimation of photosynthetic pigments

Total photosynthetic pigments; Chl a, Chl b and carotenoids, were extracted from moringa leaves by grinding in cold acetone (80%) using pre-chilled mortar and pestle. The extracted pigments were centrifugated at 3000 rpm for 15 min and quantified according to the method prescribed by Metzner et al. (1965). The quantified pigments were expressed as (µg/g dw).

Preparation of methanolic extract

The fine powdered plant tissues (5 g for each sample) were extracted with 50 ml of 95% methanol for 12 h at room temperature in an orbital shaker (Panasonic, MIR-S100, Japan). The extracts were filtered through Whatman No. 1 filter paper. The residues

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were extracted twice again as previously and extracts were combined. The combined extracts were concentrated under reduced pressure at 40 °C using rotary evaporator (Heidolph) and adjusted to 50 ml by methanol. Extracts were stored in glass vials at 4 °C until the time of analysis. All the spectrophotometric measurements were carried out using JENWAY 6315 UV/Visible Spectrophotometer (Japan).

Determination of total soluble carbohydrates

The total soluble carbohydrates content in the methanolic extract of plant powder was measured according to the phenol–sulfuric acid method using glucose as a standard sugar (Dubois et al., 1965) and expressed as mg/g dw.

Determination of total soluble proteins

Total soluble protein content of the extract was measured using bovine serum albumin (BSA) as a protein standard. The extract or the standard protein was mixed with Coomassie brilliant blue G250 reagent, the absorbance was measured at 595 nm and the results were expressed as mg/g dw (Bradford, 1976).

Lipid quantification

Total lipid content of leaf powders was extracted by the method reported by Bligh and Dyer (1959). The samples were stirred in a chloroform and methanol mixture (2:1 v/v) over 48 h, the powder was filtered off and washed with additional chloroform. This was repeated three times. The volatiles were removed under reduced pressure and the lipid content was expressed as mg/g dw.

Determination of flavonoids

Colorimetric aluminum chloride method was used for flavonoid determination (Chang et al., 2002). Leaf extract was mixed with 1.5 ml of methanol, 0.1 ml of 10% $AlCl_3$, 0.1 ml of 1M K-acetate and 2 ml of distilled water. The absorbance of the reaction mixture was measured at 417 nm. Total flavonoids were calculated as quercetin from a calibration curve and expressed as mg/g dw.

Determination of ascorbic acid (AA)

Ascorbic acid was extracted by homogenization in 5% sulfosalicylic acid. The resultant homogenate was centrifugated at 5000 rpm for 15 min and 1 ml of the supernatant was incubated with 2 ml 2% Na-molybdate, 2 ml 0.15 N H_2SO4 and 1 ml 1.5 mM Na_2HPO_4 at 60 °C for 40 min. Absorbance was measured at 660 nm and ascorbic acid content was calculated as mg/g dw using a prepared calibration curve by ascorbic acid (Oser, 1979).

DPPH radical scavenging activity

The antioxidant capacity of the obtained methanolic extracts was measured by bleaching of the purple coloured solution of 1, 1- diphenyl-2-picrylhydrazyl radical (DPPH) according to the method of Sun et al. (1988). Methanolic extracts were added to 0.2 mM DPPH and incubated at room temperature for 30 min, then the absorbance was measured against a blank at 517 nm. DPPH scavenging activity was calculated from the formula: (%) = 100 [(A blank-A sample)/A blank].

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Free amino acids estimation

Amino acids content of the methanolic extracts was estimated by ninhydrin assay using glycine as a standard amino acid (Lee and Takahashi, 1966). Samples were mixed with ninhydrin-citrate buffer-glycerol mixture (0.5 ml 1% ninhydrin solution in 0.5 M citrate buffer pH 5.5, 1.2 ml of 55% glycerol and 0.2 ml of 0.5 M citrate buffer). The mixture was then shaken and boiled in a water bath for 12 min, cooled, shaken well, and the absorbance was measured at 570 nm and the result was calculated as mg/g dw.

Determination of phenolic compounds

The total phenolic content of methanolic extracts was determined using Folin-Ciocalteu's reagent (Jindali and Singh, 1975). Aliquot of extracts was mixed with 0.1 ml Folin-Ciocalteu's reagent and 1 ml 20% Na_2CO_3, then completed up to a known volume with dist. water. Thereafter, the absorbance was measured at 650 nm after 30 min and gallic acid was used as a standard phenol and phenol content was expressed as mg/g dw.

Determination of alkaloids

Alkaloids were determined using the method of Harbourne (1984). Alkaloids were extracted by a mixture of acetic acid and ethanol (1:9 v/v). The mixture filtered and the extract was concentrated on a water bath. Concentrated ammonium hydroxide was added dropwise to the extract until the precipitation was complete. The precipitate was collected, washed with dilute ammonium hydroxide and then filtered. The residue is the alkaloid, which was dried and weighed then expressed as mg/g dw.

Estimation of saponins

Saponin content was estimated quantitatively by the method described by Hiai et al. (1975) in the methanolic extract. Aliquot of 0.5 ml extract was mixed with 0.5 ml of 8% vanilline. The mixture was placed in an ice bath and mixed with 5 ml of 72% H_2SO_4, then heated in a water bath at 60 °C for 10 min followed by cooling in the ice-cold water bath. The content of saponins was calculated as mg/g dw using a standard curve by cholesterol.

Total tannins determination

The tannin contents of methanolic extracts were determined by method of Broadhurst and Jones (1978) using tannic as a standard. Aliquot of 400 µl extract is added to 3 ml of 4% vanillin in methanol and 1.5 ml of concentrated HCl. After 15 min of incubation the absorbance was read at 500 nm. Tannin content was calculated as mg/g dw.

Total terpenoids determination

Aliquot of 1.5 ml chloroform was added to 200 µl extract, the mixture was vortexed well, then 100 µl conc. H_2SO_4 were added and incubated at dark for 2 hs. The supernatant was decanted and 1.5 ml of 95% methanol was added, followed by vortexing until all the precipitate dissolved completely and the absorbance was measured at 538 nm. Total terpenoids were calculated using a standard curve prepared by linalool and expressed as mg/g dw (Ghorai et al., 2012).

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Statistical analysis

Results were statistically analyzed by one-way ANOVA using Costat under Windows software. Significant differences among accessions were calculated by Duncan’s multiple range test at 0.05%. Results were represented as mean of three replications ± standard deviation (SD).

Results

Table 1 shows the physical properties of soil samples collected from *M. oleifera* various growth habitats (accessions). A highly significant change was observed in electrical conductivity (EC) as a measure of soluble salts in the soil and salinity level. The uppermost EC value was quantified for S4 and S1 soil samples (897 and 860 µS/cm, respectively). Meanwhile, the lowermost values were observed in soil samples of S3 and S2 (335.3 and 345.7 µS/cm, respectively). As for soil reaction, the results showed that S1 soil was slightly acidic (6.9), S2 was exactly neutral (7), however S3 and S4 soils were marginally alkaline (7.2).

Table 1. *Physical properties (EC and pH) of soil samples collected from growth accessions of M. oleifera trees in Jeddah city*

No	EC (µS/cm)	pH
S1	860.0±7.0c	6.9±0.2a
S2	345.7±0.6b	7.0±0.0ab
S3	335.3±0.6a	7.2±0.1c
S4	897.0±1.0d	7.2±0.1bc

The concentrations of various heavy metals (Cr, Pb, Cu, Fe, Mn and Zn), as well as some essential elements (P, Ca, Mg and K) in addition to Na in the soils and leaves of *M. oleifera* collected from four differentially polluted habitats, S1-S4, in Jeddah city, KSA significantly varied (*Table 2*).

Table 2. *Mineral analysis of soil samples and M. oleifera leaves (ppm) exposed to different pollution intensities in Jeddah city*

Metal concentration (ppm)	Soil samples				Leaf samples			
	S1	S2	S3	S4	S1	S2	S3	S4
Cr	1.15^{a}	0.98^{d}	1.06^{c}	1.12^{b}	0.02^{a}	0.10^{b}	0.02^{a}	0.01^{b}
Pb	0.50^{c}	3.63^{a}	1.13^{b}	0.21^{d}	0.25^{c}	1.95^{a}	0.41^{d}	0.01^{b}
Cu	0.74^{d}	1.97^{a}	1.22^{b}	1.08^{c}	0.06^{c}	0.29^{a}	0.06^{c}	0.11^{b}
Fe	865.0^{a}	818.0^{b}	766.0^{d}	785.0^{c}	6.27^{b}	18.00^{a}	0.19^{c}	6.71^{b}
Mn	15.4^{c}	26.4^{a}	15.90^{bc}	16.70^{b}	0.42^{c}	2.30^{a}	0.55^{b}	0.06^{d}
Zn	1.70^{d}	2.30^{b}	3.10^{a}	1.91^{c}	0.59^{b}	6.40^{a}	0.53^{b}	0.53^{b}
P	23.14^{a}	18.09^{c}	15.78^{d}	21.25^{b}	13.89^{c}	20.89^{a}	5.47^{d}	17.67^{b}
Ca	296.0^{a}	182.0^{b}	141.0^{c}	106.0^{d}	60.80^{b}	37.30^{c}	170.0^{a}	172^{a}
Mg	597.5^{b}	550.6^{c}	602.7^{b}	726.8^{a}	45.70^{c}	147.30^{a}	64.6.0^{b}	36.6^{d}
Na	22.23^{c}	17.30^{d}	25.47^{a}	24.05^{b}	13.92^{d}	83.73^{a}	17.61^{c}	24.65^{b}
K	84.80^{b}	73.10^{c}	73.30^{c}	116.0^{a}	53.90^{c}	70.10^{b}	90.20^{a}	54.1^{c}

Means within the same row followed by different letters are significantly different ($P < 0.05$)

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Difference in heavy metals transport from soils to leaves of *M. oleifera* was remarkable. The most pronounced concentration of metals in soil samples was recorded for Pb, Cu and Mn in S2 soil (3.63, 1.97 and 26.4 ppm, respectively), Cr and Fe in S1 soil (1.15 and 865.0 ppm respectively) and Zn in S3 soil (3.10 ppm). The results indicated that soil S4 is less contaminated with heavy metals than the rest of the soils under study. For essential elements, it has been proven that S1 soil was the richest with P and Ca (23.14 and 296.0 ppm, respectively) and S4 soil was the richest with Mg and K (726.8 and 116.0 ppm, respectively). However, the highest Na concentration was more remarkable in S3 soil (25.47 ppm).

Estimation of heavy metals and essential minerals in *M. oleifera* leaves distinguished trees grown in S2 accession (heavily polluted by flaming fires, factories waste and random waste disposal) as highly accumulative for these elements. The most prominent concentration in S2 leaves was observed for Pb, Cu, Fe, Mn, Zn, P, Mg and Na (1.95, 0.29, 18.0, 2.3, 6.4, 20.89, 147.30 and 83.73 ppm, respectively). However, the most prominent concentration of Ca and K was observed in S1 and S3, respectively (60.80 and 90.20 ppm). Therefore, *M. oleifera* could be used as a biomarker for air and soil pollution in heavily polluted areas like S2 one.

The ambient air and soil pollution profoundly affected photosynthetic pigments concentration in *M. oleifera* leaves. Chlorophyll a content significantly lowered in S2, S3 and S4 accessions (3.90, 3.31 and 3.65 µg/g dw, respectively) comparable to S1 (4.71 µg/g dw) (*Fig. 1*). The most pronounced reduction in Chl a was observed in leaves of S3. For Chl b, the same result was attained, as S1 was higher than other accessions (1.61 µg/g dw). The least Chl b content was observed in S2 and S4 (1.41 and 1.40 µg/g dw, respectively). In contrast to chlorophyll, carotenoids recorded the highest content in S2 leaves (1.46 µg/g dw).

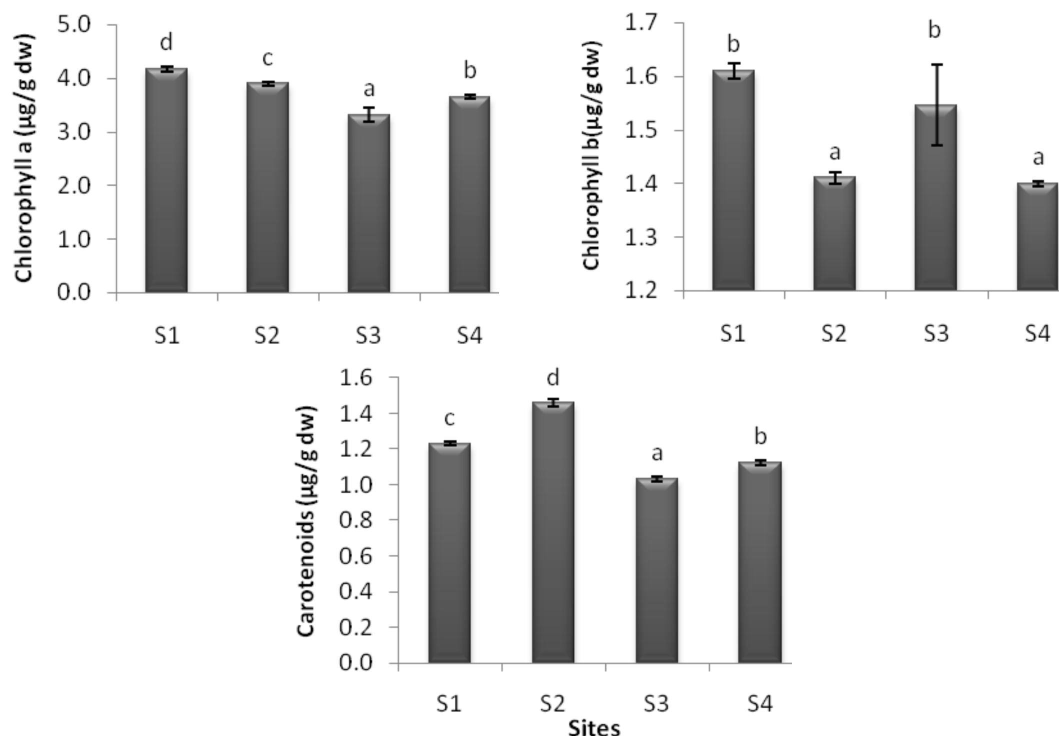

Figure 1. *Photosynthetic pigments of M. oleifera leaves (µg/g dw) exposed to different pollution intensities in Jeddah city*

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Total soluble sugars content in *M. oleifera* leaves was profoundly varied with the growth site (*Fig. 2*). The uppermost sugar content was detected in S4 leaves compared to other accessions. However, the least sugar concentration was in leaves of trees growing in S1 (77.6 mg/g dw). The same pattern of sugars concentration was perceived for total soluble proteins in the studied accessions. As for total lipids, the leaves of trees growing in S1 and S4 recorded the least content (58.0 and 61.5 mg/g dw, respectively), whereas those of S2 and S3 recorded higher levels (77.0 mg/g dw).

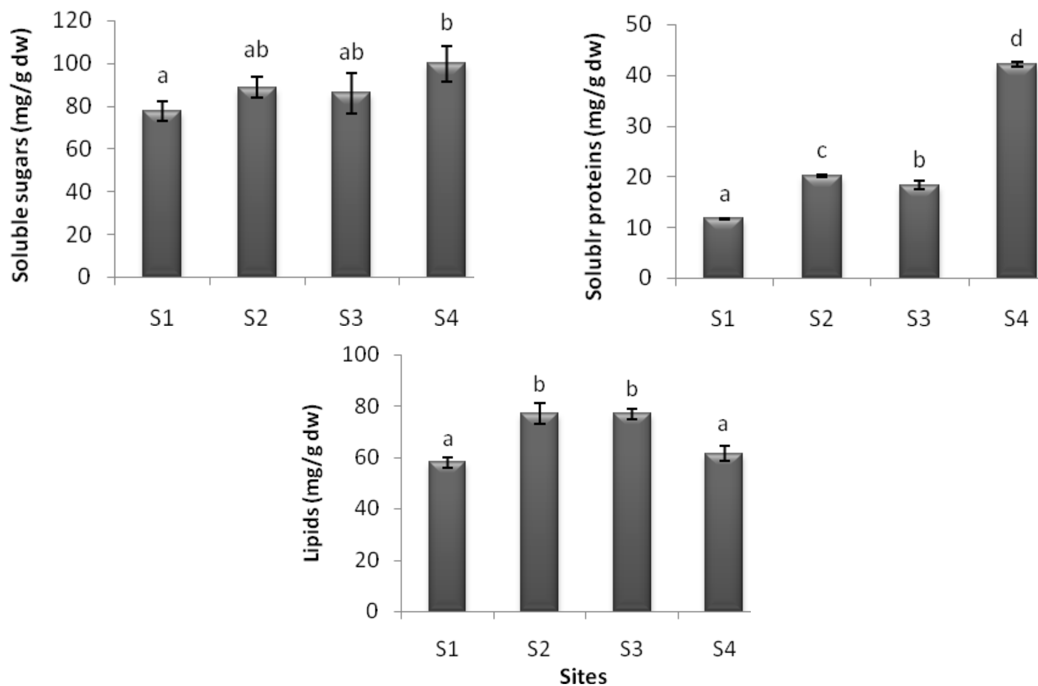

***Figure 2.** Total soluble sugars, soluble proteins and total lipids of M. oleifera leaves (mg/g dw) exposed to different pollution intensities in Jeddah city*

Data in *Figure 3* illustrate the non-enzymatic antioxidant activities of *M. oleifera* leaves growing at different accessions in Jeddah city (KSA), represented in flavonoids, ascorbic acid and total free radical scavenging activity (DPPH). The highest flavonoids content (5.76 mg/g dw) was ascertained in S2 leaves, however the least content (2.57 mg/g dw) was in S1. Ascorbic acid level ranged from lower value (19.11 µmol/g dw) in S3 to higher value (23.68 µmol/g dw) in S1 leaves. Free radical scavenging (DPPH) activity differed in plant leaves with the difference in growth position. The most pronounced DPPH activity (35.7%) was detected in S4 leaves, meanwhile the least activity (24.7%) was recorded in S1 leaves.

Accumulation of free amino acids, phenolic compounds and alkaloids in *M. oleifera* leaves in response to pollution intensity in four geographical accessions in Jeddah city is summarized in *Figure 4*. Total content of free amino acids oscillated between 61.9 and 187.0 mg/g dw in the studied plant leaves. Accumulation of free amino acids was more pronounced in S2 (highest value), nevertheless the least content was detected in S1 leaves. Study results revealed a significant difference among study accessions in *M. oleifera* leaves phenolic content. The highest phenolic content was detected in the leaves of S4 and S2 (78.39 and 76.75 mg/g dw, respectively), however the lowermost

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content was in leaves of S1 (39.29 mg/g dw). For alkaloids content, the change with the growth accession was highly significant. Accession S2 recorded the maximum rate of alkaloids accumulation (51.0 mg/g dw) in *M. oleifera* leaves, while accession S3 showed the lowest alkaloids content (25.0 mg/g dw) in *M. oleifera* leaves.

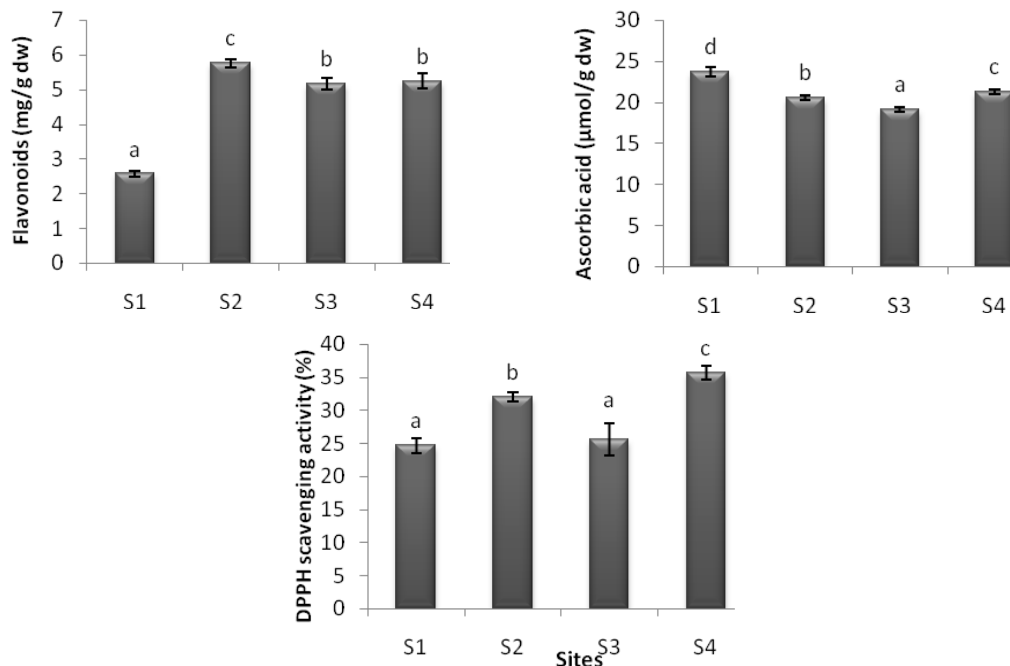

Figure 3. *Non-enzymatic antioxidant activities of M. oleifera leaves (flavonoids, ascorbic acid and DPPH activity) exposed to different pollution intensities in Jeddah city*

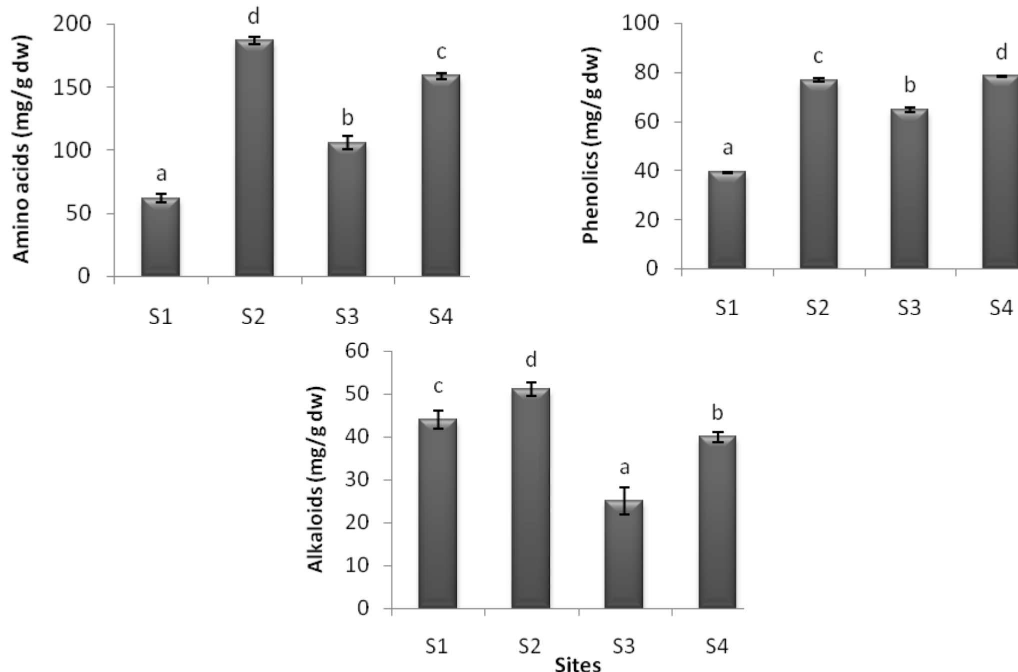

Figure 4. *Variations in amino acids, phenolic compounds and alkaloids contents (mg/g dw) of M. oleifera leaves exposed to different pollution intensities in Jeddah city*

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Variation in the content of some secondary metabolites (saponins, tannins and terpenoids) in *M. oleifera* leaves with the change in the intensity of air and soil pollution in Jeddah city, KSA, is represented in *Figure 5*. The heavy pollution in S2 by flaming fires, factories wastes and random waste disposal caused the accumulation of saponins in *M. oleifera* leaves with the uppermost value (21.3 mg/g dw) throughout this study. In contrast, the lowermost accumulation of saponins (11.6 mg/g dw) was documented in S2, which is exposed to air pollution by automobile exhaust. Tannins and terpenoids content was significantly varied with growth habitat from lowest value (4.35 and 1.59 mg/g dw, respectively) in S3, to highest one (6.06 and 5.90 mg/g dw, respectively) in S1.

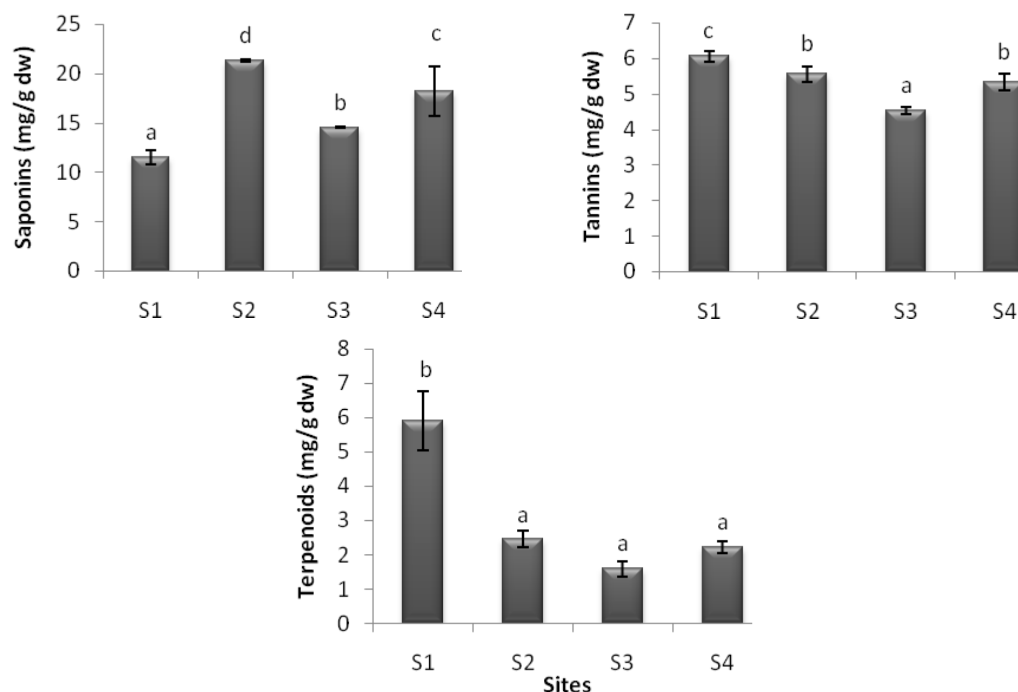

Figure 5. *Variations in secondary metabolites (saponins, tannins and terpenoids) content (mg/g dw) of M. oleifera leaves exposed to different pollution intensities in Jeddah city*

Discussion

The crisis of environmental pollution is one of the most important challenges facing man in our time. After the industrial revolution in the nineteenth century, the sources of water, air and soil pollution increased, which had a profound impact on the biosphere in which humans, animals and plants live. At the end, all sources of pollution affects human health negatively, either directly or indirectly. The plants are considered to be the most important indicators of evidence of pollution occurrence, and also contribute significantly to the removal of some environmental pollutants. In the present study, *Moringa oleifera* tree growing in differentially polluted habitats in Jeddah city, KSA was used as indicator on air and soil pollution. The variation in pollution intensity had significantly affected mineral content, photosynthetic pigments, primary and secondary metabolites and antioxidant status of *M. oleifera* leaves.

The present study showed that S2 soil (the most polluted site due to flaming fires and factories waste disposal) was more polluted with toxic metals as Pb, Cu and Mn. In the

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same time, trees growing in that area accumulated incredible quantities of heavy metals like Pb, Cu, Mn, Fe and Zn in their leaves. Anthropological activities release large amounts of pollutants due to industrial effluents leading to pollution of ground water through the infiltration by soil which is a major issue (Vasanthavigar et al., 2012). Presence of heavy metals in soil and water can cause bioaccumulation affecting the entire ecosystem and pose harmful health consequences to all life forms. The main effects of these metals are inhibition of seed germination, hindering growth and production, affecting the yield of crops and viability of soil microflora (Sethy and Ghosh, 2013).

Photosynthetic pigments of *M. oleifera* leaves were affected by the pollution intensity of the growth site. Chlorophyll a content significantly affected within study accessions, the highest Chl a content was recorded in S1 (double public road exposed to automobile pollutions). Such results are in agreement with those obtained by Giri et al. (2013), who reported that air pollution significantly lowered Chl a content in *Azadirachta indica*, *Mangifera indica*, *Nerium oleander* and *Dalbergia sissoo* leaves. On the other hand, S4 and S2 leaves showed the least Chl b content. This result suggested that Chl b was more sensitive to air pollution than Chl a in *M. oleifera* leaves. Accordingly, the reduction in Chl b content was compatible with the results of Giri et al. (2013) who concluded that polluted sites have less Chl b content than non-polluted ones. Such reduction in Chl b content in air-polluted sites could be attributed to the accumulation of some metals in leaf tissues that increase chlorophyll degrading enzymes activity, inhibit chlorophyll synthesizing activity, or replace Fe and Mn ions in chloroplast proteins reducing their activity. In addition, the non-neutralized heavy metal ions stimulate formation of an excess of free radicals and slow down the turnover of chloroplasts proteins, mostly D1 protein (Lin and Aarts, 2012).

Carotenoids accumulation in plant leaves is a stress marker in most plant species. Carotenoids recorded the highest content in S2 leaves of *M. oleifera*. As a normal consequence of air and soil pollution, generation of free radicals and active oxygen species is stimulated. To prevent the deleterious effects of these oxygen species, plants have developed antioxidative mechanisms to detoxify such molecules. Carotenoids accumulation in response to heavy metals was observed by (Pinto et al., 2011) in *Gracilaria tenuistipitata* macroalga as they play crucial role in membrane stability, specially thilakoid membranes, as well as being natural antioxidants.

Soluble sugars and proteins were significantly lowered in heavily polluted accessions, comparable to those less polluted. Such result is in agreement with those obtained by Tzvetkova and Kolarov (1996), who concluded that decrease in total sugar content of in polluted sites could be attributed to the photosynthetic inhibition or stimulation of respiration rate. Also, decrease of soluble sugars could be an indicator of starch hydrolysis disturbance or altered carbohydrate metabolism in metal and gaseous polluted sites. The reduced carbon and sugar metabolism due to heavy metals and air pollutants might result from their probable interaction with ribulose-bisphosphate carboxilase active centre (Stiborov'a et al., 1987). The reduced protein content in polluted area could be attributed to impairment of pollutants with nitrogen metabolism or disruption in protein synthesizing enzymes. Such findings are in agreement with those of Rai (2016), who attributed that the enhanced rate of protein denaturation and breakdown of existing protein to amino acids are the main causes of protein reduction in response to air pollution. In addition, prolonged exposure to environmental pollutants results in depletion of the antioxidant enzymes as a result of oxidative damage to

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biological molecules, such as protein and DNA (Bebianno et al., 2005). Furthermore, heavy metals disorder protein metabolism either by forming complex with functional side chains or by substituting essential metal ions in metalloproteins (Tamás et al., 2014).

High pollution level in S2 and S3 accession resulted in higher levels of total lipids accumulation in plant leaves comparable to other ones. The same result was obtained by Nouairi et al. (2006) in *Brassica juncea* plants exposed to cadmium stress. They attributed this to the increase in vacuolar area where toxic metals are frequently stored. Previous work of Djebali et al. (2005) revealed variable effects of heavy metals on different fatty acids, as they have negative impact on α-linolenic acid proportion, while other fatty acids were affected positively.

Our results revealed that the highest flavonoids content was found in the heavily polluted (S2) leaves. The results are in accordance with those of Robles et al. (2003) who reported that total flavonoids accumulation was indicator on ozone pollution in *Pinus halepensis* growing at five different polluted areas. Also, *Betula pubescens* leaves in strongly polluted areas significantly accumulated variability of some phenolics which might related to the effect of environmental contamination on shikimate and phenyl-propanoid pathways (Loponen et al., 2001). Such increase in flavonoids content could be associated with its defensive role under conditions of environmental stress.

Variations in ascorbic acid level with growth accession were not effective. Previous work of Zengin and Munzuroglu (2005) showed that metal-treated (lead, copper, cadmium and mercury) bean seedlings showed significant higher ascorbic acid content. Such increase in ascorbic acid in polluted areas could contribute in raising the antioxidant capacity of stressed plants. Heavily polluted areas (S2 and S3) showed moderate DPPH activity, however less polluted areas showed oscillation in DPPH activity. The variation in total antioxidant activity between growth accessions could be attributed to the variable response of plant leaves to pollution intensity, in addition to the selective impact of contaminant kind on the antioxidant molecules.

The highest leaf free amino acids content was accounted for *M. oleifera* growing in S2 accession (most polluted). The increased accumulation of amino acids under metal toxicity might be due to its role in detoxifying heavy metal through making complexes with it, consequently assisting in the protection of the leaf and photosynthetic machinery from heavy metal injury (Rascio and Navari-Izzo, 2011). A leading mechanism in metal detoxification is the chelation of metals by metabolites rich in -SH groups. Hence, the content of non-protein thiols, which include thiol-rich peptides, glutathione, or other -SH rich compounds. A recent work of Nadgórska–Socha et al. (2017) demonstrated that thiol-containing amino acids content in *Robinia pseudoacacia* and *Taraxacum officinale* leaves were higher in the high traffic and emission regions.

Phenolic content in the leaves of *M. oleifera* only slightly differed between the various contaminated study sites. The uppermost phenolic content was detected in the leaves of S4 and S2. Mir et al. (2009) reported that total flavonoids and phenolics in *Catharanthus roseus* and *Ocimum sanctum* may serve as biomarkers of urban auto pollution as they showed a positive relationship with the vehicular pollution load. Rai et al. (2007) have also reported significant increase in total phenol contents in wheat under air pollution stress. Increase in phenolics under environmental stresses was monitored in many plant species as they play a crucial role in defense mechanisms against ROS damage.

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With respect to alkaloids accumulation, accession S2 recorded the maximum rate in *M. oleifera* leaves. These results are consistent with those obtained by Singh et al. (2000), who reported that alkaloid content of *Datura innoxia* was considerably accumulated in various plant organs in response to coal-smoke pollution. They attributed the increase in alkaloid content to a chemical adaptation or a defensive/protective strategy under stress. Alkaloids accumulation under stress conditions was reported in various plant species as *Senecio longilobus* (Briske and Camp, 1982), *Lupinus angustifolius* (Jansen et al., 2009) and *Achnatherum inebrians* (Zhang et al., 2011).

On the same pattern, the heavy pollution in S2 caused the accumulation of saponins in *M. oleifera* leaves comparable to other study sites. Szakiel et al. (2011) reviewed that plants belonging to the same species in different geographical zones may differ significantly in their content of secondary metabolites. This phenomenon was observed in many saponin-containing plants, including *Panax ginseng*, *Panax quinquefolius* and *Panax notoginseng*. The accumulation of saponins and other secondary metabolites in response to environmental factors is a part of an adaptive strategy leading to enhanced tolerance (Ramakrishna and Ravishankar, 2011). Nevertheless, tannins and terpenoids along this study showed variable accumulation in response to pollution intensity.

Conclusion

Trees and plants in the surrounding environment are considered the lungs of our life, on which most other organisms rely to obtain oxygen and energy. Civilization and urban life have become a major burden on the environment as a result of pollution of water, air and soil. In this study, *Moringa oleifera* trees growing in differentially polluted accessions in Jeddah city, KSA, showed that the intense pollution has a deleterious impact on the metabolic machinery and the physiological processes of the trees. As a responsive mechanism in heavily polluted accession, *M. oleifera* showed a diminution in chlorophyll, soluble sugars, soluble proteins, ascorbic acid, and terpenoid contents. On the other hand, lipids, flavonoids, amino acids, alkaloids and saponins were accumulated in the tree leaves.

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