International Journal of Plant Production 10 (3), July 2016
ISSN: 1735-6814 (Print), 1735-8043 (Online)
www.ijpp.info

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Impact of environmental pollution on the growth and production of Egyptian clover

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Received 25 October 2015; Accepted after revision 5 February 2016; Published online 24 May 2016

Abstract

The present study investigated the impact of environmental pollution, represented in soil, irrigation water and air heavy metals, on the growth and production of Egyptian clover cultivated at south Greater Cairo, Egypt. Plants were sampled through five quadrats (0.5×0.5 m), distributed equally in four cultivated farms in unpolluted and polluted sites, at the harvesting time. In addition, soil, air and irrigation water were collected from each farm. Significant differences in air, soil and irrigation water between the polluted and unpolluted sites were recognized. Plant density, shoot and root lengths; as well as biomass and yield were remarkably lower in the polluted site. In contrast with chlorophyll b; chlorophyll a and carotenoids contents were lower in clover cultivated in the polluted site. However, chlorophyll a/b ratio was significantly higher in plants from the polluted site. It was found that, As, Cr, Ni, Zn, Ag and V were significantly higher in clover shoots than roots, while Pb, Cd, Cu, Fe, Mn and Co concentrations were higher in the roots. The bioaccumulation and translocation factors of most heavy metals were greater than unity indicating high potential of the study species for phytoremediation in polluted areas. Egyptian clover accumulated toxic concentrations of Fe, Pb, Ni, Zn, Cd, Cr and Co, which have adverse effects directly on livestock and indirectly on human health through its flow in the food chain. In order to use Egyptian clover as a forage crop, its cultivation should be avoided in polluted areas.

Keywords: Egyptian clover; Pollutants; Heavy metals; Bioaccumulation; Translocation.

Introduction

Heavy metals are significant environmental pollutants and their toxicity is a problem of increasing significance for ecological, evolutionary, nutritional and environmental reasons. They have largest availability in soil and aquatic ecosystems and to a relatively smaller proportion in atmosphere as particulate or vapors (Nagajyoti et al., 2010). Heavy metals are ubiquitous in the environment, as a result of both natural and anthropogenic activities and humans are exposed to them through various pathways (Wilson and Pyatt, 2007). Their accumulation in plants depends upon plant species and the efficiency of different plants in absorbing metals is evaluated by either plant uptake or soil-to-plant transfer factors of the metals (Khan et al., 2008; Rattan et al., 2005).

Environmental pollution by heavy metals, even if it is at low concentrations and the long- term cumulative health effects that go with it, is of major health concerns all over the world (Opaluwa et al., 2012). Accumulation of these metals in crop plants is of great concern due to the potential for food chain contamination through the soil-root interface. This food chain contamination is one of the important pathways for the entry of these toxic pollutants into the human body (Zhuang et al., 2009). Cultivation of crops for human or livestock consumption on contaminated soil can potentially lead to the uptake and accumulation of heavy metals in the edible plant parts with a resulting risk to human and animal health (Zhuang et al., 2009; McBride, 2007). Use of polluted land or water for cultivation of crops mainly accounts for decrease in the overall productivity and results in contaminated food grains and vegetables, which adversely affect human health (Singh et al., 2010).

The rapid industrialization in developing countries, though contributed to economic development, has resulted in heavy losses to economic welfare in terms of effects on agricultural activities, human health and ecosystem, through air and water pollution (Reddy and Behera, 2006). Wastewater irrigation, solid waste disposal, sludge applications, vehicular exhaust and industrial activities are the major sources of soil contamination with heavy metals and an increased metal uptake by food crops grown on such contaminated soils is often observed (Singh et al., 2010; Khan et al., 2008). Basically water pollution poses a serious challenge due to its impact on a large number of economic activities. The problem of water pollution acquires greater relevance in the context of an agrarian economy like Egypt. This is mainly due to its direct impact on human health and livelihoods (Reddy and Behera, 2006).

The serious problem of feed shortage in Egypt, especially green summer fodder suppresses the improvement of animal production. Therefore, dependence on improving local food and food resources for both animals and humans is necessary for a sound policy (Shaltout et al., 2010). Though there are a number of empirical studies on agriculture related environmental problems, such as soil degradation, wind and water erosion, only a few studies have dealt with environmental problems associated with industrial pollution and its impact on agriculture and other sectors (Reddy and Behera, 2005). Therefore, the present study aims at investigating the impact of environmental pollution, represented in soil, irrigation water and air heavy metals, on the growth and production of Egyptian clover; a common fodder plant in Egypt. It also investigates the potential accumulation of heavy metals in the plant tissues, which may cause health risk for animals and humans through the food chain.

Materials and Methods

Study species

Egyptian clover (*Trifolium alexandrinum* L.) is of central Asia-Mediterranean origin, which has been introduced to Egypt via Syria and Palestine (Galal, 2001). Its cultivation favors the temperate climate. In Egypt, it represents the main forage crop in Upper and Lower Egypt, with wide ecological amplitude. It was sawn in October and harvested in May (Abdel-Razik, 1980). As many other leguminous crops, cultivation of clover is beneficial for the land in; 1) compensating the humus loss in the soil through the process of grazing in the field, 2) its high natural fertilizing ability, especially for the other subsequent crops as it adds more nitrogenous compounds to the soil; and 3) soil decomposition, which is necessary for the growth and yield quality of the other crops (Galal, 2001). So, Egyptian clover cultivation is preferable, but at the expense of other important crops such as broad bean or wheat. It fits well in any soil type, but in wet and sandy soils, its yield is rather low and it does not grow in highly salinized lands. Cultivation of clover improves the alkaline soil (as in Egypt), which increases its productivity (Abdel Ghani and El Bakry, 1992).

Plant sampling

Four cultivated farms (one acre each) representing the growth of the Egyptian clover, were selected at the time of harvesting during May (2104) in polluted and unpolluted sites. These farms were dry sown by clover (multiple cuttings type) at the first week of October (20 kg seeds/acre) and the first cut was after 90 days and other cuttings were obtained at about 40 days interval's before flowering. After sowing first two light irrigations were given so that seed does not collect at one side of bed and subsequent irrigations were given at 15 to 20 days in winter and 10 to 12 days in summer. Polluted site was located at South Cairo Province, receiving pollutants from many industries such as steel, cook, cement, fertilizers and sewage plants, which discharge their toxic elements into air, soil and irrigation water. On the other hand, unpolluted site was located at South Giza Province and is characterized by air, soil and irrigation water receiving no industrial pollution. At each cultivated farm, five quadrats (0.5×0.5 m) were randomly selected to represent the growth of clover plants. The number of plants was counted and then the whole plant parts were harvested and transferred to the laboratory for measuring shoot and root lengths and the number of leaves / plant. Plant shoots and roots were separated and weighted before and after oven drying at 60 ºC till constant weight, to estimate their fresh weight (production) and biomass.

Plant analysis

Three composite samples of the clover roots and shoots were taken from each cultivated farm in the polluted and unpolluted sites and ground into a powder using a metal-free plastic mill for analysis. Nutrients and heavy metals were extracted from 0.5-1 g samples using mixed-acid digestion method. Total nitrogen (N) was assessed by the Kjeldahl method, while P by applying molybdenum blue method using a spectrophotometer (CECIL CE 1021). K was determined using a flame photometer (CORNING M410). Total soluble proteins were determined according to Lowry et al. (1951), while carbohydrates (total soluble sugars) according to the anthrone technique (Umbriet et al., 1959). Heavy metals; Fe, Cu, Mn, Zn, Cd, Pb, Co, Cr, As, Ag, V and Ni were determined with PyeUnicamSp 1900 Recording Flame Atomic Absorption Spectrophotometery. All these procedures for plant analysis were outlined by Allen (1989).

For analysis of plant pigments, three clover shoots from each quadrat, in the polluted and unpolluted sites, were collected and combined to make three samples. Chlorophyll and carotenoids were extracted from a known fresh weight (about 2 g) of leaves in 50% (v/v) acetone in complete darkness and kept overnight at 4 ^{o}C, which was taken and measured spectrophotometrically against a blank of aqueous acetone at the three wave lengths 453, 644 and 663 nm (Allen, 1989). The concentration of each pigment fraction was calculated from the following equations:

$$\text{Chl. a} = 10.3\ E_{663} - 0.918\ E_{644}$$

$$\text{Chl. b} = 19.7\ E_{644} - 3.87\ E_{663}$$

$$\text{Carotenoids} = 4.2\ E_{453} - (0.0264\ \text{chl. a} + 0.426\ \text{chl. b}),$$

where E is the absorbance at different wavelengths (663, 644 and 453). The values were then expressed as (mg g^{-1} fresh wt).

Soil, water and air analysis

Three composite sub-surface soil samples around the plant roots were collected from each farm. In addition, three composite surface water samples were collected from each irrigation source, supplies corresponding farm in the polluted and unpolluted sites, for chemical analysis. Atmospheric particles were collected monthly (From October 2013 to May 2014) with Ecotech Model 3000 PM_{10} High Volume Air Sampler, from the polluted and unpolluted sites. The PM_{10} inlet was used to collect all particles of less than 10 µm. Fiberglass filters of 150 mm diameter were used as collection media. Samples of airborne particles were collected in different days of the week, therefore, representing the different meteorological and environmental conditions of the study sites.

Soil water extracts (1:5 w/v) as well as water samples were used to determine pH values using a glass electrode pH meter (Model 9107 BN, ORION type) and electrical conductivity (EC) with (conductivity meter 60 Sensor Operating Instruction Corning). Total N and P were estimated using a spectrophotometer (CECIL CE 1021) by applying Indo-Phenol blue and molybdenum blue methods, respectively. K was determined using a flame photometer (CORNING M410). For heavy metals analysis, Soil, water and air particulate matter samples were digested in 20 ml tri-acid mixture of HNO_3:H_2SO_4:$HClO_4$ (5:1:1, v/v/v) for 8 h at 80 °C, then the digests were filtered and diluted to 50ml with double de-ionized water, following the method described by Allen (1989). Fe, Cu, Mn, Zn, Cd, Pb, Co, Cr, As, Ag, V and Ni were determined using PyeUnicamSp 1900 Recording Flame Atomic Absorption Spectrophotometry. The instrument setting and operational conditions were done in accordance with the manufacturers' specifications. All the above methods for soil, water and air analyses were underlined by Allen (1989).

Data analysis

The differences in the soil, water, air and plant variables among the polluted and unpolluted sites were tested using paired-sample t-test. After testing the data for normality, one way analysis of variance (ANOVA) was used to assess the significance of variations of nutrients among the different plant organs in the different sites using SPSS software version 15.0 (SPSS, 2006).

Bioaccumulation factor

Heavy metal concentrations of soils and plants were calculated on the basis of dry weight. The bioaccumulation factor (BF), an index of the ability of the plant to accumulate a particular metal with respect to its concentration in the soil substrate (Ghosh and Singh, 2005), was calculated as follows: BF = $C_{plant\ root}/C_{soil}$, where $C_{plant\ root}$ and C_{soil} represent the heavy metal concentrations in the plant root and soils, respectively.

Translocation factor

The translocation factor (TF) or mobilization ratio, assessed to determine the relative translocation of metals from belowground root to the aboveground shoot of the plant (Gupta et al., 2008), was calculated as: TF = C_{shoot}/C_{root}, where C_{shoot} and C_{root} represent the heavy metal concentrations in the plant shoot and root, respectively.

Results

Soil, water and air properties

Soil chemical analysis of the study farms indicated significant differences in most nutrients and heavy metals between soil samples from the polluted and unpolluted sites (Table 1). Potassium concentration in the unpolluted (reference) soils was higher than that in the polluted ones, while N, P and heavy metals as well as pH and salinity were higher in the polluted soils. In the reference soil, Mn had the highest concentration (25.6 mg kg^{-1}) followed by Fe, Cu and Zn (13.7, 3.1 and 2.4 mg kg^{-1}, respectively), while in the polluted one, Fe had the highest concentration (194.3 mg kg^{-1}) followed by Mn, Zn and Pb (95.1, 91.8 and 91.7 mg kg^{-1}, respectively).

Table 1. Soil characteristics (mean ± SD) of Egyptian clover (N = 3) cultivated in polluted and unpolluted areas. Means with ** and *** are statistically significant with P values of less than 0.01 and 0.001, respectively.

Soil variable	Unpolluted	Polluted
pH	6.8±0.2	8.3±0.5***
EC (µS cm^{-1})	2.0±0.1	6.5±0.6***
N (%)	119.83±3.3	295.3±15.6***
P (%)	10.2±1.2	25.8±1.2***
K (mg kg^{-1})	445.0±2.0	52.5±3. 4***
Pb (mg kg^{-1})	0.5±0.0	91.7±2.5***
Cd (mg kg^{-1})	0.03±0.0	0.5±0.0**
As (mg kg^{-1})	0.02±0.0	0.43±0.0**
Cr (mg kg^{-1})	0.14±0.0	4.3±0.6***
Cu (mg kg^{-1})	3.1±0.2	24.4±2.6***
Ni (mg kg^{-1})	0.11±0.0	2.3±0.1**
Fe (mg kg^{-1})	13.7±2.0	194.3±12.1***
Mn (mg kg^{-1})	25.6±2.1	95.1±1.1***
Zn (mg kg^{-1})	2.4±0.2	91.8±3.8**
Ag (mg kg^{-1})	0.1±0.0	0.5±0.0**
Co (mg kg^{-1})	0.1±0.0	0.7±0.0***
V (mg kg^{-1})	0.04±0.0	0.2±0.0**

The Chemical characteristics of irrigation water of Egyptian clover showed significant difference between polluted and unpolluted sites (Table 2). The concentrations of heavy metals in polluted water were higher than those of the unpolluted. Heavy metals like Zn, Fe and Cu had the highest concentrations (1.8, 1.7 and 1.7 mg L^{-1}) in the polluted water, while most investigated metals were present as traces in the unpolluted one.

Table 2. Chemical characteristics (mean ± SD) of irrigation water (N = 3) of Egyptian clover cultivated in polluted and unpolluted sites Means with *, ** and *** are statistically significant with P values of less than 0.05, 0.01 and 0.001, respectively.

Water variable	Unpolluted	Polluted
pH	7.5±1.2	7.9±1.7**
EC (µS cm^{-1})	1.7±1.1	18.6±3.9***
NO_3 (mg L^{-1})	0.2±0.0	3.2±0.6***
PO_4 (mg L^{-1})	0.1±0.0	4.2±0.8***
Pb (mg L^{-1})	0.01±0.0	0.5±0.0**
Cd (mg L^{-1})	< 0.01	0.5±0.0**
As (mg L^{-1})	< 0.01	0.1±0.0***
Cr (mg L^{-1})	< 0.01	0.2±0.1*
Cu (mg L^{-1})	< 0.01	1.7±0.1**
Ni (mg L^{-1})	< 0.01	0.3±0.0***
Fe (mg L^{-1})	0.1±0.0	1.7±0.1***
Mn (mg L^{-1})	0.04±0.0	0.5±0.0**
Zn (mg L^{-1})	< 0.01	1.8±0.1**
Ag (mg L^{-1})	< 0.001	0.2±0.0*
Co (mg L^{-1})	< 0.001	0.1±0.0**
V (mg L^{-1})	< 0.01	0.4±0.2*

The particulate matter isolated from the atmosphere around the cultivations of Egyptian clover in the polluted site was remarkably different from the unpolluted one (Table 3). Investigated metals, except Ag, had higher concentrations in the polluted than unpolluted air, with the highest concentrations of Ni, Fe and Cu (5.1, 5.1 and 4.8 mg kg^{-1}, respectively).

Table 3. Heavy metals analysis (mean ± SD) of particulate matter (mg kg^{-1}) collected from air around Egyptian clover cultivated in polluted and unpolluted areas. Means with * and *** are statistically significant with P values of less than 0.05 and 0.001, respectively.

Particulate matter	Unpolluted	Polluted
Pb (mg kg^{-1})	< 0.002	0.33±0.03***
Cd (mg kg^{-1})	< 0.002	0.21±0.04***
As (mg kg^{-1})	< 0.002	< 0.01***
Cr (mg kg^{-1})	< 0.01	0.18±0.04***
Cu (mg kg^{-1})	< 0.01	0.48±0.07***
Ni (mg kg^{-1})	< 0.01	0.51±0.09***
Fe (mg kg^{-1})	< 0.01	0.51±0.08***
Mn (mg kg^{-1})	< 0.01	0.36±0.09***
Zn (mg kg^{-1})	< 0.01	0.44±0.06***
Ag (mg kg^{-1})	< 0.01	< 0.01*
Co (mg kg^{-1})	< 0.01	0.17±0.05***
V (mg kg^{-1})	< 0.002	< 0.01***

Growth parameters

The measurements of the growth parameters of Egyptian clover showed that plant density in the unpolluted site (47.7 plants m^{-2}) was greater than that of the polluted one (29.7 plants m^{-2}) (Table 4). In addition, shoot and root lengths in the unpolluted site (63.7 and 8.2 cm) were significantly higher than in the polluted one (42.1 and 5.5 cm, respectively). Stems and leaves fresh and dry weights of Egyptian clover were considerably lower under the effect of pollution. The yield production and biomass of Egyptian clover in the unpolluted site (13641.5 and 700.0 kg $acre^{-1}$) was higher than the polluted one (8380.4 and 408.5 kg $acre^{-1}$, respectively).

Table 4. Morphological characters and biomass (mean ± SD) of Egyptian clover under the effect of pollution. Means with * and ** are statistically significant with P values of less than 0.05 and 0.01, respectively.

Plant parameter	Unpolluted area	Polluted area
Number of plants m^{-2}	47.7±2.1	29.7±6.0*
Stem length (cm)	63.7±3.1	42.1±2.4*
Root length (cm)	8.2±1.3	5.5±0.6*
Number of leaves $plant^{-1}$	10.6±1.64	9.9±0.6
Leaves fresh weight g m^{-2}	716.0±38.6	423.3±61.3*
Stem fresh weight g m^{-2}	2532.0±70.1	1572.0±301.9*
Leaves dry weight g m^{-2}	47.1±1.2	32.6±1.5**
Stem dry weight g m^{-2}	119.6±5.2	64.7±9.1*
Production (kg $acre^{-1}$)	13641.5±149.4	8380.4±1511.8*
Dry biomass (kg $acre^{-1}$)	700.0±18.0	408.5±43.9*

Plant analysis

Pigments

A significant difference in the pigments contents of the Egyptian clover leaves between polluted and unpolluted sites were recognized (Figure 1). Chlorophyll a and carotenoids contents in the polluted site (1.9 and 0.7 mg g^{-1}) were higher than those in the unpolluted one (1.6 and 0.4 mg g^{-1}), in contrast with chlorophyll b, which showed a reverse trend. Moreover, chlorophyll a/b ratio was significantly higher in plants cultivated in the polluted site.

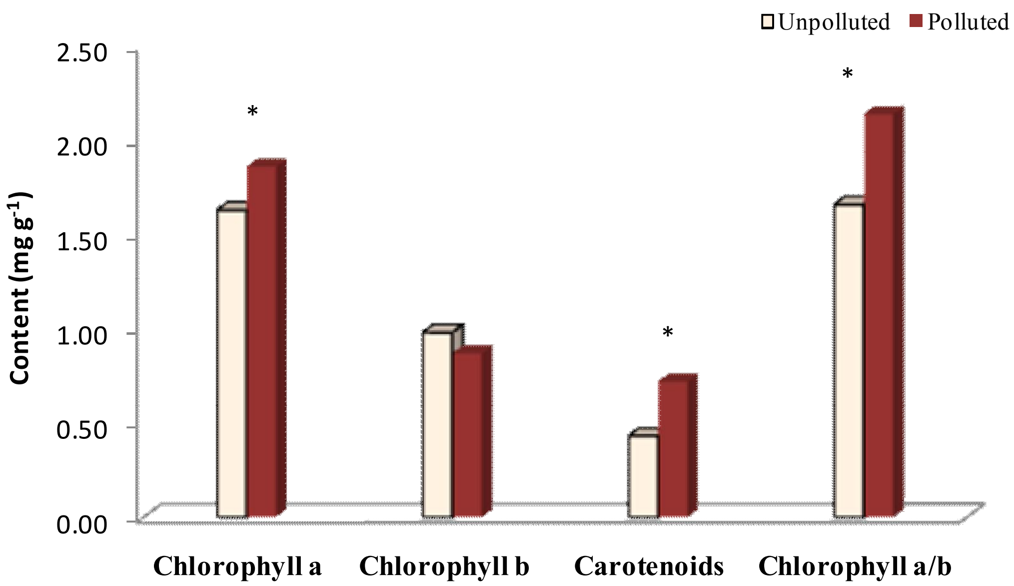

Figure 1. Pigments analysis of Egyptian clover under the effect of industrial pollution. Values of t-test are provided. Means with * are significant with P value less than 0.05.

Nutrients

Significant variations in the accumulation of nutrients and heavy metals in the above- and below-ground parts of Egyptian clover were recorded in both polluted and unpolluted sites (Table 5). Clover plants accumulated N, P and K concentrations in their roots higher than shoots in both polluted and unpolluted sites. The concentrations of these nutrients were lower in the leaves, but higher in the roots under the effect of pollution. Significant higher protein contents in the below- than above-ground parts of Egyptian clover in both polluted and unpolluted sites were recorded with the highest content (15.90%) in the plant root and the lowest (7.81%) in the leaves from the polluted site. Carbohydrates showed non-significant variations in the above- and below-ground parts as well as in the polluted and unpolluted sites.

Heavy metals

The heavy metals analysis of Egyptian clover showed significant differences between plant organs from polluted and unpolluted sites (Table 5). In the unpolluted site, heavy metals such as As, Cr, Ni, Zn, Ag and V, in Egyptian clover shoot, were significantly higher than roots, while Pb, Cd, Cu, Fe, Mn and Co concentrations were higher in the roots. Most investigated metals, except Ag and Co, were accumulated in higher concentrations in the below- than above-ground parts of clover plants cultivated in the polluted site. It worth noting that, Fe, Pb, Ni, Zn and Cd accumulated in high concentrations in the plant tissues.

Table 5. Nutrients and heavy metals (mean ± SD) of the above- and below-ground parts of the Egyptian clover from the polluted and unpolluted sites.

Nutrients	Unpolluted area		Polluted area	
	Shoot	Root	Shoot	Root
Inorganic				
N (%)	$1.9±0.2^{b}$	$2.5±0.3^{c}$	$1.3±0.1^{a}$	$2.5±0.3^{c}$
P (%)	$1.8±0.2^{b}$	$1.8±0.2^{b}$	$1.2±0.1^{a}$	$1.5±0.3^{a}$
K (mg kg^{-1})	$14.2±2.5^{a}$	$14.5±2.5^{a}$	$13.1±1.9^{a}$	$16.0±2.7^{b}$
Pb (mg kg^{-1})	$34.35±14.9^{c}$	$80.2±6.4^{b}$	$2113.3±83.9^{a}$	$2912.5±10.8^{d}$
Cd (mg kg^{-1})	$0.4±0.1^{a}$	$4.3±0.5^{c}$	$13.3±1.5^{b}$	$48.3±0.8^{d}$
As (mg kg^{-1})	$0.1±0.0^{a}$	$0.03±0.0^{a}$	$0.3±0.0^{b}$	$0.4±0.0^{c}$
Cr (mg kg^{-1})	$1.4±0.1^{a}$	$0.5±0.0^{c}$	$1.3±0.1^{a}$	$4.4±0.8^{b}$
Cu (mg kg^{-1})	$0.3±0.0^{a}$	$0.4±0.0^{a}$	$4.2±0.1^{b}$	$16.5±1.5^{c}$
Ni (mg kg^{-1})	$109.1±2.3^{b}$	$2.4±0.1^{a}$	$89.3±9.3^{b}$	$172.8±18.2^{c}$
Fe (mg kg^{-1})	$686.3±18.4^{a}$	$1060.8±23.2^{b}$	$1253.7±54.6^{c}$	$10150.0±522.0^{d}$
Mn (mg kg^{-1})	$8.1±1.8^{a}$	$16.8±2.5^{c}$	$4.1±0.1^{b}$	$23.3±1.9^{d}$
Zn (mg kg^{-1})	$102.8±5.5^{b}$	$35.8±3.8^{a}$	$158.5±11.8^{c}$	$387.2±2.0^{d}$
Ag (mg kg^{-1})	$1.6±0.1^{a}$	$1.6±0.3^{a}$	$3.6±0.2^{b}$	$3.1±0.1^{b}$
Co (mg kg^{-1})	$1.6±0.1^{ab}$	$1.7±0.4^{a}$	$4.8±0.1^{c}$	$3.4±0.2^{bc}$
V (mg kg^{-1})	$0.1±0.0^{a}$	$0.04±0.0^{a}$	$0.1±0.0^{a}$	$0.4±0.0^{b}$
Organic (%)				
Carbohydrates	$12.4±1.9^{b}$	$11.7±1.9^{ab}$	$10.0±0.9^{ab}$	$9.4±1.2^{a}$
Proteins	$11.7±1.0^{b}$	$15.4±2.1^{c}$	$7.8±1.7^{a}$	$15.9±1.8^{c}$

Means with the same letters are not significant.

Bioaccumulation and translocation factors

The bioaccumulation potential of Egyptian clover was high in both polluted and unpolluted sites (Figure 2a). The BF for most heavy metals, except As (metalloid), Cu and Mn in unpolluted and Cu, Mn and V in polluted sites, had values more than unity, indicating high bioaccumulation potential of clover for these metals. In addition, the TF of most investigated heavy metals, except Ag and Co in the unpolluted site, were less than unity. In the polluted site, some heavy metals were translocated from below-ground root to above-ground shoots in the following order: Ni > Cr > Zn > As > V > Ag (Figure 2b).

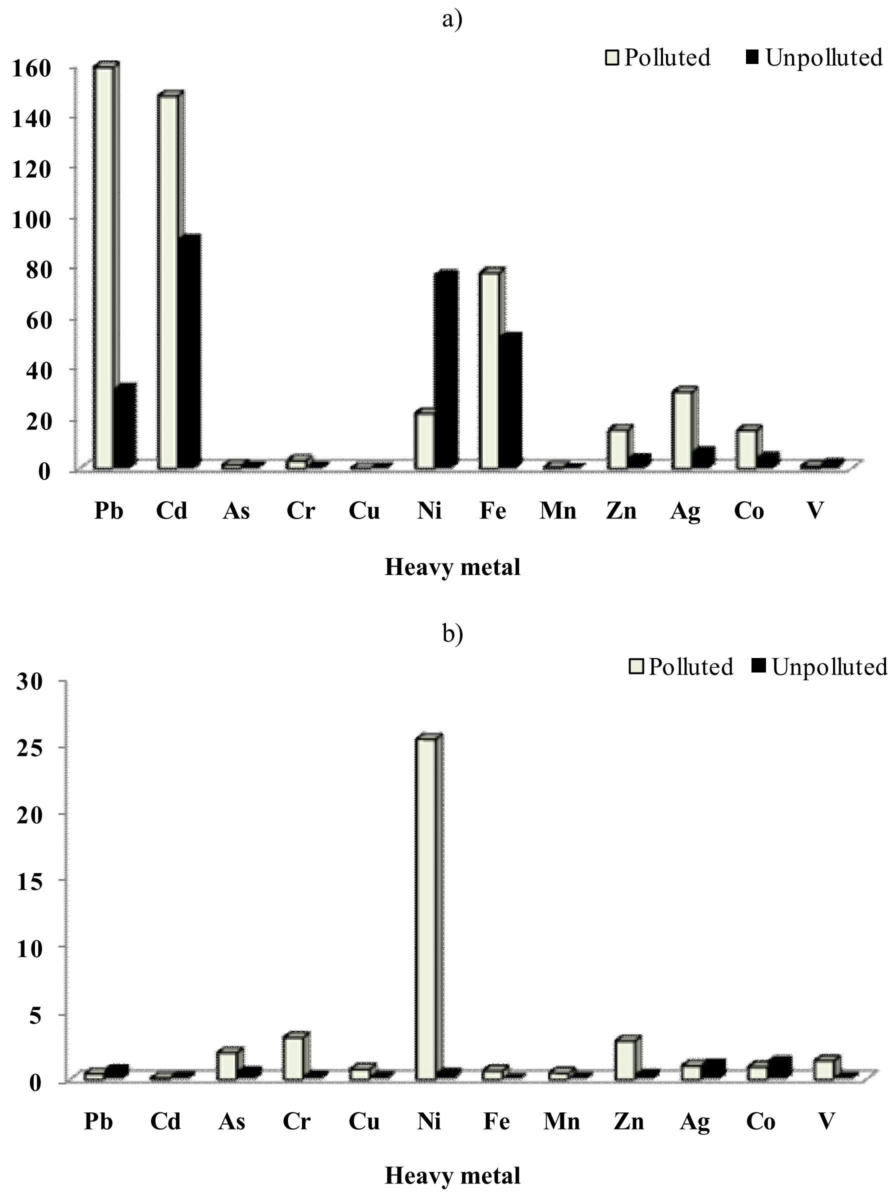

Figure 2. Bioaccumulation (a) and translocation (b) factors of heavy metals in Egyptian clover cultivated in polluted and unpolluted areas.

Discussion

The application of wastewater has led to changes in some soil physicochemical characteristics and heavy metal uptake by food crops. The soil pH changes depend on its value of the wastewater used for irrigation and the soil pH has a great influence on the mobility and bioavailability of heavy metals (Nigam et al., 2001; Khan et al., 2008). Whereas, low pH is optimal for metal availability, but is adverse to the vegetation, since in heavy metal studies, solubility has been shown to increase with decreasing pH (Nanda and Abraham, 2013). This may lead to increased metals availability in the unpolluted site with lower soil pH. In this study area, soil contamination with metals is mainly due to wastewater irrigation, application of sludge in the farm-lands and possible atmospheric deposition. However, vehicle emissions are significant source of heavy metals, particularly the traffic-related metals Pb, Cu and Zn (Omar et al., 2007). Based on the spatial analysis, it was found that areas with highly elevated metal concentrations were generally located in industrial and residential areas, roadsides and crowded commercial districts (Alyemeni and Almohisen, 2014). It worth noting that most investigated heavy metals; except Pb in the polluted soil, were in the safe level. On the contrary, all heavy metals, except Cr in the unpolluted site; exceeded the safe range of normal water (Allen, 1989).

The present study indicated remarkable differences in plant height, root length and number of leaves / plant as well as the above-ground shoot dry weight of Egyptian clover with values in the unpolluted site higher than the polluted one. These results may be attributed to the higher concentrations of heavy metals especially Pb, Fe, Zn, Ni and Cd, in the different plant organs. According to Hadi et al. (2010, 2014), heavy metals generally reduce plant growth with special reference to Pb and Cd, which significantly reduced leaf number, inter nodal distance, plant height and root length. The production of Egyptian clover was reduced by 38.57%, while the biomass was reduced by 41.65%. According to John et al. (2009) and Hadi et al. (2014), the reduction in dry biomass is one of the common symptoms of heavy metal stress on plants and several scientists have reported such effect of heavy metals on biomass. However, the lower values of stem fresh and dry weights may be attributed to the inhibition of chlorophyll synthesis (Chauhan and Joshi, 2010). According to Nagajyoti et al. (2010), decreased photosynthetic activity and poor plant growth apparently reduced the dry matter and yield.

The minimum protein content in the animal diet ranges between 6-12% depending on the animal species (Shaltout et al., 2010). According to the National Research Center (NRC, 1985), sheep are known to require 8.9% protein for maintenance. The average protein content in the above-ground shoots of Egyptian clover in the unpolluted area was 11.7% and in the polluted one was 7.8%. This figure is lower than 16.2% recorded by Chauhan et al. (1980) on the same plant. The protein contents of the clover, cultivated in the polluted site, agreed with the scale of the protein content of some rough fodder materials (Shaltout et al., 2010). Moreover, carbohydrates content was 12.4% and 9.8% in the unpolluted and polluted sites, respectively compared with 43.4% recorded by Chauhan et al. (1980). The accumulation of nutrients depends on the concentrations of N and P in the plant tissue as well as on the amount of plant biomass, whereas the amounts of elements stored in biomass are many times as the element concentration in the plant tissue per unit area (Maddison et al., 2009).

Photosynthetic pigments are fairly sensitive to air pollutants and their sensitivity may determine the response of plants to pollutants. Visible pigment loss has been observed in Egyptian clover exposed to a range of soil, irrigation water and atmospheric pollutants. Significant lower chlorophyll a and carotenoids contents were recorded in plants cultivated in the polluted site. Under pollution stress, heavy metals inhibit chlorophyll synthesis or destruct the chloroplast envelope. Pollutants have been shown to reduce the synthesis of chlorophyll and enhance its degradation (Chauhan and Joshi, 2010). Moreover, Chlorophyll a/b ratio, which could be used as a stress indicator, increased slightly with increasing metal concentration (Zeid et al., 2013). In this study, the ratio was higher under the effect of pollution that may be attributed to increased chlorophyll a concentrations rather than loss of chlorophyll b. Carotenoids in Egyptian clover exhibited great reduction under pollution stress coinciding with Joshi and Swami (2007), who reported significant reduction in carotenoid content of different plants grown at polluted sites.

The Egyptian clover accumulated high concentrations of Fe, Pb, Ni, Zn, Cd, Cr and Co in its tissues, in toxic levels (Padmavathiamma and Li, 2007; Allen, 1989). Zeid et al. (2013) reported that, Co and Cr ions negatively affect all growth parameters such as shoot and root length, shoot and root fresh and dry weight, leaf number and leaf fresh and dry weight, which may be attributed to the marked reduction in photosynthetic pigments, photosynthetic activity and consequently, the carbohydrate content. In addition, the appearance of iron toxicity in plants is related to high Fe uptake by roots and its transportation to leaves via transpiration stream. Iron toxicity was accompanied with reduction of plant photosynthesis and yield (Nagajyoti et al., 2010). Moreover, Pb exerts adverse effect on morphology, growth and photosynthetic processes of plants. The high concentration of Pb in the plant tissues is often strongly (linearly) correlated with traffic density and deposited on plant directly since its concentration in soil was relatively low (Walraven et al., 2014). Furthermore, Ni decreases plant growth due to its negative impact on the metabolism of plants such as legumes (Shahid et al., 2014).

The ability of a plant to accumulate metals from soils can be estimated using the BF, while its ability to translocate them from the roots to the shoots is measured using the TF. Both BF and TF can be used to estimate a plant's potential for phytoremediation purposes. According to Galal and Shehata (2015), TF > 1 indicates a very efficient ability to transport nutrients from roots to shoots, most likely due to efficient metal transport systems. It worth noting that Egyptian clover had TF for As, Cr, Ni, Zn, Ag, Co and V more than unity, therefore it is a suitable plant for phytoextraction for these metals. These results are in agreement with of Ali et al. (2012) on *T. alexandrinum* and Yoon et al. (2006) on native plants in contaminated site. On the other hand, some heavy metals such as Pb, Cd, Cu and Mn had TF less than one, which is suitable element for phytostabilization. This finding agreed with Ali et al. (2012) and Hadi et al. (2014), who reported an important restriction of the internal transport of Pb, Cd and Cu from roots to shoots of *T. alexandrinum* resulting in higher root concentrations rather than translocation to shoots.

Conclusion

Egyptian clover was greatly impacted by environmental pollution, which negatively affects its growth parameters and crop yield. Its nutrients contents and photosynthetic activity were significantly decreased under pollution stresses. Egyptian clover has a high potential for accumulating heavy metals, therefore its cultivation in polluted areas should be avoided. The high toxic levels of pollutants in the tissues of this common fodder plant have adverse effects directly on livestock and indirectly on human health through its flow in the food chain. Finally, in order to use Egyptian clover as a forage crop, its cultivation should be avoided in polluted areas.

Acknowledgements

The author would like to express his gratitude to Mr Omar Ebrahem Elawa for his kind help in sampling and analysis of study materials.

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