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Effects of treated wastewater irrigation on size-structure, biochemical products and mineral content of native medicinal shrubs

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ABSTRACT

The aim of this study is to evaluate the impact of treated wastewater irrigation on five native medicinal shrubs (*Artemisia monosperma*, *Zilla spinosa*, *Farsetia aegyptiaca*, *Deverra tortuosa* and *Calligonum polygonoides*) in abandoned areas of a desert plantation in Egypt. Mineral content, size-structure and biochemical products of shrub shoot samples were determined for the plantation and an adjacent, non-irrigated control site outside the forest. We found that the application of wastewater significantly increased trace metal concentrations (Cd, Cu, Mn, Ni and Pb) in the plantation-soil compared to the control, showing phytotoxic concentrations of Ni. Each shrub species showed different selectivity to accumulate specific elements in their shoots, with high concentrations of N, P, K, Mn, Zn, Ni, Cu, Cd and Pb in forest site plants. Cd and Ni concentrations in shoot samples from both the planation and control sites were about 13 and 500 times above permissible levels, respectively. Our results suggest that native medicinal shrubs irrigated by wastewater may not be safe for medicinal use or grazing purposes and represent potential risks. More efforts should be directed to monitor the potential hazards of using wastewater irrigation on all components of the ecosystem.

1. Introduction

The irrigation of agricultural fields using treated wastewater is being extensively practiced in countries faced with water shortage problems (Belaid et al., 2012). The establishment of green belts around cities and desert plantations (e.g. forests) irrigated by treated wastewater can improve air quality and increase local timber production (FAO, 2006). However, the direct application of wastewater on agriculture and desert lands can lead to contamination with trace metals, organic toxic chemicals and pathogens (Singh and Bhati, 2005; Ali et al., 2011). Most previous studies of the impact of treated-wastewater irrigation have concentrated on its effects on biomass, growth, nutrient cycling and accumulation of trace metals in tree plantations and agricultural crops, but have ignored the effects on the native flora which represent an essential component of the ecosystem (e.g. Belaid et al., 2012; Farahat et al., 2012).

Native shrubs and trees have structural and economic importance (Challenger, 1995) in arid regions. They play a significant role in soil protection and stabilization, provide a source of forage for animals, fuel for local inhabitants, and have medicinal and industrial values (Shaltout and Ahmed, 2012). Shrubs and trees of desert communities could thus be considered as keystone species, on which many other ecosystem processes depend (e.g. herbivory) (Galal, 2011). In Egypt, the desert vegetation is by far the most important and characteristic type of natural plant life. Desert vegetation covers about 95% of the total area of the country, and is mainly formed of xerophytic shrubs and sub-shrubs (Abd El-Ghani and Amer, 2003).

To increase the knowledge about the ecological impact of wastewater irrigation in forest plantations on native shrubs, five native desert shrub species growing as natural understory vegetation under the canopies of cultivated trees or in abandoned areas in a desert forest in north-western Egypt, were studied. These shrubs were: *Artemisia monosperma* Delile (F. Asteraceae), *Zilla spinosa* (L.) Prant. (F. Brassicaceae), *Farsetia aegyptiaca* Turra (F. Brassicaceae), *Deverra tortuosa* (Desf.) DC. (F. Apiaceae), *Calligonum polygonoides* subsp. *comosum* (L'Her.) Soskov (F. Polygonaceae). These shrubs were selected since they have been reported as medicinal and forage plants, both in Egypt and elsewhere. For instance, *A. monosperma* and *Z. spinosa* are used for kidney stone treatments and have strong anti-viral potential (Stavria et al., 2005;

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El-Ghazali et al., 2010; Soltan and Zaki, 2009), *F. aegyptiaca* is used in alternative medicine as anti-rheumatoid (IUCN, 2005) while *D. tortuosa* is used as a diuretic, carminative, analgesic and its seed oil showed antimicrobial activity (Abdel Ghani and Hafez, 1995). *C. polygonoides* subsp. *comosum* is a multiple purposes plant, which is used as fuel wood and a good fodder for livestock (Ghazanfar, 1994). Recently the plant extract has been used to control *Biomphalaria alexandrina* snails (the intermediate host of *Schistosoma mansoni* in Egypt) (Bakry, 2009).

The aim of the present study was to (1) evaluate the impact of treated wastewater irrigation on the mineral content, size-structure and biochemical products of shoot samples collected from the selected native medicinal plants; (2) determine the impact of wastewater on the soil chemistry; and (3) assess any potential risks of using wastewater-irrigated medicinal shrubs.

2. Methods

2.1. Site description

The study was conducted in the Egyptian-Chinese Young People Friendship forest in Sadat City, Egypt (hereafter referred to as Sadat forest). It is located at about 65 km north-west of Cairo (30°27′55″–30°28′45″ N, 30°35′1″–30° 35′31″ E). It was established in 1999, situated on reclaimed sandy desert lands, and relies on treated wastewater for irrigation. The main planted trees in the forest are: *Eucalyptus camaldulensis* Dehnh., *Eucalyptus citriodora* Hook., *Khaya senegalensis* (Desr.) A. Juss., *Dalbergia sissoo* (Roxb.), and *Casuarina* spp. These trees are cultivated as single or mixed canopies and occupy more or less equal areas. The total actual cultivated area of the forest is 210 ha, and the density of planted trees ranges from 1114 (trees ha^{-1}) for *E. camaldulensis* to 716 (trees ha^{-1}) for *D. sissoo*. Soil in Sadat forest is sandy with 79.4% sand, 10.1% silt and 10.4% clay particles (Farahat and Linderholm, 2012). The treatment of the wastewater begins by storing water in large oxidizing pools for sedimentation of suspended solids. Then, the water is filtered through a system of water pumps and finally used for drip irrigation in the forest. The forest has many abandoned areas, due to cutting of overstory trees in 1992. These are devoid of planted trees but occupied by native desert plants, which regularly receive wastewater through the irrigation network. The abandoned areas are located on the forest edges.

We assigned fifteen plots (10 m × 10 m) in abandoned areas (F) in Sadat forest and in a control site in the adjacent desert area, ca. 250 m away from the forest borders, which was not irrigated. This distance was considered to be enough to eliminate any effect of the forest trees on the desert plants. At each plot, we conducted a number of measurements presented in the following sections.

2.2. Density, cover and size structure

The density (individual per 100 m^2) and visual cover (in %) of the selected shrubs in all plots at the forested and control sites were determined. The cover was established by means of the abundance-frequency scale of Braun-Blanquet (1979), and the values were then converted into percentages in accordance with the following equivalence: 5 = 87.5%; 4 = 62.5%; 3 = 37.5%; 2 = 17.5%; 1 = 5%; += < 5% (Braun-Blanquet, 1979). The size-structure of the shrubs was estimated through its canopy architecture traits, which were measured twice during the growing season (in June and end of August 2012) and the mean values of the two measurements were calculated for each trait. For each shrub, stem height (the vertical distance from the ground to the highest apex), maximum crown diameter (m), mean crown diameter (including the minimum diameter and its perpendicular maximum diameter (m)) and crown projection area (m^2) were measured for all plants in the plots according to the methods proposed by Beaudet and Messier (1998).

2.3. Plant sampling and chemical analysis

Plant samples were collected at the end of the growing season (August 2012) from the forest and control sites. In each plot, three shoot samples from three different individuals were collected, including leaves (when present), stems and inflorescences. Initially, the plant samples were thoroughly washed with running distilled water to remove dust and other solid particles. The samples were then dried at 60 °C for 48 h, and then ground to fine powder for analysis.

The total soluble sugars (TSS, mg g^{-1} DM) content was estimated according to Umbriet et al. (1972). Eight milliliters aqueous ethanol (80%) was added to 0.1 g sample and placed in water bath at 80 °C for 30 min. Then the solution was centrifuged at 5000 rpm for 10 min and then topped up to 25 ml with additional ethanol. The clear supernatants were collected in different test tubes and 5.5 ml of Anthrone reagent was added. TSS content was determined by a spectrophotometer at 620 nm. Glucose was used as standard to correct the TSS content. To get the total carbohydrate content (mg g^{-1} DM) in the shoot samples, 30 mg of dry powdered shoot samples were hydrolyzed in 10 ml of 1 N H_2SO_4 in digestion tubes at 80 °C for 8 h. This was made up to a definite volume. Then the total carbohydrates were determined using an Anthrone reagent as described above (Jermyn, 1975).

Total soluble proteins (TSP, mg g^{-1} DM) were determined according to Lowry et al. (1951) using Bovine serum albumin as a standard. Tissue samples (0.1 g DM) were extracted in 10 ml distilled water for 2 h at 90 °C for analysis of soluble protein. The extracts were centrifuged at 5000 rpm and the clear supernatants were collected in different test tubes and assayed for protein content by addition of Folin's reagent. After 30 min, the extinction against appropriate blank was measured at 700 nm. For macronutrients and trace metal analysis, we followed the protocol recommended by Oliva and Rautio (2004) and Ukpebor et al. (2010) for plant materials analysis. The washed and dry shoot samples (0.5 g) were ashed at 550 °C in muffle furnace for 3 h and then digested with 10 ml nitric acid (2.8%) overnight. The sample volume was brought to 50 ml using ultra pure distilled water. The solutions were analyzed for P, Ca, Mg, Na, K, Mn, Zn, Ni, Cu, Cd and Pb, using ICP-MS (Agilent 7500). The results were calculated on dry weight basis. Nitrogen concentrations were determined for dry shoot samples using a Fisons EA-1108 CHNS-O Elemental Analyzer (Fisons, Milan, Italy). The analysis of shoot samples was carried out at the University of Gothenburg, Sweden.

2.4. Soil and effluent analysis

Soil samples were collected in profiles (June 2012) including the top soil (at a depth of 0–50 cm) from the forest and control sites, with three samples each at the forest and control sites. The soil samples were brought to the laboratory in plastic bags shortly after collection, spread over paper sheets; air dried, passed through a 2 mm sieve to remove gravel and debris, and then packed in paper bags ready for physical and chemical analysis. Soil extracts were prepared to meet the requirements for different determinants, 1:5 (w:v) soil (g): distilled water (ml) extract. This extract was used for pH, EC, HCO_3, Ca, Mg, Na and K analysis in H_2O suspensions. The pH values of soil samples were determined using a glass electrode pH meter (Model 9107 BN, ORION type). The electrical conductivity of the soil water extract (EC) was measured with