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Growth, gas exchanges and accumulation of inorganic matter of *Populus nigra* L. in responses to treated wastewater

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Abstract

Tunisia has made considerable efforts to solve major environmental problems. Reforestation of vast marginal spaces with appropriate species, such as poplar, is one of the economic and environmental challenges (I-488). The fast-growingof this rupicolous species has a high specific versatility in its use. However, its need for water will limit their use in reforestation in areas where water resources are scarce. To cope with this situation, the use of unconventional water resources, including wastewater treatment, is a promising way to increase domestic production of wood. Thus, the treated wastewater is valued as a source of unquestionable water, but also as a source of nutrients. These plants were raised under non-binding for four months and divided into two lots irrigated daily to field capacity with (i) potable water (control : T) and (ii) with treated waste water (TWW). Biomass production, gas exchange and some mineral ions were measured during the experiment, in summer. Our results indicate that irrigation with treated wastewater has submitted a substantially marked effect resulting in an increase of gas exchange. Furthermore, after 60 days, the accumulation of certain metal ions (Cd, Pb and Ni) has resulted in a major malfunction on gas exchange.

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Introduction

Currently, the world is facing a variety of changes, especially population growth, urbanization, desertification and climate change (FAO, 2009). Given population growth, mobilization of water for agricultural purposes are beyond our water resources (Constantino, 2009). In the countries of the southern margin of the Mediterranean, particularly in the Arab region, known for its arid to semi-arid climate, water scarcity is a marking problem. This is a particularly worrying situation for Tunisia as it is one of the regions that is most affected by climate change (FAO, 2009). Therefore, these climatic changes can have serious consequences on water resources and various economic activities such as agriculture (JORT, 1993). Tunisia is characterized by a low forest-covered area, indeed, wooded land does not exceed 7.5% of the land area (FAO, 2008). In this context, a very advantageous alternative was directed, among others, for the installation of woodland outside the forest formations to increase the marginal rate of coating where water resources are critical. Thus, the use of treated waste water is one of the strategies to successfully install new plantations in poor water areas.

Fast growing trees such as *Populus* are used for the rehabilitation of marginal areas. However, this specie requires a long growing season with adequate water supply especially during summer, so planting them in areas with scarce water causes problems. Thus, the use of treated waste water, particularly during the period of active growth will be appreciated as a source of water but also as source of nutrients. The success of these plantations has two advantages: the use of waste water and the increase of forest area.

The capacity of plants to accumulate metals in their organs has been proposed as a suitable tool for the remediation of metal-polluted substrates. This natural technique, called phytoremediation, can allow the reduction or complete removal of mild and diffuse metal contamination of waters. It also avoids the heavy impacts on ecosystems and the high costs associated with traditional techniques (Cunningham and Ow, 1996). In this context, the potentiality of Salicaceae, such as poplars, for the remediation of water from metal pollution has been highlighted (Meers *et al.*, 2007). In particular, it has been reported (Pietrini *et al.*, 2010) that metal-treated poplars showed good tolerance and a high bioconcentration factor for traits that are useful for phytoremediation. In fact, poplars are characterized by high variability and adaptability to environmental constraints in addition to having an extended root system and a low impact on trophic chains. Poplars also grow relatively fast, and they are widely used in agro forestry because of their high biomass production which can be used to generate heat or electric power. Our aim was to evaluate the impact of irrigation with treated wastewater on growth and accumulation of trace metals in Populus nigra. The objectives of this work are to (i) evaluate the eco-physiological behavior of poplar seedlings (I-488) (ii) evaluate the nutrient and/ or the toxic effect of water.

Material and methods

Plant Materials and Growing conditions

The study was carried by the National Institute for research in Rural Engineering, Water and Forests Tunis (INRGREF) in semi-controlled conditions during three months of summer. Cutting roots of one clone of *Populus nigra* (cv. I-488; *Populus* x euramericana (Dode) Guinier), aged one year were planted in a plastic pot with 38 cm of diameter and 40 cm of height with a mixture of soil and sand (2: 1, V/V) with 0.5 kg of gravel at the bottom and placed on bricks. During the experiment, the plants were distributed into two batches, the first batch was irrigated with drinking water (C) and the second batch was irrigated with treated wastewater (TWW).

Measurements

Plant growth was assessed three months after planting by measuring total dry mass, aerial part and roots part determined for leaves after 30 days of irrigation. The net photosynthetic rate (*A*), stomatic conductance (g_s) and transpiration (*E*) were determined for the leaves of the rank 5-8 which are the most exposed to the sun. All the measurements were conducted on the plant every 15 days with a portable photosynthesis system, (IRGA, Analytical Development Company Ltd. Hoddesdon, Hertfordshine, EN 110AQ, R-U.: LCA model 4). Samples of dry plant leaf mass TWW and C were used to analyze the content in minerals elements (Na, K, P, Pb, Cd and Ni). These analyses were performed after 60 and 90 days of irrigation. Samples of water used for irrigation of plants (C, TWW) were selected to quantify the concentration of inorganic ions especially traces elements (Cd, Ni, Zn, Pb), anions (No_2, No_3) and cat ions (Ca^{2+}, K^+). The Ni, Cd, Pb and Zn and for K^+, Mg^{2+} and Ca^{2+} were determined by ICP-OES. Samples of water used for irrigation of plants (C, TWW) were selected to quantify the concentration of inorganic ions especially traces elements (Cd, Ni, Zn, Pb) and cations ions (Ca^{2+}, K^+ , Mg^{2+}) (table 1). The Cu content was determined by flame spectrophotometer (SAA-F), the Ni, Cd, Pb and Zn were determined by ICP-OES and for K^+, Mg^{2+} and Ca^{2+} were measured by ion chromatography.

Table 1. Chemical composition of irrigation water.

Concentration of ions (mg/l)	Treated wastewater (TWW)	Drinking water (Control: C)
K^+	34,97	15.1
Mg^{2+}	60.8	23
Ca^{2+}	140	61
Ni	0.052	< L.Q
Zn	0.07	< L.Q
Pb	0.066	< L.Q
Cd	0.04	< L.Q

< L.Q: lower than the limit of quantification or detection.

To determine the effect of irrigation water on plant growth, the relative growth rate (RGR) of six plants of each treatment was determined for 1-30 days, 30-60 days and 60 -90 days. This parameter is used to evaluate the effectiveness of growth expressed by the ability of plants to produce biomass while eliminating the effect of the initial plant size (Kozlowski *et al*, 1991). It is defined by the relationship:

RGR_n = (Wn Ln - Ln Wn-1) / (tn - tn-1); where:

Wn: represent the total biomass of plants C,
Wn-1: represent the total biomass of plants TWW,
tn and tn-1 periods of immersion.

Statistical Analysis

The comparison of the average of the two treatments (T and TME) was performed using the Student test-Newnan-Keuls and least significant difference (LSD) at 5% using the "General Linear Models Procedure" software SAS.

Results

Morphological appearance

After 70 days, irrigation with treated wastewater manifested by epinasty and curvature of mature leaves (fig. 1) and necrosis followed by drying of older leaves after 80 days (fig. 2). Also, at 60 days, TWW induced a marked prolongation of the initial root system and development of fine roots (fig. 3).

Fig. 1. Aspect of morphological leaves of witness poplar seedlings (a) and irrigated by the wastewater (b), after 70 days of treatment.

Fig. 2. Necrosis of the leaves of poplar seedlings irrigated with treated wastewater, after 80 days of treatment.

Fig. 3. Root system of poplar seedlings controls (a) and irrigated with wastewater (b) after 60 days of treatment.

Effect of treated wastewater on soil

The pH on the soil of treated wastewater (TWW) and control plants has not changed. Moreover, the conductivity (CE) in soil irrigated with treated wastewater (TWW) was significantly decreased than in the control plants of *Populus*. At the beginning, the conductivity of soil irrigated with treated wastewater was 1.6 mS corresponding to a saline soil. In fact, after 90 days, the CE of soil irrigated with treated wastewater was significantly higher than in the beginning of irrigation. The high conductivity is the result of the gradual increase in soil salinity over time. After 90 days, the results of plants irrigated with TWW showed high levels in organic matter and minerals including potassium (P_2O_5), chlorine, sodium and potassium in treated wastewater (Table 2).Also, the concentration of Ca^{2+} and Mg^{2+} showed a high levels.

Effect of treated wastewater on the relative growth rate (RGR)

The relative growth rate (RGR) has not significantly changed after 60 days. After 90 days, the RGR deceased in the plants irrigated with treated wastewater compared to seed plants T. This reduction is 45% compared to plants T (Fig. 4).

Table 2. Value of pH, of electrical conductivity and of the levels of certain compounds from the culture substrate at the beginning and after 90 days of irrigation system in TME.

	At the beginning	After 90 days
pH $_{1/2.5}$	7.1 ± 0.2	7.7 ± 0.4
Electrical conductivity at the saturated paste (mS)	1.6 ± 0.3	4.2 ± 0.4
Organic matter (%)	0.7 ± 0.3	1.7 ± 0.5
K_2O (ppm)	138 ± 7	68 ± 11
Cl^-	8.1 ± 0.5	20.3 ± 1.4
Na^+	7.7 ± 0.6	19.9 ± 1.2
K^+	1.0 ± 0.3	8.3 ± 0.4
Mg^{2+}	3.1 ± 0.4	3.6 ± 0.5
Ca^{2+}	4.2 ± 0.3	5.8 ± 0.4
SO_4^{2-}	7.1±0.3	11.93±1.1

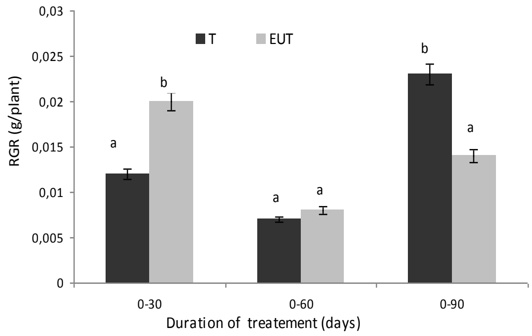

Fig. 4. Evolution of the relative growth (RGR) of poplar seedlings, control (C) and treated wastewater (TWW), n = 6 (mean ± SD).

Effect of treated wastewater on the gas exchange

Photosynthesis (*A*) of plants irrigated with treated wastewater (TWW) was significantly greater (ddl =1 ; F = 229,679 ; p < 0,0001) than in the control plants of populus (Fig. 5). In particular, the first 60 days, photosynthesis was 34% higher in treated than in control plants (fig. 1). However, the photosynthesis rate (*A*) of the plants (TWW), decreased after 90 days showing similar values to the control the plants (C).

After 60 days of irrigation, a significant difference in stomatic conductance (g_s) was found (ddl =1 ; F = 132,165 ; p < 0,0001), it was 19% higher in treated than in control plants (Fig. 6). However, the stomatic conductance (g_s) of the plants (TWW), decrease after 90 days, it was 10% lower in the treated plants (TWW) than in control plants (C).

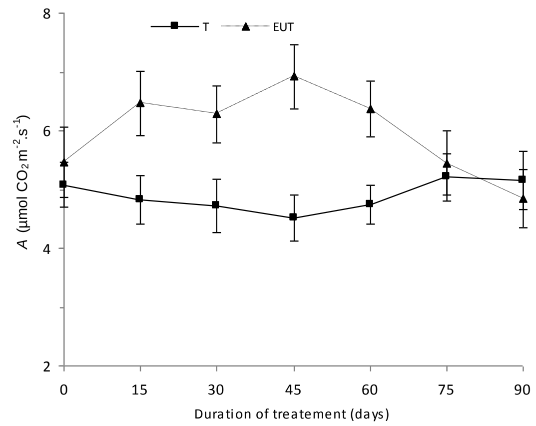

Fig. 5. Evolution of net assimilation (*A*) of poplar seedlings witnesses (C) and those irrigated with treated wastewater (TWW), n = 6 (mean ± SD).

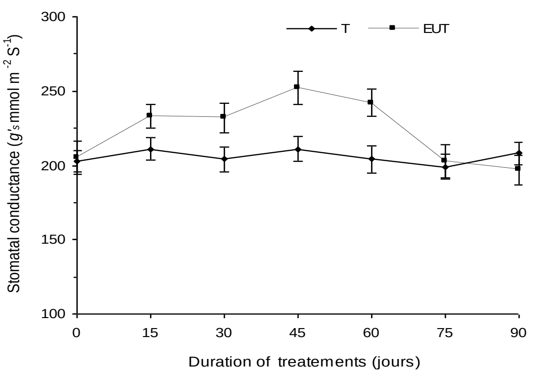

Fig. 6. Evolution of stomatal conductance (g'_s) of witnesses poplar plants (C) and those irrigated with treated wastewater (TWW), n = 6 (mean ± SD).

A significant increase was found in the rate of transpiration (*E*) in plants TWW (df = 1, F = 269.390, p <0.0001) (Fig. 7). After 60 days, a significant difference in the transpiration of treated plants was found; it was 20% in treated plants compared to control (Fig. 7). However, the rates of transpiration decreased after 90 days of irrigation. It was 6% in the plants (TWW) lower than in control plants (C).

Impact of the TWW on the integrity of membrane structures

The degree of resistance of the membrane structure is reflected by the speed of propagation of the damage and of their intensity. Indeed, the evolution of the damage of membrane structures over time shows a similar behavior with the duration of treatment.

The results in Fig. 8 showed that the damage to membranes by the TWW system was relatively low in the plants 'I-488' and not more than 10.2% after 60 days. Additional, membrane resistance was recorded in plants TWW reflected by a low damage (54%) after 90 days.

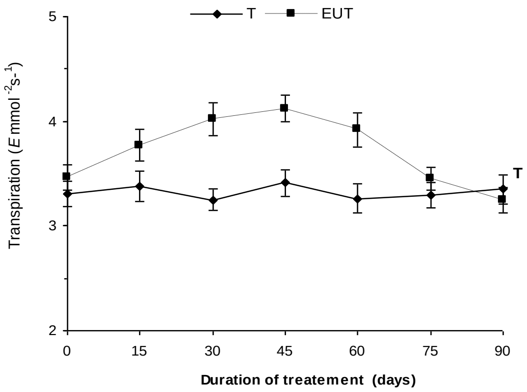

Fig. 7. Evolution of transpiration (*E*) of poplar seedlings witnesses (T) and those irrigated with treated wastewater (TWW), n = 6 (mean ± SD).

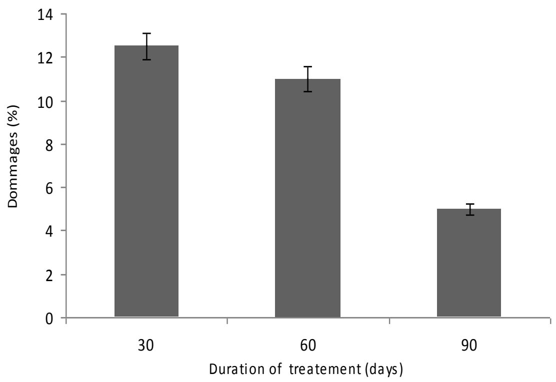

Fig. 8. Evolution of the relative damage of poplar seedlings irrigated with treated (TWW) wastewater, n = 6 (mean ± SD).

Effect of treated wastewater on the accumulation of some mineral ions

Accumulation of ions K, P and Na

The data (fig. 2) indicate a significant effect on the plants irrigated with TWW on K, P and Na over time.

In fact, minerals elements in leaves of plants irrigated with TWW followed the order K> P> Na after 60 days. The levels of K and P in treated plants were significantly higher than in the control plant (C) during the first 60 days (Fig. 9) as the concentrations for K and P increased respectively with 125 % and 20% in comparison with control plants . Besides, the result (Fig. 9) shows a significant increase of Na in treated plants which was 54% higher than in control plants after 90 days. In this period, minerals elements in leaves followed the order K> Na> P.

Accumulation of ions Cd, Pb and Ni

The concentration of metals in *Populus nigra* leaves (I-488) irrigated with treated waste water showed significant difference compared with control plants (Fig. 10). The first 60 days, the treated plants show elevated levels of Cd (0.65 ± 0.75µg/ g MS), Pb (2.5 ± 2.5µg/ g MS) and Ni (1.4 ± 1.5µg/ g Ms). Metals in leaves of treated plants followed the order Cd> Pb> Ni. After 90 days, treated plants show elevated levels of Cd (0,9 ± 1 µg/ g Ms), Pb (3,8 ±4µg/g Ms) and Ni (2 ± 2,75µg/ g Ms). Metals in leaves of treated plants follow the order Cd> Ni > Pb.

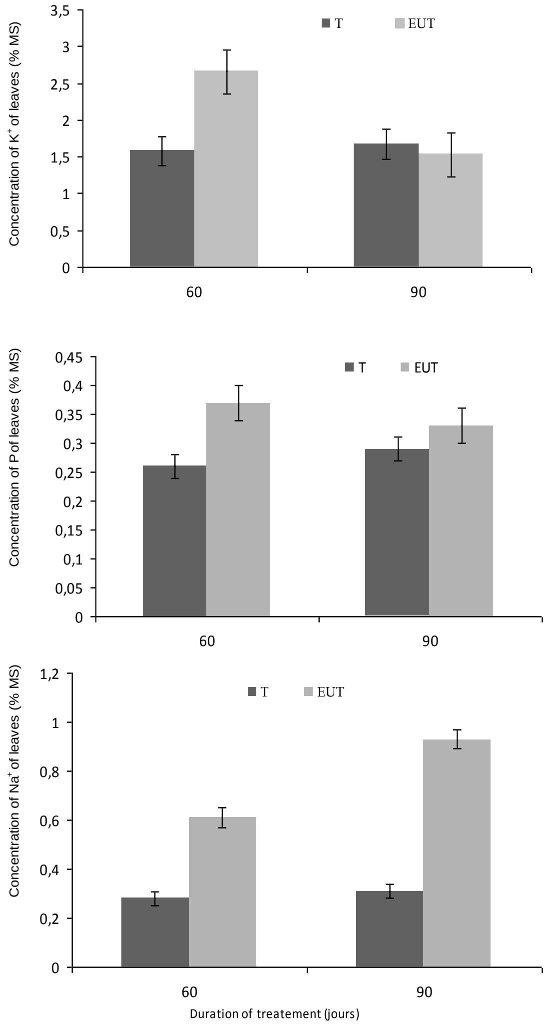

Fig. 9. Leaf content of potassium (K), of phosphorus (P) and of sodium (Na), after 60 and 90 days, of *Populus nigra* seedlings, witness (C) and irrigated with Treated wastewater Those (EUT), n = 6 (mean ± SD). The confidence intervals were calculated at the threshold of 5%.

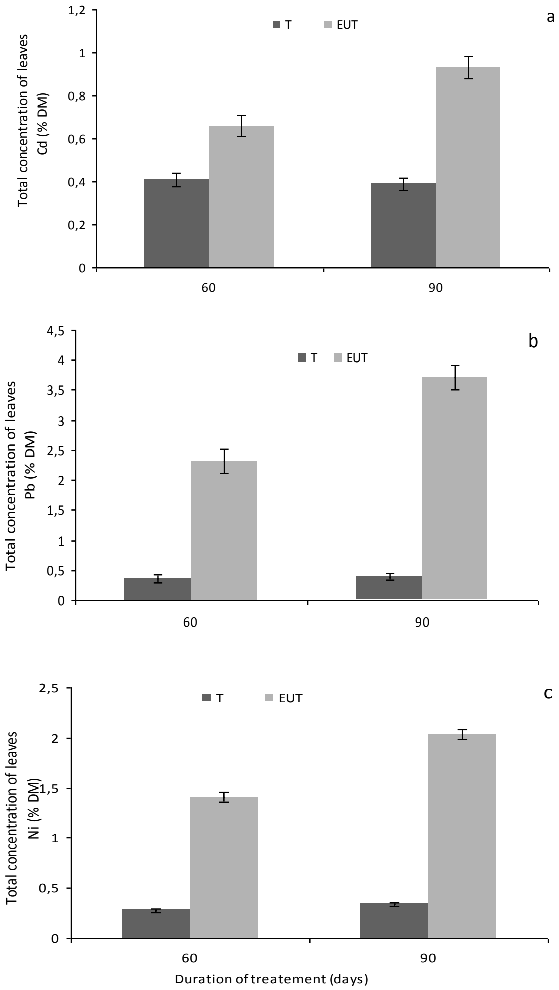

Fig. 10. Leaf content of cadminium (Cd), of plomb (Pb) and nickel (Ni), after 60 and 90 days, of Populus nigra seedlings, witness (C) and Irrigated with Treated wastewater Those (EUT), n = 6 (mean ± SD). The confidence intervals were calculated at the threshold of 5%.

Discussion

The present work studied the effect of treated wastewater on plant morphology, RGR, membrane integrity and accumulation of some minerals in *Populus nigra*. The RGR, gas exchange and content of some minerals ions depend on the duration of treatment. In the first 60 days, the irrigation with treated wastewater (TWW) on *Populus* I-488 show a significant difference on RGR. This indicates a positive fertilization effect that most likely was the result of the content of TWW on potassium, phosphorus... (Trad Rais *et al.*, 2009, Zupančič *et al.*, 2010). These nutrients seem to improve the capacity of carbon fixation and growth in the plant (Karnosky, 2003). Thus during this period, the treated wastewater stimulates *A* (fig. 5), g'_s (fig. 6) and E (fig. 7) of treated plants. The same effects of irrigation with TWW have been demonstrated in *Salix nigra* and in *Populus* X Canadensis (Borghi, 2007). After 60 days of irrigation, plants irrigated with TWW show a decrease of the RGR (32%) when compared with control plants (table 1). Other researchers have reported similar effects on RGR in *Eucalyptus corelliana* (Liang *et al.*, 1999), *Salix viminalis* (Stephens *et al.*, 2000) and *Populus* sp (Meers *et al.*, 2007). This decrease of RGR was the result of the excessive absorption of Na (fig. 6), Cd (fig. 7), Pb (fig. 8) and Ni (fig. 9) and their accumulation in the aerial parts. Indeed, foliar concentration of Ni, Pb, Cd and Na increased significantly after 60 and 90 days. These results indicate that the TWW regime has altered the nutritional balance of the seedlings of *Populus nigra* (I-488). This translocation to the aerial parts during transpiration is a possible consequence of an alteration of the selective power of roots. In fact metals, particulary Pb, Ni (Llamas *et al.*, 2007) and Cd (FAO, 2008), disrupt the assimilation of Ca, Fe, Mg and Mn (Basile *et al.*, 2003), limiting their absorption and/or transport to the leaves (Xiong, 1997). Thus, irrigation for a long time with TWW caused the absorption of Pb (fig. 8), Cd (fig. 7) and Ni (fig. 9) ions and their translocation to the aerial parts. These negative effects of metal stress have been reported in various species, including *Fraxinus pennsylvania* (Pezeshki, 1986) and *Populus* sp (Zalesny and Bauer, 2007). Also, beyond 60 days the accumulation of metal in the transpiration areas affects physiological processes including gas exchange (fig. 1). Similar results have been demonstrated in publications on Salix Sp and Populus deltoïde (Ganesh *et al.*, 2008). At the same time, increases in physiological processes (*A*, *E*, g'_s) are the consequence of translocation of metals to older leaves. These metals (Pb, Ni and Cd) have the ability to induce stomatal closure (Sergin and Ivano, 2001). This is the plant's defense mechanism against metals internally reaching toxic levels. In *Populus*, many studies showed that the transfer of Pb, Cd and Ni ions to the leaves was important and that these plants can store metal ions (Pulford *et al.*, 2001).

Conclusion

The eco-physiological behavior of *Populus nigra* (I-488) to the treated waste water (TWW) was dependent on the duration of irrigation. Beyond 60 days, the TWW biomass was significantly affected due to the decrease of photosynthesis. Also, the TWW regime caused the accumulation of toxic elements which reduced the capacity for carbon fixation. All studied parameters showed that *Populus nigra* is a tolerant plant to metal. During the first 60 days, there was a significant decrease in biomass production compared to control plants. Our results suggest that the use of *Populus* for the phytoremediation has advantages such as high biomass yield and the retention of important nutrients in the soil-plant system. Thus, poplar 'I-488' would be appropriate for future applications in the phytoremediation, especially because rhizofiltration waste water contains metal ions. In addition, the use of different clones of *Populus* would be needed to confirm the encouraging results obtained in semi-controlled conditions. Similarly, it would be interesting to do a comparative study between different clones of Poplar and other species tolerant in accumulating metal ions to reveal and define the mechanism of tolerance to metal. Before implementing an operational level, it is important to conduct research to develop long-term planting site on the frequencies of irrigation in relation to the accumulation of heavy metals in soils, evolution of fertility, texture and structure of soils and their uptake by plants of the different strata (herbaceous, shrubs etc ...).

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