Chapter 18

How Soil Nutrient Availability Influences Plant Biomass and How Biomass Stimulation Alleviates Heavy Metal Toxicity in Soils: The Cases of Nutrient Use Efficient Genotypes and Phytoremediators, Respectively

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Additional information is available at the end of the chapter

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1. Introduction

There are many factors influencing plant biomass, such as soil humidity, soil and air temperature, photoperiod, solar radiation, precipitations, genotype e.t.c. One of the most important factors influencing biomass is soil nutrient availability. Both nutrient deficiency and toxicity negatively affect total biomass and fruit production [1-10]. So, by controlling the optimum levels of nutrient availability in soil, the production of biomass and, of course, the economic benefit (fruit production) for the farmers can be maximized. In the cases of limited nutrient availability in soils, fertilization seems to be the most usual practice adopted by the farmers in order to ameliorate the low nutrient status. However, since: i) during the last two decades the prices of fertilizers have been dramatically increased, and ii) soil degradation and pollution, as well as underground water pollution, are serious consequences provoked by the exaggerate use of fertilizers, a global concern to reduce the use of fertilizers has been developed. So, the best (most economic and ecological) way in our days to achieve maximum yields is by selecting and growing nutrient efficient genotypes, i.e. genotypes which are able to produce high yields (biomass) in soils with limited nutrient availability. Many researchers studied the influence of genotype on biomass and plant growth (nutrient use efficient genotypes) and found impressive results. According to Chapin and Van Cleve (1991) [11], nutrient use efficiency is defined as the amount of biomass produced per unit of nutrient. So, nutrient use efficient genotypes are those having the ability to produce biomass sufficiently under limited nutrient availability. In our research with different olive cultivars, grown under hydroponics, or in soil substrate, we found significant differences concerning macro- and micronutrient utilization efficiency among genotypes [12-13]. Possible reasons for differential nutrient utilization efficiency among genotypes may be: i) the genetic material used, i.e. cultivar (differential nutrient uptake, accumulation and distribution among tissues, mechanisms of cultivars/genotypes), ii) differential colonization of their root system mycorrhiza fungi. Chatzistathis et al. (2011) [14] refer that the statistically significant differences in Mn, Fe and Zn utilization efficiency among three Greek olive cultivars ('Chondrolia Chalkidikis', 'Koroneiki' and 'Kothreiki') may be probably ascribed to the differential colonization of their root system by arbuscular mycorrhiza fungus (AMF) (the percentage root colonization by AMF varied from 45% to 73%).

Heavy metal (Cu, Zn, Ni, Pb, Mn, Cr, Cd) toxicity is a very serious problem in soils suffering from: i) industrial and mine activities [15], ii) the exaggerate use of fertilizers, fungicides and insecticides, iii) acidity, iv) waterlogging, v) other urban activities, such as municipal sewage sludges, vi) the use of lead in petrols, paints and other materials [16]. Under these conditions, plant growth and biomass are negatively affected [17-20]. According to Caldelas et al. (2012) [19], not only growth inhibition happened, but also root to shoot dry matter partitioning (R/S) modified (increased 80%) at Cr toxic conditions in Iris pseudacorus L. plants. Some plant species, which may tolerate very high metal concentrations in their tissues, can be used as hyper-accumulators and are very suitable in reducing heavy metal concentrations in contaminated soils [21]. These species are able to accumulate much more metal in their shoots, than in their roots, without suffering from metal toxicity [22]. By successive harvests of the aerial parts of the hyper-accumulator species, the heavy metals concentration can be reduced [23]. Phytoremediation is an emerging technology and is considered for remediation of inorganic- and organic-contaminated sites because of its cost-effectiveness, aesthetic advantages, and long-term applicability. This technique involves the use of the ability of some plant species to absorb and accumulate high concentrations of heavy metal ions [17]. Some of these species may be a few ones from Brassicaceae family, such as raya (Brassica campestris L.) [17] and Thlaspi caerulescens [23], or from other families, such as spinach (Spinacia oleracea L.) [17], Sedum plumbizincicola [24], Amaranthus hypochondriacus [25], Eremochloa ophiuroides [26], Iris pseudacorus L. [19], Ricinus communis L., plant of Euphorbiaceae family [18]. Finally, the tree species Genipa Americana L. may be used as one with great ability as phytostabilizer and rhizofilterer of Cr ions, according to Santana et al. (2012) [20]. Basically, there are two different strategies to phytoextract metals from soils: the first approach is the use of metal hyper-accumulator species. The second one is to use fast-growing, high biomass crops that accumulate moderate to high levels of metals in their shoots for metal phytoremediation, such as Poplar (Populus sp.) [27-28], maize (Zea mays), oat (Avena sativa), sunflower (Helianthus annuus) and rice (Oryza sativa L.) [25]. Generally, the more high biomass producing is one plant species, the more efficient is the phytoremediation effect. So, in order to enhance biomass production under metal toxicity conditions, different strategies, such as the application of chemical amendments, may be adopted [21]. Since Fe deficiency symptoms may be appeared under Cu and Zn toxicity conditions in some species of Brassicaceae family used for phytoremediation, a good practice is to utilize Fe foliar sprays in order to enhance biomass, thus the phytoremediation effect [29].

All the above mentioned topics, concerning the influence of nutrient deficiency and metal toxicity on plant biomass, as well as the importance of using nutrient use efficient genotypes and cultivars, are within the aim of the present review. Furthermore, the characteristics that should have the plant species used for phytoremediation (fast-growing, high biomass crops) in heavy metal polluted soils are fully analyzed, and the different strategies that should be adopted in order to enhance plant growth and biomass production under so adverse soil conditions are also discussed under the light of the most important and recent research papers.

2. Agronomic, environmental and genotypic factors influencing plant growth

Plant growth (i.e. biomass production) is influenced by many (agronomic environmental and others, such as genetic) factors. Some of the most important factors that influence biomass production are: i) soil humidity, ii) soil and air temperature, iii) air humidity, iv) photoperiod, v) light intensity, vi) soil fertility, i.e. soil nutrient availability, and vii) genotype, and are fully analyzed below.

2.1. Soil humidity

Soil humidity is a very crucial factor influencing root growth, thus nutrient uptake and total biomass. Many plant species are more sensitive in soil humidity shortage during a particular (crucial) period of their growth. In olive trees, if soil humidity shortage happens early spring, shoot elongation, as well as the formation of flowers and fruits, are negatively influenced. If the shortage happens during summer, shoot thickening, rather than shoot elongation, is influenced. Finally, soil humidity shortage reduces olive tree canopy (in order to reduce the transpiration by leaf surface) and favors root system growth (in order to have the ability to exploit greater soil volume and to search for more soil humidity), so that the ratio canopy/root is significantly reduced [30]. On the other hand, under excess soil humidity conditions (waterlogging), when soil oxygen is limited, the root system may suffer from hypoxia, thus, nutrient uptake is negatively influenced. Under extreme anaerobic soil conditions, the presence of pathogen microorganisms, such as *Phytophthora* sp. may lead to root necrosis. According to Therios (2009) [31], for olive trees the mechanism of tolerance to waterlogging is based on the production of adventitious roots near to the soil surface.

2.2. Soil temperature

Soil temperature influences root growth, thus nutrient and water uptake and, of course, biomass production. Most nutrients are absorbed with energy consumption (energetic uptake), so, low and very high soil temperatures negatively influence root growth and nutrient uptake. Furthermore, low soil temperatures induce a water deficit [32].

2.3. Air temperature

Air temperature directly influences photosynthesis, which is the most important physiological function in plants. The optimum temperature for photosynthesis depends on plant species and also on cultivar for the same species. Usually, the optimum temperature for maximum photosynthetic activity is around 25°C for most vegetative species. When temperature exceeds 35°C photosynthesis is inhibited, thus biomass production may be restrained. High temperatures are associated with a high vapor pressure deficit between leaves and the surrounding air. The same applies to fruit, where high temperatures may cause fruit drop in olive trees [31]. On the other hand, low temperatures act negatively in photosynthesis function and starch is redistributed and is accumulated in organs protected from frost, such as roots. Very low temperatures (<-12°C) damage the leaf canopy, shoot and branches of trees [31].

2.4. Air humidity

Low atmosphere humidity speeds up transpiration by leaf surface. Increase of the rate of transpiration causes reduction of vegetative tissues water content, thus depression in the rate of growth and biomass production.

2.5. Photoperiod

Photoperiod is the duration of light in 24 hours and it is one of the most important factors influencing vegetative growth. Plant species whose vegetative growth is mostly influenced by long day conditions are *Populus robusta*, *Ulmus Americana* and *Aesculus hippocastanus* [30].

2.6. Light intensity

Light, together with CO_2, are the two main factors influencing photosynthetic rate. By increasing light intensity up to an optimum limit the maximum photosynthetic rate, so the greatest biomass production can be achieved.

2.7. Nutrient availability

Limited nutrient availability influences negatively biomass production. Nitrogen deficiency strongly depresses vegetation flush. According to Boussadia et al. (2010) [8], total biomass of two olive cultivars ('Meski' and 'Koroneiki') was strongly reduced (mainly caused by a decrease in leaf dry weight) under severe N deprivation, while in an out-door pot-culture experiment with castor bean plants (*Ricinus communis* L.), conducted by Reddy and Matcha (2010) [9], it was found that among the plant components, leaf dry weight had the greatest decrease; furthermore, root/shoot ratio increased under N deficiency [9]. Phosphorus deficiency caused reduced biomass, photosynthetic activity and nitrogen fixing ability in mungbean (*Vigna aconitifolia*) and mashbean (*Vigna radiata*) [33]. Under P deficiency conditions, genotypic variation in biomass production is evident; according to Pang et al. (2010) [34], who studied in a glasshouse experiment the response of ten perennial herbaceous legume species, found that under low P conditions several legumes produced more biomass than lucerne. Nutrient deficiency may cause physiological and metabolism abnormalities in plants, which may lead to deficiency symptoms. There are two categories of symptoms: i) General symptoms, such as limited growth and inability of reproduction (flowering and fruit setting), caused by the deficiency of many necessary macro- or micro-nutrients, and ii) typical, characteristic, deficiency symptoms, such as chlorosis, i.e. yellowing (due to Fe deficiency). In both cases biomass production is depressed. In the study of Msilini et al. (2009) [10], bicarbonate treated plants of *Arabidopsis thaliana* suffered from Fe deficiency displayed significantly lower biomass, leaf number and leaf surface, as compared to control plants, and showed slight yellowing of their younger leaves. Under limited nutrient availability, arbuscular mycorrhiza fungi (AMF) may favor nutrient uptake and thus enhance biomass production. Hu et al. (2009) [35] refer that AMF inoculation of maize plants was likely more efficient in extremely P-limited soils. Generally, root colonization by AMF influences positively plant growth under N, P, or micronutrient deficiency conditions [36].

2.8. Genotypic factors (root morphology and architecture, genetic growth capacity e.t.c.)

According to Bayuelo-Jimenez et al. (2011) [3], under P deficiency, P-efficient accessions of maize plants (*Zea mays* L.) had greater root to shoot ratio, nodal rooting, nodal root laterals, nodal root hair density and length of nodal root main axis, and first-order laterals. In our experiments, we also found differential root system morphology among three Greek olive cultivars (the root systems of 'Koroneiki' and 'Chondrolia Chalkidikis' were less branched and more lateral, and with less root hair development and density, than that of 'Kothreiki', which was richly-branched and with much greater root hair development and density), something which was probably the main reason for the great genotypic variations in nutrient uptake and growth among the three cultivars (Chatzistathis, unpublished data). Singh et al. (2010) [37] found that great differences existed among 10 multipurpose tree species, grown in a monoculture tree cropping system on the sodic soils of Gangetic alluvium in north India, concerning plant height, diameter e.t.c.

3. Physiological roles of nutrients

The absolutely necessary nutrients for plant growth are the following: N, P, K, S, Ca, Mg (macronutrients), Zn, Cu, B, Mn, Fe, Mo (micronutrients). Without one of these nutrients, plant organism can not grow normally and survive. The physiological roles of these nutrients are described in detail below.

3.1. Macronutrients

Nitrogen: It is a primary component of nucleic acids, proteins, amino acids, purines, pyrimidines and chlorophyll. Nitrogen exerts a significant effect on plant growth, as it reduces biennial bearing and increases the percentage of perfect flowers. In olive trees, lack of N leads to decreased growth, shorter length of annual shoots (<10cm), fewer leaves, reduced flowering and decreased yield [31].

Phosphorus: P is a component of high-energy substances such as ATP, ADP and AMP; it is also important for nucleic acids and phospholipids. Phosphorus affects root growth and maturation of plant tissues and participates in the metabolism of carbohydrates, lipids and proteins [31].

Potassium: K plays a crucial role in carbohydrate metabolism, in the metabolism of N and protein synthesis, in enzyme activities, in the regulation of the opening and closing of stomata, thus to the operation of photosynthesis, in the improvement of fruit quality and disease tolerance, in the activation of the enzymes peptase, catalase, pyruvic kinase e.t.c. [31,38].

Calcium: It is the element that participates in the formation and integrity of cell membranes, in the integrity and semipermeability of the plasmalemma, it increases the activity of many enzymes, it plays a crucial role in cell elongation and division, in the transfer of carbohydrates e.t.c. [31,38].

Magnesium: It is part of chlorophyll molecule, it activates the enzymes of Crebs' cycle and it also plays a role in oil synthesis [38].

Sulphur: Sulphur plays role in the synthesis of some amino-acids, such as cysteine, cystine, methionine, as well as in proteins synthesis. It also activates some proteolytic enzymes, such as papaine, bromeline e.t.c. Finally, it is part of some vitamins' molecule and that of gloutathione [31,38].

3.2. Micronutrients

Iron: Iron plays an important role in chlorophyll synthesis, without being part of its molecule. Furthermore, it participates in the molecule of Fe-proteins catalase, cytochrome a, b, c, hyperoxidase e.t.c. In addition to that, it is found in the enzymes nitric and nitrate reductase, which are responsible for the transformation of NO_3^- into NH_4^+, as well as in nitrogenase, which is the responsible enzyme for the atmospheric N capturing [38].

Manganese: Manganese is activator of the enzymes of carbohydrates metabolism, those of Crebs' cycle, and of some other enzymes, such as cysteine desulphydrase, glutamyl transferase e.t.c. It also plays a key-role in photosystem II of photosynthesis, and particularly in the reactions liberating O_2. Finally, Mn acts as activator of some enzymes catalyzing oxidation and reduction reactions [38].

Zinc: Zn plays crucial role in tryptophane biosynthesis, which is the previous stage from IAA (auxin) synthesis (direct influence of Zn on plant growth and biomass production). IAA concentration is significantly reduced in vegetative tissues suffering from Zn deficiency. In addition to the above, Zn is part of some metal-enzymes [38].

Copper: Cu is activator of some enzymes, as well as it is part of enzymes catalyzing oxidation and reducing reactions, such as oxidase of ascorbic acid, lactase, nitrate and nitric reductase e.t.c. [38].

Boron: B plays role in the transfer of sugars along cell membranes, as well as in RNA and DNA synthesis. It also participates to cell division process, as well as to the pectine synthesis [38].

Molybdenum: It is part of the enzyme nitrogenase (capturing of atmospheric N) and nitric reductase (transformation of NO_3^- to NO_2^-). Mo also participates to the metabolism of ascorbic acid [38].

As it is clear from all the above physiological roles of nutrients, the deficiency of even one of them in the mineral nutrition of higher plants depresses their growth, thus biomass production. So, in order to achieve the maximum biomass production, apart from the optimum conditions of all the other environmental and agronomic factors influencing plant growth (temperature, soil humidity, photoperiod, light intensity), it should always be taken care of maintaining the optimum levels of all the necessary soil nutrients. This is usually achieved with the correct fertilization program of the different crops. For example, fruit trees have high demands in K, since fruit production is a K sink and reduces its levels in plant level. According to Therios (2009) [31], potassium plays an important role in olive nutrition. Thus, fruit trees should be periodically fertilized (usually K fertilizers applied during autumn, or winter, and are incorporated into the soils) with enhanced doses of potassium fertilizers (usually K_2SO_4). Apart from chemical fertilizers, organic amendments can be also applied under limited nutrient conditions in order to enhance plant growth. According to Hu et al. (2009) [35], stem length, shoot and root biomass, as well as crop yield of maize were all greatly increased by the application of organic amendments on a sandy loam soil. Apart from the application of chemical fertilizers, organic amendments e.t.c., another modern method to improve yields and to increase biomass is the irrigation of crops with FFC H_2O, a commercial product currently utilized by the agriculture, fishery and food industries in Japan. In the study of Konkol et al. (2012) [39], radish and shirona plants irrigated with FFC H_2O developed larger average leaf area by 122% and greater dry weight and stem length by 39% and 31%, respectively, compared to the plants irrigated with deionized H_2O. FFC H_2O offers agriculturalists a simple and effective tool for the fortification of irrigation waters with micronutrients [39].

4. Nutrient utilization efficiency (NUE): The case of nutrient use efficient genotypes

World population is expected to increase from 6.0 billion in 1999 to 8.5 billion by 2025. Such an increase in population will intensify pressure on the world's natural resource base (land, water, and air) to achieve higher food production. Increased food production could be achieved by expanding the land area under crops and by increasing yields per unit area through intensive farming. Chemical fertilizers are one of the expensive inputs used by farmers to achieve desired crop yields [40]. However, during the last years, the prices of fertilizers have been considerably increased. Furthermore, soil degradation and pollution, as well as underground water pollution, are serious consequences provoked by the exaggerate use of fertilizers during last decades. These two aspects are responsible for the global concern to reduce the use of fertilizers. The best way to do that is by selecting and growing nutrient use efficient genotypes. According to Khoshgoftarmanesh (2009) [41], cultivation and breeding of micronutrient-efficient genotypes in combination with proper agronomic management practices appear as the most sustainable and cost-effective solution for alleviating food-chain micronutrient deficiency.

Nutrient use efficient genotypes are those having the ability to produce high yields under conditions of limited nutrient availability. According to Chapin and Van Cleve (1991) [11] and Gourley et al. (1994) [42], as nutrient utilization efficiency (NUE) is defined the amount of biomass produced per unit of nutrient absorbed. **Nutrient efficiency ratio (NER)** was suggested by Gerloff and Gabelman (1983) [43] to differentiate genotypes into efficient and inefficient nutrient utilizers, i.e. **NER=(Units of Yields, kgs)/(Unit of elements in tissue, kg),** while **Agronomic efficiency (AE)** is expressed as the additional amount of economic yield per unit nutrient applied, i.e. **AE=(Yield F, kg-Yield C, kg)/(quantity of nutrient applied, kg),** where F applies for plants receiving fertilizer and C for plants receiving no fertilizer.

Many researchers found significant differences concerning nutrient utilization efficiency among genotypes (cultivars) of the same plant species [1,12,13,40,44-46] Biomass (shoot and root dry matter production) was used as an indicator in order to assess Zn efficient Chinese maize genotypes, grown for 30 days in a greenhouse pot experiment under Zn limiting conditions [1]. NUE is based on: a) uptake efficiency, b) incorporation efficiency and c) utilization efficiency [40]. The uptake efficiency is the ability of a genotype to absorb nutrients from the soil; however, the great ability to absorb nutrients does not necessarily mean that this genotype is nutrient use efficient. According to Jiang and Ireland (2005) [45], and Jiang (2006) [46], Mn efficient wheat cultivars own this ability to a better internal utilization of Mn, rather than to a higher plant Mn accumulation. We also found in our experiments that, despite the fact that the olive cultivar 'Kothreiki' absorbed and accumulated significantly greater quantity of Mn and Fe in three soil types, compared to 'Koroneiki', the second one was more Mn and Fe-efficient due to its better internal utilization efficiency of Mn and Fe (greater transport of these micronutrients from root to shoots) [12] (Tables 1 and 2). Aziz et al. (2011a) [47] refer that under P deficiency conditions, P content of young leaves in *Brassica* cultivars increased by two folds, indicating remobilization of this nutrient from older leaves and shoot. However, differences in P remobilization among *Brassica* cultivars could not explain the differences in P utilization. Phosphorus efficient wheat genotypes with greater root biomass, higher P uptake potential in shoots and absorption rate of P were generally more tolerant to P deficiency in the growth medium [6]. According to Yang et al. (2011) [48], on average, the K efficient cotton cultivars produced 59% more potential economic yield (dry weight of all reproductive organs) under field conditions even with available soil K at obviously deficient level (60 mg/kg).

The possible causes for the differential nutrient utilization efficiency among genotypes and/or species may be one, or combination of more than one, of the following: a) genetic reasons (genotypic ability to absorb and utilize efficiently, or inefficiently, soil nutrients), b) mycorrhiza colonization of the root system, c) differential root exudation of organic compounds favorizing nutrient uptake, d) different properties of rhizosphere, e) other reasons. According to Cakmak (2002) [49], integration of plant nutrition research with plant genetics and molecular biology is indispensable in developing plant genotypes with high genetic ability to adapt to nutrient deficient and toxic soil conditions and to allocate more micronutrients into edible plant products. According to Aziz et al. (2011b) [50], *Brassica* cultivars with high biomass and high P contents, such as 'Rainbow' and 'Poorbi Raya', at low available P conditions would be used in further screening experiments to improve P efficiency in *Brassica*. More specifically, a number of genes have been isolated and cloned, which are involved in root exudation of nutrient-mobilizing organic compounds [51,52]. Successful attempts have been made in the past 5 years to develop transgenic plants that produce and release large amounts of organic acids, which are considered to be key compounds involved in the adaptive mechanisms used by plants to tolerate P-deficient soil conditions [53-55]. However, differential root exudation ability in nature exists among different plant species. According to Maruyama et al. (2005) [56], who made a comparison of iron availability in leaves of barley and rice, the difference in the Fe acquisition ability between these two species was affected by the differential mugineic acid secretion. Chatzistathis et al. (2009) [12] refer that, maybe, a similar mechanism was responsible for the differential micronutrient uptake and accumulation between the Greek olive cultivars 'Koroneiki' and 'Kothreiki'. According to the same authors, differential reduction of Fe^{3+} to Fe^{2+}, or acidification capacity of root apoplast (which associates with the increase of Fe^{3+}-chelate reductase and H-ATPase activities) among three Greek olive cultivars should not be excluded from possible causes for the significant differences observed concerning Fe uptake [14]. Mycorrhiza root colonization may be another responsible factor for the differential micronutrient utilization efficiency among genotypes. According to Citernesi et al. (1998) [57], arbuscular mycorrhiza fungi (AMF) influenced root morphology of Italian olive cultivars, thus nutrient uptake and accumulation, as well as plant growth. In our study with olive cultivars 'Koroneiki', 'Kothreiki' and 'Chondrolia Chalkidikis', we found significant differences concerning root colonization by AMF (that varied from 45% to 73%), together with great differences in uptake and utilization efficiency of Mn, Fe and Zn among them (particularly, 1.5 to 10.5 times greater amount of Mn, Fe and Zn accumulated by 'Kothreiki', compared to the other two cultivars, but the differences in plant growth parameters between the three cultivars were not impressive; this is why the micronutrient utilization efficiency by 'Kothreiki' was significantly lower, compared to that of the other two ones). Finally, the different properties of rhizosphere among genotypes may be another important factor influencing nutrient uptake and utilization efficiency, and of course biomass production. According to Rengel (2001) [58], who made a review on genotypic differences in micronutrient use efficiency of many crops, micronutrient-efficient genotypes were capable of increasing soil available micronutrient pools through changing the chemical and microbiological properties of the rhizosphere, as well as by growing thinner and longer roots and by having more efficient uptake and transport mechanisms.

Soil	Cultivar	Micronutrient	Root	Stem	Leaves
Marl		**Mn**			
	Kor		50.2b	38.0a	11.8a
	Koth		74.1a	12.8b	13.1a
Gneiss schist					
	Kor		56.5b	34.2a	9.3a
	Koth		81.3a	10.8b	7.9a
Peridotite					
	Kor		44.0b	44.0a	12.0a
	Koth		76.0a	12.9b	11.1a
Marl		**Fe**			
	Kor		93.7a	3.9a	2.4a
	Koth		98.0a	0.9b	1.1b
Gneiss schist					
	Kor		94.0a	3.7a	2.3a
	Koth		98.8a	0.6b	0.6b
Peridotite					
	Kor		90.8a	7.1a	2.1a
	Koth		98.3a	0.8b	0.9b
Marl		**Zn**			
	Kor		49.3b	29.6a	21.1a
	Koth		64.4a	15.6b	20.0a
Gneiss schist					
	Kor		59.1b	26.7a	14.2a
	Koth		73.7a	14.3b	12.0a
Peridotite					
	Kor		37.3b	33.9a	28.8a
	Koth		65.3a	18.0b	16.7b

The different letters in the same column symbolize statistically significant differences between the two olive cultivars in each of the three soils, for $P \leq 0.05$ (n=6) (SPSS; t-test).

Table 1. Distribution (%) of the total per plant quantity of Mn, Fe and Zn in the three vegetative tissues (root, stem and leaves) of the olive cultivars 'Koroneiki' and 'Kothreiki', when each one was grown in three soils (from parent material Marl, Gneiss schist. and Peridotite) with different physicochemical properties (Chatzistathis et al., 2009).

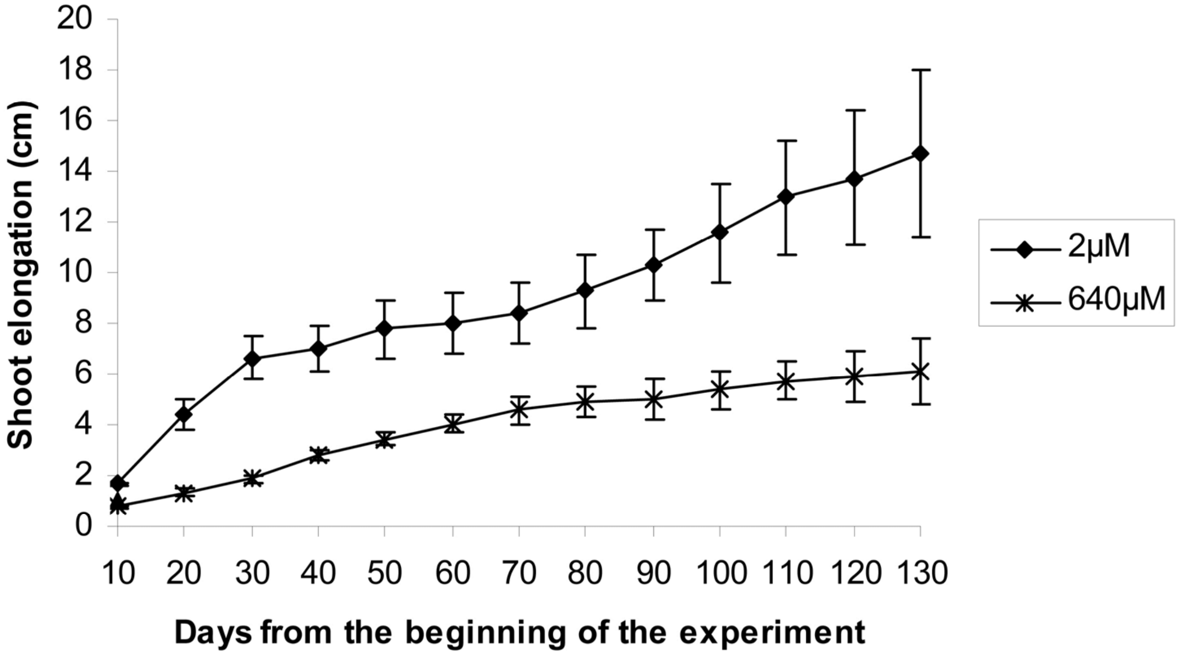
Soil	Cultivar	MnUE	FeUE	ZnUE
Marl		mg of the total plant d.w./μg of the total per plant quantity of micronutrient		
	Kor	31.85a	1.73a	77.53a
	Koth	18.68b	0.65b	68.08a
Gneiss schist				
	Kor	39.87a	1.84a	51.04a
	Koth	17.94b	0.44b	49.15a
Peridotite				
	Kor	23.33a	1.19a	61.75a
	Koth	18.00a	0.58b	72.88a

The different letters in the same column symbolize statistically significant differences between the two cultivars in each of the three soils, for $P \leq 0.05$ (n=6) (SPSS; t-test).

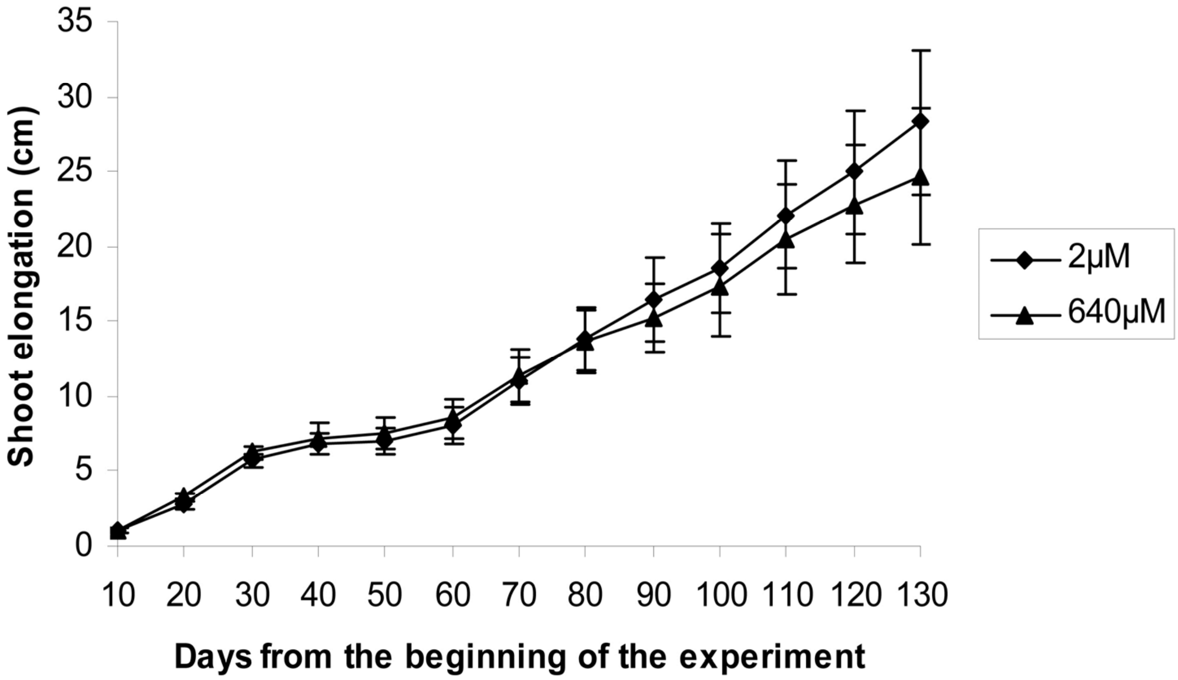
Table 2. Nutrient utilization efficiency (mg of the total plant d.w. /μg of the total per plant quantity of micronutrient or mg of the total per plant quantity of macronutrient) of the olive cultivars 'Koroneiki' and 'Kothreiki', when each of them was grown in three soils (from parent material Marl, Gneiss schist. and Peridotite) with different physicochemical properties (Chatzistathis et al., 2009).

5. The influence of heavy metal toxicity on biomass production

Soil heavy metal contamination has become an increasing problem worldwide. Among the heavy metals, Cu, Zn, Mn, Cd, Pb, Ni and Cr are considered to be the most common toxicity problems causing increasing concern. Growth inhibition and reduced yield are common responses of horticultural crops to nutrient and heavy metal toxicity [2]. Nevertheless, sometimes less common responses happen under metal toxicity conditions. For example, in the case of Pb it has been suggested that inhibition of root growth is one of the primary effects of Pb toxicity through the inhibition of cell division at the root tip [59]. Significant reductions in plant height, as well as in shoot and root dry weight (varying from 3.3% to 54.5%), as compared with that of the controls, were found for *Typha angustifolia* plants in different Cr treatments [60]. Furthermore, according to Caldelas et al. (2012) [19], not only growth inhibition happened (reached 65% dry weight) under Cr toxicity conditions, but also root/shoot partitioning increased by 80%. Under Cr stress conditions, it was found that root and shoot biomass of *Genipa americana* L. were significantly reduced [20]. The biomass reduction of *Genipa americana* trees is ascribed, according to the same authors, to the decreased net photosynthetic rates and to the limitations in stomatal conductance. The disorganization of chloroplast structure and inhibition of electron transport is a possible explanation for the decreased photosynthetic rates of trees exposed to Cr stress [20]. In contrast to the above, Cd and Pb applications induced slight or even significant increase in plant height and biomass. The fact that Cd and Pb addition enhanced Ca and Fe uptake suggests that these two nutrients may play a role in heavy metal detoxification by *Typha angustifolia* plants; furthermore, increased Zn uptake may also contribute to its hyper Pb tolerance, as recorder in the increased biomass over the control plants [60]. According to the



(A)



(B)

Figure 1. Shoot elongation of olive cultivars 'Picual' (A) and 'Koroneiki' (B), when grown under hydroponics at normal (2 μM) and excess Mn conditions (640 μM Mn) (Chatzistathis et al., 2012).

same authors (Bah et al., 2011), plants have mechanisms that allow them to tolerate relatively high concentrations of Pb in their environment without suffering from toxic effects.

Tzerakis et al. (2012) [2] found that excessively high concentrations of Mn and Zn in the leaves of cucumber (reached 900 and 450 mg/kg d.w., respectively), grown hydroponically under toxic Mn and Zn conditions, reduced the fruit biomass due to decreases in the number of fruits per plants, as well as in the net assimilation rate, stomatal conductance and transpiration rate. However, it was found that significant differences concerning biomass production between different species of the same genus exist under metal toxicity conditions; *Melilotus officinalis* seems to be more tolerant to Pb than *Melilotus alba* because no differences in shoot or root length, or number of leaves, were found between control plants and those grown under 200 and 1000 mg/kg Pb [15]. In addition to the above, genotypic differences between cultivars of the same species, concerning biomass production, under metal toxicity conditions may also be observed; Chatzistathis et al. (2012) [13] found that under excess Mn conditions (640 μM), plant growth parameters (shoot elongation, as well as fresh and dry weights of leaves, root and stem) of olive cultivar 'Picual' were significantly decreased, compared to those of the control plants (2 μM), something which did not happen in olive cultivar 'Koroneiki' (no significant differences were recorder between the two Mn treatments) (Figure 1). According to the same authors, some factors related to the better tolerance of 'Koroneiki' not only at whole plant level, but also at tissue and cell level, could take place. Such possible factors could be a better compartmentalization of Mn within cells and/or functionality of Mn detoxification systems [13]. Significant growth reductions of several plant species, grown under Mn toxicity conditions, have been mentioned by several researchers [61-65].

Nickel (Ni) toxicity, which may be a serious problem around industrial areas, can also cause biomass reduction. At high soil Ni levels (>200 mg/kg soil) reduced growth symptoms of *Riccinus communis* plants were observed [18]. According to Baccouch et al. (1998) [66], the higher concentrations of Ni have been reported to retard cell division, elongation, differentiation, as well as to affect plant growth and development. Excess Cd, which causes direct or indirect inhibition of physiological processes, such as transpiration, photosynthesis, oxidative stress, cell elongation, N metabolism and mineral nutrition may lead in growth retardation, leaf chlorosis and low biomass production [67]. According to the same authors, Cd stress could induce serious damage in root cells of grey poplar (*Populus x canescens*). Arsenic (As) toxicity may be another (although less common) problem contributing to soil contamination. Repeated and widespread use of arsenical pesticides has significantly contributed to soil As contamination [4]. According to the same authors, plant growth parameters, such as biomass, shoot height, and root length, decreased with increased As concentrations in all soils.

6. Phytoremediation

Soil pollution represents a risk to human health in various ways including contamination of food, grown in polluted soils, as well as contamination of groundwater surface soils [68].

Classical remediation techniques such as soil washing, excavation, and chelate extraction are all labor-intensive and costly [69].

Phytoremediation of heavy metal contaminated soils is defined as the use of living green plants to transport and concentrate metals from the soil into the aboveground shoots, which are harvested with conventional agricultural methods [70]. The technique is suitable for cultivated land with low to moderate metal contaminated level. According to Jadia and Fulekar (2009) [71], phytoremediation is an environmental friendly technology, which may be useful because it can be carried out *in situ* at relatively low cost, with no secondary pollution and with the topsoil remaining intact. Furthermore, it is a cost-effective method, with aesthetic advantages and long term applicability. It is also a safe alternate to conventional soil clean up [17]. However, a major drawback of phytoremediation is that a given species typically remediates a very limited number of pollutants [24]. For example, a soil may be contaminated with a number of potentially toxic elements, together with persistent organic pollutants [72]. There are two different strategies to phytoextract metals from soils. The first approach is the use of metal hyperaccumulator species, whose shoots or leaves may contain rather high levels of metals [25]. The important traits for valuable hyperaccumulators are the high bioconcentration factor (root-to-soil metal concentration) and the high translocation factor (shoot to root metal concentration) [73]. Another strategy is to use fast-growing, high biomass crops that accumulate moderate levels of metals in their shoots for metal phytoremediation [25]. Phytoextraction ability of some fast growing plant species leads to the idea of connecting biomass production with soil remediation of contaminated industrial zones and regions. This biomass will contain significant amount of heavy metals and its energetic utilization has to be considered carefully to minimize negative environmental impacts [74].

7. Plant species used for phytoremediation

Many species have been used (either as hyperaccumulators, or as fast growing-high biomass crops) to accumulate metals, thus for their phytoremediation ability. Hyperaccumulators are these plant species, which are able to tolerate high metal concentrations in soils and to accumulate much more metal in their shoots than in their roots. By successive harvests of the aerial parts of the hyperaccumulator species, the heavy metals concentration in the soil can be reduced [23]. According to Chaney et al. (1997) [21], in order a plant species to serve the phytoextraction purpose, it should have strong capacities of uptake and accumulation of the heavy metals when it occurs in soil solution. For example, *Sedum plumbizincicola* is an hyperaccumulator that has been shown to have a remarkable capacity to extract Zn and Cd from contaminated soils [75]. In addition, a very good also hyperaccumulator for Zn and Cd phytoextraction is *Thlaspi caerulescens* [23]. *Iris pseudacorus* L. is an ornamental macrophyte of great potential for phytoremediation, to tolerate and accumulate Cr and Zn [19]. Furthermore, many species of *Brassica* are suitable for cultivation under Cu and Zn toxicity conditions and may be used for phytoremediation [29]. *Phragmites australis*, which is a species of *Poaceae* family, may tolerate extremely high concentrations of Zn, Cu, Pb and Cd, thus can be used as heavy metal phytoremediator [76].

Santana et al. (2012) [20] refer that *Genipa americana* L. is a tree species that tolerates high levels of Cr^{3+}, therefore it can be used in recomposition of ciliary forests at Cr-polluted watersheds. According to the same authors, this woody species demonstrates a relevant capacity for phytoremediation of Cr. *Elsholtzia splendens* is regarded as a Cu tolerant and accumulating plant species [77]. Peng et al. (2012) [78] refer that *Eucalyptus urophylla X E.grandis* is a fast growing economic species that contributes to habitat restoration of degraded environments, such as the Pb contaminated ones. On the other hand, concerning Cd phytoextraction ability, only a few plant species have been accepted as Cd hyperaccumulators, including *Brassica juncea, Thlaspi caerulescens* and *Solanum nigrum*. Poplar (*Populus* L.), which is an easy to propagate and establish species and it has also the advantages of rapid growth, high biomass production, as well as the ability to accumulate high heavy metal concentrations, could be used as a Cd-hypaeraccumulator for phytoremediation [27-28,67]. According to Wang et al. (2012) [28], the increase in total Cd uptake by poplar genotypes in Cd contaminated soils is the result of enhanced biomass production under elevated CO_2 conditions. Furthermore, *Amaranthus hypochondriacus* is a high biomass, fast growing and easily cultivated potential Cd hyperaccumulator [25]. Another species was found to be a good phytoremediator concerning its phytoaccumulation and tolerance to Ni stress is *Riccinus communis* L. [18]. Finally, *Justicia gendarussa*, which was proved to be able to tolerate and accumulate high concentration of heavy metals (and especially that of Al), could be used as a potential phytoremediator.

Differences between species, or genotypes of the same species, concerning heavy metal accumulation have been found by many researchers. According to Dheri et al. (2007) [17], the overall mean uptake of Cr in shoot was almost four times and in root was about two times greater in rays, compared to fenugreek. These findings, according to the same authors, indicated that family *Cruciferae* (raya) was most tolerant to Cr toxicity, followed by *Chenopodiaceae* (spinach) and *Leguminosae* (fenugreek). Peng et al. (2012) [78] found that cultivar ST-9 of *Eucalyptus urophylla X E.grandis* was shown to accumulate more Pb than others of the same species, like ST-2, or ST-29.

8. Different strategies adopted in order to enhance biomass production under heavy metal toxicity conditions

Under elevated CO_2 conditions the photosynthetic rate is enhanced, thus biomass production is positively influenced. According to Wang et al. (2012) [28], the increase in total Cd uptake by poplar (*Populus* sp.) and willow (*Salix* sp.) genotypes due to increased biomass production under elevated CO_2 conditions suggests an alternative way of improving the efficiency of phytoremediation in heavy metal contaminated soils.

The use of fertilizers is another useful practice that should be adopted by the researchers in order to enhance biomass production under extreme heavy metal toxicity conditions. Some Brassica species, which are suitable to be used as phytoremediators, may suffer from Fe or Mn deficiency symptoms under Cu, or Zn toxicity conditions. In that case, leaf Fe and Mn fertilizations should be done in order to increase their biomass production [29], thus their ability to absorb and accumulate great amounts of heavy metals in contaminated soils, i.e. the efficiency of phytoremediation. According to Li et al. (2012) [25], in order to achieve large biomass crops, heavy fertilization has been practiced by farmers. Application of fertilizers not only provides plant nutrients, but may also change the speciation and mobility of heavy metals, thus enhances their uptake. According to Li et al. (2012) [25], NPK fertilization of *Amaranthus hypochondriacus*, a fast growing species grown under Cd toxicity conditions, greatly increased dry biomass by a factor of 2.7-3.8, resulting in a large increment of Cd accumulation. High biomass plants may be beneficed and overcome limitations concerning metal phytoextraction from the application of chemical amendments, including chelators, soil acidifiers, organic acids, ammonium e.t.c. [21]. Mihucz et al. (2012) [79] found that Poplar trees, grown hydroponically under Cd, Ni and Pb stress, increased their heavy metal accumulation by factor 1.6-3.3 when Fe (III) citrate was used.

Mycorrhizal associations may be another factor increasing resistance to heavy metal toxicity, thus reducing the depression of biomass due to toxic conditions. Castillo et al. (2011) [80] found that when *Tagetes erecta* L. colonized by *Glomus intraradices* displayed a higher resistance to Cu toxicity. According to the same authors, *Glomus intraradices* possibly accumulated excess Cu in its vesicles, thereby enhanced Cu tolerance of *Tagetes erecta* L. [80].

Finally, other factors, such as the influence of *Bacillus* sp. on plant growth, in contaminated heavy metal soils, indicate that biomass may be stimulated under so adverse conditions. According to Brunetti et al. (2012) [81], the effect of the amendment with compost and *Bacillus licheniformis* on the growth of three species of *Brassicaceae* family was positive, since it significantly increased their dry matter. Furthermore, the strain of *Bacillus* SLS18 was found to increase the biomass of the species sweet sorghum (*Sorghum bicolor* L.), *Phytolacca acinosa Roxb*., and *Solanum nigrum* L. when grown under Mn and Cd toxicity conditions [82].

9. Conclusion and perspectives

Biomass production is significantly influenced by many environmental, agronomic and other factors. The most important of them are air and soil temperature, soil humidity, photoperiod, light intensity, genotype, and soil nutrient availability. Soil fertility, i.e. the availability of nutrients in the optimum concentration range, greatly influences biomass production. If nutrient concentrations are out of the optimum limits, i.e. in the cases when nutrient deficiency or toxicity occurs, biomass production is depressed. Under nutrient deficient conditions, the farmers use chemical fertilizers in order to enhance yields and fruit production. However, since the prices of fertilizers have been significantly increased during the last two decades, a very good agronomic practice is the utilization of nutrient use efficient genotypes, i.e. the utilization of genotypes which are able to produce high yields under nutrient limited conditions. Although great scientific progress has been taken place during last years concerning nutrient use efficient genotypes, more research is still needed in order to clarify the physiological, genetic, and other mechanisms involved in each plant species.

On the other hand, in heavy metal contaminated soils, many plant species could be used (either as hyperaccumulators, or as fast growing-high biomass crops) in order to accumulate metals, thus to clean-up soils (phytoremediation). Particularly, the use of fast growing-high biomass species, such as Poplar, having also the ability to accumulate high amounts of heavy metals in their tissues, is highly recommended, as the efficiency of phytoremediation reaches its maximum. Particularly, since a given species typically remediates a very limited number of pollutants (i.e. in the cases when soil pollution caused by different heavy metals, or organic pollutants), it is absolutely necessary to investigate the choice of the best species for phytoremediation for each heavy metal. In addition to that, more research is needed in order to find out more strategies (apart from fertilization, the use of different *Bacillus* sp. strains, CO_2 enrichment under controlled atmospheric conditions e.t.c.) to enhance biomass production under heavy metal toxicity conditions, thus to ameliorate the phytoremediation efficiency.

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