UNIVERSITÀ DEGLI STUDI DI CATANIA

DIPARTIMENTO DI SCIENZE DELLE PRODUZIONI AGRARIE E ALIMENTARI

Dottorato di Ricerca Internazionale in Produttività delle Piante Coltivate in Ambiente Mediterraneo - XXVI Ciclo

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Adaptability, Biomass Yield, and Phytoremediation of *Arundo donax* L. on marginal lands: salt, dry and lead-contaminated soils

Tesi di Dottorato

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Triennio 2010 - 2013

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INTRODUCTION

Marginal lands have been identified as potentially suitable for production of perennial grasses and grass polycultures for use in bioenergy production.

It is thought that many environmental and societal benefits could result from conversion of marginal lands currently in row-crop to perennial biomass cropping systems.

In addition to biomass yields, it is thought marginal lands could provide environmental benefits such as wildlife habitat, flood protection, and groundwater infiltration when appropriately managed. However, conversion from row crop agriculture to dedicated biomass crop systems, even low intensity systems, may involve tillage during the establishment year, nutrient application annually and periodic use of pesticides. Therefore, careful consideration must be given to the site-specific nature of marginal lands and various management options in order to understand potential benefits and risks associated with biomass production.

Use of marginal lands for biomass production has become a contentious issue. Although marginal lands are suggested as ideal locations for growing nonfood crops for bioenergy production, the necessary biomass supply for meeting national renewable energy goals will likely require perennial plants to be grown in agronomic systems rather than as systems that mimic managed conservation areas for wildlife or other goals. It is not clear whether land use change within marginal lands, particularly intensification of biomass production through use of tillage, synthetic fertilizers and pesticide inputs will result in overall gains in benefits or overall reduction in environmental and ecological benefits. For example, it is generally acknowledged that perennial crops are a potential a benefit for their carbon sequestration capabilities compared to annual crops. However, carbon emissions rather than sequestration can result if previously unplowed lands are tilled for agronomic production of perennial energy crops. Additionally, tillage and use of heavy equipment of marginal lands risks increases in soil erosion and compaction, respectively. These impacts to marginal soils could reduce their already limited productivity over time. Additionally, use of nitrogen fertilizer on marginal lands can lead to nitrogen emissions that contribute to global climate change, and runoff into nearby waterways leading to water quality reduction within entire watersheds (www.fao.org).

The increasing demand for biomass for production of heat, power, biofuels and bio-based materials is generating land-use conflicts which are discussed in the food versus biofuel controversy (Baffes and Haniotis 2010) and the dabate about indirect land-use change effects (Wiegmann et al., 2008).

These conflicts may be solved through the integration of food and biomass production systems and/or spatial segregation of food/feed and biomass producing areas. In this context, marginal lands are suggested as having high potential for growing energy crops (Dauber et al., 2012). In fact, growing energy crops on "marginal land" is seen as a way of ensuring that biomass production involves an acceptable and sustainable use of land (Reijnders, 2009; International Energy Agency, 2010). The production of biomass on agricultural land has raised a number of interrelated controversies. Competition for land between biofuels and food crops is seen as one of the causes of food prices pikes that occurred in 2007 and 2008, leading many to conclude that biofuels production was unethical: the so called "food versus fuel" controversy (Mc Michael, 2010; Mol, 2010). There is the issue of the direct and indirect destruction of natural lands and land with high carbon stocks resulting in the release of carbon emissions (Council on Bioethics, 2011; Gamborg et al., 2012). The use of marginal land is cited as a way of overcoming land use controversies because, as the UK government states in the 2009 Renewable Energy Strategy: "Use of this "marginal", land will reduce the risk of competition with existing food crop production, and help ensure that any associated land use change does not have a significant impact on the anticipated greenhouse gas savings or pose any other significant detrimental environmental impact" (UK Government, 2009).

Water deficit could affect crop production in areas, such as semi-arid Mediterranean environment, where for a long period the low rainfalls in presence of high temperatures determine evapotraspirative stress ascribing these areas to the marginal land category. Indeed in these areas the competition between food and energy crops for the use of irrigation water is very strong. In this environment the selection of crops tolerant to drought stress, such as *Arundo donax*, may allow the cultivation of energy crops in dry Mediterranean area without the use of irrigation water.

The production of biofuels from wastes and residues is seen as another way of dealing with these

issues, as well as the production of both animal feed and biofuels from food crops (Ozdemir et al., 2009; Drax Group plc, 2011).

The pollution of soils due to accumulation of heavy metals is a global problem that may involve the loss of agricultural areas: contaminated land is no longer suited to farming, and may be especially harmful to the entire ecosystem. Plants tolerant to heavy metals, can be used to enhance sites unsuitable for biomass production, restoring the ecosystem services and providing valuable feedstocks to biorefineries, in a phytoremediation process.

The idea of putting "marginal land" in areas where farming is currently unprofitable to a more productive use while meeting energy goals is an appealing one. Energy could be locally grown, produced with few inputs, not compete with food production and give farmers an additional income (Schubert et al., 2008). Some controversy surrounds the idea of using marginal land however.

1. PERENNIAL GRASSES

Perennial grasses have been widely used as fodder crops for centuries, often contributing significantly to energy supply on farms through the use of draft animals. In the 21st century, perennial grasses may be set for a comeback through a number of different energy conversion pathways. There has been increasing interest in the use of perennial grasses as energy crops in the US and Europe since the mid-1980s. The characteristics which make perennial grasses attractive for biomass production are their high yield potential, the high contents of lignin and cellulose of their biomass and their generally anticipated positive environmental impact.

1.1.Environmental aspects

The substitution of fossil fuels or of raw materials based on fossil fuels by biomass is an important contribution to reduce anthropogenic CO_2 emissions. Compared to other biomass sources, like woody crops and other C_3 crops, C_4 grasses may be able to provide more than twice the annual biomass yield in warm and temperate regions because of their more efficient photosynthetic pathway (Clifton-Brown and Jones, 1996). The greater amount of carbon sequestered is assimilated by the plant to the growth of the various organs, and then from the atmosphere permanently. It was calculated that the effect of "carbo-retention" (ability to assimilate CO_2), for the duration of the life cycle of a perennial crop is greater than 20-30 times than that of an annual crop (McLaughlin and Walsh, 1998).

Unlike annual crops, the need for soil tillage in perennial grasses is limited to the year in which the crops are established. Nevertheless, the multi-year crops require less use of fertilizers, pesticides and herbicides, and in relation to their abilities can play a positive role in cleaning up the water, thanks to the reduction of pollutant loads from cultivated land and purification through natural processes based on the use of plants (phytoremediation).

The ecological advantages of the long periods without tilling are reduced risk of soil erosion and a likely increase in soil carbon content (Ma et al., 1999). The action of anti-erosive crops from deferred energy is manifested in a dual role, in fact, the aerial part offers a good protection of the ground as vegetation throughout the year, while the

underground through the root systems developed and active, retains the earthy masses during periods more rainy year.

Furthermore, due to the recycling of nutrients by their rhizome systems, perennial grasses have a low demand for nutrient inputs (Christian et al., 1997).

Herbaceous perennial crops were greater amount of organic carbon in the soil through the formation of extensive root systems, stores the plant in the first surface layers of the soil (crop residues, root exudates), providing benefits on the fertility of the soil, improving its structure, porosity, the ability to retain and preserve the nutrients.

Perennials are also less impactful regarding soil quality, erodibility and biological and landscape diversity because they need lower land disturbance, build up more biomass and have higher permanence periods. Impact reduction strategies are limited to crop management options, which can influence emissions, nutrient status and mineral ore depletion. (Fernando et al., 2012)

Perennials exhibit lower erodibility potential and runoff, than annual crops, owing to the greater interception of rainfall, by the deep and dense root systems, and more surface cover for a longer time period (Fernando et al., 2011; Boléo, 2010). Water scarcity is especially problematic in semi-arid and arid areas as in some parts of the European Mediterranean region, where water availability is low and varies from year to year; so, according to EEA (2006), perennial plantations can be designed to minimize negative impacts on water use. Main reasons for this are: the higher lignin and cellulose contents of perennials, which allows the plants to stand upright at low water contents (Lewandowski et al., 2003) and the high water use efficiency due to their deep and well-developed root system (Zegada-Lizarazu et al., 2010; Boléo, 2010).

The longer permanence of perennials in the soil favours also the minimization of surface run-off (Fernando et al., 2010, 2011).

Some studies indicate that perennial energy crops (e.g. reed canary grass, giant reed, cardoon, miscanthus, switchgrass) generally reduce soil compaction due to their expanded deep roots and less need of soil disturbance (EEA, 2006). It has been reported that perennial crops accumulate higher SOM than annuals (Fernando et al., 2010). Herbaceous perennials provide higher organic matter accumulation and structural enhancement related to permanence, high inputs of residues and vigorous root development (Fernando et al., 2010; Boléo, 2010) A less intensive soil amendment and

the reduced tillage or no-tillage systems (by comparison with annuals) also contributes to minimize the impact (Fernando et al., 2010; Bolé, 2010; Picco, 2010).

Perennial energy crops require lower fertilizers inputs than annuals, therefore, with positive economic and environmental feedback (Zegada-Lizarazu et al., 2010) several characteristics can explain this behavior: some of these perennial herbaceous crops may use organic nitrogen from nitrogen fixing bacteria, free or associated to root systems (e.g. giant reed and switchgrass); nutrients are recycled by the rhizome system, being translocated from aerial to underground parts in the end of the growing season, and being demobilized in spring for regrowth; their extensive root system can easily immobilize nutrients thus increasing the nutrient use efficiency (Picco, 2010) Regarding soil pH, it has been verified that herbaceous perennials systems do not affect the native status of the soil (Fernando et al., 2010). With these features the cultivation of these species provides benefits to soil fertility, such as improving its structure and porosity, increasing the field capacity, extending storage capacity and availability of nutrients. Perennials are less P and K demanding than annual crops, thus showing lower impact regarding mineral resources exploitation, although differences to most of the annual energy crops are not significant (Fernando et al., 2011).

Since they have few natural pests, they may also be produced with little or no pesticide use (Lewandowski et al., 2000). Perennials generally require lower pesticides inputs than annuals by taking advantage of the use of herbicides only during planting phase of the crop, while annual crops require year round applications (Zegada-Lizarazu et al., 2010). Moreover, some energy crops, e.g Miscanthus and giant reed, present no major illnesses requiring plant protection measures (Fernando et al., 2010).

Perennial grasses can therefore contribute to ecological values in agricultural production. They can also function as elements in landscape management and as habitat for different animals.

Perennial herbaceous can add to landscape diversity and habitat diversity, due to their different structural characteristics (Boléo, 2010; Picco, 2010). Perennial herbaceous, like switchgrass, giant reed, and cardoon, can also contribute to the ecological value of agricultural production, functioning as elements in a diversifying landscape management and as habitat for different animals (Lewandowski et al., 2003). But if native species (cardoon and reed canary grass) and colorful blossomed crops (cardoon)

contribute to the biodiversity value (Fernando et al., 2011), on the other hand, however, the establishment of a monoculture and aggressiveness of species (reed canary grass and giant reed) result in a higher impact (Fernando et al., 2011).

1.2.Biomass production

The characteristics which make the perennial species interesting for the production of biomass are their high yield potential, the high content of lignin, cellulose and hemicellulose, a greater amount of carbon sequestered in the soil, an improvement of the characteristics of the soil preventing the erosion and their environmental impact is generally positive.

Energy crops are grown for the express purpose of using their biomass for combustion or for traction, then to obtain energy. The biomass of perennial grasses has higher lignin and cellulose contents than the biomass of annual crops. There are many ecological benefits expected from the production and use of perennial grasses. The high content of lignin, cellulose and hemicellulose is a desirable aspect especially if their end use is in the form of solid biofuel or biochemically transformed as biofuel liquid; the high content of carbon in lignin (about 64%) means high heating value. The biomass obtained from lignocellulosic perennial species has a content of cellulose and hemicellulose comparable to that of annual crops. The presence of these polysaccharides makes these crops a suitable substrate for the production of second generation bioethanol (Scordia et al., 2010).

Perennial crops are also strongly lignified crops that can stand upright at low water contents; therefore, their biomass has lower water contents, this means that the biomass can dry ‘on the stem’ and a late harvest for improved biomass quality is possible (Hartmann et al., 2001).

Studies on perennial crops

Research on perennial species must start from the identification of those species that best meet the needs of bioenergy production, in terms of high returns in biomass, characteristics of biomass adequate in relation to the environment and the process of converting used.

Taking into consideration the specific chemical properties of a particular crop and the factors affecting its bioconversion, the choice of plant species for a particular area depends on factors such as the geographic and climatic conditions, the amount of rain or the water supply, the annual temperature profile, the condition of the soil and the presence of nutrients.

In both the US and in Europe, there are various candidate perennial grasses available which differ considerably in their potential productivity, chemical and physical properties of their biomass, environmental demands and crop management requirements.

Energy crops tolerant to freezing or flooding can cover those unused areas of central and northern Europe, while, in southern Europe halophytes energy crops could better adapt to the unused land , typically found in coastal areas characterized by water scarcity or breakish waters.

The research should look for those most suitable energy crops for a specific area, while the genetic improvement should provide well-adapted varieties and cultivars specific for unused land.

Lewandowski et al (2003) reported a study in which among 20 perennial grasses studied four species were selected as the most promising: Miscanthus (*Miscanthus* spp.), Giant reed (*Arundo donax* L.), Switchgrass (*Panicum virgatum* L.) and Reed canarygrass (*Phalaris arundinacea* L.) (Lewandowski and Heinz, 2003).

In Mediterranean area, there are various perennial grasses candidates for their potential productivity, due to their chemical and physical properties of the biomass produced. These perennial species are able to grow in a specific underutilized and degraded lands, representing the starting point for a future development program of biomass or to be used as genetic sources in the construction of plants in order to increase productivity and stress tolerance of bioenergy crops most widely used today.

Among the ligno-cellulosic crops, grasses deferred for semi-arid Mediterranean environments, *Arundo donax* L. has been identified as the most promising, due to the demand for reduced input, at high yields in dry biomass (Cosentino et al., 2006) and the high content of structural polysaccharides (Scordia et al., 2011; 2012; 2013). Moreover, thanks to its hardiness and the fact that it can grow in environments or on non-cultivated

land where other species are not able to grow, is believed to be suitable to be introduced on marginal land as required by European policy.

Moreover, in the same environment numerous wild species from *Cymbopogon hirtus*, *Phragmites australis, Saccharum spontaneum spp. Aegyptiacum, Ampelodesmos mauritanicus, Oryzopsis miliacea* and *Lygeum spartum* have been studied (Cosentino et al., 2011 and 2012; Copani et al., 2013).

2. GIANT REED (*Arundo donax* L.)

2.1. Importance

Giant reed (*Arundo donax* L.) has been shown by several authors as one of the most promising species for the production of energy and pulp for the environments of southern Europe (Lunnan, 1997; Anatoly et al., 2002; Lewandowski et al., 2003) thanks to some very interesting features: herbaceous perennial and easy adaptation to different environments (Christou, 2001; Cosentino et al., 2005), high biomass production and reduced input crop needs (Cosentino et al., 2005).

Certain natural, unimproved populations give dry matter biomass yields of up to 40 t ha^{-1}. This means that giant reed presents a good starting point in terms of yields, being one of the most productive among the biomass crops currently cultivated in Europe, and that it has a good chance, through selection and genetic improvement, of becoming the leading biomass crop in certain European regions.

Giant reed is also an environment-friendly plant: its robust root system and ground cover, and its

living stems during the winter, in fact, offer valuable protection against soil erosion on slopes and erosion-vulnerable soils in southern European countries. *Arundo donax*, is also a very aggressive plant, suppressing any other vegetation under its canopy. During the summer it is green and succulent, and has the ability to remain undamaged if an accidental fire, very frequent in semi arid regions in south Europe, sweeps across a giant reed plantation; it is an extremely pest (disease, insect, weed)- resistant crop, not requiring any of the chemical inputs (pesticides) that under certain conditions pollute the environment.

Giant reed is also considered to be one of the most cost-effective energy crops, because it is perennial and its annual inputs, after establishment, are very low. Only harvesting costs will occur and, depending on site and climate, irrigation and/or fertilization costs. Giant reed is also a lodging-resistant plant. All these attributes make giant reed a very attractive and promising candidate species for biomass production in European agriculture.

2.2. Taxonomy and botanical description

Giant reed or common reed (*Arundo donax* L.), also known as Provence reed or Indian grass, is a grass that belongs to the Arundo genus of the *Poaceae* (Gramineae family) *Arundinoideae* subfamily, tribe *Arundinae,* genus *Arundo.* To the genus *Arundo*, the GRIN Taxonomy (2011) ascribes four taxa: in addition to *Arundo donax* L., *Arundo donax* var. *versicolor* (Mill.) Stokes, a variant with variegated leaves, *Arundo formosana* Hack., from island of Taiwan, Japan and the Philippines and Arundo Plinii Turra, native of the Mediterranean area.

GRIN Taxonomy also lists synonyms and other designations in the past referred to the genus Arundo and now attributed to other genres.

Among them, *Arundo plinii* Turra is quite similar in appearance to *Arundo donax*; they have coarse, knotted roots, cauline, flat leaves and large, loose, plumose panicles; their spikelets are laterally compressed with few, usually bisexual, florets. The glumes are nearly equal, as long as the florets, with three to seven nerves. The lemmas have three to five nerves, with long, soft hairs on the proximal of the back. The rachilla is glabrous. However, between the two reed-like perennial species, there are certain morphological and growth differences; it differs from *Arundo donax* L. in that the plants are shorter, usually less than 2m, with stems that are always slender and with leaves that are rigid and that stick out stiffly from the stem at a right angle or less with tips sharply pointed. However, in *Arundo donax* L. the spikelets are at least 12mm long with three or four florets. The hairs of the lower lemma are almost the same length as the glumes and the lower lemma is two-pointed. In *Arundo plinii* Turra the spikelets are not more than 8 mm long with one or two florets. The hairs of the lower lemma are shorter than the glumes and the lower lemma is entire at the apex. In *Arundo donax* L. the chromosome number is 2n = 110, 112, while in *Arundo plinii* Turra the number is 2n = 72.

There are three off-type giant reeds that are used for ornamental purposes:

1 *Arundo donax 'microphylla'*, in which the leaves are even more glaucous and broader than the basic type, up to 9 cm wide.

2 *Arundo donax 'variegata'*, known in the USA as *Arundo donax 'versicolor'*, in which all plant parts are usually smaller and the plants very much more frost sensitive than the basic type, and the leaves are white striped, usually with broad white bands at the margins.

3 *Arundo donax 'variegata superba'*, a name used to distinguish a superior variegated form in which the leaves are much broader than in *Arundo donax 'variegata'* – the leaf blades are normally as much as 6.5 cm wide, about 30cm long and borne on stems that attain heights of up to 1 m, and the internodes are shorter and the leaves grow much closer on the stems.

Because giant reed is wild growing and entirely unknown as a cultivar or crop, the 'state-of-the-art'

production knowledge is missing. This means that neither selection of wild grown genotypes nor genetic improvement has been attempted so far, and the most appropriate cultural techniques for maximizing biomass yields are unknown.

Giant reed is probably the largest grass species in the cool temperate regions only exceeded in size by some of the bamboos.

It is a vigorously rhizomatous perennial species with a stout, knotty rootstock.

Rhizomes are long, woody, swollen in places, covered in the younger parts by yellowish scales, triangular, dry, representing the sheaths reduced the leaves. They embrace the rhizome and the base, the other face are covered with long white hair (Onofri, 1940), by coriaceous, scale-like sheaths; they are intricate, branched and robust and consists of buds ready to develop new stems (major buds), buds not fully developed, but destined to produce stems (secondary buds) buds intended to extend the rhizome (buds extension) (Onofri, 1940). The rhizome brings numerous roots that arise from both the upper face from the lower nodes of the rhizome.

Rhizomes are irregular in shape and can be several inches deep, so they can emerge a few centimeters from the ground. They also show a clear division into segments of a few centimeters and gems evident throughout the year, especially in the late winter and early spring, the rhizomes older, show woody texture, dark-brown coloring and no buds.

The stems, cylindrical and hollow inside are stout, up to 3.5 cm in diameter, measure on average from 3 to 4 meters (Cosentino et al., 2006), but can also reach 8-9 meters in height (Lewandowski et al., 2003).

The stems, are made up of nodes (30-40) and internodes of length and thickness decreasing from the bottom upwards. At the nodes, the epidermis of the barrels may have brownish -red hues.

The diameter at the base of the stem is 1-4 cm; stems are coated for much of their length from the leaf sheaths, which except that in the first leaves, they expand in a long lamina linear, narrow towards the apex. The sheath is crossed by several parallel measurements, and these correspond to the more robust vascular bundles cribro.

The ligule is formed by a 2 mm membrane; devoid of both fibrous and vascular bundles, formed by elongated cells in the longitudinal direction.

The leaves are regularly alternate on the stems and the leaf blades are up to 5cm wide and up to 3.3m long; they are almost smooth, green, and scabrous at the margin and also glabrous and covering the nodes. The largest leaves and most vigorous stems are produced on plants that are cut to ground level at the end of each season.

Leaves have a lanceolata shape up to 80 cm long and are green, alternately arranged , with entire margin and parallel veins The leaves are regularly alternate on the stems and the leaf blades are up to 5cm wide and up to 3.3m long. The leaf sheaths are smooth, glabrous, covering the nodes. The largest leaves and most vigorous stems are produced on plants that are cut to ground level at the end of each season.The young leaves have long hairs on the apical margins of the sheath; leaves emerged in summer are usually smaller and with a bright green color.

The stems terminates with a panicle inflorescence highly branched p to 60 cm long, erect or somewhat drooping. Its colour is initially reddish, later turning white, visible from the end of August, in a Mediterranean environment, composed of thousands of monoecious spikelets, often red streaked. In cool regions the stems will not achieve flowering size.

Individual peduncolate spikelets have two glumes, sub- equal, membranous, nearly equal to flowers, and glumette with lower bifid apex and briefly aristed between the lobes; each spikelet has mostly three flowers.

2.3. Origin and diffusion

Arundo donax has been cultivated throughout Asia, southern Europe, northern Africa, and the Middle East for thousands of years. Ancient Egyptians wrapped their dead in the leaves. The canes contain silica, perhaps the reason for their durability, and have been used to make fishing rods, walking sticks and paper.

Giant reed has several attractive characteristics that make it one of the most important biomass crops.

The widespread of common reed make its origin uncertain, there is no unanimous agreement on the location of the source area. Botanical and historical evidence are in favor of the hypothesis that the origin is to be traced back to a group of wild plants, native to the Mediterranean region (Zeven and Wet, 1982), both for *Arundo donax* and for other similar species, *Arundo plinii*, *Arundo hill* and *Arundo Mediterranean*.

An alternative hypothesis, instead proposed that the genus *Arundo* is native to eastern Asia (Polunin and Huxley, 1987): originated in the fresh waters of East Asia. *Arundo donax*, according to Lewandowski et al. (2003), it was widely in the rest of Asia, north Africa, the Middle East and southern Europe, where it was naturalized in the countries bordering the Mediterranean and where for many years has been cultivated (Perdue, 1958).

According to Perdue (1958) from this area, *Arundo donax*, was then widely distributed in subtropical and warm temperate regions of the world for multiple uses. It is believed that *Arundo donax*, has been introduced into North America from the Mediterranean in the early nineteenth century, starting from Southern California to control erosion. Since its introduction in North America, Arundo donax has escaped cultivation and has become one of the most common weed species, in areas along the rivers, displacing native species and altering ecological processes (Bell, 1994, 1997).

Giant reed, nowadays, is wildspread in Southern European regions (Greece, Italy, Spain, Southern France and Portugal) and other Mediterranean countries. It also grows wild in other parts of the world (China, southern USA etc.) (Xi, 2000). Although giant reed is a warm climate plant, certain genotypes are adapted to cooler climates and can be grown successfully as far north as the United Kingdom and Germany.

2.4. Genetic

Giant reed (*Arundo donax* L.*)* is characterized by low level of genetic variability and apparent lack of sexual reproduction, however, a recent study conducted by Ahmad et al, 2008, using molecular markers demonstrated the presence of a very small genetic variability present on American soil, which led the authors to hypothesize that all the clones in the country, are derived from a single clone.

More recent studies, carried out by Mariani et al. (2010) and confirmed by molecular results obtained by Bucci et al. (2012), show a greater genetic variability present in the Mediterranean Basin.

Despite *Arundo donax*, is able to produce flowers , non-viable seeds were observed in most of the areas where it was introduced (Perdue, 1958), including North America (Di Tomaso and Healey, 2003; Dudley 2000) and Italy where the species while producing inflorescences very large in some cases, does not produce pollen and then seeds.

The sterility implies that in nature, the common reed spreads by asexual reproduction, with a drastic reduction of genetic variability present in natural populations; the sterility of the seeds (Boose and Holt, 1999) determined by the failure of cell division mothers of megaspore (Lewandowski et al., 2003) makes it obligatory asexual reproduction that occurs in nature through the dispersion of traits of culm or rhizome, operated by water, wind, man, etc.

The sterility that distinguishes *Arundo donax* (Perdue, 1958) is a stretch advantageous for a crop energy because the photosynthetic products are used for the production of lignocellulosic biomass and not for the seeds, on the other hand , the sterility is a serious obstacle to the realization of breeding programs genetic that aim to increase the productivity and the quality of the biomass and it causes a drastic reduction of the variability of the species (Mariani et al., 2010)

Recently, Mariani et al. (2010) have suggested the monophyletic origin of this species. From Asia , center of origin, it would then spread to Europe, Africa , Asia , the Mediterranean, with no traces of hybridization with other species of the kind laid out in this area.

The results of a study by Mariani et al, (2010) aimed at assessing the phenotypic and genetic variability in a group of clones of Arundo donax, collected in 12 Italian regions, from Piemonte to Sicily, and one in Lanzarote (Spain, Canary island) have shown strong similarities between the clones investigated, for both biometric and productive characters, confirmed by DNA analysis by AFLP (Amplified Fragment Length Polymorphism) which showed a very low level of genetic diversity and no association between geographic distance and genetic distance of the clones tested. El Bassam (2011) indicates for *Arundo donax* L., a chromosome number 2n = 110.

2.5. Biology

Arundo donax is a perennial plant, the first buds emerge from the rhizome in the Mediterranean basin during the spring. If most of the canes emerge between spring and summer, the issue of new shoots from rhizomes, is observed throughout the season.

When the cane reaches about two feet tall (May), some nodes, particularly those nearest the apex vegetative shoots can develop that accrue until the autumn (branching).

The canes that emerge instead for last (June-July), tend to develop shoots from the nodes in the following spring.

The bloom, which occurs in late summer and early autumn, determines the end of the life cycle, even if the growth can only continue in the autumn. The rods remain green until winter, when the skin, drying, takes on a yellowish color; during the winter the canes lose their leaves and inflorescences.

In the warm Mediterranean regions, the aboveground giant reed parts remain viable during the winter months. If plants are not cut, in the following spring new shoots emerge at the upper part of the stem from buds located at stem nodes. After cutting a giant reed plantation, usually in autumn or winter, new growth starts early next spring. New shoots emerge from buds located on the rhizomes and they develop very rapidly.

Later in the season, in June–July, peak growth rates up to 7 cm per day have been observed. In fertile fields, new shoots continue to emerge until early August under a huge, well-developed canopy. These late shoots develop at a faster rate and attain the same height as the early ones, though the leaves are smaller and the stem diameter is much larger as much as twice as large.

The plant has a C_3 photosynthetic cycle but its photosynthetic capacity and production equal or superior to other plants with C_4 cycle (Beale and Long, 1995; Rossa et al., 1998; Christou, 2001).

By analyzing parameters such as gas exchange, fluorescence and stomatal conductance under conditions of natural growth, there has been observed an assimilation rate of CO_2 equal to 37 μmoli m^{-2} s^{-1}, about 50% more than in fiber sorghum, species to cycle C_4. Also, the leaves of Giant reed not also showed the phenomenon of saturation light.

Giant reed, by virtue of its high production capacity, the hardiness of the plant and its low input crop needs (Vecchiet and Jodice, 1996; Cosentino et al., 2005), presents a very positive energy balance (Mantineo et al., 2009).

In terms of production, the common reed in different locations over the national territory Italian has shown considerable potential (Di Candilo et al., 2005; Cosentino et al., 2005; Angelini et al., 2005). The species is distinguished by its ability to accumulation of C in the soil (McLaughlin and Walsh, 1998). Monti and Zatta (2009) found a quantity of dry matter in the underground part of Arundo donax, 6 times greater than the sorghum and two times more than in Miscanthus.

For all these productive characteristics, the common reed is considered one of the best species for the production of lignocellulosic biomass for energy use in Mediterranean countries (Lun, 1997; Foti and Cosentino, 2001; Shatalov and Pereira, 2002; Di Candilo et al., 2008).

2.6. Ecological requirements

Soil

In its wild state, giant reed is usually found along river banks and creeks and on generally moist soils, where it exhibits its best growth. Giant reed can be grown on almost any soil type from very light soils to very moist and compact soils. It s also found in relatively dry and infertile soils, at field borders, on field ridges or on roadsides, where it grows successfully.

Arundo donax prefers deep soils, medium texture, organic matter and well equipped with a good level of moisture, but the plant has capacity to adapt to all soil types, including marginal and salty (Peck, 1998) and it is able to vegetate for long periods in extreme conditions of soil water content, from the driest to the most humid (Lewandowski et al., 2003), when there is an underground water table it has the ability to absorb water from the table throughout is branched root system. Arundo tolerate pH values between 5 and 8.7 (Di Tomaso, 1998). Some recent studies have highlighted the ability of *Arundo donax* in the rehabilitation and environmental decontamination of sites highly polluted by organic substances and heavy metals (Kos et al., 2003; Mirza et al., 2010).

The adaptability of *Arundo donax* to marginal lands and lands not suited to agricultural production allows to realize the strategic goal to produce bioenergy without taking up floor space to food production.

Temperature

Arundo donax prefers high temperatures; according to Perdue (1958), the common reed has a high daily growth rate (up to 5 cm) for a long period of year (early April - late October). In Northern Italy, the plant grows from April, thanks to the wide temperature range of vegetation; the cold winter leads to desiccation of the vegetation but does not affect the viability of the rhizomes, after the vegetative growth, however, can be severely damaged by frost.

Propagation

In nature, giant reed populations spread outwards through their rhizomes' growth. Where farmers have planted giant reed on their field borders to serve as windbreaks, the plant creates problems by spreading into the fields, reducing the available cropland. In such cases, the unwanted rhizomes need to be eradicated every few years so that giant reed growth remains limited to the borders.

The system of cultivation is the most critical of the agro-energy chain, in relation to the transaction costs, and to the availability of propagation material; this is represented by rhizomes, micro- propagated seedlings, cuttings and stems.

Due to the infertility that characterizes the species outside of its origin area (Polunin and Huxley , 1987), the propagation occurs via agamic through portions of stem or rhizome.

The use of rhizomes ensures a high index of engraftment (Copani et al., 2003) but their use is expensive and difficult to implement on a large scale, while the use of stem cuttings may be easier and less expensive.

A key point in the semi-arid Mediterranean is represented by the water content in the soil at the time of transplantation, which must ensure a good water supply to the organs of propagation for the purpose of a regular settlement.

In autumn and spring, the water content of the soil is generally satisfactory, but low temperatures can affect the success of the transplant, so it is necessary to find the right combination of temperatures and soil water availability.

Rhizome propagation is implemented early in the spring before the new shoots start emerging in the mother plantation. Propagation by stem cuttings is implemented later in the season when the soil warms up and promotes mobilization of the node buds to develop new shoots.

Giant reed rhizomes are irregular in shape and variable in size and bud bearing. Rhizomes range from 1cm up to 10 cm in diameter. Their abundant reserves promote vigorous new growth. Several buds are mobilized and up to 10 stems per rhizome may emerge by the end of the first growing period. However, propagation by rhizomes is labour intensive and very expensive. After collection, rhizomes have to be cut into pieces and sorted according to their bud bearing capacity. It is much cheaper to use stem cuttings or whole stems. Stem cuttings consist of one node with sections of adjacent internodes. Stem cuttings could be either planted directly in the field or planted in plastic bags for transplanting into the field after they sprout. In the field, stem cuttings are covered to a depth of 4 to 8 cm, depending upon the soil temperature and soil moisture. Whole stems could be used instead of stem cuttings. Stems are laid down into soil furrows at a depth of 6 to 8 cm and covered by soil. However, propagation by stems or stem cuttings has not always proved successful in experiments in northern China (Xi, 2000).

Generally, the spread of common reed by stem cuttings can be carried out successfully using the ramifications that appear on stalks at the end of the first year of growth and in the second year.

The spring and summer seasons are considered the most suitable season for the reed planting (Decruyenaere and Holt, 2001).

2.7. Crop management

Planting

Giant reed has no special soil preparation requirements. A simple ploughing and/or disc harrowing is considered sufficient.

Natural populations are usually very dense: more than 50 stems m^{-2} is quite common. When establishing giant reed plantations with rhizomes, care should be taken that each piece has at least one bud in order to avoid gaps in the field.

Distances of 70 cm between rows and 50 cm within rows result in a relatively thick plantation with a stem number of up to 10 m^{-2} on average at the end of the transplanting growing period. In the subsequent two years stem density increases.

Giant reed plantations established with stem cuttings are much thinner at the end of the growing

period.

Plant survival of between 70 and 82% has been reported (Jodice et al, 1995a). The biomass yields at the end of the establishment year are much lower – less than one-third of the biomass obtained by rhizome planting. Because stem cuttings are much cheaper it is advisable to plant them closer within the rows.

Fertilization

Arundodonax in many researches conducted, did not show a significant response to mineral fertilization and in particular to that of nitrogen (Dalianis et al., 1994; Christou, 1999; Monti and Venturi, 1999; Cosentino et al., 2005). The response of the crop to nitrogen fertilization is weak, no differences were observed with doses of nitrogen fertilizer production from 60 to 120 kg ha^{-1}.

However, the needs of the crop are high only in the early years of the plant, during the period of growth and accumulation of nutrients in the rhizomes. Subsequently, the decay of rhizomes exhausted and the defoliation throughout winter, give a good return of nutrients to the soil.

Despite this, annual applications of nitrogen at up to 100 kg ha^{-1}, especially in nitrogen-poor soils, are recommended; applications should be implemented before the new sprouts start emerging early in spring. However, Dalianis et al. (1995a) reported that high nitrogen rates (240 kg/ha) have no significant effect on biomass yields compared to low rates (60 kg ha^{-1}). This leads to the conclusion that the application of reduced nitrogen rates is justified, at least during the initial growing periods.

Giant reed is a perennial crop that lasts for several decades and is also a high biomass-yielding crop, so before establishing a new plantation it is necessary to incorporate sufficient phosphorus into the soil by ploughing, more than 200 kg ha^{-1}, especially in phosphorus-deficient fields. Most fields in semi-arid Mediterranean regions are rich in potassium, so potassium fertilization is not required.

Generally, in soils of average fertility is recommended to make 150-200 kg ha^{-1} of P and K, in pre-implantation and 100-120 kg ha^{-1} of N in coverage, from the second year onwards, only the administration of 60-80 kg ha^{-1} of N is recommended.

These reduced nutrient requirements by crops are due to the peculiar characteristics of the plant, such as: a) high nitrogen use efficiency, b) translocation of nutrients in the

autumn, the leaves to the rhizomes (Bell, 1997) for use subsequently in spring the vegetative resumption; c) strong reduction of losses by leaching of nitrates, by hedging of soil for a long period of the year (Tolbert et al. , 1998 ; Pimental and Krummel, 1987).

Angelini et al. (2005), reported that fertilization positively affects all the phenotypic characteristics, and in particular on the characteristics most closely linked to the final yield, such as number of stems m^{-2}, the height and the average diameter of the plants, especially in the early years of planting.

Filed tests, conducted in Tuscany by Angelini et al., 2005 have shown that fertilization of 200-80-200 kg NPK ha^{-1}, in the first 6 years after planting, lead an increase in yield of 15% compared with a 70% increase in energy consumption.

Irrigation

Arundo donax has a remarkable ability to get to water in deep soil layers, and is enough durable to resist with severe drought conditions (Ranney and Mann, 1994).

Although giant reed can be grown without irrigation under semi-arid southern European conditions, its response to irrigation is significant. However, the effect of irrigation rates on fresh and dry matter biomass yields is insignificant. It was reported (Dalianis et al., 1995a) that fresh and dry matter biomass yields of giant reed, averaged over three years for autumn harvests, were respectively 59.8 t ha^{-1} and 32.6 t ha^{-1} for the high irrigation rate (700 mm $year^{-1}$) and 55.4 t ha^{-1} and 29.6 t ha^{-1} for the low irrigation rate (300 mm $year^{-1}$).

Under optimal soil water availability, the productive potential of the plant, it is remarkable, more than 100 t ha^{-1} of fresh matter. In numerous tests conducted in southern Europe, the production of dry matter stood on the 30 t ha^{-1} with peaks above 40 t ha^{-1} (Cosentino et al., 2005).

With reference to the water requirements of the crop, experimental tests conducted in Catania from 1997 to 2000 indicate that without irrigation, the dry biomass yield is around 10-15 t ha^{-1}, while with water volumes around 350 mm, could be reached a dry biomass yield of 30-35 t ha^{-1}.

The needs of the culture was estimated by Vecchiet et al, (1996) in 282 L kg^{-1} of dry matter.

According Christou et al., (2003), the giant reed is able to increase its water use efficiency in relation to environmental conditions: in optimal conditions of water availability, a production of dry matter equal to 2-3 g L^{-1} of water was estimated, while in water-stressed conditions, this index was higher (6-10 g L^{-1} of water).

Control of adversity- weeds

Arundo donax L., due to characteristic hardiness, doesn't show any particular susceptibility to pathogens and insects, so it does not need any treatment of the defense. Giant reed is one of the most pest-resistant plants. As reported by Jackson et al. (1964) and Perdue (1958), the culms and leaves contain several chemical compounds, including silicon and alkaloids that protect it from pests of all kinds. Occasionally during the early growth stages of the new sprouts, while they are still in a succulent condition, they may be attacked by *Sesamia* spp. and die (El Bassam, 2011). However, very soon new sprouts appear from the rhizome buds and replace the damaged ones.

Giant reed develops a huge canopy that suppresses any weed growth. Even during the establishment year there is no need for herbicide applications if rhizomes are used as planting material. However, if establishment is implemented by stem cuttings, pre-planting herbicide applications help the establishment and early growth of the giant reed plantation (El Bassam 2011).

The great vigor of the plants arising from rhizomes and their fast growth starting as early as March, allowing ground cover in a short time, competing strongly for water, light and nutrients with weeds. In some cases even the weeding is unnecessary, however, especially in the year of the transplant, a broad-spectrum herbicide, which is used in the pre-emergency, could improve the engraftment of the rhizomes and fitness of young shoots.

To prevent infestation of weeds, it is recommended an early planting of Giant reed so as to avoid any periods of drought, being poorly suited for the setting of the common reed, may slow the growth and facilitate the weeds. Late planting, may require some irrigation interventions and practices of weeding.

Installations of Giant reed from seedlings, because of less vigorous and less coverage capacity of the soil, requires a greater extent of irrigation and weeding; in this case,

good results were obtained with a volume of water equal to 250-260 mm for season (Bucci et al., 2012).

Harvesting

Giant reed can be harvested each year or every second year, depending on its use. Normally, the biomass in the case in which the product is intended for the thermo-chemical conversion (combustion) is harvested once per year, the harvest is made during the vegetative stasis of the plant (January-February) because in that time the product has a better energy content, thanks to the reduction of its moisture content and the effect of low temperatures, in this moment it also has a greater level of quality as a result of more complete translocation of assimilated to the rhizomes due to the natural loss of the leaves, the richest in ash (Monti et al., 2008). For pulp production giant reed is harvested each year and new growth starts in the spring.

In the case in which the biomass is used for the production of bioethanol is important that the product is not lignified, therefore it should better to harvest during summer or early autumn.

However, the harvest should be avoided when the soil is too wet, because the rhizomes are plagued by the compaction caused by harvesting.

In southern European regions the giant reed could be harvested either in the autumn or in the late winter. However, it should be noted that a significant reduction in biomass yield is observed between the autumn and late winter harvests. The dry matter yield reductions are the result of losses of the leaves and many of the tops, especially if hard winters are accompanied by strong winds. Dry matter losses of up to 30% were reported by Dalianis et al (1995a).

In semi-arid Mediterranean climates, the moisture content of the autumn-harvested plants ranges

between 36 and 49%, and weather conditions are suitable for natural drying in the field after cutting. These results indicate that not only is delaying harvesting time until after November useless, but there is a danger, depending on the prevailing weather, of significant biomass losses (Dalianis et al, 1995a).

However, the autumn harvest, especially in fertile fields and warm regions, may result in an early sprouting during the following spring. If a late winter frost occurs these early sprouts may die, but are quickly replaced with new ones that emerge from buds at the rhizomes.

A significant advantage of giant reed is its good storability compared to many other biomass crops. It can be stored outdoors without any shelter protection with minor losses. Storage losses occur mainly in the leaf fraction (blades and sheaths), which represents a small percentage, about 10 to 15% of the total biomass production. Stems can be stored with almost no losses.

2.8. Yields

There are only a few references to giant reed biomass yields in the world literature; in some studies conducted by Dalianis et al, 1995a; Jodice et al, 1995a, 1995b; Morgana and Sardo, 1995, giant reed's high biomass yield potential has been showed.

Despite the cultivation of Arundo donax is not yet widespread on a large scale, there are numerous tests conducted, especially in Italy. All the tests, reported dry matter yield very high: Venturi and Monti (2005) reported yields ranging averages of dry matter comprised between 15 and 35 t ha^{-1} and test conducted in Sicily by Cosentino et al. (2005) referred dry matter yields between 25 and 45 t ha^{-1}.

Angelini et al. (2009) reported for experimental tests conducted for 12 years in central Italy average yield of dry matter of 37.7 t ha^{-1}. According to the authors, the dry matter yield was highest in the second and third year of life of the system with values higher than 50 t ha^{-1}, and then the level off around 40-45 t ha^{-1} in the intermediate phase and decrease in last three years to 25-30 t ha^{-1}.

It should be underline that these high yields are obtained from unimproved wild populations with almost no crop management. This indicates the great biomass potential of this biomass plant for the future.

2.9. Destinations and uses

Giant reed has not so far been exploited on a commercial basis throughout the world.

The biomass produced by Giant reed (*Arundo donax* L.), as well as the production of paper, can be devoted to thermo-electric conversion, burning, and/or production of

second-generation bioethanol, through hydrolysis of cellulose into simple sugars and their fermentation.

Industrial use of this species has been started for the first time between 1937 and 1962 by the SNIA Viscose who patented a process for the production of viscose rayon and paper from pulp derived from Giant reed. This one, has many positive characteristics that approach to that which is to be considered the ideal plant for the production of ligno-cellulosic biomass; it is characterized by high daily growth rate, maintained for a long period of the year, from March to end of October; it is a perennial species, limiting the costs necessary for tillage and seeding, carbon accumulation in organs that will not be collected, increase organic matter in the soil; it has a well-developed root system, able to draw water at greater depths and good resistance to water stress too strict; *Arundo donax* is also poorly susceptible to pests, not needs any chemical treatment for the defense against pathogens and insects; it is also competitive towards weeds, no intervention weeding is need or only one limited to the first year of planting. Giant reed, due to the translocation of nutrients in autumns, from the leaves to the rhizomes, for later use in the spring vegetative growth, it shows a reduced need for fertilizers. It is adaptable to any type of soil including marginal lands unsuitable for food production.

For *Arundo donax* L., is recognized importance from the point of view of the environment by virtue of its role in reducing the erosion.

From the biomass of the culm , it is possible to extract pulp for the manufacture of paper and in the past was used for the production of rayon (Facchini , 1941). Giant reed stems, being tough and hollow, flexible and strong, are also used as a source of reeds for musical instruments such as the oboe, bassoon, clarinet and saxophone. Giant reed has been used to make flutes for over 5000 years.

It is also often used for the chanter and drone reeds of many different forms of bagpipes. Its resistant and stiffs stems are used as supporting poles for climbing vegetables and ornamental plants and also as support for climbing plants or for vines. Further uses are walking sticks and fishing poles. In certain windy areas it is used as a windbreak to protect other crops, while its stems are used for the construction of sun protection shelters or to make baskets.

Since *Arundo* species grow rapidly, their use has been suggested for biomass for energy and a source of cellulose for paper. Only a few references are available concerning the

possibility of exploiting giant reed for pulp production (Arnoux, 1974; Faix et al, 1989). The cellulose and hemicellulose content of its stems are about 45 per cent on a dry matter basis, while its lignin content is about 25 per cent. Because giant reed is a pithless plant (in contrast to miscanthus, for example) it is considered to be a very suitable non-wood plant for pulp production since no depithing is required. Giant reed produces an average of 25 tonnes of high-quality fibre per acre twice annually.

Recently, Giant reed has been considered as a new source of raw material for energy; initial results indicate that giant reed is a promising alternative to conventional non-wood fibre options and is a useful biofilter, C sequestration and biofuel crop. Heating values of 3600 kcal kg^{-1} were determined for *Arundo donax* (Dalianis et al, 1994). Based on these values and the dry matter yields obtained so far, the estimated energy potential is up to 11.8 t/ha/year.

It is also a kind energy as can be derived from biomass for a second generation fuels; in this context, one of its most significant uses will be to produce chips for the manufacture of high-grade biofuel pellets or dried chips. Giant reed is an ideal biofuel that produces methanol from gas diffusion as a by-product in manufacturing cellulose. The option to gasify this product is to produce a valuable energy product. It is possible to utilize new high-efficiency gasification systems to convert giant reed into a multitude of different energy sources, such as syngas, standard steam turbine electrical generation, ethanol and biodiesel.

Highly significant also is the importance of a crop with a growing cycle of over 20 to 25 years without annual replanting, and the ability to exclude many costly fertilizers and weed killers that are also an environmental concern, which will return agriculture to a more profitable basis than many crops.

Arundo donax contains molecules of potential biochemical interest, some studies (Khuzhaev and Aripova, 1998), showed the presence of alkaloids in the roots, in particular, have been identified and isolated five molecules: arundamine (Zhalolov et al., 2002), arundanine (Khuzhaev et al., 2003) , arundarine (Khuzhaev et al., 2004) arundacine (Khuzhaev et al., 2002), and arundavine

(Khuzhaev et al., 2004). Studies conducted in india, have also highlighted the presence in the rhizomes of Arundo of a large amount of lectins, proteins from proven anticancer properties (Kaur et al., 2005).

Bioethanol production

The Arundo thanks to the high yield per hectare is an excellent source of carbohydrates that lend themselves to the production of bioethanol.

In Mediterranean climates, the high yields of dry matter of *Arund*o, superior even to Miscanthus, may be assumed that the production of bioethanol from common reed shows efficiency ratios even higher than those found in other multi-year, with significant benefits in terms of economic and environmental issues.

Batch tests have shown *Arundo donax* production between 84.8 and 109.0 mg g^{-1} of ethanol per gram of dry matter in the leaves and between 44.2 and 47.8 mg g^{-1} of biofuel for stems (Anderson et al., 2008).

Studies carried out on *Arundo*, shows that the species has an intermediate behavior between herbaceous biomass and woody biomass but that as regards the production of ethanol, is characterized by a good fermentability.

Despite Arundo in various process parameters does not demonstrate exceptional performance efficiency, it has to be considered the high productivity of dry matter per hectare, much higher than that of any herbaceous crop.

A highly effective lignin degradation pre- treatment could favorably affect the efficiency of bioethanol production.

Scordia et al., 2011 report a final yield of bioethanol from *Arundo* of 57% after hydrolysis treatment with oxalic acid to 2% (w/w), carried out at high temperatures (170-190°C) for 15-40 minutes, followed by treatment with cellulase and by alcoholic fermentation to work of *Scheffersomyces (Pichia) stipitis* of the solid residue (2%). Alcoholic fermentations performed following the same treatment but with the use of *Saccharomyces carlsbergensis*, have instead found worse performance due to the inability of the latter to use xylose and arabinose that are free degraded hemicellulose.

The higher percentage of xylose detected in *Arundo* after pre- treatment can be explained only in part with the greater abundance of hemicellulose (31.2%) , compared to cereal straws (26-27%), but especially with a greater difficulty of cellulose (42.5%) to be degraded to glucose. Scordia et al. (2011) have confirmed the difficulties in obtaining a high yield because of the glucose.

Among the pre- treatments to be performed on biomass, Duff and Murray (1996) report the steam explosion (auto - hydrolysis) which consists in subjecting the biomass to high temperatures (160-260°C), at a pressure of 0.69 to 4.83 MPa, for a few seconds or a few minutes, before the material is exploded at atmospheric pressure. During this process, hemicellulose and lignin degradation undergoes a transformation due to high temperature, and during the explosive decompression, cellulose undergoes a mechanical destruction, which gives a high level of digestibility of biomass. Caparros et al. (2006), have shown that a treatment of steam explosion for *Arundo*, conducted at 150-195 °C up to 15 hours, with a liquid/solid ratio equal to 8 g/g, degrades more the fibrous fraction of hemicellulose.

Limits and perspectives

Though with many strengths, the cultivation of *Arundo* for energy, is difficulty to expand as it presents some not easy problems and with not immediate resolution, or the retrieval of nursery material and especially its cost. A major barriers to increased Giant reed cultivation is also the lack of a clear and stable legislation aimed at promoting the cultivation of the species.

3. MARGINAL LANDS

Productivity is not only based on the biophysical characteristics of land, but also depends on the socio-economic parameters of a specific environment.

The evaluation of land does not only refer to its productive potential, but also to the sustainability of its use, that is the maintenance or enhancement of its productivity over the long term, while at the same time conserving its potential as a resource base. Marginality can be the result of different combinations of constraints. The nature, composition and interaction of the factors which determine land marginality differ widely; any number of factors may lead to shifts of land from one category to another. These shifts may be upward, through applications of improved techniques, or downward as a result of land degradation or inappropriate development of lands formerly at low use levels. Hence, marginality is not a static concept. When dealing with issues of natural resources management, it is essential that these spatial and temporal dimensions be taken into account. Therefore, "marginality" has to be assessed in terms of specific types of land use. The severity of soil constraints may vary with the climatic conditions under which they occur.

Several authors define marginal lands as lands with inherent disadvantages or lands marginalized by natural land or artificial forces. These lands are generally under- used, difficult to cultivate, have low economic value, and varied developmental potential. There are many other names given to low-quality lands or lands with few or no competing uses such as abandoned, disturbed, underutilized, wasteland, limbo land, degraded, and idle. These mayor may not be interchangeable terms. The authors consider “marginal” to be the most inclusive term that encompasses all land categories mentioned. “Abandoned lands” are lands that were previously used for human- related activities (agriculture, forestry, mining, etc.) but are no longer in use due to economic, social, political, or environmental reasons. “Disturbed lands” suggest a human intervention, where the natural ecosystems have been altered or modified, as with mining or oil drilling; under- utilized lands may have the potential to be productive but for economical or physical (e.g. accessibility) reasons, they are not. “Waste-lands” are generally associated with barren lands, lack of vegetation, and often are uncultivated and desolate. “Limbo lands” imply lands with uncertain status; “degraded lands” are

defined as lands with reduced or lost biological or economic productivity and complexity; land degradation can be human-induced or result from natural processes and “idle lands” are lands currently not in use.

In accord to FAO definitions (www.fao.org), Marginal Agricultural Lands (MAL) were identified as those areas which are currently used for agriculture, grazing or agroforestry (1.8 billion ha). Such areas typically encompass mountains and tropical and sub-tropical lowlands or plateaux with low, unstable rainfall or higher rainfall areas in intensive use relative to use-capability under existing population densities, traditional technologies and institutional structures. In most cases, in absence of external inputs, they have reached or exceeded the threshold limits to maintenance or enhancement of agricultural performance. They are characterized by: poor soil fertility (nutrient deficiencies, acidity, salinity, poor moisture holding capacity, etc.), inaccessibility (poor communications, immobility with all its social and economic implications); fragility (low input absorptive capacity, high input-output ratios, limited capacity to withstand disturbance, vulnerable to irreversible damage); and heterogeneity (physically and culturally diverse with site-specific constraints and opportunities which restrict applicability of general technological or institutional measures to remove constraints or exploit opportunities). Aside from the above inherent characteristics, marginal low-productivity lands may also result from degradation of non-marginal lands or inappropriate development of lands formerly at low or zero use levels. All areas are at risk of further degradation with high expectation of negative externalities.

Land can be "marginal" depending on: its use (what is marginal agricultural land may be highly productive forest land); its natural biophysical characteristics (which can be altered by investment); its location relative to infrastructure such as roads, railroads, harbors, and cities, the institutional and policy context which influences access of inhabitants to land, water, credit, markets, outside inputs, population pressure, technology development; taking advantage of niche opportunities. Because of the wide variety of ways in which "marginal" lands can be defined, the term is used to mean quite different things, depending on the context.

Define a land "marginal" is, however, very difficult and complicated, because there is a meaning unique to define it. The term "marginal" can refer both to its agronomic characteristics (inadequacy of physical, chemical and biological properties of the soil in

order to obtain a production qualitatively and quantitatively suitable for the market) to both its economic characteristics, refers in terms of profitability.
A recent study carried out by Kang et al. (2013) on the assessment of marginal land has been examined a new approach , proposing a hierarchical framework on the basis of accessibility, productivity , environment, ecosystem and economy.
Consideration exact justified by the fact that the economic marginality of an area depends very much on the management of the land and the technology used.

3.1.Cause of marginality

Desertification

Desertification is the extreme phase of the degradation process on fertility of the soil; in fact, it can adversely affect the balance of ecosystems causing changes often can not be modified.
When it comes to desertification refers to those social and economic processes, unsustainable for poor land management, which degrade the vital potential of the soil and reduce the natural resources (Di Fabbio and Fumanti, 2008).
Human activities (agriculture, animal husbandry, urbanization, tourism, landfills and mining activities, water consumption) are the causes of increased incidence of the phenomenon, although other causes , this time of natural origin (climate change, drought, erosivity of rain), contribute to accentuating desertification. However some aspects that predispose a certain area to be more susceptible to this phenomenon, such as lithology, morphology, vegetation have been considered.

Salinization

Each floor has a natural salt content that are essential for plant growth. When natural or anthropogenic factors lead to an accumulation of salts in the soil up to a level that would compromise the vegetative activity and production of crops and also to determine unwanted effects on soils are defined as "saline" (Dazzi, 2006).
The salinization of soils, due to irrigation, is a problem that is bound to get worse not only for pushing competition in water use for the overexploitation of aquifers and the

use of water in agriculture always less adequate (water saline, civil and industrial waste water), but also due to the planned climate change, increasing aridity, would lead to a lower leaching resulting in an increase in the levels of salts in the soil (Di Fabbio and Fumanti, 2008). Particularly vulnerable are the climate areas tend to be hot and dry, such as those of southern Europe.

The problems to be addressed for the improvement of saline soils are not easily solved and are complicated by the water-soil relationships that are established depending on the nature of the soil, its particle size and structure from erosion, from cultivation techniques, irrigation methods by, the quality of the water used (Dazzi, 2006).

The saline soils now account for about 10% of the cultivable areas and most are located in arid and semi-arid . In Europe, the Mediterranean countries (Italy , Spain, Portugal and Greece) those which have the highest presence of saline soils.

In Italy the areas most affected by the problem turn out to be the lower Po valley, the Tyrrhenian and Adriatic coastal areas and islands. The widespread increase in Sicily is considerable soils affected by salinization cover about 10 % of the region (about 250,000 ha) (Di Fabbio and Fumanti, 2008).

Contamination

For widespread soil contamination is the set of phenomena contribution of pollutants to soils that do not always identifiable origin. Detailed soil contamination phenomena occur as a result of spills, leaks and improper waste management due to the presence of anthropogenic activities.

In most cases these alterations do not affect only the soil, since the contaminants are transported to other environmental matrices. The impacts due to the phenomenon of contamination, be it of a widespread basis or soil, then cover the passage of the pollutant to other environmental media, such as groundwater and air, and this also leads to a series of significant social, economic and health. Intensive agriculture can be considered a source of diffuse pollution because it involves the generous use of pesticides, chemical fertilizers and manure, which can leach into the groundwater, and transported over great distances.

Remediation techniques should focus on the adoption of tools compatible with the environment, bioremediation technologies such as constructed wetlands (phytoremediation).

4. WATER AND SALINITY STRESS

An important branch of environmental physiology is concerned with how plants and animals respond to environmental conditions that deviate significantly from those that are optimal for organisms in general. As a division of physiological ecology, the stress physiology can contribute to understanding of what limits plant distribution and how adverse environmental conditions limit agricultural yields.

The stress concept is often expressed in an improper way, and because of the terminology is sometimes confusing, it is good to give some definitions.

Stress is usually defined as an external factor that exerts a disadvantageous on the plant. In many cases, stress is considered in relation to the survival of the plants, the crop yield, growth, and the accumulation of biomass or assimilation processes (CO_2 and nutrients), in general related to growth.

Since stress is defined almost exclusively in terms of the response of plants, the concept of stress is closely associated with that of stress tolerance, which is the plant's way to adapt to the unfavorable environment. An environment that is stressful for a plant, it may not be stressful for another one.

If the tolerance to stress increases as a result of previous exposure to stress, the plant is called acclimatized, this should be distinguished from adaptation that is a certain level of resistance to genetically acquired during this process, through the generations.

Plants are constantly exposed to stress both in natural agricultural conditions, some environmental factors such as air temperature, can become stressful for plants within a few minutes, while others, like the water of the soil, can take days or weeks or, in the case of nutrients even months.

Stress plays also a key role in the determination of the limit of the distribution of the plant species in function of the soil and the climate; under natural and agricultural conditions, plants are constantly exposed to adverse environments that cause a certain degree of stress.

The main stress factors that limit the growth of the plants are water deficit, freezing, cooling, heat stress and heat shock, salinity, oxygen depletion and pollution of air and soil. As consequence of these factors, crop yields and biomass production at the end of the season, express only a fraction of the genetic potential of the plant.

The ability of the plant to combat the hostile environments is known as stress resistance. Adaptations of plants that confer resistance to stress, are genetically determined (Taiz and Zeiger, 1998).

4.1.Water stress

Arid and semiarid regions are defined as areas where perspiration reaches 50 % or less of perspiration that would occur with unlimited availability of water. The water deficit is however not limited to these regions areas classified as arid or semi-arid, but in any area in which the evaporative demand greatly exceeds rainfall during the growing season (Day, 1981), since even in the most humid climates, where the uneven distribution of rainfall, leading to periods when water availability limits the growth (Boyer, 1982).

Water is involved in all physiological process in plants; at the cellular level is the major medium for transporting metabolites and nutrients.

The water status of a plant is described by measuring water potential and relative water content.

If the water balance in plants is not enough, due to an insufficiency of water, the plants experience water deficit or suffer from water stress In literature water stress is also described as “dehydration” or “drought”. Water deficit is not only caused by lack of water but also by environmental stresses like low temperature or salinity.

The availability of water determines the distribution of plants and their productivity. Water stress leads directly to changes in the physical environment of the crops, and these changes may subsequently affect crop physiology. As the soil dries, the soil water potential decreases, so leading to a decrease in plant water potential and so does the soil hydraulic conductivity. Thus it is more difficult for plants to extract water (Gardner, 1960) and as a consequence, the plant water potential tends to decrease. This decrease may directly affect the physical aspect of some physiological processes. The plant water potential does not depend only on the soil water potential but also on plant structure and transpiration rate. The availability of some nutrients decrease as the soil dries, although the importance of this decrease depends on the nutrients amount in the soil; as the soil is dried, the soil water potential decreases.

Plant water potential is a measure of water stress, but its importance may be complicated by changes in the component potentials and by changes in crop structure. Osmotic adjustment decreases in the osmotic potential under stress, which lead to the maintenance of turgor at lower water potential, is a mechanism whereby plants to adapt to water stress conditions (Turner, Begg and Tonnet, 1978). For example, turgor

pressure in the cells will decrease, and turgor forces play a role in the process of leaf expansion (Hsiao and Acevedo, 1974). Loss of turgor can cause leaves to wilt, thus decreasing their light interception and reducing photosynthesis rates. Under these conditions stomatal closure has a great effect upon photosynthesis rates. Direct and indirect effects are important and have to be considered in decreasing of water potential in plants.

Decreased leaf expansion and stomatal closure both restrict photosynthesis and slowing dry matter accumulation; this reduction in assimilate supply may affect many physiological processes including the differentiation and expansion of a new tissue.

Shortage of assimilates at the roots may not only decrease root growth but, as a consequence of this decreased growth, the roots may be less able to utilize all the soil's reserves of water.

When water supply to a crop is stopped, the most immediate effect is that the soil in the rooting zone begins to dry, and eventually transpiration by the crop will decrease as a consequence of this drying.

Water stress also leads to differences in the aerial environment of crops. Because of the difference in the leaf area in stressed and unstressed crops, the amount of light intercepted differ markedly between crops. As a consequence of decreased transpiration, temperature in a stress crops is generally higher and humidity lower than in unstressed crops. These differences lead to an increased water vapour pressure deficit in stressed crops, which may have a direct influence on stomatal resistance (Rawson, Begg and Woodward, 1977).

Leaf extension is particularly sensitive to water stress; leaf area can be influenced by changes in the time of leaf appearance, in the rate and duration of leaf expansion, and in leaf senescence.

According to Hsiao (1973) cellular growth appears to be the most sensitive response to water stress. Decreasing the external water potential (Ψ) by only 0.1 MPa or sometimes less, results in a perceptible decrease in cellular growth and thus root and shoot growth (Neumann, 1995). Hsiao suggested that this sensitivity is responsible for the common observation that many plants grow mainly at night when water stress is lowest.

The inhibition of cell expansion is usually followed closely by a reduction in cell-wall synthesis. Protein synthesis may be almost equally sensitive to water stress. Many

studies indicate that activities of certain enzymes, especially nitrate reductase, decrease quite quickly as water stress increases. A few enzymes, such as α-amylase and ribonuclease, show increases activities. Nitrogen fixation and reduction also decrease with water stress.

At Ψ= -0.3 to -0.8 MPa, cytokinins decrease in leaves of some species; at slightly more negative water potentials, the amino acid prolin begins to increase sharply. Depending on species, other amino acids and amides, especially betaine, also accumulate when the stress is prolonged.

At higher levels of stress (Ψ = -1.0 to -2.0 MPa), respiration, translocation of assimilates, and CO_2 assimilation drop to levels near zero. Hydrolytic-enzyme activity increases considerably, and ion transport can be slowed. In many species, respiration often increases until water stresses of -5.0 MPa are reached. Plants usually recover if watered when stresses are -1.0 to -2.0 MPa; because growth is especially sensitive to water stress, yields can be considerable decreased whit even moderate drought. Cells are smaller and leaves develop less during water stress, resulting in reduced area for photosynthesis.

Plants water response

If it would be classify plants according to their response to available water, they can be distinguished in hydrophytes, mesophytes and xerophytes. All the name derived from Greek: -phyte , “plant”, hydro, “water”, meso, “middle”, xero, “dry”; The first group (hydrophytes) is referred to plants that grow where water is always available, the second one (mesophytes) grow where water availability is intermediate and xerophytes grow where water is scares most of the time.

Since solutes strongly influence water potential and can have specific toxicities, ecologists futher classify plants that are sensitive to relatively high salt concentration as glycophytes (from Greek: glycol, “sweet”) and those that are able to grow in the presence of high salt concentration as halophytes (from halo, “salt”).

All the plants of the desert are called xerophytes, but different species survive the drought in various ways. Homer LeRoy Shantz (1927) used four terms in classifying xerophytes: escape, resist, avoid and endure. Plants such as the palms that grow at an oasis, where their roots reach the water table, or other plants such as alfalfa (*Medicago*

sativa) that have roots that extend as much as 7 to 10 m down to the water table, never experience extremely negative water potential, and because of this they are "water spenders" and they certainly avoid the drought.

The so called "desert ephemerals" are annual plants that escape the drought by existing only as a dormant seeds during the dry season. When enough rain falls to wet soil to a considerable depth, these seeds often germinate.

Succulent species such as the cacti and various crassulacean-acid metabolism (CAM) plants, are water collectors; they resist the drought by storing water in their succulent tissues. Enough water is stored, and for their tick cuticle and stomatal closure during daytime, its rate of loss is so extremely low, that they can exist for long periods without added moisture.

Because their protoplasm is not subjected to extremely negative water potentials, succulents are drought avoiders and not truly drought-tolerant.

Some species that are subjected to periodic drought can switch from CAM, which conserves water because stomates are closed during the day, to C_3 photosynthetic pathway when water becomes available.

Many no-succulent desert plants have other adaptations that reduce water loss; they are water savers. For example, its common for desert shrubs and other plants to have small leaf blades. This condition increases heat transfer by convection, lowering leaf temperature and thus reducing transpiration. Other adaptations that reduce transpiration include sunken stomates, shedding of leaves during dry period and heavy pubescence on leaf surfaces.

Although these modifications may indeed reduce the loss of water, they never completely prevent it and they are by themselves insufficient protection against extreme drought.

As water evaporates from plants, salts in the protoplasm could reach levels that could damage crucial enzymes. An important adaptation found in many organism subjected to water and other stresses is the accumulation of certain organic compounds such as sucrose, amino acids (especially proline) and several others that lower the osmotic potential and thus the water potential in cells without limiting enzyme function. As water stress increases, such compounds appear in the cells of many xerophytes; the

resulting drop in osmotic potential is called osmotic adjustment or osmoregulation (Morgan, 1984).

Among xerophytes there are plants that simply endure the drought: they lose large quantitaties of water, so their protoplasm is subjected to extremely negative water potentials, without achieve the death; these plants are called euxerophytes (true xerophytes) and exhibit dehydration tolerance or hardiness. In this group of plants, the last weapon against drought is the ability to endure it to be drought-tolerant.

Plants have developed many mechanisms to adapt their growth to limited water conditions (Black and Pritchard, 2002). The plant's molecular response to water scarcity is dependent on the severity and duration of the water deficit but also on the developmental stage and morphological and anatomical parameters of the plants. Many papers on plant responses to drought have been published. The mechanisms of resistance to drought change in relation of climate and soil conditions. The leaves expansion inhibition is one of the first responses to water stress; it occurs when the decrease of turgor, as a result of water deficit reduces or eliminates the driving force for the cell and leaves expansion. Additional mechanisms implemented by plants in response to water stress include leaf abscission, the extension of the root in deeper and wet soils and the stomata closure. Plants possess acclimation mechanisms that are activated in response to water stress, but also some possess adaptations such as C_4 and CAM metabolisms that allow them to take advantage of the most arid environments.

The movement of water molecules is determined by the water potential gradient across the plasma membrane, which in turn is influenced by the concentrations of solute molecules inside and outside the plant cell. Fluctuations in the availability of extracellular water cause transmembrane water and solutes fluxes that perturb cellular structures, alter the composition of the cytoplasm, and modulate cell function (Bartels and Souer, 2004).

As the water content of the plant decreases, the cells shrink and the cell wall collapses; this decrease of cell volume results in a lower hydrostatic pressure or turgor. As the water loss and contraction of the cells goes on, the solutes become more concentrated in the cells, the plasma membrane becomes thicker and more compressed since it covers a smaller area than before.

The cell expansion is a process dependent on the turgor and since the loss of the latter is the first biophysical significant effect due to the loss of water, also the expansion is extremely sensitive to water deficit. The leaf expansion depends primarily on cell expansion; inhibition of this, occurs with a slowdown foliar that occurs at the beginning of water deficit. Leaf area smaller transpires less water, preserving effectively in soil and using it for a longer period. The limitation of the leaf can be considered a first line of defense against drought.

Water stress not only limits the size of individual leaves, but also the number of leaves of a plant to increase indefinitely, because it reduces both the rate of growth that the number of branches. If plants are stressed by lack of water, after which it has developed a substantial leaf surface, the leaves undergo senescence and eventually fall out. This calibration leaf area is an important long-term change that improves the ability of a plant to adapt to an environment with limited water availability. Many desert plants are deciduous, they drop all their leaves during the drought and make it sprout other after a period of rain, this cycle can occur two or more times during a single season. The process of abscission during stress is caused by increased synthesis and plant response to ethylene. Moderate water deficits also affect the development of the root system.When the absorption of water decreases, the process of leaf expansion is affected early.

The inhibition of the expansion foliar reduces the consumption of carbon and energy and a greater proportion of the compounds assimilated by the plant can be distributed to the root syste, where ensures further growth. Root tips placed in dry soils loose turgor.

As the water deficit increases, the surface layers of the soil to dry out first, it is common to see a shallow root system when all the layers of the soil are rich in water and notice a loss instead of a proliferation of surface roots and deep roots when is a loss of water; the growth of the root in a moist soil deeper can be considered a second line of defense against drought.

The closing of the stomata can be considered the third line of defense against drought, the entrance and the exit of water from the guard cells changes its turgor and modulates the opening and closing of stomata; the responses of stomata to leaf dehydration can vary widely both between species and within the same species.

The speed of the photosynthetic leaf, more rarely responds to moderate water stress than they face the leaf expansion; this because photosynthesis is much less sensitive to turgor than it is the leaf expansion; water stress usually affects both the stomatal conductance that on photosynthetic activity of the leaf. Adaptations to osmotic cell, help maintain the water balance leaf, the osmotic regulation develops slowly in response to dehydration of the tissue. It must be understood as acclimatization which increases the tolerance to dehydration.

According to Turner and Jones (1980) the maintenance of turgor allows the continuation of elongation cell and facilitates stomatal conductances highest to lowest water potential.

The osmotic regulation, or accumulation of solutes by the cells, is the process by which the water potential can be lowered without a corresponding decrease of turgor.

It must not be confused with the increase in the concentration of solutes that occurs during dehydration and sagging cell; it is the net increase in the amount of solute per cell, regardless of changes in volume that occur due to the water loss.

The osmotic regulation can also occur in the meristems radicals, increasing the turgor and maintaining the growth of the root and is important in the changes of the patterns of root growth during periods of drought.

The water deficit alters the energy dissipation from the leaves; when water stress limits the transpiration, the leaf heats up, unless other processes outweigh the loss of cooling.

To maintain a leaf much more fresh air, is required the evaporation of a large amount of water. In this way, adaptations which cool the leaves through other strategies, increase the effectiveness of water conservation. When the transpiration lowers and raises the temperature of the leaf, a certain extra amount of energy is dissipated as foliar loss of perceptible heat.

Numerous plants of the arid zones, have very small leaves, which minimizes the resistance to the boundary layer in order to transfer the heat of the leaf to air. Leaves small, because of their poor resistance of the boundary layer, tend to remain closed at room temperature, even when the breathability is greatly reduced. Leaves large possess thick boundary layers and dissipate less heat per unit of leaf area, by direct transfer.

This limitation may be offset by the movement of the leaf that allow for further protection from heat during water stress. The leaves are turning away from the sun are

called “paraeliotrope”, while those who buy energy orienting perpendicular to the sunlight are called “diaeliotrope”.

Other factors that can alter the interception of the radiation include the drying, which changes the angle of the leaf and in the Gramineae, the rolling-up, which minimizes the amount of tissue exposed to the sun. The energy absorption can also be dampened by the hairs of the leaf surface and outer layers of epicuticular waxes reflective. The leaves of some plants have an appearance due grayish-white hairs accumulating to reflect a large amount of light; this pubescence, maintains the fresh leaves reflecting the radiation, but also the wavelength of the visible active in photosynthesis, thereby diminishing the gain carbon.

According to Blizzard and Boyer 1980, the resistance in the plant is greater than that present in the soil. As during the dehydration of the soil, the extension radical slows down, the outer layers of the cortex overlap more easily suberin, a lipid impermeable to water, thus increasing the resistance to the flow of water.

A common response of development during water stress is the production of a thick cuticle that reduces the cuticular transpiration. A thick cuticle also blocks the passage of CO_2, but this does not affect the photosynthesis as leaf epidermal cells. The cuticular transpiration, it still represents only 5-10% of the total leaf transpiration, becoming significant only if the stress is extremely pronounced, or if the cuticle is damaged.

The water deficit may induce the Crassulaceae acid metabolism, which is an adaptation of plant which open the stomata at night and close them during the day. The difference in vapor pressure in the layer leaf - air which allows the traspiration, is much reduced at night, when the leaf is that the air is fresh. As a result, the efficiencies of water use of CAM plants are among the highest that can be measured in higher plants. A plant may acquire CAM 1 g dry weight for each 125 g of water used, from three to five times larger than the ratio of a plant with C_3 metabolism.

The phenomenon is characteristic of CAM succulent plants such as cacti and some succulent species are proving optional CAM, CAM triggering mechanism when subjected to water deficit or saline conditions (Hanscom and Ting, 1978). Moreover, increases in leaf temperature during the day can still be more pronounced in plants of arid and semiarid regions, where drought is accompanied by a strong solar radiation.

4.2. Salinity stress

Salinity is one of the most serious factors limiting the productivity of agricultural crops, with adverse negative effects on the germination, plant vigour, and crop yield (Munns and Tester, 2008).

Salinization affects many irrigated areas mainly due to the use of brackish water.

Worldwide, more than 45 million hectares of irrigated land have been damaged by salt, and about 1.5 million hectares are set aside each year due to high levels of salinity of the soil (Munns and Tester, 2008). Conditions of high salinity affect plants in different ways: water stress, ion toxicity, nutritional disorders, oxidative stress, alteration of metabolic processes, membrane disorganization, reduction of cell division and expansion, genotoxicity (Hasegawa et al., 2000; R. Munns ,2002; Zhu, 2007); together, all of these effects, reduce plant growth, development and survival.

Salt injury depends on species, variety, growth stage, environmental factors and nature of the salts.

Over the onset and development of salt stress within a species, all the main processes, such as photosynthesis, protein synthesis and energy and lipid metabolism are influenced (Parida and Das, 2005). During initial exposure to salinity, plants experience water stress, which reduce leaf expansion. The osmotic effects of salinity stress can be observed immediately after the application of salt and it persists throughout the duration of exposure, resulting in inhibition of cell division and cell expansion, as well as the stomatal closure (Flowers, 2004; Munns, 2002). During long-term exposure to salinity, plants can experience stress ionic, which can lead to premature senescence of adult leaves, and therefore a reduction in the photosynthetic area available to support a continued growth (Cramer and Nowak, 1992).

The excess of sodium and especially chloride can affect plant enzymes and cause cell swelling, resulting in reduced energy production and others physiological changes (Larcher, 1980).

Ionic stress, cause premature senescence of older leaves and toxicity symptoms (chlorosis, necrosis) in mature leaves due to high concentration of Na^+ that affects plants by disrupting protein synthesis and interfering with the enzymatic activity (Hasegawa et al., 2000; Munns, 2002; Termaat and Munns, 1986).

4.2.1. Soil salinity

The most widely accepted definition of a saline soil has been adopted from FAO (1997) as one that has an electrical conductivity of the saturation extract (Ece) of 4 dS m^{-1} or more, and soils with Ece's exceeding 15 dS m^{-1} are considered strongly saline.

Saline soil is characterized by toxic levels of chlorides and sulphates of sodium. The common cations associated with salinity are Na^+, Ca^{2+} and Mg^{2+}, while the common anions are Cl^-, SO_4^{2-} and HCO_3^-. Among these, Na^+ and Cl^- ions are considered the most important, since Na^+ causes deterioration of the physical structure of the soil and both Na^+ and Cl^- are toxic to plants (Dudley, 1994; Hasewaga et al., 2000).

According the USDA salinity laboratory, saline soil can be defined as soil having an electrical conductivity of solution extracted from the water-saturated soil paste Ece (Electrical Conductivity of the extract) of 4 dS m^{-1}, where 4 dS $m^{-1} \approx$ 40 mM NaCl or more (Chinnusamy et al., 2005; Kotuby-Amarcher et al., 2000).

Salt-affected lands occur in practically all climatic regions, from the humid tropics to the polar regions. The saline soils are found at different altitudes, from below sea level (e.g. around the Dead Sea) to mountains rising above 5000 meters, such as Tibetan Plateau or the Rocky Mountains.

Furthermore, the presence of saline land is not limited to desert conditions (Singh and Chatrath, 2001). The saline soils now account for about 10% of the cultivable areas (in the world) and most of these are located in arid and semi-arid areas.

In Europe, some countries of the Mediterranean are experiencing increasing problems resulting from the intrusion of salt stress marine aquifers, and irrigation with brackish water (Rana and Katerji, 2000). In particular, Spain, Portugal and Greece, which have the highest presence of saline lands in Europe, accompanied by some regions in southern Italy that are approximately 400000 hectares, about 2% of the lands farmed by dry-land agriculture have been already damaged by salts (Lauchli, et al., 2008).

The salt can be either native or non-native origin, in the first case is formed directly in the place where it is present, during the soil formation that has dented rocks capable of releasing salts or favored reactions that produced them; in the second case, the salinity has arrived from another environment due to the transport and subsequent deposition by water or, to a lesser extent, of the air. In addition, the salinity may be temporary if, as in soils of marine origin or coastal lands affected by flooding, in the presence of adequate

drainage, it takes a few years and a sufficiently wet to reduce it to tolerable limits; it may be on the other hand, permanent, if indigenous or source or localized flooding in the lowlands receive brackish waters from the areas above, or if it is in arid or semiarid areas where the poor percolation water cannot flush enough salts that are deposited on the surface by evaporation.

In Italy, the origin of salinity is mostly constitutional, even if the improper use of irrigation water characterized by an excessive ion content, over the years assumes increasing importance.

Salinization is a process of accumulation of soluble salts , very frequent in arid and semi -arid regions where the amount of water that achieve the soil by the rains is less than the amount of water lost from the soil due to the evapotranspiration phenomenon.

The main source of salinization are the salts derived from the alteration of rocks and minerals during soil formation.

The human activity is considered significant because of the salinity of the soil, not only from the point of view of irrigation water with a high concentration of salts but also for fertilization techniques and shedding of waste.

The accumulation of salts in soils can also occur in places near the coast due to the infiltration of seawater or in cases of abnormal drainage.

In areas with a humid climate, rains leach the soluble salts to lose them along the soil profile and groundwater, while in areas with low rainfall , the salts are not completely leached , accumulate and the soils become saline . In dry seasons, the saline soils are easily recognizable by the formation in the surface layer of whitish efflorescence.

An additional, important source of salts in many landscape soils comes from ice melters used on roads and sidewalkers. Among the various source of soil salinity, irrigation, combined with poor drainage is the most serious, because it represents losses of once productivity agricultural land (Zhu, 2007).

4.2.2. Causes of salinity

Most of the saline-sodic soils are developed due to natural, geological, hydrological and pedological processes. Climatic factors and water management may accelerate salinization. In arid and semi-arid lands, evapotranspiration plays a very important role in the pedogenesis of saline and sodic soils. Wanjogu et al. (2001) reported that most of

these lands receive less than 500 mm of rainfall annually and this, coupled with an annual potential evapotranspiration of about 2000 mm, leads to salinization. Another types of salinity occurs in coastal areas subject to tides and the main cause is intrusion of saline waters into rivers (Cyrus et al., 1997) or acquifers (Howard and Mullling, 1996).

Secondary salt affected soils are those that have been salinized by human-caused factors, mainly as a consequence of improper methods of irrigation. Poor quality water is often used for irrigation so that eventually salt builds up in the soil unless the management of the irrigation system is such that salts are leached from the soil profile.

Ponnamperuma (1984) reported that anthropic salinization occurs in arid and semi-arid areas due to waterlogging brought about improper irrigation.

Secondary salt-affected soils can also be caused by human activities other than irrigation, and include, deforestation, accumulation of air-borne or water-borne salts in soil, salinization caused by contamination with chemicals and overgrazing. The kind of salinization more often occurs in modern intensive agricultural system, particularly in greenhouses and intensive farming systems

Deforestation is recognized as a mayor cause of salinization and alkalinization of soils as a result of the effects of salt migration in both upper and lower layers.

Chemical accumulation from industrial emissions may accumulate in the soil, and if the concentration is high enough, can result in salt accumulation in the upper layer of the soil. Similarly, as reported by Bond (1998) and Bouwer (2002) water characterized by a considerable salt concentration such as a waste water from municipalities and sludge may contaminate the upper soil causing salinization and/or alkalinization.

Because of overgrazing, the natural vegetation becomes sparse and progressive salinization develops, and sometimes as the poor pasture diminishes, the process ends up in desertificaton.

All soils contain salts as well as all the waters of irrigation, both from canals, and groundwater pumping, including those considered to be of excellent quality. The salts are a common and necessary component of the soil, and many salts (including potassium and nitrate) are essential nutrients for the plants. The mineral salts come from atmospheric agents, inorganic fertilizers, soil amendments and irrigation water (Kotuby-Amacher, 2000; Koenig and Kitchen, 2000).

The problem of soil salinity is increasing due to the use of poor quality water for irrigation, improper drainage, entry of seawater during cyclones in costal areas, and salt accumulation in the root zone in arid and semi-arid regions due to high evaporative demand and insufficient leaching of ions as the rainfall is inadequate (Chinnusamy and Zhu, 2003)

The process of soil salinization is indeed dramatically aggravated and accelerated by irrigation of crops, combined with poor drainage, involves the loss of productive agricultural land (Zhu, 2007).

The irrigation water contains calcium (Ca^{2+}), magnesium (Mg^{2+}), and sodium (Na^{+}). When the water evaporates, Ca^{2+} and Mg^{2+} often precipitate as carbonates, leaving a concentration of Na+ dominant in soil (Serrano et al., 1999). In consequence, the concentration of Na^{+} is often superior to that of most of macronutrients (even of one or two orders of magnitude, or more in the case of micronutrients).

High concentrations of Na^{+} in the soil solution may depress the activity of the nutrient ions and produce extreme ratios of Na^{+}/Ca^{2+} or Na^{+}/K^{+} (Grattana and Grieve, 1999). Increases of cations and their salts, in particular NaCl, in soils, generates an osmotic potential outside that can prevent or reduce the inflow of water in the root. The resulting water deficit is similar to drought conditions and is also aggravated by the presence of Na^{+} ions (Bohnert, 2007).

Improper handling of salinity can lead to conditions of sodicity soil; damaging the structure of the soil, in particular, the action of Na+ ions, when they occupy the cation exchange complex of clay particles, cause soil aggregates to break down, increase bulk density, make the soil more compact and decrease the total porosity, thereby hampering soil aeration.

Soil salinity is often accompanied by a wide range of pedospheric (low soil fertility, high exposure to erosion processes), atmospheric (high air temperature, low precipitation and air humidity) and hydrospheric (water scarcity) constrains, which negatively influence agricultural production.

Secondary consequences of soil salinity may cause permanent soil degradation because of dispersion of soil aggregates. Sodium (Na^{+}), as the most frequent causative agent of salinity, is the most pronounced destructor by dispersion of secondary clay minerals.

Dispersed clay particles undergo through the soil and may accumulate and block pores, especially in fine-textured soil horizons (Burrow et al, 2002), i.e. cause pedospheric waterlogging.

Furthermore, dispersion of clay usually induces topsoil crusting, thus reducing infiltration, enhancing surface runoff and other related degradation processes such as soil erosion or desertification processes.

Over time, in saline especially sodic soils, crusted surface layer constrains hydraulic properties (water permeability, infiltration rate) as well as aeration of topsoil horizons and the root zone.

During the dry periods, salts accumulated in the soil profile or on the soil surface may further affect soil properties. Metternicht and Zinch (2003) reported ground observations and radiometric measurements that confirmed that quantity and quality of salts, together with soil moisture, color and roughness, affect the soil surface reflectance and consequently influence the topsoil physical properties such as the warming.

Increased salinity in soil solution, especially increased concentrations of Na^+ and Cl^- ions, significantly influence solubility and mobility of potential toxic trace elements (Helal et al., 1999; Weggler et al., 2004). Excessive salinity may cause desorption of particular metal and other cations from the soil adsorption matrix, increasing concentration of bioavailable forms in the soil solution. In particular, an exposure to increasing NaCl salinity increased concentration of trace elements (e.g. Cu, Zn, Cr and/or Cd) in the rhizosphere soil solution (Khoshgoftar et al. 2004). Organic and inorganic surfaces, mostly negatively charged, compete with salt anions (Cl^-, SO_4^{2-}) for metal cations (Cu^{2+}, Al^{3+}) via adsorption and complexation reactions (Adriano et al., 2004). Therefore, under excessive concentrations of Na^+, it is really expected displacement of weakly bound metal forms (Cd^{2+}, Zn^{2+}) and their accelerate release from soil solids to solution and enhance their mobility.

4.2.3. Salinity in plants

One of the major factor that limits the yield of agricultural crops, jeopardizing the capacity of agriculture to sustain the burgeoning human population increase is the soil salinity (Flowers, 2004; Munns and Tester, 2008; Parida and Das, 2005). At low salt concentrations, yields are mildly affected or not affected at all (Maggio et al., 2001).

Salinity is detrimental to plant growth as it causes nutritional constraints by decreasing uptake of phosphorous, potassium, nitrate and calcium, ion citotoxicity, mainly due to Na^+, Cl^- and SO_4^- and osmotic stress (Zhu, 2001; 2002). Na^+ competes with K^+ in biochemical reactions, which is adverse to cellular processes. Under salinity, ions like Na^+ and Cl^- penetrate the hydratlon shells of proteins and interfere with the function of these proteins. Ion toxicity, osmotic stress and nutritional defects under salinity, lead to metabolic imbalances and oxidative stress (Zhu, 2001).

High salinity affects plants in two main ways: high concentrations of salts in the soil disturb the capacity of roots to extract water, and high concentrations of salts within the plant itself can be toxic, resulting in an inhibition of many physiological and biochemical processes such as nutrient uptake and assimilation (Hasegawa et al., 2000; Munns, 2002; Munns et al., 1995; Munns and Tester, 2008). Together, these effects reduce plant growth, development and survival.

The initial and primary effect of salinity, especially at low and moderate concentration, is due to its osmotic effects (Munns and Termaat, 1986; Jacoby, 1994) as a result of lowering of the soil water potential due to increasing solute concentration in the root zone. At very low soil water potential, this condition interferes with the plant's ability to extract water from the soil and maintain turgor. At high soil water potential (low or moderate salt concentrations), plant adjust osmotically by internal solutes accumulation, and maintain a potential for the influx of water (Gurrier, 1996; Ghoulam et al., 2002).

High ionic concentration may disturb membrane integrity and function, interfere with internal solute balance and nutrient uptake, causing nutritional deficiency symptom (Grattan and Grieve, 1999). At high salinity levels some specific symptoms of plant damage such as necrosis and leaf tip burn due to Na^+ and Cl^- ions, can be observed (Wahome et al., 2001).

The degree to which growth is reduced by salinity differs greatly with species and with varieties (Bolarin et al., 1991; Ghoulam et al., 2002).

The severity of salinity response is also mediated by environmental interactions such as relative humidity, temperature, radiation and air pollution (Shannon et al., 1994).

The cultivated plants on saline soils suffer from a form of water stress called "physiological dryness", due to the fact that the salts make difficult the intake of water by the roots , this is due to osmotic effect exerted by the liquid phase of the soil, more

concentrated than the internal to the plant. When the osmotic pressure exceeds certain levels, the plants have difficulties in absorbing and it can be observed the plasmolysis.

The effect of salinity on the plant appears to be related primarily to the displacement of energy from the processes of growth than those required to maintain the osmotic differential between the interior of the cell and the liquid phase of the soil.

One of the first processes by which this energy is diverted is that from elongation cell, that is, the tissues continue to divide but not to elongate; the continued growth in the number of cells per unit of leaf area, gives the leaves a deep green color, typical symptom of osmotic stress in plants.

Often, accompanied with salt stress, there is also an imbalance in the plant nutrition, due to the high sodium concentration that leads to the deficiency of calcium and magnesium (Sequi, 2005).

The various cultivated species show a different tolerance to salinity , as well as genetic characteristics also dependent on the conditions of moisture that are found in the soil during the crop cycle and atmospheric agents that can lead to frequent rains and dilution of salts.

In general, the plants in saline soils not only suffer from high levels of Na^+ , but are also influenced by a certain degree of hypoxia (Singh and Chatrath, 2001; Tisdale et al, 1993).

Even the type of terrain and environmental factors, such as water vapor pressure deficit, radiation and temperature can alter the salt tolerance of crops (Chinnusamy et al., 2005).

In cultivated land, in fact, the salt levels fluctuate seasonally and spatially, and the change occurs because of the circumstances that affect each plant (Estes, 2002). In addition, the continuous use of the same soil for cultivation results in an increase in salinity.

Salt accumulation in leaves causes premature senescence, reducing the supply of assimilates to the growing regions and thus decreasing plant growth (Munns et al., 1995).

Sodium ions may enter passively in the roots, moving along the gradient of the electrochemical potential, in this way the cells radicals must use energy for the active transport to move back to the Na^+ in the external solution. In contrast, the Cl^- is excluded because of the low permeability of the plasma membrane radical against this

ion. The movement of Na^+ in the leaves is limited further by the absorption of this ion from the transpiration stream (xylem sap) during its motion from the roots to the shoots. Some plants that are resistant to salt, as the salt cedar (*Tamarix* sp.) and salt bush (*Atriplex* sp.) not extrude the ions at the level of the roots, but possess of salt glands on the leaf surface. The ions are transported in these glands, where the salt crystallizes and is no longer harmful.

Plant cells can calibrate their water potential in response to osmotic stress through two distinct processes: the accumulation of ions in the vacuole and the synthesis in the cytosol of compatible solutes, including the betaine, proline, sorbitol and sucrose.

According Wyn Jones and Gorham, 1983, the plant families tend to use preferentially one or two of this compound compared to others.

Salt stress affects all the mayor processes such as growth, water relations, photosynthesis and mineral uptake.

Growth and water relations

The main cause of reduction in plant growth may result from salinity effects on water status. According to Sohan et al. (1999) and Romero-Aranda et al., (2001), increase of salt in the root medium, can lead to a decrease in leaf water potential and hence, may affect many plant processes.

Several authors found that water potential and osmotic potential of plants, become more negative with an increase in salinity, whereas turgor pressure increased (Meloni et al., 2001; Romero-Aranda et al., 2001; Gulzar et al., 2003).

According some studies, under high salt concentration, plant sequester more NaCl in the leaf tissue than normally occurs; increases in NaCl within the leaf tissue, the result in lower osmotic potentials and a more negative water potentials and also the reduction in root hydraulic conductance reduces the amount of water flow from the roots to the upper portion of the canopy, causing water stress in the leaf tissue. Salinity has been reported to cause also leaf anatomical changes in a lot of studied plants.

Shoot growth is more sensitive than root growth to salt- induced osmotic stress probably because a reduction in the leaf area development relative to root growth would decrease the water use by the plant, thus allowing it to conserve soil moisture and prevent salt concentration in the soil (R Munns & Tester, 2008). Reduction in shoot growth due to

salinity is commonly expressed by a reduced leaf area and stunted shoots (Läuchli and Epstein, 1990). Moreover the salt-induced inhibition of the uptake of important mineral nutrients, such as K^+ and Ca^{2+}, further reduces root cell growth (Larcher, 1980) and, in particular, compromises root tips expansion. Apical region of roots grown under salinity show extensive vacuolization and lack of typical organization of apical tissue. A slight plasmolysis due to a lack of continuity and adherence between cells is present with a tendency to the arrest of growth and differentiation. Otherwise, control plants root tips are characterized by densely packed tissues with only small intercellular spaces.

Photosynthesis

Growth of plants is dependent on photosynthesis and therefore, environmental stresses affecting growth, also affect photosynthesis (Salisbury and Ross, 1992; Dubey, 1997; Taiz and Zeiger, 1998).

The reduction in photosynthetic rates in plants under salt stress is mainly due to the reduction in water potential; the main aim of salt tolerance is, therefore, to increase water use efficiency under salinity. To this effect, some plants shift their C_3 mode of photosynthesis to C_4 pathway or to CAM metabolism, in response to salinity. These changes allows the plant to reduce water loss by opening stomata at night, thus decreasing transpiratory water loss.

The effect of salinity on photosynthesis rate depends on salt concentration and plant species.

Studies conducted by several authors with different plant species showed that photosynthetic capacity was suppressed by salinity (Dubey, 1997; Kao et al., 2001; Ashraf, 2001; Romero-Aranda et al., 2001). The reduction in photosynthetic rates in plants under salt stress is mainly due to the reduction in water potential; the main aim of salt tolerance is, therefore, to increase water use efficiency under salinity. To this effect, some plants shift their C_3 mode of photosynthesis to C_4 pathway or to CAM metabolism, in response to salinity. This changes allows the plant to reduce water loss by opening stomata at night, thus decreasing transpiratory water loss.

Iyengar and Reddy (1996) attributed decreases in photosynthetic rate as a result of a salinity to a number of factors: (1) dehydration of cell membranes which reduce their permeability to CO_2, high salt concentration in soil and water create high osmotic

potential which reduces the availability of water to plants; decrease in water potential causes osmotic stress which reversibly inactivate photosynthetic electron transport; (2) salt toxicity caused by Na^+ and Cl^- ions; (3) Reduction of CO_2 supply because of closure of the stomata. The reduction in stomatal conductance results in restricted availability of CO_2 for carboxylation reactions (Brugnoli and Bjorkman, 1992). Higher stomatal conductance in plants is know to increase CO_2 diffusion into the leaves and thereby favor higher photosynthetic rates; (4) enhanced senescence induced by salinity; (5) changes of enzyme activity induced by changes in cytoplasmic structure and (6) negative feedback by reduced sink activity.

In photosynthetic tissues, in fact, Na^+ accumulation affects photosynthetic components such as enzymes, chlorophylls, and carotenoids (Davenport et al., 2005).

The derived reduction in photosynthetic rate in the salt sensitive plants can increase also the production of reactive oxygen species (ROS). Normally, ROS are rapidly removed by antioxidative mechanisms, but this removal can be impaired by salt stress (Allan and Fluhr, 1997; Foyer and Noctor, 2003). ROS signalling has been shown to be an integral part of acclimation response to salinity.

Garatt et al. (2002) and Mittova et al. (2002 and 2003) reported increased activities of the antioxidative enzymes in plants under salt stress. The mechanism by which salinity affects the antioxidant responses is not yet clear. ABA plays an important role in the response of plants to salinity and ABA-deficient mutants perform poorly under salinity stress (Xiong et al., 2001). The levels of plant hormones such as ABA and cytokinins increase with high salt concentration (Aldesuquy, 1998; Vaidyanathan et al., 1999). Popova et al. (1995) reported that the inhibitory effect of NaCl on photosynthesis, growth and translocation of assimilates was alleviated by ABA. Although the nature of ABA receptors remains unknown,

Chen et al, (2001) reported that the increase of Ca^{2+} uptake is associated with the rise of ABA under salt stress and thus contributes to membrane integrity maintenance, which enables plants to regulate uptake and transport under high levels of external salinity in the longer term.

Other plant hormones found to accumulate in the presence of salt, include jasmonates; these have been reported to have important roles in salt tolerance. According to Hilda et al. (2003), jasmonates are generally considered to mediate signaling such as defense

responses, flowering and senescence; however factors and mechanisms involved in the jasmonate signal transduction pathway remain still unclear.

Mineral uptake

Salinity stress has stimulatory as well as inhibitory effects on the uptake of some nutrients by plants. According to Vîllora et al. 1997 and Grattan and Grieve, 1999), nutrient imbalance may result from the effect of salinity on nutrient availability, competitive uptake, transport or partitioning within the plant, or may be caused by physiological inactivation of a given nutrient in the plant's internal requirement for that essential element.

High salt uptake competes with the uptake of other nutrients ions such as K^+, Ca^{2+}, N and P, resulting in nutritional disorders and eventually reduced yields and quality (Grattan and Grieve, 1999). Increased NaCl concentration has been reported to induce increases in Na+ and Cl- and decreases in Ca^{2+}, K^+, Mg^{2+} level in a number of plants (Perez-Afocea et al., 1996; Khan et al., 2000; Bayuelo-Jiménez et al., 2003). Under salt stress conditions, the uptake of N by plants is generally affected. A number of studies have shown that salinity can reduce N accumulation in plants (Feigin et al., 1991; Pardossi et al., 1999; Silveira et al., 2001). Several authors have attributed this reduction to Cl^- antagonism of NO_3^- (Bar et al, 1997).

The effect of salinity on P concentration has been reported by Grattan and Grieve (1994) to be highly dependent on plant species, plant development stage, composition and level of salinity, and the concentration of P in the substrate. In most cases, salinity decreased the concentration of P in plant tissue (Sonneveld and de Kreij, 1999; Kaya et al., 2001), but the results of some studies indicate salinity either increased or had no effect on P uptake (Ansari, 1990). Sharpley et al. (1992) suggested that the reduction in P availability in saline soils was a result of ionic strength effects that reduce the activity of phosphate, and the low solubility of Ca-P minerals.

Plants response to salinity

Plant sensitivity to salt levels in the soil is also highly dependent on environmental factors (Shannon et al., 1994), plant species, cultivars within a species (Greenway and

Munns, 1980; Ashraf, 2002), as well as the stage of plant development (Vicente et al., 2004).

The ability of plants to tolerate salinity depends on the interaction between salinity and environmental factors such as soil, water, and climatic conditions (Shannon et al., 1994).

Many crops, as reported by Mass and Hoffman (1977) are less tolerant to salinity when grow under hot and dry conditions than under cool and humid conditions, mainly due to decreased ion accumulation and/or improved plant water relations in these latter conditions (Salim, 1989).

The response of plants to salinity varies with growth stage at which salinization is initiated. Vicente et al. (2004) demonstrated that the reaction to salt stress varies with the stage of plant development and that a given cultivar may be tolerant at one stage and sensitive to another.

The available data, generally agree that the early seedling stage of growth is the most salt sensitive for most crops (Maas and Poss, 1989; Vicente et al., 2004). Consequently, high soil salinity during this stage can severely affect final seed yield.

Although salt stress delays germination and emergence, most crops are able to germinate at higher salinity levels than they would normally tolerate at the vegetative or reproductive stage of growth (Maas and Grieve, 1990).

On the basis of their sensivity to salinity, plants have been classifies in two large categories: the halophytes and glicophytes (Levitt, 1980; Shannon et al.; 1994). Glycophytes are plants that are sensitive to relatively low salt concentrations. Almost all major crop species as well as most wild species are glycophytes; halophytes, on the other hand, are plants that can grow in the presence of high concentrations of salt, even higher than that of seawater, and have a competitive advantage over non-halophytes in these environment

The amount of ion- radical movement towards the xylem which must be limited to protect the plant, depends on the stress and the plant's ability to compete with the ions of the shoot. It is believed that halophytes have a greater ability to accumulate ions into the cells of the shoot, when compared to glicophytes. Although individual responses to high salinity may differ, several studies suggest that all plants use the same general salt tolerance regulatory mechanisms, and the difference between halophytic and

glycophytic species are of a quantitative rather than qualitative nature (Greenway and Munns, 1980; Zhu, 2001).

Depending on their salt-tolerating capacity, these plants can be either obligate or facultative, characterized by physiological diversity and found in saline and non-saline habitats (Parida and Das, 2005).

At low salt concentrations, glycophytes yields are mildly affected or not affected at all (Maggio et al., 2001). As the concentrations increase, the yields move towards zero, since most plants, glycophytes, including most crop plants, will not grow in high concentrations of salt and are severely inhibited or even killed by 100-200 mM NaCl. The reason is that they have evolved under conditions of low soil salinity and do not display salt tolerance (R Munns and Termaat, 1986). On the contrary halophytes can survive salinity in excess of 300-400 mM. Halophytes are known to have a capability of growth on salinized soils of coastal and arid regions due to specific mechanisms of salt tolerance developed during their phylogenetic adaptation. Measurements of ion contents in plants under salt stress revealed that halophytes accumulate salts whereas glycophytes tend to exclude the salts (Zhu, 2007).

In general sense, halophytes are plants that tolerate salt concentrations that kill 99% of other species. Altough the most common definition of a halophyte involves growth and survival under saline conditions, the effect of salinity on growth varies amongst halophytes.

4.2.4. Salt tolerance

Salt stress as one of the most widespread abiotic constrains in food production may also result in the negative ecological, social and economic outcomes.

Successful remediation of salt-degrated area for food production, besides using relatively salt-tolerance genotypes, is highly dependent on sustainable management practices that are usually costly, time consuming and may be difficult or impossible to implement fully in certain situations.

Recent advances in plant breeding and molecular biology technology, suggest that increasing salt tolerance in cultivated plants could be one of the most promising and effective strategies for food production in salt-affected environments.

Plant salt stress resistance has been defined by Shannon and Grieve (1999) as the inherent ability of plants to endure the effects of high salt concentrations in the root zone or on the leaves without a significant adverse effect.

Levitt (1980) characterized these mechanisms as avoidance and tolerance, and has used the term "salt resistance" to refer a combination of tolerance and avoidance strategies.

Example of salt avoidance mechanisms include delayed germination or maturity until favourable conditions prevail; the exclusion of salt at the root zone or preferential root growth in non saline areas; compartmentalization of salt into vacuoles; secretion from specialized organelles such as salt glands and salt hairs, or storage in old leaves (Hasewaga et al., 1986).

A variety of mechanisms contribute to salt tolerance and salt resistance (Gorham, 1995); among them, 1) selective accumulation or exclusion of ions; 2) synthesis of compatible solutes; 3) control of ion uptake by roots and transport into leaves; 4) changes in photosynthetic pathway (under salinity); 5) induction of antioxidative enzymes (by salinity); 6) induction of plant hormones (by salinity).

Both glycophytes and halophytes cannot tolerate large amounts of salts in the cytoplasm and therefore, under saline conditions they either restrict the excess salts in the vacuole or compartmentalize the ions in different tissues to facilitate their metabolic functions (Iyengar and Reddy, 1996; Zhu, 2003).

In general, exclusion mechanisms are effective at low to moderate levels of salinity, whereas ion accumulation is the primary mechanism used by halophytes at high salt levels, presumably with the contemporary capacity to compartmentalize ions in the vacuole (Jeschke, 1984). Glycophytes limit sodium uptake, or partition sodium in older tissues, such as leaves, that serve as storage compartments which are eventually abscissed (Cheeseman, 1988).

Inclusions of ions in the cytoplasm can lead to osmotic adjustment that is generally accepted as an important adaptation to salinity (Guerrier, 1996).

The presence of salt in the growth media often results in accumulation of low-molecular-mass compounds, named compatible solutes, which don't interfere with the normal biochemical reactions (Hasewaga et al., 2000; Zhifang and Loescher, 2003). These compatible solutes include mainly proline and glycine betaine (Ghoulam et al., 2002; Girija et al., 2002; Khan et al., 2000; Wang and Nii, 2000).

The proposed function of proline under stress conditions include osmotic adjustment, protection of enzymes and membranes, as well as acting a reservoir of energy and nitrogen for utilization during exposure to salinity (Bandurka,1993; Perez-Alfocea et al., 1993a).

Exposure to saline stress results in accumulation of nitrogen-containing compounds such as aminoacids, amides, proteins, polyamines and their accumulation is frequently correlated with plant salt tolerance (Mansour, 2000). These compounds have been reported to function in osmotic adjustment, protection of cellular macromolecules, storage of nitrogen, maintenance of cellular pH, detoxification of the cells and scavenging of free radicals. Other compatible solutes that accumulate in plants under salt stress include carbohydrates, such as sugars (glucose, fructose, sucrose, fructans) and starch (Parida et al., 2002; Kerepesi and Galiba, 2000) which major functions have been reported to be osmotic adjustment, carbon storage and radicle scavenging, and polyols that serve as scvengers of stress-induced oxygen radicals and are also involved in osmotic adjustment and osmoprotection (Bohnert et al., 1995).

Plant regulate ionic balance to maintain normal metabolism; uptake and translocation of toxic ions such as Na^+ and Cl^- are restricted, while uptake of metabolically required ions such as K^+ is maintained or increased. This is possible by regulating the expression and activity of K^+ and Na^+ transporters and of H^+ pumps that generate the driving force for transport (Zhu et al., 1993). It is documented by several authors that a greater degree of salt tolerance in plants is associated with a more efficient system for the selective uptake of K^+ over Na^+ (Noble and Rogers, 1992; Ashraf and O'Leary, 1996).

The salt tolerance, in fact, can generally be assessed as the percentage of biomass production in saline conditions compared to conditions of "control" for a prolonged period of time (usually this correlates with yield) or in terms of survival, which is very appropriate for the perennial species (R. Munns, 2002).

Plant salt tolerance mechanisms can be grouped into cellular homeostasis (ion homeostasis and osmotic adjustment), stress damage control (repair and detoxification) and growth regulation (Zhu, 2001). According to R. Munns & Tester (2008), plants have evolved several mechanisms to acclimatize to salinity. It is possible to distinguish three different types of plant response or tolerance: a) the tolerance to osmotic stress; b) the Na^+ exclusion from leaf blades and c) tissue tolerance.

Osmotic tolerance

Osmotic tolerance involves the plant's ability to tolerate the drought aspect of salinity stress and to maintain leaf expansion and stomatal conductance (Rajendran et al., 2009). If the accumulation of salts overcomes the toxic concentrations, the old leaves die and the young leaves, no more supported by the export of photosynthates, undergo a reduction of growth and new leaves production. For this reason increased osmotic tolerance involves an increased ability to continue production and growth of new and greater leaves, and higher stomatal conductance. The resulting increased leaf area would benefit only plants that have sufficient soil water, such as in irrigated food production systems where a supply of water is ensured, but could be undesirable in water-limited systems (Munns and Tester, 2008).

Exclusion of Na^+

Another essential mechanism of tolerance involves the ability to reduce the ionic stress on the plant by minimizing the amount of Na^+ that accumulates in the cytosol of cells, particularly those in the transpiring leaves. This process, as well as tissue tolerance, involves up- and downregulation of the expression of specific ion channels and transporters, allowing the control of Na^+ transport throughout the plant (Munns and Tester, 2008; Rajendran et al., 2009).

Exclusion of Na^+ from the leaves is due to low net Na^+ uptake by cells in the root cortex and the tight control of net loading of the xylem by parenchyma cells in the stem (Davenport et al., 2005). Na^+ exclusion by roots ensures that Na^+ does not accumulate to toxic concentrations within leaf blades. A failure in Na^+ exclusion manifests its toxic effect after days or weeks, depending on the species, and causes premature death of older leaves (Munns and Tester, 2008).

Tissue tolerance

The third mechanism, tissue tolerance entails an increase of survival of old leaves. It requires compartmentalization of Na^+ and Cl^- at the cellular and intracellular level to avoid toxic concentrations within the cytoplasm, especially in mesophyll cells in the leaf (Munns and Tester, 2008) and synthesis and accumulation of compatible solutes

within the cytoplasm. Compatible solutes play a role in plant osmotolerance by various ways, protecting enzymes from denaturation, stabilising membrane or macromolecules or playing adaptive roles in mediating osmotic adjustment (Ashraf and Foolad, 2007). Compatible solutes are small molecules, water soluble and uniformly neutral with respect to the perturbation of cellular functions, even when present at high concentrations (Sakamoto and Murata, 2002; Yancey et al., 1982). They comprise nitrogen containing compounds such as amino acids, amines and betaines, but also organic acids, sugars and polyols (Mansour, 2000). These solutes also function to protect cellular structures through scavenging ROS (Hasegawa et al., 2000; Zhu, 2001).

Among the best known compatible solutes, proline and glycine betaine (GB) have been reported to increase greatly under salt and drought stresses (Munns, 2002; Sakamoto and Murata, 2002) and constitute the major metabolites found in durum wheat under salt stress, as in other Poaceae (Ashraf and Foolad, 2007; Carillo, et al., 2005; Sairam and Tyagi, 2004).

The mechanisms of genetic control of salt tolerance in plants have not yet fully understood because of its complexity. There are in fact several genes controlling salinity tolerance in the different species whose effect interacts strongly with environmental conditions. Thus, genetic variation can only be demonstrated indirectly, by measuring the responses of different genotypes. salt tolerance, can vary greatly with the genetic characteristics of the species; the salinity sensitivity of a given species can change during ontogeny, salinity tolerances in fact may increase or decrease depending on the plant species and/or on environmental factors. For some species, the salt sensitivity can be stronger during the germination phase, while for other species, the sensitivity can increase during reproduction (Howat, 2000; Marschner, 1986).

Several efforts have been made to improve salt tolerance of crops by means of conventional and more recently genetic breeding program. However, the genetic complexity of salt tolerance makes the task extremely difficult.

Several efforts have been undertaken to enhance the salt tolerance of economically important plants by traditional plant breeding as well as by biotechnological approaches. One of the main strategies for improving plant salt tolerance has been through the overexpression of single genes that are either induced by stress and/or have been shown to be required for normal levels of tolerance.

Transgenic plants overexpressing the genes participating in the synthesis or accumulation of osmoprotectants that function for osmotic adjustment, such as proline (Kishor et al., 1995), glycinebetaine (Holmström, et al., 2000) or other osmolytes show increased salt tolerance. Other genes that encode enzymes that are involved in oxidative protection, such as glutathione S-transferase, peroxidase, superoxide dismutase, ascorbate peroxidases, and glutathione reductases, can also be modified to improve plant salt tolerance (Yang et al., 2009).

Overexpression of regulatory genes in signalling pathways, also increases plant salt tolerance (Chen et al., 2010). The overexpression of the vacuolar Na^+/H^+ antiport has shown to improve salinity tolerance in several plants (Silva and Gerós, 2009).

4.2.5. Sustainable agricultural management in saline soil

Sustainable agricultural management in salt-affected conditions is principally based on two main approaches: prevention and remediation management (Biggs et al., 2010).

Saline lands can be converted to more productive croplands by preventing the influx of salt water through proper farm management practices, correcting soil toxicity and nutrient deficiency, and leaching the salts out of the root zone.

It is estimated that about one third of irrigated land on earth is affected by the presence of salts, a very big problem in agriculture is the use of irrigation water is not suitable for the accumulation of salts. When it is in the presence of a poor quality of irrigation water, i.e. when the water contains a high concentration of solutes, and there is the possibility to flush the salts with appropriate drainage system, the salts can reach concentrations harmful to species sensitive to salt.

Munns et al. (2002) proposed that irrigated agriculture could be sustained by better irrigation practices such as adoption of partial root zone drying methodology, and drip or micro-jet irrigation to optimize use of water. They suggested that salinity could also be contained by reducing the amount of water passing beyond the roots by re-introducing deep rooted perennial plants that continue to growth and use water during the seasons that don't support annual crop plants.

Salinity causes nutrient imbalances. Mainly resulting in lower concentrations of the macro-elements (N, P, K and Ca) in plant tissues; hence, the most direct way to recover

the normal nutrient concentrations within the plant, would be by raising their concentrations in the root zone by higher fertilizer doses.

Many studies have shown that salt stress may be mitigated by an increased supply of calcium to the growth medium (Raush et al., 1996; Ebert et al., 2002; Kaya et al. 2002). Depending on the concentration ratio, sodium and calcium can replace each other from the plasma membrane, and calcium might reduce salt toxicity (Raush et al., 1996).

Adams and Ho (1995) reported that increased Na^+ in the growth medium, generally decreases the K^+ content, suggesting an antagonism between Na^+ and K^+; addition of K^+ to the nutrient solution has been found to raise K^+ concentrations in the leaves and improve salinity stress effects (Lopez and Satti, 1996; Kaya et al., 2001). Similarly, under salt stress conditions, the uptake of N by plants is generally affected, and application of supplementary N has been found to improve the deleterious effects of salinity (Gómez et al., 1996). The effect of salinity on P in plants depends on P concentration in the nutrient solution: at low P concentration in the root medium, supplementary P applied to saline growth medium enhanced the capacity of the plant to regulate Na^+, Cl^- and K^+ distribution, and improved plant growth (Awad et al., 1990; Kaya et al., 2001). At high P concentrations, leaf injury has been interpreted by Awad et al., (1990) as P toxicity induced by salinity.

The most effective method known to reclaim saline soil by removing soluble salts is the leaching of soil by accurate drainage. This method, requires good permeability of the soil and good quality irrigation water. Accord to Dregne, (1976), removal of salts by leaching reduces salt hazard for plants but might cause permeability to decrease and pH to increase, resulting in decomposition of roots as soil is changed from saline-sodic to sodic. However this process is expensive in developing countries the water scarcity represents a problem.

As reported by Shannon (1984), salinity can be possibly also managed through biologically manipulating the plants. Identification of plant genotypes with tolerance to salt and incorporation of desirable traits into economically useful crop plants, may reduce the effects of salinity on productivity. Developing crop plants tolerant to salinity has the potential of making an important contribution to food production in many countries.

Great effort is being directed toward the development of salt-tolerant crop genotypes through the use of plant-breeding strategies involving the introgression of the genetic background from salt tolerant wild species into cultivated plants (Shannon, 1984; Pitman and Laüchli, 2002).

4.3.Heavy metal stress

Heavy metal contamination of soil is a growing problem that raises environmental and public health concerns in numerous areas of the world.

Environmental pollution, especially by chemicals, is one of the most effective factors in the destruction of biosphere components. The most detrimental anthropogenic impact on the biosphere is related to emissions of various acids (e.g., H_2SO_3, H_2SO_4, HF, HNO, HNO_3) and radionuclides. Energy and mineral consumption by man is the main cause of trace element pollution in the biosphere.

The problem of environmental pollution with heavy metals is becoming increasingly urgent. Many of these metals manifest high affinity for sulfur-containing ligands and strongly bind the latter.

Therefore, when such heavy metals enter the cell, they interact with SH-groups, inactivate many enzymes and disturb many metabolic processes. The idea that all heavy metals are highly toxic is misleading: this series comprises such elements as copper, zinc, manganese, iron, and other micronutrients essential for all animals and plants. Among non-nutrient heavy metals, Cd and Pb are the most widespread. Most of Pb and Cd contamination results from four human economic activities such as burning liquid and solid fuels, smelting and foundry works, high Pb and Cd sewage, and soil-applied chemicals, including fertilizers.

Bowen94 has suggested that when the rate of mining a given element exceeds the natural rate of its cycling by a factor of ten or more, the element must be considered a potential pollutant.

Thus, the potentially most hazardous trace metals to the biosphere may be Ag, Au, Cd, Cr, Hg, Mn, Pb, Sb, Sn, Te, W, and Zn, however, only Be, Cd, Cr, Cu, Hg, Ni, Pb, Se, V, and Zn are present in the list of elements considered to be of great risk to environmental health.

The urgent environmental problem at the present time is closely associated with pollution in which trace metallic pollutants play a significant role.

Most air pollution has arisen from the burning of coal and other fossil fuels and from smelting of iron and nonferrous metals. In general, elements that form volatile compounds, or are present at a lower particle radius, may be readily released into the atmosphere from the burning of coal and other industrial processes.

The atmospheric deposition of trace elements, mainly the heavy metals, contributes to contamination of all other components of the biosphere (e.g. waters, soils, and vegetation).

Trace elements are present in natural waters (ground and surface), and their sources are associated with either natural processes or man's activities. The basic natural processes contributing trace elements to waters are chemical weathering of rocks and soil leaching.

The anthropogenic sources of trace elements in waters are associated mainly with mining of coal and mineral ores and with manufacturing and municipal waste waters. Water pollution by trace elements is an important factor in both geochemical cycling of these elements and in environmental health. Most trace elements, especially heavy metals, do not exist in soluble forms for a long time in waters. They are present mainly as suspended colloids or are fixed by organic and mineral substances.

Soil is a very specific component of the biosphere because it is not only a geochemical sink for contaminants, but also acts as a natural buffer controlling the transport of chemical elements and substances to the atmosphere, hydrosphere, and biota. However, the most important role of soil is its productivity, which is basic for the survival of humans. Two terms - *soil contamination* and *soil pollution* - have been defined differently in various publications. The recent definition given by Knox et al. (1999) refers to soil contamination as soil whose chemical state deviates from the normal composition but does not have a detrimental effect to organisms. Pollution occurs when an element or a substance is present in greater than natural (background) concentrations as a result of human activity and has a net detrimental effect on the environment and its components.

Trace elements originating from various sources may finally reach the surface soil, and their further fate depends on soil chemical and physical properties and especially on their speciation.

The persistence of contaminants in soil is much longer than in other compartments of the biosphere, and contamination of soil; metals accumulated in soils are depleted slowly by leaching, plant uptake, erosion, or deflation.

Bowen (1979), estimated the residence time of trace elements in soils of temperate climate as follow: for Cd, 75 to 380 years; for Hg, 500 to 1000 years; and for Ag, Cu, Ni, Pb, Se, and Zn, 1000 to 3000 years. In soils of tropical rainforests, the rate of leaching of the elements is much shorter and is calculated at about 40 years.

Elevated concentrations of trace elements can also be of lithogenic (geogenic) origin.

The fate of these metals in soils depends upon many soil processes such as: dissolution, sorption, complexation, migration, precipitation, occlusion, diffusion, binding by organic substances, absorption and sorption by microbiota, volatilization. All these processes are governed by several soil properties, of which soil pH and redox potential are known to be the most important parameters.

The solubility of trace metals is often shown as a function of pH affected by the amount and kind of organic matter factors, such as CEC, carbonates, Fe and Mn hydrous oxides, clay minerals, and fine granulometric fractions are known to play significant roles in the behavior of trace elements.

Although trace elements are mainly inherited from the parent rocks, their distribution within the soil profiles and their partitioning between the soil components reflect various pedogenic processes as well as the impact of external factors (e.g., agricultural practices, pollution).

The main soil parameter governing processes of sorption and desorption of trace elements can be presented as follows: pH, fine granulometric fraction (<0.02 mm), organic matter, oxides and hydroxides (mainly Fe, Mn and Al) and microorganisms.

Microorganisms are very important ecologically because they are the producing, consuming, and transporting members of the soil ecosystem and therefore are involved in the flow of energy

and in the cycling of chemical elements. Reduction of microbial growth and enzymatic activity is often reported for soils contaminated by heavy metals (88,733,814).

Solubility of heavy metals

The mobility of the elements during weathering processes is determined first by the stability of the host minerals and second by the electrochemical properties of the elements. Trace element distribution is usually a parameter that is very sensitive to changes of weathering environments.

Each element can also be quite readily precipitated and/or adsorbed, even under a small change of the equilibrated conditions. In soils, solubility equilibrium may change significantly within a few centimeters, even millimeters, at both horizontal and vertical soil gradients. the most mobile fractions of ions occur at a lower range of pH and at a lower redox potential, with increasing pH of the soil substrate, the solubility of most trace cations will decrease. Indeed, the concentration of trace elements is lower in soil solutions of alkaline and neutral soils than in those of light acid soils.

Chuan et al. (1996) described that acidic and reducing soil conditions are more favorable for trace metal solubilization, and the effect of pH is more significant than that of redox potential.

Metal solubility is dependent on a number of soil characteristics and is strongly influenced by soil pH (Harter, 1983) and complexation with soluble ligands (Norvell, 1984). Blaylock et al. (1997) and Huang et al. (1997) have shown the effectiveness of applying chelates to the soil to increase lead solubility and plant uptake as part of the remediation process. In their studies, EDTA was substantially more effective than the other chelates tested at increasing Pb solubility in the soil solution and increasing Pb concentrations in the plans shoots.

The solubility of trace elements in soil has great significance in their bioavailability and their migration. Heavy soils, both neutral and alkaline, provide good storage for trace elements and will

supply them to plants at a slow rate. This slow release may, however, cause deficiency effects of certain micronutrients to develop in plants. Light soils, on the other hand, can be a source of easily

available trace elements during a relatively short period of time. These soils can also lose their pool of available micronutrients at quite a high rate.

Hodgson et al. (1966) indicated that appreciable quantities of trace elements are present as complexes, mainly with organic ligands. Rainfall, evaporation, and plant transpiration can change trace element concentrations in soil solutions more than tenfold.

The solubility of trace elements in soils evidently depends on complex formation.

The calculation made by Kabata-Pendias (1972) showed that the total content of trace cations generally ranges from 10 to 100 g L^{-1} in normal soil solutions, while in contaminated soils these values can be much higher. When soluble compounds of trace metals are added to soils, their concentrations in equilibrated solutions increase with increasing doses of added metals.

The transport of dissolved trace elements may take place through the soil solution (diffusion) and also with the moving soil solution (mass flow, leaching). Generally, in soils formed under a cool and humid climate, the leaching of trace elements downward through the profiles is greater than their accumulation, unless there is a high input of these elements into the soils. In warm, dry climates, and also to some extent in humid hot climates, upward translocation of trace elements in the soil profiles is the most common movement.

Impoverishment of soils in trace elements is due mainly to their mobility downward with percolating waters through the profiles of freely drained acid soils and also to trace element uptake by plants. On the other side trace metals enter the soil layers by a number of pathways, including aerial deposition and by leaching or decomposition of the above-ground parts of plants; by the utilization of wastes and by pesticide and fertilizer application; and by river waters and dredged sediment disposal, and by the atmospheric precipitation that allows their accumulation in particular soil horizons.

Rhizosphere bacteria, may be expected to play an important role in the bioavailability of metals to the plants. They can produce metal-chelating agents, like siderophores, that possess a high affinity for Fe^{3+}. Siderophores production can be stimulated by the presence of heavy metals. Since most siderophores also show a lower but significant affinity for bivalent heavy metal ions, they affect the bioavailability of the heavy metals.

Plant heavy metal uptake

In general, plants readily take up the species of trace elements that are dissolved in the soil solutions in either ionic or chelated and complexed forms.

In general, the uptake of trace elements by plants is affected, in addition to plant-specific ability, by soils factors, of which the most significant are pH, Eh, water regime, clay content, organic matter content, cation exchange capacity, nutrient balance, and concentration of other trace elements. Also, climatic conditions are shown to influence the rate of trace metal uptake, which may be partly an indirect impact due to the water flow phenomenon. Generally, a higher ambient temperature influences a greater uptake of trace elements by plants.

Plant ability to take up chemical elements from growth media is evaluated by a ratio of element concentration in plants to element concentration in soils and is called with different names: Biological Absorption Coefficient (BAC), Index of Bioaccumulation (IBA), Transfer Factors (TF) or Concentration Factor (CF). Some elements are more susceptible to phytoavailability than others.

A number of extraction methods have been suggested in recent years for the evaluation of trace element concentrations in soils. In general, they can be classified into separate groups as follows: acids (HCl, NHO3, aqua regia), chelating agents (EDTA, DTPA), buffered salt solutions, and unbuffered salt solutions ($CaCl_2$, $MgCl_2$, $NaNO_3$, NH_4NO_3). Acid extractants, depending on the strength and soil mineralogical composition, can extract nearly total amounts of trace metals. Chelating agents and buffered salt solutions are believed to extract potentially mobile portions of metals. Neutral salt solutions have been introduced as simulating the natural soil solutions and therefore are useful to evaluate the ecological relevance of metals.

The ability of different plants to absorb trace elements varies greatly; the specific properties of plants are very significant in determining the bioavailability of trace elements and are quite variable with changing soil and plant conditions. Conditions of plant growth media also have a significant impact on the absorption of trace elements by roots.

The content of the heavy metals in various plant organs decreased in the following series: roots > leaves > stems > inflorescences > seeds; however, this order sometimes varied with plant species.

Root uptake

The uptake of elements by plant roots is restricted to the liquid phase, therefore the content of metals in the soil solution is of primary importance.

The absorption of trace elements by roots can be both passive (non-metabolic) and active (metabolic). Passive uptake is the diffusion of ions from the external solution into the root endodermis. Active uptake requires metabolic energy and takes place against a chemical gradient. Several data support the suggestion that, at the concentration generally present in soil solutions, the absorption of trace elements by plant roots is controlled by metabolic processes within roots.

When roots absorb heavy metals, they accumulate primarily in the rhizodermis and cortex; mechanisms of uptake differ, depending on the given element. Pb and Ni are preferably absorbed passively, while Cu, Mo, and Zn are preferably absorbed actively. When biological and structural properties of root cells are altered, however, all elements are taken up passively.

Much evidence indicates that roots exhibit great activity in the mobilization of trace elements that are bound by various soil constituents. Roots and associated microorganisms are known to produce various organic compounds which are very effective in releasing the trace elements from firmly fixed species in soil. The trace elements most readily available to plants are, in general, those that are adsorbed on clay minerals. The mechanisms of uptake of trace elements by roots involve several processes; changes in the pH of the root ambient solution may play an especially significant role in the rate of availability of certain trace elements. Roots can also act as a “barrier” in the uptake or transport of trace elements.

Foliar uptake

Foliar uptake is believed to consist of two phases non-metabolic cuticular penetration, which is generally considered to be the major route of entry, and metabolic mechanisms, which account for element accumulation against a concentration gradient. The second process is responsible for transporting ions across the plasma membrane and into the cell protoplast.

Trace elements taken up by leaves can be translocated to other plant tissues, including roots where the excesses of some metals seem to be stored. The rate of trace element movement among tissues varies greatly, depending on the plant organ, its age, and the element involved.

A fraction of the trace elements absorbed by leaves may be leached from plant foliage by rainwater. Differences in leaching of trace elements can be related to their function or metabolic association. Little and Martin (1972) observed that the easy removal of Pb by washing suggests that the metal was largely a superficial deposit on the leaf surface. In contrast, the small fraction of Cu, Zn, and Cd that can be washed off indicates a greater leaf penetration of these metals than was noted for Pb. The absorption of trace metals, directly from wet (and dry) deposition by aboveground parts of plants, has been often reported. Morphology of the surface of leaves is an important factor governing foliar uptake of trace elements. Some plants (e.g., lichens, mosses, mushrooms, etc.) are especially susceptible to absorb elements and some compounds from aerial sources.

Such plants are very suitable for the phytoindication of atmospheric pollution. Also, cereal tops show a relative sensitivity to aerial pollution, revealing the variation in trace metal contents (especially Pb and Ni) mainly due to the absorption from aerial particles. Dalenberg and van Driel studied the uptake and translocation of ^{210}Pb from atmospheric deposition; their results have indicated that 73 to 95% of the total Pb content in plants are taken up by leaves and transported to other plant organs. This was observed not only for leafy plants, such as spinach, but also for cereals.

Translocation

The transport of ions within plant tissues and organs involves many processes: a) Movement in xylem; b) Movement in phloem; 3) Storage, accumulation, and immobilization.

The chelating ligands are most important in the control of cation translocation in plants. However, numerous other factors such as pH, the oxidation-reduction state, competing cations, hydrolysis, polymerization, and the formation of insoluble salts (e.g., phosphate, oxalate, etc.) govern metal mobility within plant tissues.

The transport of trace elements among plant organs also depends on the electrochemical variables of elements. In general, easily transported from roots to above-earth parts are

Ag, B, Li, Mo, and Se; moderately mobile are Mn, Ni, Cd, and Zn; and strongly bound in root cells are Co, Cu, Cr, Pb, Hg, and Fe.

Toxicity

Trace elements are involved in key metabolic events such as respiration, photosynthesis, and fixation and assimilation of some major nutrients; although many are essential for growth, they can also have toxic effects on cells at higher concentrations.

Both deficiencies and toxicities of trace elements for plants most commonly result from complex factors that vary with the specific environment

Although plants adapt rather readily to chemical stress, they also may be very sensitive to an excess of a particular trace element. The "upper critical level" of an element is the lowest tissue concentration at which it has toxic effects.

Toxic concentrations of these elements in plant tissues are very difficult to establish. Visible symptoms of toxicity vary for each species and even for individual plants, but most common and nonspecific symptoms of phytotoxicity are chlorotic or brown points of leaves and leaf margins, and brown, stunted, coralloid roots.

Lower plants especially, such as microorganisms, mosses, liverworts, and lichens, reveal an extremely high level of adaptation to toxic concentrations of certain trace elements.

Although the higher plants are believed to be less tolerant of increased concentrations of trace elements, they are also widely known to accumulate these elements and to survive on soils contaminated by large quantities of various trace elements. Tyler et al.1989 attempted to summarize and define what is implied by the term "tolerance" of plants. This term refers to both the population occurring in an area highly contaminated by trace elements, and to individual plants or species which are able to withstand greater levels of toxicity than others.

Metal tolerance

Many authors summed up the possible mechanisms involved in metal tolerance, among them Antonovics et al. (1971); Bradshaw (1975) and Foy et al. (1978) distinguished external factors, such as low solubility and mobility of cations surrounding plant roots, as well as effects of metal ion antagonisms. The real tolerance, however, is related to

internal factors. This is not a mechanism of tolerance in a simple sense, but consists of several metabolic processes: 1) selective uptake of ions; 2) decreased permeability of cell walls or other differences in the structure and function of membranes; 3) immobilization of ions in various organs 4) alteration in metabolic patterns 5) adaptation to toxic metal replacement of a physiological metal in an enzyme; 6) release of ions from plants by leaching from foliage, guttation, leaf shedding, and excretion from roots; 7) release of volatile organic metal compounds (e.g., Hg, Pb, and Sn) and 8) excretion from leaf tips in the form of salts.

Selective uptake of ions is related to a capability of plants for active selective sorption and discrimination of available ions or compounds in the soil. Selective transport of ions to the tops taken up by roots is also observed.

Decreased permeability of cell walls is closely associated with the immobilization; synthesis of immobilizing compounds and/or fixation by charged ligands are responsible for the removal of trace ions from plant metabolism by deposition (storage) in fixed and insoluble forms in various organs and organelles. Roots are the most common storage for the excess of trace metals, but also foliage and seeds are known to accumulate deposited forms of different trace elements.

The tolerance of some plants to elevated concentrations of trace metals in growth media and in tissues creates a health risk to humans and animals. Therefore, health-related limits for certain elements in food plants are carefully controlled by national and international legislation. Tolerant plants, due to their ability to grow in contaminated substrates, and due to the accumulation of extremely high amounts of trace metals, may create a great health risk by forming a polluted link in the food chain.

Plants species and even plant populations within a species considerably differ by their sensitivities to heavy metals. Species of higher plants that show a tolerance to trace elements belong most commonly to the *Caryophyllaceae*, *Cruciferae*, *Cyperaceae*, *Gramineae*, *Leguminosae*, and *Chenopodiaceae* families.

The uptake and transport of heavy metals differ in their sensitivity towards these metals; in the tolerant populations, plants confine heavy metals preferentially in the cell wall and the vacuole, whereas a considerable amount of heavy metals enters the cytoplasm of the susceptible plants; the tolerant and susceptible plants differ in the rates of heavy-metal transport from roots into shoots and heavy-metal accumulation in the particular

root tissues; tolerant plants synthesize the enzymes-resistant to heavy metals and in the end the mechanisms for excluding heavy metals from the cells are activated in the tolerant plants.

Plants develop different mechanisms to protect against their excess. These mechanisms are, in general, related to the root (mainly root tips, meristems) exudates containing polygalacturonic acid that fix metals outside or within root cells, and to the production of phytochelates (various derivatives of glutathione) fixing metals and displacing either in vacuoles or on cell membranes.

In plants, tolerance can be defined as the ability to survive in a soil that is toxic to other plants of the same of different species. The toxicity of the soils is defined by reference to its effects on other plants. Tolerance is manifested as a genotype x environment interaction.

A variety of tolerance and resistance mechanisms have evolved, including exclusion or active efflux systems to minimize the cellular accumulation of metals. These are effecting protective strategies of plants that result in low concentrations of metal ions in the organism, the opposite outcome of that desidered from phytoremediation, where the goal is to maximize metal accumulation in plant material. Physiological mechanisms that are based on tolerance rather than avoidance of metals are likely to be important for phytoremediation, as these will allow plants to survive while accumulating high concentration of metals.

In the mechanism of tolerance of plants to heavy metals, are evolved a lot of compounds, among them two different types of metal ligands: phytochelatins and metallothioneins.

The metabolic capacity of plant-associated bacteria may be used to develop new phytoremediation strategies. Plants stimulate the growth of microorganisms due to secretion of organic molecules by their roots. This results in higher populations densities of bacteria in the rhizosphere (Anderson and Coats, 1995; Rovira et al., 1979). In addition, endophytic bacteria colonize the interior of root and stem tissues. Plant species from metalliferous soil (metallophytes) are characterized by desiderable properties which might be transferable to more productive species by means of genetic engineering or interspecific crossing. Metalliferous soils are often enriched in

combinations of different heavy metals and therefore, local metallophytes often exhibit combined tolerance to different metals.

High levels of tolerance to specific heavy metals are known to occur in wildtype populations from heavy metals enriched substrates such as mine-waste deposits. A minority of the species concerned, the so-called hyperaccumulators, exhibit extremely high rates of foliar metal accumulation.

Hyperaccumulators are however, in most of cases, insufficiently productive in terms of harvestable aboveground biomass to be useful in phytoremediation. The concentrations of metals in plant parts depend both an intrinsic (genetic) and extrinsic (environmental) factors and vary greatly for different species and for different metals. Baker (1981) proposed two basic strategies by which higher plants can tolerate the presence of large amounts of metals in their environment: 1) exclusion, whereby transport of metal is restricted, and low, relatively constant metal concentrations re maintained in the shoot over a wide range of soil concentrations; and 2) accumulation, whereby metals are accumulated in nontoxic form in the upper plant parts at both high and low soil concentrations. He suggested that accumulators can be characterized by a leaf:root metal concentration ratio >1, because of the tendency to translocate metals from root to shoot, whereas in excluders the ratio is <1. Plants which accumulate very high concentrations of metals are called hyperaccumulators.

Hyperaccumulation

The word hyperaccumulator was explained by several authors during the time; Peterson (1971) defined metal accumulation in two ways: 1) accumulation of an element within an organism to concentration greater than those found in the growth medium and 2) possession of greater quantities of an element than is usual for that organism. However, these definitions create some problems for the interpretation of metal-accumulating patterns in plants because only in laboratory studies, using nutrient solutions, is possible to know the metal concentration of a precisely defined growth medium, while in soil it is necessary to base the discussion on total metal concentration, or one of a variety of measures of “available” metal concentrations.

Brooks et al. (1977a) used the term hyperaccumulators to describe plants with Ni concentrations > 1000 μg/g (0.1%) in their dried leaves.

Reeves (1992) further elaborated on this definition, including only those species which accumulated such concentrations when growing in their natural habitats. This criterion was also considered appropriate to specify hyperaccumulation of copper (Brooks et al., 1980) and lead (Reeves and Brooks, 1983a).

Recent studies have further extended the range of species known to be capable of metal hyperaccumulation; in agreement with the accumulator strategy proposed by Baker (1981), hyperaccumulator species typically maintain high tissue metal concentrations across a wide range of soil metal concentrations,

Metal concentrations in leaves, and often stems, of hyperaccumulators greatly exceed those of roots and other storage organs (Rascio, 1977; Hajar, 1987; Homer et al., 1991). Thus the roots, which are the perennial organs of many of these plants, are protected from very high metal levels, while there is the possibility of eliminating a considerable amount of accumulated metals through leaf fall (Vergnano Gambi et al., 1982; Schlege et al., 1991). Little is known of the mechanism of transport of metals in hyperaccumulator plants.

Many of the European hyperaccumulator plants are of small biomass, although considerable natural variation exists within populations (Lloyd-Thomas, 1995; Chaney et al., 1997).

Selection trials are needed to identify the fastest growing (largest potential biomass and greatest nutrient responses) and most strongly metal-accumulating genotypes.

Hyperaccumulator plants offer a very important opportunity to achieve economic phytoexctraction to decontaminated polluted soil.

4.4 Phytoremediation

The use of plants in environmental remediation has been called “green remediation”, “phytoremediation”, “botanical remediation”, “phytoextraction”, etc.

This new technology is being developed for the cleanup of both soil metals and xenobiotics.

Because metals cannot be biodegraded, remediation of soil metal risks has been a difficult and/or expensive goal (Chaney et al., 1995, 1997a).

The general strategies for phytoremediation of soil metals is to either: 1) phytoextract the soil elements into the plant shoots for recycling or less expensive disposal; 2)

phytovolatilize the soil trace elements or 3) phytostabilize soil metals into persistently non bioavailable forms in the soils.

The third method is usually called “in situ remediation” by which incorporation of soil amendments rich in Fe, phosphate are used to transform soil Pb into forms with lower biovailability and/or phytoavailability. Sometimes soil Pb and some other elements become much less phytoavailable or bioavailable to organisms which consume soils; plants can contribute to this process by hastening the formation of phyromorphite an insoluble and non-biovailable Pb compound (Ma et al., 1993; Berti and Cunningham, 1997; Zhang et al., 1997; Brown et al., 1998).

Phytoremediation employs plants to remove contaminants from polluted soils which require decontamination. The commercial strategy is to use phytoremediation as a lower cost alternative to current expensive engineering methods (Benemann et al. 1994; Salt et al., 1995).

Soil remediation technology is needed to reverse risk to humans or the environment from metals in soil, both geochemical enrichment and anthropogenic soil contamination.

Soil metals have caused phytotoxicity to sensitive plants at numerous locations, especially where mine wastes and smelters caused contamination of acidic soils with Zn, Ni or Cu (Chaney et al., 1998b). Although some of these situations can be remedied by soil amendments (Brown et al., 1998), phytoremediation offers an alternative whereby the contaminant would be removed from soils and either recycled or safely disposed.

The combination of the need to prevent adverse environmental effects of soil contaminants, and to do so at lower cost than existing technologies, has brought increased attention to phytoremediation.

Recovery of energy by biomass burn or pyrolysis could help make phytoextraction cost effective.

The possibility of effective phytoremediation of heavy metal-contaminated soil depends on the availability of plants varieties with high rates of accumulation and tolerance of the metals to be exctracted.

Phytoremediation is a green technology that uses specially selected metal-accumulating plants to remediate soil contaminated with heavy metals and radionuclides; it also offers

an attractive and economical alternative to currently practiced soil removal. The integration of specially selected metal-accumulating crop plants with innovative soil amendments allows plants to achieve high biomass and metal accumulation rates from soils. The use of plants to remove toxic metals from soils is being developed as a method for cost-effective and environmentally sound remediation of contaminated soils (Baker et al., 1994; Chaney, 1983; Raskin et al., 1994). This process of extracting metals from the soils and accumulating and concentrating metals in the aboveground plant tissues enables plants to be used as a part of a soil clean-up technology. The metal-rich plant material can be swathed, collected, and removed from the site using established agricultural practices. Post-harvest biomass treatments may also be employed to reduce the volume and weight of biomass for disposal.

The metal bioaccumulation of the plants shoots above that of the soil concentration coupled with subsequent biomass reduction processes can greatly reduce the amount of contaminated material requiring disposal compared to soil excavation, thereby decreasing the remediating costs.

Successful implementation of phytoremediation in the fields depends on a significant quantity of metal being removed from the soil through plant uptake to effectively decrease the soil metal concentration.

Several conditions must be met in order for phytoremediation to be effective. The availability of metals in the soil for root uptake is the first critical factor for metal uptake. Soil containing metal contaminants that cannot be solubilized or made available for plant uptake will limit the uptake and therefore the success of phytoremediation (Blaylock, 2000).

The plants selected for phytoremediation must also be responsive to agricultural practices and produce sufficient biomass coupled with high rates of metal uptake. The plant must also be adapted to the wide variety of environmental conditions that exist in contaminated soils and waste sites.

One crop plant that produces high rates of biomass under filed conditions and also has the capacity to accumulate substantial metal concentrations in its shoots is *Brassica juncea* also known as Indian mustard (Kumar et al., 1995; Blaylock et al., 1997), which also been used successfully to decrease the selenium content of soils in central California (Bañuelos et al., 1993).

4.4.1. Mechanisms involved

Plants can be used in a number of fundamentally different ways to assist in bioremediating metal contaminated soils and waters.

There are two potential method available for metal-polluted soils, both of which are designed to reduce the size of the bioavailable soil metal pool: 1) phytostabilization, or in situ metal immobilization by means of revegetation, either with or whitout nontoxic metal binding or fertilizing soil amendments (Czupyrna et al., 1989) and 2) phytoextraction, or metal bioextraction by means of hyperaccumulating plants.

In phytostabilization, plants are used to stabilize the land and reduce or eliminate the movement of the toxic elements from the contaminated soil to the general environment. Plants used for phytostabilization will need to be tolerant of the metals present in the particular site, but the accumulation of metals in their aerial parts may be positively disadvantageous. If the objective is purely to prevent erosion and improve the visual amenity of a derelict site, then the accumulation of metals in the plants may be irrelevant; if on the contrary, the site is to be used by the public or cropped for biofuels or timber, then the presence of toxic metals in the plants or crop might render the site less valuable for this purpose (Cunningham et al;1995)

In phytoextraction, plants are used to extract metals from the soil. The objective may be to clean up mildly contaminated soils, so that the metal is removed from the site in situ, or to biomine metals from heavily contaminated soils. It is important that plants are tolerant of the soils in which they are to grow, but in this case, it is also important that they translocate significant amounts of metal and accumulate in their aerial parts. In practice, only plants that accumulate more than 1% (dry weight) of metals are going to be useful in either context (Chaney et al., 1997).

Phytovolatilization is the volatilization of an element (for example Selenium) by plants (Terry et al., 1995); it is a highly attractive technique for the phytoremdiation of Se pollution because it removes Se completely from the ecosystem while at the same time minimizing entry of the toxic selenium into the food chain.

Once present in soils and waters at high concentrations, Se is very complicated and highly expensive to remove with conventional physical and chemical techniques. This techniques (chemical or electrochemical treatments), moreover, may also produce large amounts of highly polluted sludges. Plants are highly effective in removing Se from

contaminated sites, With their big root systems, plant may scavenge large areas and volumes of soil, removing Se as selenite or selenite. Once absorbed by plant roots, Se is translocated to the shoots where it may be harvestes and removed from the site. In addition to their uptake and immobilization of Se in their tissues, plants have the capacity to remove Se from contaminates substrates and metabolize it into a non-toxic volatile gas.

4.5 Lead stress and phytoremediation of lead

According to the Environmental Protection Agency (EPA), lead (Pb) is the most common heavy metal contaminant in the environment (Watanabe, 1997). Human activities such as mining, smelting, burning of fossil fuels, dumping of municipal sewage sludge, and the manufacture of pesticides and fertilizers are the primary causes (Kabata-Pendias and Pendias, 1992).

Lead is a nonessential element in metabolic processes and may be toxic or lethal to organisms even when absorbed in small amounts (Walker et al., 1996). Given its potential hazard and widespread contamination, there is a high level of interest in methods aimed at cleaning up Pb at minimal costs with the fewest environmental side effects. Traditional methods of remediating Pb contaminated sites include a variety of physical, thermal, and chemical treatments (Cunningham et al., 1997).

Lead (Pb) as an environmental pollutant is particularly important (Salt et al., 1998) because it is toxic to many plants and organisms, and causes harmful effects on the health of children and adults (Lanphear, 1998). Lead is not an essential element for the growth of plants, nor does it participate in the process of cell metabolism. Lead pollutions in the environments are mainly brought about by industrial activities (mining and smelting activities and so on), motor vehicles and also by the use of chemical fertilizers and municipal sewage sludge (Jackson and Watson, 1977; Levine et al., 1989). Many fertilizers contain Pb and therefore, it is found that vast areas of agricultural land contain high concentrations of Pb (Shull, 1998).

Lead contamination may bring about a serious problem for agriculture, for example, the primary effect of Pb toxicity on plants is a rapid inhibition of root growth due to the inhibition of cell division in the root tip (Eun et al., 2000).

Plants are poisoned by Pb, and Pb-contaminated soil may result in a great decrease in crop productivity (Buchauer, 1973; Johnson and Eaton, 1980).
Furthermore, Pb is also accumulated in the human body through our foodstuff and damages the brain and the nervous system (Body et al., 1991).

Source of lead

Lead (Pb) is a naturally occurring element and, as a result of anthropogenic activities, a ubiquitous environmental contaminant. Elevated Pb levels in the soil cause concern for human health and the environment.
Relatively well documented sources of Pb contamination include Pb mining and smelting activities, the widespread use of Pb compounds in the automotive industry, land application of sewage sludge, Pb-containing paint pigments, and the deposition of Pb shot and sinkers in wetland and aquatic environments; the manufacturing and texting explosives, manufacturing and combustion of antiknock agents in gasoline and occasionally with Pb bullets or shot. Another source of Pb is the demolition of industrial buildings containing Pb based paint, Pb pipes and Pb linings used as antispark coatings.
Lead contaminants originated in a number of different forms including Pb metal, inorganic salts, and organic Pb compounds such as the antiknock agents tetraethyl and tetramethyl Pb. These Pb materials entered a variety of different environments including soils and sediments, each with its own characteristic chemistry that may further alter the chemistry of the Pb contaminants once deposited. Lead in soils may be present, also, in different oxidation states, and associated with different complexation states on soil surfaces. Divalent Pb is often complexed with organic matter, adsorbed onto cation exchange sites on the soil surface, or precipitated as relatively insoluble salts. The various forms present in soils have different solubilities and bioavailiabilities.
Natural background concentrations of Pb in soil range from 10 to 30mg Pb kg^{-1} soil however, where low-level contamination has occurred due to anthropogenic release of Pb into the environment, soil Pb concentrations can be expected to lie within the range of 30 to 100 mg kg^{-1} (Davies, 1990). Due to the relatively low solubility of Pb, the metal has a long residence time in soils: estimates of the half-life of Pb in soil range from 740 to 5900 years (Kabata-Pendias and Pendias, 1992).

Lead uptake

Lead absorption is regulated by pH, cation exchange capacity of the soil, as well as by exudation and physicochemical parameters (Alloway, 1992; Parker et al., 1995; Lasat, 2000). Absorption by roots from the soil occurs via the plasma membrane, probably involving cationic channels such as calcium channels. Roots are capable of accumulating significant quantities of this heavy metal and simultaneously restrict its translocation to the shoot (Lane and Martin, 1977).

The retention of Pb in roots involves binding to the cell wall and extracellular precipitation, mainly in the form of lead carbonate, which is deposited in the cell wall. At low concentration, Pb can move through root tissue, mainly via the apoplast and radially through the cortex where it accumulates near the endoderm. When entering the root, Pb moves by apoplast until they reach the endodermis. At this stage, the rate of transport depends on the chemical composition of the cell walls. The accumulation of heavy metals in the cell walls may reduce the plasticity of the latter and in this way reduce cell elongation. The endoderm acts as a partial barrier to the translocation of Pb through the root to the shoot. This may be one of the reasons for the much greater accumulation of Pb in roots than in shoots (Jones et al., 1973; Verma and Dubey, 2003). Roots usually manifested the maximum content of Pb; leaves vary with age in their abilities to accumulate Pb. Salt et al.(1998) noted that Cd accumulated preferably in the youngest leaves of *Brassica juncea* and *Thlaspi caerulescens*, whereas, in other species, the maximum Pb content was found in the senescing leaves.

Lead toxicity

The plasmalemma is a primary site of the toxic effects of Pb. The changes in its permeability and, therefore, the ionic balance may result from the inhibition of H+-ATPase and the changes in the membrane lipid composition.

Excess Pb causes a variety of toxicity symptoms in plants, such as reduced growth, chlorosis and darkening of the root system. Inhibition of root growth appears to result from Pb-induced inhibition of cell division of the root meristem (Eun et al., 2000). Lead inhibits photosynthesis, alters the mineral nutrition and water balance, modifies hormone levels and affects the structure and permeability of the plasma membrane (Sharma and Dubey, 2005).

Lead exert numerous and diverse changes in the metabolic processes; similar changes are produced when plants are exposed to such diverse stresses as high and low temperatures, salinity, anoxia, etc.

Many researchers reported a decline in transpiration rate and water content in plants treated with Lead (Pb^{2+}). Various mechanisms underlie these effects. First, growth retardation results in the reduced area of leaves, the major transpiring organ. Second, the guard cells are sometimes smaller in the plants treated with lead, whereas, in other cases, the guard cells are relatively more numerous, because these heavy metals generally affect leaf growth to a greater extent than the particular differentiation of stomata. Third, lead lower the contents of the compounds maintaining cell turgor and cell wall plasticity and thus lower the water potential; the latter effect becomes an important factor of growth inhibition. Fourth, these metals increase the content of ABA, thus inducing stomata closure. Fifth, disordered respiration and oxidative phosphorylation can also cause disarray in the plant water regime; moreover, the water stress incurred by the heavy metals promotes super-production of proline, an osmoregulating, antioxidant, and stress-protecting substance (Singh et al., 1997).

Lead disrupt photosynthesis in different ways. The decline of the photosynthetic rates results from the distorted chloroplast ultrastructure; the restrained synthesis of chlorophyll, plastoquinone, and carotenoids; the obstructed electron transport; the inhibited enzyme activities of the Calvin cycle; and CO_2 deficiency due to stomatal closure; lead change also the lipid composition of thylakoid membranes.

All these metabolic changes produced by lead dramatically modify plant growth and development.

The toxic effects of lead on cell division and elongation are typical of other metals.

The inhibition of most enzyme activities by Pb results from its direct binding to the functional SH-groups and from the disordered ionic balance due to the competitive inhibition of the absorption and transport of such nutrients as Zn, Fe, and Cu. The dramatic changes in the secondary metabolism exerted by Pb depend on the inhibition of enzymes and respiration.

Lead Hyperaccumulation

Hyperaccumulation of lead is particularly rare. The low solubility of most lead compound in a circum-neutral media and the ready precipitation of lead by sulfate and phosphate at the root system may partly explain this. Despite of this, *Thlaspi rotundifolium* spp. *Capaeifolium* from a lead/zing mining area of "Cava del Predil" (Northern Italy) has been found with lead up to 8200 μg/g of dry weight (Reeves and Brooks 1983a). *Alyssum wulfenianum* Schlecht from the same location, also contained remarkable high Pb concentrations, reaching 860 μg/g in leaf dry matter.

A concentration of up to 2740 μg/g Pb was also reported by Shimwll and Laurie (1972) in *Thlaspi caerulescens* colonizing a lead mine district in the Pennines, England.

Deram and Petit (1977) reported of lead hyperaccumulation in the grass *Arrhenatherum elatius*, belonging to Poaceae family, growing on calamine soils in France.

Barry and Clarck (1978) recorded shoot Pb values from 11, 75 to 130 μg/g in *Festuca ovina* and Williams et al (1977) found a mean shoot Pb value of 13,488 μg/g in pasture species growing on mining waste in the U.K.

High average Pb concentrations can be achieved by uptake through the root system: Baker et al. (1994) found that seedlings of *Thlaspi caerulescens* exposed to a nutrient solution containing 20 μg/ml Pb, contained 4500-7000 μg/g Pb after 21 days (roots 29,000 μg/g; shoots 280 μg/g).

Addition of synthetic chelates such as EDTA (ethylenediaminetetraacetic acid) to the root medium can dramatically increase lead uptake and accumulation in *Zea mays* and *Pisum sativum*, giving shoot concentrations in excess of 1% of dry weight (Huang and Cunningham, 1996; Huang et al., 1997). Similarly, shoot concentrations of 1.5% lead by dry weigh have been observed in *Brassica juncea* growing in contaminated soil amended with EDTA (Blaylock et al. 1997).

Lead remediation

Lead is an extremely difficult soil contaminant to remediate because it is a "soft" Lewis acid that forms strong bonds to both organic and inorganic ligands in soil.

For the most part, Pb-contaminated soils are remediate through civil engineering techniques that require the excavation and landfilling of the contaminated soil.

Phytoextraction, also called, biomining, is a site decontamination technique in which lead is gradually removed from the site by plant uptake and harvesting. In contrast, phytostabilization is a site stabilization technique that does not remove lead from the soil, but may reduce environmental and health risks by an alternative strategy. This technique, also called in situ inactivation or natural land restoration, uses soil amendments to sequester lead in such a manner that it does not interact biologically with target organism.

Both of these techniques are relatively simple and low-cost: however, each resolves the risk to the environment and human health through contrasting approaches.

In phytoextraction, soil lead is taken into plant roots, translocated into the top of the plants and removed by plants harvesting. Several techniques parameters affect how efficiently this process functions.

First, soil lead must be in a form that is available to the plant root. More often, at a site, the total lead level is quite high, but the fraction of lead availability for root uptake is exceedingly low. Second, the plant must be able to transfer lead in the roots to the xylem stream to be carried to harvestable plant tissues. In most plants, this is difficult due to chemical and physical environment in their tissues. Finally, the lead-containing harvested plant material must be processed.

Several different pathways have been proposed to separate the organic plant material from the Pb, including the removal of the organic material by microbial degradation, incineration, low temperature ashing, or direct smelting from which the Pb could be removed. Along with these technical concerns there are still a number of regulatory, economic and logistic questions that must be answered adequately before phytoextraction is accepted as a cost-effective and viable remediation technology. At the present, no Pb-contaminated site has been fully remediated using phytoextraction (Cunningham and Berti, 2000).

The literature defines as a Pb-hyperaccumulating plants as one that can accumulate at least 1000 mg of lead. These concentrations of lead are substantial when compared to non-hyperaccumulators that rarely accumulate more than 100 mg Pb kg^{-1} regardless of the soil concentration.

Plants differ widely between their ability to take up lead from the solution and accumulate in the roots. Some plants, such as corn, may accumulate at most a few

hundred mg Pb/kg in the roots. Other plants grown under identical controlled conditions, such as *Thlaspi rotundafolium* L., may accumulate over 3% in their roots.

Relatively little amount of lead is transferred from the roots to shoots. A shoot to root lead concentration ratio of over one would greatly increase the effectiveness of phytoextraction.

Lead is only sparingly soluble in solution, and even at the most contaminated sites, lead in the soil solution is often less than 4 mg L^{-1}. At these low concentrations, many plants can effectively remove soil solution lead into root tissues; however very little lead is translocated to aerial tissues that can be harvested.

When lead enters the plant root, it immediately comes in contact with high phosphate concentrations, relatively high pH, and high carbonate-bicarbonate concentrations in the intracellular spaces.

Under these conditions, lead precipitates out of solution as phosphates or carbonates that can be seen in electron micrographs of roots from plants grown hydroponically in lead solutions. These plants roots show inclusion bodies of these forms of lead in the tissue, resolving the question of the cause of limited lead translocation in plants.

The the low solubility of lead in soil causing lead to be unavailable for plant uptake; the poor lead translocation in plants to harvestable plant portions, and the toxicity of lead to the plant tissue, represent the main phytoextraction limits.

A technique should be developed to increase soil lead availability in plant roots and increase internal plant translocation from root-to-shoot by sequestering lead in a such way that is not precipitated either in the soil or in the root tissue. Recent research suggest that certain organic chelates may directly address all three all the factors mentioned above and mobilize lead into aerial plant tissues. Many chelates have a great affinity for lead and form strong bonds with the elements, increasing soil solution concentrations.

Research date indicates that the effectiveness of phytoextraction depends on agronomic practices, plant selection, chelate selection soil pH, ionic balance of the soil solution, climatic factors, and the use of fertilizers. Mycorrhizal infection of the plants roots may also influence phytoextraction. Under low Pb levels, mycorrhizae increase Pb uptake into the plant, but they actually decrease Pb uptake under higher Pb levels.

Even by altering some of these parameters, to optimize Pb removal, phytoextraction at relatively contaminates sites may require a decade or longer to remediate a site. Given the typical yields of plants grown on contaminated soils and the Pb concentrations of the harvestable plant tissues in most plants, it has been estimated that 300 to 1000 mg Pb/kg soil may be removed in 7-10 years. From this estimation, it appears that there is an upper Pb concentration limit for this technique, and that phytoextraction is perphaps most applicable at low to moderately contaminated sites where restoration of a clean soil may have additional benefits (Cunningham and Berti, 2000).

The phytostabilization of lead is a in-situ inactivation that stabilizes soil lead both chemically and physically through the use of soil amendments and a vegetative cover. Soil amendments alter the existing lead chemistry in the soil and reduce the biological availability of lead by inducing the formation of very insoluble species.

In spite of phytostabilization is becoming an attractive possibility for the remediation of many different sites, however, critical questions remains and they must be resolved before this technique will be widely accepted by regulatory agencies and public opinions. The first of these questions concerns what simple test more appropriately measures Pb bioavailability while adequately reflecting risk. The second question regards the longevity of inactivation treatments and the possible need for re-treatments or restrictions on future land use of the site following phytostabilization. The third question concerns the practicality of optimizing treatment effectiveness. The fourth question concerns the concentration limit above which phytostabilization is not practical or effective. Currently, most research concentrates on soils in the 1000 to 3000 mg kg^{-1} range. The practical upper soil lead concentration limit for phytostabilization is unknow. Until these questions are adequately answered, regulatory and public acceptance of phytostabilization will be unattainable.

Economic are also a necessary consideration in the choice of remediation technique for any site.

Researches investigating phytoextraction and phytostabilization are actively working with state and federal regulators at many sites to push these techniques forward and demonstrated them on a full-scale basis. Hopefully, with sufficient validation and some changes in current regulatory concepts regarding lead bioavailability, these techniques will soon be available as alternatives to current practices (Cunningham and Berti, 2000).

EXPERIMENTAL PART

1. AIM OF THE WORK

Water shortage, especially during spring and summer, is the main factor limiting crop production (Araus et al, 2002; Passioura, 1977), moreover, in Mediterranean area, inadequate irrigation practices may also exacerbate soil salinity.

The improvement of drought and salinity tolerance are therefore some of the main objectives in the management of crops whose cultivation area is in Mediterranean environment (Araus et al, 2002).

Species of great interest, especially for biomass production, are the perennial and lignocellulosic crops, widespread and adapted to Mediterranean environment; however, despite an increasing number of studies on these species, there are still many information necessary to optimize their cultivation for energy purposes.

It is therefore of strategic importance for the introduction of perennial species in Mediterranean marginal areas the identification of species (i) with high yields and (ii) well suited to the limiting factors of the Mediterranean environment, such as limited availability of water resources, high concentrations of salts in the soil due to pedogenic factors or brackish waters utilization as irrigation practic.

Limited rainfall in the Mediterranean area and brackish or saline waters could take a considerable importance for the cultivation of biomass for energy; brackish aquifers, very common in these areas, it may therefore be used for irrigation of tolerant species. However, the high concentration of inorganic salts and organic content in the irrigation water or in soils, originates obvious problems to soil structure, as the deterioration of the stability of the aggregate, reduction of the hydraulic conductivity, surface runoff, soil erosion, soil compaction, etc.

In addition to the problems of the soil structural degradation, the soil salt concentration may influence the morphology, physiology, ontogeny of the plants and consequently the productivity. Under sufficient water stress conditions, the plant closes its stomata to conserve water and therefore photosynthesis is inhibited and the growth is reduced. (Bresler et al., 1982).

The response of perennial species, the duration and the level of salt stress, however, is not completely understood, especially at the root level. It would therefore be advisable to use genotypes with improved tolerance to salinity (Munns et al., 2002).

The pollution of soils due to accumulation of heavy metals is a global problem that may involve the loss of agricultural areas: contaminated land is no longer suited to farming, and may be especially harmful to the entire ecosystem. Plants tolerant to heavy metals, can be used to enhance sites unsuitable for biomass production, restoring the ecosystem services and providing valuable feedstocks to biorefineries, in a phytoremediation process.

Among the perennial species used for energy purpose in the Mediterranean environment, *Arundo donax* L. could be a suitable species for marginal lands including dry areas, salt and lead contaminated soil. This last issue is a current topic because the lead concentration in soils is in a continuous increase due to the human activities.
On the basis of these premises the following research lines were carried out with the aim of assess the possibility of cultivate *Arundo donax* on dry, salt and lead contaminated marginal lands:

- Line 1: Giant reed screening to salinity levels
- Line 2: Response of *Arundo donax* L. clones at increasing levels of salinity and at different soil water content
- Line 3: Phytoremediation of *Arundo donax* L. in lead-contaminated soils with different water levels of the soil
- Line 4: Phytoremediation of different *Arundo donax* L. clones in lead-contaminated soils

2. MATERIAL AND METHODS

2.1.Research line 1: Giant reed screening to salinity levels

The research was carried out at the department of Agricultural and Food Science (DISPA) of the University of Catania from May to December 2012 with the aim to compare different clones of *Arundo donax* (L.) in order to identify information on contrasting clones tolerant and sensitive to increasing salinity levels.

The experiment was performed in pots with a diameter of 24 cm and with a capacity of 9.5 liters, arranged in a randomized block experimental design with two replication.

The transplanted rhyzomes were taken from the collection of *Arundo donax* present at the experimental farm of Catania University, established within the Project Giant reed Network (Cosentino et al., 2006).

The substrate used was the sphagnum peat with the following characteristics: 34% Corg, Norg 0.2%, dry substance 68%, pH 5.0 to 6.5.

The studied factors were:

- Giant reed clones: 40 ecotypes collected through Sicily and south Calabria, Italy (Cosentino et al., 2006), as shown in Table 1;
- Salinity (NaCl) of irrigation water:
  - natural salinity of tap water for civil use no salt added (control) (S_0),
  - 4 dS m^{-1} (S_1)
  - 8 dS m^{-1} (S_2).

At each irrigation NaCl has been added to 17 liter of tap water for a total amount of 21 g of NaCl for S_1 treatment and 52 g of NaCl for S_2 treatment, respectively. All the pots were maintained in well watered condition performing irrigation twice a week.

Throughout the growing season the following measurements have been performed:

- on soil:
  - electrical conductivity (EC), (GS3 sensor, ProCheck (Decagon Devices, Inc)).
  Date of measurement on soil: at transplanting and then after 36, 45, 75 and 115 days after transplant (DAT);
- on plant:
  - biometric: number of stems, stem height and non-destructive leaf area index (LAI).

These measurements were performed 28, 63, 93 and 115 DAT.

- physiological: net photosynthesis, transpiration and stomatal conductance by infra red gas analyzer (IRGA) (LICOR 6400 , LI-COR Bioscience) and SPAD unit (SPAD 502, Konica Minolta). These measurements were performed 63, 80 and 116 DAT.

Biomass harvest took place in October 2012 (140 DAT).

At harvest were measured:

- aboveground biomass: dry matter yield, biomass partitioning, specific leaf area (SLA), specific leaf weight (SLW), main stem height and number of stems.
- belowground biomass: size of rhizomes and root weight.

A two-way analysis of variance was performed to tests statistical significance among genotypes and treatment. The Student-Newman-Keuls test was used to separate means with a significance level ≤ 0.05.

Table 1 - List of collected clones, geographic coordinates and altitude, according to Cosentino et al., 2006.

Clones (n°)	Name	Geographic coordinates		Altitude
		Lat N	*Long E*	*a.s.l.*
1	S.S. 417 BR	37°20’	14°45’	200
2	S.S. 417 Caltagirone	37°14’	14°31’	608
3	Caltagirone	37°07’	14°32’	313
4	Piazza Armerina	37°23’	14°22’	697
5	Bivio Ramata	37°31’	14°18’	670
6	Piedimonte Etneo	37°48’	15°10’	348
7	Passopisciaro	37°50’	15°08’	550
8	Maniace	37°53’	14°47’	787
9	Bicocca	37°30’	15°04’	7
10	Fondachello	37°45’	15°11’	1
11	Santa Tecla	37°37’	15°10’	22
12	Fontane Bianche	36°57’	15°12’	17
13	Lentini	37°17’	15°00’	53
14	Vittoria	36°57’	14°32’	168
15	Modica	36°50’	14°46’	296
16	Pozzallo	36°43’	14°51’	20
17	Gela	37°04’	14°13’	46
18	Biancavilla	37°38’	14°52’	515
19	Tortorici	38°01’	14°49’	450
20	Capo D'Orlando	38°08’	14°43’	8
21	S. St.di Camastra	38°00’	14°20’	70
22	Cefalù	38°01’	14°00’	16
23	Roccalumera	37°58’	15°23’	7
24	Villafranca	38°14’	15°26’	22
25	Milazzo	38°13’	15°14’	1
26	Caltanissetta	37°29’	14°02’	568
27	Agrigento	37°19’	13°35’	230
28	Ribera	37°29’	13°15’	223
29	Menfi	37°36’	12°58’	119
30	Licata	37°06’	13°56’	8
31	Trabia	38°00’	13°38’	50
32	Capaci	38°10’	13°14’	51
33	Castellammare	38°01’	12°53’	26
34	Birgi	38°01’	12°32’	3
35	Mazara	37°39’	12°35’	8
36	Gioia Tauro	38°25’	15°53’	29
37	Lamezia	38°58’	16°18’	216
38	Catanzaro	38°53’	16°65’	320
39	Val di Neto	39°05’	17°07’	8
40	Tellaro	36°47’	15°03’	40

2.2. Research Line 2: Response of *Arundo donax* L. clones at increasing levels of salinity and at different soil water content

Based on the results of the salinity screening carried out in the Research Line 1, in 2013, 12 clones of Giant reed (*Arundo donax* L.) have been studied with the aim of evaluate their response to increasing levels of salinity and soil water content.

The research was carried at the department of Agricultural and Food Science (DISPA) of the Catania University from June to November 2013.

The rhizomes of the selected genotypes were transplanted in 25 l pots (diameter 40 cm and height 30 cm).

The substrate used was the sphagnum peat with the following characteristics: 34% Corg, Norg 0.2%, dry substance 68%, pH 5.0 to 6.5. The pots were arranged in factorial experimental design with two replications.

The studied factors were:

• Giant reed clones: 12 ecotypes (Table 2) collected through Sicily and south Calabria, Italy (Cosentino et al., 2006) and selected on the basis of salinity screening carried out in 2012

• Salinity of irrigation water: 3 levels of salinity:

- S_0, natural salinity of drinking water for civil use, without added of salt (control);
- S_1, 6 dS m^{-1}
- S_2, 12 dS m^{-1}

To reach the required salinity level at each irrigation different amount of sodium chloride (NaCl) were added to the irrigation water.

• Irrigation: 2 restoration levels of maximum evapotranspiration (Etm):

- 25% (I_{25})
- 100% (I_{100})

During the experiment, the irrigations were carried out twice a week. The amount of water was determined by filling two pots of I_{100} treatment for each salinity levels until the pot field capacity was reached. The water amount of the I_{25} treatments was quantified as 25% of the corresponding I_{100} treatments.

Throughout the growing season the following measurements have been performed:

- On soil:
  - Electrical conductivity (EC), by GS3 sensor (ProCheck, DecagonDevices, Inc.).

- Soil water content, by EC_5 probe (ProCheck, DecagonDevices, Inc.)

Measurements of electrical conductivity and soil water content were performed at the transplant and after 33, 47, 54, 75, 104, 125 and 138 DAT, while soil moisture concurrently on physiological measurements.

- On plants:

  - Morphobiometric: stem number (#) , height of the main stem (cm), number of green and senescent leaves (#) and leaf area index (LAI).

The morphobiometric measurements were performed at 14, 33, 48, 75, 95 and 112 DAT.

  - Physiological: (i) net photosynthesis, transpiration and stomatal conductance by infra red gas analyzer (IRGA) (LICOR 6400 , LI-COR Bioscience), (ii) chlorophyll content at leaf level, measured in SPAD units (SPAD 502, Konica Minolta) and (iii) foliar fluorescence (Handy Pea Hansatech).

Photosynthesis, transpiration and stomatal conductance were measured at 53, 104, 125 and 138 DAT, while the SPAD units after 28, 45, 54, 74, 105, 115 and 126 DAT; the fluorescence after 48, 55, 75, 95, 119 and 126 DAT.

The whole biomass was harvested 150 days after transplant collecting all aboveground and belowground biomass.

At harvest the following measurements were carried out:

- On aboveground biomass: stems number (#), height of the main stem (cm), stem diameter (mm), number of green and senescent leaves (#), biomass partitioning (%) and dry matter yield (g).

  For the dry matter determination, samples of stems and leaves were oven dried at 70°C until constant weight.

- On belowground biomass: size and weight (fresh and dry) of the rhizomes, weight (fresh and dry) of primary and secondary roots.

The data collected were subjected to a three-way analysis of variance (ANOVA) using the software CoStat 6.003. The means were separated by the Student-Newman-Keuls test (SNK) when $p \leq 0.05$.

Table 2 - List of collected clones, geographic coordinates and altitude, according to Cosentino et al., 2006.

Clones (#)	Name	Geographic Coordinates		Altitude
		Lat N	*Long E*	*a.s.l.*
2	S.S. 417 Caltagirone	37°14’	14°31’	608
6	Piedimonte Etneo	37°48’	15°10’	348
7	Passopisciaro	37°50’	15°08’	550
10	Fondachello	37°45’	15°11’	1
13	Lentini	37°17’	15°00’	53
14	Vittoria	36°57’	14°32’	168
18	Biancavilla	37°38’	14°52’	515
20	Capo D'Orlando	38°08’	14°43’	8
24	Villafranca	38°14’	15°26’	22
34	Birgi	38°01’	12°32’	3
40	Tellaro	36°47’	15°03’	40

Instruments used

Chlorophyll Meter SPAD-502

Unit Spad measurements was performed using a Chlorophyll Meter SPAD-502, a compact, lightweight meter which can be used to determine the amount of chlorophyll present in plant leaves.

The measurements were carried on the third fully-expanded leaves, the same used for the physiological measurements. For each leaf three different measurements were performed and the final value was the average of the three values. The values were calculated based on the amount of light transmitted by the leaf in two wavelength regions in which the absorbance of chlorophyll was different. The wavelength ranges chosen to be used the measurement were the red area, where absorbance is high and unaffected by carotene and the infrared area, where absorbance is extremely low. The leaf was be inserted in the receiving window; during the measure extremely thick parts of the leaves, damaged parts and veins were avoid.

IRGA analyzer

The measurement of plants gas exchange was carried out by IRGA (Infrared Gas Analyzer). On each plant three different measurements were made and the final value was the average of the three values recorded. The measurements on leaves were performed at the moment of maximum intensity of solar radiation, from 12 a.m. to 2 o clock p.m.

The measurements were carried out on the upper surface of the third fully expanded leaf for each plants, in the median portion of the leaf, avoiding veins and damaged parts of the leaves. The leaves were fully exposed to light and the instrument for the measuring was held parallel to the solar radiation and avoiding shadows.

Handy PEA Fluorimeter

The Chlorophyll Fluorescence was measured on the youngest fully expanded leaves on the adaxial leaf surface using a continuos fluorescence portable fluorimeter (Handy PEA Hansatech, UK) that uses the principle of continuous excitation. The first step of the measurement process was to cover the sample area to be analyzed, with a small,

plastic lightweight leafclip that contains a sliding window to obscure the sample that must remain in the dark for several minutes (20) to adapt. During this period, the white surface of the clip and the shutter stainless steel reflect incident sunlight reducing the heating of the sample. The locating ring section of the clip which interfaces with the fluorimeter optical assembly is constructed from black plastic. This ensures that the measurements is unaffected when measuring during conditions of high light intensity environment.

To make a measurement, the sensor of the Handy-PEA was placed on the leaf clip with the shutter open. With the simple press of a button the LED light High intensity (maximum 3000μmol $m^{-2}s^{-1}$) was actived; this light is sufficient to reach the saturation with most plants of the plant species, and thus allowing an accurate measurement of the maximum fluorescence (Fm).

During dark adaption, all the reaction centres are fully oxidized and available for photochemistry and any fluorescence yield is quenched. This process takes a variable amount of time and depends upon plant species, and if the plant is stressed or not. Typically, 15-20 minutes may be required to dark adapt effectively.

The parameters Fo, Fm, Fv, Fv/Fm, Tmax, and the area under the curve of fluorescence between Fo and Fm were automatically calculated and displayed immediately after the measurement.

After 20 minutes, the measurement was repeated on the same leaf clip, with shutter closed, to detect the parameters of dark adaptation of the leaf.

The system stores all recorded points taken during the measurement interval; these one, once saved were transferred to a PC via a software that allows sophisticated data processing.

Measured parameter:

Fv'/Fm'- maximum photochemical efficiency of PSII in a light-adapted sample;

Fv/Fm- maximum photochemical efficiency of PSII in a dark-adapted sample;

With:

Fv/Fm is presented as a ratio of variable fluorescence (Fv) over the maximum fluorescence (Fm).

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2.3.Research line 3: Phytoremediation of *Arundo donax* L. in lead-contaminated soils with different water levels of the soil

The research was conducted at the "Department of Science and Technology of Biomass", belonging to the "Faculty of Science and Technology" of the “New University” of Lisbon during the year 2012-2013, under the supervision of Dr. Ana Luisa Fernando.

The aim of this research was to study the behavior of the species *Arundo donax* (L.) in lead contaminated soils with different concentrations of lead and at different soil water levels.

The test was performed in pots with a diameter of 30 cm and with a capacity of 12 liters, by adopting a factorial experimental design with two replicates.

The pots were filled with loam soil previously analyzed (Table A), by determining the following parameters: moisture, pH, conductivity, organic carbon, total nitrogen, ammonia content (NH_4^+), nitrate and nitrite content (NO_3^-, NO_2^-), phosphorus, total phosphates (PO_4^-), number of total microorganisms eco-toxicity essay and metal content, with particular attention to the lead content,

Before the transplant, in addition to the soil, tap water for irrigation was analyzed (Table B), through the characterization of the following parameters: pH, conductivity, organic matter content, ammonia nitrogen (NH_4^+), nitrate and nitrite content (NO_3^-, NO_2^-), phosphates (PO_4^-) and metal content.

The two studied factors were the level of contamination with lead in the soil and the water level of the soil.

The sludge containing lead, and added to the pots with the aim of simulate the two levels of contamination, was a waste product derived from a manufactury of batteries, the "Sociedade Portuguesa do Acumulador Tudor, “LDA”, located in Castanheira do Ribatejo, near Lisbon.

Even the sludge, before being added and mixed with the soil in the pots , was characterized for the following parameters (Table C): moisture, pH, conductivity, organic matter, ash , total nitrogen (N), ammonia content (NH_4^+) , nitrate and nitrite content (NO_3^- , NO_2^-), total phosphorous (P), phosphates (PO_4^-), metal content, with particular attention to the lead content.

Table A – Soil characterization

Parameters	Soil Pb_0	Soil Pb_1	Soil Pb_2
Moisture	7%	8%	8%
pH	7.9	7.8	7.5
Conductivity (μS/cm)	100	126	150
N total (g/Kg)	0.28	0.39	0.28
$N\text{-}NH_4^+$ (mg/Kg)	n.d.	3.5	4.2
$N\text{-}NO_2^-$ (mg/Kg)	n.d.	0.005	0.048
$N\text{-}NO_3^-$ (mg/Kg)	0.63	0.25	0.63
P total (mg/Kg)	643	750	873
$P\text{-}PO_4^-$ (mg/ Kg)	n.d.	n.d	n.d

Table B - Tap-water characterization

Parameters	Irrigation water
pH	7.6 ± 0.6
Conductivity (mS cm^{-1})	0.35 ± 0.04
Oxidability (mg dm^{-3} O_2)	1.5 ± 0.3
$N\text{-}NH_4$ (mg dm^{-3} N)	0.14 ± 0.03
N-Total (mg dm^{-3} N)	0.24 ± 0.03
Nitrates (mg dm^{-3} N)	9.1 ± 0.3
Nitrites (mg dm^{-3} N)	0.010 ± 0.003
Phosphates (mg dm^{-3} P)	0.048 ± 0.003
Total Phosphorus (mg dm^{-3} P)	0.078 ± 0.007
Al (mg dm^{-3})	<0.037
As (μg dm^{-3})	<0.32
Cd (mg dm^{-3})	<0.004
Cr (mg dm^{-3})	<0.007
Cu (mg dm^{-3})	0.092 ± 0.007
Fe (mg dm^{-3})	<0.007
Hg (μg dm^{-3})	<0.89
Mn (mg dm^{-3})	<0.003
Ni (mg dm^{-3})	<0.007
Pb (mg dm^{-3})	<0.009
Zn (mg dm^{-3})	1.01 ± 0.03

Table C - Pb-Sludge characterization

Parameters	Pb sludge
Moisture	16%
pH	9.1
Conductivity (μS/cm)	160
Organic matter	98% (dry matter)
Ash content	2% (dry matter)
N total (mg/Kg)	84
$N\text{-}NH_4^+$ (mg/Kg)	n.d.
$N\text{-}NO_2^-$ (mg/Kg)	n.d.
$N\text{-}NO_3^-$ (mg/Kg)	n.d.
P total (mg/Kg)	149
$P\text{-}PO_4^-$ (mg/Kg)	42
Pb (g/Kg)	106

Compared to the control, which was not added any quantity of lead (Pb0), in the thesis PbI 450 mg Pb DM kg-1, was added, and in the thesis PbII 900 mg Pb DM kg-1, corresponding respectively to the maximum quantity of lead in soil permitted by Portuguese Law and reported in Annex I of the Decree Law No. 276 /09 (2009), and to the double concentration of the same in order to simulate a high level of contamination. With regard to the second factor, the study predicted 3 water levels: 238 mm (I1), 475 mm (I2) and 950 mm (I3), corresponding respectively to the optimum water requirement of Giant reed (Arundo donax L.) (475 mm) (Dalianis et al., 2005), the double (950 mm) and the middle (238 mm).

In each pot two rhizomes of Arundo donax L., taken from the University Campus of the Faculty and selected based on the number of buds present lives, in order to make more homogeneous the experiment were planted on May 2012.

After transplanting, the pots were fertilized with 3 g N m-2 (Urea, 46% N) , 3 g N m-2 (Nitrolusal, mix NH4NO3 + CaCO3, 27% N), 17 g K2O m-2 (potassium sulphate, 51 % K_2O), 23 g P_2O_5 m^{-2}.

Irrigation was carried out weekly from the month of May 2012 (transplant) and up to January 2013 (harvest of aboveground part of plants), at a rate of 120% , 60% and 30 % capacity field of each pot.

At the end of the growing season (January 2013), the plants were harvested and on them, were determined morpho-biometric characters and yield. In particular were determined: the number of stems, the height of the main stem, the basal diameter, the number of nodes per stem and the weight of stems and leaves for the determination of the aerial biomass.

On the fractions stems and leaves were also carried out chemical analyzes on, ash content, total content of nitrogen (N) and phosphorous (P) and metal content, with particularly attention for lead content. The latest parameters were determined through the use of the atomic absorption spectrometer after digestion with nitric acid of the ash obtained.

After the first year of the experiment an amount of soil was taken from the pots of each thesis, at two different depths (0-20 cm and 20-40 cm), in order to identify any differences and the degree of mobility of the lead, following the various water content of the soil.

On the soil sample from each pots were made the following analysis: moisture, pH, conductivity, organic matter, total content of nitrogen (N) and phosporous (P), ammonia content (NH_4^+), nitrate and nitrite content (NO_3^-, NO_2^-), phosphates (PO_4^-), metal content, total microorganisms content and eco-toxicity essay through the system Microtox ®.

On September, 2013, the plants were cut and re-analyzed according to the same parameters determined in the previous year. The soil was taken from each pot with the same criteria used in the first year, at two different depths (0-20 cm and 20-40 cm) from each thesis and characterized through the same analysis carried out in the first year.

To understand the real phyto-extractive capacity of *Arundo donax* L., at the end of the second growing cycle (September 2013), the rhizomes of each thesis were taken, and for each of them length and weight for the determination of the root biomass were determined and at the same time on the same, chemical analyzes concerning ashes content, total nitrogen (N) and phosphorous (P) content and the metals content , with particularly regard to the concentration of lead present, were performed.

At the beginning of the vegetative cycle of the plants, and at the moment of the harvest of the plants, leachates from each pot were collected, with the aim of monitoring any loss of elements. On collected and filtered leachates were analysed the following parameters: pH, conductivity, ammonia nitrogen (NH_4^+), nitrate and nitrite content (NO_3^-, NO_2^-), phosphates (PO_4^-), metal content and especially lead content, were determined. In addition to this analysis, on the same collected samples, the number of microorganisms present and the essay of eco-toxicity were performed.

Regarding to the tools used during the test, they are summarized in Table 1, while Table 2, 3, 4 and 5 show the protocols followed for the determination of analysis required for the soil (Tables 2 and 3) and for the leached (Tables 4 and 5) and for for biomass and sludge (Table 6 and 7).

The data obtained were subjected to statistical analysis of ANOVA by means of CoStat software (version 6.0) and the averages were separated according to the test of Student-Newman-Keuls (SNK) for $p \leq 0.05$.

2.4. Research lines 4: Phytoremediation of different *Arundo donax* L. clones in lead-contaminated soils

This research was carried out at the "Department of Science and Technology of Biomass", belonging to the "Faculty of Science and Technology" of the "New University" of Lisbon during the year 2013, using four genotypes studied in Catania experiments and selected from a previous screening concerning the salinity resistance or susceptibility (Research line 1). The aim of this research was to evaluate the response of different *Arundo donax* clones to increasing lead contamination.

The adopted methodology was the same described in Research line 3 with the exception of the irrigation level; in these research line, it was adopted only the maximum water level (I_3) corresponding at 950 mm.

Protocols followed in the analysis performed on soil, sludge, tap water, leachates, biomass

Determination of pH

- Soil and Sludge (D. Baize, 2000)

Experimental procedure:

1) Calibrate the instrument through the use of standard solutions of pH;
2) Weigh 5 grams of soil/sludge in a beaker and add 25 mL of distilled water. Shake for about 30 minutes and read immediately with a suitable pH glass electrode (micropH2001, Crison).

Alteration of the Protocol: add 50 mL of distilled water instead of 25 mL.

- Tap-water and Leachates (APHA, AWWA and WPC, 1985)

Experimental procedure:

1) Calibrate the instrument through the use of standard solutions of pH;
2) Read the value on the pH glass electrode (micropH2001, Crison), immersed in the water sample.

Determination of Electrical conductivity

- Soil and sludge (D. Baize, 2000)

Experimental procedure:

1) Weigh 5 g of soil/sludge in a beaker and add 25 mL of distilled water. Shake for about 30 minutes and read the conductivity with a conductivity electrode (MC226, Mettler Todelo - InLab 730 Conductivity).

Alteration of the Protocol: add 50 mL of distilled water instead of 25 mL.

- Tap water and leachates (ISO 7888, 1985)

Experimental procedure:

1) Immerse the conductivity electrode in the liquid sample and read the value

Determination of moisture content

- Soil and Sludge
- Biomass

Experimental procedure:

1) Calibrate weighting filters in the oven (WTC binder 7200 TUTTLINGEN Germany) at 101 °C±1 °C for 1 hour. Allow to cool 1 hour or more if necessary. Weigh into the analytical balance (Ohaus Explorer Pro Libra);
2) In the filter weighing, weigh accurately (with the precision of 0.0001 g) 1 g of a soil sample (or approximately 2 g of a plant sample). Weigh the weighing filter with the sample;
3) Put the filter in an oven with the sample and leave it overnight at 101 °C±1 °C. The next day, transfer the weighting filter with the sample from the oven to the dryer and allow it to cool for at least half an hour or more if necessary and weigh in analytical balance (Balance Ohaus Explorer Pro).

Expression of results:

$$H20\ \% = \frac{P2-P3}{P2-P1} * 100$$

where:

P1 = weighing filter tare

P2 = weight of the sample of moist soil/sludge (wet plant sample)

P3 = weight of dry soil/sludge (dry plant sample)

Determination of organic carbon (Walkley, A. and Black, IA, 1934)

- Soil

Experimental procedure:

1) Weigh 1 g of soil (Balance Ohaus Explorer Pro), add 10 mL of K_2Cr_2O 0.25 N, 15 mL of concentrated H_2SO_4 (95-97 % , Sigma -Aldrich) , a pinch of $HgSO_4$, and some regulatory spheres boiling;

2) Digest for two hours at 135 ° C in the digester;

3) Transfer the contents into a erlenmeyer flask , add deionized water to 100 ml , 3 drops of ferroin indicator and titrate with 0.25 N ammonium ferrous sulfate (Panreac) until reaching a red- chestnut color. Make a " white " at the same time.

4) Determining the exact title from the solution of ferrous sulfate ammonia: in a erlenmeyer flask, add 10 ml of $K_2Cr_2O_7$ (Panreac), 100 mL of deionized water, 25 mL of concentrated H_2SO_4 , 3 drops of ferroin (1.485 g 1-10 phenanthroline-monohidratata) and titrate with ammonium ferrous sulfate.

$$\text{Ferrous sulphate ammonia title(N)} = \frac{\text{0,25 N} * \text{10 mL}}{\text{Volume ferrous sulphate}}$$

Alteration of the Protocol:

In step 1) in some situations , the amount of soil or the volume of $K_2Cr_2O_7$ ore the amount of H_2SO4 were modified.

Expression of results:

$$\%C = \frac{(\text{Volume title white sample} - \text{Volume title sample}) * \text{Title ferrous sulphate} * 0{,}3}{\text{Soil weight (g)}}$$

$$\text{Organic matter} = 1{,}724 * (\%\ \text{C})$$

Reagents used:

- $K_2Cr_2O_7$ 0.25 N: dissolve 12.3 g of potassium dichromate in 1000 mL of distilled water
- Ferrous sulphate ammonia 0.25 N: Dissolve 98 g of $Fe\ (NH_4)_2(SO_4)2.6H_2O$ in distilled water , add 20 mL of concentrated H_2SO_4. Allow to cool and dilute to 1000 mL with distilled water.
- Ferroin : add 1.485 g of 1.10- phenanthroline-monohidrata to 0.695 g of $FeSO_4.7H_2O$ and dilute in 100 mL of distilled water.

Extraction for ammonia nitrogen (NH_4^+), (Haigh, M. and Dyckhoff, C, 1996), **nitrate** (NO_3^-)) **and nitrite** (NO_2^-) **content** (Palintest ® test instructions) (Jenkis, P. et al ., 1996)

- Soil and Sludge

Experimental procedure:

1) Weigh 40 g of soil, add 100 mL of 1 M KCl solution, shake for 1 hour and filtered. The sample is ready for determination of ammoniacal nitrogen according to the experimental protocol for the determination of nitrogen. The nitrates and nitrites are determined through the use of Palintest.

Alteration of the Protocol:

Weigh 20 g of soil and not 40 g.

Determination of ammoniacal nitrogen (NH_4^+)

- Soil and sludge (After previous extraction) (Haigh M. and Dyckhoff C., 1996)
- Leachates (ISO 5664, 1984)

Experimental procedure:

1) In a distillation tube add a measured volume of sample (100 mL), three drops of phenolphthalein (solution at 1 % , Panreac). (The volume of the sample may also be another on condition that the total is equal to 100 mL);

2) Subsequently, proceed with the alkalinization of the medium through the addition of a solution of sodium hydroxide (NaOH) 6N (Merck p.a.), up to which the solution reach a pink color.

3) In a 250 mL erlenmeyer flask, add 50 mL of boric acid (Riedel-de-Haën 99,8% p.a.) and 0.5 mL of indicator solution (0.2 g of methyl red Merck) in 100 mL of 95 % alcoholic solution+0.1 g of methylene blue (Panreac 82%), in 50 mL of alcoholic solution); (the alcoholic solution was prepared from a solution of ethanol, Panreac 96%);

4) Place the tube of distillation and the erlenmeyer flask in the distiller (Kjeltec 1002 Distilling System Unit de Tecator) and distilled until a volume of distillate in the erlenmeyer flask of 200 mL (During the distillation, in the case of nitrogen is present in the sample, the color of the sample in the flask turns from purple to green);

5) Titrate the distilled with sulfuric acid (0.02 N, Carlo Herba Reagent; 37%) until the solution change from green to purple again. If after the distillation, the distillate remains purple, is not necessary to titrate.
6) Record the volume of the titrant spent during the titration.

Chemical reactions:

The sample is steam distilled , the distillate is collected in a erlenmeyer flask containing a solution of boric acid indicator. When heating up to boiling, the ammoniacal nitrogen is released in the form of gas and transported through the steam, as shown in the following equation:

$$NH_4^+ + H_2O \rightarrow NH_3 + H_3O^+$$

After the condensation of the steam , the ammonia reacts with boric acid to form the ammonium ion and borate ion, as is shown in the following equation:

$$NH_3 + H_3BO_3 \rightarrow NH_4^+ + H_2BO_3^-$$

Alteration of the protocol:

In step 5 the distillate was titrated with chloridric acid (0.02 N)

Expression of the results:

- For leached :

$$\text{Ammoniacal nitrogen } \left(\frac{\text{mg}}{L}\text{N}\right) = \frac{0{,}02 * 14{,}01}{v2} * 1000 * v1$$

Where:

v1 = Volume of titrant spent in titration (mL)

v2 = volume of the sample used in the distillation (mL)

- For soil and sludge :

$$\text{Ammoniacal nitrogen } \left(\frac{\text{mg}}{\text{Kg}}\text{N}\right) = \frac{0{,}02 * 14{,}01}{v2} * 1000 * v1 * v3/m$$

where:

v1 = the volume of titrant spent in titration (mL)

v2 = volume of the sample used in the distillation (mL)

v3 = volume of 1M KCl solution (mL, 100mL)

m = mass of soil/sludge (g)

Determination of Nitrate (NO_3^-) **and Nitrite** (NO_2^-) (Palintest instructions, ISO 6777, 1984)

The method is based on a first reduction of nitrate to nitrite, and the resulting nitrite is determined by a reaction that leads to the formation of a pinkish color. The reduction step occurs when zinc and a tablet of nitratest are added to the water sample, which leads to a rapid flocculation after one minute of contact. The nitrite resulting from the reduction phase is determined for the reaction of sulphanilic acid ($C_6H_7NO_3S$) in the presence of N-(1-naphthyl)-ethylenediamine (NED) with formation of a blue dye color. The reagents are contained in the tablet nitricol.

Nitrite (NO_2^-)

- Soil / Sludge (extract)
- Leached (waters)

Experimental procedure:

1) Pipette 10 mL of sample extract (soil/sludge) or water collected directly from the pots into two cells of Palintest.

2) In one of the two Palintest cells, add a tablet nitricol (Palitest), crush and wait 10 minutes for the dissolution of the color.

3) Record the concentration in mg/L N in the molecular absorption spectrophotometer (Interface Photometer 7000) at a wavelength of 520 nm.

Expression of the results:

- For waters, leached

$$\text{Nitrites } (\frac{\text{mg}}{\text{L}}, \text{N}) = read\ value\ (\frac{mg}{L})$$

- For soils / sludge

$$\text{Nitrites}\left(\frac{\text{mg}}{\text{Kg}},\text{N}\right) = \frac{read\ value\ \left(\frac{mg}{L}\right)}{m} * v1$$

where:

v1 = volume of 1M KCl solution (mL, 100mL)

m = mass of soil/sludge (g)

Nitrates (NO_3^-)

Experimental procedure:

1) In a 50 mL flask, pipette 20 mL of sample and add a pinch of zinc (Palitest);

2) Add one tablet of nitratest (Palitest) and mix for about 1 minute until complete dissolution; wait for about 2 minutes;

3) Filter in a glass and pipette 10 ml of the filtrate into a cell of Palintest. Add one tablet of nitricol, crush and wait 10 minutes for the dissolution of the color;

4) Record the concentration in mg / LN in the spectrophotometer molecular absorption (Interface Photometer 7000) at a wavelength of 570 nm.

Expression of the results:

Nitrates content was determined by the difference with the determination of nitrites content.

Determination of Total Phosphorus (Watts, S. and Halliwell, L., 1996)

Preparation of solutions and reagents :

- Phosphorus stock solution : Weigh 219.5 mg of anhydrous KH_2PO_4 (Riedel-de-Haën; 99,5%) in a volumetric flask and dilute to 1000 mL with ultra-pure water, resulting in a solution of 50 mg (P) L^{-1} L. Store in a refrigerator.
- Phosphorus Standard Solution: Dilute 2 ml of the stock solution (50 mg (P) / L) in a 100 mL volumetric flask with ultra-pure water (1 mg (P)/L). This solution must be prepared on the same day of the determination.
- Solution of ammonium molybdate: Dissolve 20 g of ammonium molybdate $(NH_4)6Mo7O_{24}.4H_2O$) in a volumetric flask with ultra-pure water. Store in a refrigerator.

- H_2SO_4 - Dilute, 70 mL of concentrated sulfuric acid (H_2SO_4) in distilled water in a volumetric flask of 500 mL.
- Solution of Potassium Tartarato and Antimony - Dissolve 1.4 g of potassium solution, tartarato and antimony ($K(SbO)C_4H_4O_6.0.5H_2O$) in a volumetric flask of 500 mL with ultra-pure water.
- Reducing agent - Dilute 100 mL of 5N H_2SO_4 , 30 mL of ammonium molybdate solution, 10 mL of a solution of potassium, antimony and tartarato and dissolving 1.04 g of ascorbic acid in a volumetric flask of 200 mL with ultra-pure water.
- NaOH - Dissolve 240 g of NaOH in a 800 mL of distilled water. Cool to room temperature and dilute to 1000 mL with ultra-pure water.
  - Biomass
  - Soil
  - Sludge

Experimental procedure:

1. Digestion:
   a) Weigh strictly in an analytical balance (Ohaus Explorer Pro Libra) about 1 g of sample in a tube of digestion;
   b) Add 10 mL of H_2SO_4 (95-97%, Sigma Aldrich) and a dose of catalyst mix composed of selenium and potassium sulphate (2 g of selenium and 200 g K_2SO_4, Riedel-Haen 99%, Panreac 99.0%, respectively) and some regulatory spheres boiling;
   c) Put tubes of digestion under hood and let to warm the digester (Tecator 2006 digestor) at a temperature of 360 ° C until the sample becomes clear or colorless;
   d) Turn off the heat and leave to cool the tube at room temperature;
   e) Add 50 mL of distilled water and transfer the solution with the sample digested in a volumetric flask of 100 mL;
   f) Rinse the tube digestion and make up to volume with distilled water.

2 . Calibration

a) For the calibration curve it is necessary to prepare a series of standard solutions of phosphates with different concentrations: 0, 0.10, 0.20, 0.30 mg/L (P), from the standard solution of phosphates;

b) Transfer through the use of pipettes 10.0, 20.0, and 30.0 mL of standard solution of phosphorus
(1 mg (P)/L) in a series of 100 mL volumetric flasks;
c) Add 8.0 mL of reducing agent; dilute to 100 mL with ultra-pure water. Also prepare a zero with only 8 mL of reducing agent and diluted with ultra-pure water.
The standard solutions correspond to 0.0, 0.10, 0.20, 0.30 mg (P) L^{-1}.
d) Measure the absorbance of each solution in the molecular absorption spectrophotometer (Shimadzu Spectrophotometer UV-120-11) after 20 minutes at a wavelength of 880 nm.
e) Draw a graph of the absorbance as a function of phosphorus content in mg/L (P), for the standard solutions.
The relationship between absorbance and concentration is linear: Y = ax + b,
where:
y = absorbance at a wavelength of 880 nm
and
x = concentration in mg/L

3. Preparation of samples
a) Put a dose of the digested sample (<40 mL , V0) in a 100 mL volumetric flask and add a small volume of ultra-pure water (20 mL) and a few drops of phenolphthalein solution.
b) Carefully add 6N NaOH until achieving a pink color; add 8.0 mL of reducing agent and dilute to 100 mL with ultra-pure water.
c) After 20 minutes, read the absorbance at a wavelength of 880 nm, using the zero reference cell (A_1). Proceed as described for the determination of phosphorus in a blank test (A_0).

Expression of results:

$$\% \text{ phosphorous} = \frac{(x1 * v1 * b1)}{(v2 * p1)}]/10^4$$

where,
v1 = the volume of the flask used in measuring the absorbance (mL);

v2 = volume of the digested sample (mL) and used in the reaction with the reducing agent;

x1 = the value in mg/L (P) obtained from the calibration curve, using the absorbance value (880 nm) measured; determination by difference with the white;

b1 = volume of the flask where the sample has been stored the sample digested;

p1 = dry mass of the sample used in the digestion (g).

Determination of phosphates (PO_4^-) (FS Watanabe and SR Olsen, 1965; ISO 6878-1)

- Waters and leached

The phosphates (PO_4^-) are determined in the waters or in leachets by molecular absorption spectrometry, through the formation of a colored complex with a solution of ammonium molybdate in the presence of ascorbic acid and potassium tartarato and antimony.

In an acid medium and in the presence of ammonium molybdate, the ortho-phosphates form a complex of phospho-molybdate, which reduced for the presence of ascorbic acid, develops a blue color, susceptible of colorimetric test.

The readings from the molecular absorption spectrophotometer and the calibration curves were carried out in agreement with the experimental procedure of phosphate extractable, with the exception of the extraction procedure (which is not performed in this protocol).

Experimental procedures:

1. Preparation of reagents

- Reducing agent: for 500 mL, add 250 mL 5N H_2SO_4 (Fisher Chemical, 95%), 75 mL of antimony molybdate (Riedel-de-Haën), 2.6 g of ascorbic acid (Fisher Scientific) and 25 mL of potassium tartarato and antimony (Chem-Lab), make up to volume with ultra-pure water.
- Stock solution of phosphate: Weigh 219.5 mg of KH_2PO_4 (Riedel-de-Haën; 99,5%) and dilute to 1000 mL with ultra-pure water.
- Phosphate standard solution: Pipette 10 mL of the stock solution of phosphates and dilute to 500 mL with ultra-pure water.

2. Preparation of the calibration curve

a. For the calibration curve, prepare different standard solutions of phosphates with different concentrations: 0, 0.10, 0.20, 0.30 mg/L (P) , from the standard solution of phosphates;

b. In a 100 mL volumetric flasks , pipette a volume corresponding to the standard solution, add 8 mL of reducing agent and bring to volume with ultra-pure water;

c. After 20 minutes, read the absorbance of each solutions at the molecular absorption spectrophotometer (Shimadzu Spectrophotometer UV -120 -11) at 880 nm.

d. Plot a graph of the absorbance as a function of the concentration, in mg/L (P) , for the standard solutions. The relationship between absorbance and concentration is linear.

3. Preparation of samples:

a) In a 100 mL volumetric flask , add a measured volume of sample, 8 mL of reducing agent and bring to volume with ultra-pure water. Wait 20 minutes for the development of the blue color.

b) Reading the absorbance in a molecular absorption spectrophotometer (Shimadzu Spectrophotometer UV -120- 11) at a wavelength of 880 nm.

Expression of results:

$$\text{Phosphates (mg/L (P))} = \frac{x * v1}{v2}$$

Where:

v1 = volume of the flask (mL);

v2 = volume of the sample used (mL);

x = concentration in mg L-1 (P) obtained from the calibration curve;

Determination of extractable phosphates (Olsen, SR et al. , 1954 Watanabe, FS and Olsen, SR , 1965)

Experimental procedure:

1 . Extraction:

Weigh 0.5 g of soil or sludge in an analytical balance (Scales Ohaus Explorer Pro), put it in an erlenmeyer flask and add 100 mL of sodium bicarbonate (Panreac). Shake for about 30 minutes and filter.

2 . Determination:

The phosphates are determined in the sample , by molecular absorption spectrometry, through the formation of a colored complex with a solution of ammonium molybdate, in the presence of ascorbic acid, potassium tartarato and antimony according to the following phases:

a) In a 100 mL volumetric flask , add a measured volume of sample, 8 mL of reducing agent (250 mL of 5N H_2SO_4 + 75 mL of ammonium molybdate (Riedel-de-Haën ultra-pure, 99.9% p.a., 40 g L^{-1}) + 2.6 g of ascorbic acid (Merck 99.79 %) + 25 mL of potassium tartarato and antimony (Merck pure, 2.8 g/L in 500 mL) and dilute to volume with ultra-pure water . Wait for about 20 minutes for the dissolution of the blue color.

b) Read the absorbance of each sample in the molecular absorption spectrophotometer (Shimadzu Spectrophotometer UV -120- 11) to a wavelength of 880 nm after the preparation of the calibration curve.

3. Preparation of the calibration curve:

a) From a stock solution of 1 mg/L (P), prepare standard solutions at different concentrations: 0, 0.05, 0.10 , 0.15, 0.20 , 0.25 mg/L (P);

b) In a 100 volumetric flask , pipette a volume corresponding to the standard solutions, add 8 mL of reducing agent and bring to volume with ultra-pure water;

c) After 20 minutes, read the absorbance with the molecular absorption spectrophotometer (Shimadzu Spectrophotometer UV-120-11) for each solution at a wavelength of 880 nm;

d) Plot a graph of absorbance as a function of the concentration, in mg/L (P) for the standard solutions. The relationship between absorbance and concentration is linear.

Alteration of the protocol:

The sample and the reducing agent were added in a 50 mL volumetric flask instead of 100 mL volumetric flask.

Expression of results:

$$\text{Extracts Phosphates}\left(\frac{\text{mg}}{\text{Kg}}(\text{P})\right) = \frac{x * v1}{v2} * v3/m$$

Where,

v1 = volume of the flask (mL)

v2 = volume of the sample used (mL)

x = concentration in mg L^{-1} (P) obtained from the calibration curve

v3 = volume of sodium bicarbonate (mL) (100 mL)

m = mass of soil/ sludge (g) (0.5 g)

Determination of total nitrogen (Watts, S. and Halliwell, L., 1996)

Solutions:

- 6N NaOH - Dissolve 240 g of NaOH in about 800 mL of distilled water. Cool to room temperature and dilute to 1000 mL with distilled water.
- 0.02 N H_2SO_4 - Dilute 200 mL of 0.1 N H_2SO_4 in a container volume of 1000 mL with distilled water. This solution should be standardized by normal analytical procedures
- H_2SO_4 0.1 N - This solution must be prepared by diluting 2.8 mL of concentrated sulfuric acid in a 1000 mL volumetric flask. This solution should be standardized by normal analytical procedures.
- Boric Acid - Dissolve 20 g ± 1 g of boric acid (H_3BO_3) in hot distilled water . Allow to cool to room temperature. Dilute to 1000 mL with distilled water in a volumetric flask of 1000 mL.
- Indicator solution - Dissolve 0.2 g of methyl red in 100 mL of a solution of ethylene content (95%). Dissolve 0.1 g of methylene blue in 50 mL of ethylene alcohol to 95%. Mix the two solutions. Store in a refrigerator.

- Soil
- Sludge
- Biomass

Experimental procedure:

1. Digestion

a) Weigh strictly in an analytical balance (Ohaus Explorer Pro Libra) about 1 g of dry sample into a digestion tube .

b) Add 10 mL of H_2SO_4 (95-97 % , Sigma Aldrich) and a dose of catalyst mix composed of selenium and potassium sulphate (2 g Se and 200 g K_2SO_4, Riedel - Haen 99% , Panreac 99.0% , respectively) and some regulatory spheres boiling.

c) Put tubes of digestion under a hood and let to warm the digester (Tecator 2006 digestor) at a temperature of 360 ° C until the sample becomes clear or colorless.

d) Turn off the heat and leave to cool the tube at room temperature.

e) Add 50 ml of distilled water and transfer the sample digested in a 100 mL volumetric flask. Rinse the tube digestion, cool, dilute to the mark with distilled water and mix thoroughly.

2. Determination

a) Put a dose of digested sample in the distillation tube (± 50 mL , or x) , add a volume of distilled water to reach 100 mL (± 50 mL, or (100 -x)). Add a few drops of phenolphthalein solution (solution at 1 %, Panreac).

b) In a 250 mL erlenmeyer flask , add 50 mL of boric acid (Riedel-de-Haën 99,8% p.a.) and 0.5 mL of indicator solution (0.2 g of methyl red (Merck) in 100 mL of 95 % alcoholic solution + 0.1 g of methylene blue (Panreac 82%), in 50 mL of alcohol solution); alcoholic solution was prepared from a solution of ethanol (Panreac 96%).

c) Subsequently, proceed with the alkalinization of the medium through the addition of a solution of sodium hydroxide (NaOH) 6N (Merck p.a.), up to which the solution achieve a pink color.

d) Place the tube of distillation and the erlenmeyer flask in the distiller (Kjeltec 1002 Distilling System Unit de Tecator) and distilled until a volume of distillate in the erlenmeyer flask of 200 mL (During the distillation, in the case of nitrogen is present in the sample, the color of the sample in the flask turns from purple to green).

e) Titrate the distilled with sulfuric acid (0.02 N, Carlo Herba Reagent; 37%) until the solution change from green to purple again . If after the distillation, the distillate remains purple, is not necessary to titrate.
f) Record the volume of the titrant spent during the titration.

Proceed as described for the digestion and determination of nitrogen for the preparation of the blank test.

Expression of results:

$$\%\ \text{nitrogen} = \frac{(V1 * N * b1)}{(V2 * m1)} * 14.01$$

where:

V1 = volume of H_2SO_4 0.02 N, used in titration (mL) by subtracting the volume in mL of acid used for titration of the blank test

V2 = volume of the digested sample used in the distillation (mL)

b1 = volume of the flask where it was digested (mL)

N = normality of titrant

m1 = mass of dry sample, which is used in digestion (g)

Determination of ash content:

- Biomass
- Sludge

Experimental procedure:

1) Preparation of the dish - put a clean ceramic capsule in a muffle furnace at 550 ° C ± 50 ° C for about 1 hour. Cool in a desiccator until the use. Weigh immediately before use in an analytical balance capable of weighing up to 0.1 mg (P1);
2) Preparation of sample - put a certain amount of dry material (usually 1 g) in a ceramic capsule before prepared and weigh the whole in an analytical balance (Ohaus Explorer Pro Libra) (P2);
3) Transfer the weighed sample in the capsule in the muffle furnace and burning at 550 ° C ± 50 ° C for 2 hours. Cool in desiccator and weigh (P3);

4) Repeat the cycle of burn, cooling, drying and weighing until it reaches a constant weight or until the loss in weight is less than 4% of the previous weight.

Expression of results:

$$\% \text{ ashes } = \frac{P3 - P1}{P2 - P1} * 100$$

Extraction of metals in aqua regia

- Soil and Sludge

Experimental procedure:

1. Digestion with aqua regia: weigh 1 g of soil in a tube of CQO and add 10 mL of aqua regia . Each tube is placed in the heating block at 140 ° C for about 4 hours. Every 30 minutes, mix the tubes placed in the block to ensure that the soil is well digested. Make a white at the same time.
2. At the end of the 4 hours, add 20 mL of ultra-pure water and carefully filtered in a flask of 50 mL.
3. Wash all the material and the filter with ultra-pure water . Store in a plastic flask for subsequent reading of metals using atomic absorption spectrophotometer.
4. Determination of metals in either brute or in the dilutions with Atomic absorption spectrophotometer.

Preparation of aqua regia: 3 parts of HCl and 1 part of concentrated HNO_3 (eg. in 10 mL of solution, 7.5 mL of concentrated HCl and 2.5 mL of concentrated HNO_3.

Dilutions:

1/10 - Dilute 5 mL of the digested sample to 50 mL in a volumetric flask with ultra-pure water

1/100 - Dilute 5 mL of 1/10 dilution to 50 mL in a volumetric flask with ultra-pure water

Determination of metals

- Waters and leached (APHA , AWWA and WPCF , 1985) (only to read the filtrates the solutions or dilutions)
- Biomass

Experimental procedure :

1. Preparation of solutions :
   a) HNO_3 1:1 - Dilute 500 mL of concentrated HNO_3 to 1000 mL with ultra-pure water
   b) 3.25% HNO_3 - Dilute 25 mL of concentrated HNO_3 to 500 mL with ultra-pure water

2. Digestion:

a) Dissolve the ash in 25 mL of 1:1 HNO_3 . Transfer the capsules containing the ashes , covered with a glass disk in a steam bath and allowed to evaporate to about 5-10 mL (approximately 20 minutes).

b) Strain into a volumetric flask of 100 mL , rinse the capsule containing the ashes and the glass disc with ultra-pure water and add the residues of washing in the volumetric flask.

c) Allow to cool and make up to volume with ultra-pure water. Mix throughly.

3. Dilutions of the solution obtained

1/10 - Dilute 5 mL of the digested sample to 50 mL in a volumetric flask with ultra-pure water

1/100 - Dilute 5 mL of 1/10 dilution to 50 mL in a volumetric flask with ultra-pure water

Use parts of this solution or the diluted for the determination of metal required.

4. Determination of the metal concentration

Reading of the concentration of metals through the atomic absorption spectrophotometer (Espectrofotómetro de absorção Atómica Solaar Thermo Electron Corporation M series) with acetylene flame and acetylene/ nitrous oxide, depending on the metal to be analyzed.

The calibration was performed by measuring standard solutions of metals with mono - elementary.

These solutions were prepared from standard solutions of 1000 mg L^{-1}, certified.

Expression of results:

$$\text{Concentration of metal } (\frac{\text{mg}}{\text{kg}}) = \frac{A * B}{C}$$

where:

A = concentration of the metal in the digested solution, mg L^{-1}

B = final volume of the digested solution, mL

C = g of sample, (P2-P1), obtained by the determination of ash.

Counting of Total vital microorganisms at 22 ° C

- Soil (on dilutions L / S = 10 with sterilized water) (ISO 6222 , 1999; EN ISO 87-1 , 1999)
- Waters and leached (on sample) (ISO 8199, 2005; ISO / TC 11133-1, 2000; ISO TC/11133-3, 2003; NP 1828, 1982; NP 1829, 1982; NP 2079, 1989)

Experimental procedure :

1 . Collect samples for microbiological analysis, according to the requirements laid down by the Portuguese legislation NP 1828 (1982), using for that tools by made of inert, cleaned and sterilized material. The collection must be done with necessary precaution under aseptic conditions and in a way to maintain the microbiological characteristics of the samples. These ones must be analyzed in a space of time less than 24 hours, during which they must be stored at 0-4 ° C , as reported in the legislation NP 1828 (1982). The sample preparation is performed in accordance with the Portuguese legislation NP 1829 (1982) using inert, cleaned and sterilized material and in order to ensure a perfect uniformity of distribution of the microorganisms and with extreme accuracy and aseptic conditions with the aim to avoid any kind of contamination.

2 . Counting of vital microorganisms at 22 °C: sowing by incorporation of a certain amount of suspension of the respective mother and decimal dilutions , in an

appropriate medium (Plate Count Agar, Becton, Dickinson and Company) . Incubation of seed plates for 68 hours ± 4 hours at a temperature of 22 °C± 2 °C in aerobic conditions. Calculation of the number of vital microorganisms per g of soil, or for mL solution of water /leached from the number of colonies developed in the plates selected.

Determination of biochemical oxygen deficiency after 5 days (BOD5) (ISO 5815-1, 2003; ISO 5815-2, 2003)

- Soil respiration after five days (BOD5) - Respirometric method (ISO 1672 , 2002)

The shortage(deficiency) biochemical oxygen demand (BOD) is defined as the amount of dissolved oxygen , expressed in mg L^{-1} of O_2 , which is consumed during a certain number of days from aerobic microorganisms (inoculated or already present in the test solutions) to decompose (oxidize) in the dark and at a temperature of 20 °C the organic substances present in a liter of water or aqueous solution.

It is an indirect measure of the content of biodegradable organic matter present in a sample of water or aqueous solution and is one of the most commonly used parameters to estimate the pollutant load of wastewater.

It is normally expressed in mg O_2 L^{-1}, consumed in five days (120 hours) by aerobic microorganisms; the test is normally performed in the dark to prevent the developing of photosynthetic processes, at a temperature of 20 °C and for a period of time of 5 days (120 hours).

For both measurement of BOD_5 (leached and soil), has been used the method respirometric (System OxiTop ®). This system provides the oxygen consumption, measured by measuring the decrease in pressure of oxygen, and the absorption of carbon dioxide , in a certain number of days (5), generated in the containers of the samples for the addition in them of sodium hydroxide (NaOH).

Experimental procedure:

1. The sample is placed in a container equipped with a differential pressure gauge and tightly closed to prevent the exchange of O_2. Screwing like a top on the appropriate dark glass bottle, The BOD meter OxiTop , detects the pressure in the gap between

the gap and the interface of the liquid, using a small transducer connected to a microprocessor. In the course of biological degradation of the organic content it has O_2 consumption, and this generates a depression in the gas , measured by the manometer. If previously calibrated, the gauge immediately returns the value of BOD of the sample. In this test interference is present linked to the production of carbon dioxide; with the aim to remedy this, sodium hydroxide, which removes the CO_2 chemically, is added to the gaseous phase.

2. The bottle , is positioned on a magnetic stirrer suitable to be introduced in an incubator , where the sample remain for a certain number of days determined (5) at a temperature of 20 °C.

The value that appears on the display present on ' OxiTop is expressed in mg L^{-1} , and refers to a volume of sample equal to 432 mL , for a measuring range of 0-40 mg L^{-1}. For ranges above, it should make use of specific correction factors multiplied by the different quantities of sample.

Every 24 hours, OxiTop system stores the values of BOD, viewable through the end of the determination of buttons placed next to the display.

Expression of results:

- For waters:

$$\text{BODn original sample } \left(\frac{\text{mg}}{\text{L}}\right) = (\text{A}) * (\text{FD}) - (\text{B})$$

where:

A = BODn analyzed sample (mg/L) = beginning dissolved O_2 - end dissolved O_2

B = BODn white (mg/L) = beginning O_2 dissolved - end dissolved O_2

FD = Dilution factor

- For soils:

$$\text{BODn original sample} \left(\frac{\text{mg}}{\text{Kg}}\right) = \big((\text{A}) * (\text{FD}) - (\text{B})\big) * \text{V/m}$$

where:

A = BODn analyzed sample (mg/L) = beginning dissolved O_2 - end dissolved O_2

B = BoDN white (mg/L) = dissolved O2 beginning - end of dissolved O2

FD = Dilution factor

V = volume of the soil occupied in the beaker + water (432mL)

m = mass of soil (g)

Ecotoxicity Assay (De Vetter et al. , 2008; EN 12457-4 , 2002)

- Water and Leached (determined directly in the solutions)
- Soil and sludge

Experimental procedure:

To test the eco- toxicity of the samples, it is necessary to obtain the aqueous extracts of soil samples according to EN 12457-4 (2002). For a ratio of 10 L/kg , the samples are shaken at 150 rpm for 24 hours at 20 ° C. After this time, they are left to decant and the supernatant is filtered and the aqueous phase is filtered for the assay of acute toxicity using the Microtox® system.

The Microtox system is a bio-analyzer that uses luminescent bacteria *Vibrio fisheri* NRRL B- 11177 as tester organisms. Bioluminescence by *V. fischeri* is measured at self-calibrated spectrophotometer M500 (Microtox ®), at a temperature of 20 °C. The light signal is recorded initially and after 30 minutes of exposure to *V. fisheri* at different concentrations of aqueous extracts of soil samples, or waters and leachets. The luminous intensity after incubation is compared with that of the pure bacteria.

The toxicity is identified by reducing the intensity of light. The results are normalized , and the value EC_{50} (concentration that produces a 50% reduction of luminescence) is calculated with the Software MicrotoxOmni.

The EC50 value can be subsequently transformed into units of ecotoxicity (UT 's), in agreement with the formula $UT = 100/EC_{50}$.

In terms of eco-toxicity , the soils can be classified into non-toxic (UT <2), low toxicity (UT 's 2-4), slightly toxic (UT 's 4-8), toxic (UT 's 8-16) and very toxic (UT 's> 16) (De Vetter et al. , 2008).

Determination of Oxidability (COD) (APHA , AWWA and WPCF , 1985)

The test of the chemical oxygen deficiency (COD) is widely used in the quantification of the organic load of domestic and industrial waste waters , which is measured in terms of total amount of oxygen required for its oxidation to carbon dioxide and water.

The test is precise and accurate for samples with COD>50 mg/L. For samples with lower contents , as is the case of irrigation water, the alternative procedure is the hot determination of the oxidability with the potassium permanganate method, in an acid medium.

Through this method, the organic substances present in the water are oxidized by an excess of potassium permanganate in a hot acid medium, to carbon dioxide and water, reducing the permanganate to Mn^{2+} .

At the end of the digestion , the excess of permanganate is reduced by the excess of oxalic acid.

This excess of oxalic acid is quantified by titration with a solution of potassium permanganate, as the following equation:

$$5C_2O_4^{2-} + 2MnO_4^- + 16H^+ \rightarrow 10CO_2 + 2Mn^{2+} + 8H_2O$$

Experimental procedure:

1) Place in an erlenmeyer flask with a date volume of sample and distilled water so that the final volume is 100 mL. Prepare a blank using 100 mL of distilled water.
2) Add 10 mL of 1:3 sulfuric acid (Fisher Scientific p.a. > 95%) and bring to a boil. When the sample or blank come into boiling , add 5 mL of 0.01 N potassium permanganate (Merck) and boil for 10 minutes, at the end of this time let cool.
3) Add 5 mL of 0.01 N oxalic acid (Merck) and shake until the color disappears.
4) Titrate with 0.01 N potassium permanganate (Merck) until the achieving of pink color. Record the volume of 0.01 N potassium permanganate consumed.

Expression of results:

$$\text{Oxidability } \frac{\text{mg}}{\text{L}}O_2 = \frac{(v1 - v2) * N * 8000}{v3}$$

where:

v1 = the volume of titrant consumed in the sample (mL)

v2 = volume of titrant consumed in the blank (mL)

v3 = volume of the sample

N = normality of the solution of potassium permanganate

Table 1 Instruments used in the laboratory to perform on the samples the analyzes described.

Lab tools used	Analysis
Analytical balance (Bilancia Ohaus Explorer Pro)	Weighing of samples and reagents
Oven (WTC binder 7200 TUTTLINGEN Germany)	Moisture determination
Muffle furnace(marca)	Ashes content determination
Dryer(marca)	Moisture elimination
pH-glass electrode (micropH2001, Crison)	pH determination
Conductivity electrode (MC226, Mettler Todelo – InLab 730 Conductivity)	Electrical conductivity determination
Digestore (marca)	Samples digestion
Kjeldall (Kjeltec 1002 Distilling System Unit de Tecator)	Total Nitrogen (N) and Ammonia (NH4+) determination
Interface photometer 7000 (Palintest Ltd)	Nitrates and Nitritis determination (NO_3^-, NO_2^-)
Spettrofotometro ad assorbimento molecolare (Shimadzu Spectrophotometer UV-120-11)	Phosphates determination
Atomic Absorbance Spectrophotometer (Espectrofotómetro de Absorçáo Atómica SOLAAR Thermo Electron Corporation M series)	Metals content determination
Microtox ® M500 system	Ecotoxicity essay
OxiTop ® system	BOD_5 determination

Table 2 Physico-chemical characterization of soil

Analysis	Methods used
Moisture	Determination made through the loss of weight after drying in a oven at 105 ° C ± 2 ° C, until reaching constant weight, expressed in relation to the wet sample (Baize, 2000).
Organic matter	Determination made by the method Walkley-Black (1934). The organic carbon content is calculated from the ratio (organic matter) / 1.724 (Costa, 1999; Baize, 2000).
pH	Determination made by the potential difference, measured in a suspension of ground in distilled water with a glass electrode, according to a ratio L / S = 1/ 2.5 (APHA, AWWA and WPC, 1985).
Electrical conductivity	Determination made with a glass conductivity electrode, in a suspension of soil in distilled water, in the ratio L / S = 1/ 2.5 (APHA, AWWA and WPC, 1985)
Totale nitrogen (N) content	Determination through digestion with hot HNO_3 and HCl. Determination of the nitrogen content of the digested sample (Watts S. and L. Halliwell, 1996)
Ammonia content (NH_4^+)	Extraction with 1N KCl at a ratio L / S = 2.5, (Haigh M. and C. Dyckhoff, 1996). Extract distillation and titration of the distillate with 0.02 N H_2SO_4 (Haigh M. and C. Dyckhoff, 1996)
Nitrates (NO_3^-) e Nitrites (NO_2^-) content	Extraction with 1N KCl at a ratio L / S = 2.5, (Haigh M. and C. Dyckhoff, 1996). Reduction of nitrate to nitrite, through contact of the filtered extract with a little 'of zinc (Palintest, Ltd.). Nitrite is determined by molecular absorption spectrometry, through the formation of a purple-red, for the combination of diazotized sulfanilic acid with NED (P. Jenkins et al., 1996).
Total phosphorous (P) content	Digestion with hot HNO_3 and H_2SO_4. Determination of phosphate in digested (Watts S. and L. Halliwell (1996).
Extractable phosphates ($PO4^-$)	Extraction with 0.5 M $NaHCO_3$, the ratio L / S = 200 (SR Olsen et al., 1954). Determination of phosphate in the extract filtered to molecular absorption spectrometry, through the formation of a colored complex with a solution of ammonium molybdate, in the presence of ascorbic acid and potassium tartarato and antimony (Watanabe FS and SR Olsen, 1965).
Metals content (Na, K, Ca, Mg, Fe, Mn, Al, Zn, Cu, Ni, Cd, Cr, Pb)	Digestion with aqua regia in accordance with ISO 11466 (1995). Determination of metals in digested by atomic absorption spectrometry (ISO 11466, 1995).

Table 3 Biological characterization of soil

Analysis	Methods used
Soil respiration	Determination made through the respirometric method (System OxiTop) (ISO 16072, 2002).
Counting of total microorganisms at a temperature of 22 ° C	Determination made through "seeding" by incorporation of a certain amount of mother suspension and of the respective decimal solutions in an appropriate medium (Plate Count Agar, Becton, Dickinson and Company). Incubation of the seed plate for 68 ± 4 hours at a temperature of 22 ± 2 ° C, in aerobiosis. Calculation of the number of microorganisms per gram of soil, starting from the number of colonies developed in the plates selected (ISO 6222, 1999).
Eco-toxicity Essay	Extraction with H_2O in the ratio L / S = 10 (EN 12457-4, 2002). Determination made on the aqueous extracts, using the Microtox ® system, bio-analyzer that uses luminescent bacteria *Vibrio fischeri* NRRL B-11177 as test organisms (L. De Vetter et al., 2008).

Table 4 Physico-chemical characterization of leachates collected from the pots

Analysis	Method used
pH	Determination made by the potential difference, measured in a water sample with a glass pH electrode (APHA, AWWA and WPC, 1985
Electrical conductivity	Determination made measured in a water sample with a glass conductivity electrode (ISO 7888, 1985).
Oxidability	Hot Oxidation of the organic matter present in the sample with potassium permanganate in an acid medium. Determination of permanganate consumed for the addition of oxalate in excess, followed by titration with permanganate (APHA, AWWA and WPC, 1985).
Ammonia content (NH_4^+)	Extract distillation and titration of the distillate with 0.02 N HCl (Haigh M. and C. Dyckhoff, 1996, ISO 5664, 1984)
Nitrates (NO_3^-) and Nitrites (NO_2^-) content	Reduction of nitrate to nitrite, through contact of the filtered extract with a little 'of zinc (Palintest, Ltd.). Nitrite is determined by molecular absorption spectrometry, through the formation of a purple-red, for the combination of diazotized sulfanilic acid with NED (P. Jenkins et al., 1996). (P. Jenkins et al., 1996, ISO 6777, 1984)
Extractable phosphates ($PO_4^{-)}$	Determination of phosphate in the extract filtered by molecular absorption spectrometry, through the formation of a colored complex with a solution of ammonium molybdate, in the presence of ascorbic acid and tartarato potassium and antimony (Watanabe FS and SR Olsen, 1965). (Watanabe FS and Olsen SR, 1965, ISO 6878-1, 1986)
Metals content (Na, K, Ca, Mg, Fe, Mn, Al, Zn, Cu, Ni, Cd, Cr, Pb)	Determination of metals by atomic absorption spectrophotometry. (APHA, AWWA and WPC, 1985)

Table 5 Biological characterization of leachates collected from the pots.

Analysis	Method used
BOD_5	Determination of the consumption of dissolved oxygen after 5 days of incubation at 20 ° C in the absence of light with the addition of a nitrification inhibitor (ISO 5815-1, 2003; ISO 5815-2, 2003).
Counting of total microorganisms at a temperature of 22 ° C	Determination made through "seeding" by incorporation of a certain amount of mother suspension and of the respective decimal solutions in an appropriate medium (Plate Count Agar, Becton, Dickinson and Company). Incubation of the seed plate for 68 ± 4 hours at a temperature of 22 ± 2 ° C, in aerobiosis. Calculation of the number of microorganisms per mL of leachate, from the number of colonies developed in the plates selected. (ISO 6222, 1999, EN ISO 6887-1, 1999 ISO 8199, 2005 ISO / TC 11133-1, 2000 ISO / TC 11133-2, 2003 NP, 1828, 1982, NP 2079, 1989)
Eco-toxicity essay	Determination performed on leachates, adopting the Microtox ® system, bio-analyzer that uses luminescent bacteria Vibrio fischeri NRRL B-11177 as a test organism (L. De Vetter et al., 2008).

Table 6 Chemical characterization of the biomass collected from each pots.

Analysis	Methods used
Ashes	Determination through a cycle of burning, cooling, drying and weighing of the sample
Total Nitrogen (N)	Determination through digestion with hot HNO_3 and HCl. Determination of the nitrogen content of the digested sample (Watts S. and L. Halliwell, 1996)
Total Phosphorous (P)	Digestion with hot HNO_3 and H_2SO_4. Determination of phosphate in digested (Watts S. and L. Halliwell (1996).
Metals content (Na, K, Ca, Mg, Fe, Mn, Al, Zn, Cu, Ni, Cd, Cr, Pb)	Determination of metals by atomic absorption spectrophotometry. (APHA, AWWA and WPC, 1985)

Table 7 Chemical characterization of the sludge.

Analysis	Methods used
Moisture	Determination made through the loss of weight after drying in a oven at 105 ° C ± 2 ° C, until reaching constant weight, expressed in relation to the wet sample (Baize, 2000).
Organic matter	Determination made by the method Walkley-Black (1934). The organic carbon content is calculated from the ratio (organic matter) / 1.724 (Costa, 1999; Baize, 2000).
pH	Determination made by the potential difference, measured in a suspension of ground in distilled water with a glass electrode, according to a ratio L / S = 1/ 2.5 (APHA, AWWA and WPC, 1985).
Electrical conductivity	Determination made with a glass conductivity electrode, in a suspension of soil in distilled water, in the ratio L / S = 1/ 2.5 (APHA, AWWA and WPC, 1985)
Ashes	Determination through a cycle of burning, cooling, drying and weighing of the sample
Totale nitrogen (N) content	Determination through digestion with hot HNO_3 and HCl. Determination of the nitrogen content of the digested sample (Watts S. and L. Halliwell, 1996)
Ammonia content (NH_4^+)	Extraction with 1N KCl at a ratio L / S = 2.5, (Haigh M. and C. Dyckhoff, 1996). Extract distillation and titration of the distillate with 0.02 N H_2SO_4 (Haigh M. and C. Dyckhoff, 1996)
Nitrates (NO_3^-) e Nitrites (NO_2^-) content	Extraction with 1N KCl at a ratio L / S = 2.5, (Haigh M. and C. Dyckhoff, 1996). Reduction of nitrate to nitrite, through contact of the filtered extract with a little 'of zinc (Palintest, Ltd.). Nitrite is determined by molecular absorption spectrometry, through the formation of a purple-red, for the combination of diazotized sulfanilic acid with NED (P. Jenkins et al., 1996).
Total phosphorous (P) content	Digestion with hot HNO_3 and H_2SO_4. Determination of phosphate in digested (Watts S. and L. Halliwell (1996).
Extractable	Extraction with 0.5 M $NaHCO_3$, the ratio L / S = 200 (SR Olsen

phosphates ($PO4^-$)	et al., 1954). Determination of phosphate in the extract filtered to molecular absorption spectrometry, through the formation of a colored complex with a solution of ammonium molybdate, in the presence of ascorbic acid and potassium tartarato and antimony (Watanabe FS and SR Olsen, 1965).
Metals content (Na, K, Ca, Mg, Fe, Mn, Al, Zn, Cu, Ni, Cd, Cr, Pb)	Digestion with aqua regia in accordance with ISO 11466 (1995). Determination of metals in digested by atomic absorption spectrometry (ISO 11466, 1995).

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3. RESULTS

3.1. **Results Research line 1**

3.1.1. Soil salinity

Electrical conductivity (EC) is the most common measurement of soil salinity and is indicative of the ability of an aqueous solution to carry an electric current.

It is commonly expressed in units of deciSiemens per meter (dS m^{-1}). By agricultural standards, soils with an EC greater than 4 dS m^{-1} are considered saline. Salt-sensitive plants may be affected by conductivities less than 4 dS m^{-1} and salt tolerant species may not be impacted by concentrations of up to twice this maximum agricultural tolerance limit.

During the days after transplant (DAT) the salinity in the soil increased also in the S_0 treatment for the effect of salt concentration in the tap water. In all treatments EC started at less than 1 dS m^{-1} to reach 2.2 dS m^{-1} in the S_0, 6.3 dS cm^{-1} in the S_1 and 9.1 dS cm^{-1} in the S_2 treatment following 115 DAT (Fig. 1).

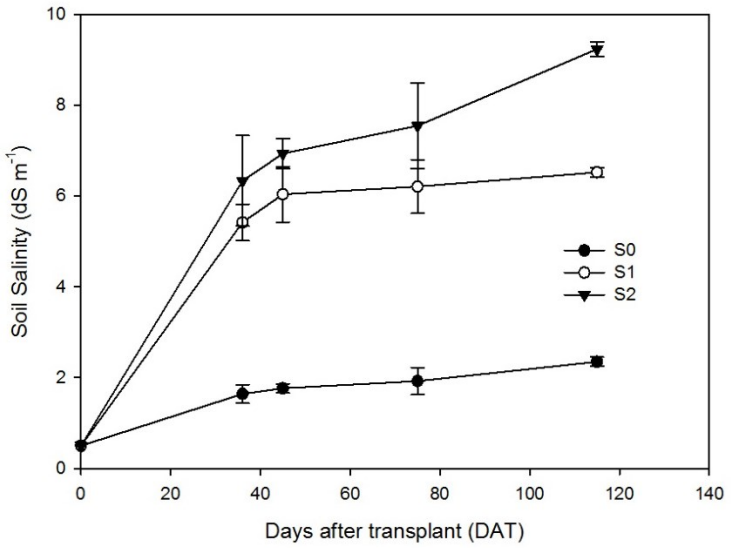


Figure 1. Soil salinity concentration in the average of the genotypes per treatment (S_0 irrigation with tap water, S_1 irrigation with 4 dS m^{-1} and S_2 irrigation with 8 dS cm^{-1}) during the experimental period. Vertical bars represent the standard deviation.

3.1.2. Stem number

The number of stem per pot at harvest resulted significantly higher in S_0 than S_1 and S_2 (in the average 5.5, 4.9 and 3.9) (Fig.2). Genotypes 34, 13 and 26 reported 9 and 8 stems, while genotypes 31, 36 and 12 only 2.5, 3.5 and 3.5 stems per pot in S_0.

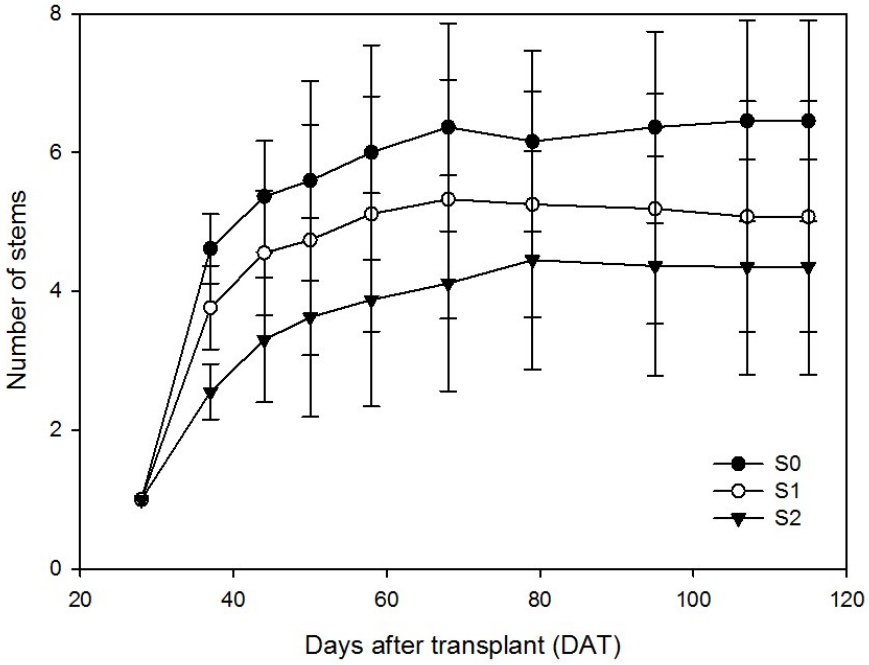
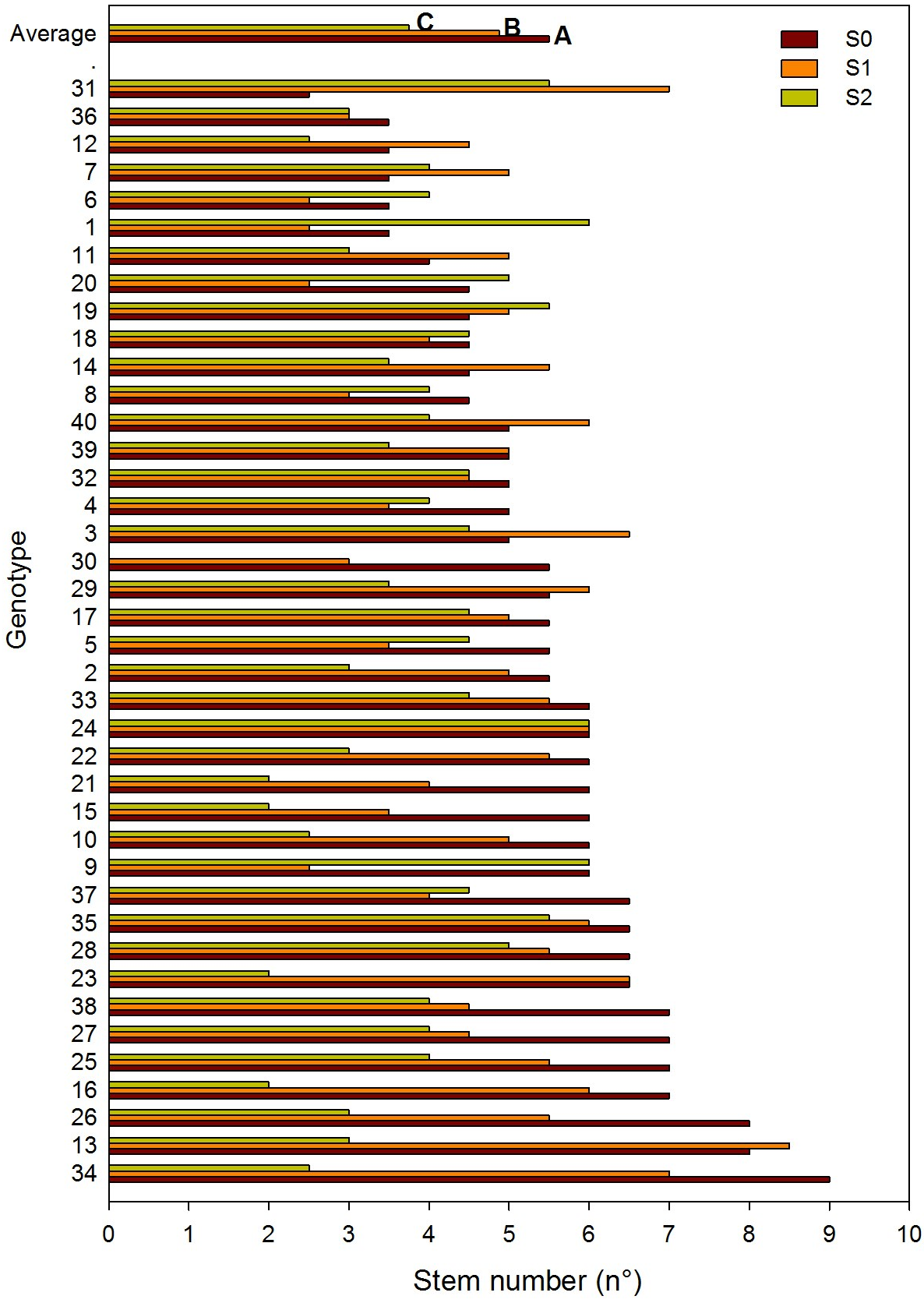


Figure 2. Number of stems per pot in the average of the genotypes per treatment versus days after transplant (DAT). Vertical bars represent the standard deviation of 40 genotypes per treatment.

In S_1 treatment, genotypes 13, 34 together with 31 and 29 showed the highest number of stems (8.5 and 7.0), while the lowest were genotypes 6, 1 and 20 (3.5, 3.5 and 4.5, respectively). In the S_2 treatment the highest number of stems were observed in genotypes 24, 9 and 1 (6.0) while the lowest number were recorded in genotypes 15, 21, 16 and 23 (2.0), as shown in Fig. 3.


C
B
A
S0
S1
S2
Average
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36
12
7
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23
38
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25
16
26
13
34
Genotype
0
1
2
3
4
5
6
7
8
9
10
Stem number (n°)

Figure 3. Stem number of the studied genotypes at harvest in the three treatments (S_0, S_1 and S_2). Different letters in the average bars represent statistical significance per $p \leq 0.05$.

3.1.3. Main stem height

No differences in stem height were observed between S_0 and S_1 treatment at 115 DAT (119 cm), while only 90 cm stem height were recorded in S_2 treatment, as shown in Fig. 4.

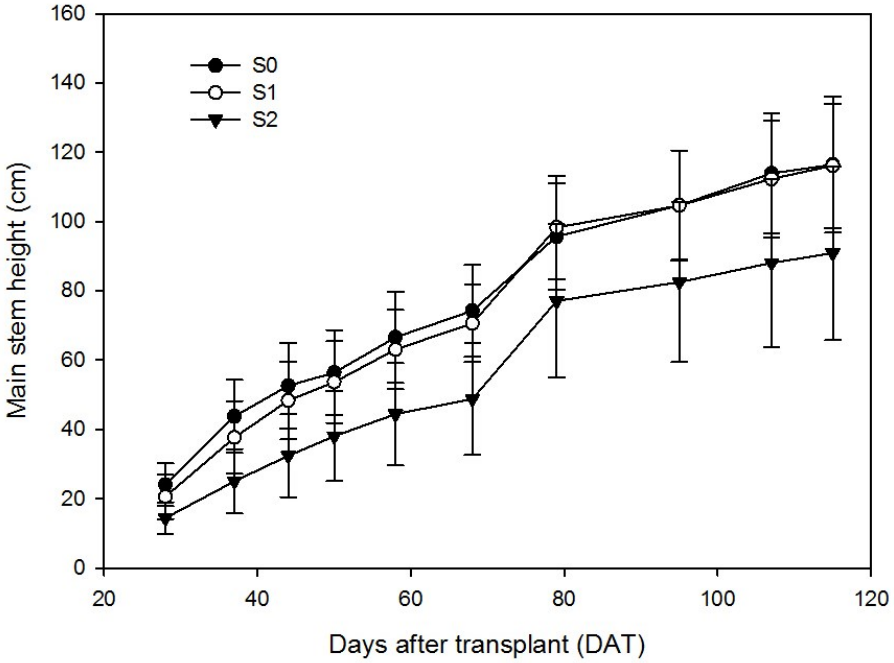

Figure 4. Height of the main stem versus days after transplant (DAT) in the average of genotypes per treatment (S_0, S_1 and S_2). Vertical bars represent the standard deviation of 40 genotypes per treatment.

In S_0 treatment the genotypes 13, 7, 11 and 31 showed a main stem height equal to 188.0, 164.5, 160.0 and 157.7 cm, while genotypes 22, 12, 21 and 38 only 92.5, 91.5, 87 and 83 cm

In S_1 treatment genotype 18 showed the highest height of the main stem (184.5 cm) followed by genotypes 6 and 20 (164.5 and 156 cm, respectively), while the lowest were genotypes 9, 4 and 24 (64, 58 and 57 cm, respectively).

In the highest salinity treatment (S_2) the highest values were observed in genotypes 18, 26 and 22 (156.5, 137.0 and 134.0 cm, respectively) while the lowest in genotypes 28, 15 and 29 (56.0, 49.5 and 45.0 cm, respectively), as shown in Fig. 5.

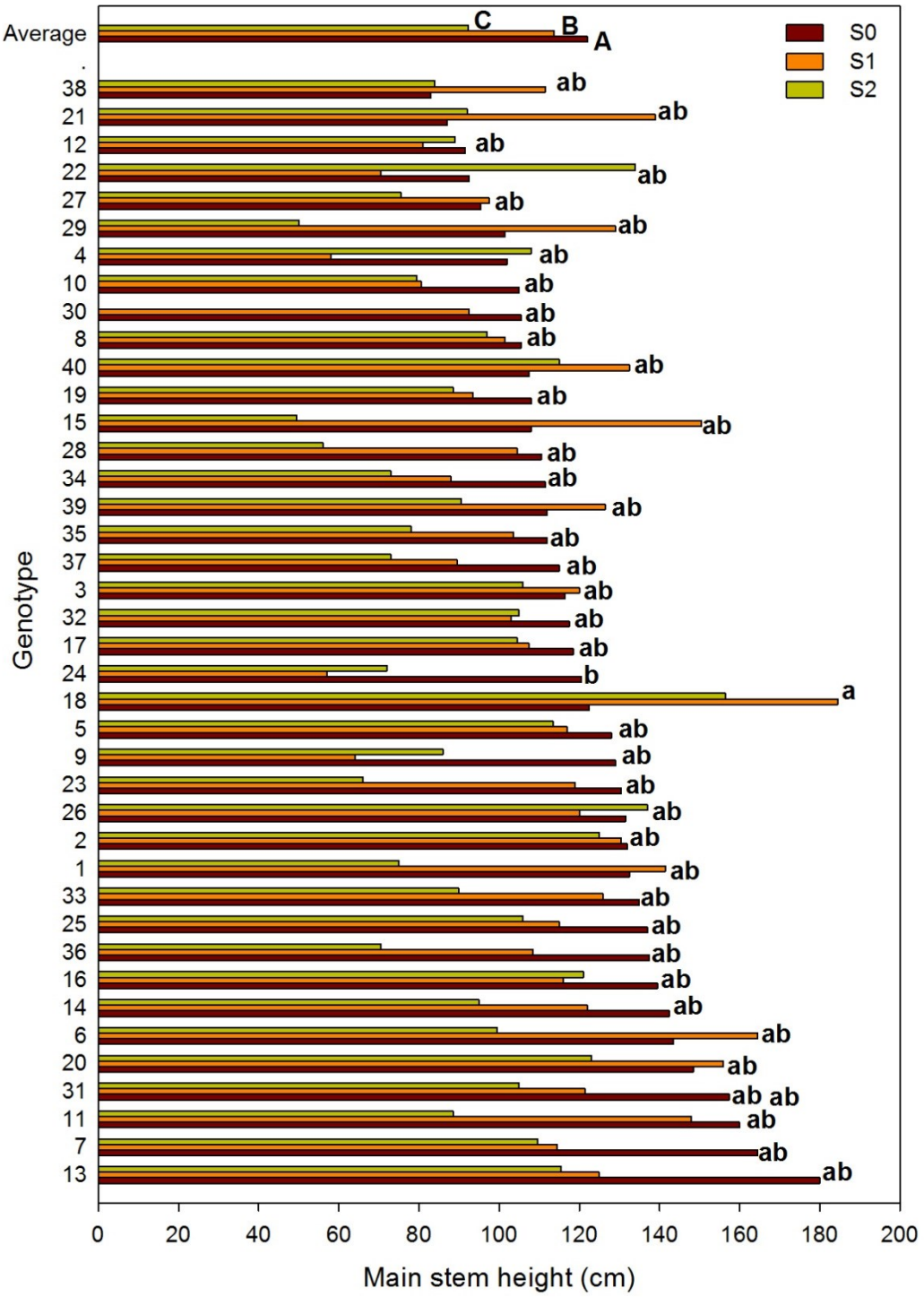

Figure 5. Main stem height of the studied genotypes at harvest in the three treatment (S_0, S_1 and S_2). Small letters, for averaged values of salinity level within each genotype; capital letters for averaged values of all genotypes within each salinity level. Different letters indicate significant differences at $P \leq 0.05$ by SNK Test.

3.1.4. Leaf Area Index (LAI)

LAI, at first increased and then decreased after certain DAT. The same trend was observed in all treatment following DAT, however, LAI was higher in the treatment S_0 and S_1 than S_2; the highest value was reached after 63 DAT in S_0 and S_1, while at 105 DAT in S_2 treatment (Fig. 6).

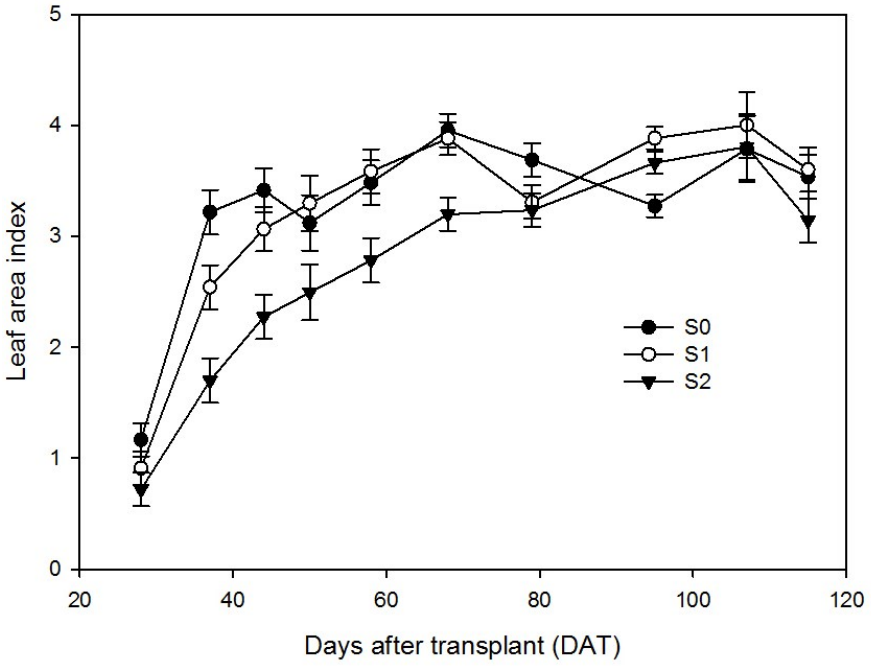

Figure 6. Leaf area index (LAI) versus days after transplant (DAT) in the average of genotypes per treatment (S_0, S_1 and S_2). Vertical bars represent the standard deviation of 40 genotypes per treatment.

In the average of the genotypes, S_1 provided higher LAI values (3.6) than S_0 (3.4) and S_2 (3.2). In S_0 treatment genotypes with higher LAI were 20 and 6 with 5.3 and 5.1, respectively, while the lowest was genotype 40 with LAI equal to 1.9. In S_1, genotypes which provided the highest leaf area index were genotypes 1 and 30 with 5.7 and 5.4, respectively, while the lowest was genotype 4 that showed a LAI equal to 1.7. In S_2

treatment highest values of LAI were observed in genotypes 26 and 16 (4.4 and 4.2, respectively), while the lowest (1.6) in genotype 23 (Fig.7)

In the average of the salinity treatments genotypes 1 and 30 showed the significantly highest LAI value (4.5) while the lowest was observed in genotype 4 (2.6).

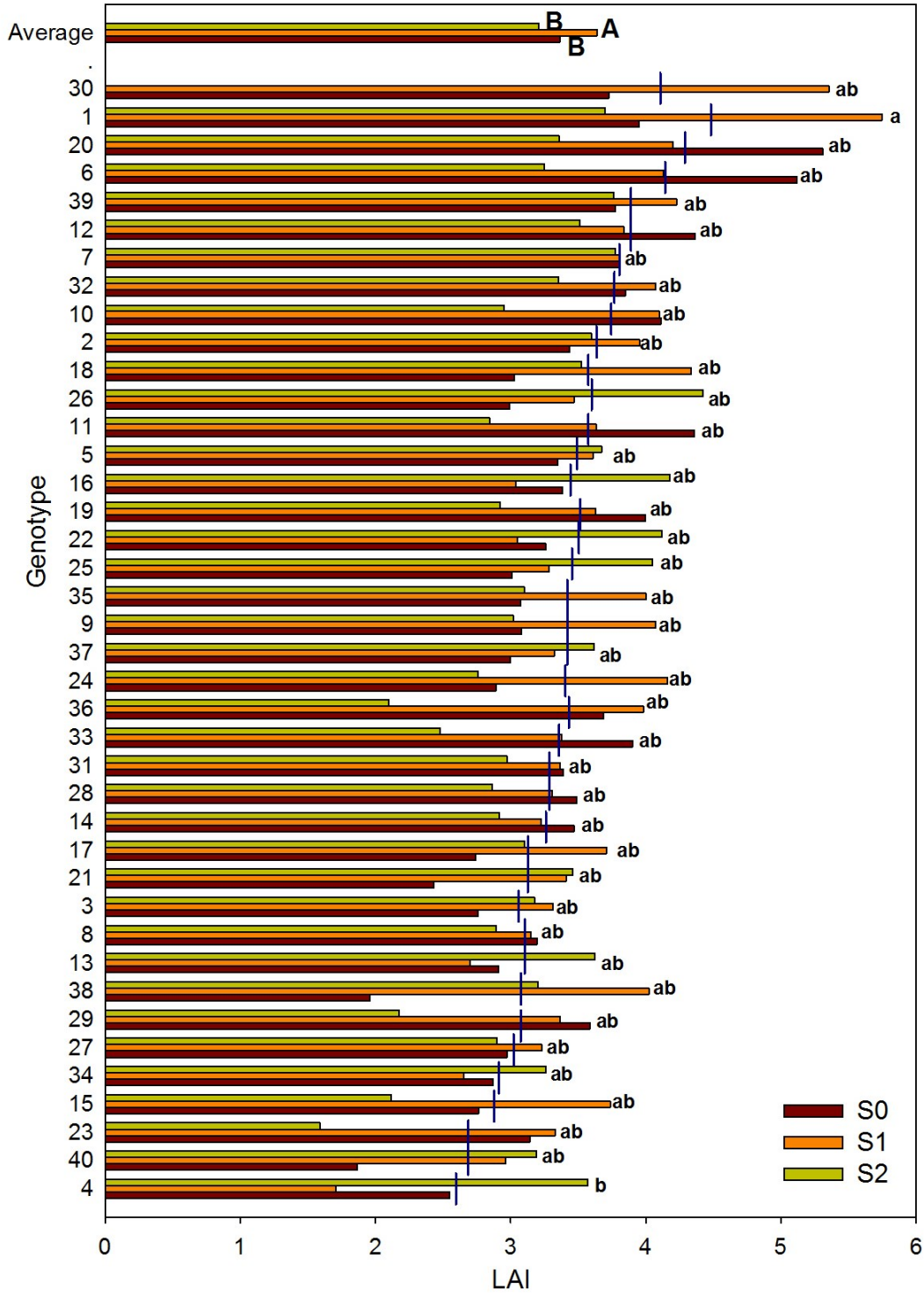

Figure 7. Leaf area index (LAI) of the tested 40 genotypes at harvest in the three treatment (S_0, S_1 and S_2). Vertical line represent average value of the three treatments (S_0, S_1 and S_2). Small letters, for averaged values of salinity level within each genotype; capital letters for averaged values of all genotypes within each salinity level. Different letters indicate significant differences at $P \leq 0.05$ by SNK Test.

3.1.5. SPAD unit

The SPAD unit was measured on the third fully expanded leaf on 50, 80 and 115 DAT in all genotypes and treatment.

The amount of chlorophyll present in plant can serve as an indicator of the overall conditions of the plants itself. In general, healthier plants, will contain more chlorophyll than less healthy ones.

The leaf SPAD unit decreased as the plant growth increased which may be related to the nutrient uptake in the substrate, mainly nitrogen content. In the average of the studied genotypes, the highest value was observed in S_0 treatment, ~50 SPAD unit at 50 DAT to decrease up to 38 SPAD unit at 115 DAT (Fig.8). Similar trend and value was observed in S_1 treatment. A different trend was, instead observed in S_2 treatment; SPAD unit at first slightly increased (from 42 to 43) from the first to the second measurement, and then decreased until 35 at the final measurement time (115 DAT).

This different trend may be related to the lower uptake of the root system of S_2 treatment as compared to the reduced salinity treatments due to osmotic adjustment. In fact at the end of the experiments a lower biomass of the root system (rhizomes, primary and secondary roots) was observed in S_2 treatment.

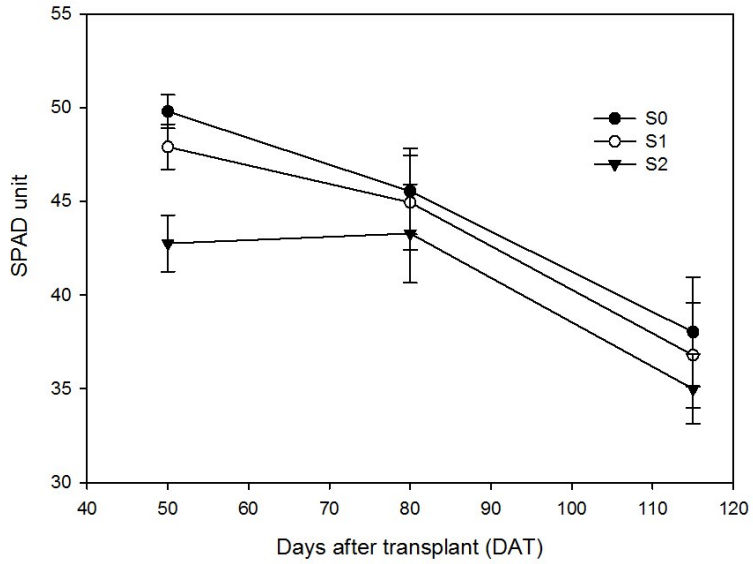

Figure 8. SPAD unit in the average of the genotype per treatment (S_0, S_1 and S_2). Vertical bars represent the standard deviation of 40 genotypes per treatment.

In the average of the studied genotypes, higher SPAD unit were observed in the S_2 (38.3) treatment than S_1 and S_0, which were equal to 36.8 and 35.0, respectively. Among the genotypes in S_0 treatment, genotypes 28, 9, 33 and 16 showed the highest SPAD unit (38, on average), while the lowest values were observed in genotypes 22 and 31, in which SPAD unit were equal to 31.9 and 31.5, respectively. In S_1 treatment, genotypes 2 and 32 provided the highest SPAD unit, 42.3 and 42.2, respectively, followed by genotypes 38 and 1 (41.6 and 41.1, respectively); the lowest SPAD unit was observed in genotype 8 (30.8).

In S_2 treatment, in almost all genotypes, the highest SPAD unit of the trial were observed, probably due to the stress which caused the lowest translocation in the reproductive organs. In fact in S_2 treatment, plant were greener than S_1 and S_0 plants and without flowers.

Genotypes 9 and 28 provided the highest SPAD unit in S_2 treatment, 45.5 and 44.4, respectively, while the lowest were observed in genotypes 27 and 10, 32.4 and 33.3, respectively.

In the average of the salinity treatments genotype 9 showed the significantly highest SPAD unit (40.7) while the lowest values were observed in genotypes 22 and 40 (33.9 and 33.8, respectively).

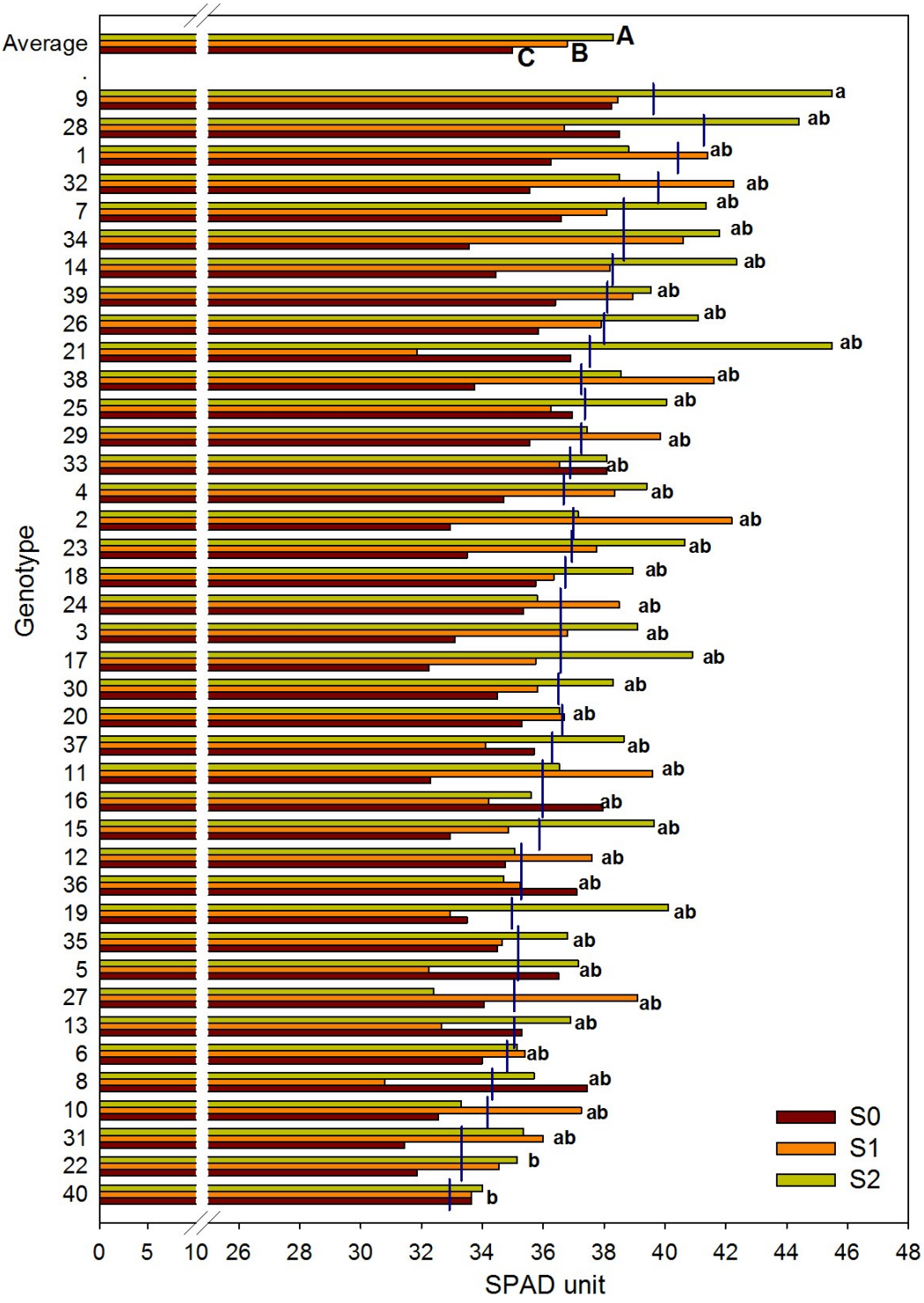

Figure 8. SPAD unit of the studied genotypes at harvest in the three treatment (S_0, S_1 and S_2). Vertical line represent average value of the three treatments (S_0, S_1 and S_2). Small letters, for averaged values of salinity level within each genotype; capital letters

for averaged values of all genotypes within each salinity level. Different letters indicate significant differences at P≤0.05 by SNK Test. LSD (genotype x treatment) = 5.9

3.1.6. Aboveground biomass yield

Genotypes 13, 20, 6, 16, 2, 7, 14, 30, 23, 18, 11, 12, 21 and 5 yielded over the average value in S_0 (from 131.2 to 88.7 g), the others genotypes under the average value (Figure 9). The highest biomass was observed in genotype 13 (131.2 g), while the lowest in genotype 40 (56.2 g).

In S_1 treatment, genotypes 24, 11, 40, 6, 31, 3, 18, 2, 20, 19, 14, 35, 33, 13, 39, 17, 25, 34, 23, 16, 26, 38 and 10 yielded over the average (from 124.1 to 76.5 g), the other genotypes under the average value. In genotype 24 was observed the highest biomass yield (124.1 g), while the lowest in genotype 9 (36.7 g).

In S_2 treatment, genotypes 6, 18, 24, 39, 22, 20, 30, 8, 26, 12, 31, 5, 4, 2, 19, 37, 25, 7, 1, 38, 17 and 14 yielded over the average (from 76.9 to 49.6 g), the other genotypes under the average value. Genotype 6 was the most productive (77.0 g) and genotype 21 the least one (20.2 g).

In the average of the genotypes, in S_0 was observed the significantly highest biomass, followed by S_1 and S_2 (88.5, 77.2 and 51.1 g, respectively).

Among genotypes, in the average of salt treatment, genotype 6 showed the significantly highest biomass (96.0 g) while the lowest in genotype 9 (52.1 g).

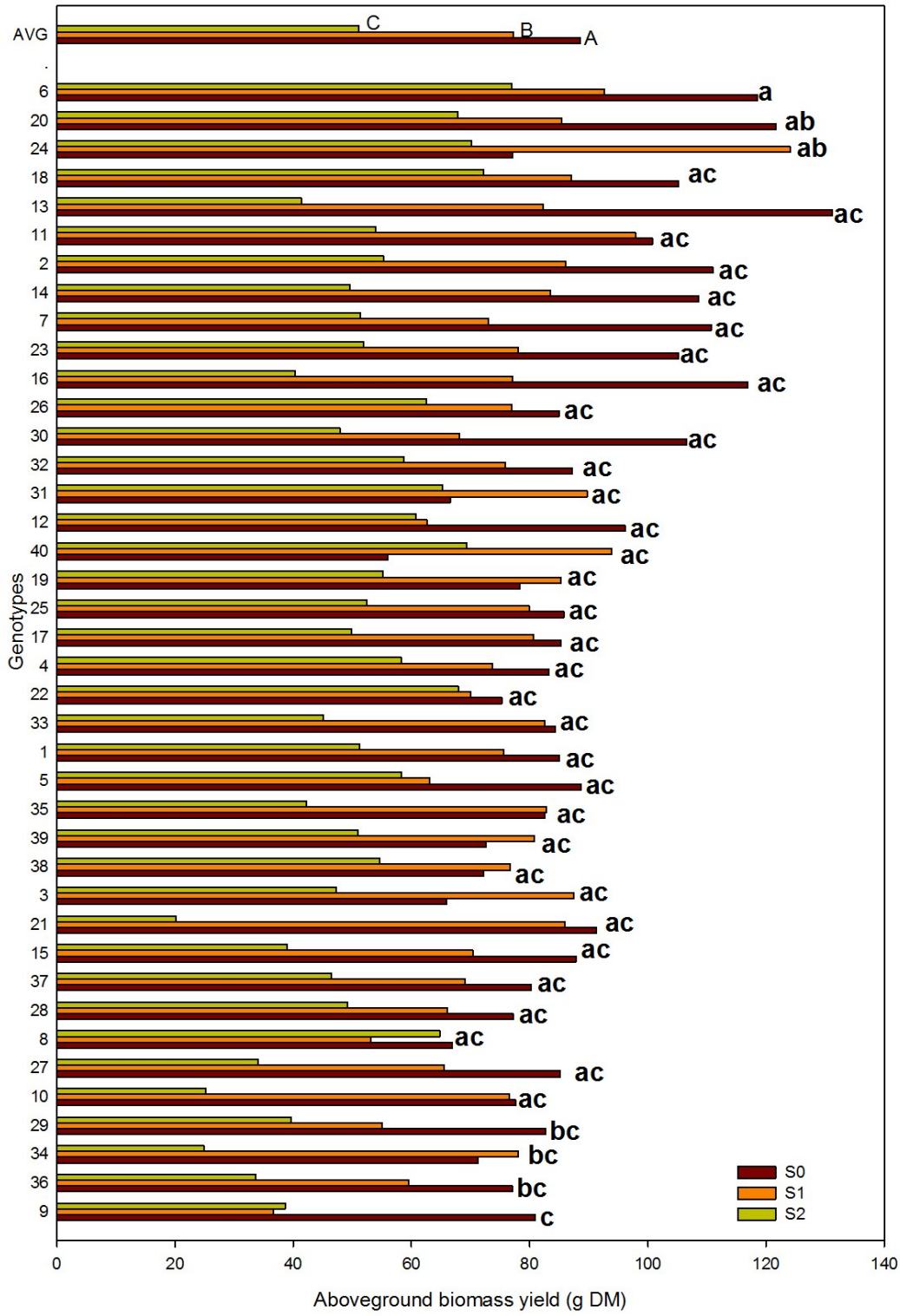

Figure 9. Biomass yield (g DM) of of the studied genotypes at harvest in the three treatment (S_0, S_1 and S_2). Vertical line represent average value of the three treatments (S_0, S_1 and S_2). Small letters, for averaged values of salinity level within each genotype; capital letters for averaged values of all genotypes within each salinity level. Different letters indicate significant differences at $P \leq 0.05$ by SNK Test. LSD (genotype x treatment) = 31.84.

The aboveground biomass was partitioned, in the average, by 66.5% stems and 33.5% leaves in S_0, 61.7% stems and 38.3% leaves in S_1 and 55.9% stems and 44.1% leaves in S_2 (Fig. 10).

Surprisingly, stems incidence tended to decrease, while leaves incidence on total aboveground biomass increased as the salinity levels were raised.

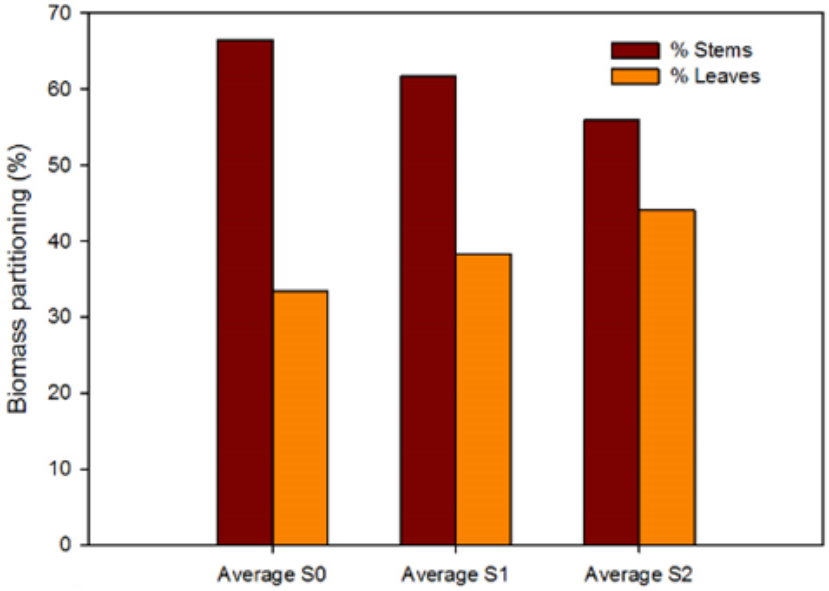

Figure 10. Biomass partitioning (%) in the average of the genotypes per treatment (S_0, S_1 and S_2).

Indeed, specific leaf weight (SLW), in the average of genotypes per treatment, increased as the salinity levels were raised. However, specific leaf area (SLA), in the average of genotypes per treatment, was larger in S_0 (3.90 mm^2 mg^{-1}), followed by S_1 and S_2, with 3.54 and 2.08 mm^2 mg^{-1} (Table 3). SLA of a species grown in environments with some resources limitation or stress (heat stress, drought stress, salinity, etc.) tends to be smaller than the same species grown in resource rich environments. The leaf water content (LWC % w/w) was measured on individual genotypes per treatment. However, in the average of genotypes, S_0 and S_1 reported very similar values (35.8 and 35.3 %) while S_2 tended to decrease (31.7%). Less pronounced was the stem water content at harvest with 48.7 in S_0, 47.9 in S_1 and 46.3 % w/w in S_2 treatment.

Table 3: Specific Leaf Area (mm^2 mg^{-1}), Specific Leaf Weight (1/SLA), Leaf water Content (% w/w) and Stem Water Content (% w/w) of the average of 40 clones in the same treatment (S_0, S_1 and S_2)

Treatment	*SLA*	*SLW*	*LWC*	*SWC*
Average (S_0)	3.90	0.29	35.8	48.7
Average (S_1)	3.54	0.34	35.3	47.9
Average (S_2)	2.08	0.52	31.7	46.3

3.1.7. Belowground biomass

Regarding belowground biomass in the average of the treatment, S_1 yield was slighty higher than S_0 and S_2 which yields were equal to 170.6, 162.9 and 136.0 g, respectively; among genotypes, in the average of salt treatment, genotype 6 yielded the most (192 g) while genotype 30 the lowest (117.2 g) (Fig.11). Among genotypes, in the average of salt treatment no significantly difference were observed (Fig.11).

Belowground biomass was partitioned in rhizome and roots which fractions were weighted separately.

The highest weight was observed in S_1 treatment which was slightly higher than S_0 and S_2. In the average of the treatment, S_1 yielded 119.9 g of rhizome dry weight, S_0 116.7 g and S_2 97.5 g (Fig.12). However, salinity levels resulted not significant in the three treatments. It is worth to mention that during the experimental period, the rhizomes increased their fresh weight 300-fold in S_0, 319-fold in S_1 and 265-fold in S_2, respectively. The moisture content was, in the average of the genotypes per treatment, 71.3% in S_0, 72.5 in S_1 and 75.6% in S_2.

In roots, both primary and secondary, the highest dry weight was obtained with S_1, which was slightly higher than S_0 and S_2 (Fig. 13). In the average of the treatment, S_1 yielded 50.8 g of roots dry weight, S_0 46.2 g and S_2 38.6 g. The moisture content was, in the average of the genotypes per treatment, 79.4% in S_0, 80.2 in S_1 and 83.7% in S_2.

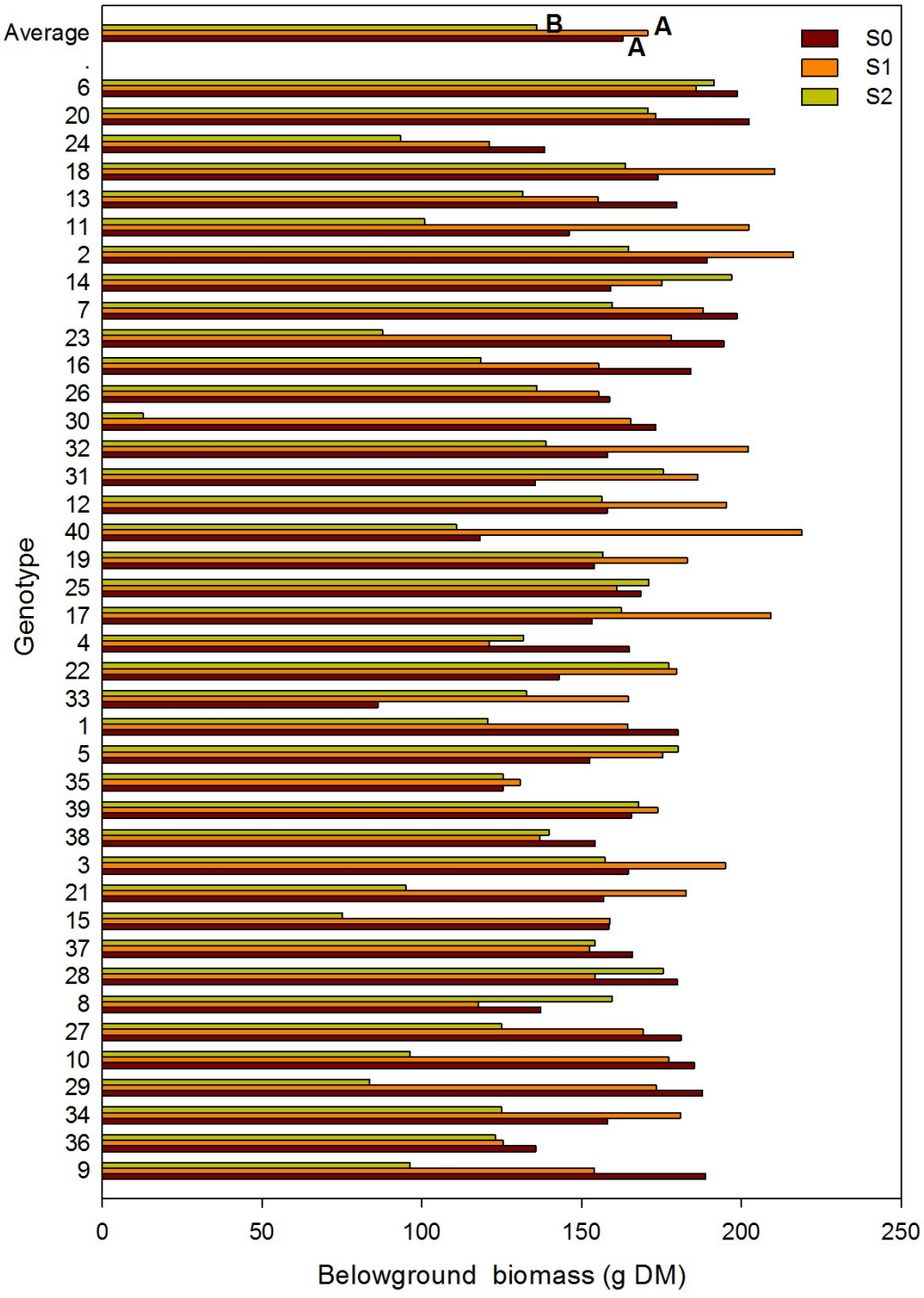

Fig.11 Belowground biomass (g DM) of each genotype in the three treatment (S_0, S_1 and S_2). Different letters in the average bars represent statistical significance per $p \leq 0.05$. LSD (genotype x treatment = 65.50).

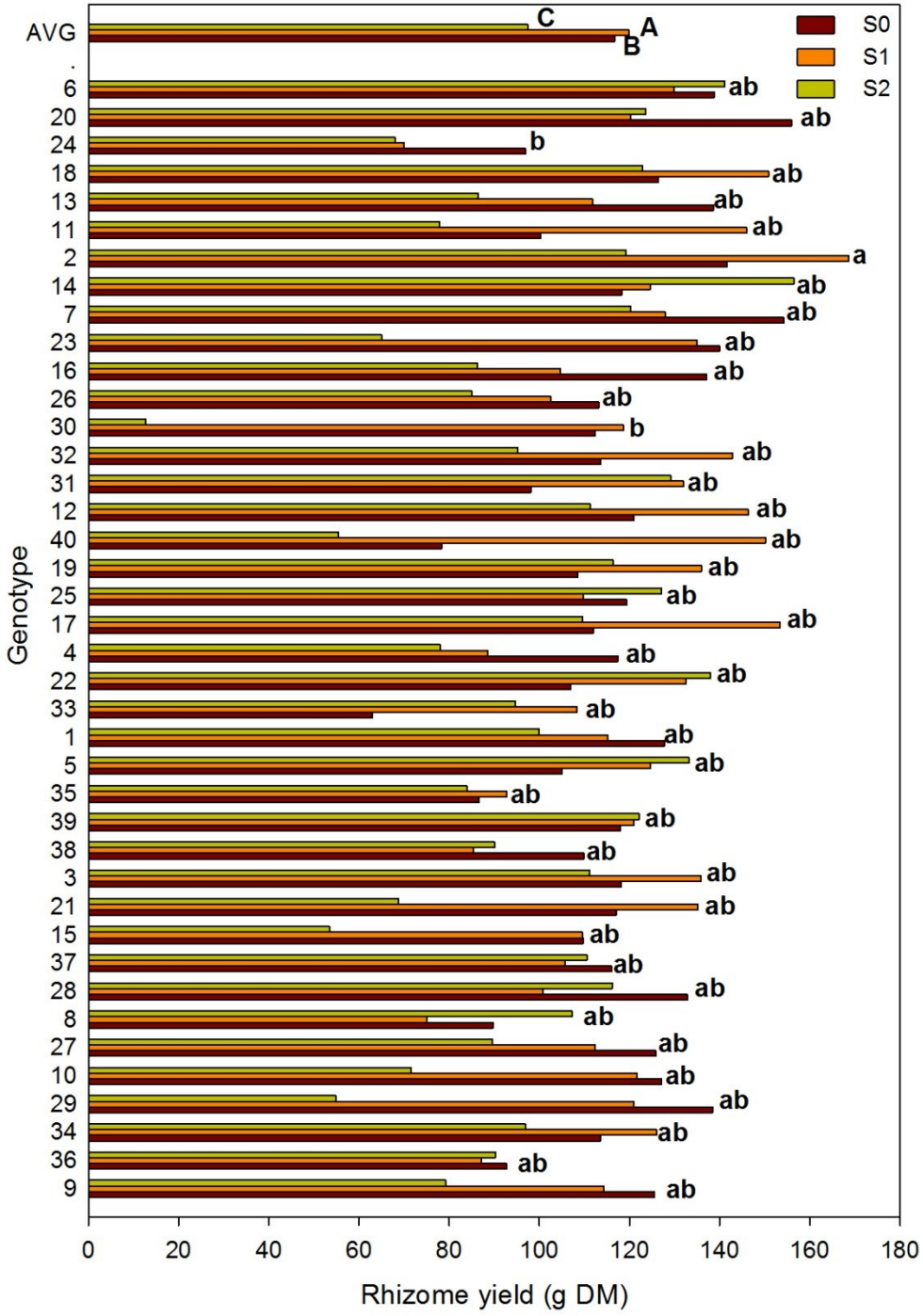

Fig.12 Dry rhizome weight of each genotype in the three treatment (S_0, S_1 and S_2). Different letters in the average bars represent statistical significance per $p \leq 0.05$.

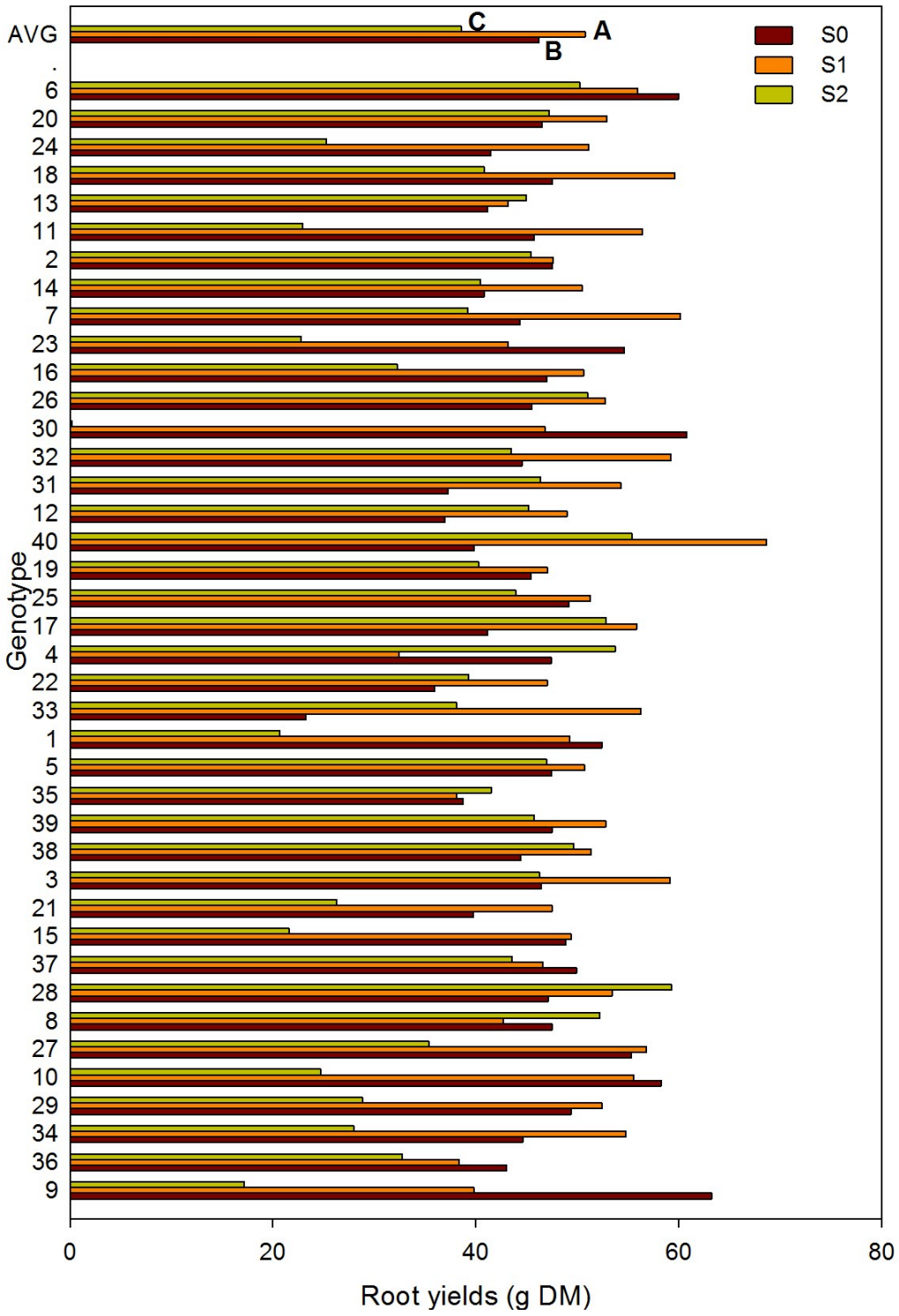

Figure 13 Dry roots weight of each genotype in the three treatment (S_0, S_1 and S_2). Different letters in the average bars represent statistical significance per $p \leq 0.05$. LSD (genotype x treatment) = 21.29

3.1.8. Physiological measurements

On July 20, August 3 and September 12, respectively at 63,80 and 116 days after transplant, Net photosynthesis (µmol CO_2 m^{-2} s^{-1}), transpiration rate (mmol H_2O m^2 s^{-1}) and stomatal conductance (mol H_2O m^2 s^{-1}) have been measured.

A decrease in the three parameters was observed during the measurement time, which is ascribable to the effect of environmental variables. For instance, solar radiation was, in the average of the measurements, 1765 MJ m^{-2} on July 20, 1551 MJ m^{-2} and 1442 on August 3 and September 12, respectively.

This is one of the most important environmental variables and as reported by may authors, photosynthesis is positively correlated to solar radiation. Moreover, higher temperatures were encountered after 63 days after transplant leading to stomata closure and therefore lower values of gas exchanges between canopy an atmosphere.

Net photosynthesis, in the average of genotypes, in S_0 decreased from the first data of measurement to the third one from 27.0 to 22.2 µmol CO_2 m^{-2} s^{-1}, in S_1 from 22.7 to 19.4 µmol CO_2 m^{-2} s^{-1}, and in S_2 from 17.7 to 14.0 µmol CO_2 m^{-2} $s^{-}1$ (Fig.14).

Stomatal conductance, in the average of genotypes and from the first data of measurements to the third one decreased from 0.48 to 0.39 mol H_2O m^2 s^{-1} in S_0 treatment, from 0.24 to 0.20 mol H_2O m^2 s^{-1} in S_1, and from 0.19 to 0.17 in S_2 treatment, respectively (Fig. 15).

The assimilation rate, followed the trend observed for the other physiological parameters: from July to September and in the average of the genotypes in S_0 treatment transpiration rate decreased from 11.3 to 9.05 mm H_2O m^{-2} s^{-1} from 8.5 to 6.9 mm H_2O in S_1 and from 6.8 to 6.09 mm H_2O in S_2 treatment (Fig. 16).

Overall, in the average of the three data of measurements, S_0 treatments showed higher net photosynthesis values, stomatal conductance and transpiration rate than saline treatments S_1 and S_2. This is justified by the fact that the plants in S_0 treatment were not subjected to salt stress and thus they had higher physiological parameters than plants subjected to saline treatments.

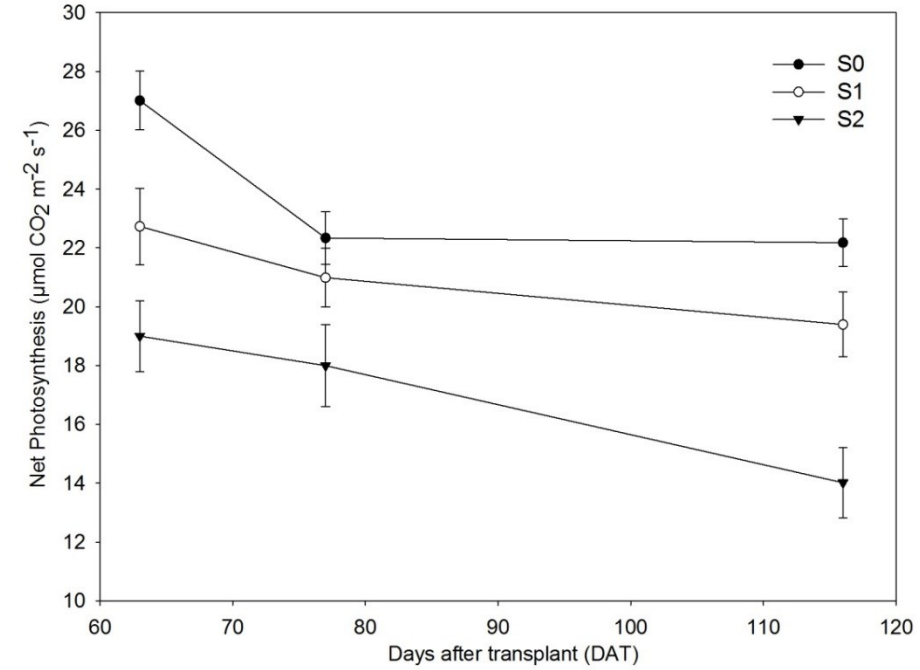

Fig. 14. Net photosynthesis in the average of 40 giant reed clones. S_0 (control), S_1 (4 dS m^{-1}) and S_2 (8 dS m^{-1}).

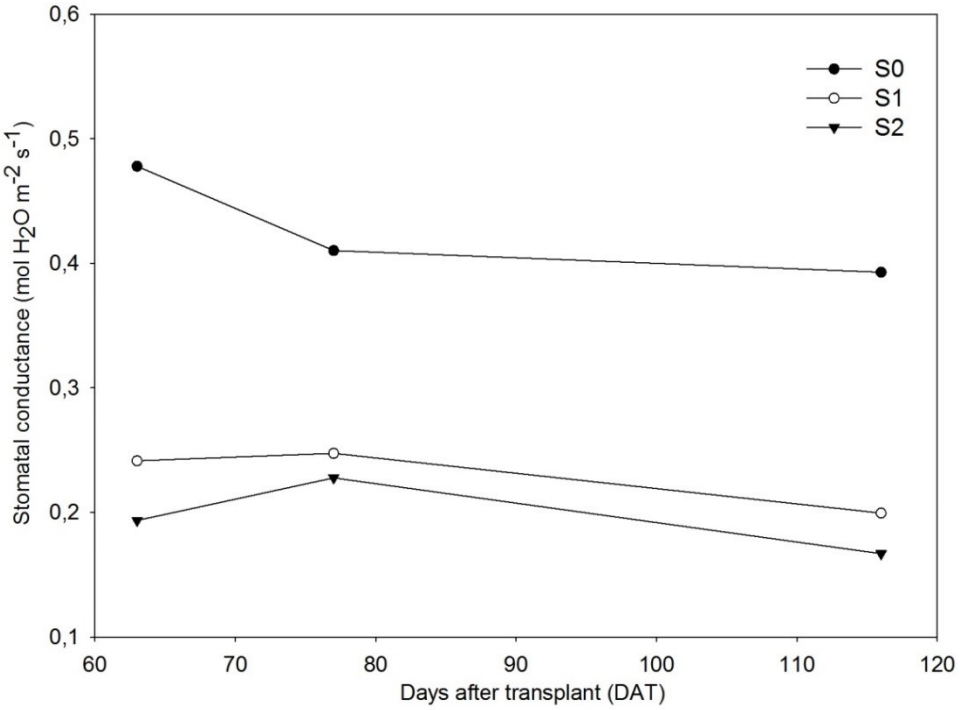

Fig. 15. Stomatal conductance in the average of 40 giant reed clones. S_0 (control), S_1 (4 dS m^{-1}) and S_2 (8 dS m^{-1})

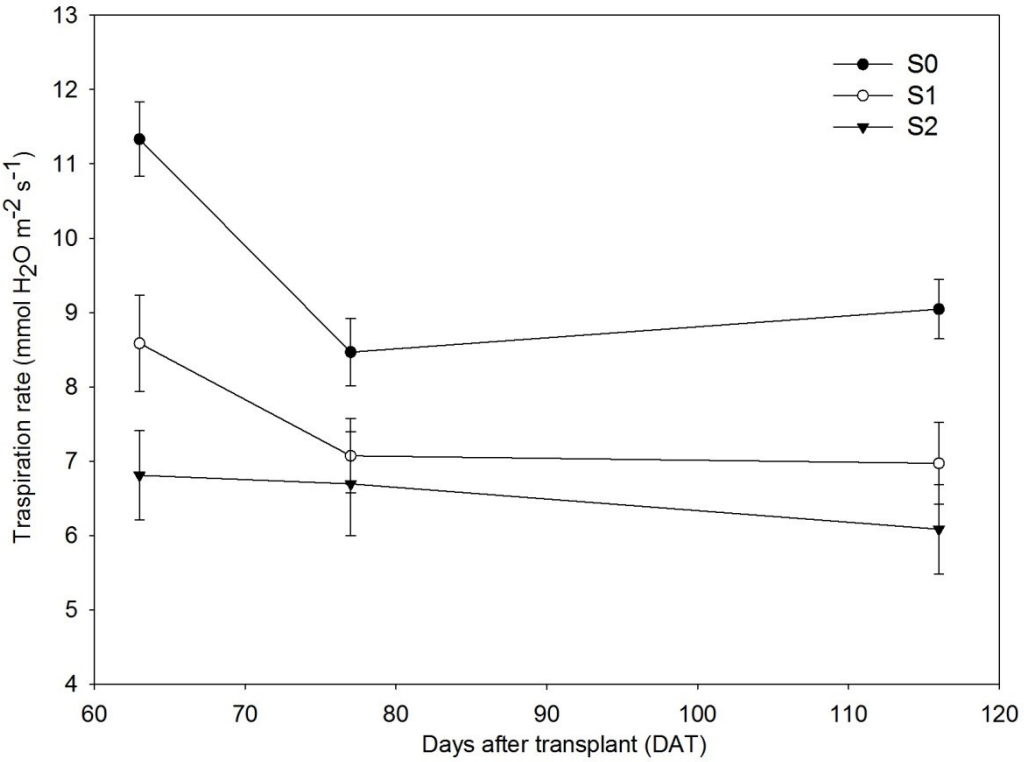

Fig. 16 Transpiration in the average of 40 giant reed clones. S_0 (control), S_1 (4 dS m^{-1}) and S_2 (8 dS m^{-1}) .

Although, physiological measurements have been carried out on July 20th, August 8th and September 9th, however only the second data is shown and discussed. The other measurements dates are available in the annex.

Within a single genotype, net photosynthesis carried out on 03 August 2012, shown a statistical significance in the average of the salt treatment, with S_0 being the highest and S_2 the lowest. Genotype 18 shown the significantly highest net photosynthesis (26 µmol CO_2 m^{-2} s^{-1}), while the lowest was clone 33 (~14 µmol CO_2 m^{-2} s^{-1}), with the others showing intermediate values.

The same trend was observed for transpiration rate and stomatal conductance (fig. 17). Previous and further measurements dates revealed more or less the same trend.

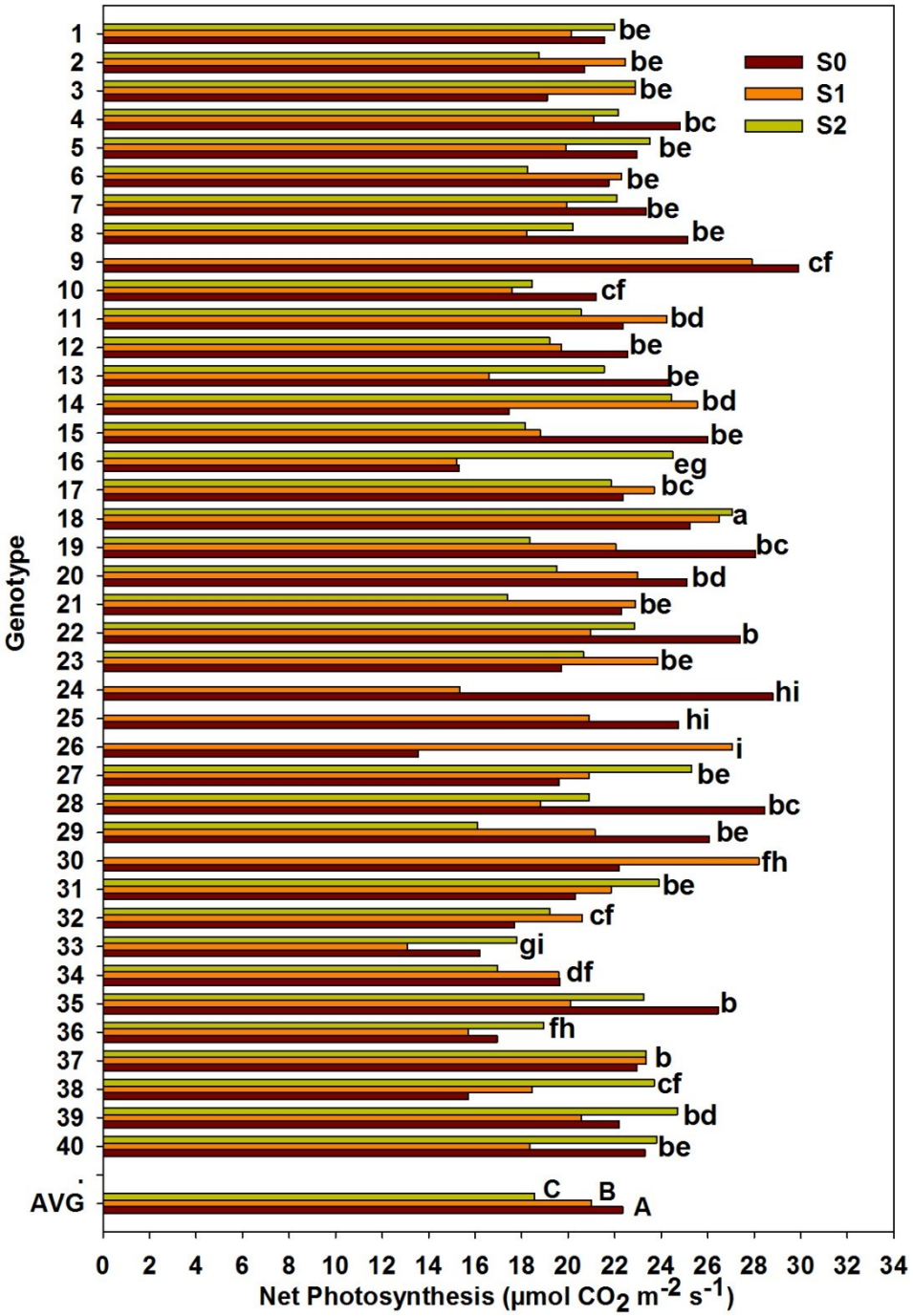

Figure 17. Net photosynthesis messured on 08/03/2012 of each genotype in the three treatment (S_0, S_1 and S_2). Small letters, for averaged values of salinity level within each genotype; capital letters for averaged values of all genotypes within each salinity level. Different letters indicate significant differences at P≤0.05 by SNK Test. LSD (genotype x treatment) = 3.54

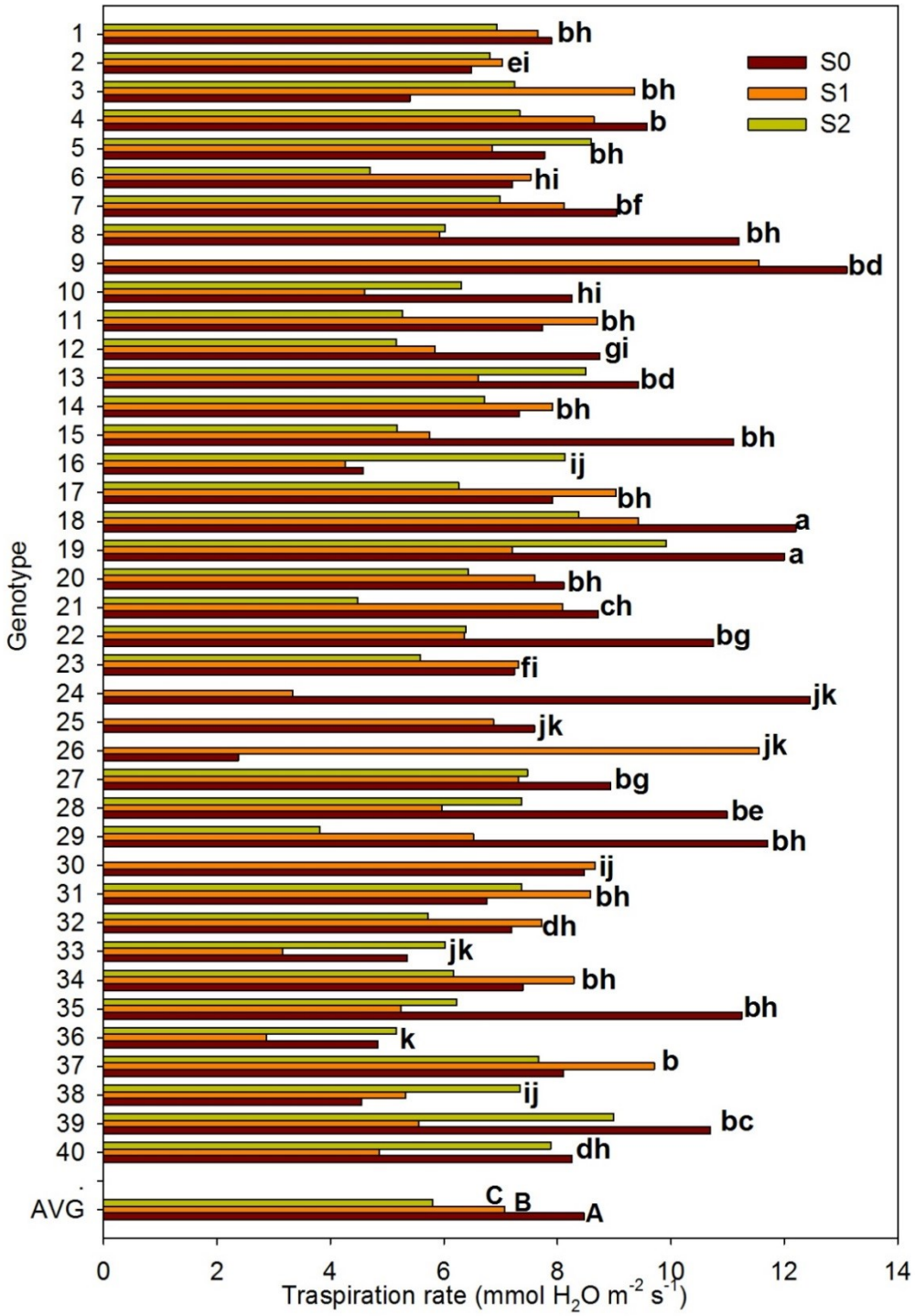

Figure 18. Transpiration rate (08/03/2012) of each genotype in the three treatment (S_0, S_1 and S_2). Small letters, for averaged values of salinity level within each genotype; capital letters for averaged values of all genotypes within each salinity level. Different letters indicate significant differences at $P \leq 0.05$ by SNK Test. LSD (genotype x treatment) = 1.26

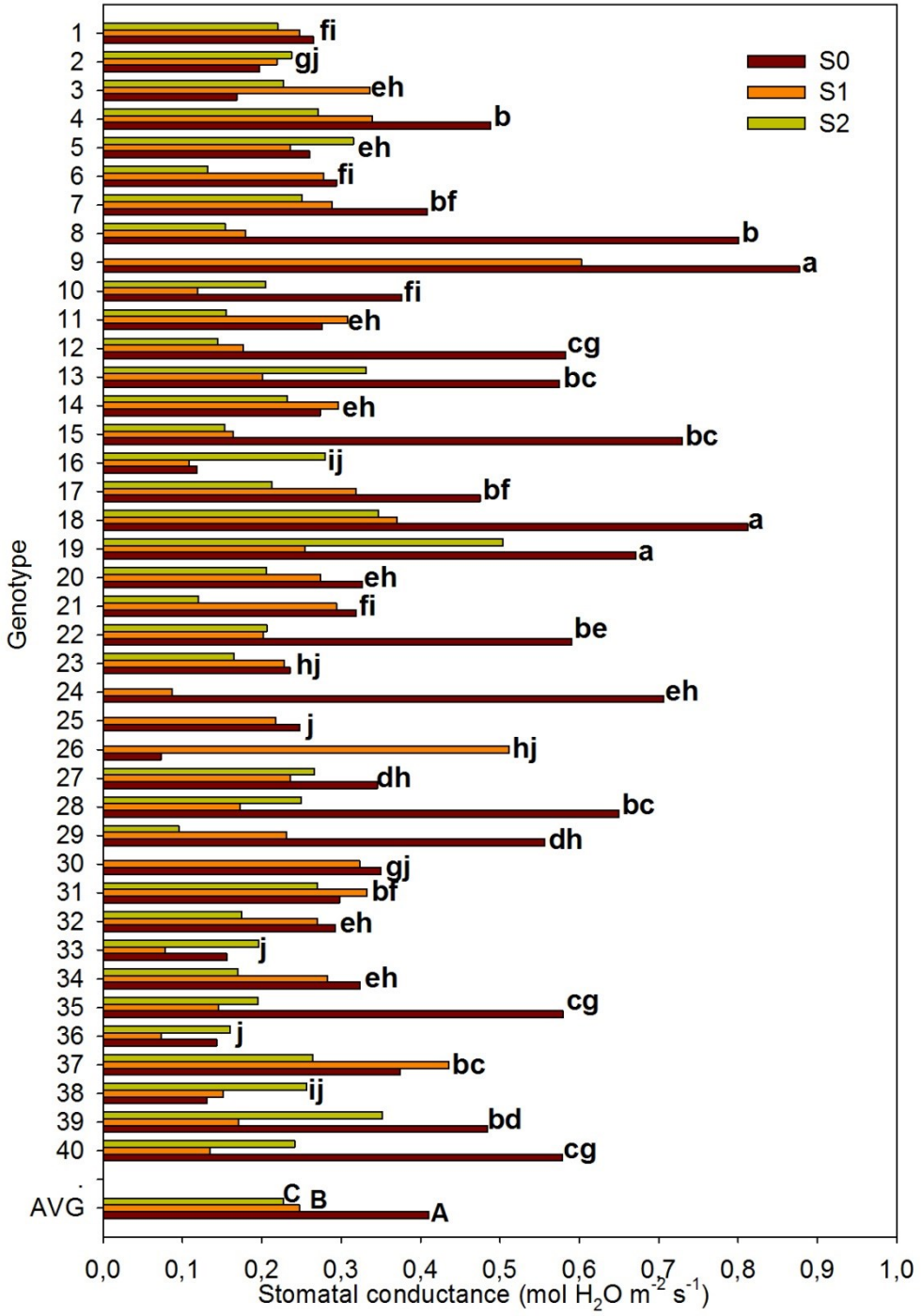

Figure 19. Stomatal conducatance (08/03/2012) of each genotype in the three treatment (S_0, S_1 and S_2). Small letters, for averaged values of salinity level within each genotype; capital letters for averaged values of all genotypes within each salinity level. Different letters indicate significant differences at $P \leq 0.05$ by SNK Test. LSD (genotype x treatment = 0.08).

The relation between photosynthesis and stomatal conductance, taking into account all salt treatments and clones, highlights how the crop is able to increase net photosynthesis as stomatal conductance increase with a coefficient of determination equal to 0.61 (figure 20).

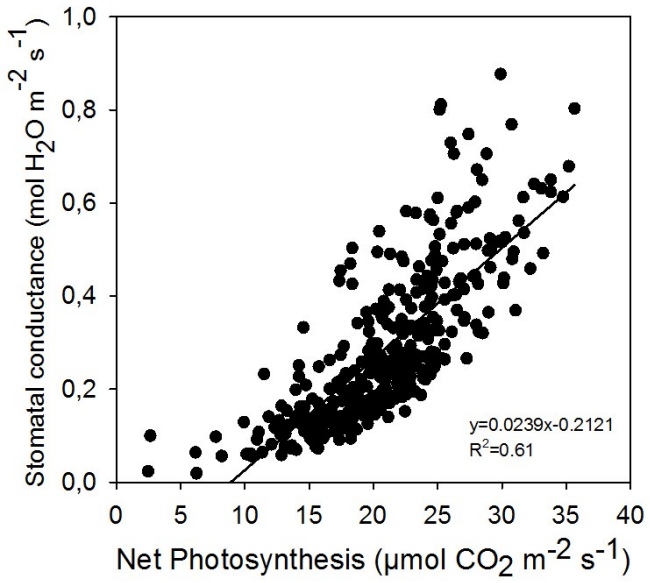

Figure 20. Net photosynthesis vs stomatal conductance (all data).

Correlation analysis also showed a relationship between net photosynthesis and transpiration rate (R^2= 0.69), as shown in in figure 21.

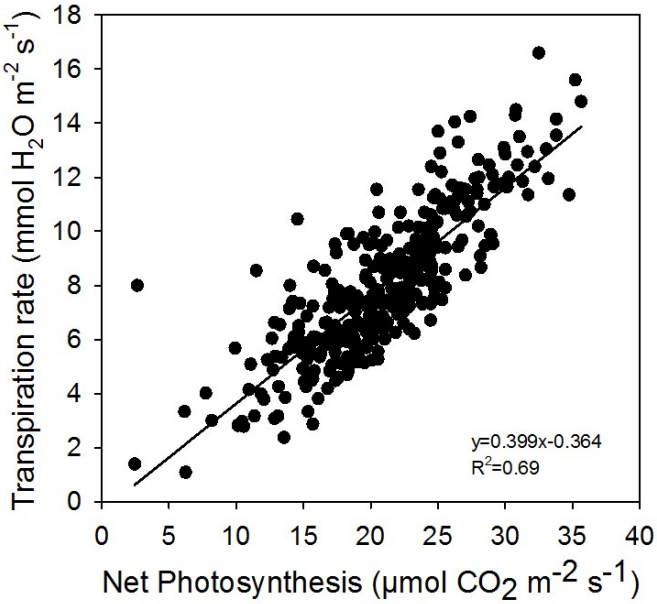

Figure 21. Net photosynthesis vs transpiration rate (all data).

Positively correlated resulted also transpiration rate and stomatal conductance, as shown in figure 22.

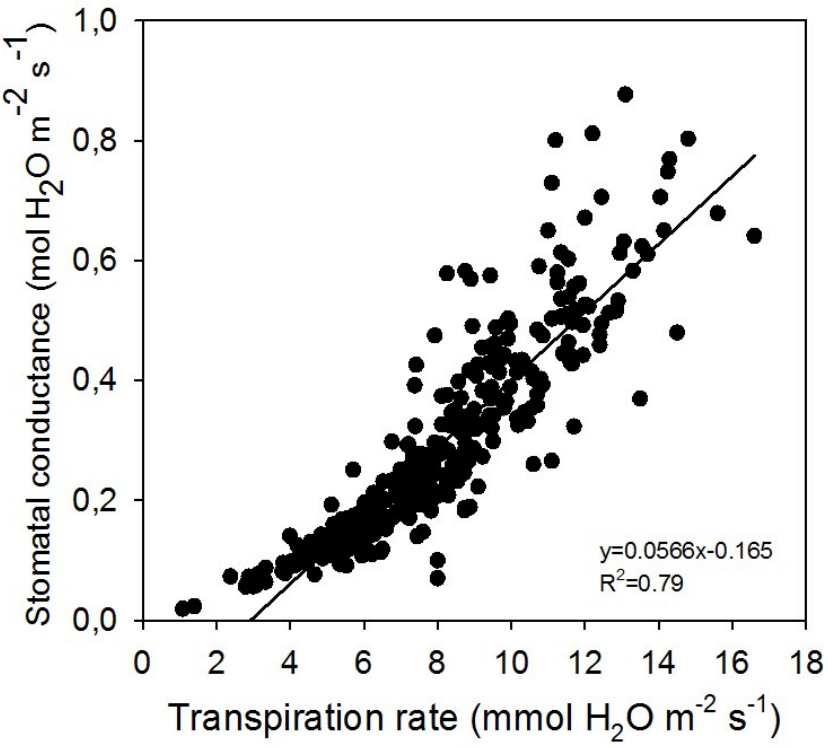

Figure. 22 Transpiration rate vs stomatal conductance (all data).

3.1.9. Genotype screening

In order to carry out a preliminary screening with the measurements obtained so far the following steps have been attempted: (i) calculate the average of the treatment in the same, (ii) calculate the standard deviation and then (iii) the coefficient of variability (CV). Plotting the CV versus the aboveground biomass yield in a scatter plot it is possible to split the area of the plot in four sub-areas (A, B, C, and D). Clones laying in the area where there is the highest level of aboveground biomass yield with the lowest CV can be considered the most tolerant to salinity level (A), vice versa the less ones (C) with (B) and (D) showing high biomass yield but low tolerance and high tolerance but low biomass yield, respectively. Therefore, a screening of the different clones, with respect of the aboveground biomass yield, by averaging the three NaCl levels within the same genotype, is shown (Fig. 23).

According to this screening method, genotypes 6, 18, 20, 24, 2 and 11 are considered the most tolerant to salinity levels (A), while genotypes 21, 34, 9, 10, 36, 27, 29, 15, 30 and 35 the sensitive ones (D), having the highest coefficient of variability.

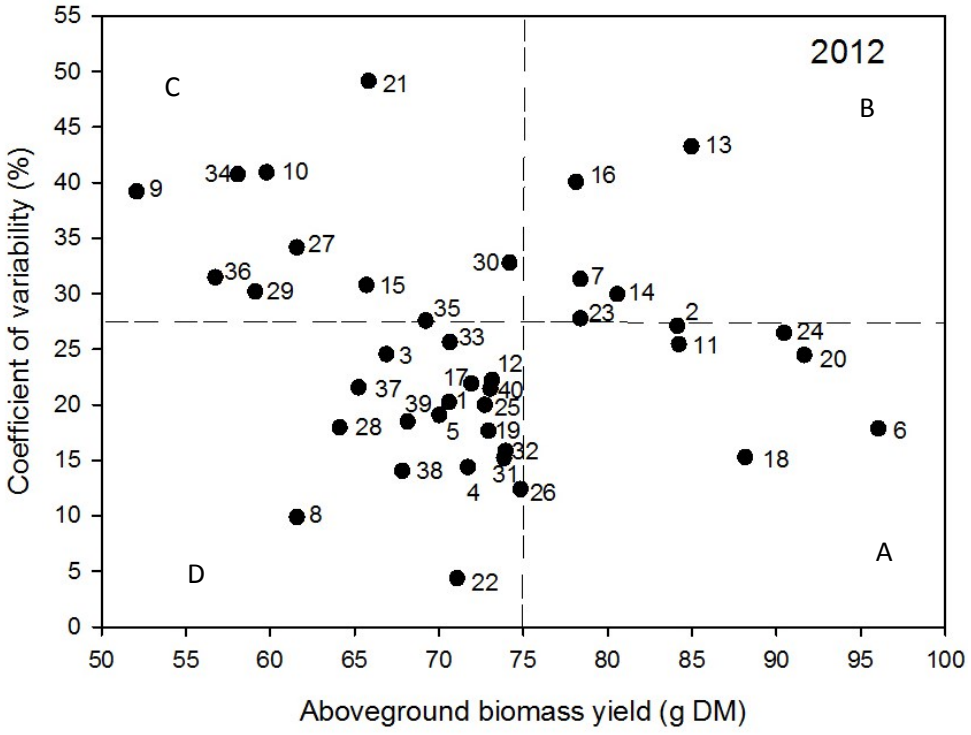

Figure 23. Screening of 40 giant reed clones taking into account aboveground biomass dry matter and coefficient of variability (by averaging a single clone in the three treatments). (A) represents the highest tolerant to salinity, (C) the lowest tolerant with (B) and (D) showing high biomass yield but low tolerance and high tolerance but low biomass yield respectively.

3.2. Results Research Line 2

3.2.1. Soil salinity

The electrical conductivity (EC) is the most commonly adopted measure to determine the salinity of the soil and is indicative of the ability of an aqueous solution to be passed through by an electric current. It is commonly expressed in units of deci-Siemens per meter (dS m^{-1}) for standard agricultural soils with an EC greater than 4 dS m^{-1} are considered saline. Plants sensitive to soil salinity can be affected by an electrical conductivity of less than 4 dS m^{-1}, while tolerant or resistant species are not affected by concentrations of concentrations double or even triple the upper limit of tolerance farm.

The salinity of the soil was measured in the pots of the different compared treatments at 33, 47, 54, 75, 104, 125 and 138 DAT (Fig. 24).

In the average of soil salinity levels, the I_{100} treatment showed, at the end of the cycle, higher level of soil salinity than the I_{25} (6.9 dS m^{-1} with 6.5 dS m^{-1}, respectively) .

In the average of the irrigation water, soil salinity increased linearly with the passing of days after transplanting to settle at the highest values of 10.6 dS m^{-1} in the S_2 treatment, followed by the S_1 treatment (8.0 dS m^{-1}) and the control (1.59 dS m^{-1}) (Fig. 24).

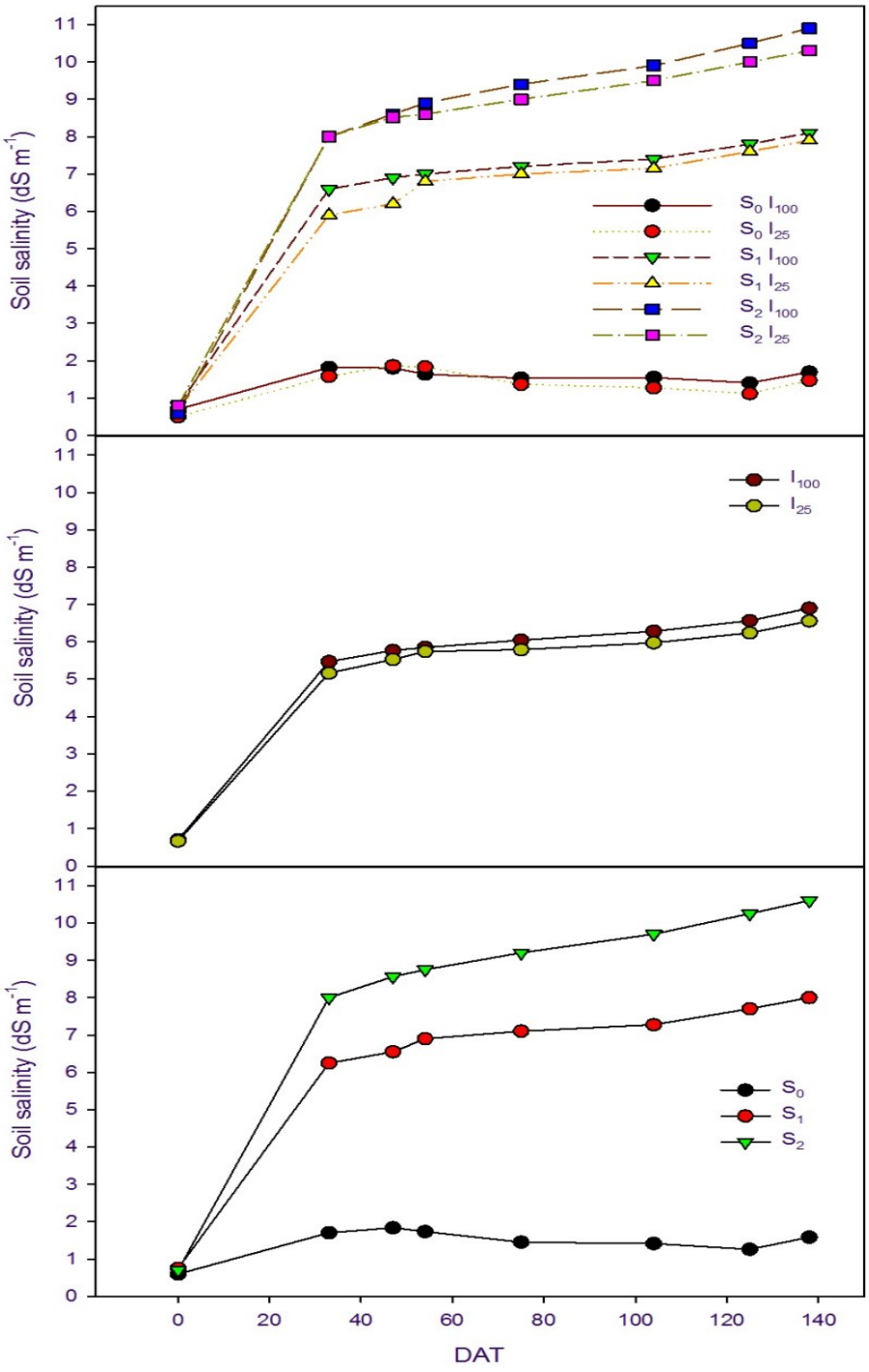

Figure 24 - Evolution of soil salinity in relation to different treatments in the studied of genotypes on average.

3.2.2. Main stem height

The height of the stem, measured at the last survey (112 DAT) was higher in the treatment without salt added (S_0), in both water levels (I_{25} and I_{100}). The treatment with the medium salt concentration (S_1) gave, for both water levels, higher values than the treatment with the highest salt concentration (S2) (Figure 25) .

In particular, the control (S_0), shoved the highest height in the average of the studied genotypes, and in both the water levels (I_{25} and I_{100}) tested. Observing the single treatments in the average of the genotypes, S_0I_{100} and S_0I_{25}, showed heights of 182.9 cm and 153.1 cm, respectively, while S_1I_{100} and S_1I_{25} treatment showed an heights of 156.8 cm and 144.2 cm, higher than those observed in S_2I_{100} and S_2I_{25} treatment (126.8 cm and 139.0 cm, respectively).

The not saline treatment (S_0), provided an average height of the stem higher than those provided by the saline treatments (S_1 and S_2) with values observed of 168.0 cm, 150.5 cm and 132.9 cm, respectively.

The restoration of 100 % of the evapotranspirated water (I_{100}) pointed out in the average of the genotypes, an height of stems greater than that provided by the treatment that involved the lower restoration water (I_{25}) with values of 155.5 and 145.0 cm and 125.0 cm, respectively.

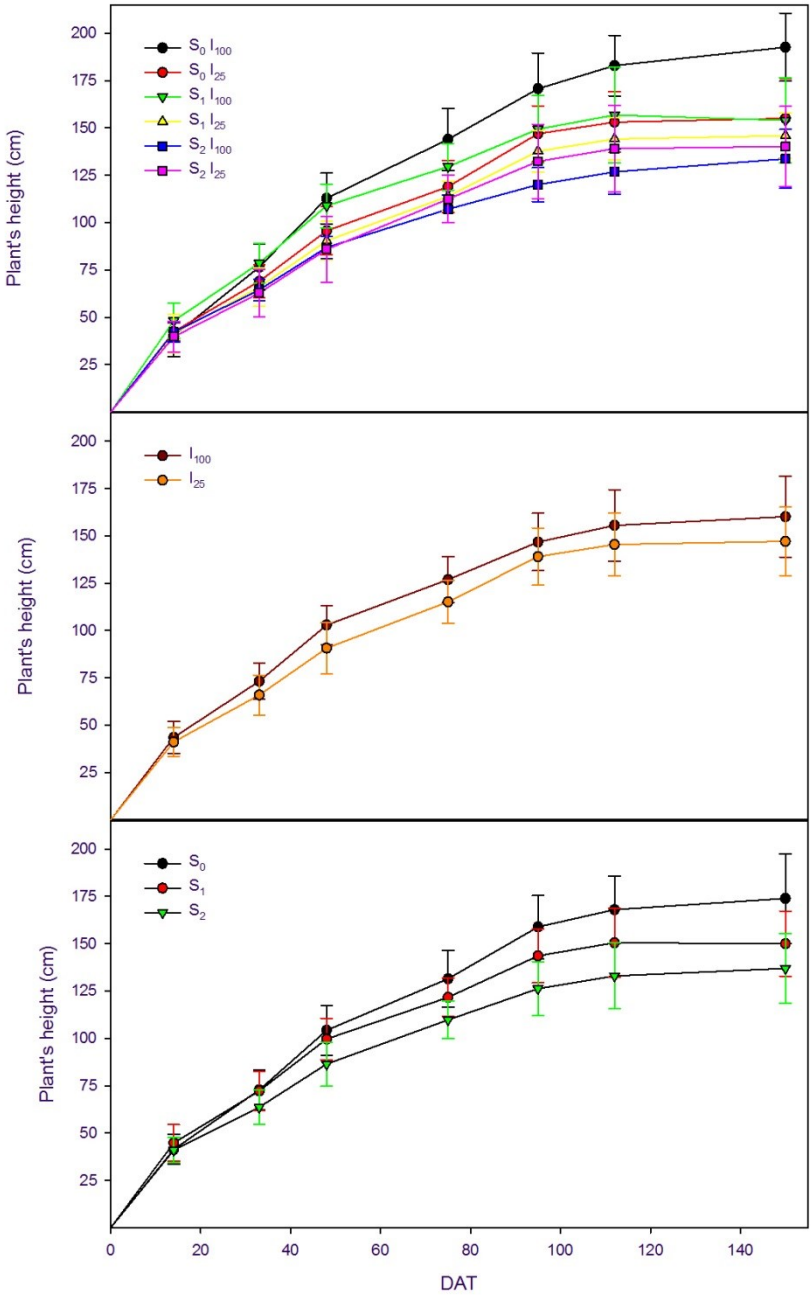

Figure 25. - Height of the main stem in relation to different treatments in the average of the studied genotypes

In the S_0I_{100} treatment the average height of the main stem was equal to 183.0 cm. Among the genotypes 6, 24 and 14 attained the greatest height (216, 207 and 203 cm, respectively) while the lowest was the genotype 20 (135 cm). All the other genotypes showed heights close to the average (figure 26). Genotypes 14 and 24 also in S_0I_{25}

treatment showed the highest height of the main stem (179 and 180 cm, respectively). In this treatment the average height was equal to 153 cm. The lowest height was observed in the 34 genotype (114 cm), while in the other genotypes were observed heights between 127 cm (18) and 174 cm (6).

In the treatment with the intermediate salinity level (S_1) and with the highest water restoration (I_{100}), the average height of the main stem was equal to 157 cm. The highest height were observed in the genotypes 18 and 16 (207 and 188 cm, respectively). In the treatment S_1I_{25} the highest height was observed in the genotype 18 (204 cm) followed genotype 24 (179 cm), while the lowest height was observed in genotype 14 (105 cm).

In the treatment with the highest salt concentration and water restoration (S_2I_{100}), the genotypes that reported the best heights were 2 (176 cm) and 18 (156 cm), while the lowest was 34 (71 cm); the average value was equal to 127 cm. In the same salinity level but in I_{25} restoration the genotypes 24 and 14 were characterized by the highest height (200 and 173 cm) while the lowest value was provided by genotype 34 with 90 cm.

In the average of all salinity and water treatments the significantly highest height were observed in genotypes 24 and 18 (178 and 173 cm, respectively), while the lowest were 20 and 34 (124 and 121 cm, respectively).

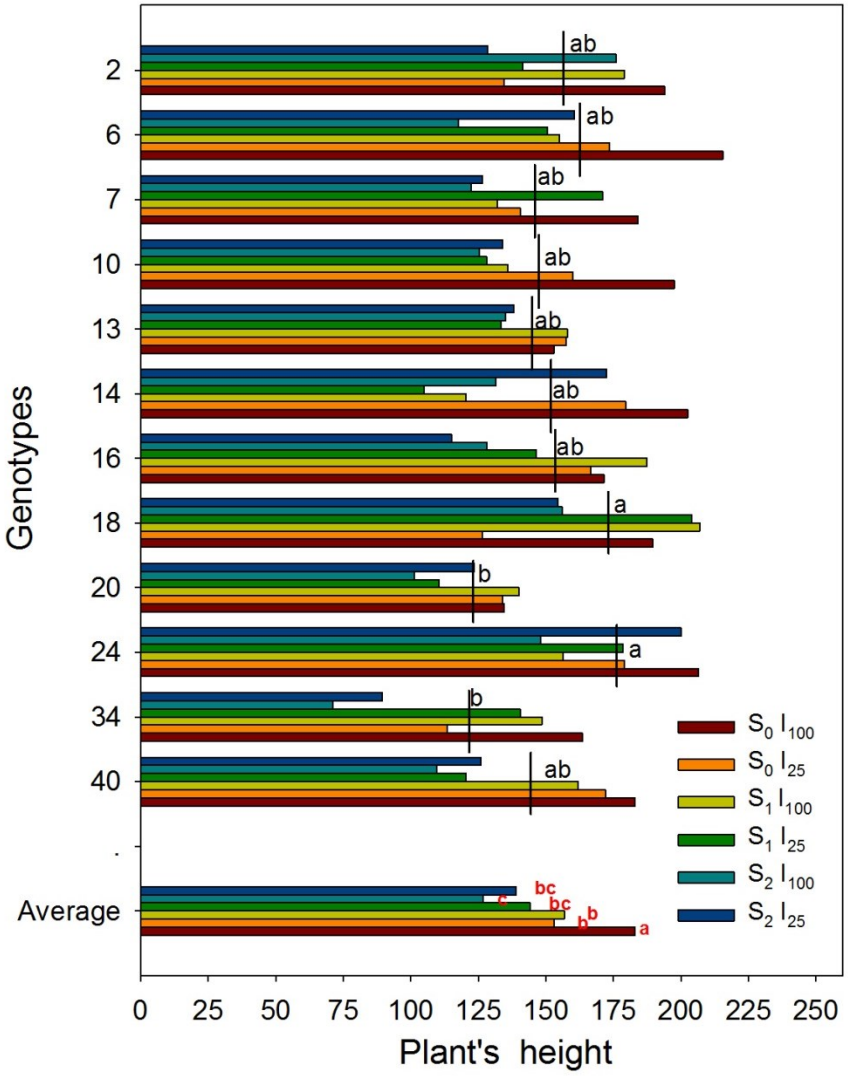

Figure 26 - Height of stems released among the different genotypes in relation to the studied factors. Vertical line represent the average value of the six treatments. Small letters, for averaged values of salinity and irrigation levels within each genotype; red letters for averaged values of all genotypes within each salinity and irrigation levels. Different letters indicate significant differences at P≤0.05 by SNK Test.

3.2.3. Stem number

The stems number per pot, observed on the last field survey (112 DAT) was higher in the S_0 treatment (9.3) in both water levels tested (I_{100} and I_{25}, 10.3 and 8.3 stems, respectively) (Figure 27).

In reference to the high salinity level (S_2) the highest number of stems was observed in the I_{100} treatment (9.2) while the lowest in the I_{25} treatment (7.9). Even the medium salinity level (S_1) showed, again in the average of the genotypes, a higher number of stems in the treatment with a maximum water restoration (8.4 to 7.5 stems).

In reference to the salinity of the soil and in the average of the irrigation levels and genotypes, the treatment S_0 showed a value of stems of 9.3, higher than that recorded in treatments S_1 and S_2 that reported a stems number, between the studied genotypes, equal to 8.0 and 8.6, respectively.

With regard to the water content, as expected, the restoration of 25% of the evapotranspirated water (I_{25}) showed a stems number less (7.9) than that provided by the restoration of 100 % of the evapotranspirated water (I_{100}) (9.3).

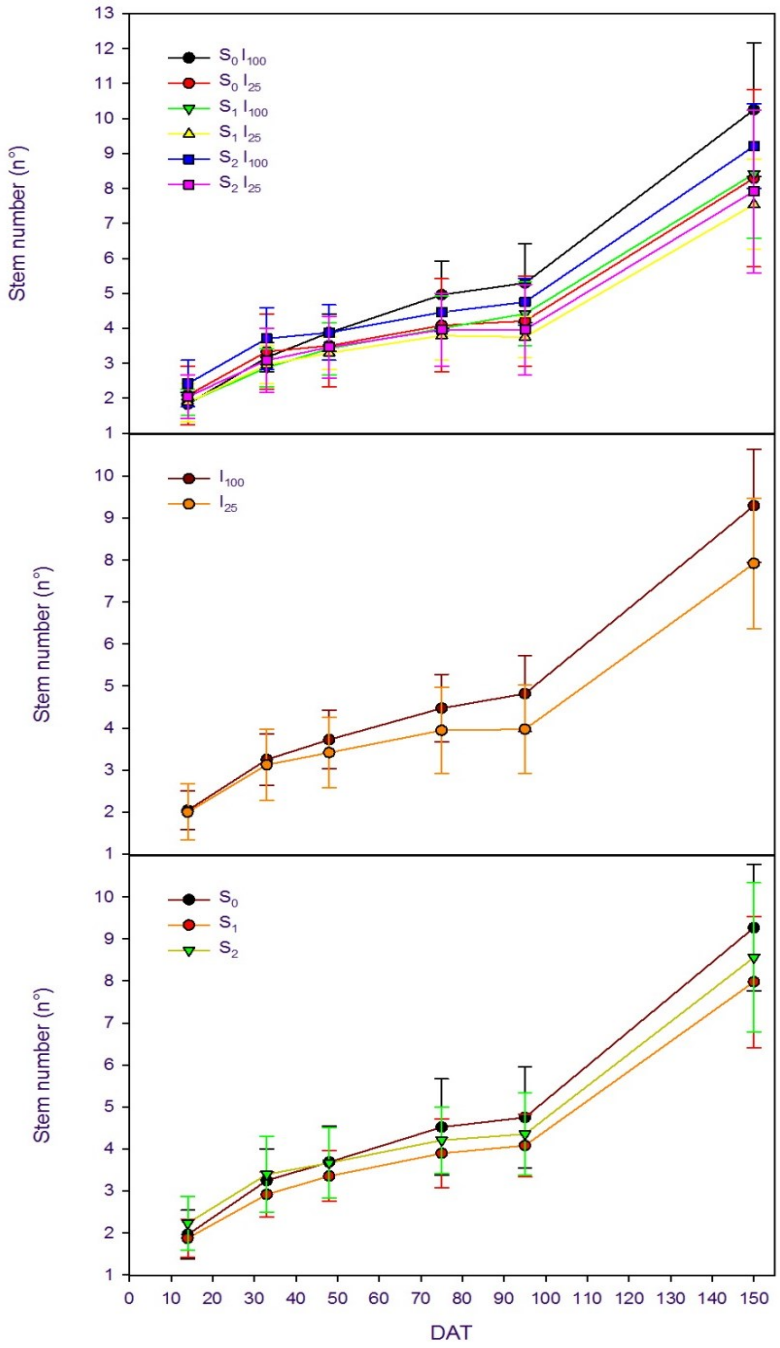

Figure 27 - Trend in the number of stem issued in relation to the different treatments in the average of the studied genotypes.

The genotypes that provided a greater stems number in the S_0I_{100} treatment, whose average was equal to 10.2, were 34 and 10 with values of 14.0 and 13.5, respectively. The genotype with the lowest number of stems was 20 with a stem number equal to 6 (figure 28). In the treatment without salt added and with the lower water restoration

(S_0I_{25}), the stems number was equal to 8.3; in this case the genotypes that provided a higher number of stems were 34 and 13 with values equal to 12.0, and 5.0, while the lower was the genotype 24 (4). The S_1I_{100} showed an average stem number of 8.4. In this treatment the genotypes with highest stems number were 40 and 7 with an average of 10.5 and 10.0 stems, respectively, while the genotype with the lowest was 13 with 5 stems.

In the treatment S_1I_{25} the genotype with the lowest stem number was 24, with 3 stems per pot, while the genotypes that showed the highest stem number were 34 and 40 with 13 and 10 stems per pot, respectively.

In the treatment, which provided both the highest salt concentration and the greatest water restoration (S_2I_{100}), genotypes that showed the highest stem number were 40, 34 and 13 with 6, 11 and 11 stems, respectively, while genotypes that showed the lowest number were 16, 13 and 10 with a 7 stems per pot. The high stressed treatment (S_2I_{25}) generally showed the lowest number of stems. In this treatment the lowest numbers of stem were observed in genotypes 10 and 2 with 10.5 and 10.0 stems per pot, respectively. The lowest number of stems (5) was in this case observed in genotypes 16 and 24.

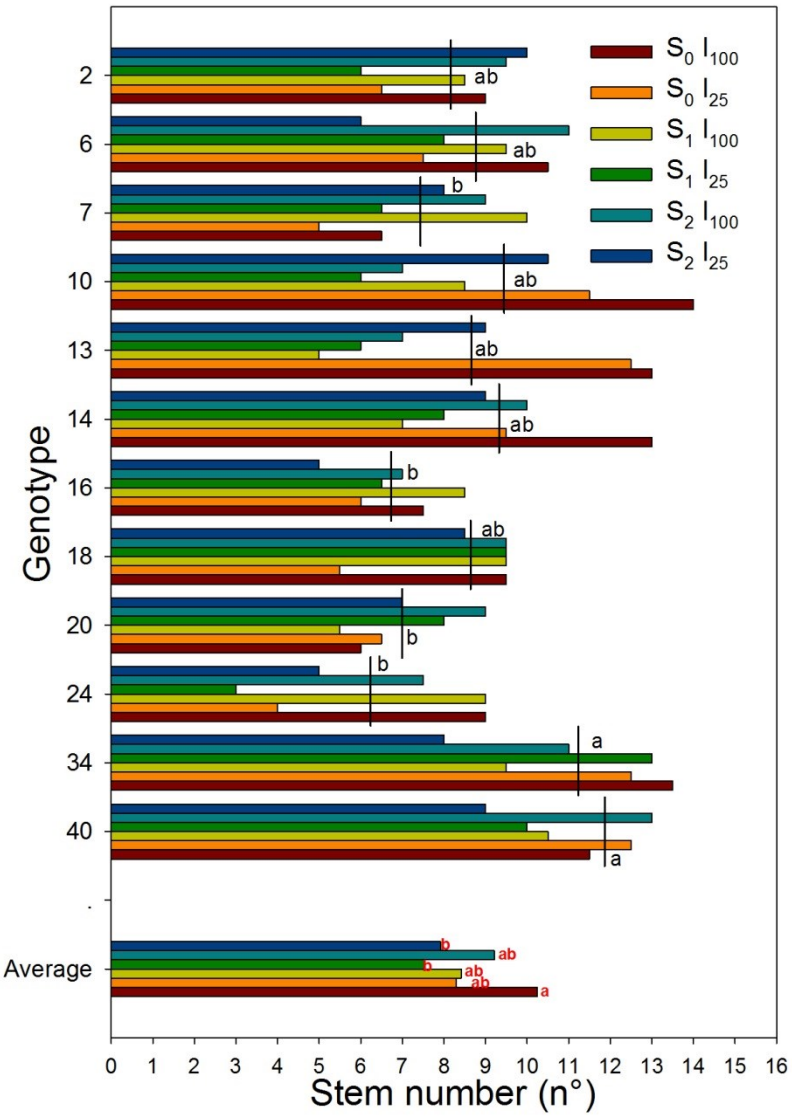

Figure 28 - Number of stems released during the different genotypes in relation to the studied factors. Vertical line represent the average value of the six treatments. Small letters, for averaged values of salinity and irrigation levels within each genotype; red letters for averaged values of all genotypes within each salinity and irrigation levels. Different letters indicate significant differences at P≤0.05 by SNK Test.

3.2.4. Leaf number

The number of leaves per stem, observed at 112 days after transplantation, followed in the average of the studied genotypes and in the average of the irrigation levels, the trend of soil salinity. With increasing of soil salinity the number of leaves decreased (30.4, 29.4 and 28.3, respectively for the thesis S_0, S_1 and S_2) (Figure 29).

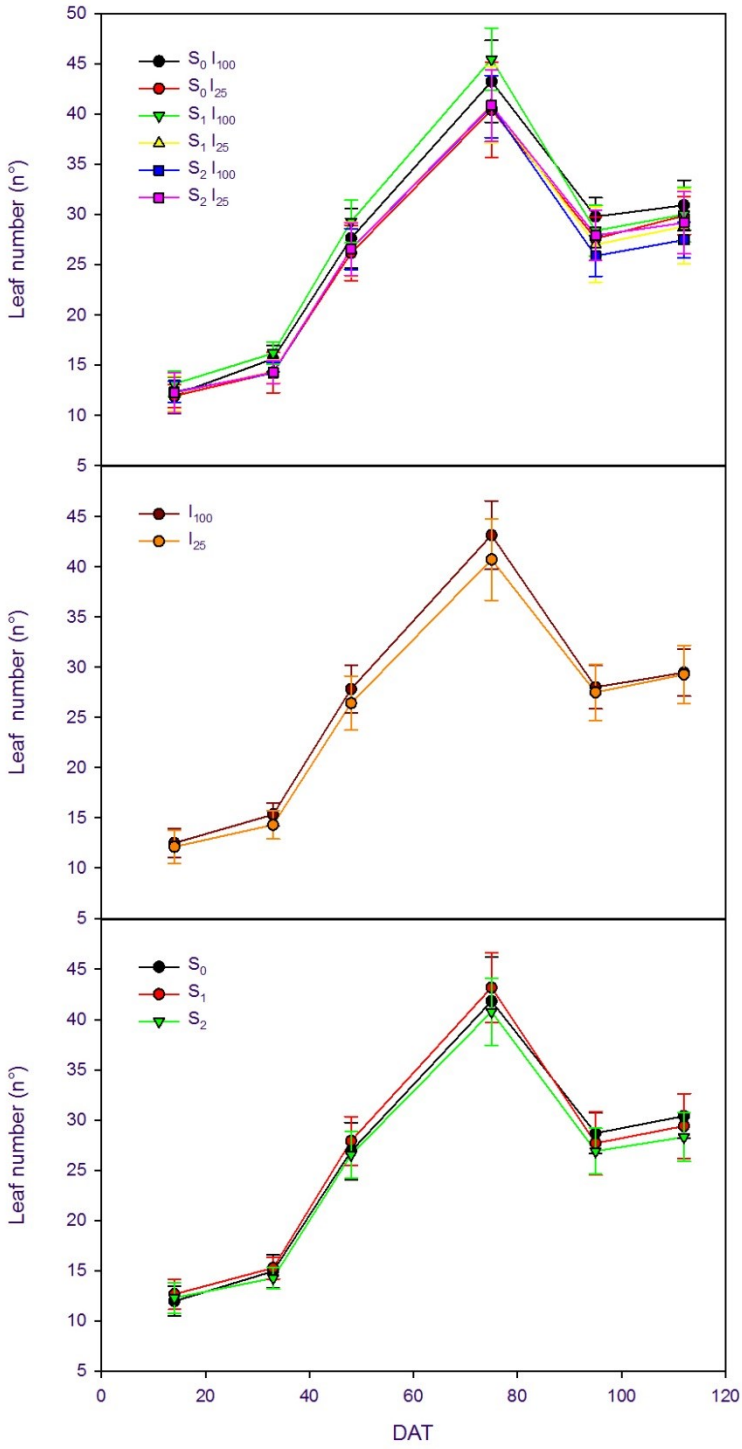

Figure 29 - Trend in the number of leaves issued in relation to the different treatments in the average of the studied genotypes.

The water levels did not affect the number of leaves per stem, 29.5 and 29.3 respectively for the thesis I_{100} and I_{25}.

In the treatment without sal added and with the greatest water restoration (S_0I_{100}) , genotypes that are distinguished for the highest number of leaves produced were 2 and 38 with an average number of 38 and 35.5, respectively. The lowest number of leaves was observed in genotype 13 with 24.5 leaves per stem. The other genotypes showed values close to the average (30.9) (Figure 30).

In the treatment S_0I_{25}, the average number of leaves was equal to 29.9, within this treatment genotype 6 pointed out the maximum number of leaves (34.5), followed by genotype 13 (33), while the lowest value was observed in genotype 14 (21.5). Even in the treatment S_1I_{I00} genotype 2 confirms the high number of leaves issued (35.5). The genotype 14 also confirms in the S_1I_{25} treatment the lowest number of leaves (24.5).

The treatment with the higher salt concentration and higher water restoration (S_2I_{100}), produced the lowest number of leaves (27.5) on average. Among the genotypes the highest number was observed in genotypes 18 and 2 (34 and 33.5 leaves per stem), while the lowest number was observed in 34 with a number of leaves equal to 24.5 .

In the average of the studied treatment, the genotypes with the highest number of leaves were 2 and 18, with 33.3 and 32.7 leaves per stem, while the genotype with the lowest number of leaves was 14, with 25.4 leaves per stem.

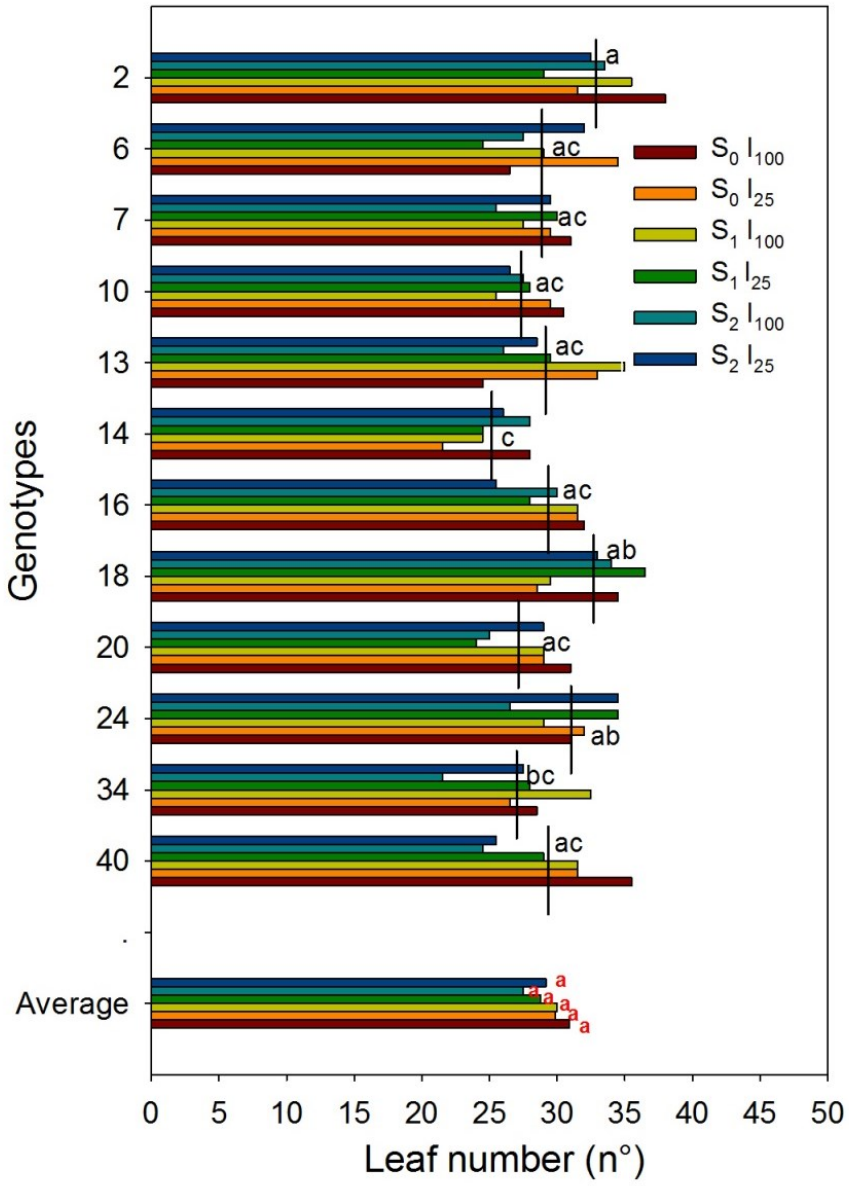

Figure 30 - Number of leaves in the different genotypes in relation to the studied factors - Vertical line represent the average value of the six treatments. Small letters, for averaged values of salinity and irrigation levels within each genotype; red letters for averaged values of all genotypes within each salinity and irrigation levels. Different letters indicate significant differences at P≤0.05 by SNK Test.

3.2.5. LAI

The leaf area index (LAI), for all treatments, increased in the first two months after transplant and then decreased until the date of the last measurement made.

The treatment without salt and with the water restoration equal to 100% (S_0I_{100}) provided in the average of the studied genotypes an average value of LAI equal to 2.7, higher than that observed in the saline treatments S_1 and S_2, that showed LAI values equal to 2.1 and 2.0 respectively (Figure 31).

The treatment without salt added and with the lowest water level (S_0I_{25}), provided in the average of the genotypes, a higher value of LAI (2.9) compared to the saline treatments S_1 and S_2 (2.6).

Analyzing the saline treatments in the average of the irrigation levels, treatment S_0, as for the other characters previously discussed, recorded the highest value compared to higher salinity levels (S_1 and S_2). The highest LAI value (3.5) was reached at 95 days after transplant in the treatment S_0I_{100}, the time after which suffered a decrease, falling at 112 days after transplant (04.10.2013) at 2.8.

Treatment S_1, on average reached the highest LAI (3.2) at 75 days after transplant and then decreases up to a value of 2.4, in the average of the genotypes observed at 112 days after transplant. Even the treatment with the highest salt concentration (S_2) reached the highest value of LAI (2.8) at 75 days after transplant, time after which showed a decrease up to a value of 2.3, detected at 112 days after transplant.

As for the water treatment, contrary to what was observed in the other characters, the treatment which provided the lowest water restoration (I_{25}) presented from 48 days after transplant, higher values than the treatment with the maximum water level (I_{100}).

In the average of salinity levels, during the time of maximum leaf expansion, which occurred at 75 days after transplant, the value of LAI observed was equal to 3.3 in both the water levels (25% and 100% of water restoration).

At the last measurement carried out, at 112 days after transplant, the average of the treatment I_{25} showed LAI values on average of 2.7, while in the treatment I_{100} the average value was equal to 2.3.

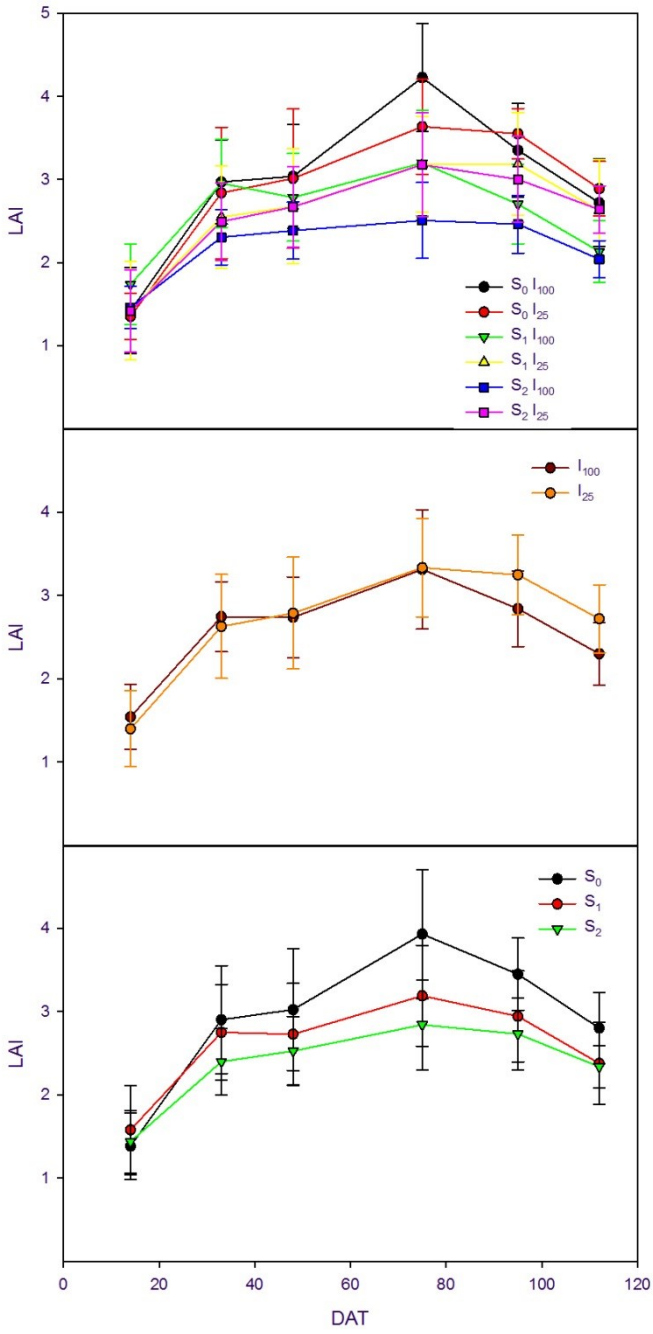

Figure 31 - Evolution of leaf area index in relation to different treatments in the average of the studied genotypes.

The leaf area index (LAI) showed values in a range between 2 (S_2I_{100}) and 2.9 (S_0I_{25}). In the treatment S_0I_{100}, the average LAI was equal to 2.7 (Figure 32). The genotypes that showed a highest values were 10, 18 and 34 with values of 3.8, 3.6 and 3.5, respectively, while the genotype that showed the lowest LAI was 6 (1.8). In the

treatment without salt added, and which the lowest water restoration (S_0I_{25}) genotypes that had a higher LAI were 10 and 40 with a value of 3.8, while the lowest LAI was observed in genotype 2.1; the others genotypes provided a LAI value close to the average that was equal to 2.9.

In the average of the saline treatment with the higher water restoration (S_1I_{100}), the average LAI was equal to 2.1; the best genotypes were 18 and 34 with respective values of 3.4 and 3, while the worsts were 14 and 10 with LAI values of 1.5 and 1.6, resepectively. In the treatment S_1I_{25}, among the genotypes, 14 and 20 showed the lowest LAI (1.6 and 1.5, respectively), while genotypes 7 and 2 showed the highest LAI values equal to 4 and 3.4, respectively.

The treatment with the highest salt concentration and with the highest water level (S_2I_{100}), had in the average of genotypes a LAI value equal to 2; among the genotypes that have provided an higher LAI, genotypes 2 and 18, with values above the average and equal to 2.7 and 2.6, respectively, were distinguished from the others, while the lowest LAI was provided by the genotype 34 (1.2).

In the treatment S_2I_{25}, whose average LAI was equal to 2.6, the best genotypes were 18 and 6 with LAI values of 4.2 and 3, the highest values observed among the studied genotypes, while the lowest LAI was observed in the genotype 14 (2).

In the average of the studied treatments, the genotype that showed the highest LAI was genotype 18, with a LAI value of 3.3 on average while the genotype that observed the lowest value of LAI was genotype 20 with a value below the average (2.5) and equal to 2.

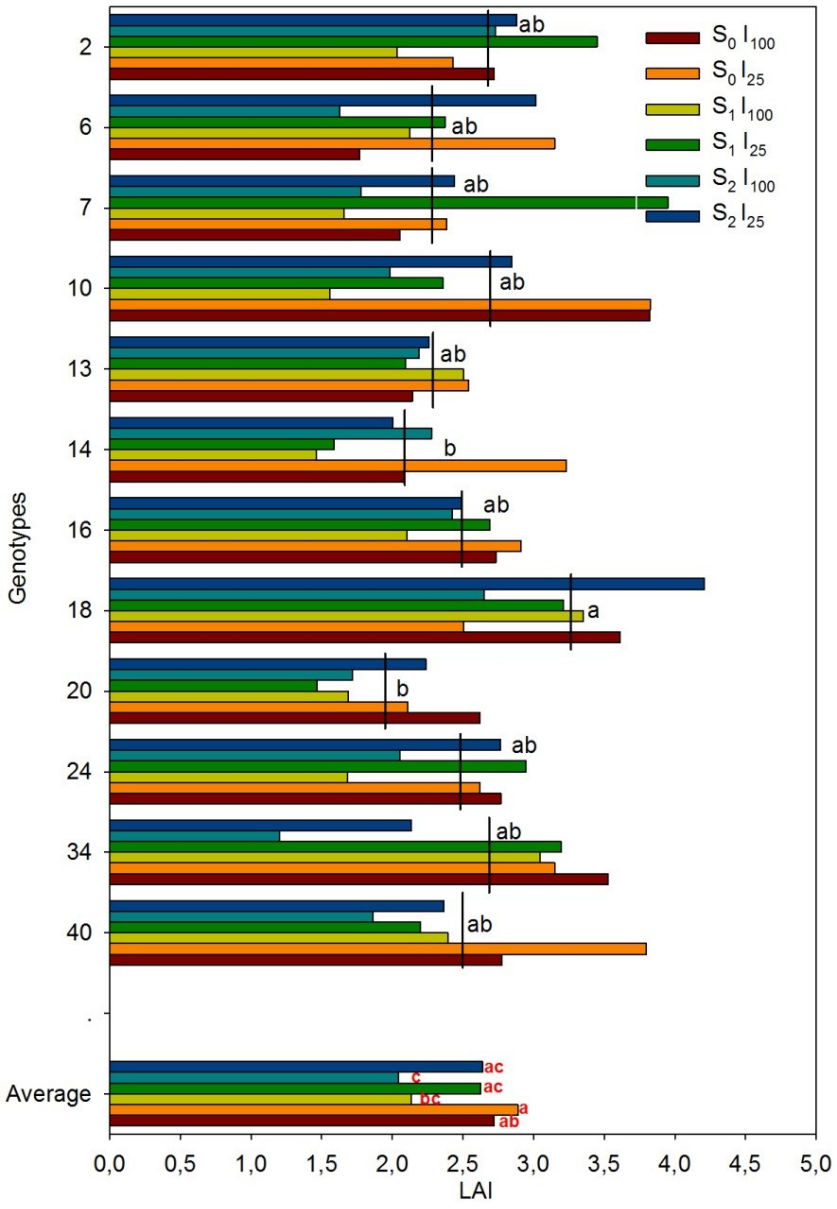

Figure 32 - Leaf area index recorded in the last survey date before the final harvest. Vertical line represent the average value of the six treatments. Small letters, for averaged values of salinity and irrigation levels within each genotype; red letters for averaged values of all genotypes within each salinity and irrigation levels. Different letters indicate significant differences at P≤0.05 by SNK Test.

3.2.6. SPAD unit

The SPAD unit, measured at leaf level, allows to have an estimate of chlorophyll content of leaves. The measurements were made on the third leaf fully expanded at 28, 45, 54, 74, 105, 115 and 126 DAT in each genotype compared and in all treatments tested in the study.

The trend observed decreased over time in all the compared treatments, but in the middle of the stage of plant growth, the SPAD values stabilized for about 60 days before decreasing in all the studied treatments.

In the average of the studieded genotypes, the observed SPAD values were not much differed from each others, in fact in the last measurement performed (on 30 September), they varied between the lowest value observed in the treatment S_0I_{100} (35.2) and the highest value observed in the treatment S_2I_{25} (Figure 33). In relation to soil salinity, in the average of irrigation levels, the treatment with the higher concentration of salt showed higher values of SPAD .

The value of SPAD units is reduced in relation to the plant's growth, its decrease may be due not only to the development stage of the plant, even to the variation of the nutritional status of the soil and in particular to the nitrogen content.

With regard to the different levels of soil salinity, in the average of irrigation treatments, the treatment with the highest salt concentration (S_2) showed higher SPAD values than the S_1 treatment and the control (S_0).

In the average of salinity levels, the treatment with the highest level of water restoration showed slightly higher values in SPAD units.

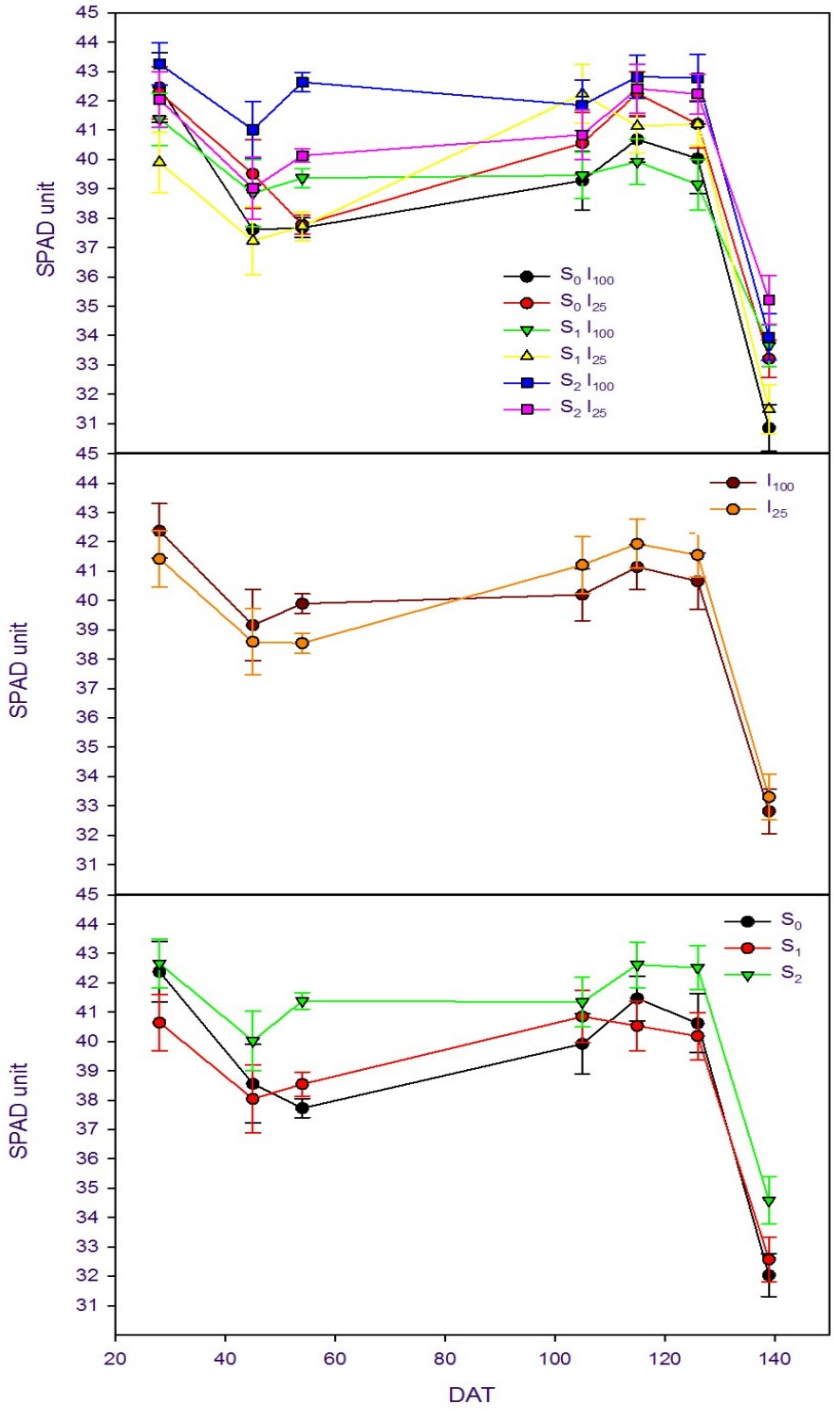

Figure 33 - SPAD units in relation to the different treatments in the studied genotypes on average.

3.2.7. Aboveground biomass yield

In the average of the studied genotypes S_0 treatment yielded more in both levels of water restoration adopted (I_{100}, I_{25}), than treatments S_1 and S_2. In particular, the non-saline treatment provided in the average of the genotypes, dry biomass production equal to 200.0 g in the

I_{100} treatment, and 115.2 g in the treatment with a lowest water restoration (I_{25}); the saline treatments (S_1 and S_2) yielde in the average of the genotypes 139.7 and 96.3 g in the I_{100} treatment and 99.0 g and 90.8 g in the I_{25}, treatment, respectively (Figure 34). The restoration of 100% of the water supplied in the average of the saline treatments and in the average of the genotypes, provided dry biomass production higher than that provided by the treatment with the lowest water restoration (I_{25}).

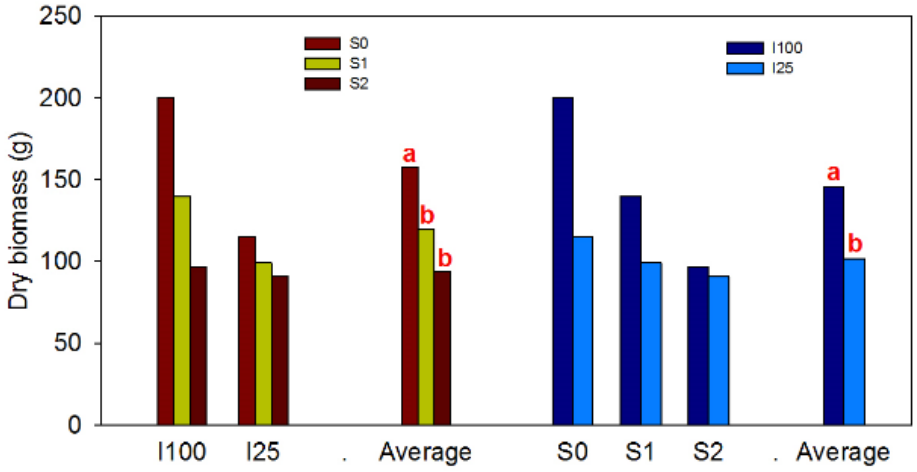

Figure 34. Dry biomass (g) in the average of the irrigation levels (I_{25} and I_{100}), of the saline treatments (S_0, S_1 and S_2) and of the genotypes. Different letters indicate significant differences for $P \leq 0.05$ (SNK test).

About genotypes, in the treatment S_0I_{100}, the genotypes 34, 10 and 6, provided the highest dry biomass production, with yields of 274.1 g, 236.2 g and 231.3 g, respectively, while the genotype that provided the lowest production of aerial biomass was 20 with 153.4 g. In the S_0I_{25} treatment, , the genotype 10 yielded the most (141.2 g), while the genotype 7 was the less productive (67.0 g).

In the S_1I_{100} treatment, the highest dry biomass production among all genotypes and among all the treatments was recorded: it was provided by the genotype 40 and it was

equal to 221.5 g; in the same treatment, a good production has also been provided by the genotype 18, whose dry biomass was equal to 210.0 g, while the genotypes that have provided the lowest dry biomass production were 20 and 14 with 86.2 g.

In S_1I_{25} treatment genotype 7 yielded the most (170.0 g), followed by genotype 18 was biomass was equal to 144.0 g. The genotypes that in this treatment had the lowest dry biomass production were 20 and 14 with 56.3 and 59.9 g, respectively.

In the most saline treatment (S_2), the genotypes that have provided a highest dry biomass production in the I_{100} treatment were the genotypes 18 and 2 with respective productions equal to 159.7 g and 141.2 g, while the genotype that provided the lowest values of dry biomass was 34 with 69.5 g.

The S_2I_{25} treatment pointed out as the most productive the genotypes 18 and 10 with a dry biomass production amounted to 135.4 g and 119.2 g, respectively , while the genotypes that gave the lowest values were 34 and 7 with a production of 69.5 g and 50.9 g, respectively.

In the average of the compared treatments, the genotype that has provided the highest production of dry biomass was genotype 18 with 148.9 g , while the less productive was the genotype 20 with a dry biomass production equal to 85.9 g (figure 35).

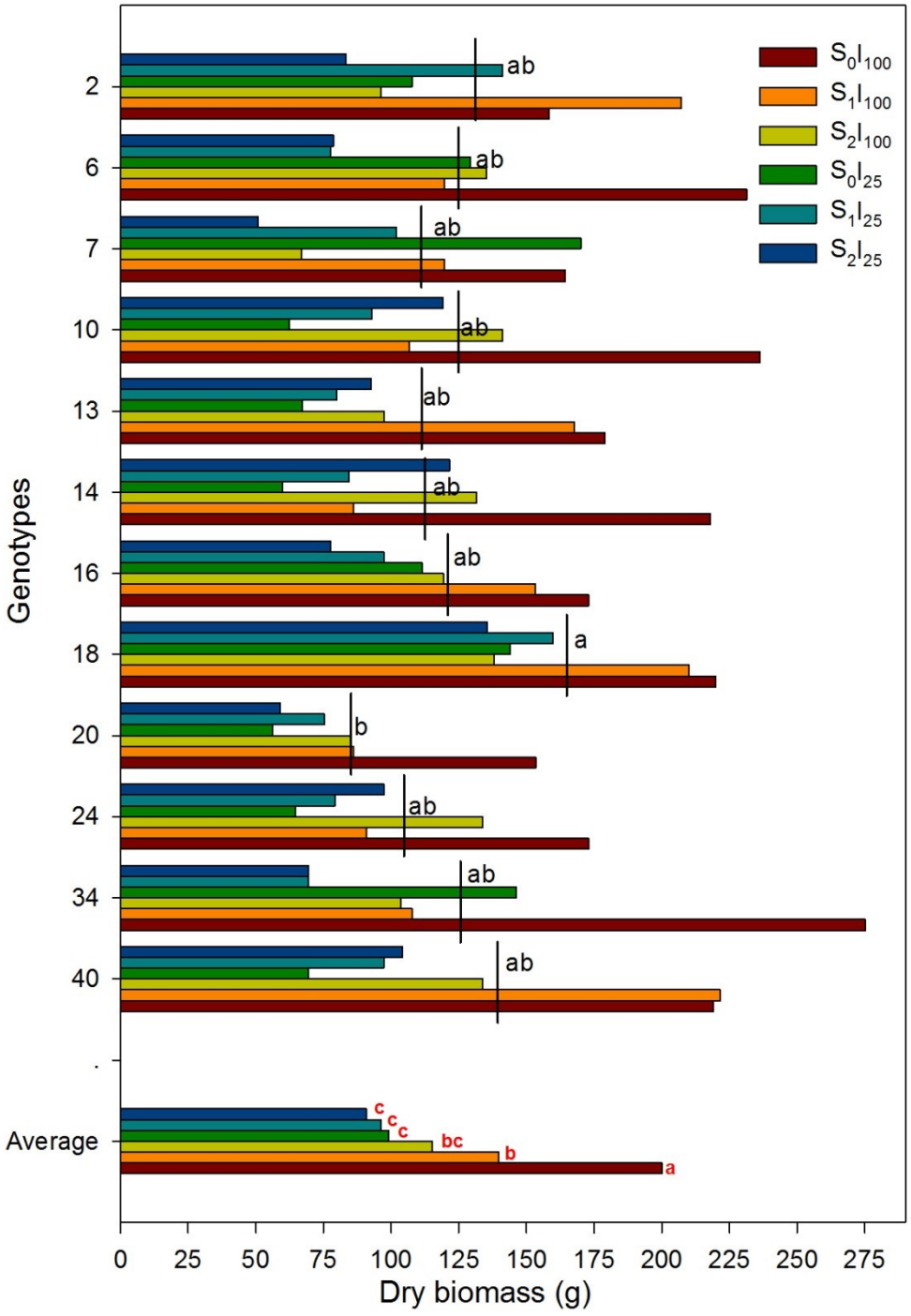

Figure 35. Dry biomass (g) of the different genotypes, irrigation treatments (I_{25} and I_{100}) and saline treatments (S_0, S_1 and S_2). Vertical line represent the average value of the six treatments. Small letters, for averaged values of salinity and irrigation levels within each genotype; red letters for averaged values of all genotypes within each salinity and irrigation levels. Different letters indicate significant differences at $P \leq 0.05$ by SNK Test.

3.2.8. Physiological measurements

Physiological measurements have been carried out at different dates (August 6th, September 26th, October 17th and October 30th 2013), however only, September 26th is shown and discussed below. The other measurements dates are available in the annex.

Photosynthesis

Gas exchange between the atmosphere and the different genotypes of *Arundo donax* in the different studied treatments (S_0I_{100}, S_1I_{100}, S_2I_{100}, S_0I_{25}, S_1I_{25} and S_2I_{25}), were measured after 53, 104, 125 and 138 days after transplanting, using an infrared gas analyzer system (LICOR 6400).

Regarding the net photosynthesis, in all salt levels, the greater water content showed higher values than those measured using the 25% water restoration, in the average of the saline treatments and genotypes; in the 100% water restoration the values of net photosynthesis were equal to 21.39 µmol CO_2 m^{-2} s^{-1}, compared to those provided by the 25% water restoration (19.67 µmol CO_2 m^{-2} s^{-1}) (Fig.37).

Regarding the net photosynthesis, in all the studied different water levels, the control treatment (S_0), observed in the average of all the studied genotypes, showed the higher photosynthetic rate than that measured in S_1 and S_2 treatments; the net photosynthesis values observed in the studied treatments were equal to 25.53, 19.81 and 16.24 µmol CO_2 m^{-2} s^{-1}, for S_0, S_1 and S_2, respectively.

In the average of the studied treatments, and among the genotypes, those who showed the highest photosynthetic rate were genotypes 20, 24 and 40 with 22.2 µmol CO_2 m^{-2} s^{-1} and 21.9 µmol CO_2 m^{-2} s^{-1}. In the S_0I_{100} treatment, whose net photosynthesis on average was equal to 26.4 µmol CO_2 m^{-2} s^{-1}, genotypes 40 and 10 showed the best performances with values of net photosynthesis equal to 31.1 and 30.9 µmol CO_2 m^{-2} s^{-1}, respectively, while genotype 13 showed the lowest photosynthetic rate in the same treatment, reaching a value equal to 21.1 µmol CO_2 m^{-2} s^{-1}.

In the S_1I_{100} treatment, genotype 34 provided the highest photosynthetic rate (24.5 µmol CO_2 m^{-2} s^{-1}), while the lowest values were detected by genotypes 6 and 18 with values equal to 16.9 µmol CO_2 m^{-2} s^{-1}, and 17.4 µmol CO_2 m^{-2} s^{-1}, values below the average, which was equal to 20.7 µmol CO_2 m^{-2} s^{-1}.

In the treatment S_2I_{100}, whose average of photosynthetic rate between genotypes was equal to 17.7 µmol CO_2 m^{-2} s^{-1}, the genotype 10 was that with the highest photosynthetic rate with observed value of 21.5 µmol CO_2 m^{-2} s^{-1}, while the genotype 6 with 11.6 µmol CO_2 m^{-2} s^{-1}, showed the lowest value.

The treatment without added salt and with the lowest water content (S_0I_{25}), had on average a photosynthesis rate among the genotypes equal to 23.7 µmol CO_2 m^{-2} s^{-1}, the genotype 24 whose that has provided the highest value with 28 µmol CO_2 m^{-2} s^{-1}, while those who have provided the lowest values were genotypes 16 and 7 with respective values of 21.6 µmol CO_2 m^{-2} s^{-1} and 21.4 µmol CO_2 m^{-2} s^{-1}. In the S_1I_{25} treatment whose average of photosynthesis rate among the studied genotypes was equal to 18.4 µmol CO_2 m^{-2} s^{-1}, the genotype 20 was distinguished with 26.8 µmol CO_2 m^{-2} s^{-1}, while the genotype 18 observed the lowest photosynthesis rate equal to 12.4 µmol CO^2 m^{-2} s^{-1}. In the treatment with the highest salt concentration and with the lowest water content (S_2I_{25}), the net photosynthesis rate among the genotypes was equal to 14.4 µmol CO_2 m^{-2} s^{-1}, on average; genotypes 14 and 6 were distinguished among the others with net photosynthesis value equal to 16.5 µmol CO_2 m^{-2} s^{-1} , while genotypes 10 and 18 had the lowest rate of photosynthesis and provided values equal to 10.8 µmol CO_2 m^{-2} s^{-1} and 10.6 µmol CO_2 m^{-2} s^{-1}, respectively (Figure 36).

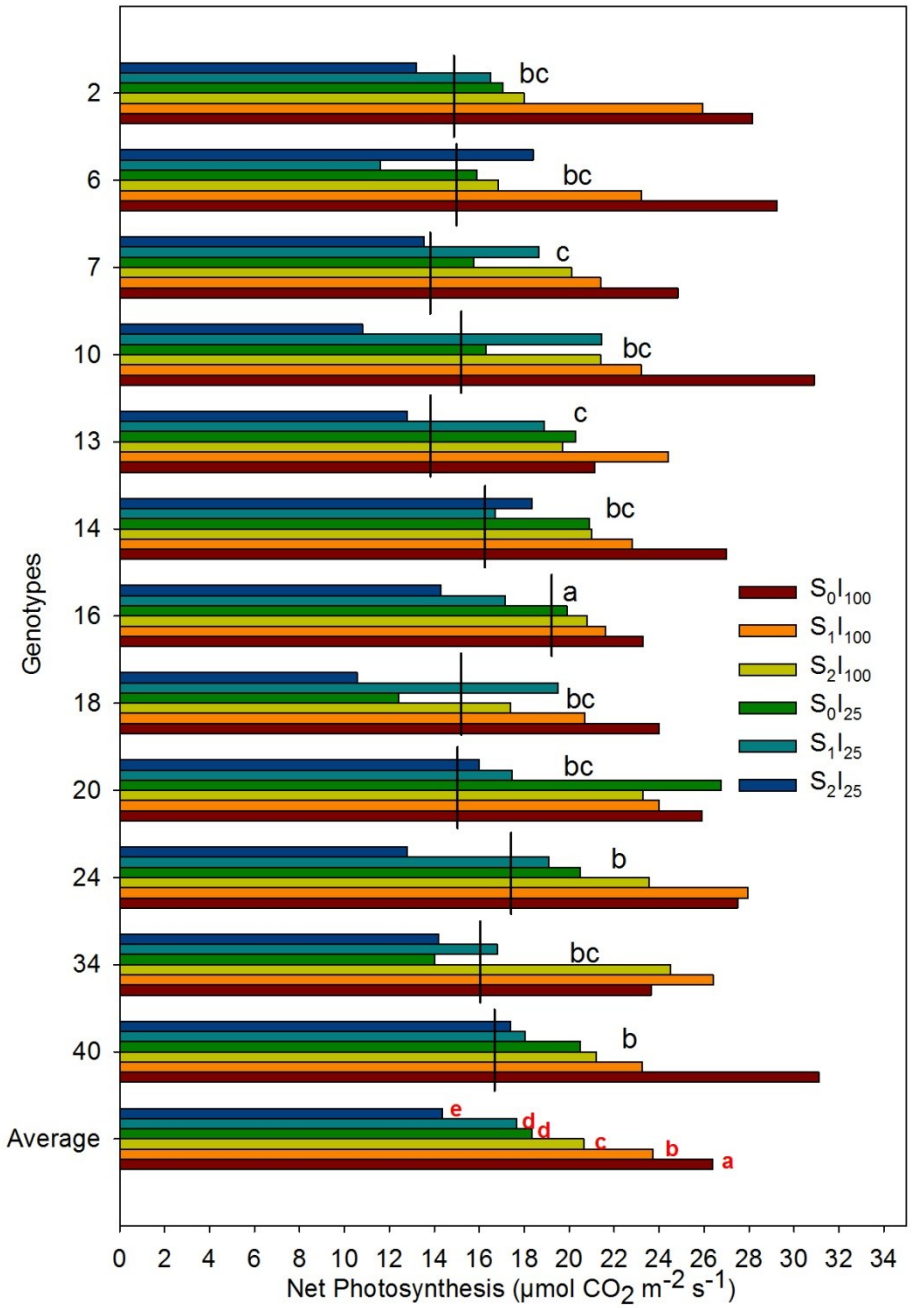

Figure 36 - Photosynthesis (µmol CO_2 s^{-1} m^{-2} s^{-1}) detected on 26 September 2013 in the average of the studied genotypes and in relation to different treatments in the study. S_0 (control), S_1 (salt concentration equal to 6 dS m^{-1}), salt concentration S_2 equal to 12 dS m^{-1}, I_{100} (100% restoration of evapotranspirated water), I_{25} (25% restoration of evapotranspiration). Vertical line represent the average value of the six treatments. Small letters, for averaged values of salinity and irrigation levels within each genotype; red letters for averaged values of all genotypes within each salinity and irrigation levels. Different letters indicate significant differences at P≤0.05 by SNK Test. LSD (genotype x salinity level x water level) = 4.77

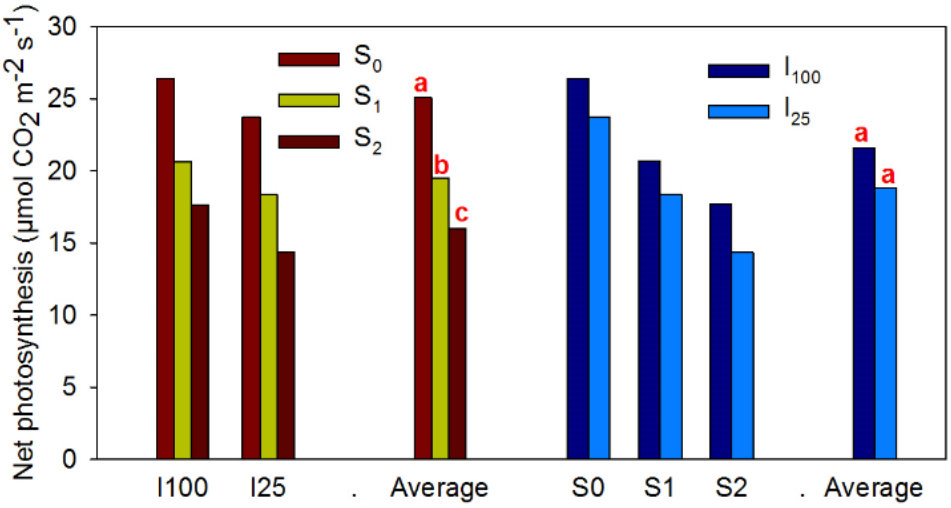

Figure 37 - Photosynthesis (µmol CO_2 m^{-2} s^{-1}) detected on 26 September 2013 in the different studied genotypes in relation to different tested treatments. Different letters indicate significant differences for $P \leq 0.05$ (SNK test).

Transpiration rate

The leaf transpiration, gave the highest average values in the treatment that provided the highest water content (I100), compared to the treatment I_{25}, specifically, the average values of leaf transpiration observed in the average of the treatments were 5.6 mmol H_2O m^{-2} s^{-1}, observed in the I_{100} treatment and 4.9 mmol H_20 m^{-2} s^{-1} in the I_{25} treatment. Regarding to the salt levels adopted, the treatment without added salt (S_0) have noted higher values compared to saline treatments (S_1 and S_2) in both restoration water, with a mean value of leaf transpiration measured equal to 7.8, 4.9 and 3.0 mmol H_2O m^{-2} s^{-1}, in treatment S_0, S_1 and S_2, respectively (Figure 39).

Regarding to the genotypes compared in the study, in the treatment S_0I_{100}, whose average value of leaf transpiration was equal to 8.7 mmol H_2O m^{-2} s^{-1}, the genotypes that provided a greater value were 40, 20 and 7 with respective values of 10.8 (40 and 20) and 10.4 (7) mmol H_2O m^{-2} s^{-1}, while the genotype that provided the lowest value (5.6 mmol H_2O m^{-2} s^{-1}) was genotype 13. In the treatment S_1I_{100}, the genotype 24 provided the highest value of leaf transpiration and equal to 7.4 mmol H_2O m^{-2} s^{-1}, compared to the other genotypes that showed leaf transpiration values close to the average (4.8 mmol H_2O m^{-2} s^{-1}); however, among these genotypes, the worst genotype was found to be genotype 2 with a transpiration value equal to 1.5 mmol H_2O m^{-2} s^{-1}.

The S_2I_{100} treatment showed an average value of leaf transpiration between genotypes equal to 3.3 mmol H_2O m^{-2} s^{-1}; genotypes that are distinguished for the highest values observed were 18 and 10 with respective values of 4.3 and 4.1 mmol H_2O m^{-2} s^{-1}, while the genotype that provided the lowest value was the 6 with a leaf transpiration value equal to 1.9 mmol H_2O m^{-2} s^{-1}.

In the treatment without salt added and with the lowest water restoration (S_0I_{25}), the average rate of leaf transpiration, among the genotypes was equal to 6.9 mmol H_2O m^{-2} s^{-1}, the highest values were provided by genotypes 20, 16, 34 with 11.6, 9.9 and 9.5 mmol H_2O m-2 s^{-1}, respectively, while the genotype that provided the lowest value was 10 with a value of leaf transpiration equal to 1.5 mmol H_2O m^{-2} s^{-1} (Figure 38).

In the S_1I_{25} treatment, genotypes 16 and 40 provided the highest rate of leaf transpiration (9.6 and 8.0 mmol H_2O m^{-2} s^{-1}, respectively), while the lowest value was observed in genotypes 18 and 34 (2.3 and 2.1 mmol H_2O m^{-2} s^{-1}, respectively); the other genotypes showed leaf transpiration rate close to the average value (5.0 mmol H_2O m^{-2} s^{-1}).

In the S_2I_{25} treatment, whose average rate of leaf transpiration was equal to 2.7 mmol H_2O m^{-2} s^{-1}, the genotypes that are distinguished for the highest values of leaf transpiration measured were 14, 7 and 10, with values of 3.7 mmol H_2O m^{-2} s^{-1}, 3.6 and 3.5 mmol H_2O m^{-2} s^{-1}, respectively, while the genotype 24 was that one that provided the lowest value of leaf transpiration with 1.6 mmol H_2O m^{-2} s^{-1}.

In the average of the studied treatments, the highest values of leaf transpiration were provided from genotypes 20, 16 and 40 with values of 7.0, 6.6 and 6.4 mmol H_2O m^{-2} s^{-1}, respectively, while the genotypes 2 and 6 provided the lowest values (4.2 and 4.6 mmol H_2O m^{-2} s^{-1}, respectively).

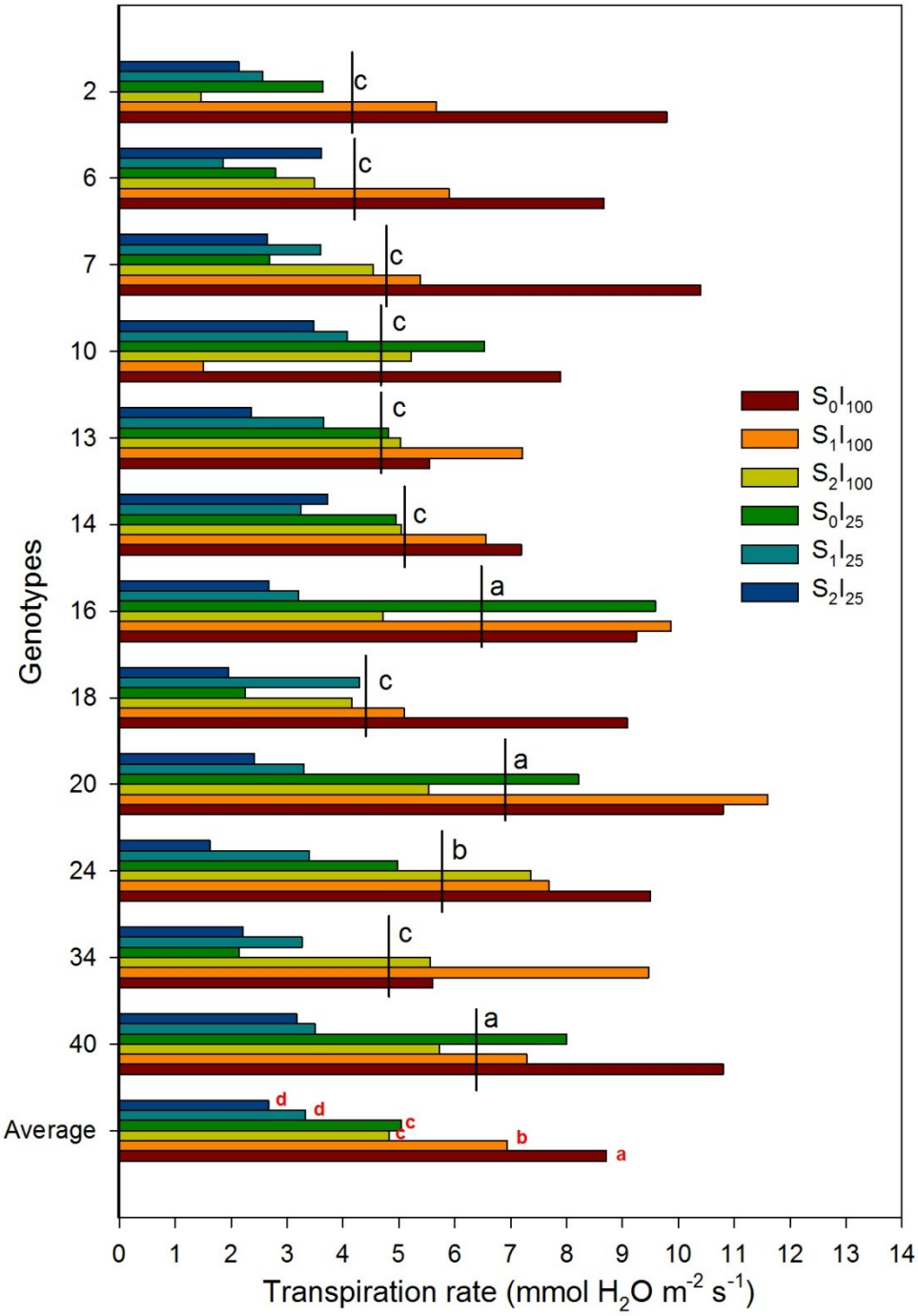

Figure 38 - Transpiration (mmol H_2O m^{-2} s^{-1}) detected on 26 September 2013 in the average of the genotypes in the study and in relation to different treatments in the study. S_0 (control), S_1 (salt concentration at 6 dS/m), salt concentration S_2 at 12 dS/m, I_{100} (100% restoration of evapotranspiration), I_{25} (25% restoration of evapotranspiration). Vertical line represent the average value of the six treatments. Small letters, for averaged values of salinity and irrigation levels within each genotype; red letters for averaged values of all genotypes within each salinity and irrigation levels. Different letters indicate significant differences at P≤0.05 by SNK Test. LSD (genotype x salinity level x water level = 0.09).

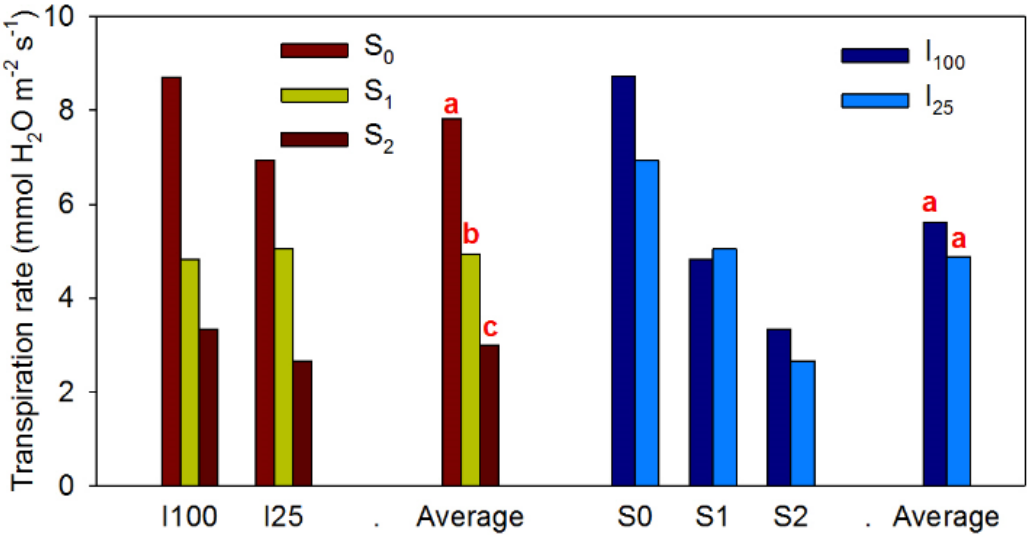

Figure 39 - Transpiration (mmol H_2O m^{-2} s^{-1}) detected on 26 September 2013 in the different studied genotypes in relation to different tested treatments. Different letters indicate significant differences for $P \leq 0.05$ (SNK test).

Stomatal conductance

Stomatal conductance, like photosynthesis, was higher in the treatment with increased amounts of water, compared to those which provided the restoration of 25% of water restoration. The I_{100} treatment, on average, showed a value of stomatal conductance equal to 0.27 mol m^{-2} s^{-1}, while the I_{25} treatment showed values in saline treatments equal to 0.23 mol m^{-2} s^{-1} (Figure 41).

S_0 treatment, in both water restoration (I_{100} and I_{25}), showed values of stomatal conductance higher than those measured in the saline treatments (S_1 and S_2). The stomatal conductance in S_0 was equal to 0.46 mol H_2O m^{-2} s^{-1} on average, while those observed in S_1 and S_2 were equal to 0.19 and 0.10 mol H_2O m^{-2} s^{-1}, respectively.

Among the genotypes, in the treatment S_0I_{100}, whose average value of stomatal conductance was equal to 0.5 mol H_2O m^{-2} s^{-1}, the genotype 20 provided the highest value (0.9 mol H_2O m^{-2} s^{-1}) while the lowest value was provided by genotypes 34 and 13 (2 mol H_2O m^{-2} s^{-1}, respectively).

In the S_1I_{100} treatment the average value of stomatal conductance recorded between genotypes was equal to 0.2 mol H_2O m^{-2} s^{-1}. The studied genotypes showed small

differences from the average, the only genotype which gave a value higher than average, was genotype 24 (0.3 mol H_2O m^{-2} s^{-1}) (Figure 40).

In the treatment with the highest salt concentration and greatest water restoration (S_2I_{100}), all genotypes have shown values of stomatal conductance equal to each other and equal to 0.1 mol H_2O m^{-2} s^{-1}, the only genotype that has provided a different value but at the same time higher than one tenth of a percentage point was 18 with a value of stomatal conductance equal to 0.2 mol H_2O m^{-2} s^{-1}. In the treatment without salt and 25% of water restoration (S_0I_{25}), the stomatal conductance was equal to 0.4 mol m^{-2} s^{-1}, on average, and genotypes 20 and 13 have been distinguished for conductance stomatal values equal to 0.8 and 0.6 mol m^{-2} s^{-1}, respectively, the genotype 10 was instead the one that provided the lowest value equal to 0.1 mol m^{-2} s^{-1}.

In the S_1I_{25} treatment, whose stomatal conductance was equal to 0.2 mol H_2O m^{-2} s^{-1}, on average, the genotypes did not provide values much diverged from the average, however, the highest values were provided by the genotypes 20 and 16 (0.4 mol H_2O m^{-2} s^{-1}, respectively). The treatment with the most salt concentration and lowest water restoration (S_2I_{25}) provided in all genotypes stomatal conductance values equal to 0.1 mol H_2O m^{-2} s^{-1}.

In the average of the studied treatments, the genotypes compared in the experiment provided stomatal conductance values between a range of 0.2 and 0.4 mol H_2O m^{-2} s^{-1}, and the genotype 20 provided the highest value equal to 0.4 mol H_2O m^{-2} s^{-1}.

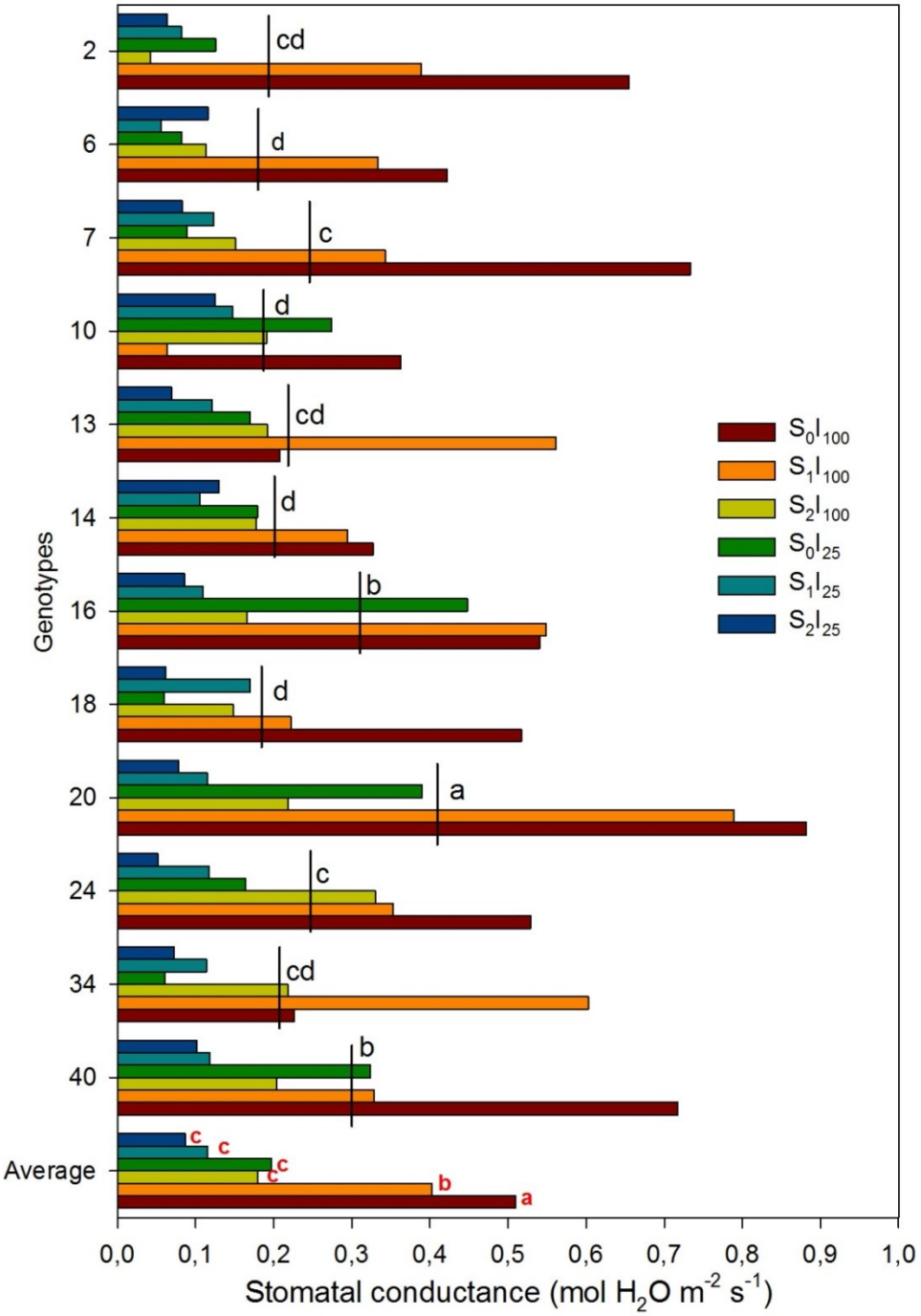

Figure 40 - Stomatal conductance (mol H_2O m^{-2} s^{-1}) detected on 26/09/2013 in the average of genotypes in the study and in relation to different treatments in the study, S_0 (control), S_1 (salt concentration equal to 6 dS m^{-1}), salt concentration S_2 equal to 12 dS m^{-1}, I_{100} (100% restoration of evapotranspiration), I_{25} (25% restoration of evapotranspiration). Vertical line represent the average value of the six treatments. Small letters, for averaged values of salinity and irrigation levels within each genotype; red letters for averaged values of all genotypes within each salinity and irrigation levels. Different letters indicate significant differences at P≤0.05 by SNK Test. LSD (genotype x salinity level x water level = 1.74).

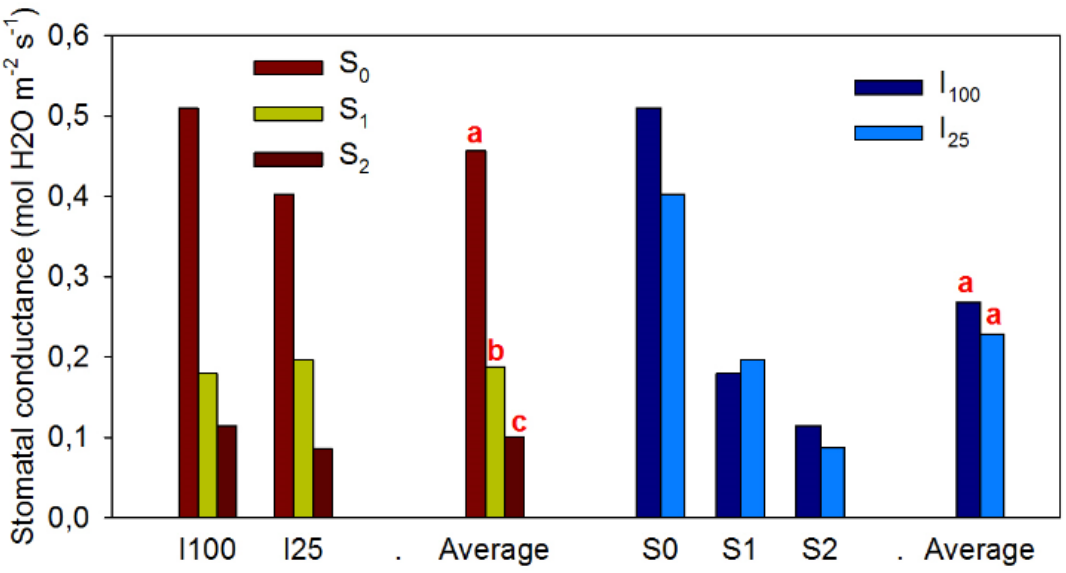

Figure 41 - Stomatal conductance (mol H_2O m^{-2} s^{-1}) detected on 26/09/2013 in the different studied genotypes in relation to different tested treatments. Different letters indicate significant differences for $P \leq 0.05$ (SNK test).

As far as the physiological trend is concerned, net photosynthesis, stomatal conductance and transpiration rates were always higher in S_0I_{100} treatment, followed by S_0I_{25}, S_1I_{100}, S_1I_{25}, S_2I_{100} and S_2I_{25}. (Fig.42, 43, 44).

In the last measurement, however, S_0I_{100} showed quite fairly close values to S_1I_{100}, while S_0I_{25} shown the unexpected lowest value.

It is worth to mention that last measurement was carried out just before biomass harvesting and the plants did not receive regular irrigation before that measurement. It has been noted that S_0 treatment used more water than S_1 and S_2, both in I_{100} and I_{25} treatment. It is well-known that high salt level in the soil leads to plant osmotic stress, lowering water uptake by roots and as consequence S_1 and S_2 pots had higher water content than S_0.

For this reason S_0 decreased the most at the last measurement, while S_1 and S_2 treatment kept their trend.

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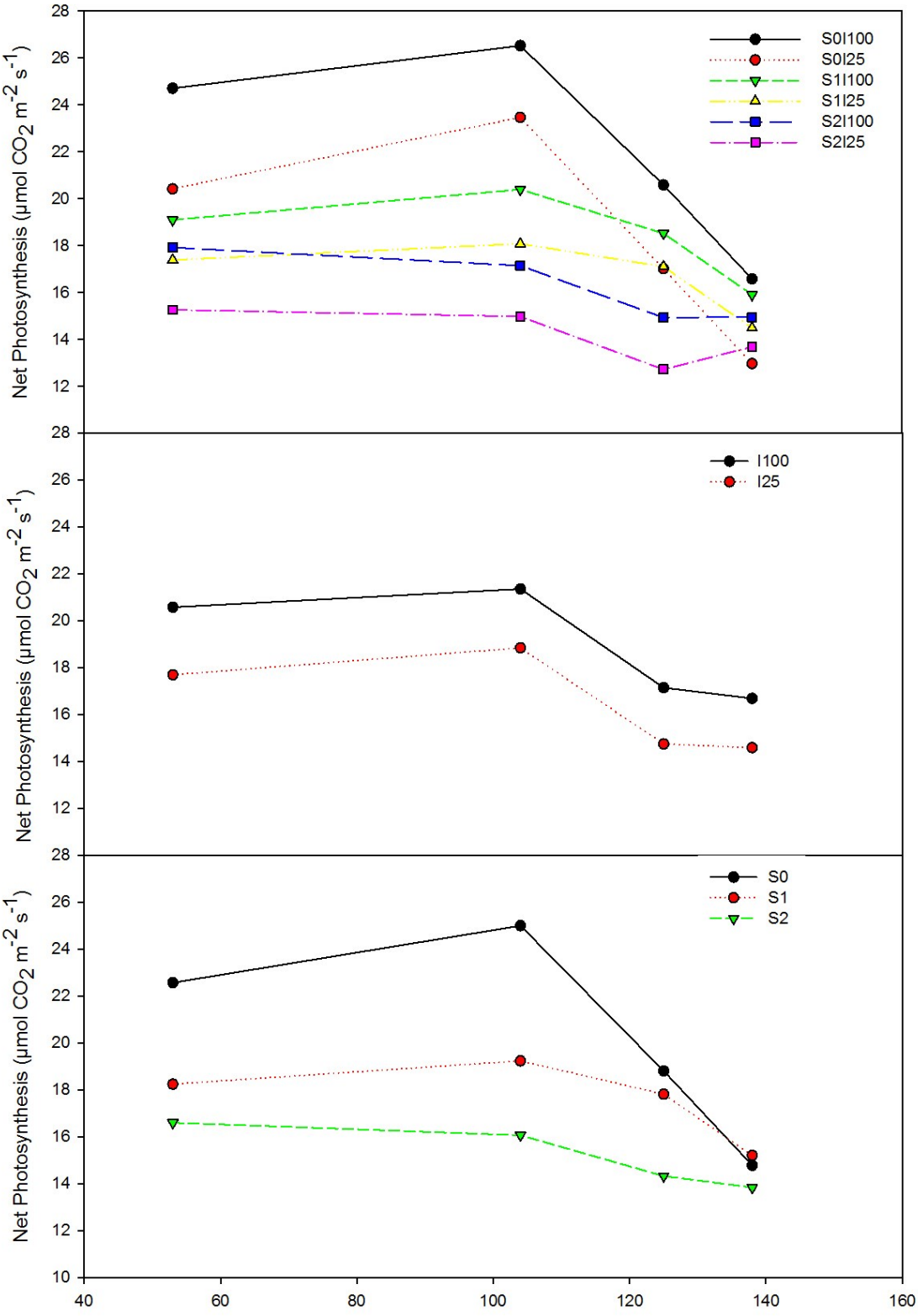

Figure 42 – Net photosynthesis trend in the average of the studied genotypes in relation to different treatments.

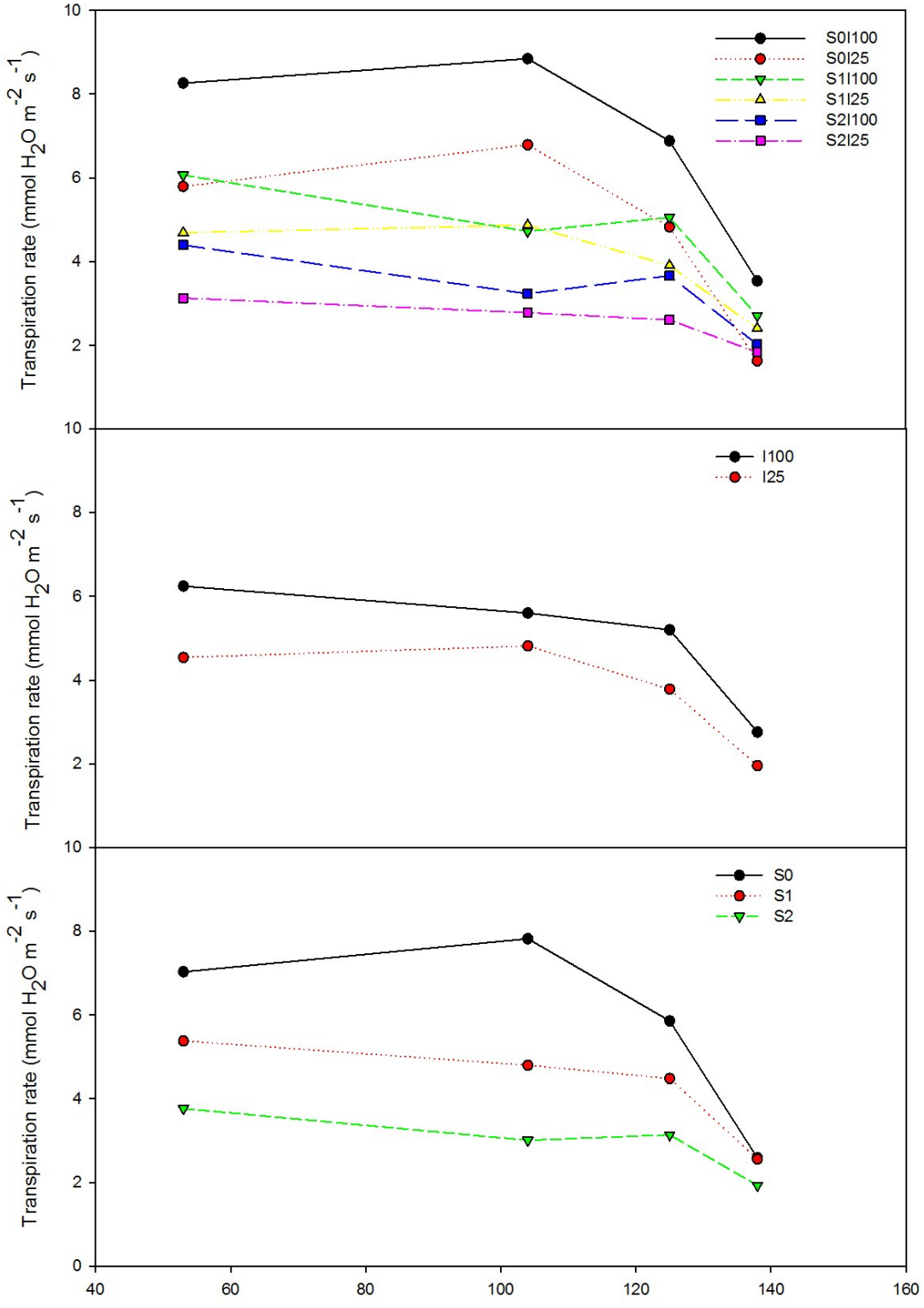

Figure 43 – Transpiration rate trend in the average of the studied genotypes in relation to different treatments.

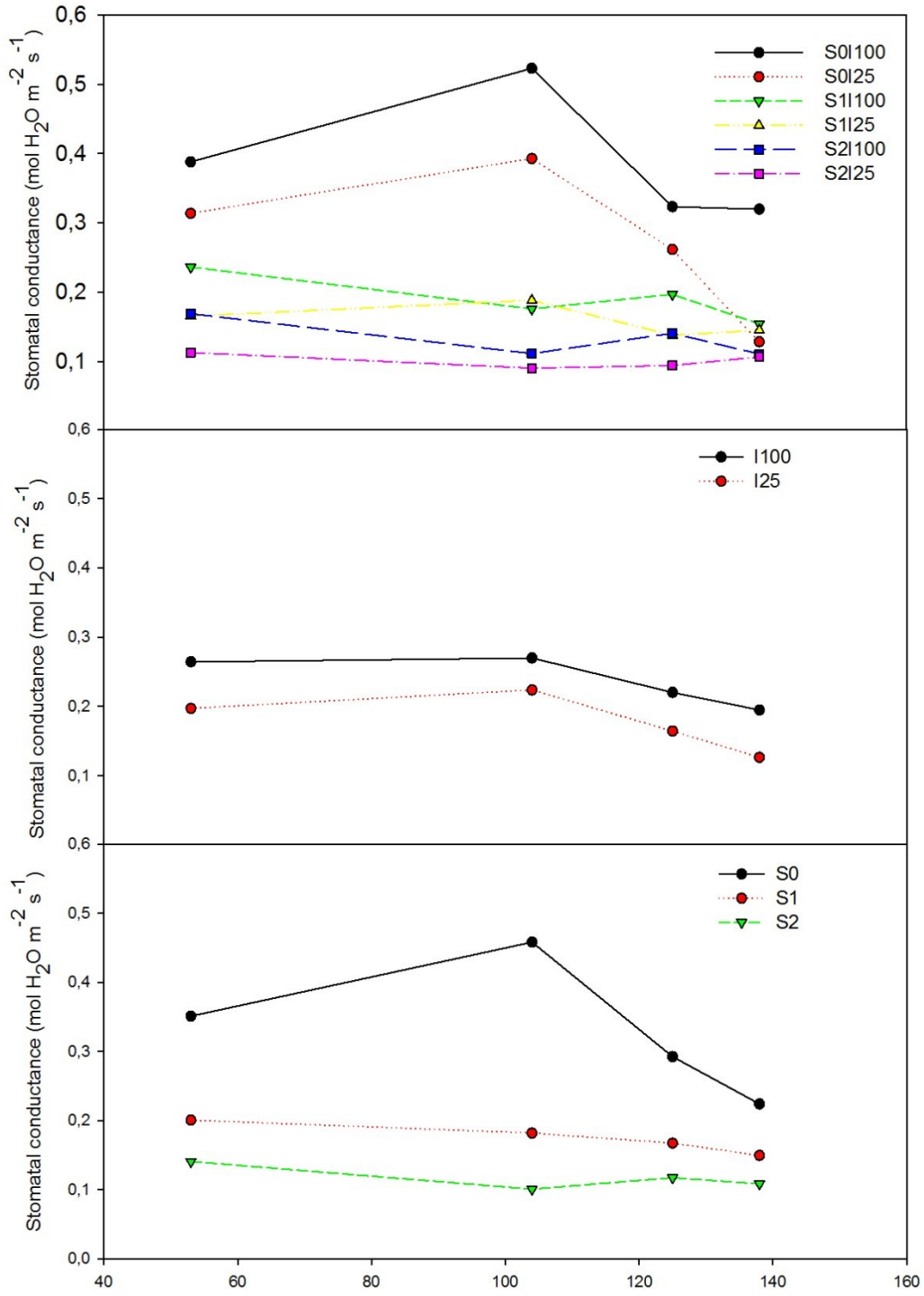

Figure 44 – Stomatal conductance trend in the average of the studied genotypes in relation to different treatments.

The relation between photosynthesis and stomatal conductance, taking into account all salt treatments and clones, highlights how the crop is able to increase net photosynthesis as stomatal conductance increase with a coefficient of determination equal to 0.50 (Figure 45).

The same linear trend was observed by plotting net photosynthesis and transpiration rate, which showed a positive correlation (R^2= 0.63), as shown in figure 46.

Positively correlated resulted also transpiration rate and stomatal conductance (R^2=0.79), as shown in figure 47.

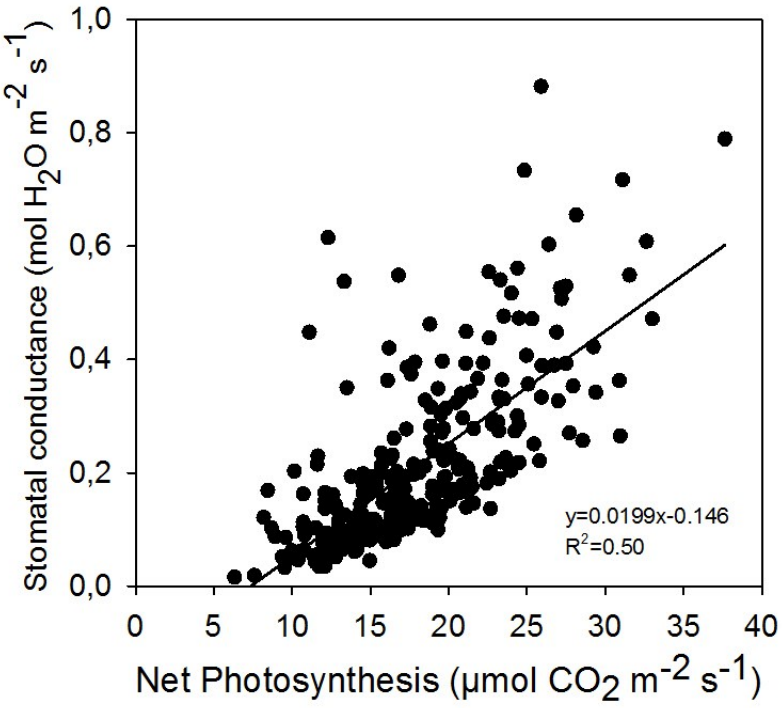

Figure 45 –Net photosynthesis vs stomatal conductance (all data).

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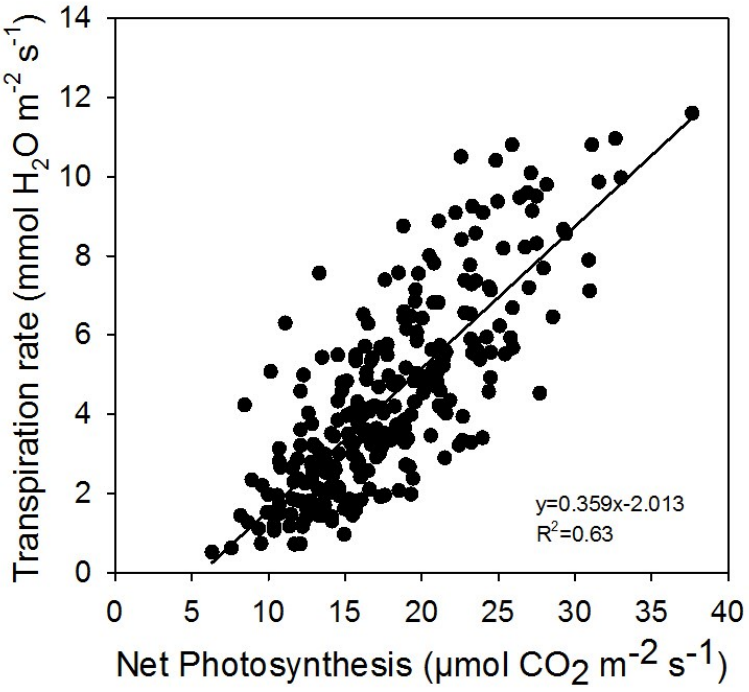


Figure 46 –Net photosynthesis vs transpiration rate (all data).

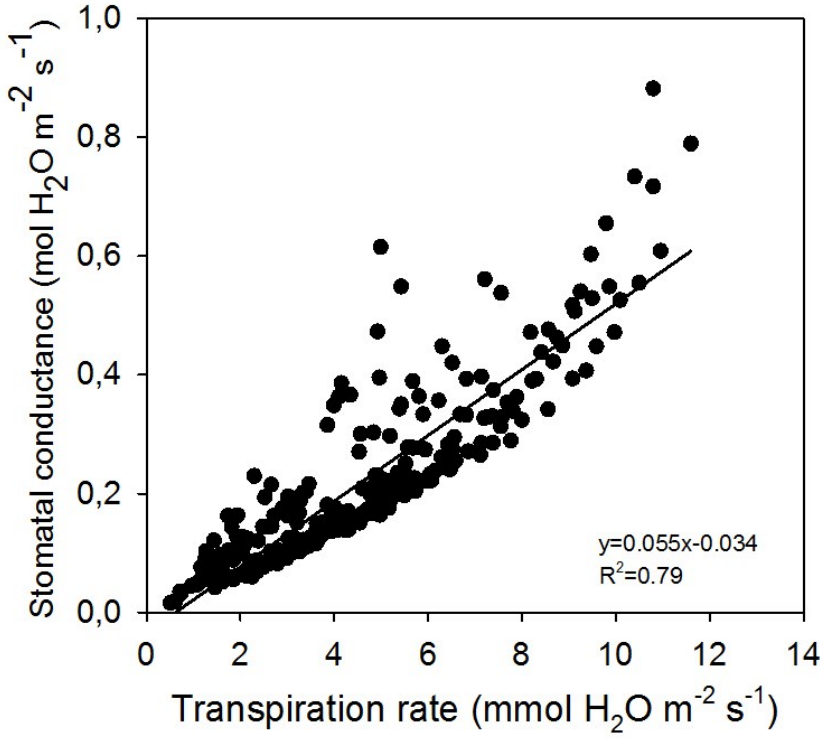


Figure 47 –Transpiration rate vs stomatal conductance (all data).

Fluorescence

During the salt and water experiment the chlorophyll fluorescence was measured by using a continuous fluorescence portable fluorimeter (Handy PEA, Hansatech, UK). It was estimated Fv'/Fm' parameter, which provides an estimate of the photosystem II (PSII) maximum efficiency within light-adapted material and Fv/Fm value, which is the PSII maximum efficiency within dark-adapted material. The fluorescence is a technique employed in the physiology of stress. Through the analysis of the fluorescence of chlorophyll can be achieved information on the efficiency of the photosynthetic apparatus, allowing to assess the physiological state of the photosynthetic samples examined. Any forms of biotic or abiotic stress which have an effect on the photosynthetic performance on the sample, for example, heat, cold, drought, salinity, atmospheric pollutants, alter the intensity of the chlorophyll fluorescence emission. In fact, energy dissipation via chlorophyll fluorescence, increases due to a decrease in dissipation via photochemical pathways. Consequently, measurement of changes in the extent of fluorescence emissions can be used to infer informations about changes in the efficiency of light use for photochemistry (Krause and Weis 1991; Govindjee 1995). The parameter $F_V/F_M = [(F_M-F_0)/F_M]$, in dark-adapted samples is a parameter widely used to indicate the maximum quantum efficiency of Photosystem II and is the parameter most commonly used as a reliable indicator photochemical activity of the photosynthetic apparatus. Theoretically , the values of F_V/F_M can vary from 0 to 1 , but the optimum value of this parameter for healthy samples is 0.83 (Björkman and Demmig, 1987). Values lower than this will be observed if a sample has been exposed to some typic of biotic or abiotic stress factors which show damage or reduced efficiency of the reaction center of PSII. The reduction in the ratio F_V/F_M involves the increase in energy dissipation the aerial in the form of heat and is index of photoinhibition.

In the experiment, the light-adapted PSII maximum efficiency (Fv'/Fm') was slightly affected by salt treatments (Figure 48). Overall the experiment, in the average of the genotypes, S_0 treatment showed a mean value of Fv'/Fm' equal to 0.702, higher than that provided by S_1 treatment (0.667) and S_2 (0.644)., Relative to salt effect, S_2 treatment decreased the Fv'/Fm' (about 8%). Despite this the PSII physiological functions were preserved up to high level of stress as confirmed by Morant-Avice et al.

(2004) and Jimenez et al. (1977), whose study found no changes in fluorescence parameters in salt-stressed roses.

Soil water content showed higher mean value of Fv'/Fm' I_{25} treatment than that showed by I_{100}, 0.681 and 0.675, respectively (Figure 50). In general, the soil water content did not affect the value of Fv'/Fm' as detected in the salt treatments. The dark-adapted PSII maximum efficiency (Fv/Fm) was not affected by the different salt treatments (Figure 49) neither by the different water content of the soil (Figure 51).

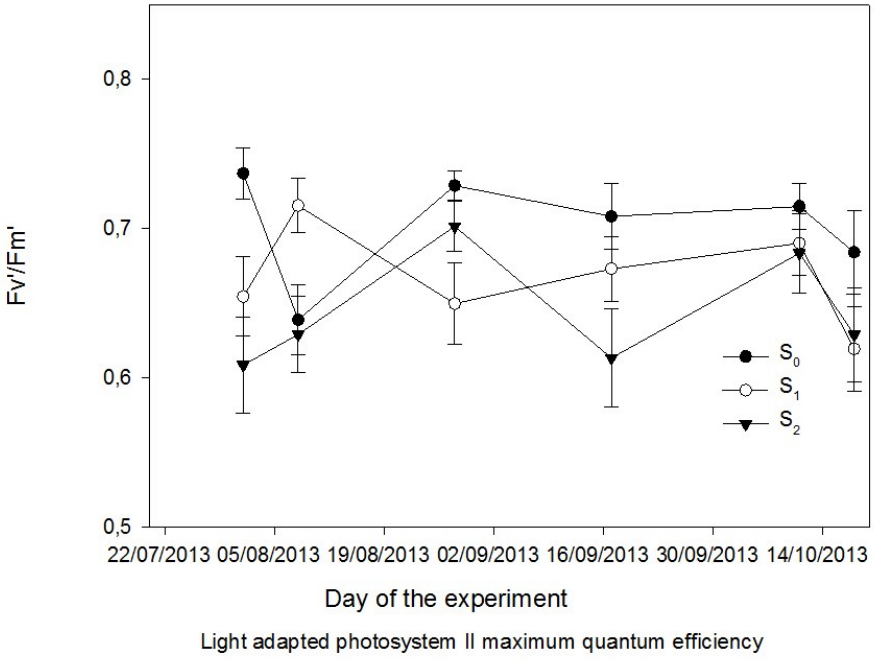


Fig 48 Light adapted photosystem II maximum quantum efficiency (Fv/Fm), for all the three salt treatments (S_0, S_1, S_2). Values are means, ± standard error of the mean.

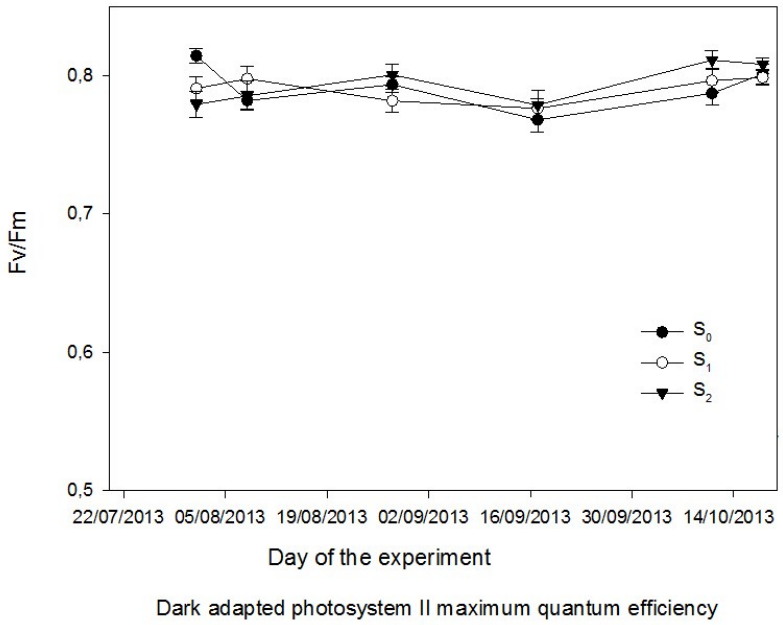


Fig. 49 Dark adapted photosystem II maximum efficiency (Fv/Fm), for all the three salt treatments (S_0, S_1, S_2). Values are means, ± standard error of the mean.

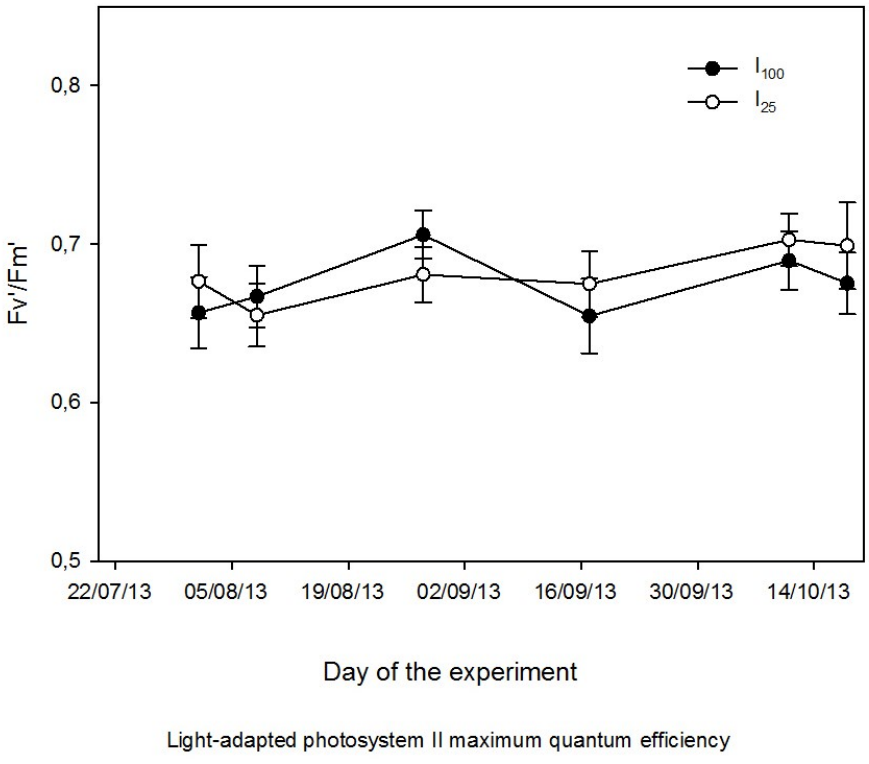
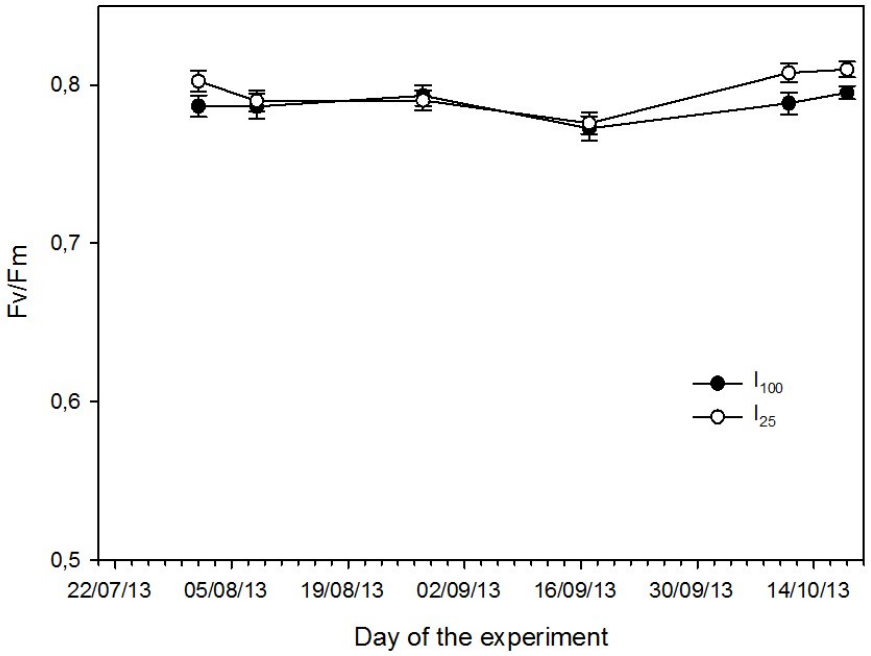


Fig. 50 Light adapted photosystem II maximum quantum efficiency (Fv'/Fm'), for all the three salt treatments (S_0, S_1, S_2). Values are means, ± standard error of the mean.



Dark-adapted photosystem II maximum quantum efficiency

Fig. 51 Dark-adapted photosystem II maximum quantum efficiency (Fv/Fm), for all the three salt treatments (S_0, S_1, S_2). Values are means, ± standard error of the mean.

3.2.9. Genotypes screening

The screening of the studied genotypes was conducted by calculating the coefficient of variation (CV%) plotted at the average dry biomass of treatments within the same genotype.

The genotypes that fall within the "A" area, may be considered resistant to salinity and water scarcity, as they have a low CV and high dry biomass. Conversely, genotypes that fall within the quadrant "C" are considered to be sensitive to salinity and water scarcity as they have a high CV and low dry biomass. The genotypes falling in quadrants "B" and "D" have characteristics intermediate "B" having a high dry biomass and high CV , so with substantial differences when the same genotype is grown under salt or water stress.

In the case of the quadrant "D" , the genotypes in question having a relatively low dry matter production but at the same time a low CV can be cultivated under conditions of water or salt stress. The choice of the optimal genotype to be used on marginal land or fertile, respectively , will depend on the characteristics of the environment cultivation.
The genotypes 18, 2, 6 and 16 can be considered "best" because they respond positively to all types of stress, genotypes 14, 7 , 13 and 20 on the contrary the worst ones. The genotypes 10, 40 and 34 can give good productions if not cultivated in environments with low stress, while the genotype 24 although at lower production compared to 18, 16, 2 and 6 may be grown in environments equally stressed by salinity or water scarcity (Figure 52).
By comparing the screening carried out in 2012 with the current one, genotypes 18, 6 and 2 confirmed their high biomass yield under salt stress, while 20 and 24 the opposite trend: best in 2012, worst in 2013. Genotypes 34, 10, 7 and 14 confirmed their low adaptability when grown under salt stress, even if high biomass content is achieved.
When analyzed separately by irrigation treatment (I_{25} and I_{100}, respectively) taking into account only salt stress (as compared to 2012 trial) different behaviors were observed: in the average of salt treatment and I_{25} only, the most tolerant genotypes were 6, 16, 24 and 2. High biomass production was achieved by 18, 34, 10, 40, 14 and 7, even if these latter were in the quadrant with high CV and so with low stress to salt tolerance. Genotypes 20 and 13 resulted the lowest yielding but tolerant (Figure 53).
When I_{100} treatment was averaged by salt treatments, genotype 18, 40 and 2 showed the highest yield and tolerance, while 6 and 10 high yielding but not tolerant. Genotypes 13, 7, 16 and 20 showed a low yield but good tolerance, while 14, 24 and 34 low yielding and low tolerant to salt stress.
Some genotypes, as 34, 16 and 18 adversely respond to salt stress when the water is reduced, decreasing or increasing biomass yield or tolerance as water become a limiting factor. In particular 18 seems more tolerant as water stress is reduced, while 6, on the other hand become less tolerant (Fig. 54).

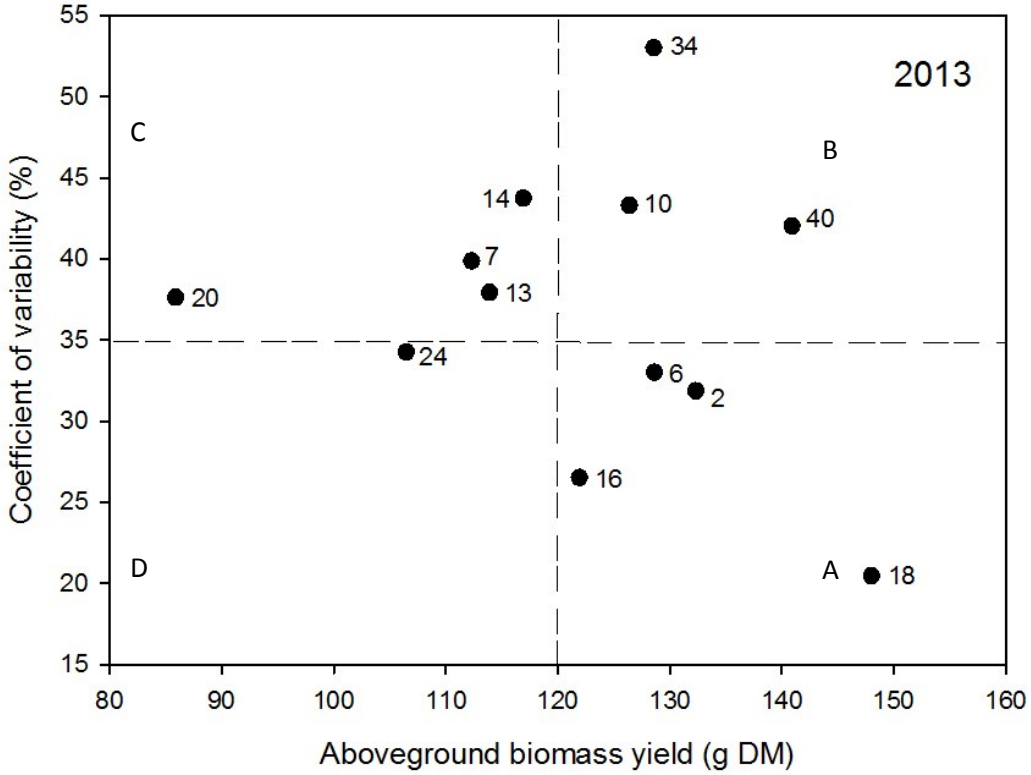


Figure 52. Screening of Giant reed clones taking into account aboveground biomass dry matter and coefficient of variability (by averaging a single clone in the three treatments). (A) represents the highest tolerant to salinity, (C) the lowest tolerant with (B) and (D) showing high biomass yield but low tolerance and high tolerance but low biomass yield respectively.

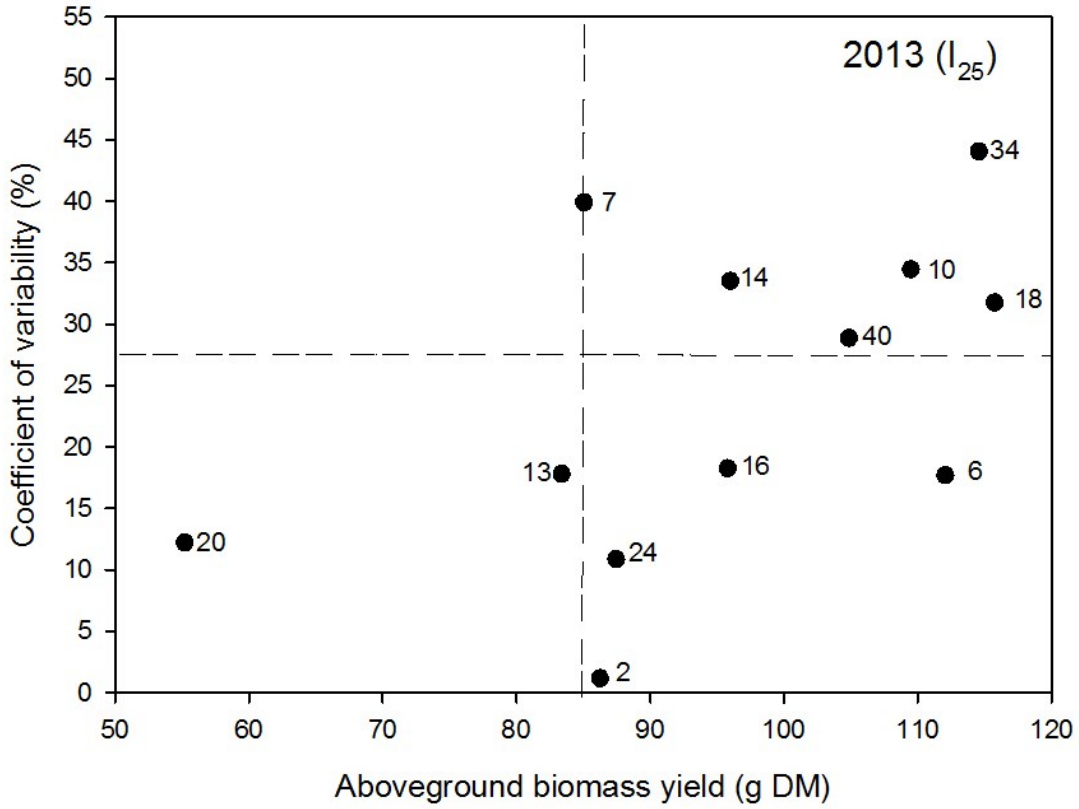


Figure 53 Screening of Giant reed clones taking into account aboveground biomass dry matter and coefficient of variability (by averaging a single clone in the three salt treatments and I_{25}). (A) represents the highest tolerant to salinity, (C) the lowest tolerant with (B) and (D) showing high biomass yield but low tolerance and high tolerance but low biomass yield respectively.

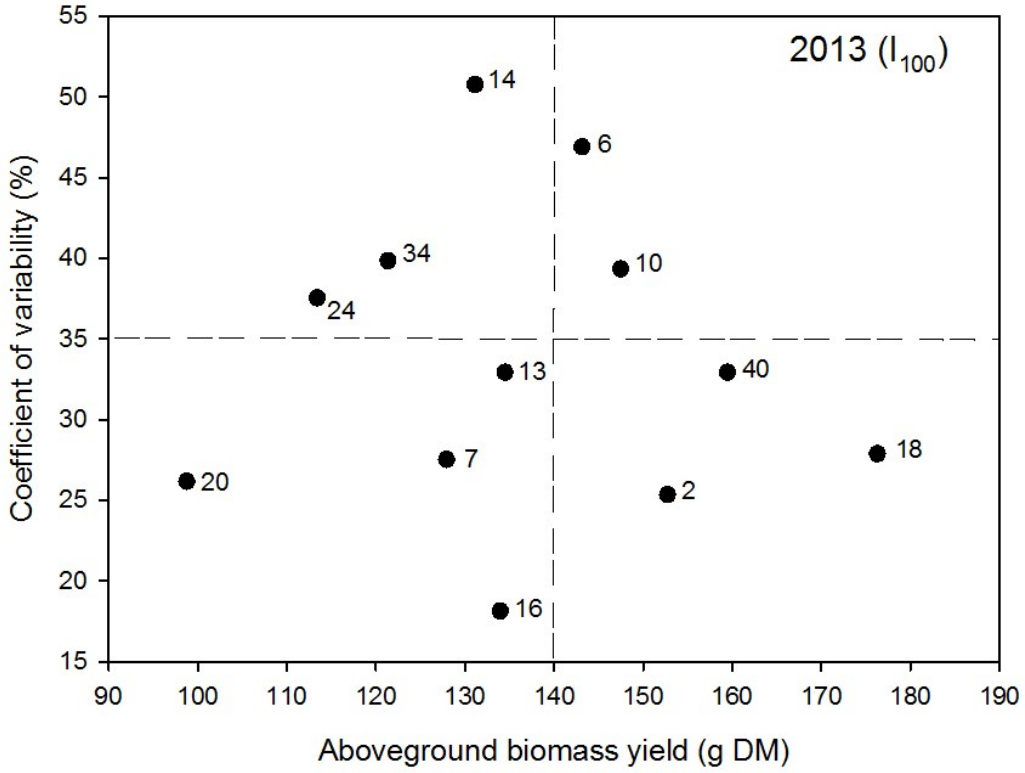


Figure 54 Screening of Giant reed clones taking into account aboveground biomass dry matter and coefficient of variability (by averaging a single clone in the three salt treatments and I_{100}). (A) represents the highest tolerant to salinity, (C) the lowest tolerant with (B) and (D) showing high biomass yield but low tolerance and high tolerance but low biomass yield respectively.

3.3. Research line 3

3.3.1. First year results

Productivity

From the results relating to the production of aboveground biomass, it can be assumpted that the contamination due to the presence of lead in soil has reduced the productivity of Arundo donax L. in the presence of high (I_3) and medium (I_2) water content of the pots but had no effect at lower quantities of water (I_1). The reduction of the water content, has reduced the production of aboveground biomass in the pots without the addition of lead (Pb_0) and in those with a high level of contamination of 900 mg DM kg^{-1} (Pb_{II}), but not in those with a concentration of lead of 450 mg DM kg^{-1}, Pb_I) (Fig.55).

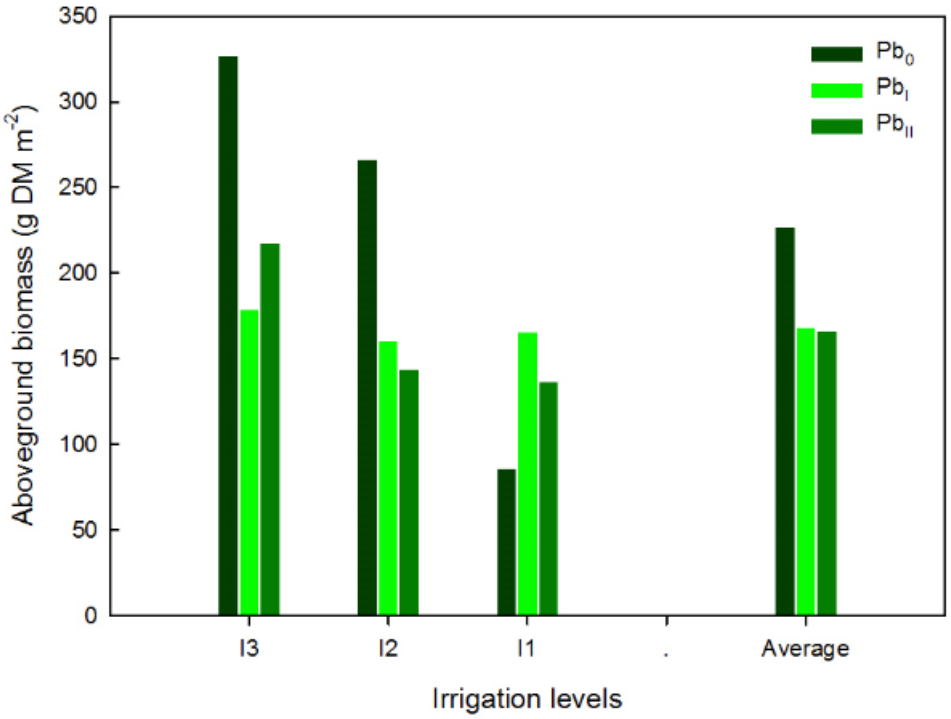


Fig. 55 Aboveground biomass (g DM m^{-2}) in relation to the studied treatments: irrigation levels, (I_3, I_2, I_1) and lead contamination (Pb_0, Pb_I and Pb_{II}).

Morfo-biometric characters

About the studied morpho-biometric characters, the contamination of the pots with increasing doses of lead has resulted in a lower number of culms in all water levels tested.

The height of the stems was significantly decreased in relation to the reduction of the water content of the pots; analyzing the levels of contamination in the two water levels which provided the highest water return (I_2 and I_3), the height of the stem decreased with increasing of contamination level; vice versa in the thesis with the lowest water return (I_1), the presence of lead has increased the height of the stems, factor by which it can be deduced that for this character water stress is less favorable to the development of the plant than that imposed by contamination with lead.

With regard to the basal diameter of the main stem, in the presence of contamination with lead, this decreased, especially in pots with a higher water content (I_3); different water levels of soil appear to affect the basal diameter of the stems: the decrease in water availability, in fact, decreases the stem basal diameter (Tab.4).

Regarding to the number of nodes in all the studied treatments, with decreasing water availability, it has been reduced from 19 (I_3) to 14 (I_2), and to 11 (I_1), but the lowest number of nodes has been detected in all the theses in the presence of the highest level of contamination (Pb_{II}).

Tab.4 Morpho-biometric characters in relation to the studied treatments: irrigation levels, (I_3, I_2, I_1) and lead contamination (Pb_0, Pb_I and Pb_{II}).

Water level	Stems (n°)				Height (cm)				Basal diameter (mm)				Nodes (n°)			
	Pb0	PbI	PbII	*Av*	Pb0	PbI	PbII	*Av*	Pb0	PbI	PbII	*Av*	Pb0	PbI	PbII	*Av*
I_3	3,0ab	1,5ns	1,5ns	*2,0ns*	93,2a	61,0a	52,3a	*69,0a*	9,1a	6,1ns	6,3a	*7,2a*	20,0a	20,0a	17,0a	*19,0a*
I_2	4,5a	1,0ns	1,5ns	*2,3ns*	47,5b	50,6ab	31,8ab	*43,0b*	6,9b	6ns	4,7b	*5,8b*	14,0b	17,0a	12,0b	*14,0b*
I_1	2,0b	2,0ns	1,5ns	*1,8ns*	26,3c	35,4b	39,4b	*34,0c*	5,8b	4,8ns	4,6b	*5,1b*	11,0c	12,0b	10,0c	*11,0c*
Average	*3,2 a*	*1,5b*	*1,5b*		*56,0a*	*49,0a*	*41,0b*		*7,3a*	*5,6b*	*5,2b*		*15,0a*	*16,0a*	*13,0b*	

Lead content of biomass

The lead content of the biomass, related to the plant ability to extract the metal from the soil and its role in the phytoremediation of heavy metal contaminated sites, this was

higher in the leaf fraction than that recorded in stems, especially in contaminated pots (Tab.5). In stems the different water content of the pots had no significant effect on the content of lead, while the leaf fraction in the thesis Pb_0 and Pb_I, with higher water return water, the lead content decreased.

Tab.5 Lead content detected in stems and leaves in relation to the studied treatments: irrigation levels, (I_3, I_2, I_1) and lead contamination (Pb_0, Pb_I and Pb_{II}).

	Water level	Pb_0	Pb_I	Pb_{II}	*Average*
Stems	I_3	0,000 ns	1,635 ns	0,282 ns	*0,639 ns*
	I_2	0,969 ns	1,005 ns	0,375 ns	*0,783 ns*
	I_1	0,879 ns	0,000 ns	0,295 ns	*0,391 ns*
	Average	*0,616 ns*	*0,880 ns*	*0,317 ns*	
Leaves	I_3	0,130 ns	2,140 b	10,077 a	*4,116 a*
	I_2	0,818 ns	2,735 b	1,599 c	*1,718 c*
	I_1	1,305 ns	5,990 a	2,854 b	*3,383 b*
	Average	*0,751 c*	*3,622 b*	*4,844 a*	

Biomass Nitrogen content

About nitrogen content, leaves showed higher content than stems. Lead contamination and the decrease in the irrigation level affect nitrogen content – it increases it, especially in the leaves, due to a concentration effect of the low productivity. Higher value were recorded with the highest lead content of the soil (900 mg kg^{-1}, Pb_{II}) at lower and medium water regime (I_1 and I_2 respectively) (Fig 56) (Fig.57).

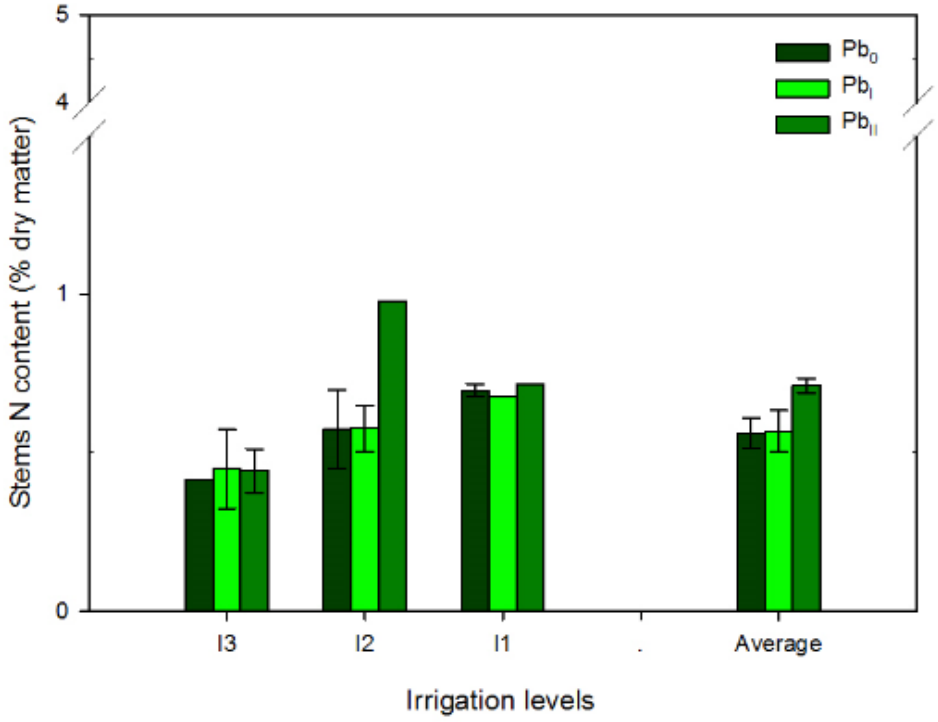


Fig. 56. Stems Nitrogen content in relation to the studied treatments: irrigation levels, (I_3, I_2, I_1) and lead contamination (Pb_0, Pb_I and Pb_{II}).

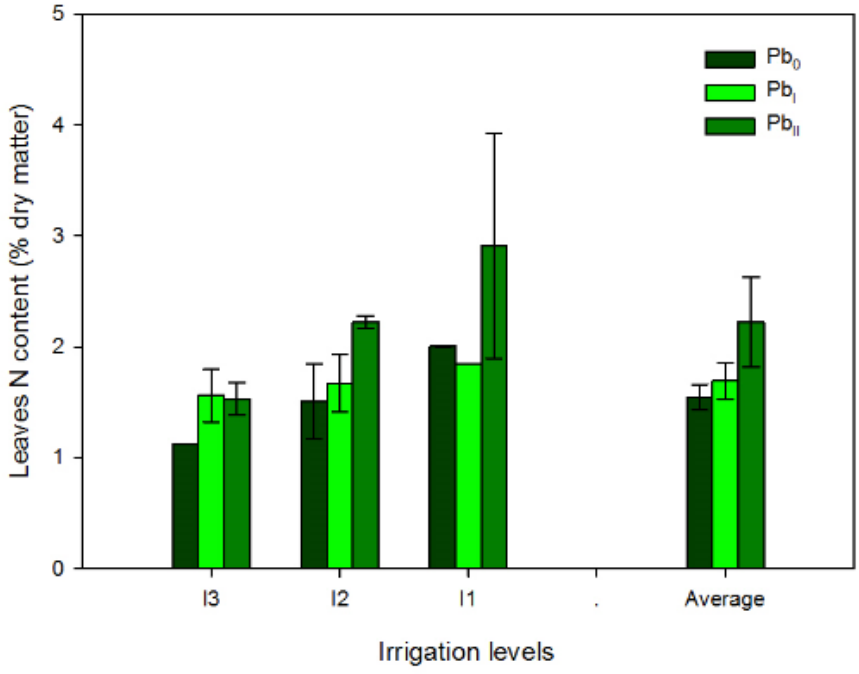


Fig.57. Leaves Nitrogen content in relation to the studied treatments: irrigation levels, (I_3, I_2, I_1) and lead contamination (Pb_0, Pb_I and Pb_{II}).

Biomass Phosphorous content

Results about phosphorus content, showed that leaves had higher (I_3 and I_2) or similar (I_1) content than stems.

Pb contamination did not affect the phosphorus content. Lowering the water regime did not also affect the phosphorus content, although a trend to an increase was observed with the lower amount added. At lower water supplies (I_1), phosphorus content was higher both in stems and leaves than at medium (I_2) and high (I_3) water regimes (Fig. 58) (Fig.59).

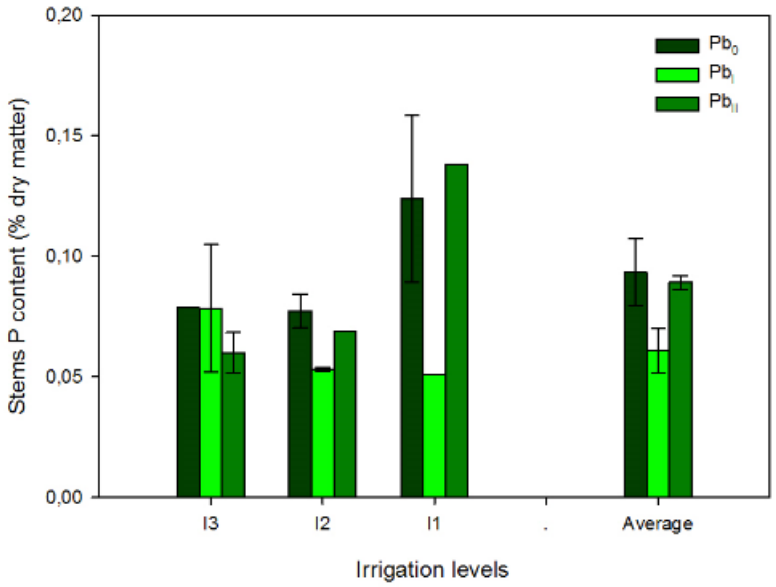


Fig. 58 Stems phosphorous content in relation to the studied treatments: irrigation levels, (I_3, I_2, I_1) and lead contamination (Pb_0, Pb_I and Pb_{II}).

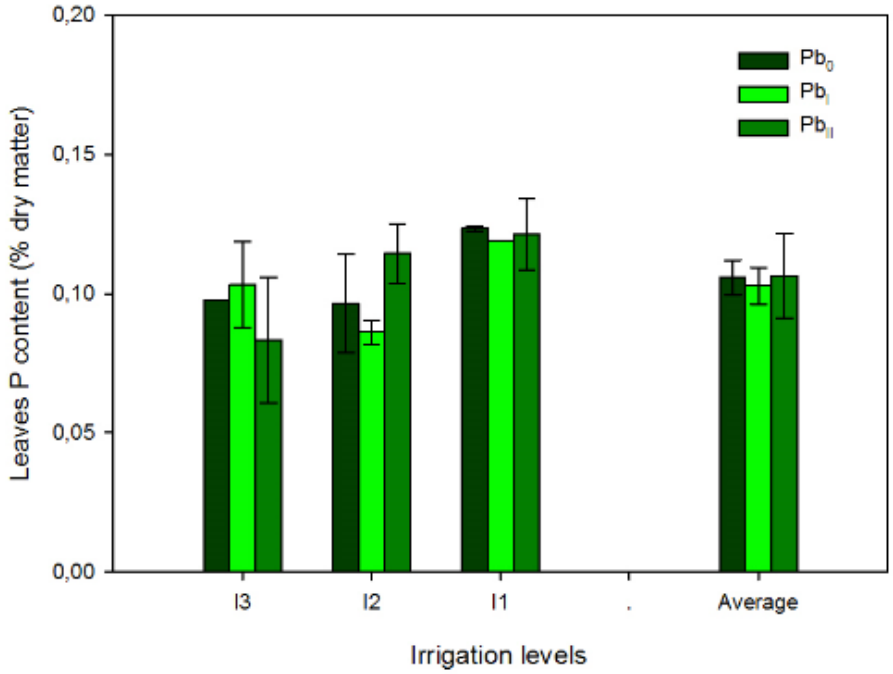


Fig.59 Leaves phosphorous content in relation to the studied treatments: irrigation levels, (I_3, I_2, I_1) and lead contamination (Pb_0, Pb_I and Pb_{II}).

Ash content of biomass

Results about ash content showed higher % in leaves than stems (Fig. 60) (Fig.61). The different water regimes didn't affect the ash content in stems and leaves. On the contrary, lead contamination, led to some interesting results; indeed, high lead concentration (900 mg kg^{-1}, Pb_{II}) increased ash content in the biomass, especially at highest water level content of the soil (I_3); good results were obtained in leaves at highest (I_3)and lowest (I_1)water content of the soil(13,8% DM) (13% DM), respectively.

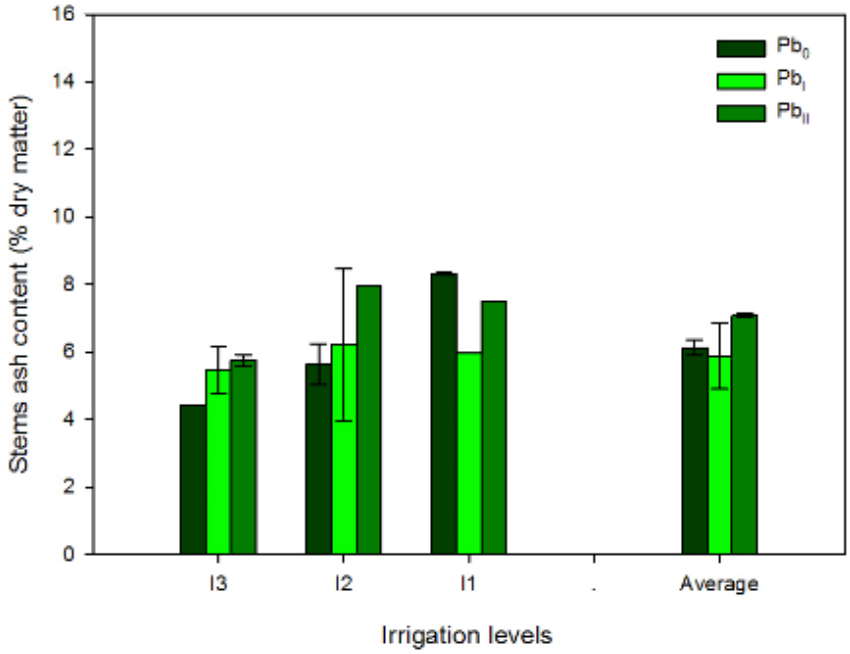


Fig. 60 Stems ash content in relation to the studied treatments: irrigation levels, (I_3, I_2, I_1) and lead contamination (Pb_0, Pb_I and Pb_{II}).

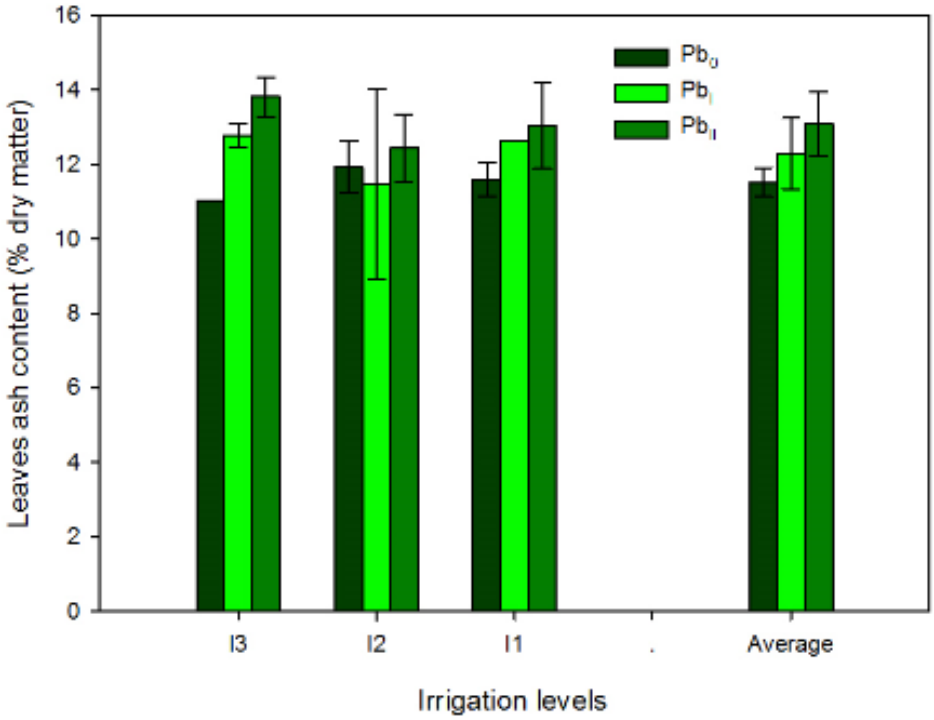


Fig. 61 Leaves ash content in relation to the studied treatments : irrigation levels, (I_3, I_2, I_1) and lead contamination (Pb_0, Pb_I and Pb_{II}).

Soil Lead content

At the end of the first year results about soil Pb content in pots showed no differences among irrigation levels; Pb content varied, in fact, from 208 mg kg^{-1} DM, detected in I_3 water level, to 219 mg kg^{-1} DM in I_1 water level, observed in pots with plants; while in pots without plants the amount of lead in soil, in the average of the contamination levels, decreased from 251 mg kg^{-1} DM to 240 mg kg^{-1} DM, from I_3 to I_1, respectively.

No differences between pot with plants and pots without plants were observed; the lead content in these pots was equal to 211 mg kg^{-1} DM and 244 mg kg^{-1} DM, for pots with plants and for pots without plants, respectively. Despite of this, there was a trend for higher Pb content in soils without plants, thus showing a slight phytoremediation effect on the pots with biomass.

No differences were observed also between the soil layers in the pot: 459 mg kg^{-1} for superior and 429 mg kg^{-1} DM for the inferior, respectively.

The only differences observed were among the contamination levels, in fact, with the highest contamination the highest Pb content was observed, in accordance with the contamination addedd. The lead content in Pb_{II} contaminated pots was equal to 902 mg kg^{-1} DM in pots with plants and 932 mg Kg^{-1} DM in pots without biomass. If compared to the Pb_I pots, the Pb content observed in Pb_{II} pots was twice as the lead sludge amount added in soil before the transplant (Fig.62)

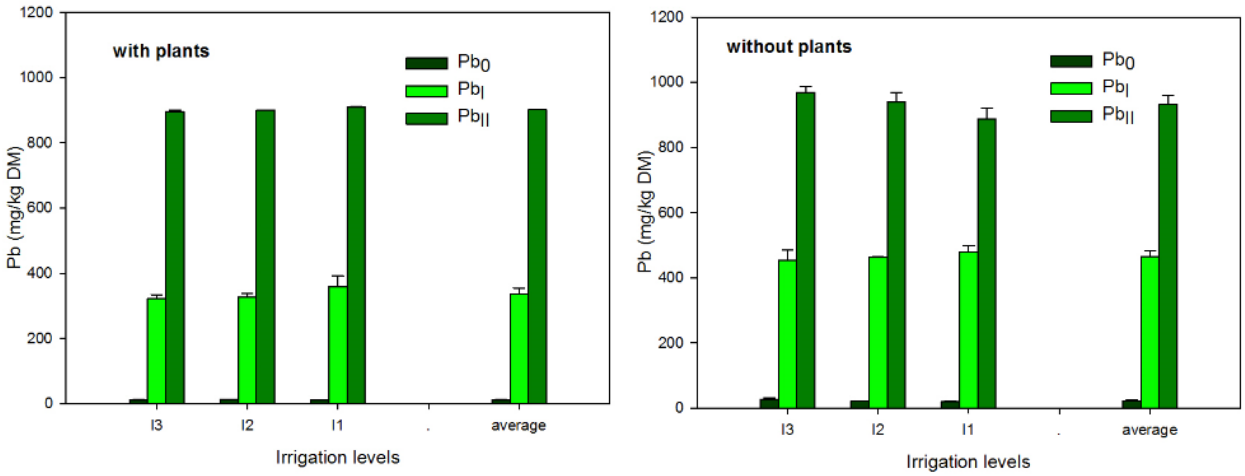


Fig.62 Lead content in soil, detected after the first year from transplant, in pots with and without plants in relation to the different studied treatments (irrigation and contamination levels).

3.3.2. Second year results

Productivity

Results about the production of aboveground biomass showed that the presence of lead in pots reduced the productivity of *Arundo donax*, mainly with I_3 and I_2 water content of the soil. Productivity. in the average of contamination levels tested was reduced from 676.17 g m^{-2} obtained with I_3 water level to 281.68 and 204.16 g m^{-2} with I_2 and I_1, respectively (Fig. 63)

The reduction of the water content reduced the aboveground biomass with all contamination levels tested except with higher contamination (Pb_{II}) which from I_2 to I_1 showed a light increment.

Compared to the first year, the aboveground biomass production, as expected, increased twice in all water content of the soil and in P_0 and Pb_I pots, the increase obtained in Pb_I pots was less marked; in the same pots (Pb_I) with I_2 water level, on contrary, a light decrease of production was observed. In the average of the water content of the soil, the aboveground biomass production was reduced from 469.31 g m^{-2}, detected in Pb_0 pots to 383.40 and 309.29 g m^{-2}, obtained in Pb_{II} pots.

Results about stem production, showed a reduction in response to a decrease of water level; specifically in the average of the water levels tested, the stem production varied from 290.26 g m^{-2} to 51.57 g m^{-2} from the highest water level (I_3) to the lowest (I_1) (Fig.64). The contamination with lead didn't affect the production; Pb_0 pots showed higher stem production (188.41 g m^{-2}) than that provided by Pb contaminated pots (132.99 and 111.28 g m^{-2}, for Pb_I and Pb_{II}, respectively). The production of leaves was decreased in response to the amount of lead in pots and with the reduction of water level. Specifically, in the average of the contamination level, the leaf production was reduced from 359.50 g m^{-2}, observed with I_3 water level to 135.43 g m^{-2}, obtained in I_1 and in the average of water level the production was reduced from 272.15 to 177.67 g m^{-2}, in Pb_0 and in Pb_{II} respectively (Fig.65).

About the belowground biomass production, the results showed a reduction with increasing of Pb doses in all water content of the soil (Fig.66). The reduction of the production was observed also with less water restitution, mainly in pots without contamination(Pb_0) and with high level of contamination (Pb_{II}). In the average of contamination level, the belowground biomass production showed a decrease from

4056.03 g m^{-2}, obtained with I_3 water level to 2618.47 and 1654.01 g m^{-2} observed with I_2 and I_1, respectively.

In the average of water levels tested the belowground biomass obtained decreased from 3878.43 g m^{-2}, detected observed in Pb_0 pots to 2650.22 g m^{-2} and 1799.86 g m^{-2} obtained in Pb_I and Pb_{II} respectively.

About belowground biomass production both the increase of contamination level and decrease of water level of soil, reduced the rhizomes and roots production. In the average of contamination roots production varied from 1310.31 g m^{-2}, provided with the highest water level (I_3) to 442.61 g m^{-2}, value recorded with the lowest water content (I_1) while in the same water level, the rhizome production was reduced from 2745.72 g m-2 (I_3) to 1240.06 g m^{-2}.

In the average of water levels, the increase of lead contamination in pots provided a reduction of root and rhizomes production (Fig.67) (Fig.68); specifically, from Pb_0 to Pb_{II} roots were decreased from 1408.56 g m^{-2} to 615.53 g m^{-2}, while rhizomes production was reduced from 2469.88 g m^{-2}, to 121.99 g m^{-2} in Pb_0 and Pb_{II}, respectively.

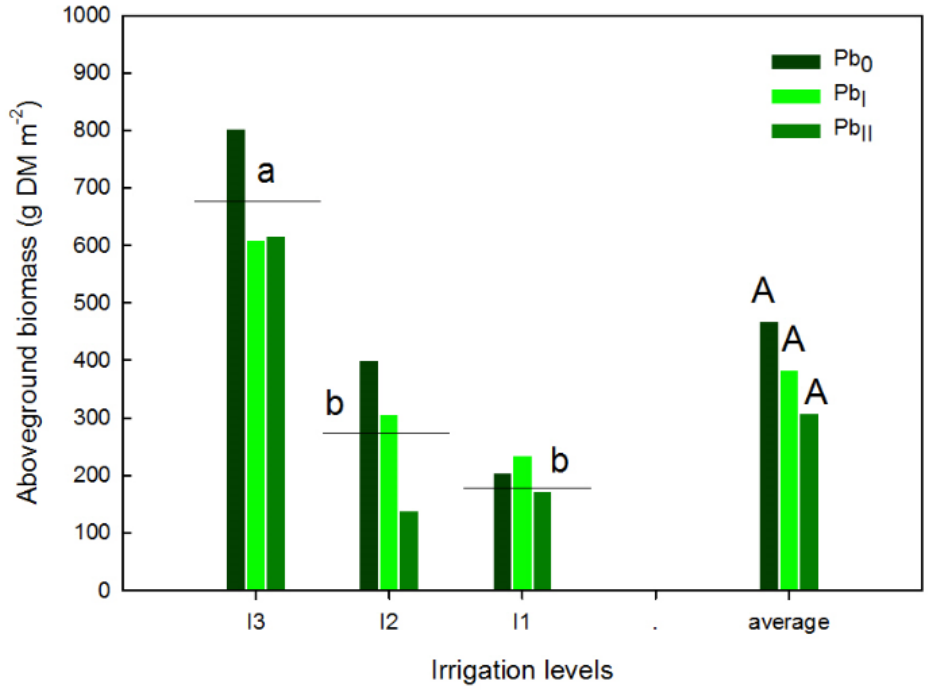


Fig. 63. Aboveground biomass (g DM m^{-2}) in relation to the studied treatments: irrigation levels, (I_3, I_2, I_1) and lead contamination (Pb_0, Pb_I and Pb_{II}).

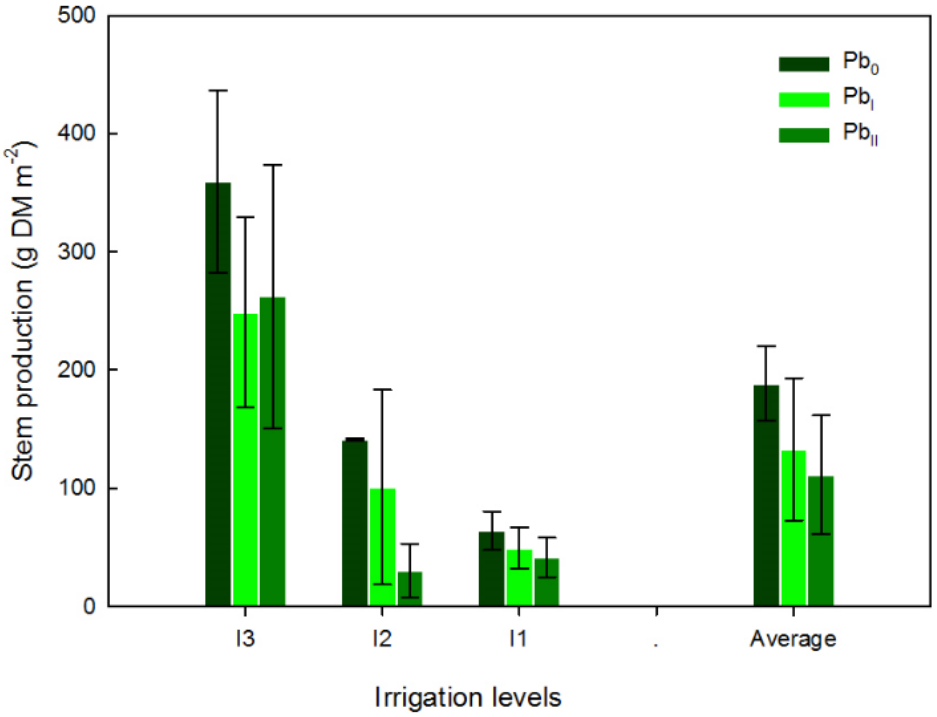


Fig.64 Stem production (g DM m^{-2}) in relation to the studied treatments: irrigation levels, (I_3, I_2, I_1) and lead contamination (Pb_0, Pb_I and Pb_{II}).

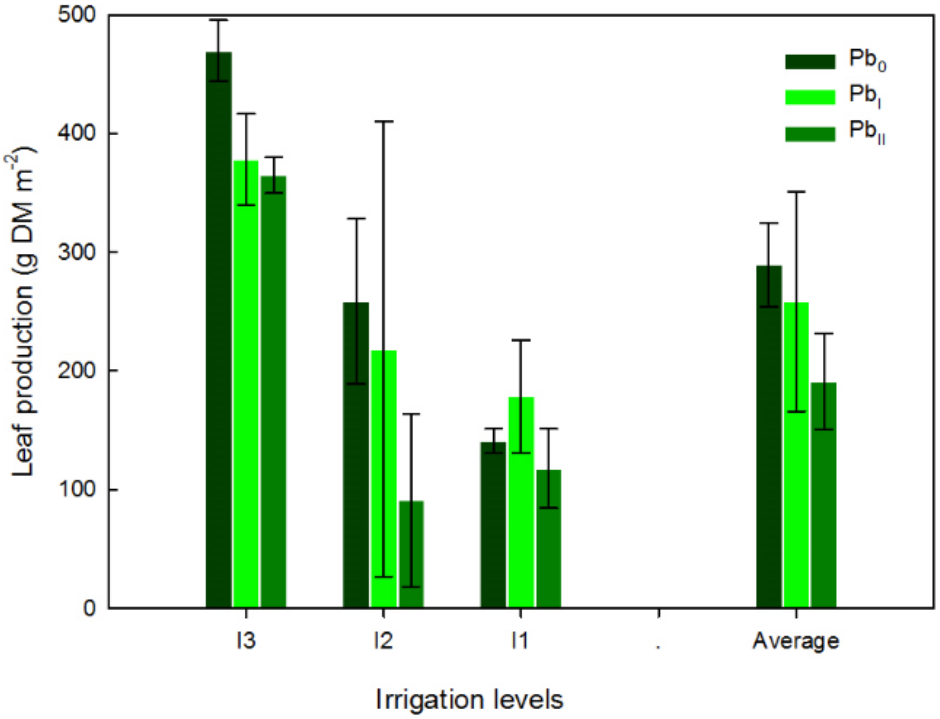


Fig. 65 Leaf production (g DM m^{-2}) in relation to the studied treatments: irrigation levels, (I_3, I_2, I_1) and lead contamination (Pb_0, Pb_I and Pb_{II}).

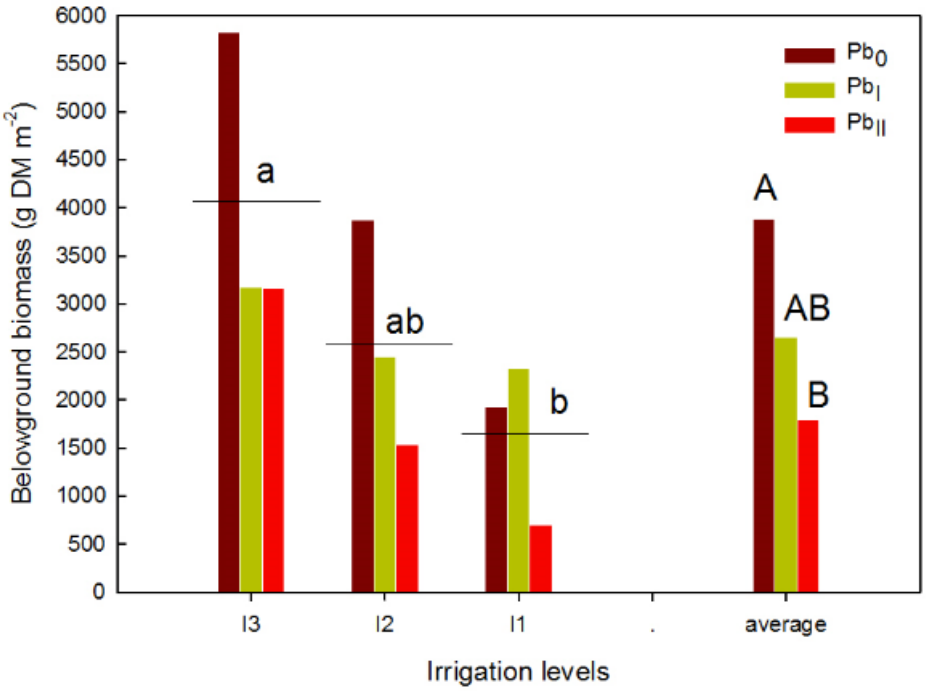


Fig. 66 Belowground biomass (g DM m^{-2}) in relation to the studied treatments: irrigation levels, (I_3, I_2, I_1) and lead contamination (Pb_0, Pb_I and Pb_{II}).

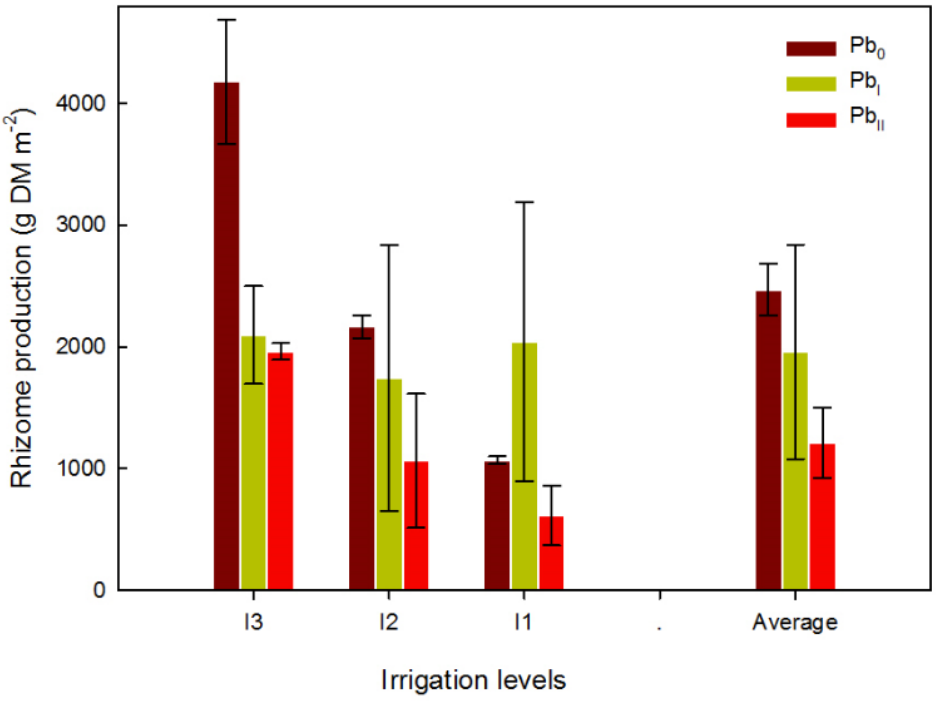


Fig. 67 Rhizome production (g DM m^{-2}) in relation to the studied treatments: irrigation levels, (I_3, I_2, I_1) and lead contamination (Pb_0, Pb_I and Pb_{II}).

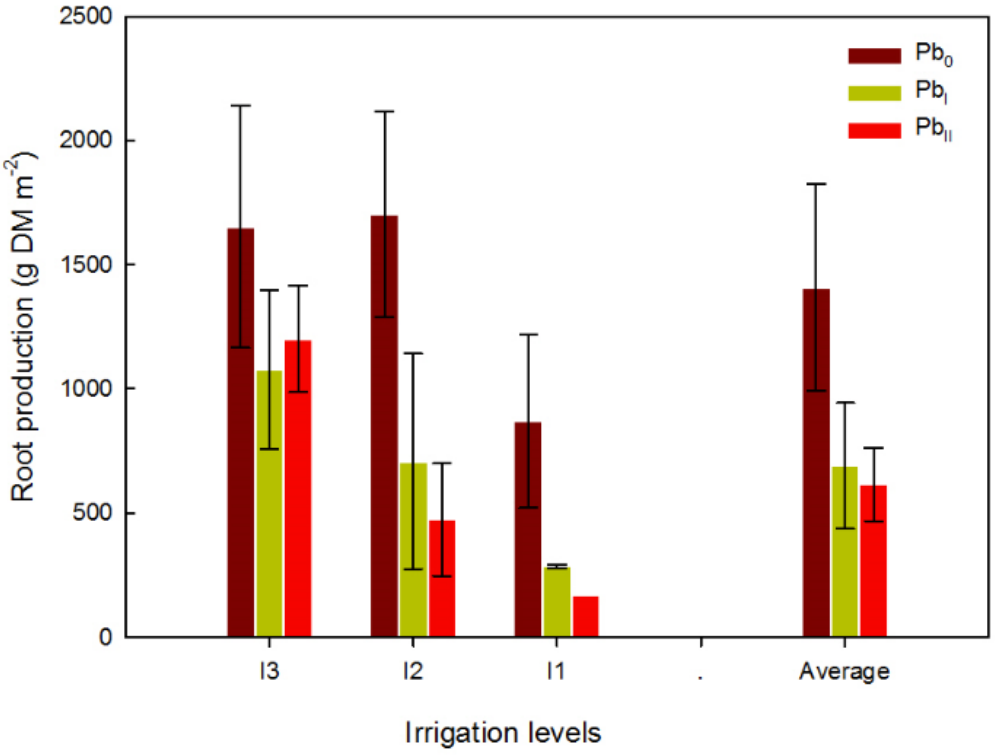


Fig. 68 Root production (g DM m^{-2}) in relation to the studied treatments: irrigation levels, (I_3, I_2, I_1) and lead contamination (Pb_0, Pb_I and Pb_{II}).

LAI

Results on leaf area index (LAI) showed a decrease with the reduction of water level content of soil, from 3.8 to 2.2 and to 1.6 in the average of contamination level for I3, I2 I1, respectively (Fig. 69). The contamination with Pb, in the average of water levels tested led to a reduction of LAI, especially from pots without contamination (Pb_0) to pots with higher content of lead (Pb_{II}).

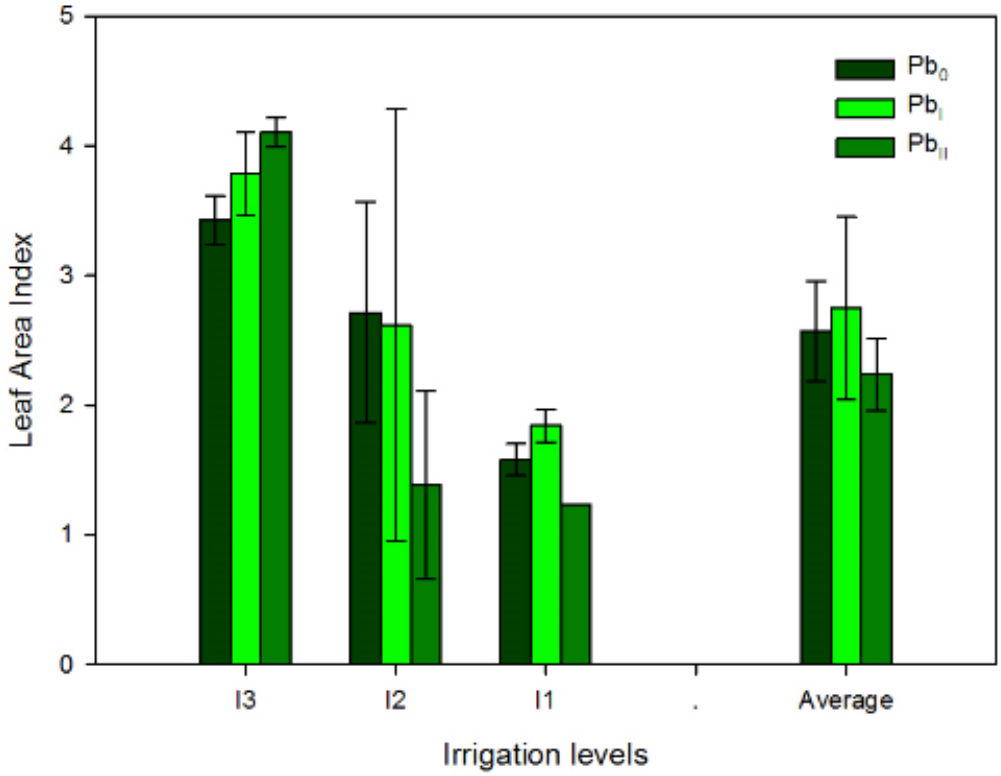


Fig.69 Leaf area index (LAI) measured in pots in the different studied treatments (water and contamination levels).

Morpho-biometric characters

The height of stems was decreased in relation to the reduction of water content of the pots, as observed in the first year; in the average of the contamination level, the height was reduced from 96.40 cm, to 48.82, values measured in I_3 and in I_1 water content, respectively. The height of the stem decreased also with the increase of contamination level with lead, in the average of water level, the height was reduce from 80.72 cm, value measured in pots without contamination to 59.67 cm, height observed in pots with the highest contamination level without any significant difference.

The heigh of stems increased twice from the first year to the second year (Tab.6).

The increase of contamination level provided a lower number of stems with medium (I_2) and low (I_1) water content of soil With the highest water level (I_3) the contamination increased the number of stems. The reduction of water level resulted in lower number of stem especially in Pb contaminated pots (Tab.6).

The number of nodes per stem was reduced with the increasing of contamination level and with the decreasing of water level; as the first year, the highest number of nodes

(20) was recorded in the pots without contamination (Pb_0) and with the highest water level (I_3), vice versa, the lowest number of nodes (10) was recorded in the pots with the highest contamination level (Pb_{II}) and with the lowest water level (I_1).

In the average of water level, the number of nodes varied from 21.3 to 13.4, observed in Pb_0 and in Pb_{II}, respectively; while in the average of the contamination levels this was reduced from 19.25 to 13.08 in I_3 and I_1, respectively (Tab.6).

About the basal diameter of the main stem, the reduction of water led to a decrease of the parameter in all the contamination levels tested, mainly in pots without contamination; the highest contamination (Pb_{II}) provided a lower basal diameter than that one's obtained in pots without contamination (Pb_0), except with the lowest water content (I_1) in which was observed an increase of the parameter in response to higher contamination (Tab.6).

Tab.6. Morpho-biometric characters in relation to the studied treatments: irrigation levels, (I_3, I_2, I_1) and lead contamination (Pb_0, Pb_I and Pb_{II}).

Water level	Stems (n°)				Height (cm)				Basal diameter (mm)				Nodes (n°)			
	Pb_0	Pb_I	Pb_{II}	***Av***	Pb_0	Pb_I	Pb_{II}	***Av***	Pb_0	Pb_I	Pb_{II}	***Av***	Pb_0	Pb_I	Pb_{II}	***Av***
I_3	2.0	2.5	3.0	***2.5ns***	109.6	96.3	83.3	***96.4a***	14.2	8.3	10.5	***11.0ns***	26.0	14.8	17.0	***19.3a***
I_2	3.0	1.5	2.0	***2.2ns***	75.7	58.2	56.1	***63.3b***	6.9	8.4	5.8	***7.0ns***	20.8	17.8	13.3	***17.3ab***
I_1	2.0	1.5	1.0	***1.5ns***	56.9	50.0	39.6	***48.8b***	5.4	11.5	9.5	***8.8ns***	17.0	12.3	10.0	***13.1b***
Average	***2.3ns***	***1.8ns***	***2.0ns***	***2.1***	***80.7ns***	***68.2ns***	***59.7ns***	***69.5***	***8.8ns***	***9.4ns***	***8.6ns***	***8.9***	***21.3a***	***15.0b***	***13.4b***	***16.6***

Nitrogen II year pots

Results about nitrogen content in stems, equal to 0.48% DM on average, in the average of water levels showed higher lead contamination (Pb_{II}) provided higher N content than Pb_I and Pb_0 pots, 0.85, 0.71 and 0.5 % DM, respectively; this was due to higher sludge in the soil of Pb_{II} pots, rich in nitrogen. In the average of lead contamination it was also observed an increment of N content with less water; specifically I_3 water level provided nitrogen content equal to 0.69, while N content in I_2 and I_1 were equal to 0.42 and 0.33, respectively (Fig.70).

In leaves, N content on average of contamination and water levels was equal to 1.52% DM. None effects on N due to different water levels and contamination levels were observed (Fig.71).

In rhizomes, N content was equal to 0.74% DM on average; none effects of lead contamination on N content was observed, while the reduction of water levels provided a small N accumulation: from 0.46 % DM to 0.77 % in the average of contamination levels from I_3 to I_1 water level, respectively (Fig.72).

Roots showed the lowest N content, 0.17% DM on average. The reduction of water, in the average of contamination level showed from I_3 to I_1 a small N accumulation with content equal to 0.16 and 0.19 % N for I_3 and I_1, respectively (Fig.73).

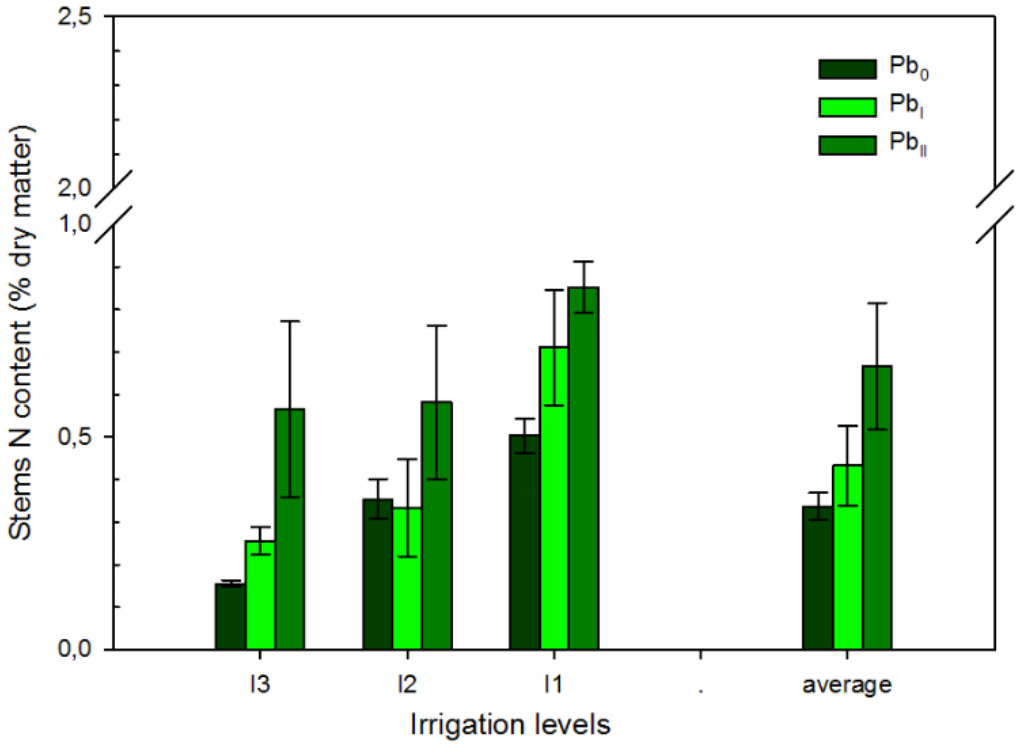


Fig. 70 Stems N content (% DM) in relation to the studied treatments: irrigation levels, (I_3, I_2, I_1) and lead contamination (Pb_0, Pb_I and Pb_{II}).

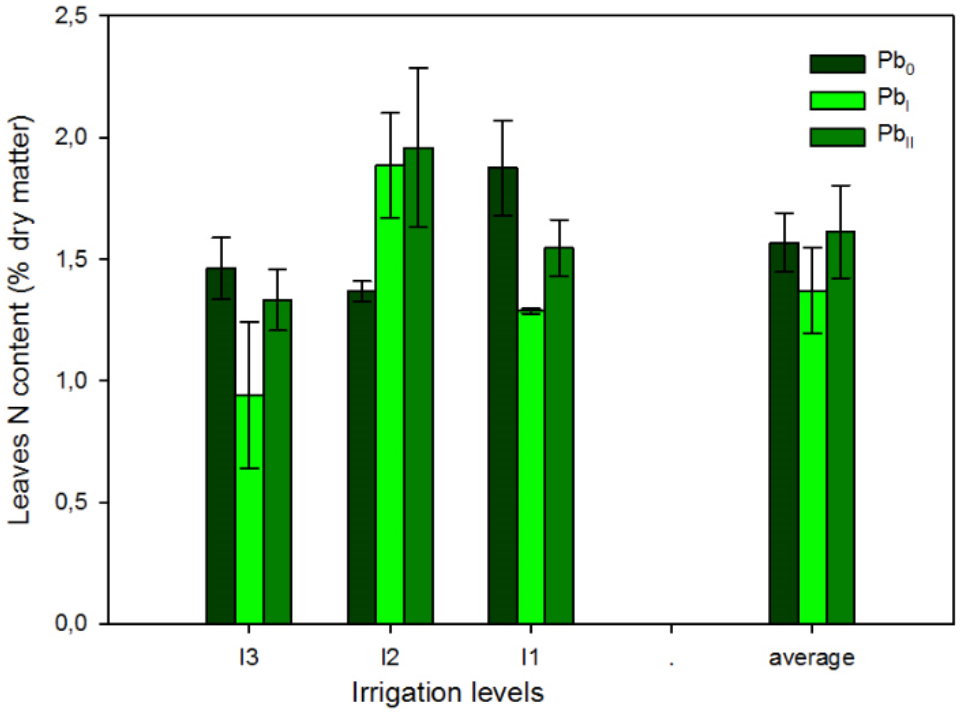


Fig. 71 Leaves N content (% DM) in relation to the studied treatments: irrigation levels, (I_3, I_2, I_1) and lead contamination (Pb_0, Pb_I and Pb_{II}).

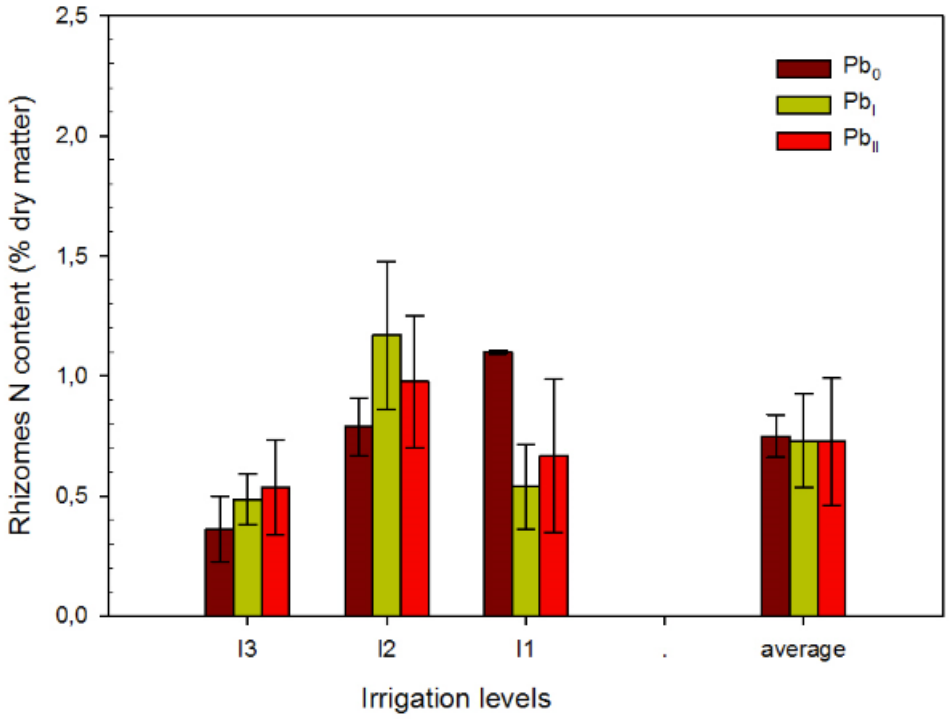


Fig. 72 Rhizome N content (% DM) in relation to the studied treatments: irrigation levels, (I_3, I_2, I_1) and lead contamination (Pb_0, Pb_I and Pb_{II}).

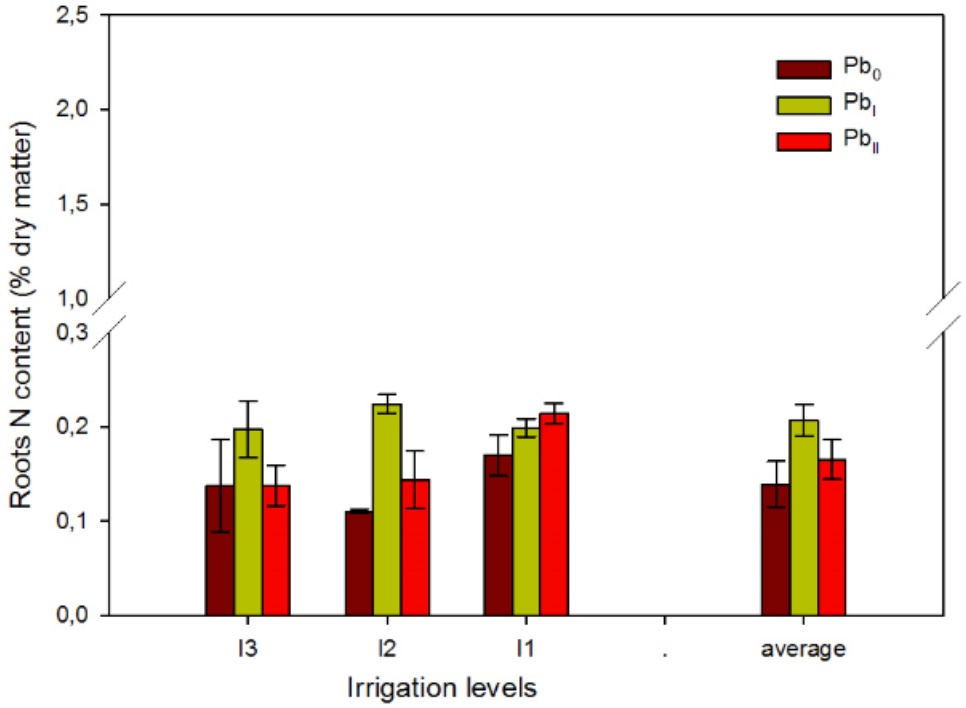


Fig. 73 Roots N content (% DM) in relation to the studied treatments: irrigation levels, (I_3, I_2, I_1) and lead contamination (Pb_0, Pb_I and Pb_{II}).

Phosphorous II year pots

Results about phosphorous content showed similar values for the different biomass fractions analyzed.

In stems, which P content was equal to 0.09 on average, an increment of P content was observed with the reduction of water and with the increasing of contamination levels; specifically, in the average of contamination levels, P content varied from 0.075 to 0.127% DM from I_3 to I_1, respectively and in the average of water levels P content varied from 0.053 to 0.116 % DM from pots without contamination (Pb_0) to pots with higher contamination level (Pb_{II}), mainly due to higher sludge in the soil of these latter pots, rich in phosphorous (Fig.74).

In leaves, P content was equal to 0.1 % DM on average and none effects due to the reduction of water and the different level of contamination were observed (Fig.75).

In rhizomes P content was equal to that of measured in leaf fraction, 0.1% DM on average, none effects on P content was observed due to the contamination level and the

reduction of water level (Fig.76). The same happens with roots, which P content was on average equal to 0.06 % DM (Fig.77).

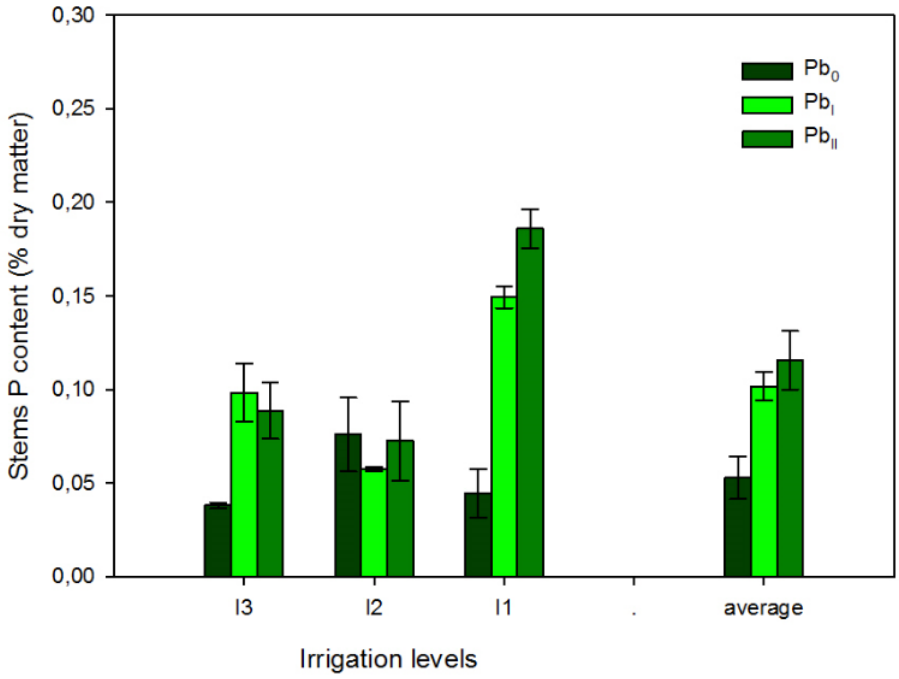


Fig. 74 Stems P content (% DM) in relation to the studied treatments: irrigation levels, (I_3, I_2, I_1) and lead contamination (Pb_0, Pb_I and Pb_{II}).

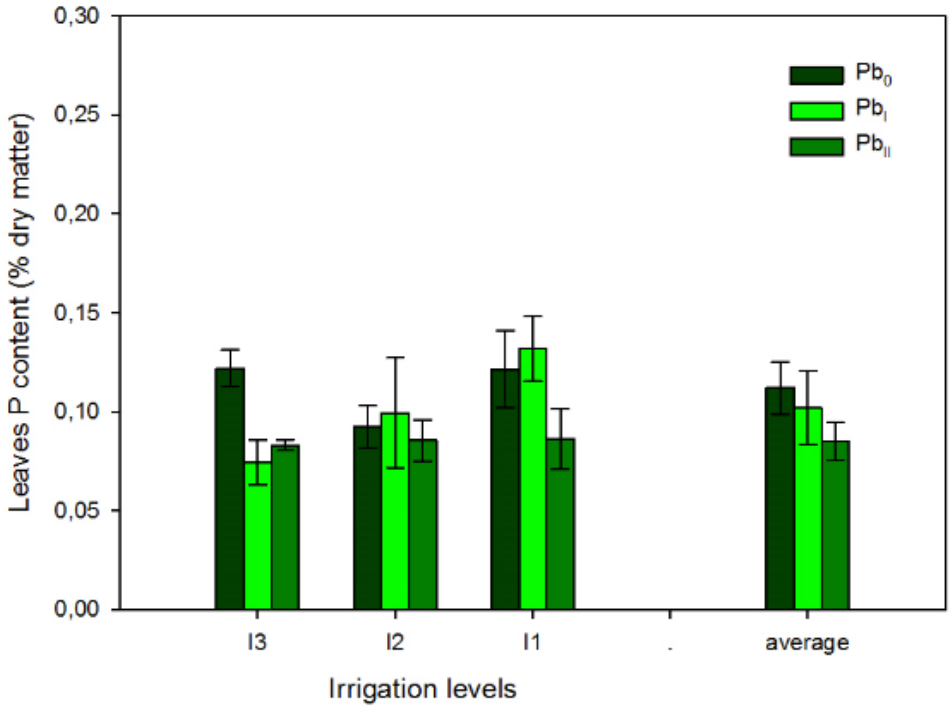


Fig. 75 Leaves P content (% DM) in relation to the studied treatments: irrigation levels, (I_3, I_2, I_1) and lead contamination (Pb_0, Pb_I and Pb_{II}).

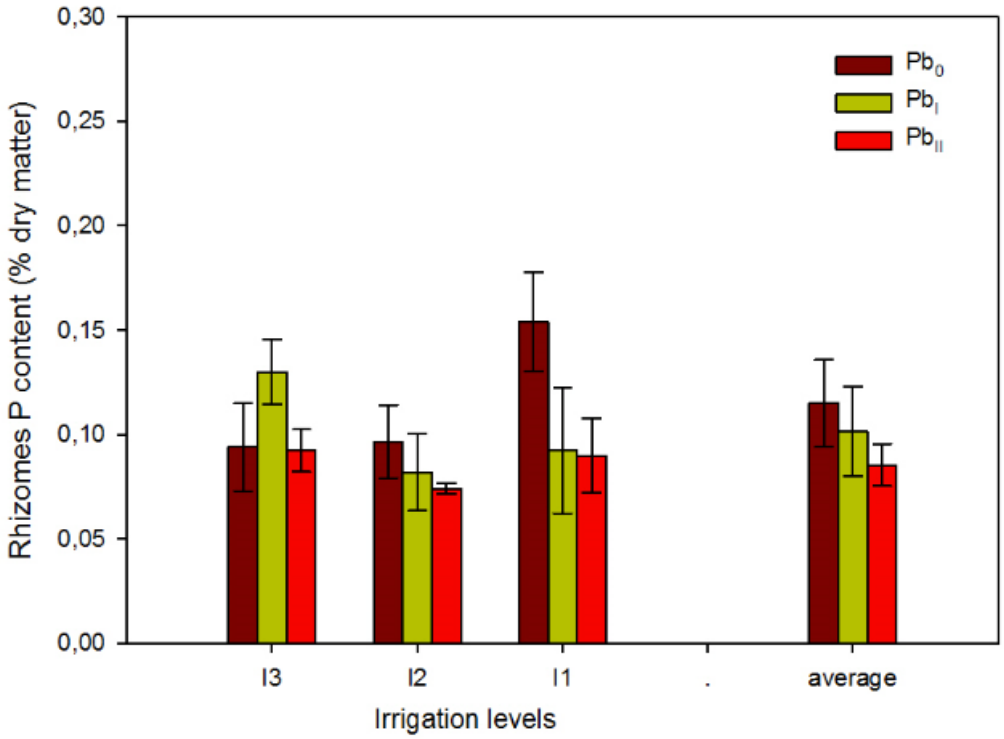


Fig. 76 Rhizome P content (% DM) in relation to the studied treatments: irrigation levels, (I_3, I_2, I_1) and lead contamination (Pb_0, Pb_I and Pb_{II}).

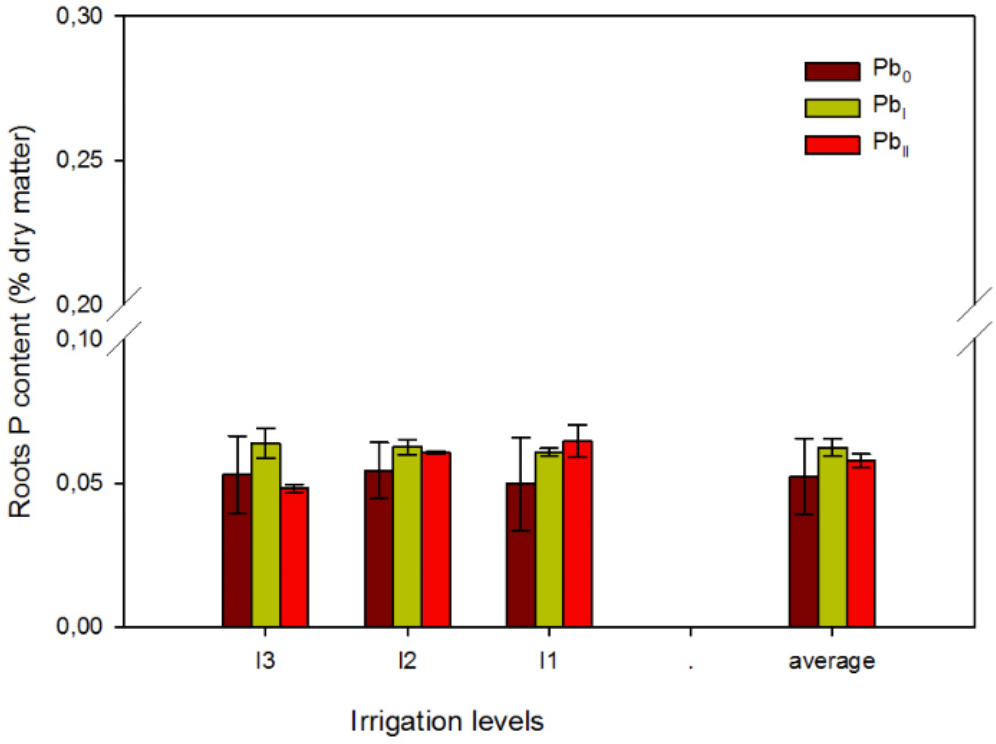


Fig. 77 Roots P content (% DM) in relation to the studied treatments: irrigation levels, (I_3, I_2, I_1) and lead contamination (Pb_0, Pb_I and Pb_{II}).

Ash content

Results about ash content showed in all plants fractions analyzed an increment of ash content with the lead contamination, except in roots where pots with higher contamination showed a reduction of ash content than pots without contamination.

The reduction of water level did not affect ash content in rhizomes and leaves (Fig.79) in all contamination levels, while in stems (Fig.78) and roots an increment of ash content was recorded with the lowest water level (I_1).

The belowground fractions (roots and rhizomes) (Fig.80) (Fig.81) showed the highest ash content than that analyzed in stems and leaves. In the average of all studied treatments ash content in roots and rhizomes was equal to 76.90 and 23.65%, respectively, while in stems and leaves ash content was lower and equal to 5.50 and 10.96%.

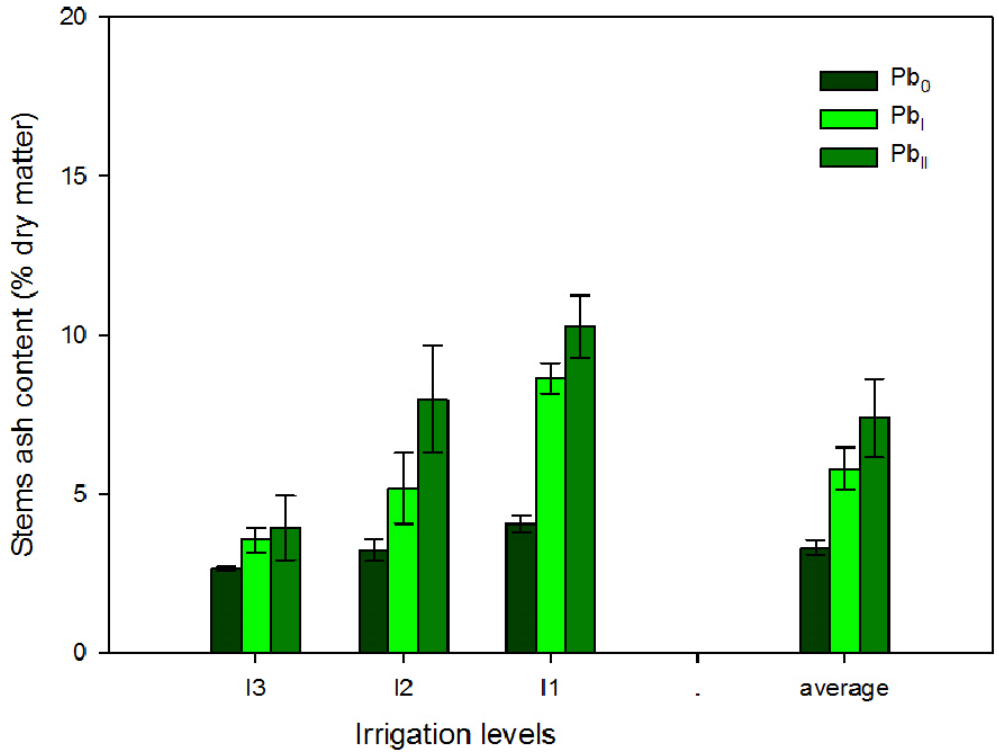


Fig. 78 Stems ash content (% DM) in relation to the studied treatments: irrigation levels, (I_3, I_2, I_1) and lead contamination (Pb_0, Pb_I and Pb_{II}).

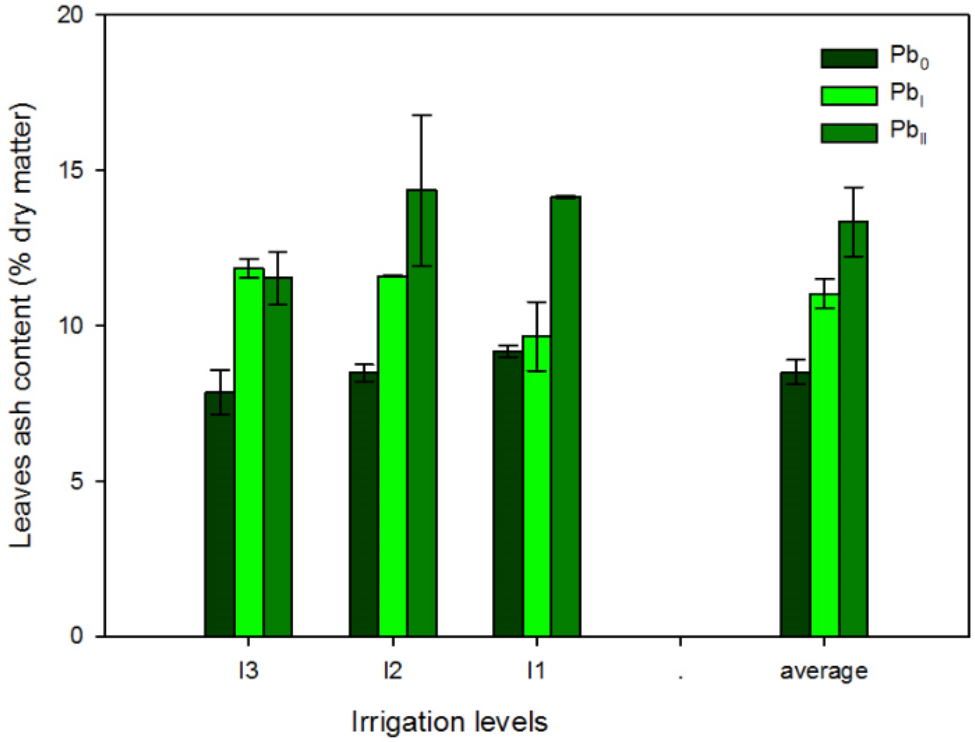


Fig. 79 Leaves ash content (% DM) in relation to the studied treatments: irrigation levels, (I_3, I_2, I_1) and lead contamination (Pb_0, Pb_I and Pb_{II}).

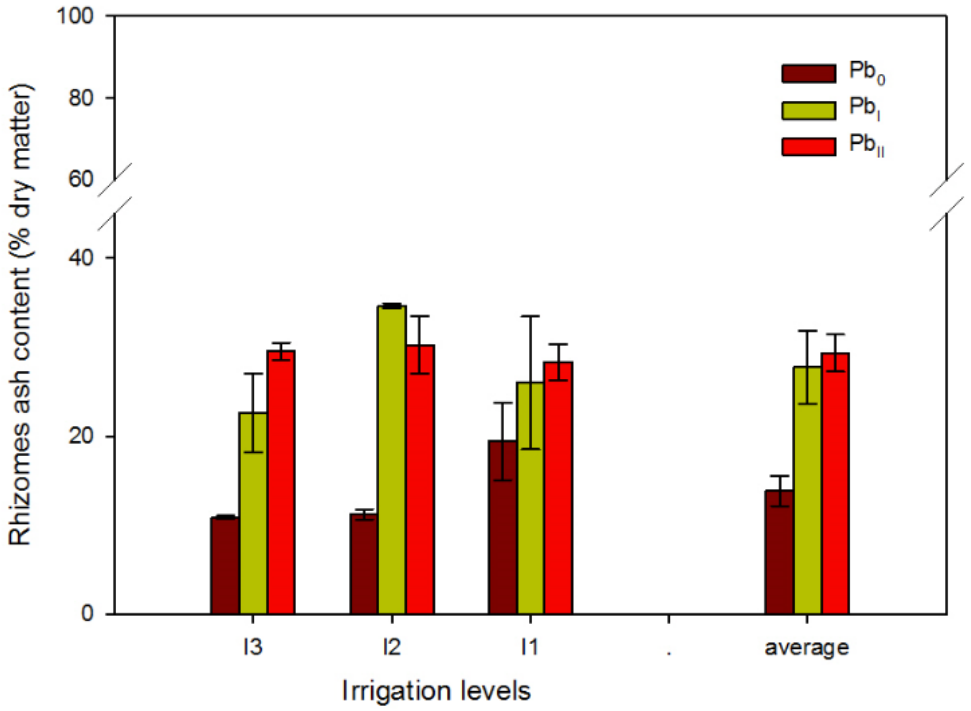


Fig. 80 Rhizome ash content (% DM) in relation to the studied treatments: irrigation levels, (I_3, I_2, I_1) and lead contamination (Pb_0, Pb_I and Pb_{II}).

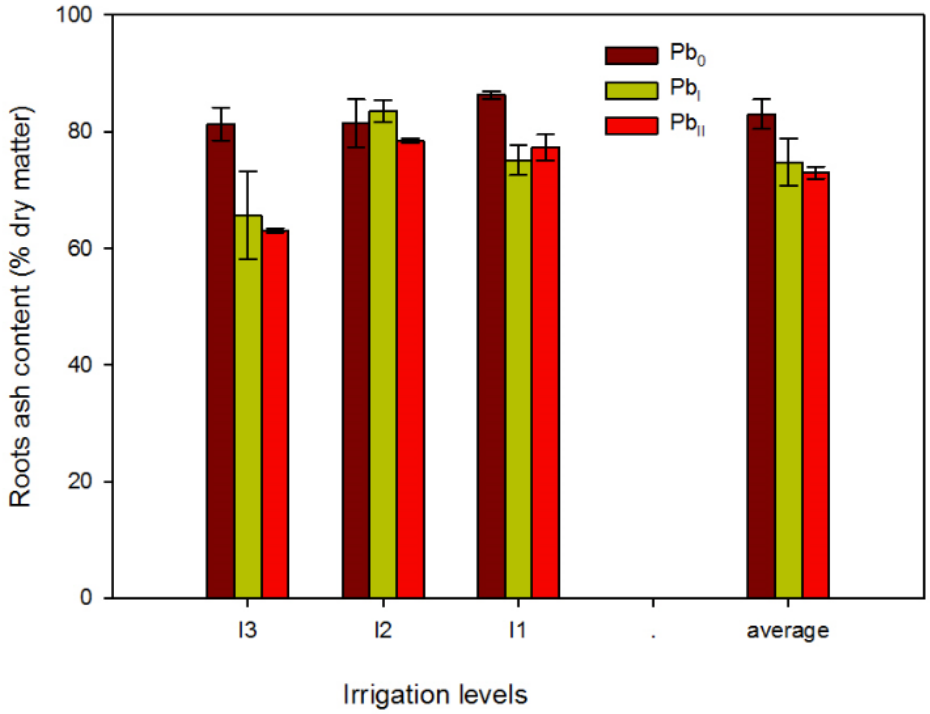


Fig. 81 Roots ash content (% DM) in relation to the studied treatments: irrigation levels, (I_3, I_2, I_1) and lead contamination (Pb_0, Pb_I and Pb_{II}).

Lead content

The lead content of biomass as reported from literature, was higher in roots and rhizomes fraction than that recorded in leaf and stem fractions (Tab.7).

Leaves as showed in the first year, presented a higher Pb content than stems; the amount of lead recorded was equal to 5.03 mg kg^{-1} DM and 2.35 mg kg^{-1} DM on average, for leaves and stems, respectively.

No significant differences among water level were observed both in stems and leaves, while contamination level provided in both analyzed fractions an increment of Pb content. Specifically, in the average of water levels Pb content in leaves, varied from 1.18 to 9.03 mg kg^{-1} DM from pots without contamination (Pb_0) to pots with higher contamination (Pb_{II}) while in stems varied in the same contamination level from 0.6 to 4.10 mg kg^{-1} DM (Tab.7)

Roots presented higher Pb content than rhizomes with amount of lead equal to 22.18 and 16.21 mg DM kg^{-1} on average for roots and rhizomes, respectively. In both belowground fractions, an increment of Pb content was observed with contamination; in the average of water levels, lead content, varied from 0.72 to 37.91 mg DM kg^{-1} in rhizomes and from 2.09 to 48.50 mg DM kg^{-1} in roots on average (Tab.7)

No significant differences were observed among water level in Pb content of roots, while in rhizomes with a decrease of water content an accumulation of lead was observed; specifically, lead content in rhizomes, in the average of Pb contamination levels, varied from 9.3 mg DM kg^{-1}, observed with the highest water level (I_3), to 21.97 mg DM kg^{-1}, detected with the lowest water content (I_1) (Tab.7)

The results showed that Pb amount was mainly concentrated in roots with a quite low translocation to aerial organs, in agreement with Zhao-hui and Xu-feng (2010) who found the limited capacity for Pb translocation from roots to shoots in A. donax.

This also confirmed what reported by Obarska-Pempkowiak and Klimkowska (1999) and by Vymazal et al. (2009) that trace elements concentrations decreased in *A. donax* according to the order of root>rhizome>leaf > stem. This last issue is in agreement with the general trend of element mobility in rooted macrophytes where the primary source of nutrients in leaves are generally roots.

Tab.7 Lead content detected in different fractions of the plants in relation to the studied treatments: irrigation levels, (I_3, I_2, I_1) and lead contamination (Pb_0, Pb_I and Pb_{II}).

	Water level	Pb_0	Pb_I	Pb_{II}	***Average***
Stems	I_3	0.73	1.16	4.30	***2.06ns***
	I_2	0.69	0.99	3.72	***1.80ns***
	I_1	0.38	4.89	4.29	***3.19ns***
	Average	***0.60c***	***2.35b***	***4.10a***	***2.35***
Leaves	I_3	1.21	7.05	8.41	***5.56ns***
	I_2	1.12	4.73	10.22	***5.36ns***
	I_1	1.21	2.89	8.46	***4.19ns***
	Average	***1.18c***	***4.89b***	***9.03a***	***5.03***
Rhizomes	I_3	*0.94*	*6.25*	*20.71*	**9.30ns**
	I_2	*0.77*	*14.49*	*36.80*	**17.35ns**
	I_1	*0.45*	*9.24*	*56.23*	**21.97ns**
	Average	***0.72b***	***9.99b***	***37.91a***	***16.21***
Roots	I_3	*3.07*	*24.56*	*55.25*	**27.63ns**
	I_2	*1.17*	*16.25*	*34.94*	**17.45ns**
	I_1	*2.02*	*7.08*	*55.31*	**21.47ns**
	Average	*2.09b*	*15.96b*	*48.50a*	**22.18**

Soil lead content

At the end of the second year, results about lead content in soil, showed differences among contamination levels: as in the first year, the highest Pb contamination in pots, provided the highest Pb content: 910 mg kg^{-1} DM, and 915 mg Kg^{-1} DM for pots with and without plants, respectively.

No differences were observed among the irrigation levels, although between pots with *Arundo* and pots without *Arundo* a contrary trend was noted. In pots with plants, the amount of lead in soil increase in response to the reduction of water level, in fact from I_3 to I_1, lead content in soil increased from 409 mg kg^{-1} DM to 426 mg kg^{-1} DM; while in pots without plants the reduction of water caused a reduction of lead content in soil

which varied from 465 mg kg^{-1} DM to 455 mg kg^{-1} DM, from I_3 to I_1 water level, respectively. But those differences are not significant.

Overall, no differences were observed between pots with and without plants, although there is a trend for higher Pb in soils without plants: in the average of contamination levels: 416 mg kg^{-1} DM and 457 mg kg^{-1} DM, for pots with plants and without, respectively.

No differences were observed also between the layers of soil analyzed: 448 mg kg^{-1} DM and 426 mg kg^{-1} DM, respectively, superior and inferior layer. In this respect, the same trend was observed between layers, as it was for soils in 2012. The bottom layer presented a lower Pb concentration than the top layer. This difference was also attributed to the higher phytoextraction performed by the higher densified below ground biomass present in the pots (Fig.82).

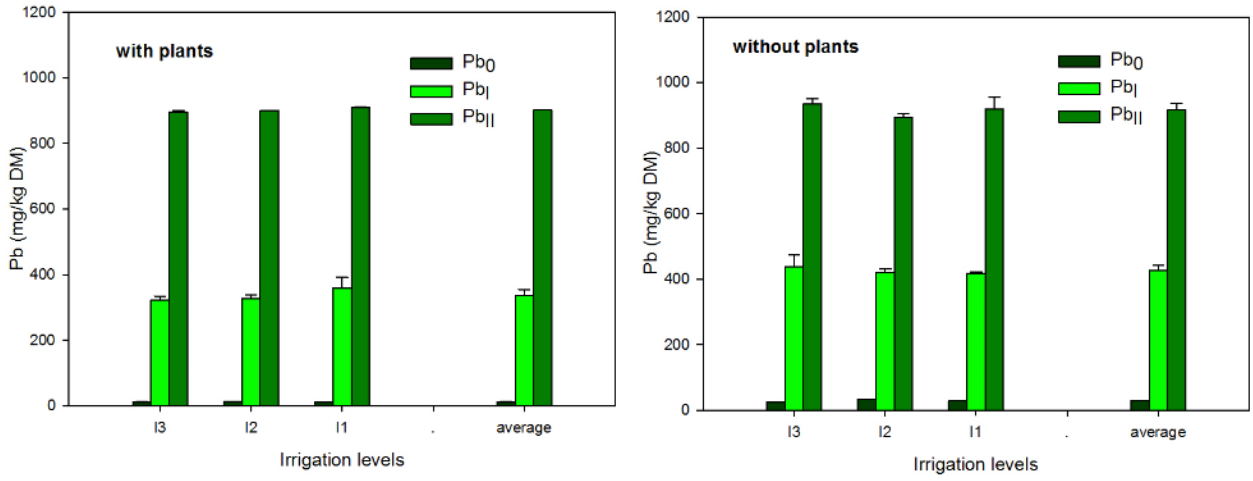


Fig. 82. Lead content in soil, detected after the second year from transplant, in pots with and without plants in relation to the different studied treatments (irrigation and contamination levels).

Leached lead content

At the harvest of the plant, results about lead content in leachates collected from the pots with biomass showed no differences among contamination levels; on the contrary, differences were found in pots without biomass, specifically, Pb_I and Pb_{II} contaminated pots leached higher amounts of lead: 0.023 mg/L on average, compared to pots without

contamination (Pb_0) that recorded lead content equal to 0.010 mg/L. No differences were observed among the irrigation level.

In the experiment, differences were observed between pots with and without plants due to the fact that pots without biomass leached higher Pb and this could be important for the use of *Arundo* to accumulate and stabilize the lead and to prevent the "run-off" and leaching of lead into groundwaters (Fig.83).

The amount of Pb leached is however not problematic in every pot, once it is below the limit value of Pb (mg/L) when wastewaters are discharged into waters, according to the Portuguese Law (Dec Lei 236/97).

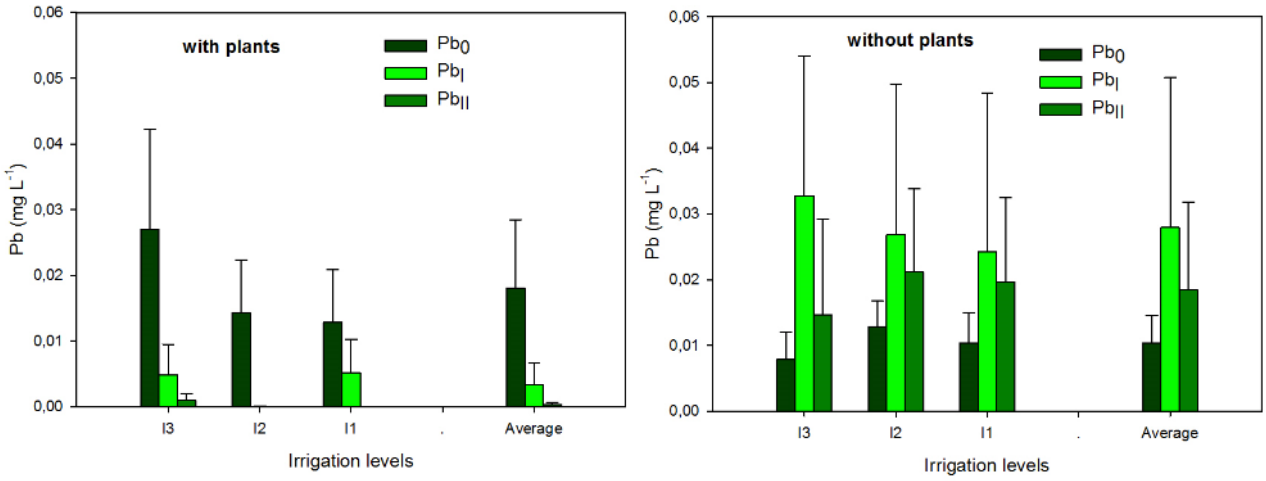


Fig.83. Lead content leached in relation to the different studied treatments: irrigation and contamination levels.

3.4. Research Line 4

Productivity

Among the studied genotypes, the results about biomass productivity showed that genotype 27 and genotype 30 were the most productive with 700.97 g m^{-2} and 705.35 g m^{-2}, respectively, on average of the lead contamination, while genotype 22 and genotype 19 showed the lowest aboveground biomass with 476.08 and 459.74 g m^{-2} on average. Lead contamination didn't affect production (Fig.84).

Among the studied genotypes, genotypes 30 and 27 provided the highest stem production in the average of lead contamination with 306.77 g m^{-2} and 304.14 g m^{-2}, respectively, while the least stem productivity was provided by genotype 19 and genotype 22 with 172.66 g m^{-2} and 192.71 g m^{-2}, respectively. Lead contamination didn't affect the stem productivity (Fig. 85).

In the average of lead contamination and among genotypes, genotypes 27 and 30 were the most productive with leaf production of 401.20 and 394.19 g m^{-2}, respectively, while the less productive were genotypes 19 and 22 with 279.72 and 272.73 g m^{-2}, respectively.

Lead contamination didn't affect the leaf production, Pb0 showed in fact, the highest leaf production (352.90 g m^{-2}) in the average of the studied genotypes while Pb contaminated pots gave a production equal to 317.51 and 340. 49 g m^{-2} for Pb_I and Pb_{II}, respectively (Fig.86).

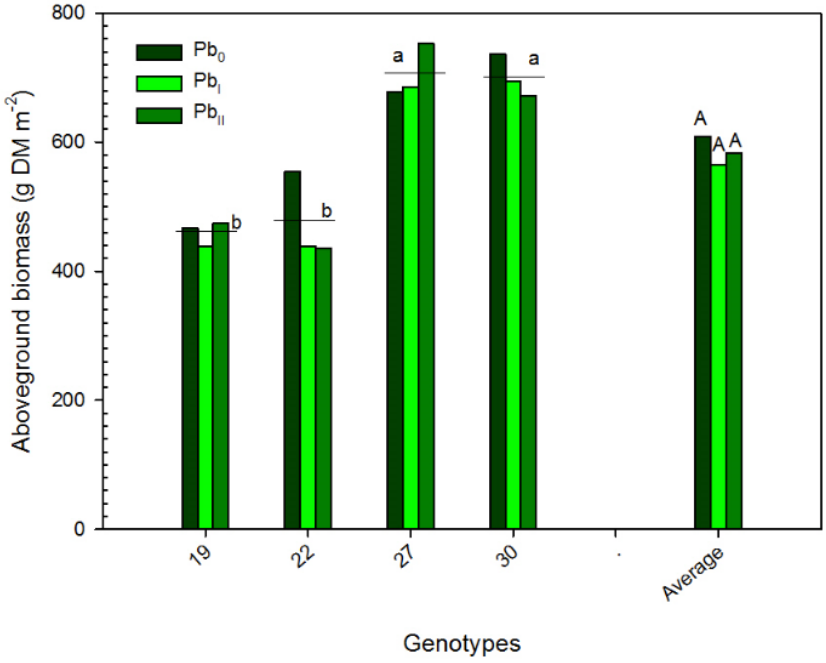


Fig.84 Aboveground biomass (g DM m^{-2}) in the different studied genotypes in relation to different lead contamination levels (Pb_0, Pb_I and Pb_{II}).

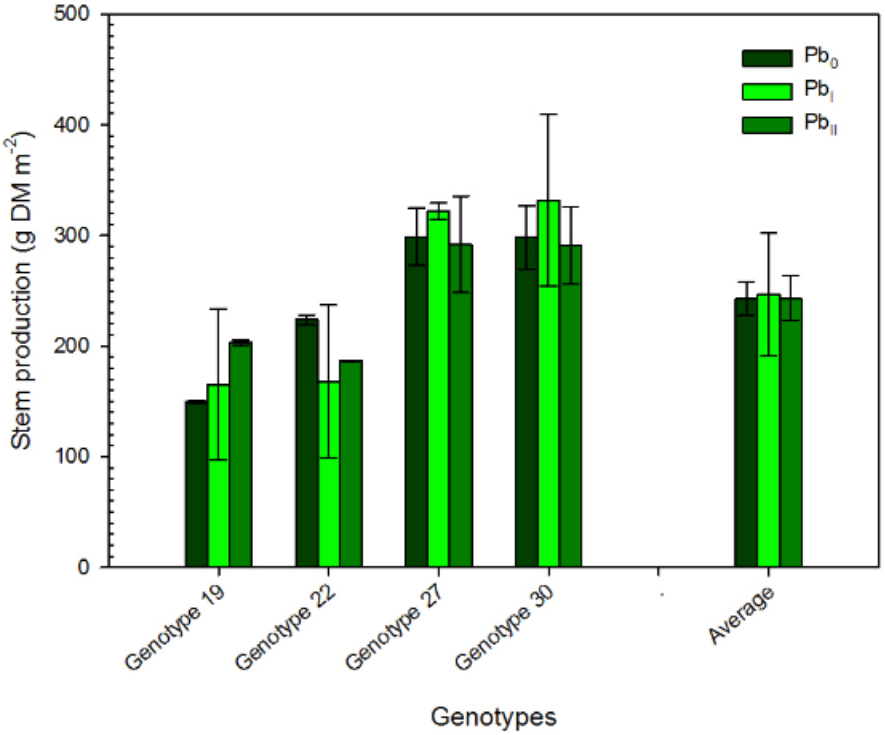


Fig. 85 Stem production (g DM m^{-2}) in the different studied genotypes in relation to different lead contamination levels (Pb_0, Pb_I and Pb_{II}).

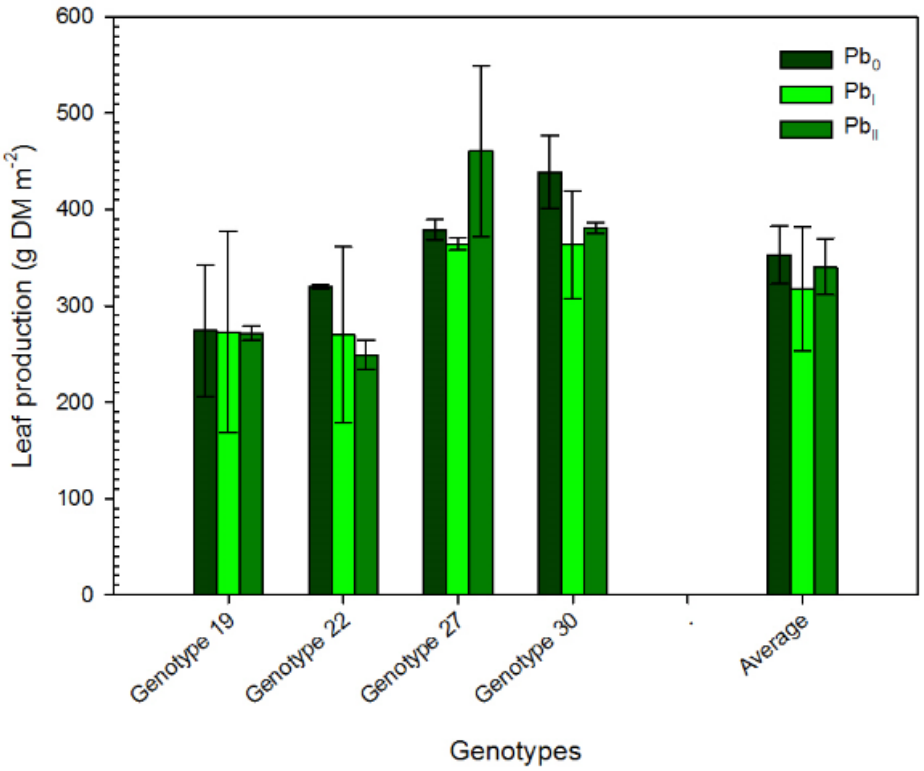


Fig.86 Leaf production (g DM m^{-2}) in the different studied genotypes in relation to different lead contamination levels (Pb_0, Pb_I and Pb_{II}).

Regard to the belowground biomass, genotype 27 was the most productive with 2020.34 g m^{-2}, while the less productive was genotype 19 with 1334.76 m^{-2}, on average of lead contamination.

In all studied genotypes contamination with lead enhanced root and rhizome production, in fact the productivity of the contaminated treatments were always higher than that provided from Pb_0 treatment (Fig.87).

About rhizome production, among the genotypes and in the average of lead contamination levels, genotypes 27 and 30 were the most productive with 1951.06 g m^{-2} and 1641.73 g m^{-2}, respectively, while the less productive were genotypes 19 and 22 with a rhizome production equal to 1271.63 and 1360.21 g m^{-2}, respectively. Lead contamination didn't affect the rhizome production (Fig.88).

Among genotypes, genotype 30 and genotype 27 provided the highest root production with values equal to 243.80 g m-2 and 207.82 g m^{-2}, respectively; while the less productive were genotype 19 and genotype 22 with 63.16 and 190.68 g m^{-2}, in the average of lead contamination levels.

Lead contamination didn't affect the root production (Fig.89).

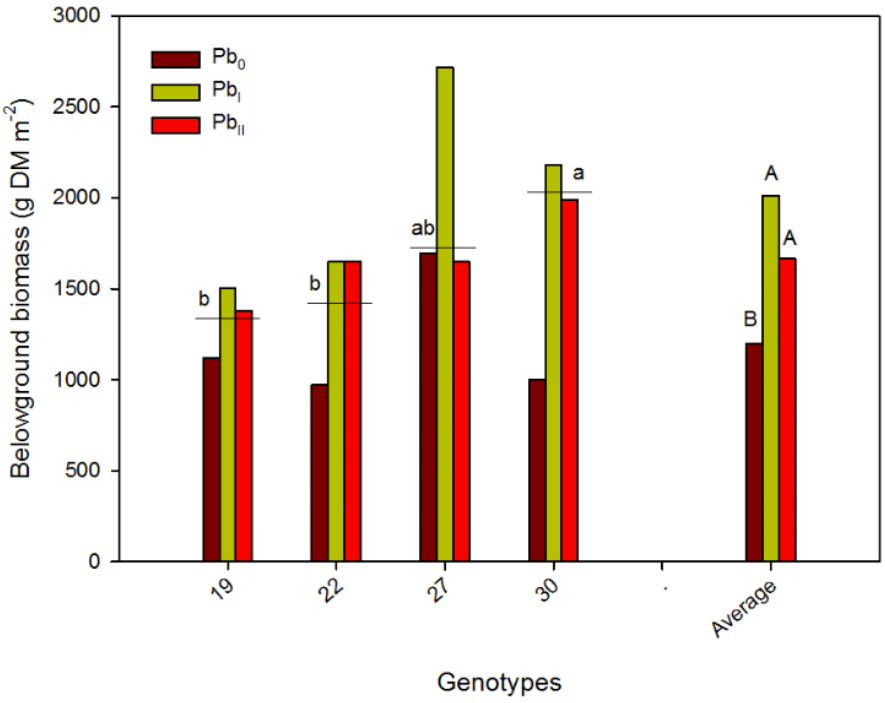


Fig. 87 Belowground biomass (g DM m^{-2}) in the different studied genotypes in relation to different lead contamination levels (Pb_0, Pb_I and Pb_{II}).

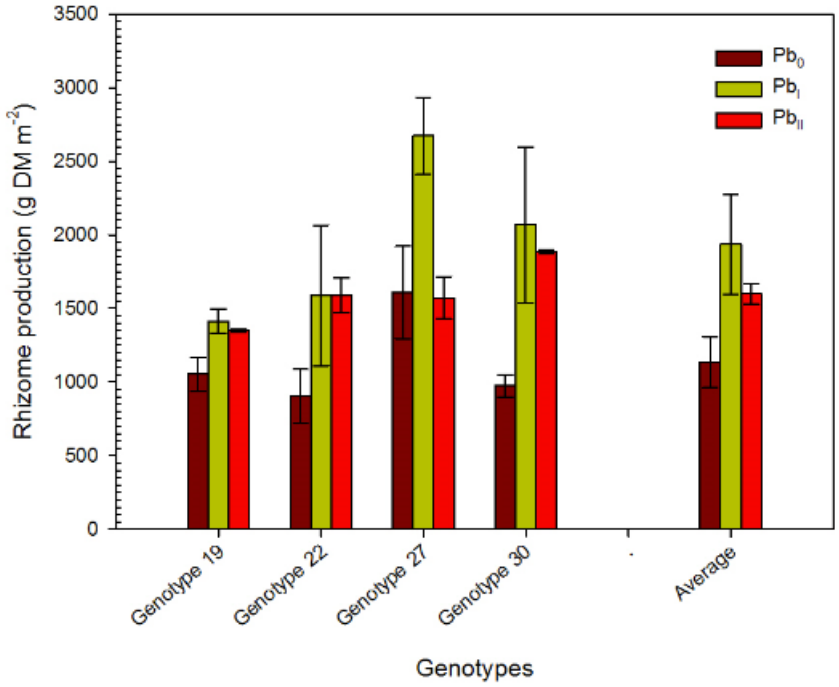


Fig.88 Rhizome production (g DM m^{-2}) in the different studied genotypes in relation to different lead contamination levels (Pb_0, Pb_I and Pb_{II}).

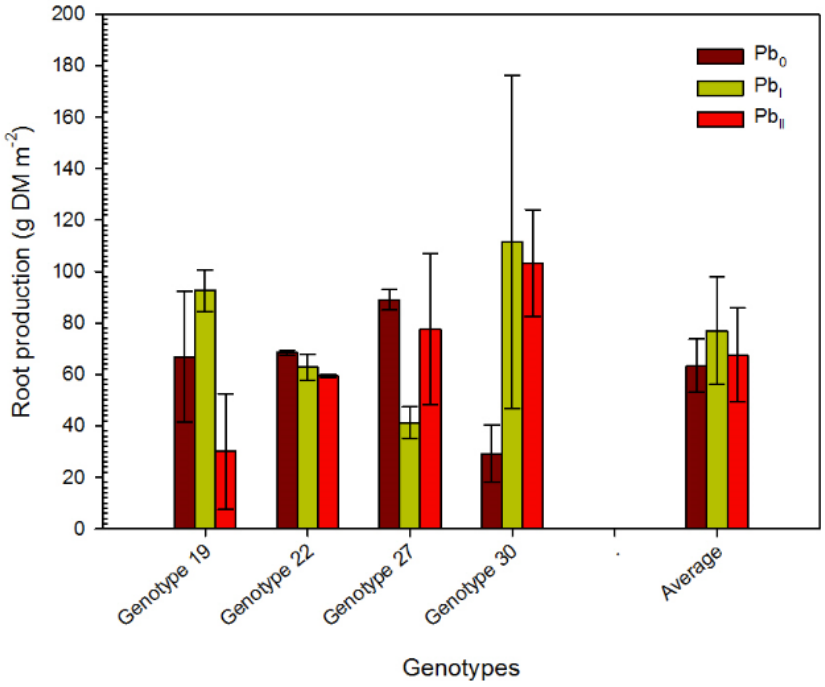


Fig.89 Root production (g DM m^{-2}) in the different studied genotypes in relation to different lead contamination levels (Pb_0, Pb_I and Pb_{II}).

Morpho-biometric characters

Results about the morpho-biometric characters showed that genotype 19 provided the highest value among the compared genotypes among the lead treatments, about the height of the main stem (120.28 cm, on average), while the genotype that provided the lowest height was genotype 30 with 90.31 cm, on average (Tab.8).

Lead contamination affected positively the height of the stem because the height recorded in Pb_{II} treatment were always higher than that provided from Pb_I and from Pb_0 pots, in particular Pb_{II} pots provided in the average of the studied genotypes a height of the main stem of 117.62 cm, while Pb_I and Pb_0 showed average height of 106.94 and 93.76 cm, respectively.

Genotype 27 showed the highest stem diameter among the studied genotypes equal to 7.8 mm, while other genotypes provided similar diameter of 6.7 mm (genotype 19 and 30) and 6.9 mm (genotype 22) in the average of lead contamination (Tab.8). Pb_0 pots provided in the average of the genotypes slighty higher stem diameter than Pb contaminated pots; in this case lead contamination seems didn't affect the stem diameter.

Among the studied genotypes, genotype 27 provided the highest nodes number in the main stem (16.6) in the average of lead contamination, followed from genotypes 19 and 30 (15.1 and 15, respectively) and in the end, from genotype 22 that with 14,9 nodes showed the lowest number (Tab.8).

In the average of the genotypes, Pb contaminated pots provided the highest nodes number, in particular, Pb_{II} pots recorded 16.3 nodes number and Pb_I 15.5, while Pb_0 pots showed the lowest number of nodes, equal to 14.5; because of that seems that lead contamination affected the nodes number.

On contrary than nodes number, lead contamination didn't affect the stem number: in the average of the genotypes, Pb_0 showed the highest value with 3,8 number of stems, while Pb_I and Pb_{II} recorded 3.4 and 2.9 number of stems, respectively (Tab.8).

Among genotypes in the average of lead contamination, genotype 30 provided the highest stem number (4), while the lowest number of stems was provided by genotype 22 with 2.8 stems.

Tab.8 Morpho-biometric characters in relation to the studied treatments: irrigation levels, (I_3, I_2, I_1) and lead contamination (Pb_0, Pb_I and Pb_{II}).

Genotype	Stems (n°)				Height (cm)				Basal diameter (mm)				Nodes (n°)			
	Pb_0	Pb_I	Pb_{II}	**Av**	Pb_0	Pb_I	Pb_{II}	***Av***	Pb_0	Pb_I	Pb_{II}	***Av***	Pb_0	Pb_I	Pb_{II}	***Av***
19	3.5	3.5	3.0	***3.3ns***	119.8	113.0	128.0	***120.3a***	7.1	6.4	6.7	***6.7ns***	15.3	13.9	16.3	***15.2ns***
22	4.0	3.0	1.5	***2.8ns***	94.6	109.8	123.0	***109.1ab***	7.4	6.5	7.0	***7.0ns***	13.8	15.3	15.6	***14.9ns***
27	3.0	3.0	3.5	***3.2ns***	87.8	108.1	119.8	***105.2ab***	8.3	7.7	7.5	***7.8ns***	15.5	17.3	17.2	***16.7ns***
30	4.5	4.0	3.5	***4.0ns***	72.9	99.3	99.6	***90.6b***	6.5	6.7	7.1	***6.8ns***	13.3	15.6	16.1	***15.0ns***
Average	***3.8ns***	***3.4ns***	***2.9ns***	***3.3***	***93.8b***	***107.6ab***	***117.6a***	***106.3***	***7.3ns***	***6.8ns***	***7.1ns***	***7.1***	***14.5ns***	***15.5ns***	***16.3ns***	***15.4***

Moisture

The stem moisture at the harvest was higher in the average of the studied genotypes in Pb contaminated pots, 70.7 % and 70.6% for Pb_{II} and Pb_I, respectively, than Pb_0 pots that showed the least humidity with 65.68% on average. Among genotypes, genotype 19 and genotype 22, in the average of lead contamination showed the highest moisture (78.07% and 74.07 %, respectively) while genotype 27 and genotype 30 recorded the littlest stem water content.

At harvest, no differences in moisture of leaves among contamination levels were recorded; all treatments achieved an average moisture of 73.20%; also among genotypes no differences were observed; only genotype 30 showed the least moisture because of it was the last genotype to be measured and its leaves were more dried.

The root moisture content, in the average of the studied genotype was higher in Pb_{II} pots that recorded 31,87% of moisture, than Pb_I pots (28.47%) and Pb_0 pots (27.41%). Among the studied genotypes and in the average of lead contamination levels, genotype 27 provided the lowest water content (25.17%) while the highest root water content was observed in genotype 22 (31.95%).

Lead contamination didn't affect the rhizome moisture; in the average of lead contamination level the rhizome water content was 62.54%. Among the genotypes, genotype 22 provided the highest rhizome water content (66.49%) while the lowest water content was measured in genotype 27 (57.13%).

LAI

The leaf area index was not affected from lead contamination, among the genotypes the average LAI value observed was equal to 4.67 for Pb_0 and Pb_I pots and 4.4 for Pb_{II} pots; genotype 27 provided the highest LAI value (5.5) in the average of lead contamination levels while the lowest LAI value was observed in genotype 30 (3.8). Among genotypes, there was not difference with lead contamination for genotypes 19 and 27, while in genotypes 22 and 30 and it seems that lead contamination reduced LAI values because the LAI values recorded were 4.8, 4.3 and 4.1 for Pb_0, Pb_I and Pb_{II} pots, respectively in genotype 22 and 4.8, 3.9 and 2.6 in genotype 30 (Fig.90).

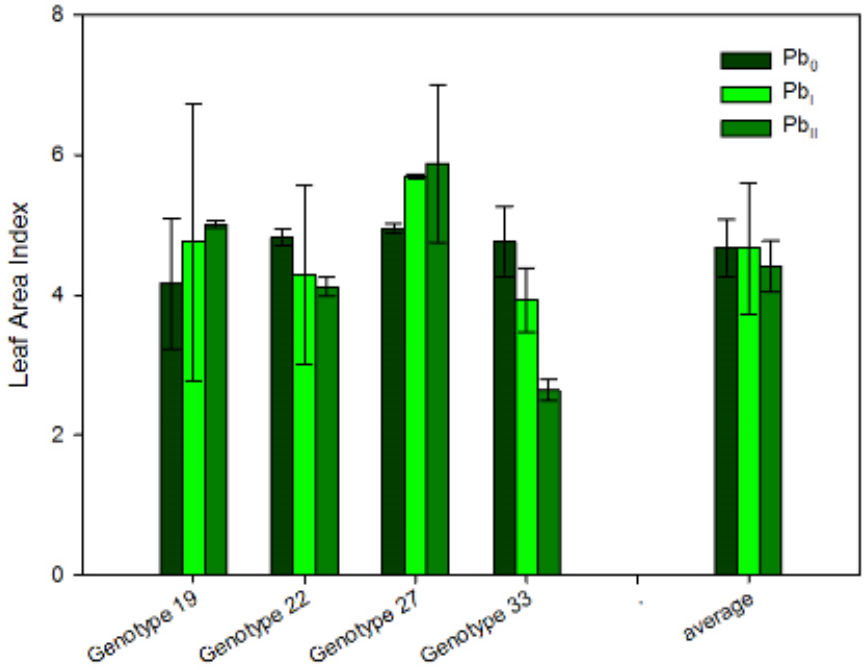


Fig.90 Leaf area index of the different studied genotypes in relation to different level of contamination

Nitrogen content

Among the different studied fractions of plants, leaves showed the highest N content; in the average on genotypes, this was equal to 1.55 % DM . Among genotypes, genotype 22 presented higher N content than others (2.00 %), while genotypes 19, 27 and 30 showed value below the average and ranging between 1.25 % (genotype 27) and 1,51 % (genotype 19).

Increment in contamination level induced reduction in N accumulation in all the studied genotypes; in particular, in the average of the genotypes N content in leaves varied from 1.86 %, measured in Pb_0 pots to 1.67 % and to 1.13% observed in Pb_I and Pb_{II} pots, respectively (Fig.92).

About stem fraction, genotypes 19 and 22 presented in the average of contamination higher N content than others: 0.62 % and 0.65 %, respectively, while genotypes 27 and 30 showed similar N content (0.36% and 0.33%) and above the average (0.49%) (Fig.91).

Among genotypes, no differences were observed in genotypes 19 and 22 while in genotypes 27 and 30 an opposite effect was detected; in genotype 27 a lower N content was observed with higher lead contamination (from 0.51%, in Pb_0 pots to 0.27% in Pb_{II} pots), while in genotype 30, a higher N content was noted with a higher Pb contamination (from 0.21% in Pb_0 pots to 0.44% in Pb_{II} pots).

Rhizomes presented N content, similar to stems; in the average of genotypes, N content was equal to 0.58% DM (Fig. 93). Among genotypes, genotype 22 presented higher N content than others 0.79 % in the average of contamination, while the other studied genotypes showed similar N content each others and close to the average value; only genotype 27 showed N content above the average and equal to 0.43%.

About lead contamination, in rhizomes, lead induced a light increment in N accumulation, especially with the highest lead concentration (Pb_{II} pots); only in genotype 27 the contamination led to a decrease of N accumulation (from 0.48 %, measured in Pb_0 pots to 0.41%, observed in Pb_{II} pots).

Roots were the fraction with the lowest N content, compared to the others: 0.24 % on average (Fig.94).

Among genotypes, genotype 19 presented a lower N content than others (0.19% in the average of contamination), while genotypes 22, 27 and 30 showed content close to the average.

In the average of the genotypes, increment in contamination level induced increment in N accumulation, in particular N content varied from 0.19%, measured in Pb0 pots to 0.27% in PbII pots, on average. Genotypes 19 and 22 showed the biggest N content variation in response to an increasing of lead contamination: from 0.14% measured in Pb_0 pots to 0.20 % observed in Pb_{II} pots and from 0.17 % to 0.27%, in the same pots, for genotype 19 and 22 respectively.

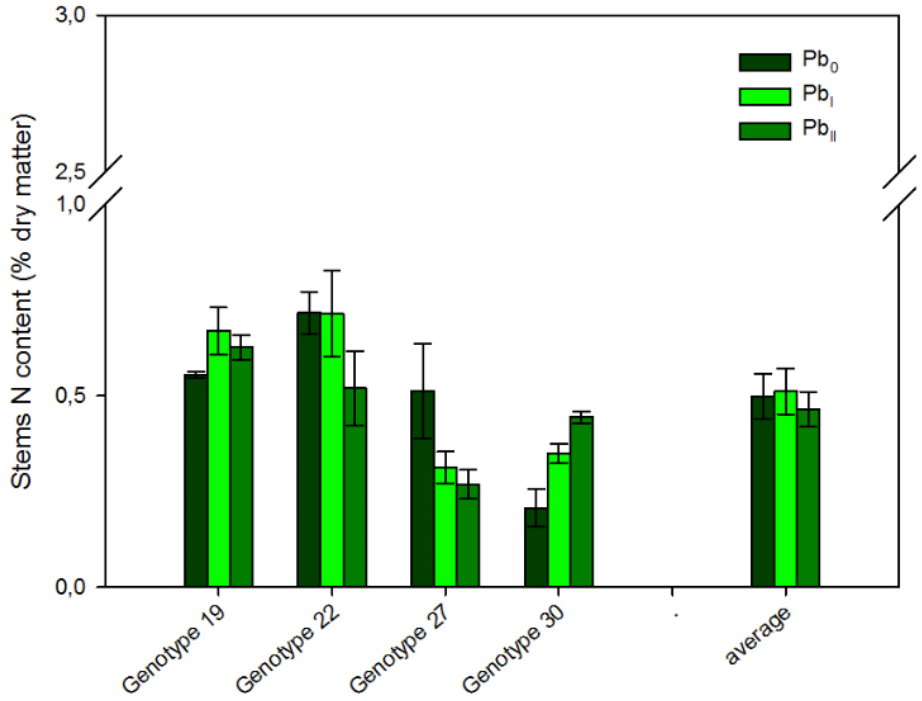


Fig. 91. Stems N content (% DM) in the studied genotypes in relation to different lead contamination levels (Pb_0, Pb_I and Pb_{II}).

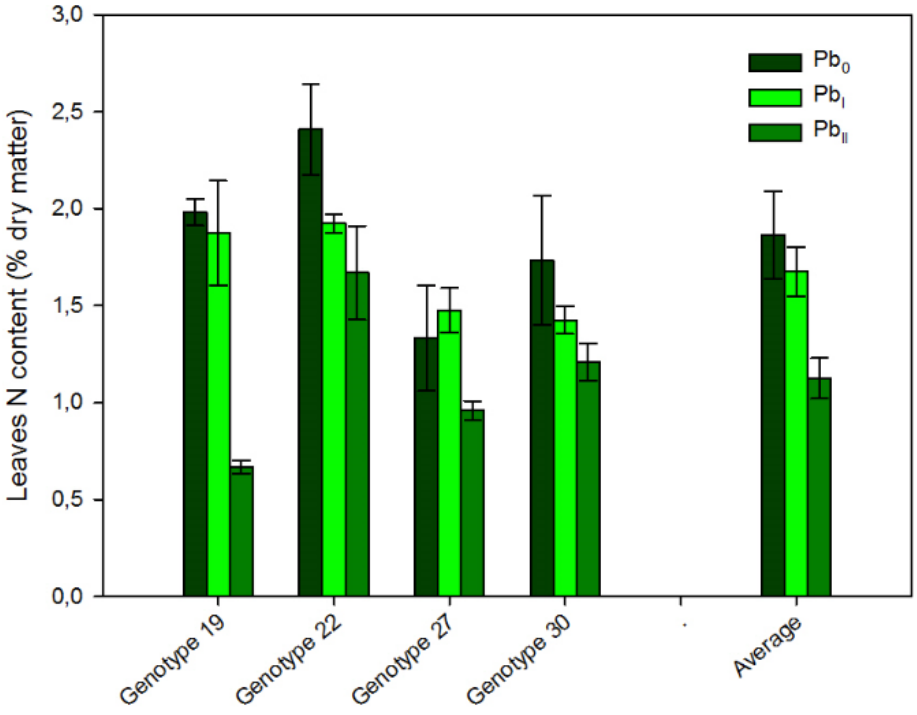


Fig. 92. Leaves N content (% DM) in the studied genotypes in relation to different lead contamination levels (Pb_0, Pb_I and Pb_{II}).

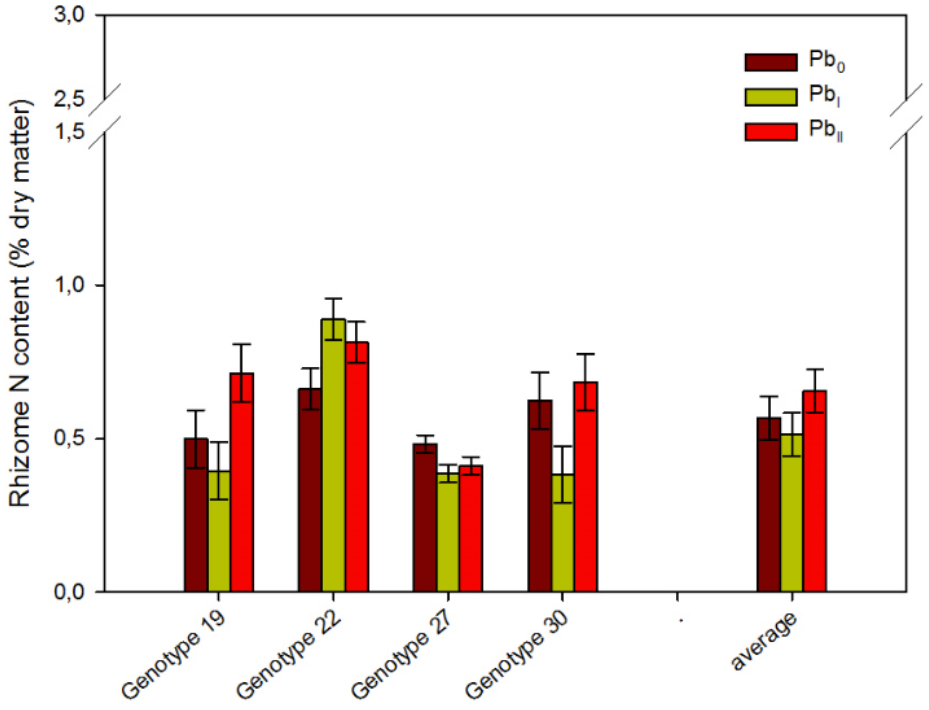


Fig. 93. Rhizome N content (% DM) in the studied genotypes in relation to different lead contamination levels (Pb_0, Pb_I and Pb_{II}).

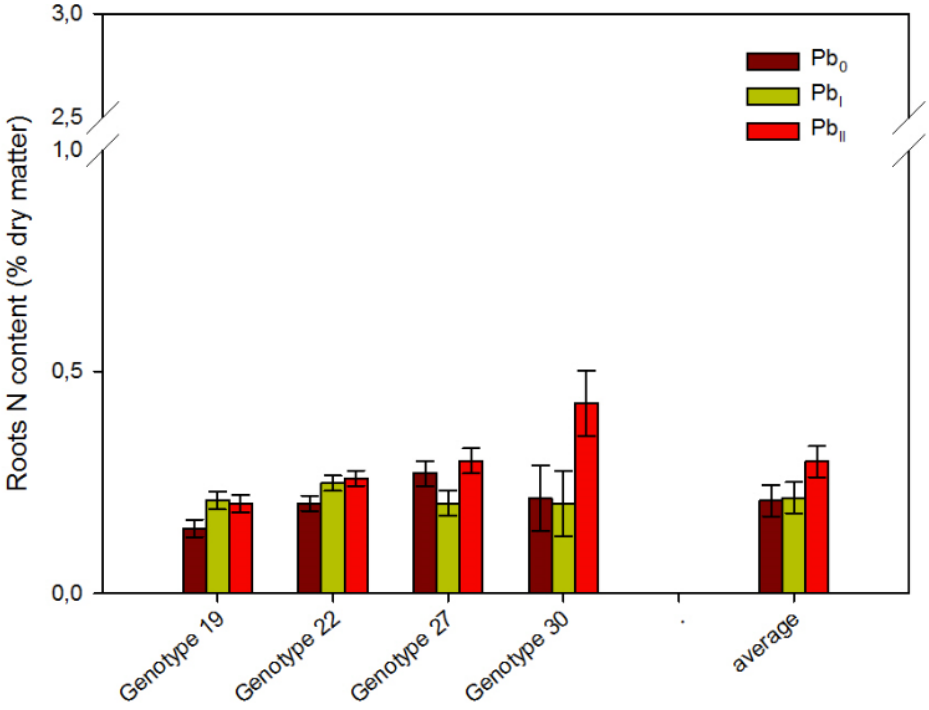


Fig. 94. Roots N content (% DM) in the studied genotypes in relation to different lead contamination levels (Pb_0, Pb_I and Pb_{II}).

Phosphorous content

In the average of the studied genotypes, the phosphorous content measured in stem was equal to 0.13% DM. Among genotypes, 19 and 22 presented higher P content than others, with 0.19 % and 0,16% DM, respectively, while genotypes 27 and 30 showed similar values of P content (0.09%).

Pb contamination didn't affect phosphorous content in genotypes (Fig.95).

About phosphorous content measured in leaves no differences were observed among genotypes whose P content was on average equal to 0.11% DM and at the same time, no differences were observed among contamination levels (Fig.96).

In rhizomes, P content was 0.08% DM on average, among genotypes, genotype 22 presented higher P content than others, specifically this was equal to 0.18% DM , while in genotypes 19, 27 and 30 P content was similar and ranging between 0.07% (genotype 27) and 0.12 (genotype 19).

With increasing of contamination level, no effect was observed on P content in rhizomes (Fig.97).

In the average of genotypes, P content in roots was equal to 0.07% DM (Fig.98); if compared to the other fractions, roots showed the lowest P content. Among genotypes, no differences were observed but small differences were on contrary noted with different lead contamination level. In particular, the increasing of contamination level induced light P accumulation, especially in Pb_{II} pots and for genotypes 27 and 30 that showed P content values equal to 0.08%, while in Pb_0 pots, the same presented only 0.06 and 0.07% DM P content.

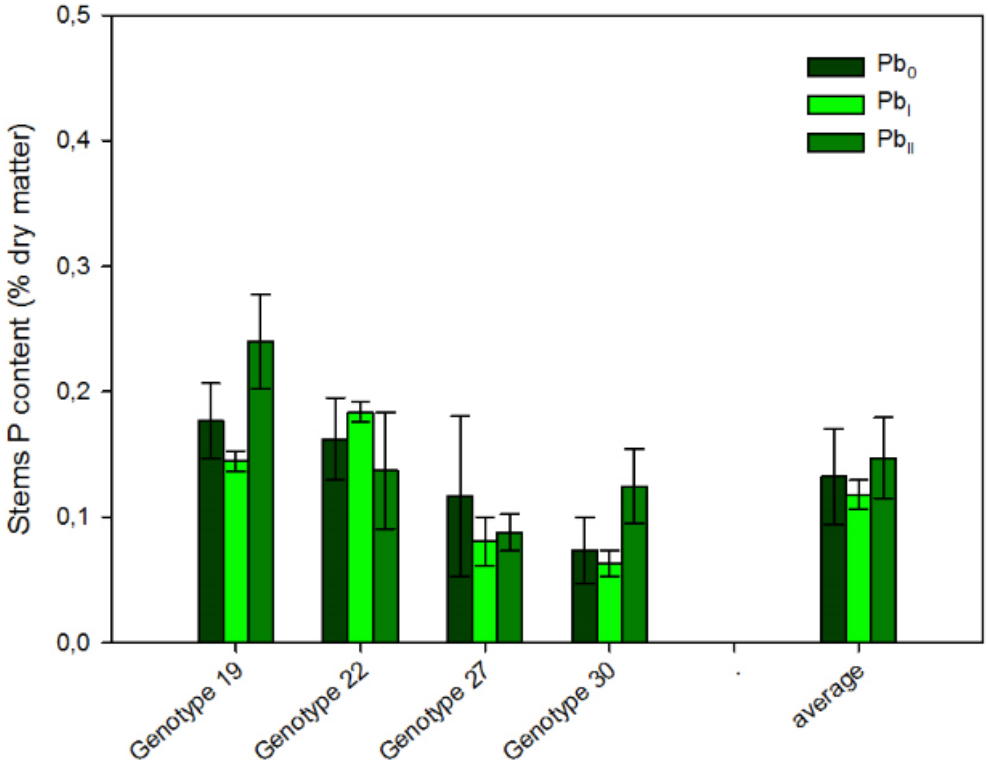


Fig. 95. Stems P content (% DM) in the studied genotypes in relation to different lead contamination levels (Pb_0, Pb_I and Pb_{II}).

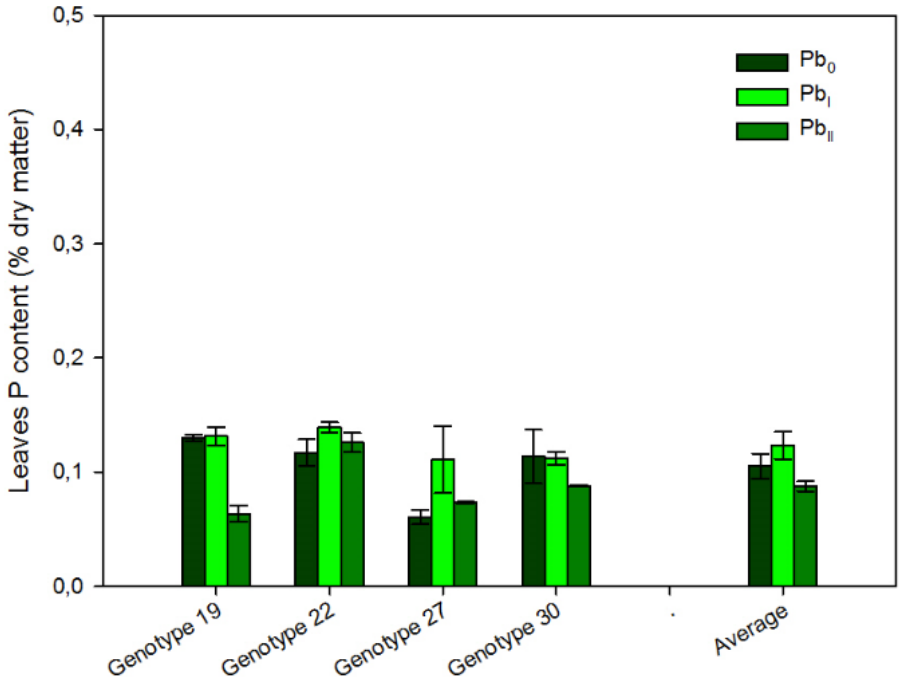


Fig. 96. Leaves P content (% DM) in the studied genotypes in relation to different lead contamination levels (Pb_0, Pb_I and Pb_{II}).

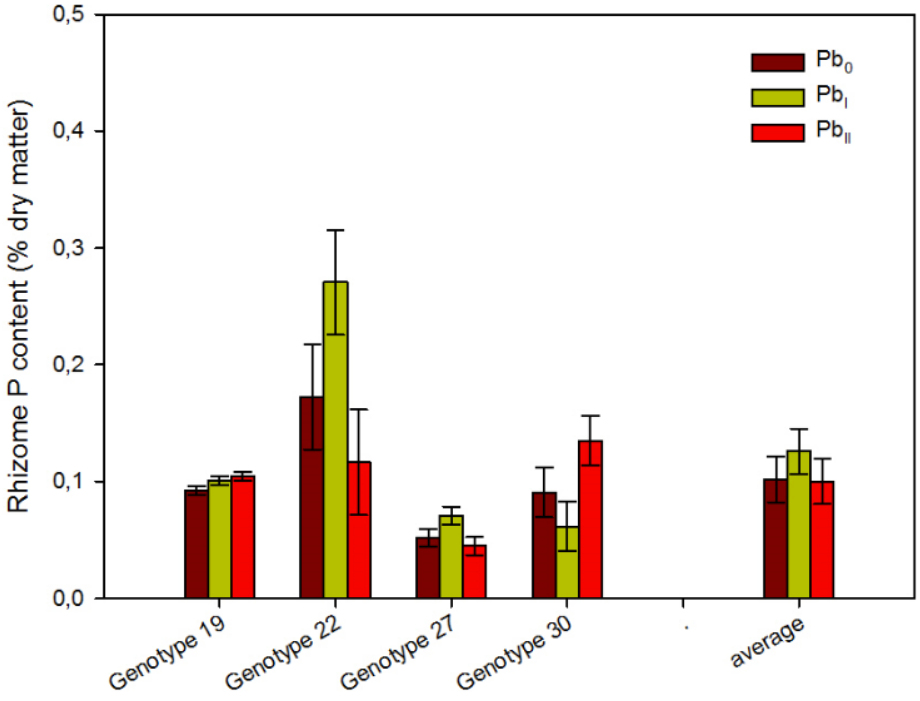


Fig. 97. Rhizome P content (% DM) in the studied genotypes in relation to different lead contamination levels (Pb_0, Pb_I and Pb_{II}).

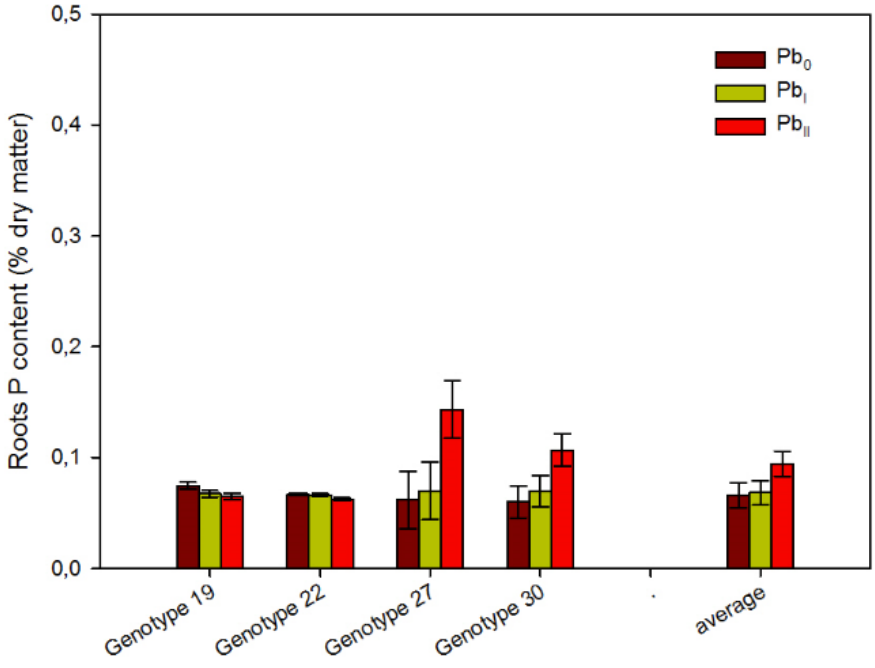


Fig. 98. Roots P content (% DM) in the studied genotypes in relation to different lead contamination levels (Pb_0, Pb_I and Pb_{II}).

Ash content

About ash content in genotypes, results showed that in the belowground part was detected the highest ash content. Specifically, the ash content in roots and in rhizome was equal to 76.6% DM and 16.62% on average, while in stems and leaves ash content was equal to 5.62 and 13.7 % DM, respectively. As expected, roots were the fraction with the highest ash content, probably because of the contact with soil, while stems provided the lowest ash content.

In the stems of genotypes, genotype 19 presented higher ash content (7.74 % DM) than others while genotype 22, 27 and 30 provided similar ash content among them and close to the average (5.62%). Genotypes 19 and 22 showed higher ash content in Pb_{II} pots than Pb_0 and Pb_I pots, while genotypes 27 and 30 showed no differences among contamination levels (Fig.99).

In leaves no significant differences among contamination levels were recorded; genotype 22 presented higher ash content than others (16.5 % DM), while genotypes 19, 27 and 30 showed ash content close to the average (13.7% DM) (Fig.100).

In rhizomes, it was detected much contamination with soil particles and this probably resulted in high variability among genotypes ash content and among contamination level. Among genotypes, genotype 19 and 22 presented higher ash content than others (22.0 and 17.9 % DM, respectively) while genotypes 27 and 30 showed ash content values below the average that was equal to 16.6 % DM (Fig.101).

In roots, as mentioned above, there was a lot of "contamination" of soil and this resulted in very high ash content; among genotypes and among contamination levels no differences were detected (Fig.102).

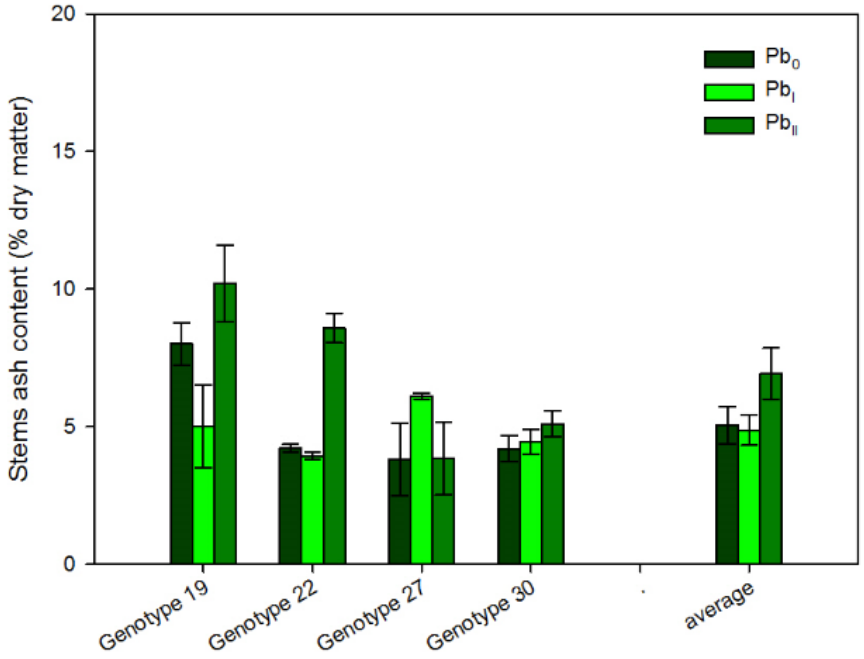


Fig. 99. Stems ash content (% DM) in the studied genotypes in relation to different lead contamination levels (Pb_0, Pb_I and Pb_{II}).

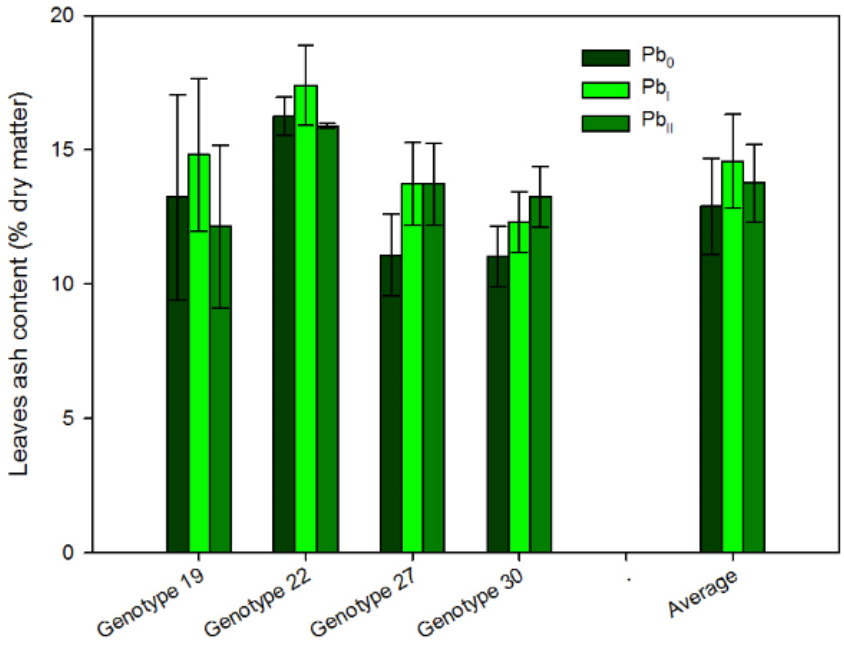


Fig. 100. Leaves ash content (% DM) in the studied genotypes in relation to different lead contamination levels (Pb_0, Pb_I and Pb_{II}).

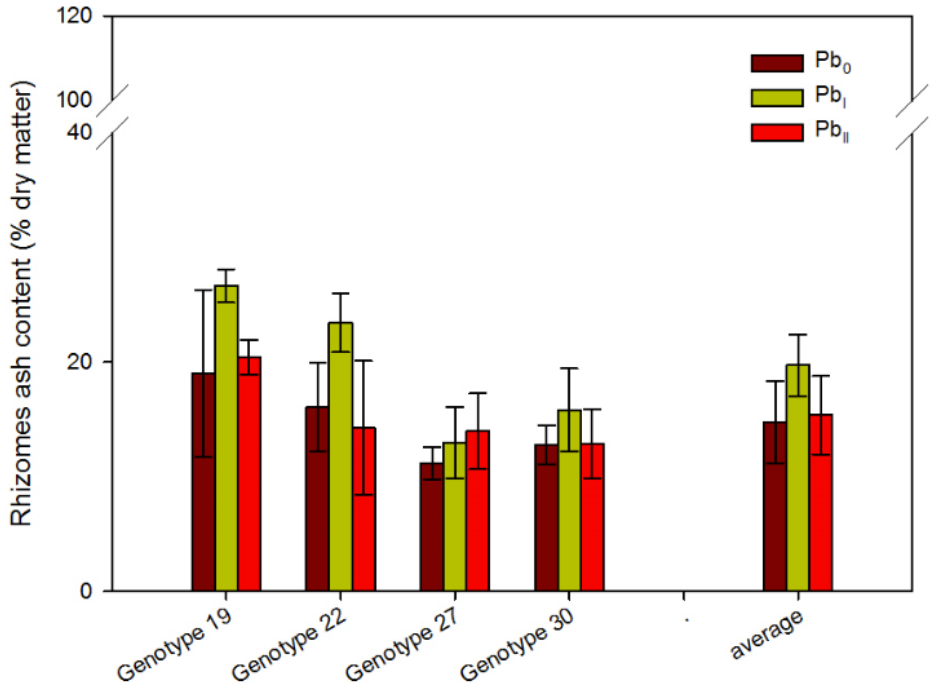


Fig. 101. Rhizome ash content (% DM) in the studied genotypes in relation to different lead contamination levels (Pb_0, Pb_I and Pb_{II}).

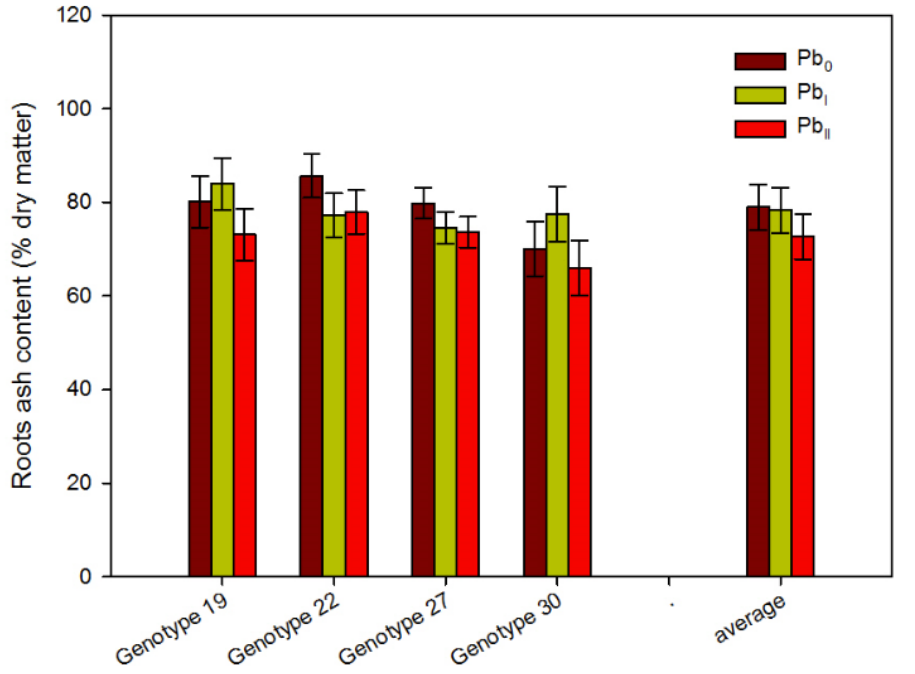


Fig. 102. Roots ash content (% DM) in the studied genotypes in relation to different lead contamination levels (Pb_0, Pb_I and Pb_{II}).

Lead content

Results about lead content showed the highest accumulation in the belowground part of the plants (roots and rhizomes) whose average content among genotypes and contamination level were equal to 68,2 and 8.61 mg kg^{-1} DM, respectively; while stems and leaves showed much lower lead content: 2.89 mg kg^{-1} DM (stems) and 2.34 mg kg^{-1} DM (leaves).

In the average of genotypes higher Pb content was observed with increased contamination with lead, from 0.52 mg kg^{-1} DM detected in pots without contamination, to 8.20 mg kg^{-1} DM measured in Pb_{II} pots.

Genotypes 27 and 30 provided high lead accumulation in stems (Fig.103), from 0.44 to 5.22 mg kg^{-1} DM (genotype 27) and from 1.34 to 4.77 mg kg^{-1} DM (genotype 30), respectively. In both genotypes the accumulation of lead in Pb_I pots was similar to Pb_{II} pots accumulation, meaning that these genotypes are also able to accumulate Pb in less contaminated soils.

In genotypes 19 and 22, lead content in stems was much higher in Pb_{II} pots than Pb_I and pots without contamination (Pb_0). Specifically, lead content in Pb_{II} pots was equal to 8.20 mg kg^{-1} DM and 4.48 mg kg^{-1} DM in genotypes 19 and 22, respectively, compared with Pb_I lead content of the same that was equal to 0.68 and 0.04 mg kg^{-1} DM, respectively.

In leaves, higher Pb content was detected in response to increased contamination with lead), in the average of genotypes, lead content increased from 0.22 mg kg^{-1} DM to 5.53 mg kg^{-1} DM, in Pb_0 and Pb_{II} pots, respectively (Fig.104).

Among genotypes, genotype 22 was that one that accumulated the highest content of lead, in the average of contamination levels, this was equal to 5 mg kg^{-1} DM. Specifically, genotype 22 showed to be able to accumulate lead at lower levels of Pb soil: lead content in genotype 22 varied, in fact, from 1.13 mg kg^{-1} DM (Pb_0) to 5.62 mg kg^{-1} DM in Pb_I pots.

The other genotypes (19, 27 and 30) showed a higher lead concentration in Pb_{II} pots than pots without contamination (Pb_0) and pots whit lead concentration equal to 450 mg kg^{-1} DM (Pb_I).

In rhizomes, higher Pb concentration was observed with increased lead contamination: in the average of genotypes this varied from 1.99 mg kg^{-1} DM, detected in Pb_0 pots, to 16.2 mg kg^{-1} DM, measured in Pb_{II} pots.

Among genotypes there were no differences, although genotype 30 showed the highest lead content in the average of contamination levels (9.78 mg kg^{-1} DM); the other studied genotypes showed rhizome lead content close to the average that was equal to 8.61 mg kg^{-1} DM (Fig.105).

Roots, as expected were the fraction with the highest lead content, 68.2 mg kg^{-1} DM, on average Higher Pb content was observed in response to increased contamination with lead, in the average of genotypes, from 4.70 mg kg^{-1} DM to 120 mg kg^{-1} DM, detected in Pb_0 pots and Pb_{II} pots, respectively.

In the average of lead contamination, among genotypes, genotype 27 showed the highest lead content in roots, much higher (123 mg kg^{-1} DM) than the average that was equal to 68.2 mg kg^{-1} DM; in the average of lead contamination, genotype 19 showed lead content above the average (78.4 mg kg^{-1} DM), while genotypes 22 and 30 provided lead content in roots below the average: 24.5 and 47.3 mg kg^{-1} DM, respectively (Fig.106).

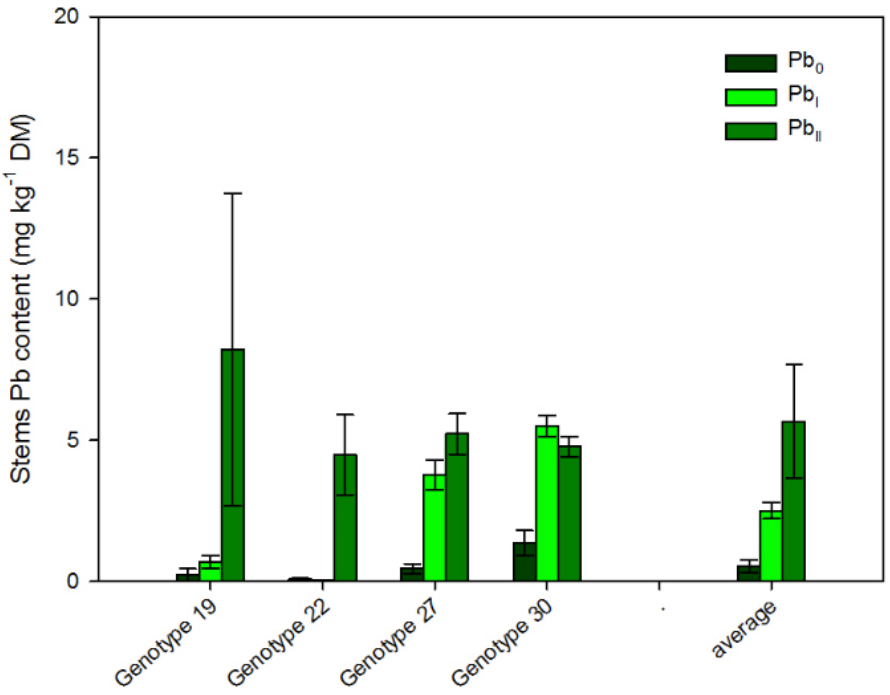


Fig. 103. Stems Pb content (mg DM Kg^{-1}) in the studied genotypes in relation to different lead contamination levels (Pb_0, Pb_I and Pb_{II}).

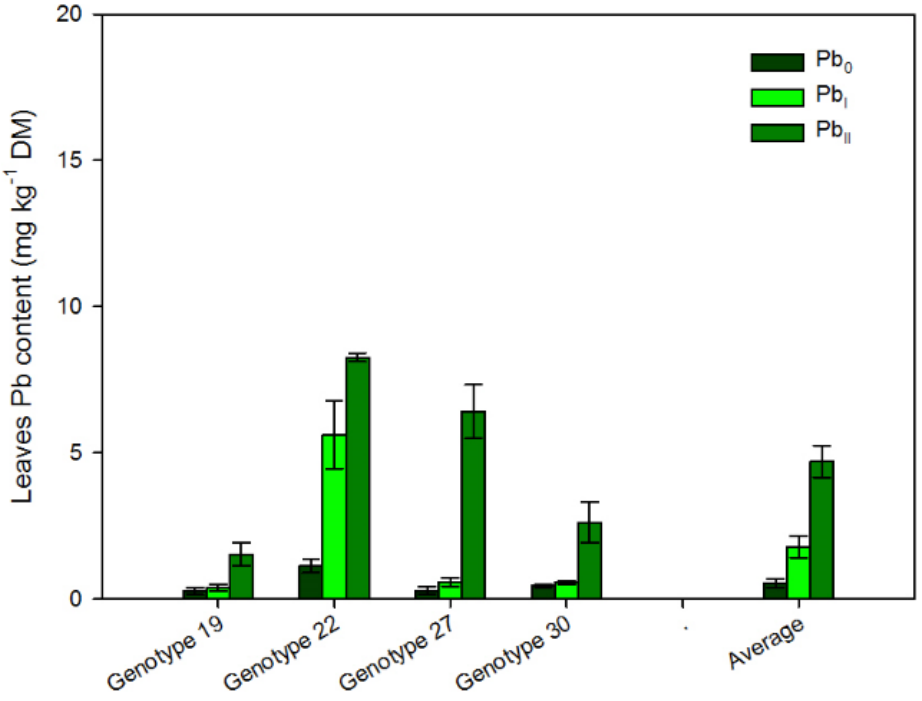


Fig.104. Leaves Pb content (mg DM Kg^{-1}) in the studied genotypes in relation to different lead contamination levels (Pb_0, Pb_I and Pb_{II}).

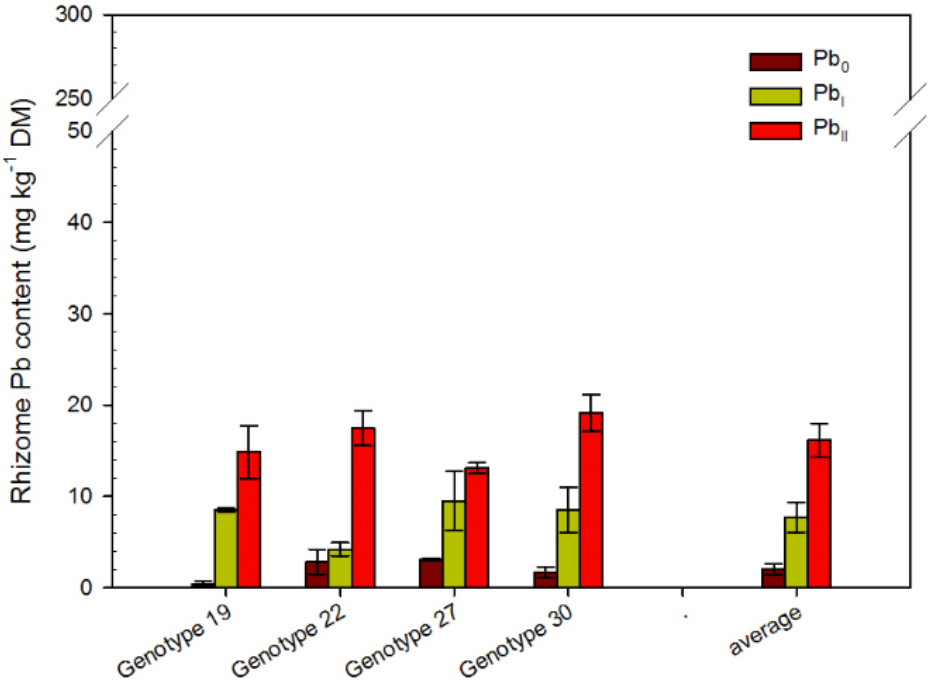


Fig.105. Rhizomes Pb content (mg DM Kg^{-1}) in the studied genotypes in relation to different lead contamination levels (Pb_0, Pb_I and Pb_{II}).

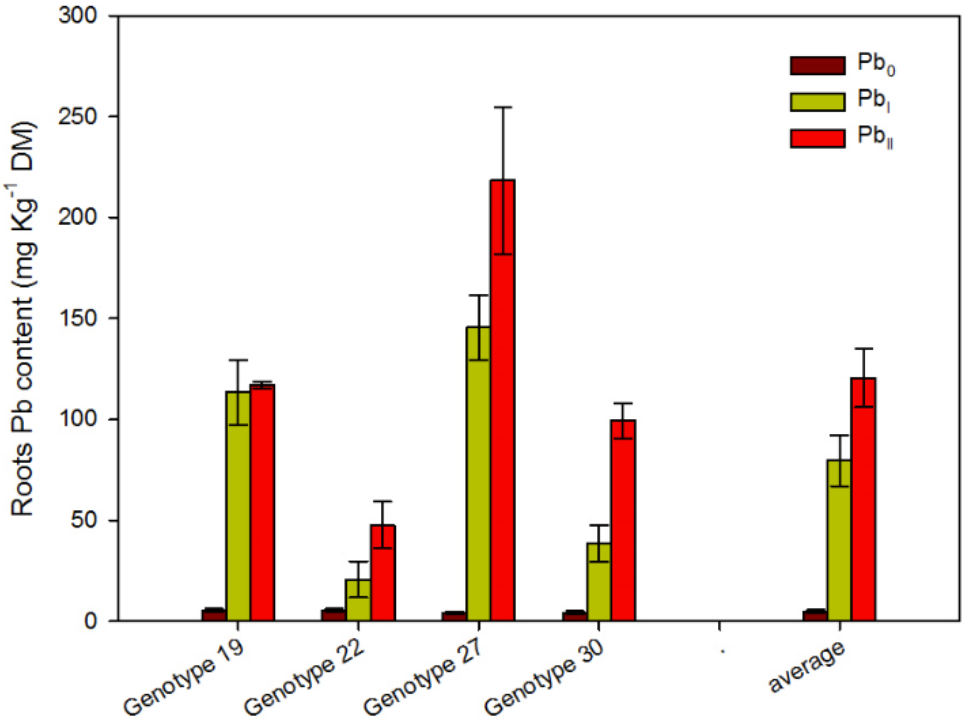


Fig. 106. Roots Pb content (mg DM Kg^{-1}) in the studied genotypes in relation to different lead contamination levels (Pb_0, Pb_I and Pb_{II}).

Soil Lead content

About lead content in soil no differences were observed among genotypes, whose average value was equal to 460 mg kg^{-1} DM. At the same time, differences among contamination levels were observed, specifically, as expected, the highest Pb content was observed in the most contaminated pots (Pb_{II}) which lead content was equal to 969mg kg^{-1} DM twice than lead content detected in Pb_I pots (432 mg kg^{-1} DM) (Fig.107).

The inferior layer of the pots, showed a Pb content less than that observed in the superior layer, the amount of lead in these was equal to 433 and 487 mg kg^{-1} DM, respectively on average (data not shown). This difference can be attributed to the effect of the rhizosphere. The lower Pb content of the bottom layer can be due to the higher phytoextraction once a higher density of roots and rhizomes was verified in the bottom of the pots compared to the top layer of the pots.

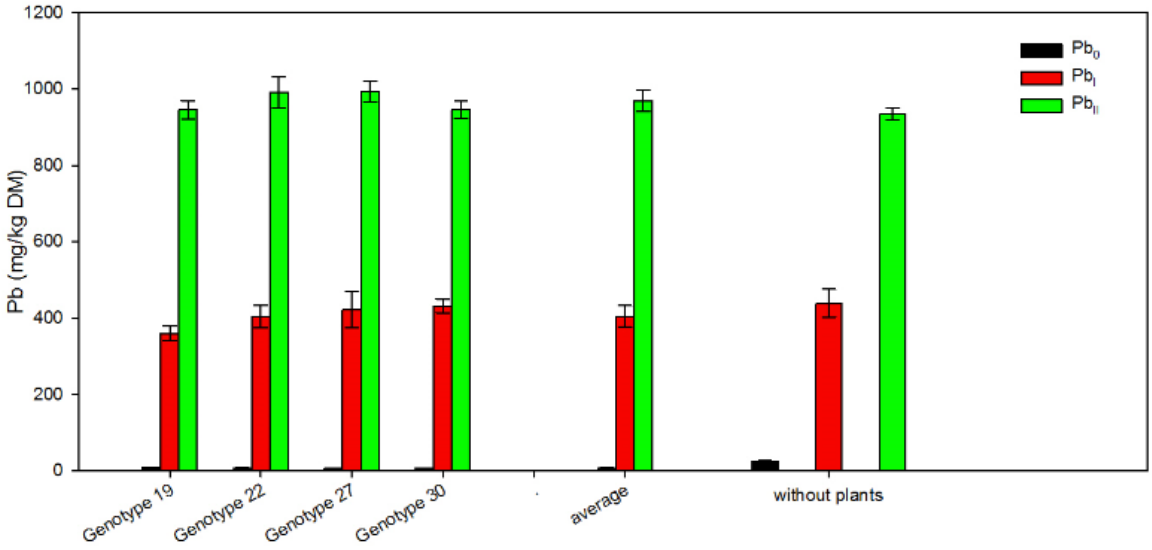


Fig. 107. Soil lead content observed in pots with genotypes compared with soil lead content in pots without plants.

Lead content in leachates

Results about lead content in leachates from genotype pots showed no differences among contamination levels, this content was equal to 0.007 mg/L on average; no differences among genotypes and no differences between pots with (0.007 mg/L, on average) and without (0.006 mg/L, on average) plants were also observed (Fig. 108).

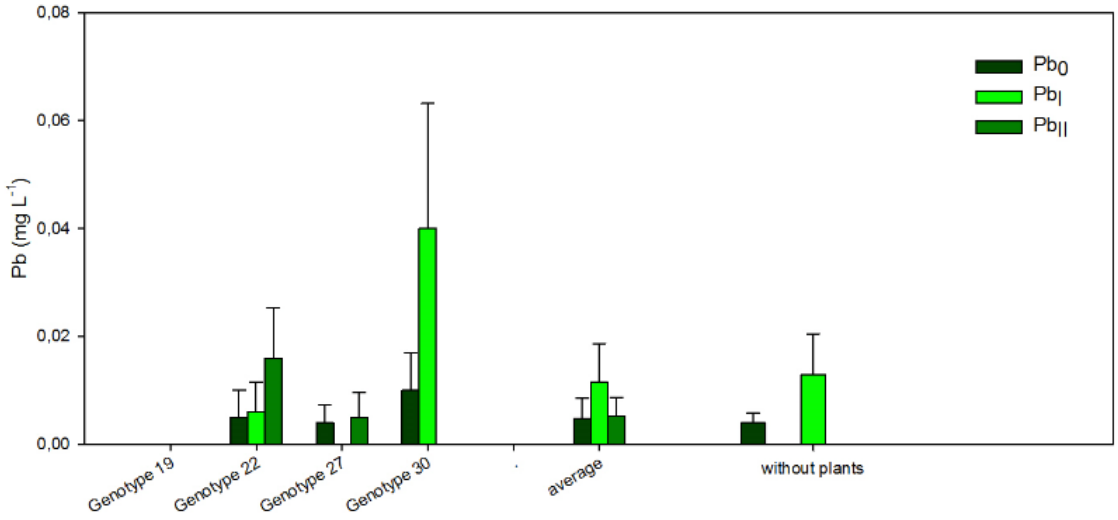


Fig. 108. Lead leached in pots with and without plants in the different genotypes and in relation to the contamination levels.

2.5.Common results of Researc Lines 3 and 4

Phytoremediation of *Arundo donax*

But the main question of this work is: which is, in practice, the potential of *Arundo* for phytoremediation of Pb contaminated soils?

The phytoremediation potential of *Arundo* can be associated with the capacity of this plant to adsorb on the radicular system, the Pb in the soil, stabilizing and immobilizing the contaminant, that otherwise could be leached. But, the phytoremediation potential can be also associated with the release of exudates and enzymes to soil by the rhizosphere, that can stimulate the remediation of pollutants, by the stabilization/immobilization of Pb. In effect, the radicular fraction of the biomass releases CO_2 to the soil, which, dissolved in the water solution of the soil and in alkaline pH, can precipitate the metal, as carbonates. The phytoremediation potential of *Arundo* can be also associated with the stimulation of the stabilization/immobilization of metals by fungi or other microorganisms in the soil-rhizome-roots interface.

These phytoadsorption/phytostabilization mechanisms were particularly visible in the analysis of Pb contents of leachates. Indeed, it was observed that the leachates from pots with plants showed lower amounts of Pb than the leachates from pots without plants. This means, therefore, that the presence of *Arundo* has a retainer/stabilizer effect on the Pb fraction most easily leached, probably due to phytoadsorption/phytostabilization mechanisms mentioned. This effect can be considered as a remedial effect, since it does not allow these Pb fractions to be leached into groundwater, thus preventing contamination of water resources.

However, in terms of treating contaminated soils, the phytoextraction perspective is the most interesting, both from an environmental point of view, and from an economic standpoint. Indeed, the processing of the extractor plant may represent a form of economic recovery.

Considering the phytoextraction perspective, the metal accumulation can be defined in two ways, according to Peterson (1971):

(1) - presence of larger quantities of a given element than is usual, and

(2) - the accumulation of a given element in concentrations higher than the growth medium..

Considering the definition given in (1), it was found that *Arundo* from contaminated pots showed levels of Pb, in the below and aerial fractions, significantly higher than the Pb content of biomass in the control pots. Figure (109) and (110) show this difference.

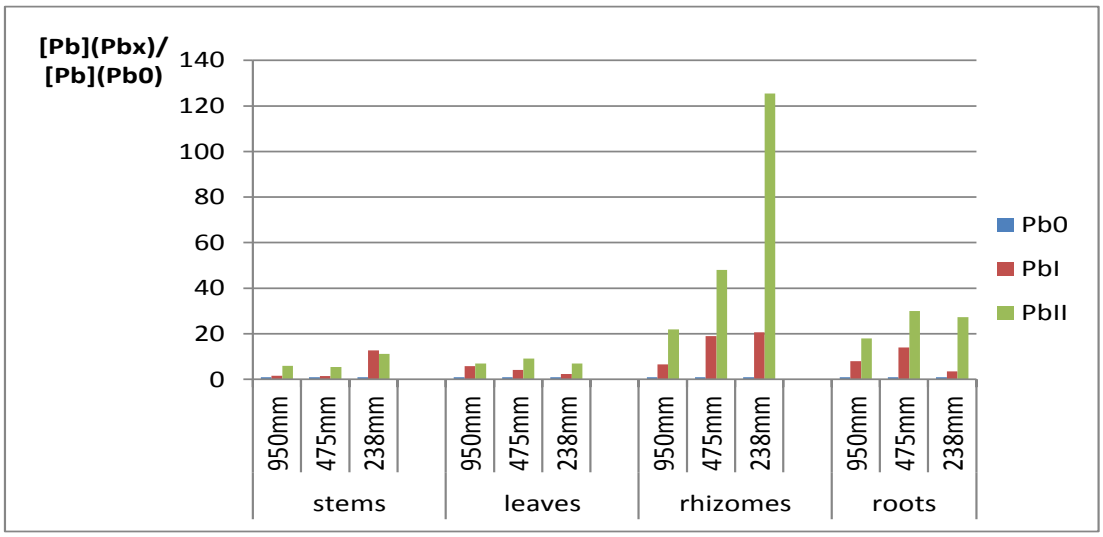


Figure (109) – Effect of the Pb contamination on the ratio $[Pb](Pb_x)(mg.kg^{-1})/[Pb](Pb_0)(mg.kg^{-1})$, for the several biomass fractions (*x*, represents 0, 450, or 900 mg/kg (Pb) in the soil) in the research line 3 results (results from the 2nd year).

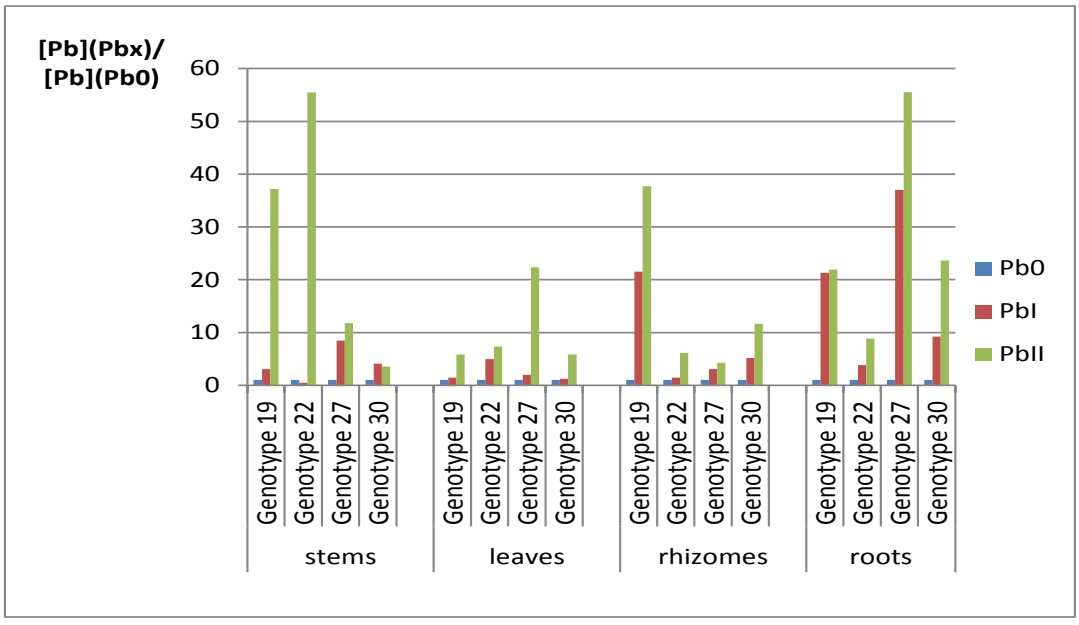


Figure (110) – Effect of the Pb contamination on the ratio $[Pb](Pb_x)(mg.kg^{-1})/[Pb](Pb_0)(mg.kg^{-1})$, for the several biomass fractions (x, represents 0, 450, or 900 mg/kg (Pb) in the soil) in the research line 4 results (genotype research line).

In all pots with contamination, biomass fractions presented values higher than unity. In accordance with these results, *Arundo* can accumulate in the several biomass fractions more than the usual Pb amounts.

Results also show that in the research line 3, the rhizome fraction is the one with higher phytoremediation/phytoextraction capacity, since it presents the higher ratio. In this fraction, highest accumulation was obtained with the highest contamination and the lowest irrigation level (238mm). Regarding the genotype essays, globally, roots presented the highest phytoextraction capacity, followed by stems. Globally, considering the several biomass fractions, Genotype 19 and genotype 27 presented the best phytoextraction capacities. Genotype 19, accumulating more in the stems, rhizomes and roots fraction, and Genotype 27, in the leaves and roots fraction. Genotype 22, presented a lower ration regarding control pots, and accumulates higher Pb in the stems. Genotype 30 accumulates more Pb in the roots.

Furthermore, considering the definition given in (1), one may wonder if the plants of the pots with sludge exported larger quantities of Pb than plants grown in pots without sludge. In terms of phytoextraction treatment, this calculus is even more interesting for phytoremediation purposes. Figures 111 and 112 show the difference in relation to the several fractions and the total (below and aerial) exported for the research line 3 and research line 4.

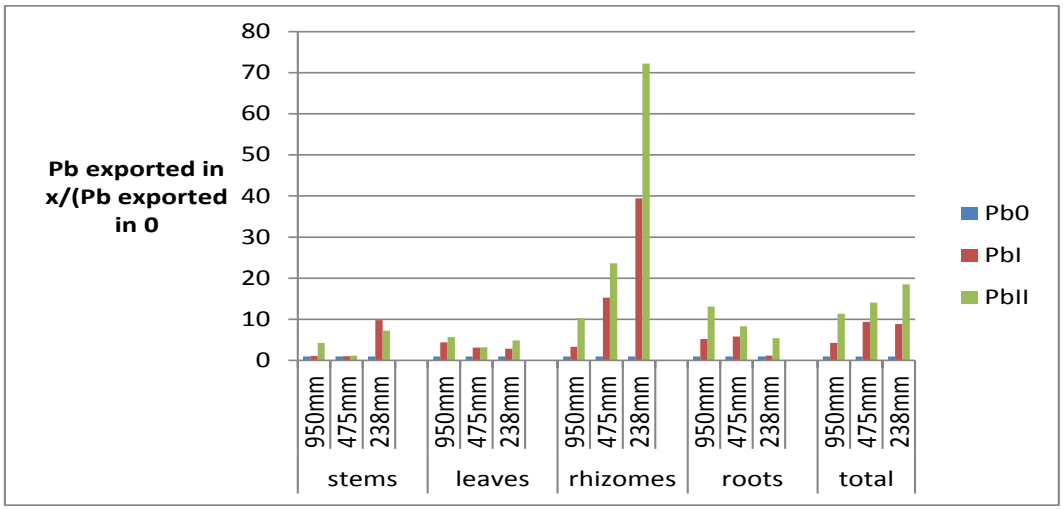


Figure (111) – Effect of the Pb contamination on the ratio $[Pb](Pb_x)(mg.m^{-2})/[Pb](Pb_0)(mg.m^{-2})$, for the Pb exported by the several biomass fractions (*x*, represents 0, 450, or 900 mg/kg (Pb) in the soil) in the research line 3 results (results from the 2nd year).

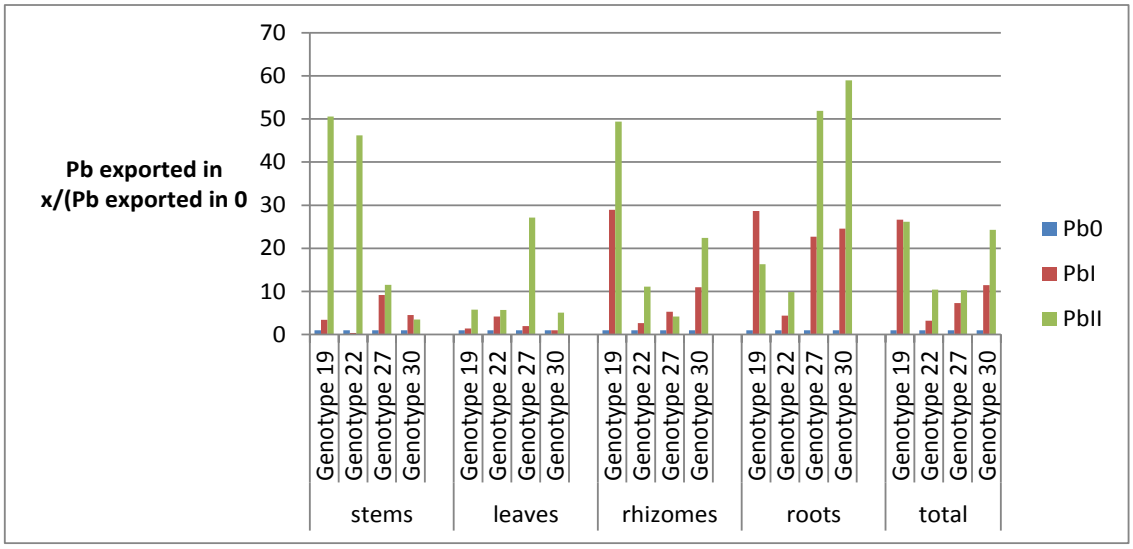
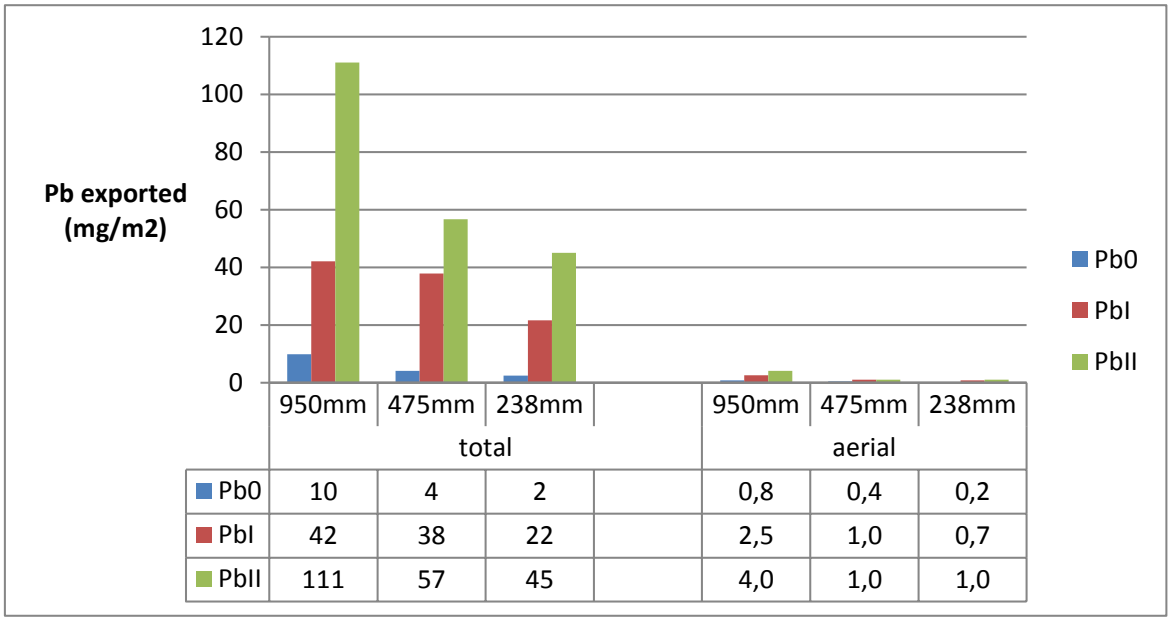


Figure (112) – Effect of the Pb contamination on the ratio [Pb](Pb_x)($mg.m^{-2}$)/ [Pb](Pb_0)($mg.m^{-2}$), for the Pb exported by the several biomass fractions (x, represents 0, 450, or 900 mg/kg (Pb) in the soil) in the research line 4 results (genotype research line).

As can be seen from the previous figures, in all pots with contamination, Pb exported by biomass fractions presented values higher than unity. In accordance with these results, *Arundo* can export, due to contamination, in the several biomass fractions more than the usual Pb amounts.

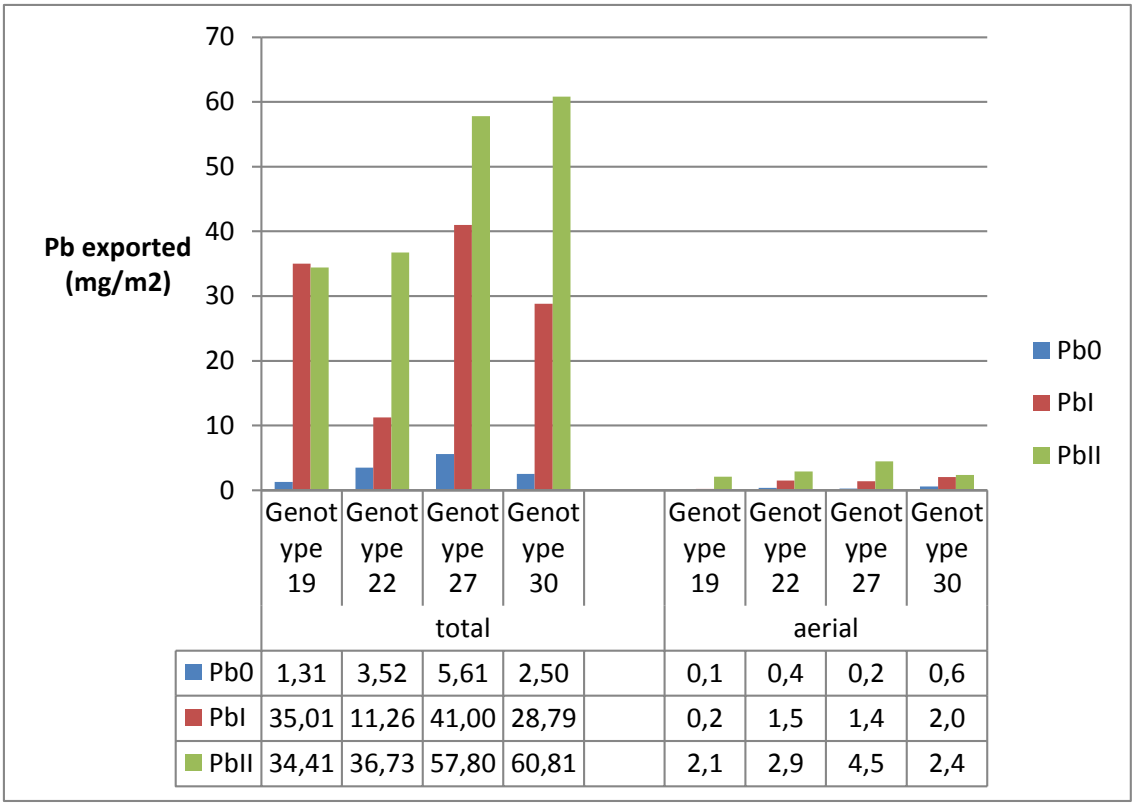
Results also show that in the research line 3, the rhizome fraction is the one with higher phytoremoval capacity, since it presents the higher ratio. In this fraction, highest ratio was obtained with the highest contamination and the lowest irrigation level (238mm). Considering the total Pb that can be exported by the total biomass (stems+leaves+roots+rhizomes), the same pattern was observed: highest ratio is obtained in pots with the highest contamination and the lowest irrigation level (238mm). Regarding the genotype essays, globally, Genotype 19 and genotype 30 are the ones with higher ratio. Stems, rhizomes and roots are the biomass fractions of genotype 19 that accomplish a higher ratio. Phytoremoval Pb ratio of genotype 22 is higher with stems fraction and genotype 27 with leaves and roots fraction. Highest removal ratio of Pb for genotype 30 is given by the belowground biomass (roots and rhizomes).

But, regarding the results obtained, if we consider that only that the aerial fraction is harvested, than, the Pb that can be exported annually will be much less since Pb exported by stems and leaves are significantly lower than what can be exported by the below ground biomass. Figures 113 and 114 show the removal capacity if we consider only the harvest of the aerial biomass compared with the removal capacity done by the totality of the biomass.



	total				aerial		
	950mm	475mm	238mm		950mm	475mm	238mm
Pb0	10	4	2		0,8	0,4	0,2
PbI	42	38	22		2,5	1,0	0,7
PbII	111	57	45		4,0	1,0	1,0

Figure 113 – Pb exported by the totality of the biomass and by the aerial fraction of biomass in the research line 3 (results from the 2nd year).



	total					aerial			
	Genotype 19	Genotype 22	Genotype 27	Genotype 30		Genotype 19	Genotype 22	Genotype 27	Genotype 30
Pb0	1,31	3,52	5,61	2,50		0,1	0,4	0,2	0,6
PbI	35,01	11,26	41,00	28,79		0,2	1,5	1,4	2,0
PbII	34,41	36,73	57,80	60,81		2,1	2,9	4,5	2,4

Figure 114 – Pb exported by the totality of the biomass and by the aerial fraction of biomass in the research line 4 (genotype trial).

Regarding the phytoremoval capacity of Pb from soil, this will be higher when a combination of higher content and higher productivity is observed. Concerning the research line 3, Figure 63 shows that either if we consider the totality of the biomass or only the aerial biomass, the highest removal is observed at higher irrigation and at higher contamination. But aerial removal is only 3.6% of the Pb that can be removed by the total biomass. This is mainly because Pb remains in the belowground fraction and it does not mobilize for the aerial fraction. Concerning the research line 4, Figure 84 shows that if we consider the totality of the biomass, the highest removal is observed with genotypes 27 and 30 (due to higher productivities), at higher contamination. But aerial removal is higher with genotypes 22 and 27, at higher contamination, although this removal only represents 7.8-7.9% of the total Pb accumulated and removed by the totality of the biomass. Again, this is mainly because Pb remains in the belowground fraction and it does not mobilize for the aerial fraction.

4.CONCLUSIONS

The results of the three years researches carried out with the aim to evaluate the adaptability and phytoremediation of *Arundo donax* L. on marginal salt, dry and lead-contaminated soils, allowed to outline the following remarks:

Research line 1

The research carried out with the aim to evaluate the effects of salinity stress on different clones of *Arundo donax* L., allowed to draw the following conclusions:

- The increase of salinity level in the soil for the effect of NaCl concentration during the irrigations affected plant morphology and physiology. It was shown that under salt stress the plant decreased stomatal opening and thus photosynthesis was inhibited. All these lead to reductions in plant growth.
- Highest level of net photosynthesis and SPAD unit were recorded in control (S_0) compared to the treatment irrigated with water at 4 dS m^{-1} (S_1) and 6 dS m^{-1} (S_2). The same trend was observed for aboveground dry biomass, main stem height and number of stems at harvest. The highest rhizome and roots dry weight were obtained with S_1, which was slightly higher than S_0 and S_2.
- According to the first year screening genotypes 6, 18, 20, 7, 2 and 14 are considered the most tolerant to salinity levels, while genotypes 34, 29, 10, 15, 21, 23 and 3 the sensitive ones.

In summary, soil salinity at 4 to 6 dS m^{-1} slightly affected growth, morphology, physiology and yields. Giant reed was able to grow at soil salinity level up to 8-9 dS m^{-1}. However, if this parameter is taken into account for soil marginality classification, then marginal lands lead to marginal yields (reduction of 44% in S_2 treatment respect to the control S_0).

Research line 2

The research carried out with the aim to evaluate the effects of water and salinity stress on different clones of *Arundo donax* L., allowed to draw the following conclusions:

- The effect of NaCl concentration in the soil induced by salt irrigation water, influenced the morphology and physiology of the studied clones.

- Under salt stress plants reduce the opening of the stomata and consequently photosynthesis is lower compared to the not stressed plants. This lead to a reductions in plants growth and aboveground dry biomass yield.
- The water stress affected morphology, physiology, dry biomass yield; in fact the treatment with 100% of the evapotranspiration restoration (I_{100}) showed the highest values of dry biomass yield.
- According to second year screening genotypes 18, 6, 2 and 16 can be considered the most tolerant to salinity and water stress, while genotypes 14, 7 and 13 the most sensitive.
- In summary the irrigation water to 6 dS m^{-1} has slightly affected the growth, morphology, physiology and yields of the studied *Arundo donax* clones. Giant reed was able to grow with irrigation water up to 12 dS m^{-1}. However, if this parameter would be considered to classify a soil as marginal, it is clear that marginal land would be obtained marginal yields (in S_2I_{100} treatment there was a reduction in aboveground dry biomass of 51%, compared to the control treatment S_0I_{100}) and reductions up to 55% in the treatment with water and saline stress (S_2I_{25}).

Research lines 3 and 4

These contamination essays with Pb, were installed in pots in order to study the effect of Pb contaminated soils in the production and also in the quality of the biomass in order to evaluate the capacity of *Arundo* to growth in this type of marginal land. It also allowed quantifying the Pb exported by plants, analyze the state of soil and assess the risks of soil and groundwater contamination and toxicity to plants. Along with contamination several irrigation levels were also studied in order to identify patterns of response to Pb contamination under different water levels.

- In terms of biomass production, it can be concluded that the contamination level essayed affected the biomass production, especially at higher irrigation levels, with the *Arundo* collected at the FCT fields. This decrease in productivity of *Arundo* can be attributed to the amount of Pb released into the soil by the industrial sludge. This amount of Pb was removed and accumulated by the plant since not high amounts of Pb were lost through leaching. This accumulation and phytoextraction was more significant in the belowground biomass than in the

aerial biomass thus compromising the phytotreatment yield. But, the phytostabilization of the Pb in the belowground biomass is also an alternative process to the use of contaminated land, thus contributing to the recovery of soils and landscape. Nevertheless, in two years, the majority of the Pb added to the soils, remained accumulated in the soils. Regarding the study of different clones of *Arundo* in the phytoremediation of Pb contaminated fields, it was concluded that no phytotoxicity effects were observed for those clones. This indicates that screening tests and breeding tests to identify and improve the ability of this energy crop to growth and tolerate heavy metals contaminated fields is a mandatory effort in the framework of the EU energy policies.

- As a phytotreatment technology, globally, concerning the research line 3, the highest removal is observed at higher irrigation and at higher contamination.
- Concerning the research line 4, the highest removal, if we consider the totality of the biomass, is observed with genotypes 27 and 30 (due to higher productivities), at higher contamination. But aerial removal is higher with genotypes 22 and 27, at higher contamination.
- Associated with phytoextraction, the phytostabilization effected by the belowground fraction of *Arundo*, also contributed to the phytoremediation process of Pb contaminated soils. Indeed, the presence of *Arundo* had a retainer/stabilizer effect on the Pb associated with soluble fractions, which could be easily leached.
- Nevertheless it is considered that, for the establishment of generalized conclusions, pilot studies in Pb contaminated fields should be carried out.
- Finally, although the *Arundo* plants are not hyperaccumulators, high phytoextraction yields can be achieved in contaminated soils, due to a conjugation of phytotolerance and high yields. This allows us to consider that *Arundo* can be more efficient than certain hyperaccumulator plants but with very low yields. On the other hand, the viable growth of *Arundo* in contaminated soil and subsequent soil revegetation, ensures the long term stability of the surface, reducing the leachates, the amount of potentially toxic elements released into watercourses and groundwater and the development of a vegetative landscape or ecosystem in harmony with the surrounding environment.

- Future reasearches may evaluate the possibility of studying the potential of Giant reed in soils contaminated with explosives and organic pollutants. Improving knowledge on phytoremediation contributes to the recognition of this technique as an environmental cleanup technology. The development of phytoremediation requires, however, an integrated multidisciplinary research effort that combines the knowledge of plant biology, chemistry of soils, soil microbiology, and agricultural and environmental engineering.

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ANNEX

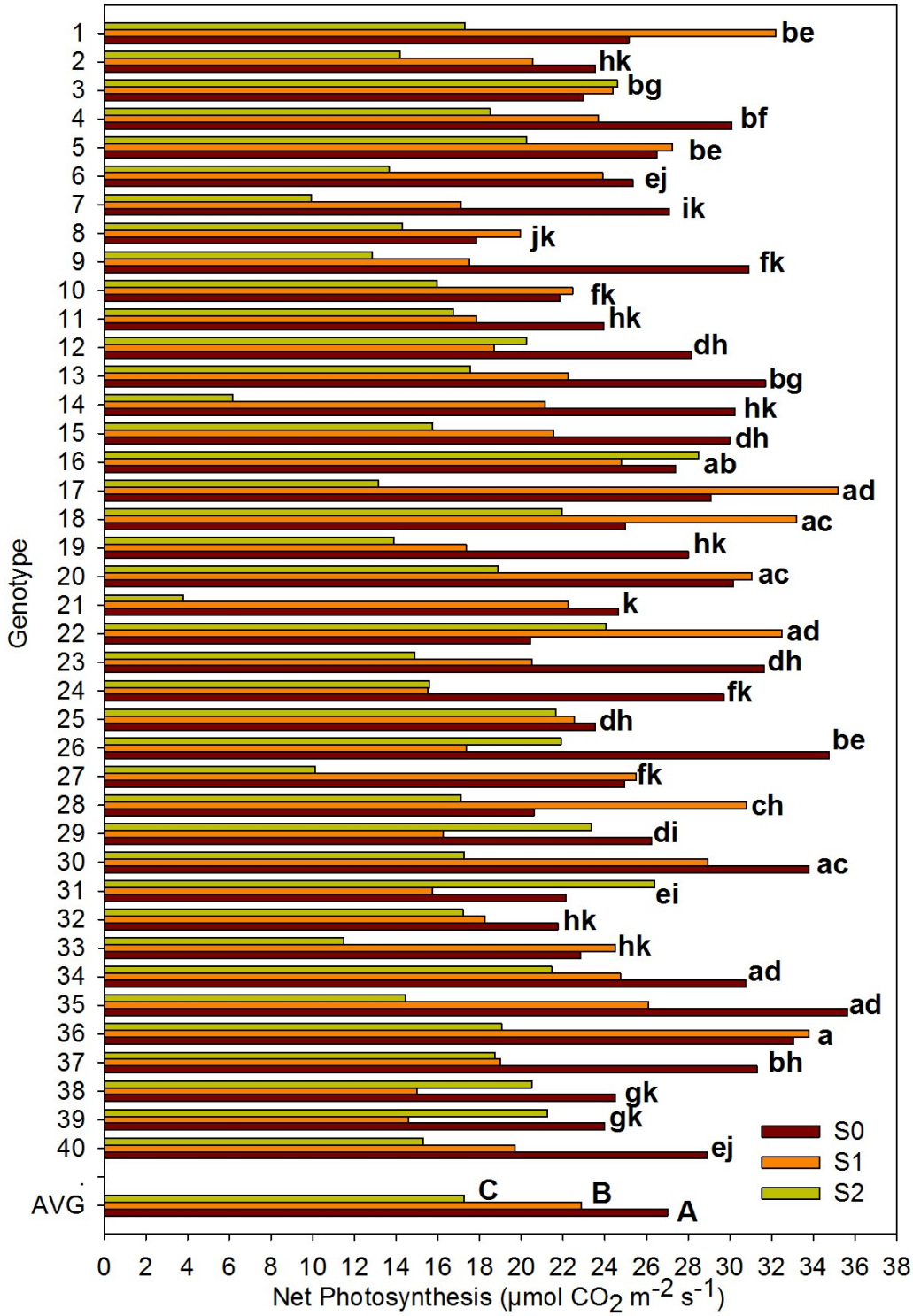

Fig. 1 - Net photosynthesis (20/07/2012) of each genotype in the three treatment (S_0, S_1 and S_2). Small letters, for averaged values of salinity level within each genotype; capital letters for averaged values of all genotypes within each salinity level. Different letters indicate significant differences at $P \leq 0.05$ by SNK Test. LSD (genotype x treatment) = 3.92

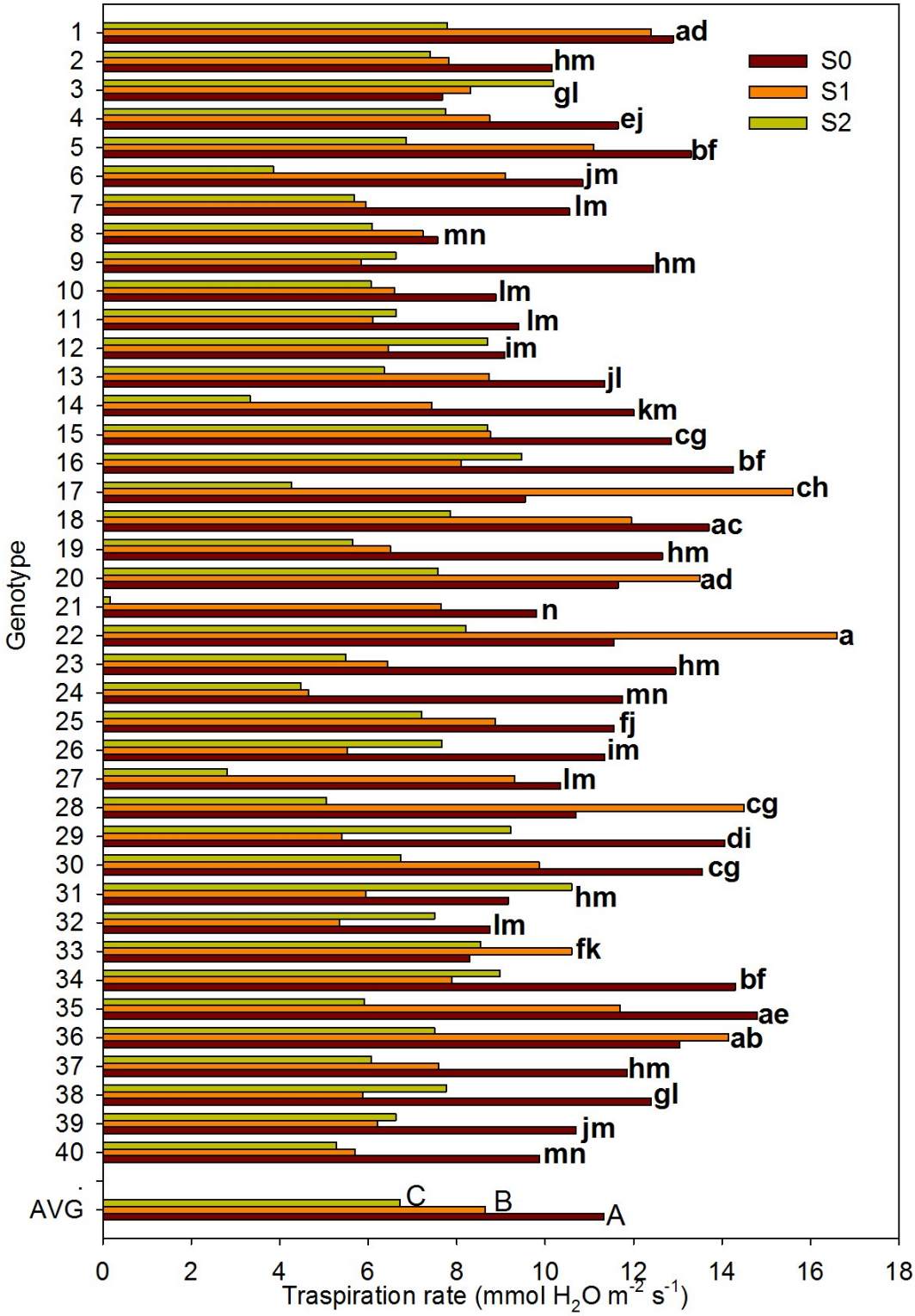

Fig. 2 - Transpiration rate (20/07/2012) of each genotype in the three treatment (S_0, S_1 and S_2). Small letters, for averaged values of salinity level within each genotype; capital letters for averaged values of all genotypes within each salinity level. Different letters indicate significant differences at $P \leq 0.05$ by SNK Test. LSD (genotype x treatment) = 1.57

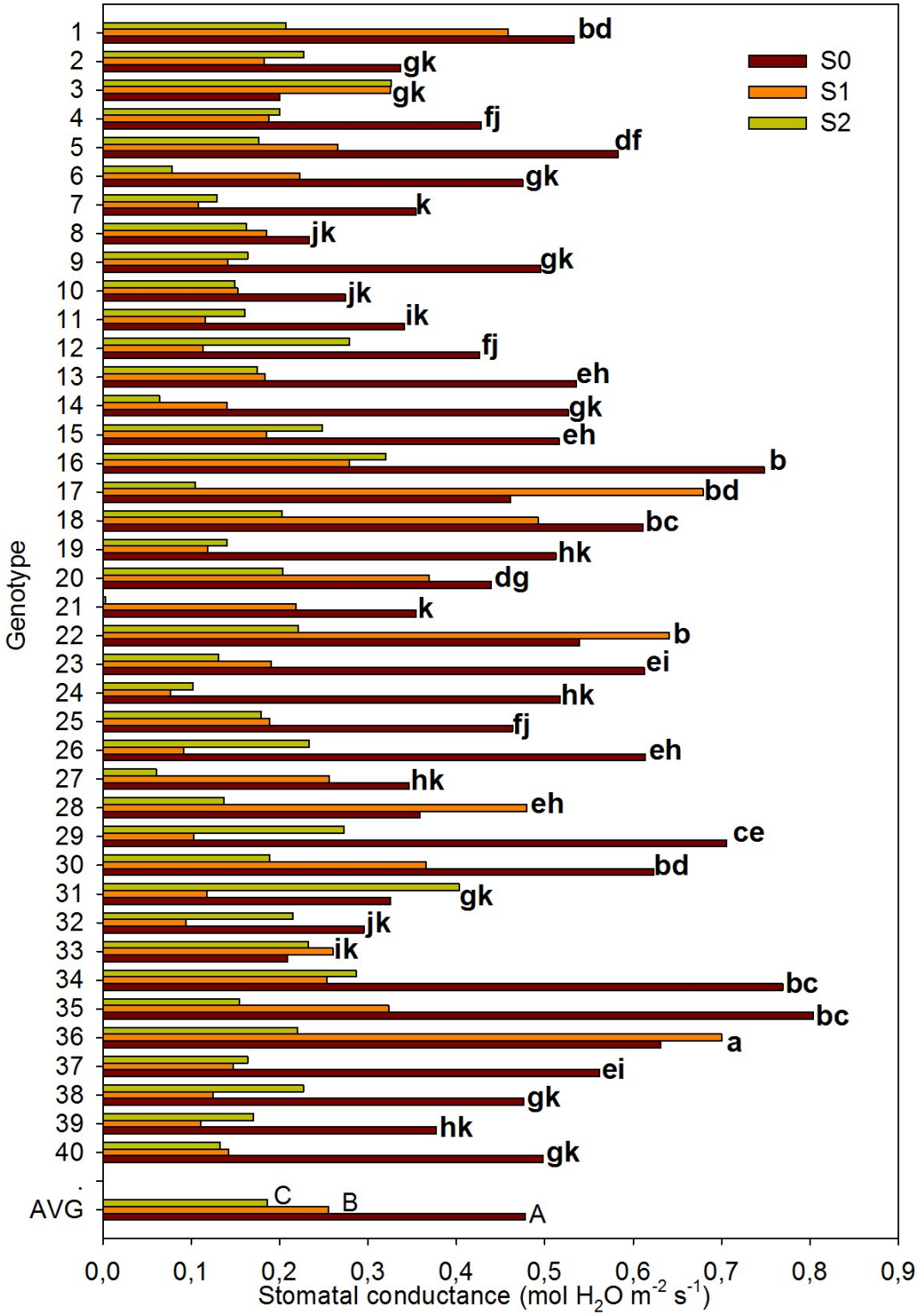

Fig. 3 - Stomatal conductance (20/07/2012) of each genotype in the three treatment (S_0, S_1 and S_2). Small letters, for averaged values of salinity level within each genotype; capital letters for averaged values of all genotypes within each salinity level. Different letters indicate significant differences at $P \leq 0.05$ by SNK Test. LSD (genotype x treatment) = 0.08

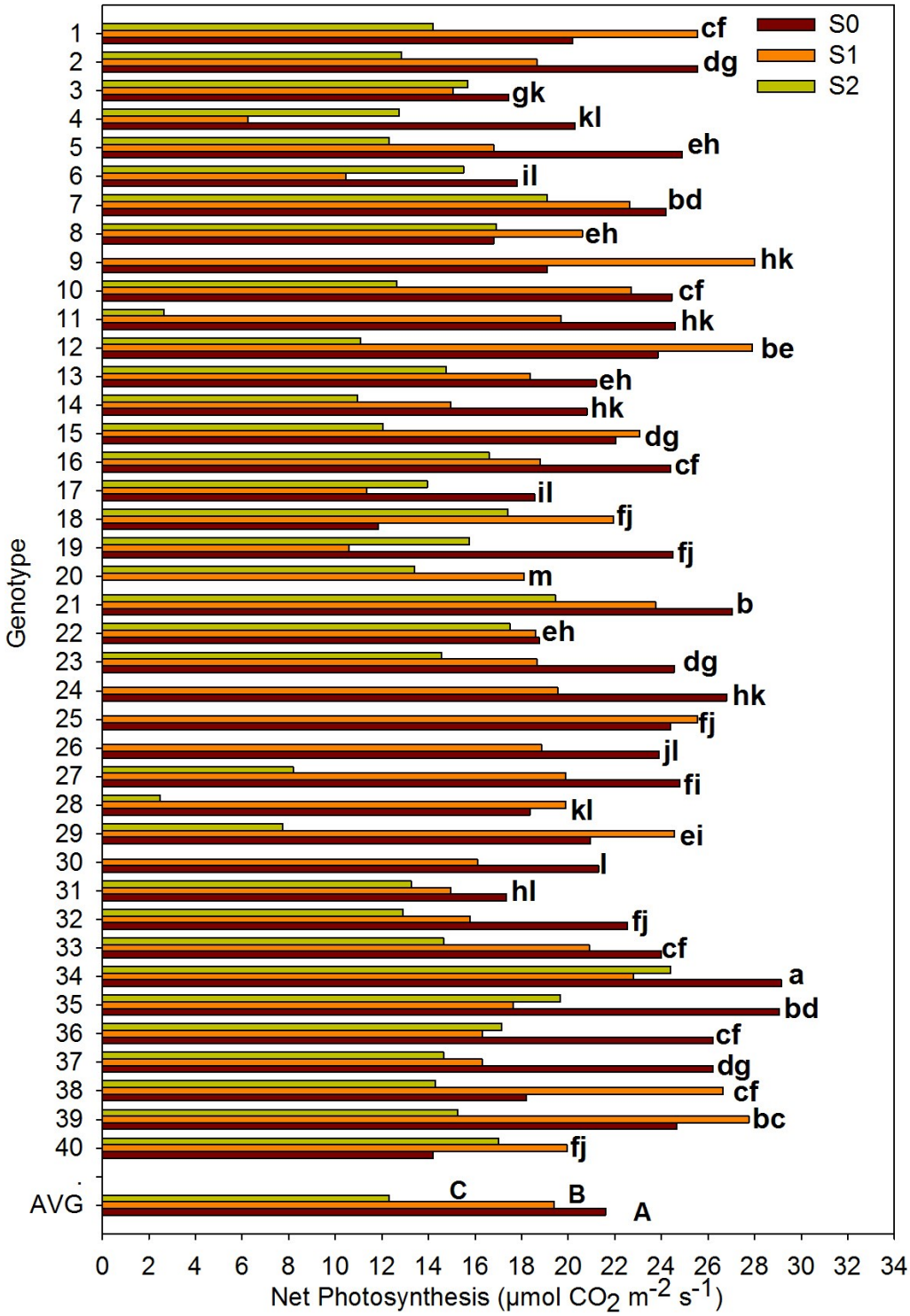

Fig. 4 - Net photosynthesis (12/09/2012) of each genotype in the three treatment (S_0, S_1 and S_2). Small letters, for averaged values of salinity level within each genotype; capital letters for averaged values of all genotypes within each salinity level. Different letters indicate significant differences at P≤0.05 by SNK Test. LSD (genotype x treatment) = 3.17

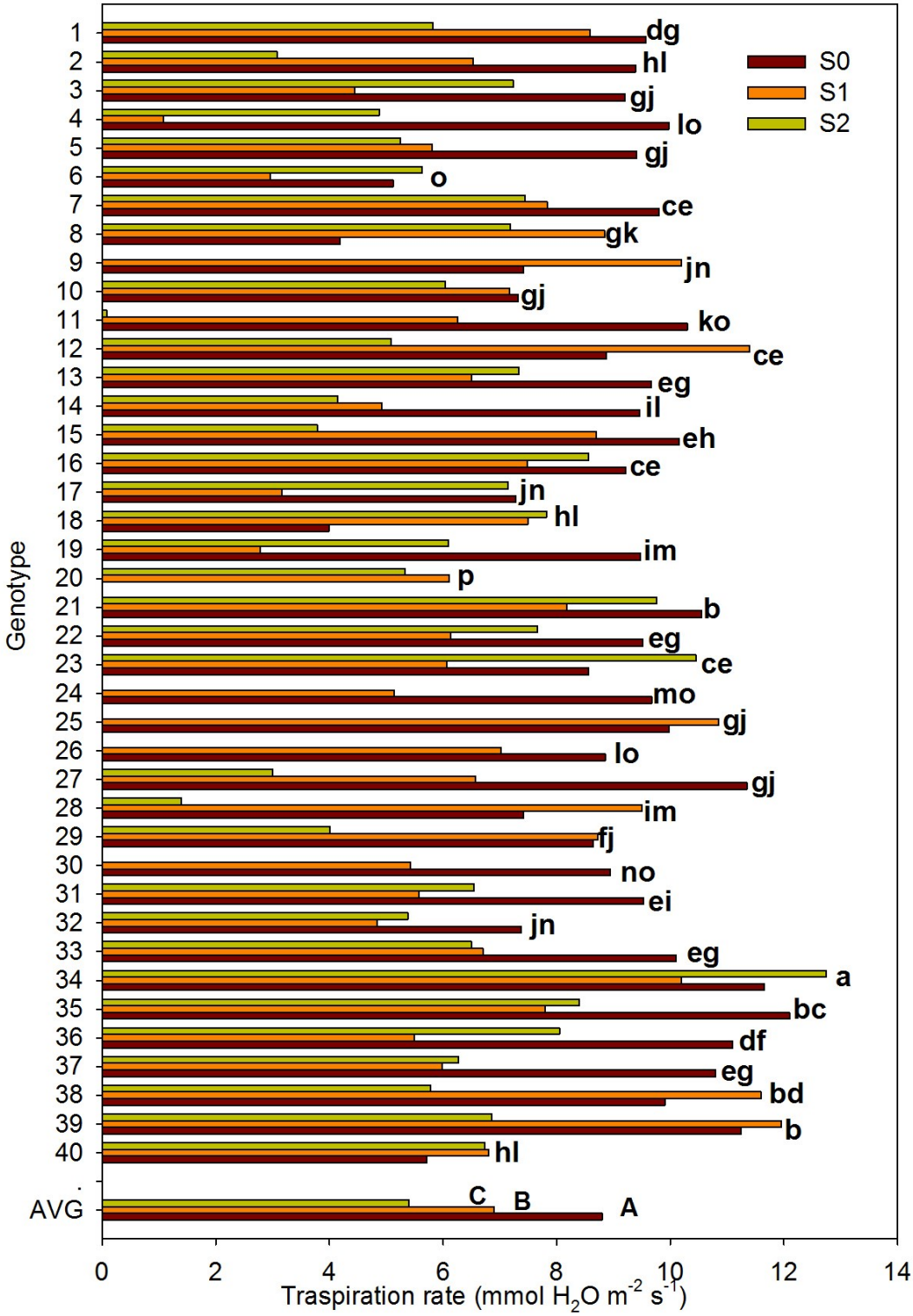

Fig. 5 - Transpiration rate (12/09/2012) of each genotype in the three treatment (S_0, S_1 and S_2). Small letters, for averaged values of salinity level within each genotype; capital letters for averaged values of all genotypes within each salinity level. Different letters indicate significant differences at $P \leq 0.05$ by SNK Test. LSD (genotype x interaction) = 1.27

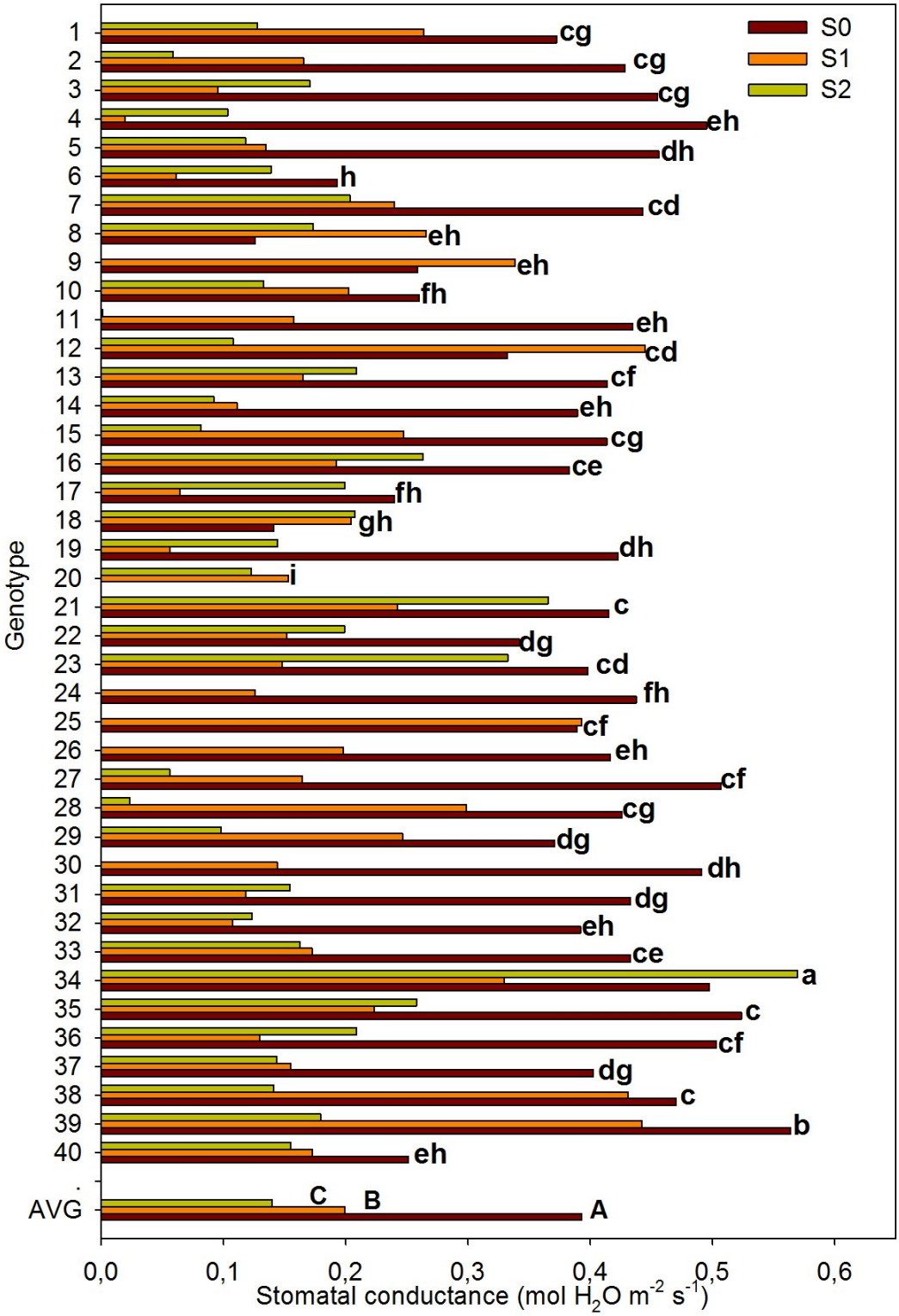

Fig. 6 - Stomatal conductance (12/09/2012) of each genotype in the three treatment (S_0, S_1 and S_2). Small letters, for averaged values of salinity level within each genotype; capital letters for averaged values of all genotypes within each salinity level. Different letters indicate significant differences at $P \leq 0.05$ by SNK Test. LSD (genotype x treatment = 0.08).

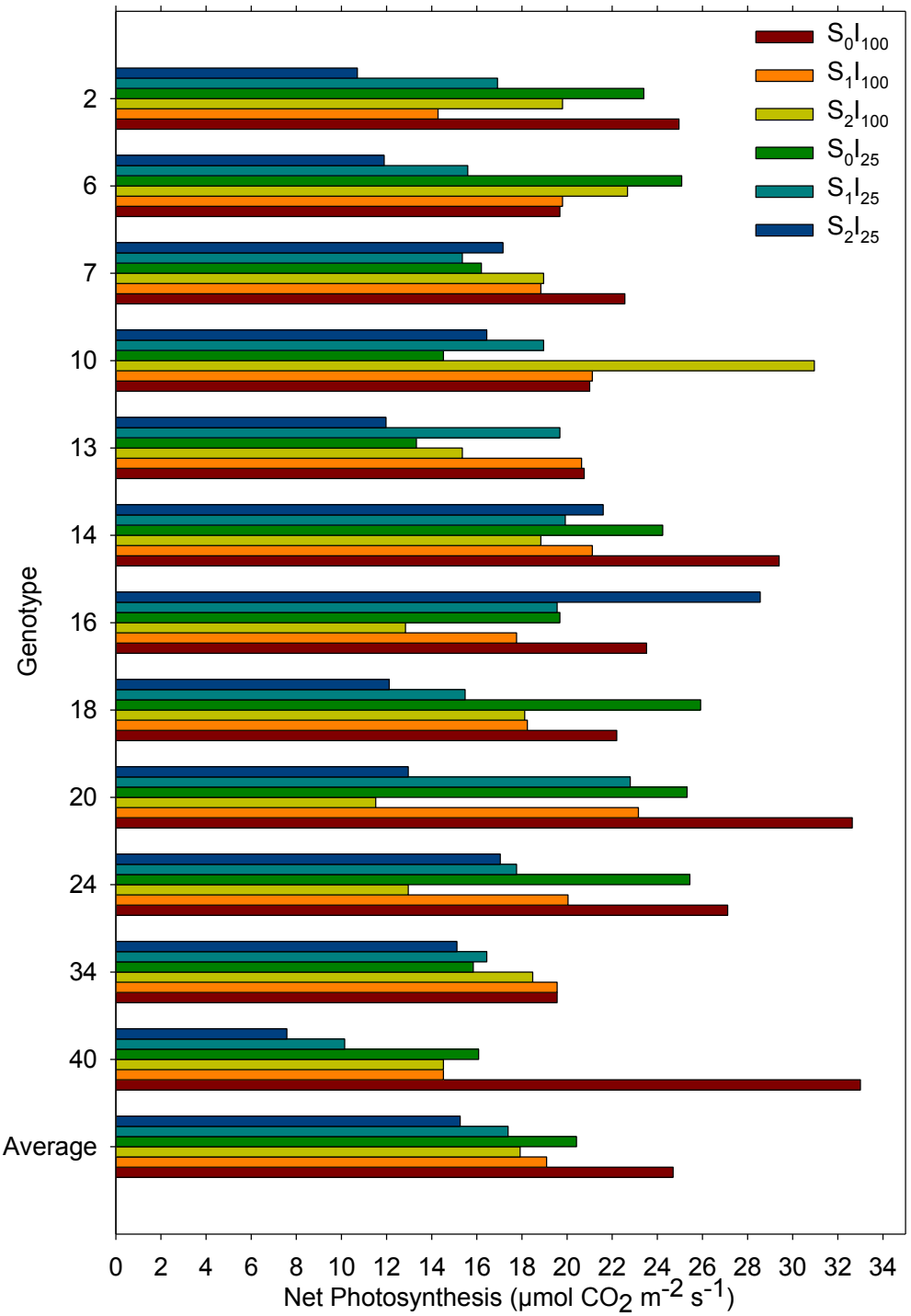

Fig. 7 - Net photosynthesis (µmol CO_2m^{-2} s^{-1}) detected on 06.08.2013 in the average of genotypes in the study and in relation to different treatments in the study, S_0 (control), S_1 (salt concentration equal to 6 dS m^{-1}), salt concentration S_2 equal to 12 dS m^{-1}, I_{100} (100% Etm restoration), I_{25} (25% Etm restoration). LSD (genotype x salinity level x water level = 1.74). LSD (Genotype x salinity level x water level) = 5.85

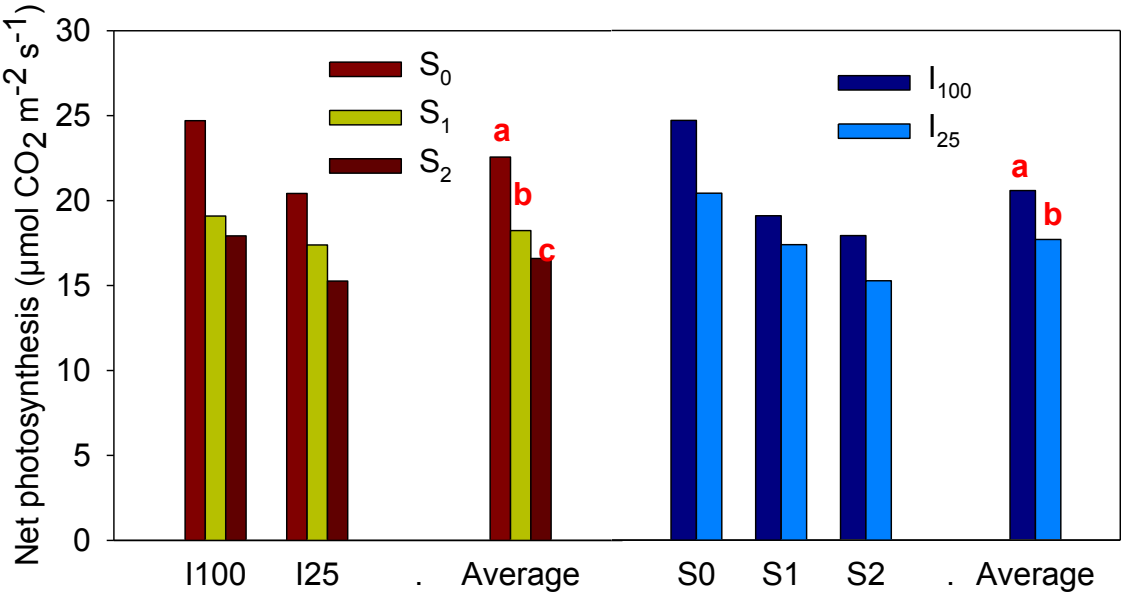

Fig.8 - Photosynthesis (µmol CO_2 m^{-2} s^{-1}) detected on 06/08/2013 in the different studied genotypes in relation to different tested treatments. Different letters indicate significant differences for $P \leq 0.05$ (SNK test).

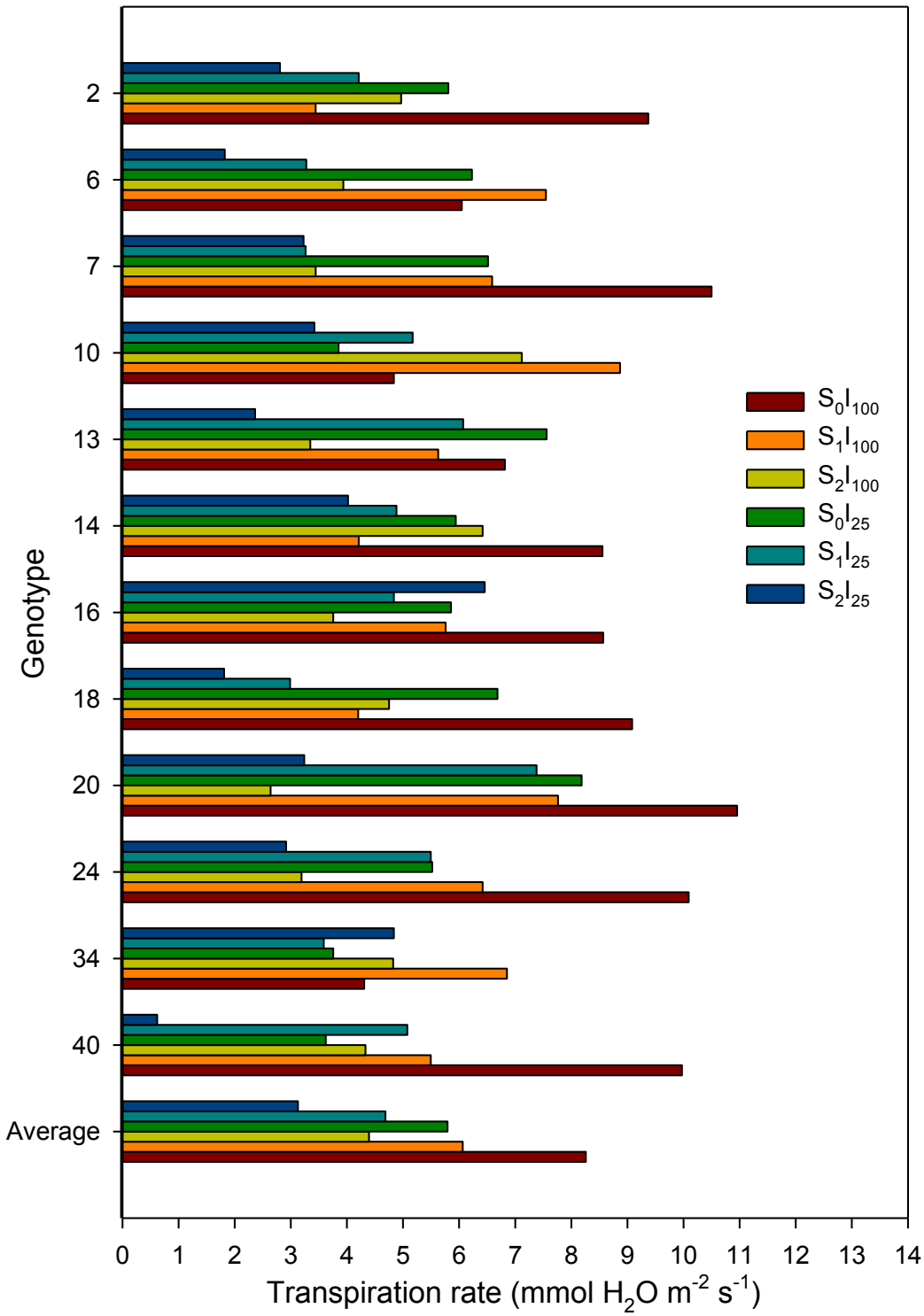

Fig. 9 - Transpiration rate (mmol H_2O m^{-2} s^{-1}) detected on 06.08.2013 in the average of genotypes in the study and in relation to different treatments in the study, S_0 (control), S_1 (salt concentration equal to 6 dS m^{-1}), salt concentration S_2 equal to 12 dS m^{-1}, I_{100} (100% Etm restoration), I_{25} (25% Etm restoration). LSD (genotype x salinity level x water level = 1.74). LSD (Genotype x salinity level x water level) = 1.71

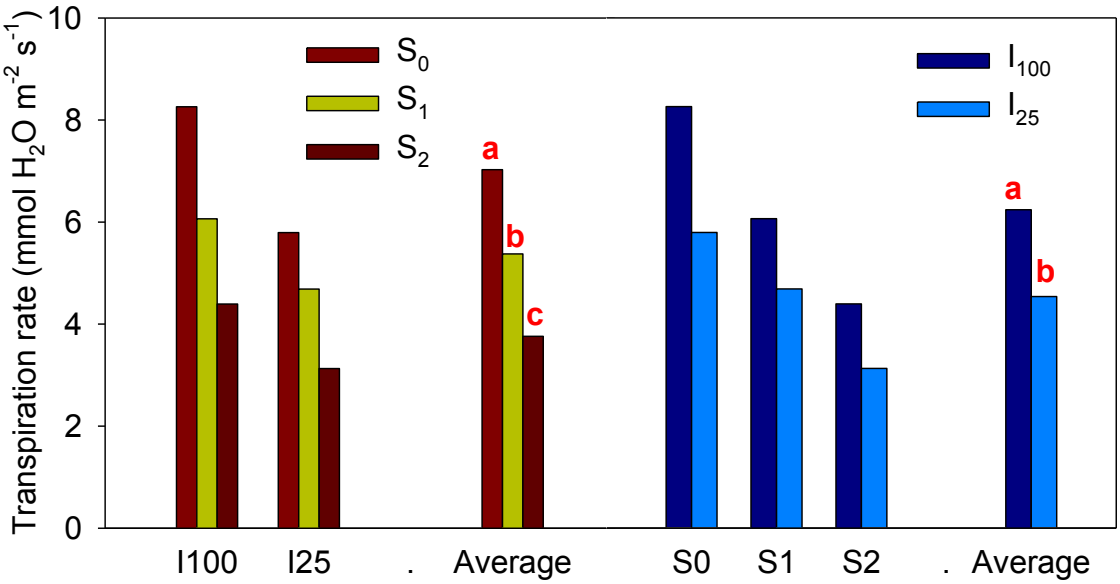

Fig. 10 - Transpiration (mmol H_2O m^{-2} s_{-1}) detected on 06.08.2013 in the different genotypes studied in relation to different treatments in the study. Different letters indicate significant differences for $P \leq 0.05$ (SNK test).

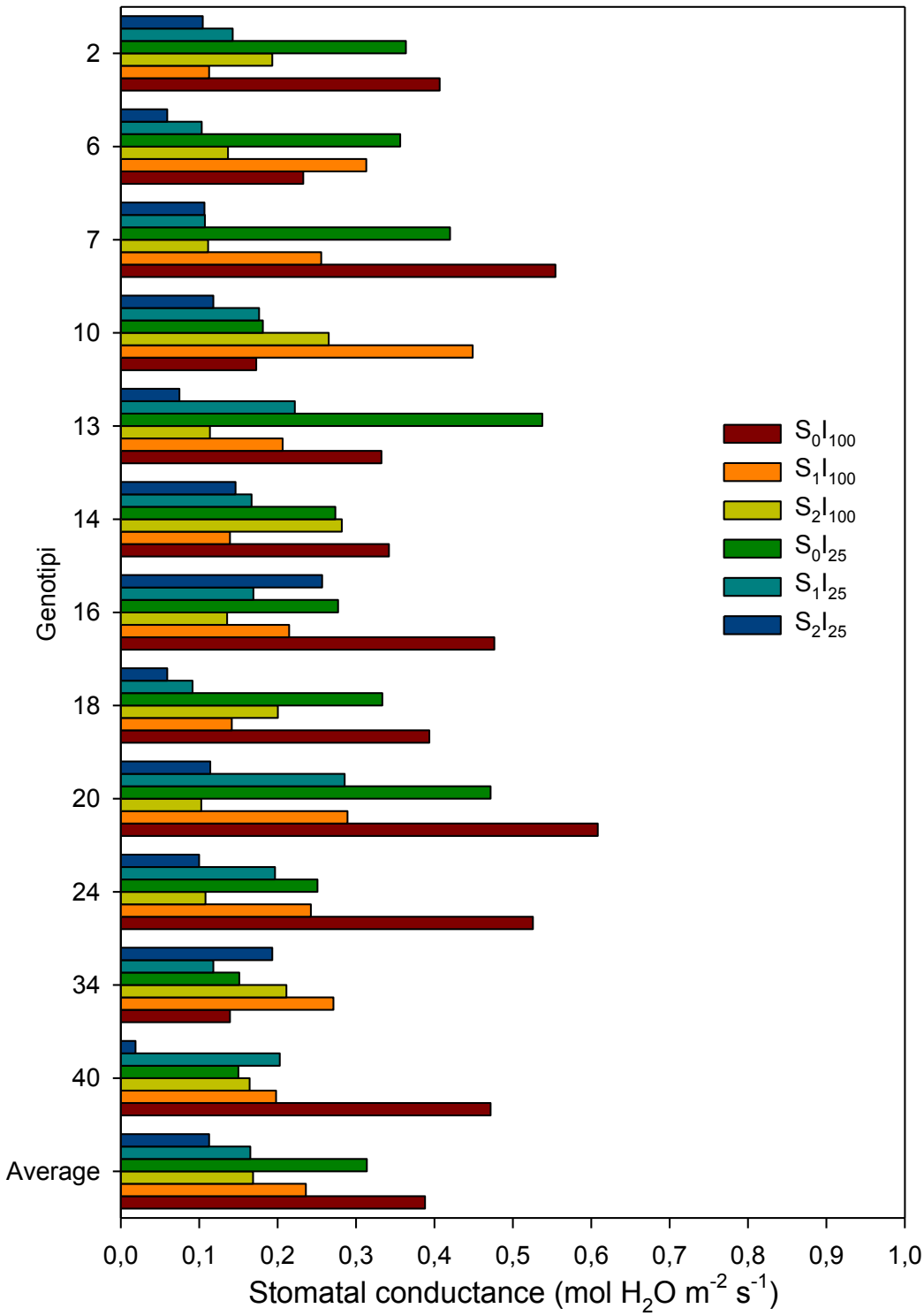

Fig. 11 - Stomatal conductance (mol H_2O m^{-2} s^{-1}) detected on 06.08.2013 in the average of genotypes in the study and in relation to different treatments in the study, S_0 (control), S_1 (salt concentration equal to 6 dS m^{-1}), salt concentration S_2 equal to 12 dS m^{-1}, I_{100} (100% Etm restoration), I_{25} (25% Etm restoration). LSD (genotype x salinity level x water level = 1.74). LSD (Genotype x salinity level x water level) = 0.09

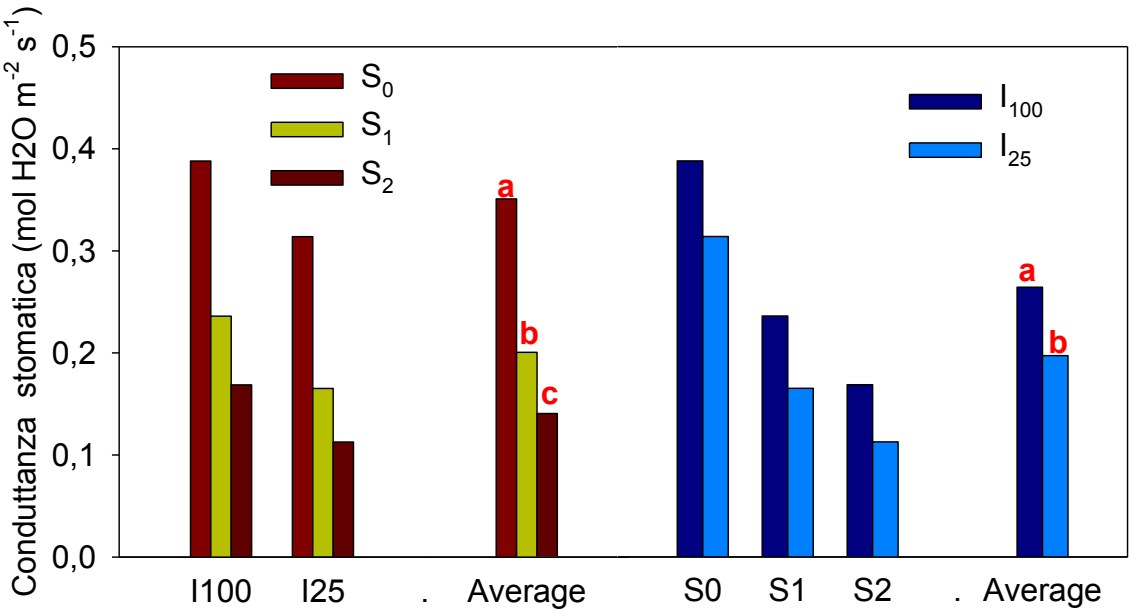

Fig. 12 -Stomatal conductance (mol H_2O m^{-2} s^{-1}) detected on 06.08.2013 in the different genotypes studied in relation to different treatments in the study. Different letters indicate significant differences for $P \leq 0.05$ (SNK test).

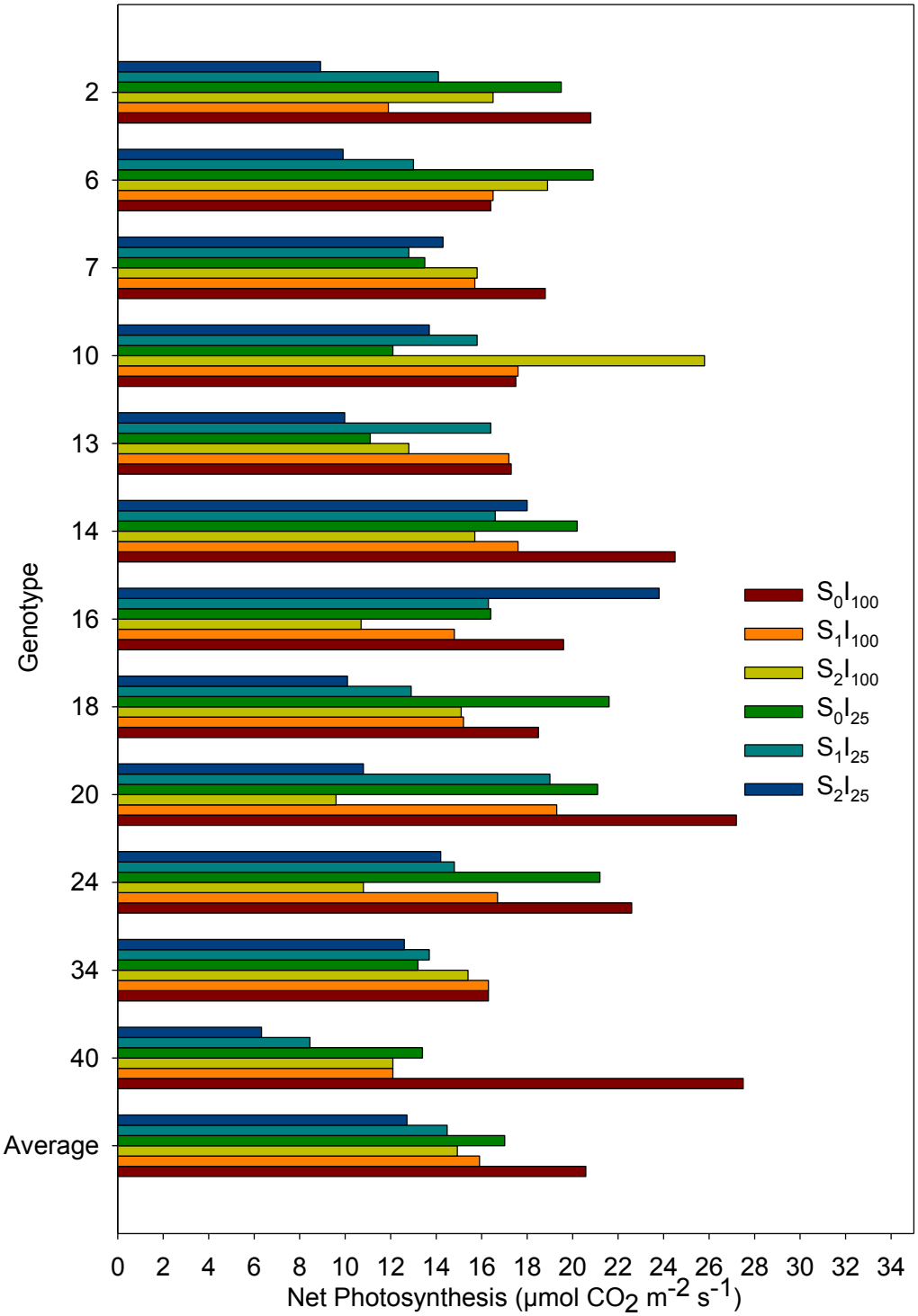

Fig. 13 - Net photosynthesis (µmol $CO_2 m^{-2} s^{-1}$) detected on 17.10.2013 in the average of genotypes in the study and in relation to different treatments in the study, S_0 (control), S_1 (salt concentration equal to 6 dS m^{-1}), salt concentration S_2 equal to 12 dS m^{-1}, I_{100} (100% Etm restoration), I_{25} (25% Etm restoration). LSD (Genotype x salinity level x water level) = 2.67

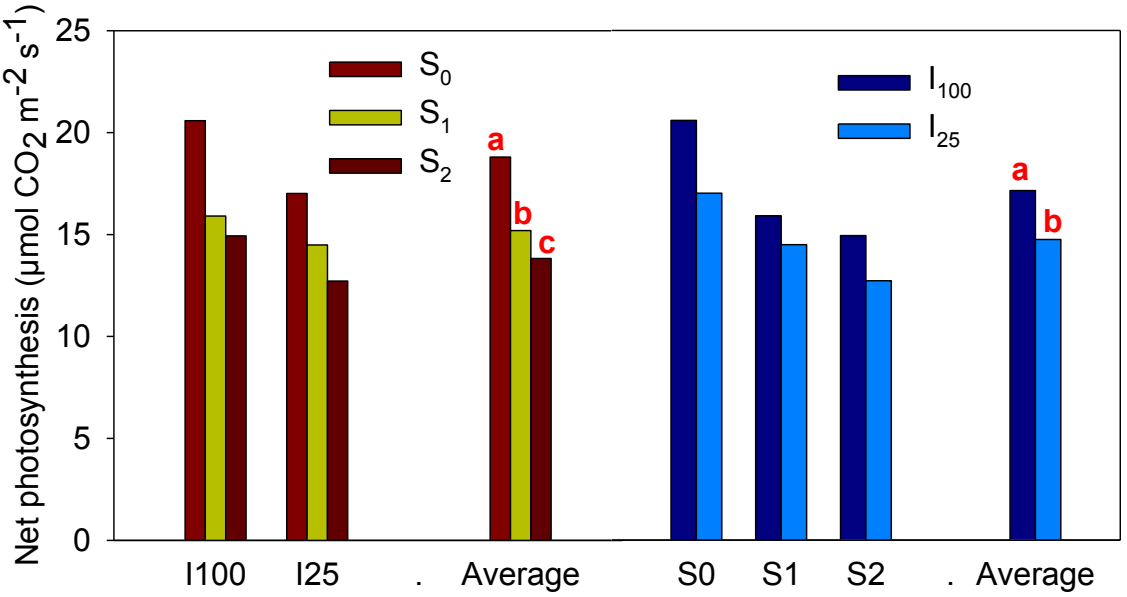

Fig. 14 - Net Photosynthesis (µmol CO_2 m^{-2} s^{-1}) detected on 10/17/2013 in the different studied genotypes in relation to different tested treatments. Different letters indicate significant differences for $P \leq 0.05$ (SNK test).

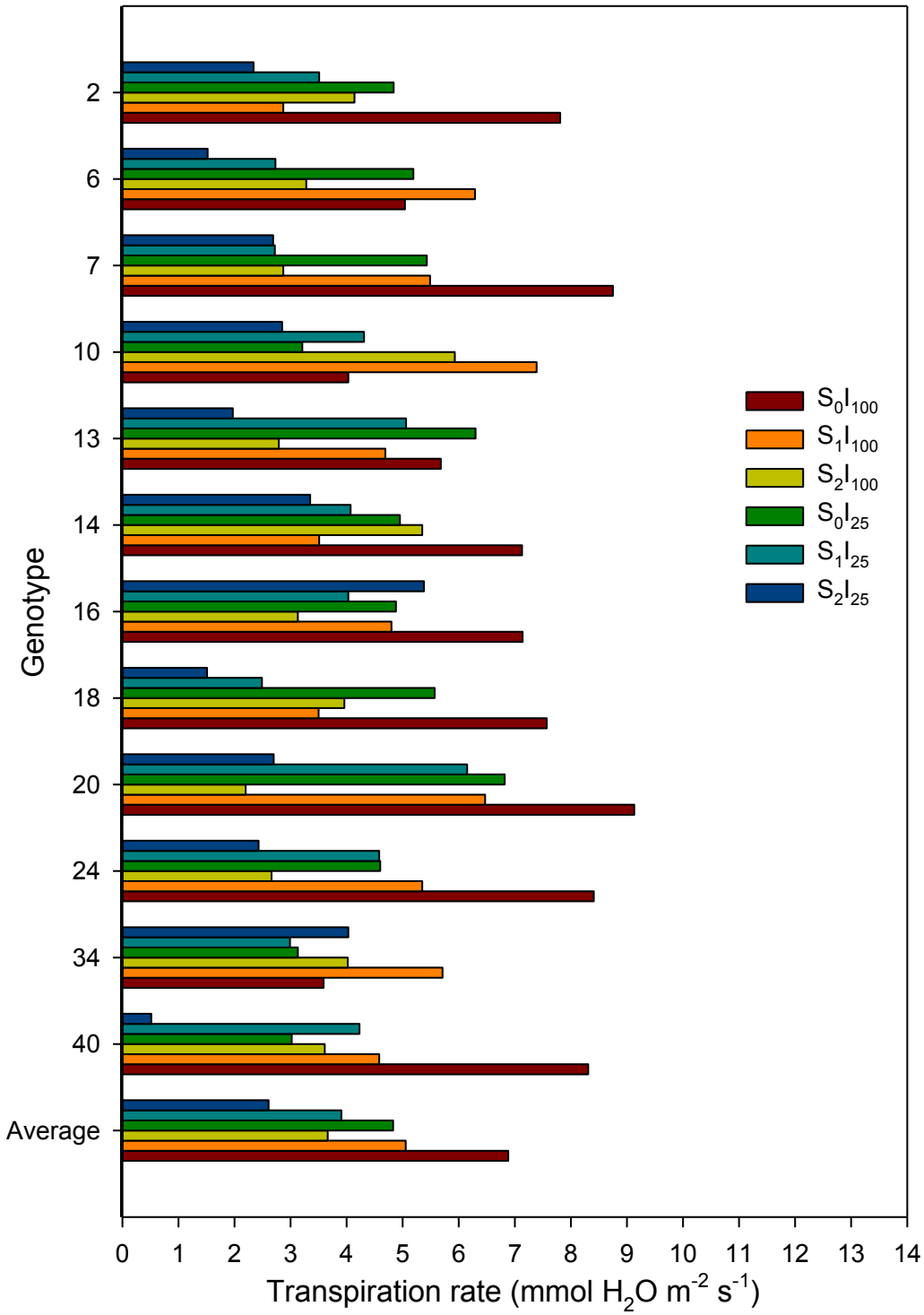

Fig. 15 - Transpiration rate (mmol H_2O m^{-2} s^{-1}) detected on 17.10.2013 in the average of genotypes in the study and in relation to different treatments in the study, S_0 (control), S_1 (salt concentration equal to 6 dS m^{-1}), salt concentration S_2 equal to 12 dS m^{-1}, I_{100} (100% Etm restoration) , I_{25} (25% Etm restoration). LSD (Genotype x salinity level x water level) = 0.94.

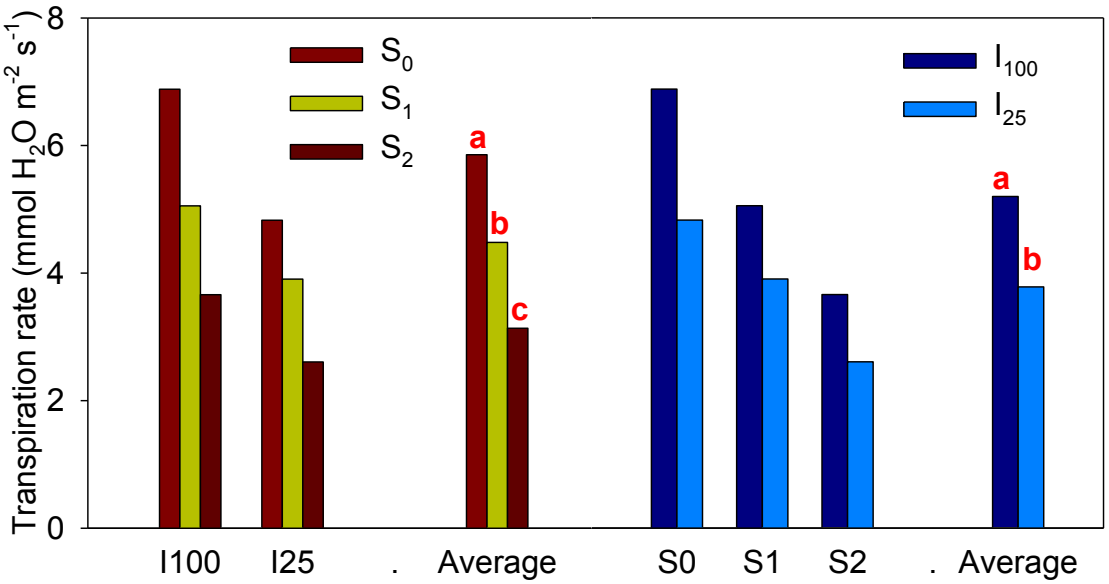

Fig. 16 Transpiration rate (mmol H_2O m^{-2} s_{-1}) detected on 17.10.2013 in the different genotypes studied in relation to different treatments in the study. Different letters indicate significant differences for $P \leq 0.05$ (SNK test).

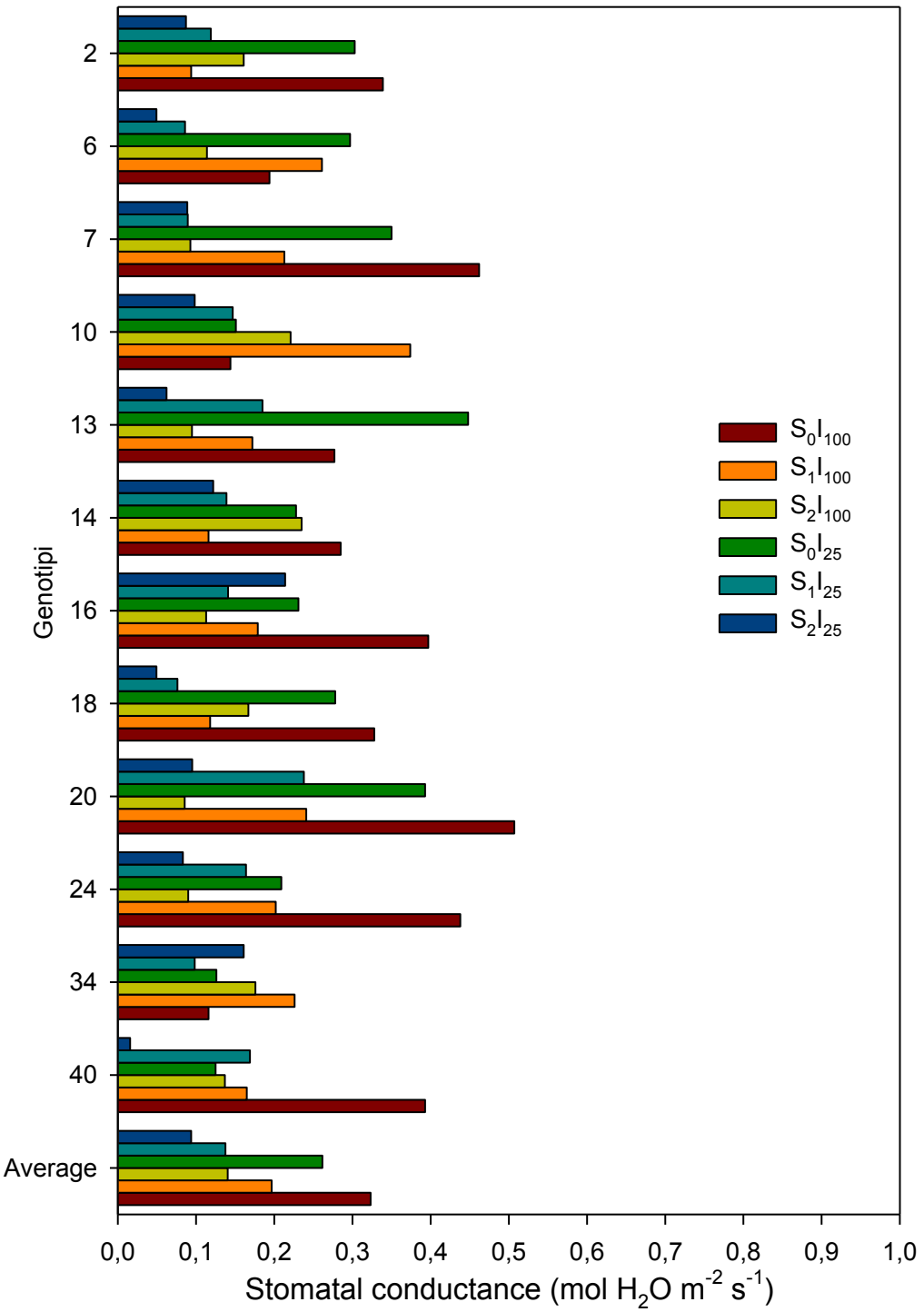

Fig. 17 - Stomatal conductance (mol H_2O m^{-2} s^{-1}) detected on 17.10.2013 in the average of genotypes in the study and in relation to different treatments in the study, S_0 (control), S_1 (salt concentration equal to 6 dS m^{-1}), salt concentration S_2 equal to 12 dS m^{-1}, I_{100} (100% Etm restoration), I_{25} (25% Etm restoration). LSD (Genotype x salinity level x water level) = 0.05

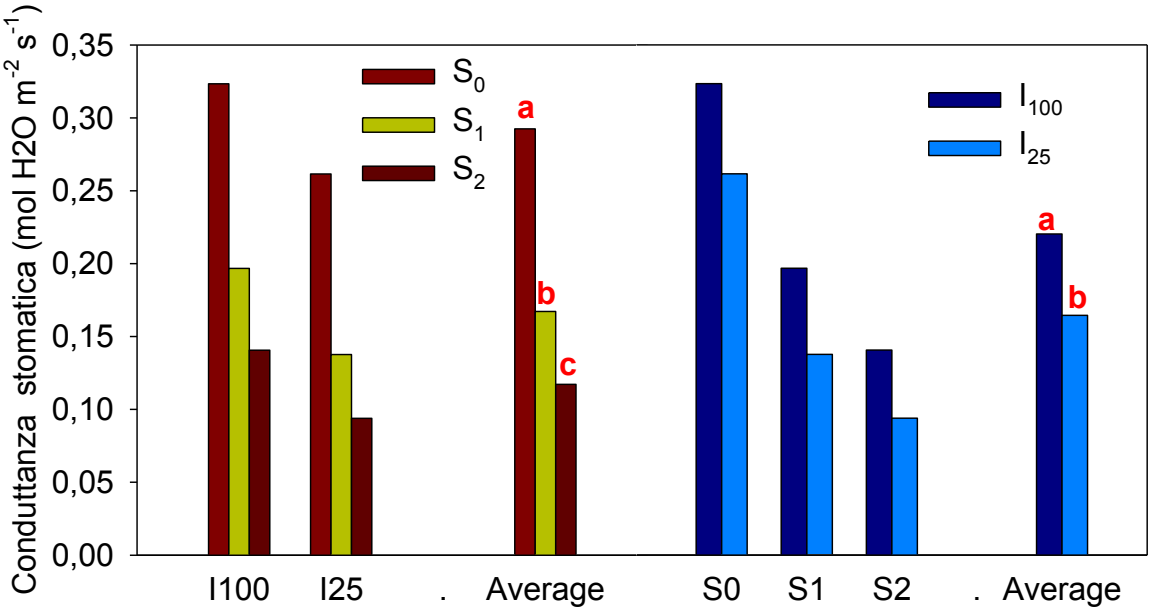

Fig. 18 - Stomatal conductance (mol H_2O m^{-2} s^{-1}) detected on 17.10. 2013 in the different genotypes studied in relation to different treatments in the study. Different letters indicate significant differences for $P \leq 0.05$ (SNK test).

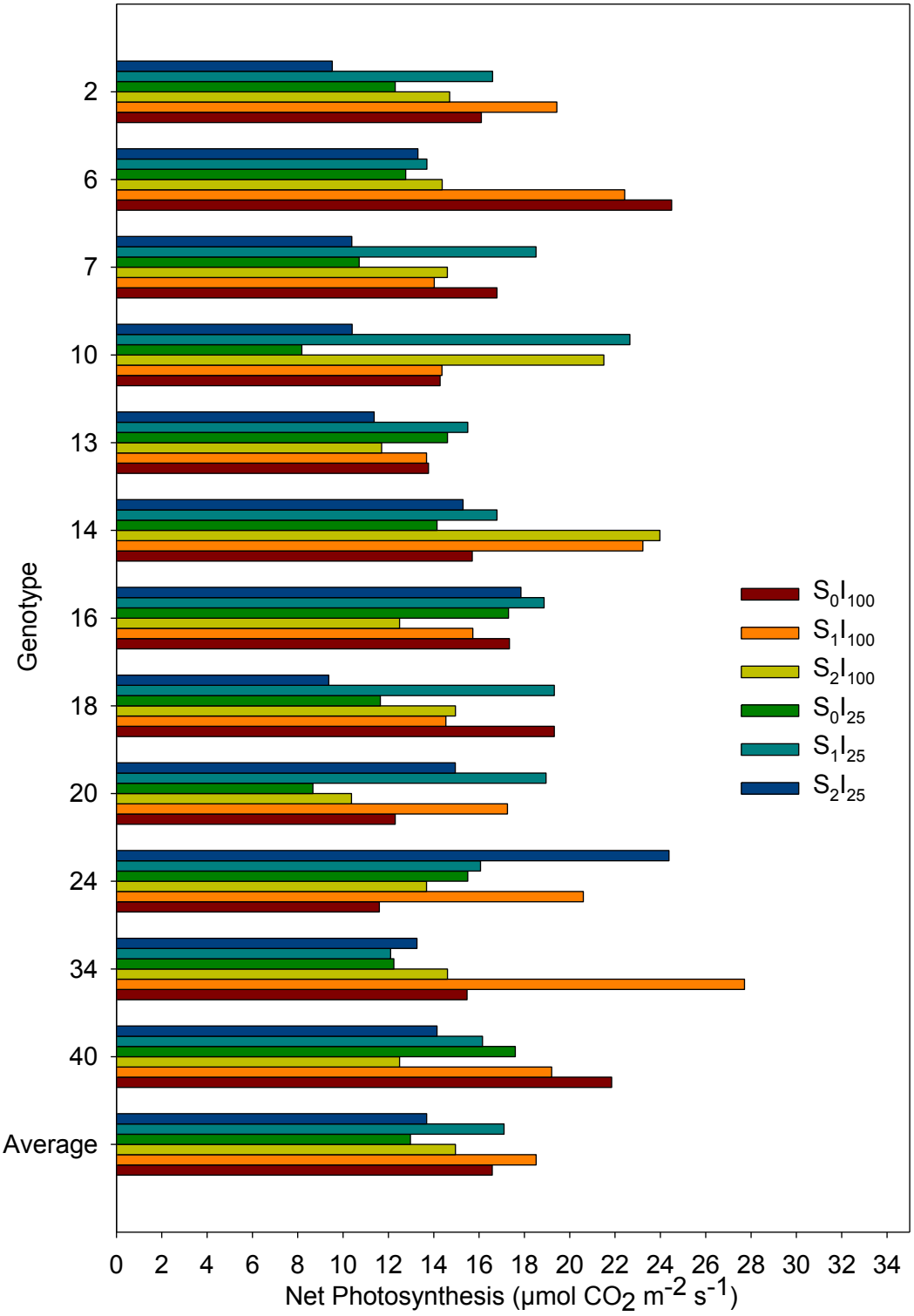

Fig. 19 - Net photosynthesis (µmol $CO_2 m^{-2} s^{-1}$) detected on 30.10.2013 in the average of genotypes in the study and in relation to different treatments in the study, S_0 (control), S_1 (salt concentration equal to 6 dS m^{-1}), salt concentration S_2 equal to 12 dS m^{-1}, I_{100} (100% Etm restoration), I_{25} (25% Etm restoration LSD (Genotype x salinity level x water level) = 4.77

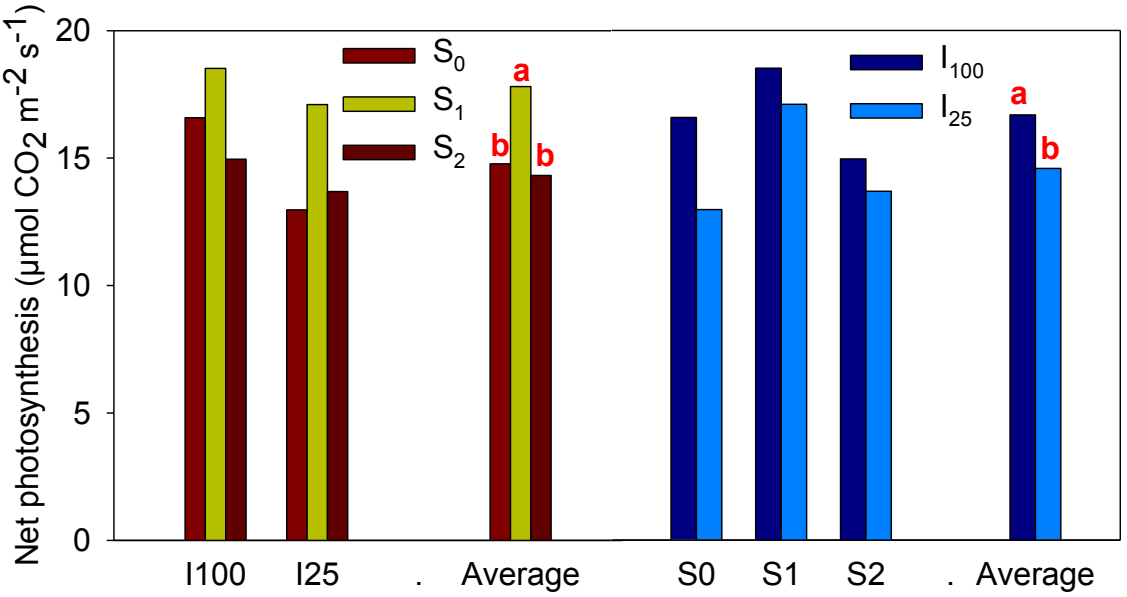

Fig. 20 - Net Photosynthesis (µmol CO_2 m^{-2} s^{-1}) detected on 30/10/2013in the different studied genotypes in relation to different tested treatments. Different letters indicate significant differences for $P \leq 0.05$ (SNK test).

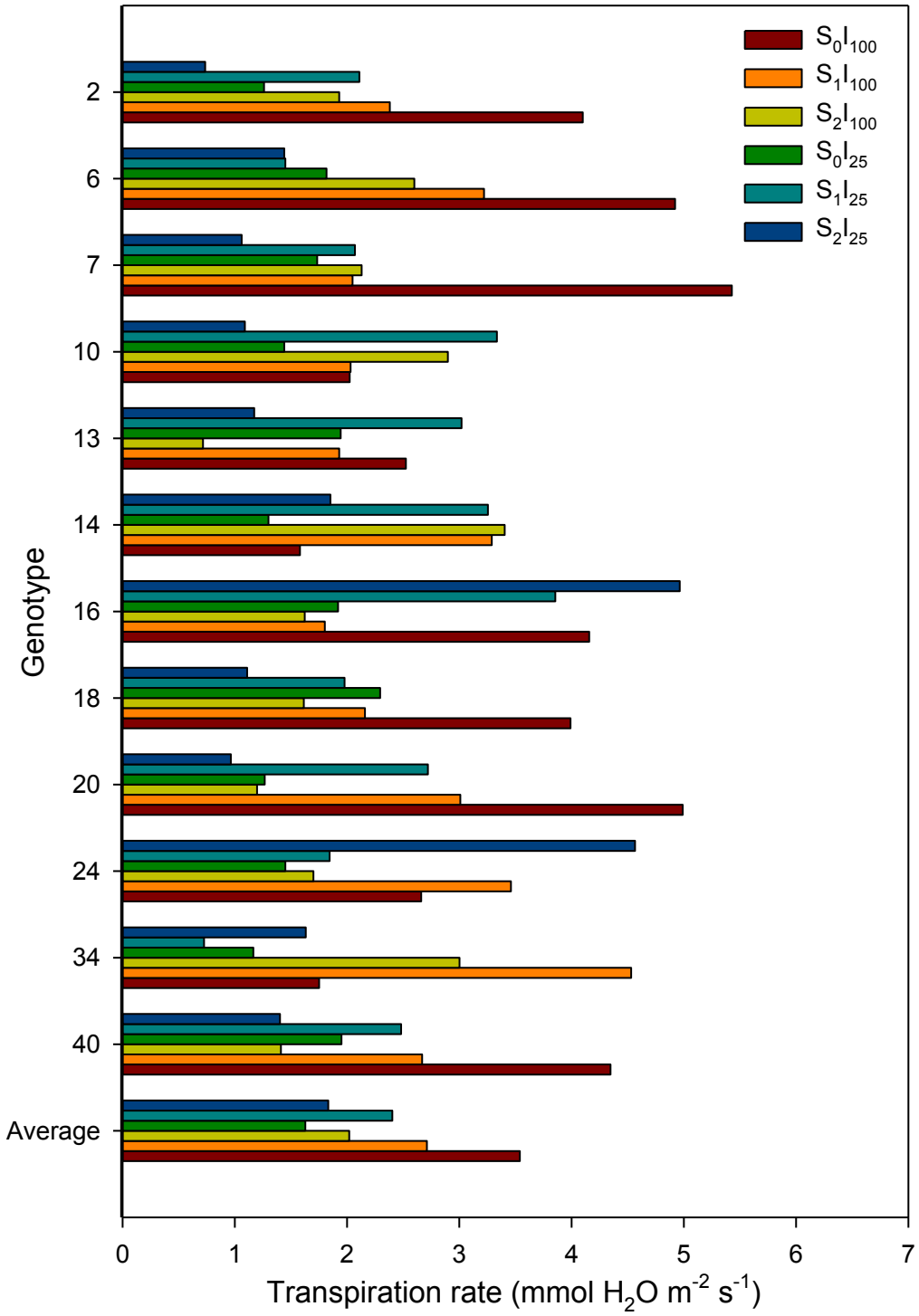

Fig. 21 - Transpiration rate (mmol H_2O m^{-2} s^{-1}) detected on 30.10.2013 in the average of genotypes in the study and in relation to different treatments in the study, S_0 (control), S_1 (salt concentration equal to 6 dS m^{-1}), salt concentration S_2 equal to 12 dS m^{-1}, I_{100} (100% of Etm restoration), I_{25} (25% of Etm restoration). LSD (Genotype x salinity level x water level) = 0.77

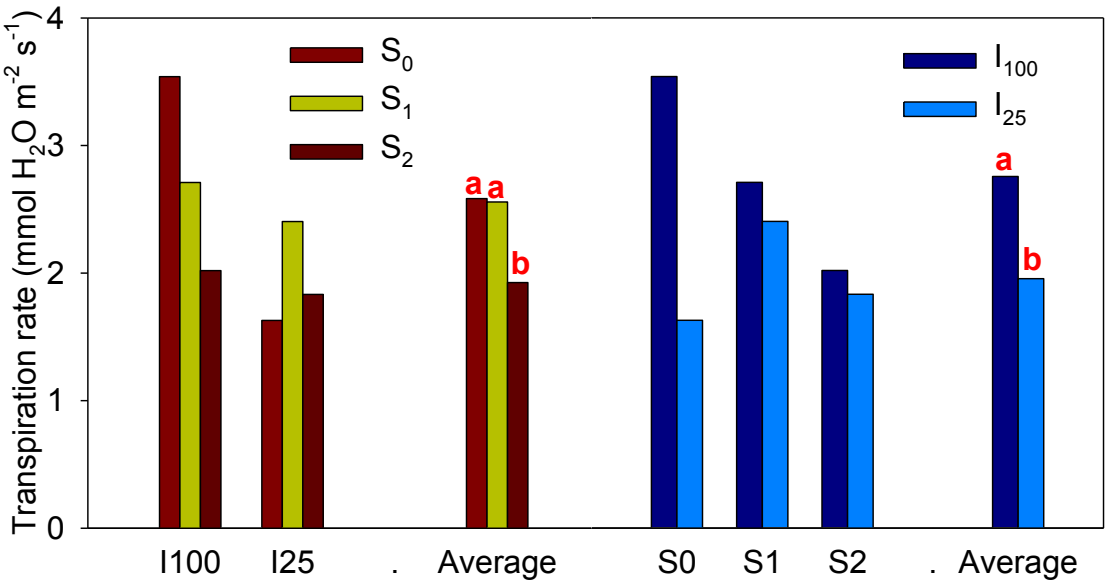

Fig. 22 - Transpiration (mmol H_2O m^{-2} s_{-1}) detected on 30.10.2013 in the different genotypes studied in relation to different treatments in the study. Different letters indicate significant differences for $P \leq 0.05$ (SNK test).

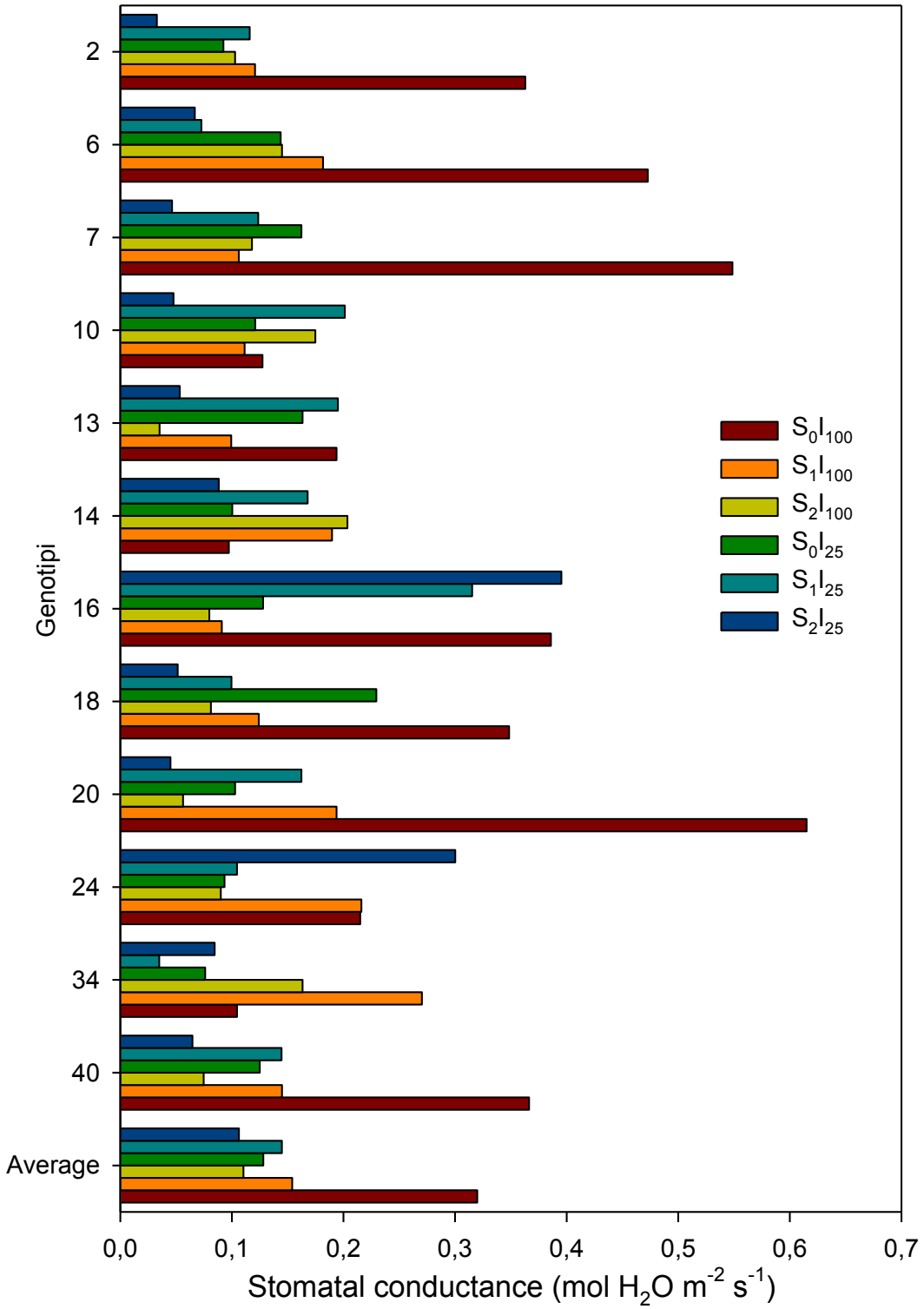

Fig. 23 - Stomatal conductance (mol H_2O m^{-2} s^{-1}) detected on 10.30.2013 in the average of genotypes in the study and in relation to different treatments in the study, S_0 (control), S_1 (salt concentration equal to 6 dS m^{-1}), salt concentration S_2 equal to 12 dS m^{-1}, I_{100} (100% Etm restoration), I_{25} (25% Etm restoration). LSD (Genotype x salinity level x water level) = 0.06.

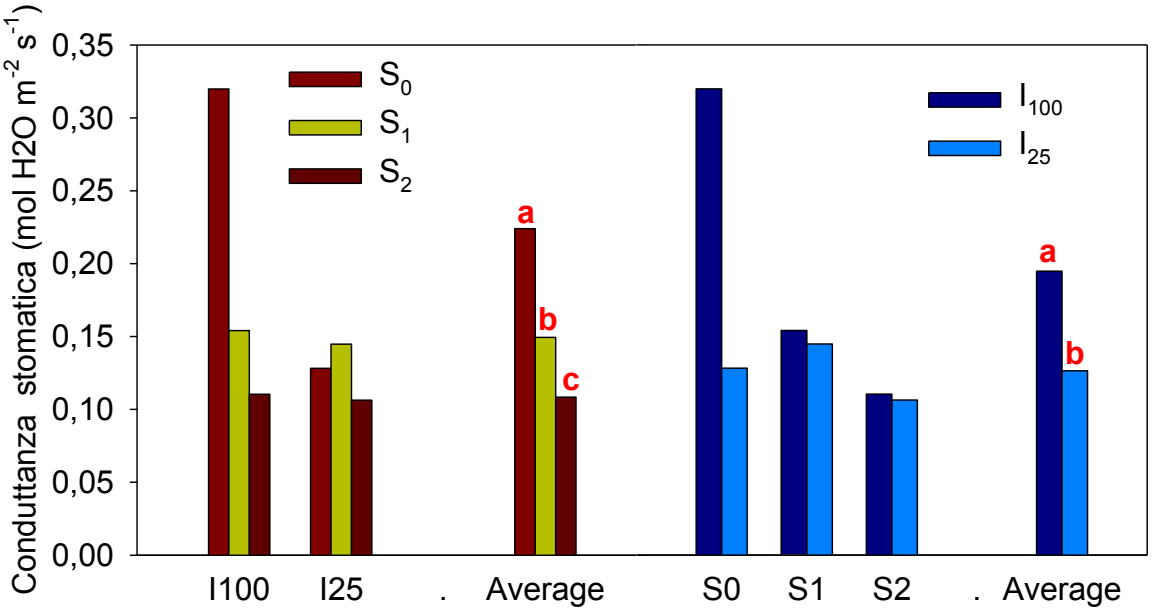

Fig. 24 - Stomatal conductance (mol H_2Om^{-2} s^{-1}) detected on 30.10.2013 in the different genotypes studied in relation to different treatments in the study. Different letters indicate significant differences for $P \leq 0.05$ (SNK test).