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PHYTOEXTRACTION AND HYDROLOGICAL PHYTOCONTROL IN AN INDUSTRIAL SITE CONTAMINATED BY HEAVY METALS AND ARSENIC

(FITOESTRAZIONE E FITOCONTROLLO DEL BILANCIO IDROLOGICO IN UN SITO INDUSTRIALE CONTAMINATO DA METALLI PESANTI E ARSENICO)

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A mio padre
per le volte che aveva ragione e che ho capito solo adesso,
per le cose che non gli ho mai detto e valeva la pena di dire,
per ogni volta che guarda da questa parte e continua ad aiutarmi

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GENERAL

INTRODUCTION

The world decontamination needs and the phytoremediation

More than two hundred years of industrialisation have left their trace on the status of soil. In fact Europe has a problem of historical contamination of soil due to the use and presence of dangerous substances in many production processes. Moreover, soil contamination is still currently being produced by inadequate practices and accidents.

Soil contamination is an unresolved problem, due to the widespread presence of contaminated soils and to technical and economic drawbacks of current remediation techniques. Worldwide there are many sites contaminated, to various extent, by heavy metal, hydrocarbons and organic chemical substances. In Italy, for example, more than 3% of the whole National territory with more than 4,000 sites have been indicated as contaminated above threshold risk limit for human health. Because of their high number and of the high costs of the de-contamination activities the authorities on these sites are limited to take concrete actions to cope with this problem.

Soil degradation has a direct impact on water and air quality, biodiversity and climate change; it can also impair the health of European citizens and threaten food and feed safety. Furthermore, the conventional methods currently used have other consequences besides the high costs: they can lead to an alteration of the ecosystem where they are applied that persists for long time.

Phytoremediation is a recently developed and sustainable innovative technologies that is defined as the use of plants to remove pollutants from the environment or to render them harmless (Salt et al., 1998). As reported by Rai et Pal, 1999 the basic idea that plants can be used for environmental remediation is quite old. However it was not until 1948 that some Italian researchers first reported Nickel hyperaccumulation in the serpentine plant *Alyssum bertolonii* Desv.. This finding was all but forgotten until 1977, when in new

Zealand researcher made similar observation. This time the concept caught on. So over past decades phytoremediation emerged as a new technology opposed to conventional methods.

The advantages and disadvantages of phytoremediation are reported below according to Pilon-Smith, 2005 and Ghosh et Singh, 2005 .

Advantages:

- Amended to a variety of organic and inorganic compounds
- Used for solid, liquid and gaseous substrates
- Defined as clean sun-power technology, with no emission of greenhouse gasses
- Relatively inexpensive: the conventional methods of remediation may cost from $10 to 1000 per cubic meter, while phytoremediation costs are estimated to be as low as $0.05 per cubic meter
- *In situ* applications show a minimum disturbance of soil and environment.
- Plants can stimulate microorganisms through the release of nutrients and the transport of oxygen to their roots so to allow a quick restoration of the biological activities of contaminated soil
- Plants produce biomass that can be used for energy production.
- Appealing visual results

Disadvantages/Limitations:

- Restricted to sites with low contamination concentrations and with shallow contamination within rooting zone of remediative plants
- Process may take up many growing season to remediate a contaminated site
- In situ soil proprieties, toxicity level and climate should affect the plant growth
- The food chain could be adversely affected by the degradation of chemicals

- Harvested plant biomass from phtoextration may be classified as a hazardous waste hence disposal should be proper

In the phytoremediation process different plant species can be used and once moved up along the plant, metals should be preferentially stored in the woody parts that are less physiologically active with respect to foliage, not renewable and can be easily harvested and burned to produce bioenergy (Pietrini et al., 2009).

In this context, research should be focused on increasing knowledge of the mechanisms by which plants can tolerate, accumulate and translocate metals, as well as exploring the variability in these traits in spontaneous and cultivated species. Then, it is important to screen out the spontaneous and cultivated plant populations growing in contaminated sites to individuate the specific capabilities of these plants to tolerate or accumulate a particular pollutant or a combination of them.

Phytoremediation methodology and its applications

Phytoremediation is potentially applicable to remove various contaminants from soils, sludge, sediments, ground and surface waters and waste waters. Many plants absorb from the rhizosphere, organic and inorganic xenobiotic molecules that have similarities (hydrophobicity, polarity, molecular weight and molecular sizes) to the nutrients essential for their growth. Arsenic, for instance, has similarities with phosphate and is absorbed likely using the same specialised transporters, while Selenium has similarities with sulphur and cadmium with calcium and zinc (Salt et al., 1998).

Potentially, a large variety of contaminants, including heavy metals, hydrocarbons, halogenated compounds, radionuclides, nutrients, pentachlorophenol and polycyclic aromatic hydrocarbons may actively or with some degree of passivity, enter roots and thus be transferred from soil or water to plants. The key action of this removal process is the transport of contaminants from soil or water into roots. Many absorbed xenobiotic molecules or contaminants remain in the roots tightly bound to wall and membrane structural molecules, or move through the epidermis to the Casparian strips where they face a strong specific selection for passing through, and eventually reach, the above ground organs via the xylem vessels. The high shoot accumulation and tolerance of contaminants, heavy metals in particular, are considered as traits useful to select among species and genotypes for some activities required in phytoremediation of contaminated environments (Baker et al., 2000).

Some plants displayed an extreme form of tolerance, allowing to accumulate exceptionally high amount of heavy metals in their tissues, so to reach a metal concentration in the aboveground biomass higher than that in the soil. These plants are called hyperaccumulators (Cai et Ma., 2003). Studies carried out in recent years on species hyper-accumulating and tolerating heavy metals and on those able to colonize sites contaminated by organic substances have produced some important indications on their characteristics. Specifically, for the removal of heavy metals (some species do it between 0.1-1% of their dry weight), useful plants should apparently move the absorbed metal ions from roots to shoots and neutralize their toxicity through the sequestration into the large volume of vacuoles therein. Further, only hyperaccumulators with a high and rapid biomass production could be effectively used for phytoremediation.

Currently, at least 45 families of higher plants are known to include species which hyper accumulate heavy metals in shoots, and more than 88 different species of plants and trees are reported to be able to take up and accumulate/degrade or transpire 70 organic and inorganic xenobiotic chemicals, representing many classes of compounds.

An example of hyperaccumulator plant species is *P. vittata* L., a fern that can accumulate extremely large concentrations (up to 23000 mg/kg) of this toxic element in its above ground biomass. Several other fern species have been reported by Zhao et al. (2002) to hyperaccumulate As to concentrations similar to *P.vittata* L. (P. cretica, P. longifolia, P. umbrosa).

P. vittata L. shows the common traits associated with metal/metalloid hyperaccumulation: enhanced root uptake, efficient root-to-shoot translocation and a far elevated tolerance through internal detoxification (Caille et al., 2005).

Some plants show considerable promise in the remediation of both chlorinated hydrocarbons and heavy metals. The soil and water rhizosphere of these plants is the optimum environment for the growth and the metabolic activities of useful micro-organisms (e.g. bacteria and mycorrhizae) that co-degrade organics and increase heavy metal bioavailability for plant extraction. These activities are naturally sustained by plant root exudation of photosynthates and many other stimulating molecules into the rhizosphere.

Studies on this natural process have allowed to enhance this rhizosphere remediation activities by amending the soil with specific substances. This practice is now defined as enhanced rizoremediation. Various clones of poplars, willows, eucalyptus are currently under investigations for evaluating if they are suitable for rhizoremediation. All these plants are fast growing, are highly symbiotic in their extended rhizosphere with mycorrhizae and many bacteria, have large root systems able to explore the soil down to few meters, and represent a rich germplasm with high genetic variability that make them adaptive to most pedo-climatic conditions. These plants are also tolerant and itself able to degrade chlorinated hydrocarbons even under sterilized conditions

Salicaceae, such as Poplar and Salix spp, have been, for example, used because associate high biomass accumulation and transpiration rates, to the ability of decontaminating soils from organics such as, for example, atrazine, polychlorinated biphenyls, heavy metals, hydrocarbon, herbicides and trichloroethylene.

Numerous studies are currently under development to explore plant biodiversity for various phytoremediation abilities and these studies show that plants can really be a sustainable solution to cope with many environmental problems. Modern analytical tools based on identification of genetic diversity could contribute to a future increase of the number of eligible species. Anyway most of the time phytoremediation applications have been successful when the evolution of the decontamination have been monitored and when the selection of the plants and the modality of their applications have been studied very thoroughly in the site to decontaminate.

However, there is not yet available a universally applicable phyto-solution to the problem of contamination. Only few species can be applied for phytoremediation schemes and most of them are trees that has to be often used in association with herbaceous plants more effective in decontaminating soils from a given contaminants.

Furthermore, Phytoremediation is a general term for referring to many forms and processes based on plant application (Ghosh et Singh, 2005; Peer et al., 2006). Thus, it is difficult to have a single process as the most effective in different sites. To remove specific types of contaminants and some contaminated sites may require different types of plants. Often different plant activities such as extraction, degradation, immobilization, volatilisation etc., combine to deliver these various forms of phytoremediation. A brief description of these applications and of their use in soil and sludge decontamination is given in Fig.1 and following delineated.

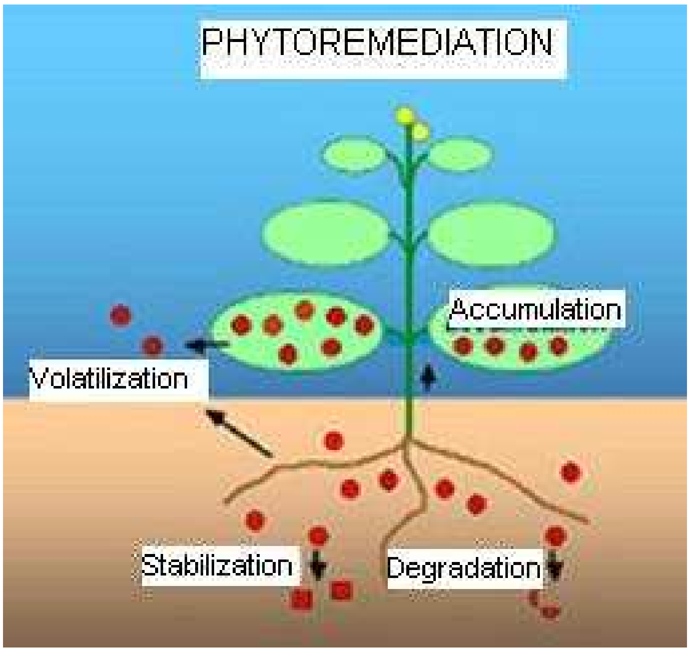


Fig.1 – Various forms of phytoremediation

Specifically:

a) Phytoextraction and Rhizofiltration

The extraction activity of plants (phytoextraction), concerns the uptake of metals (Ag, Cd, Co, Cr, Cu, Hg, Mn, Mo, Ni, Pb, Zn), metalloids (As and Se), and radionuclides (90Sr, 137Cs, 234U, 238U) by roots and transfer into the harvestable portions of the plant. Laboratory based experiments have been extensive but only a few field-scale test applications have been successfully conducted, most of them in USA. Notably, it has been reported that the extraction coefficients under field conditions were lower than those determined in laboratory. Generally, a reduced bioavailability of metals due to metal sorption by soil has been regarded as the main cause of this difference between laboratory and field results. In effect, the extraction takes place in the root zone of plants, which can generally offer a limited surface contact with soil. Further, many chemical substances in the soil, particularly relevant are humic substances, can bind and trap some contaminants impeding their diffusion to the rhizosphere. The addition of chelating agents seems to help with this problem. It should be underlined that phytoextraction of organic contaminants is not as used as phytoextraction of metals.

In this process, detailed investigation are needed, when the technology include the final combustion of the contaminated biomass, to envisage the disposal of ashes and to ensure that products that will be released in the atmosphere with fumes are not toxic.

Rhizofiltration is the removal by root trapping, of some contaminants from surface water, waste water, or extracted ground water. This process is very similar to the phytoextraction but has the advantage of an easier harvest of all contaminated plant parts, specially roots for the final disposal. It is eligible to remove metals and radionuclides from large volume of water using plants with greater and faster growing root systems. Interestingly, this process can be used either “ex situ” or “in situ”. Rhizofiltration using sunflower has been applied to remove 137Cs and 90Sr radionuclides from a small pond near the Chernobyl reactor in Ukraine.

b) Phytostabilisation/immobilisation and hydrologic control

Plants can efficiently be used to contain/stabilize soil contaminants in situ. Phytostabilisation is as relevant a strategy as phytoextraction, and for many of the metals the only practical option, since there are limited plants available which can extract them from the soil or water. Phytostabilisation does not clean the soil but rather, it prevents the loss of contaminants from a site through wind or water erosion, or where pertinent, through leaching, such that the site no longer represents a risk to the wider environment. Phytostabilisation may be used for both metals and organic contaminants. Plants seem to be able to significantly modify the chemical, biological, and physical conditions in some soils. Roots can release exudates that can induce precipitation of contaminants in solution, their complexation, or the metal valence reduction within the rhizosphere. This phyto-application is, unfortunately, a long-term process and requires to maintain an active vegetation in an unfavourable environment. When employing phytostabilisation as a strategy, it is desirable to select varieties which will tolerate metal contaminants but exclude them from their above ground tissues, while also producing economically viable amounts of biomass. This also overcomes the need to make special provision for the subsequent handling of biomass which contains contaminants collected during growth.

To date, much effort has been focused on identifying varieties which will take up large quantities of contaminant, most notably, heavy metals, with mixed success. Cd, Zn and to a more limited extent, Cu and Ni may be remediated successfully in a reasonable timescale.

A phyto-application that is considered in some tests on field is the hydraulic control of deeply contaminated soils by using Salicaceae. Willow and poplar, classifiable as phreatophytes or water-loving plants, seem far more preferable for these applications than other trees since between 100 and 1000 liters/day can be transpired by a single poplar or willow tree.

c) Rhizodegradation and Phytodegradation.

Plant roots are also able to enhance naturally occurring biodegradation of some organic chemicals by bacteria, fungi (mycorrhizae), and actinomycetes in soil. Significantly higher populations of denitrifiers, BTX (benzene, toluene, xylenes) degraders, and atrazine degraders have been identified as colonizers of the rhizosphere soil around hybrid poplar trees in a field plot. Probably, root exudates such as sugars, amino acids, organic acids, fatty acids, sterols, growth factors, nucleotides, flavanones, enzymes, and other compounds stimulate microbial growth and degradation activities in the rhizosphere. This process of rhizodegradation is a very promising and convenient process when applicable. Contaminant destruction, in fact, takes place “in situ” and, as a consequence, eliminate the necessity of disposing of contaminated material. However, an impediment can be that sometimes only part of the contamination is in contact with roots, whose further growth into new areas may be impeded by the inhospitable conditions. As for phytoextraction, trees with a large and tolerant root systems, such as some Poplar or Salix clones, can be used to explore a deep soil with high contaminant diffusion and interaction of bacteria with poorly-hydrosoluble organics. Most of organic contaminants are removed with rhizodegradation, as indicated by a variety of greenhouse, laboratory, and growth chamber studies. Nevertheless, some species have been reported to be useful for uptake, metabolizing, and degradation of contaminants within the plant. Phytodegradation, contrarily to rhizodegradation, is not dependent on associated microorganisms and fungi in the rhizosphere. Some explosives, chlorinated solvents, herbicides, and insecticides can be degraded by this way. A typical example is the metabolization using poplar of organic compounds like TCE , TNT and atrazine.

In some cases degradation reactions can lead to toxic products that can be released to the atmosphere through stomata transpiration. This process is known as phytovolatilization and is sometimes used to transfer contaminants, without any transformation, to the atmosphere.

It is important to consider that all these forms of phytoremediation can be often combined and deployed in a phytoremediation site simultaneously or in sequence for a particular contaminant and that different forms may act on different contaminants or at different exposure concentrations.

Currently used decontamination techniques

Besides the phytoremediation, which is a relatively new technique for soil decontamination, there are other methodologies (RTDF, 1998) that are used in sites characterized by a higher contaminant concentration.

Generally conventional methods include one of the following techniques:

Excavation with off-side disposal: removal of the source contamination from the site transporting the soil to landfills designed to accept hazardous wastes.

Off site and on site soil incineration: contaminated soils are burned at temperatures high as much as to destroy the toxic contaminant.

Thermal desorption: generally this remediation technology is applied to remove chlorinated compounds from soils or other substrates, that are subjected to high temperature to volatilise the chemicals absorbed on particles.

Vaporous extraction: through the usage of particular chemical solvents and liquids the contaminants in the soil react and pass into a vapour state. In this way they left the soil and go on air. This in situ technique move the contamination from soil to air, thus implying the necessity of analysing the vapours that are released.

Soil Washing: the soil to be decontaminated is exported in specific site where it is washed with chemical solutions or solvents and it is cleaned. Once the cleaning operation is ended it is brought back to its original place and the entire site is decontaminated. This ex-situ technique anyway is applicable only to little site and is very expensive, due to the transportation of the soil and to the washing operations. Obviously, this way other contaminated substrates are obtained such as washing solution that need to be fatherly cleaned and disposed This ex-situ technique has the further disadvantage of modifying the soil chemical composition, thus increasing the time that the soil takes to get back to its normal state (e.g. the state in which it was before the contamination took place).

On site stabilisation/solidification: with this technology soil contaminants are converted into less soluble or less mobile forms. Solidification, in particular, involves the use of a polymer to encapsulate the soil or the vitrification by passing electricity through the melted soil. This way the contaminant is stabilised but not destroyed.

Pump and treat: this remedy of contaminated ground water can involve various treatments such a selective barrier filtration or absorption, air sparging (this remedy can be also applied in situ without pumping) that is based on volatilisation and further condensation of chemical substances.

With respect to Phytoremediation, the other decontamination techniques have the following disadvantages:

- they have higher costs;
- they can be performed only in site with a small extension. The transportation and the operation that have to be performed on the soil imply in fact a large usage of machineries that are expensive;
- they are destructive. Once one of the mentioned above decontamination technique is performed, the soil loses its natural composition and its basic components and it takes a long time to go back in a healthy state again;
- their ex-situ characteristic implies a complete modification of the landscape for all the time that the decontamination takes to be completed;
- they are not preventive. The usage of a decontamination method based on the phytoremediation enables a preventive function, that is related with the deployment of the plants in the sites to be decontaminated. In the case that a new contamination appears the dead of the plants is a clear sign to prevent the enlargement of the contamination.

ORGANIZATION OF STUDY

AND GENERAL AIM

Studies were performed:

- *in situ*, investigating the utility of some spontaneous and cultivated plants in a phytoremediation strategy and analysing the capability of a poplar plantation in remediate soil and in water control of an industrial contaminated site
- *ex situ*, making growth P. vittata L. in greenhouse to investigate the tolerance and accumulation capability of this plant to elevated Arsenic concentration with in order to introduce this specie in the island.

It's possible divide this work in 3 parts. Each of them is organized as monothematic chapter, with an identical internal partition: an "Abstract", where all chapter is summarised, an "Introduction" where the subject treated in the chapter is analysed by the literature, a "Materials and Methods", where methods of sampling and analyses are reported, a "Results", where all results obtained are listed, a "discussion", where results are explained and at last a "Conclusion", where the sense of the chapter is explained.

The three parts are:

1- **PART I - A SURVEY ON THE METAL AND METALLOID PHYTOEXTRACTION ABILITY OF SPONTANEOUS AND CULTIVATED PLANTS GROWING IN THE LANDFILL OF AN INDUSTRIAL SITE IN THE VENICE LAGOON:** to investigate the capability of different spontaneous and cultivated plant species growing on island, to examine their potential use for a phytoremediation strategy.

2- **PART II - SITE REMEDIATION AND HYDROLOGICAL CONTROL IN LANDFILLS: STUDY ON A POPLAR PLANTATION IN AN INDUSTRIAL SITE IN THE VENICE LAGOON**: to verify if in an industrial island in Porto Marghera a poplar plantation was useful both in site reclamation strategy and in the hydrological control. The hydrological control of this site became necessary since year 2008, when island was wholly separated from surrounding sea to avoid environmental contamination by a waterproof barrier.

3- **PART III - A LONG-TERM EXPERIMENT TO TEST *P. vittata* L. UNDER HIGH AS CONCENTRATION** : to investigate the As tolerance and accumulation capability of *P.vittata* exposed to a remarkable As concentration in a long-term hydroponic experiment, evaluating some implications at physiological and biochemical level.

A detailed description of the study locations (both in situ and ex situ) will be done in each chapter.

The experimental field (*in situ* location) is an Island of the industrial zone of Porto Marghera (Venice lagoon, Italy) classified as one of the most important "contaminated sites of national interest"(SIN) in Italy and considered as an area of high environmental risk which needs to be reclaimed. The island, called "Isola dei Petroli" is 12 ha and has been used as an industrial landfill. Previous study described the island as highly contaminated by As, Cd and Zn mainly in soil depth range of 1-2 m.

Since 2008 the island was wholly separated from surrounding sea by a waterproof barrier to avoid environmental contamination. This manufacture bounds the contamination scattering bat also it modifies the water balance of the island, annulling all exchanges island-sea.

In this context a vegetation cover could be useful to remove water in excess, but soil toxicity could affect the growth plants. So an investigation of species both spontaneous and cultivated grown in the island are performed to analyse their utility in a phytoremediation and phytocontrol of hydric state strategy and their capability to survival in the contaminated site.

Spontaneous plants are mainly herbaceous and shrub species, probably transported in the island by the wind or presented in the cleaned soil, used to made a unpolluted top layer.

Planted species consist in some herbaceous and tree species previous introduced in the island. Among these species the most interesting are three clones of poplar: Dvina and Lena (*Populus deltoides* Bartr.Ex Marsch) and Neva (*Populus x canadensis* Moench), planted in 2004 in a high density plantation design. The plantation is studied both for phytoextraction function and for water phytocontrol, according to knowledge that poplar species usually show a high transpiration capability.

The laboratory analyses and the *ex situ* study location are performed in the IBAF, in the CNR centre in Montelibretti.

PARTE I:

A SURVEY ON THE METAL AND METALLOID PHYTOEXTRACTION ABILITY OF SPONTANEOUS AND CULTIVATED PLANTS GROWING IN THE LANDFILL OF AN INDUSTRIAL SITE IN THE VENICE LAGOON.

Abstract

This work aimed to evaluate the capability of spontaneous and cultivated plants growing in an industrial landfill island in Porto Marghera (Venice lagoon, Italy) to take up heavy metals and metalloids such Pb, Cd, Zn and As and bioconcentrate them in their leaves and stems. Specifically, it focused on the most abundant tree, shrub and herbaceous species to identify useful plants to for the reclamation of this area. In soil of different depths and in the groundwater table, a large degree of heterogeneity in metal contamination, with values close to or higher than Italian guideline values (by Italian law D.Lgs 152/06), was found. Plants differed in pollutant accumulation. All spontaneous species, except for *Amaranthus retroflexus* L. towards zinc, showed a higher ability to exclude rather than accumulate heavy metals compared with cultivated species, making them unsuitable for the *in situ* phytoremediation of a contaminated industrial area. On the contrary, in cultivated species a valuable heavy metal concentration in above-ground organs was detected. In particular, good performances were evidenced for *Chrysopogon zizanioides* (L.) Roberty to bioconcentrate Pb and for both *Salix matsudana* Koidz. and *Salix alba* L. to bioconcentrate Cd and Zn, allowing the evaluation of the possible utilization of these species for the phytoremediation of contaminated sites.

Introduction

Heavy metals and metalloids are natural constituents of the Earth's crust. Their release into the environment can arise through the waste of many human activities such as mining, industry and agriculture in different chemical forms (i.e., chloride, sulphate and fluoride). If this waste is not properly stored, air, soil and water pollution can pose a serious threat to human and animal health. In fact, most of metals and metalloids are toxic, mutagenic and carcinogenic. Low cost, effective and sustainable methods need to be further developed to remove these contaminants from the environment or detoxify them (Bozkurt et al., 2000; LeDuc et Terry, 2005). Conventional methods developed to remediate contaminated substrates by *in situ* or *ex situ* treatment are cost intensive, technically complex and environmentally invasive procedures. In this context, the possibility of using plants and microorganisms to reduce soil and water pollution is an old and fascinating idea. Over past decades the concept of phytoremediation emerged as a new technology based on the use of vascular plants for cleaning or decreasing the toxicity of soils, sludges, sediments and wastewaters contaminated by metals, organic xenobiotics, explosives or radionuclides (Salt et al., 1998; Barcelò et Poschenrieder, 2003). Phytoremediation has numerous advantages. First, it is a solar-driven process that minimises energetic input and thereby represents an environmentally friendly technique. Furthermore, the establishment of a vegetation cover over a contaminated site can limit soil erosion, create an aerobic environment in the rhizosphere that favours microorganism colonisation and activity, enhance soil organic matter and increase soil aggregation. Moreover, phytoremediation is considered a low cost technology, estimated to be 50–80% less expensive than conventional applications (Pulford et Watson, 2003; Vamerali et al., 2009). To be even more attractive, phytoremediation should be performed by plants selected for their enhanced capability to tolerate the elevated concentration of heavy metals in soil and water and to absorb metals by roots and translocate them in above-ground organs (Robinson et al., 2000). Once moved up along the plant, metals should be preferentially stored in the woody parts that are less physiologically active with respect to foliage, not renewable and can be easily harvested and burned to produce bioenergy. In this context, research should be focused on increasing knowledge of the mechanisms by which plants can tolerate, accumulate and translocate metals, as well as exploring the variability in these traits in spontaneous and cultivated species. Then, it is important to screen out the spontaneous and cultivated plant populations growing in contaminated sites to individuate the specific capabilities of these plants to tolerate or accumulate a particular heavy metal or a combination of them. This information is useful for using these plants both as biomonitors for the evaluation of the distribution of heavy metal pollution over large territories (Djingova et al., 1999; Madejón et al., 2004) and to phytoremediate contaminated sites (Rosselli et al., 2003; Nagendran et al., 2006; Komárek et al., 2008).

The aim of this study was to investigate the capability of different spontaneous and cultivated plant species growing on an industrial landfill island in Porto Marghera (Venice lagoon), one of the most important Italian industrial sites to accumulate arsenic and heavy metals, to examine their potential use for a phytoremediation strategy.

Materials and methods

Study area

Porto Marghera is one of the main chemical districts in Italy, located in the Venice lagoon (45° 24′ 47″ N, 12° 17′ 50″ E), a shallow transitorial environment in the northern basin of the Adriatic Sea (Fig. 2). This industrial zone was created in 1917 on the border of the

Venice lagoon, as an extension of the Venice Port, to sustain oil- and coal-related activities.

Considerable industrial activities, in particular chemical production, oil refining and storage, shipbuilding, metal extraction and metallurgy, energy production and distribution, wastewater treatment and hazardous waste incineration, caused the extensive contamination of air, soil, groundwater and inner tidal canals. Moreover, industrial plants were built on marshlands, previously filled with contaminated materials. The industrial zone of Porto Marghera is now classified as one of the most important “contaminated sites of national interest” (SIN) in Italy and is considered an area of high environmental risk that needs to be reclaimed (Bellucci et al., 2002; Zonta et al., 2007).

Previous studies on the sediments of the Venice lagoon have evidenced a diffuse contamination of both organic compounds and heavy metals, with a prevalence of Zn over Pb, As and Cd, caused by activities involving the use of minerals, metals and catalysts (Argese et Bettiol, 2001; Giusti et Zhang, 2002; Libralato et al., 2008).

The study area is an island located inside the oil refinery of Porto Marghera (Fig. 2) in a macro-area called the “Area dei Petroli” (oil area). In this macro-area, studies (www.ambiente.venezia.it/suolo.asp?sub=chimicasuolo&suo=petroli#sette) have shown that 90% of soil contamination is caused by heavy metals, in particular As, Zn and Cd, with concentrations higher than the Italian law limits, mainly in the soil depth range of 1–2 m. This island, called the “Isola dei Petroli” (oil island), is 12 hectares and until recently was used as an industrial landfill. Approximately half of its surface is now taken up by oil storage containers.

Fig. 2 – Location of study area. A) Venice lagoon in Italian peninsula B) Porto Marghera C) "Isola dei Petroli"

Plant, water and soil sampling

Plants, soil and groundwater were sampled in autumn 2006 at the end of the vegetation phase. Regarding plants, this study focused on the most abundant tree, shrub and herbaceous species growing on the island (Tab.1). Plant species were spontaneous or planted but all were characterised by a good adaptation to the site conditions. For each species, three to five individuals were sampled depending on their abundance. For herbaceous and shrub species, pooled leaves from each individual were taken. Similarly, for tree species pooled leaves or stems were collected from each individual. Thirteen soil sampling points were cored in a randomised block design, covering the whole island area, and two samples for three depth layers (0–40 cm; 40–80 cm and 80–100 cm) were collected. Groundwater samples were collected from 12 piezometers randomly distributed over the study area.

Tab.1 - Plant species sampled in "Isola dei Petroli", Venice lagoon, Italy.

Plant species	Family	Origin •	Type ••
Amaranthus retroflexus L.	*Amaranthaceae*	S	H
Blackstonia perfoliata (L.) Hudson	*Gentianaceae*	S	H
Cornus sanguinea L.	*Cornaceae*	S	S
Eleagnus angustifolia L.	*Elaeagnaceae*	S	S
Ulmus minor Miller	*Ulmaceae*	S	T
Paulownia tomentosa Steud.	*Scrophulariaceae*	P	T
Phragmites australis (Cav.)Trin.	*Poaceae*	S	H
Rubus caesius L.	*Rosaceae*	S	S
Verbena officinalis L.	*Verbenaceae*	S	H
Chrysopogon zizanioides (L.) Roberty	*Poaceae*	P	H
Salix matsudana Koidz.	*Salicaceae*	P	T
Salix alba L.	*Salicaceae*	P	T
Eucalyptus camaldulensis Dehnh.	*Myrtaceae*	P	T

• Origin - S: spontaneous ; P: planted; ••Type - H: herbaceous ; S: shrub ; T: tree

Sample preparation

Soil samples were oven-dried at 60°C and passed through a 2 mm stainless steel mesh, separating the skeleton fraction. The sieved fraction was used for granulometry, pH, carbon and nitrogen determination and heavy metal and metalloid detection. Groundwater samples were stored in polypropylene bottles. Conductivity measurements were taken *in situ* by a Hydrolab QuantaG (Loveland, Colorado, USA) multiparameter probe. All plant samples, leaves and stems were oven-dried at 60°C until a constant weight was reached. Before analysis, samples were ground into a fine powder.

Heavy metal and metalloid analysis

Arsenic, cadmium, zinc and lead concentrations were determined in the plant, soil and water samples. Plant materials (0.5 g d.w.) were digested with 5 ml of HNO_3 65% (v/v) and 2 ml of $HClO_4$ 60%, whereas soil samples (1 g d.w.) were digested with 3 ml of HNO_3 65%, 9 ml of HCl 37% and 3 ml of H_2O_2 35% using the TMD20 digesting system (Velp Scientifica, Milano, Italy). The metal and metalloid concentrations on filtered extracts were determined by ICP-MS (Thermo Jarrell, Ash Iris Advantage, Thermo Electron Corp., Milford, MA, USA) in soil samples and by Zeeman Graphite Tube Atomic Absorption Spectroscopy **(**Varian SpectrAA-800**,** Mulgrave, Victoria, Australia) in plant and groundwater materials. Reagent blanks and internal standards were used where appropriate to ensure accuracy and precision in analysis.

Soil and groundwater properties and chemical analysis

To determine the soil granulometry and consequent texture, the physical separation of soil particles was carried out. In particular, a wet sieving procedure was performed to determine the amount of sand. The finer fractions, i.e. silt and clay, were separated on the basis of the fall rate in a liquid, followed by the pipette method as described by Fontaine et al. (2000). For pH analysis (HI9321 pH meter, Hanna Instruments, Woonsocket, RI, USA), 10 g of sieved and oven-dried soil was placed into a beaker with 25 ml of 1M KCl and stirred for 2 h. Carbon and nitrogen analysis was performed with a Elemental analyser (Carlo Erba CHNS 1108,Rodano, Italy). An oven-dried (60°C) sieved aliquot was ground and directly analysed on a tin cup. Groundwater samples were filtered through a 0.45 µm filter (Polypropylene Filter Media, Whatman) and analysed for pH determination. To preserve water characteristics, samples were acidified with HNO_3 to pH< 2.

Data analysis

Statistical analyses of the heavy metal concentrations in samples were performed using analysis of variance (ANOVA), and means were compared by Tukey's test (SPSSWIN software, Chicago, IL, USA). Results were evaluated on the basis of homogenous group at a given significance level ($p<0.05$).

Results

General characteristics of the site: soil and groundwater

Soil characterisation (Tab.2) showed a soil sandy clay loam with an approximately neutral pH, and low contents of total C and total N, delineating a poor soil characterised by slow biological and mineralisation processes.

Concentrations of As, Cd, Pb and Zn at sampling sites and at different depths are summarised in Tab.3. A large degree of heterogeneity in contamination at all depths was found. The levels of heavy metals were generally higher at greater depths (80–100 cm), exceeding considerably the Italian guideline values (IGV, by Italian law D.Lgs 152/06) for an agricultural soil. Values up to five times higher than the IGV were found for As, 13 times for Cd, eight times for Zn and two times for Pb considering the law limits for agricultural soils. Instead considering IGV for industrial soils mean values found in the site were lower for all pollutants, while maximum values found were up approximately to two times higher only for As and Cd.

Characteristics of the groundwater are listed in Tab.2 .Water table depth varied from 86 to 150 cm and pH was approximately neutral. Electrical conductivity analysis allowed to evaluate the depth of seawater infiltration.

Tab.2 – Characteristics of soil and groundwater of "Isola dei Petroli", Venice lagoon

SOIL CHARACTERISTICS (Mean ± St.Err.)		
Granulometry:	**S**keleton (Ø >2 mm)	0%,
(according to USDA classification)	Sand (Ø: 2.0-0.05 mm)	48%,
	Silt (Ø: 0.05-0.002 mm)	23%
	Clay (Ø: less than 0.002 mm)	29%
pH:		7.05 ± 0.05
Total C (%):		6.16 ± 0.14
Total N (%):		0.07 ± 0.02

GROUNDWATER CHARACTERISTICS (Mean ± St.Err.)		
Depth (cm) of water table (min-max)	121 ± 5.9 (86-150)	
pH	7.3 ± 0.0	
Depth (cm) average of mixing zone (fresh and salt water)	32.3 ± 8.1	
Conductivity (mS)	up mixing border	8.7 ± 1.1
	down mixing border	18.8 ± 1.5

Tab.3– Concentration (mg/kg dry matter) of arsenic, cadmium, zinc and lead in soil of "Isola dei Petroli", Venice lagoon: average and values in different depth layers.

Heavy Metals	Depth soil	Concentration min-max	mean	St.Err.	Average in Italian soil [mg/kg] • (0-80/>80 cm)	IGV•• Agr.	IGV•• Ind.
As	**average**	**3.75 - 105.15**	**20.05**	**4.88**	**10.2/10.9**	**20**	**50**
	0-40	4.70 – 41.33	13.53 b	2.76			
	40 – 80	3.75 – 18.77	6.82 b	1.01			
	80 – 100	78.86 – 105.15	97.33 a	6.24			
Cd	**average**	**1.51 – 27.70**	**8.36**	**1.18**	**0.44/0.4**	**2**	**15**
	0-40	**1.84 – 26.80**	**7.73 a**	**1.94**			
	40 – 80	**1.51 – 27.70**	**9.17 a**	**2.12**			
	80 - 100	**3.93 – 13.35**	**8.26 a**	**1.29**			
Zn	**average**	**123.60 – 1183.80**	**447.16**	**50.19**	**86.2/78.3**	**150**	**1500**
	0-40	**133.42 – 859.19**	**363.97 b**	**53.52**			
	40 – 80	**123.60 – 812.05**	**345.92 b**	**47.76**			
	80 - 100	**795.34 – 1183.80**	**1050.33 a**	**66.95**			
Pb	**average**	**22.15 – 199.52**	**75.60**	**7.75**	**31.6/25**	**100**	**1000**
	0-40	**30.55 – 154.94**	**64.43 b**	**9.34**			
	40 – 80	**22.15 – 123.33**	**58.12 b**	**7.31**			
	80 - 100	**88.91 – 199.52**	**152.79 a**	**16.84**			

The "average" are data from the mean of whole samples (n=78) while for each depth layers n=26. For comparison of means, ANOVA followed by Tukey's test, with 95% confidence level, were performed. Values in columns followed by different letter are significantly different ($p < 0.05$) among depth layers for each heavy metal analysed. • : APAT – yearbook 2009 – (Data 2005) • •: IGV- Italian Guideline Values (D.Lgs 152/06) in: Agr. - soil for agricultural uses and Ind.- soil for industrial uses.

Metal and metalloid concentrations in groundwater are depicted in Tab.4. The mean concentration values were under the IGV threshold except for As. High scattered values of metal concentration were detected among piezometers, with the upper values of the range 3–4 times higher than the maximum limit.

Tab.4 - Concentration (µg/kg) of arsenic, cadmium, zinc and lead in groundwater samples In "Isola dei Petroli", Venice lagoon.

	As	**Cd**	**Zn**	**Pb**
IGV •	**10**	**5**	**3000**	**10**
Groundwater samples Mean ± St.Err. (min-max)	15 ± 1 (12-20)	4 ± 2 (0-14)	1500 ± 659 (70-3668)	6 ± 1 (3-16)

n= 12; • IGV- Italian Guideline Values in groundwater (D.Lgs 152/06)

Heavy metals in plants

Thirteen plant species, chosen among the most abundant tree, shrub and herbaceous species, spontaneous or cultivated, growing on the island, were sampled. The concentration of different heavy metals in the leaves of the sampled plant species is shown in Tab.5. Large variations among species in metal concentration and distribution occurred, following the order Zn > Pb > Cd and As.

For most species analysed, the concentration of As was within the normal range (1.0–1.7 mg/kg) reported for leaf tissues of plants grown in unpolluted areas (according to Kabata-Pendias and Pendias, 2001). Values exceeding this range were found in *Blackstonia perfoliata* (L.) Hudson (4.67 mg/kg), *Verbena officinalis* L. (2.13 mg/kg) and *Ulmus minor* Miller (1.87 mg/kg). However, these values were below the threshold of phytotoxicity (5 mg/kg, Madejon et al., 2004).

Tab.5 – Concentration (Mean ± Err.St.) of arsenic, cadmium, zinc and lead (mg/kg dry matter) in leaves of 13 species sampled in "Isola dei Petroli", Venice lagoon.

Plant species	As		Cd		Zn		Pb	
Amaranthus retroflexus L.	1.16 ± 0.19	bc	1.16 ± 0.19	c	841.7± 147.8	a	9.20 ± 1.25	c
Blackstonia perfoliata (L.) Hudson	4.67 ± 0.30	a	0.78 ± 0.15	c	200.6 ± 42.1	c	56.59 ± 9.44	b
Cornus sanguinea L.	1.06 ± 0.04	c	0.12 ± 0.03	c	52.5 ± 5.6	c	13.46 ± 1.56	c
Eleagnus angustifolia L.	0.95 ± 0.03	c	0.65 ± 0.07	c	103.7 ± 5.0	c	12.16 ± 0.66	c
Ulmus minor Miller	1.87 ± 0.23	bc	0.54 ± 0.02	c	81.5 ± 21.2	c	8.27 ± 0.86	c
Paulownia tomentosa Steud.	0.90 ± 0.04	c	0.18 ± 0.08	c	381.4 ± 164.3	bc	8.54 ± 0.02	c
Phragmites australis(Cav.) Trin.	1.29 ± 0.17	bc	0.11 ± 0.02	c	65.8 ± 13.7	c	21.72 ± 1.49	c
Rubus caesius L.	1.31 ± 0.15	bc	0.63 ± 0.16	c	184.2 ± 57.0	c	8.98 ± 1.44	c
Verbena officinalis L.	2.13 ± 0.82	b	0.40 ± 0.16	c	186.2 ± 34.0	c	20.95 ± 2.88	c
Chrysopogon zizanioides (L.) Roberty	0.95 ± 0.02	c	0.82 ± 0.21	c	100.8 ± 15.2	c	263.20 ± 23.25	a
Salix matsudana Koidz.	1.09 ± 0.03	bc	10.93 ± 0.67	a	698.3 ± 75.7	ab	7.32 ± 0.78	c
Salix alba L.	1.13 ± 0.05	bc	10.73 ± 0.89	a	1079.3 ±378.8	a	6.06 ± 0.24	c
Eucalyptus camaldulensis Dehnh.	1.15 ± 0.08	bc	5.60 ± 1.15	b	351.69 ± 31.5	bc	37.07 ± 5.24	bc
NORMAL RANGE •	1.0 - 1.7		0.05 - 0.2		27 -150		5 – 10	
PHYTOTOXIC RANGE ••	5 - 100		5 - 700		500 - 1500		30-300	

For comparison of means, ANOVA followed by Tukey's test, with 95% confidence level, were performed. Values in columns followed by different letters in the columns indicate significantly differences ($p< 0.05$) among species for each heavy metals analyzed. • Normal values (range) for trace elements in plants, according to Kabata-Pendias and Pendias (2001) – • • Phytotoxic range according to Alloway (1990) except for As (Peer et al. 2006).

Cadmium and zinc concentrations in leaves were higher than the normal values in 10 and eight of the 13 plants, respectively. Both *Salix* species showed the highest values of Cd concentration among sampled plants. Moreover, *Salix alba* L. also showed the highest detected values for Zn concentration. Beyond *S. alba* L., *Salix matsudana* Koidz. and *Amaranthus retroflexus* L. both showed Zn concentration values higher than the phytotoxic threshold reported for plants by Alloway (1990).

Lead concentration exceeded the normal value in seven out of the 13 plants, reaching the highest value in *Chrysopogon zizanioides* (L.) Roberty leaves. Nevertheless, all sampled plants revealed Pb concentrations in the leaves below the phytotoxic threshold.

Tab.6 reports the concentration of heavy metals in leaves and stems in the planted woody species (*S. alba*, *S. matsudana* and *Eucalyptus camaldulensis* Dehnh.)**.** In these woody plants, stems accumulated a higher concentration of As and Pb than leaves, except for *E.camaldulensis* where no significant difference in lead concentration between leaves and stem was found. On the contrary, Cd and Zn concentrations were higher in leaves than stems. The two *Salix* species were not significantly different in cadmium concentrations in both stems and leaves; the latter values were higher than those detected in *E. camaldulensis* leaves. By contrast, *Eucalyptus* plants revealed notably higher Pb concentrations in leaves compared with the two *Salix* species.

Tab.6 – Concentration (Mean ± Err.St.) of arsenic, cadmium, zinc and lead (mg/kg dry matter) in leaves and stems of the tree species found in the island and ANOVA results for species and part plants (leaves and stems) in tree species analysed.

Tree species		As		Cd		Zn		Pb	
Salix matsudana Koidz.	**Leave**	1.09 ± 0.03	b	10.93 ± 0.67	a	698.27 ± 75.67	ab	7.32 ± 0.78	b
	Stem	1.89 ± 0.08	a	3.23 ± 0.14	bc	118.96 ± 8.71	c	52.64 ± 8.34	a
Salix alba L.	**Leave**	1.13 ± 0.05	b	10.73 ± 0.89	a	1079.3± 378.8	a	6.06 ± 0.24	b
	Stem	1.96 ± 0.09	a	3.20 ± 0.20	bc	130.65 ± 10.02	c	50.19 ± 4.0	a
Eucalyptus camaldulensis Dehnh.	**Leave**	1.15 ± 0.08	b	5.60 ± 1.15	b	351.69 ± 31.48	bc	37.07 ± 5.24	ab
	Stem	2.22 ± 0.16	a	0.84 ± 0.11	c	143.47 ± 15.34	c	58.57 ± 5.59	a
ANOVA results		**As**		**Cd**		**Zn**		**Pb**	
Species		ns		***		***		***	
Part Plants		***		***		*		**	
Species x Plant Parts		ns		ns		*		ns	

For comparison of means, ANOVA followed by Tukey's test, with 95% confidence level, were performed.. Different letters in the columns indicate significatively differences ($p< 0.05$) among species for each heavy metals analyzed. In ANOVA results: ns not significant; * $p< 0.05$; ** $p<0.005$; *** $p<0.001$, significance of the main effects and their interaction.

Bioconcentration factor (BCF)

The BCF is defined as the ratio between total metal concentration in leaves and soil (Zayed, 1998) and describes the capability of a plant to extract heavy metals from a contaminated substrate. Tab.7 reports the BCF of leaves and stems in analysed species. The BCF of arsenic was rather low (0.04–0.1) in all investigated plants, except for *B. perfoliata* where a BCF three times higher than the mean value calculated for all the species was observed.

The leaf BCF for cadmium and zinc was highly variable among species, ranging from 0.01 to 1.31 for Cd and from 0.12 to 2.41 for Zn. The highest BCF values were found in the *Salix* species (*S. matsudana* 1.31 for Cd and 1.56 for Zn; *S. alba* 1.28 for Cd and 2.41 for

Zn). A notable BCF value (1.88) for zinc was also observed in *A. retroflexus* The BCF values for Pb ranged between 0.08 and 3.8, with the value of *C. zizanioides* more than sevenfold higher than the mean value calculated for all species.

Stem BCF values in the three planted woody species were similar for the different metals analysed, except for Cd where a lower value in *E. camaldulensis* compared with the two *Salix* species was observed.

Tab.7 - Bioconcentration factor (BCF) for different heavy metals in leaves of thirteen species and in stems of three woody plants sampled in "Isola of Petroli"

Plant species	As	Cd	Zn	Pb
LEAVES				
Amaranthus retroflexus L.	0.06 ± 0.01 bc	0.23 ± 0.105 c	1.88 ± 0.33 a	0.12 ± 0.017 c
Blackstonia perfoliata (L.) Hudson	0.23 ± 0.015 a	0.09 ± 0.018 c	0.45 ± 0.094 c	0.75 ± 0.125 b
Cornus sanguinea L.	0.05 ± 0.002 bc	0.02 ± 0.007 c	0.12 ± 0.012 c	0.16 ± 0.021 c
Eleagnus angustifolia L.	0.05 ± 0.002 c	0.08 ± 0.009 c	0.23 ± 0.011 c	0.16 ± 0.009 c
Ulmus minor Miller	0.09 ± 0.015 bc	0.06 ± 0.002 c	0.18 ± 0.047 c	0.11 ± 0.011 c
Paulownia tomentosa Steud.	0.04 ± 0.002 c	0.02 ± 0.010 c	0.85 ± 0.367 bc	0.11 ± 0.0003 c
Phragmites australis (Cav.) Trin.	0.06 ± 0.008 bc	0.01 ± 0.002 c	0.15 ± 0.031 c	0.29 ± 0.02 c
Rubus caesius L..	0.07 ± 0.008 bc	0.08 ± 0.020 c	0.41 ± 0.127 c	0.12 ± 0.019 c
Verbena officinalis L.	0.11 ± 0.041 b	0.05 ± 0.019 c	0.42 ± 0.076 c	0.28 ± 0.038 c
Chrysopogon zizanioides (L.) Roberty	0.05 ± 0.001 c	0.10 ± 0.026 c	0.23 ± 0.034 c	3.48 ± 0.308 a
Salix matsudana Koidz.	0.05 ± 0.002 bc	1.31 ± 0.081 a	1.56 ± 0.169 ab	0.10 ± 0.010 c
Salix alba L.	0.06 ± 0.002 bc	1.28 ± 0.107 a	2.41 ± 0.847 a	0.08 ± 0.003 c
Eucalyptus camaldulensis Dehnh.	0.06 ± 0.004 bc	0.67 ± 0.137 b	0.79 ± 0.070 bc	0.49 ± 0.069 bc
STEMS				
Salix matsudana Koidz.	0.09 ± 0.004 a	0.39 ± 0.017 a	0.27 ± 0.019 a	0.70 ± 0.11 a
Salix alba L.	0.10 ± 0.004 a	0.38 ± 0.024 a	0.29 ± 0.022 a	0.66 ± 0.053 a
Eucalyptus camaldulensis Dehnh.	0.11 ± 0.008 a	0.10 ± 0.013 b	0.32 ± 0.034 a	0.77 ± 0.074 a

For comparison of means, ANOVA followed by Tukey's test, with 95% confidence level, were performed both in leaves and in stems. Different letters in the columns indicate significatively differences ($p < 0.05$) among species for each heavy metals analyzed, in leaves and stems separately.

Discussion

Industrial sites are known to cause severe heavy metal pollution to adjacent ecosystems. In the past few years, numerous studies have highlighted the role of plants in metal polluted areas regarding environmental stabilisation and pollution control. In fact, it was reported that a vegetation cover can limit the dispersion of metal containing soil particles exerted by water and wind erosion, and reduce the water transfer along the soil profile towards the groundwater table (Vangronsveld et al., 1995; Yanqun Z. et al., 2005; Yang et Ye, 2009; Migeon et al., 2009).

A successful reclamation of degraded industrial sites is conditioned by the choice of selected plant species that can efficiently colonise and grow in metal contaminated soils (Evanylo et al., 2005; Dongmei et Changqun, 2008). In this context, natural or assisted-selection can represent a valuable tool for identifying metal accumulating plants and improving them for characteristics useful for more efficient metal phytoextraction. The physiological and biochemical characterisation of plants growing in contaminated areas can then single out plant species or genotypes with a particular ability to tolerate, extract and bioaccumulate metals in their organs. Accordingly, this work was focused on assessing the ability of plants, spontaneous or cultivated, in an industrial landfill island in Porto Marghera (Venice lagoon, Italy) to accumulate arsenic and heavy metals to evaluate their potential use for phytoremediation.

Previous studies characterising the contamination of the sediments and waters in the Venice lagoon have highlighted the role played by the Porto Marghera industrial area as the main source of heavy metals (Apitz et al., 2007; Bernardello et al., 2006; Mattiuzzo et al., 2007).

On the "Isola dei Petroli", an area used as landfill for industrial waste, a considerable patched contamination of soil and groundwater by As, Cd, Zn and, to a lesser extent, Pb was detected (Tab.3 and Tab.4). These results are consistent with a previous study in this area that reported that As, Zn and Cd were the main soil and water contaminants with concentrations higher than the Italian law limits, especially in the 1–2 m soil depth layer (http://www.ambiente.venezia.it/suolo.asp?sub=chimicasuolo&suo=petroli#sette).

Patched contamination detected in the area could be ascribed to the different composition of the industrial waste that, covered by an unpolluted soil layer, gave rise to the island over time. Moreover, the different degree of contaminant leaching because of both groundwater table movement and the origin of soil contributed to soil contamination.

Several studies have focused on the factors that affect the movement of trace elements along the soil profile and cause groundwater pollution. These studies underlined that metal mobility depends on the geochemical properties of soil as well as other environmental factors (Prokop et al., 2003; Maddocks et al., 2009; Zhao et al., 2009). Garcia et al. (2009) showed that the mobility of As, Cd, Zn and Pb in a sandy-loam textured soil, similar to that on the "Isola dei Petroli", followed the order Pb < As < Zn and Cd. Data reported in Tab.3 evidence a homogeneous Cd distribution in all soil layers because of the high mobility of Cd. On the contrary, lesser mobile elements such as As and Pb accumulated preferentially in the deeper layers sampled. Zn distribution along the soil profile could be the result of the particular soil composition, i.e., industrial waste that could have reduced the mobility of this metal despite the movement of seasonal groundwater to the top layer.

Significant groundwater contamination by heavy metals was detected (Tab.4). This aspect is a particular environmental pollution hazard, especially on an island. In fact, seawater intrusion, a common phenomenon on the island and in coastal waters, represents a serious risk factor for pollutant diffusion to marine ecosystems. Scattered data regarding metal presence in the groundwater samples might be able to reflect the patched distribution of soil contaminants, leaching processes exerted by rainfall and degree of seawater intrusion.

On the “Isola dei Petroli”, the vegetation cover consists of spontaneous species, mainly herbaceous and shrubbery (Tab.1). Adult plants of *E. camaldulensis, S. alba, S. matsudana*, *Paulownia tomentosa* Steud. and *C. zizanioides* were all recognised as planted species in the study area.

Analysis of metal concentration in leaves (Tab.5) revealed that, as commonly found (Yanqun Z. et al., 2005, Dongmei L. et al., 2008), plants growing on polluted soils showed different adaptive responses depending on the heavy metal element. In fact, these plants exhibited different capabilities to exclude or concentrate metals into their organs. As a general trend, spontaneous species showed lower heavy metal concentration than planted species. The former evidenced a good ability to exclude contaminants, (Madejon et al., 2004), except for *B. perfoliata* and *A. retroflexus* for As and Zn, respectively. On the contrary, planted species seemed to express a greater ability to concentrate metals in their leaves, even if the metal concentration degree depended on the species and metal analysed.

On the “Isola dei Petroli”, the range of arsenic concentration detected on spontaneous and planted leaf species varied from 0.9 to 4.67 mg/kg, in agreement with that found by Burgos et al. (2008) in spontaneous grasses growing in mine spill-contaminated soil and by Del Rio et al. (2002) in analyses of 99 spontaneous plant species on a polluted site in Mexico. A notable variability of metal concentrations among plant species occurred, varying from 0.11 to almost 10 mg/kg for Cd and 52.5 to 1079.3 mg/kg for Zn**.** These data are in accordance with those previously reported by Del Rio et al. (2002), but are higher than those found by Burgos et al. (2008) and Brunetti et al. (2009). Lead concentration in the analysed plants ranged from 7.32 to 263.2 mg/kg, in agreement with the values reported by Del Rio et al. (2002) and Migeoen et al. (2009). The high concentration value detected for Pb in *C. zizanioides* confirms the capability of this plant to extract this metal from different contamination conditions such as hydroponics (Andra et al., 2009), pot (Wilde et al., 2005; Chiu et al., 2006) and field (Yang et al., 2003).

Among tree species, heavy metal concentration in leaves and stems differed markedly (Tab.6). Cd and Zn concentrations in leaves were higher than in stems, whereas As and Pb showed the opposite behaviour, as previously reported by Unterbrunner et al. (2007) and Tlustos et al. (2007). This feature is in accordance with the mobility of metals along plant axis (Boruvka et al., 1997; Ko et al., 2007) that follows the order Zn > Cd > Pb and As. Cd and Zn concentrations detected in willow leaves are similar to those found by French et al. (2006) for plants growing on industrial waste and higher than those reported by Vamerali et al. (2009) in pyrite mine waste near Venice.

No differences in metal concentration in stems were found among species. By contrast, metal concentration in leaves varied markedly, being higher in willow species than in *E. camaldulensis* except for lead. The significance of a preferential accumulation in woody plant tissues instead of foliage has been discussed by Zacchini et al. (2009) and Pietrini et al. (2009). The storage of metals in non-renewable parts of plants (i.e., stems and branches) represents a goal of the phytoremediation strategy because it allows for the more effective removal of metals from soils without recycling them in the top soil by leaf shedding. Moreover, woody biomass obtained by tree plantation can also be used for energy production (Puldorf et al., 2003; Rockwood et al., 2004) by appropriating pyrolysis and burning processes, with metal remaining in the ashes. These arguments should be considered when choosing the plants to be used to reclaim heavy metal-polluted sites.

To better evaluate the capability of plants growing on the “Isola dei Petroli” to extract metals, the BCF calculation for leaves and stems was performed (Tab.7). In all plants analysed, the BCF value for As was below one, a recognised threshold for assessing the ability of a plant to hyperaccumulate metals (McGrath and Zhao, 2003; Yanqun et al., 2005). However, the BCF values obtained in this investigation were 10 times higher compared with some herbaceous species growing on mine tailing (Chang et al., 2005), even if these values are notably lower than those of hyperaccumulating ferns (Ma et al., 2001). In woody plants, stem tissues exhibited a higher BCF value than leaves because of the low mobility of this metal inside plants.

S. alba and *S. matsudana* leaves showed BCF values for Cd and Zn higher than one, confirming the good ability of these *Salicaceae* plants to accumulate these metals. In fact, many authors pointed out the great capability of willows to accumulate Cd and Zn in their organs under different pollution and growing conditions (Landberg and Greger, 1996; Rosselli et al., 2003; Lunackova L. et al., 2003; Zacchini et al., 2009). According to Chehregani et al. (2009), the zinc accumulation capability of *A. retroflexus* found on the "Isola dei Petroli" characterises this species as a Zn, Pb, Cu, Cd and Ni accumulator.

All BCF values for Pb in the analysed plants were below one except for *C. zizanioides*, which showed a notable capability to bioaccumulate this metal in the aerial parts. This plant has been investigated for its special affinity and tolerance to toxic heavy metals (especially Zn and Pb) and metalloids and its capability to tolerate several environmentally critical conditions such as temperature, salinity, alkalinity, floods and the presence of xenobiotics (Srivastava et al., 2008). In this work, *C. zizanioides* exhibited a BCF value several times lower than that reported by Boonyapookana et al. (2005) in hydroponics, even if this growing system could have exploited the maximum potentiality for this plant to concentrate lead in its tissues.

Conclusion

The survey of the most abundant plant species growing on the "Isola dei Petroli" and analysis of their ability to bioconcentrate metals show a different behaviour between spontaneous and planted species. All spontaneous species, except for *A. retroflexus* towards zinc, exhibited a higher ability to exclude rather than accumulate heavy metals compared with planted species, resulting not suitable for the *in situ* phytoremediation of a contaminated industrial area. Nevertheless, for an appropriate evaluation of the role of a natural vegetation cover in contaminated industrial soil, it should be considered that naturally growing plants limit pollutant leaching and metal mobility along soil profiles and modify the rhizosphere to favour biological processes. Planted species seemed more suitable for reclaiming metal-contaminated sites. In particular, *C. zizanioides* confirmed its valuable capability to mitigate lead contamination in an industrial site, whereas *S. matsudana* and *S. alba* showed the potential to reclaim Cd- and Zn-contaminated soils. The suitability of these *Salicaceae* for the phytoremediation of metal-polluted sites is worthy of further investigation. Further studies should consider other interesting traits such as adaptability to harsh soil conditions, high biomass productivity, effective nutrient uptake and high evapotranspiration that can limit water movement in the soil.

PART II:

SITE REMEDIATION AND HYDROLOGICAL CONTROL IN LANDFILLS: STUDY ON A POPLAR PLANTATION IN AN INDUSTRIAL SITE IN THE VENICE LAGOON

Abstract

This work aimed to verify if, in an industrial site located on a small island (Porto Marghera, Venice, Italy), a poplar plantation was useful both in site reclamation strategy and in the hydrological control of the site that, further to reduce transfer of contaminated water down to the water table, became fundamental since year 2008, when island was wholly separated from the surrounding sea by a waterproof barrier to avoid environmental contamination. The plantation consisted of three poplar clones: two *Populus deltoides* Bartr. Ex Marsch clones (Dvina, Lena) and one *Populus x canadensis* Moench clone (Neva). P..To investigate the poplar capability in accumulate As, Cd and Zn plant material, soil and groundwater table were sampled from 2006 to 2008 while to study the hydrological control by the plantation, plant transpiration was measured by Granier sensors during the three years.

The results showed as the poplar plantation was able to regulate the hydrological balance of the site during growing season, with the exception of occasional heavy thunderstorms at the end of summer. Differences among clones were found for extraction efficiency of Cd, Zn and As: Cd and Zn are more bioconcentrated and clone Neva seems to show the best performances.

Introduction

Trees and forests play an important role in the hydrological cycle. In recent years, several studies on the quantification of the effect of the land use on soil water dynamics and on soil characteristics have been performed (Yunusa et Newton, 2003; Jackson et al., 2005; Gyenge et al., 2009;).

During their normal physiological process plants pump large amount of water, but also solutes and organic matter. This pumping action can be exploited to improve degraded environments by stabilizing, removing or breaking-down contaminants in the substrates (Al-Yousfi et al., 2000; Robinson et al., 2003). The bio-pump characteristic is related to the transpiration process: the large water –potential difference between soil-plant-atmosphere drives the flow of sap from the roots to the leaves. The sap flow movement depends on the water-potential in the leaves and meteorological parameters as well as on soil moisture. (Raven 1990, Canny, 1998; Roberts, 2007)

The direct measurements of sap flow is the most used way to follow the water consumption of trees in their natural environment. Much attention has been given to water use at the scale of individual leaves and to how different species are adapted morphologically and physiologically to drought in controlled environment conditions. The development of techniques for measuring sap flow in whole trees (Cermák et al., 1973; Granier, 1987) has made it possible to study transpiration and water relations at the whole trees or stand scale (Zhang et al., 1999; Kubota et al., 2005). There are different methods of sap flow monitoring: each of them has specific advantages and all o f them are based on heat diffusion in the xylem (Granier et al., 1996).

Scaling-up is often required in ecosystem studies in order to extrapolate local measurements to larger areas. Extrapolation of sapflow can be done at different spatial scales: from sensor to tree, from tree to stand and even up to a regional scale (Granier et al., 1996).

Numerous studies have highlighted the role of plants in areas polluted by metals in environmental stabilization and pollution control. In fact, it has been reported that vegetation covers can limit the dispersion of soil particles containing metals exerted by water and wind erosion, and reduce the water transfer along the soil profile towards the groundwater table (Vangronsveld et al., 1995; Yanqun et al., 2005; Yang et Ye, 2009).

Pollutants are taken up by plants generally by macro and micro nutrient transport systems, that are often metal chemical analogues and are accumulated in plant organs. Ernst et Nelissenl (2008) found that bleeding sap and leaves of silver birch can be considered as bioindicators of metal contaminated soils.

Hence, plants can be used for treating environmental contaminants such as heavy metals, trace elements, organic or radioactive compounds in soils, groundwater and industrial waste in an emerging cleanup technology indicated as phytoremediation. (Baker et al., 1991; Raskin et al., 1997)

The aim of this study was to verify if in an industrial site located on a small island (Porto Marghera, Venice, Italy), a poplar plantation was useful both in site reclamation strategy and in the hydrological control of the site that, further to reduce transfer of contaminated water down to the water table, became fundamental since year 2008, when the island was wholly separated from the surrounding sea by a waterproof barrier to avoid environmental contamination.

Materials and methods

The study area

Porto Marghera is one of the main chemical districts in Italy, located in Venice lagoon (45°24′47″N, 12°17′50″E), a shallow transitorial environment in the northern basin of Adriatic Sea. This industrial zone was created in 1917 on the border of the lagoon, as an extension of the Venice Port, in order to sustain activities related to oil and coal.

The considerable industrial activities, in particular the production of different basic chemicals, oil refining and storage, shipbuilding, metal extraction and metallurgy, energy production and distribution, wastewater treatment and incineration of hazardous waste, caused the extensive contamination of air, soil, groundwater and inner tidal canals. Moreover, industrial plants were built on marshlands, that were previously filled with materials, frequently contaminated. The industrial zone of Porto Marghera is now classified as one of the most important "contaminated sites of national interest" (SIN) in Italy and it is considered as an area of high environmental risk which needs to be reclaimed (Bellucci et al., 2002; Zonta et al, 2007).

Previous studies on the sediments of Venice lagoon have evidenced a diffuse contamination by both organic compounds and heavy metals, with a prevalence in Zn over Pb, As and Cd, caused by activities involving the use of minerals, metals and catalysts (Argese et Bettiol, 2001; Giusti et Zhang, 2002; Libralato et al., 2008).

The study area is an island located inside the industrial zone of Porto Marghera, in a macro-area called "Area dei Petroli" (Oil area). In this macro-area some studies (www.ambiente.venezia.it/suolo.asp?sub=chimicasuolo&suo=petroli#sette) showed that 90% of soil contamination is caused by heavy metals, in particular As, Zn and Cd, with concentrations higher than the Italian law limits, mainly in the soil depth range of 1-2 m.

This 12 hectares island is called “Isola dei Petroli” (Oil Island) and it was used as landfill of industrial wastes until few years ago. Approximately, half of its surface is now taking up by oil storage containers.

To avoid environmental contamination, since 2008 the island was physically separated from surrounding sea by a waterproof barrier.

Meteorological parameters

Some meteorological variables were monitored continuously throughout the year with an automatic weather station. All the measured parameters and relative sensors are listed in Tab.8.

Tab. 8 - Measured parameters and relative sensors used for meteorological monitoring

Parameters	Sensor type	Unit of measurement
Air temperature	Rotronic MP-100	°C
Air umidity	Rotronic MP-100	%
Photosynthetically active radiation	LiCor 190	$\mu mol\ m^{-2}\ s^{-1}$
Global incoming radiation	Kipp&Zonen CM5	$W\ m^{-2}$
Global reflected radiation	Kipp&Zonen CM5	$W\ m^{-2}$
Net radiation	Radiometer REBS	$W\ m^{-2}$
Radiative flux in soil	Plate REBS	$W\ m^{-2}$
Precipitation	Pluviometer ARG100	mm
Soil water content (5-20 cm) (three sensors in total)	TDR CS 616 Campbell	$m^3\ m^{-3}$ (converted to %)
Groundwater depth	Piezometer TecnoEl	Cm

Data are recorded each 10 seconds and averaged (or summed for precipitation) each 30 minutes on a CR10 datalogger (Campbell Scientific, Logan, UTAH, USA).

The potential evapotranspiration of the site was calculated according to the Penman-Monteith equation – FAO method equation (Allen et al., 1998)

The plantation

The studied plantation, established in 2004, consisted of three poplar clones: two *Populus deltoides* Bartr. Ex Marsch clones (Dvina, Lena) and one *Populus x canadensis* Moench clone (Neva). Planting density was 5880 stems/ha (spacing 1.7 x 1 m) and clones were disposed in randomized rows. Experimental area was composed by four parts: three repeated blocks with plantation and one with only spontaneous vegetation used as control.

Biomass sampling

To assess biomass of poplar clones, the average-tree approach was used, involving destructive sampling of trees that best represent the mean size of the plantation and using the number of trees in the plantation to expand mean-tree values to a plot surface. The selection of sample trees was based on multiple characteristics, i.e. the average diameter at breast height (DBH) and height of the plantation. Plant DBH and heights of all trees were measured annually. Three "average" trees, with good form and vigour, were selected for destructive sampling every year. Each sampled tree was divided into stem, branches and leaves. Green weights of all components were determined in the field, and samples were collected to assess their dry-to-fresh weight ratio.

Heavy metals content in trees, soil and water

Plant components, soil and groundwater were sampled at the end of every growing season in 2006, 2007 and 2008. For each clones, leaves, branches and stem were taken, from plants of each row in the experimental plots. Samples taken from single rows were pooled for subsequent analysis. Nine soil sampling points were cored in a randomised block design, covering the whole experimental area, and samples for three depth layers (0–40 cm; 40–80 cm and 80–100 cm) were collected. Groundwater samples were collected from 12 piezometers randomly distributed over the study area. Soil water was sampled by 9 lisimeters (6 under the plantation and three under the control area) placed grouped in three at 15, 30 and 45 cm depth.

Soil samples were oven-dried at 60°C and passed through a 2 mm stainless steel mesh, separating the skeleton fraction. The sieved fraction was used for granulometry, pH, carbon and nitrogen determination and heavy metal and metalloid detection. Groundwater and soil water samples were stored in polypropylene bottles. Conductivity measurements in groundwater were taken *in situ* by a Hydrolab QuantaG (Loveland, Colorado, USA) multiparameter probe. All plant samples, leaves and stems were oven-dried at 60°C until a constant weight was reached. Before analysis, samples were ground into a fine powder.

Arsenic, cadmium and zinc concentrations were determined in the plant, soil and water samples. Plant materials (0.5 g dry weight) were digested with 5 ml of HNO_3 65% (v/v) and 2 ml of $HClO_4$ 60%, whereas soil samples (1 g d.w.) were digested with 3 ml of HNO_3 65%, 9 ml of HCl 37% and 3 ml of H_2O_2 35% using the TMD20 digesting system (Velp Scientifica, Milano, Italy). The metal and metalloid concentrations on filtered extracts were determined by ICP-MS (Thermo Jarrell, Ash Iris Advantage, Thermo Electron Corp., Milford, MA, USA) in soil samples and by Zeeman Graphite Tube Atomic Absorption Spectroscopy **(**Varian SpectrAA-800**,** Mulgrave, Victoria, Australia**)** in plant and groundwater materials. Reagent blanks and internal standards were used where appropriate to ensure accuracy and precision in analysis.

The bioconcentration factor (BCF) was used to analyse the capability of poplar clones to extract heavy metals from the contaminated substrate. This index was defined by Zayed (1998) as the ratio between total metal concentration in leaves and soil.

Measurements of tree sap flow

Xylem sap flow density was monitored continuously throughout the growing season using the heat dissipation method according to Granier (Lu et al., 2004). The Granier system consists of two sensor probes (Fig.3) to measure the quantity of sap moving around the sensors for a given sapwood area. Each probe consists of a heating element (which also represents the effective sensing part of the probe, typically 20 mm long), wound around a steel needle containing a T-type thermocouple (Copper-Constantan) with the tip located in the middle of the heating element. The constantan ends of the two thermocouples are connected to measure the temperature difference between the two probes at the ends of the copper wires. The two probes are typically inserted radially into the stem 10-15 cm apart, in pre-inserted heat-distributing aluminium tubes. The upper probe is continuously heated at constant power (approx 0.2 W) while the lower probe is left unheated to measure the ambient temperature of the wood tissue and acts as a reference probe. Temperature difference between the two probes is influenced by the heat dissipation caused by the sap flowing in the vicinity of the heated probe (Lambs et Muller, 2002; Lu et al., 2004). The difference is maximum during night, with no sap flowing, and minimum at the hour of maximum sap flow.

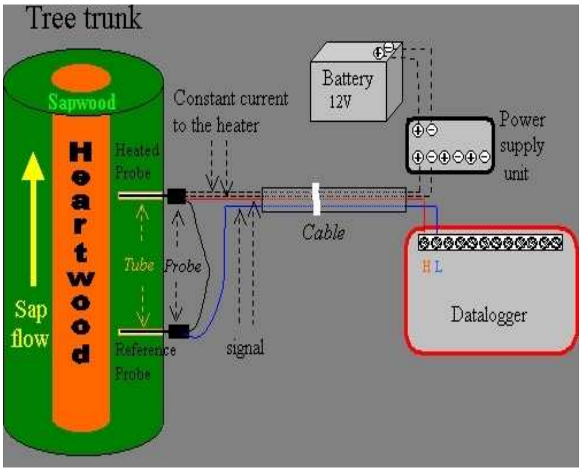


Fig. 3 – Granier system diagram

A preliminary test installation was made at the end of growing season in 2006 (September) to test the sensors and the clones variability. In this test only two poplar clones (Dvina and Lena) were considered.

From 2007 to 2009, the trees to be measured were selected among the 30% bigger and healthy plants, with the aim to investigate the contribution of a mature population to hydrological control of a mature population in this site.

The sensors were installed before the starting of the growing season and were generally removed after leaves had fallen to avoid damage in winter. Every years, six trees, two for each clones, were monitored for sap flow.

The output value of each sensor was measured every 10 s and averaged over 30 minutes.

This study utilized environmental and sap flow density data measured respectively from beginning of September 2006 to November 2009. The duration of the growing period was generally from April to October, with earlier start in mid March and later closing in early November. Transpiration of the measured trees was upscaled to the whole plantation using the average transpiration of each clone multiplied by the number of plants of that clone present in the plantation. For days when measured sap flow data were missing, transpiration at stand scale was calculated using the relationship between measured data and global incoming radiation.

Since the main interest is in seasonal and long-term trends, meteorological variables and sap flux density were aggregated to *daily, monthly and seasonal* values depending on circumstances.

Statistical analysis

Statistical analyses of the heavy metal concentrations in samples were performed using analysis of variance (ANOVA), and means were compared by Tukey's test (SPSSWIN software, Chicago, IL, USA). Results were evaluated on the basis of homogenous group at a given significance level ($p<0.05$).

Results

Climate during the study period

Meteorological variables were measured from July 2006 to November 2009 and their monthly trend are shown in Fig.4.

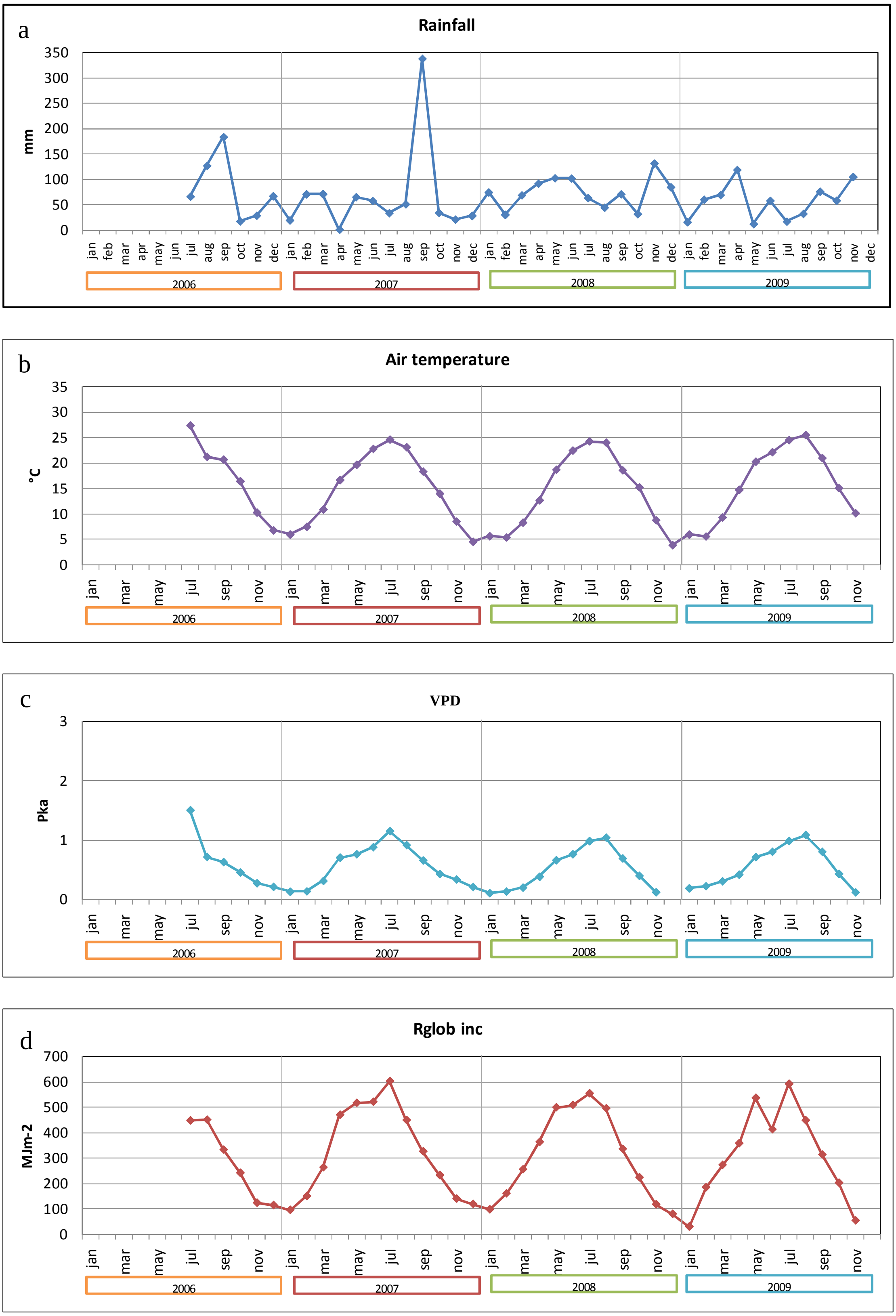
Mean temperatures across sampling period were 17.1 °C in 2006 (June-December), 14.7 °C in 2007, 14.0 °C in 2008 and 15.8° C in 2009 (January-November). Seasonal trends in temperatures were similar across the sampling years exhibited as well as those of incident radiation.

Overall, similar trends in precipitation were observed from 2006 to 2009. Total annual precipitations during the period of study were 485.8 mm (June-December), 783.2 in 2007, 888.0 in 2008 and 614.4 in 2009 (January-November); end-of-summer thunderstorms occurred in September 2006 and 2007, the latter particularly relevant.

Mean annual vapour pressure deficit (VPD) was 0.63 kPa (June-December), 0.55 kPa, 0.49 kPa and 0.55 kPa (Jan-Nov) respectively in 2006, 2007, 2008 and 2009. The seasonal trends in monthly VPD were comparable among years.

Soil water content measured at the same depth showed a similar trend in planted and control plot. However, the seasonal variations in soil water content were affected by the presence of plant canopy, with the planted plot showing lower values at growing seasons' peak in 2006 and 2007 due to evapotranspiration and slightly higher values in Autumn, when soil was temporarily covered by leaf litter. The difference generally disappeared in Winter.

Groundwater table was oscillating in time reaching maximum values in spring and winter and minimum values at the end of each summer. Very low values were measured in summer 2007. In that year, a relatively warmer and drier spring and the limited amount of rain of summer months (the thunderstorm was at end of summer) resulted in higher evaporative demand, reaching maximum VPD and minimum air and soil water content values.


a
Rainfall
mm
350
300
250
200
150
100
50
0
jan feb mar apr may jun jul aug sep oct nov dec
2006
2007
2008
2009
b
Air temperature
°C
35
30
25
20
15
10
5
0
jan mar may jul sep nov
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2007
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c
VPD
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3
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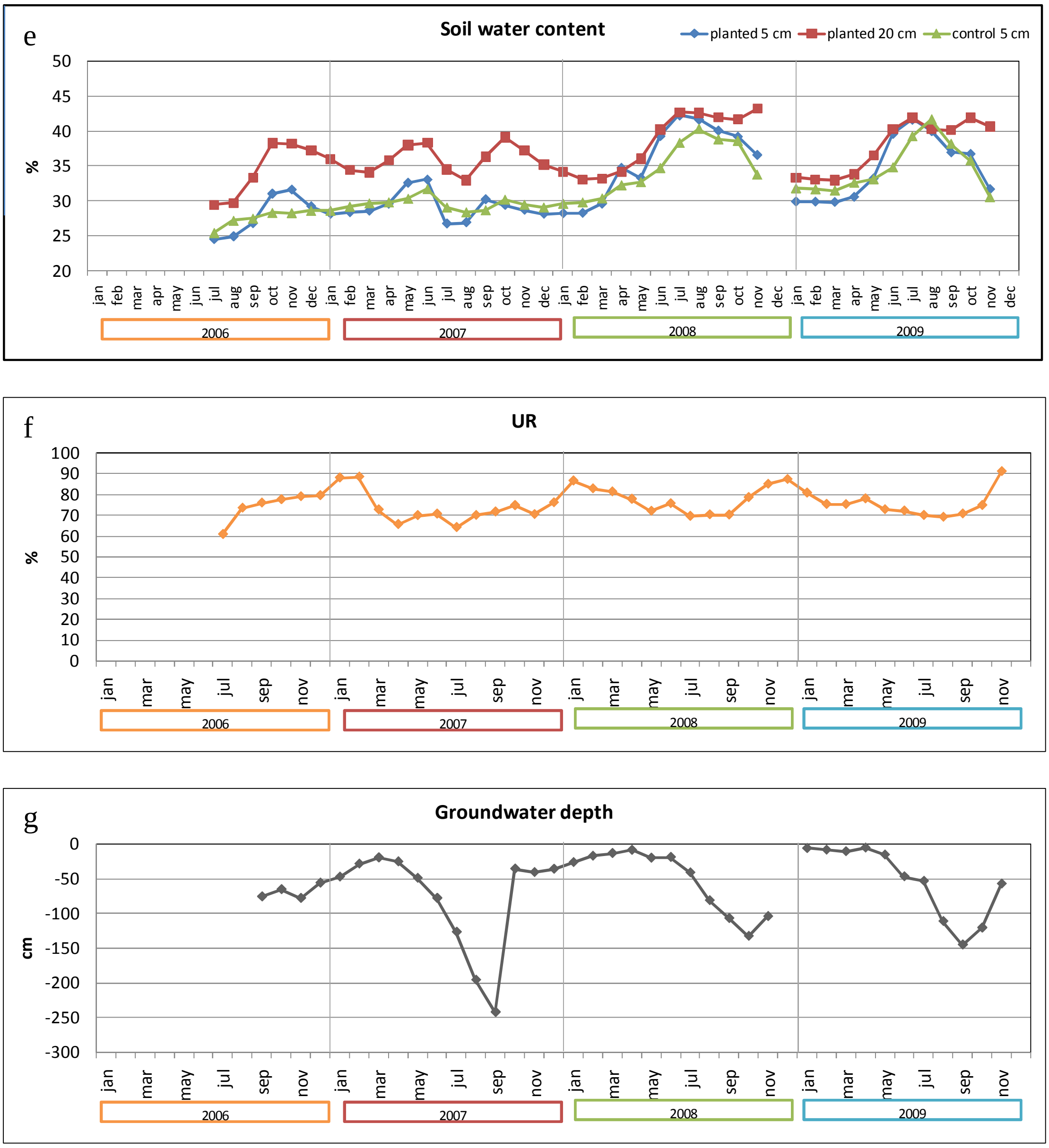


Fig. 4 - Meteorological variables, measured from July 2006 to November 2009. a) Rainfall (mm); b) Air temperature (°C); c) Vapour Pressure Deficit (VPD, kPa); d) Global incoming radiation, total monthly; e) Soil water content, main monthly; f) Air humidity (UR, %); g) Groundwater depth (cm from surface). b), c), e), f), g) are monthly means; a) and d) are total monthly data

Plant biomass

At the beginning of this study the plantation was 2 years old. Diameter at breast height (DBH) and plant average height (H) during the three years of study are reported in Tab.9.

Tab. 9 – Living plants, diameter (D) and breast height (DBH) for each clones and during the three years of study

clone		2006	2007	2008
Dvina	Living DBH (cm) H (m)	57 4.2 ± 0.3 5.6 ± 0.3	55 5.3 ± 0.4 6.7 ± 0.3	53 6.7 ± 0.4 7.4 ± 0.2
Lena	Living DBH (cm) H (m)	62 4.8 ± 0.4 6.5 ± 0.3	54 6.1 ± 0.5 7.6 ± 0.4	50 7.8 ± 0.5 8.3 ± 0.3
Neva	Living DBH (cm) H (m)	63 5.6 ± 0.4 6.5 ± 0.3	63 6.3 ± 0.5 7.6 ± 0.4	63 9.0 ± 0.6 8.7 ± 0.3

The distribution of dry biomass in tree components (stem, branch and leaves) are presented in Fig.5. The aboveground mean tree dry weight increases with stand age but was also different among the clones. At the beginning of study period trees weighed, on average, 3.2±0.5 Kg for clone Dvina, 3.9±0.6 kg for clone Lena and 5.5±0.7 kg for clone Neva, reaching at the end of study period 8.0±1.12 kg for Dvina, 11.6±1.5 kg for Lena, 14.7±1.86 kg for Neva.

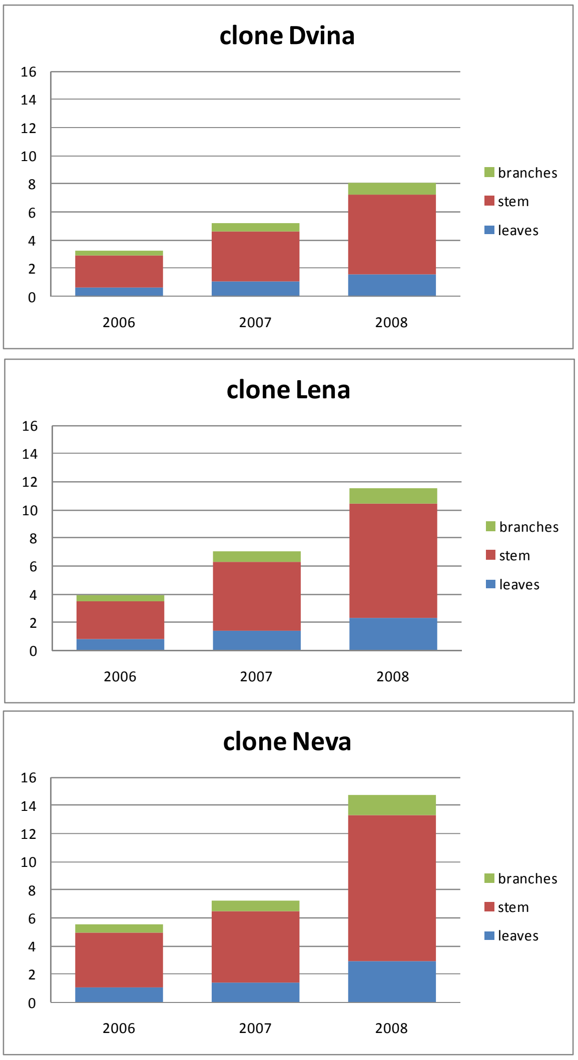


Fig. 5 - Distribution of dry biomass (Kg d.w./plant) in tree components for each clones and during the three years of study

Heavy metals in soil, water and trees

Concentrations of As, Cd and Zn at sampling sites and at different depths are summarized in Tab.10. A large heterogeneity in contamination was found during all three years and at all depths. The concentrations of heavy metals were generally higher at greater depths and frequently exceeding the Italian guideline values for an industrial soil (IGV, by Italian law D.Lgs 152/06) particularly for As and sometimes for Cd. If the comparison is made against the legal limits for agricultural soils (IGV, by Italian law D.Lgs 152/06), the soil can be considered as contaminated for Cd and Zn, with concentration were always higher than legal limits, while for As the contamination was variable, depending on soil depth (Tab.10).

Tab.10 - Concentration (mg/kg dry matter) of arsenic, cadmium, zinc in soil of "Isola dei Petroli", Venice lagoon: average and values in different depth layers and during the three years of study.

	Control Mean ± Er.St. min-max			Planted Mean ± Er.St. min-max		
Soil depth	**As**	**Cd**	**Zn**	**As**	**Cd**	**Zn**
	2006					
0-40	13.8 ± 4.3 4.7 - 41.3	7.3 ± 2.0 2.4 - 23.3	438.4 ± 80.2 210.1-859.2	13.3 ± 3.6 4.9-32.1	8.2 ± 3.7 1.8-26.8	271.0 ± 55.6 133.4-561.0
40-80	6.3 ± 1.0 3.8 - 11.8	7.2 ± 3.0 1.5 - 23.0	334.9 ± 89.4 123.6 - 812.1	7.2 ± 1.7 3.8 - 18.8	10.9 ± 3.0 2.0 - 27.7	355.6 ± 50.3 132.9 - 492.2
80-100	89.8 ± 10.9 78.9 -100.7	10.0 ± 0.2 9.8 – 10.2	1153.7 ± 30.1 1123.5 -1183.8	104.9 ± 0.3 104.6 – 105.2	7.5 ± 1.7 3,9 – 13.3	981.4 ± 93.3 795.3 – 1086.3
	2007					
0-40	20.6 ± 0.4 17.9 – 24.4	7.0 ± 0.3 3.8 – 10.5	322.2 ± 13.4 185.6 – 435.5	21.7 ± 1.5 14.2 – 29.4	8.3 ± 1.1 3.2 – 16.9	390.2 ± 45.2 186.5 – 621.9
40-80	18.1 ± 0.4 17.3 – 18.8	19.9 ± 3.7 14,6 – 27.1	629.2 ± 95.4 478,1 – 805.5	18.5 ± 1.0 15,4 – 23.5	17.4 ± 5.1 6,9 – 55.5	518.4 ± 69.2 301,7 – 945.0
	2008					
0-40	36.4 ± 1.6 31.9 – 39.3	9.3 ± 3.4 0.5 – 15.6	379.6 ± 80.6 244.0 – 582.9	39.9 ± 2.6 30.1 – 52.7	8.2 ± 1.2 5.8 – 16.3	319.3 ± 50.0 162.8 – 596.5
40-80	34.5 ± 1.8 29.7 – 37.9	8.4 ± 1.6 5.7 – 11.9	282.9 ± 58.5 163.8 – 415.8	32.1 ± 1.9 21.5 – 37.6	12.7 ± 4.0 3.4 – 36.9	339.0 ± 92.4 77.5 – 808.9
80-100	53.9 ± 10.4 43.5 – 64.6	6.2 ± 1.0 5.2 – 7.2	442.4 ± 13.2 429.2 – 455.5	29.0 ± 2.6 23.6 – 36.0	9.9 ± 2.9 4.7 – 17.1	371.4 ± 110.7 113.6 – 654.0
IGV (D.Lgs 152/06) - mg/kg -						
Industrial soil	50	15	1500			
Agricultural soil	20	2	150			

Metal and metalloid concentrations in soil water are presented in Tab.11 while pollutants contamination in groundwater in Tab.12. The mean values in groundwater were below the IGV threshold except for As in 2006 and Cd and As in 2008. It is worth to mention that values of metal concentration detected were very variable among piezometers, with the upper values of the range 3–4 times higher than the maximum limit.

Tab. 11 - Metal and metalloid concentrations in soil water, under poplar plantation and control part

2007 µg/l	***As***	***Cd***	***Zn***
Control	7.78 ± 0.78	1.25 ± 0.46	126.68 ± 16.04
Planted	8.35 ± 0.43	0.69 ± 0.11	113.55 ± 5.04

2008 µg/l	***As***	***Cd***	***Zn***
Control	8.42 ± 1.16	0.12 ± 0.01	83.79 ± 16.07
Planted	6.15 ± 0.68	0.34 ± 0.08	100.00 ± 15.04

Tab. 12 - Metal and metalloid concentration in ground water table

µg/l Mean ± St.Err. (min-max)	**As**	**Cd**	**Zn**
IGV [a]	**10**	**5**	**3000**
2006	15 ± 1 (12 - 20)	4 ± 2 (0.2 – 14)	1500 ± 659 (70 - 3668)
2008	10 ± 4 (0.1 – 54)	7 ± 1 (0.7 – 13)	2254 ± 639 (73 – 7714)

In Tab**.**13, the concentrations of heavy metal in different plant components during the study period are presented. During all of the three years, Cd and Zn concentrations followed a trend as leaves>>branches>stem in all clones, while the concentration of As was similar in all organs. Among clones, no clear significant differences were found in time. The bioconcentration factors (BCF) for each clone and for whole plants are reported in Tab.14.

Tab. 13- As, Cd and Zn concentrations (mg/kg d.w.) in different parts of three poplar clones and during the three years of study

2006 As	leaves	stem	Branches
Dvina	1.1 b	2.1 a	0.9 a
Lena	1.2 ab	1.8 a	1.0 a
Neva	1.4 a	1.1 a	1.0 a

2006 Cd	Leaves	stem	branches
Dvina	18.4 b	2.8 a	6.7 a
Lena	23.8 a	4.3 a	6.3 a
Neva	24.9 a	3.9 a	8.2 a

2006 Zn	leaves	stem	branches
Dvina	1384 b	143 a	195 a
Lena	1677 a	156 a	202 a
Neva	1664 a	151 a	284 a

2007 As	leaves	stem	Branches
Dvina	0.9 a	1.0 a	0.9 a
Lena	1.2 a	1.1 a	0.8 a
Neva	1.2 a	0.9 a	0.9 a

2007 Cd	Leaves	stem	branches
Dvina	20.4 b	5.3 a	9.9 b
Lena	26.7 ab	5.2 a	13.6 a
Neva	27.2 a	5.2 a	13.2 a

2007 Zn	leaves	stem	branches
Dvina	1430 a	124 b	229 b
Lena	1469 a	147 ab	284 b
Neva	1445 a	197 a	408 a

2008 As	leaves	stem	Branches
Dvina	0.6 a	0.8 a	0.6 a
Lena	0.6 a	0.7 a	0.7 a
Neva	0.7 a	0.7 a	0.5 a

2008 Cd	Leaves	stem	branches
Dvina	18.4 a	7.1 a	10.0 a
Lena	16.9 a	8.7 a	14.4 a
Neva	16.7 a	6.6 a	9.9 a

2008 Zn	leaves	stem	branches
Dvina	900 a	96 a	216 b
Lena	868 a	125 a	277 a
Neva	835 a	100 a	294 a

For comparison of means, ANOVA followed by Tukey's test, with 95% confidence level, were performed. Values in columns followed by different letters in the columns indicate significantly differences ($p < 0.05$) among plant organs for each clones analyzed, for a single element and during each years of study.

Tab. 14 - Bioconcentration factor for the three poplar clones during the three years of study

	BCF		
2006	As	Cd	Zn
Dvina	0.04	0.71	0.74
Lena	0.04	0.94	0.87
Neva	0.03	0.96	0.87
2007			
Dvina	0.05	0.68	0.87
Lena	0.05	0.81	0.94
Neva	0.05	0.81	1.03
2008			
Dvina	0.02	0.94	0.78
Lena	0.02	1.06	0.84
Neva	0.02	0.87	0.78

Transpiration of the tree plantation

In Fig.6 monthly values of stand-scale transpiration and potential evapotranspiration (Et_o, Penman equation) are presented.

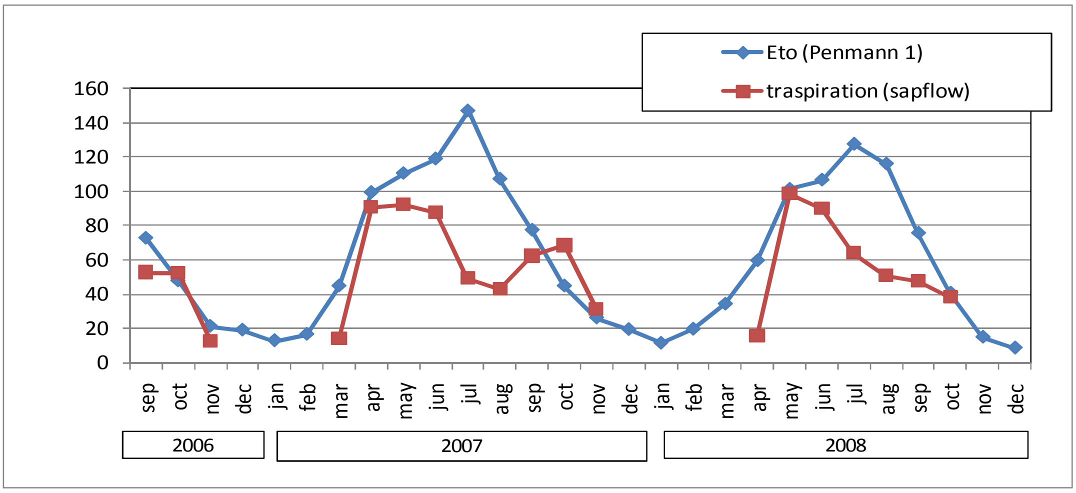


Fig. 6 –Trend of poplar plantation transpiration (by Granier sensor) and potential evatranspiration (Et_o by Penman equation)

Transpiration was maximum in spring and early summer, peaking in May and June, while in late July, August and September was affected by a more limited availability of soil water, particularly evident in 2007. In Spring and early summer, stand transpiration was close to potential evapotranspiration, indicating the efficiency of the poplar plantation in regulating the hydrological balance of the site in non-limiting conditions. This is confirmed when monthly stand transpiration is compared to monthly precipitation (Fig.7).

In 2007, the plantation transpired amount of water higher or similar to precipitation from April to August and then in October and November (when incoming rain was unusually low), while in 2008 the same occurred from May through October (Fig. 5).

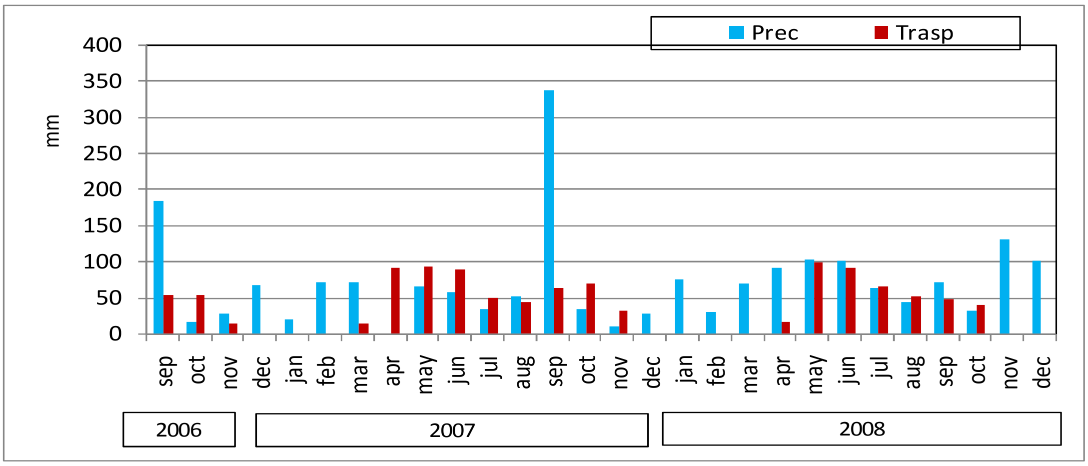


Fig. 7 – Trend of total monthly precipitation and poplar plantation transpiration during the three years of study

Summing precipitation, transpiration and the potential evapotranspiration for the whole year and for the growing season are presented in Tab.15. Transpiration was close to precipitation (80-85%) for the growing seasons of 2007 and 2008. Over the whole year, precipitation was more than 1.4 times transpiration in 2007 and more than 2.2 times in 2008. In 2006, from September to December, precipitation was 2.5 times transpiration.

Tab. 8 Precipitation, Transpiration and Potential evapotranspiration over the whole year and over the growing season during the three years of study

Full year	2006 *	2007	2008
Precipitation	294	772	904
Transpiration	118	541	405
Potential Evapotranspiration	161	826	717

Growing season (Apr - Oct)	2006 *	2007	2008
Precipitation	200	576	502
Transpiration	105	496	405
Potential Evapotranspiration	121	706	628

(*: study period from September to December)

Discussion

Growth and biomass data can be related to the different tolerance of the poplar clones to the pollutants present in the contaminated site. During the three year of the study, the clones, that were initially selected for their capability to adapt to mean climate condition of the area, showed inter-clonal differences both in height and in diameters (Tab.9): clone Neva exhibited the best growth performance, a lower mortality rate and reached higher total aboveground biomass of clone respect Dvina ad Lena (Fig. 4).

The heavy metal concentrations in soil (Tab.10), circulating water (Tab.11) and groundwater table (Tab.12) indicated a heterogeneous pollutants distribution and a degree of contaminant leaching in soil profile, particularly for Arsenic, found in high concentration also in circulating water.

The use of plants to decontaminate soils polluted by heavy metals has received considerable attention in recent years. Poplars (*Populus* spp.) can accumulate relatively high levels of certain metals and numerous study were performed to investigate the responses to heavy metals conditions in terms of uptake, transport and tolerance for different clones (Vandecasteele et al., 2003; Giacchetti et Sebastiani, 2006; Hermle S. Et al., 2007).

In the three analysed clones (Dvina, Lena and Neva), Zn and Cd presented a higher mobility in plants (according to Boruvka et al., 1997) (Tab.13). For this two heavy metals the lowest concentrations were found in stem, while the highest concentrations were found in leaves, suggesting that, in accordance with Laureysens et al. (2004), removal and treatment of fallen leaves should be necessary if medium- to long-term decrease of pollutants in soil has to be sought. On the other hand, As showed a similar concentration in all plant parts, that could be indicative of a limited mobility into the plants. Moreover Cd and Zn were most efficiently taken up respect to As: the bioconcentration factors (BCF –

Tab.14) for Cd and Zn were close to 1, while BCF for As was very low during all period and for all clones.

This study reported seasonal transpiration by a three clones poplar plantation of 118 mm in 2006 (considering only September to November), 541 mm in 2007 (Mid March – Mid November) and 405 mm in 2008 (April – October) with a daily mean (± standard deviation) of 1.42±0.79 mm day^{-1} in 2006, 2.15±0.98 mm day^{-1} in 2007 and 2.3±0.98 mm day^{-1} in 2008. The stand transpiration values of this study are close to those available in the literature for poplar plantation. For example Meiresonne and colleagues (1999) reported a daily mean transpiration of 1.9 mm day^{-1} for a plantation of hybrid poplars in Belgium, Anselmi (1982) observed values of 158 mm over 120 days for *Populus* x *euramericana* in Italy, while a transpiration range of 2.7-3.8 mm day^{-1} was reported for in trees of *Populus trichocarpa* Torr&A.Gray x *P.tacamahaca* L. (clone TT32) in Southeastern England (Zhang et al, 1999).

The potential evapotranspiration (Tab.14) during the study periods was always higher than the annual poplar stand transpiration, in accordance with review data from several studies, that found annual transpiration totals substantially lower than potential transpiration (Hall and Roberts, 1990).

In the specific conditions of the present study, the transpiration process was effective in regulating the hydrological balance of the Porto Marghera site in spring and summer and, generally, along the growing season when only in case of heavy thunderstorm, the plantation was not able to transpire back the precipitation amount.

Conclusions

This study analysed the capability of a poplar plantation in reclamation strategy and in controlling the hydrological balance in an polluted island in Porto Marghera. The hydrological control is a necessity since the island was completely separated from surrounding sea by a waterproof barrier to avoid environmental contamination. In this respect, the poplar plantation was able to regulate the hydrological balance of the site during growing season, with the exception of occasional heavy thunderstorms at the end of summer. Differences among clones were found for extraction efficiency of Cd, Zn and As: Cd and Zn are more bioconcentrated and clone Neva seems to show the best performances.

PART III:

A LONG-TERM EXPERIMENT TO TEST *P. vittata* L. UNDER HIGH As CONCENTRATION

Abstract

Pteris vittata L. is the first plant reported to be a hyperccumuator of arsenic (As). Several studies, aimed at evaluating the ability of *Pteris vittata* L. in the As hyperaccumulation, were carried out in the field or pot conditions while only a few in a hydroponic system. Moreover, hydroponic studies were commonly performed either at low As concentrations for long time or at high concentrations but only for 3 to 10 days. This study was aimed to investigate the tolerance and accumulation capability of *P. vittata* exposed to a remarkable As concentration in a long-term semi-hydroponic experiment, evaluating some implications at physiological and biochemical level.

After 17 weeks in pre-treatment condition, $Na2HAsO4 \cdot 7H2O$ was added to solution in two times to reach a concentration of 7 mg/l in the acclimatizing phase of 13 days and then of 21 mg/l in the treatment phase for seven weeks. At the end of treatment plants were sampled and divided into fronds, rhizome and roots. Biomass, As concentration, Cys, GSH, PCs, chlorophyll fluorescence image and chlorophyll content analysis were performed. It can be highlighted that organs developed during As treatment can maintain a valuable physiological status despite of the elevated As concentration in their tissues and that the main changes in the chlorophyll fluorescence parameters due to As treatment were the large reduction in ΦPSII (45%) and the notable increase in NPQ (350 %).

Introduction

Arsenic (As) is a natural element, classified as a toxic and carcinogenic metalloid and distributed ubiquitously throughout earth crust, soil, sediments, water, air and living organisms. The terrestrial abundance of this element is around 1.5-3.0 mg/kg. Since the 20t^{h} century the anthropogenic activities caused a remarkable increase of arsenic and other pollutants in the environment compromising the natural resources and affecting also the residue levels in plants and animals (Leist et al., 2000; Mandal et Suzuki, 2002).

Living organisms faced such environmental changes by modifying metabolic pathways through adaptive processes. In this context, plant metal tolerance can be considered as one of the best examples of an evolution process driven by anthropogenic activities (Bondada et Ma, 2003). Metal tolerance in plants can be achieved by different mechanisms (Baker et al., 1988) among which the enhanced metal accumulation, followed by metal sequestration or compartmentalisation, characterise some plants that can be so considered as metal accumulators.

Plants can also displayed an extreme form of tolerance called hyper-tolerance allowing to accumulate exceptionally high amount of heavy metals in their tissues, so to reach a metal concentration in the aboveground biomass higher than that in the soil. These plants are called hyperaccumulators (Cai et Ma., 2003). To evaluate the capability of a plant to hyperaccumulate metals, some descriptive indexes, such as the Bioconcentration Factor (BCF: ratio of metal concentration within plant tissues to that in soils) and the Translocation Factor (TF: ratio of metal concentration in the aboveground plant tissues to that in the roots) have been proposed (Ma et al., 2001). For example, it is generally accepted to define a plant an arsenic hyperaccumulator if it shows a BCF>1 and a TF>1 as well as it accumulates > 1,000 µg/g arsenic in plant biomass (Cai et Ma., 2003).

Arsenic is a nonessential element for plants and it is taken up via the phosphate transport system, being phosphorous its chemical analogous (Meharg 1994; Santos et al 2008). At high concentrations, if not safely sequestered, this metalloid interferes with basic plant metabolic processes, by reacting with sulfydril groups of enzymes and tissue proteins, leading to severe damages for plants ranging from root growth inhibition to plant death (Meharg et al., 2002; Tu et Ma, 2002).

The discovery of an As hyperaccumulator plant species is quite recent. In 2001 Ma et al. reported that a fern, *Pteris vittata* L., can accumulate extremely large concentrations (up to 23000 mg/kg) of this toxic element in its above ground biomass. Several other fern species have been reported by Zhao et al. (2002) to hyperaccumulate As to concentrations similar to P. vittata (P. cretica, P. longifolia, P. umbrosa) with significant differences in their accumulation ability linked to the growth conditions (Wang et al., 2007).

P. vittata shows the common traits associated with metal/metalloid hyperaccumulation: enhanced root uptake, efficient root-to-shoot translocation and a far elevated tolerance through internal detoxification (Caille et al., 2005, Tu et Ma., 2002).

Detoxification of arsenate by plants is reported to be carried out by means of an intracellular chelation by ligands followed by compartmentation (Meharg 1994). Inside plant cells, arsenate may be detoxified through reduction to arsenite and its sequestration in vacuoles by thiol-containing compounds as glutathione (GSH) and phytochelatins (PCs), that are considered to be the main ligands of many metals and metalloids (Lombi, 2002; Zhang et al., 2004).

PCs are a family of sulfur-rich peptides characterized by a general structure as $(\gamma\text{-GluCys})_n\text{-Gly}$ (n=2 to 11) and they are synthesized from glutathione (GSH), a tripeptide containing cysteine (Cys) (Grill et al.,1989; Mendoza-Cozatl, 2005). These compounds play a key role in constitutive and adaptive tolerance to As in several nonhyperaccumulating plant species (Zhao et al., 2002; Caille et al., 2005; Kim et al., 2009). As far as concerns hyperaccumulating plants, several authors reported that even if arsenate exposure induces PC synthesis and an increase of thiols in P.vittata leaflets (Vetterlein et al., 2009; Zhao et al., 2003), PC induction did not appear as the main process responsible for As hypertolerance, as reported by Zhang et al., 2004. In this regard, Raab et al., (2004) showed that less than 1% of the As accumulated in fronds of the hyperaccumulator *P. cretica* was complexed with PCs, while Cao et al. (2004) proposed that in *P. vittata* an important role in detoxification was exerted, at low levels of As (up to 20 mg/kg), by enzymatic antioxidants (SOD,CAT,APX, and GPX) whereas at high As concentrations (50-200 mg/kg) by non-enzymatic antioxidants (GSH and other –SH compounds).

Several studies, aimed at evaluating the ability of *P. vittata* in the As hyperaccumulation, were carried out in the field or pot conditions while only a few in a hydroponic system. Moreover, hydroponic studies were commonly performed either at low As concentrations for long time, i.e 145 μg/l of As for 45 days (Santos et al., 2008) or at high concentrations (10-20 up to 208 mg/l) but only for 3 to 10 days (Zhao et al., 2003; Kertulis et al., 2005; Luongo and Ma, 2005; Singh et Ma, 2006).

Hydroponics is a very suitable growth system to exploit the potentiality of a plant towards metal tolerance and accumulation and more in general for the evaluation of its phytoremediation ability (Lunackova et al., 2003; Pilipovic et al., 2005; Dos Santos Utmazian et al., 2007; Zacchini et al., 2009).

Then, this study was aimed to investigate the tolerance and accumulation capability of *P.vittata* exposed to a remarkable As concentration in a long-term hydroponic experiment, evaluating some implications at physiological and biochemical level.

Materials and method

Experiment setup

Three-month-old P.vittata L. plants were obtained from a nursery (IBAF - CNR Legnaro) . At the 3-4 frond stage, plants were transferred from pots to a semi-hydroponic growth system, washing carefully the roots with water to remove soil residues. Semi-hydroponic growth system was chosen as a growth system module consisted in two polyethylene tanks (0,81 m^2): the tank A contained sand to anchor plants while the tank B was used as water drainage collector. A pumping system forced the drained water to move continuously from tank B to A to assure oxygen for roots and to avoid the formation of pollutant and nutrient gradients. A nutritive solution was prepared with 0.7 gr/l of soluble fertilizer NPK 20-20-20 (Nutri-Leaf, Miller, Hannove, Pennsylvania, U.S.A.) especially formulated for hydroponics and added every ten days to tank B. The circulating solution volume was 130 l and pH was maintained around 7. Two similar modules were created to establish a control (module 1) and a treatment (module 2) thesis. Fifteen plants, homogeneous in size and in root development, were assigned to each module.

These modules were placed inside a greenhouse and plants were exposed to controlled air temperature (25°C) and photon flux density of 100 μmol m^{-2} sec^{-1}.

After 17 weeks in pre-treatment condition, $Na_2HAsO_4 \cdot 7H_2O$ was added to tank B in two times to reach a concentration of 7 mg/l in the acclimatizing phase of 13 days and then of 21 mg/l in the treatment phase for seven weeks. Every ten days nutrient solution was replenished. At the end of the experimental period control and treated plants were collected and separated into fronds, rhizomes and roots. In treated plants fronds were also divided into old and young fronds, depending on their completed or uncompleted unrolling at the beginning of the treatment. In particular, the fronds already unrolled were marked and indicated as old. Control plants were collected without separation in young and old fronds because similar As concentration was found between them in preliminary experiments (data not shown).

Part of samples were oven-dried for 3 days at 60°C, weighted and ground to fine powder for arsenic concentration analysis and the remaining was stored at -80°C for thiol and PC analysis.

Before treatment water and sand As concentration was 14.1 ± 0.98 µg/l and 1.7 ± 0.15 mg/kg respectively.

Chemical and biochemical analysis

For element concentration analysis all samples were digested: the different parts of plants with a mixture of concentrated HNO_3 (65 % v/v), H_2O_2 (35% m/v) and bi-distillate H_2O; water with 3 ml of HNO_3 and sand with 2 ml of H_2O_2 (35% m/v) and 5 ml of HNO_3 (65 % v/v). Digestion was carried out with a closed-vessel microwave system (Excel-Shanghai EU, Microwave Chemistry Technology Co.Ltd.).

Arsenic analysis were performed with a Zeeman Graphite Tube Atomic Absorption Spectroscopy (Varian SpectrAA800, Mulgrave, Victoria, Australia). The concentrations of micro and macro nutrients in extracts were carried out using a Plasma Atomic Emission Spectroscopy (ICP-MS, Thermo Jarrell, Ash Iris Advantage, Thermoelectron, USA).

Reagent blanks and internal standards were used where appropriate, to ensure accuracy and precision in analysis.

For Cys, GSH and PCs analysis samples were homogenised in liquid N_2 with three volumes of extraction buffer (0.1 N HCl, 1 mm EDTA, 400 mg/ml PVPP) and centrifuged (15,000 g) for 25 min at 4°C. Supernatant aliquots (100 µl) were mixed with 150 µl 0.2 M HEPES and 25 µl 3mM DTT, then some drops of 2 N NaOH were added to correct pH to

8.0. After 1 h of dark incubation at room temperature, 15 µl 30 mM monobromobimane were added to the assay buffer that had been successively incubated in darkness for 15 min at room temperature. To stop the reaction, 75 µl 10% acetic acid were mixed into the solution, centrifuged (15,000 g) for 5 min at 4°C and filtered (0.2 µm) ready for injection into an HPLC system (Beckman-Coulter, Fullerton, CA, USA). Phytochelatins (PCs) were separated on a C18 reverse-phase column (250x4.6 mm, 5 µm pore size; Grace Davison, Deerfield, IL, USA). Elution was performed with a linear gradient (A = MeOH with 0.25% acetic acid, pH corrected to 4.3 with NaOH; B = H_2O with 0.25 % acetic acid, pH corrected to 4.3 with NaOH) as follows: 90–60 % B in 15 min, 60–20 % in 5 min, 20–90 % in 1 min, re-equilibration in 90 % B for 5 min, at a flow rate of 1 ml/min). Chromatograms were recorded and integrated using the 32 KARAT™ Software 5.0 (Beckman-Coulter). PCs were identified by comparison with standard PC samples purified from *Silene vulgaris* (Moench). Quantification was performed with a Jasco fluorescence detector (model FP 2020 Plus; Tokyo, Japan; excitation 380 nm, emission 480 nm).

Indexes used

Bioconcentration Factor (BCF) was calculated as the ratio of arsenic concentrations in fronds to concentration in soil (Tu et Ma, 2002). To describe the ability of a plant to translocate pollutants Translocation Factor (TF) was measured. This index is defined as the ratio of arsenic concentrations in fronds to those in roots (Wei et al., 2006). In addition to frond/root, TF was expanded to rhizome/root and frond/rhizome to better understand arsenic distribution in the plants (Singh et Ma., 2006)

Chlorophyll fluorescence image and chlorophyll content analysis.

Chlorophyll fluorescence image of fern leaves was performed using a MINI-Imaging PAM (Walz, Effeltrich, Germany) in order to investigate the heterogeneity of chlorophyll fluorescence parameters under metal stress conditions. The MINI-Imaging-PAM employs blue LEDs, with a peak wavelength at 450 nm, for pulse modulated measuring light, continuous actinic illumination and saturation pulses. The charge-coupled device (CCD) camera has a resolution of 640 × 480 pixels. Pixel value images of the fluorescence parameters were displayed using a false colour code ranging from black (0.000) through to red, yellow, green, blue and pink (1.000) (Berger et al., 2004). Leaves were dark adapted for at least 30 minutes before determining F_o and F_m. The maximum quantum yield of PSII photochemistry (F_v/F_m), was determined as $(F_m - F_o)/F_m$. Leaves were then adapted to a light intensity of 100 µmol m^{-2} s^{-1} and a saturating pulse was applied to determine the maximum fluorescence (F'_m) and steady-state fluorescence (F_s) during the actinic illumination. Saturation pulse images and values of various chlorophyll fluorescence parameters were captured. The quantum efficiency of PSII photochemistry, ΦPSII, was calculated according to Genty et al. (1989) using the formula $(F'_m - F_s)/F'_m$. The coefficient of photochemical quenching, qP, is a measurement of the fraction of open centres calculated as $(F'_m - F_s)/(F'_m - F'_o)$ (Schreiber et al., 1986). The value of F'_o was estimated by the approximation of Oxborough et Baker (1997), namely $F'_o = F_o/(F_v/F_m + F_o/F'_m)$. Calculation of quenching due to non-photochemical dissipation of absorbed light energy (NPQ) was determined at each saturating pulse, following the equation $NPQ = (F_m - F'_m)/F'_m$ (Bilger et Björkman 1991). The measured values of NPQ were divided by four to display values less than 1.000. Chlorophyll fluorescence determinations were obtained from n = 5 leaves.

The chlorophyll content of the leaves was rapidly assessed using the SPAD-502 chorophyll meter (Konica Minolta, Tokyo, Japan). Plants were harvested immediately after these measurements were completed to assess dry matter.

Data analysis

All results were expressed as an average of 5 replicates at least. Normally distributed data were processed with analysis of variance (ANOVA) using the SPSS software tool (Chicago, IL, USA).. Correlations were evaluated using bi-variation method, with two-tailed significance and Pearson correlation coefficients. Student t-test were used to compare data from treatment and control conditions.

Results

Plant biomass

Total biomass at the beginning of the experiment was 2.07±0.33 g D.W. per plant, while at the end of the experiment it reached 14.16±1.76 for control and 15.14±2.43 g D.W. for treated plants. Biomass distribution among fronds, rhizome and roots analysed at the end of the experiment is shown in Fig.8. Data revealed a significant biomass reduction in the roots of As-treated plants compared to control.

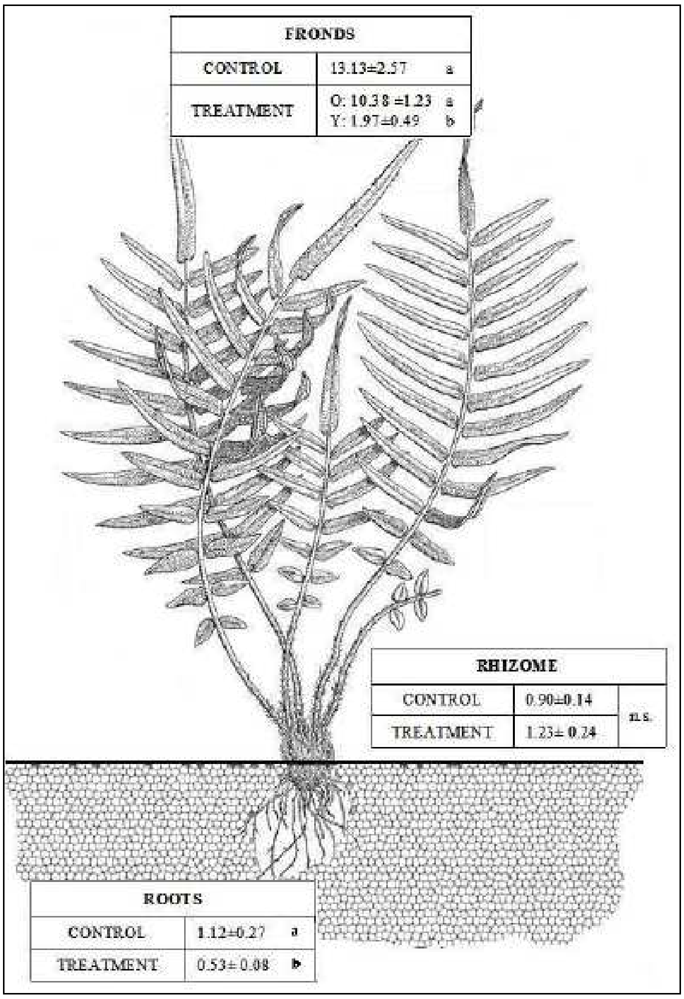


Fig. 8– Biomass distribution in plants. Old and young ..see M&M For comparison of means ANOVA was performed by followed by Tukey's test, with 95% confidence level. Different letters indicate significatively differences ($p< 0.05$) among treated and control for each heavy metal analyzed in each plant parts.

Arsenic in plants, BCF and TF

After 9 weeks of growth in control or As-spiked solution plants showed different arsenic concentrations in the sampled organs. Both in control and in treated plants the metalloid concentration followed a trend such as root = rhizome < frond (Fig 9). In As-treated plants a highly significant difference between the young and the old fronds in As accumulation was found, being the concentration of the metalloid in the former more than 13 times higher compared to the latter, already completed unrolled after the adaptation step.

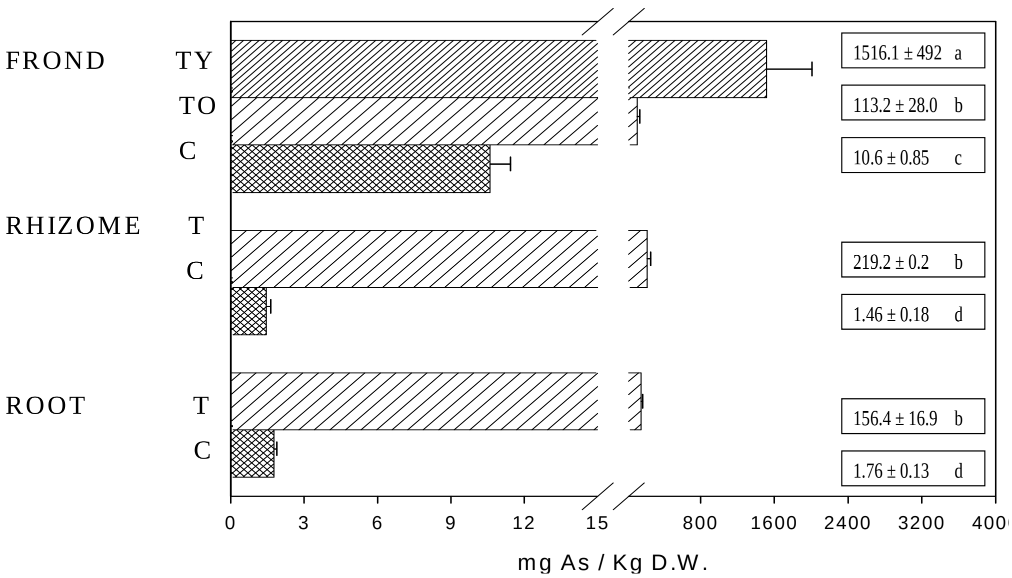
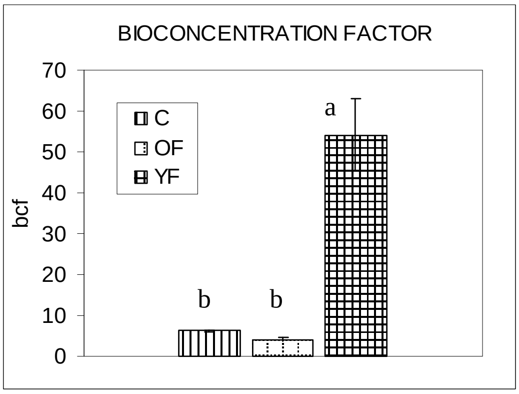


Fig. 9 – Arsenic concentration in different plant parts. For comparison of means was performed by ANOVA followed by Tukey's test, with 95% confidence level. Different letters indicate significatively differences (p< 0.05) among plant parts.

The Bioconcentration factor (BCF) in *P. vittata* fronds was higher than 1 (Fig.10), as commonly found in hyperaccumulating plants. In control plants, BCF was 6.2 while in treated plants young fronds exhibited a much higher value than old fronds. Old fronds in treated plants showed a similar BCF value than fronds of control plants.

Translocation factor (Tf – Fig. 10) was calculated to evaluate the capability of the plants to move the metalloid among the three organs of fern plants (root, rhizome and fronds). Tf values were always higher than 1 except for As-treated plants where the ratio old frond/root and old frond/rhizome was 0.7 ± 0.1 and 0.5 ± 0.1 respectively.

In As-treated plants Tf calculation revealed that, both in frond/root and in frond/rhizome, Tf values of young frond/roots were notably higher compared to old frond/root, while no statistical differences were observed between Tf values related to young frond/root in treated plant and total frond/root in control plant. Tf values related to rhizome/root were not significant.



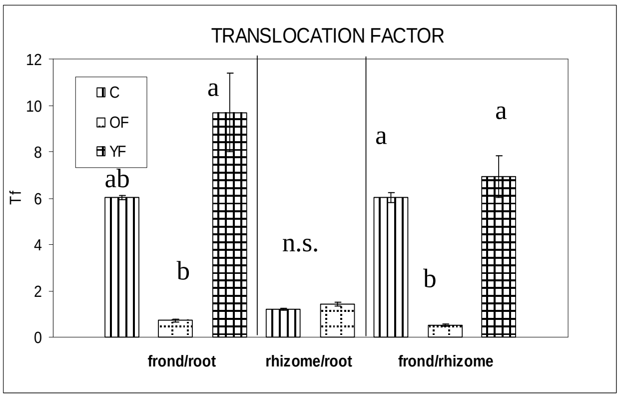


Fig. 10 – Bioconcentration (left) and Translocation factor (right) for treated and control plants

Analysis of other elements in plants

Macronutrient (Ca, Mg, K, P), micronutrient (B, Cu, Fe, Mn, Zn) and metal (Al, Cd, Cr, Na, Ni, Pb) concentrations within plant parts were measured and a comparison between control and treated plant was performed (Tab.16). A significant increase in B, Cd, Cr, Cu, Fe, Ca, K, Pb concentrations and a decrease in Na concentration in roots of treated plants with respect to control plants were observed. In rhizome, an enhancement of Cu concentration and a decrease of Ni and P characterised the treated plants from the control plants. In treated plants, only the concentration of K was higher in young fronds compared to old fronds being Al, B, Fe, Ca, Na, Mn and Mg concentrations higher in old than young fronds. In general, in control fronds, the concentrations of most analysed element were similar to treated old fronds except for Cd, Cu, Ca, Na concentrations that were significantly lower in control plants.

Tab.16– Macronutrient, micronutrient and some metal concentrations within plant parts. About root and rhizome: comparation of means between treated and control was performed by t-student test with n.s.: not significant * p< 0.05; ** p<0.005; *** p<0.001. About fronds comparison of means was performed by ANOVA followed by Tukey's test, with 95% confidence level. Different letters indicate significatively differences (p< 0.05) among control, treated young and old fronds for each elements .

mg/kg St.Err.	Root C	Root T	t-test	Rhizome C	Rhizome T	t-test	Frond C	Frond T Old	Frond T Young
Al	**2271** 1277	**4485** 470	n.s.	**2101** 211	**2262** 557	n.s.	**500** a 62	**527** a 42	**276** b 30
B	**74** 19	**173** 9	***	**170** 35	**189** 29	n.s.	**81** ab 3,7	**107** a 6,9	**70** b 8,5
Cd	**1,6** 0,4	**2,6** 0,1	**	**1,8** 0,07	**2,3** 0,4	n.s.	**0,8** b 0,1	**1,4** a 0,1	**1,3** a 0,2
Cr	**10,5** 1,1	**17,9** 0,8	***	**5,6** 0,1	**8,5** 1,6	n.s.	**4,6** a 0,7	**6,9** a 1,2	**5,7** a 0,2
Cu	**290** 77	**801** 22	***	**99** 13	**257** 43	*	**8,3** b 0,4	**12,6** a 0,9	**11,6** a 0,6
Fe	**4279** 996	**6365** 412	*	**2257** 25	**2037** 197	n.s.	**318** a 72	**332** a 61	**50** b 2,6
Ca	**35518** 10373	**56723** 3196	*	**38322** 4643	**45222** 5867	n.s.	**10972** b 465	**18211** a 254	**4559** c 450
Na	**1623** 241	**776** 214	*	**5569** 466,9	**4556** 492,3	n.s.	**5108** b 708	**12496** a 824	**2512** c 232
Mn	**354** 93	**454** 28,0	n.s.	**264** 36	**264** 52	n.s.	**33** a 3,9	**31** a 3,4	**6,1** b 0,3
Mg	**5887** 1054	**5210** 323	n.s.	**3859** 380	**3223** 573	n.s.	**4358** a 253	**5660** a 382	**2055** b 145
K	**151** 39	**231** 5,3	*	**300** 26	**276** 14	n.s.	**302** b 6,7	**300** b 7,6	**353** a 11,4
Ni	**24** 5,5	**17** 0,8	n.s.	**10,8** 1,5	**6,2** 1,2	*	**1,9** a 0,6	**2,1** a 0,3	**2,2** a 1,2
P	**6890** 1893	**7086** 813	n.s.	**10956** 1969	**4962** 830	*	**1643** b 127,2	**2114** ab 134,9	**2356** a 117,7
Pb	**9,9** 1,7	**16,0** 1,3	*	**9,9** 1,1	**13,1** 2,2	n.s.	**3,9** a 0,2	**6,0** a 1,3	**6,2** a 1,0
Zn	**344** 100,5	**204** 37	n.s.	**308** 59	**237** 78	n.s.	**43** a 8,4	**33** ab 3,1	**18** b 2,9

Analysis of CYS, GSH and PCs concentrations

The content of phytochelatins (PCs) and of two thiols, cysteine (Cys) and gluthatione (GSH), was investigated in roots and fronds of control and treated plants (Fig.11).

Thiol and PC content showed lower concentrations in roots respect to fronds; Cysteine content under arsenic treatment increased significantly in fronds compared to control with the old fronds that showed a Cys content higher than young fronds. No significant differences in roots were observed between control and As-treated plants. GSH content showed in treated plants a decrease in old fronds compared to young fronds. This decrease was significantly observed also in comparison with control fronds. Similarly to Cys, GSH content did not evidence significant differences in roots between control and As-treated plants.

PC concentration was higher in treated young frond in comparison with old treated and control fronds, being the content of old fronds unaffected by the As treatment. PCs were not detected in control roots while an induction of these polythiols was observed as a consequence of As treatment.

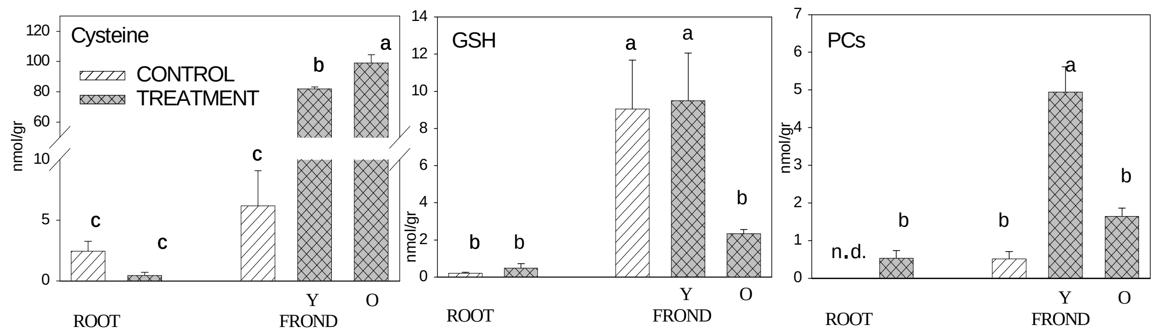


Fig.11– Cysteine (Cys), Gluthatione (GSH) and phytochelatins (PCs) concentrations in different plant parts. For comparison of means was performed by ANOVA followed by Tukey's test, with 95% confidence level. Different letters indicate significatively differences ($p < 0.05$) among plant parts among each biochemical compound .

Chlorophyll fluorescence image and chlorophyll content analysis

Chlorophyll a fluorescence parameters in control and As treated pinnae were shown in Tab.17. A representative image of chlorophyll fluorescence in two control and metal treated pinnae measured at the end of As treatment is reported in Fig.12. All parameters were affected by treatment. In particular arsenic treatment compared to control caused an increase of Fo and Fm values by around 37 % and 14 %, respectively. On the contrary, Fv/Fm and ΦPSII showed a decrease by around 13 % and 46%, respectively. Moreover, qP was significantly reduced with respect to control, while NPQ showed an increase of 3.5 times.

Tab. 7 - Chlorophyll fluorescence parameters in two control and arsenic treated leaves of *Pteris vittata* measured at the end of the experiment. F_o, minimum chlorophyll fluorescence yield obtained with dark-adapted leaf; F_m, maximum chlorophyll fluorescence yield obtained with dark-adapted leaf; F_v/F_m, maximal quantum efficiency; ΦPSII, quantum efficiency of PSII photochemistry; qP, photochemical quenching; NPQ, non-photochemical quenching. Values are means of 5 samples. For comparison of means, ANOVA followed by Tukey's HSD test, calculated at 95% confidence level, were performed. Values followed by the same letter indicate no significant differences.

Species	Treatment	F_o	F_m	F_v/F_m	ΦPSII	qP	NPQ
P.vittata	**Control**	0.093 b	0.371 b	0.750 a	0.458 a	0.843 a	0.731 b
	Arsenic	0.149 a	0.432 a	0.655 b	0.248 b	0.720 b	2.584 a

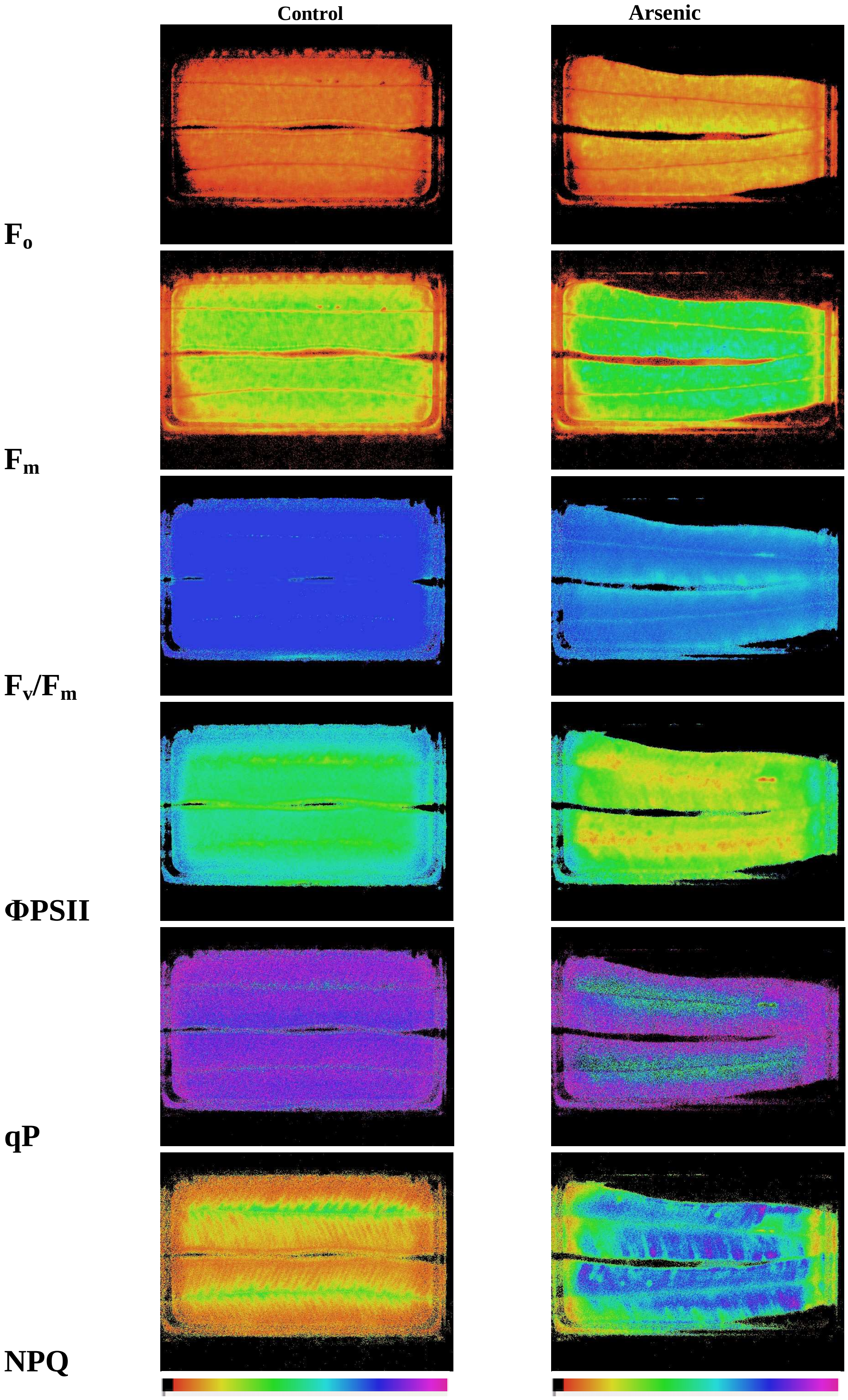


Fig.12 – Chlorophyll fluorescence images of Fo, Fm and Fv/Fm in two dark-adapted pinnae and PSII, qP and NPQ at steady-state with actinic illumination of 100 µmolphotons m-2s-1 measured at the end of the experiment. The false colour code depicted at the bottom of each image ranges from 0.000 (black) to 1.000 (pink)

Pinnae chlorophyll content (Fig.13), assessed by SPAD, showed a significant lower value in treated plants (37.5±0.5) compared to control plants (43.9±0.9).

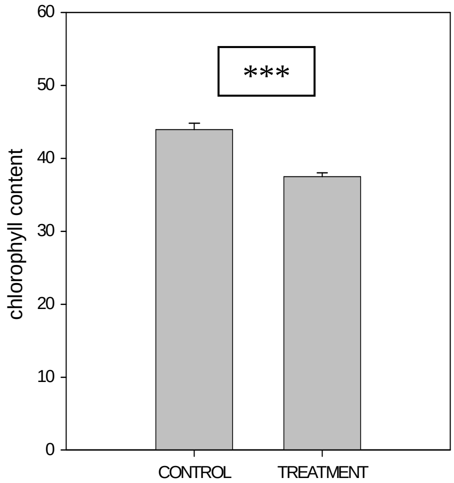


Fig.13– Chlorophyll content in conterol and treated pinnae. Comparation of means between treated and control was performed by t-student test and *** p<0.001

Discussion

P. vittata can tolerate and accumulate high levels of As in its organs. For this reason this plant is considered an As-hyperaccumulator able to remove efficiently this metalloid from soil (Ma et al., 2001). Most part of the information about this aspect has been obtained in soil studies where the environmental conditions other than pollution could represent a limiting factor. To overcome this problem and to exploit and compare the potentiality of different plant species to phytoextract metals, hydroponic studies are commonly conducted (Dos Santos Utmazian et al., 2007; Baldwin et Butcher., 2007; Zacchini et al., 2009). Few studies regarding the evaluation of the *P. vittata* capability in the As phytoextraction were carried out under hydroponics. Almost all were performed either at low As concentrations for long time or at high As concentration in short time experiments

(Santos et al. 2008; Zhao et al. 2003; Kertulis et al. 2005; Luongo and Ma 2005; Singh et al. 2006).

With this in mind this study was designed to investigate some physiological and biochemical responses, associated to arsenic accumulation and tolerance, in a long-term and high As concentration experiment in hydroponics. The *rationale* of this experimental design was to evaluate the potentiality of this plant species to tolerate and remove the metalloid from a substrate in extremely toxic conditions.

Arsenate toxicity on plants has been well described (Paliouris et Hutchinson, 1991; Tu et Ma, 2002). In particular, toxic symptoms such as plant growth and crop yield reduction, a decrease in root functionality, a wilting and a necrosis of leaf tips and leaf margins and a decrease in photosynthetic capacity have been reported (Bondada et Ma, 2003; Shaibur et Kawai, 2009).

In this study, *Pteris vittata* plants exposed for 9 weeks to 28 mg/l of As exhibited some visible toxic symptoms (not shown). In particular, at the end of treatment period, in old fronds already developed before As exposure, necrosis of the tips and margins in some leafleats and leaf withering were noticed, whereas young fronds, emerging during As treatment, showed no toxic symptoms. This observation can suggest that newly formed leaves have developed some tolerance mechanisms allowing plant to cope to high concentration of As in the growing medium.

Biomass production is an another important parameter that can be used to evaluate the capability of plant to tolerate the presence of heavy metals in the growing solution. In this study a significant reduction in root biomass in treated plants compared with control occurred, while biomass of other plant parts was not affected (Fig.8). The reduction of root biomass following As treatment represents a typical toxic symptom induced in plants by this metalloid, as already reported by Wang et al., 2002 and Zhang et al., 2004.

The As absorbed by the plants was accumulated prevalently in the fronds, followed by the rhizome and the roots (Fig 9). The same behaviour was also observed in control plants. The As concentration in the fronds showed a significantly difference between old and young fronds being 113.20 ± 28.02 and 1516.20 ± 492.31 respectively. These results were in agreement with Tu et Ma (2005), that reported the highest As accumulation in the young fronds, followed by mature and old frond, in plants exposed to <30 mg/kg As.

Rhizome showed a notable concentration of arsenic without a decrease in biomass under our treatment condition (Figgs. 8 and 9). This plant part could be very important in limiting the traslocation of the toxic element from roots to fronds, accumulating a large amount of As especially when plants grow in heavily contaminated soils. Liao et al. (2004) suggest that rhizome in *P.vittata* can act as a "buffer-storage" for As restricting As translocation to aerial parts to avoid the toxic effects on the physiological processes occurring in the fronds.

BCF is a useful index to evaluate the potentiality of a plant to phytoextract metals from a substrate and concentrate them into its organs. It is commonly utilised to identify hyperaccumulating plants that can be so defined if the BCF value is higher than 1. In literature, the BCF value reported for *P.vittata* fronds were significantly higher than 1 (Gonzaga et al., 2008), reaching also a value of 184 in Santos et al. (2008). In the present work, BCF was 13.5 times higher in young than in old fronds of As-treated plants, ranged from 4 in the former to 54 in the latter. This finding underlines the ability of this plant to bioconcentrate the toxic element especially in fronds completely developed during the treatment (Fig 10). An accumulation of As in the above ground biomass in un-polluting conditions was also observed. In fact, due to the residue levels of As in the sand, in control plants, total plant BCF was 6.23 ± 0.5, proving the capability of this fern to accumulate the toxic element also in very low arsenic presence.

Beyond to extract arsenic from soil, *P. vittata* efficiently translocates this element from roots to fronds. In this work, the Tf calculation (Fig.10) showed a higher capability of young fronds to translocate arsenic from roots compared to old fronds, evidencing the capability of the organs developed under treatment to activate metabolic processes to act as a sink of the absorbed arsenic.

It has been reported that toxic metals may interfere with the uptake of other elements by plants causing a notable detrimental effect on plant growth. In fact, among these chemical elements there are some essential macro- and micro-nutrients beyond other non essentials metals (Cao et al., 2004; Gonzaga et al., 2007; Oliveras et al., 2009). In this study the chemical element concentrations within plant organs were modified differently by the As treatment (Tab.16). In fact, in roots of treated plants an increase for all elements, except for Na, was observed, particularly for B, Cr and Cu, even if only eight out of 15 element evidenced statistically significant difference respect to control.

In rhizome the concentration of the elements was substantially unaffected by the As treatment since a difference between control and treated plants was only ascertained in three out of 15 elements. On the contrary, in fronds the concentrations of the nutrients were notably altered by the exposure of the plants to the metalloid. In fact, only four out of 15 elements did not change their titre between control and young/old treated fronds. Particularly interesting resulted the increase of the macronutrient P and K concentrations occurring in young treated fronds respect to control. Conversely, the young treated fronds showed a remarkable reduction compared with control in Ca, Mg and in micronutrients such as Al, Na, Mn, Fe and Zn. Under As treatment the old fronds did not reveal a remarkable change in element composition compared with control except for an enhancement in Na, Ca, Cu and Cd. An enhancement of Ca concentration in mature fronds of *P. vittata* following As treatment was shown by Fayiga et al. (2008), supporting the hypothesis of a role in As detoxification.

Considering the whole plant, a general trend of increase in nutrient absorption in response to arsenate could be evidenced.

In fronds different behaviours has been notice depending on element analyzed. Cao et al. (2004) reported that along with As uptake in P. vittata there was enhanced uptake of plant nutrients including K, Zn, Mn, Fe and especially P. This study confirms the increase of elements concentrations in treated plants especially in roots.

In the present study, in fern plants exposed to arsenate in a long-term hydroponic experiment an induction of thiols and PCs occurred (Fig.11). This finding agrees with previously reported data (Zhao at al, 2003; Zhang et al., 2004; Cai et al., 2004) obtained in different experimental conditions than those adopted in the present work. In particular, PCs were induced in roots by arsenic treatment and their content enhanced markedly in young treated fronds in comparison with old treated fronds and control fronds. These observations are in agreement with the As content detected in the different plant parts, confirming the role of PCs as bio-ligands for As even in plants exposed for several weeks at a notable arsenate concentration. In this context, it was argued by different authors (Zhang et Cai, 2003; Vetterlein et al., 2009) that in arsenic hyperaccumulating plants the capacity to tolerate high concentration of this metalloid is not strictly related to the induction of thiols. In fact, it was suggested that in As-hypertolerant plants an adaptive PC-independent sequestration in vacuole occurs as a main detoxification mechanism, supplemented by a PC-dependent detoxification process that activates once the former is saturated.

Beyond many other important functions in plant cell, Cys and GSH are involved in PC formation and changes of their concentration in plant exposed to metals have been extensively reported (Schmöger et al., 2000; Cao et al., 2004). In old fronds a higher Cys content and a lower GSH content compared with young fronds occurred (Fig.11). Moreover, old fronds showed a lower PC content as above discussed. In this regard, a preferential accumulation of a low molecular thiol such as Cys could be an adaptive mechanism to reduce As transport and storage, explaining the lower As accumulation in old fronds in comparison to young fronds.

GSH content did not vary both in roots and in young fronds of arsenic-treated plants compared with control as previously reported by Zhao et al (2003).

Chlorophyll fluorescence imaging allowed to investigate the effects of heavy metal on the photosynthetic activity in young fronds of P. vittata that showed a remarkable as-concentration exposed for 9 weeks to 28 mg/l of As. No study about the utilisation of this technique on P. vittata was found in literature. Chlorophyll fluorescence images indicated a reduction of the photosynthetic activity in treated leaves of P. vittata with respect to control (Tab.17 and Fig.12). In particular, arsenic treatment increased the values of Fo and Fm. According to Gilmore et al. (1996), Fo increases when the photochemical apparatus is damaged or, more specifically, when the number of functional chlorophylls not connected to the reaction centres of PSII increases. Stoeva et al (2003) showed both an increase of Fo and a considerably decrease of Fv/Fm in Zea mays plants exposed to arsenic. In this study arsenic treatment reduced Fv/Fm values of about 13% with respect to control. Nevertheless, the main changes in the chlorophyll fluorescence parameters due to arsenic treatment were the large reduction in ΦPSII (45%) and the notable increase in NPQ (350%). The quantum efficiency of PSII photochemistry, ΦPSII, can be used to estimate the photosynthetic activity, while NPQ reflects heat-dissipation of excitation energy in the antenna system and is a good indicator for excess light energy (Demmig-Adams et al.,1996; Rohacek 2002). Little is known about the effect of arsenic on photosynthesis, but some authors consider that the limiting step on photosynthesis is due to the inhibition effect of this element on the chlorophyll synthesis (Krupa and Moniak, 1998; Stoeva et al. 2003). According to these authors this study showed a significant reduction of chlorophyll content in arsenic treated plants in comparison to control plants (Fig.13). Moreover, Miteva and Merakchiyska (2002) reported that arsenic concentrations higher than 25 mg/kg significantly reduced the chlorophyll content and inhibited the photosynthetic process by 40% in bean plants.

Conclusions

This study shows that P. vittata can activate physiological and biochemical processes targeted to As hyperaccumulation and tolerance even in extremely toxic growth conditions such as the exposure of elevated concentrations of arsenate in hydroponics for a long period. The relationship between the bio accumulation of the metalloid and the capability to tolerate its toxic effects varied remarkably among plant organs. As expected, to cope with the metalloid in the growth solution plants notably reduced their biomass production, probably due to the metabolic efforts carried out to induce and maintain tolerance processes. Particularly, it can be highlighted that organs developed during As treatment, such as young fronds, can maintain a valuable physiological status despite of the elevated As concentration in their tissues. A role for PCs in the safe accumulation of As in these organs was highlighted.

The main changes in the chlorophyll fluorescence parameters due to arsenic treatment were the large reduction in ΦPSII, used usually to estimate the photosynthetic activity , and the notable increase in NPQ indicator for excess light energy. Also chlorophyll content was significantly reduced under arsenic condition.

Therefore P. vittata showed some damage during the long exposure to 28 mg/l of As, but it proved able to tolerate this concentration with a reduction of less than 50% of the photosinthetic activity.

GENERAL CONCLUSION

This study show some different capability of different species to survival in the contaminated site of Isola dei Petroli in the Venice Lagoon and their utility in a phytoremediation and phytocontrol of hydric state strategy.

The survey on the metal and metalloid phytoextraction ability of spontaneous and cultivated plants growing in the landfill of the industrial site of Isola dei Petroli in the Venice Lagoon, show a different behaviour between spontaneous and planted species. All spontaneous species, except for *A. retroflexus* towards zinc, exhibited a higher ability to exclude rather than accumulate heavy metals compared with planted species, resulting not suitable for the *in situ* phytoremediation of a contaminated industrial area. Nevertheless, for an appropriate evaluation of the role of a natural vegetation cover in contaminated industrial soil, it should be considered that naturally growing plants limit pollutant leaching and metal mobility along soil profiles and modify the rhizosphere to favour biological processes. Planted species seemed more suitable for reclaiming metal-contaminated sites. On the contrary, planted species seemed more suitable for reclaiming metal-contaminated sites. In particular, *C. zizanioides* confirmed its valuable capability to mitigate lead contamination in an industrial site, whereas *S. matsudana* and *S. alba* showed the potential to reclaim Cd- and Zn-contaminated soils.

The study on a poplar plantation in the contaminated site of Isola dei Petroli showed as this plantation was able to regulate the hydrological balance of the site during growing season with the exception of occasional heavy thunderstorms at the end of summer. This is an important result because hydrological control of the site is a necessary since the island was completely separated from surrounding sea by a waterproof barrier to avoid environmental contamination. Differences among clones were found for extraction efficiency of Cd, Zn and As. In fact Cd and Zn are more bioconcentrated and clone Neva seems to show the best performances both in pollutant extraction and in biomass increase.

The ex situ study on the capability of Pteris vittata L. to tolerate a long exposure to a high arsenic concentration proved that this species can activate physiological and biochemical processes targeted to As hyperaccumulation and tolerance even in extremely toxic growth conditions. This study, with in order to introduce this species in the Isola dei Petroli, shows that P.vittata suffers some damage during the long exposure to 28 mg/l of As, but it proves able to tolerate this concentration with a reduction of less than 50% of the photosynthetic activity. In this contest this specie could be planted in situ even if some studies on tolerance of particular soil should be performed.

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CURRICULUM VITAE
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  Terminato e in attesa della data di discussione, prevista presso l'Università degli Studi della Tuscia, Viterbo, nell'anno 2010.
  Titolo tesi: **Phytoextraction and hydrological phytocontrol in an industrial site contaminated by heavy metals and arsenic**
  Temi di ricerca: studio, in un sito industriale contaminato di Porto Marghera-VE, sia delle capacità di estrazione di metalli pesanti e arsenico di specie vegetali presenti spontaneamente o introdotte sia delle capacità di controllo dello stato idrico del sito da parte di una piantagione di cloni di pioppi.

- **Laurea in Scienze Forestali e Ambientali:** 20 Giugno 2005 - Università degli Studi della Tuscia (Voto: 110/110 e Lode)

  Tesi di laurea sperimentale: "**Analisi dell'accrescimento di una faggeta danese in rapporto ai fattori ambientali mediante la tecnica degli isotopi stabili del carbonio**"-

  Relatore: Prof.Giuseppe Scarascia Mugnozza

  Tirocinio pratico-applicativo: Tetouan (Marocco) – Viterbo

  Il tirocinio è stato svolto nell'ambito di uno "**studio dendrocronologico e dendroecologico su *Abies maroccana* Trab. del Rif**".

  Tutore: Prof. Bartolomeo Schirone, D.A.F. Università della Tuscia in collaborazione con l'Università di Tetouan e Movimondo ong

Attività professionale e fruizione di borse di studio e fellowship:

nov 2006 – nov 2009	*Borsa di studio* presso l'Istituto di Biologia Agro-Ambientale e Forestale (IBAF) del CNR di Monterotondo Scalo (Bando N.010.BS.2/06 del 02/08/2006 prot:408/06) nell'ambito progetto della Regione Lazio "Modelli applicativi di agricoltura multifunzionale nello sviluppo sostenibile di alcune aree della regione Lazio". Il lavoro svolto rientra nella tematica del fitorimedio e in particolare si è studiato l'uso di colture forestali, quali pioppi e salici, nel fitorimedio di alcuni metalli pesanti come As, Cd, Zn, Pb.
lug 2006 – sett 2006	*Contratto di prestazione d'opera in regime di lavoro autonomo occasionale* presso l'Istituto di Biologia Agro-Ambientale e Forestale (IBAF) del CNR di Monterotondo Scalo riguardante attività di analisi delle caratteristiche fotosintetiche su diverse varietà di cotone e in condizioni di campo al fine di valutare differenze nella tolleranza alla siccità di tali varietà. In particolare lo studio svolto rientra nell'ambito di un progetto EU-INTAS, una collaborazione tra task provenienti da Svizzera,Italia, Uzbekistn e Kazakistan, per la selezione di genotipi di grano e cotone resistenti alla siccità
mar 2006 – lug 2006	*Marie Curie Fellowship* presso Institute of Forest Botany, Georg-August-University, Gottingen, Germania, con tema di ricerca **"**Impact of elevated CO_2 and fertilization on some biochemistry wood property of P.euramericana". In particolare studio del contenuto in proteine solubili, zuccheri e amido, Klason lignina e potere calorifico in campioni di legno di Populus euramericana provenienti dal sito sperimentale EURO-FACE di Tuscanica (VT, Università della Tuscia).

dic 2005 - feb 2006 *Contratto di prestazione d'opera in regime di lavoro autonomo occasionale* presso presso l'Istituto di Biologia Agro-Ambientale e Forestale (IBAF) del CNR di Monterotondo Scalo riguardante attività di studio e ricerca per la gestione sostenibile delle risorse forestali e faunistiche della Riserva Naturale Monte Navegna e Cervia, con particolare attenzione allo studio sulle potenzialità di assorbimento del carbonio da parte della stessa riserva per 'applicazione del Protocollo di Kyoto a livello regionale

Idoneità:

gen 2006 Idonea ma non vincitrice per il conferimento di una borsa di studio per l'approfondimento delle tematiche di particolare rilievo per il settore della pesca e dell'acquacoltura, indetta con bando su G.U. n°3 del 13 gennaio 2006 dal Ministero delle Politiche Agricole, Alimentari e Forestali, Dipartimento delle filiere agricole ed agroalimentari, Direzione generale della pesca marittima e dell'acqua coltura.

Attività scientifico-divulgativa:

PUBBLICAZIONI ISI

- Pietrini F., Zacchini M., Iori V., Pietrosanti L., Ferretti M. & Massacci A. - **Spatial distribution of cadmium in leaves and its impact on photosynthesis: examples of different strategies in willow and poplar clones –** Plant Biology, 2010, 12:355–363

- Pietrini F.; Zacchini M.; Iori V.; **Pietrosanti L**.; Bianconi D.; Massacci A. - **Screening of poplar clones for cadmium hytoremediation using photosynthesis, biomass and cadmium content analyses** – International Journal of Phytoremediation, 2010, 12:105-120

- Zacchini M., Pietrini F.,Scarascia-Mugnozza G., Iori V., **Pietrosanti L**., Massacci A.- **Metal tolerance, accumulation and translocation in poplar and willow clones treated with cadmium in hydroponics** – Water Air Soil Pollut, 2009, 197: 23-34

- Massacci A., Nabiev S.M., **Pietrosanti L.**, Mematov SK., Chernikova T., Leipner J.,- **Response of the photosynthetic apparatus of cotton (Gossypium hirsutum) to the onset of drought stress under field conditions studied by gas-exchange analysis and chlorophyll fluorescence imaging** - Plant Physiology and Biochemistry, 2008, 46:189-95

PROCEEDINGS NAZIONALI ED INTERNAZIONALI

- **Pietrosanti L.**, Matteucci G., Pietrini F., Stivanello S., Capotorti G., Molinari M., Magnani E., Santarelli G., Zuin M.C., Aromolo R., Massacci A. -**" Hydrological control and phytoremediation by poplar and willow clones in a contaminated industrial site in Venice Lagoon"**. In Book of Abstract (pag. 53) and in CD e-proceedings (ID138) – 4° European Bioremediation Conference, Chania, 3-6 sett 2008 - PRESENTAZIONE ORALE

ABSTRACT

- De Paolis M.R., **Pietrosanti L**., Capotorti G., Molinari M., Masacci A. and Lippi D. – **Cadmium-resistant bacterial communities in a contaminated industrial site in Venice Lagoon** – 14th International Biodeterioration and Biodegradation Symposium – IBBS-14, pag. 174, (Sant'Alessio siculo, Messina, Italy, 6-11 ott 2008)

- Capotorti G., Nardella A., Molinari M., Massacci A., **Pietrosanti L**., Matteucci G., Zuin M.C. - **Phytoremediation Test for Arsenics Pollution Control in contaminated Industrial Soil in Porto Marghera-** *Paper B-11*, in: A.R. Gavaskar and C.F. Silver (Symposium Chairs), *In Situ and On-Site Bioremediation—2007.* Proceedings of the Ninth International In Situ and On-Site Bioremediation Symposium (Baltimore, Maryland; May 7–10,2007). ISBN 978-1-57477-161-9, published by Battelle Press, Columbus, OH, www.battelle.org/bookstore.

ALTRO

- POSTER DI PRESENTAZIONE "**Mesocosmi sperimentali per lo studio dei processi fisiologici di specie arboree forestali nell'ambito del fitorimedio.** - Responsabili: Massacci A., Scarascia-Mugnozza G.; Collaboratori: Pietrosanti L., Iori V., Barchetti A., Rosati S., Pietrini F., Zacchini M.".
  Meeting "Modelli applicativi di agricoltura multifunzionale nello sviluppo sostenibile di alcune aree della Regione Lazio" PRAL – progetto codice 2003/92, Regione Lazio, Direzione Agricoltura, CRA, Unità di ricerca per la climatologia e la meteorologia applicata all'agricoltura, 14 marzo 2008

- POSTER DI PRESENTAZIONE “**Relazione tra ecosistemi terrestri ed inquinanti: ripristino ecologico, biorimedio e biomitigazione** – Massacci A., Bianconi D., Pietrini F., Pietrosanti L., Iori V., Zacchini M.”
  REMTECH, I Salone sulle bonifiche dei siti contaminati, Ferrara, 26-28 sett 2007

<u>Altre partecipazioni a corsi e meeting</u>

giu 2009	Corso di formazione ambientale - Siti contaminati : caratterizzazione, bonifica e analisi di rischio – I modulo: Caratterizzazione ambientale” – ISPRA, Istituto Superiore per la Protezione e la Ricerca Ambientale, Roma
mag 2008	Giornata Internazionale della Biodiversità ed Agricoltura- Bioversity International, Roma
mag 2006	Corso di Metodologia Sperimentale in Agricoltura – Prof. R.Casa, Università delle Studi della Tuscia, Dipartimento di Produzione Vegetale, Viterbo
ott 2003	Corso R.Y.L.A.: “Ambiente e territorio: problematiche dello sviluppo sostenibile in un territorio costiero soggetto a forti pressioni antropiche” Parco Nazionale del Circeo , Sabaudia-LT

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Lucia Pietrosanti

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