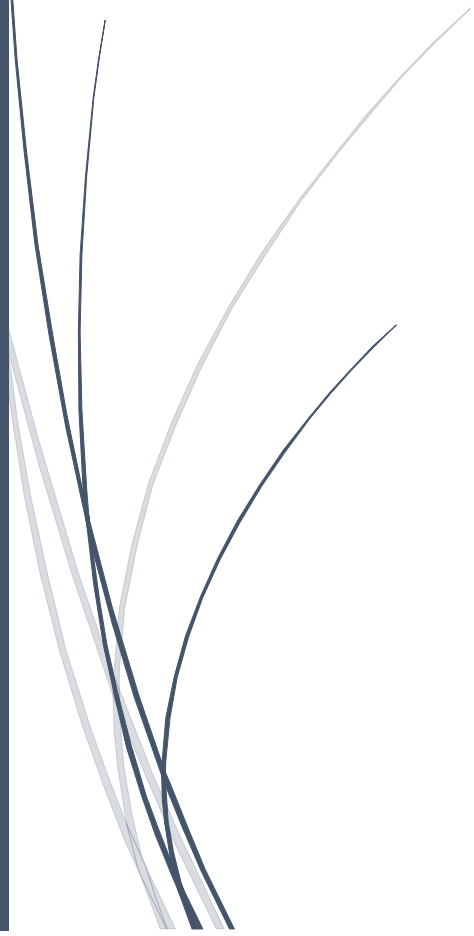
Phyto-portfolio

Research Institute on Terrestrial Ecosystems (IRET)

National Research Council (CNR)



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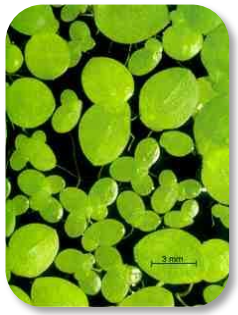
Intro

The activity of the Research Institute on Terrestrial Ecosystems (IRET-CNR) arises from the experience gained from over 20 years of base research on plant physiology, on the biological functions at roots and leaf level, and mainly on photosynthesis. The development of these research lines led to study the active role of the plant towards the environment and therefore their potential in the uptake, degradation and accumulation of contaminants and in the stimulation of the microorganism activities within the rhizosphere. Over 50 International publications have been produced on these topics highlighting the high quality of the professional expertise of the research group.

Besides the long research activity on phytotechnologies, the IRET focused also on knowledge spreading (the first International conference on Phytoremediation in Italy has been organised by IBAF in Sorrento, 2000), on educational issues (coordinating Thesis and PhDs) and on technology transfer (implementation of a spin-off which is currently under definitive approval). Additionally, in order to reach a larger audience, IRET produced several dissemination material on phytotechnologies such as a video and a database, accessible from IRET website (https://www.iret.cnr.it/phytoremediation/phytoremediation.pdf).

In 2015 a pilot project on phytoremediation developed in Taranto (Fact Sheet IV) has been awarded the prize of Legambiente as “Remarkable Good Environmental Practice”.

Process goal: Remediation of ORGANIC COMPOUNDS

Tested plant species	 Poplar	 Willow	 Duckweed
Lab. / Field studies	Poplar *in vitro* culture with **Ibuprofen** (pharmaceutical pollutant, 0.03-30 mg/l) Taranto (**PCB** contamination, fact sheet IV) Valle del Sacco (**Lindane** contamination, fact sheet II) Papigno Project (**PCB** contamination, fact sheet I)	Willows in hydroponics with **Ibuprofen** (pharmaceutical pollutant, 3-30mg/l) Papigno Project (**PCB** contamination, fact sheet I)	The effects of 1 mg/l , 0.2 mg/l and 0.02 mg/l (hight and environmentally relevant levels) of **Ibuprofen** on *Lemna gibba* L. were monitored in an 8-day lab. test
Main outcomes	• Poplar cells showed a remarkable tolerance to ibuprofen (Ref. 13) • Ibuprofen complete removal in 3 weeks. (Ref. 13) • Poplar clone Monviso demonstrated to well tolerate PCB and Lindane pollution and to enhance biodegradation in soil. This poplar allows to successfully associate soil cleaning by rhizoremediation with an economically sustainable biomass for energy production of large poplar plantations.	Specific willows clones removed 37-81% of Ibuprofen from the solution in 2 weeks. (Ref. 15)	• The role of *L. gibba* in Ibuprofen metabolism has been proven. (Ref. 32) • 11 Ibuprofen metabolites were detected in plants. (Ref. 6) • A growth stimulation of *Lemna* was observed in presence of Ibuprofen. (Ref. 6, 32) • About 90% of Ibuprofen was removed in 8 days. (Ref. 6, 32)
Potential for phytotechnologies	Wastewater reclamation Rhizodegradation	Wastewater reclamation Hydrological control	Wastewater reclamation Biomonitoring

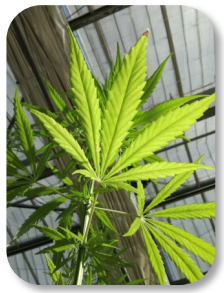
Process goal: Remediation of HEAVY METALS

*BCF= Bio Concentration Factor; Contaminant Concentration in plant/concentration in soil or water

Tested plant species	 Poplar	 Willow	 Eucalyptus	 Amaranth
Lab. / Field studies	Tests on different poplar clones tested in hydroponic with **Cadmium** (10 mg/l $CdSO_4$ and with zinc (1500-4700 mg/l $ZnCl_2$) Outdoor pot experiment for two poplar clones with **Cd** (Ref.7) Papigno and Taranto **multi-contaminated** sites (Fact sheets I, IV) Isola dei Petroli **As** contaminated site (Fact sheet V)	Tests on different willow clones tested in hydroponic with **Cadmium** (10 mg/l) and zinc (1500-4700 mg/l) Isola dei Petroli **As** contaminated site (Fact sheet V) Papigno **multicontaminated** site (Fact sheet I)	Tests in hydroponics with **Cadmium** (10 mg/l $CdSO_4$)	Tests in hydroponics with **Nickel** (1.5-8.5 mg/l NiCl). Plants grown in soil mixed with industrial slag (steel work factory) for 6 weeks in greenhouse
Main outcomes	• Cadmium BCF* in hydroponics: 160 –300. (Ref. 37, 41) • Zinc BCF* in hydroponics: 1500 (Ref. 7,9) • Poplar clone Monviso showed to well tolerate the multicontamination of heavy metals and organics. (Ref. 4, 47, 54) •Trees have been efficient in hydrological control during the vegetative season. (Ref. 36, 43, 44, 53)	• Cadmium BCF* in hydroponics: 80 (Ref. 37) • Trees have been efficient in transferring rainwater from soil to the atmosphere during the vegetative season, restoring the hydrological balance. (Ref. 36, 43, 44, 53)	Cadmium BCF* in hydroponics: 33-200 (Ref. 35, 45)	• Amaranth removed about 60% of Ni from the solution after 1 week. (Ref. 14) • Amaranth tolerated up to 5% of slag added to soil. (Ref. 34)
Potential for Phyto-technologies	Wastewater reclamation Phytostabilisation Hydrological control	Wastewater reclamation Phytostabilisation Hydrological control	Wastewater reclamation Hydrological control	Wastewater reclamation Phytoextraction

Process goal: Remediation of HEAVY METALS

***BCF**= Bio Concentration Factor; Contaminant Concentration in plant/concentration in soil or water

Tested plant species	 *Duckweed*	 *Common reed*	 *Ladder brake*	 *Cannabis sativa*
Indoor / Field studies	Plants of *L. minor* were exposed to different **Cadmium** concentrations (0, 1.5, 2.5 and 5 mg L^{-1} Cd) for periods of 24, 48 and 72 h at ambient and at elevated CO_2	Plants exposed to a high concentration of **$CdSO_4$** (50 µM) for 21 days were analysed with respect to the distribution of metal its effects on antioxidants, the antioxidant enzymes and the redox status in leaves, roots and stolons.	Tests in hydroponics with **Arsenic** (28 mg/l Na_2HAsO_4) Isola dei Petroli **As** contaminated site (Fact sheet V)	Greenhouse experiment in pots filled with soil from "Valle del Sacco" contaminated site (As: 22.6 ppm ; Pb: 115 ppm ; V: 106.7 ppm; Zn: 92.8 ppm)
Main outcomes	Lemna proved to be a useful organism for water treatment systems because of its high accumulation capacity and the ease of culture. It accumulated up to 4800 mg kg^{-1} DM; adverse effects of Cd were significant at 5 mg L^{-1} Cd. (Ref. 33)	•The highest accumulation of Cd^{2+} occurred in roots followed by leaves.(Ref. 11) •Glutathione exerted a direct important protective role on photosynthesis in the presence of Cd. (Ref. 27)	• Arsenic BCF* in hydroponics: 60. (Ref. 36) • Ladder brake easily propagates in greenhouse, efficiently concentrates As from soil to vegetal tissues. (Ref. 36, 43, 44)	• Hemp plants cv. Codimono can satisfactorily grow in a soil moderately contaminated by metal (loid)s, producing biomass, inflorescence and seeds • The toxic metal(loid)s were not translocated in the above ground organs: leaves, inflorescences and stem free from toxic metals • Presence of metal(loid)s in roots
Potential for Phyto-technologies	Wastewater reclamation Biomonitoring	Wastewater reclamation High tolerant plant vs heavy metals Phytoextraction	Phytoextraction	Heavy metal containment Production of safe bio-resources on slightly contaminated soil

DEVICES FOR ON-SITE TREATMENT (Mesocosms)

Outdoor facility, sited in the experimental field of the "Area della Ricerca di Roma 1", CNR-Italy, it is used for long term studies on plant physiological responses to nutrients and contaminants. The device works in semi-hydroponic conditions and comprises plastic containers in which agriperlite is used as anchorage and draining material for the plants.

Mesocosms, lateral view

It is a modular system that can work in parallel or in sequence, easily adapting to the treatment targets. The system can be placed in the contaminated sites for the on-site treatment of the contaminated matrices (water/sediments/soil).

External tank and pump with float

Mesocosm, top view

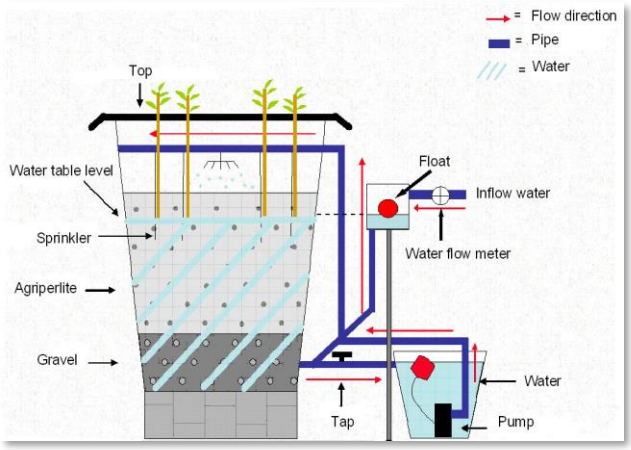
Mesocosms front view

Plant scheme

The facility consists of 18 circular 1 m3 plastic containers (0.785 m2 x 1.3 m depth) arranged in two rows. Each tank can contain up to 4 plants.

The tanks are filled with a constructed medium profile including two layers from bottom up: gravel (0-0.25 m) and agriperlite (0.25-1.00 m). Each tank contains 500 l of water.

The water flow is performed by pumping water from an external tank (50 l) into the big container and then allowing it to flow back into the external tank via tap valve. The operation of the drain-refill cycle takes ~ 150 minutes. An automated switching device controlled the maintenance of the water table level in the container.



FACT SHEET I

PAPIGNO FORMER INDUSTRIAL LANDFILL

Project founded by Italian Government.

Partners: IRET-CNR

Umbria Environmental Protection Agency (ARPA)

University of TUSCIA

Location - Papigno (Terni), Umbria region, Italy

Site description – Ex-Industrial area for the production of chemicals (calcium carbide and calcium cyanamide); the contaminated area was used for 60 years as a landfill of waste from production. Later the area has been used to discharge inert wastes. Then, one part of the site became a public park with sport facilities. In 2001 the area has been included within the perimeter of a National Interest Polluted Site (SIN).

Climate- mediterranean, continental climate

Size - 3,5 ha

Critical issue: the risk factors are related to: the pollution of the adjacent river as a result of sediment transport (the aquifer is not contaminated), the improper use of the land for crops, the dust transport to the village and the direct contact.

Contamination – irregular distribution of the contaminants in spots, near surface (0-2 m). PCB (up to 3,5 mg/Kg, legal limit:0,06 mg/Kg), hydrocarbons C>12 (up to 360mg/Kg; legal limit 50mg/Kg) and heavy metals (Zn, Cu, Pb, Ni, Cr)

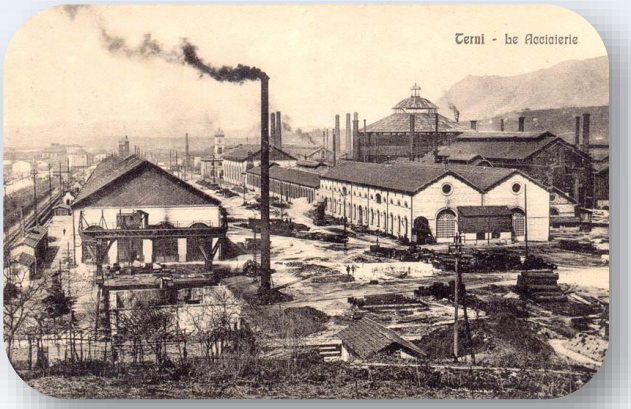
Goals: remediation of the contaminated area, quick return of the area to the local communities for access and usability, landscape rehabilitation.

Phytotechnology system – The area will be splitted in several sub-areas of treatment: i) Green Area, which will be promptly remediated through the removal of the contamination hot-spot; ii) Area for Extensive Phytoremediation, where a short rotation forestry plantation will be created, iii)Intensive Phytoremediation Area, where the contaminated soil removed from the green area will be phyto-treated in treatment tanks.

Vegetation type–poplar, willows and local vegetation.

Project status – the preliminary project was approved, bureaucratic procedures in progress.

Aerial view with delimitation of the treatment area



Electrochemical Industry in Papigno during the production activity

Detail of the preliminary project, on site soil treatment tanks

FACT SHEET II

VALLE DEL SACCO

Project founded by the Special Commissariat for Emergencies

Partners: IRET-CNR

IRSA-CNR

ISB-CNR

Location – Valley of the Sacco river (Frosinone), Lazio region, Italy

Site description – The Sacco River has been polluted with chemicals due to the uncorrected disposal of wastes of a industry manufacturing plant protection products. Subsequent to flooding events and to crop irrigation with water from the river, the riparian strip along the river has been seriously contaminated for several kilometres. Organic contaminants have been found into the milk of the cows fed with the contaminated forage; this event raised the case: the site has been registered as Polluted Site of National Interest (SIN).

Climate- Mediterranean, continental climate.

Size – 4000 m^2 (experimental area), 1000 ha (total polluted area)

Critical issue - any agricultural and zootechnical utilisation of the contaminated area has been forbidden. This pose a serious harm to the local economy. The large extension of the polluted area imposes a socio-economically and ecologically sustainable approach.

Contamination - medium-high concentrations of hesachlorociloexane (HCH, lindane) have been measured in the soil of the experimental area. Among the three isomers: αHCH: up to 0,02 mg/Kg; βHCH: up to 0,06 mg/Kg; γ-HCH: 0,02 mg/Kg; legal limit for each isomer 0,01 mg/Kg.

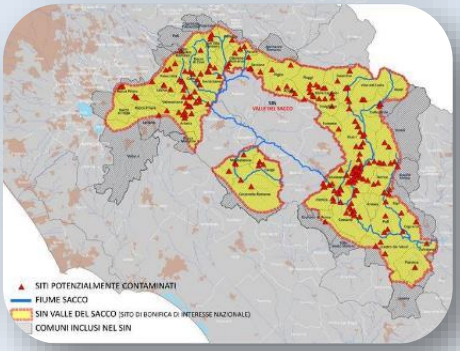
Goals: to remediate the area, avoiding the misuse of the land for crops and grazing and creating a source of revenue for farmers

Phytotechnology system – Establishment of a Short Rotation Forestry plantation (SRF), with high-density poplars, in order to stimulate the contaminant degradation (rizhoremediation) and to ensure a source of income for local farmers through biomass production. The possibility of growing *Cannabis sativa* in the polluted fields is currently being evaluated.

Vegetation type – A particular poplar genotype (Monviso) has been selected for its tolerance towards the contaminant and effectiveness in the stimulation of the rhizosphere degrading activity. An Italian cultivar of *C. sativa* (Codimono) is under test.

Project status – the pilot plan has been realized in 2009 and important reductions in Lindane content have been registered (57% after 2 years from planting time).

Main outcomes –important reductions in Lindane content have been registered (57% after 2 years from planting time).



First perimeter of the polluted site (SIN)

Prohibition of agricultural and zootechnical utilisation of the area

High density poplar plantation

Poplar plantation, distance between trees

ALCANTARA INDUSTRIAL LANDFILL

FACT SHEET III

Project founded by
Alcantara SpA.
Partners:
University of TUSCIA
IRET-CNR

Location - Nera Montoro (Terni), Umbria region, Italy

Site description –Industrial site (Alcantara SpA) for the production of a covering material. The landfill area of the Alcantara factory includes sealed landfills and a landfill currently in use, producing the major part of the leachate.

Climate- Mediterranean, continental climate

Size - 1400 m^2 (field 1), 2100 m^2 (field 2) and 1900 m^2 (field 3).The surface of the landfill "in use" is 2800 m^2

Critical issue – the treatment of the leachate produced by the landfills represents a high cost for the Industry. The leachate production varies over the year, with a minimum in summer.

Contamination – The leachate has high DOC and is rich in Sulphates and Chlorides.

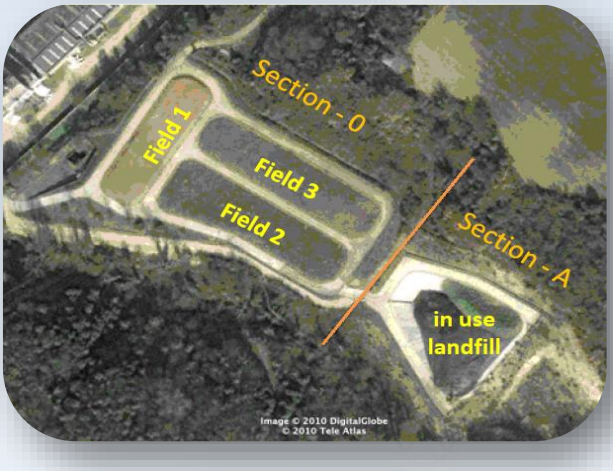
Goals: reducing or completely avoiding the need of transfer of the landfill leachate to external treatment systems.

Phytotechnology system - different plant species were planted on the top of the sealed landfills in order to consume the water faction of the leachate. To cope with the seasonality of the leachate production, the waste is stored in tanks and additional source of water are used during summer. The additional water is produced in the factory and is characterised by high concentration of nitrogen (so acting as fertiliser). The leachate is provided to the plants by an automated irrigation system, based on the continuous monitoring of the soil water content and conductivity.

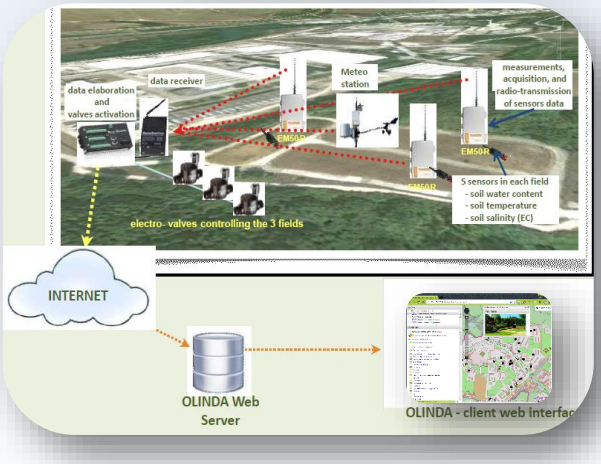
Vegetation type–.Three different evergreen woody shrub species (*Nerium oleander, Viburnum tinus, Prunus laurocerasus*) were selected, according to the local climate conditions, and tested comparatively to evaluate the best compromise between the leachate consumptions and management costs. As additional constraints for the plant selection, the limited soil depth and the seasonality of leachate production have been considered.

Project status – the system has been realized in 2012 and is currently working efficiently.

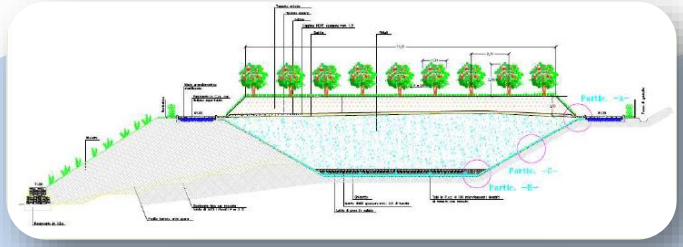
Main outcomes – the project enabled to interrupting the cycle of waste production (leachate).



Aerial view of the landfills



Schematic representation of the control system



Section of the landfill with phytocapping

The vegetated cover pf the landfills

TARANTO CONTAMINATED SITE

FACT SHEET IV

Project founded by CISA SpA.
Partners: IRSA-CNR
IRET-CNR

Location - Taranto, Puglia region, Italy

Site description – the site was employed as armoury of the Italian Navy. Subsequently the area was used as an uncontrolled disposal of different kinds of waste. Currently the site has been granted under concessions to a local non-profit organisation (CEM).

Climate- Mediterranean

Size - 1600 m^2 (experimental area), 5000 m^2 (total polluted area)

Critical issue – the area is contaminated and thus it is not suitable for industrial, commercial or residential use. The proximity to the sea increases the risk of contamination spreading to other environmental matrices.

Contamination – High heavy metal concentrations were found into the soil (**Cr**: 34-284 mg/Kg, legal limit: 150 mg/Kg; **Zn**: up to 556mg/Kg, legal limit: 150 mg/Kg; **As**: up to 36 mg/Kg, legal limit 36 mg/Kg; **Cd**: 11mg/Kg, legal limit: 2mg/Kg; **Pb**: up to 794mg/Kg, legal limit: 100mg/Kg). A PCB pollution has been detected, probably the result of some transformer oil spillage (Total PCBs: up to 0,31 mg/Kg, legal limit: 0,06 mg/Kg). Metal and **PCB** pollution has a typical irregular dispersion into the soil matrix (up to 300 µg/Kg, legal limit: 60 µg/Kg).

Goals: cleaning up of the area, ensuring the involvement of local communities.

Phytotechnology system – Part of the site has been organized in two experimental plots, planted with a hybrid poplar genotype and with *Tamarix gallica*, respectively. The plots have been equipped with an automatic irrigation system. The plantation was supposed to have a role in PCB degradation through microbial population enhancement (rhizoremediation) and in metal containment (phytostabilisation). The participants to an employment assistance project coordinated by the non-profit organisation CEM assured a careful maintenance of the vegetation.

Vegetation type– a particular poplar clone (Monviso) has been selected for his hardness, his tolerance towards organic chlorinated contaminants and for its proved effectiveness in the remediation of contaminated soil through rhizoremediation. Tamarisk species has been chosen because it is an autochthonous of Mediterranean regions, well adapted to chalky soil and salt spray resistant. The poplars showed grate survival and growth rates; the tamarisks did not survived, probably due to a delay in planting.

Project status – The pilot plant has been realized in 2013. In 2015 the project was awarded by Legambiente as "Remarcable Good Environmental Practice"

Main outcomes – To date, important reductions in PCB (below the legal limit) and metal content have been registered in the soil surrounding roots (rhizosphere).

Location of the contaminated area

Poplar plantation after planting (above), and after 18 months (below)

Vegetation in the polluted site 18 months after planting.

FACT SHEET V

ISOLA DEI PETROLI

Project founded by ENI SpA.
Partners: IRET-CNR

Location - Porto Marghera (Venice), Italy

Site description – Artificial island (called "Isola dei Petroli", "oil island"), in past it was used as landfill for wastes from petrochemical industry. From 2008 in order to prevent the contamination of surrounding waters, an impermeable barrier has been constructed around the island. The island is included into the industrial zone of Porto Marghera, classified as one of the most important "contaminated sites of national interest "(SIN) in Italy.

Climate –Mediterranean climate with very high humidity (57-90 mm rainwater per month)

Size –1000 m^2 (experimental area); 12 ha (total polluted area)

Critical issue: Following the construction of an impermeable barrier, built to avoid the contamination of the surrounding water, the rainwaters raise the water table, transferring the contaminants up to the topsoil. After rainfalls, the Island is flooded. The contaminated fine particulate is transferred from soil to the surrounding areas by wind.

Contamination - Arsenic (max. conc.105 mg/Kg, Ldl: 50 mg/Kg), Zn, Pb, Cd (max. conc.27 mg/Kg; Ldl: 15 mg/Kg). The contamination is unevenly distributed and concentrated in the top 1-2 m of the soil.

Goals – phyto-hydrological control and heavy metal phyto-immobilisation in the top-soil.

Phytotechnology system – A portion of the Island has been planted with poplar and willow in order to test the potentiality in heavy metal immobilisation and in the hydrological control of the site (the trees work as a "biological pump", extracting water from soil through evapotranspiration). Another experimental plot has been planted with *Pteris vittata*, in order to test the suitability of this species for Arsenic extraction.

Vegetation type – Poplar and willow species were chosen for their high transpiration potential and for their tolerance towards heavy metals in soil. Pteris vittata is a fern well known for its extraction potential of Arsenic (hyperaccumulator species).

Project status – pilot plan execution.

Main outcomes – The poplar and willow vegetation has transferred to the atmosphere all the rainfall during the vegetative season. During winter, the hydrological balance has not been compensated. Priority in selection should be given to high transpiring evergreen species (e.g. Eucalyptus). The fern can be easily propagated in greenhouse, grows well under the tree canopy and efficiently concentrates As from soil to vegetal tissues, however it does not adapt to winter temperature of the site; it needs annual re-planting.

Isola dei petroli, aerial view

Isola dei petroli, side view

Evidence of sediment contamination on the island

ARTICLES PUBLISHED BY IRET-CNR GROUP ON PHYTOREMEDIATION

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