Executive Summary:

The overall goal of UMBRELLA was to use microorganisms to develop cost-efficient and sustainable measures for soil remediation at heavy metal contaminated sites throughout Europe. The work packages on microbiology (WP1), plant uptake (WP2) and (hydro)geochemistry (WP3) produced data on microbial influence on biogeochemical cycling of metals from six European sites (Germany, Italy-Sardinia, Poland, Romania, Sweden, Wales-GB) in all climatic conditions which can be used as input for modeling of the processes and their influence on large-scale landscape levels (WP 4). The transfer of technologies (WP 5) includes a tool-box to end-users with protocols for acquisition of microbes for remediation actions in different European climatic, geological and biological settings that will allow low-cost, sustainable, on-site bioremediation of metal contaminations. Dissemination (WP 6) of results was ensured by participation in international congresses and publications; at the same time, an internationalized education of interdisciplinary trained PhD students provides a sustainable education profile with relevance to soil remediation. The impact of the data provided for use in soil and water protection has been addressed with involvement of government agencies (WP 7). The project focussed on ecotoxicological risks resulting from metal contamination on-site as well as by transport through water paths in ground water and international water ways, provide rules to overcome the current practise of regulation by individual European agencies for soil and water protection. This proposal aims at the development of innovative techniques that will provide a milestone in biotechnology for soil remediation by establishing optimal techniques for coupling microorganisms and plants for the remediation of soils influenced by mining activities; improving and geochemically monitoring metal stabilization by mineralization depending on biotic and abiotic factors; producing mathematical models of key processes at ecosystem and landscape scale relevant to the distribution of contaminants which, taken together will lead to a desig of integrated bioremediation methodologies for different landscapes across the European Union influenced by mining. The project's landscape approach explicitly connects soil remediation with the reduction of the risk for other environmental compartments (mainly water, but implicitly relevant also for biodiversity). A broad EU dimension is ensured by the development of a remediation methodology using mathematical models using data sets obtained from contaminated sites distributed across large latitudinal and longitudinal gradients. The ultimate goal, a technique for soil remediation allowing unrestricted land-use after recultivation of former mining sites or other areas with metal contamination, has been addressed by providing guidelines and rules of best practise.

Project Context and Objectives:

Phytoremediation is an in situ procedure by using vegetation to treat contaminated soils. It provides an alternative method to the expensive and soil destroying technical, thermal, chemical in-situ or ex situ treatments. Cornerstone of phytoremediation is plant transpiration by altering the water balance of the location (Robinson et al., 2003). Plant growth limits the movement of contaminants off site by reducing the erosion by wind, run off, and leaching. This requires the growth of plants which needs to be possible in spite of the site contamination. Phytoextraction then can be performed by translocation of contaminants from soil via plant roots into easily harvestable boimass. Or else, phytostabilization allow fixation of contaminants preventing erosion, groundwater contamination, and introduction of contaminants into the nutritional nets. This includes metal stabilization through complexation, sorption or precipitation (Ghosh and Singh, 2005).

The overall goal of UMBRELLA was to use microorganisms to develop cost-efficient and sustainable measures for soil remediation with plants at metal contaminated sites throughout Europe based on the general knowledge of microbe-plant interactions. Thus, work packages on microbiology, plant uptake and transfer, (hydro)geochemical characterization of metal mobilization and geomorphological modeling were combined to investigate the microbial influence on plant growth and metal biogeochemical cycles. Dissemination was achiebed by compiling rules of best practise within a guideline for remediation to be used by end-users like land owners, federal, state, and municipal governments, as well as EU for legislation.

The formulated objectives of the UMBRELLA project are establishing of techniques for coupling microorganisms and plants for remediation of soils influenced by mining activities; improving of metal stabilization by mineralization depending on biotic and abiotic factors and geochemical monitoring; producing mathematic models of key processes at ecosystem and landscape scale relevant to the distribution of contaminants and designing of an integrated bioremediation methodology for different landscapes across the European Union influenced by mining.

The risk characterization determines the qualitative or quantitative probability of occurrence of known and potential adverse effects of an agent in a given organism under defined exposure conditions (World Health Organisation, 2004). Risk characterization combines the results of exposure assessment and hazard assessment and can be expressed as the quotient between site-specific exposure and critical exposure. This risk index serves as a basis for priority rankings.

In contrast to human health as protection target risk assessment in terms of soil ecosystem combines exposure assessment and hazard assessment into one operation. From an ethical point of view it is generally accepted that the investigation of the influence of contaminants to the ecosystem can be performed in a direct way. In ecological risk assessment the total soil concentration plays only a subordinate role. The bio-available fraction is the more prominent measure for exposure. As a basis for all further actions within the remediation process the investigation of the contaminated site is a very important step. The results of this investigation deliver data with impacts on the risk assessment, the potential remediation method, and also on the site's future use.

A Preliminary Investigation (= common approach) comprises a desk study and also site visits. The conceptual model should be developed, adjusted, and improved in the course of site investigation until a desirable degree of detail has been reached and after defining the remediation strategy the first risk management measures can be performed. According to UMBRELLA's innovative approach (= additional approach) and the improvement of phytoremediation techniques by using microbes in order to promote plant growth or to enhance metal extraction at contaminated sites, respectively, site investigation for applying UMBRELLA methods requires extensive knowledge about their biocoenosis. Hence, a concept for the survey of the current vegetation cover, its species and distribution, as well as the investigation of microbes within the soils of contaminated areas should be prepared in the Preliminary Investigation. The results of these examinations provide useful information for the application of UMBRELLA's remediation tools. A fieldwork to collect the soil and groundwater samples is the main part of the Exploratory Investigation. The sampling strategy needs to be adjusted to the respective site by taking into account the results of the Preliminary Investigation. During the Exploratory Investigation, the last step of the site investigation concentrates on the improvement of knowledge about the contamination's spatial distribution. Finally, risk management leads to definition of UMBRELLA remediation strategy.

Development of rhizosphere consortia including AM fungi and bacteria is an important prerequisite of the successful site restoration. Phytostabilization and phytoextraction are chosen as remediation strategies in the UMBRELLA project. Phytostabilization as the main phytoremediation strategy is chosen to stabilize the soil by developing a vegetation cover. The other strategy, phytoextraction, is well suited as a polishing step for already rehabilitated areas with still high concentrations of metals and radionuclides. As generation of a vegetation cover and enhancing plant growth usually requires fertilization, or potentially even harmful substances being added like EDTA, the beneficial character of microorganisms like rhizobacteria and arbuscular mycorrhizal fungi on plant uptake of metals, degradation of organic pollutants or increasing the bioavailability of the metals is exploited; however, phytoremediation in combination with soil microorganisms without conventional soil amendments is still poorly investigated and understood (Salt et al., 1995; Ma et al., 2001).

Phytostabilization: Metal and radionuclides contaminated soils are mostly sparse covered by vegetation, because of the toxic effects of these pollutants. Hence, soils and the polluters are expected to disperse by water and wind erosion (Salt et al., 1995). Phytostabilization means to hold contaminated soils and sediments in place by establishing a vegetation cover. Thus, a viable plant for phytostabilization should tolerate high concentrations of various metals. The challenge is to identify regional-specific native plants that do not accumulate metals in their above-ground tissues (Mendez and Maier, 2008), so strong native plants with an extensive root system are preferred. In temperate regions grass and in the arid regions drought-, salt- and metal-tolerant plants are the most used species (Mendez and Maier, 2008). Plant families for phytostabilization purposes are, for example, Asteraceae, Euphorbiaceae, and Poaceae as summarized by Mendez and Maier (2008). Vegetation, in general, lead to immobilization of metals by precipitation of less soluble forms, metal reduction, complexation and sorption onto roots as summarised by Salt et al. (1995) and Mendez and Maier (2008). Primary purpose of the plants is to stabilize the soil, to provide hydraulic control and suppress the vertical migration of leachate to groundwater (Salt et al., 1995). Since, a major impact in temperate environments is the formation of AMD as a result of lowering pH and mobilization of metals. Typical plants are phreatophyte trees for hydraulic control, grasses with fibrous roots to prevent soil erosion, and dense root systems are needed to fix contaminants. Phytostabilization may be more cost effective and easy to implement in contrast to other phytoremediation strategies in case of metal contaminated sites where ex situ methods are not practical. Additionally, phytostabilization may also be a polishing step where concentrations of contaminants are below regulatory action levels.

Phytoextraction: The best plant for phytoextraction should tolerate a high level of metals, be able to accumulate metals in high amounts in easy harvestable plant parts, have a fast growth rate and produce high biomass (Salt et al., 1995). Metal accumulation by absorbing, concentrating, and precipitating inorganic contaminants in plant's roots, above-ground shoots, and leaves by translocate them from soil is the main process. Plant species that can concentrate metals in their tissues are known as hyperaccumulators (Ghosh and Singh, 2005). Most known hyperaccumulating plants are, for example, species from following families: Euphorbiaceae, Asteraceae, and Brassicaceae. It is the best approach to clean up soils without destroying the soil structure and fertility. Concentration of metals in plants is caused by coordination of several processes including enhanced metal uptake, efficient root-to-shoot translocation and effective detoxification in leaves (Zhao and McGrath, 2009). Phytoextraction, also phytoaccumulation, is well suited for diffuse contaminated soils where the contaminant concentrations is low and superficially. Typical plants are sunflowers, Indian mustard, rape seed plants, barley, hops, crucifers, serpentine plants, nettles and dandelions. Normally, wild metal hyper-accumulating plants are characterized by shallow root growth, small in size and have a slow growth rate, so that those plants need treatment in order to growth faster and produce high amounts of biomass (Salt et al., 1995; Prasad and Freitas, 2003). Treatment in UMBRELLA project means to bring hyper-accumulating native plants together with rhizosphere bacteria and mycorrhizal fungi which are identified to have beneficial effects. As a result of this microbial treatment plants shall be strong growing (> 3 tons dry matter/acre-yr) and intake large amounts of metals in the above-ground biomass in an envisaged time frame (approximately ten times the level in soil) as Schnoor (1997) reported.

Project Results:

Within the work-package of microbiology, four groups have been working on the development of plant growth promoting microbial consortia (Prof. Kothe, Germany, speaker of WP 1), physiological and molecular characterization of microbial communities (Prof. Sprocati, Italy), development of microbially controlled remediation processes (Dr. Hallberg, U.K.), and isotope fractionation to follow metal uptake into living systems (Prof. Öhlander, Sweden). They were closely interlinked and working on the same samples to find microbial populations from different sites with applicability to different European settings. The methods used are inter-dependent to optimally describe microbial strains and communities for remediation purposes. The research is cutting-edge with the use of tools like CHIP technology, high-throughput cultivation and physiological screening and molecular identification of heavy metal retention molecules. All methods are highly advanced and oriented towards molecular understanding of processes. All results will be combined into a comparative geographic analysis.

The WP identified and characterized microbes which were then used in planting regimes to test for bioaugmentation in remediation actions. To achieve this goal different microbial communities were isolated from each of the six sites across Europe using specific techniques provided by the 4 partners included in this WP. All goals within the time period outlined in the proposal could be achieved.

The goal was to identify strains and consortia from the six sites. To achieve comparability of the data obtained from the involved partners, protocols were set up and supplied via the intranet of the website. The experimental protocols used for the strains characterization are according to Umbrella Protocols established during the kick-off meeting (October 2009) and revised during later meetings. Heavy metal resistance has been tested using a mininal medium amended with soil extracts obtained from each soil, according to the UMBRELLA protocol. In addition, heavy metal resistances were tested on individual metals, added to the growth medium at known concentration.

Ten strains were to be pooled for each site as a tool-box after assessing their plant growth promoting activity, metal resistance, community structures at the site and physiological characterization. The site characterizations were connected to WP3. Four taks were formulated in the proposal and the results to each of these tasks are summarized below.

The results obtained for the six sites investigated throughout Europe allow to assess the approach which can be applied in any new site. Thus, the main results of the work packages 1 through 4 are summarized here. 14 theses or thesis-like reports of each of the involved partners have been delivered which will allow for a much more detailed overview on the work performed.

The aim of the Umbrella Project was to establish optimal techniques for coupling microorganisms and plants for the remediation of soils influenced by mining activities around Europe. Three sites were under investigation: Mica Valley's tailing dam (Zlatna municipality, Romania), Ronneburg (Thuringia, Germany) and Kopparberg mining area near Ljusnarsberg (Örebro County, Sweden). Excursions at the sites were carried out in order to collect soil samples for microbial isolation, soil characterization and as a substrate to perform small scale experiments.

One of the tasks of WP1 was to isolate bacteria from the mining areas and characterized them physiologically by investigating plant growth promoting traits and taxonomically via 16S rDNA sequencing. On the base of the characteristics determined for all the strains isolated, so-called PGPR, a consortium for each site was created, with the intent to establish optimal techniques for coupling microorganisms and plants for the remediation of soils influenced by mining activities.

Plant growth promoting bacteria are able to exert a beneficial effect upon plants growth via direct and indirect mechanisms; the first include traits as phosphate mobilization and nitrogen fixation, elements which could be limited in polluted low fertile soils, and the production of phytohormones.

Indirect mechanisms include the release of chelating compounds such as siderophores, which make Iron soluble and so available to plant uptake.

The microbiological investigation has been structured with the followings activities:

1. Extraction of the native microbial community from the soil
2. Count of the total microbial load
3. Isolation and purification of the colony morphotypes
4. Taxonomic identification of the strains isolated (16S r-DNA-sequencing)
5. Heavy metals resistance on soil extract medium
6. Plant Growth Promoting traits:
7. N2 fixing
8. PO4 mobilization
9. Siderophores production
10. Phytohormones production
11. Creation of Microbial Consortia with the bacterial strains considered plant growth promoting (PGPB)

The time table could be met, all activities were performed as planned, involving all partners. The established consortia were provided to WP2 to be tested in pot experiments in association with one selected plant species, originating from the corresponding remediation site. The pot experiments aimed to establish a microbial assisted phytoremediation process, for the remediation of soils influenced by mining activities. These consortia, together with the correspondent selected botanic species (identified by WP2), were used for field trials. This created a tool-box to provide new tools for new approaches to remediation of soils influenced by mining activities. The deliverable D1.2 for this WP, 4 PhD theses or thesis-like publications, are uploaded with the report. D1.3 (Tool-box for microbial strains) has been delivered, all strains are deposited in culture collections and the sequences for identification were uploaded to the Pubmed database.

The work-package of applied botany was involved in establishing plant uptake of heavy metals and the response of plants to stressors like heavy metals in the presence of rhizosphere microbes. The microbial strains were provided from WP 1. Plant selection has been established on each site after mapping the plant communities in the contaminated catchment and detailing the structure of the community in the contaminated areas. Plant growth promoting rhizobacteria and mycorrhizal fungi have been studied, however, their influence is not fully exploited for the use in bioremediation and biomonitoring. Four groups with expertise in plant uptake of heavy metals (Dr. Neagoe, Romania, speaker of WP 2), mycorrhizal alteration of heavy metal uptake (Prof. Turnau, Poland), heavy metal response in plants (Prof. Lichtscheidl, Austria), and investigation of speciation of metals in soil and in plant biomass (Dr. Arnold, Germany) participated to achieve a molecular understanding of metal uptake and plant response in phytoremediation approaches. The data were be used to extrapolate these results to ecosystem and catchment levels.

The use of plants in combination with rhizosphere microorganisms and in combination with legumes for nitrogen fixation by symbiotic bacteria is monitored for the enhancement of metal uptake into plant biomass. In addition, the use of aminoalcohols as stimulants for plant metal uptake is tested in small lysimeter experiments. At the same time, the percolation water was sampled and tested for the quantification of metal dispersion with the ground water. In parallel with the experimental studies, ecological field studies on the plant communities have been performed. The experimental and field data produced within this WP will support the calibration and validation of bioaccumulation and eco-physiological models (WP4). They are then used for the optimization of soil management of contaminated sites in a landscape context.

During the second period, a complete set of pot experiments and field trials were performed. Indirect effects of microorganisms may be inferred from patterns of measured variables at different scales in microcosms, mezocosms, or field experiments. Multiscale experiments are needed for upscaling because they provide information about net effects and their heterogeneity at a larger scale than those of the processes supporting them. Thus, different scales are studied.

The work package compiled a data base which will be available for the public after publication of the data in a journal. The primary goal was to establish a tool for all researchers to include their plant diversity at contaminated sites such as to make it more easy for future application to select a plant. For this reason, an evaluation of species distribution at different sites was performed. The selection of plants has been performed with a 6-plant set as pre-trial using plant pots in greenhouses. Ecotypes are validated for some sites (I, T) and interaction with microbes is tested in one set of first pot experiments as a proof-of-concept. Best performing plant of the set is chosen for the field trial subsequently conducted.

The methods used are inter-dependent to optimally describe plant uptake and strategies for metal detoxification in the plants tested. All tests were performed in different ecotypes and re-evaluated with respect to microbial communities inoculated in pot experiemtns and in the field. The research is cutting-edge. All methods are highly advanced and oriented towards molecular understanding of processes. All results are be combined into a comparative geographic analysis. The output of data from this WP is relevant for approaches describing metal distribution in WP3.

The point of this WP was to provide information about the effects of the interaction between microorganisms and plants (preferably native ones in order to eliminate the invasiveness risk) on the mobility of metals from contaminated sites. This is a difficult problem because of its multi-scale character. What we have to assess is the role of the microorganisms in metals mobility at site and catchment scale. While the direct roles of microorganisms, organic carbon or minerals in the mobility of metals in contaminated sites are well documented, their indirect roles (in particular the effects at larger scales) are much less clear. Understanding the indirect roles is crucial because the scale for the management of contaminated areas is not the very small scales specific to microorganisms. The selection of plants for has been achieved based on thorough field studies.

The WP underwent no deviation. All goals were successfully met.

The deliverable 2.2 for this WP, 4 PhD theses or thesis-llike reports, has been delivered (uploaded with the report). D2.3, tool-box plants, have been selected and their performance was described in detail.

In the work-package (hydro)geochemistry, the transport medium water was investigated with regard to flow paths of heavy metal contaminated acid mine drainage waters and the importance of such transport paths for uptake into the biopath. Thus, the experimental sites were characterized with respect to flow paths, waters sampled was from soil, surface and ground water wells in dependence of microbial inoculation (WP1) and investigated for metal content, speciation, reactive transport and mineralization/mineral solution processes. Percolation waters from lysimeter experiments (WP2) were included to identify processes. The objective was to combine different approaches - the water ways and the use of rare earth elements to define sources and sinks, the use of radioisotope probing to follow water paths in the scale of catchment areas, the characterization of mineral dissolution and precipitate formation and the investigation of mineral phases developing under microbial impact - with microbiological experiments to understand the impact of microbes on mobilization and immobilization from single sites to large catchment areas. Thus, the four partners of WP3 provided their expertise to characterize the six sites.

For the six sites involved, detailed site descriptions are available. The results of (hydro)geochemical characterization are provided for WP4 and WP6 to ensure distribution of results to the public. These then will be the basis of integration of all results in policy making to support EU's public awareness.

The descriptions of the contaminated areas include former and recent use and remediation activities performed, geographical and geological setting including landscape, plant and animal communities, climate, ore deposit, etc. For hydrogeology (including groundwater), discharge pathways are defined. In case of ground- and surface water, pH value, redox potential, electrical conductivity, contents on main cations and anions, metals and radionuclides, hydraulic conductivity of aquifers were studied, while for soil characterization, pH value, cation exchange capacity, total content on nutrients, metals and radionuclides, amount of bioavailable metals and radionuclides, grain size distribution, soil type and mineralogical composition were obtained. The six sites were described with great detail.

The results of the (hydro)geochemical characterization of metal mobilization were provided for WP6 (Dissemination of results an educational issues) to ensure their distribution to the public. These were the basis of integration of all results in policy making to support EU's public awareness. The needs for georeferenced maps for modeling have been met, and these, with extensive descriptions of the six sites, were added in the workspace of UMBRELLA.

All goals have been met. The deliverable D3.2 from this WP (4 PhD theses or thesis-like reports) were delivered and are uploaded with the report. D3.3 (geochemical for modeling) were provided above for WP 4.

The integrated modelling has been achieved and forecasts were performed for distribution of heavy metals at landscape and ecosystem scale. The general objective of WP4 was the development of contaminant dispersal modeling. According to the goal of the project, modeling covered scales from plant to catchments in order to link local remediation measures with their regional consequences, and not only separately describe them. Within this WP, a nonlinear correlation model at the landscape level on the impacts of contamination on soil and water ecosystems has been developed and long-term distribution of contaminants at catchment scale are predicted for use by stake-holders and the EU.

An integrated delivery in terms of an integrated biogeochemical model was set up for this purpose, with biological and geo-morphological processes across scales, that did not exist. Developing such a model is a research activity with a larger risk than running existing models at separate scales. In order to minimize these risks and to ensure that modelling tools will exist in the tool-box, our strategy was to implement two activities with lower and with higher risk. The existing CAESAR-TRACER model coupled with a stochastic rainfall generation model is run on the population of European catchments (low risk, no integration). A knowledge base of existing plant bioaccumulation models enriched with models developed from the plant-soil data-base generated by the European partners is developed (low risk, no integration). And software CAESAR-TRACER was re-written in order to improve its speed performances requested for integration across scales, to include the effects of plants at local scales in the slope area, and to include a module for bioaccumulation in plants in the floodplain area (high risk, integrated delivery).

Within the UMBRELLA project, hazard assessment procedures at the catchment (i.e., non site-specific) scale encompass the estimation of historical and future soil heavy metal concentrations in the catchment. Based on a general assessment protocol for catchments affected by mines (Macklin et al. 2006), the objectives of WP4.1 include the prediction of hazard for different future remediation and climate change scenarios. Under these restrictions, approaches purely based on current contamination levels need to be complemented with forecasting models of contamination at catchment scales. Under WP4, the protocols defined involve:

1. Field surveys of heavy metal concentrations.
2. Forward simulation soil contamination model.
3. Validation of the soil model via comparison with the field surveys.
4. Forecasting future catchment contamination with remediation and climate change scenarios.

The focus sites have been restricted to three climatically contrasting sites within the UMBRELLA project: Ampoi river (Romania), Naracauli river (Sardinia), and Ystwyth river (Wales). Essentially, protocol 1 defines the current conditions of contamination at the catchment scale, and serves as a basis for validation of the forward simulation model in protocol 2. The model, based on a modification of CAESAR/TRACER, requires detailed information of mining activities during the past 300 years. Such information was only available for the Naracauli and Ystwyth catchments. Hence, validation (protocol 3) only took place on these sites. Finally, protocol 4 developed forward simulations with different scenarios based on the current conditions in the three contrasting catchments.

Soil contamination is modelled with the use of CAESAR/TRACER (Coulthard et al. 2000, Coulthard 2001, Coulthard & Macklin 2003). CAESAR is a cellular automaton model of landscape evolution able to simulate processes of erosion and deposition in mid-size catchments at high spatial and temporal resolution. TRACER is a complementary module simulating the spatiotemporal patterns of contamination through simple sedimentation mechanisms of materials produced by mining activities.

The biogeochemical/ecotoxicological models and the adapted TRACER are established providing a tool for exploring the effects of soil remediation scenarios on water protection and protection of biodiversity at catchment scale. The hydrogeochemical part of modeling explains sorption/desorption activities for reactive transport, while the biological/ecological part focusses on bioaccumulation, ecotoxicological effects, and biologically mediated effects on biogeochemical metal cycles and fluxes. The data were provided including all data sets of WP1 though 3.

One of the very novel aspects of this project is the modeling of long-term (decades/centuries) and catchment-scale sediment associated contaminant dispersal. The output of the modeling is used to guide remediation plans, especially in terms of identifying contamination hot spots' and how these might change over both space and time in a catchment.

All goals have been met. The deliverable D4.2 from this WP (2 PhD theses or theses-like reports) are integrated and uploaded with the report. Models for the sites are included in these theses.

Two major targets were defined for the socio-economic impact of UMBRELLA: the incorporation of the knowledge gained in this project to establish a tool-box for use in remediation actions, and proof-of-concept. Mycorrhizal fungi are commonly occurring soil fungi that colonize the roots of most terrestrial plants including various crops. The benefits of the symbiosis include improved uptake of immobile soil mineral nutrients, water relations, disease resistance, increased stability of soil aggregates (Smith and Read 2008). The utilization of the mycorrhizal symbiosis is an important tool not only in sustainable agricultural systems but also in ecosystem restoration. There are two ways for the utilization of these fungi. The first is to stimulate the indigenous population already present in the soil. The second is to inoculate plants with effective isolates of AM fungi.

The main parts were to establish mycorrhiza (partner KWAZAR) in pot experiments associated with the plant and microbe selection, and to perform a proof-of-concept in field trials.

The aim of the pot experiment was to evaluate the contribution of microorganisms (bacterial consortia and / or mycorrhiza) to the process of phytoremediation of soils contaminated by heavy metals, in order to identify some tool boxes for subsequent field applications. Autochtonous botanic species were selected, one for each site: Agrostis capillaris (Romany), Deschampsia flexuosa (Sweden), Euphorbia pithyusa (Italy), Festuca rubra (United Kingdom), Helianthus annuus (Germany) and Verbascum thapsus (Poland). The experimental design included the following: The experiment was designed to test each plant in each of the six soils. Soil was inoculated with the native microbial consortium, as a bioaugmentation agent. The experiment was conducted in 5 replicates for each condition.

The test field at the former mining district of Ronneburg in Thuringia, East Germany, had been affected by heap leaching and was remediated from 1991. In 2004, the Friedrich Schiller University installed a test field, 'Gessenwiese', on the basement area of the former uranium leaching heap Gessenhalde, covered during technical remediation with approx. 40 cm of an allochthonic soil substrate (Grawunder et al., 2009).

Three different plots (K, containing 5 cm of compost addition applied in 2004; MB, containing 5 cm top-soil addition; C, control plot without soil amendment). The field trial was carried out from June to September, 2011. An area of 7.50 m x 7.50 m was divided into nine subplots, and in addition two external plots were used, each 2.50 m x 2.50 m. Rows of seeds 30 cm apart were inoculated with mycorrhizal fungi (M) or mycorrhizal and/or the bacterial consortium (B), in addition to non-inoculated control subplots. Before inoculation, the soil was mixed, mycorrhizal Glomus intraradices was inoculated, the seeds were distributed and bacteria were applied (for preparation of inocula, see above). A proper inoculation was achieved by applying the bacteria with a watering can at the time of sowing.

At the end of each experiment, soil and plants analysis were performed in order to investigate the metal load and the bioavailable fractions of metals in soil, plant uptake and the effects of the applied inoculation. The soil substrates were separated from the roots and dried at 30 °C. Homogenized samples were used for soil pH, EC, sequential extraction (bioavailable fractions, F1 and F2), TC content and LOI as described above. In case of pot experiments, analyses were done for each pot separately; in the field trial, five soil samples of each subplot were collected in plastic bags and pooled in one representative sample.

At the time of harvest, weeds were harvested as well, and measured for fresh and dry weight. Weeds dry weight was observed in the following order: C>B2> C1> B1, M2> MB3> MB2> M3> C3> C2, where C showed a weight of 0.52 kg. Interestingly, where the bacterial consortium was applied, in general higher dry weight of all plants was found.

The uptake into plant biomass for Al, Cu, Mn, Ni, Zn and Fe was determined. In the control subplots, the metal content in plant tissues was found to be very high, much higher than those of the plots inoculated with bacteria, with mycorrhiza, or both. For Fe, Mn, Al, Zn, uptake was higher than for Ni, Cu and U (with values between 0.05 and 0.001 µg/g, data not shown). This means that the inoculation was able to preserve plants from metal uptake, unlike in the control where all elements have been taken up into plant biomass.

The BCF for the total content in soil could be calculated just for Cd, Cu and Mn as the other elements were not detectable. A strategy of phytoextraction was thus employed in case of Cd at subplot C2, whereas stabilization strategy has been employed by plants in presence of bacteria, mycorrhiza and their combination. For bioconcentration with regard to bioavailable element concentrations, the BCF(F1+F2), shows high divergence among subplots, with a maximum of accumulation at the subplot C1. Among all variants, Pb and U are excluded by sunflower (BCF<1), although the exclusion process is enhanced in presence of the different inoculants. In case of Mn, just the control subplots C1 and C2 show an accumulation (BCF>1), whereas all others tend to exclude this element. Concerning Zn, the accumulation is important in the controls C1 and C2, where values higher than 30 and 40 are reached, unlike the inoculated ones showing a BCF between 9 and 2, but still the element is strongly taken up. Similar to Zn are Fe and Cu, with a BCF>1 for Ni found in all variants but MB2 and MB3.

The case of Cd, the plants use two strategies: in presence of mycorrhiza and bacteria, and with bacteria alone, the element is excluded, while in the control plants it is accumulated, although not as much as it was assessed for pot experiments. In the pots, a higher BCF and metal uptake into plant biomass was registered, although the biomass was comparatively small and no other plants were growing. Among all plots, the highest values of BCFbioavailable were shown by C2, where plants accumulated all metals. This confirms that sunflower was the best plant for a multi-metal contamination: extraction in case of no inoculum and stabilization in case of addiitona of bacteria, mycorrhiza and their combination. Thus strategies fitting to each objective can be devised.

Chlorophyll fluorescence measurements were performed in order to assess the stress due to metal presence for all subplots. The photochemical efficiency was lower than 0.800, the value universally considered for healthy, non-stressed plants, with slightly higher values only for the inoculated subplots M3 and MB2 t. This allows to speculate that the presence of mycorrhiza and the combination with bacteria could have preserved the plants from metal stress.

At a second test field, the achievements possible using UMBRELLA tools were tested in a completely different climatic setting in Sardina, Italy. The overall goal of UMBRELLA is to use microorganisms to develop cost-efficient and sustainable measures for soil remediation at heavy-metal contaminated sites. Thee toolbox consists of symbiotic microorganisms and higher plants, preferentially endemic to the contaminated soil. They are used to revegetate the heavy-metal contaminated, and often sulfatic, barren soil in order to prevent erosion (Phytostabilisation) and/or to extract metals by regularly harvesting the metal-accumulating plants (Phytoextraction). Proof of concept consists of observing improved plant growth, soil stabilization and metal extraction for field-plots treated with bacterial consortia and/or mycorrhizae, as compared to field-plots that have not been treated. Practicality of concept consists of demonstrating that the toolbox is a potential asset in the re-habilitation strategies of environmental engineering companies that remediate heavy-metals-contaminated sites. This report describes preliminary activities related to trial site selection and organisation (site preparation, field trial area division into sub-plots and following treatment) at the abandoned Ingurtosu mining site; and monitoring and control actions performed since planting and sowing, in order to assess degree of plants growth and seeds germination. This report discusses the preliminary results as concerns the demonstration of practicality of the various Umbrella toolbox treatments, in particular in combination with the commercial mineral-reagent of the ViroMineTM technology.

In order to verify the homogeneity of the experimental field, before proceeding to the bioaugmentation and the introduction of the plants, a triplicate sampling within each subplot was performed in the soil top layer and the composite sample thus obtained was used for analysing the metal content for all 27 subplots. The distribution of metal within the different subplots is fairly uniform

On the same samples the analysis of the metabolic profile using the Biolog Ecoplate (CLPP) was performed. The soil microbial activity was extremely low, both for the range of substrates used (functional diversity) and for kinetics of use, so as to be almost absent. The values at time 0 are shown. The application of bioaugmentation has definitely improved the metabolic profile, in particular the functional diversity of the soil, greatly expanding the range of substrates used. The subsequent introduction of the plants of Euphorbia pithyusa has further enriched the soil.

Field trial area set up (roughly 200 m2) has included the following preliminary activities:arrangement of existing roads for the transit of vehicles and delimitation of the testing area, staked out by means of pickets; arrangement of rainwater drainage system; preparation of test site without removing the surface layer, while adding 5 cm of mud taken from adjacent area as indicated by DISTER (University of Cagliari, Italy); homogeneously mixing of the upper 30 cm of the plots with the added 5 cm of mud; division of the field trial area into 27 subplots; fencing of the area with metal gauze to protect plants from larger grazers. An area of about 7.50 m x 22.50 m was divided into 27 subplots of 2.50 m x 2.50 m. For each subplot the inner part of 2.0 m x 2.0 m has been used, leaving a service strip of 0.50 m between plots

Close to the plots, three pots containing garden soil have been placed to be used as positive control to verify the growing of the Euphorbia plants and seeds in a contamination-free soil.

A preliminary laboratory test phase has been carried out in order to determine the range in variation of pH and electrical conductivity (EC) for leachate of samples (5 parts de-ionized water per part of solid, mixed in a tumbler during 24 hours) of several different areas in the site of Naracauli, in order to estimate possible weight percentages of use of ViroMineTM reagent. These results have been used to choose the area for the trial and to define the best soil conditions to Euphorbia pythiusa growth and the dosing of the reagent in the test.

Tool-box microbial consortia have been selected for different European sites. For inoculation, the Sardinia strains were used. The inoculation of bacteria has been carried out by ENEA and on 11/10/2011 1.5 litres of suspension containing bacteria has been diluted in a total of 150 litres of water. 13 subplots have been watered with 10 litres of the diluted bacterial suspension. Monitoring and control occurred since planting and sowing, gathering data on plant growth and seed germination. Consequently, health and growth of plants was determined.

In the month of December, samples were also taken from each of the 3 'Control' subplots and 3 'ViroMineTM' subplots. In order to define the characteristics of heavy metal release of the soil, samples taken from the two differently treated subplots, were subjected to elution tests for 24 hours, using deionized water (10 times the weight of the solid fraction), in containers rotating at 5 RPM. The leachate was then filtered through 0,45 µm filter paper, acidified with 1 ml/100 ml nitric acid at 65%, and then analysed for Al, As, Cd, Cu, Fe, Mn, Ni, Pb, Zn, Co and total Cr by means of ICP-MS.

A decrease in the concentration of As and Cd is observed in all of the three samples taken from subplots treated with ViroMine, as compared to samples taken from 'Control' subplots. A decrease in the concentration of Mn and Zn in two of the three 'ViroMineTM' samples and a decrease of Cr and Cu in one of the three 'ViroMineTM' samples are observed as well. The decrease in the concentration of metals and semi-metals in the leachate of ViroMineTM samples is due to immobilization of contaminants. The higher concentration of Al and Fe in the leachate of the ViroMineTM samples is consistent with the presence of transformed Bauxite refinery residue in the reagent. Furthermore, it should be noted that the initial concentration of metals in the different subplots is not yet known (samples to be analysed by ENEA). The analysis of the leachate 2 LS of soil samples, taken from C1 (Control) and C9 (ViroMine reagent) plots in order to define the calcium and sodium release of the two different types of plots. Significant differences in concentrations of calcium and sodium were found in the leachate of the plots treated with ViroMineTM and Control. In general, the subplots Bacterial Consortium, Mycorrhizae and Control show the highest number of living plants, while the lowest number can be detected in the subplots ViroMineTM and, above all, in one of the "Mycorrhizae and ViroMineTM'. It should be noted that in subplot A2, a number of plants higher than planted has been detected; this can be due to spontaneous growth of new plants, due to the presence of seeds in the soil and to the irrigation of the plots. The average plant growth, as compared to the month of December, was approximately 1 cm in January, with the exception of subplots A4 "Bacterial consortium and ViroMineTM' where a 5 cm growth has been observed. As already detected in the tests of previous months, subplots with the same treatment may show different results in plant growing (number of plants, health and height). In general, subplots of line A seem to show a better growth than those of the other two lines.

Based on monthly monitoring results on the plants health, it is reasonable to assume that bacteria and mycorrhizae can be effective, when mixed with contaminated soil, in promoting Euphorbia pythiusa growth. It is believed that the suffering and death of some plants in these plots is likely due to the low temperatures of January and February, rather than their composition. Moreover, since the last control (March 2012), a vegetative growth has been observed, that may lead to more detailed consideration after the foreseen additional monitoring. In general, subplots 'Bacterial Consortium' and 'Mycorrhizae' show the highest number of plants, while the lowest number can be detected in subplots 'ViroMineTM' and in 'Mycorrhizae and ViroMineTM'. However, as far as health plants are concerned, it was observed that plots with the same composition did not have homogeneous behavior. The different leaching of the soil by rain water may explain the different behaviour of subplots with the same composition. Row 1 (A1, B1, C1 plots) has shown the best results: no dead plants, plants with a wider branching and a more developed biomass.

The goal of the trial was to evaluate the possible use of Euphorbia pythiusa as remediation plant in the area of Sterile Valley, using several combinations of tools and evaluating the plant health, metal accumulation, and growth promotion after bioinoculation, yield of biomass and transfer factors and soil treatment reagent (ViroMineTM). Bacterial consortium used is formed by the best ten strains, selected by ENEA of the project for the aptitude to associate the capacity to grow in the presence of heavy metals with metabolic traits for plant growth promotion. Also the bacteria are selected and combined starting from the typical bacteria of the site.

A mixture of species/strains obtained from Ingurtosu area have been studied by another partner of the project and used as mycorrhizae inoculum pellets, containing spores, pieces of roots with spores and mycelium, and soil mycelium. An inorganic reagent (a special mixture of chemicals and minerals) has been used to stabilize the metals and to improve the water retention of the tailings.

The WP did perform an integrated, cross-cutting task which was achieved according to plan. He goals could be reached as planned with incorporation of end-users.

The deliverable, a proof-of concept, is delivered and both partners involved in this WP have delivered their results which were uploaded with the report.

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The successful completion of WP6 with continued dissemination of findings depends on the integration of results from all WPs. Especially education and training of young scientists in an established network of excellent laboratories will ensure the distribution of knowledge and research results within the scientific community. The cooperation with, and information of government partners and public bodies will improve knowledge, support the establishing of relevant guidelines and show best practices. The implementation of the tool box strategy in academic and non-academic remediation approaches is based fundamentally on the dissemination of knowledge (contact to stakeholders), awareness in the scientific community (publication in peer reviewed journals) and maintenance of the scientific infrastructure within the field of bioremediation by exchange of PhD students, conduct of workshops and organization of meetings.

The success of WP6 includes:

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In addition, all UMBRELLA university partners regularly attended scientific meetings on subjects related to the topics of the consortium. For further dissemination of results, meetings of the consortium were organized.

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The UMBRELLA webpage (http://www.umbrella.uni-jena.de) delivers the essential web based portal for both the interested public, unltimately for potential users and the scientific colleagues and cooperators. The open access area of the webpage informs on the overall UMBRELLA goal and tasks of the single WPs. Description of all test sites included and information on all academic and non-academic partners can be found. The intranet serves as an exchange platform to share protocols on methods, provision of literature and last not least updates on field trips and meetings. In addition, a plant web site is established which will be released in 2013, after all publications from UMBRELLA are released.

The dissemination activities of the project led to publications through multiple channels. In addition to the scientific output, young researchers were trained in this important topic for EU.

All goals for dissemination have been reached.

Guidelines for governments and politics were one of the specific aims of the project which will be laid down in rules for best practise to inform political decision making. It is combining the socio-economic aims of soil and water regulations. For that, WP 7 included governmental collaborators. The outcome of this WP is the development of processes for remediation of metal contaminated soils. As contamination of groundwater and of cross-boundary river systems poses a threat to the European Union, the development of guidelines which have both soil and water protection aims would be an urgent call. Thus this project seemed ideally suited to suggest guidelines to help unify the water and soil protection laws within the European Union.

One problem long known, but never addressed, is the definition of metal loads which necessitate action for remediation. In both soil and water guidelines, total contents rather than bioavailable contents are the basis on which decisions that imply measures for remediation action are taken. From an ecological and ecotoxicological point of view, this is not supported by scientific data, and the differences in geogenic contents of metals in soils have led to problems with determination of relevant limits. The inclusion of government collaborators allowed to develop feasible guidelines that can be proposed to the European Union to develop a combinatory guideline including both soil and water protection issues.

For optimal output, the speakers of the basic research work packages 1 through 4, as well as the two representatives of small and medium enterprises were combined with the governmental partners in this work package. Speaker of this work package will be the representative Andreas Baumgarten, head of the institute for soil health and plant nutrition at the AGES and executice secretary of the council for soil fertility and soil protection at the ministry for agriculture, forestry, environment and water management.

Guideline proposal for integrated soil and water protection acts

1 Introduction

- For low contaminated sites, the cost effective and sustainable technique of phytoremediation using vegetation for in-situ treatment is recommended.

1.1 Phytoremediation

- Phytoremediation is an in situ procedure by using vegetation to treat contaminated soils - either to decontaminate or to stabilize it in order to minimize negative effects on the environment; an alternative method to the expensive and soil destroying technical, thermal, chemical in-situ treatments

1.2 Range of application

- Phytoremediation requires some pre-existing basic factors e.g. contamination should be below or near risk thresholds or a large-scale removal action is required, low mobile fraction, shallow depth of the contaminant's spatial distribution; Phytoremediation is not applicable on sites with sensitive land use; Soil properties must support sufficient growing of plants.

2 Risk assessments at site scale

- Risk assessments are divided in three different steps: exposure assessment, hazard assessment and risk characterisation.

Uniform definitions of terms like contaminated sites, values and thresholds are needed for remediation actions. Standard methods are recommended to determine necessary data for risk assessment and future remediation action.

2.1 Pathways

- Potential exposure pathways are the base for a project's risk assessment, so it's necessary to identify all of them at the beginning of a remediation action.

3 Remediation strategy

- Microbial mediated phytostabilisation and phytoextraction are preferred, as the beneficial character of microbes like rhizobacteria and arbuscular mycorrhizal fungi (AMF) on plant uptake of metals, degradation of organic pollutants or increasing the bioavailability of the metals is known; both can be used as polishing step for already rehabilitated areas with still high metal concentrations.

3.1 Principles of phytoremediation

- using native, regional-specific plants that do not accumulate (phytostabilisation) or do accumulate (phytoextraction) metals in their above-ground parts as well as identifying and characterising plant promoting microbes such as rhizobacteria and AMF from the area of interest

- selected plants and microbes have to be tested individually for the use for remediation purposes and then in combination together with the contaminated soil

4 UMBRELLA tool box

4.1 Site characterisation and soil properties

- Information about following issues is necessary: former and recent usage as well as mining situation, current remediation activities, geological and geographical setting including also hydrogeology, climate, flora, fauna etc.; identification of discharge pathways

- soil characterisation with standard methods: pH value, cation exchange capacity, total content and bioavailable fraction on nutrients and metals/radio nuclides, grain size distribution, soil type, mineralogical composition, hydraulic conductivity, water content

- ground/surface water characterisation: pH value, redox potential, electrical conductivity, contents on main cations and anions/radio nuclides

- A characterisation is useful for judging the success of the remediation activity

4.2 Screening and selection of plant species

4.2.1 Plant survey

- suitable plant species: naturally occurring vegetation colonizing (contaminated) site of interest, seasonal surveys are necessary in order to identify and select native species, ecotype or genotype; estimation of the plant cover

4.2.2 Selection criteria

- non-food crops; plant species appropriate to the climatic and topographic conditions; plants producing viable seeds in sufficient quantity; positive results in germination tests; disease-free plants; special care must be focused on large biomass production, root morphology and depth - fibrous root system; tolerant to drought; usage of seeds without fungicides; screening of phytotoxicity

- grasses are well suited - phytoremediation processes even during the winter season, capability to exude metal chelats and organic ligands

- annual/ephemeral plants due to their rapid germination, fast growing; perennial plants have to be installed only once; halophytes used for groundwater desalinisation; phreatophytes used for groundwater remediation; selected plants should be tolerant to drought that is the biggest problem at degraded sites, metallophytes tolerate and take up high amounts of metals (hyperaccumulators)

4.3 Screening and selection of microbes

4.3.1 Plant growth promoting bacteria

- Plant growth promoting bacteria are native bacteria/microorganisms that are able to positively associate with plants enhancing plant growth and metal resisting properties by favourable metabolic processes.

- microbial characterisation:

i) Extraction and isolation of the native microbial community by sampling soil/substrate from the rhizosphere of endemic botanical or in some case from pioneer species

ii) Taxonomic identification of the isolated strains performed by DNA sequencing

iii) Metal resistance (tested by applying following methods: e.g. agar diffusion assay testing the growth in presence of bioavailable soil metals; test for growth on soil extract and a quantitative test determining the minimal inhibitory concentration

- Plant growth promoting bacteria tested on base of metabolic features like nitrogen fixation, phosphates mobilisation, siderophores or phytohormones production

- Analysis of the physiological profile at community-level (BIOLOG-ECOPlates)

- Bioaugmentation by using microbes or pre-cultured microorganisms as additional for the soils/substrates to be remediated to enhance intrinsic activities of soil and to improve the remediation capacities of contaminated sites. To be successful, the bacteria need to be inoculated properly, which necessitates the estimation of the colony forming units and the produce a proper volume of cell suspension depending on the aims for inoculation.

4.3.2 Arbuscular mycorrhizal fungi

- The selection of AMF is done individually for each site which shall be remediated by screening the site after plant species (e.g. Plantago which is a common plant growing on every habitat and is known to have a high diversity on mycorrhizal fungi such as Glomus on their plant roots. Glomus provides a lot of benefits to their plant hosts like drought and disease resistance.)

4.3.2.1 Arbuscular mycorrhizal fungi trap culture is a conventional method of inoculum production where the fungal isolates should originate from the area of interest for remediation purposes.

4.3.2.2 Criteria of selection of AMF

- spore germination (e.g. ISI Spore Germination Test); efficiency to enhance root absorption area; efficiency to provide access to nutrients and water; efficiency to enhance and stabilise the substrate structure due to formation of the hyphal net; ability to accumulate potentially toxic metals; efficiency to develop abundant mycorrhizal colonisation on the soil/substratum; interactions with e.g. nitrogen-fixing bacteria, endophytic fungi and bacteria should be taken into account

4.3.2.3 Estimation of mycorrhizal parameters

- parameters are: mycorrhizal colonisation, frequency of mycorrhiza, mycorrhiza activity

- discriminating between mycorrhizal, endophytic and parasitic fungi

4.4 Coupling of competent associations

- Small scale experiments (e.g. pot experiments) in order to find out the best combination of the selected native strains of bacteria, fungi and plants for future field trials

UMBRELLA site-specific applications comprise the dominant best native plant, best native bacterial strains and AMF.

Both guidelines and best practise rules have been devised and were delivered. This includes the work of all consortium members and is the final result of the project for use with EU authorities. The meetings including stake holders have been organized in Örebro and Vienna.

Potential Impact:

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List of Websites:

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