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Soil Phytoremediation—A Case Study in Greece

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Abstract: At the present manuscript, a brief report is given concerning heavy metals soil bioremediation methods and particularly phytoextraction by means of hyper-accumulator plants. The picking of the plants i.e. *Thlaspi caerulescens* should meet certain requirements according to already conducted experiments. Cultivations' data of the aforementioned Cd and Zn phytoaccumulator species are demonstrated. The last part incorporates a case study of a heavy metal-contaminated area nearby an abandoned lead-zinc mine located somewhere in NE (Northeast) Greece and a fundamental operational & cost analysis of the whole remediation project.

Key words: Phytoremediation, bioremediation, heavy metals hyperaccumulators, soil decontamination, phytoextraction coefficient, chelate agents.

1. Introduction

For the remediation of soils from chemical pollution, various techniques are used that are roughly divided into two main categories: physicochemical (i.e. physical/chemical separation of pollutants by sieving, wet sieving, using classifiers, magnetic separation, floating, hydrolysis, oxidation, oxidation neutralization, electrolysis, photolysis, use of electrokinetic methods) and biological. In the 2nd main category, the majority of methodology variations are based on the beneficial use of microorganisms that either exist in the soil or are transplanted to the place of contamination, as a later stage (e.g. in the form of compost), to achieve biological assimilation, inactivation, transformation, biodegradation, hydrolysis, oxidation of hazardous substances to other inert or less hazardous substances. Pollutants that are highly biotoxic or difficult to biodegrade, present inherent obstacles to implement decontamination techniques.

Bioremediation techniques can be applied both in situ in the field and locally or industrially (ex situ) by transferring part of the contaminated soil to suitable bioreactors. The above practice also includes the extraction of pollutants with the beneficial use of microorganisms. A promising biological technique is considered to be the use of hyperaccumulator plants [1]. The cultivation of hyperaccumulator plants is widely used mainly for the removal of heavy metals from the soil. The selection of plants is mainly based on the local flora (endemic plants). Such species meet certain experimental specifications showing excellent resistance to high concentrations of pollutants (heavy metals and/or toxic organic compounds). Reducing soil pollution is achieved in many ways. Through release of substances, mainly enzymes, from the root system of the plant that directly affects the stability of pollutants (Phytostabilization), through enzymes' release that enhances the bioactivity of the microbial population (stimulators) which live synergistic in the rhizosphere. Other techniques are by hydrolysis/dissolution of pollutants and then absorption (in ionic form) and later bioaccumulation in specific plant tissues (phytoextraction, rhizofiltration) or even release in the atmosphere through the processes of foliar evaporation (phytovolatilization) [1-4]. Table 1 provides a comprehensive summary of all available bioremediation

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Table 1 Phytoremediation categories [4].

Terminology	Remediation
Phytoextraction	Removal of pollutants mainly Me from the soil and then biomass harvesting
Phytofiltration	Removal of contaminants through the root system in aquatic environment or soil moisture
Phytostabilization	Use of plants to reduce the bioavailability of pollutants to the environment
Phytovolatilization	Pollutants enter the plant biomass and then evaporate through the foliage into the environment
Phytodegradation	Use of plants for the biodegradation of organic pollutants
Phytotransformation	Use of plants for the transformation of organic pollutants and change of Me complexation
Phytostimulation	
Rhizodegradation	
Plant-assisted bioremediation	Use of microorganisms of the root system of plants for better bio-binding or degradation of pollutants
Plant-assisted degradation	
Plant-aided in situ biodegradation	
Enhanced rhizosphere degradation	Use of microorganisms of the plant root system for the decomposition of pollutants and chelates for better and faster bio-binding in plant biomass

Table 2 Hyperaccumulator species [4].

Pollutant	Plant species
Zinc (Zn)	*T. caerulescens*
Cadmium (Cd)	*T. caerulescens*
Nickel (Ni)	*Berkheya coddii*
Selenium (Se)	*Astragalus racemosus*
Thallium (Tl)	*Iberis intermedia*
Copper (Cu)	*Ipomoea alpine*
Cobalt (Co)	*Haumaniastrum robertii*
Arsenic (As)	*P. vittata*

alternative methods. In addition, Table 2 presents certain plant species, being proven heavy metal (Me) hyperaccumulators, and their special binding capacity against specific pollutants. The criteria for designating plants as hyperaccumulators of pollutants as for Ni are the value of 1,000μg/g dry biomass base, as for Zn and Mn the threshold of 10,000 μg/g and as for Cd 100 μg/g dry biomass base [4]. As regards Co, Cu, Pb and Se the threshold is 1,000 μg/g in shoot dry matter [4]. Bioremediation requires plants that are bio-tolerant in receiving high amounts of specific pollutants and with the ability to accumulate Me or other toxic compounds in their biomass. Such plants develop extensive root subsoil systems. They grow rapidly and show resistance and tolerance to different bioclimates and a variety of soil characteristics. The high biomass production of these plants is considered to be necessary for the methods of removal of pollutants through their accumulation in biomass and then through harvesting and removal.

An advanced method of phytoextraction is the enhanced/induced phytoextraction. A key difference from the previous to the newer method is the addition to the soil of the cultivation of chemical agents that stimulate/facilitate the biosorption processing of pollutants from plant biomass. It is common practice for the heavy Me bio-binding evolution, the addition of small amounts of chelates e.g. EDTA (Ethylenediaminetetraacetic Acid), HEDTA (Hydroxyethylethylenediaminetriacetic Acid) etc. in the last stages of plant growth before harvesting. Table 3 demonstrates certain chelate agents, the usefulness of which are established through experimental studies and are now employed quite frequently in cases of soil bioremediation. Many chemical agents are not environmentally friendly per se when applied on the field. Therefore, they should be used after careful study on a case-by-case basis as they may lead to the

Table 3 Acronyms/names of chelates used in phytoremediation [5].

Acronyms	Chemical agent’s name
EDTA	ethylenediaminetetraacetic acid
HEDTA	N-hydroxyethylethylenediaminetriacetic acid
DTPA	diethylenetriaminopentaacetic acid
CDTA	trans-1,2-diaminocyclohexane-: N,N,N’,N’-tetraacetic acid
EGTA	ethylenebis(oxyethylenetrinitrilo)- N,N,N’,N’-tetraacetic acid
EDDHA	ethylenediamine-di (o-hydroxyphenylacetic acid)
HEIDA	N-(2-hydroxyethyl)iminodiacetic acid
EDDS	ethylenediaminesuccinate
NTA	nitrilotriacetic acid
HBED	N,N-di(2-hydroxybenzyl)ethyleneamide N,N’-diacetic acid
Citric acid	Citric acid
Malic acid	Malic acid

opposite of the desired results (e.g. increased mobility of pollutants in the soil, percolation/infiltration, faster pollutants’ transfer via dissolution, unpredictable deterioration of groundwater quality). Some of the chelates degrade rapidly in the soil after application. This is, in general, undesirable. It is notable that EDTA is considered to be toxic in high concentrations even to hyperaccumulator plants. Compared to other listed chelates, the compound EDTA has a high chemical affinity for Cd [5].

2. Soil Pollution & Phytoremediation

Soil pollution is mainly due to dry deposition or wet precipitation of gaseous pollutants from industrial activities. Physicochemical extraction is the main technique used for severe soil pollution and is a high-cost method. In addition, it is applicable to small areas of pollution and causes, during the application period, the destruction of the natural micro-fauna of the soil. European countries are suffering from light to severe soil pollution in large scale on account of heavy metals mobilization as a result of long time lasting industrial activities, brown field sand intense mining industry with negative effects of the henceforth land use changes and significant restrictions if arable land the crops dedicated for human consumption.

When heavy metals are deposited on the soil surface, they are bound to the crystal lattices of the soil constituent compounds and reach a depth of up to a few tens of cm. The characteristics of the soil and the region microclimate determine up to a certain extent the fate of the pollutants. The development and maintenance of hyperaccumulator plants in organized crops is a very good, alternative antipollution method, in terms of efficiency and cost effectiveness. Decontamination is achievable relatively superficial i.e. in 20-30 cm depth, which corresponds to the depth growth of plants’ root systems [6-8].

In the present work, field bioremediation test scenarios shall be formulated by using hyperaccumulator plants (in situ crops). A remediation case scenario was built based on plants, properly selected and transplanted aiming to soil decontamination from Cd and Zn. The goal is to reduce the Cd concentration in the soil down to 1.0 mg/kg as designated in Joint Ministerial Decision 80568/4225of the Greek legislation regarding deactivated sludge specifications derived from wastewater treatment plants before being applied to arable crop soils.

In Table 4 given below, data on the yield of the plant (*Thlaspi caerulescens*) in field crops are presented. This plant is well testified and known as Cd and Zn hyperaccumulator within the last 20 years of numerous completed and published relative studies and field experiments. It thrives mainly in cold climates; however, it has remarkable adaptability and resistance to climatic variations as well as to different soil qualities (acidic, alkaline-calcareous). It is usually

Table 4 Biomass yield from decontamination and sowing of Thlaspi caerulescens cultivation [8].

Plant species	Yield at harvest (2nd year, dry matter) (t/ha)[a]	Decontamination yield (kg Zn/ha/year)	Decontamination yield (kg Cd/ha/year)	Sowing data (kg seeds/ha)
Thlaspi caerulescens	5.0[b]	20.0[c]	0.10-0.54	8

[a] 1 ha= 10.000 m^2.

[b] Mean yields since in bibliography occurs significant fluctuation i.e.1.0-13.4 t/ha/year [8].

[c] Yield in acidic soil such as in the case study and in moderately contaminated soil.

harvested every two years and its biomass production is comparable to other species of the same use. An indicative yield is about 5.0 t of dry mass/ha/year. Its root system does not exceed a depth of 30 cm. However, there are publications that report biomass production that amounts to only 0.5 t/ha/year, whereas in certain cases harvesting is appliable up to three (3) times a year [6-8].

The hyperaccumulator plant *Thlaspi caerulescens*, grows after sowing in the selected plots of land intended for decontamination. The given decontamination capacity values require the sowing of 8 kg of seeds in a plot of one hectare [7]. However, it is also achievable soil remediation by planting small plants of the species. The rate of Cd removal by the phytoextraction method—plant removal—is much higher in the 1st year of appliance and monitoring compared to the 2nd growth year. The degree of phytoextraction also kwon as transfer coefficient, is well defined and measured by transfer ratio concept. That is the mg of heavy Me bound per kg of dry plant tissue to mg in heavy Me per kg of dry soil substrate and is reported to be 3.4. An increase in the 2nd year biomass yield is expected compared to the yield of the first year of implementation [6]. *Thlaspi caerulescens* is a very good solution for its combined anti-pollution bioactivity against the presence of a significant concentration of Cd and Zn. The transfer coefficient for Zn in the small branches of the aforementioned plant equals 3.1 [6]. Furthermore, its proven storage capacity amounts up to 25,000 mg Zn and 560 mg Cd per kg of dried matter according to the literature reports. However, normal design values are significantly lower [6].

The incremental bioaccumulation rate of heavy Me in plants' tissues through the soil and mostly the root system, is determined by the ability of the dissolved Me compounds to be bio-bound during plants' physiology. The addition of EDTA solution during the irrigation phases increases the bioaccumulation yields [5]. In the subsoil zone of the rhizosphere, the organic compounds' release from the root system, in the context of the manifestation of the physiology of the plant, transforms the rheological data of the micro-area and has a direct effect on the assimilation of soil nutrients.

The main sources of atmospheric emissions of zinc are large fossil fuel combustion plants and the plating industries and they are presented in figure 1. Zn is abundant in sulfur rocky soils, sedimentary and basalt rocks, geogenic inorganic and organic fertilizers, pesticides, brownfields, deposits of mining industry and biological treatment sludge deposition in arable areas serving as soil conditioner.

The main sources of Cd atmospheric emissions are the non-ferrousmetal/ferrousmetal ores industries, the iron industries and the large fossil fuel combustion plants e.g. for power generation. Cd compounds may be airborne by fly ash or other microparticles upon adsorption on their surface. This is followed by the deposition and gradual pollution of the soil, superficially at the beginning and later in the subsoil. Extensive pollution, due to anthropogenic pressure, among others, is caused by the intensive use of phosphate fertilizers and fungicides.

2.1 Phytoremediation Needs in Greece

There are certain candidate areas, at least theoretically, in which potentially, a mild to moderate

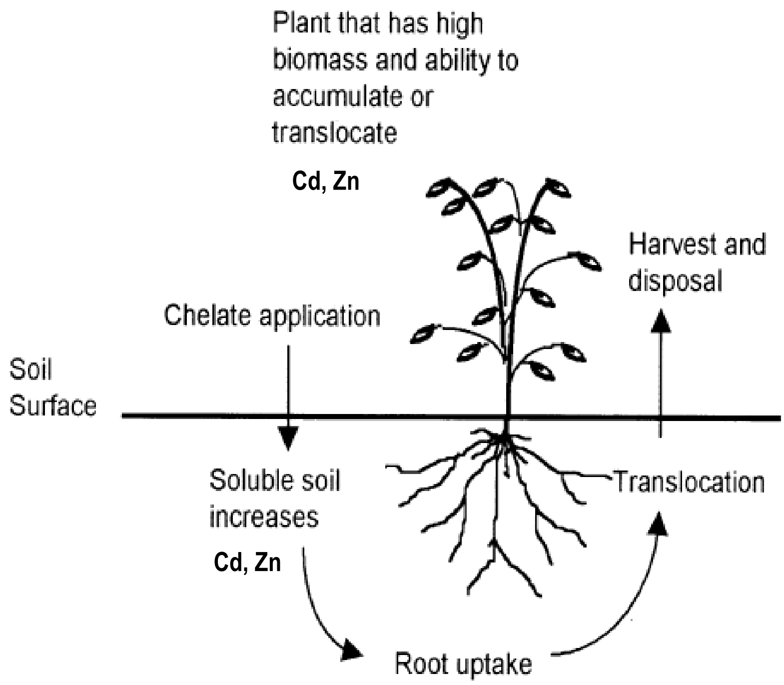

Fig. 1 Schematic representation of processing stages during phytoremediation by implementing phytoextraction [9].

gradual soil decontamination program might be applicable over a period of a few years with extensive use of hyperaccumulator plants. The phytoextraction methodology could be applied even on well-defined plots, intense cultivated and cropped. The soil remediation process might also be applied for precautionary reasons, if it was prejudged that the presence of soil pollutants reaches critical values, by applying complementary special species crops simultaneously with the existing systematic crops for commercial purposes.

Potential areas of application of phytoremediation techniques could be those that encounter minor or moderate heavy metal problems in the aftermath of intense prior industrial activities. Such characteristic sites are the arable areas near designated Industrial Areas in Greece (e.g. industrial areas of Inofyta, Patras, Ioannina, Sindos, Heraklion, Volos, Komotini etc.). The transfer of gaseous pollutants from exhaust fumes from industrial activities and subsequent dry deposition in the soil in relation to the local climatic conditions, favor the mild pollution of the soil mainly in heavy metals and hazardous hydrocarbons. Candidate areas of application are also extensive areas nearby abandoned mines e.g. post lignite era vast sites in NW (Northwest) Greece. Due to former mining activities in certain cases the barren material was dumped in areas in vicinity to the mining activities. In numerous cases the resulting dust covers large adjacent areas.

3. Case Study Formulation of Phytoremediation in Greece

The mine of Aghios Filippos in Kirki region in NE (Northeast) Greece remains inactive for many years. Prior, until 1998, a Pb and Zn mine was in full action, therefore it is considered to be ideal for any phytoremediation effort. Field measurements published in previous years consolidate the fact that the broader area is suffering from soil high heavy Me concentration (see Table 5). The soil pollution load in descending order is given as follows i.e.: Cd > Pb > Zn > As.

Table 5 Soil quality data in the Kirki area of the St. Philip of the NE Greece (prefecture of Evros). Observed concentrations (mg/kg) at six (6) control points in proximity to the extraction point in the range 300-950 m [10].

Control spots	Mn	Fe	Zn	Cu	Pb	Cd	As
A_1	450.5	36.551	345.1	28.3	80.4	3.2	8.9
A_2	878.3	27.586	474.0	42.7	219.0	5.8	14.1
A_3	1,212.0	22.517	256.3	37.7	195.4	2.0	11.4
A_4	511.2	26.324	103.6	12.8	28.5	3.1	7.1
A_5	607.4	22.455	56.9	17.4	36.3	1.2	4.7
A_6	342.1	30.101	47.4	14.9	31.0	0.9	5.4

The indices employed to assess the degree of soil pollution are the *Geoaccumulation Index* and the *Enrichment Factor* [10, 11]. Furthermore, Table 6 lists data on the soil/sedimentation pollution classification according to Müller's introduction [10, 12-14].

Müller (1969, 1979 & 1981) introduced seven classes of the geo-accumulation index (I_{geo}) (see Eq. (1)) to identify the heavy metals' pollution magnitude in sediments. Values lower or equal to zero indicate practically non-polluted soil. On the other hand, values greater than 5 indicate sediments extremely polluted. Table 6 presents the pollution classes. I_{geo} calculation formula is given below [12, 13]:

$$I_{geo} = \log_2\left(\frac{C_n}{1.5 * B_n}\right) \quad (1)$$

where C_n is the measured conc. of element Pb and B_n designates the background preindustrial shale concentration of the same element. Each element has its own background shale/crust values. Geo-accumulation index (I_{geo}) in several sampling areas of our interest, were estimated and listed in Tables 6-8.

Tables 8 and 9 present the values of the geoaccumulation indicator and the enrichment factor and thereof determine the gravity of heavy Me pollution at six (6) control points. Limit value data derived from Tables 6 and 7, consolidate that the six

Table 6 Geoaccumulation index, Müller's classification [12, 13].

Classification	Index value	Soil sediment quality
0	$I_{geo} \leq 0$	unpolluted
1	$0 < I_{geo} < 1$	unpolluted to moderately polluted
2	$1 < I_{geoc} < 2$	moderately polluted
3	$2 < I_{geo} < 3$	moderately to strongly polluted
4	$3 < I_{geo} < 4$	strongly polluted
5	$4 < I_{geo} < 5$	strongly to extremely polluted
6	$5 < I_{geo}$	extremely polluted

Table 7 Enrichment factor degrees [15].

Enrichment factor	Class designation of soil/sediment quality
< 1	No enrichment
1 ≤ 3	Minor enrichment
3 ≤ 5	Moderate enrichment
5 ≤ 10	Moderately severe enrichment
10 ≤ 25	Severe enrichment
25 ≤ 50	Very severe enrichment
> 50	Extremely severe enrichment

Table 8 Geoaccumulation indices in the Kirki area of St. Philip of the NE Greece (prefecture of Evros). Estimated values at six (6) control spots in proximity to the extraction point in the range 300-950 m [10].

Control spots	Cu	Mn	Zn	Pb	Cd	As
A_1	4.7	0.0	7.5	8.2	9.9	7.1
A_2	-0.4	-1.0	1.7	1.4	4.4	2.0
A_3	0.2	0.0	2.2	2.9	5.3	2.7
A_4	0.0	0.4	1.3	2.7	3.8	2.3
A_5	-1.6	-0.8	0.0	-0.1	4.4	1.7
A_6	-1.1	-0.6	-0.9	0.3	3.0	1.1

Table 9 Enrichment factors in the Kirki area of the St. Philip ore mines of the NE Greece (prefecture of Evros). Estimated values at six (6) control spots in proximity to the extraction point in the range 300-950 m [10].

Control spots	Cu	Mn	Zn	Pb	Cd	As
A_1	1.1	0.7	4.7	3.8	31.7	5.7
A_2	2.2	1.9	8.5	13.9	42.1	11.9
A_3	2.3	3.1	5.6	15.2	19.1	11.8
A_4	0.7	1.1	1.9	1.9	10.7	6.3
A_5	1.1	1.6	1.2	2.8	11.4	4.9
A_6	0.7	0.7	0.8	1.8	17.7	4.2

checkpoints near the mining area are considered to be polluted, particularly in Cd and secondarily in Pb, As and Zn. In fact, the closer the checkpoints are located at the former mining activities, the more serious the encountered pollution problems.

3.1 Field Testing before Phytoremediation Application

The systematic planting and cultivation of *Thlaspi caerulescens*—the proposed species—in a hypothetical phytoremediation project entails a prior sampling and testing in laboratory soil quality, in terms of nutrients sufficiency, alkalinity, pH, Eh (Redox Potential), and thorough examination of the prevailing climatic conditions.

Any remediation action presupposes stakeholders' consent and willingness to pay attitude for any income loss in the near future. Covenants should be arranged with the landowners of the zones of project's interest, assumptions should be made of low budget site monitoring and the acceptance of the commitment of the land use for at least two years period. All necessary inquiries should be carried out to ensure the favored and uninterrupted plant development conditions. The species is not endemic in Greece. However, scientific studies have shown its high resistance in different soil types [7].

The project benefits are undoubtedly cost effective in every aspect. Cultivated land fields, former impropriate for any type of cultivation due to extensive soil pollution, after systematic phytoremediation implementation are becoming arable for human consumption agro-products. The proposed plant is a first-class Cd & Zn hyperaccumulator.

The deposition of barren soil piles "*tailings*" from the prior mining activity, on a large area of 2.2 km^2 coverage (see Fig. 2), favors the geological AMD (Acid Mine Drainage) phenomenon to occur. Thus, tailings resulting from the mining activity are deposits rich in metal sulfides, and more particularly, in iron sulfides i.e. pyrite (FeS_2), as the most abundant sulfide in nature undergoes a progressive oxidation in the presence of bacteria groups that act as catalysts. As regards iron sulfides, AMD geological effect is described through the following reactions [16-21]:

(α) oxidation of divalent Fe of the sulfide

$$FeS_2 + 3.5O_2 + H_2O \rightarrow Fe^{2+} + 2SO_4^{2-} + 2H^+ \qquad +1.409\ kJ/mol$$

(β) oxidation of divalent iron (Fe^{2+}) to trivalent (Fe^{3+})

$$Fe^{2+} + 0.25O_2 + H^+ \rightarrow Fe^{3+} + 0.5H_2O$$

(γ) hydrolysis of trivalent iron (Fe^{3+})

$$Fe^{3+} + 3H_2O \rightarrow Fe(OH)_3\ (s) + 3H^+$$

Since oxidation reactions release heat which has been observed to reach up to 70 ℃, it incurs oxygen reduction in the mine tailings and the formation of trivalent iron ions and acidification in the extraction soil. Acidification brings about increased solubility and therefore metal ions mobility in both in surface and subsoil. Various acidophilic bacteria, mainly autotrophic, inter alia, Ferrooxidans, *Leptospirillum ferrooxidans*, *Thiobacillus ferrooxidans* etc. oxidate iron sulfides to divalent at the first stage and later to trivalent ions. Such bacteria have the ability to catalyze ferrous substrates [16-21].

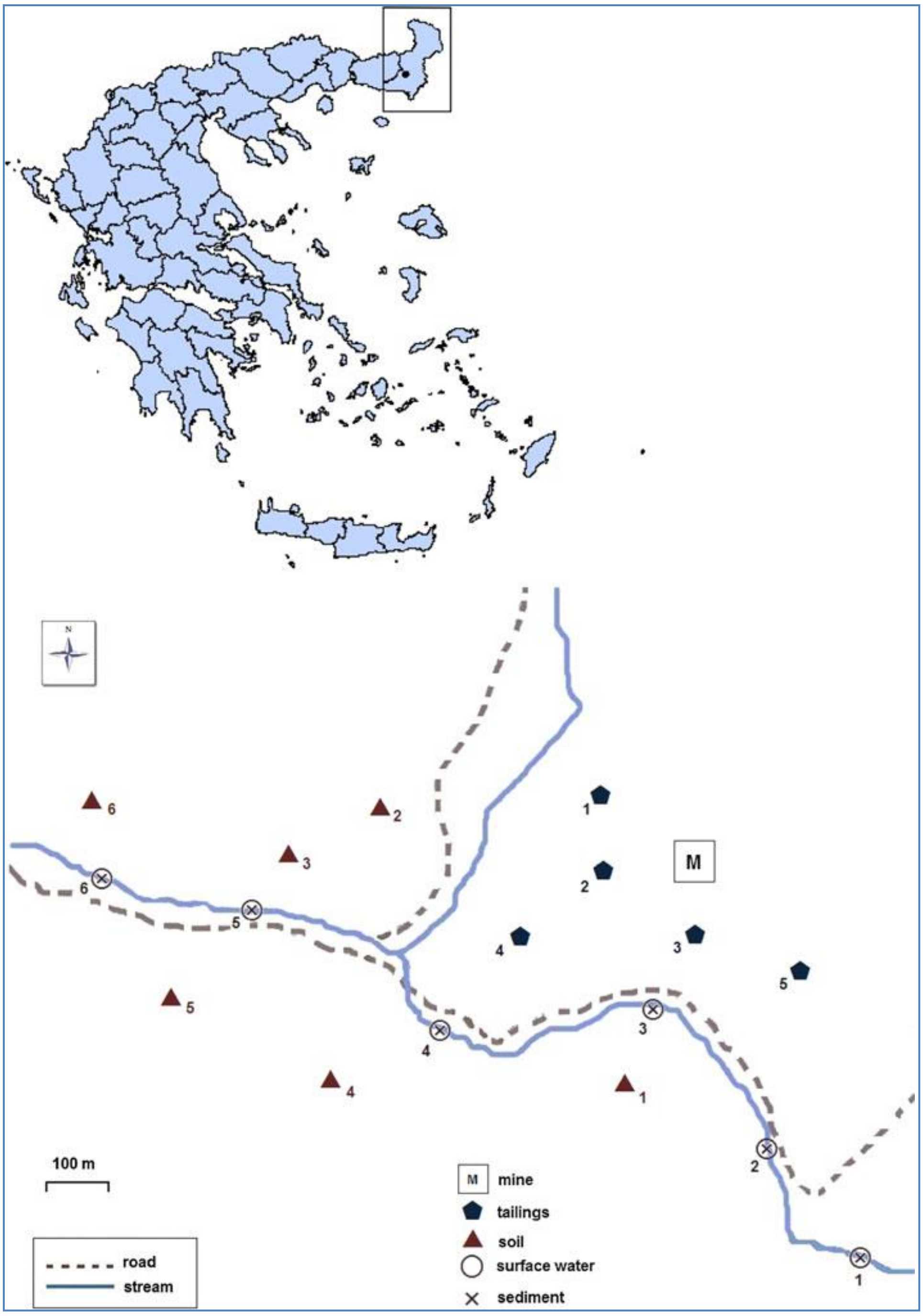

Fig. 2 Location of the case study in the minefield of Aghios Philippos. The denoted checkpoints are depicted by small deep red triangles. The mining point is located in the square box marked (M) [10].

An environmentally friendly intervention in the major area is needed to alleviate the problem of heavy Me ions mobility occurring in high concentration. It is enhanced through rainwater drainage and percolation/infiltration phenomena to distant and potentially cultivated plots of land. It also incurs aquifer degradation in terms of the presence of undesirable hazardous chemical agents. The increased acidity motivates and eases ion contaminants' fate, released from their binding molecules/crystals in the soil and favors the transition to solutions in the area of the root system of plants (rhizosphere). The processing facilitates the uptake of minerals into the body of plants first in the area of roots and secondarily in parts of the plant above the soil (shoots, foliage, clones).

The Joint Ministerial Decision (JMC), 80568/4225 published in the Governmental Gazette 641/B/7.8.1991 defines the terms and heavy Me limit values in arable soil. This JMC is the integration of Directive 86/278/EEC (Sewage Sludge Directive in agriculture) into the Greek legislation. The given limits listed in Table 10 are those defined by JMC 80568/4225. JMC determines the pollutants' limit value measured in the top soil, after harvesting occurs in a phytoremediation project.

A potential heavy Me contained mass value exceedance per unit weight of the plant biomass, entails an increase in the decontamination cost. The reason is the fact that the polluted biomass is characterized henceforth as environmentally hazardous solid waste and the whole treatment until the final disposal and deactivation of metal ions is very costly.

In such cases the phytoextraction technique is likely to be taken under consideration along with other conventional techniques in a decision-making mechanism to qualify and promote the most suitable decontamination method.

Decontamination plants support bioremediation projects in an eco-safe, cost effective and a non-intensified way. JMC 80568/4225 determines heavy metal content values in the soil. Harvested biomass contains hazardous chemical agents within certain limits well defined by the scientific bibliography.

In order to accelerate the uptake process and compress the bioremediation period, the use of chelate agents such as EDTA (see Table 3), might be adopted to achieve high-rate heavy metal binding in the parts of the plants, along with the soil control of pH and redox with the use of fertilizers in a limited scale that are proven accelerators in plant's growth rate.

Comparing the listed values of Tables 5 & 10, the conclusions are summarized in Table 11. Regarding Cd, an excess of concentration values was observed with reference to the established limits of Directive 86/278 / EEC at control points A_1, A_2 and A_3. At point A_4 there is a potential excess related to the pH of the soil while at points A_5, A_6 no excess whatsoever was detected. As for Zn, at the control points A_1 and A_2 limit values were exceeded. At the check point A_3 there is a potential excess related to the pH of the soil while at points A_4-A_6 no excess was detected. As for

Table 10 Heavy metals' limit value in the soil as well as limit values for imported quantities of heavy metals in cultivated soils based on an average ten years' period [22].

Chemical agent	Soil limit values mg/kg in dry matter (with pH 6-7 and depth sampling of 10-25 cm)	Appliance limits to crops (kg/ha/year)
Cd	1-3[a]	0.15
Cu	50-140	12.00
Ni	30-75	3.00
Pb	50-300	15.00
Zn	150-300	30.00
Hg	1-1.5	0.10

[a] mg/kg dry matter in a representative soil sample.

Table 11 Heavy Me values exceedances as determined in Directive 86/278/EEC (JMD 80568/4225) at the six (6) nearby check points at a 300-950 m distance away from the ore mine field of Aghios Philippos [10, 22].

Check point	Cd	Zn	Cu	Pb[a]	Cr[b]	Hg[b]
A_1	Out of limit	Out of limit	Within limits	Potentially out of limits	-	-
A_2	Out of limit	Out of limit	Within limits	Potentially out of limits	-	-
A_3	Out of limit	Potentially out of limits	Within limits	Potentially out of limits	-	-
A_4	Potentially out of limits[c]	Within limits	Within limits	Within limits	-	-
A_5	Within limits	Within limits	Within limits	Within limits	-	-
A_6	Within limits	Within limits	Within limits	Within limits	-	-

[a] mg/kg dry matter in a representative soil sample.

[b] There are no measurement data.

[c] Limits applied to soil pH 6-7.

Table 12 Assumptions for the case study phytoremediation.

Cd upper limit value	1.0 mg Cd/kg [22]
Zn upper limit value	150mg Zn/kg [22]
Plant species	*Thlaspi caerulescens*
Plant growth expectancy	Two years (2nd year harvesting)
Decontamination rate (Cd)	0.5 kg/ha/year [8]
Decontamination rate (Zn)	20.0 kg/ha/year [8]
Decontaminated area	2.2 km^2
Topsoil parameters	Compatible to the species cultivated
1 m^3 of soil weighs approximately	1,200-1,500 kg of bulk soil [6, 8]

Table 13 Contaminants in 7,150 $\times 10^5$ kg bulk soil.

Chemical agent	Quantity contained (kg)	Quantity (kg) to be removed	Remediation period (years)
Cd	715	3,432	31
Zn	107,250	231,660	52

Cu, no exceedance was observed at any control point. As regards Pb at points A_1-A_3, there is a potential excess related to the pH of the soil while at points A_4-A_6 all measurement values were within limits.

The area of interest is shown in Fig. 2, with the located six (6) checkpoints, which cover, an area of 2.2 km^2. Considering in a rough approach, that 1 m^3 of soil corresponds to 1,200-1,500 kg [6, 8], and the bulk soil for the seedlings to be cultivated reaches up to 25 cm depth in the subsoil (Directive 86/278/EEC), then the mass of soil to be rehabilitated is easily calculated. Namely:

$$2.2\times10^6\ m^2 \times 25\times10^{-2}\ m = 5.5\times10^5\ m^3$$

and $5.5\times10^5\ m^3 \times 1,300\ kg/m^3 = 7,150 \times 10^5$ kg bulk soil to be decontaminated.

In the present manuscript the whole remediation project planning is going to meet the environmental requirements of the control points with the highest acceptable concentration load as regards Cd and Zn pollutants in accordance with Directive 86/278 /EEC incorporated in the Greek legislation as JMD 80568/4225.

It is noteworthy that the neighboring area has no cultivated crops whatsoever directed to human consumption. The highest concentrations that occurred at control point A_2 as regards Cd & Zn amounts to 5.8 mg/kg and 474 mg/kg respectively. The desired decontamination goal amounts to 1.0 mg Cd/kg and 150 mg Zn/kg of bulk soil respectively. A reduction of 4.8 mg/kg for Cd and 324 mg/kg of bulk soil for Zn will be sought so as the aforementioned limit values can be achievable. Tables 12 and 13 list design parameters and the estimated quantities to be removed in a rehabilitation project.

Given that the upper accepted limit value is 150 mg Zn/kg, according to the Greek legislation, soil restoration by employing plant extraction method in controlled cultivation of *Thlaspi caerulescens* species, considering a decontamination rate at 20.0 kg/ha/year [8], or 4,400 kg/year in the preselected area of 2.2 km^2, is estimated ~52 years.

Likewise, regarding Cd removal by the plant extraction method at 500.0 g/ha/year by utilizing the same plant species *Thlaspi caerulescens* [8], to achieve the remediation of the same surface i.e. bulk soil, at the predetermined level at 1.0 mg Cd/kg soil, a period of ~31 years is required for the final soil restoration up to the acceptable levels according to the national legislation.

Thlaspi caerulescens demonstrates the highest biomass production in the second growth year. Sowing is carried out in April and biomass extraction (uprooting) takes place in November. It is a perennial plant by nature. Therefore, after the end of two (2) years period, uprooting and re-sowing of field under remediation is required. For simplicity reasons it is considered the same decontamination rate in two years period.

During the first weeks of the plant's life, due to the fact that selected species demonstrate a reduced competitiveness with weeds, it is considered necessary to use herbicides along with uprooting, selective cleaning and removal of weeds. In addition, the right phytoremediation strategy should be applied, in slightly and moderately contaminated soils. The addition of chelating agents is indicated on the basis of groundwater measurements of the area since the values in Cd and Zn are already high and possible, albeit limited, use of such compounds would potentially incur greater metal ions mobilization and thus a further environmental degradation.

The decontamination area is considered to be an area with low average temperatures and intense annual precipitation. This climate is fairly similar to the thriving zones of the species. This is considered to be important issue, regarding biomass yield expectancy. In general, however, it has been studied and proved that the species shows high adaptability and sufficient growth rate even in different climates [7]. During the period of reduced rainfall, it is necessary to water the hyperaccumulators using a water tank (at least up to 20 times) during the dry months (May-September).

The resulting crops biomass cannot be disposed in sanitary landfills. Crops in remediated zones do not follow the life cycle of agricultural biomass. That is, to be transformed to biofuel, to be composted, to be applied as a soil conditioner etc. high concentrations of heavy Me are restrictive reasons for any of the aforementioned utilizations. *Thlaspi caerulescens* stalks and leaves accumulate up to 12.0 mg Cd/kg and 2,600 mg Zn/kg plant biomass respectively [7]. The above concentrations vary of those given in Table 4.

Expenditures in Tables 14 and 15 include sowing, soil preparation (hoeing), cleaning, tank irrigation in

Table 14 Economic data of heavy Me soil remediation by implementing plant extraction technique [7].

Actions	Task analysis	Repetitions (within 52 years)	Cost within remediation period (estimations in 2006 as a reference year) (€)
Crops field work	Digging, cleaning, soil preparation	26	3,935
	Sowing, 8 kg/ha	26	2,383
	Harvest[a]	26	1,600
Chemical additives (if necessary)	Herbicides, pesticides	26	2,942
Electrical supply, water supply	Irrigation, ploughing, 5.11 €/ha/year	52	265
Fuel consumption, emergency measurements[b]	Transportation, machinery operation 25.56 €/ha/year	52	1,329
Overall			12,454

[a] 12.31 €/Mg dry matter, biomass yield (Table 4) is estimated to be 5.0 t/ha/year (see Table 4) namely 12.31 × 5.0 = 61.55 €/ha/year.

[b] Fixed cost not related to the number of crops.

Table 15 Economic data of hazardous biomass disposal cost [7].

	Value
Yield of biomass treatment(t/ha/year)	5.0
Yearly mass production (t)	1,100
Crops (years)	26
(% w/w) ash in biomass	2
Hazardous ash disposal (€/t)	60
Final disposal cost (€)	68,640

dry months, growth monitoring, soil quality control, harvesting, biomass management, *Thlaspi caerulescens* species biannual replanting, monitoring measurements, use of herbicides, chelates if required, etc. The overall cost incorporates the final management cost of the stalks that should be treated as hazardous material when handed over to a properly licensed company. No use of fertilizers for low biomass yields was adopted in the present case study.

4. Conclusions

Phytoextraction technique is a cost-effective solution to obtain rehabilitation in vast areas that undergo significant pollution on account of hazardous chemical agents. It is appliable to a small to moderate degree of contamination, in areas of low environmental risk and lack of requirement for rapid rehabilitation. The application of the method entails implantation of hyperaccumulator species that are tolerant to the uptake of high amounts of pollutants and with the ability to accumulate heavy Me ions or other toxic compounds in their biomass. Extensive subsoil root systems and rapid growth of the preselected plant species assure high rate of biomass production and significant heavy metals binding as a prerequisite for the proper application of the method. The chemically/chelate enhanced phytoextraction technique can be applied on a case-by-case basis, using chelates mainly EDTA. Pollutants' concentration level in biomass is regulatory in the final application cost of the method [4-8].

Specific plant species applications as hyperaccumulators are carefully selected after previous soil sampling, testing and comparison with scientific literature. Decision making tools determine the final technique to be applied. In any case, the plant growth rate and physiology should be favored in the areas to be cultivated taking into consideration the local microclimate. It is much preferable the choice of indigenous species even if their metal ions binding capacity and performance are significant lower. The interaction of alien species with the endemic fauna is another point of serious study before making final decisions. In the present case-study the recovery time was much higher than usual which is normally up to fifteen (15) years. This is due to the fact that in the present case, seeds were used instead of transplanted species. In the latter way it is possible to extract biomass up to three times a year which drastically reduces the recovery time. *Thlaspi caerulescens*, though found mostly in northerly European regions, still demonstrates remarkable adaptivity in relative warmer areas.

The engagement of the land use for such a long restoration period is a problem of minor importance, since the land uses around the mine activities render the land for the next decades improper for crops directed to the food industry or other development activities (ecotourism). The lack of infrastructure in the area (electricity, water, sewerage networks, developed road network, distance from ports, airports) discourages any investment/industrial project initiative which incurs heavy environmental impact. Thus, any temporary land deprivation for decontamination's sake is of minor gravity.

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