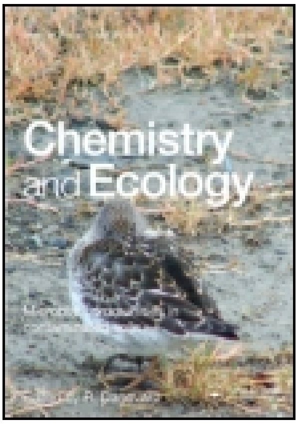

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Phytoremediation of contaminated sediments: evaluation of agronomic properties and risk assessment

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This study evaluates the possibility of reusing marine sediments in land management. The sediments, dredged from Livorno port (Italy), had previously been phytotreated, using a salt-tolerant plant cover and earthworms, with the aim of reducing the salt level and improving the texture and microbiology. In this study, sediments were investigated in order to: (1) test their capability to be used as a revitalised soil-like substrate (techno-soil), and (2) assess the human exposure risks associated with sediment management. Results obtained after 6 months of experiments performed with biological indicators composed of an association of graminae grass (*Paspalum vaginatum*), legumes (*Trifolium alexandrinum*) and earthworms (*Eisenia foetida*), showed that the substrate behaves like a natural soil capable of supporting biological life (total N $=$ 0.2%; total P $=$ 0.7%; EC $= 1.5\,\mathrm{mS}\cdot\mathrm{cm}^{-1}$; $\beta -$ glucosidase $= 20\,\mu\mathrm{g}$ PNP $\cdot\, \mathrm{g}^*\mathrm{h}^{-1}$). It was also found that plants accumulate small amounts of heavy metals in shoot tissues ($120\,\mathrm{mg\,Zn}\cdot\mathrm{kg}^{-1}$; $25\,\mathrm{mg\,Cu}\cdot\mathrm{kg}^{-1}$). In detail, risk analysis was performed considering: (1) sediment storage in a sealed disposal basin inside the Livorno port area, and (2) off-site phyto-remediation. The maximum hazard index was found for workers inside the port area, with values of 1.7 and 25 for dermal contact and vapour inhalation risks, respectively.

Keywords: marine sediments; heavy metals; phytoremediation; risk assessment

1. Introduction

The various logistic and management requirements of port areas and artificial internal water ship-canals, together with the increasing awareness of environmental issues, prompt the need to develop and carry out infrastructural work, which requires preliminary studies on safeguarding and/or reclamation of bottom sediments. At the same time, the volumes of sediments to be removed are increasing significantly, so that more suitable logistic solutions capable of managing the reclamation in a technical, economical and environmental way are needed. The amount of contamination often requires dedicated dredging projects, even in cases in which this is not required for shipping [1]. This is due to the very strict standards for river and sea water quality in developed countries. Many of these problems exist in Italy, and require adequate technical and cost-effective treatment procedures. Nowadays, reclamation studies of contaminated matrices refer mainly to soils. The results obtained from these studies are sometimes extended to sediments.

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First, the main differences between soil and sediments results from their origins. Soil represents unconsolidated mineral or organic material on the immediate surface of the earth that serves as a natural medium for the growth of land plants [2]. Sediments derive from soil due to desegregation factors, such as climate (including water, wind and temperature effects), and also macro- and microorganisms, conditioned by relief, acting on the parent material over time. A product-soil (i.e. sediment) differs from the material from which it is derived in many of its physical, chemical, biological and morphological properties and characteristics [2,3].

At present, the treatment of contaminated sediments is aimed at the partial or total reclamation of sediments, so that they can be reused as land replenishment materials for the construction of roadbeds or land-fill basins [4] and/or the partial decontamination of sediments for disposal in specific and more economical landfills [5]. However, the choice of treatment typology depends on three principal parameters: (1) the granulometry of the sediment (percentage of gravel, sand, silt and clay); (2) the type and concentration of the different contaminants (heavy metals, hydrocarbons, organic compounds, etc.); and (3) the amount of sediment to be treated.

Transformation of a sediment into a reusable soil represents, when suitable, the most convenient and economical solution. With this aim, it is possible to use plants and their associated rhizosphere microorganisms to treat the contaminants present in a contaminated matrix [6], through natural techniques that transform the sediment into soil. The advantages of natural techniques are the possibility of treating huge quantities of sediments, the ease of maintenance of the plants and low management costs. The disadvantages are the need for large areas (difficult to locate within urban districts), the long time required for the treatment (often incompatible with customer demand) and the sometimes unsatisfactory level of contaminant abatement (satisfactory results are not found for all types of contaminants). Recent results achieved by applying phytoremediation techniques to soil [7,8], encourage the extension of this technique to different matrices. Actually, very few applications are currently used with sediments, and none on a large scale. Previous mesoscale studies have demonstrated the efficiency of an agronomic technique, similar to phytoremediation, applied to contaminated marine sediments [9,10]. The described systems also carried out the degradation of organic pollutants, particularly hydrocarbons, due to the presence of microorganisms in the rhizosphere [11]. The inorganic pollutants (e.g. heavy metals) could be accumulated in vegetal tissues, or blocked in the rhizosphere [12], due to root exudates and humic substances. In the case of phytoremediation techniques applied on a field-scale, there is also concern about metal-accumulating plants providing an exposure pathway for toxic elements to enter the food chain [12]. Furthermore, the addition of chelating agents to enhance plant metal uptake, invariably increases the risk of metal leaching [12,13]. For these reasons, an ecological risk exists and needs careful evaluation. Risk assessment is associated with estimating the probability of negative effects on human health, because humans are an integrated part of ecosystems [13]. Because of the low contamination content when applying natural reclamation techniques, and the low heavy metal content usually found in plant tissues [10], the risks associated with phytoremediation techniques are often negligible.

This study proposes the use of plants, earthworms and agronomic amendments (TRIAS) [14] for the transformation of sediments into a fertile soil (techno-soil). This is possibly reusable for environment restoration purposes, without posing risks for human health.

2. Materials and methods

2.1. *Description of the mesoscale pilot system*

The marine sediment (dredged from Livorno port) used in the mesoscale pilot system was originally a heterogeneous and complex matrix. It was formed of solid-aggregated particles

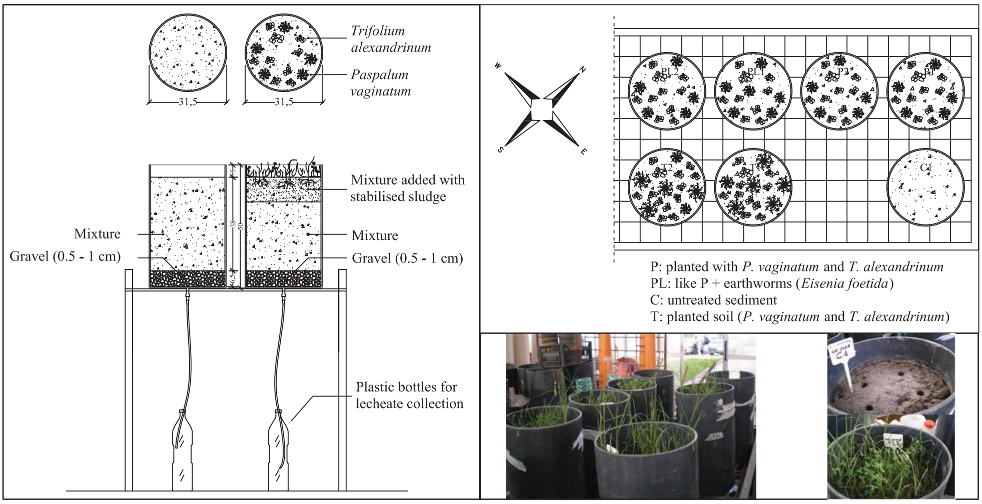

Figure 1. Mesoscale pilot system for the evaluation of sediment agronomic qualities using biological indicators (plants and earthworms).

contaminated by heavy metals and hydrocarbons, biologically inactive and poor in nutrients [15]. The sediments used in this study were previously pre-conditioned with a calcareous-sandy inert residue and compost, then phytotreated using a salt-tolerant plant cover association (*Paspalum vaginatum* and *Tamarix gallica*) and earthworms, simulating a rainfall drainage aimed at reducing the salt level and improving texture and microbiology [9,14].

The pilot system consists of seven hydraulically equipped mesocosms (vertical plastic pipes, 50 cm in height and 30 cm in diameter) placed in the greenhouse of the CNR, Institute of Ecosystem Studies (ISE) in Pisa (Figure 1). Four different treatments were tested:

(1) P: two mesocosms planted with *Trifolium alexandrinum* and *Paspalum vaginatum*;
(2) PL: two mesocosms planted like P, with the addition of earthworms (*Eisenia foetida*);
(3) C: one mesocosm without plants and earthworms, used as a control;
(4) T: two mesocosms filled with an agronomic soil and planted with *Trifolium alexandrinum* and *Paspalum vaginatum*.

The sediment (dredged from Livorno port) was derived from a previous experiment, based on the phytoremediation technique [9,14]. The sediment was then mixed with calcareous sand (30% weight) and planted again as described above. Samples from each mesocosm were taken at the beginning of the experiment and after 6 months, from two different depths (0–15 and 15–25 cm). Analyses of plants (*Paspalum vaginatum* and *Trifolium alexandrinum*) were performed after 6 months.

2.2. ***Laboratory analyses***

2.2.1. *Chemical parameters*

Electrical conductivity (EC) and pH were measured in a 1 : 10 (w/v) aqueous solution. The total organic carbon (C) was determined by oxidation using a RC412 Multiphase Carbon (Leco, USA). Total nitrogen (N) was determined by flash combustion using a FP-528 Protein/nitrogen Determinator (Leco, USA) and total phosphorus (P) by using the method reported by Olsen and Sommers [16]. Water-soluble carbon (WSC) was determined according to the method reported by Yeomans and Bremner [17]. Available P was determined by the method of Murphy and Riley [18]. Total heavy metals, total sodium (Na) and total potassium (K) were determined by means of a high-resolution continuous atomic absorption spectrometer (Analytical Jena, Contraa).

2.2.2. *Physical parameters*

The particle size distribution was evaluated by means of laser screening (Microtrac ASVR device). The $BaCl_2$ method was use to determine the cation-exchange capacity (CEC) [19]. Soil bulk density was measured on undisturbed cores [20].

2.2.3. *Biochemical parameters*

Respiration (expressed in terms of CO_2 release) was measured in the absence of organic substrates, according to the Cheng and Coleman method [21].

The methods used to assay hydrolase activities (phosphatase, β-glucosidase and urease) are described by Garcia et al. [22].

2.3. ***Statistical analysis***

Statistical analyses of the experimental data were performed using the Statistica 6.0 software package. All data were reported as the mean ±SEM. Statistical differences between treatment groups were determined by one-way analysis of variance (ANOVA) with the Student's independent t-test. The level of significance was $p < 0.05$.

2.4. ***Risk analysis***

Risk analysis was performed using the RISC 4.0 software package, which provides a complete set of tools for calculating the risk to human health and surface water. Receptors are considered as belonging to the contaminated site. Probabilistic (Monte Carlo) exposure capabilities are provided to calculate the risk (*forward* analysis). From the calculated risk, the software calculates the site-specific target levels (SSTLs), performing a *backward* analysis.

3. Results and discussion

3.1. ***Meso-scale pilot system: comparison with an agronomic soil***

In this study, biological indicators composed of an association of salt-tolerant graminae grass (*Paspalum vaginatum*), legumes (*Trifolium alexandrinum*) and earthworms (*Eisenia foetida*), were used to test the ability of the substrate (the treated sediment) to behave like a natural soil. Results reported in Table 1 show that, after a six-month experiment, the treated sediment was capable of supporting biological life, also showing good agronomical and biochemical properties. The nutrient content (total organic C, total N, total K, total and available P) shows appreciable values due to the presence of the graminae grass–leguminous plant association, which releases large amounts of root exudates via processes mediated by the simultaneous action of earthworms [23]. The possibility of plants growing and earthworms surviveing was related to the electrical conductivity, which expresses the salinity level reached after sediment treatment (Table 1).

The physical improvement of the sediment, as shown by the texture and bulk density, allowed better circulation of water–air and nutrients inside the pores at the root zone (Table 1). Sandy-loam textured soils better support microbial life proliferation in the rhizosphere [24].

Table 1. Characteristics proposed as basic indicators of soil quality and comparison with the treated sediment (after 6 months) – values are means of PL and P performance.

Parameters	Units	Measured in treated sediment	Value ranges for agronomic soils	Ref.
pH	–	8.20–8.56	7.3–8.1 sub-alkaline; <8.2 alkaline	[37,38]
Electrical conductivity	($\mu S\cdot cm^{-1}$)	1540–3625	<2000 viable for all cultivations 2000–4000 risk for sensitive cultivations 4000–8000 risk for all cultivations 8000–16,000 not tolerated by any cultivations	[37,39]
Tot organic C	($g\cdot kg^{-1}$)	13–20	For a sandy loam soil: <7 low; 7–9 normal; 9–12 good; >12 very good	[38,39]
Total N	(%)	0.08–0.19	0.15–0.4 mean value	[37,38]
Total P	($g\cdot kg^{-1}$)	0.18–0.77	0.2–5 mean value	[37,38]
Total K	($g\cdot kg^{-1}$)	5.5–6.5	0.8–40	[37,39]
Total Na	($g\cdot kg^{-1}$)	9.5–25	0.8–25	[38,39]
C/N	–	9.8–16	<8 low; 8–12 medium; >12 high	[38,39]
Soluble P	($mg\ P_2O_5\cdot kg^{-1}$)	100–230	>70 required content	[39]
Bulk density	($g\cdot cm^{-3}$)	0.90–0.92	1–1.4 structured soil; 1.2–2 non-struct. soil	[39]
C.E.C.	($meq\cdot 100\ g^{-1}$)	16–18	<10 low; 10–20 medium; >20 high	[38,39]
Soil texture	–	Sandy-loam	Sandy-loam	[40]
β-Glucosidase	($\mu g\ PNP\cdot gh^{-1}$)	15–30	20–200 degraded soils; 140–700 natural soils	[41]
Phosphatase	($\mu g\ PNP\cdot gh^{-1}$)	120–200	100–200 cultivated soils	[41,42]
Urease	($\mu g\ N4+\cdot gh^{-1}$)	10–30	20–40 natural soils	[24]
Cu	($mg\cdot kg^{-1}$)	20–30	5–17	[43]
Zn	($mg\cdot kg^{-1}$)	110–130	20–40	[43]
Ni	($mg\cdot kg^{-1}$)	0–1	0–1	[43]

Therefore, the simultaneous action of plants, earthworms and microorganisms (biological indicators) was efficient for the revitalisation and restoration of the microbiological functions of the treated sediments, thus confirming the need for sediments to undergo a previous phytotreatment and bio-physical conditioning [25]. This is necessary to create a link between function and indication exhibited by these organisms. In the rhizosphere, plant roots release exudates, microorganisms mineralise them releasing nutrient for plants, and earthworms feed on microorganisms [14].

Biological conditioning was measured through enzyme tests currently used to assess nutrient cycling in soils, i.e. β-glucosidase (involved in the organic-C cycle), phosphatase (organic-P cycle) and urease (organic-N cycle). These enzymes are considered indicators of microbial activity [26,27] and also express a biochemical energy resource capable of revitalising heavily degraded soils [28]. The results achieved, shown in Table 1, are very satisfactory, especially for phosphatase and urease, whereas β-glucosidase corresponded to a range of degraded soils. However, this represents a very promising result, considering the original characteristic of the sediments, which were geologically and biologically degraded.

Metabolic activity in the system was directly measured through sediment respiration and the CO_2 released was related to the WSC, which represents an easily decomposable organic substrate for microorganisms. Figure 2(a) reports the WSC detected in each mesocosm, at the beginning and after 6 months of incubation. The results show that the sediment of trials P and PL behaved like control soil T, showing decreasing values with time, especially in the top layer 0–15 cm, which coincided with the rhizosphere depth ($p < 0.05$).

Microbiological activity is also described by the CO_2 released by microbial respiration. Figure 2(b) shows the respiration for each mesocosm, after 6 and 24 h. Values confirmed the similar behaviour between PL and T, whereas a complete absence of respiration was shown by the sediment in control trial C, which never underwent phytotreatment ($p < 0.05$).

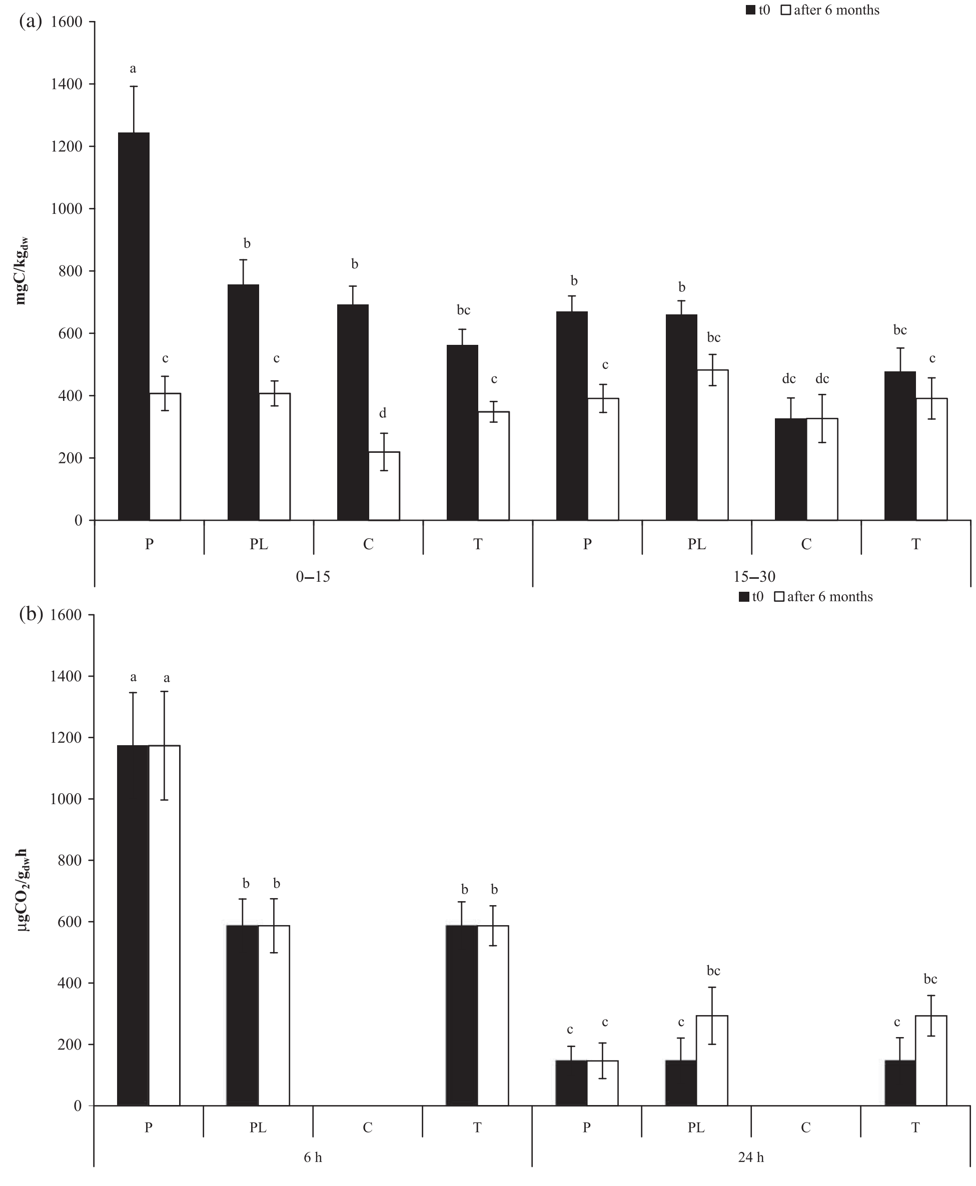

Figure 2. (a) Water-soluble carbon (WSC) detected in each mesocosm (P, PL, C, T) at the beginning and after 6 months, in the top (0–15 cm) and bottom layer (15–25 cm). (b) Respiration measured in terms of CO_2 release, in each mesocosm, after 6 and 24 h. Values with different letters are statistically significant at $p < 0.05$.

Table 1 reports the heavy metals accumulated in the shoots of *Paspalum vaginatum* and *Trifolium alexandrinum* after 6 months. The values were higher than those usually found in crops, except for the Ni. Zn and Cu represent essential nutrients for plants: 15–30 mg·kg^{-1} of dry plant matter satisfies the physiological needs of plants [29]. The higher concentrations found for Zn possibly highlight the capability of the plants used here to phytoextract and accumulate Zn from the sediment, without showing toxicity symptoms. Although the concentrations detected are small compared with those usually found in metal-accumulating plant species [30], the possibility of metal accumulation in plant shoots might represent an exposure pathway for toxic elements to enter the food chain.

3.2. *Risk assessment*

Two different scenarios have been evaluated to determine the possible risk associated with the management of dredged sediments from port areas [31–33]:

(A) The dredged sediments are stored in a sealed accumulation basin within Livorno port (currently adopted situation). The conceptual model is shown in Figure 3. Workers have been considered as target points.
(B) The sediment is treated on a restoration site, applying an off-site phytoremediation technique. In this case, the target points are children and adults, living in a nearby area [34]. The conceptual model is shown in Figure 4.

The results for both the scenarios are reported in Tables 2 and 3. Results have shown that the best solution is associated with the low level of total risk for each exposure pathway, described in scenario (B), in which dredged sediments are phytoremediated off-site.

The detected pollutants are heavy metals (Cd, Cr, Ni, Cu, Pb, Zn) and hydrocarbons ($C > 12$ and $C < 12$). The value of each contaminant found in the accumulation basin in the port of Livorno (CRS) was compared with the limit values of Italian legislation DLgs 152/06, issued relative to industrial areas (Table 2). The risk is expressed in terms of the Hazard Index (HI), because these compounds are considered non-carcenogenic for human health [35], and the maximum admissible value is 1. In scenario (A), total $HI = 2.5$. Referring to Table 2, the major cumulative HI considering the surface sediment as an exposure pathway, was associated with dermal contact (1.7). The maximum HI (25) was found to be related to the outdoor air exposure pathway (Table 2). This means that workers inside port areas are exposed to a high health risk, due to the possible inhalation of vapours and particulate matter released by wind erosion.

However, considering scenario (B), the HI value associated with the outdoor air exposure pathway was 0.98 for children (Table 3) and 0.28 fro adults (data not reported). In Table 3 the HI values refer only to children (the worst case scenario). The off-site phytoremediation technique prompted a decrease in vapour inhalation, probably because of the presence of the plants. The

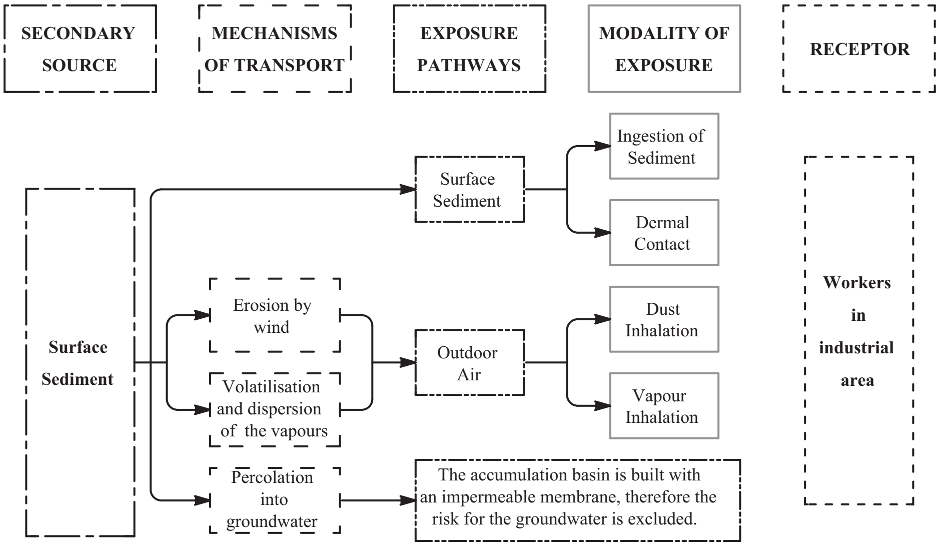

Figure 3. Conceptual model for scenario (A). The dredged sediments are stored in a sealed accumulation basin inside Livorno port. Workers were considered to be target points.

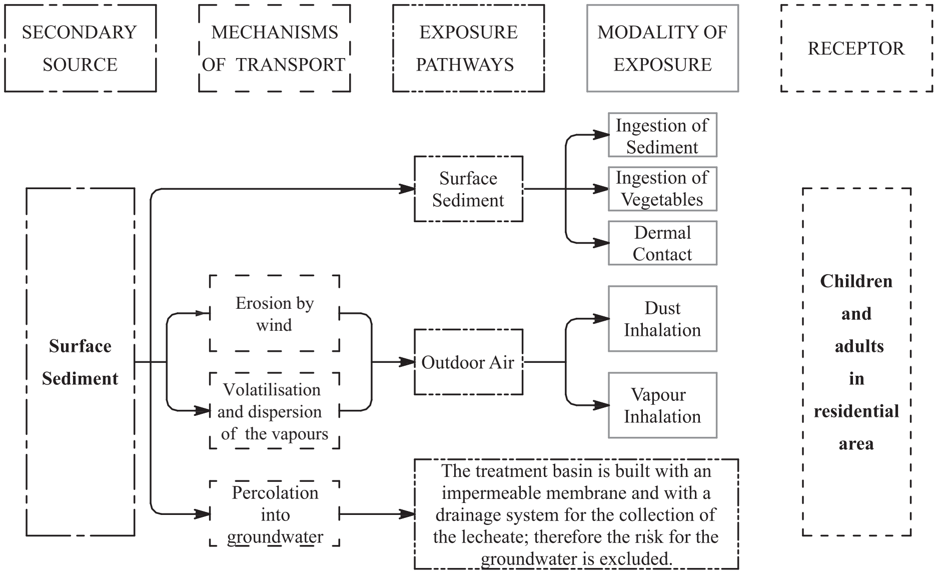

Figure 4. Conceptual model scenario (B). The sediment is treated on a restoration site, applying the off-site phytoremediation technique. The target points are children and adults living in a nearby area.

Table 2. Hazard Index (HI) for scenario (A): workers inside the Livorno port area.

			Exposure pathways			
			Surface sediment			Outdoor air
Pollutants	Limits DLgs 152/06 Industrial area	CRS $mg \cdot kg_{dw}^{-1}$	Ingestion of soil	Dermal contact soil	Total HI	Vapour inhalation
Cd	15	13.2	2.6E−02	6.8E−04	2.7E−02	–
Cr	800	123	8.0E−05	2.1E−05	1.0E−04	–
Ni	500	81	1.3E−03	3.5E−04	1.7E−03	–
Cu	600	85	1.5E−01	3.9E−02	1.9E−01	–
Pb	1000	541	4.0E−03	1.0E−03	5.0E−03	–
Zn	1500	1456	4.7E−03	1.2E−03	5.9E−03	–
$C < 12$	250	3191	7.8E−02	2.0E−01	2.8E−01	2.5E+01
$C > 12$	750	16461	5.4E−01	1.4E+00	2.0E+00	4.6E−02
Total HI			8.0E−01	1.7E+00	2.5E+00	2.5E+01

Table 3. Hazard Index (HI) for scenario (B): adults and children in a residential area near the off-site phytoremediation area.

			Exposure pathways				
			Surface sediment				Outdoor air
Pollutants	Limits DLgs 152/06 Residential area	CRS $mg \cdot kg_{dw}^{-1}$	Ingestion of soil	Dermal contact soil	Vegetable ingestion	Total HI	Vapour inhalation
Cd	2	3	2.5E−02	1.0E−04	2.7E−01	3.0E−01	–
Cr	250	58	1.6E−04	6.6E−06	2.4E−05	1.9E−04	–
Ni	120	56	1.2E−02	4.8E−04	9.3E−03	2.2E−02	–
Cu	120	55	9.0E−03	3.6E−04	7.0E−02	8.0E−02	–
Pb	100	102	1.2E−01	4.8E−03	0.0E+00	1.2E−01	–
Zn	150	1035	1.5E−02	5.9E−04	4.3E−01	4.4E−01	–
$C < 12$	10	101	1.1E−02	4.3E−03	7.1E−02	8.6E−02	9.4E−01
$C > 12$	50	1000	2.1E−02	8.5E−03	5.3E−01	5.6E−01	3.8E−02
Total HI			2.1E−01	1.9E−02	1.4E+00	1.6E+00	9.8E−01

Table 4. Cleanup values (SSTLs) for scenario A and B (CRS: pollutant concentrations in sediment).

	A		Surface sediment	Outdoor air	**B**		Surface sediment	Outdoor air
Pollutants	Limits DLgs 152/06 Industrial area	CRS mg/kg$_{dw}$	SSTLs mg/kg$_{dw}$	SSTLs mg/kg$_{dw}$	Limits DLgs 152/06 Residential area	CRS mg/kg$_{dw}$	SSTLs mg/kg$_{dw}$	SSTLs mg/kg$_{dw}$
Cd	15	13.2	5.7	–	2	3	1.9	–
Cr	800	123	50	–	250	58	36	–
Ni	500	81	33	–	120	56	35	–
Cu	600	85	22	–	120	55	53	–
Pb	1000	541	220	–	100	102	63	–
Zn	1500	1456	590	–	150	1035	640	–
$C < 12$	250	3191	1300	95	10	101	62	–
$C > 12$	750	16461	6700	490	50	1000	640	–

possibility of interaction with the surface sediment on the part of adults and/or children living in a nearby residential area (through ingestion of soil or vegetable, and dermal contact) was assessed considering the possible migration of plant leaf due to the wind, or to animals that might pass through the area and export the contaminated matrices. This was simulated reducing the receptors' exposure frequency in RISC 4.0 software.

The pollutant concentrations (CRS) refer, in this case, to the values obtained by mixing the sediment with the excavation soil belonging to the off-site restoration area (data from a previous study [15], just before application of the phytotreatment; Table 3). The cumulative HI considering the surface sediment as an exposure pathway was associated with the ingestion of vegetables (1.4). Results from a previous mesoscale study [9], showed that the accumulation of metals in plant tissues (especially leaves) is negligible. This was confirmed by the results found for metals in this study (refer to the mesoscale pilot system). This means that there is a possible overestimation of the human risk associated with the ingestion of vegetables growing in the contaminated matrix.

Table 4 shows the site-specific target levels (SSTLs) to be reached in both scenario (A) and scenario (B), in order to guarantee human health, when applying reclamation techniques [36]. The SSTL values could be reached only for scenario (B), in which sediment treatment and reclamation is contemplated, when applying the phytoremediation technique. Scenario (A) referred instead to storage of sediment inside a sealed accumulation basin within Livorno port (real situation), without considering any other possibility of sediment reuse or reclamation (the SSTL values could not be reached in this case).

4. Conclusions

The proposed study has demonstrated the possibility of applying phytoremediation techniques to sediments with low-level contamination, transforming them into a soil-like substrate (technosoil). This is reusable in the environment, without posing risks to human and ecosystem health. The 6-month experiment supported by biological indicators (plants and earthworms), showed that the substrate behaves like a natural soil capable of supporting biological life, also showing good final agronomical and biochemical properties.

The study also assessed that the risk to human health related to application of the phytoremediation technique on a full-scale site is practically nonexistent. The possible ingestion of vegetables growing in the contaminated site was justified by the low accumulation level of metals, usually found in plant leaves. Therefore, the proposed technique represents a valid alternative to the usual final destination of the contaminated sediments (considered as refuse), which is disposal in a controlled landfill, since it allows recovery and revitalisation of a huge amount of sterile geo-resource that can be used in land management systems.

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