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Investigation of the suitability of marine remediated sediments as growing media for food crops

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Abstract

In accordance with the principle of the sustainable reuse of environmental resources, and with the final objective to tend towards a circular economy, this PhD thesis deals with the reuse of dredged marine sediment in horticulture. The dredging of sediments is a routine engineering activity carried out to guarantee the navigability of water bodies. Dredging, however, generates a great amount of material usually containing several inorganic and organic pollutants. Accordingly, contaminated sediments are not frequently relocated in other processes and are destined to landfill.

Horticulture is essential for human nutrition, but, at the same time, intensive horticulture entails some environmental impacts, partly due to the large use of peat. In fact, the use of peat in nursery and plant growth activities is characterized by environmental implications related to its extraction and long-way transport, no longer sustainable due to the exhaustion of peat bogs.

Phytoremediated sediments could be one of the possible new waste-derived soilless substrates proposed for plant cultivation. Phytoremediation can lower the level of contaminants in the sediments, converting them in a fertile matrix. Remediated sediments have been successfully used as an alternative substrate to peat for producing ornamental plants. However, as compared to ornamental plant, recycling dredged sediments for growing food crops rises additional concerns related to human health. As far as is known, no data are available on the quality and safety of the edible products cultivated in remediated dredged sediments.

In this thesis, phytoremediated sediments, dredged from Leghorn port, were used alone or mixed with a peat-based commercial substrate as growing media for plant cultivation. As model plants, lettuce, strawberry and pomegranate were chosen as they have different morphology and physiology patterns. The main aim of the present PhD project was to investigate the effect of the sediment on plant yield, quality and safety. The sediments were characterized from a physical, chemical and toxicological point of views. Notwithstanding remediation, sediments showed concentrations of Zn, aliphatic hydrocarbons and polycyclic aromatic hydrocarbons exceeding the limits established by the Italian L.D. 152/2006, regulating the contamination of soil in green areas. However, no evidence of plant contamination by toxic heavy metal was highlighted. Among organic contaminants, only dioxin-like polychlorinated biphenyls were determined in the edible products, at toxic equivalent concentrations fourfold lower than the limit established by the European Union. Plants grown on the sediment-based substrates showed a significant increase in ascorbic acid and sugar (i.e. in strawberries), antioxidant activity and organic acids (i.e. lettuce heads), anthocyanins, ellagitannins and polysaccharides (i.e. in pomegranates) in the edible products. Considering plant productivity, a general decrease of lettuce, strawberry and pomegranate yield grown on pure sediment was detected due to its unsuitable physical properties, whereas the use of mixture sediment–peat and pure peat resulted in the same yield. Nevertheless, in the strawberry case, the plant productivity was increased with the second year of re-cultivation, indicating an improvement of the physicochemical condition of the sediment. In conclusion, the whole set of data obtained in these experiments (i.e. data regarding the transfer of inorganic and organic contaminants from the substrate to plant, but also the quality and quantity of the final product obtained) indicates that also in case the pure sediment (i.e. the worst scenario in terms of substrate native contamination) is adopted for the soilless growth, the edible products obtained are safe and of good quality.

1. Introduction

1.1. The problem of dredged sediments

Sediment is a heterogenous material composed by sand, silt, clay and organic matter, originated in the catchment through erosion processes and transported in river systems in the direction of the coast, with oceans being the final sink (SedNet, 2004). Estuaries and coastal zones are among the most productive ecosystems in the world, with both high ecological and economical values (Council of the European Union, 2012), and sediments are integrated part of those areas, sustaining the hydrological system and hosting plants and animal biodiversity (SedNet, 2004). An excessive load of deposited sediments in deltas, wetlands and harbors, can change their functioning through filling the rivers and channels (Martins, 2002) as well as compromise sediment ecological function respect to animals and plants. Meanwhile, a shortage of accumulated sediments, promotes beaches and riverbank erosion, loss of wetland and river profile degradation (Martins, 2002). Moreover, during the last decades human activities have determined sediments contamination with various organic and inorganic pollutants (Bert et al., 2009). Thus, to avoid any modification of the ecological equilibrium of these habitats, a good management of sediment is essential.

Management of the sediment refers to the dredging, activity that provides the excavation and reallocation through dredging vessels in another part of the water. Briefly, the process can be summarized in four phases: excavation, vertical and horizontal transport and placement or use of the material dredged. Dredging is necessary to improve navigation system and maintenance the channels, harbours and waterways. Also ensures there is enough water for drinking, irrigation and shipping (SedNet, 2004). This activity is successively repeated until a target volume of sediments is dredged and it is generally accomplished by relevant projects requiring expansive capital investments and competent operators. Moreover, the sediment disposal and placement represent a big effort facing the dredging project due to the great amount of excavated material. In the European Union, it is estimated that 200 million t of sediments are dredged every year for various purposes, with Italy contributing with about 5 million m^3 (Bortone, 2007).

The management of dredged materials is also concerned with the fate of the removed sediments. The placement of dredged sediments has traditionally been considered disposal in open water, regardless of sediment quality (Darmody and Marlin, 2002), causing a biological impact in the environment especially when sediments are contaminated. Nowadays, contaminated sediments are located in open-water placement and isolated trough a capping in order to avoid any pollutants dispersal to the water column. There are different remediation technologies available, but relatively few are applicable. There are two possible remediation process (Peng et al., 2009): a) immobilization, by increasing the stabilization of contaminants on sediment particles; b) ex situ extracting or separating contaminants from sediment (e.g. washing, flotation). The choice of the best remediation technology depends from the overall costs which are greatly influenced by specific factors, such as dredging site, material volume and sediment physicochemical characteristics (Bortone et al., 2004). In fact, the high sediment salinity and the unsuitable characteristics (e.g. high bulk density, the presence of different type of contaminants) make the disposal in landfill the most diffused practice.

Relation with the research

This study used a marine sediment dredged from Leghorn port in the 2008. Leghorn port, as each port, needs to carry out continuous dredging activities to guarantee its full operation. With the denotation of Leghorn port as a Site of National Interest (SIN) (highly contaminated site), the port authorities encounter several difficulties to find adequate sediment destination, due to the presence of various pollutants. In order to reduce the level of contaminants through the application of biotechnologies, the sediment was subjected to three years of phytoremediation under the frame of AGRIPORT project (see paragraph 1.2. for full details). Phytoremediation allowed to convert dredged sediment into a fertile “technosoil” (Doni et al., 2015; Masciandaro et al., 2014). The PhD activities began with the physical, chemical and toxicological analysis of the phytoremediated sediment. After the preliminary characterization, the sediment underwent to 3 months of landfarming, a biological decontamination treatment consisting in the periodical sediment aeration by mechanical moving. This treatment allowed to homogenize the matrix, increase the microbiological fertility and further decrease the toxic organic compounds.

1.2. The reuse of dredged sediments in agriculture

Dredged sediments, being a natural resource derived from natural processes, can be relocated as a topsoil in urban green, in environmental restoration works or in horticulture as growing medium (IMO, 2013). The first studies on the reuse of dredged sediments as topsoils are available in the unofficial literature (also known as "grey literature"), such as documents of the U.S. Army Corps of Engineers or private industries and companies. For example, the Cooperative Research and Development Agreements (CRDA) developed sediment-based topsoils for commercial sale throughout the United States (Krause and Mcdonnell, 2000). Similarly, also the Naval Facilities Engineering and Expeditionary Warfare Center (NAVFAC EXWC) of USA created different blends of dredged sediment and compost. However, these reports deal with engineering issues and are often difficult to interpret.

A research on Scopus and Google Scholar was performed using "dredged sediment, agriculture, food crop" as keywords and a total of 39 scientific articles were found. Among these studies, the 40% was produced from the 1970 to 2000; the 29% from the 2001 to 2010; the remaining 31% from 2011 to 2019. This trend underlines that most of the scientific production is represented by early studies. Moreover, the 64% of the early studies related to this topic was produced in USA. In fact, the first studies evaluating the agricultural potential of dredged sediments, were conducted in USA to improve the fertility of marginal and unproductive croplands adjacent to waterways. Gupta et al., (1979) observed that these marginal soils, limited by factors such as drainage, texture and structure were enhanced by increasing the depth of the aerated soil zone and reducing wind and water erosion upon incorporation of dredged material. Gold (1971) observed a relevant increase of plant growth after the addition of silty-clay sediment to coarse-textured soil. The addition of dredged sediment to the farmland on the lake watershed, determined an increase of the corn yield compared to the original farmland thanks to the greater water holding capacity which resulted in a larger water supplied during the summer moisture-stress season (Lembke et al., 1983).

The main concern of reusing dredged sediment on land is related to the presence of persistent pollutants. The risk is the establishment of contaminated but fertile sites with few beneficial uses (Vervaeke et al., 2001). Mudroch (1974) was the first author studying the sediment-to-plant metal transferability with edible crops, concluding that element uptake depended mainly from the plant species and less to the type of contaminant or its concentration in the sediment. This latter aspect is very important since there are plant species more suitable to absorb and accumulate toxic heavy metals due their morphology and physiology. For example, leafy vegetables are considered as potential heavy metals hyperaccumulators (Malandrino et al., 2011). Beside the plant species, differences in heavy metals uptake can also occur among cultivars.

The transferability of heavy metals to plant depends by several physicochemical properties of the sediment. For example, cultivating lettuce, barley, tomato, snap bean and radish on river sediment, Darmody et al., (2004) observed that plants accumulated similar element concentrations to those cultivated in a reference unpolluted topsoil. The authors concluded that the alkaline sediment pH may have limited metal solubility and bioavailability (Darmody et al., 2004). In fact, pH is the most important factor affecting element availability in both soil and sediment (Peng et al., 2009; Antoniadis et al., 2017). Although sediment chemical composition can greatly vary depending on the origin, most of the scientific papers reported a pH >7, underlining the alkaline nature of the sediment (Canet et al., 2003; Ebbs et al., 2006; Tozzi et al., 2020; Ugolini et al., 2018). As a consequence, the majority of heavy metals such as Pb, Cd, Ni, Cu, Mn and Fe are less soluble at increasing pH (Zeng et al., 2011; Kabata-Pendias and Mukherjee, 2001) and this reduce the potential plant uptake (Mattei et al., 2018; Tozzi et al., 2020, 2019). The mobility of heavy metals is highly sensitive even at slight pH variations (Gundersen and Steinnes, 2003). Several mechanisms can modify the element bioavailability. For example, metals mobilization can occur upon pH-acidification and complexation by carboxylic acids present in root exudates (Renella et al., 2006). Roots can also induce changes in the ionic concentration altering the redox conditions and the heavy metal mobility in the rhizosphere (Marschner, 1995). In the case of dredged sediment, Marseille et al., (2000) observed that rape, a species able to excrete proton in the rhizosphere for nutrient mobilizing, accumulated an excessive amount of Zn and Cd (3600 and 120 mg kg^{-1} dry weight, respectively) in the root. On the contrary, a great accumulation of Fe was found in ryegrass shoot, suggesting that these species opted for another strategy, releasing organic ligands and particularly phytosiderophores to complex Fe III (Marseille et al., 2000).

When it comes to deal with dredged sediments, food safety is a crucial aspect, due to the risks related to human contamination through the food chain (Tozzi et al., 2020; 2019). Most of the studies have been focused on heavy metal accumulation from sediment to plant. Nevertheless, sediments can contain a wide range of organic contaminants such as PCBs, PAHs, dioxins and chlorinated pesticides such as dieldrin, aldrin and DDT (Fattore et al., 2002; Fetters et al., 2019; Marziali et al., 2017; Mattei et al., 2016; Tozzi et al., 2020). Organic contamination has been studied with the aim to assess sediment ecological and environmental role within the salt marsh ecosystem (Nunes et al., 2014; Watts et al., 2006), while, regarding food crops, there is only one early article (Sawhney and Hankin, 1984) reporting the presence of PCBs in bean, turnips and beet from soil amended with dredged sediment. The authors concluded that the uptake may be occurred through sorption of vapours from volatilization of PCBs or through root absorption and translocation (Sawhney and Hankin, 1984). Therefore, the presence of organic compounds in the sediments should not be neglected as these compounds can cause adverse effects to human health, and therefore be even more harmful than heavy metals.

The publications of the last 10 years mainly dealt with sediments, undergone to a previous bio-treatment, before their used as plant growing-medium. These scientific articles were carried out in the frame of European projects. The European Eco-innovation Agriport project (ECO/08/239065) demonstrated the environmental and economic benefits of phytoremediation processes in recycling polluted dredged sediments from ports into reusable soil. Sediments were mixed with a sandy agronomic soil (30% v/v) to improve sediment porosity and a layer of compost (4 kg m^{-2}) was added on the top of the soil-sediment mix before planting. The results of the project demonstrated the efficiency of this phytotechnology in establishing a good nutrient profile (i.e. total carbon, nitrogen and calcium) and to increase the microbial population (Doni et al., 2015; Masciandaro et al., 2014). In a pilot experiment, Mattei et al. (2017) cultivated seven ornamental crops using the sediment treated by Agriport technology. Plants showed good performance in terms of growth and development and no symptoms of toxicity was detected (Mattei et al., 2017). In a subsequent EU project, namely Life Cleansed project (ENV/IT/000652), sediments were dredged from Navicelli waterways and treated according to the aforementioned Agriport technology. The remediated sediments were mixed with alluvial soil of the Pistoia plain and used as growing media for the cultivation of three ornamental species (*Photinia x fraseri, Viburnum tinus* L., *Eleagnus macrophylla*). The mixed substrates produced good results in terms of water drainage and were similar to the control in terms of soluble nutrients, guaranteeing and enhancing the aboveground and belowground growth of all the three species (Ugolini et al., 2017). Moreover, the same remediated sediment showed germination levels of holm oak comparable to those in the traditional peat-based substrate (Ugolini et al., 2018).

Relation with the research

This PhD was carried out in the frame of the last European project, Life Hortised (ENV/IT/000113), aimed at demonstrating the suitability of dredged sediments, remediated using the above-mentioned Agriport technology, for the cultivation of edible crops. The choice of edible crops was justified by the fact that most of the previous researches were based on forest and ornamental crop and thus, few information was available. Compared to ornamental species, producing food crop on dredged sediment, raises important concerns on food safety. Three species were chosen as model plant (lettuce, strawberry and pomegranate) since they have different patterns in contaminant uptake and different vegetative and reproductive performances in response to the presence of the sediment a substrate.

1.3. End of a waste

A waste ceases to be such when it has been subjected to a recovery treatment and meets the specific criteria to be followed under the following conditions (Article 184 L.D. 152/2006):

a) the substance or object is commonly used for specific purposes;
b) there is a market or an application for this substance or object;
c) the substance or object meets the technical requirements for specific purposes and complies with the existing legislation and standards applicable to the products;
d) the use of the substance or object will not lead to overall negative impacts on the environment or human health.

According to these above-mentioned criteria, 4 important and interconnected aspects (i.e. legal issues, technical aspect, food quality and social acceptability) were designated in order to understand the pathway necessary to convert sediment into a valuable and reusable resources.

1.3.1. Legal issues

The main important aspect that precludes the use of sediments in agriculture is related to the current Italian laws. Therefore, the following paragraphs reports the main Italian laws and criteria concerning the sediment reuse and reallocation on land and regarding the characteristics of the growth substrates allowed in agriculture. These two legislative aspects put the barriers to the use of sediment in agriculture if several physical and chemical parameters are not satisfied.

Legislative issues for the sediment reuse: the Italian case
Until the seventies, the management of dredged material has been lacking a dedicated legislation. Indeed, there were not specific norms and rules regarding dredging operations, treatments and disposal. The first international conventions and treaties (i.e. London, OSPAR, Barcelona) were aimed at safeguarding the waterbodies from irregular dumping and indiscriminate wastes disposal. However, these conventions indicated principles and guidelines that each national framework should have followed, without the imposition of sanctions whether the recommendations were not fulfilled. At European level, there is still not a unique and specific directive on dredged material. The management of dredged sediment in EU fall under three important European Directives: EU water (2000/60/EC), EU waste (75/442/EEC,91/156/EEC) and EU protected Habitat (92/43/EEC).

The management of dredged sediment in Italy instead depends on whether the sediment is dredged from a Site of National Interest (SIN). So, there are two decrees ministerial (D.M.):

- D.M. 172/2016: regulates management of sediment dredged within SIN. This decree regulates the "*priority dredging needs*" and identifies the appropriate sediment reallocation: i) *flown back to the original water basin, or reused for sandy shore, coastal plot reconstruction or for enhancing seabed through capping;* ii) *inland reused;* iii) *disposed in containment structure*. The provisions "*inland reused*" refers to the "temporary deposit" before sending sediment to "*an authorized landfill or to a treatment plant for subsequent reuse according to the management options provided*". For the "*inland reused*", the sediment must not exceed the threshold of contaminants concentration (C.S.C.) reported in Table 1 of Annex 5 to Part IV of L.D. 152/2006 (see **Table 1**).
- D.M. 173/2016: regulates the management of sediment dredged outside SIN areas and sediment dredged within SIN which can be reuse outside SIN. This regulation provides two possible destination: i) *deliberate immersion in sea*; ii) *nourishment and immersion in environment bordering with SIN area*.

Moreover, according to the Italian legislations, dredged sediment can i) be excluded from the scope of waste legislation, ii) be a by-product, or iii) be considered and managed as waste. Below, the main regulations are summarized:

- Article 185, comma 3, L.D. 152/2006: sediment cease to be considered and treated "waste" if its movement is carried out for specific purposes (e.g. waterways management, shore consolidation, floods preventing, or of land reclamation) and if it does not contain hazardous substances. With this article, dredged sediment is excluded from the scope of waste legislation.
- Article 184-bis, L.D. 152/2006: sediment not excluded by the above-mentioned article (Article 185, comma 3, L.D. 152/2006), can be qualified as "by-product", with the following conditions: i) *it originates from a production process, whose primary purpose is not the production of it*; ii) *it is certain that the substance or the object will be used, during the same or a subsequent process of production or use*; iii) *the substance or object can be used directly without any further processing other than normal industrial practice*; vi) *the use of the substance or object will not lead to overall negative impacts on the environment or on human health*. Hence, the end of waste categorization is conditioned not only by the decontamination treatment but also by the identification of a specific use of treated sediments.

When the sediment, does not satisfy the Article 185 L.D. 152/2006 and does not meet the conditions for its reuse as "by-product", this material is considered as "waste". According to the European Waste Catalogue (2000/532/EC), dredged sediments receive code 170505, if they contain hazardous substances, or 170506, otherwise (Annex D, Part IV of L.D. 152/2006).

Italian regulation on growing media

The production and marketing of growing media are regulated by L.D. 75/2010. This regulation defines the substrates as "*the materials other than the soils in situ where the vegetables are grown*" and foresees two types of product "basic growing media" and "mixed growing media". Among the raw organic materials, there are peats (acid, neutral and humified), plant improvers not composted (such as coconut fiber, rice husk, wood fiber), green composted soil conditioners, obtained from various vegetable wastes. These can be used alone or mixed with other organic and mineral materials, corrective and fertilizer products. Physical and chemical requirements (pH, electrical conductivity, organic carbon, bulk density, heavy metal) are indicated in the regulation for each kind of material (**Table 1**).

Relation with the research

Since the sediment used in this study derived from the SIN of Leghorn port, the D.M. 172/2016 was considered for determining the suitability to the "inland reuse". Although the remediation processes (i.e. phytoremediation and landfarming), the sediment had some parameters such as Zn, C>12 (hydrocarbons with a number of carbon atoms in the range of C12-C40) and PAHs (polycyclic aromatic hydrocarbons) still higher than the legal limit for the civil reuse (Table A), even if much lower than the limit for industrial reuse (Table B) (**Table 1**). This means that sediment cannot be allocated on land for environmental and agricultural purposes. However, these laws refer to the Italian situation, while, for example, considering the Spanish laws, the considered sediment could be used in agriculture since all the chemical and toxicological parameters fulfilled the Spanish laws (i.e. Royal Decree 715/2012). For these reasons, a unique and specific regulation for the management of dredged sediment at European level is highly advisable in order to bypass dissimilarities and discordances along the European countries. Furthermore, since the allocation of contaminated sediment on land is prohibited, alternative management solutions for this material, such as its relocation as substrate for pot is necessary to avoid the contact between the soil matrix and the sediment.

Considering the physical and chemical parameters of the dredged sediment used in this experimentation, bulk density, pH and total organic carbon did not comply the Italian regulation for agronomic substrates (**Table 1**). However, these parameters can easily be improved by using the sediment mixture with materials with a high content of organic substance in order to further reduce the pH and bulk density. Furthermore, all the heavy metals concentrations respected the limits established by this law, thus indicating that the sediment could be suitable as soilless substrate from the metal content point of view. In this regard, it should be noted that for Cd, Cu, Hg, Ni, Pb and Zn the limits laid down by the above-mentioned L.D. 152/2006 are lower than those established by the L.D. 75/2010. This indicates a non-parallelism between laws concerning the environment (i.e. L.D. 152/2006) and agriculture (i.e. L.D. 75/2010), which should be overcome given the current interconnectedness that exists between the environment and agriculture.

Table 1- Main physicochemical characteristics, metal and organic contaminant concentrations of sediment after landfarming. Limits reported in the Italian Legislative Decrees (L.D.) 152/2006 regulating the properties of soil in public, private and residential green areas (Table A) and industrial area (Table B) and L.D. 75/2010 regulating the properties of agronomic growing media.

Parameter	Landfarmed sediment	(L.D. 152/2006)		(L.D. 75/2010)
		Table A	Table B	Agronomic base substrate
Bulk density (g/cm^3)	1.52 ± 0.14	-	-	0.45
pH	8.6 ± 0.1	-	-	3.5-7.5
EC (dS/m)	0.28 ± 0.02	-	-	<0.7
TOC (%)	1.3 ± 0.1	-	-	>8
TN (%)	0.09 ± 0.01	-	-	<2.5
P_2O_5 (%)	0.11 ± 0.02	-	-	<1.5
Cd (mg/kg)	0.96 ± 0.06	2	15	1.5
Cu (mg/kg)	48 ± 5	120	600	230
Hg (mg/kg)	0.075 ± 0.001	1	5	1.5
Ni (mg/kg)	37.5 ± 0.6	120	500	100
Pb (mg/kg)	40 ± 7	100	1000	140
Zn (mg/kg)	206 ± 12	150	1500	500
C> 12 (mg/kg)	86 ± 10	50	750	-
PAHs (mg/kg)	47 ± 6	10	100	-
PCBs (mg/kg)	0.04 ± 0.00	0.06	5	-

EC= electrical conductivity; TOC= total organic carbon; TN= total nitrogen; C>12 = hydrocarbons with a number of carbon atoms in the range of C12-C40; PAHs = polycyclic aromatic hydrocarbons; PCBs = polychlorinated biphenyls indicators.

1.3.2. Growing media technical aspects

Soilless plant production is a method applied worldwide by the horticulture and nursery industry. The total volume of peat and other growing media used by European countries is about 35 million m^3, with Italy contributing to about the 15% of the total volumes (Schmilewski, 2009). Soilless system has seen an increasing successful due to the several economic and practical advantages. Differently from the typical plant production in soil, soilless cultures allow a precise supply of plant nutrient and water and a reduction of labour requirements and spaces, maintaining high plant growth rate and yields, thus, optimizing the efficiency of the spend resources (Giuffrida and Michel, 2010). The choice of growing medium is based on an equilibrium between the physical and chemical qualities which have to be maintained during the entire plant cycle (Pardossi and Bibbiani, 2004). A plant growing media has to (Serra, 1992):

- guarantee the best anchoring of the root system and the maximum stability of the vase-plant system;
- be equipped with a satisfactory water retention capacity;
- be free of animal, plant and potentially phytotoxic residues;
- have high pH buffering power;
- maintain apparent bulk over time;
- have a good ease of absorbing water but also good drainage;
- be homogeneous and have constant characteristics over time;

- have a low cost compared to the cost of the plant nautical miles.

The most adopted material for both professional and hobby use is peat. Peat derives from the incomplete decomposition of different plant residues in conditions of high humidity and lack of oxygen. In water-saturated environments, lack of oxygen causes inhibition of degradation process and organic matter mineralization, leading to a positive carbon balance which results in accumulation of partially decomposed organic matter (Fisher, 1985). Peat formation is affected by different factors such as time, temperature, seasonal water balance, nutrient elements availability and plant species as well as the decomposition speed of plant residues (Cattivello, 2009). Hence, depending on the environments and the species, peats have different characteristics in terms of degree of decomposition, particle size, reaction, salt content, etc.

Among the soilless available substrates, peat is the mostly used, alone or as a component of pot mixture due to the optimal physicochemical characteristic (slow degradation rate, low bulk density, high porosity, high water holding capacity, relatively high cation exchange capacity) that makes peat suitable for a large number of vegetables and ornamentals (Bohlin and Holmberg, 2004). Moreover, peat is the only substrate available in industrial amounts, suitable to offer the same uniformity and quality to the grower. For example, for the European nursery industry, peat has been easily locally accessible, since the main peat suppliers, based in Northern Europe (i.e. Baltic Countries) provided substantial quantities of pure peat at relative low cost. All these characteristics have made peat as irreplaceable with other materials. Therefore, peat use in horticulture increased during the last decades, resulting in raised costs (Abad et al., 2001) and generating doubts about availability of this material in the near future (Fascella, 2015). Peat is not considered a renewable resource due to its long regeneration time. Moreover, the intensive overexploitation of peatland has led to a depletion of peat resources and consequently to an increase of prices and a decreasing of the quality (Rea, 2005). This situation principally goes to the detriment of the final users (i.e. nursery and horticulture companies). Moreover, due to the concern about the disturbance of peatlands, these ecosystems are under the safeguard of the directive 92/43/EC for natural habitats and wild fauna and flora. Progressively, peat use has become a matter of debate between European policies and nursery associations, holding environmental and economic issues in the spotlight. In addition, the European Commission has excluded all the peat-based products and their derivatives from the ecological certification “Ecolabel” (2001/688/EC). There are alternative materials aiming to produce “peat-free” substratum, possibly made of composted material originated by organic residues such as municipal civil solid wastes, green or agro-industrial residues (Abad et al., 2001). However, peat replacement is currently not always feasible because of large variability in local availability, unsuitable physicochemical properties, potential presence of plant pathogens (Landis and Morgan, 2009) and poor plant quality (Abad et al., 2001; De Lucia et al., 2013).

Relation with the research

A growing media should be preferably sourced close to the market since its transport is subjected to high cost (Alexander et al., 2008). In addition, reduced distances between the source and the final users allow to reduce the CO_2 emissions and thus, making the process more sustainable (De Lucia et al., 2013). The Pistoia District, where it is placed the biggest Italian nursery production, could take advantages of sediment dredged from Leghorn port, once remediated. In fact, economical cost and emissions should be reduced since the distance between Pistoia and Livorno is about to 90 km, while Pistoia's nurseries import peat from Baltic Countries (about 2000 km) (Lazzerini et al., 2016).

1.3.3. Food quality

Food quality is a complex issue since it is not a single, recognizable characteristic. Quality is a very broad term and can be defined using very different terms (Grunert, 2005). The following paragraphs discuss the main aspects of the food quality: the commercial aspect and the food safety.

Commercial aspect of the quality

Food quality is made by several objective physical characteristics built into the final product (Grunert, 2005) which are measurable through a multitude of standardizes and instrumental test able to quantify food quality. Quality is also made by a “subjective” part, the one perceived by consumers (Grunert, 2005). Therefore, quality has a product- and a consumer-dependent dimension. Moreover, also the producers have their quality definition which is a combination of good parameters such as high yield, good appearance, easy to harvest, and must withstand long-distance shipping (Kader, 1999). It should be point out that the main factor affecting the quality of a product intended for the fresh consumption is the maturity (Kader, 1999), which influences the external aspect, the organoleptic and sensorial characteristic as well as the nutraceutical profile. Here a list of the main qualitative parameters:

<u>External qualities</u>: size and shape, color, presence of malformation, general appearance (Schnitzler and Gruda, 2003)
<u>Sensorial qualities</u>: texture, pH, total acidity, total soluble solid, flesh firmness, aroma and volatile compounds (Kader, 1999)
<u>Nutraceutical qualities</u>: sugar and organic acid (also influencing the final taste), antioxidants (polyphenols, tannins, anthocyanins, flavonols), vitamins and minerals.

Food safety

Food safety can assume several definitions as in the case of quality. In a narrow sense, food safety is a concept based on the risk assessment derived from the consumption of a certain food from the scientists and food experts’ point of view. In a broad sense, food safety can encompass food nutritional qualities and some relative concerns (i.e. in the case of genetically modified organism) which are not desirable by consumers (Grunert, 2005). These paragraphs discuss the definition of food safety relate to the risk assessment.

- Foodstuff legislation issues: The Food Law

The ‘General Food Law Regulation’ (EC 178/2002) contains the foundations of the European Food Law, laying down the general principles, requirements and procedures in matters of food production, processing, distribution and safety. It aims at “*adopting measures aimed at guaranteeing that unsafe food is not placed on the market*” and “*providing the basis for the assurance of a high level of protection of human health and consumer interest”.* Article 6 of the EC 178/2002 establishes the definition of hazard, risk, and risk analysis which included risk assessment, risk management and risk communication. The whole process of the risk analysis is responsibility of European Food Safety Authority (EFSA), which was legally established under this regulation. EFSA will “*provide scientific advice, opinion and technical support for the Community's legislation and policies in all fields which have an impact on food and feed safety”.* Moreover, after the diffusion of the mad cow disease, the Food Law introduces the *“precautionary principle*” which is a provisional risk management measures necessary in the specific circumstances where, “*following an assessment of available information, the possibility of harmful effects on health is identified but scientific uncertainty persists, provisional risk management measures necessary to ensure the high level of health protection*”.

- Foodstuff legislation issues: The Codex Alimentarius

After the World War II, the need to improve the health and food control was high because of the rapid international commercial trades. In 1962, a Joint FAO/WHO Food Standard Program was established, and a joint subsidiary body was created: The Codex Alimentarius Commission (CAC). CAC’s main objectives are to protect the health of consumers and ensure fair practices in food trade and achieve international harmonization in food quality and safety requirements. Codex committees develop guidelines and standards that become effective when adopted by the CAC (Codex Alimentarius Commission, 2008). Codex guidelines and standards are important as templates for many Codex member governments but have no direct regulatory authority except through recognition of Codex as the presumptive international authority on food by the

World Trade Organization (WTO). All the regulations concerning maximum levels for certain contaminants in foodstuffs take into account the Codex Alimentarius. The reference legislation, concerning contaminants in foodstuff is represented by the Reg. 1881/2006 amended with Reg. 629/2008, Reg. 420/2011, and Reg. 1259/2011. These regulations set the maximum limits for a large part of different contaminants: nitrates, toxins, heavy metals, PAHs, dioxins and PCBs.

- Chemical risk assessment

Chemical risk assessment has been defined as ‘the evaluation of the potential for adverse health effects in humans from exposures to chemicals’ (RATSC, 1999). Chemical risk assessment is used when direct measurements of exposure or biological monitoring data are not available or where these techniques are not appropriate for the exposure assessment situation (Fryer et al., 2006). The exposure assessment stage is crucial and consists of quantifying the level of chemicals to which human populations, population subgroups and individuals are exposed, in terms of magnitude, duration and frequency. The process of chemical risk assessment can be divided into four distinct stages: (1) hazard identification; (2) exposure assessment; (3) dose-response characterisation; (4) risk characterisation. In this regard, there are impact-assessment models aimed at evaluating and quantifying human exposures to chemicals via contact with the surrounding natural environment. Two broad categories of environmental exposure models can be distinguished: (1) environmental exposure models, and (2) human intake models. Environmental models simulate likely scenarios where environmental processes (air inhalation, soil and dust ingestion and dermal contact) generate contaminants to which humans may come into contact. Human intake models aim at quantifying human chemical intake from contact with the relevant environmental media. Among human intake models, there are dietary exposure models, developed to predict human exposures to chemicals resulting from the consumption of contaminated food and water (Fryer et al., 2006). Human intake models require parameters relating to human activities (e.g. contact rates) and physiology (e.g. bodyweight) in addition to parameters representing the chemical and environmental conditions.

Relation with the research

The sediment used in this study contained several heavy metals and organic compounds. From the analysis of organic pollutants in strawberry and lettuce, only PCBs dioxin like were detected in both plant species (see Article 1 and 2). However, the concentrations found were three- and four-fold (for lettuce and strawberry respectively) lower than the recommended threshold established by the European Commission Recommendation 2014/663/EU. Regarding heavy metals, none of the heavy metals regulated by the Codex Alimentarius (Cd and Pb) have been detected in the edible parts of lettuce and strawberry (see Article 1, 2 and 3). However, for the remaining metals such as Fe, Mn, Cr, Ni, Zn, Cu, models and indicators based on the dietary intake were necessary to predict the health risk derived from the exposure of contaminated foodstuff. In Article 1, attention was paid to the toxicological impact that would has resulted from the consumption of sediment-grown lettuce. Results showed that even in the in worst case, that is when the remediated sediment is adopted pure (100%), the toxicological impact that would has resulted from the consumption of lettuce is negligible.

1.3.4. Social acceptability

Among the possible discussions that the topic of reuse of dredged sediments in agriculture might raise, social acceptability represents a delicate issue. Consumers can raise concerns about the “unfamiliar food” (Yeung and Morris, 2001), being critical about the quality and safety of food. For example, it is commonly believed that consumers have a negative attitude respect biotechnology and certain production techniques (like in the case of OMG or food irradiance) since they perceive them as unsafe (Boccaletti and Moro, 2000; Grunert, 2005). Food-borne illnesses caused by contaminants and residues, that were subjected to intensive mass media coverage, are examples of the overestimation of a relatively small actual risk (Miles et al., 2004). In some cases, little relation is seen between the perceived hazard of a food safety issue and its actual, scientifically proven, hazard (Verbeke, 2005). It should be also pointed out that mass media are consistently ranked by the public as a primary source of food safety information, influencing individual risk judgments (Pisano and Woods, 2002). The often-seen gap between scientific reality and human perception is determined by many factors, including individual characteristics and food properties (Drichoutis et al., 2005), together with information and communication. Therefore, public acceptance is influenced by perceived credibility of data, rigor of regulatory policy, impartial action of regulators, and demonstrated responsibility of industry (Bruhn, 2007).

A policy and marketing strategy to overcome consumer's low confidence lies in the certification system which allows to communicate to consumers the safety characteristics of the labelled product. The use of credible labels allows firms to signal quality and safety to consumers. From the producer's viewpoint, in the case of agricultural products, a quality certification (either it is a certification of traceability or a quality label) is a tool that adds value to their products and justifies higher prices for them (Botonaki et al., 2006).

The use of labelling has become increasingly important since it allowed to differentiate and valorise healthy, safer and environmentally-friend food products (McCluskey and Loureiro, 2003a). Several publications, which based their scientific approach on surveys, observed an increasing consumer willing to pay for green product such as organic, pesticide-free and green-labelled food (Boccaletti and Moro, 2000; McCluskey and Loureiro, 2003b). Individuals are encouraged to pay for eco-friendly product since they care environmental issues in terms of waste and energy reduction and preservation of natural resources (Royne et al., 2011). All this information indicate that the sustainability is recognized as a major public health and environmental issue of the twenty-first century.

Relation with the research

The social acceptability of the use of remediated sediments as growing media in horticulture was tested by the preparation of a questionnaire. A survey (n=500) was distributed during different events, such as conferences, workshops and seminars in Italy and Spain. The main attendants were mainly students and scientists. Interviewees were asked to consider the possibility to consume the food products cultivated on remediated dredged sediment. The results of the questionnaire indicated that the 82% of the interviewed would be willing to eat and purchase the sediment-grown products, only in the case all the contaminants values, regulated by the Law, were below the recommended level. Furthermore, the 89% of the participants were in favour for the presence of a certification/label indicating the origin of the products. These preliminary results point out that consumers are willing to buy foods that, despite being produced using unconventional methods, have been originated by substituting non-renewable natural resources. Another interesting result emerged from this survey, indicates that consumers want to be aware of the food origin and, at the same time, protected by certification/labelling.

Object of the research

The aim of this PhD research was to assess the technical suitability of remediated sediment used as substrate for the production of food crops. The specific objects were:

- Sediment physicochemical and toxicological characterization
- Assessment of the vegetative growth and productive performance of three plant species (i.e. lettuce, strawberry and pomegranate)
- Measurement of concentration and assessment of impact of heavy metals and organic contaminants on plants and fruits
- Evaluation of the quality in terms of nutraceutical compounds.

The PhD research was mainly carried out at the Department of Agricultural, Food, Environmental and Forestry Sciences of the University of Florence and at the Department of Plant Science and Microbiology of the Universidad Miguel Hernández de Elche in Orihuela (Spain). The period of study in Spain allowed to achieve the qualification of European Doctorate.

During these three years, the activities concerned field trials, laboratory measurements and data analysis. The results are reported in four scientific papers. Articles 1 and 2 (i.e. Tozzi et al., 2019; Tozzi et al., 2020) report pilot-scale experiments where lettuce and strawberry plants were cultivated in pot. The aims of these experiments were to investigate the effect of the remediated sediment on plant yield, quality and safety. Article 3 (in preparation) reports a long-term experiment where strawberry plants were cultivated for two consecutive productive years on the same sediment-based substrate in order to verify whether the sediment-based growing media maintain a long-term fertility which can support the horticulture production for several agronomic seasons. Article 4 (submitted) reports an in-depth investigation of the nutraceutical compounds of different parts of the pomegranate fruit (peel and juice) from plants grown on the remediated sediment. In detail, the influence of the sediment-based substrate on the anthocyanin composition of juice was studied, while the peel was analyzed for its phenolic (i.e. ellagitannins) and polysaccharidic compounds.

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Article 1

Remediated marine sediment as growing medium for lettuce production: assessment of agronomic performance and food safety in a pilot experiment

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Remediated marine sediment as growing medium for lettuce production: assessment of agronomic performance and food safety in a pilot experiment

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Abstract

BACKGROUND: The use of reclaimed dredged sediments as growing media may offer a profitable alternative to their disposal as a waste and at the same time meets the need of peat-substitute substrates in horticulture. When sediments are reused to cultivate food crops, issues related to human health rise due to potential accumulation of contaminants in the product. This pilot study aimed at verifying the suitability of a reclaimed dredged port sediment, used pure or mixed with peat, as a growing medium for lettuce cultivation.

RESULTS: The pure sediment caused a reduction in crop yield, probably due to its unsuitable physical properties, whereas the mixture sediment – peat and pure peat resulted in the same yield. Although the sediment contained potentially phytotoxic heavy metals and some organic pollutants, no symptoms of plant toxicity were noted. Besides, no organic contaminants were detected in lettuce heads, and heavy metals amounts were not hazardous for consumers. Conversely, plants grown in the sediment were particularly rich in minerals like Ca, Mg and Fe, and showed higher concentrations of organic acids and antioxidants.

CONCLUSION: The use of the sediment as a growing medium for lettuce was shown to be safe for both inorganic and organic contaminants. Nevertheless, considering crop yield results, the mixture of the sediment with other materials is recommended in order to produce a substrate with more suitable physicochemical properties for vegetable cultivation.

Keywords: reclaimed sediments; innovative substrates; *Lactuca sativa*; dietary intake; health risk assessment; resource recycling

INTRODUCTION

Marine sediments are regularly dredged to maintain an adequate navigation depth of docks and waterways and to remove contaminated sediments, which can act as a secondary source of pollution for the aquatic environment. In fact, sediments are a sink of pollutants directly released into waters or reaching water bodies through leaching and runoff from contaminated land. In the European Union (EU), it is estimated that 200 million m^3 of sediments are dredged every year, and Italy contributes about 5 million m^3.[1] While the main international conventions on sea protection (e.g. OSPAR, 1992) suggest considering dredged sediments as a resource, these materials are mostly managed as a waste, and stockpiled near the dredging areas or landfilled in confined facilities, when disposal at sea is not possible. Physical and chemical decontamination technologies (e.g. thermal treatment, physical fractionation, solvent extraction) are potentially available, but high economic and energetic costs prevent their application.[2] Conversely, phytoremediation has been proposed as an ecologically and economically sustainable management option for reclaiming dredged sediments.[3]

Phytoremediation leads to degradation of organic pollutants and chemical stabilization of heavy metals, improves structure and water retention and increases the microbiological activity, converting the dredged sediments into a biologically active matrix.[4–6] Phytoremediated sediments have already been successfully used as growing media in ornamental and forest tree nurseries.[7–9] Moreover, life cycle analysis (LCA) applied

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to the production process of ornamental plants in the Pistoia district (central Italy) demonstrated that replacing the conventional growing media (mainly peat) with reclaimed sediments significantly reduced the environmental impact of nursery activity.[8]

In the present study, we hypothesized that phytoremediated sediments could be used as growing media for the production of leafy vegetables. In order to verify this hypothesis, lettuce plants were grown in a phytoremediated sediment, used pure or mixed with peat, and crop response was evaluated for both yield and quality. Lettuce was chosen as being one of the most pollutant-sensitive crops, whose growth can be compromised even in moderately polluted or remediated soils.[10,11] Besides, lettuce is a good source of various bioactive compounds and provides considerable amounts of several minerals,[12] which are susceptible to high variations in response to growing conditions and environmental stress.[13] For this reason, the main nutritional quality parameters (minerals, sugars, organic acids and antioxidants compounds) of lettuce cultivated in remediated sediment were evaluated in comparison with those of lettuce grown in a peat-based medium. Furthermore, considering that the cultivation of vegetables in reclaimed sediments raises food safety concerns owing to possible contaminant accumulation in the edible products, attention was paid to the toxicological impact that would result from the consumption of sediment-grown lettuce.

MATERIALS AND METHODS

Lettuce cultivation, yield and quality

Sediments dredged from the port of Livorno (central Italy; 43° 33′ 25″ N, 10° 17′ 39″ E) in 2008–2009, and subjected to phytoremediation for 3 years,[4,6] were used in the experiment. The phytoremediated sediment underwent landfarming (periodical aeration by mechanical handling) for 3 months prior to their use for lettuce cultivation. After landfarming, the treated sediment had a sandy loam texture (56% sand, 25% silt, 19% clay). The treatments consisted of the following growing media: treated sediment (TS100), treated sediment mixed with a peat-based medium (1:1 v/v, TS50) and the peat-based medium used as control treatment (TS0). As preliminary evaluation of possible mixtures of peat–sediment, a ratio of 1:1 was chosen since it is one of the most commonly used when peat is mixed with mineral materials in the formulation of growing media for pot cultivation in horticulture.[14] The main physical and chemical characteristics of the three growing media are shown in Table 1.

Table 1. Main physicochemical proprieties, element concentrations and organic contaminants in TS0, TS50 and TS100

Parameter	TS0	TS50	TS100
Bulk density (g cm^3)	0.19	1.01	1.52
Total porosity (vol. %)	95	89	76
Water capacity pF = 1 (vol. %)	81	76	64
Air capacity pF = 1 (vol. %)	14	13	11
Easy available water (vol. %)	42	29	20
pH	5.6	7.8	8.6
EC (dS m^{-1})	0.68	0.52	0.28
TOC (%)	20	2.5	1.3
TN (%)	0.37	0.14	0.09
Ca (g kg^{-1})	4.75	26.2	38.7
K (g kg^{-1})	4.41	4.43	3.83
P (g kg^{-1})	0.33	0.51	0.54
Mg (g kg^{-1})	2.18	8.07	10.4
Na (g kg^{-1})	1.22	0.54	0.50
Cu (mg kg^{-1})	7.45	40.3	48.1
Fe (g kg^{-1})	3.90	12.4	14.5
Zn (mg kg^{-1})	9.83	193	206
Mn (mg kg^{-1})	85.7	202	281
Ni (mg kg^{-1})	2.75	30.5	37.5
Cr (mg kg^{-1})	12.7	33.7	42.9
Pb (mg kg^{-1})	7.75	36.4	39.6
Cd (mg kg^{-1})	0.20	0.52	0.96
PAHs (mg kg^{-1})	<0.01	32.9	46.9
$C > 12$ (mg kg^{-1})	31.5	43.6	86.1
DL-PCBs (μg kg^{-1})	0.11	1.97	3.25
PCDD/F (ng TEQ kg^{-1})	0.037	0.565	1.541

PAHs, polycyclic aromatic hydrocarbons; $C > 12$, hydrocarbons > 12; DL-PCBs, dioxin-like polychlorinated biphenyls; PCCD/F, polychlorinated dibenzo(p)dioxins and furans.

Lettuce (*Lactuca sativa* L. 'Ballerina') was cultivated outdoors at the Department of Agriculture, Food, Environment and Forestry (DAGRI, University of Florence; 43° 81′ 68″ N, 11° 19′ 99″ E) from 20 June 2017 to 29 July 2017. The average daily maximum and minimum temperatures were 38.3 and 18.8 °C, respectively, and relative humidity ranged between 40% and 50%. No precipitation occurred during the whole cultivation period. Lettuce plantlets ready for transplant were planted in pots 5 L in volume (one plant per pot), fertilized with 12 g $CaNO_3$, 2 g KH_2PO_4 and 5 g K_2SO_4 per pot, and irrigated daily by sprinkle irrigation (250 mL d^{-1} per pot). Pots were arranged in three blocks, each consisting of 10 pots per treatment, for a total of 90 pots (10 pots × 3 treatments × 3 blocks). When plants had reached the harvest stage, four plants per block and per treatment were randomly chosen to evaluate the following head parameters: fresh and dry weight, head diameter, leaf number, leaf area, leaf blade colour and chlorophyll content (expressed as SPAD values). Leaf colour was measured with an electronic colorimeter (Minolta chromameter CR200, Konica Minolta, Osaka, Japan) and *a* and *b* coordinates were transformed into the chroma index $(a^2 + b^2)^{1/2}$. SPAD was measured using a Minolta chlorophyll meter (SPAD-501). Leaves were scanned, and the total leaf area was calculated using Tomato analyser 3.0 software. Dry weight was measured after oven-drying at 70 °C until constant weight.

Three heads per block and per treatment were freeze-dried and ground for use in the assessment of sugars, organic acids, polyphenols and antioxidant activity. Sugars and organic acids were quantified according to Serna *et al.*[15] Briefly, 3 g freeze-dried material were homogenized in 10 mL deionized water using a polytron homogenizer (IKA Labotechnik, Staufen, Germany) and centrifuged at $15\,000 \times g$ for 20 min at 4 °C. The supernatant (10 μL) was injected into a high-performance liquid chromatograph (series 1100, Hewlett-Packard, Waldbrom, Germany) equipped with a column (Supelcogel C-610H, 30 cm × 7.8 mm) at 30 °C, a refractive index detector (for sugar analysis) and an absorbance detector (210 nm UV for acid analysis). The elution system consisted of 0.1% H_3PO_4, running isocratically at a flow rate of 0.5 mL min^{-1}. Standards from Sigma (Poole, UK) were used. Results were expressed as grams per kilogram fresh weight (FW). The same supernatant was used for the determination of antioxidant activity and total soluble polyphenols (TSP). Antioxidant activity was tested by ABTS (2,2-azinobis-3-ethylbenzothiazoline-6-sulfonic

Table 2. Head parameters (leaf fresh and dry weight, head diameter, leaf number, leaf area, leaf blade Chroma and SPAD) in lettuce grown in TS0, TS50 and TS100

Treatment	Leaf fresh weight (g)	Leaf dry weight (g)	Head diameter (cm)	Leaf number	Leaf area (cm^2)	Chroma	SPAD
TS0	120.7a	8.7ab	33.3a	32.3a	2097.8a	36.8a	33.8a
TS50	124.0a	10.2a	29.9ab	28.9a	2014.6a	36.5a	35.0a
TS100	62.5b	4.6b	26.9b	24.8a	1084.2b	35.9a	32.7a

Different letters in the same column indicate significant differences between means for $P \leq 0.05$ ($n = 12$).

acid radical cation) assay according to Re *et al.*[16] The results were expressed as milligrams of Trolox equivalent (TE) per gram FW. Total soluble polyphenols were determined using the Folin–Ciocalteu reagent according to Wood *et al.*[17] Results were expressed as milligrams of gallic acid equivalent (GAE) per gram FW.

Elemental analysis (Ca, K, P, Mg, Na, Cu, Fe, Zn, Mn, Ni, Cr, Pb, Cd) was performed on the oven-dried leaf samples. For each treatment, the samples of each block were ground together in a ceramic mortar. An aliquot of 500 mg ground material was mineralized by microwave (Ethos 1 Milestone, FKV, Italy) and analysed by inductively coupled plasma (ICP) (Liberty AX, sequential ICP-OES; Varian, Palo Alto, CA, USA).

For each treatment, a leaf sample from nine plants (three plants per block) was used for the determination of organic pollutants by an accredited laboratory (PH srl, Florence, Italy). Seventeen polychlorinated dibenzo(*p*)dioxins and furans (PCDD/F) and 12 polychlorinated biphenyl (PCB) congeners were analysed according to EPA 1613B 1994 and EPA 1668C 2010, respectively, by isotope dilution technique using high-resolution gas chromatography–high-resolution mass spectrometry (HRGC-HRMS). Hydrocarbons > 12 ($C > 12$) and 13 polycyclic aromatic hydrocarbons (PAHs) were extracted according to EPA 3545A 2007 and analysed according to EPA 8015C 2007 and EPA 8270D 2014, respectively, by HRGC.

Lettuce contribution to mineral intake and health risk assessment

The estimated dietary intake (EDI, mg day^{-1}) of minerals resulting from the consumption of lettuce was calculated using the following formula:

$$\mathrm{EDI} = C_{\mathrm{metal}} \times (\mathrm{DC}_{\mathrm{lettuce}}/1000)$$

where C_{metal} is the element concentration (mg kg^{-1} FW) and $\mathrm{DC}_{\mathrm{lettuce}}$ is the average daily consumption of lettuce in Europe, corresponding to 22.5 g per person.[18,19] In order to evaluate the contribution of lettuce to human mineral requirements, EDI was expressed as percentage (EDI%) of the recommended dietary intake (RDI, mg d^{-1}) for Ca, P, Mg, Cu, Fe and Zn or adequate intake (AI, mg d^{-1}) for K, Mn and Cr, as defined by the Italian Society of Human Nutrition (SINU), considering RDI/AI values referred to an adult male.[20]

Health risk related to lettuce consumption was assessed for Cu, Fe, Zn, Mn, Ni, and Cr, calculating the health risk index (HRI) using the following the formula:

$$\mathrm{HRI} = \mathrm{EDI}_{\mathrm{Bw}}/\mathrm{RfD}$$

where $\mathrm{EDI}_{\mathrm{Bw}}$ is the EDI per kilogram body weight (BW) and RfD (mg kg^{-1} body weight d^{-1}) represents the oral reference dose, which is an estimate of daily exposure of humans to heavy metals having no hazardous effect during the whole lifetime according to USEPA.[21] BW (the average body weight for an adult) was assumed to be 55.9 kg, as previously reported.[22] If HRI values are <1, the exposed population is assumed to be safe.[21]

Statistical analysis

Head parameters (leaf fresh and dry weight, head diameter, leaf number, leaf area, leaf colour and SPAD), sugars, organic acids, polyphenols, antioxidant activity and elemental data were subjected to statistical analysis with SPSS version 24 software (IBM®, Armonk, NY, USA). A one-way analysis of variance (ANOVA) was conducted according to a randomized block design with three replicates, and means were compared by Duncan's test for $P \leq 0.05$. A principal component analysis (PCA) was also performed.

RESULTS

Lettuce yield and quality

Head parameters are shown in Table 2. Lettuce heads grown in TS0 and TS50 showed a fresh weight and leaf area about twice the value of plants grown in TS100. Considering the dry weight, the trend was: TS50 = TS0 ≥ TS100. Head diameter was higher for lettuce grown in TS0 (33.3 cm) and lower for lettuce grown in TS100 (26.9 cm). No significant differences were noted for leaf number, leaf blade Chroma index and SPAD.

Table 3. Sugars (sucrose, glucose and fructose), organic acids (citric, malic and tartaric), antioxidant activity (ABTS) and total soluble polyphenols (TSP) in lettuce grown in TS0, TS50 and TS100

Treatment	Sucrose	Glucose (g kg^{-1} FW)	Fructose	Total	Citric	Malic (g kg^{-1} FW)	Tartaric	Total	ABTS (mg TE g^{-1} FW)	TSP (mg GAE g^{-1} FW)
TS0	1.42a	19.2a	0.84a	21.5a	2.83b	3.36b	0.03b	6.23b	0.80b	3.06b
TS50	1.29a	19.4a	0.76a	21.4a	3.36a	5.51a	0.08a	8.95a	0.89a	3.34a
TS100	1.31a	18.9a	0.75a	21.0a	3.28a	5.56a	0.09a	8.92a	0.92a	3.39a

Different letters in the same column indicate significant differences between means for $P \leq 0.05$ ($n = 3$).

Table 4. Element concentrations in lettuce grown in TS0, TS50 and TS100

Element	TS0	TS50	TS100
Ca (g kg^{-1} FW)	0.85b	0.84b	1.90a
K (g kg^{-1} FW)	2.78b	3.45a	3.54a
P (g kg^{-1} FW)	0.37a	0.27b	0.25b
Mg (g kg^{-1} FW)	0.23a	0.17b	0.24a
Na (g kg^{-1} FW)	0.14a	0.09b	0.15a
Cu (mg kg^{-1} FW)	0.31b	0.33b	0.54a
Fe (mg kg^{-1} FW)	6.62b	6.67b	21.1a
Zn (mg kg^{-1} FW)	2.56a	2.43a	3.01a
Mn (mg kg^{-1} FW)	13.0a	1.35b	2.70b
Ni (mg kg^{-1} FW)	0.08a	0.08a	0.09a
Cr (mg kg^{-1} FW)	0.03b	0.04b	0.10a

Different letters in the same row indicate significant differences between means for $P \leq 0.05$ ($n = 3$).

Table 3 shows sugars (sucrose, glucose and fructose), organic acids (citric, malic and tartaric), antioxidant activity (ABTS) and total soluble polyphenols (TSP) results. Total and individual sugar concentrations were not affected by the growing media. Conversely, organic acids (i.e. malic, citric, tartaric), ABTS and TSP were similarly affected by the growing media and the trend was: TS50 = TS100 > TS0.

Element concentrations are reported in Table 4. Cadmium and Pb were not detected in lettuce, regardless of the growing medium. The highest concentrations of Ca, K, Cu, Fe and Cr were found in lettuce grown in TS100, whereas P and Mn were mostly accumulated by control lettuce (TS0). Lettuce cultivated in TS50 showed the lowest values for Mg and Na. No differences were observed for Zn and Ni, and the average values were 2.67 and 0.08 mg kg^{-1} FW, respectively.

Organic contaminants were not detected in lettuce heads, with the exception of dioxin-like polychlorinated biphenyls (DL-PCBs). For all treatments, DL-PCB values, based on the 2005 WHO re-evaluation of TEF (toxic equivalency factors) using the upper bound method[23,24] were 0.03 pg TEQ g^{-1} FW.

The PCA biplot is shown in Fig. 1. The two first PCs showed eigenvalues equal to 19.7 and 8.3, representing 70.3% and 29.7% of the total variability, respectively. PC1 was highly correlated ($r > 0.8$) with several parameters (positively associated with head fresh weight, leaf chroma index, head diameter, leaf number, leaf area, fructose, total sugars and P concentrations, and negatively associated with malic, tartaric acid, total organic acids, ABTS, TPS, Ca, Cr, Cu, Fe, K, Ni and Zn concentrations). PC2 was positively associated with Mg and Na concentrations and negatively correlated with SPAD and glucose. Lettuce grown in TS0 was located in the quadrant corresponding to high values of yield and high values of Na and Mg concentration, SPAD and glucose content (PC2). Lettuce grown in TS100 showed lower yield parameters and higher content of elements (Zn, Fe, Ca, Ni, Cu, Cr, and K), with the exception of Mn and P. Lettuce cultivated in TS50 had an intermediate position between TS0 and TS100 in PC1, and a very low value for PC2.

Lettuce contribution to mineral intake and health risk assessment

Table 5 shows the EDI% values resulting from a daily lettuce consumption of 22.5 g per person. The highest contribution to RDI/AI was found for Mn in lettuce grown in TS0 (10.9%). This growing medium resulted also in the highest EDI% for P (1.2%). The lowest contribution was observed for Na, with an average value of 0.2%. For all the other minerals (Ca, K, Na, Mg, Cu, Fe, Zn and Cr) the highest EDI% was observed for lettuce cultivated in TS100.

As regards the health risk assessment, for all the elements the calculated EDI_{BW} was below the oral reference dose (RfD) established by USEPA[21] and the HRI values were far below 1.0 (Table 6).

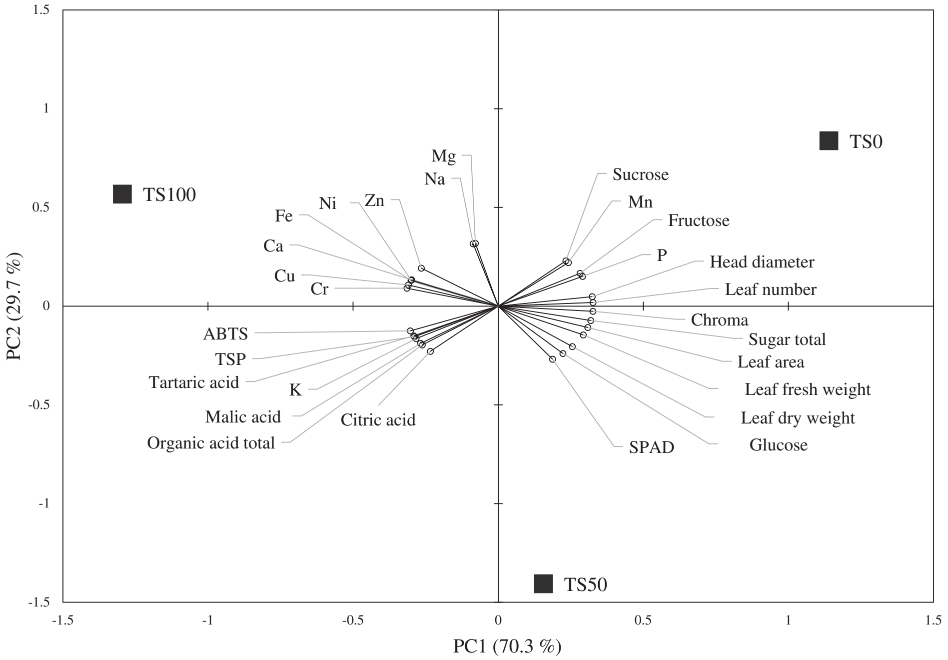

Figure 1. Principal component analysis (PCA) of all the investigated parameters in lettuce grown in TS0, TS50 and TS100.

Table 5. Estimated dietary intake expressed as percentage (EDI%) of the recommended dietary intake (RDI) or adequate intake (AI) resulting from the consumption (22.5 g d^{-1}) of lettuce grown in TS0, TS50 and TS100

Element	TS0	TS50	TS100	RDI/**AI**[a]
Ca	1.92	1.89	4.28	1000
K	1.60	1.99	2.04	**3900**
P	1.20	0.86	0.82	700
Na	0.21	0.14	0.22	**1500**
Mg	2.18	1.56	2.28	240
Cu	0.79	0.81	1.35	0.9
Fe	1.49	1.51	4.75	10
Zn	0.48	0.45	0.56	12
Mn	10.9	1.12	2.25	**2.7**
Cr	2.46	2.33	6.48	**0.035**

[a] RDI (regular type) and AI (bold type) according to SINU.[20]

DISCUSSION

While the possibility of using reclaimed sediments as growing media in nursery production of ornamental shrubs and trees has been demonstrated,[7–9] to our knowledge this is the first study assessing the suitability of reclaimed sediments for growing a food crop. As a first approach to this issue, lettuce was chosen among food crops, being a good indicator of the physical and chemical soil/substrate quality.[25] The experiment was intended to be a pilot study aiming at testing the hypothesis that reclaimed sediments could be safely used as ingredients of peat-free growing media for vegetable plants.

The sediment used in this study contained higher amounts of heavy metals and organic compounds potentially detrimental for plants compared to the traditional peat-based growing medium (Table 1), but no phytotoxic effect of TS50 and TS100 was noted on lettuce. In fact, plants did not show any symptoms of toxicity, suggesting that, as observed in previous studies, heavy metals were not available to plants.[4,5,8] Nevertheless, lettuce grown in TS100 showed lower head fresh weight, dry weight and leaf area in comparison with plants cultivated in TS0 and TS50, as shown also by PCA. The number of leaves, although without reaching significant differences, was lower in the TS100 as well, revealing again a stress induced by the sediment. The detrimental effect of TS100 on lettuce growth may be in part related to the high pH of the sediment, much over the optimal pH range for this species.[26] In addition, the physical properties of the sediment were probably detrimental for yield. In fact, TS100 showed high bulk density, low macro-porosity, low drainage capacity and superficial crust. Such characteristics, observed also by Macía *et al.*,[27] made the sediment used in this research unsuitable as a growing medium. However, when the sediment was mixed with peat, lettuce yield did not differ from control plants (Table 2). Similar results were reported by Canet *et al.*,[28] who did not observe differences in biomass accumulation of lettuce grown in pots with different mixtures of lake sediments and soil.

Table 6. Estimated daily intake per kilogram body weight (EDI$_{BW}$, mg kg^{-1} body weight d^{-1}) and health risk index (HRI) resulting from the consumption (22.5 g d^{-1}) of lettuce grown in TS0, TS50 and TS100

Element		TS0	TS50	TS100	RfD[a]
Cu	EDI$_{BW}$	1.3×10^{-4}	1.3×10^{-4}	2.2×10^{-4}	0.04
	HRI	3.2×10^{-3}	3.3×10^{-3}	5.4×10^{-3}	
Fe	EDI$_{BW}$	2.7×10^{-3}	2.7×10^{-3}	8.5×10^{-3}	0.7
	HRI	3.8×10^{-3}	3.9×10^{-3}	1.2×10^{-2}	
Zn	EDI$_{BW}$	1.0×10^{-3}	9.8×10^{-4}	1.2×10^{-3}	0.3
	HRI	3.4×10^{-3}	3.3×10^{-3}	4.0×10^{-3}	
Mn	EDI$_{BW}$	5.2×10^{-3}	6.9×10^{-4}	9.9×10^{-4}	0.033
	HRI	1.6×10^{-1}	2.1×10^{-2}	3.3×10^{-2}	
Ni	EDI$_{BW}$	3.6×10^{-5}	3.3×10^{-5}	3.6×10^{-5}	0.02
	HRI	1.8×10^{-3}	1.7×10^{-3}	1.8×10^{-3}	
Cr	EDI$_{BW}$	1.5×10^{-5}	1.5×10^{-5}	4.1×10^{-5}	1.5
	HRI	1.0×10^{-5}	9.7×10^{-6}	2.7×10^{-5}	

[a] Oral reference dose (RfD) (mg kg^{-1} body weight d^{-1}) according to USEPA.[21]

Quality parameters were also influenced by the sediment. Lettuce soluble sugar content is relatively low in comparison with other plant species (about 3 g 100 g^{-1} FW);[26] nevertheless, they are important as a source of energy for maintaining cell metabolism and for their role in osmoregulation.[29] After harvest, sugars are essential for keeping cells alive and to ensure a long shelf life of the final product.[30] In this study, lettuce sugar concentration was in accordance with those reported by Tesi[26] and Mou[31] and was not affected by the growing medium (Table 3). Conversely, malic, citric and tartaric acids, TSP and antioxidant activity were all significantly higher in lettuce cultivated in TS50 and TS100 than in control plants, revealing a stress condition caused by the sediment. Organic acid metabolism has been associated with plant adaptation to soil stress.[32] Also antioxidant compounds, such as polyphenols, are well known to provide tolerance in plants exposed to moderate stress conditions,[33] in addition to being secondary metabolites affecting organoleptic and nutritional properties of vegetables.[34]

PCA clearly illustrated that lettuce cultivated in TS100 accumulated the greatest concentration of elements (Fig. 1). In particular, the significantly higher concentration of Ca, Cu, Fe and Cr found in lettuce grown in TS100 is consistent with the high content of these elements in the sediment. Lettuce grown in TS0 accumulated a higher amount of P and Mn compared to the other treatments, as evidenced also by PCA. Low content of P in lettuce grown in TS50 and TS100 may be due to the high pH and Ca concentration in the sediment, which promoted P precipitation.[5] The high accumulation of Mn in control lettuce could be explained considering the acidic pH value of the peat, or its high concentration in low-molecular-weight organic ligands entailing the formation of soluble complexes that can increase the Mn availability to plants.[35,36] Consequently, the EDI% for this element from control lettuce was particularly high (10.9%) in comparison with that from plants cultivated in TS50 and TS100 and for any other elements.

Regardless of the growing media, all the element concentrations and their contribution to the RDI/AI (EDI%) were in accordance with values reported by Pinto *et al.*[19,37] for Ca, K, Mg, Cu, Fe, Zn, Ni and Cr, and lower for P and Na. However, EDI% of P and Na from lettuce were within the range indicated by Kim *et al.*[12] However, since an excess of Na in the diet is responsible for hypertension, a reduced intake is widely recommended.[38]

Several data are reported in the literature on heavy metal uptake by plants grown in polluted sediments, with different results depending on sediment contamination level.[39,40] In this study, the assessment of food safety due to the consumption of lettuce produced in TS50 and TS100 showed no potential risks for human health. In fact, EDI$_{BW}$ as compared with RfD as well as

HRI values (Table 6) demonstrated that element concentrations in the sediment were not hazardous. Thus the toxicological impact that would result from the consumption of lettuce grown in TS50 and TS100 would be negligible. Among organic contaminants, only DL-PCBs were detected in lettuce heads, regardless of the growing medium. Due to their hydrophobicity, DL-PCBs in soil are mostly unavailable for plant uptake,[41] so contamination may have been occurred through resuspension from soil of dust containing DL-PCBs, followed by deposition on leaf tissues.[24,42] In any case, the measured value (0.03 pg TEQ g^{-1} fresh weight) was below the recommended threshold reported in the *Codex Alimentarius* for DL-PCBs in foodstuff (0.1 pg TEQ g^{-1} fresh weight; Reg. 2014/663/EU). The other organic pollutants analysed and found in the growing media were not detected in plants. Nevertheless, their presence in the sediment-based growing media may be a hindrance to the reuse of sediment for horticulture purposes.

CONCLUSION

This pilot study showed that the phytoremediated marine sediment tested in the described experiment can be safely used as a growing medium for lettuce cultivation, but a mixture with peat (or other materials) is necessary in order to guarantee agronomic results. A ratio of sediment – peat of 1:1 showed an agronomic performance similar to the peat-based medium; nevertheless further studies on different sediment proportions are necessary in order to identify a sediment mixture resulting in the best agronomic performance with the lowest peat content. Moreover, considering that plants' response to different growing media may vary in different genotypes, the use of sediment as growing medium should be tested in other cultivars and species. The reuse of sediments for horticultural purposes offers a profitable alternative to sediment disposal as waste, in line with European and worldwide policies which support the idea of sediments as a resource, and, at the same time, complies with the current need to reduce the use of peat in horticulture.

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Article 2

Use of a remediated dredged marine sediment as a substrate for food crop cultivation: sediment characterization and assessment of fruit safety and quality using strawberry (Fragaria x ananassa Duch.) as model species of contamination transfer

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HIGHLIGHTS

- The use of sediment/peat 50/50 *v/v* (TS50) did not affect yield of Camarosa fruits.
- Cr, Pb and Cd were not detected in fruit from TS50, nor from pure sediment (TS100).
- Cu, Zn, and Ni were comparable in fruits from TS50, TS100 and control (TS0).
- DL-PCBs in fruits from TS50 and TS100 were fourfold lower than threshold values.
- Fruit grown on TS50 and TS100 had comparable/higher quality indexes than TS0 fruit.

GRAPHICAL ABSTRACT

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ABSTRACT

A harbour sediment, previously remediated, was tested for soilless strawberry cultivation (Camarosa and Monterey cultivars), as an innovative, cost-effective and environment-friendly approach of sediment management. Sediments were tested as such (TS100) and mixed 1/1 (*v/v*) with a peat-based commercial substrate (TS50), using the peat-based medium as control (TS0). Substrates were characterized for some physicochemical properties (e.g. density, porosity and water capacity). Minerals (P, Ca, K, Na and Fe), heavy metals (Cu, Zn, Mn, Ni, Cr, Pb and Cd), aliphatic hydrocarbons ($C > 12$), polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), dibenzodioxins and dibenzofurans were analysed in substrates and fruits. Sugars and organic acids, including the ascorbic, were also determined in fruits, as quality indicators. Notwithstanding remediation, sediments showed concentrations of Zn ($206\ mg\ kg^{-1}$), $C > 12$ ($86\ mg\ kg^{-1}$) and PAHs ($47\ mg\ kg^{-1}$) exceeding the limits established by the Italian L.D. 152/2006, regulating the contamination of soil in green areas, thus making its relocation in the

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Polycyclic aromatic hydrocarbons
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Dioxins

environment not permitted as such. No evidence of fruit contamination by Cr, Pb and Cd was highlighted. Moreover, Cu, Zn and Ni fruit concentrations were comparable among treatments. Conversely, Mn showed statistically higher concentrations in TS0 fruits (56–57 mg kg^{-1}) compared to those grown in sediment-based substrates (8–20 mg kg^{-1}). Among organic contaminants, only dioxin-like PCBs were determined in fruits, at toxic equivalent concentrations fourfold lower than the limit established by the European Union. TS100 fruits showed a yield reduction from 40 to 70% for Camarosa and Monterey, but higher sugar and ascorbic acid contents.

1. Introduction

The management of harbour sediments undoubtedly represents a significant environmental problem, both because of the large quantities of material that are dredged annually from ports and waterways, and the high concentrations of inorganic and organic contaminants that are commonly found in these matrices (Masciandaro et al., 2014). At the EU level, sediment management is addressed fragmentarily, being it only covered by EU policies and directives for very specific issues (Bortone, 2007). Hence, in EU countries, the management of dredged sediments follows national regulations. In Italy, dredged sediments are classified based on the Legislative Decree 152/2006, part IV, annex D and part V, annex 5 (Government of Italy, 2006), which regards the restoration of soil in contaminated sites and refers to concentration limits of selected metals and organic compounds. In particular, according to this legislation, soils are destined to public, private and residential green use ("A" limits) or commercial and industrial use ("B" limits), allowing the relocation of dredged sediments in the marine environment only in case "A" limits are met. Alternatively, the sediments must necessarily be treated to lower metal and/or organic compound concentration below the "A" limits or sent to landfills specifically authorized by the European Union.

The relocation of marine sediments in sectors of production is promoted worldwide through multiple international treaties such as the "Oslo Convention" (Government of Norway, 1972), the "OSPAR Convention" (OSPAR Commission, 1992) and the "Barcelona Convention" (European Union, 1995). However, the physicochemical characteristics of these materials often make their use unsuitable even for industrial applications such as the production of bricks or cements (Cappuyns et al., 2015), which provides their substantial inertisation with consequent minimal environmental impact.

The relocation of dredged sediments in sectors of production with a potentially greater environmental impact, such as agriculture, appears even more problematic due to the possible transfer of contamination to environmental matrices, plant products and humans. The treatment of sediments for lowering their level of contamination is therefore a fundamental step for possible relocation in the agricultural sector. Among the different available restoration techniques, phytoremediation and landfarming have proven to be a sustainable management option for the treatment of dredged marine sediments, leading to the degradation of organic contaminants and the decrease in the bioavailability of heavy metals (Doni et al., 2015; Brown et al., 2017).

The successful application of phytoremediation for sediment decontamination clearly suggests the suitability of sediment as unconventional plant growth substrate in the nursery sector, which is currently based on the massive use of peat. In fact, among the commercially available substrates, peat is widely employed as the main component, due to its relatively low price and good agronomic characteristics. However, the current implications related to peatland exploitation have led to the exploration of other innovative and renewable materials, including dredged sediments, to integrate environmental issues in the nursery sector. In this regard, some studies demonstrated the suitability of using phytoremediated harbour sediments for growing forest and ornamental plants (Mattei et al., 2017; Ugolini et al., 2017). Conversely, as far as we know, only very few papers focused on the use of phytoremediated marine harbour sediments for fruit production (Melgarejo et al., 2017, 2019). In these studies, strawberry and pomegranate were used as model species, evidencing no significant impact of different sediment-to-peat ratios on fruit quality attributes. However, to the best of our knowledge, no information about food safety of fruits grown in contaminated sediment-based media has been reported, thus limiting the overall significance of data. Actually, food safety is a topic of paramount relevance when polluted substrates, even underwent a remediation process, are used for growing edible crops. In fact, reclaimed resources may still contain significant concentrations of a wide range of inorganic and organic pollutants, thus increasing the risks related to human contamination through the food chain. Furthermore, it should be considered that bioavailability of pollutants may strongly vary due to the plant-soil interactions (Antoniadis et al., 2017). In this regard, among fruit species, strawberry represents a good model. In fact, strawberry is a species suitable for soilless cultivation, generally demanding high-quality growth substrates (Lieten, 2013) and characterized by a fast and abundant fruiting. In addition, stems are short and fruits translocate high amounts of water, thus potentially favouring the contamination transfer. Strawberry (*Fragaria x ananassa* Duch.) is also one of the most important fruit species worldwide, with an European production of about 1.7 million tons, nearly 11% of which cultivated in Italy (Food and Agriculture Organization (FAO), 2017). This fruit is also characterized by unique organoleptic characteristics as well as overall fruit nutritional and nutraceutical (Giampieri et al., 2014) attributes, reasons why strawberry is widely appreciated by consumers.

Based on the aforementioned considerations, in this study, dredged harbour sediments previously underwent phytoremediation and landfarming were characterized for a number of physicochemical characteristics as well as inorganic and organic contamination. The sediment as such and mixed with a common peat-based commercial substrate were reused for strawberry cultivation and fruits were carefully analysed for the transfer of several toxic metals and organic pollutants. Additionally, a number of quality attributes (i.e. sugars, organic acids and minerals) were assessed. Results obtained with sediment-based substrates were compared with those achieved using the aforementioned peat-based medium as control.

2. Materials and methods

Full details of materials, reagents and standards used in this study were reported in section S.1 of the *Supplementary materials*.

2.1. Design of the study

Sediments were dredged in Leghorn harbour (Italy, DMS coordinates 43°33′25″N, 10°17′39″E) and decontaminated during three years of phytoremediation (Masciandaro et al., 2014). Afterwards, remediated sediments as such underwent to three months of landfarming, which consisted in a periodical aeration by mechanical overturning and irrigation, in order to homogenise the matrix and further reduce the concentration of organic pollutants.

In this study, the phytoremediated and landfarmed sediment was used as such or mixed with a peat-based commercial substrate to obtain the following treatments: TS100 (100% treated sediment), TS50 (1/1 peat-based substrate/treated sediment *v*/*v*), and TS0 (100% peat-based substrate) considered as the control treatment. More in detail, both pure sediments and the peat-based commercial substrate were separately mixed in a concrete mixer before to be used for pot filling. For the preparation of TS50, equal volumes of previously homogenized pure sediments and peat-based commercial substrate, were inserted in the concrete mixer and carefully homogenized.

Young fridge stored certified plants of *Fragaria* × *ananassa* (Camarosa and Monterey cultivars) were organically grown outdoor (DMS coordinates: 43°81′68″N, 11°19′99″E) during the season 2016 (March–September). For each cultivar, 60 plants were grown and each combination "cultivar x substrate" was replicated in 2 blocks, consisting of 10 plants (10 plants × 2 blocks x 3 treatments x 2 cultivars = 120 plant). Plants were grown in 106-L plastic pots (length 80 cm, width 39 cm and height 34 cm, Leroy Merlin mall, Campi Bisenzio, Italy) and treated with 100 g of NPK fertilizer (see the paragraph S.1 of the *Supplementary materials* for full details of the fertilizer composition) per pot. For all the growing media, plants were irrigated manually to maintain the soil moisture in the range of about 80% of the field capacity.

2.2. Fruit and plant samples

Fruits were harvested throughout the crop cycle when considered "marketable" (characterized by a red colour all over the fruit) and weighted, thus determining the fruit yield as total production over the experimental period. Fruits harvested from each plant were separately stored at −20 °C until fruit production ended, thus obtaining at least 20 fruit samples for each cultivar and treatment. Afterwards, for each cultivar and treatment, six fresh strawberries were randomly selected and used as such for ascorbic acid determination, whereas the remaining fruits were separately freeze-dried and then stored at −20 °C until they were used for the other analyses. Within each cultivar and treatment, six freeze-dried fruits were randomly selected and used for the analysis of inorganic and organic contaminants. Further six samples were destined to the analysis of primary metabolites.

When fruit production ended, all plants ($n = 20$ for each cultivar and treatment) were harvested and six plants were randomly selected for the evaluation of leaf area. Leaves were scanned, and the mean leaf area was calculated with Tomato Analyser 3.0 software (van der Knaap Labs, University of Georgia). Further six plants were randomly selected and used for the calculation of aerial wet weight (w.w.) and dry weight (d.w.), the latter obtained after oven drying at 70 °C until constant weight.

2.3. Substrate analysis

TS0, TS50 and TS100 were analysed after pot filling, before plant cultivation, for the parameters reported in Table 1. More in detail, for each substrate, about 500 g-aliquots were sampled from each pot (two sampling points per pot) containing the same substrate (four pots), thus obtaining 2 kg-samples for each kind of substrate.

A representative TS100 sample was sieved for texture determination following the USDA methodology (USDA Soil Science Division Staff, 2017).

Soil bulk density was measured on undisturbed cores (Blake and Hartge, 1986). Substrate water parameters (porosity, water and air capacity, easy available water) were determined through a water retention curve obtained using the gravitational drainage technique for soil improvers and growing media (European Committee for Standardization (CEN), 2011). Electrical conductivity (EC) and pH were measured in a 1:15 (*v*/*v*) aqueous solution (AG 8603 Seven- Multi, Mettler Toledo, and Orion model 150). Total organic C (TOC) and total N content (TN) were determined by dry combustion with RC-412 multiphase carbon and a FP-528 protein/nitrogen analyser (LECO, Saint Joseph MI, USA). Chloride was determined by using the silver nitrate titration method with potassium chromate as indicator (ISO, 1989), after extraction with ultrapure water (Edwards et al., 1981).

Element concentrations (P, Ca, Mg, Na, K, Fe, Cu, Zn, Mn, Ni, Cr, Pb, Cd) were analysed by ICP (iCAP 7400 DUO ICP-OES; Thermo Fisher Scientific, Waltham, MA, USA), after microwave (model Ethos 1, Milestone, Bergamo, Italy) assisted acidic digestion (see section S.2 of the *Supplementary materials* for full details).

The determination of Cr(VI) was performed by ion chromatography (IC) with post-column derivatization and spectrophotometric detection (Bruzzoniti et al., 2017). Method quantification limits and recovery were found to be 0.02 mg kg^{-1} d.w. and 34%, respectively.

Organic micropollutants were analysed by an external accredited laboratory, following official USEPA analytical protocols. Aliphatic hydrocarbons with carbon numbers in the range 12–40 ($C > 12$) and polycyclic aromatic hydrocarbons (PAHs) were analysed according to the extraction method EPA 3545A (EPA, 2007) and the instrumental determination methods EPA 8015D (EPA, 2003) and EPA 8270D (EPA, 2014). Polychlorinated biphenyls (PCBs), as well as polychlorinated dibenzo(p)dioxins and furans (PCDDs and PCDFs) were analysed according to the EPA 1668C (EPA, 2010) and EPA 1613B (EPA, 1994) methods, which are based on an isotope dilution technique and gas chromatographic-high-resolution mass spectrometric determination. Method quantification limits and apparent recoveries for the investigated organic micropollutants are reported in Table S2 of the *Supplementary materials*.

2.4. Fruit analysis

Concentrations of elements and organic contaminants ($C > 12$, PAHs, PCBs and PCCD/F) were determined according to the methods previously mentioned in "substrate analysis". Method quantification limits and apparent recoveries of organic micropollutants were reported in Table S2 of the *Supplementary Material* section.

The extraction and the analysis of ascorbic acid, citric and malic acids, and sugars (i.e. glucose, fructose and sucrose) were performed as elsewhere reported (Doumett et al., 2011). Details of these analytical protocols were reported in section S.4 of the *Supplementary materials*.

2.5. Data analysis

The multiple comparison tests of the mean concentration values were performed by using the non-parametric Games-Howell (Minitab®17.1.0, Minitab Inc., State College, PA, USA), which makes it possible to compare mean values and corresponding standard deviations without assuming equal variances of the measured

Table 1
Main physicochemical characteristics, metal and organic contaminant concentrations of TS0, TS50 and TS100 growing media used for the experimentation. Values are the means ($n = 3$) and standard deviations (in bracket). Within a same row, different letters mean statistically significant differences ($P < 0.05$). Limits reported in the Italian Legislative Decrees (L.D.) 75/2010 and 152/2006, regulating the properties of growing media (base and mixed) and soil in public, private and residential green areas, respectively, are also shown for a comparison.

Parameters	TS0	TS50	TS100	L.D. 75/2010		L.D. 152/2006
				Base	Mixed	
Physicochemical characteristics						
Texture	n.a.	n.a.	Sandy/Loam	n.a.	n.a.	n.a.
Bulk density (g cm^{3})	0.19 (0.02) a	1.01 (0.1) b	1.52 (0.14) c	≤0.45	≤0.95	n.a.
Porosity (%)	95 (4) a	89 (2) a	76 (3) b	n.a.	n.a.	n.a.
Water capacity (%)	81 (3) a	76 (1) a	64 (2) b	n.a.	n.a.	n.a.
Air capacity (%)	14 (1) a	13 (1) ab	11 (1) b	n.a.	n.a.	n.a.
Easy available water (%)	42 (4) a	29 (2) b	20 (2) c	n.a.	n.a.	n.a.
pH	5.6 (0.1) c	7.8 (0.2) b	8.6 (0.1) a	3.5–7.5	4.5–8.5	n.a.
EC (dS m^{-1})	0.68 (0.01) a	0.52 (0.03) b	0.28 (0.02) c	≤0.70	≤1.0	n.a.
Chloride (mg kg^{-1})	39 (4) a	20 (3) b	14 (2) c	n.a.	n.a.	n.a.
TOC (%)	20 (2) a	2.5 (0.1) b	1.3 (0.1) c	≥8	≥4	n.a.
TN (%)	0.37 (0.01) a	0.14 (0.01) b	0.09 (0.01) c	n.a.	n.a.	n.a.
Phosphorus (g kg^{-1})	0.33 (0.01) b	0.51 (0.01) a	0.54 (0.00) a	n.a.	n.a.	n.a.
Metals						
Ca (g kg^{-1})	4.8 (0.5) c	26 (1) b	39 (4) a	n.a.	n.a.	n.a.
Mg (g kg^{-1})	2.2 (0.2) c	8.1 (0.7) b	10 (1) a	n.a.	n.a.	n.a.
Na (g kg^{-1})	1.2 (0.1) a	0.5 (0.1) b	0.5 (0.1) b	n.a.	n.a.	n.a.
K (g kg^{-1})	4.4 (0.2) a	4.4 (0.4) a	3.8 (0.3) a	n.a.	n.a.	n.a.
Fe (g kg^{-1})	3.9 (0.2) b	12.4 (0.2) a	15 (2) a	n.a.	n.a.	n.a.
Cu (mg kg^{-1})	7.5 (0.9) c	40 (6) a	48 (5) a	≤230	≤230	≤120
Zn (mg kg^{-1})	10 (1) b	193 (28) a	206 (12) a	≤500	≤500	≤150
Mn (mg kg^{-1})	86 (8) c	202 (24) b	281 (10) a	n.a.	n.a.	n.a.
Ni (mg kg^{-1})	2.7 (0.4) b	30 (6) a	37.5 (0.6) a	≤100	≤100	≤120
Cr (mg kg^{-1})	12.7 (0.7) c	34 (2) b	43 (3) a	n.a.	n.a.	≤150
Cr (VI) (mg kg^{-1})	<0.02 b	<0.08 a	<0.10 a	≤0.5	≤0.5	≤2
Pb (mg kg^{-1})	8 (1) b	36 (3) a	40 (7) a	≤140	≤140	≤100
Cd (mg kg^{-1})	0.20 (0.04) c	0.52 (0.04) b	0.96 (0.06) a	≤1.5	≤1.5	≤2
Organic contaminants						
C > 12 (mg kg^{-1})	32 (5) c	44 (7) b	86 (10) a	n.a.	n.a.	≤50
PAHs (mg kg^{-1})	<0.01 c	33 (3) b	47 (6) a	n.a.	n.a.	≤10
PCBs (μg kg^{-1})	<0.1 c	10.7 (0.6) b	15.1 (1.5) a	n.a.	n.a.	≤60
DL-PCBs (μg kg^{-1})	0.11 (0.01) c	1.97 (0.01) b	3.25 (0.02) a	n.a.	n.a.	n.a.
PCDD/PCDF (ng kg^{-1})	3.5 (1) c	93 (7) b	192 (12) a	n.a.	n.a.	n.a.
PCDD/PCDF (ng TEQ kg^{-1})	0.037 (0.002) c	0.565 (0.005) b	1.541 (0.008) a	n.a.	n.a.	≤10

n.a. = not applicable/not available; EC = electrical conductivity; TOC = total organic carbon; TN = total nitrogen; C > 12 = hydrocarbons with a number of carbon atoms in the range of C12-C40; PAHs = polycyclic aromatic hydrocarbons; PCBs = polychlorinated biphenyls indicators non dioxin-like; DL-PCBs = dioxin-like polychlorinated biphenyls; PCDD/PCDF = polychlorinated dibenzo(p)dioxins and furans.

variables.

3. Results and discussion

3.1. Physicochemical characteristics of growing media

Main physicochemical characteristics of the growing media are reported in Table 1, where they are also compared to the Italian Legislative Decree (L.D.) 75/2010, which regulates base (single-materials) and mixed (mixed materials) media to be used as growing substrates for food crops (Government of Italy, 2010).

Texture analysis of pure sediment showed a composition with 57% sand, 24% silt and 18% clay, thus identifying a sandy-loam structure. Bulk density showed statistically significant differences among treatments, with the following trend: TS100 > TS50 > TS0. The high values found for both TS50 and TS100 (1.01 and $1.52\ \text{g cm}^{-3}$, respectively) were over the limits set by the L.D. 75/2010. This finding is the consequence of the aforementioned textural composition of the sediment, which also resulted in a significantly lower porosity, water and air capacity, as well as easy available water, of the TS100 compared to the TS0. TS100 showed the highest pH value (i.e. 8.6), followed by TS50 and TS0, denoting the alkaline condition of the sediment. Differently from TS100, the TS50 complied the limit of the mixed substrate category. The trend found for EC was TS0>TS50 > TS100, with all values below the limit established by the L.D. 75/2010. The water washing procedure performed on the sediment during the landfarming process can explain this trend, which was the same found for chlorides. TOC and TN showed significantly higher values in control substrate (TS0), compared to the sediment-based ones (TS50 and TS100), owing to the organic nature of peat. As regards TOC, both TS50 and TS100 did not meet the limits for base and mixed agronomic substrates. Conversely, the sediment-based substrates showed higher P-concentrations than peat, thus representing a positive attribute from the agronomic viewpoint.

Considering the element concentrations, the trend found for Ca, Mg, Mn, Cr and Cd followed the order TS100 > TS50 > TS0, with statistically significant differences among treatments. Significantly higher concentrations were also found in sediment-based substrates than in peat for Fe, Cu, Ni, Cr(VI), Pb and especially Zn, even though the TS50 and TS100 exhibited quite similar values, probably due to local inhomogeneity of the samples analysed. Conversely, data obtained for Na highlighted a trend similar to those previously discussed for EC and Cl^{-}, notwithstanding the marine origin of the sediment, thus confirming the role of landfarming in this regard. Interestingly, the sediment-based substrates showed concentrations of K very similar to those determined in peat, compared to all the other investigated elements. This finding, together with the aforementioned considerations regarding phosphorus, highlights the potential suitability of the sediment as agronomic substrate.

Concentrations of Cu, Zn, Ni, Cr, Cr(VI), Pb and Cd can be compared to the limits reported in L.D. 75/2010 and/or L.D. 152/2006, the latter regulating the contamination of soil in public, private and residential green areas (Government of Italy, 2006) (Table 1). All metals showed concentrations fully included in the limits established for agronomic growing media (L.D. 75/2010), thus evidencing that even the sediment as such is suitable as soilless substrate from the metal content point of view. The metal concentrations determined in the growing media were also lower than the limits reported in the L.D. 152/2006 with the only exception of Zn in both TS50 and TS100. In this regard, it should be noted that for Zn, Cu and Pb the limits laid down by the L.D. 152/2006 are lower than those established by the L.D. 75/2010.

Organic contaminants followed the same concentration trend previously shown for most metals, i.e. TS100 > TS50 > TS0. Concentrations of organic contaminants herein investigated can be compared only to the limits established by the L.D. 152/2006. As shown in Table 1, PAHs were not detected in TS0, whereas TS50 and TS100 had mean PAHs values of 33 and 47 mg kg^{-1}, respectively, approximately three to five times higher than the maximum permitted concentration (i.e. 10 mg kg^{-1}). As regards hydrocarbons with C > 12, only TS100 showed a concentration over the limit, whereas for PCBs and PCDD/PCDF the values determined were well-below the maximum acceptable concentrations. These data highlighted that, notwithstanding the long remediation process, the relocation of this sediment in the environment is not permitted by the Italian legislation. Accordingly, alternative management solutions for this material, such as its relocation as substrate for pot cultivation, are necessary.

3.2. *Effect of the sediment on plant biomass and fruit yield*

Fig. 1A–F illustrates the mean values of leaf area, dry weight and water content of the plant aerial part, as well as yield, dry weight and water content of fruits, found in Camarosa and Monterey varieties cultivated in TS0, TS50 and TS100 growing media. The sediment had a clear effect on all the plant aerial parameters. In fact, such parameters exhibited a general decrease with increasing the sediment percentage in the growing media, thus suggesting that plants were stressed when grown on sediment-based media. More in detail, the influence of the sediment was particularly evident for the leaf area (Fig. 1A), since plants of both cultivars grown in TS50 and TS100 media showed statistically lower values when compared to control treatments. Aerial dry weight resulted significantly affected by the presence of the sediment only for Monterey grown in TS100 substrate (i.e. M-TS100) (Fig. 1B), whereas in both varieties a significantly lower water content in the presence of 100% sediment as growing media (i.e. C-TS100 and M-TS100), was observed (Fig. 1C). Because plant growth greatly depends on leaf area development, which guarantees light interception and thus production of assimilates, a limited leaf area development compromises the accumulation of dry matter in the aboveground tissues of plants cultivated in sediment-based media. Interestingly, this effect is more evident for Monterey than Camarosa cultivar, evidencing one more time different behaviours due to genotype in response to a stress factor (Del Bubba et al., 2016). As regards the shoot water content (Fig. 1C), since both cultivars behaved similarly, it can be stated that the significant differences observed by comparing TS0 and TS100 (about 14% and 8% for Camarosa and Monterey, respectively) were mostly due to the sediment. In fact, the sediment is characterized by higher bulk density, as well as lower porosity, water capacity and easy available water compared to peat (Table 1). Hence, roots of plants cultivated in the sediment-based growing media have probably suffered from a limited water availability, which has in turn affected plant growth and leaf area development.

In this regard, it should be noted that even mild water stress can greatly reduce fruit expansion and thus fruit productivity (Grant et al., 2010). As regards fruit yield, the effect of the water deficit, as well as the other sediment-induced consequences, appears much more evident in Monterey than in Camarosa. In fact, the presence of 50% sediment in the growing media decreased fruit yield of more than 50% in Monterey, whereas it did not produce any yield variation in Camarosa (Fig. 1D). Hence, the fruit production of the two cultivars, which were very different in TS0, became very similar in TS50. The use of 100% sediment further lowered the productivity of both cultivars that remained statistically comparable.

The presence of the sediment in the growing substrate gave rise to a decrease of fruit water content, compared to control, with statistically significant differences in both varieties (Fig. 1F). This finding was in agreement with the lower water percentage found in aboveground tissues of plants grown in sediment-based media. Both cultivars exhibited also a statistically significant variation in the fruit dry weight, which was generally higher in strawberries from plants grown in TS50 and TS100 substrates (Fig. 1E). Hence, an opposite trend was found for dry matter in shoot compared to fruit (Fig. 1B and E). In this regard, it has been elucidated that during flowering and fructification processes, strawberry plants effort to allocate the resources in the reproductive organ, rather than in vegetative tissues (Fernandez et al., 2001). Furthermore, the preferential apportionment of limited resources towards reproductive organs has been highlighted for wild strawberries (*F. vesca* L.) in the presence of stress or disturbance events (Jurik, 1983).

3.3. *Heavy metals and organic contaminants in strawberry fruits*

Cr, Pb and Cd were not detected in fruit tissues and therefore their presence must be considered lower than the method detection limits, which were assessed as 0.20 mg kg^{-1}, 0.11 mg kg^{-1} and 0.10 mg kg^{-1}, respectively. To the best of our knowledge, only the presence of lead is regulated in small berries, even though without explicitly quoting strawberry (Food and Agriculture Organization and World Health Organization, 2018). A recommended Pb concentration limit of 0.1 mg kg^{-1} has been established, which is comparable with the MDL herein obtained.

Table 2 reports the concentrations of Cu, Zn, Mn and Ni found in Camarosa and Monterey fruits cultivated in TS0, TS50 and TS100 growing media. No significant differences were found in Zn and Ni concentrations among the investigated treatments. Analogously, Cu showed very similar concentrations in fruits obtained on sediment-based substrates and peat, even though for Camarosa a statistically higher Cu level was determined in TS0 than in TS100. A very different behaviour was observed for Mn, which showed for both cultivars statistically higher concentrations in control fruits compared to those grown in sediment-based substrates. This trend was the opposite to the one found in substrates and can be ascribed to the alkaline pH value of sediment (Table 1), which is the key factor in attenuating metal availability by precipitation of poorly soluble salts, such as carbonates or hydroxides (Renella et al., 2004). Indeed, a very low heavy metal availability in remediated sediments has been elsewhere reported in the same sediment herein investigated, before its landfarming (Mattei et al., 2018).

Taking into account organic contaminants, C > 12, PAHs, PCCD/PCDF and non-dioxin like PCB indicators were never quantified and their presence must therefore be considered below the MQLs reported in Table S2 of the *Supplementary Material*. In this regard, it should be emphasized that aliphatic hydrocarbons and non-dioxin like PCB indicators have not yet been included in any regulation or recommendation concerning their presence in fruits or other foods.

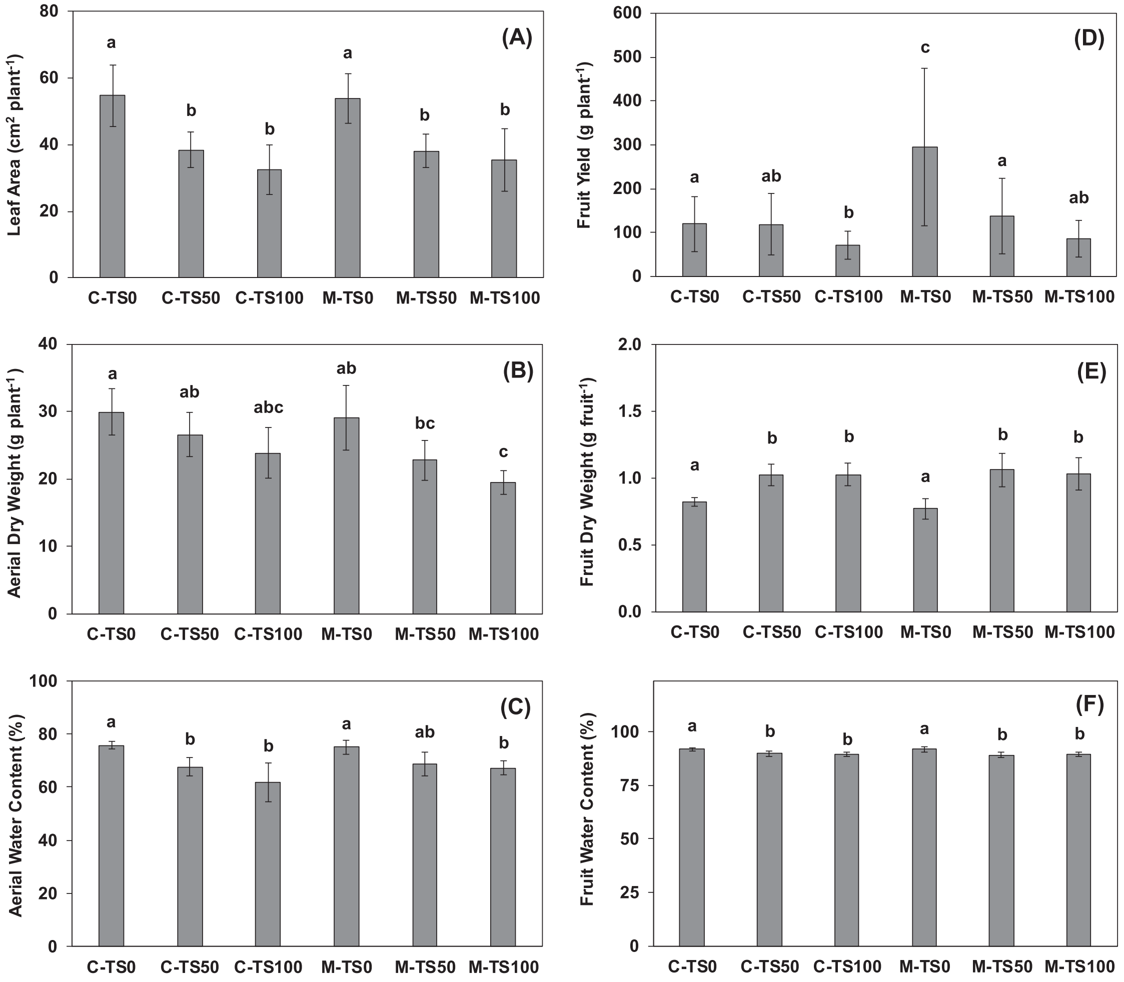

Fig. 1. Mean values of leaf area (A, n = 6), plant aerial dry weight (B, n = 6), plant aerial water content (C, n = 6), fruit yield (D, n = 20), fruit dry weight (E, g) (n = 20) and fruit water content (F, %) (n = 20) in Camarosa (C) and Monterey (M) cultivars grown in TS0, TS50 and TS100 growing media. Error bars represent standard deviation. Different letters mean statistically significant differences ($P < 0.05$).

Table 2
Concentration of Cu, Zn, Mn, Ni (mg kg^{-1} d.w.) found in Camarosa (C) and Monterey (M) fruits grown in TS0, TS50 and TS100 growing media. Values are the means (n = 6) and standard deviations (in bracket). Within a same column, different letters mean statistically significant differences ($P < 0.05$).

Treatment	Cu	Zn	Mn	Ni
C-TS0	3.8 (0.8) a	15 (4) a	56 (7) a	0.37 (0.03) a
C-TS50	3.2 (0.5) ab	11 (3) a	14 (1) c	0.38 (0.04) a
C-TS100	2.3 (0.4) b	13 (3) a	8 (1) d	0.35 (0.03) a
M-TS0	3.9 (0.9) a	15 (4) a	57 (6) a	0.39 (0.04) a
M-TS50	3.5 (1.1) ab	12 (2) a	20 (2) b	0.41 (0.07) a
M-TS100	3.6 (0.7) a	10 (2) a	9 (1) d	0.36 (0.05) a

Conversely, the presence of PAHs, PCDDs and PCDFs in certain foodstuffs is regulated by the European Commission Regulations 835/2011 (European Commission, 2011) and the European Commission Recommendation 2014/663/EU (European Commission, 2014). As regards PAHs, no maximum concentration levels have been established in fruits. It should be however remarked that the limits set by the European Community for other foodstuffs (i.e. oils, molluscs and meat, 2–6 μg kg^{-1} for benzo(a)pyrene and 10–35 μg kg^{-1} for the sum of benzo(a)pyrene, benzo(a)anthracene, benzo(b)fluoranthene and chrysene) (European Commission, 2011) are comparable with the method sensitivity herein achieved (see Table S2 of the *Supplementary Material*), thus ensuring that PAHs are kept at concentrations that do not cause health concern in strawberries.

Differently from findings observed for C > 12, PAHs, PCCD/PCDF and non-dioxin like PCB indicators, all the individual DL-PCBs were determined in strawberry fruits (Table 3). Overall occurrences of DL-PCBs were in the ranges 4.3–27 and 4.9–44 pg g^{-1} w.w. for Camarosa and Monterey varieties, with statistically significant increments in the order TS0<TS50 < TS100 for both genotypes, in agreement with the concentration trend found in substrates (see Table 1).

DL-PCB individual concentrations were in-between hundreds of fg g^{-1} to tens of pg g^{-1} (expressed on a w.w. basis), depending on compound and growing substrate considered. The most abundant congeners found in all fruit samples, including those grown on sediment-free substrates, were PCB 105 and PCB 118 (Table 3). More in detail, these analytes accounted together for about 50%, 70% and 80% of total DL-PCBs content in TS0, TS50 and TS100 fruits, respectively. Interestingly, these DL-PCB congeners were also by far the most abundant found in both peat-based substrate and media containing sediments, representing together about 70–83% of the whole content of DL-PCBs of substrates and thus explaining their much higher occurrence in strawberries. More in detail, PCB-105 and PCB-118 concentrations in growing substrates averaged 0.03

Table 3

Concentrations of individual and total dioxin-like PCBs congeners expressed as pg g^{-1} w.w. found in Camarosa (C) and Monterey (M) fruits grown in TS0, TS50 and TS100 growing media. TEQ were also expressed as pg g^{-1} f.w. Values are the means ($n = 6$) and standard deviations (in bracket). Within the row regarding the sum of dioxin-like PCBs expressed as TEQ, different letters mean statistically significant differences ($P < 0.05$).

Congeners	C-TS0	C-TS50	C-TS100	M-TS0	M-TS50	M-TS100
PCB-77	0.23 (0.02) a	0.84 (0.07) bc	2.3 (0.9) b	0.4 (0.1) c	0.61 (0.07) bc	1.4 (0.6) bc
PCB-81	0.21 (0.02) a	0.20 (0.01) a	0.20 (0.01) a	0.20 (0.01) a	0.19 (0.01) a	0.20 (0.01) a
PCB-105	0.61 (0.06) a	2.9 (0.8) b	5.2 (0.8) c	0.7 (0.1) a	1.8 (0.6) b	10 (1) d
PCB-114	0.20 (0.01) a	0.30 (0.07) a	0.90 (0.07) b	0.20 (0.01) a	0.20 (0.01) a	1.3 (0.5) b
PCB-118	1.6 (0.5) a	6.1 (0.9) b	15.7 (0.9) c	2.0 (0.5) a	4.9 (0.8) b	27 (4) d
PCB-123	0.20 (0.02) a	0.30 (0.03) b	0.40 (0.07) bc	0.20 (0.01) a	0.20 (0.01) a	0.5 (0.1) c
PCB-126	0.21 (0.01) a	0.21 (0.01) a	0.21 (0.01) a	0.20 (0.01) a	0.21 (0.01) a	0.22 (0.01) a
PCB-156	0.21 (0.01) a	0.70 (0.06) b	0.99 (0.07) c	0.20 (0.01) a	0.43 (0.09) d	1.4 (0.8) abcd
PCB-157	0.20 (0.02) a	0.30 (0.05) b	0.20 (0.02) a	0.20 (0.01) a	0.21 (0.02) a	0.20 (0.01) a
PCB-167	0.24 (0.04) ac	0.30 (0.01) a	0.60 (0.11) b	0.20 (0.01) c	0.28 (0.04) a	0.61 (0.08) b
PCB-169	0.21 (0.01) a	0.20 (0.02) a	0.19 (0.01) a	0.22 (0.01) a	0.20 (0.01) a	0.20 (0.01) a
PCB-189	0.21 (0.01) a	0.21 (0.01) a	0.30 (0.05) b	0.20 (0.01) a	0.18 (0.01) a	0.21 (0.01) a
Σ DL-PCBs	4.3 (0.5) a	13 (2) b	27 (2) c	4.9 (0.5) a	9 (1) d	44 (4) e
Σ DL-PCBs (as TEQ)	0.028 (0.001) a	0.0270 (0.0007) a	0.0274 (0.0009) a	0.0266 (0.0007) a	0.0267 (0.0007) a	0.0275 (0.0004) a

and 0.06 μg kg^{-1} in TS0, 0.42 and 0.96 μg kg^{-1} in TS50, 0.69 and 1.62 μg kg^{-1} in TS100 (data not shown). The sediments (but not the peat-based substrate) contained also remarkable concentrations of PCB-77 and PCB-156 (TS50: 0.035–0.044 μg kg^{-1}; TS100: 0.22–0.37 μg kg^{-1}), which actually showed fourfold to tenfold higher concentrations in fruits grown in pure sediments than in peat-based substrates (Table 3).

The DL-PCBs occurrence determined in the two investigated strawberry varieties were particularly different in the TS100 samples, being their total concentrations in Monterey about twofold higher than in Camarosa, whereas an opposite trend was observed in TS50, even though with smaller differences.

Considering the toxic equivalent concentrations (TEQ) of DL-PCBs, all treatments showed similar concentrations, resulting about fourfold lower than the suggested threshold established by the European Commission Recommendation 2014/663/EU (European Commission, 2014).

3.4. *Effect of remediated sediment on fruit quality*

Total and individual concentrations of sugars (sucrose, glucose and fructose), organic acids (ascorbic, citric and malic), and concentrations of minerals (P, Ca, K, Na and Fe) found in Camarosa and Monterey fruit grown in TS0, TS50 and TS100 growing media, are illustrated in Table S3 of the *Supplementary materials*.

3.4.1. *Sugars*

When concentration values of sugars found in TS0 and TS50 were compared, the two cultivars showed similar increasing trends, even though the extent of the increment was much more evident for Monterey than Camarosa. More in detail, the increase of sucrose concentration was statistically significant in both the investigated cultivars, whereas for glucose and fructose, as well as for total sugars, the variations were significant only in Monterey fruits. Based on the comparison of fruit yield in TS0 and TS50 (Fig. 1D), Monterey cultivar seems the one more affected by the stress due to the presence of sediment and the consequent lower water availability.

Many morphological and physiological variations may occur in plant organs in order to tolerate water deficit. Fruit can be involved in the adaptation process through an overexpression of primary and secondary metabolites (Navarro et al., 2010; Pastenes et al., 2014). Hence, the overexpression of sugars represent an effort of plants to maintain the osmotic adjustment, thus counteracting the lower percentage of easy available water in the sediment-based substrates. It should also be underlined that the increasing trend of sugars in fruits cultivated in TS50 compared to control, is in accordance with the aforementioned accumulation of dry matter observed in these fruits (Fig. 1E).

The use of 100% sediment gave rise to a statistically significant decrease in sucrose concentration, compared to TS50, which was a common finding for both genotypes. Conversely, for glucose and fructose, no significant variations were observed passing from TS50 to TS100. The pure sediment as growth medium can indeed induce a greater stress level for plants compared to the sediment-peat mixture (TS50), due to both the higher level of chemical contamination and the lesser water capacity that characterize the sediment (Table 1). The combination of the chemical and physical effects exerted by the sediment on the plant have resulted in a stress level high enough to interfere with the synthesis of osmolytes, such as sugars. Furthermore, the translocation of assimilated carbon into a particular plant organ, such as fruit, depends on its sink strength, which seems to be lowered in fruits grown in the presence of sediment, as deduced by the significantly lower water content of fruit (Fig. 1F). The only study published so far on the use of remediated sediments as substrates for growing strawberries reported data on sucrose, glucose and fructose concentrations expressed on a w.w. basis (Melgarejo et al., 2017). These data showed a general concentrations increase in response to the presence of growth media containing sediment. More specific comparisons of the results of these studies could however be not relevant, since different percentages of water in fruits can significantly influence the trend of data expressed on a w.w. basis, compared to what is observed on a d.w. basis (Terry et al., 2007).

3.4.2. *Organic acids*

In strawberry, the most abundant organic acids have been identified as citric, malic and ascorbic (Pérez et al., 1997) and a number of researches focused their attention on these compounds (Terry et al., 2007; Giné Bordonaba and Terry, 2010; Del Bubba et al., 2016). In this study, citric was found to be the most abundant acid, with an overall mean of 59 mg g^{-1} d.w., while malic and ascorbic accounted for 33 and 7 mg g^{-1} d.w., respectively. The concentration herein observed for all the organic acids are in line with those reported in several strawberry cultivars (Terry et al., 2007; Giné Bordonaba and Terry, 2010). The presence of the sediment did not affect the concentration of malic, citric and total organic acids. Conversely, ascorbic acid showed statistically significant differences among treatments, with higher concentrations in fruits cultivated on sediment-based substrate.

Ascorbic acid is an antioxidant and redox buffer enzyme cofactor, covering multiple roles associated to plant response to abiotic stresses, respiration and photosynthesis (Smirnoff, 2011). In addition, ascorbic acid, being an organic solute like soluble sugars, can be also involved in osmotic adjustment and its synthesis in fruit is enhanced when water availability is low (Navarro et al., 2010). In this regard, a significant increase of ascorbic acid concentrations in water stressed strawberry genotypes was observed, being but the extent of this effect different among genotypes (Giné Bordonaba and Terry, 2010). Another factor contributing to the synthesis of ascorbic acid is the fruit irradiance and a higher light exposure was positively correlated with ascorbic acid concentration in apple and tomato fruits (Gautier et al., 2008; Li et al., 2009). Additionally, the role of light exposure in the overexpression of ascorbic acid was suggested in strawberry plants (Pincemail et al., 2012). The correlation between light exposure of fruits and accumulation of ascorbic acid may provide a further explanation of the higher concentrations of ascorbic acid determined in the fruits grown on the sediment. In fact, these fruits were obtained from plants characterized by a limited leaf area (Fig. 1A) and therefore it can be reasonably assumed that they have undergone a higher light exposure than those grown on the control plants that have conversely shown a more vigorous canopy.

3.4.3. *Minerals*

Besides the taste-related compound (i.e. sugar and organic acids), strawberry can be considered an important source of minerals (Giampieri et al., 2014), which have a relevant impact on the overall fruit quality. The most abundant minerals were K, Ca, Mg and P with concentration values in the g kg^{-1} d.w. order of magnitude, whereas Na and Fe were found at much lower concentrations (i.e. mg kg^{-1} d.w., levels). Independently from the growing media, the concentration herein reported are in line with the range indicated in literature (Akhatou and Fernández Recamales, 2014; Giampieri et al., 2014). Considering the effect of the growing media, P concentration was found significantly higher in fruit cultivated in peat substrates (i.e. C-TS0 and M-TS0), even though TS50 and TS100 substrates had higher P concentration than peat (Table 1). This result can be explained considering that P availability in the sediment-based media is limited by the combination of high pH and Ca concentration which promotes P precipitation (Del Bubba et al., 2003).

Taking into account Ca, both varieties showed higher values of Ca in fruits cultivated in sediment-based medium than those found in control fruit. The high Ca content observed in fruit cultivated in TS50 and TS100 substrates is linked to the high Ca content within the sediment (Table 1). Sodium accumulation was found generally similar among treatments except for Camarosa grown in TS50 substrates, which showed lower values. Considering Mg, K and Fe, both varieties displayed similar concentrations, evidencing that for these minerals plant pattern of accumulation behaved similarly independently form the investigated substrates.

4. Conclusions

The results of this research may provide a significant impact in the sector of management of marine sediments, proposing their possible relocation in the agricultural field, in agreement with the principles of circular economy. In fact, this study demonstrated for the first time that phytoremediated sediments, in mixture 1/1 (*v/v*) with commercial peat-based substrates or even pure, can be safely used for the soilless cultivation of strawberry, which represent a fruit model particularly sensitive to cultural practices. In fact, as indicated by the whole dataset regarding the transfer of inorganic and organic contaminants from the substrate to the fruit, also in case TS100 is adopted for the soilless growth of strawberry (i.e. the worst scenario in terms of substrate native contamination) the sediment-based cultivation approach can be considered suitable from a food safety viewpoint.

Furthermore, the overall quality of fruits grown in TS50 and TS100 media was comparable or even higher than that observed in control fruits.

As a future perspective, studies performed over a longer timescale and/or focused on other fruit species, may provide additional information about the safety of this non-conventional management strategy of contaminated sediments.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.chemosphere.2019.124651.

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Article 3

Long-term effect of dredged remediated sediment as growing media for strawberry cultivation

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ABSTRACT

A marine sediment, previously undergone to phytoremediation and landfarming process, was used as plant substrate for the soilless cultivation of two strawberry cultivars (i.e. Camarosa and Monterey). Plants were re-grown for two consecutive productive seasons on sediment-based media without removing the growing media from the pots. Physico-chemical and toxicological parameters of the treated sediment (TS100) and of its mixture (1:1, in volume; TS50) with a peat-based substrate (also used as control treatment; TS0) were monitored. Strawberry biomass allocation, fruit yield parameters and element concentration in leaves and fruits were assessed. The first year of cultivation, plants grown on sediment-based media showed a significantly limited biomass allocation and fruit yield compared with peat mainly due to low sediment nitrogen content and unsuitable physical characteristics. The following year, plant re-cultivation improved the sediment aeration, increasing NO_3^- and soluble nutrient availabilities. As a result, plants showed similar biomass allocation and significantly higher fruit productivity in response to an amelioration of the physico-chemical condition of the sediment-based substrates (TS50 and TS100). Moreover, leaves of plant-sediment grown were richer in carbon and micro-element (i.e. Fe, Cu and Zn) necessary for better vegetative and reproductive performances. No detectable toxic heavy metals were found in both leaves and fruits due to the low heavy metal availability caused by the alkaline sediment pH. Health risk assessment confirmed the lack of risks for human health, supporting the food safety of strawberry via consumption. In conclusion, phytoremediated sediment could be one of the possible new waste-derived soilless substrates proposed for plant cultivation and a re-evaluation of sediments as a suitable component material in the ambit of the new EU Regulation 2019:1009 on soil fertilizers and amendments is advisable.

Keywords: dredging; heavy metal; long-term cultivation; health risk assessment;

INTRODUCTION

Sediments are heterogeneous materials composed by sand, silt, clay and organic matter formed by the erosion processes, and transported by wind and rivers to their final sinks such as plains and coastal areas (Salomons and Brils, 2004). In urban and industrial areas, sediments are periodically excavated to keep suitable port docking depth and for preventing flooding from rivers, canals and lakes. In industrialized areas, sediments dredging is also a measure for preventing water pollution, as contaminated sediments act as secondary pollutant sources for the water bodies due to changes in water chemical properties or during physical disturbance of the bottom (Eggleton and Thomas, 2004). According to the environmental legislation of several countries, unpolluted dredged sediments can be used as inert materials for e.g. beach nourishment, embankments, filling of dismissed quarries, road construction materials. Differently, contaminated sediments are managed as waste, therefore stored in confined sea tanks or landfilled because the current physico-chemical technologies for sediment decontamination are often not sustainable and, therefore, not extensively practiced for sediment remediation (Alvarez-Guerra et al., 2008).

Phytoremediation is a sustainable management biotechnology to remediate sediments contaminated by inorganic and organic pollutants potentially allowing their productive re-use (Bert et al., 2009). In fact, plants growing on dredged sediments enrich them with nutrients, such as C and N, increase their microbial biomass and activity, stabilize the heavy metals, and allow the degradation of the organic contaminants (Doni et al., 2015). The result of the cooperative actions of the microbial biomass and plant growth on dredged sediments leads to a general amelioration of their physical, chemical and biological fertility, reduce their eco-toxicity, converting them into technosols suitable as growing media for ornamental plants (Mattei et al., 2017). Phytoremediation of dredged sediments is therefore in line with the indications of all major international conventions on sediment management (i.e. OSPAR 1992), that urge all the stakeholders to consider the dredged sediments as a resource, not a waste.

Horticulture is essential for human nutrition at worldwide level, but it is among the most intensive agricultural practices, resulting in environmental impact due to the large use of fertilizers and pesticides, and to high water consumption. In soilless horticulture, a large part of the overall economic and environmental impact is represented by the use of peat and coir pith as growing media. It is estimated that 60 million m^3 of peat are annually used in EU (Bos et al., 2011), and Italy imports ca. 1 million m^3 of peat from Baltic Countries for producing technical growing media (Schmilewski, 2009). While peat is the horticultural growing media of choice, owing to its physical and chemical properties, its use is no longer sustainable due to impacts caused by extraction, drying and bulking operations, and transportation over long distances. Life cycle analysis (LCA) applied to the plant nursery sector indicated that peat based growing media are major contributors to the whole production process (Lazzerini et al., 2016; Mattei et al., 2018). Moreover, peat is used for a single production cycle due to its loss of physico-chemical fertility, and disposal of exhausted growing media is a major factor causing environmental impact and additional costs in the plant nursery industry and horticulture (Diara et al., 2012). Similar problems are envisaged for the coir pith, a by-product of coconut production mainly produced in South-West India, although there is still no sufficient knowledge from case studies. Although several Countries have long planned phasing out from the use of peat for soilless horticulture, such materials have been included as component material categories in the ambit of the new EU Regulation 2019:1009 on soil fertilizers and amendments, due to the difficulties of peat replacement with alternative materials, such as compost or sludge, affected by unsuitable and variable physico-chemical properties causing poor plant quality (Abad et al., 2001; Chong and Purvis, 2011; De Lucia et al., 2013; Noguera et al., 2003). Differently, dredged sediments have been excluded from the component material categories of EU Regulation 2019:1009, mainly due to the lack of sufficient knowledge on their potential beneficial use and safety.

We hypothesized that phytoremediated sediments could be used as ingredients for preparing growing media suitable for horticultural plants. We tested our hypothesis by growing strawberry plants on remediated sediments alone, commercial peat-based growing media and a mix of sediment and peat. Strawberry plant was selected as model plant because it is highly sensitive to quality and contamination of growing media, and it is a high added value plant produced only on soilless media (Tozzi et al., 2020). We evaluated the growth and productivity of two strawberry cultivars with different production cycles, and the fertility parameters of the peat- and sediment-based growing media. We also determined the potential impacts on human health related to consumption of strawberry cultivated on both peat- and sediment-based growing media. We hypothesized that sediment-based growing media could maintain their fertility and support the plant growth and productivity longer than the peat-based growing media. We therefore monitored the growth of the strawberry cultivars on the peat- and sediment-based growing media over two productive seasons.

MATERIALS AND METHODS

Experimental design

Sediments were dredged from the Industrial Port of Livorno (Central Italy, 43°33′25″N, 10°17′39″E) in 2008, and subjected to three years of phytoremediation using mixed herbs and shrubs from the native flora as previously reported (Masciandaro et al., 2014). After phytoremediation, sediments were homogenised and further reclaimed by three months of landfarming. Landfarmed sediments were used alone or mixed with a peat-based commercial substrate as it follows: sediment only (TS100), peat:sediment 50:50 v:v (TS50), and commercial peat only (TS0) considered as the control treatment. Certified strawberry plants (*Fragaria x ananassa* Duch.) of Camarosa, a cultivar blooming only once per season and Monterey, a re-blooming cultivar, were cultivated outdoor in 106 L rectangular plastic pots containing the treated substrates (i.e. TS100, TS50 and TS0) during the spring-summer periods of the years 2016, according to Tozzi et al. (2020), at the Department of Agriculture, Food, Environment and Forestry (DAGRI, University of Florence, 43°81′68″N, 11°19′99″E). After the first year, the growing media were not removed from the pots, and were covered with polyethene film and stored indoor to be reused for the 2017 growing season. Each year, the agronomic season was from the end of February to early September when fruit harvest was completed. Every year at plant transplanting, all pots were fertilized with NPK fertilizer (100 g per pot) (Nitrophoska Gold Compo). Each cultivar/substrate combination was replicated in 2 blocks, consisting of 10 plant replicates (10 replicates x 3 treatments x 2 blocks). A completely randomized block design was applied, while each treatment (cultivar-substratum) was independently run in two blocks. As a result, 120 strawberry plants each year were grown.

Analysis of the growing media

At the end of the cultivation all growing media were collected from each block and treatment for analyses. The sediment pH value was measured in 1:2.5 (*w:v*) aqueous suspensions by a GLP 22 (CRISON, Spain) pH-meter. Electrical conductivity (EC) were measured in a 1:5 (*w:v*) aqueous solution (AG 8603 Seven-Multi, Mettler Toledo, and Orion model 150). Total organic C (TOC) and total N content (TN) were determined by dry combustion with RC-412 multiphase carbon and a FP-528 protein/nitrogen analyzer (LECO, Saint Joseph MI, USA). NH_4^+ and NO_3^- were measured in the water extract with selective electrode (Sevenmulti Mettler Toledo). Available P concentrations were determined by the colorimetric method (Murphey and Riley, 1962) on water extracts (1:5 *w:v*). For macro and micro-nutrients (K, Ca, Mg, Fe and Cu), samples were diluted in deionized water, centrifuged for 20 min and filtered prior to analysis. Heavy metal availability (Cd, Cr, Ni, Pb and Zn) was determined by extractions with 1M NH_4NO_3 neutralized with concentrated ammonia as described (Renella et al., 2004). Nutrients and heavy metals concentrations were measured by inductively coupled plasma (ICP) (iCAP 7400 DUO ICP-OES; Thermo Fisher Scientific, Waltham, MA, USA). The sediment toxicity to microorganisms was evaluated by the BioTox™ Flash Test (Aboatox Oy, Turku, Finland) based on the inhibition of the luciferase activity of the bioluminescent *Vibrio fisheri* bacteria, according to the method of Lappalainen et al. (Lappalainen et al., 2001). Bacterial bioluminescence was determined using a Sirius Luminometer (Berthold, Germany), and the bioluminescence inhibition percentage (inh%) according to the formula:

$$\text{inh\%} = 100 - 100 \times [(IT_{15}/(KF \cdot \times IT_0)] \quad (1)$$

where, IT_{15} and IT_0 are the luminescence values of samples at time 15 min and zero, respectively, and KF is a correction factor given by the ratio between the luminescence after 15 minutes and that at zero time in control samples. Samples were considered toxic when the inhibitory effects were >20% (Persoone et al., 2003).

Plant and fruit analyses

Fruit sampling were performed according to Tozzi et al. (2020). Briefly, fruit yield was determined as total production over the experimental period. Fruits harvested from each plant were separately freeze-dried and stored at -20°C until analysis. Within each cultivar and treatment, six freeze-dried fruits were randomly selected and used for the analysis of elements. At the end of fruit production, 20 plants for each cultivar and treatment were harvested and six were randomly selected and used for the calculation of plant fresh and dry weight by oven-drying at 70°C until constant weight. Concentrations of Ca, K, P, Mg, Cu, Fe, Zn, Ni, Cr, Pb, and Cd were determined on oven-dried leaf samples and freeze-dried fruit samples. Briefly, an aliquot of 500 mg of dry leaves or fruits were grinded in a ceramic mortar and mineralized by microwave-assisted digestion (model Ethos 1, Milestone, Bergamo, Italy) using 25 mL of H_2O_2:HNO_3 1:3 (*v/v*) and analysed by ICP. Concentrations of C and N in leaves were determined on the same samples using a C N analyser (Thermo Scientific EA 1112 series).

Health risk assessment

Health risk related to strawberry consumption was assessed for Cu, Fe, Zn, Ni, and Cr by calculating the Estimated Dietary Intake (EDI) based on element concentration in fruit and the daily intake of it using the formula:

$$EDI = (C_{metal} \times D_{food\ intake})/B_W \qquad (2)$$

where C_{metal} is the element concentration (mg kg^{-1} on a fresh weight basis) in fruit, $D_{food\ intake}$ is the daily average strawberry intake assumed to be 4.9 g day^{-1} (US EPA, 2018) and B_W is the body weight for an adult, assumed to be 70 kg as in previous studies (Antoniadis, et al., 2019b).

The Health Risk Index (HRI) was calculated with the formula:

$$HRI = EDI/Rfd \quad (3)$$

where R*f*D (mg kg^{-1} body weight day^{-1}) represents the reference oral dose, which is an estimate of daily exposure of humans to heavy metals having no hazardous effect during the whole lifetime (USEPA IRIS, 2013).

Data analysis

Normality and homogeneity of parameters were tested prior to ANOVA, and data were normalized by transformed as needed. One-way ANOVA followed by the Fisher multiple range test at the level of significance of 95% ($P < 0.05$) was used for substrate (pH, EC, TOC, TN, NO_3^-, NH_4^+, macro and micro-nutrients and heavy metal availability concentration) and plant parameters (biomass and productivity and element concentration in leaves and fruits).

RESULTS

Analysis of the growing media

The pH value of the sediment-based growing media (i.e. TS50 and TS100) ranged between 6.94 and 7.69, which were significantly higher than that of the peat-based growing media (4.69-6.61) (**Table 1**). The EC value was significantly higher in TS100, and lower in TS0 for both cultivars. The highest values of TOC and TN were found in the peat-based growing media (TS0) as compared to sediment-based media in both years. Considering NO_3^- availability, the two cultivars behaved similarly, showing significant higher NO_3^- concentration in TS0 substrates, followed by TS50 and TS100 for 2016 season, and, in 2017, higher values for the two sediment-based substrates (22-25 mg L^{-1}) and lower for control substrates (8-9 mg L^{-1}). Regarding NH_4^+, only the growing media of the Monterey cultivar showed significant differences, showing higher values in the sediment-based media (TS50 and TS100) for 2016. In 2017, the TS100 growing media of the Camarosa cultivar showed higher NH_4^+ concentration, followed by TS50 and TS0, whereas for the Monterey cultivar, the TS0 showed the highest values. All growing media showed no significantly differences in P availability for the 2016, with values ranging from 1.92 to 2.47 mg kg^{-1}, while in 2017, the highest P concentration was found in the peat-based growing media (1.62 and 1.65 mg kg^{-1} for Camarosa and Monterey respectively).

Availability of heavy metals was generally low in all the investigated growing media, especially for the TS0 which showed values always below the detection limit (**Table 2**). Availability of Cd and Ni showed similar trends, with significantly lower values in the TS100 than in TS50 growing medium for both cultivars in years 2016, while in 2017 their availability was below the detection limit. Available Pb was detected only in TS50 and TS100 growing media in 2016, with similar values ranging from 0.024 to 0.027 mg kg^{-1}, while the Zn availability was significantly higher in the TS50 than in TS100 growing media for both cultivars and seasons (**Table 2**). Both cultivars showed in 2016 significant higher K and Fe availability for TS50 and TS100 substrates, while the trend found in 2017 was: TS100>TS50>TS0 (**Table 3**). Peat-based substrates showed the lowest Mg availability, while the TS50 substrates showed the highest Ca concentration generally for both cultivars and years. Cu availability was found higher in TS50 and TS100 in 2016 and 2017 seasons, respectively.

The BioTox test assays resulted in bioluminescence inhibition lower than 20% for both cultivars in all the growing media and investigated season (**Fig. 1S A and B**).

Table 1- Main physico-chemical properties found in TS0, TS50 and TS100 growing media at the end of 2016 and 2017. Values are the means (n=3) and standard deviations. Different letters in a column indicate significant differences (*P<0.05*).

Cultivar	Season	Substrate	pH$_{(H20)}$	EC (dS m^{-1})	TOC (%)	TN (%)	N-NO$_3^-$ (mg L^{-1})	N-NH$_4^+$ (mg L^{-1})	P (mg kg^{-1})
Camarosa	2016	TS0	6.61 (0.03) b	0.22 (0.01) c	22 (3) a	0.61 (0.08) a	27 (4) a	0.43 (0.16) a	2.01 (0.30) a
		TS50	7.67 (0.13) a	0.28 (0.01) b	6.7 (1.5) b	0.20 (0.06) b	8.0 (1.4) b	0.52 (0.11) a	2.47 (0.77) a
		TS100	7.67 (0.02) a	0.30 (0.01) a	1.7 (0.2) c	0.15 (0.04) b	1.8 (1.1) c	0.67 (0.21) a	2.29 (0.43) a
	2017	TS0	4.69 (0.16) b	0.14 (0.01) c	10 (3) a	0.35 (0.08) a	9 (1) b	0.013 (0.005) c	1.62 (0.002) a
		TS50	7.02 (0.47) a	0.32 (0.01) a	6 (1) b	0.16 (0.02) b	24 (2) a	0.024 (0.001) b	0.72 (0.08) b
		TS100	7.00 (0.03) a	0.30 (0.01) a	1.4 (0.5) c	0.11 (0.03) b	24 (1) a	0.143 (0.003) a	0.59 (0.05) b
Monterey	2016	TS0	6.50 (0.07) b	0.17 (0.01) c	25 (4) a	0.74 (0.06) a	22 (2) a	0.39 (0.13) b	1.92 (0.34) a
		TS50	7.60 (0.04) a	0.28 (0.01) b	3.8 (0.7) b	0.19 (0.06) b	7.6 (2.1) b	0.82 (0.07) a	2.36 (0.62) a
		TS100	7.69 (0.12) a	0.30 (0.01) a	1.5 (0.3) b	0.14 (0.03) b	2.8 (1.1) c	0.89 (0.15) a	2.36 (0.39) a
	2017	TS0	5.02 (0.25) b	0.19 (0.01) c	6.5 (0.9) a	0.20 (0.06) a	8 (1) b	0.111 (0.041) a	1.65 (0.22) a
		TS50	6.94 (0.34) a	0.29 (0.01) b	3.2 (0.3) b	0.17 (0.02) b	25 (5) a	0.016 (0.003) b	0.59 (0.03) b
		TS100	7.24 (0.57) a	0.35 (0.01) a	1.5 (0.3) b	0.10 (0.03) b	22 (1) a	0.019 (0.008) b	0.52 (0.07) b

Table 2- Heavy metal availability (Cd, Cr, Ni, Pb and Zn, mg kg^{-1}) found in TS0, TS50 and TS100 growing media at the end of 2016 and 2017. Values are the means (n=3) and standard deviations. Different letters in a column indicate significant differences (*P<0.05*).

Cultivar	Season	Substrate	Cd	Cr	Ni	Pb	Zn
Camarosa	2016	TS0	<0.01 c	<0.01 a	<0.01 c	<0.01 b	<0.01 c
		TS50	0.020 (0.005) a	<0.01 a	0.039 (0.01) a	0.027 (0.005) a	0.24 (0.02) a
		TS100	0.016 (0.005) b	<0.01 a	0.029 (0.01) b	0.025 (0.00) a	0.18 (0.03) b
	2017	TS0	<0.01 a	<0.01 a	<0.01 a	<0.01 a	<0.01 c
		TS50	<0.01 a	<0.01 a	<0.01 a	<0.01 a	0.632 (0.02) a
		TS100	<0.01 a	<0.01 a	<0.01 a	<0.01 a	0.55 (0.08) b
Monterey	2016	TS0	<0.01 c	<0.01 a	<0.01 c	<0.01 b	<0.01 c
		TS50	0.018 (0.003) a	<0.01 a	0.037 (0.012) a	0.026 (0.004) a	0.24 (0.02) a
		TS100	0.014 (0.001) b	<0.01 a	0.027 (0.006) b	0.024 (0.003) a	0.19 (0.02) b
	2017	TS0	<0.01 a	<0.01 a	<0.01 a	<0.01 a	<0.01 c
		TS50	<0.01 a	<0.01 a	<0.01 a	<0.01 a	1.07 (0.46) a
		TS100	<0.01 a	<0.01 a	<0.01 a	<0.01 a	0.52 (0.15) b

Table 3- Macro and micro-nutrients availability (K, Mg, Ca, Fe and Cu, mg L^{-1}) found in TS0, TS50 and TS100 growing media at the end of 2016 and 2017. Values are the means (n=3) and standard deviations. Different letters in a column indicate significant differences (*P<0.05*).

Cutivar	Season	Substrate	K	Mg	Ca	Fe	Cu
Camarosa	2016	TS0	8 (1) b	1.9 (0.2) b	10 (1) a	2.9 (0.3) b	0.004 (0.001) c
		TS50	12 (1) a	4.8 (0.1) a	11 (1) a	7.5 (0.2) a	0.026 (0.002) a
		TS100	13 (1) a	4.6 (0.3) a	9 (1) a	6.2 (0.3) a	0.014 (0.001) b
	2017	TS0	1.5 (0.2) c	1.1 (0.1) b	2.4 (0.6) c	3.3 (0.3) c	0.017 (0.002) c
		TS50	22 (5) b	9 (2) a	10 (1) a	27 (6) b	0.064 (0.008) b
		TS100	31 (2) a	11 (1) a	8.4 (0.4) b	39 (5) a	0.086 (0.004) a
Monterey	2016	TS0	5 (2) b	2.1 (0.6) b	7.6 (0.3) b	2.5 (0.6) b	0.005 (0.002) c
		TS50	13 (1) a	5 (1) a	12 (1) a	7 (1) a	0.029 (0.002) a
		TS100	15 (3) a	4.4. (0.1) a	8 (1) b	7.5 (0.4) a	0.016 (0.001) b
	2017	TS0	2.4 (0.4) c	1.2 (0.2) b	3.6 (0.6) b	3.2 (0.8) c	0.016 (0.001) c
		TS50	17 (1) b	8 (1) a	9.1 (0.4) a	22 (4) b	0.056 (0.001) b
		TS100	27 (3) a	10 (2) a	9 (1) a	31 (3) a	0.082 (0.003) a

Plant biomass and fruit yield

Plant biomass and plant water content were significantly reduced in 2016 for both cultivars grown on sediment-based growing media, whereas no differences were detected in 2017 (**Table 4**). The cultivars displayed significantly lower fruit weight and yield when grown on TS50 and TS100 in 2016, and significantly higher weight and yield on TS50 substrates in 2017 (**Table 4**).

Table 4- Mean values of total plant dry weight (g) and total plant water content (%), fruit weight (g) and fruit yield (g plant$^{-1)}$ of Camarosa and Monterey grown in TS0, TS50 and TS100 growing media at the end of 2016 and 2017. Values are the means (n=6) and standard deviations. Different letters in a column indicate significant differences (*P<0.05*).

Cultivar	Season	Substrate	Total dry weight (g)	Total water content (%)	Fruit weight (g)	Fruit yield (g pant^{-1})
Camarosa	2016	TS0	33 (4) a	77 (2) a	8.2 (4.1) a	120 (63) a
		TS50	30 (4) ab	69 (4) b	7.6 (3.6) b	119 (79) a
		TS100	27 (4) b	66 (5) b	5.7 (2.6) c	72 (32) b
	2017	TS0	38 (5) a	75 (8) a	6.4 (2.9) c	55 (33) b
		TS50	42 (10) a	77 (6) a	10.9 (3.4) a	131 (75) a
		TS100	39 (6) a	73 (6) a	8.1 (3.2) b	100 (62) a
Monterey	2016	TS0	33 (5) a	75 (2) a	10 (4.8) a	295 (129) a
		TS50	26 (3) b	71 (5) ab	7.1 (4.0) b	138 (85) b
		TS100	23 (2) b	70 (2) b	6.6 (3.9) b	86 (42) b
	2017	TS0	30 (7) a	74 (3) a	8.7 (4.1) b	217 (104) a
		TS50	40 (5) a	76 (4) a	10.5 (4.8) a	268 (105) a
		TS100	37 (6) a	70 (5) a	8.2 (4.4) b	148 (71) b

Leaf elemental concentration

Carbon concentration in leaves of Camarosa and Monterey cultivars (**Fig. 1A and C**) were similar in year 2016, whereas it was higher for plant cultivated on TS50 and TS100 substrates for both 2017. The N concentration of the Camarosa leaves showed higher concentrations on TS0 in 2016 and 2017 (**Fig. 1B**), whereas, for the Monterey cultivar, significant differences occurred in 2017, with higher values for TS0 and TS50 (**Fig. 1D**).

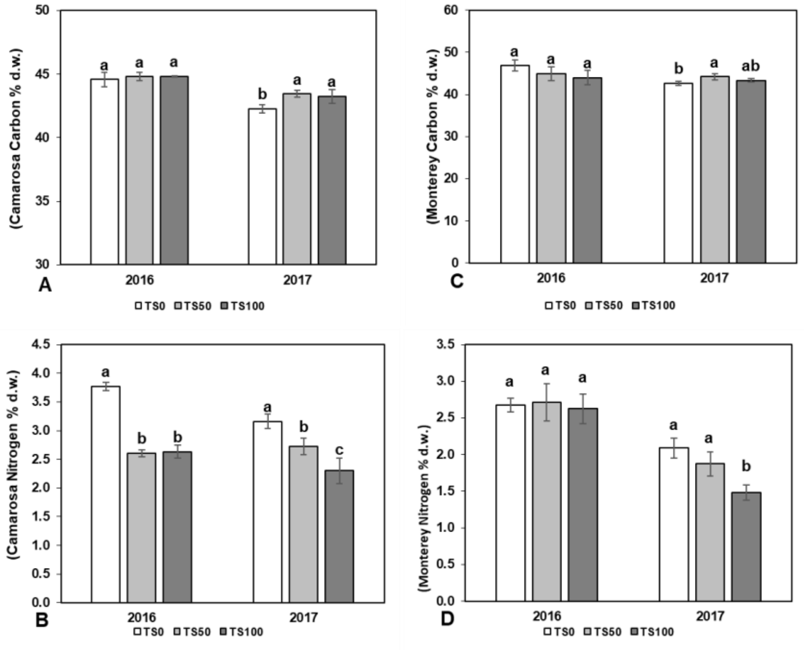

Figure 1- Leaf carbon and nitrogen (% d.w.) concentration of Camarosa (A-B) and Monterey (C-D) grown in TS0, TS50 and TS100 growing media at the end of 2016, 2017 and 2018. Error bars represent standard deviation (n=6).

Calcium in leaves of plants cultivated on TS50 and TS100 growing media showed higher concentrations during the cultivation seasons 2016 for both cultivars, while the only differences detected in 2017 regarded Monterey leaves grown on TS50 substrates exhibiting the highest Ca concentration (27 mg kg^{-1}) (**Table 5**). The K concentration in Camarosa leaves showed no differences in 2016 and higher concentration when grown on TS50 in 2017, while for the Monterey cultivar, K concentrations were significantly higher when grown on TS0 and TS50 in 2016 and 2017. Potassium and Mg concentrations in leaves of the two strawberry cultivars were always higher for plants on TS0 growing media regardless of the cultivation year (**Table 5**). Iron and Ni concentrations in leaves were similar for all cases in 2016, whereas in 2017 it was lower for leaves of plants grown on TS0. Zinc concentrations in the Camarosa and Monterey leaves were significantly higher when grown on TS0 in 2016. In the 2017, for the Monterey cultivar, the Zn concentration in leaves was significantly higher when grown on TS50 and TS100 in 2017, while Camarosa and displayed higher values for TS0 (**Table 5**). Leaf Cu concentrations were significantly lower for both cultivars grown on TS0 as compared to sediment-based growing media in all two cultivations seasons (**Table 5**). The Cr leaf concentrations were below the instrumental detection limit in 2016, whereas in 2017 foliar Cr concentrations were detected with higher values for plants grown on sediment-based growing media, except for the Monterey plants of 2017 in which the trend was: TS100>TS0>TS50. Cadmium and Pb concentration were below the detection limit in leaves of both cultivars regardless the cultivation season and the growing media.

Table 5- Leaf element concentration (Ca, K, P, Mg expressed as g kg^{-1} d.w., and Fe, Cu, Ni, Zn, Cr expressed as mg kg^{-1} d.w.) of Camarosa and Monterey cultivated in TS0, TS50 and TS100 growing media during the season 2016 and 2017. Different letters in a column indicate significant differences ($P<0.05$) among mean values (n=6).

Cultivar	Season	Substrate	Ca	K	P	Mg	Fe	Cu	Ni	Zn	Cr
Camarosa	2016	TS0	7 (0.4) b	12 (2) a	1.9 (0.2) a	2.6 (0.3) a	53 (9) a	1.9 (0.3) b	0.6 (0.1) a	27 (3) a	<0.1 a
		TS50	10 (1) a	12 (1) a	0.9 (0.01) b	2.1 (0.3) b	46 (5) a	4.4 (1.1) a	0.6 (0.1) a	20 (3) b	<0.1 a
		TS100	10 (1) a	12 (1) a	0.9 (0.1) b	2.1 (0.3) b	49 (5) a	3.7 (0.7) a	0.6 (0.1) a	20 (3) b	<0.1 a
	2017	TS0	14 (1) a	20 (1) b	5.4 (0.3) a	5.5 (0.3) a	82 (5) b	2.9 (0.8) b	0.4 (0.1) b	29 (2) a	0.2 (0.1) b
		TS50	12 (1) a	23 (2) a	2.4 (0.2) b	2.6 (0.6) b	94 (8) a	6.6 (0.5) a	0.6 (0.1) a	18 (2) c	0.7 (0.2) a
		TS100	12 (2) a	20 (1) b	2.5 (0.1) b	2.4 (0.1) b	93 (10) a	6.6 (0.8) a	0.4 (0.1) b	23 (1) b	0.4 (0.1) a
Monterey	2016	TS0	16 (1) b	12 (1) ab	3.5 (0.3) a	3.73 (0.65) a	50 (7) a	2.7 (0.7) b	1.3 (0.2) a	37 (6) a	<0.1 a
		TS50	22 (1) a	13 (1) a	1.5 (0.2) b	2.79 (0.17) b	41 (7) a	4.3 (1.1) a	0.2 (0.1) b	25 (5) b	<0.1 a
		TS100	22 (2) a	11 (1) b	1.2 (0.1) c	2.75 (0.20) b	41 (6) a	4.3 (1.4) a	0.2 (0.1) b	22 (5) b	<0.1 a
	2017	TS0	19 (1) c	15 (1) a	5.3 (0.2) a	3.01 (0.11) a	71 (6) b	2.5 (0.8) c	0.3 (0.01) b	15 (5) b	0.8 (0.3) b
		TS50	27 (1) a	14 (1) b	1.5 (0.2) b	2.23 (0.10) b	85 (7) b	5.1 (0.6) b	0.4 (0.1) b	25 (8) a	0.4 (0.1) c
		TS100	23 (2) b	11 (1) c	1.4 (0.01) b	2.21 (0.09) b	125 (17) a	6.6 (0.9) a	1.2 (0.2) a	33 (5) a	1.3 (0.1) a

Heavy metal concentrations in fruits

No significant differences were found in fruit Fe concentrations of both cultivars in 2016 regardless of the growing media, whereas significantly lower Fe values were found in fruits of Camarosa and Monterey grown in TS0 and TS50 in 2017 (**Table 6**). The only difference detected for Cu concentration regarded Camarosa fruits exhibiting significantly higher values for TS0 and TS50 in 2016 and higher values for TS100 in 2017. Ni concentrations were similar regardless of the cultivar, growing media and cultivation season, whereas Zn concentration showed higher values for TS0 fruits in 2016 and 2017 seasons in both cultivars. Fruit Cr concentrations were below the detection limits in both cultivars in 2016 and not significantly different in fruits of the Camarosa cultivar in 2017. For Monterey cultivar, significantly lower Cr concentrations were found for fruits of plants grown on TS50 and T100 in 2017 (**Table 6**). No detectable Cd and Pb concentrations were observed in fruits of the two strawberry cultivars regardless of growing media and cultivation seasons.

Table 6- Fruit micro-nutrient (Fe, Cu, Mn, Ni, Zn and Cr, mg kg^{-1} d.w.) concentrations of Camarosa and Monterey grown in TS0, TS50 and TS100 growing media at the end of 2016 and 2017. Values are the means (n=6) and standard deviations. Different letters in a column indicate significant differences ($P<0.05$).

Cultivar	Season	Substrate	Fe	Cu	Ni	Zn	Cr
Camarosa	2016	TS0	66 (8) a	3.8 (0.8) a	0.33 (0.08) a	15 (4) a	<0.1 a
		TS50	60 (7) a	3.2 (0.5) a	0.25 (0.03) a	11 (2) a	<0.1 a
		TS100	64 (10) a	2.3 (0.4) b	0.31 (0.09) a	13 (2) a	<0.1 a
	2017	TS0	22 (2) b	1.8 (0.6) c	0.25 (0.02) a	22 (3) a	0.20 (0.08) a
		TS50	23 (2) b	3.5 (0.7) b	0.28 (0.21) a	17 (5) b	0.18 (0.02) a
		TS100	29 (4) a	5.3 (0.7) a	0.21 (0.03) a	14 (7)b	0.24 (0.05) a
Monterey	2016	TS0	69 (9) a	3.9 (0.9) a	0.27 (0.05) a	16 (3) a	<0.1 a
		TS50	62 (12) a	3.5 (1.0) a	0.35 (0.12) a	12 (2) b	<0.1 a
		TS100	62 (9) a	3.6 (0.7) a	0.31 (0.06) a	10 (3) b	<0.1 a
	2017	TS0	20 (3) b	1.5 (0.7) a	0.54 (0.05) a	17 (4) a	0.31 (0.04) a
		TS50	22 (3) b	2.3 (0.7) a	0.42 (0.14) a	11 (2) b	0.15 (0.10) b
		TS100	42 (5) a	2.6 (0.8) a	0.47 (0.19) a	10 (3) b	0.14 (0.02) b

Health risk assessment

The two cultivars showed similar EDI values, with the highest intake resulting for Fe and Zn, and the lowest intake for Cr and Ni (**Table S1**). For each heavy metal, the calculated EDI values resulted below the reference oral dose (R*f*D) (**Table S1**). In all cases, the calculated Health Risk Index (HRI) values were below 1.0 (**Table S2**), that is the threshold considered for the health protection of life-time risk for humans (USEPA IRIS, 2013).

DISCUSSION

The sediment-based growing media allowed the cultivation of two strawberry cultivars grown for two consecutive years despite their physico-chemical properties were different from those of commercial peat (**Table 1**). The reduced plant growth and lower fruit yield in both strawberry cultivars grown on the sediment-based growing media observed in year 2016 (**Table 4**) was ascribed to the high bulk density and low porosity of these growing media which hampered the root development, and induced stress in plants, as previously reported in Tozzi et al., (2020). Strawberry plants generally grow with poor root-soil contact, with shallow roots displaying a morphology which depends on the growing media, spanning from 90% to 50% in the top 15 cm in clayey and sandy loam soils, respectively (Trejo-Tellez and Gomez-Merino, 2014). Growing media fertility, water supply, and aeration at depths greater than 15 cm represent major factors favouring strawberry root proliferation (Trejo-Tellez and Gomez-Merino, 2014). These results paralleled those of Tozzi et al., (2019) on reduced growth and yield of lettuce grown on sediment-based growing media, mainly due to high bulk density of the growing media, although it can not be excluded that the lower plant growth recorded in the 2016 could be also related to the initial lower sediment N content (**Table 1**). Nitrogen is among the main nutrient limiting the plant growth, and for strawberry adequate N content is essential for reaching the full plant growth and fruit size (Santos and Chandler, 2009; Tagliavini et al., 2005). Since the significant reduction of plant biomass and fruit yield of both cultivars grown on sediment-based growing media was only observed in 2016 (**Table 4**), an improvement of the physical and chemical fertility of the sediment occurred in the subsequent year. The re-cultivation has improved the sediment structure by increasing the aerobic condition and consequently supporting a major organic matter mineralization which have released N and soluble elements. In fact, along with the better growing media structure, also the significant increase of $N\text{-}NO_3^-$ (**Table 1**) and soluble macro and micro-nutrients (i.e. K, Ca, Fe and Cu) (**Table 3**) recorded in the growing media of both cultivars after the first season of cultivation may have supported higher fruit productivity (**Table 4**). Generally, Fe, Cu, and Zn concentrations were significantly higher in leaves of both varieties cultivated on sediment-based substrates at the end of 2017 (**Table 5**). Micronutrients play an essential role in supporting both strawberry vegetative and reproductive (flowering and fruiting) development (Chaturvedi et al., 2005; Lieten, 2003). Overall, these results indicated that, after the first year of cultivation, the sediment-based growing media ensured sufficient nutrient availability for optimal plant productivity in spite of their alkaline pH values, and overall no nutrient deficiency symptoms such as marginal yellowing, browning and chlorosis nor necrosis and also fruits were observed.

Both strawberry cultivars grown on peat showed a strong decrease in productivity passing from the first to the second year of cultivation. Such decrease in yield could be related to the peat acidification observed in 2017 (**Table 1**), to levels below the optimal pH values for horticultural growing media (Abad et al., 2001). The acidification of the peat recorded in 2017 could be due to a greater plant absorption of physiologically active cations (e.g. K^+, Mg^{2+}, Ca^{2+}) and to the release of H^+ by plant roots (Marschner, 1995), and to $N\text{-}NO_3^-$ released from the organic matter mineralization followed by nitrification, as discussed before.

The sediment-based growing media showed a relatively low heavy metal availability (**Table 2**), except for Zn that was the only metal showing an increase availability throughout the two seasons in both TS50 and TS100 growing media for the two cultivars. This result could be explained by the alkaline pH value of the sediment-based growing media (**Table 1**) and the release of Zn absorbed onto carbonates that have reduced the metals solubility by precipitation (Shaheen et al., 2018) induced by plant growth. The low solubility and bioavailability of heavy metals could be the main factor explaining the lack of eco-toxicity during the cultivation of Camarosa and Monterey plants (**Figure S2A and B**). The absence of detectable Cd and Pb transfer to leaves and fruits paralleled the results of Tozzi et al. (2019) who did not observe heavy metal accumulation by lettuce grown on sediment-based growing media.

Strawberry plants grown on TS50 and TS100 media absorbed Ni, Cr and Zn in leaves and fruits at concentrations (**Table 5 and 6**) similar to those found in strawberry cultivated on commercial growing media (Akhatou and Fernández Recamales, 2014). The health risk assessment based on the human exposure pathway through strawberry consumption calculated through the estimated daily intake (EDI) and health risk index (HRI) for Fe, Cr, Ni, Zn, Cu, (**Table S1 and S2**), showed the consumption of strawberry cultivated on sediment-based media do not represent a risk for human health, since the EDI found for all the investigated metals were far below the reference oral dose (R*f*D). Plant grown on contaminated soil can cause human exposure *via* accumulation of contaminants in the edible products (Antoniadis et al., 2019). Our results were in line with previous ones showing that lettuce and tomato grown on dredged sediments did not accumulate excessive concentrations of potentially toxic metals (Pb, Ni, Cr and Cd) in plant tissues (Canet et al., 2003), and that mixing of dredged sediments to biosolids improved barley plant growth and reduced heavy metal accumulation by plants (Ruiz Diaz et al., 2010). Globally, our results indicated the lack of risks for human health from strawberry plants grown on a phytoremediated sediment, which represent an extreme scenario for eventual sediment reuse in agriculture.

CONCLUSIONS

Marine sediments reclaimed by phytoremediation and landfarming proved to be potential suitable ingredients for growing media in horticulture, both in terms of plant productivity and food safety. An increasing plant productivity over two cultivation years was observed in plants grown on sediment-based respect to the peat-based growing media, due to the progressive improvement of physical and chemical fertility of the sediment matrix. This is interesting in the circular economy perspective, because it can lead to a reduced use of peat and less waste production in horticulture. For this reason, a re-evaluation of reclaimed sediments as a suitable category of component material in the ambit of the EU Regulation 2019:1009 on the fertilizing and amendments is advisable.

Limit of the research

This article was prepared considering data obtained on plants (i.e. biomass, productivity, elements in leaves and in fruits) and data analysed in the investigated substrates (i.e. chemical and toxicological parameters). The article is still in a preparatory phase as some analyses for the substrate characterization (i.e. bulk density and enzymatic activities) are still in progress. Furthermore, the statistical analyses, already carried out, will be integrated with other additional analyses, highlighting any significant trend over the two productive seasons. Therefore, the results here presented are to be considered preliminary as they will be studied in depth and discussed once all the analyses have been completed.

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SUPPLEMENTARY MATERIAL

S1. Analysis of the growing media

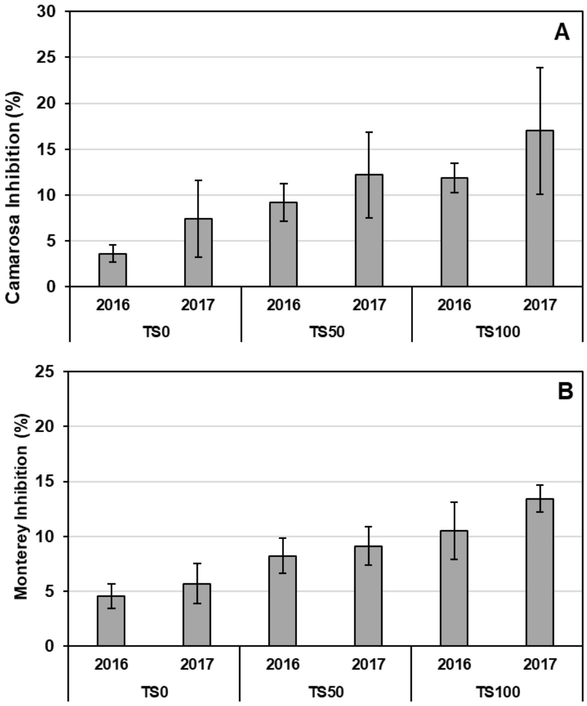

Figure S1- Bioluminescence trends in the Biotox text on leachates of the growing media (TS0, TS50 and TS100) of Camarosa (A) and Monterey (B) at the end of 2016 and 2017. Bioluminescence inhibition values below 20% indicate no toxicity. Error bars represent standard deviation (n=4).

S2. Health risk assessment in fruit

Table S1- Estimated Daily Intake (EDI) (mg kg^{-1} day^{-1}) resulting from the consumption of Camarosa and Monterey strawberry fruit cultivated on the growing media (TS0, TS50 and TS100) at the end of 2016 and 2017.

Element	Cultivar	2016			2017			R*f*D[a]
		TS0	TS50	TS100	TS0	TS50	TS100	
Cu	Camarosa	2.14E-05	2.26E-05	1.59E-05	1.32E-05	2.82E-05	4.32E-05	0.04
	Monterey	2.18E-05	2.67E-05	2.66E-05	1.31E-05	1.98E-05	2.19E-05	
Fe	Camarosa	3.72E-04	4.22E-04	4.50E-04	1.67E-04	1.88E-04	2.35E-04	0.70
	Monterey	3.90E-04	4.73E-04	4.62E-04	1.68E-04	1.87E-04	3.55E-04	
Ni	Camarosa	1.85E-06	1.73E-06	2.21E-06	1.85E-06	2.25E-06	1.74E-06	0.02
	Monterey	1.53E-06	2.70E-06	2.27E-06	3.47E-06	3.66E-06	4.51E-06	
Zn	Camarosa	8.44E-05	8.04E-05	8.86E-05	1.68E-04	1.39E-04	1.16E-04	0.30
	Monterey	8.71E-05	9.04E-05	7.27E-05	1.44E-04	9.89E-05	8.43E-05	
Cr	Camarosa	n.a.	n.a.	n.a.	1.52E-06	1.46E-06	1.94E-06	1.50
	Monterey	n.a.	n.a.	n.a.	2.66E-06	1.30E-06	1.15E-06	

[a] Reference oral dose, expressed as mg kg^{-1}day^{-1} (USEPA IRIS, 2013).

Table S2- Health Risk Index (HRI) resulting from the consumption of Camarosa and Monterey strawberry fruit grown on growing media (TS0, TS50 and TS100) at the end of 2016 and 2017.

Element	Cultivar	2016			2017		
		TS0	TS50	TS100	TS0	TS50	TS100
Cu	Camarosa	5.4E-04	5.7E-04	4.0E-04	3.3E-04	7.1E-04	1.1E-03
	Monterey	5.5E-04	6.7E-04	6.7E-04	3.3E-04	5.0E-04	5.5E-04
Fe	Camarosa	5.3E-04	6.0E-04	6.4E-04	2.4E-04	2.7E-04	3.4E-04
	Monterey	5.6E-04	6.8E-04	6.6E-04	2.4E-04	2.7E-04	5.1E-04
Ni	Camarosa	9.3E-05	8.7E-05	1.1E-04	9.2E-05	1.1E-04	8.7E-05
	Monterey	7.7E-05	1.4E-04	1.1E-04	1.7E-04	1.8E-04	2.3E-04
Zn	Camarosa	2.8E-04	2.7E-04	3.0E-04	5.6E-04	4.6E-04	3.9E-04
	Monterey	2.9E-04	3.0E-04	2.4E-04	4.8E-04	3.3E-04	2.8E-04
Cr	Camarosa	n.a.	n.a.	n.a.	1.0E-06	9.7E-07	1.3E-06
	Monterey	n.a.	n.a.	n.a.	1.8E-06	8.6E-07	7.7E-07

Article 4

Purple Queen® fruits of Punica granatum L.: a relation between reclaimed sediments and nutraceutical properties

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ABSTRACT

BACKGROUND**:** Purple Queen® is a new and early ripening pomegranate cultivar growing well in soilless cultivation. Plant substrates have direct effects on plant development and, due to progressive peatland exhaustion, the request for new materials suitable for plant growth and production is high. The objective of this work was to verify the effects of a new potential substrate obtained from the remediation of marine port sediments on the nutraceutical profile of Purple Queen® fruits, using 50% and 100% of reclaimed sediment. The study was carried out determining ellagitannins and polysaccharides obtained from peel after decoction, and anthocyanins in aril juice on fruits from two agronomic seasons.

RESULTS: The presence of 100% of the sediment had a partial detrimental effect on fruits size and weight due to its high sediment bulk density. Compared to a peat-based commercial substrate (control), the remediated port sediment preserved ellagitannin content and increased the anthocyanin amount (up to 40% higher) and polysaccharide content up to 34% on dry fruit. High molecular weight polysaccharides (<2000 kDa) were identified in all the fruits with glucose and galacturonic acid as the major sugars.

CONCLUSION: Fruits from plants cultivated only on sediment or in a mixture with 50% of peat showed significant increases of bioactive compounds in two consecutive seasons. Our results highlighted an increased production of bioactive compounds as stress response induced by dredged sediments.

Keywords: dredged sediments; ellagitannin; punicalagin; anthocyanin; pectin; polysaccharide.

INTRODUCTION

Pomegranate (*Punica granatum L.*) is classified among the top seven fruits with the highest beneficial properties for human health (Pereira et al., 2016). The fruits, usually consumed fresh or in form of juice, are widely appreciated by consumers and known as good source of nutraceutical compounds (Tomás-Barberán et al., 2017; Hussein et al., 2018). To date, it has been demonstrated that the health benefits from pomegranate are related to the intake of compounds present both in juice, mainly anthocyanins, and in peel (mesocarp + esocarp), mainly ellagitannins and polysaccharides. Pomegranate peel represents the 40-50% of the whole fresh fruit weight, and it is the main by-product obtained after juice extraction (Sing et al., 2018). The main bioactive molecules of pomegranate are ellagitannins as α punicalagin and β punicalagin, together with other minor constituents as ellagic acid and their derivatives (Fisher et al., 2011; Khatib et al., 2017). The ellagitannins of pomegranate are water soluble, consequently easily extractable by polar media as hydro-alcoholic solutions or a simple decoction, being these molecules also thermally stable (Joseph et al., 2013). Furthermore, nowadays, polysaccharides of pomegranate peel, are gaining growing scientific interest thanks to the their antioxidant effects (Joseph et al., 2013), antiglycation and tyrosinase inhibition properties (Rout et al., 2007), *in vitro* prebiotic properties (Khatib et al., 2017), immunomodulatory and anti-inflammatory effects (Ahmadi et al., 2018). It has been observed that a simple water decoction from mesocarp of pomegranate guaranteed the extraction of both ellagitannins and polysaccharides (Khatib et al., 2017), and that this extract showed the ability to counteract initial, intermediate and late stages of colon carcinogenesis in rats, suggesting a possible use of the decoction in primary and secondary prevention of human colon cancer (Tortora et al., 2018). Purple Queen® is greatly appreciated for its productivity and early ripening (second half of August in Alicante and Murcia, Spain). These peculiarities are notably functional for economic and marketing strategies since they broaden the availability of pomegranate fruits for both fresh consumption and processing. Registration has been requested for Purple Queen in various countries outside the European Union, especially in the southern hemisphere to respond to the demand for pomegranate fruit which has strongly increased over recent years.

Peat is one of the main components of substrates used in agriculture. However, due to the progressive exhaustion of peatland, the demand for alternative substrates with suitable physico-chemical properties is increasing. To meet sustainability criteria in the plant nursery industry, attention is currently focused on the reutilization of waste-derived substrates. Among these, dredged remediated sediments have already been proposed as soilless growing media for the cultivation of ornamental and food crops (Mattei et al., 2018; Tozzi et al., 2019).

Dredging, normally performed to guarantee navigability, involves the excavation of sediment from the bottom of water basins and its reallocation in another place (DelVall et al., 2004). This process generates a huge volume of dredged spoils which must be appropriately managed following national regulations. Unpolluted sediments are generally re-used for beach nourishment and embankments while contaminated sediments, being classified as waste, are placed in landfill. Phytoremediation has proven to be a sustainable technology for reclaiming highly polluted sediments (Bert et al., 2009) and for converting sediment into a “techno-soil” able to support vegetation (Masciandaro et al., 2014; Doni et al., 2015). Hence, the potential reallocation of remediated dredged materials in agriculture could provide an alternative solution to the disposal in landfills and, at the same time, reduce the intensive use of raw material as in the case of the requested peat. As reported by Mattei et al. (2018), the cultivation of an ornamental crop in remediated sediments showed a significantly lower environmental impact with respect to the use of traditional peat. Recently, dredged sediments were used as growing media for soilless cultivation of Purple Queen® evaluating the plant productivity and the main nutritional characteristic of the juice were evaluated (Melgarejo et al., 2018). The authors observed that fruit yield was partially reduced due to the high sediment bulk density which limited overall plant development. At the same time, fruits cultivated on sediment showed a significant increase of soluble solids, fructose and glucose content in the arils, indicating that the presence of sediment promoted greater dry matter accumulation in this tissue. The present study was focused on the characterization and quantification of the bioactive components of whole fruits (aryls and peel) obtained from Purple Queen® trees cultivated as previously described by Melgarejo et al. (2018). An evaluation of the nutraceutical quality of the fruits from two agronomic seasons was carried out determining anthocyanins in juice as well as polysaccharides and ellagitannins in peel after extraction by decoction. To the best of our knowledge, this is the first study investigating the effect of innovative substrates on the main nutritional components of the arils and peel from pomegranate fruits obtained in soilless cultivation.

MATERIALS AND METHODS

Experimental design and fruit sampling

Purple Queen® plants were cultivated in a remediated dredged sediment as described by Melgarejo et al. (2018). Briefly, the sediments were dredged from the Livorno port (Italy) and were subjected to three years of phytoremediation as described by Masciandaro et al. (2014). Afterwards, the sediment underwent three months of landfarming, a bioremediation consisting in periodic aeration and irrigation of the sediment in order to homogenize the matrix. The remediated sediment was used to prepare two growing substrates: PQ-100 (PQ2017-100 and PQ2018-100 samples), the remediated sediment alone, and PQ-50 (PQ2017-50 and PQ2018-50 samples) derived from the remediated sediment mixed with a traditional peat-based commercial substrate (1:1, v/v). The same peat-based commercial substrate present in PQ-0 was used as control treatment (PQ2017-0 and PQ2018-0 samples). Moreover, samples of Purple Queen® fruits purchased in local markets were also analysed (PQ-C) as further control samples. The main physico-chemical parameters of the substrates are presented in **Table 1**.

Table 1- Main chemical parameters of the commercial peat substrate (0), mixture sediments: peat v/v 1:1 (50), and remediated sediment used as pure (100). The values are the mean and standard deviation (n=3).

Parameters	0	50	100
pH	6.2 (0.2)	7.9 (0.2)	8.21 (0.3)
EC (µs cm^{-1})	1129 (7)	596 (14)	352 (16)
NH_4^+ (mg l^{-1})	1.03 (0.19)	0.25 (0.03)	0.08 (0.03)
NO_3^- (mg l^{-1})	304 (1)	190 (18)	26 (0.1)
TN (%)	1.3 (0.2)	0.29 (0.01)	0.12 (0.02)
PO_4 (mg l^{-1})	16.8 (0.2)	0.4 (0.0)	0.4 (0.0)
Chloride (mg kg^{-1})	20.5 (0.2)	12.4 (2.1)	11.4 (0.5)
K (mg kg^{-1})	65 (5)	19 (0.5)	9 (0.1)
Mg (mg kg^{-1})	22.1 (0.3)	11.4 (0.2)	8.4 (1.5)
Ca (mg kg^{-1})	114 (1)	67 (2)	31 (4)
Cu (mg kg^{-1})	14.1 (0.3)	43.7 (0.9)	55.4 (1.2)
Zn (mg kg^{-1})	13 (1)	170 (8)	194 (7)
Ni (mg kg^{-1})	0.2 (0.0)	35 (2)	39 (2)
Pb (mg kg^{-1})	1.4 (0.0)	34 (3)	38 (6)

The fruit samples used in this experiment were harvested at full ripening at the end of two growing seasons (2017 and 2018). All plants (PQ-0, PQ-50 and PQ-100) received a complete Hoagland nutrient solution, composed of KNO_3, NH_4NO_3, K_2SO_4, HNO_3, H_3PO_4, and a complex mix of microelements[18]. The arils and peel (mesocarp + exocarp) used for the analyses are presented in **Table 1S**.

Standards and reagents

All solvents were of analytical HPLC grade from Sigma Aldrich (St. Louis, Missouri, USA). Ultrapure water was obtained by the Milli-Q-system (Millipore SA, Molsheim, France). Ellagic acid (purity ≥ 95%) and punicalagin (purity≥ 98%) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Oenin chloride (purity ≥ 95%) was purchased from Extrasynthese (Genay, France). Dextrans at different molecular weights (2000, 1100, 410, 150, and 50 kDa) and sucrose (360 Da) used for SEC analyses were from Sigma-Aldrich, USA. The ellagitannins were quantified according to their maximum absorption at either 380 nm using a five-point calibration curve of a racemic mixture of α punicalagin and β punicalagin (purity ≥ 99%, linearity range 2 - 5 µg, R^2=1.000) or 370 nm using a five-point calibration curve of ellagic acid (purity 95%) (linearity range 0-1.7 µg, R^2=1.000). The anthocyanins were quantified at 520 nm with a four-point calibration curve of oenin chloride (purity ≥ 95%; linearity range 0-2.6 µg, R^2=0.999).

Anthocyanins from arils

Arils from PQ2018 (0; 50; 100) and Purple Queen® commercial sample (PQ-C) were used for the preparation of juices using a domestic Hurom extractor which guarantees the preparation of juices through a rapid process at low temperatures. The arils derived from fruits collected from the different soils were divided into three aliquots to obtain a triplicate. Each juice was diluted (1:1 v/v) with ethanol (2% HCOOH) to better stabilize the solution before analysis. The samples were then centrifuged at 14000 rpm for 5 min. The supernatant was recovered and analyzed by HPLC-DAD-MS.

Ellagitannins and polysaccharides from peel

The peel and arils from fruits (n=10) were manually separated, weighted and freeze-dried until constant weight was reached. The freeze-dried peel (5 g) was boiled in 200 mL of ultrapure water, for 1 h as for Khatib et al. (2017). The supernatant was recovered after cooling and centrifugation (4500 rpm, 8 min, 4 °C) and taken to a final volume of 200 mL with ultrapure water. Ten mL were used for the analysis of ellagitannins. The remaining amount of supernatant was treated with 300 mL of ethanol and kept at 0° C to induce polysaccharides precipitation. After centrifugation (4500 rpm, 8 min, 4 °C) the recovered polysaccharides were freeze-dried and weighed to calculate the yield.

HPLC-DAD analysis of phenolic extracts

The ellagitannins in peel and juice and the anthocyanins in juice were analyzed using a HP 1200L liquid chromatograph equipped with a DAD detector (Agilent Technologies, Palo Alto, CA, USA) after removing suspended solids by centrifugation at 14,000 rpm for 10 min. A Kinetex, 100, EC-C18 (30 x 3 mm, 2.6 µm, Agilent, USA) column was used to determine the two phenolic subclasses by a single chromatographic run; solvent A was CH_3CN and solvent B was H_2O acidified by HCOOH (3% v/v). The following linear gradient was applied: solvent A varied from 5% to 25% in 8 min, then was kept for 10 min at A 25%, in 2 min it reached 95%, and finally was kept in this condition for 6 min. Total time of analysis was 28 min, equilibration time 10 min, and flow rate 0.4 mL/min. Injection volume: 2 µL for ellagitannins extracts (decoction) and 10 µL for anthocyanins extracts (centrifuged juices). Chromatograms were recorded at 370 nm, 380 nm and 520 nm.

MS analysis

The extracts from decoction and juices from arils were analyzed on a quadrupole ionic trap LTQ (Thermo Finnigan) coupled to an HPLC (Thermo Finnigan Surveyor, San Jose, CA, USA); the HPLC conditions were the same used for the HPLC-DAD analysis. The analyses were conducted with the following ESI parameters (electrospray ionization): Sheath Gas Flow Rate: 35; Aux Gas Flow Rate: 10; Sweep Gas Flow Rate: 7; Spray Voltage: 4.20 V; Capillary temperatures: 280 °C; Capillary Voltage: -23 V; Tube Lens: -53. Acquisition for mass analysis was performed in negative and positive ions in full spectrum scan in the range of m/z from 100 to 1800.

Size Exclusion Chromatography (SEC) for polysaccharides

The samples containing the total polysaccharides were analyzed by SEC to determine the apparent molecular weight of the main constituents. Briefly, after freeze drying the samples were dissolved in distilled water at a final concentration close to 0.5 mg mL^{-1}. The samples were analyzed according to as Chamizo et al. (2018) using a ProStar HPLC Chromatograph (Varian USA) equipped with a refractive index detector (mod 355), using two columns, PolySep-GFC-P 6000 and PolySep-GFC-P 4000 from Phenomenex, USA, connected in series. The columns (700 mm length and 7.8 mm internal diameter) had separation ranges of 100 kDa to 15 MDa and 0.3 to 400 kDa. HPLC-grade water was used for the isocratic elution, with a flow of 0.6 mL min-1, and total time of 70 min. Blue-dextrans at various molecular weights ranging from 50kDa to 2000 kDa were used as internal standards to determine the hydrodynamic volume of the main polymers.

Sugars analysis

The two samples (PQ-C and PQ2018-100) were dialysed (cut-off 12–14 kDa), freeze-dried and treated according to Nunes et al. (2001) for the determination of neutral sugars after acid hydrolysis (H_2SO_4 72%) and conversion to the corresponding alditol acetates. Gas chromatography was performed using a Hewlett-Packard 5890 with a split injector (split ratio 1:60) and FID detector. A 25-m column CP-Sil-43 CB (Chrompack, Holland) with 0.15 mm i.d. and 0.20-µm film thickness was used. With the injector and detector operating at 220 °C, the following temperature program was used: 180 °C for 5 min and 200 °C for 20 min, with a rate of 0.5 °C min^{-1}; linear velocity of the carrier gas (H2) was set at 50 cm s^{-1} at 200 °C. In addition, uronic acids were colorimetrically determined using m-phenylphenol as previously reported (Nunes et al., 2001).

Statistics

Results were expressed as mean ± SD using EXCEL software (version 2013) in-house routines. One-way ANOVA and F-test ($p < 0.05$) by Microsoft Excel statistical software and Fisher's LSD (DSAASTAT software v. 1.1, Onofri, Pisa, 2007) were used to point out significant differences.

RESULTS AND DISCUSSION

Morphological characteristics of Purple Queen® fruits

As reported in **Table 1S**, all the fruits showed a similar mean weight for both 2017 and 2018; control fruits (PQ-0), although the plants were cultivated in pots, showed a mean weight of 298.05 g which was comparable to that of the commercial fruits (PQ-C) at 303.1 g. The presence of sediments in both the tested percentages negatively affected fruit size. Whole fruits, arils and peel total weight followed a common trend with PQ-0>PQ-50>PQ-100 in both the years. These findings clearly indicate that the physical and chemical composition of the sediments (**Table 1**), different from peat, induced plant stress resulting in a lower plant productivity as previously highlighted by Melgarejo et al. (2018).

Anthocyanins in juice

In order to investigate on specific differences in the amount of the single compounds, the qualitative and quantitative evaluation of anthocyanins was carried out by HPLC-DAD. The anthocyanin chromatographic profiles of fruits collected in 2017 showed similar patterns for the produced juices independently from the substrate (data not shown). In light of these preliminary findings, the fruits collected in 2018 were used also to carry out a quantitative evaluation. As expected, the anthocyanin fingerprints were similar to those found in Purple Queen® from 2017 and to profiles reported in the literature for other varieties (Fisher et al., 2011) (**Figure 1Sa**).

The absence of α and β punicalagins in our samples is linked to the method applied to obtain the juices from arils only, unlike most commercial pomegranate juices which are usually made by pressing half of the whole fruit, meaning a co-extraction of some ellagitannins from peel (Fisher et al., 2011). The identified anthocyanins are summarized in **Table 2S**. The use of the sediments particularly influenced the concentration of cyanidin-3,5- diglucoside which showed the highest values (140 mg L^{-1}) in the PQ2018-100 sample; the control (PQ2018-0) showed the lowest value (90 mg L^{-1}). Moreover, **Figure 1A** shows that also cyanidin-3-glucoside was significantly increased in 2018 fruits cultivated on sediment-based media (100% and 50%) with respect to the control (PQ2018-0) and commercial fruit (PQ-C). It should be pointed out that an environmental factor, such as the growing media, positively affected the content of cyanidin, an important molecule with beneficial qualities such as neuroprotective, antioxidant and antidiabetic properties (Casedas et al., 2019). An opposite trend was observed for delphinidin-3,5-diglucoside with resulted statistically higher in control fruit compared to the other treatments, although with slight differences (**Figure 1A**). The total anthocyanins ranged from 171 to 233 mg L^{-1} in PQ2018-0 and PQ2018-100, respectively (**Figure 1B**).

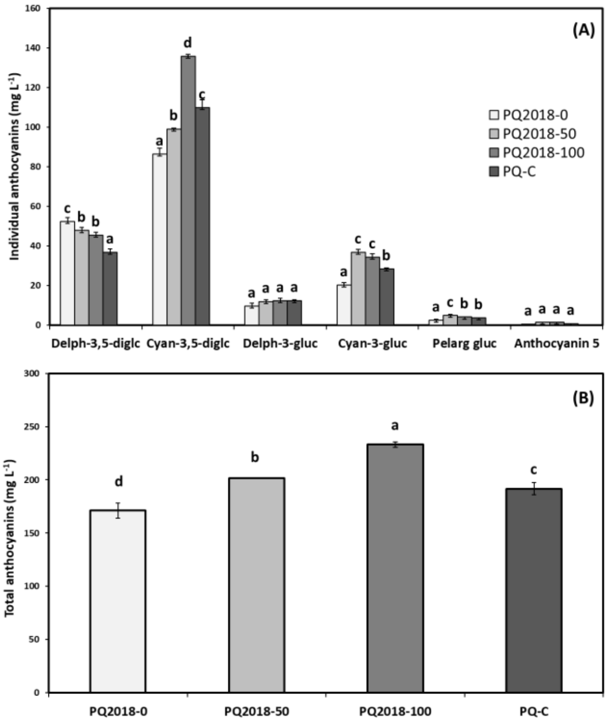

Figure 1- Anthocyanins in juices (mg L^{-1}) obtained from fruits PQ-0, PQ-50, PQ-100 and PQ-C collected in 2018: **A)** distribution of the main compounds, and **B)** total anthocyanins content. Error bars represent the standard deviations (n=3). Different letters mean statistically significant differences ($p< 0.05$).

It is noteworthy that both PQ-100 and PQ-50 from 2018 samples were richer in anthocyanins than the control and commercial fruit (PQ2018-0 and PQ-C respectively). Based on these results, plants PQ2018-100 and PQ2018-50 synthetized a higher anthocyanins content in response to a greater stress, presumably caused by the sediment. Previous data regarding anthocyanins content in Purple Queen grown under the same reclaimed sediment did not evidence significant variations, probably due to a different extraction procedure and the application of analytical methods based on the spectrophotometric determination of the total anthocyanins content (Chamizo et al., 2018).

Findings from fruits of fifteen Iranian varieties of pomegranate indicated 252.2 mg L^{-1} as the highest anthocyanin content determined by HPLC-DAD (Alighourchi and Barzegar, 2018). Comparing these results with the amount measured in Purple Queen®, it turns out that this variety is a good source of anthocyanins, with values comparable to those of the richest Iranian varieties even when grown under only remediated dredged sediments (PQ2018-100).

Anthocyanins are well-known to have a strong positive impact on human health (Sing et al., 2018) and, although there are no fixed values for anthocyanins intake, in 2013 the Chinese Nutrition Society, suggested providing a daily intake of at least 50 mg. Taking into account that red pomegranate juice is the main or only part of the fruit usually consumed fresh, the importance of finding new substrates for cultivation suitable to maintaining or even better to improving the phenolic expression, in particular of the anthocyanins, is certainly of great interest.

Ellagitannins from peel

The peel is known to be as the richest tissue of pomegranate fruit in terms of ellagitannins and polysaccharides, two classes of bioactive compounds that are well soluble and chemically stable in hot water (Khatib et al., 2017). Due to these properties, in our work peel extraction was carried out by applying a decoction because this procedure resulted suitable to efficiently recover both ellagitannins and polysaccharides with high yields. The possibility of using only water as extractive solvent can be strategic to facilitate the valorisation of this by-product obtained in large quantities during the production of juice (Khatib et al., 2017). Applying this process, it is also possible to propose the use of the dry decoction, almost representing the 70% of the dry weight of the peel, as a new functional ingredient to enrich different foods with ellagitannins and pomegranate polysaccharides.

The components detected in Purple Queen® decoction were the same as those previously found in other varieties (Fisher et al., 2011) (**Table 2S**), with α+β punicalin (**2**), α punicalagin (**3**), β punicalagin (**4**), and ellagic acid (**8**) resulting as the principal ellagitannins (**Table 2**).

Table 2- Ellagitannins in the decoction of peel of Purple Queen® (PQ) samples collected in 2017 and 2018 grown in pots in different conditions (PQ-0; PQ-50; PQ-100); PQ-C was a commercial sample from 2018. Data (mg g^{-1} d.w.) are means of the triplicates; the values of relative standard deviation (RSD) were below <5% for all the components.

	PQ-0		PQ-50		PQ-100		PQ-C
Compounds	2017	2018	2017	2018	2017	2018	2018
ellagitannin der. (1)	1.8	0.8	2.1	2.6	2.6	2.2	1.9
α+β punicalin (2)	12.5	7.1	18.6	22.5	19.1	22.5	19.3
α punicalagin (3)	8.0	7.2	11.6	11.7	8.8	9.0	17.5
β punicalagin (4)	21.9	14.7	27.9	27.5	24.5	23.9	39.2
ellagitannin der. (5)	1.2	0.7	0.7	1.3	0.6	0.5	1.9
ellagic acid hexoside (6)	1.5	0.9	0.9	1.5	0.7	0.8	2.0
ellagic acid pentoside (7)	1.1	2.3	1.4	1.7	1.4	1.4	1.3
ellagic acid (8)	1.8	3.9	4.7	3.3	3.8	3.9	2.2
ellagic acid der. (9)	0.1	0.7	0.1	0.2	0.1	0.1	0.2
ellagic acid der. (10)	0.1	0.1	0.1	0.2	0.1	0.1	0.3

All the chromatographic profiles related to the ellagitannins found in peel grown in 2017 and 2018 in the different substrates resulted very similar and almost completely superimposable (**Figure 1Sb**). It can be said that the presence of remediated dredged sediments did not induce changes in the biosynthetic pathways of these phenolic compounds. The ellagitannins content in the fruits harvested in the two seasons showed a similar trend (**Figure 2**): the presence of sediment mixed with peat (i.e. PQ-50) determined the maximum increase in both years, with a more pronounced effect on the fruits of 2018, while the highest concentration of ellagitannins was found in the commercial sample (PQ-C). The amount of α and β punicalagins in Purple Queen®, cited as major ellagitannins responsible for the majority of the biological properties of pomegranate, ranged from 54% to 60% of total phenols for all the fruits grown in pots, while the percentage increased in the commercial sample (PQ-C) with approximately 70% of the total content (**Table 2**).

The values reported in the literature for total ellagitannins in pomegranate peel range from 67 mg g^{-1} to 262 mg g^{-1} d.w. depending on the different varieties, geographical and environmental factors (Fisher et al., 2011; Khatib et al., 2017; Singh et al., 2018). Findings from a previous work on Wonderful and Laffan varieties showed that the total ellagitannins extracted with a hydroalcoholic medium (ethanol 70 %) and hot water gave very similar amounts (Khatib et al., 2017). In our samples, the mean values for total ellagitannins extracted by decoction ranged from 38 to 85 mg g^{-1} d.w. (**Figure 2**) confirming that Purple Queen® is a variety with a medium content of ellagitannins. To the best of our knowledge, this is the first study to evaluate ellagitannins in peel of the Purple Queen® variety and compare the content obtained from pomegranate plants grown in pots on different substrates grounds containing peat and remediated dredged sediments.

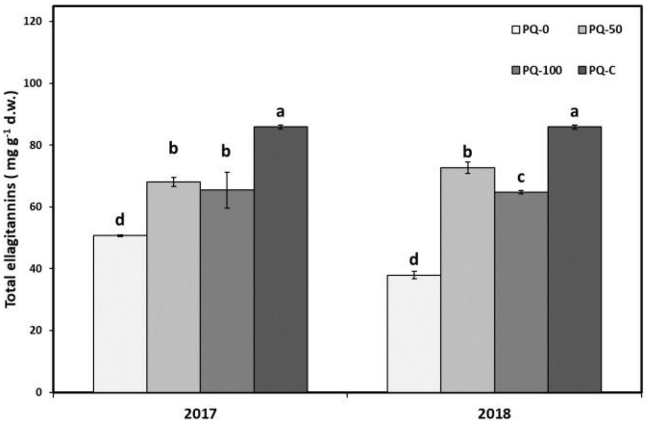

Figure 2- Total ellagitannins (mg g^{-1} d.w.) in decoction of peel: samples PQ2017 and PQ2018 grown with different media (sediments % of 0; 50; 100) compared to Purple Queen® commercial sample (PQ-C). Error bars represent the standard deviations (n=3). Different letters mean statistically significant differences ($p<0.05$).

Polysaccharides from peel

Pomegranate peel has been recognized to be a good source of polysaccharides, representing approximately 10-12% of the fruit dry weight and being mainly present as pectin (Joseph et al., 2013; Khatib et al., 2017; Ahmadi et al., 2018; Shakhmatov et al., 2019). Several biological properties concerning the polysaccharides of pomegranate have been recently highlighted in the literature: immunomodulatory (Ahmadi et al., 2018), activity, scavenging properties, and ability to reduce the growth of tumors in mice in combination with doxorubicin (Rout et al., 2007), and *in vitro* prebiotic activity (Khatib et al., 2017) contributing to maintain the health of human microbiota.

In light of these studies, although polysaccharides of pomegranate can be considered a part of the bioactive molecules of the fruit, scarce data are available on their structure and no information is available on Purple Queen® till now. In this study, initially, the yields in total polysaccharides were gravimetrically evaluated after precipitation induced by ethanol from decoction. As shown in **Figure 3**, similar values were obtained for the 2017 and 2018 samples: the increased content of polysaccharides resulted proportional to the percentage of remediated sediment in the substrate. The amount of polysaccharides found in fruits of plants cultivated only in remediated sediment (PQ-100) showed a strong increment in both years with respect to the values measured for the commercial sample (PQ-C). The total percentage of polysaccharides expressed in the dry weight of peel ranged from approximately 12% in PQ2017-0 to a maximum of 32% in PQ2017-100. These amounts are clearly greater with respect to other pomegranate varieties (Khatib et al., 2017). In general, the increase of polysaccharides production in plants has been associated to abiotic stresses, like water deficit otherwise no data are available regarding the effect of reclaimed sediment. A greater accumulation of total soluble solids has been observed also in strawberries and pomegranates fruits from plants grown on the same reclaimed sediment (Melgarejo et al., 2019; Tozzi et al., 2020), associated to the stress induced by the sediment with unsuitable physical characteristics, such as high bulk density and low porosity. Therefore, the increase of polysaccharides contributes to the enhanced accumulation of dry matter within the fruit peel, in agreement with previous observation (Melgarejo et al., 2019).

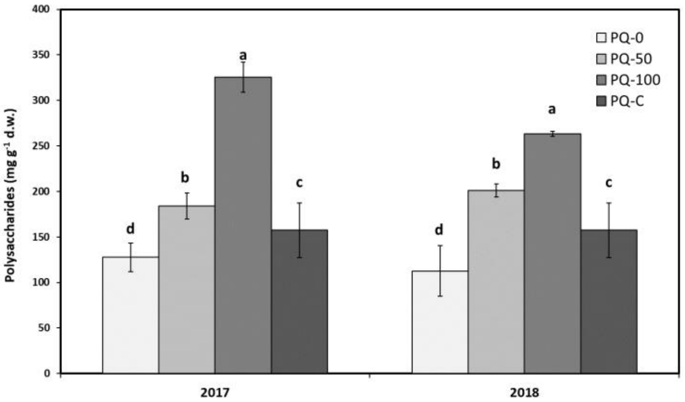

Figure 3- Total polysaccharides (mg g^{-1} d.w.) in Purple Queen® fruit samples collected in 2017 and 2018 from the three treatments compared with a commercial sample (PQ-C). Error bars represent the standard deviations (n=3). Different letters mean statistically significant differences (p<0.05).

To evaluate possible changes in the apparent molecular weight (hydrodynamic volume) of the main polysaccharides, the samples were analyzed by SEC, using a pool of dextrans to determine the hydrodynamic volume of the main polysaccharides recovered after decoction from the peel of Purple Queen® samples. **Figure 4**, in reference to the two samples of 2017 and 2018 without the presence of sediment (PQ-0), shows very similar profiles with about 50% constituted by oligosaccharides (white column, <0.36 kDa) and approximately the other 50% by polysaccharides at high molecular weight (black column) with values > 2000 kDa. On the other hand, the samples grown on 100% remediated dredged sediment show different profiles, particularly for the fruits of 2017. On the opposite, samples of 2018 have a similar polysaccharides distribution for all pot-grown plants with an almost superimposable profile of PQ2018-50 compared to the commercial sample (PQ-C). In other words, concerning the biosynthesis of polysaccharides, the major changes in terms of molecular weight distribution were observed for the younger plants from 2017, while the older plants were less susceptible to the effect of the sediment.

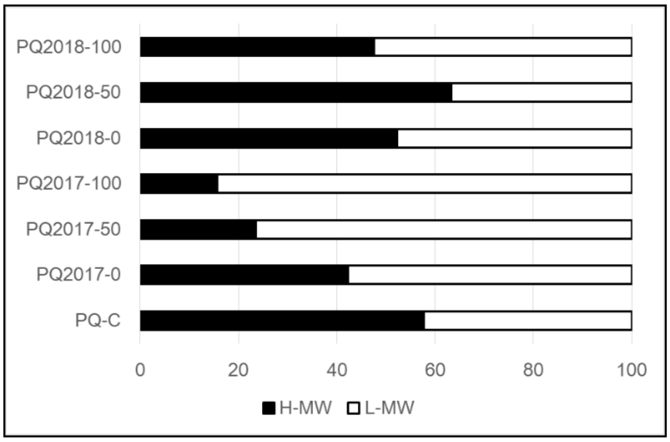

Figure 4- Distribution of the apparent molecular weight determined by SEC for the whole polysaccharide fractions recovered after decoction by ethanol addition; data are expressed as peak area % on total areas; *M, thousand Dalton and K, kilo Dalton; L, low molecular weight and H, high molecular weight.*

To further investigate the sugar composition of the total polysaccharide fractions of PQ-C and PQ2018-100, these samples were dialysed and subjected to acidic hydrolysis. We wanted to verify if the similarity in their profiles after SEC could also be confirmed in terms of sugar content. Our findings highlighted the presence of glucose as major neutral sugar: 45% and 52% in PQ-C and PQ2018-100, respectively (**Figure 5**). This result was predictable because of the presence of cellulose, which is reported as close to 20% of the total dietary fiber in pomegranate (Hasnaoui et al., 2014). According to previous data on the absence or a very low content of starch in pomegranate fruit (Gupta et al., 2015), the presence of glucose cannot be ascribable to the presence of starch. Galacturonic acid was 35% and 31% in PQ-C and PQ2018-100, respectively: galacturonic acid reported as the main polysaccharides in pomegranate (Abid et al., 2017; Zhai et al., 201; Shakhmatov et al., 2019) is linked to pectin structure. In Purple Queen, the percentage of galacturonic acid was lower than previous values obtained for other pomegranate varieties (Khatib et al., 2017) and it could be related to the fact that the analysis was carried out on the total crude polysaccharides and not on a purified extract containing only pectin. Other sugars, such as rhamnose, fucose, xylose, arabinose and galactose, are present in smaller quantities, less than 10% in both the samples.

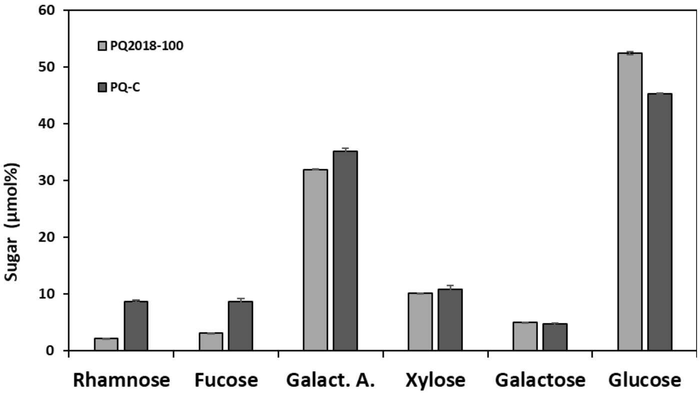

Figure 5- Sugar composition of Purple Queen® commercial (PQ-C) and Purple Queen® 2018 with 100% of sediment (PQ2018-100). The results are expressed in µmol% as a mean of a duplicate.

CONCLUSIONS

In this work, the nutraceutical profile of pomegranate fruits from trees cultivated on reclaimed dredged sediments was studied for the first time. The presence of the sediment was confirmed having a detrimental effect on fruit size and weight due to its unsuitable physical-chemical characteristics. However, this negative effect has been reduced by limiting the percentage of sediments added to the soil of older plants already adapted to growth on this new mixture of peat and sediment. Conversely, fruits from plants cultivated only on sediment or in a mixture with peat showed significant increases of bioactive compounds both in arils and in peel in the two consecutive seasons studied. The juice and peel showed significant higher concentrations of anthocyanins and ellagitannins, respectively. A similar trend was also found for the polysaccharide fraction, which was notably increased proportionally to the percentage of the remediated sediment with glucose and galacturonic acid as main sugars.

Although our findings pointed out a positive impact on the nutraceutical profile of the fruits, further studies are needed to better elucidate the physiological mechanisms behind the biosynthesis of the bioactive compounds in pomegranate in relation to the use of sediment in cultivation.

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SUPPLEMENTARY MATERIAL

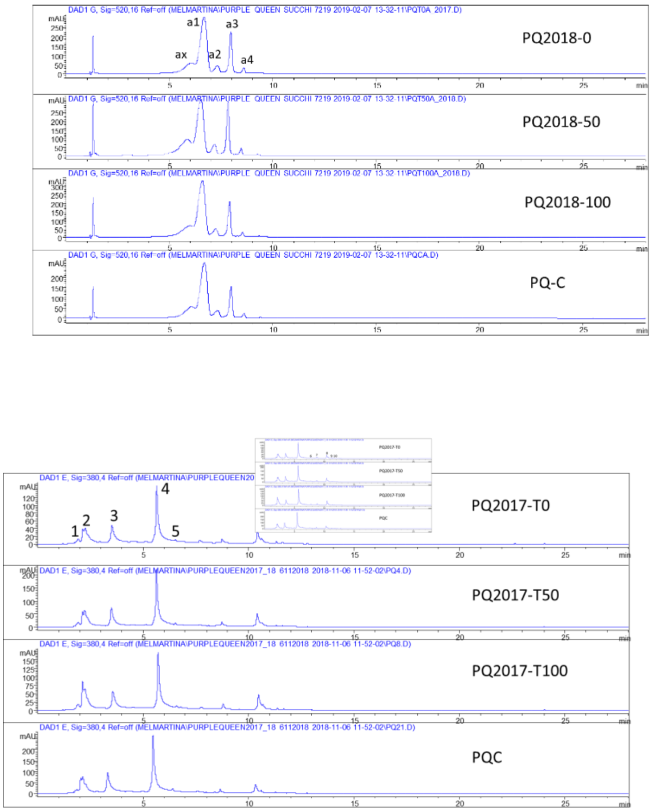

Figure 1S. HPLC-DAD profiles at 520 and 380 nm of Purple Queen sample, 2018 fruits: **a**) Anthocyanins from juice; **b**) Ellagitannins from decoction.

Table 1S- Weight of the different tissues of fruits from Purple Queen® grown on different substrates. Values are the mean of 10 fruits (n=10) and standard deviation (in brackets).

Samples	Year	Fruit total weight (g)	Aril total weight (g)	Peel total weight (g)
PQ-0	2017	302.6 (37)	139.1 (45.8)	163.5 (23)
	2018	293.5 (27)	125.2 (42.6)	168.3 (17)
PQ-50	2017	250.4 (29)	108.5 (43.3)	141.9 (24)
	2018	264.6 (35)	112.3 (42.44)	152.3 (19)
PQ-100	2017	207.3 (31)	86.8 (41.9)	120.5 (23)
	2018	216.7 (25)	88.1 (40.7)	128.6 (16)
PQ-C	2018	303.1 (39)	132.8 (43.8)	170.3 (27)

Table 2S- Main identified compounds in Purple Queen samples juices and decoction.

Analytes	rt	$[MH]^-$	Identified compounds
3	3.9	1083	alpha-punicalagin
4	5.9	1083	beta-punicalagin
7	10.4	301	ellagic acid
Anthocyanins	rt	$[MH]^+$	Identified compounds
AX	6.1	627	delphinidin-3,5- diglucoside
A1	6.8	611	cyanidin-3,5-diglucoside
A2	7.5	465	delphinidin-3-glucoside
A3	8.1	449	cyanidin-3-glucoside
A4	8.8	433	pelargonidin-3- glucoside

Conclusions

Reusing phytoremediated sediment as plant substrate paves the way for a new ecological waste management and, at the same time, represents an attempt to reduce the intensive use of peat needed by the nursery industry. In fact, the investigated sediment presented some contaminants (i.e. Zn and PAHs and C> 12 concentrations) exceeding the limits of the D.L. 152/2006 regulating the quality of soil in green areas, thus making its relocation in the environment not permitted as such. Accordingly, alternative management solutions for this material, such as its relocation as substrate for pot cultivation, are necessary. All metals showed concentrations fully included in the limits established for agronomic growing media (L.D. 75/2010), thus evidencing that even the remediated sediment as such is suitable as soilless substrate from the metal content point of view. Regarding the parameters not complying with this regulation (i.e. bulk density, pH and TOC), the use of sediment mixed with peat or other organic materials, allows to fulfil the legal limits. In addition, mixing sediment enables plants to better adapt to the presence of sediment as the ratio sediment–peat of 1:1 showed consistently the best productive performance for all the three considered species during the pilot experiments. Nevertheless, in the case of the strawberry, the re-cultivation has provided an improvement in the physical-chemical characteristics of the sediment, thanks to which plants yield was significantly increased in the successive productive seasons compared to the peat-based commercial substrates which differently possessed a short-term fertility. Accordingly, the re-cultivation of plants on sediment-based substrates could represent a strong point against the disposal of exhausted commercial growing media and, therefore, increases the sustainability of future horticulture.

The fact that no noteworthy transfer of inorganic and organic contamination from the substrate to the edible products occurred, indicated that the sediment-based cultivation approach can be considered suitable from a food safety viewpoint, even when the pure sediment is adopted, which represents an extreme scenario for eventual sediment reuse in agriculture. The quality of the edible products was comparable or even higher that obtained on peat. Furthermore, in the pomegranate case, the non-edible part of the fruit (i.e. the peel), being the one usually discharged from the juice industry, was enriched of bioactive compound when fruits were harvested from sediment.

In conclusion, the results of this research provide a significant impact in the sector of management of marine sediments, proposing their possible relocation in the agricultural field, avoiding their unsustainable disposal sine die. Moreover, in agreement with the principles of circular economy, the recycle of sediment as plant substrate, may contribute to overcome the environmental implications and limits due to the continuous degradation of natural resources, as in the peat case.

As a future perspective, studies performed on other mixture of sediment and/or focused on other food crop species and cultivars should be tested in order to provide additional information about the reuse of this non-conventional management strategy of contaminated sediments.