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Potential utilization of orange juice industrial production's residues for the immobilization of metals in contaminated soils

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Abstract

Soil contamination with potentially toxic elements (metals and metalloids) may affect the crops in several undesirable ways, and, consequently, the human food chain. The use of organic amendments to treat the contaminated soil is a possibility that cannot be excluded and thus must be taken into account when addressing this problem, since the organic matter has a sequestration potential for metals and, additionally, has the possibility to improve the physical, chemical, and biological properties of the amended soil.

The application doses of the residue evaluated equivalent to 20 and 40 t/ha were not reflected in a significant variation of the OM content in both-contaminated and non-contaminated soils, contrarywise to what was verified in the application of orange residues in a rate of 80 t/ha, which evidenced significant differences when in comparison with the non-treated samples, in both contaminated and non-contaminated soils. Concerning the pH of the soils, in the non-contaminated soil only the highest rate application registered statistical differences when in comparison with the non-treated evidence, whereas for the contaminated soil none of the application rates registered significant differences.

The obtained results showed that the application of orange juice residues caused significant differences in the pseudo-total and bioavailable contents of Cu but not of Cd, in both contaminated and non-contaminated soil. However, the Cd bioavailable contents are not considered high, varying between 5.67% and 6.45% of total Cd content. Further experiments should be made in order to assess the effects that higher doses of residue can produce in the bioavailable quantities of Cd, and also to evaluate how the application of the orange juice residues can influence the extractability of other potentially toxic elements, as well as validate the possibility of using this method to the remediation of soils in a real situation under non-controlled circumstances.

Keywords: orange juice residues; contaminated soils; cadmium; copper; organic matter; bioavailability.

Resumo

A contaminação dos solos com elementos potencialmente tóxicos (metais e metalóides) pode afetar as culturas agrícolas de diversas formas indesejáveis, não apenas do ponto de vista da produtividade, mas também da entrada deste tipo de elementos na cadeia alimentar do ser humano, para o qual são também nocivos. A utilização de corretivos orgânicos para remediação de solos contaminados é uma possibilidade que não deve ser excluída e, como tal, deve ser levada em consideração na planificação da abordagem a este problema, uma vez que a matéria orgânica tem potencial para actuar no sequestro de metais e, adicionalmente, torna também possível o melhoramento das propriedades físicas, químicas e biológicas do solo ao qual são aplicados.

Neste trabalho os resíduos utilizados como corretivo orgânico foram resíduos provenientes da produção industrial de sumo de laranja e foram aplicados nas diferentes doses de 20, 40 e 80 t/há, das quais apenas a última, corresponde à maior dose de aplicação, evidenciou diferenças significativas do ponto de vista estatístico quando comparado com a testemunha não tratada no que diz respeito ao teor de matéria orgânica do solo, tanto no solo contaminado como no não contaminado. Em relação ao pH dos solos, no solo não contaminado apenas na maior dose de aplicação se registaram diferenças significativas quando comparando os resultados com a testemunha não tratada, enquanto que no solo contaminado estas diferenças não se verificaram em qualquer uma das modalidades.

Os resultados obtidos mostraram ainda que a aplicação do resíduo de sumo de laranja provocou diferenças significativas nos teores totais e extraíveis de Cobre mas não nos de Cádmio, tanto no solo contaminado como no não contaminado, em qualquer uma das modalidades em comparação com a testemunha não tratada. No entanto, os teores de Cádmio extraíveis não são considerados elevados, variando entre 5,67 % e 6,45 % do seu teor total no solo. Mais ensaios deverão ser realizados com o objetivo de avaliar eventuais efeitos que a aplicação de resíduo em maiores quantidades possa ter na biodisponibilidade de Cádmio e para avaliar ainda outras possibilidades, tais com a possibilidade de a aplicação do resíduo influenciar a biodisponibilidade de outros elementos potencialmente tóxicos não considerados no procedimento experimental deste trabalho, bem como da sua extensão. Seria também pertinente a experimentação da aplicação destes resíduos com o objetivo de remediação de solos com elementos potencialmente tóxicos numa situação real e em ambiente não controlado.

Resumo Alargado

Os solos são um recurso não renovável e indispensável para a atividade e mesmo para a própria existência dos seres humanos, dada a sua importância para a atividade agrícola e, consequentemente, para a produção de alimentos. Por este motivo, a conservação dos mesmos é um assunto de extrema importância. Embora a contaminação dos solos com elementos potencialmente tóxicos seja inevitável, face ao nível de intensidade de certas atividades humanas, sobretudo a industrial, devem ser tomadas medidas para que, dentro dos possíveis, a saúde dos solos seja assegurada a longo prazo.

A agricultura deve, no geral, ser entendida como uma atividade económica e, assim sendo, como qualquer outra, tem como objetivo não só a obtenção, mas também a maximização do lucro dos seus agentes. No entanto, as práticas muitas vezes utilizadas para este fim, tais como a utilização intensiva dos solos e a aplicação, também ela intensiva, de materiais fertilizantes, com o objetivo de maximizar as produções e consequentemente os lucros, pese embora seja uma alternativa interessante a curto prazo, acaba por, a longo prazo, hipotecar a longevidade e a utilidade dos solos. A tomada de medidas para que tal não aconteça deve então começar por quem, à partida, terá maior interesse em manter a integridade deste recurso: quem faz dos solos a base do seu sustento.

O objetivo deste trabalho foi avaliar a possibilidade de utilização de resíduos provenientes da indústria de produção de sumo de laranja, mais concretamente das cascas dos frutos, enquanto corretivo orgânico dos solos. Estes resíduos foram recolhidos numa loja da cadeia de *A Padaria Portuguesa,* para a qual, à semelhança de muitas outras lojas ou indústrias que produzam os mesmos resíduos, estes não têm qualquer finalidade económica enquanto subproduto, sendo que o seu descarte acaba por constituir um encargo indesejado.

Foram utilizados solos recolhidos no *Instituto Superior de Agronomia,* que foram artificialmente contaminados com 12 mg/kg de cádmio (Cd) e 600 mg/kg de cobre (Cu). A estes solos foram adicionados os resíduos orgânicos provenientes da produção de sumo de laranja para uma avaliação do efeito na biodisponibilidade dos elementos contaminantes, Cu e Cd. O pressuposto da utilização destes resíduos e a sua aplicação aos solos foi o de avaliar o seu potencial para melhorar as suas características, enquanto solos agrícolas contaminados, tendo em conta a possibilidade de contribuírem para o aumento do teor de matéria orgânica dos solos, bem como influenciarem outras características, tais como o pH, e consequentemente a biodisponibilidade dos elementos potencialmente tóxicos.

Os resíduos da produção do sumo de laranja foram triturados, secos e moídos, e de seguida foram quimicamente caracterizados, nomeadamente em relação ao pH, teor de carbono, quantidade total de azoto (Kjeldahl), teor de proteínas e teor de cinza. Com base nesta caracterização foram calculadas as quantidades a utilizar nos solos e procedeu-se à sua aplicação.

Para este efeito foi realizado um ensaio de incubação, com a duração de 30 dias. Foram colocadas 250 gramas de solo em cada uma de 24 caixas, 12 delas contendo os solos contaminados artificialmente e as outras 12 contendo os solos originais. Para ambos os solos, foram utilizadas 3 modalidades diferentes, consoante a dose de resíduo orgânico aplicado, sendo essas doses correspondentes as 12 t/ha, 24 t/ha e 48 t/ha, para além de uma testemunha, com a finalidade de comparar os solos tratados com os não tratados. Durante o ensaio as caixas foram armazenadas numa camara a temperatura constante de 25°C e foram semanalmente pesadas para comparação com os pesos inicialmente registados e consequente reposição da quantidade de água perdida por evaporação. Todas as modalidades foram realizadas em triplicado.

No final do ensaio, em todas as modalidades, foram medidos o teor em matéria orgânica, pH e condutividade elétrica dos solos, bem como os teores totais e biodisponíveis dos elementos que foram utilizados para contaminar artificialmente os solos: Cu e Cd. Esta avaliação permitiu estimar os efeitos da aplicação do resíduo ao solo, para as diferentes quantidades aplicadas. Foi, ainda, realizado o tratamento estatístico adequado ao ensaio, com o objetivo de avaliar a existência de diferenças significativas entre as diferentes modalidades no ensaio e a existência ou não-existência de grupos homogéneos. Este tratamento estatístico consistiu numa análise de variância a um factor (ANOVA) com o teste de Tukey, recorrendo ao *software* GDM Solutions® Agriculture Research Management 2021.1.

Pela análise dos resultados obtidos foi possível concluir que a aplicação dos resíduos resultou na existência de diferenças significativas em muitas das variáveis sujeitas a análise, nomeadamente do pH dos solos, teor de matéria orgânica, teores totais de Cu e Cd e teor extraível de Cu. Em relação ao teor de matéria orgânica, as doses de aplicação de resíduos conduziram a efeitos significativos, excepção feita à modalidade R3. Os aumentos neste parâmetro verificaram-se na ordem dos 43% em relação ao teor inicial do solo contaminado e dos 45% em relação ao teor inicial do solo não contaminado, aumentos esses referentes à maior dose de aplicação dos resíduos. Em relação ao pH, a maior dose de aplicação dos resíduos conduziu a um aumento de 6,57 para 6,73 e de 6,83 para 7,13 no solo contaminado e no solo não contaminado, respetivamente, não havendo diferenças significativas para este parâmetro no solo contaminado, ao contrário do que sucedeu no solo contaminado. A aplicação de resíduos da produção de sumo de laranja a solos, contaminados ou não

com Cd ou Cu, também provocou diferenças significativas nos teores totais e biodisponíveis de cobre, mas não nos de cádmio. No entanto, no caso do cádmio, o teor biodisponível não é elevado, tendo variado entre 5,67 e 6,45% do total presente no solo.

De forma geral, os resultados obtidos foram bastante positivos e em consonância com os objetivos iniciais do trabalho. Com excepção feita à biodisponibilidade do cádmio e à condutividade elétrica dos solos, todas as restantes variáveis sujeitas a análises e tratamento estatístico evidenciaram diferenças significativas do ponto de vista estatístico. E inexistência destes efeitos nas respetivas variáveis pode dever-se a aplicação dos resíduos em quantidade insuficiente para que tal acontecesse, ou por um eventual menor grau de susceptibilidade a alterações do cádmio no que diz respeito à sua biodisponibilidade, quando em comparação com o cobre. Tendo em conta as tendências demonstradas na variabilidade dos parâmetros sujeitos a análise, devem ser feitas novas experiências utilizando o mesmo material, mas em doses de aplicação diferentes das aqui utilizadas, bem como avaliar a sua ação com outros elementos potencialmente tóxicos não considerados neste trabalho, pois podemos estar na presença de uma alternativa bastante viável para a melhoria das características dos solos do ponto de vista agronómico e cuja aplicação pode permitir uma utilização mais sustentável dos mesmos. Por último, e no caso de esses resultados serem positivos, deverá ser ainda sujeito a avaliação *in-field*, no qual as condições são menos controláveis e, como tal, os resultados poderão diferir de forma substancial dos que aqui foram obtidos.

Palavras-chave: resíduos de laranja para sumo; solos contaminados; cádmio; cobre; matéria orgânica; biodisponibilidade.

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Abbreviations

APA – Agência Portuguesa do Ambiente

C – Carbon

CEC – Cation Exchange Capacity

CT – Contaminated

DM – Dry Matter

EC – Electrical Conductivity

FAO – Food and Agriculture Organization

GWC – Garden Waste Compost

ICP-OES – Inductively Coupled Plasma Optical Emission Spectroscopy

ISO – International Organization for Standardization

MSWC – Municipal Solid Waste Compost

NCT – Non-Contaminated

OM – Organic Matter

PTEs – Potentially Toxic Elements

ROS – Reactive Oxygen Species

SS – Sewage Sludge

1. General Introduction

The contamination of edible plants, or plant parts (e.g., leafs, seeds, fruits) with potentially toxic elements (PTEs) is a subject transversal to several scientific areas, being a serious cause of concern not only for agriculture, but also for the environment, the food production sector and the public health.

For this reason, it is of capital importance to develop strategies to cope with this problem and reduce the risk of PTEs absorption by edible plants. This can be accomplished through the addition of amendments that contribute to the immobilization of these elements in the soil, avoiding their uptake by plants. Their immobilization ability is generally based on the soil's pH control achieved by their application and/or by the incorporation of molecules with PTEs chelating potential, reducing their availability and thus, their absorption by plants.

The organic residues from urban waste and wastewater treatment (e.g., sludge or compost) or from agro-industrial activities can fulfil the role of soil organic amendments, allowing their valorisation and avoiding their landfill deposition. This is the usual destination for the residues generated in the orange juice production process, for which it is important to equate sustainable valorisation options. Their incorporation in soil can represent an alternative option for these residues and simultaneously contribute to the remediation of the PTEs problematics previously, by aiding in their immobilization in contaminated soils and, consequently, decreasing their phytotoxicity to the cultures and their accumulation in edible plants. Bearing this framework in mind, the goals of this work will be described in further depth in the next chapter.

2. Goals

The main goal of this work was to evaluate the effects of the addition of a specific organic residue, in this case orange juice residues, mostly composed by orange peel, to a soil contaminated with PTEs, aiming their immobilization and decreasing their bioavailability, avoiding or reducing their absorption by plants.

The specific goals were:

- To assess the composition and possible utilization of the orange juice industry waste products as soil organic amendments.
- To evaluate their capacity to modify the contaminated soils properties, by decreasing PTEs extractability, measured using PTEs chemical extraction with solutions, as a surrogate measure of their bioavailability.

To achieve these goals, the materials will be characterized, both the residue and the contaminated soils, and the effect of the waste application to soil properties will be assessed through an incubation experiment that will also evaluate the possibility of their use in the sequestration of PTEs, decreasing their availability to plants.

3. Literature Review

3.1. Potentially Toxic Elements

In the recent decades there has been an increasing concern about the environmental pollution. The large extension of toxic heavy metals pollution is more and more being considered as a serious problem. This concern is the natural response to the increase of environmental pollution caused by the industrial expansion, the continuous growth of the human population and the continuous disturbance of the biogeochemical cycles (Ali *et al.*, 2013; Bilal *et al.*, 2018).

In this work, it will be used the designation potentially toxic elements (PTEs) including not only heavy but also other chemical elements that are toxic and can have environmental, biological and toxicological negative effects. This group includes the usually called heavy metals (Cd, Cr, Cu, Ni, Hg, Pb and Zn), metalloids (As and Sb), and other toxic or hazardous elements, and even certain micronutrients, linked with adverse consequences to both living beings and the environment.

With respect to plant nutrition the mineral elements are divided in two groups: the essential elements and the non-essential elements that can be toxic. There are also two very distinct groups of nutrients: the macronutrients and the micronutrients, and both macro and micronutrients are essential. The main difference between macro and micronutrients is that the macronutrients are required in much higher concentrations relatively to micronutrients. For an element to be considered as essential to plants, three conditions must be verified: a given plant must be incapable of completing its lifecycle in the elements absence, the function of the given element cannot be replaced by other element, and, finally, it must be directly involved in the plants metabolism or, at least, required for a specific metabolic process (Kirkby, 2011; Manara, 2012).

The PTEs designation has already been used to describe these elements by Pourret & Bollinger (2018) and it is the most adequate one, not only because of all the issues regarding the definition of these elements, but mostly because even though many of them are micronutrients and are vital to the development of plants, their presence in a certain dose or level of exposure can cause toxicity (Pourret & Hursthouse, 2019). Therefore, PTEs is probably a fairer term that can expose all the extension of the effects that these elements have, without the strictly negative connotation that a term like heavy metal carries. Some examples that are going to be considered in this study are cadmium (Cd), copper (Cu), chromium (Cr), lead (Pb), manganese (Mn), nickel (Ni) and zinc (Zn), with particular focus on the first two mentioned, since the experimental part of this work will involve soil artificially contamination with these two elements: Cd and Cu.

As previously mentioned, some PTEs like Fe, Zn, Cu, Ni, and Mn are essential, i.e, are needed for vital biochemical and physiological functions in living organisms, such as cell defense, gene regulation, energy metabolism, participation in redox reactions and being part of the constitution of several enzymes (Baldantoni *et al.*, 2020; Kisku *et al.*, 2000; Manara, 2012; Sharma, 2014). But even for these essential elements, their available quantity for the plants is a crucial matter. It is a fact that their absence or deficiency can cause serious disease symptoms in plants and even their eventual destruction, and in excess it might even affect the human health by entering the food chain. Nevertheless, it is also true that their presence and availability in higher doses can also have deleterious effects, a dose that depends on the element and on plant species. In other words, their essentiality or toxicity is dependent on their presence being comprehended within a certain range, among other specificities (Arif *et al.*, 2016; Vatansever *et al.*, 2017).

Although the relevance of some nutrients has been well set and established, there are some that, not being essential or yet proved to be so, can have benefic effects on plant development and life cycles, by possibly replacing some mineral nutrients in their functions, being essential for N fixation and improving insect and disease resistances (Peters & Laboski, 2011; Vatansever *et al.*, 2017). On the other hand, others, like Pb, Cd, Al, Cr and As, are non-essential and can be toxic even at very low concentrations (Baldantoni *et al.*, 2020; Kisku *et al.*, 2000; Manara, 2012; Leitão *et al.*, 2018; Shanker *et al.*, 2005; Suzuki *et al.*, 2001). Metals like Pb and Cd have no known physiological roles (Wuana & Okieimen, 2011), and are among the elements that cause bigger concerns and pose higher risks (D'Amore *et al.*, 2005).

Beyond this, the unbalance of micronutrients can also implicate indirect damages since they have interactive effects and an unbalance of only one of them can compromise their balance altogether. So, adding to the direct toxic effects, the quantity of an essential PTE can also switch the plant's ability to absorb and store non-essential elements (Arif *et al.*, 2016; Singh *et al.*, 2019). Since the micronutrients play a critical role in the development of the plants, and, thus, in the final results of the yield, it is necessary to make sure they are properly balanced and each one's presence is at least close to its optimum level or range.

3.2. Sources of Contamination and Environmental Impact

Even though the use of metals by mankind has brought some benefits, it can also represent a great deal of negative consequences (D'Amore *et al.*, 2005). The contamination of the environment, and more specifically, of the soils, with high concentrations of PTEs, often caused by industrialization, is getting very serious and one of the most severe consequences is the disturbance of biogeochemical cycles (Ali *et al.*, 2013;Khalid *et al.*, 2017). The soil pollution with PTEs can occur naturally, as a consequence of the proximity to areas that are natural sources of these elements, such as mineral outcrops, or due to anthropogenic activities, especially those included in the industrial sector (Ali *et al.*, 2013; Ferson., 1995; Pacyna *et al.*, 2007).

Despite the fact that these elements occur naturally in geologic formations of the Earth's crust, natural and anthropogenic activities contribute exponentially for their presence in the biosphere (Sharma, 2014). The most significant natural causes are the effects the climate produces on the minerals, erosion, volcanic activity, atmospheric deposition of aerosol particles and raindrops that contain PTEs or gaseous forms of the elements. The main anthropogenic activities that are responsible for this kind of contamination are wastewater irrigation, application of agricultural fertilizers and pesticides, solid waste disposal (such as organic materials that include sewage sludges, livestock manures and food wastes), smelting, mining, vehicular exhaust and other industrial activities (Ali *et al.*, 2013; Khan *et al.*, 2008; Wuana & Okieimen, 2011). According to Xiangdong *et al.* (2001) the activities that have more impact on the contamination via atmospheric deposition are the most significant, from which the car emissions also play an important role, at least in the urban environment (Adamiec *et al.*, 2016). Because of these activities and/or natural occurrences, the soils located in the areas where they take place, as well as in its proximities, have proportional higher levels and concentrations of PTEs, which can also compromise the health of the water courses and groundwaters in the vicinity of these contaminated areas (Madeira *et al.*, 2012; Zhuang *et al.*, 2009).

In addition to the industrial sources of contamination, some agricultural practices are also responsible for polluting the soils with PTEs. Due to the lack of purification of fertilizers upon their production, mainly for economic reasons, many of them end up containing a great deal of impurities, with PTEs figured among them (Gimeno-García *et al.*, 1996). Although many farmers are not environmentally aware and prioritize their production and yield maximization, these products have trace quantities of metals like Pb and Cd that, after continuous application, may increase their quantity in the soil in a very substantial way (Martos & Jost, 2019; Wuana & Okieimen, 2011). One important example is the phosphorus (P) fertilization, that must be seen cautiously because, even though it is an nutrient, the P fertilizers contain many contaminants derived from the phosphate rocks (Loganathan *et al.*, 2003). This is of course aggravated because the P mobility in the soils is very low, which commonly makes it a limiting nutrient, forcing P inputs to make crop production viable (Alam & Ladha, 2004).

In the case of pesticides, the ones with a more considerable content of PTEs were more common in the past. However, even recently in the UK an average of 10% of the approved fungicides and insecticides contain either Cu, Hg, Pb, Zn or Mn, or any of these combined (Wuana & Okieimen, 2011). Even though the use of pesticides containing these type of elements has been restricted to specific cultures and/or areas, the residual contamination they are accountable for is usually much more considerable when compared with fertilizers (McLaughlin *et al.*, 2000).

Many other application organic products like biosolids produced from municipal wastewaters, livestock manures and other composts also contain many PTEs and can also represent a source of contamination and of their accumulation in the soils (Basta *et al.*, 2004; Wuana & Okieimen, 2011).

Soils are probably the major sink of the released PTEs into the environment, since they receive them from many types of sources, restrict their mobility and, therefore, the transition to other compartments (Gil *et al.*, 2004; Wuana & Okieimen, 2011). This represents a great problem for agriculture, especially near urban areas, due to the heavier traffic volume (Xiangdong *et al.*, 2001; Chen *et al.*, 2003). The adverse environmental impacts from the excessive quantity of PTEs can include phytotoxicity, water pollution, soil erosion and loss of biodiversity (Kidd *et al.*, 2015; Zhuang *et al.*, 2009), and also the contamination of the edible plants cultivated in either rural or urban environments. Altogether, soil contamination with PTEs can have a negative impact on both human health and the environment (Orsini *et al.*, 2013).

3.3. Impact of PTEs on Plants and Humans

Taking the definitions of essential and non-essential elements clarified earlier in this work as ground-zero, in this sub-chapter the potential risks and benefits of both of them will be discussed in a deeper and more specific way. These effects are of course dependent on the element in question, as well as on its concentration in soil and uptake by crops, especially for the essential ones, given their duality. Asides from the element itself, it is also important to consider that the effects vary according to the specific plant or crop, because different plants obviously have different properties that can make the PTEs effects, as well as their optimum and toxic ranges or quantities, vary significantly from plant to plant.

The most common visible symptoms of phytotoxicity are chlorosis, necrosis, stunted growth, shorter root length, narrow leaves and darkening of the root tips, they can vary for different plant species and even for different plants of the same species (Kisku *et al.*, 2000; Moreira *et al.*, 2020). Also, for having such an undifferentiated symptomatic board, they can be confused with other issues in the plants, like an eventual iron deficiency, for example, that can also display chlorosis as a symptom (Kisku *et al.*, 2000; Prasad & Djanaguiraman, 2016).

The experimental part of this work will not feature the establishment and development of any culture in a soil contaminated with PTEs in a way that is more than merely suggestive, if we consider the fact that cultures established in a contaminated soil will also be affected by this soil condition. However, this subject's importance is, in any way, undervalued by this author. For this reason, if the results of this experiment are positive, it is of the author's opinion that they should be taken a step further and include the establishment and development of a culture in a soil treated with orange juice residues, in order to be able to verify their viability in a more complete and specific manner. In the author's opinion, the lettuce would be an appealing crop for this experiment, given its short development cycle. With that being said, the effects of each of these elements will be, as much as possible, directed to that specific crop. That means, of course, that all the potential consequences of the presence of a certain dose of a certain element can represent to some plants other than lettuce might not be mentioned, since that would be, in some way, a nuisance.

The description of the effects will be focused on the analysis of the consequences that the presence of these elements can produce to specific parameters of growth that will of course determine variations on the value and consequent profitability of the yield. Since the metal bioaccumulation affects biochemistry, physiology and structure of vegetables (Cheng, 2003; Guo *et al.*, 2007; Wolf *et al.*, 2017), this analysis will of course include effects the PTEs can produce on physiological activities, such as photosynthesis and antioxidant enzymes activity, since they are vital and indispensable for the mentioned palpable results (Hernández *et al.*, 2003; Hikawa *et al.*, 2019). Plus, the excess quantity of some PTEs can display inhibitory effects to the roots growth, which leads to increased activity of superoxide dismutase (Kopyra & Gwóźdź, 2003), an enzyme that takes part in reactive oxygen species (ROS) generating systems (López-Cruz *et al.*, 2017). Although these ROS have important tasks in the processes of growth as the development and defense against pathogens, among others, they are more reactive than O_2 and in excess they can cause oxidative damage of cell components and structures causing oxidative stress. The generation of these ROS is a common and perhaps the most concerning consequence of PTEs contamination in soils to plants (Liu *et al.*, 2017; Yan *et al.*, 2015) but in excess they can harm proteins, phospholipids, nucleic acids, Indole-3-Acetic Acid and other enzymes, as well as cause DNA injuries. So, ultimately, the PTEs can indirectly affect the growth of the plants and the results of the yield by affecting ROS (Apel & Hirt, 2004; Cakmak, 2000; Schützendübel *et al.*, 2002; Yang *et al.*, 2010).

These elements are systemic toxicants known to cause not only environmental impact, but also to represent danger to humans by entering the food chain: they enter the plants via roots or atmospheric deposition and translocate to the edible parts, from where they represent not only the reported harms to themselves, but also to the humans who consume the products (Leitão *et al.*, 2018). Examples of adverse health effects in humans are heart, kidney and blood diseases, neurologic/neurobehavioral, hematologic and immunologic disorders, diabetes, hearing loss and various types of cancer, among many other (Lyu *et al.*, 2017; Tchounwou *et al.*, 2012). The main pathways of exposure include ingestion, inhalation, and through dermal contact, via skin absorption. In the cases of ingestion and inhalation, it can also apply to plants and crops that are produced in contaminated soils, i.e, it can occur in an indirect way, since plants that grow in this kind of environment can accumulate PTEs from the soil, and even from the air (Kisku *et al.*, 2000; Larese *et al.*, 2007; Tchounwou *et al.*, 2012; Zhuang *et al.*, 2009). This chain-type contamination is perhaps the most relevant way of entrance of these kind of elements into the human body (Khan *et al.*, 2008) and the risks they can represent toward mankind will also be briefly described, since it is also a contamination parameter that must be considered (Kabata-Pendias, 2004).

3.3.1. Micronutrients

3.3.1.1. Copper

Copper can benefit plants by exhibiting antifungal properties (Wei *et al.*, 2010). However, and despite being an essential element, toxic quantities of Cu can induce growth problems and affect other physiological processes on many plants. For instance, in lettuce this contamination can represent a major concern since it has considerable tolerance to Cu, and it is considered as a hyperaccumulator crop to this element (Martins & Mourato, 2006; Shams *et al.*, 2019; Yruela, 2005). Analyzing the results attained by Liu *et al.* (2016) and Xiong & Wang (2005), it is possible to conclude that the presence of Cu in soils affects the lettuce growth and production, more specifically in root dry matter (DM), germination rate, shoot length and leaf area. The general results prove that the increase of Cu results in a decrease leaf area, which can help to explain other displayed side-effects, such as the decrease of shoots and roots size (Liu *et al.*, 2016; Shams *et al.*, 2019). However, the shoot elongation results are more dependent on the Cu concentration, that can stimulate it at some point, and inhibit it after a certain limit (Shams *et al.*, 2019). These results are consistent with the ones obtained by Sheldon & Menzies (2005) for Rhoades Grass (*Chloris gayana Knuth)* and by Upadhyay & Panda (2009) for Water Lettuce . In the lettuce crop, Cu also produces effects on the chlorophyll: while the presence of Cu may influence the chlorophyll content in a desirable way, i.e, it can increase along with the Cu increase (Xiong & Wang, 2005), after a certain limit, the Cu concentration can cease to cause positive effects and start degrading the photosynthetic pigments (Shams *et al.*, 2019). The excess of Cu can also produce negative effects in the balance of other micronutrients and even macronutrients, decreasing the contents of N, P and K in the leaves and N, P, K, Mg, Zn, and Na in the roots (Shams *et al.*, 2019).

This affectation of other nutrients can help explain how Cu contaminations affect the plants growth (Wolf *et al.*, 2017). It can also accelerate the formation of free radicals (Upadhyay & Panda, 2009), which can lead to oxidative stress, and, of the micronutrients considered, it is the one that has more effect in the seedlings vigor index and production of biomass (Moreira *et al.*, 2020).

3.3.1.2. Zinc

Zinc is also an essential trace element and it is required, even if in small amounts, by plants, animals and humans, as it is important for cell physiological processes in many living organisms, being one of the most relevant transitional metals, alongside with iron (Alloway, 2009; Sagardoy *et al.*, 2009). Similarly to Mn and Fe, Zn is one of the micronutrients that plays a part in the electron transport chain (Roosta *et al.*, 2018). It can also protect and restore the chlorophyll levels that can be affected by the presence of other metals, particularly Cd, antagonizing its toxicity and improving the chlorophyll biosynthesis, contributing to the maintenance of the whole photosynthetic activity (Aravind & Prasad, 2004). An adequate supply is of utmost importance for the cell protection against damages caused by ROS, but, in excess, Zn can induce their occurrence (Cakmak, 2000; Sagardoy *et al.*, 2009). The results of the experiments of Wolf *et al.* (2017) proved that Zn toxicity has negative effects on the lettuce growth, production of biomass (Rouphael *et al.*, 2016) and the final quality of the products. This is applicable not only to lettuce but also to other plants and vegetables, as Sagardoy *et al.* (2009) evidenced for sugar beet. It can represent also a risk of unbalancing the concentrations of all the nutrients in general, especially of Iron and Mg mainly because of the identical ion radius (Rouphael *et al.*, 2016). The results of the experiments conducted by Moreira *et al.* (2020) showed that excess Zn caused a decrease in shoot fresh weight of the green leaf cultivar of lettuce (Golden Spring), stating that it can also be responsible for inhibition of growth, even though it is, along with Mn, the PTE that has evidenced lower adverse effects to the growth of the roots on this particular cultivar.

3.3.1.3. Manganese

Manganese is the 12th most abundant element and the 5th most abundant metal in the Earth's crust of all the PTEs considered in this work. Manganese is also, probably, the one with the lower toxicity levels (Kopittke *et al.*, 2010; Moreira *et al.*, 2020). Even though Mn has a role in the photosynthesis, enabling the water splitting in photosystem II (Roosta *et al.*, 2018), higher dosage can imply reduction of stomatal conductance and photosynthesis intensity on lettuce, and consequently decrease its growth. However, it can also cause very substantial positive effects on the chlorophyll content (Przybysz *et al.*, 2017). Still focusing on the results obtained by Przybysz *et al.* (2017), we can conclude that these elevated concentrations of Mn are dependent of the cultivar of lettuce in the parameters regarding the biomass production. Although Mn acts as a cofactor for enzymes that play an important role on the defense against oxidative stress, such as glutamine synthetase and oxidoreductases, its toxicity effects also include an increase in the generation of reactive oxygen species, especially in the leaves, which is probably the source of the reduction it can represent to the photosynthesis rate and plant growth by the exacerbation of oxidative stress ((Cunha Martins, 2020); Przybysz *et al.*, 2017). However, by analyzing the results of (Moreira *et al.*, 2020), Mn is, of the elements considered, the one that less affects the length of the roots. Still according to the author, Mn only starts having negative repercussions on the seedling vigor index after an exposure of the plants to very high doses.

3.3.1.4. Nickel

Despite not being physiologically as relevant as Cu or Zn, Ni is another essential element for plants and plays an important role in their growth, particularly by interfering in the nitrogen uptake. So, at least a minor quantity of Ni is necessary to improve the yield (Ameen *et al.*, 2019; Seregin & Kozhevnikova, 2006; Shahid *et al.*, 2014). It is also important for the activation of urease, being indirectly linked to the seed germination index on some cultures (Seregin & Kozhevnikova, 2006), as Kumar (2018) evidenced for several cultivars of wheat. On the other hand, higher concentrations increase the risk of ROS formation, causing oxidative stress. The other way Ni can represent toxicity toward plants is by interfering with the uptake of other essential metals, depending of course on the plant species (Ameen *et al.*, 2019; Poulik, 1999; Zhao *et al.*, 2019). This variation between benefits/toxicity due to different concentrations has been proved to apply to lettuce crops, regarding biomass accumulation responses for both roots and shoots (Zhao *et al.*, 2019). It was also evidenced by Yadav (2016) that Ni exhibits anti-fungal properties, inhibiting the mycelial growth of *Fusarium oxysporum* not only for lettuce, but also for other cultures, such as tomato. In alignment with the ambiguous effects generally produced by the micronutrients, the effects produced by Ni in lettuce are dependent on its availability and concentration, being able to stimulate growth at some point, but also to inhibit it at higher dosages (Moreira *et al.*, 2020). As for the nutrients balance for lettuce specifically, the results of Poulik (1999) demonstrate an unmistakable negative correlation between Ni and Fe. Even though the results obtained by the latter author did not show an excess of Ni maximum admissible contents in the lettuce edible parts when cultivated in Ni contaminated soil, this value was exceeded in the tomato culture. This can represent a problem since Ni exposure is a well-known cause of several acute and chronic respiratory and cardiovascular health problems in humans, as well as cancer and several allergies (Lippmann *et al.*, 2006; Zambelli *et al.*, 2016; Zhang *et al.*, 2009).

3.3.2. Non-Essential Elements

3.3.2.1. Cadmium

Cadmium is highly toxic to plants and its presence in production soil can have harmful effects in plants germination (either by reducing, stimulating or affecting its rate), vigor of the plants, especially when regarding the growth of the shoots and roots, and, for being so poisonous to metal sensitive enzymes, Cd can even cause the plants death (He *et al.*, 2014; Moreira *et al.*, 2020; Sharma, 2014; Yang *et al.*, 2004). In fact, still according to the results of the experiments conducted by Moreira *et al.* (2020), Cd is one of the PTEs that affects these aspects in a more considerable way in lettuce, especially the root growth: its presence can inhibit it at some concentrations, but also stimulate it if it is contained within a certain range. It can also be responsible for generating abnormal seedlings and reducing the amount of viable ones, despite not affecting the germination rate (Lamb *et al.*, 2010; Moreira *et al.*, 2020). Most of the PTEs tend to accumulate more in the roots. According to Bi *et al.* (2010), the accumulation of Cd in the roots is suspected to diminish the plants ability to intake other metal ions, especially of Fe and Zn, which appeared to evidence some sort of antagonism with Cd, possibly due to similarity in the mobility of the cations. Cadmium also interferes with the water intake of the lettuce seeds and decreases the crop growth in general, especially the root elongation (Bautista *et al.*, 2013). All of the negative impacts of Cd are catalyzed and of special concern since the experiments of Moreira *et al.* (2020) made clear that for higher metal concentrations, Cd is the one that accumulates the most in lettuce crop.

3.3.2.2. Chromium

Chromium is the most common contaminant in the environment. This element is present in the environment in two different forms: Cr (VI), which is proved to be toxic, and Cr (III), the most important and biologically active form. Even though the first mentioned state is more toxic, mobile and biologically available than the second, they can interconvert in the environment (García-Gonzalo et al., 2017; Taylor et al., 1999; Wang et al., 2017). Chromium toxicity is dependent on the metal speciation and can cause a reduction in yields by affecting leaf and root growth, and inhibition or reduction of some of the plants physiological activities such as the photosynthesis and antioxidant mechanisms, and also of water and nutrients uptake, compromising all of their balance in the plants (Dixit *et al.*, 2002; Shanker *et al.*, 2005; Singh *et al.*, 2013). In lettuce specifically, Cr(VI) stress can decrease the growth and have negative impacts on pigments, proteins, sugar contents and photosynthesis reactions (Dias *et al.*, 2016). Chromium can stimulate growth of the roots up to some point, but, at higher concentrations it can cause a decrease in its fresh weight, as well as of the shoots, and also affect negatively the seeds development and vigor (Moreira *et al.*, 2020). This decrease in biomass accumulation in response to Cr excess is also applicable to other cultures, like Link *et al.* (2000) proved to happen with his experiments on *Echinochloa colona (L).* However, the latter author verified a condition that, according to Moreira *et al.* (2020), doesn't apply to lettuce, and that it that Cr excess affects the germination. Regarding the damages in humans, and even though it is proved to be necessary for human health, namely for diabetics, that excrete big quantities of Cr in their urine, it has been identified a long time ago as a carcinogen and can represent a cancer risk for a several number of organs (Costa & Klein, 2006; Suh *et al.*, 2019; Vincent, 2018), even though it participates in some important biochemical processes (Nielsen, 2000).

3.3.2.3. Lead

Lead contamination represents a major concern because of its constant increase in the environment. Lead contaminated soils display a major decrease in crop productivity and the general symptoms are stunt in growth, blackened roots and chlorosis (Farooqi *et al.*, 2009; Sharma & Dubey, 2005). Although in low concentrations Pb can stimulate respiration and ATP formation, its toxicity produces the opposite effects and can be responsible for decreasing the photosynthetic rate by obstructing activities such as the electron transport and stages of the Calvin cycle, affect seed germination, seedling vigor and total biomass (Farooqi *et al.*, 2009; Moreira *et al.*, 2020; Sharma & Dubey, 2005). The increase of its uptake by plants can also induce oxidative stress, as Verma & Dubey (2003) confirmed, regarding rice crops growing under toxic quantities of Pb. For the lettuce crop specifically, it can also reduce the photosynthetic rate even at concentrations below the admissible maximum (Pinto *et al.*, 2017) and cause genotoxicity in the roots in the same conditions (Silva *et al.*, 2017), as well as decrease the shoots and roots fresh weight, and, since its quantity increase leads also to an increase in its mobility and uptake by plants (Moreira *et al.*, 2020), it can also represent more danger to the consumers. In humans it can affect the nervous, renal, hematopoietic and reproductive systems (Carocci *et al.*, 2015) and also cause cardiovascular risks, fertility problems (Mitra *et al.*, 2017) and oxidative stress (Batool *et al.*, 2017; Liu *et al.*, 2017).

3.4. Bioavailability

The bioavailability of an element in a soil matrix is defined as the element's capacity to influence the soil organisms and the mobility refers to its transport throughout the soil (Temminghoff, 1998). The transfer of PTEs from the soil to the plants is a complex process affected by several factors. The mobility and availability of elements in soil are of course dependent of the concentration of the element in the soil solution (Temminghoff, 1998), but are also influenced by a large number of variables, so to understand their uptake by plants several factors should be taken into consideration (Kabata-Pendias, 2004). Sorption-desorption reactions, as well as soil complexation and precipitation-dissolution, are the most influent processes regarding the mobility and bioavailability of metals and metalloids in soil (Caporale & Violante, 2016).

The soil quality is a complex definition, but it is usually determined by its physical, chemical and biological properties and characteristics (Janoš *et al.*, 2010). As for soil quality, the PTEs bioavailability is also influenced by many physical factors such as soil texture, temperature, phase association, adsorption, and sequestration, as well as by chemical factors, like pH and cation exchange capacity (CEC), and also by pedogenic factors, like the concentration of organic and inorganic acids, the binding by OM, and the adsorption by clay minerals (Caporale & Violante, 2016; Kabata-Pendias, 2004; McBride et al., 2004; Tchounwou et al., 2012). The ones that will be considered in further detail are those considered by Carrillo-González *et al.* (2006) and Kabata-Pendias & Mukherjee (2007) as being the most relevant for this matter: soil pH, OM content, redox potential and soil texture and structure.

3.4.1. Physical Factors Affecting Bioavailability

The soil type and its texture exert influence in the metals mobility (Richards *et al.*, 2000). The soils that contain higher clay contents retain larger amounts of PTEs when compared with the sand-textured ones (Sherene, 2010), with the reason being the higher surface area and metal binding sites of the clay-sized particles (Rakshit *et al.*, 2017). In association with clay minerals occur oxides and hydroxides of Fe and Mn that play an important role in the PTEs behavior due to their chemical properties (Kabata-Pendias, 2004; Rakshit *et al.*, 2017), as does the presence or absence of certain cations and anions, that can either improve or inhibit the sorption of specific metals or metalloids (Caporale & Violante, 2016).

The soil water content also represents an important factor, since it is required for chemical reactions and metal mobility and circulation throughout the soils (Violante *et al.*, 2010).

Soil texture alterations also affect the water retention and hydraulic conductivity and, since the solubility of PTEs only manifests on their mobility if there is sufficient water in the soil pores, this is another relevant point regarding the soil texture influence on PTEs (Carrillo-González *et al.*, 2006) (Rakshit *et al.*, 2017). Structured soils have reduced solid-solute interaction, which increases the probability of PTEs to "escape" from the soil matrix (Carrillo-González *et al.*, 2006). Another subject to bear in mind, regarding the availability of PTEs to plants, is the dimension of the root system, since for many soils their availability varies along the soil layers (Milićević *et al.*, 2018). Although this is not a physical aspect, a well-structured soil facilitates the establishment and expansion of the root system.

3.4.2. Chemical Factors Affecting Bioavailability

3.4.2.1. Soil pH

Soil pH is considered by some authors as the most important factor affecting PTEs bioavailability (Zhao et al., 2010). It is widely seen as the main factor interfering with the PTEs mobility, and their solubility can be seriously affected by the slightest pH alteration (Carrillo-González *et al.*, 2006). Along with CEC, the soil pH is a very important factor to their mobility. The importance of CEC is related to the fact that the PTEs mobility in the soils is increased by salinity, depending the intensity of the mobilization on the PTE, its total content in the soil and type of salt. The mechanisms influenced by/related with CEC that affect the PTEs degree of mobilization are competition with Ca and/or Mg for sorption sites, complexation with chlorides and complexation with sulphates (Acosta *et al*., 2011; Iqbal *et al.*, 2017). In the case of pH, its importance is due to its interference in the weathering processes of minerals in soils, such as oxidation and reduction (Kabata-Pendias, 2004; Kabata-Pendias & Mukherjee, 2007), as is shown in **Table 1**. It also exerts influence on the bioavailability by controlling the precipitation-dissolution reaction and the adsorption processes. Although the precipitation of metals in typical soils is unlikely, in highly contaminated soils it can play an important part in the immobilization of these elements (which may reduce environmental risks), likely leading to a greater retention when the pH values are higher. The solubility is also related to the mobility and bioavailability of PTEs (Duraisamy & Bolan, 2003; Alvarenga *et al.*, 2008; Rakshit *et al.*, 2017). Most of the times, the metals solubility and the pH follow an inversed correlation, since one generally increases as the other decreases, and vice versa (Carrillo-González *et al.*, 2006), and the metal uptake by plants is commonly diminished by neutral pH values. A pH increase may also be responsible for an increase in cation absorption by affecting the surface negative charges, as well as it may be responsible for the formation of hydroxyl species of metal cations with greater affinity for adsorption sites. Also, the soil pH, just like the redox potential, has influence on the time that the PTEs remain on the surface soil (Kabata-Pendias & Mukherjee, 2007; Alvarenga *et al.*, 2008).

3.4.2.2. Redox Potential and Electrical Conductivity

The redox potential is other component that exerts a major influence in the mobility of PTEs, and thus, in the control of their toxicity, especially of Cr, Pb, Ni and Cu, (Violante *et al.*, 2010). These reactions involve the transference of electrons from a reducing agent to a oxidizing agent and they also play a role in the decomposition of OM, reason why it influences the mobility of many elements such as Zn, Cu and Pb (Caplat *et al.*, 2005). Its variations, which involve microbial activity, have influence on the metal solubilization degree. Its degree is dependent on the soil porosity and water content, as high redox potential is verified on well aerated and dry soils (Rakshit *et al.*, 2017). The metals that are more susceptible to redox reactions, such as Cr, Fe and Mn, play a major role in the dissolution and precipitations that occur in the soil (Kabata-Pendias & Mukherjee, 2007). As for the electrical conductivity (EC), this parameter act as an indicator of the quantity of soluble nutrients in the form of anions and cations (Eigenberg et al., 2002) and its variations influence the soils salinity levels, like evidenced in **Table 1**. The results obtained by some authors seem to point to a direct relationship between soil salinity and availability of some PTEs, like Cd and Pb (Marvast et al., 2013; Usman et al., 2005), maybe because with high soluble salt concentration there is a higher competition for adsorption sites, with the concomitant liberation of adsorbed PTEs cations. **Table 1** features the different degrees of mobility for some PTEs, under certain redox potential and soils' pH (Kabata-Pendias, 2001).

Table 1 - Mobility of PTEs according to different soil redox potentials and pH values. Adapted from Kabata-Pendias, 2001.

Mobility	**Soil redox potential and pH**	**Elements**
High	. Oxidizing and Acid	. ---
	. Neutral or Alkaline	. Zn
Medium	. Reducing	. ---
	. Oxidized and Acid	. Cd, Cu, Zn
Medium	. Mainly Acid	. Cd, Cu, Ni
	. Reducing with variable potential	. Cd, Cr, Mn
Low	. Oxidizing and Acid	. ---
	. Neutral or Alkaline	. Cu, Mn, Ni, Pb
Very Low	. Oxidized and Acid	. Cr
	. Neutral or Alkaline	. Cu, Ni
	. Reducing	. Cd, Cu, Ni, Pb, Zn

3.4.2.3. Soil organic matter content

The solubility and speciation of PTEs in soil determines their toxicity, availability for plant uptake, and their downward mobility (Robinson *et al.*, 2009). This is another factor that influences the PTEs availability, along with the CEC (Liang *et al.*, 2017), that is dependent on the soil pH (Munera-Echeverri *et al.*, 2018) and of the OM content, among others (Saidian *et al.*, 2016). Also, the OM has a protective role because of its CEC and the capacity to form chelate compounds with metals in soils. In other words, and even though their concentration remains untouched, the OM content of the soil can considerably decrease the PTEs bioavailability (Kinniburgh *et al.*, 1999; Kwiatkowska-Malina, 2018).

3.4.3. Biological Factors Affecting Bioavailability

The accumulation of OM is originated from the decomposition of animals and plants and its content influences the physical, chemical and biological conditions of soils (Rakshit *et al.*, 2017). There are two types of compounds that are believed to be particularly involved in weathering processes: carbonic acid and organic chelates (Kabata-Pendias & Mukherjee, 2007), that can act as PTEs sequestrators (Wuana *et al.*, 2010).

The active soil organic components include the soil microbial biomass and labile organic inputs, since their activity is dependent on the OM content, more concretely on the organic carbon that is used as an energy source for the decomposition process (Ansari & Mahmood, 2019; Diacono & Montemurro, 2010; Kabata-Pendias & Mukherjee, 2007). Therefore, the effects of soil microorganisms in the PTEs availability will also be considered here. Microorganisms generally display a high capacity of sorption regarding PTEs (Kabata-Pendias & Mukherjee, 2007) and influence their availability by redox transformation of inorganic to organic forms and vice-versa. They can also alter the PTEs oxidation state in their metabolic activities, such as anaerobic respiration (Rakshit *et al.*, 2017) and influence the uptake and release of elements from cells and their mobility state, the latter often through methylation (Kabata-Pendias & Mukherjee, 2007). The action of some soil microorganisms can also contribute to the reduction of metal toxicity by decreasing their mobility through reduction action (Violante *et al.*, 2010). Depending on their chemical speciation, the PTEs can even migrate into the ground water and create another serious contamination problem (Rakshit *et al.*, 2017).

3.5. Soil Remediation Methods

Soils are considered as non-renewable resources since their degradation takes place at a much faster rate that its processes of formation and remediation do (Ali *et al.*, 2013). There are certain difficulties in evaluating soil contamination by PTEs since there is no consensus regarding criteria for their concentration that could be hazardous to plants, animals, or human life. Because of this and of all the already described problems that the contamination with PTEs can cause, remediation of these kind of occurrences is needed, at least to considerably reduce their impact (Ali *et al.*, 2013; Kwiatkowska-Malina, 2018). The public awareness of the negative effects that this type of contaminants represent to the environment, as well as their adverse effects to human health, has increased the interest in developing remediation techniques (Yang *et al.*, 2014). So far, different physical, chemical and biological approaches have been employed for the remediation of affected soils and/or to prevent the spread of the contaminants to unpolluted areas (Pavel *et al.*, 2014). Despite being highly efficient, most of these methods suffer from limitations like high cost, intensive labor and time, may cause irreversible changes in soil properties, disturbance of native soil microflora and create secondary pollution problems, not to mention that they might also represent some level of danger to the operators, and the need for constant monitoring after application (Ali *et al.*, 2013; Khalid *et al.*, 2017; Tordoff *et al.*, 2000). Taking that into consideration, it is crucial to develop remediation techniques that are simultaneously cheap, environmentally-friendly, and effective (Ali *et al.*, 2013; Wan *et al.*, 2016). That is the case for the biological methods of soil remediation, bioremediation and phytoremediation, which are considered to be efficient, depending on the contaminated soils situations, while verifying these premises.

Phytoremediation is included in the group of the gentle remediation options and consists of a series of techniques and methods, such as phytoextraction and phytostabilization (Cheraghi *et al.*, 2011; Pavel *et al.*, 2014), that will be furtherly explained, and in where the soil-plant systems are manipulated to influence the fluxes of PTEs in the environment, with the goal of remediating contaminated soils, but with some coupled benefits, such as the recovery of some important metals in some circumstances (i.e., phytomining, (Ansari et al., 2017)), or the increase of the micronutrient concentrations in crops (i.e., biofortification, (A. A. Ansari et al., 2017)).

Phytoremediation includes biological, chemical, and physical processes and, if successfully executed, it should be less costly than other remediation technologies or, at least, be a profitable operation that produces plant biomass products that can, potentially, be valorized (McIntyre, 2003; Robinson *et al.*, 2009; Yang *et al.*, 2014). Not only it is usually cheaper than other remediation methods and treatments, such as the physical, chemical and thermal, as it is also eco-friendly, and a more conservative approach regarding the fertility of the soils (Ali et al., 2013; McIntyre, 2003).

The efficiency of phytoremediation is dependent on numerous plant and soil factors such as the physico-chemical properties of the soil, the bioavailability of metals in soil, as well as of microbial and plant exudates, and also of the capability of living organisms to uptake, accumulate, sequester, translocate and detoxify metals (Khalid *et al.*, 2017). Since water is the main transportation vehicle of many PTEs into the soil–plant system, the soil water retention capacity and its water content highly affects the effectiveness of the process (Robinson *et al.*, 2009).

3.5.1. Phytoextraction

Phytoextraction guarantees a permanent removal of PTEs from the contaminated soils, lowering their concentrations in soil, leading to a consequent soil restoration by the employment and action of plants (Bhargava *et al.*, 2012). These plants are preferably hyperaccumulators, that have considerably high biomass production and, like the name suggests, accumulate large amounts (at levels 100-fold greater that “common plants”) of one or more target PTEs in the aboveground tissues, and then translocate

them to the more easily harvested parts (e.g., shoots) (Lasat, 1999; H. M. Liang *et al.*, 2009; Robinson *et al.*, 2009; Bhargava *et al.*, 2012; Kidd *et al.*, 2015). This high biomass production, along with the ratio of metal concentration of shoots and soil, known as the bioconcentration factor (McGrath & Zhao, 2003), determines the amount of time for the plants to decontaminate the soil (Bhargava *et al.*, 2012) and, consequently, determine the efficiency of the phytoextraction (McGrath & Zhao, 2003). In fact, hyperaccumulator plants do not only have the ability of storing high quantities of essential micronutrients. They can also absorb significant amounts of non-essential elements, such as Cd (Lasat, 1999). Repeated cropping of plants that accumulate contaminating PTEs in soil can lower the soil's PTE concentrations to acceptable levels, provided that the harvested amounts of PTEs exceed further inputs (Robinson *et al.*, 2009).
It is a process driven by the solar energy, which makes it much more economical in comparison with the physical methods, such as electroremediation, or soil washing, that are not only more expensive, but also contribute to the infertility of the soil and the destruction of its structure.

Another perk of this type of remediation is the fact of plant biomass being much easier to recycle, dispose and treat than the soil itself (Liang *et al.*, 2009; MacNair, 2003; Robinson *et al.*, 2009). Plants with a high bioconcentration factor are the ones that are more adequate and suitable for phytoextraction (Cheraghi *et al.*, 2011). This technique also allows the extraction and reutilization of market value metals such as gold (Au), thallium (Tl) and Ni (Ali *et al.*, 2013).

The main downside of this technique is the fact that the hyperaccumulator plants are not only rare, but are also, generally, specific to one or two metals and have a slow growth rate. These factors turn their use in heavily contaminated sites sometimes unrealistic, considering the large amount of time needed to attain the desired remediation, sometimes hundreds of years (Yang *et al.*, 2014), not to mention that this is a decontamination method only viable in low to moderately contaminated soils (Vassilev *et al.*, 2004).

3.5.2. Phytostabilization

Phytostabilization is an *in situ* technique (Vassilev *et al.*, 2004) that is suited for heavily contaminated soils (Shackira & Puthur, 2019) and consists in the manipulation of the fluxes of contaminants by exploiting the plants metabolism and development processes, such as transpiration and roots growth (Robinson *et al.*, 2009). It does not actually remove the contaminants from the soil: its purpose is to stabilize the toxic ions within the roots or near the rhizosphere and then deactivate and immobilize them to prevent their entrance in the food chain and, thus, reducing the risk of PTEs contaminations pose for humans (Robinson *et al.*, 2009; Shackira & Puthur, 2019). The use of vegetation reduces

leaching, being its effectiveness dependent on the climate and soil erosion, since the roots bind the substrate, not to mention it can also reduce the water pollution and wind-spreading of the contaminants (Robinson *et al.*, 2009; Tordoff *et al.*, 2000). The roots also play a part in the maintenance of aerobic conditions that prevent the occurrence of reduced PTE species that are often more toxic than the oxidized ones (Robinson *et al.*, 2009).
This technique may also incorporate the addition of organic and/or mineral amendments into the polluted soils, that lead to alterations in the properties of the soils with the aim of lowering PTEs mobility, that may be even furtherly affected by the PTEs-excluding crops that are used in this method and limit the quantity of PTEs translocated from roots to shoots and help maintaining their low levels in the aerial parts (Yadav *et al.*, 2018; Pavel *et al.*, 2014; Tordoff *et al.*, 2000). This complement, and also the possibility of conducting the phytostabilization in stages, with different crops or treatments, is usually useful when there are multiple contaminants (Vassilev *et al.*, 2004). These PTEs-excluding plants do not translocate high concentrations of these elements into the edible parts, at least not surpassing a critical value (Bhargava *et al.*, 2012), an important factor since this means they do not necessarily represent a loss of production value and consequent income to the producers, because they can be harvested and valorized. There's also the possibility of developing biological processes, especially regarding the nutrient cycling, in order to achieve a low-maintenance vegetation that would reduce the need of fertilization and organic amendments addition (Tordoff *et al.*, 2000).

The plants used for phytostabilization must verify some conditions: they must be tolerant to a certain level of PTEs concentration, as well as some edaphic conditions like drought, salinity, extreme pH values and low nutrient levels (Yang *et al.*, 2014). They must also be poor translocators of metals to above-ground tissues, have rapid growth, big rooting systems and large biomass (Pavel *et al.*, 2014; Yang *et al.*, 2014). Some plants that are used for phytostabilization, like *Miscanthus* spp., have yet another perk: not only do they serve the purpose of remediation for contaminated soils and areas, as they also have potential use as biofuel material, a very useful trait if we consider the predictable increase on the pressure to substitute fossil fuels (Chou, 2009; Pavel *et al.*, 2014). There are also physical and chemical methods of stabilization, but it is widely acknowledged that the use of vegetation is a far more desirable method and of wider utilization, even though it needs to be backed up by an evaluation of the site regarding geology, climate and other parameters (Tordoff *et al.*, 2000).

Despite the many advantages of using these remediation techniques, there are still many ways to enhance their performance to attain better results (Lone *et al.*, 2008; Muthusaravanan *et al.*, 2018) like using complementary agronomic techniques, such as crop rotation, intercropping/row cropping, different planting methods and densities, harvest management, pest and weed control, and irrigation management (Kidd *et al.*, 2015). Other factors that must be considered to improve the efficiency of both phytoextraction and phytostabilization are pH adjustments (since most chemical immobilisation reactions are pH dependent), addition of fertilisers and addition of chelating agents, that can increase metal bioavailability, or contrarywise, amendments to lower the PTEs bioavailability (Alvarenga *et al.*, 2008; Yadav *et al.*, 2018).

3.6. Residues Used as Amendments in Soil Remediation Strategies

Even though the mineral fertilization is able to provide readily available nutrients for the plants development, it does not really contribute to the improvement of soils health (Ferreras *et al.*, 2006). In fact, the intensive use of mineral fertilizers, along with other poor cultural techniques used to face the need of feeding an increasing global population, like continuous cropping and inappropriate replacement of nutrients, contribute to the reduction of the soils OM content and, thus, negatively affect their physical, chemical and biological properties, having a degrading role instead of improving it (Mondini & Sequi, 2008; Tejada & Gonzalez, 2003). Because of this, it is very important to address the soils fertility issue without disregarding the sustainability and try to minimize the environmentally hazardous approaches, especially to the land and water, that are precious resources in which the agriculture and thus the mankind's subsistence depend (Amadi *et al.*, 2017; Diacono & Montemurro, 2010; Tejada & Gonzalez, 2003).

There are many kinds of wastes and residues, both organic and inorganic, that can be applied for agronomic purposes, more specifically as amendments, reclaiming degraded soils, but that can also, in some cases, act as fertilizers by supplying nutrients to the plants. Some examples of cost-effective organic amendments are the use of sewage sludge that may be co-composted with other green-derived residues, like agricultural wastes and palm tree residues, or municipal solid waste compost (Alvarenga *et al*., 2008). Other strategy is the use of certain legumes, that make associations with rhizobium species in their roots, fixing nitrogen, being these useful when nitrogen inputs are needed (Lemage & Tsegaye, 2020; Pérez-Gimeno *et al.*, 2019; Tejada & Gonzalez, 2003). In fact, regarding the utilization of sewage sludge compost, the results of the experiments led by Montemurro *et al.* (2007), in which the application of a mixed treatment (50% mineral fertiliser and 50% organic compost) did not lead to different responses than those of the highest mineral N treatment provided, which helps sustaining that premise. More so, and contrarywise to the mineral fertilizers, this addition of OM can contribute to the improvement of the soils fertility by positively affecting the physical properties such as bulk density, aggregation and stability and even decrease the erosion susceptibility and improve the water retention capacity (Celik, 2005). Much of the benefits come from the high content of humic substances in these wastes, which are very active compounds in the soils that have much higher anion and CEC than clay, not to mention all the biological properties that were already described.

Furthermore, and still in the regard of the sustainability's concern, their utilization prevents the use of non-renewable sources, like fossil fuel, and also the decrease of the energy expenses with fertilizers (both production and application), tillage and irrigation (Diacono & Montemurro, 2010).

The pollution of the environment with PTEs as a consequence of their release by mining and other industrial activities, allied with the erosion agents, such as water and wind, result in the emergence of areas where vegetation is very scarce, since the soils have undesirable characteristics like low pH, poor nutrients content and high concentrations of toxic elements (Alvarenga *et al.*, 2008).

These residues can play an important role in the renewal/reestablishment of the vegetation that, by acting as a cover to the soils is, according to Tordoff *et al.* (2000), indispensable and the only way to achieve a long-term rehabilitation of the soils. The residues are also important by adding nutrients for plant growth, increasing the water holding capacity, raising the pH, rendering the metals less soluble/bioavailable and increasing the OM content, which mineralization can be translated into better yield parameters for certain cultures (Alvarenga *et al.*, 2008; Tejada & Gonzalez, 2003). They can be of an extreme usefulness in the Mediterranean area where the climate, particularly the high temperatures and the poor distribution of rainfall throughout the year and between seasons, as well as the use of traditional high intensive cereal cultivation systems, increase the rate of OM depletion (Montemurro *et al.*, 2007).

In the experiments led by Alvarenga *et al.* (2008), different types of organic residues were tested as possible amendments, in the context of evaluating if they could be used to immobilize PTEs in contaminated soils, namely Cu, Pb and Zn. The residues used were anaerobically digested sewage sludge (SS), the organic fraction of unsorted municipal solid waste (MSWC) and garden waste compost (GWC). The results attained for each were significantly different, but the GWC was the only that was considered adequate for this purpose, decreasing the metal solubility in the soil and increasing both pH and OM content.

The results attained through the experiments of Kwiatkowska-Malina (2018), for the same purpose, regarding the use of brown coal, more specifically the humic substances that this material contains, were equally satisfying, leading to an effective immobilisation of Cd and Zn in a higher rate, but also of Pb.

The remediation techniques that recycle organic residues can be very useful and inexpensive soil conditioners and sources of nutrients to the plants, and are most likely the best option for remediation of soils contaminated with PTEs, especially if the area to treat is large and with high PTEs concentrations (Alvarenga *et al.*, 2008; Janoš *et al.*, 2010). However, the specific properties and effects caused by the amendments used, either organic or inorganic, must be evaluated, since their addition is likely to change and affect other soil properties like pH and CEC, that, by their turn, are already known to affect the PTEs bioavailability (Burgos *et al.*, 2009; Pérez-esteban *et al.*, 2013). Nevertheless, and even considering the unfeasibility of some amendments to the remediation of PTEs contaminated soils, their utilization should not be underestimated since, like previously stated, they generally have a lot of other benefits (Diacono & Montemurro, 2010).

3.7. Orange Juice Production Residues and Potential Application as Contaminated Soils Amendment

The orange is the most cultivated citrus fruit in the world, accounting for about 50-60% of the total citrus species production, whose processing industry plays an important role in the agro-industrial sector (FAOSTAT, 2020; Zema et al., 2018). The processing of citrus fruits in the food industry produces large amounts of waste materials, like peels and seeds, accounting for 55-65% of the processed fruits total weight (Andiloro et al., 2021). For this reason, a big piece of the value of this industry comes from the by-products, i.e., other products rather than orange juice, such as essential oils and cattle feed. After processing the orange juice, the agro-industry produces wastewater and solid/semi-solid residues, the citrus peel waste, that has low pH and high content of many value-added organic compounds such as flavonoids, sugars, essential oils and carotenoids, which give these residues a very high potential economic value (Fernández-López *et al.*, 2004; Goodrich & Braddock, 2006; Ortiz *et al.*, 2020; Sharma *et al.*, 2017; Zema *et al.*, 2018).

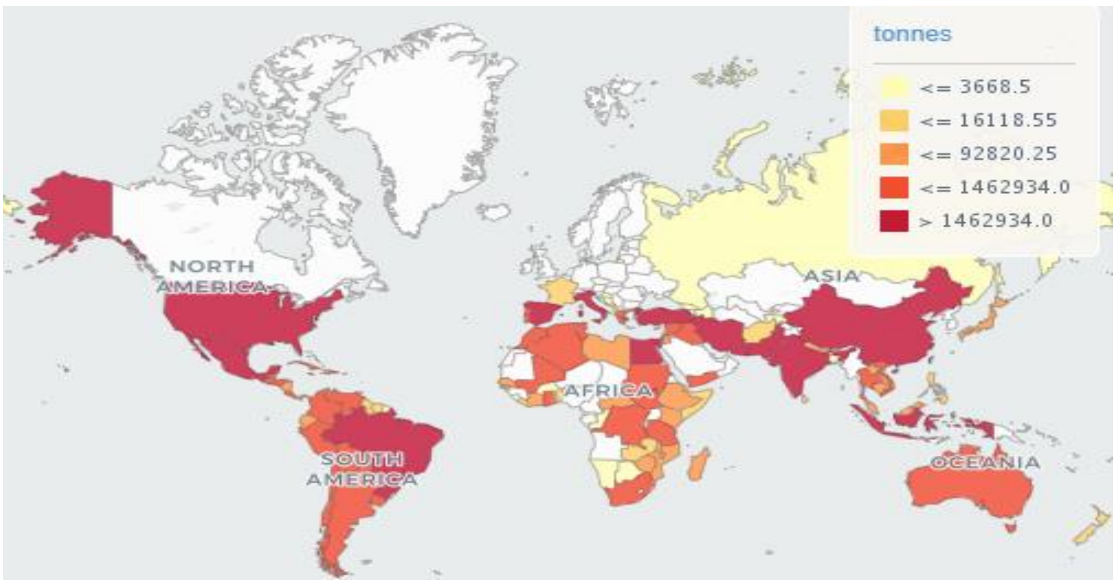

Figure 1 – Orange production average from 2000-2019 per country. Source: Adapted from FAOSTAT, 2020.

Only about 50% of the fruits are used for the juice production, whose demand has risen in the later years. This is a major problem within this industry since the residues cause clogging of the tanks and create pollution in the residual waters, which treatments and/or disposal are considerably costly to the industry itself. However, it is also a business opportunity, since the minimization of these expenses is the justification of the by-products existence, as they represent an attempt to make the orange juice more cost-effective, even though it is not always as profitable as desired (Cypriano *et al.*, 2018; Kalderis *et al.*, 2019). Still according to Cypriano *et al.* (2018), one way this goal could be accomplished would be through the utilization of these industrial residues to produce energy in the forms of biomethane and bioethanol. In the case of bioethanol production process, three high added value products can be obtained – phytochemical (hesperedin), biomaterial (nanocellulose) and biofuel (ethanol) -, which can turn the existence of these residues profitable, even considering the expenses that the implementation of a new industry represents. However, this utilization of the residues as energy sources, even though promising, has a few constraints attached, since the high levels of essential oils limit the anaerobic and alcoholic fermentation processes necessary to produce biomethane and bioethanol respectively (Andiloro et al., 2021).

In **Table 2**, **Table 3** and **Table 4** (Aravantinos-Zafiris et al., 1994; Garcia-amezquita et al., 2019; Park et al., 2018) have summarized these residues physicochemical characteristics, fibre content and also some nutrients composition. Given the described chemical composition of these residues, it is fair to say that they can be effectively used as organic amendments or as part of controlled release fertilizers (Amadi *et al.*, 2017; Flores-Rojas *et al.*, 2020). Their application can improve soil physical constraints, decreasing bulk density, increasing porosity and hydraulic conductivity, as well as their chemical and biochemical properties, leading to the increase of soil OM content and, thus, of their fertility in general, and of their enzymatic activities in particular (Kalderis *et al.*, 2019; Sial *et al.*, 2019).

Table 2 - Orange peel fibre physicochemical characterization and respective components approximate value (Source: Garcia-Amezquita *et al.*, 2019).

Component	**Content (g/100g)**
Protein	4.9 ± 0.1
Fat	1.5 ± 0.1
Ash	4.2 ± 0.1
Digestible Carbohydrates	40.6 ± 0.3
Dietary Fibre	48.7 ± 0,6
Insoluble Fraction	42.7 ± 0.5
Soluble Fraction	6.4 ± 0.3

Table 3 - Dietary fibre components content (Source: Adapted from (Aravantinos-Zafiris *et al.*, 1994).

Component	**Content (%)**
Celullose	45.7
Hemicelullose	18.8
Uronic Acids	34.7
Lignin	0.0074

Table 4 - Nitrogen, P and K content of orange juice residues (Source: Park *et al.*, 2018).

Nutrient	**Content (mg/L)**
Nitrogen	
Nitrate	<1
Nitrite	<1
Ammonium	<1
Phosphate	96.0 - 115
Potassium	1302.2

Other potential use for these residues can be their ability to adsorb chemical compounds and toxic metals (Park et al., 2018), since it has already been proved that the orange residues utilization for the removal of heavy metals from contaminated sites might be a reasonable idea (Rezzadori *et al.*, 2012). However, the management of the optimum pH values has to be smartly and consciously made, since the acid pH the orange fruits display has to be taken into account to this matter because the heavy metals are more likely to be completely released under circumstances of extreme acidic conditions. (Annadurai *et al.*, 2018). Still, according to Annadurai *et al.* (2018), the orange peels can be used for the removal of heavy metals from synthetic solutions. In addition to this, the citric acid can also serve as low cost potential chelating agent of heavy metals when used at a larger scale (Yadav *et al.*, 2018).

In the experiments led by Ricci *et al.* (2019), the pH of the orange residues was very close to 4. This value might be explained by the citric acid present in the orange juice residues, raising some red flags over the purpose of immobilizing PTEs, since it is considerably low. In fact, the experiments carried out by Amadi *et al.* (2017) showed that the phytoaccumulation of Zn by *Cyperus iria,* an accumulator plant, was improved by the addition of orange peels as an organic amendment, which is in accordance of the fact that the residue contributed to an increase in Zn availability to the plant.

The properties displayed by the orange juice residues, alongside with many others already mentioned for other waste-streams and justifies the importance of evaluating the possibility of their application to the soil as an organic amendment. This possibility can create an alternative to the disposal of the residues from the orange juice industry, when they are not valorised to create added value by-products, in a sustainable economic and ecological approach, which is one of the foundations of the circular economy (Kalderis *et al.*, 2019).

The main aim of this study relies in this aspect, the perspective of using the residues from the orange juice industry as an organic soil amendment, eventually contributing to the immobilization of PTEs when the soil is contaminated.

4. Materials and Methods

The organic material used in the experimental work was orange juice residue collected at *"A Padaria Portuguesa"*, a chain of pastry shops, in which these residues represent a burden due to the lack of a proper valorisation process for this kind of waste. Given that, the producer simply makes its disposal as a waste.

After collection, the orange peels were torn into smaller fragments and then crushed and shredded using a *Bimby* equipment. The described sequence of actions turned the peels into a compact and moist paste, from which a sample was taken and put into a sealed hermetic bag that was kept at 4°C at a laboratory in *Instituto Superior de Agronomia* until further analysis (humidity, pH, OM content and mineral composition). The remaining part of the material was oven-dried to be subsequently tested as a soil amendment.

4.1. Physico-Chemical Analysis of the Orange Juice Residues

4.1.1. pH and Electrical Conductivity

The pH and EC values were determined in an aqueous extract with a residue/distilled water ratio of 1:5 in weight, at 20 °C.

The pH determinations were made in potentiometer (*Metrohm model 632) with* a combined-glass electrode while the EC value was obtained with a conductivimeter *(Metrohm model 660)*.

4.1.2. Dry Matter

The DM determination was done via the gravimetric method, which is based in the difference of the weight values before and after the samples are submitted to drying in an oven at 102±2°C until constant weight is reached. The DM percentage can be calculated with expression:

$$\textbf{Dry Matter \%} = \frac{dry\ sample\ mass}{wet\ sample\ mass} \times 100$$

4.1.3. Organic Matter

The method used to determine the OM content was the Walkley-Black (1934) method, which determines the total organic carbon. This method is based on the oxidation of the soil's OM in an acid medium (concentrated sulfuric acid H_2SO_4, 98% d=1.83) and with an excess of potassium dichromate 1 N. The oxidant agent in excess is titrated with ammoniacal ferrous sulphate (Mohr's salt). Assuming that the OM contains 58% of carbon, the OM content is calculated by multiplying the total organic carbon by the 1.724 factor (the inverse of 0.58) (Walkley & Black., 1934).

% Organic Matter (g of OM / 100 g sample) = % Organic Carbon x 1.724

4.1.4. Nitrogen

The total N content was determined with the Kjeldahl method. This method is based in the mineralization of the organic compounds contained in the sample in an acidic medium, hot and in the presence of a metallic catalyst. This digestion leads to the conversion of all the N into ammonium ion, which is then converted to ammonia, distilled in steam and collected in a boric acid solution, where it is dosed by titration with a HCl solution acting as the titrant. The total content of N is then calculated through the following expression:

N % (g N/100 g of sample) = $\frac{1.4 \times [HCl] \times (VA - VB)}{m} \times \frac{Volume\ (Volumetric\ flask)}{Volume\ (Pipetted\ for\ distillation)}$

from which:

[HCl] = concentration of the HCl solution (molarity);
VA = volume of HCl used in the titration of the sample (mL);
VB = volume of HCl used in the titration of the blank test (mL);
m = mineralized sample mass (g).

The protein content was calculated based on the total N content value obtained through the application of the method and calculations presented above. These indicate us that 1.13 % of the residues composition is N. If we multiply that for the standard conversion factor of N to proteins, 6.25 (Mariotti et al., 2008), we are able to come up with the content of proteins within the residues, that in this case corresponds to 7.05 % of its total composition.

4.2. Physico-Chemical Analysis of the Soil

The soils were characterised regarding their texture, pH, EC, OM content and mineral composition, with methods that will be explained in this sub-chapter.

4.2.1. Electrical Conductivity and pH Determination

Both pH and EC were determined with the methods described by Póvoas & Barral (1996). The soil pH (H_2O), sometimes called soil reaction, was measured in a soil water suspension, using a soil-distilled water ratio of 1:2.5 (w/w) at 20°C. The measurements were made using a pH meter equipped with a combined glass electrode, calibrated to pH standards of 7.0 and 4.0 and using a *Metrohm model 632* pontentiometer. The classification of the soil reaction can be made using the **Table 5** (Chesworth, 2008).

Table 5 - Soil classification by pH values. (Source: Chesworth, 2008).

Classification		Values
Acidic	Extremely	0-4
	Strongly	4-5
	Moderately	5-6
	Slightly	6-6.5
Neutral		6.5-7.5
Alkaline	Slightly	7.5-8
	Moderately	8-9
	Strongly	9-10
	Extremely	10-14

The EC is directly related to the soil's salinity, which makes it conveniently measured in soil suspensions in distilled water, in a ratio of 1:2.5 in dS/m (mmhos/cm), with a conductivimeter (*Metrohm model 660*) and by inserting its conductivity cell in the water suspension. The soil salinity can be interpreted with the values presented in **Table 6** (Isaac & Donohue, 1983).

Table 6 - Soil salinity classes and respective EC intervals. Source: Adapted from Isaac & Donohue 1983).

Salinity Class	Electrical Conductivity (µS cm^{-1})
Not Saline	< 400
Very Slightly Saline	400-800
Slightly Saline	800-1600
Moderately Saline	1600-2400
Strongly Saline	2400 - 3200
Very Strongly Saline	>3200

4.2.2. Organic Matter Determination

The method used to determine the soils OM content was the same that was used and described earlier for the determination of this content in the orange juice residues, which consisted of calculating the content of organic CT and multiplying this value with the 1.724 factor. The content of soil OM can be classified in accordance with **Table 7** (Quelhas dos Santos, 1996).

Table 7 - Soil classification according to organic matter content. Source: Addapted from (Quelhas dos Santos, 1996).

Organic Matter Percentage		
Sandy Soils	Loamy and Clayish Soils	Classification
< 0.5	< 1.0	Very Low
0.6 - 1.5	1.1 - 2.0	Low
1.6 - 5.0	2.1 - 7.0	Medium
5.1 - 10.0	7.1 - 15.0	High
> 10.0	> 15.0	Very High

4.2.3. Mineral Elements Content Determination

The determination of the mineral composition of the soil, in the beginning and after the treatment, was made by digestion to destruct the OM and solubilise of the metals. This was accomplished by using aqua regia, that consists of a mixture of 21 mL of concentrated HCl (37%, d=1.19) and 7 mL of HNO_3 (65%, d=1.3) (ratio of 1:3 v/v) and is the method recommended by the *International Organization for Standardization,* ISO 11466 (1995). Even though it does not actually provide the total metal content, but yet the pseudo-total (Kaasalainen & Yli-Halla, 2003), it is considered as an adequate method for quantifying the total recoverable metals in soils, since the majority of the residual elements that are not released by aqua regia (bound to silicate minerals) is considered irrelevant to the estimation of the elements mobility and behaviour (Chen & Ma, 2001; Niskavaara *et al.*, 1997).

After the digestion of the dried soil sample (3 g) at room temperature during 16 hours, the suspension is digested for 2 h at 130 °C and under reflux conditions, filtered through a Whatman ash-free filter, diluted to 100 mL with 0.5 M HNO_3 and stored in polyethylene bottles at 4 °C for elementary analysis. The mineral elements Cd and Cu were analyzed in an ICP-OES (Inductively Coupled Plasma Optical Emission Spectroscopy, Thermo Scientific iCAP™ 7200).

4.2.4. Evaluation of the bioavailable fraction of the mineral elements

Only a fraction of the total content of the soil's elements are in an available form to be absorbed by plants, which is usually tentatively analysed after performing an adequate extraction with an appropriate solution. In order to determine the bioavailable metal content of Cu and Cd the method used was based in the one described by Houba *et al.* (1996), in which 10 g of soil were mixed with 100 mL of a $CaCl_2$ solution (0.01 M) in an end-over-end shaker, for 2 h, at 30 rpm and room temperature (20 ± 2 °C). The extract was separated by centrifugation at 3000 *g* for 10 minutes. The fraction of mineral elements which were obtained were considered the bioavailable fraction and were also measured by ICP-OES.

4.3. Characteristics and Pre-Treatment of the Soil Used in the Experiment

In the experiment the soil which was used was from Tapada da Ajuda, collected at *Instituto Superior de Agronomia*. These soils are, regarding their texture, classified as clayish soils, which corresponds to a fine texture (García-Gaines & Frankenstein, 2015). Part of the soil was artificially contaminated with 12 mg of Cd and 600 mg of Cu per kg of soil (CT), and the rest was kept at the same conditions to serve as a control non-contaminated soil (NCT). These concentrations were chosen because, as it is possible to see in **Table 8,** they clearly surpass the legal limits defined by APA (Agência Portuguesa do Ambiente), for both Cu and Cd. This way, the effects of the application of the residues becomes easier to analyse and is tested in a situation of extreme contamination (APA, 2019). The CT and NCT soils pre-experimental characterization is summarized in **Table** 9, and their main characteristics were classified in accordance with the criterions referred in the previous sub-chapters.

Table 8 - Legal limits for Cd and Cu concentrations in soil for Agricultural use. (Source: APA, 2019).

Contaminant	Reference Values for Agricultural Use(mg/kg dry weight)
Cadmium	1
Copper	62

Table 9 - Pre-experimental soils characterization.

Parameter		Control (NCT)	12 mg Cd/kg + 600 mg Cu/kg (CT)
Field Texture		Clayish	Clayish
Electrical Conductivity (1:2) (µS cm-1)		475.53	871.23
pH (H2O)		6.33	6.25
Organic Matter (%)		0.64	0.65
Total/Real	Cd (mg/kg)	1	10.5
	Cu (mg/kg)	41	618.3

4.4. Experimental Design and Procedure

The incubation test was performed in plastic boxes (**Figure 2**), in triplicate, using 250 g of non-contaminated and of contaminated soil, evaluating three application doses: R1, corresponding to 12 t/ha (0.45% m/m, all in a DM basis), R2, corresponding to 24 t/ha (0.90% m/m) and R3, corresponding to 48 t/ha (1.80% m/m). To achieve this application doses, the dry mass of residues which were applied were 1.13 g, 2.26 g and 4.52 g of orange juice residues per box, in each modality, plus a box with each soil without residue application (control). The calculations made to obtain these application doses are detailed explained in **Annex A**.

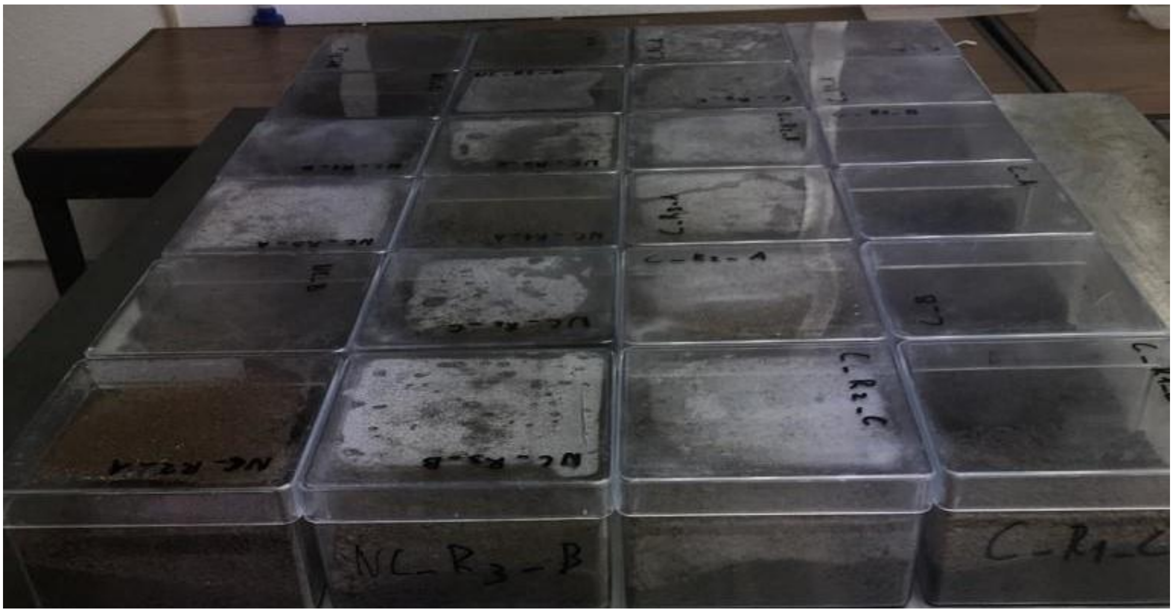

Figure 2 – Boxes and respective content used during the trial.

The description of all modalities regarding de code, soil type and quantity of each material, are summarized in **Table 10**. The soil plus the orange juice residues were thoroughly mixed. After that, distilled water was added to achieve a 60% water holding capacity (28.5 g of water per 250 g of soil). The calculations made to ascertain the quantity of water needed to apply to each box are also calculated in **Annex A**. After that, the boxes were covered, weighed and left in a acclimatized room at the laboratory, at room temperature, to incubate for one month. The boxes were weekly weighed to restore the quantity of water that was loss by evaporation by adding distilled water. At the end of the incubation experiment, the soils were analysed in order to evaluate the effects that the residues application had in the soil properties using the above-mentioned methodologies.

Table 10 - Description of the different content for each of the 24 boxes and respective weighed values (n = 3). NC: non-contaminated soil; CT – contaminated soil; R1, R2 and R3: the different orange juice residues application doses.

	Tretament	Soil (g/box)	Residue (g/box)	Application rate (t/ha)	Application rate (% m/m)
Non-contaminated Soil	NC	250	0	0	0
	NC_R1	250	1.13	12	0.45
	NC_R2	250	2.26	24	0.90
	NC_R3	250	4.51	48	1.80
Contaminated soil	C	250	0	0	0
	C_R1	250	1.13	12	0.45
	C_R2	250	2.26	24	0.90
	C_R3	250	4.51	48	1.80

4.5. Characterization of the Orange Juice Residues

The orange juice residue was milled, treated and analysed using the methods described I chapter 4, and the results are presented on **Table 11**.

Table 11 - Orange residues properties determined in the original material (n=3).

Parameter	Value
pH	3.56
Moisture content (%)	82.45
Ash (%)	4.39
CT (%, in DM)	37.80
Kjeldahl N (%, in DM)	1.13
Total Protein (%, in DM)	7.05

The orange juice residue was initially a moist yellow paste with a very high moisture content, acid pH and high CT content **(Table 11** and **Figure 3 A**). For drying, the residues were spread into metal trays and put to dry for 7 days in an oven at about 75-80 °C. This drying process led to a material more compact and fibrous (**Fig. 3 B**), brown-coloured, which was milled to obtain a more homogeneous material (**Fig. 3 C**). These processes were carried out not only to obtain a residue which was more adequate to be applied and mixed with the soil but also to facilitate the calculations of the application rates, which in this case are independent of the sample moisture content.

Figure 3 – Orange juice residues appearance alterations throughout the processes carried out for their application to the soils.

4.6. Statistical Treatment

With the purpose of evaluating and analysing the statistical differences between all the modalities and the existence or non-existence of homogeneous groups, a two factors variance analysis (ANOVA) with the Tukey test was performed. The level of significance used in this statistical treatment was 5%, which means the probability of rejecting the null hypothesis when it is true is 5%.

The statistical treatment was made resorting to the GDM Solutions® Agriculture Research Management 2021.1 *software* and considered four different modalities (R0, R1, R2 and R3) in two soils: one that was artificially contaminated (CT) and the other that was not contaminated (NCT). The soil parameters that were subjected to analysis were pH, EC, OM content and total and bioavailable contents of Cu and Cd.

In the presentation of the results, the lowercase letters at the top of each column of the graphics represent the significant differences between the means of the different modalities. Whenever a repetition of the same letter is verified across two or more different columns, the meaning is that the average values are statistically equal ($p < 0.05$). The error bars figured on the columns of the graphics represent the standard deviation (SD) of the mean values of the samples.

5. Results and Discussion

The results presented in this chapter represent the effects of three different doses of orange juice residues to two different types of soil: NCT and contaminated CT with two different of PTEs, 12 mg Cd/kg soil and 600 mg Cu/kg soil. As already mentioned, these concentrations are well above the legal limits for soils with agricultural use, being almost ten times the legal concentration for Cd and twelve times the legal concentration for Cu.

5.1. Effects of the Orange Juice Residues on Soil Properties

Like previously stated in chapter 3, the uptake of PTEs by plants varies according to many factors, which include soil chemical properties such as pH, EC and OM content. For that reason, the next sub-chapters will focus on the way each of these variables were affected by the addition of the orange juice residues to the NCT and CT soil.

5.1.1. Organic Matter

Comparing the OM content obtained in the different application doses, both CT and NCT soils registered an increase in OM content relatively to the non-amended controls, from 0.912% to 1.319% and from 0.975% to 1.393%, respectively with the application of the maximum amount of residue used in this experiment (80 t/ha) **(Figure 4)**. These changes of OM content turn both CT and NCT soils from “Very Low” to “Low” in the classification of OM content of loamy and clayish soils **(Table 7)**, which indicates that the addition of the orange juice residues can lead to an increase of the soil OM content, reinforcing the effect that the residues application had in the OM content.

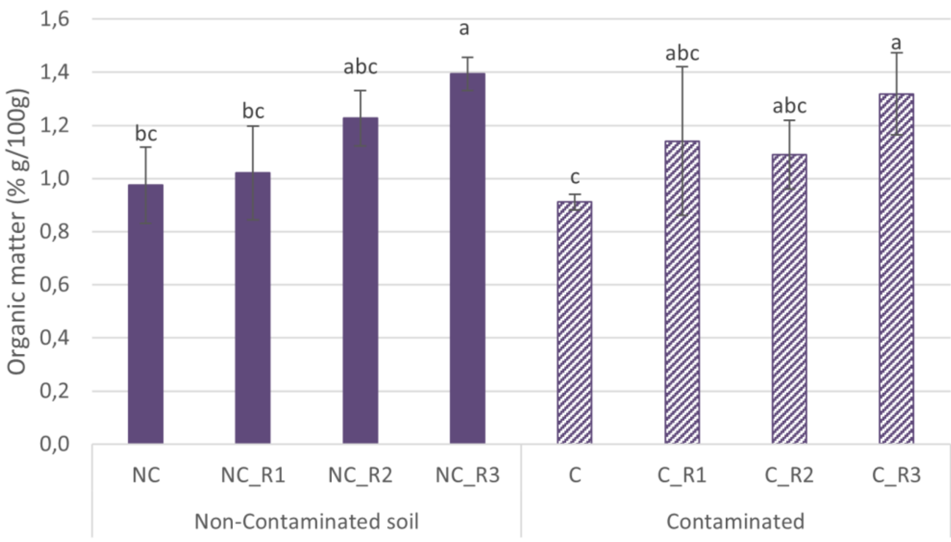

Figure 4 - Organic matter content in the non-contaminated soil (NCT) and contaminated soil (CT) exposed to the different doses of orange juice residues (R1, R2 and R3) (mean values, n=3). The error bars represent the standard deviation values. Columns marked with different letters indicate results with significant differences (Tuckey HSD test, p <0.05).

Nevertheless, the statistical treatment of the data indicates that there were no significant differences regarding the OM content of the soil between all of the four modalities considered in the experiment, neither in CT nor in NCT soil, at a 5% significance level ($p < 0.05$). In fact, these differences were only verified for the R3 modality. According to Albiach et al. (2001) and Tejada & Gonzalez (2003), the lack of significant differences in might be due to the composition of the amendment, soil type or rate of application, factors that influence the intensity of OM content increase, being the latter one a valid reason for modalities R1 and R2 not verifying significant differences when compared with the control, in opposite to what happens in modality R3.

However, like previously stated, there seems to be a tendency, in both CT and NCT soils, for the OM content to increase as the amount of residue applied increases too. Regardless of how slight these OM content increases may have been, they were to be expected, since the addition of organic amendments usually leads to increases in soil OM and microbial activities (Alvarenga et al., 2008; Goyal et al., 1999; Tejada & Gonzalez, 2003). This tendency to increase the OM content in the soils is also sustained by the high value of CT (37.8%) contained in the residues. In the NCT soil this so-called increase follows a direct proportionality between OM content and the quantity of residue added, while in the CT soil the R2 modality registered a lower value of OM when compared with the R1 modality. According to González-Ubierna et al (2012), the most effective way to increase the OM content in soils is to combine the application of organic amendments with fertilizers, which may help explaining the lack of significant differences that were verified.

In an experiment described by Bouajila & Sanaa (2011), organic compost and manure were applied to a soil in order to evaluate the effects they could produce in its OM content. According to the authors, both the applications resulted in significant differences in the organic carbon content ($p < 0.05$) when compared with the control. However, for both materials, the quantities applied to the soil were of 40 ton/ha, 80 ton/ha and 120 ton/ha, while the three modalities used in this experiment consisted of applying 12, 24 and 48 ton/ha of orange juice residues. The higher quantities applied by the authors might be a valid justification for why they have produced significant differences in the organic carbon content, while the same cannot be said for this experiment's results. Nevertheless, the application of 120 ton/ha of manure resulted in an increase from 0.69 % to 1.09 % of organic carbon content, while in our case, the highest application rate (48 t/ha) caused the OM content to increase from 0.975 % to 1.393 % in the NCT soil and from 0.912 % to 1.319 % in the CT soil.

5.1.2. Soil pH

The application of the orange juice residues led to different results in the CT and NCT soils, since modalities R2 and R3 registered significant differences when compared with the control in the NCT soil, while no significant differences were registered in CT soil **(Fig. 5)**. Nevertheless, the different doses of residue applied to the soil lead to an apparent increase of the CT soil's pH. This tendency appears to follow a direct proportionality between the quantity of residue applied and the final soil pH value **(Fig. 5)**.

These results were not expected, considering the pH value of the orange juice residues (3.56), determined in the original wet material, which was very low **(Table 5)**. However, it is important to say that the material which was applied to soil resulted from the orange juice residues dehydration in a drying oven, followed by their milling to finer particles. This process may have changed the behavior of the material regarding its pH. Despite that, by matching the values obtained with the intervals figured in **Table 5** we can see that, even though the increases in the dosage applied seemed to lead to increases in the soil pH in a linear way, no differences were verified in this chapter either, being that all the modalities of both CT and NCT soils fall into the same category, neutral soils.

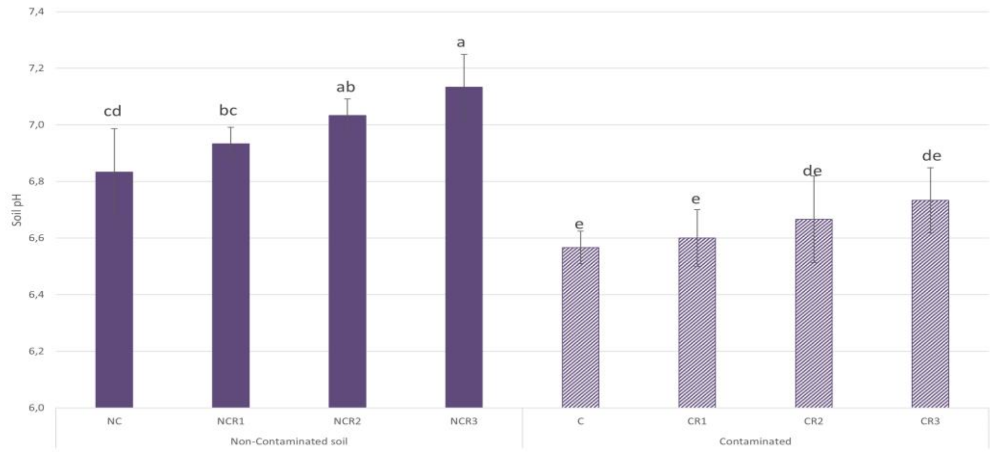

Figure 5 – Soil pH values in the non-contaminated soil (NCT) and contaminated soil (CT) exposed to the different doses of orange juice residues (R1, R2 and R3) (mean values, n=3). The error bars represent the standard deviation values. Columns marked with different letters indicate results with significant differences (Tuckey HSD test, p <0.05).

Similar results were obtained by Sial et al. (2019b) in an experiment that consisted of adding orange juice residues and waste biochar to soil. The results also verified a tendency to increase soil pH with increasing application rates, and were also significant from a statistical point of view.According to Lehmann et al. (2011), these increases might be explained by the ash contained in the biochar, which lead to the dissolution of alkaline minerals. In fact, many organic amendments produce this liming effect. In cases where there may be some economic constraints to soil remediation, organic amendments can act both as fertilizer and liming agents, being their liming efficiency dependent on the alkalinity of the ash (Naramabuye & Haynes, 2006).

5.1.3. Electrical Conductivity

Considering the EC of the soils as a consequence of the orange juice residues application, it is possible to see that, even if the differences were not significant from a statistical point of view (p>0.05), the application of different quantities of the orange waste seem to lead to slightly different values in this variable, in this case, to an overall decrease with increasing application rates **(Fig.6).**

However, the results are not the same for NCT and CT soil, since in the first case every quantity applied apparently leads to a decrease of EC when compared with the control, which does not happen in the CT soil, which has a EC higher than the NCT soil, a consequence of the application of the metals salts to artificially contaminate the soil. However, and maintaining the focus in comparing the control with the respective three modalities, despite the fact that R1 seems to lead to a slight increase of EC, the decrease verified for R2 and R3 appears to be much more consistent. Therefore, it is important to emphasize that these organic residues do not contribute to an increase in the EC of the soils, at least considering the application rates used.

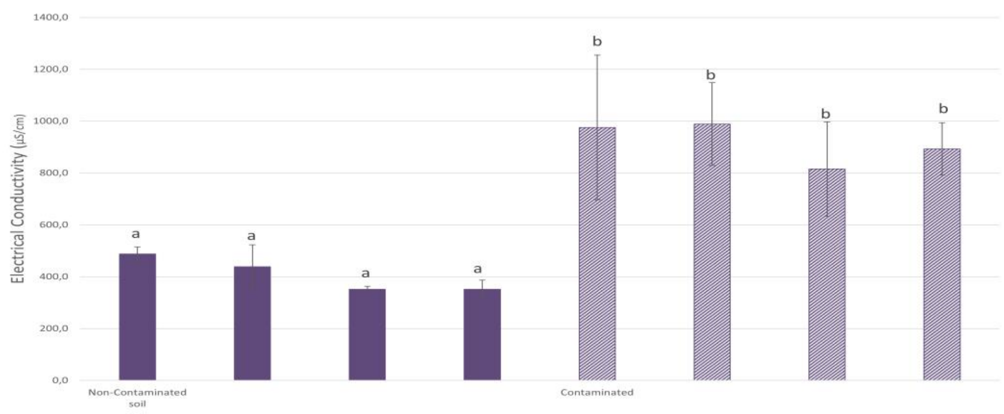

Figure 6 – Electrical conductivity values in the non-contaminated soil (NCT) and contaminated soil (CT) exposed to the different doses of orange juice residues (R1, R2 and R3) (mean values, n=3). The error bars represent the standard deviation values. Columns marked with different letters indicate results with significant differences (Tuckey HSD test, $p < 0.05$).

This results are consistent with the ones obtained by Sial et al. (2019), in which the orange juice residues application did not produce significant differences in the soils EC. According to Barbosa & Overstreet (2011), it is very difficult to conclude what might be the variations of a soil's EC, because it depends on its initial value. Nevertheless, and despite these apparent variations in EC value when comparing control with the three different modalities, they all fall in the same salinity class (not saline), as we can see by comparing the obtained values **(Figure 6)** and the values presented for EC for all different soil salinity classes **(Table 6)**.

5.2. Soil PTEs Content

The soil Cd and Cu pseudo-total concentrations were obtained in the samples and the effect of the application of the orange juice residues to the soils, both to NCT and CT soils will be evaluated in this sub-chapter.

As previously stated, the CT soil was contaminated to a level of contamination above the limit value recommended by APA for soils meant to be used for agricultural purposes, which is of 1 mg/kg for Cd and 62 mg/kg for Cu.

However, even though the total concentration of an element in a soil may be valuable information to assess the level of contamination of the same soil, this data is not enough and/or sufficiently accurate for that purpose, since the uptake of those elements by the plants will depend on other factors, such as the individual traits of each element that might affect its behavior and mobility in the soil and the soils characteristics. For this reason, this sub-chapter will include the estimated bioavailable fractions of the elements, whose values were obtained using the $CaCl_2$ extraction method, described previously in chapter 4.

5.2.1. Cadmium

By the analysis of **Figure 7**, it is possible to observe that there are no significant differences for the values of pseudo-total Cd content ($p>0.05$) among the results for each type of soil and their amended counterparts, which is in accordance with the fact that they have different levels of contamination (NC and C), which were not affected by the treatments, because the orange juice residues are not a contaminated residue. This scenario was already expected, since the orange juice residues application were not expected to produce effects in the total content of PTEs, only eventually on their extractability/mobility/bioavailability.

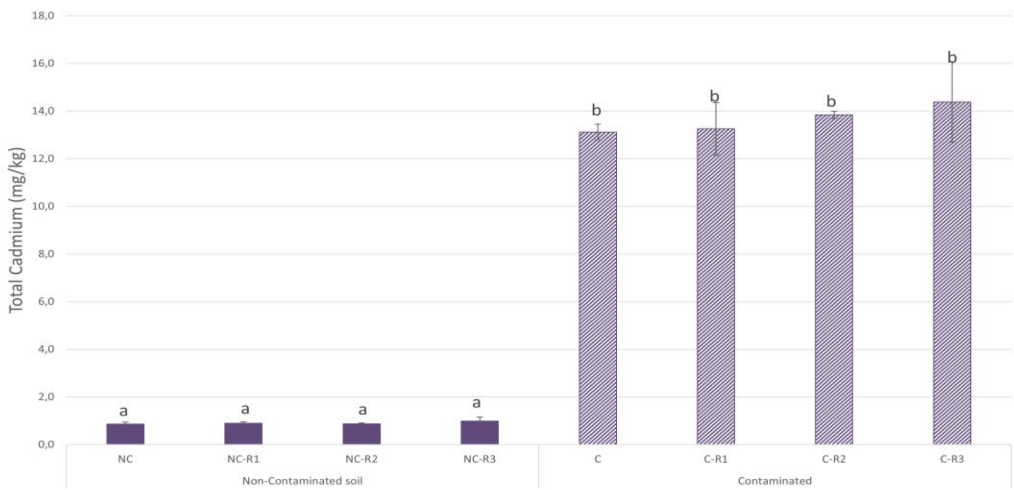

Figure 7 – Cadmium pseudo-total content (mg/kg) in the non-contaminated soil (NCT) and contaminated soil (CT) exposed to the different doses of orange juice residues (R1, R2 and R3) (mean values, n=3). The error bars represent the standard deviation values. Columns marked with different letters indicate results with significant differences (Tuckey HSD test, $p < 0.05$).

In regard of the legal values for the element concentration in soils, none of the values registered in the NCT soil exceed the 1 mg/kg DM maximum limit established by APA (2019) for soils with agricultural purposes. In the case of the CT soil, all modalities were above this limit, as previously referred.

Focusing on Cd's bioavailability **(Figure 8)**, the application of the orange juice residues has produced different results for CT and NCT soils. In the NCT soil, all modalities and control exhibit residual contents of extractable Cd. The same cannot be said for the CT soil, for which the values obtained were considerably higher when compared to those of the NCT soil. Regardless, all the modalities exhibit lower values that the control. There is not legislation for the regulation of the bioavailable contents of PTEs. However, in the CT soils, where the total contents exceed the legal limit established by APA (2019), the bioavailable Cd content represents a small fraction of its total content (**Table 6**), ranging from 5.67% to 6.45%. Also, like is evidenced in **Figure 8**, the addition of the residues did not affect the PTEs extractability, because the differences between the results were not significant at a 5 % level of significance (p-value < 0.05).

This could have happened not only because these preliminary application rates tested need to be increased, if more marked effects are intended, but also because the extractant solution might not be the most adequate. As stated before, it is only a surrogate measure of the bioavailability, which depends in many other different factors which might not be well evaluated by a simple extractant solution.

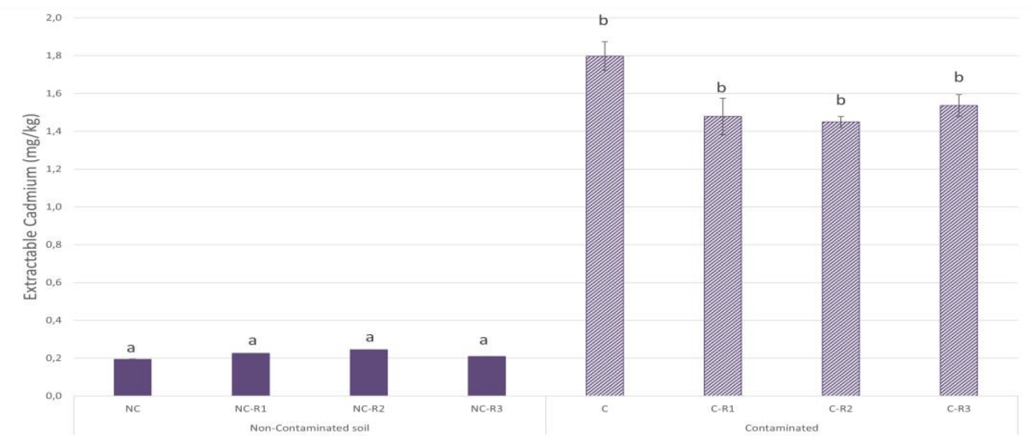

Figure 8 – Cadmium extractability (mg/kg) in the non-contaminated soil (NCT) and contaminated soil (CT) exposed to the different doses of orange juice residues (R1, R2 and R3) (mean values, n=3). The error bars represent the standard deviation values. Columns marked with different letters indicate results with significant differences (Tuckey HSD test, p <0.05).

Similar results were obtained by Xu et al. (2016), in which a Cd contaminated soil was treated with bamboo biochar. According to the authors, the organic amendment did not have much influence on the immobilization of the element, even though it increased the soil pH. The mechanisms involved in the immobilization of metals by organic amendments are: 1) the adsorption of metal ions to the surface by complexes and ion exchange; 2) an increase in soil pH and EC; 3) increase of organic carbon content (Beesley & Marmiroli, 2011; Lu et al., 2014; Xu et al., 2016). In the opinion of Xu et al. (2016), the absence of significant differences in the immobilization effects produced for the element was related to the lack of significant effects produced by the amendment application in the soil OM content. However this not being relatable to our case in which the highest rate application produced significant differences when compared with the control soil, the absence of significant differences in our experiment regarding the Cd's availability in CT soil might be related to the absence of significant differences soil Ph and EC, even though there seems to be a tendency to do so for pH. These similar results might be related to the quantities of organic amendment, which were in our case of 12, 24 and 48 t/ha, and in the bamboo biochar was of 31.2 t/ha. Nevertheless, the application of the biochar resulted in a significant decrease of the plants Cd uptake, namely in the shoots, when in comparison with the non-treated soil. This is interesting since, similarly to our results, the application of the organic amendment did not reveal a significant decrease in the element's immobilization. However, it still had significant, practical and relevant effects in the element's uptake by plants.

5.2.2. Copper

By analysis of **Figure 8**, the Cu pseudo-total concentration was not significantly affected by the residues application except in the case of the highest application dose to the CT soil, with a significant increase relatively to the control soil of that group. However, if we consider the legal limit of 62 mg/kg of Cu, applicable for soils meant to be used for agricultural purposes, established by APA (2019), we were already working with concentrations way over the legal limit in all of the modalities.

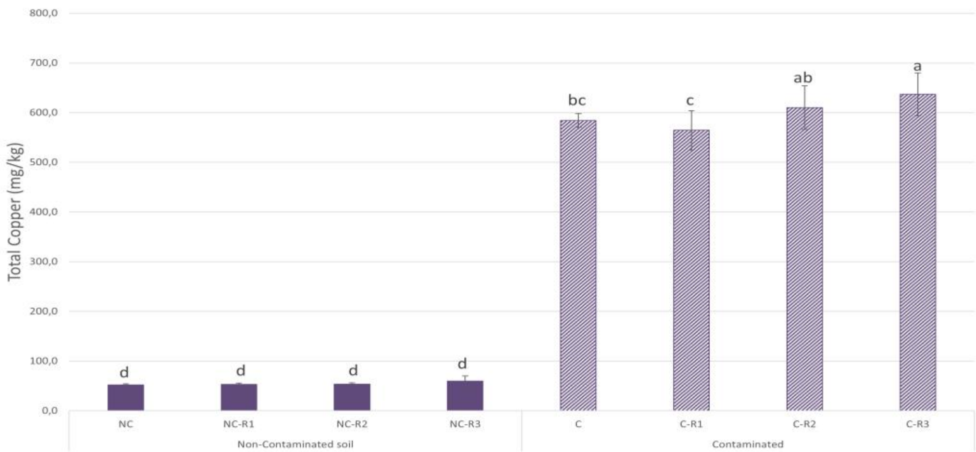

Figure 9 – Copper pseudo-total content (mg/kg) in the non-contaminated soil (NCT) and contaminated soil (CT) exposed to the different doses of orange juice residues (R1, R2 and R3) (mean values, n=3). The error bars represent the standard deviation values. Columns marked with different letters indicate results with significant differences (Tuckey HSD test, p <0.05).

As for the extractable Cu content **(Figure 10)**, all rates of application of the orange juice residues produced significant differences in the element's extractability, at a 5% level of significance. Even though there is no legislation for the bioavailable content, the extractable contents are only residual, ranging from 0.24% to 0.31% of its total content in the soil.

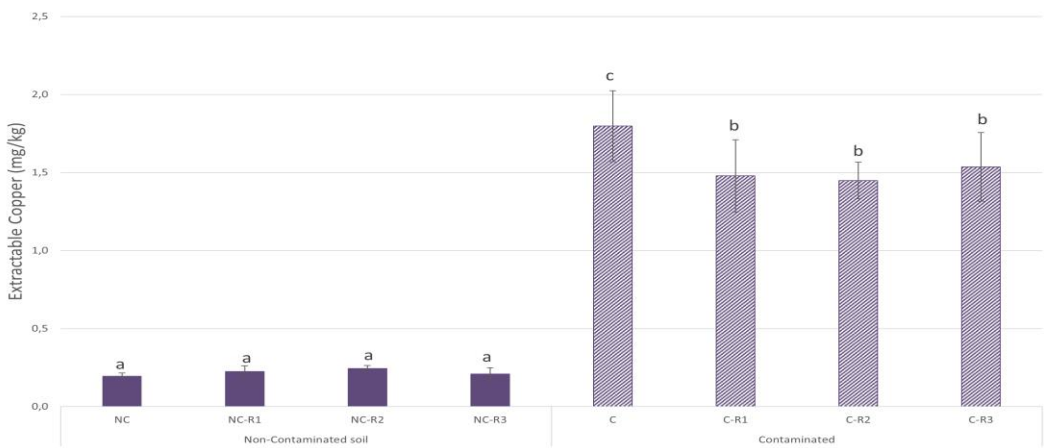

Figure 10 - Copper extractability (mg/kg) in the non-contaminated soil (NCT) and contaminated soil (CT) exposed to the different doses of orange juice residues (R1, R2 and R3) (mean values, n=3). The error bars represent the standard deviation values. Columns marked with different letters indicate results with significant differences (Tuckey HSD test, $p < 0.05$).

In the experiments led by Karaca (2004), organic waste (composted grape marc) was added to Cu contaminated soil in order to assess its effects on the element's extractability. The results showed a negative correlation between extractable Cu and OM content added. However, the quantities of OM added in the author's experiment were 2, 4, and 8%, while in this work the addition of OM, in percentage, in any of the modalities, was substantially lower (0.45, 0.90, and 1.80%). Furthermore, in this case the maximum rate of organic waste addition was also the one that verified the most considerable decrease in Cu extractability, a value of content that is much higher than 1.80%, the highest value of OM added in this experiment.

The presented results show us that, while the application of the orange residues revealed very interesting tendencies, did not produce, in all of the parameters that were subjected to analysis, significant differences between any of the three modalities and the control, allowing us to conclude, from this preliminary experiment that the quantity of orange juice residue added to the soils were not sufficient to produce the desired marked effects in all the parameters, namely in the Cd extractability.

6. Conclusions

The application of organic residues to contaminated soils could be one strategy to soil amendment and remediation. In the experiment carried out and described throughout this work, the material used as an organic amendment was the residue from the industrial production of orange juice. The statistical treatments made showed there were significant differences at a 5% level of significance in both soil pH and OM content, in both CT and NCT soils for OM content and in NCT soil for ph value, when comparing the results from the non-treated samples with the samples treated with the highest application rate.

In the case of the soils pH, which was expected to decrease with the application of the orange juice residues, what happened was the exact opposite: in both CT and NCT soils a linear tendency was verified between the quantity of residue applied and pH value, being that the maximum dose increased it from 6.57 to 6.73 and from 6.83 to 7.13, respectively. The drying and milling that the residues were subjected to might have contributed to this increase. In the case of the OM content, the maximum dose applied increased the content of the untreated samples by 43% and 45%, in NCT and CT soil respectively.

As already explained during this work, the variations registered for pH, EC and OM content influence the availability of PTEs. With the increasing concerns, not only for the environment but also for good nutrition, these results look very promising. However, in relation to the total and bioavailable contents of Cd, none of the modalities registered significant differences at a 5% significance level when in comparison with the non-treated samples. However, the bioavailable contents are not high, ranging from 5.67% to 6.45% of Cd's total content in the CT soil. Despite the non-existence of significant differences for this element, there seems to be a tendency for the residues application to decrease its extractability, considering that every modality registered a lower value than the untreated samples, in both CT and NCT soils. With that being said, and facing the results we obtained during this experiment, we conclude that, even though the orange juice residues application did not produce significant effects in all the soil properties considered, overall, the results were still very satisfying.

7. Future Considerations

Soils are a non-renewable resource on which our food and its quality depend and, consequently, our survival and quality of life. For this reason, it is of human's responsibility to manage this precious resource in the best possible way. Nevertheless, many of the agricultural practices used today, such as the mass application of certain fertilizers, have as their main purpose the maximization of crop productivity and consequently the profit of producers in the short term, completely disregarding the negative consequences to the soils and the environment in general, especially in the long run. There are, however, certain methods that allow these objectives to be achieved without jeopardizing the soils integrity, like the application of organic amendments. This practice, by being able to promote positive alterations in the soils properties, can help achieving better yields while at the same time representing a more sustainable use of the soils.

The results obtained for this trial were undoubtedly very promising. However, and without ignoring this fact, we must clarify that, being this a preliminary attempt to innovate, regarding the utilization of these specific residues to this purpose, there are still some issues that can and should be revised, in order to make this practice not only promising, but actually applicable.

In fact, even though the results show that the application of the residues to the soils may result in positive changes, both in its properties and in the availability of PTEs, the statistical treatments performed tell us that the registered differences were not considered significant (p-value < 0.05) in all the parameters considered. As we already know by now, the availability of PTEs normally increases as the soils pH decreases. Since the pre-experimental analysis of the residues showed that this material has a very low pH value, the quantities applied per ton of soil were chosen taking this information into account, so that the action of (OM) on the availability of PTEs was not canceled by the possible and expected drop in soil pH.

However, with the information that we now have available, not only the application of the residues did not cause a decrease in soil pH, as it also caused a slight increase in this variable. In this way, and in order to cause more significant effects and whose differences may be statistically relevant, in a possible next related experiment, larger amounts of residue per hectare can and should be applied to soils, knowing in advance that they will not produce undesired effects on soil pH and will, most likely, based on the direct relation between quantity of residue applied and intensity of the effects, produce results closer to what is desirable.

These practices are, in fact, quite interesting from the point of view of preserving the environment and human health. However, it must be understood that these interests must be combined with the interests of producers. Thus, it is necessary to consider the costs associated with the transformation of the residues and their consequent transport to the places where they will be applied. As we saw in the previous paragraph, the amounts of residue applied per ton of soil can, in fact, be increased to provide even more satisfactory results. However, this increase will naturally be accompanied by an increase in costs.

This problem can be overcome by the possibility of working in a circular economy context, in which the orange producers are also responsible for the production of the final product. In case this circumstance does not apply, another possibility to consider in order to at least cause a substantial reduction in transport costs is the eventuality of the two different sites of production being relatively close to each other.

As a final conclusion, we reinstate that the results obtained were very promising from a theorical point of view. Nevertheless, further research must be done in order to more precisely quantify the ideal amounts of residue necessary to obtain the best possible results. In addition, it should be put into practice in order to understand the impact it can have on a real situation, especially regarding the feasibility between costs and benefits, and to evaluate the effects in soil contaminated with other PTEs than Cd and Cu.

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Annex A

Calculations

In this sub-chapter will be featured the reasons and theorical justification for the quantity of soil, water and residue within the boxes used in the experimental trial.

Water quantity

The soils used in this experiment have a field capacity of 19%. The quantity of water to be applied in each of the boxes is the necessary amount to put the soils at 60% of their field capacity, which is the stipulated value in this type of essays. To accomplish it in a soil sample of 100g, the quantity of water to apply is obtained by 0,19 x 0,60 x 100g = 11,4 g of water. Proportionally, since the soil mass added to each box was of 250g, 28,5g of deionized water were added to each one of the 24 boxes.

Residue quantity

Since the soils used in this experiment have low porosity and high apparent density, the estimated bulk density value was of 1,4 ton/m3. And, since the application of the residues only concerns the top layer of the soil (20cm), the volume of soil contained in 1 hectare is, therefore, of 2000m3:

1 ha = 10000 m2

V = 10000m2 x 0,2m = 2000m3

Now applying the estimated bulk density of 1,4 ton/m^3 to this soil to calculate the mass of soil in 2000 m^3 comes:

1,4 ton ---------------------- 1 m^3

X ---------------------- 2000m^3

X = 2800 tonnes of soil

Assuming these soils have 95% of fine elements (and 5% of coarse elements, that are unvalued in the experiment), then the real value for the soil mass is given by

0,95 x 2800 ton = 2660 tonnes of soil

The different modalities regarding the quantity of residue applied to the soil are R1 (12 tonnes/hectare), R2 (24 tonnes/hectare) and R3 (48 tonnes/hectare). Since the mass of soil value equivalent to one hectare was calculated above (2660 tonnes), the residue/soil ratio for R1, R2 and R3 are given respectively by $\frac{\mathbf{12}}{\mathbf{2660}} = \mathbf{0,45}\%$, $\frac{\mathbf{24}}{\mathbf{2660}} = \mathbf{0,90}\%$ and $\frac{\mathbf{48}}{\mathbf{2660}}$ **= 1,80%.** However, the soil mass contained in each box is of 250g, so the quantity of residue applied will be:

For the R1 modality (0,45% dose):

0,0045 x 250 g = 1,13 g of residue

For the R2 modality (0,90 % dose):

0,0090 x 250 g = 2,26 g of residue

For the R3 modality (1,80 % dose):

0,0180 x 250 g = 4,51 g of residue