DEPARTMENT OF CHEMISTRY

Production of *Arundo donax* and *Panicum virgatum* in Heavy Metals contaminated soils

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BSc in Energy Engineering

DOCTORATE IN BIOENERGY

NOVA University Lisbon

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ABSTRACT

The development of alternative feedstocks to substitute petroleum is a challenge. Biomass production and utilization in industrial processes is a growing practice representing a renewable and more sustainable feedstock when compared with petroleum. The cultivation of energy crops on degraded soils reduces the risks associated with land use change, and biomass may represent additional revenue as a feedstock for bioenergy. Switchgrass and giant reed were tested under 450 and 900 mg Zn kg^{-1}, 450 and 900 mg Pb kg^{-1}, 200 and 400 mg Cu kg^{-1}, 300 and 600 mg Cr kg^{-1}, 110 and 220 mg Ni kg^{-1}, and 4 and 8 mg Cd kg^{-1} contaminated soils, in a two-year pot experiment. After the second year of harvest, the yield of Switchgrass (average aerial 320 g.m^{-2} and below ground 540 g.m^{-2}) was not affected by Zn, Pb, Cu, and Cd contamination, and 110 mg Ni kg^{-1} but 220 mg Ni kg^{-1} significantly affected the yields (55–60% reduction). A total plant loss was observed in Cr-contaminated pots. Giant reed aboveground yields (control: 410 g.m^{-2}), in the second year harvest, were significantly affected by all metals and levels of contamination (30–70% reduction), except in 450 mg Zn kg^{-1} and 110 mg Ni kg^{-1} pots. The tested metals did not affect the belowground biomass yields (average 1850 g.m^{-2}). The HHV of both crops (18.5 MJ/Kg of dry biomass) were similar and were not affected by contamination at any level, indicating that the biomass can be exploited for bioenergy. The fiber content (average, 74% dry matter) was also not affected by soil contamination. Nevertheless, the increased contents in ash, N and Na, K, Ca, and Mg, derived from the contamination, can limit its application in thermochemical processes.

Keywords: *Arundo donax*; *Panicum virgatum*; energy crops; phytoremediation; heavy metals contaminated soils; low ILUC crops

RESUMO

O desenvolvimento de matérias-primas alternativas para substituir o petróleo é um desafio. A produção de biomassa e a sua utilização em processos industriais é uma prática crescente, representando uma matéria-prima renovável e mais sustentável quando comparada ao petróleo. O cultivo de culturas energéticas em solos degradados contribui para reduzir os riscos associados à mudança de uso da terra, e a biomassa produzida pode representar uma receita adicional como matéria-prima para bioenergia. O switchgrass e a cana foram testados em solos contaminados individualmente com 450 e 900 mg Zn kg^{-1}, 450 e 900 mg Pb kg^{-1}, 200 e 400 mg Cu kg^{-1}, 300 e 600 mg Cr kg^{-1}, 110 e 220 mg Ni kg^{-1}, e 4 e 8 mg de Cd kg^{-1}, num ensaio em vasos, com uma duração de dois anos. Os rendimentos de switchgrass (média aérea 320 g.m^{-2} e radicular de 540 g.m^{-2}), após a colheita do segundo ano, não foram afetados pela contaminação de Zn, Pb, Cu e Cd e 110 mg Ni kg^{-1}, mas 220 mg Ni kg^{-1} afetou significativamente os rendimentos (redução de 55–60%). Uma perda total de plantas foi observada em vasos contaminados com Cr. Os rendimentos de cana gigante (parte aérea, controlo: 410 g.m^{-2}), no segundo ano de colheita, foram significativamente afetados por todos os metais e níveis de contaminação (redução de 30-70%), exceto nos vasos contaminados com 450 mg Zn kg^{-1} e 110 mg Ni kg^{-1}. Os rendimentos de biomassa abaixo do solo (média de 1850 g.m^{-2}) não foram afetados pelos metais testados. O poder calorífico de ambas as culturas (média de 18,5 MJ/kg de biomassa seca) foram semelhantes e não foram afetados pela presença da contaminação, indicando que a biomassa pode ser explorada para bioenergia. O teor de fibra (média, 74% de matéria seca) também não foi afetado pela contaminação do solo. Os maiores teores de cinzas, N e Na, K, Ca e Mg, decorrentes da contaminação, podem limitar a sua aplicação em processos termoquímicos.

Palavas chave: *Arundo donax*; *Panicum virgatum*; Culturas energéticas; Fitorremediação; solos contaminados com metais pesados; culturas com baixo ILUC

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1

INTRODUCTION

1.1 Contextualization and Motivation

The development of human society is associated with the ability to work with heavy metals. This ability is still necessary nowadays with the utilization of electronic equipment, the mechanization of agriculture, and the modernization of medicine. Every aspect of our current life uses processed metals, and this dependence on processed heavy metals causes an alteration of their natural biogeochemical cycle (Kabata-Pendias, 2011).

Heavy metals are present in all human activities, becoming a problem when discarded in nature without proper treatment. Furthermore, the processing methods of metal extraction from mineral ores and the subsequent conversion into raw materials can contaminate soils and water by metal leaching. Once in soils, heavy metals can be absorbed by plants, entering the food chain, accumulating in organisms, and having severe implications in the biosphere. Despite some metals that can act as micronutrients (Alloway, 2013) and are therefore needed in trace amounts, the absorption of high concentrations can produce serious consequences (Maret, 2016; Pandey et al., 2016; Zoroddu et al., 2019).

The anthropogenic origin of heavy metals in soils, which can be originated from various sources like fertilizers, pesticides, and industrial residues, is a concern due to their potential to cause problems in human health when in high concentrations. Heavy metals are characterized by having high specific weight, and when discarded in the environment, they can be absorbed by plants causing damage at different levels of the food chain depending on their oxidation state (Appenroth, 2010). Various techniques can be applied to remove these contaminants from the soil using physical, chemical, and/or biological methods (Kavamura et al., 2019).

Physical and chemical processes often are not the best option due to the associated costs and the risk of damaging the remaining properties or even the structural integrity of the soil. In most cases, a biological set of techniques can be more appropriate for soil remediation (Lim et al., 2014). Biological processes (bioremediation) use microorganisms and plants to remove heavy metals from the soil (Lim et al., 2014). Bioremediation can occur naturally without human intervention in a process called intrinsic bioremediation (Chiu et al., 2017), which is more likely to succeed for low contamination levels. However, for higher levels of heavy metal contamination in the soil, the bioremediation process can be designed to maximize metal extraction in engineered bioremediation (Dangi et al., 2019). Phytoremediation is an engineered biological technique that uses specific plant species to remove contaminants according to soil and contamination characteristics. Although phytoremediation needs more time to remediate contaminated sites, it is economically more viable when compared to physical or chemical methods, considering the process itself and the prospect of using the biomass produced as raw material in industrial processes. The selection of the ideal plant for phytoremediation depends on some parameters, such as the edaphoclimatic characteristics of the site, the tolerance to the contaminants to be extracted, and the productivity of the crop (Pandey et al., 2016).

Energy crops fulfill these requisites, and the produced biomass can be converted into energy or renewable and sustainable products. Another desired characteristic is the capacity of energy crops to quickly provide a high amount of biomass, mitigating the amount of time needed to decontaminate a site. Thus, perennial crops are a good option for this technique once they produce a high volume of biomass and usually have other characteristics of interest as tolerance to water stress and low need for fertilizers and pesticides.

Due to high productivity, the well-developed technology, and the possibility to use its biomass to produce biofuels and bioproducts, *Panicum virgatum* L. (switchgrass) and *Arundo donax* L. (giant reed) are two promising perennial crops for phytoremediation processes and can be used in biorefinery processes (Aderholt et al., 2017; Fernando et al., 2016).

Switchgrass phytoremediation potential has been studied in several conditions, such as multicontaminated soils (Balsamo et al., 2015; Kavamura et al., 2019; Shrestha et al., 2019) or combined with AMF (Arbuscular mycorrhizal fungi)(Ali et al., 2013; Arora et al., 2016). The results of these studies have shown that this crop presents an excellent potential for phytoremediation processes, becoming an option to decontaminate soils and wastewater while producing biomass that could generate a wide range of bioproducts.

Giant reed is a potentially high-yielding non-food crop that could meet European Union (EU) market requirements for energy and advanced biofuels, paper, pulp, and building materials (Liu et al., 2017). In addition, this C3 perennial rhizomatous grass belonging to the Gramineae family (Poaceae), widespread in Mediterranean environments, can stabilize heavy metals in contaminated soil due to its rapid growth rate and can even achieve high yields in soils polluted with multiple metals (Alshaal et al., 2015).

In this way, the main goal of this work was to understand the effects of different heavy metals in switchgrass and giant reed productivity and biomass quality for energy, along with the potential to use these crops to promote soil decontamination through phytoremediation. Moreover, the cultivation of these energy crops on degraded/contaminated soils reduces the risks associated with land use change.

Indeed, biomass production and utilization in industrial processes is a growing practice representing a renewable and more sustainable feedstock when compared with petroleum (Scordia et al. 2012). The versatility of biomass allows the production of fuels in different states: solid, liquid, or gaseous. In addition, the production of fibers and chemicals can replace petroleum with minor adaptations in industries or refineries, becoming an excellent alternative to change to a more sustainable feedstock (Carlsson et al., 2011; Jia et al., 2018; Lucia, 2008; Tilman et al., 2009; Ubando et al., 2020; Wang et al., 2020; Pires et al., 2019). Moreover, utilizing biomass in the energy sector can reduce greenhouse gas emissions, helping the European Union (EU) to achieve its goal of no net emissions by 2050, a compromise firmed in the European Green Deal (European Commission, 2019). Although the advantages of using biomass in different industries comprise an attractive choice, the cultivation and conversion of biomass could be harmed by the Indirect Land Use Change (ILUC) effects (European Commission, 2019; Scordia et al., 2022). Cultivation of non-food crops increases the demand for soil, which can cause a cascade effect, elevating the cost of food production and consequently increasing its price (Graham-Rowe, 2011; Zhang et al., 2010). To avoid a scenario that can threaten the food supply in the EU, especially for low-income families, unsuitable lands for food production come out as an opportunity for the cultivation of non-food crops (Abreu et al., 2020; Von Cossel et al., 2019). Soils contaminated with heavy metals are examples of unsuitable soils that can be used to cultivate dedicated crops, as experienced in Germany with industrial hemp (Linger et al., 2002), in Italy with giant reed (Cristaldi et al., 2020), or in the USA with switchgrass (Novak et al. 2019).

Biomass cultivation depends on factors that must be considered, such as climate conditions, cultivation methods, soil, and water quality (Collins et al., 2020). Regarding soil and water quality, the contamination of these resources constitutes a growing problem and represents a challenge for industrial crop cultivation. Yields and biomass quality can be affected, and contaminated biomass and residues can be generated, damaging equipment or retarding processes like fermentation (Barbosa et al., 2018; Cai et al., 2019; Motuzova et al., 2014). In addition, the accumulation of heavy metals in the biomass requires special attention once this is a via for its accumulation in the food chain. Despite some heavy metals acting as micronutrients in some organisms, they can cause severe health issues in humans

(Adimalla et al., 2018; Ávila et al., 2017). However, although contaminated soils may affect yields and biomass quality (Papazoglou et al., 2017), industrial crops cultivated in soils containing high concentrations of heavy metals can have an essential role from an environmental point of view. Besides being a renewable raw material, industrial crops can tackle the problem of soils contamination, accumulating or stabilizing the heavy metals in a phytoremediation technique while producing feedstock for industrial processes (Barbosa et al., 2016; Barbosa et al., 2018; Corno et al. 2014).

Therefore, the current study aims to add knowledge on how the cultivation of these two energy crops, giant reed and switchgrass, in soils polluted with different heavy metals can exert a phytoremediation action, merged with its possible exploitation for bioenergy, thus contributing to a resource-efficient bioeconomy. In this sense, the study was planned to be carried out for more than one year. This will provide more information on how those two perennial crops behave in contaminated soil during several growing seasons. These crops reach maturity only after the second harvest year, once they take the first growing season to develop their root system and rhizomes (El Bassam, 2010). Consequently, extending the study to a second year will provide more reliable information on the effect of contamination on biomass productivity and quality, information that few studies have addressed so far.

In addition, this study will evaluate the quality of the harvested biomass, e.g., ash content, nitrogen content, and calorific value, providing data that will allow a better assessment of the value of this biomass for energy, something that is also poorly evidenced in existing studies (and with these two crops, no such studies have ever been done). The contribution of the data presented in this work will help to identify the challenges and the opportunities of the production of clean bioenergy through the use of lignocellulosic non-food energy crops (listed in Annex IX of the RED II recast (European Commission. Renewable Energy n.d.)) cultivated on contaminated land and how these biomasses can help to cover the global energy demand expected until 2050 (IRENA, 2018).

These crops will be tested in soils contaminated with zinc (Zn), lead (Pb), chromium (Cr), nickel (Ni), cadmium (Cd), and copper (Cu) in soils presenting two different contaminant concentrations for each metal, using as reference the limit established in the Portuguese Decree-Law No 276/09, and twice the values. The decision to study these crops emerged from the MAGIG project – Marginal Lands for Growing Industrial Crops – supported by the European Union, which aimed to promote the utilization of industrial crops in marginal lands due to their potential to ensure profits through a sustainable and renewable alternative to fossil resources.

This experiment intends to achieve the following objectives:

- Analyze the effect of Zn, Pb, Cr, Ni, Cd, and Cu contaminated soils in switchgrass and giant reed productivity and biomass quality along two growing cycles.
- Analyze the phytoremediation potential of giant reed and switchgrass regarding Zn, Pb, Cr, Ni, Cd, and Cu contamination.
- Study the effects of the different heavy metals on the quality of both bioenergy applications.
- To perform an environmental analysis of giant reed and switchgrass utilization in heavy metal contaminated soils.

To achieve the aims of the work, a state-of-the-art on the subject was made. First, a brief introduction was made about energy crops and the potential of giant reed and switchgrass to produce bioenergy. Secondly, the phytoremediation process was addressed, and the possibility of using the studied crops in heavy metals contaminated soils was also revised, as also the opportunities for using those biomasses for bioenergy. In addition, a revision was made to the studied heavy metals, addressing the contamination in Europe, to understand the available soils contaminated with those heavy metals.

1.2 Energy crops

To achieve the goals of zero emissions proposed in the European Green Deal, new strategies must be adopted, such as utilizing a more sustainable and renewable feedstock for energy, fuels, and products. As such, the cultivation of energy crops appears as an alternative. Furthermore, biomass cultivation and use present several advantages such as these crops present resistance to weather variations, the dependence on external inputs is reduced (these crops are efficient in nutrient cycling), and they are efficient in the use of natural resources, such as water and soils nutrients, helps to keep the land producing for several years, even decades, helps in the control of diseases and pests (due its natural resistance), and in this way, increases yields. Directly and indirectly, its production and use create jobs in rural areas, improving the community's economic structure, and its renewability and biodegradability enhance the sustainability of energy and product feedstock (Zegada-Lizarazu et al., 2011).

Regarding the energetic utilization of energy crops, their biomass is usually explored through biofuels, heating, and electricity. In terms of biofuels production, energy crops are used to produce biodiesel (Vasudevan et al., 2008) (e.g., the oil from *Brassica carinata* seeds once submitted to transesterification (Bouaid et al., 2005)), jet fuel (Wei et al., 2019) (e.g., the utilization of *Jatropha curcas* oil to produce jet biofuel, JBF) (Alherbawi et al., 2021)), ethanol (Robak et al., 2020) (e.g., the utilization of corn, sugar beet, sugarcane, for ethanol production through fermentation, (Manochio et al., 2017), or green gasoline (Joensen et al., 2011).

The conversion of energy crops into heat occurs through combustion, which can be done in domestic heaters or combustion power plants (Ozgen et al., 2021). Using energy crops in thermochemical power plants is also expected and increasing through combustion, pyrolysis, and gasification. In these processes, however, the conversion processes are indirect, through the production of steam generation or another energy vector such as H_2 or syngas (Freiberg et al., 2018; Lepage et al., 2021; Ozgen et al., 2021). The combustion of biomass releases CO_2 (carbon dioxide) into the atmosphere. However, the crops capture a huge amount of CO2 through photosynthesis while growing. In this way, biomass can be considered a carbon-neutral feedstock (Wang et al., 2018), essential for the EU to achieve its zero-emission goal.

Among all the energy crops, perennial grasses appear in a highlight position. These energy crops have a high resource use efficiency, meaning that regarding light exposure, water, and nutrients' availability, the crops can have a reasonable yield with low input needs (Scordia et al., 2019). Furthermore, the recalcitrance of these crop fibers also provides resistance against diseases and pests, removing the need for pesticides (Himmel et al., 2007; Zegada-Lizarazu et al., 2010). Regarding the environmental benefits, energy crops can also contribute to avoiding erosion processes (Lo Papa et al., 2020), and their potential to be used in marginal lands also contributes to reducing the competition for agricultural lands with food crops, reducing the risks associated with indirect land use changes (ILUC) effects (Scordia et al., 2019).

1.2.1 *Arundo donax* L. (giant reed)

Among the industrial crops, *Arundo donax*, commonly known as giant reed, stands out due to its high productivity. In addition, this perennial crop presents a high tolerance to several stress conditions suitable to phytoremediation processes. Giant reed is a herbaceous crop belonging to the *Poaceae* family and the *Arundineae* tribe. It is a robust plant, and despite having a C3 cycle, it achieves photosynthetic rates that can be compared to C4 crops (di Nasso et al., 2011; Webster et al., 2016). This crop, presented in Figure 1.1, is believed to be originated in Asia, but the plant is admirably adapted to the Mediterranean basin due to its subtropical climate with warm temperatures and being present in southern Europe, North Africa, and the Middle East. However, some varieties can also present high yields under excellent conditions, usually in the United Kingdom and Germany (El Bassam, 2010).

Figure 1.1. *Arundo donax* field (Danelli et al., 2020)

The giant reed growth cycle usually occurs from early spring to autumn or winter. The vegetative growth in the northern hemisphere usually occurs in June and July, sometimes reaching 7cm per day, and from August to November, when the inflorescence (panicle) appears. From November to February, the leaves begin to enter in senescence, the panicle breaks, and the steam starts to lose moisture. Despite its ability to produce seeds, the crop usually reproduces by rhizome propagation (El Bassam, 2010).

Arundo donax can grow on humid soils, near watercourses, dry or marginal soils, and near roads or industrial sites (Papazoglou et al., 2005). Crop propagation usually occurs through the spread of the rhizome system. Belowground structures of *Arundo donax* consist of a robust woody rhizome that grows near the ground's surface and roots with a length of up to 100 cm. On the aboveground, a dense group of stems from the same rhizome usually forms a green wall that can reach 10 m in height, with alternate leaves and an apical panicle. The plants' high tolerance to stress conditions allows them to grow in different soils, including marginal soils, that might be contaminated with heavy metals or other chemicals. The climate condition that favors this crop's growth includes annual precipitation ranging from 300 to 4000 mm and a temperature variation ranging between 9 to 28°C. At lower temperatures, the plant is usually dormant (El Bassam, 2010).

Arundo donax is a very tolerant crop, resistant to different pests, making herbicides and pesticides unnecessary (Barney et al., 2008). Although *Arundo donax* is not cultivated on a large scale, all these advantages can make it a very profitable crop. Field experiments found that the margin for profit can be extremely high since the crop is perennial and the input necessities after every harvest are low (Giudicianni et al., 2014). Furthermore, the energy potential of dry biomass can reach an average of 637 GJ.ha^{-1}.year^{-1}, considering a yield of 28.7 t.ha^{-1}.year^{-1}, assuming 12 years of *Arundo donax*. Cultivation, where the annual yield in dry biomass can reach 37.7 t.ha^{-1} (Angelini et al., 2009; Cosentino et al., 2006).

However, the productivity of *Arundo donax* can be different in different Mediterranean environments. For example, the productivity (dry matter) reached 34.2 t.ha^{-1} in Sicily, Italy (Cosentino et al., 2006). In Portugal, *Arundo donax* productivity can reach 30 t.ha^{-1} (Barbosa et al., 2012), while in central Spain, the productivity reached 20 t.ha^{-1} using N fertilization (Garcìa-Galindo et al., 2016). However, it varied from 12.7 to 22.2 t.ha^{-1} in several other regions of Spain (Hidalgo and Fernandéz 2001; Sánchez et al. 2017). In Greece, the productivity varied from 12.7 to 24.1 t.ha^{-1} for the third year of cultivation, depending on the irrigation (Christou et al., 2005).

Giant reed harvest is usually made in one of the two possible periods of the year, in autumn or at the end of the winter. The harvest in autumn gives biomass with higher moisture and lower lignin content, facilitating the hydrolysis process and easing the conversion processes that use cellulose or hemicelluloses, like fermentation. On the other hand, harvesting at the end of the winter gives biomass lower moisture content, increasing the energy density, which is better for use in thermochemical energy processes (Fernando et al., 2016). Therefore, depending on the biomass utilization, the harvest can take place annually for energy production, or every two years for its incorporation in buildings and construction materials (El Bassam, 2010).

The main applications of biomass depend on its composition. The holocellulose content of *Arundo donax* ranges from 55% to 77%, with the lignin content around 25% and ash content ranging from 1.9% to 6.7% (Fiore et al., 2014; Yang et al., 2020). The large amount of holocellulose testifies to the excellent potential for conversion processes like anaerobic digestion and alcoholic fermentation (Pilu et al., 2013). Experiments under different conditions have different fiber characterization for *Arundo donax,* and these values can be seen in Table 1.1.

Table 1.1. Fiber composition of *Arundo donax*

Cellulose (% dw)	Hemi-celluloses (% dw)	Lignin (% dw)	Extractives (% dw)	Ash (% dw)	Reference
42.3	20.5	17.2		1.9	(Fiore et al., 2014)
29.20	35.9	23.32		5.35	(Saikia et al., 2015)
35	19			4.5	(Ververis et al., 2004)
37.8 - 41.4	26.4 - 29.0	18.5 - 20.3		2.6 - 4.8	(Giudicianni et al., 2014)
31.10	35.27	18.49			(Silva et al., 2015)
46	26				(Szabó et al., 1996)
43.56	12.94	33.65		3.56	(Krička et al., 2017)
36.14	32.09	10.66	18.85	1.78 - 2.26	(Oginni et al., 2019)
35.83	31.12	18.86	4.71	6.77	(Yang et al., 2020)
43.1	21.9	22.4	9.3		(Ramos et al., 2018)

dw – dry weight

Its elevated high heating value (HHV) and low ash content also give this crop the potential to be used in thermochemical conversion processes, primarily when the harvest occurs in the late winter, which decreases its content in silica and potassium (Nassi et al., 2010). The ultimate and proximate analysis can be seen in Table 1.2 and Table 1.3, respectively. These characteristics show the potential of *Arundo donax* for combustion, pyrolysis, and gasification processes (Ghetti et al., 1996; Hoffmann et al., 2010).

Table 1.2. Ultimate analysis of *Arundo donax*

C (% dw)	H (% dw)	O (% dw)	N (% dw)	S (% dw)	Reference
42.05	6.24	36.37	1.54		(Saikia et al., 2015)
45.67	6.17	47.13	0.74	0.29	(Krička et al., 2017)
42.7	7.5	48.7	0.8	0.2	(Jeguirim et al., 2009)
49.3	6.0	44.4	0.3		(Vernersson et al., 2002)
42.47	6.28	50.02	0.65	0.58	(Oginni et al., 2019)
44.76	5.28	38.69	0.77	0.45	(Yang et al., 2020)
49.3	44.4	6	0.3		(Basso et al., 2005)

dw – dry weight

Table 1.3. Proximate analysis of *Arundo donax*

Fixed Carbon (%)	Volatile Material (%)	Ash (%)	HHV (MJ.kg^{-1})	Reference
11.72	74.36	5.35	17.14	(Saikia et al., 2015)
11.47	76.06	3.56	17.48	(Krička et al., 2017)
18.4	68.4	5	17.2	(Jeguirim et al., 2009)
24.1	71.3	4.6		(Vernersson et al., 2002)
18.52	79.70	1.78 -2.26	18.96	(Oginni et al., 2019)
15.35	74.59	6.77		(Yang et al., 2020)
24.1	71.3	4.6	17.2	(Basso et al., 2005)

dw – dry weight

The ultimate and proximate analysis shows the potential of *Arundo donax* to be used in thermochemical processes, having a high HHV, low ash content, low nitrogen and sulfur, low NOx emissions, and reduced corrosion problems associated with sulfur content.

The utilization of *Arundo donax* goes further than the energy field. It is also used as raw material to manufacture musical instruments (Obataya et al., 1999), in buildings (Barreca, 2012), to produce chipboards and particle boards (Antonio et al., 2012; Ferrández-García et al., 2012), in the paper industry (Caparrós et al., 2007), to resins (Caparrós et al., 2007), bio-oil (Temiz et al., 2013), activated carbon (Sun et al., 2013), xylooligosaccharides (Caparroä et al., 2007), xylose (Shatalov et al., 2012), among other applications.

Ahmed reviewed the potential of *Arundo donax* to produce activated carbon, which showed excellent potential for organic and inorganic pollutants removal, mainly when applied to wastewater treatment (Ahmed, 2016). Ammari, on the other hand, studied the utilization of the leaves as a Cd absorbent. As a result, the wastewater containing Cd^{2+} showed a reduction of 92% of Cd with the implementation of *Arundo donax* leaves, which were added in powder (Ammari, 2014).

Due to *Arundo donax* fibrous structure, the implementation of this plant in construction materials has also been tested, aiming to implement a renewable and more sustainable material in the construction field. Karahancer et al. tested adding *Arundo donax* fibers in hot mix asphalt. Adding these fibers to the mixture increased the tensile strength ratio, improving the strength of the mixture (Karahancer et al., 2016).

The utilization of *Arundo donax* for biofuels is not new. However, some processes have been tested to improve their conversion ratio. The application of pre-treatments in biomass (using the acid pretreatment with sulfuric acid (H_2SO_4) at 65°C, followed by an alkaline pre-treatment, using sodium hydroxide

(NaOH) at 127°C) in *Arundo donax* fibers showed to improve the concentrations of xylose and glucose, increasing the glucose concentration by 3.5 times, improving the efficiency of the fibers fermentation and reducing costs in the biomass fermentation for ethanol production (Lemões et al., 2018). The bio-ethanol production using *Arundo donax* as feedstock goes from 11,000 to 15,228 L.ha^{-1}, majorly enhanced by its high biomass productivity per ha, ensuring a great potential wgen compared with other crops (Corno et al., 2014).

The potential for *Arundo donax* utilization in biogas production is also high. This crop has an excellent anaerobic biogasification potential (ABP), and when compared to corn, rye, triticale, and sorghum, the ABP corresponds to 75%, 94%, 77%, and 120% of the ABP of those biomasses, respectively. Despite this, due to its high biomass productivity, the bio-methane production potential per hectare of giant reed is higher than the other energy crops, reaching 19,440 Nm³ CH_4. ha^{-1} (Corno et al., 2014).

Arundo donax was also tested to produce hydrogen (Chen et al., 2022). In the study, the biomass of giant reed was pre-treated with three different ionic liquids and loadings (2-16 g/L). The pretreated biomass, followed by enzymatic hydrolysis, reached a sugar yield of 7.9 g/L and a hydrogen yield of 106.1 mL/g total solids during the photo-fermentation, which were 68.8 % and 35.3 % higher than those of untreated *Arundo donax*, respectively. Acetic acid was the main by-product of hydrogen production with ionic liquids pretreated *A. donax* (Chen et al., 2021).

Regarding its application for bioenergy, *Arundo donax* can be used in combustion, pyrolysis, and gasification processes. Therefore, Ba and collaborators promoted a study to evaluate the potential of *Arundo donax* pyrolysis. The model established a pyrolysis temperature in the range of 600-700°C, holding a maximum of 20% moisture, considering 10% of thermal losses in the pyrolysis reactor, and the study concludes that in an industrial scale pyrolysis (2000 t/d), the output could reach 28MW power, along with 51.36 t/d bio-oil, 555.04 t/d vinegar, and 511.36 t/d biochar (Ba et al., 2020).

The application of *Arundo donax* to pyrolysis was also studied by Zheng et al. (2018), and besides energy and biochar, the authors observed that when in the range of 300 – 600 °C, 25 different chemical products can be produced. Furthermore, it was also observed that the temperature is inversely proportional to biochar production per mass biomass since the temperature increase from 300 to 600 °C decreases the biochar production from 43.6% to 29% (Zheng et al., 2018).

The energy potential of *Arundo donax* is high (Pari et al., 2016) and can reach 637 GJ.ha^{-1} through thermochemical processes, considering the productivity and the HHV mean of 12 years experiment, as reported in the work of Angelini and collaborators (Angelini et al., 2009). However, the valorization of this potential through combustion processes can present some problems due to the composition of the leaf and stem tissues, which present high ash content. In addition, some corrosive elements also present in significant amounts, such as K, Cl, and Si can cause problems in the machinery used in the thermochemical process. Moreover, the combustion of giant reed, due to its composition, leads to the release of harmful compounds, such as NO_x, HCl, SO_2, and CO (Corno et al., 2014).

Alessina and collaborators tested the utilization of *Arundo donax* in gasification plants on a pilot scale using a gasifier of $20kW_{el}$ using a mixture of 50 – 50 percent wood chips and *Arundo donax* with a biomass-specific consumption of 2.72 $kg_{dry}.kWh^{-1}$, producing biochar (5% on a dry basis) and power (considering a biomass consumption of 1.2 kg_{dry} . kWh^{-1}, the power production can reach 21,586 MWh). The process showed promising to tackle GHG emissions, sequestrating up to 150 kg of CO2 for every Mg of Arundo donax submitted to gasification despite the electrical efficiency of only 7.72% (Allesina et al., 2018).

Correia and collaborators also studied the torrefaction and low-temperature carbonization of Arundo donax, submitting the crop in processes where the temperature varied from 200 – 350°C for 15 to 90 min. It was noticed that the temperature was the most significant parameter to influence mass and energy yields as well as biochar composition. Torrefaction reduced moisture, volatile matter, and O/C and H/C ratios of the biomass while increasing heating value, ash content, and fixed carbon. For torrefaction at 250°C or higher temperatures grind ability of the biochars was significantly improved. These biochars' low volatile matter contents and high ash contents restrict their use as solid fuels but can be valorized otherwise (Correia et al., 2017).

The hydrothermal carbonization of *Arundo donax,* performed in a 2-L Parr reactor, produced a material with a lower percentage of volatile matter, 54%, compared with a raw giant reed (78.5%). Therefore, hydrothermal carbonized giant reed presents lower reactivity and is more stable, not igniting even at low temperatures. Moreover, the hydrothermal carbonization process reduced the ash content of the material, from 6.3% (raw) to 4.8% (hydrothermal carbonized material), due to the dissolution in water of some fractions of ash (Nawaz et al., 2022). Ash content is directly related to the calorific value of the fuel. Therefore, lower ash content is linked to higher heating value, and it also means a lower possibility of slagging and fouling during boiler operation due to its higher alkaline nature.

The potential applications of *Arundo donax* make this versatile crop an excellent alternative to biorefinery applications. Furthermore, *Arundo donax* can help the EU achieve its environmental goals, with socio-economic improvements as collateral, being a feedstock that can reduce fossil resources.

1.2.2 *Panicum virgatum* L. (switchgrass)

Panicum virgatum, also known as switchgrass, is a perennial crop native to North America, well adapted to the south of the United States and northern Mexico (Mitchell et al., 2012). This C4 crop, presented in Figure 1.2, can reach up to 2.5 m in height, depending on the growing conditions and the cultivated variety (El Bassam, 2010). Switchgrass is a high-value forage crop used as a bioenergy feedstock (McLaughlin et al., 2005).

Figure 1.2. *Panicum virgatum*'s field (Samson et al., 2016)

The establishment of the crop occurs by seeds, and the germination rate can be improved using some techniques to break the dormition state of the seeds. One alternative to increase the germination rate of switchgrass is heating the seeds at temperatures around 50°C, which can break the dormancy process (Rinehart et al., 2006). The seedling process should be around 1 cm deep, and the density can be up to 400 seeds.m^{-2} (El Bassam, 2010).

Switchgrass seeds can tolerate pH variations, but the higher germination rates occur in the 4.0 – 8.0 pH range. Despite the efforts, switchgrass usually does not achieve its full yield potential during the first two growth cycles, only reaching around 33% to 66% of the yields obtained in the third year. This occurs because switchgrass root systems are in development during the first two growing cycles, limiting the absorption of nutrients (McLaughlin et al., 2005).

The cultivation of switchgrass is connected to the temperature and the cultivation period. In good conditions, with proper water availability and fertilization, the yields can reach 15 $t.ha^{-1}$, but some varieties allow two harvests per year, increasing the output (Parrish et al., 2007). However, despite the two harvest possibilities, it is common to make only one switchgrass harvest per year, usually in the winter after the growing cycle finishes and the plants dry and complete the whole growing cycle, providing higher productivity (Rinehart et al., 2006).

Mitchell et al. studied the switchgrass growing cycle. They observed that the vegetative cycle occurred basically in the first 20 days. The elongation of the plant was higher between days 23 and 37, while the reproductive cycle started on day 30 and finished on day 83, being higher from day 56 to day 69. On day 69, the appearance of seeds started (Mitchell et al., 1997).

The advantages of switchgrass as an energy crop are the possibility of cultivation in different conditions, high yields, tolerance to stress conditions, and the development of different cultivars for other purposes, targeting the final products (Stahlheber et al., 2020). For example, the productivity of switchgrass in the United States varies from 13.4 – 21.3 $t.ha^{-1}$ in North Caroline, using the Alamo variety, and can reach 16 $t.ha^{-1}$ in Nebraska, using the variety Cave-in-Rock (McLaughlin et al., 2005). While in Europe, it achieves yields an average of 12.3 $t.ha^{-1}$ in Italy and 17.3 $t.ha^{-1}$ in Greece (Alexopoulou et al., 2008).

Several studies related switchgrass as a feedstock for ethanol production (Parrish et al., 2007; Pimentel et al., 2005; Schmer et al., 2008; Tadeusz et al., 2010), and the reason is the interesting composition of this crop's fibers.

The fiber composition of switchgrass can be seen in Table 1.4, evidencing the potential of switchgrass in the cellulose and hemicellulose valorization process. Furthermore, the high amount of these two components favor fermentation processes such as ethanol production and can also be an attractive raw material for advanced materials such as nanocellulose.

Table 1.4. Fiber composition of *Panicum virgatum*

Cellulose (% dw)	Hemicelluloses (% dw)	Lignin (% dw)	Extractives (% dw)	N (% dw)	Ash (% dw)	Reference
32.9 - 38.5	25.0 - 32.8	17.4- 18.4			18.6- 18.8	(David et al., 2010)
32	19.2	18.8	18.5			(Imam et al., 2012)
38.5	28.4	21.4	4.7	6.1	1.3	(Allison, 2018)
38.77	31.67	6.9			4.8	(Lemus et al., 2008)
36.4 - 38.1	30.7 - 33.5			4.6 - 5.9	5.2 - 7.0	(Brummer et al., 2002)
71.9 - 77.1 (holocellulose)		22.7 - 24.9			2.1 - 2.5	(Yan et al., 2010)

dw – dry weight

Despite some exceptions in specific experiments, the ash content of switchgrass is usually low and associated with its ultimate analysis, seen in Table 1.5, and its proximate analysis, seen in Table 1.6, switchgrass biomass can also be suitable for thermochemical conversion processes, such as gasification, combustion, or pyrolysis.

Table 1.5. Ultimate analysis of *Panicum virgatum*

C (% dw)	H (% dw)	O (% dw)	N (% dw)	S (% dw)	Reference
42.33 – 48.00	5.30 – 6.81	37.6 – 42.5	0.03 – 1.16		(David et al., 2010)
42	6.1	47.7	0.4	0.1	(Imam et al., 2012)
46.82 – 47.41	5.47 – 5.67	40.4 – 42.4	0.35 – 0.70	0.17–0.24	(Ogden et al., 2010)
49.2	6.1	44.5	0.2	0.29	(Allison, 2018)
47.58	4.45	42.17	0.40	0.063	(Lemus et al., 2008)
47.8	5.4	41.4	0.46	0.14	(Brummer et al., 2002)

dw – dry weight

Table 1.6. Proximate analysis of *Panicum virgatum*

Fixed Carbon (%)	Moisture (%)	Volatile Material (%)	Ash (%)	HHV (MJ.kg^{-1})	Reference
11.9	8.4	84.2	3.9		(Imam et al., 2012)
13.15 – 20.26	7.22 – 7.84	73.57 – 82.93	3.92 – 6.21		(Ogden et al., 2010)
16.43		79.21	4.36	18.22	(Lemus et al., 2008)
13.6		81.6	4.8	16.4	(Brummer et al.,, 2002)
				17.6 – 21.9	(Wright 2014)

Panicum virgatum biomass primarily uses energy by burning it in power plants or co-firing with coal to generate heat and electricity (Parrish et al., 2007). To produce liquid fuels, switchgrass is submitted to direct or indirect fermentation to produce ethanol, butanol, acetate, and butyrate (Datar et al., 2004). Still, in biochemical conversion processes, switchgrass can be used in anaerobic digesters to produce methanol, ethanol, and other fuels (Parrish et al., 2007).

Brassard and collaborators analyzed the pyrolysis of *Panicum virgatum* through a life cycle analysis in two different scenarios, changing the temperature and the residence time, concluding that when the selection of process parameters is carefully made, the pyrolysis of *Panicum virgatum* for biochar and bio-oil can have a harmful emission of GHG (Brassard et al., 2018).

Larnaudie et al. also, through a life cycle analysis, studied bioethanol production using *Panicum virgatum* biomass. It was observed that 249 L of bioethanol /t *Panicum virgatum* biomass could be produced in a good scenario. In this scenario, *Panicum virgatum*'s bioethanol prices can reach prices lower than US$ 1.1/L, becoming a viable alternative to fossil fuels (Larnaudie et al., 2022).

The gasification of *Panicum virgatum* was studied by Sarkar and collaborators. The efficiency and the quality of the gasification process increased at high temperatures. Best results also can be obtained if *Panicum virgatum*'s biomass passes through pretreatments. The combined torrefaction and/or biomass densification increased the gasification's yield (Sarkar et al., 2014).

Uma et al. studied the co-digestion of *Panicum virgatum* and food waste and observed that despite the digestion of food waste alone produced a higher methane amount than the digestion of *Panicum virgatum* alone, the best results were obtained when a mixture using equal amounts of food waste and

Panicum virgatum's biomass was tested, making this biomass suitable to biogas production in co-digestion processes (Uma et al., 2020).

The applications of *Panicum virgatum* in bioenergy and biofuel production make this crop an essential renewable feedstock to reduce the dependence on fossil fuels.

1.3 Production of energy crops in heavy metal contaminated soils

The contamination of European soils deserves eminent attention because 3 million sites are estimated to be contaminated mainly with high levels of mineral oils and heavy metals, from which 250,000 need to be treated urgently (UWE, 2013). Moreover, considering that the EU wants to avoid the cultivation of energy crops in fertile soils that are suitable for food production, minimizing the energy security threat through the indirect land use change (ILUC) (Scordia et al., 2022), the cultivation of energy crops in heavy metals' contaminated soil can not only help to remediate the soil, but also contributes to provide a renewable and sustainable feedstock that can be valorized in biofuels, bioenergy, and bioproducts.

The benefits of energy crops in marginal lands are associated with ecosystem services and benefits of soil properties such as fertility, structure, and organic matter (Fernando et al., 2015; Stewart et al., 2015). Furthermore, the mechanics of biomass cultivation allows this feedstock becomes a neutral emission alternative once the emissions from the biomass utilization can be absorbed by the subsequent growing cycle, corroborating achieving the zero-emission target, firmed by the EU in the European green deal (Pires et al., 2019). Consequently, biomass production in contaminated soils also promotes soil decontamination once the crops uptaken pollutants. In the social field, the generation of jobs, the exploitation of contaminated areas that limit competition with food production, and the contribution to a more sustainable mentality make the alternative of using marginal soils to produce viable energy crops (Fernando et al., 2018).

However, the cultivation of energy crops in marginal lands also faces a few challenges, namely the loss in biomass productivity and changes in biomass quality. The decrease in energy crops' productivity can decrease their environmental benefits, reducing the amount of renewable energy produced and the amount of captured CO_2 from the atmosphere during the photosynthesis process (Kabata-Pendias., 2011). The reduction in biomass quality, on the other hand, can not only directly reduce the viability of contaminated biomass utilization (e.g., through the reduction of HHV due to the increase of ash content), but the contaminants present in biomass can cause damage in the biorefinery machinery (Paunov et al., 2018; Vassilev et al., 2013). From the phytoremediation side, the main challenge is time. In order to achieve soil decontamination, decades are necessary. This way, phytoremediation is slow and may not achieve the current society-needed results (Fernando et al., 2018; Do Nascimento et al., 2006).

The advantages and challenges regarding the cultivation of energy crops in marginal lands contaminated with heavy metals depend on which heavy metal, its oxidation state, and its concentration. Therefore, understanding the effects of heavy metals on soils and the environment is necessary.

1.3.1 Heavy metals in soil

Soil is a non-renewable natural resource used in many ways to support human development. However, the necessary infrastructure, agricultural land, and land for city expansions have decreased soil availability and increased the available regions' contamination. As a result, soils contaminated by metals are increasing worldwide among all pollutants, and heavy metals are the most hazardous (Xu et al., 2019).

Soil contamination by heavy metals is increasing around the world. For example, in the United Kingdom, the concentration of heavy metals in rural soils for Cd, Cr, Cu, Pb, Ni, and Zn are 1.15, 73, 51, 138, 7.28, and 195 mg.kg^{-1}, respectively. However, these concentrations increased drastically in urban soils, up to 134 and 4286 mg.kg^{-1} for Cd and Cr, 14714, 1038, and 23238 mg.kg^{-1} for Pb, Ni, and Zn (Alloway, 2010).

European Union has also contaminated soils, with almost 137,000 km^2 of agricultural soils contaminated by heavy metals, becoming an urgent crisis for the environment and healthy sides (Tóth et al., 2016).

In Portugal, Cu and Zn were detected in fishes in Esmoriz - Paramo's lagoon on the northwest coast of the country (Fernandes et al., 2008), and high concentrations of Zn, Pb, Cu, and Cr were detected in Aveiro’s river (Martins et al., 2013). In another study, authors indicated that mining activity was responsible for contaminating individuals with As, Cr, Mn, and Ni, after eating vegetables cultivated in contaminated soils (Ávila et al., 2017).

In Sicily, the smoke emitted by vehicles is responsible for the deposition of high levels of Cu, Pb, and Zn in the soil. Simultaneously, the petrochemical company contaminated the soil in the region around the plant with Ni and Cr (Manno et al., 2006). Soil contamination with Cu, Zn, and Cd is also a problem in Naples (Imperato et al., 2003) and Bologna (Morselli et al., 2003).

Mining is a huge source of contamination in Georgia, responsible for the deposition of Cu, Zn, and Pb in the soil, increasing the levels of these contaminants to values up to 15 times higher than the European

Union norms. In addition, heavy metals enter the food chain through the grass that feeds the animals, contaminating humans through dairy products. (Bakradze et al., 2018).

In Spain, excess of Cr and Pb was identified in regions close to forests (Cutillas-Barreiro et al., 2016). Agricultural activities were also linked to the contamination of Spanish soils with Cd, Pb, Zn, and partially Cu (Martín et al., 2013). In another study, 32 different samples of soils were analyzed, showing that where human activities are present, the concentration of Cd, Zn, Pb, Cr, Cu, Ni, and other contaminants are significantly higher, sometimes exceeding the limits, becoming an environmental problem (Roca-Perez et al., 2010).

Pb, Cr, and Zn were detected in Arc River, Provence, France. These heavy metals in the river were correlated to human activities (Desenfant et al., 2004). In La Rochelle, Ni was detected in vegetables in the local supermarkets (Cherfi et al., 2016). In the Saine River basin, the contamination of the river sediments with Pb, Cd, Zn, and Cu is massive, putting the river among the most recorded contaminated (Meybeck et al., 2007).

In Germany, sites with high levels of potentially toxic elements, including heavy metals, were detected in urban and agricultural regions in Saxony. The contamination was attributed to industrial dust deposition in the area (Rachwał et al., 2017).

In Bukowno, Poland, mining activity was linked to the contamination of the topsoil by Zn, Pb, and Cd (Verner et al., 1996), and in the Roztocze National Park forest, the concentration of Pb, Zn, and Cu was considerably higher than the regulated soil limits set for these metals (Mazurek et al., 2017)

Europe is not the only place that has problems with heavy metals contaminations. Heavy metal contamination is also a problem in Africa, contaminating rivers and soils and entering the food chain. One example occurs in Nigeria, where heavy metals were detected in vegetables cultivated in soil contaminated by mining activities (Obiora et al., 2016).

In South Africa, Cd was found in soils, and the source was traced back to industries operating in the region (Clark et al., 2015). Another study conducted in South Africa and Mozambique found Cr, Pb, and Ni in vegetables and water resources from both countries, representing a risk for humans and ecosystems in the region (Genthe et al., 2018). In Angola, domestic and industrial activities (using wood and fossil fuels) produce particulate matter contaminating the soils (Ferreira-Baptista et al., 2005).

In Asia, soil contamination is also increasing and reaching dangerous levels for the population. With a vast population attending, the demand for products and essential goods is challenging and produces many residues. China, India, and Pakistan are examples of Asian countries with soil contamination problems.

In the north region of India, a critical level of contamination was detected regarding Cr, while for Ni, Zn, and Pb, moderated levels of contamination were detected. However, Adimalla and Wang emphasize that their combination represents risks to the local population (Adimalla et al., 2018).

China also has soils contaminated with heavy metals, highlighting the high levels of Cd and Hg and the presence of Cd, Pb, Zn, Cu, and Cr in farmlands, which is a risk considering that the studied region is a significant food producer (Cai et al., 2019; Liang et al., 2017).

In Karachi, Pakistan, the levels of Pb are high, becoming a threat, especially for Pb potential to cause cancer, mainly among children, which are the most vulnerable group (Karim et al., 2014)

Australia also detected Cd, Zn, Pb, and Cu in farmlands, usually used for food production (Kachenko et al., 2006). While in South America, Brazil measured heavy metals in agricultural soil. Not just industrial activities cause the contamination of Brazilian lands, but the collapse of a mining company damn caused the contamination with As, Ba, Co, Cr, Cu, Ni, and Zn in Minas Gerais state (Davila et al., 2020). The south and northeast soils of Brazil are also affected by heavy metals (Demarco et al., 2019; França et al., 2017).

The heavy metals definition is not truly clear. They can be defined as metals with an atomic number higher than 20 and a density higher than 5 $g.cm^{-3}$ (Barson 1998), using just the density above 6 $g.cm^{-3}$ criteria (Alloway, 2010), or using families of metals for classification (Appenroth et al., 2009; Appenroth et al., 2010), but always representing the hazardous potential of these compounds. Due to their potential to harm humans, we use heavy metals to express these elements' toxicity potential.

Heavy metals are an essential part of the environment, naturally accumulated in reservoirs and distributed on earth according to their geological and chemical cycle. They can be found in soils in different forms: as free heavy metals, as a soluble metal complex, associated with the organic matter in the soil, as oxides, hydroxides, carbonates, or incorporated into silicate mineral structures (Kabata-Pendias, 2011; Motuzova et al., 2014). However, despite their natural presence, the concentration of metals in the soil is entirely modified by human activities, making use of metals such as Zn, Cr, Pb, Cd, Ni, and Cu for industrial and agricultural activities, generating contaminated residues (Tchounwou et al. 2012), which ends up spreading these components and increasing their concentration in the soil to levels that are a threat to the live organisms in the biome. Some sources of contamination can be seen in Table 1.7.

Table 1.7. Sources of heavy metals (Alloway,2010)

Geological Sources of heavy metals									
	Upper crust mg.kg^{-1}	Granite, granodiorite mg.kg^{-1}	Gabbro basalt mg.kg^{-1}	Ultramafic rocks mg.kg^{-1}	Sandstone mg.kg^{-1}	Shales mg.kg^{-1}	Black/Oil Shales mg.kg^{-1}	Limestones mg.kg^{-1}	Coal mg.kg^{-1}
Cd	0.1	0.1	0.2	0.05	<0.04	0.25	<240	0.1	1
Cr	35	10	250	2300	35	100	<700	5	20
Cu	14	12	90	40	2	45	<300	6	20
Ni	19	5	130	2000	2	70	<300	5	20
Pb	17	20	4	0.05	10	22	<100	5	20
Zn	52	50	100	60	20	100	<2314	40	50
Agriculture									
	Phosphatic Fertilizers mg.kg^{-1}		Nitrogen Fertilizers mg.kg^{-1}		Lime Fertilizers mg.kg^{-1}		Manure mg.kg^{-1}		
Cd	0.1 - 170		0.05 - 8.5		0.04 - .01		0.3 - 0.8		
Cr	66 - 600		3 -19		10 -15		5.2 - 55		
Cu	1 -300		1 - 15		2 - 125		2 - 60		
Ni	7 - 38		7 -38		10 - 20		7.8 - 30		
Pb	7 - 225		2 - 1450		20 -1250		6.6 - 350		
Zn	50 - 1450		1 - 42		10 - 450		15 - 250		

Despite Cr and Ni also being naturally released in massive amounts, as seen in Table 1.7, all the studied heavy metals have been released in tremendous amounts due to anthropogenic activities, with a significant influence on agricultural activities, due to the presence of these metals in fertilizers, pesticides, herbicides, among other products. It is also evidenced that human activities in generating heavy metals contaminated residues overcome the natural sources, having a higher concentration for all studied heavy metals.

The production of heavy metals is mainly concentrated in Asia. However, the importance of these metals in the economy is so significant that according to London Metals Exchange, Zn, Pb, Ni, and Cu achieved the highest price in the last two years, could be costing US$3,576.05, US$ 2,255.00, US$ 18,620.00, and US$ 6,810.00 respectively (LME, 2020).

The economic potential of these heavy metals is responsible for their vast exploration. Seeking to slow the rate of soil contamination, countries implemented specific legislation for this problem. For example, the European Union implemented a series of regulations to decrease the contamination of its territory by heavy metals.

In 1998 (receiving an amendment in 2012), the Aarhus Protocol on Heavy Metals targeted the contamination of three substances: Cd, Hg, and Pb, aiming to reduce the emissions in different industrial sectors and thermochemical conversion processes (combustion power plants and waste incineration) (UN, 2015). In 2001, the EU Directive 2001/80/EC targeted the emission of heavy metals in the atmosphere caused by large combustion plants (LCP) (European Commission, 2001). In 2010, agricultural and industrial activities were included in the control through EU Directive 2010/75/EU (European Commission, 2010), which also controlled water contamination. Furthermore, European Pollutant Release and Transfer Register (E-PRTR) Regulation (166/2006/EC) also created a survey of industrial facilities aiming to fulfill Kyiv's protocol goals regarding the reduction of pollutants (European Commission, 2006).

1.3.1.1 Zinc (Zn)

Zn is a heavy metal with the atomic number 30, a melting point of 419.5 °C, and a boiling point of 907 °C. It is a good conductor of electricity and malleable at temperatures ranging from 100 °C to 150 °C (Los Alamos National Security, 2021). In Table 1.8, the major Zn production countries can be observed.

Table 1.8. World's zinc production (USGS, 2021)

Country	Mine (Thousand metric tons) 2019	2020	Reserves
United States	753	670	11,000
Australia	1,330	1,400	68,000
Bolivia	520	330	4,800
Canada	336	280	2,300
China	4,210	4,200	44,000
India	720	720	10,000
Kazakhstan	304	300	12,000
Mexico	677	600	22,000
Peru	1,400	1,200	20,000
Russia	260	260	22,000
Sweden	245	220	3,600
Other countries	1,950	2,000	34,000
World Total	12,700	12,000	250,000

With the second-largest reserve and the most significant Zn mining production in 2019 and 2020, China is the most crucial country for mining this heavy metal, which is responsible for more than one-third of the world's output. Australia has the world's most significant Zinc reserves, being the second-largest producer, followed by China, Mexico, Russia, and Peru. Significant reserves and mining rates can also be seen in the United States, Kazakhstan, and India.

Zn is widely used in industries, especially in galvanization processes which, applied as a layer, helps avoid metal parts' corrosion. In addition, it is heavily involved in automobile industries, construction, and shipbuilding (Asgari et al., 2007). Another prevalent utilization is in battery manufacture. As the negative electrode, in association with other elements, Zn can be a part of different battery systems, such as zinc-carbon, zinc-manganese dioxide, zinc-nickel, and zinc-air (Li et al., 2014).

Agriculture is one of the main activities that cause environmental contamination by Zn. The intensive application or untreated disposal of fertilizers, and the application of herbicides and pesticides that have Zn in their composition, despite increases in the productivity of the crops, causes Zn to accumulate in the environment (Eriksson, 2001). Zn can also accumulate in the environment through deposition after waste and coal combustion, but the primary source of Zn contamination in soil and atmosphere is by metal process, both in mining and smelting activities (Desaulty et al.,2020).

In plants, Zn has essential functions in its metabolism as a micronutrient. It is a component for enzymes (Kabata-Pendias, 2011), metabolizes carbohydrates, proteins, and phosphates, and has a role in RNA formation (Lindsay, 1972). The need for Zn varies from plant to plant, but the excess of this heavy metal can be toxic for the crops.

Despite its functions in plant metabolism, excess Zn can interfere with nutrient uptake, be hazardous to enzymatic activities, affect photosynthesis, and cause oxidative stress (Tsonev et al., 2012).

To avoid the hazardous effects caused by the excess of Zn, the plants accumulate the metal in cell walls and vacuoles, both belowground and aboveground (Longnecker et al. 1993). This mechanism allows crops to tolerate high amounts of zinc in the soil.

Not just for plants but also in humans, Zn has essential functions as a micronutrient. The deficiency of Zn in humans can cause problems in brain functions, thymus, skin, and reproductive systems, retard growth and affect the immunologic system. On the other hand, excess Zn in the organisms can also cause brain damage, affect the respiratory system and the gastrointestinal tract, and elevate prostate cancer risk, among other problems (Plum et al. 2010).

1.3.1.2 Chromium (Cr)

Cr is a heavy metal with atomic number 24, a melting point of 1907 °C, and a boiling point of 2671 °C. Discovered in 1797, this heavy metal is steel-grey and takes hard polish (Los Alamos National Security, 2021). Cr-producer countries can be seen in Table 1.9.

Table 1.9. World's chromium production (USGS, 2021)

Country	Mine (Thousand metric tons)		Reserves
	2019	2020	
United States	---	---	620
Finland	2,415	2,400	13,000
India	4,139	4,000	100,000
Kazakhstan	6,700	6,700	230,000
South Africa	16,395	16,000	200,000
Turkey	10,000	6,300	26,000
Other countries	5,110	4,800	NA
World Total	44,800	40,000	570,000

It is possible to notice that the three largest Cr producers are South Africa, Turkey, and Kazakhstan, which are responsible for almost 60% of the Cr world's production in 2020 and have 80% of the Cr world reserves. However, India also has an essential share in mining this metal and still can increase its stake, having the third biggest Cr reserves on the planet.

Among all the uses of Cr, stainless steel production is one of the most important due to stainless steel applications in the chemical, energy, and manufacturing sectors (Pariser et al., 2018).

The sources of Cr contamination in the environment are usually linked to industry-related processes, such as electroplating, tanning, water treatment, coal combustion, oil combustion, steel and iron manufacturing, and cement production. However, there are also natural sources of Cr contamination, such as volcanos and soil suspension (Testa, 2005).

It is observed from Figure 1.3 that Europe has some spots where the concentration of Cr in the soil is high. For example, Greece, north of Italy, central Germany, and central France have Cr concentrations higher than 150 mg.kg^{-1}.

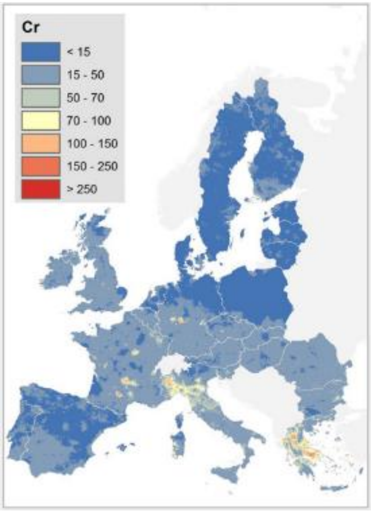

Figure 1.3. Cr-contaminated soils in Europe (Tóth et al., 2016)

The toxicity of Cr in the plant depends on the concentration of the metal and its oxidation state. Cr is found in the environment as Cr(III) or Cr(VI), being the last one highly toxic to plants (Shanker et al., 2005). Cr can affect the plant in different forms. For example, it can reduce or even inhibit the seeds' germination and the growth of roots, stems, and leaves. In addition, the Cr uptake mechanism carries essential nutrients such as Fe, S, and P inside the plant, reducing crops' nutrient absorption (Turner et al., 1970; Wallace et al., 1976).

Even though Cr(III) is an essential micronutrient in humans and animals, high amounts of this component or even introducing Cr(VI) in the food chain can cause different hazardous effects. The contamination by Cr occurs through cutaneous absorption, inhalation, or ingestion. Consequently, it can cause dermatological, lung cancer, respiratory pathologies, and hemorrhages. In some circumstances, it can cause death (Baruthio, 1992; Shekhawat et al., 2015).

1.3.1.3 Lead (Pb)

Pb is a heavy metal with atomic number 82 that belongs to group IV and the 6th period of the periodic table. The density of this metal is 11.4 g.cm^{-3}, with a melting and boiling point of 327.4 and 1725 °C for melting and boiling point, respectively. It is one of the most frequent metals in industrial production, being soft, highly malleable, ductile, has slight conductivity, and is also very resistant to corrosion but tarnishes upon exposure to air (Wuana et al., 2014). The risk associated with humans has led to the second position in the Substance Priority List – ATSDR in 2019 (ATSDR, 2019). The world major's Pb countries production can be seen in Table 1.10.

Table 1.10. World's lead production (USGS, 2021)

Country	Production		Reserves
		(Thousand metric tons)	
	2019	2020	
United States	270	290	5,000
Australia	509	480	36,000
Bolivia	88	65	1,600
China	2,000	1,900	18,000
India	200	210	2,500
Kazakhstan	56	30	2,000
Mexico	259	240	5,600
Peru	308	240	6,000
Russia	230	220	4,000
Sweden	69	70	1,100
Tajikistan	65	65	NA
Turkey	71	72	860
Other countries	591	520	5,000
World Total	4,720	4,400	88,000

When compared to Cd, Pb production is five times lower. China also appears as a significant producer, with more than 40% of total world production in 2020, 4 times more than Australia, the second largest producer. Despite being second in the mining activity, Australia has the most extensive Pb reserves, with more than 40% of world reserves. China, the United States, Mexico, Peru, and Russia also have abundant Pb reserves, potentially increasing their mining activity of this metal.

The primary use of Pb is in Pb-acid batteries, which accounts for 92% of its utilization (USGS, 2021), but it also can be found in pesticides and herbicides as lead arsenate (Meza-Montenegro et al., 2013). It can also be used in several different applications, including paints (Jacobs et al., 2002), dyes (Kar et al., 2017), ceramic glazes (Azcona-Cruz et al., 2000), pesticides (Murphy et al., 1998), ammunition (Arnemo et al., 2016), pipes (Rabin, 2008), sheets used for radiation protection (Saeedi-Moghadam et al., 2021), and was used for a long time as a gasoline additive (Potra et al., 2018).

The Pb can be present in the soil in different forms, associated with clay minerals, Mn oxides, Fe and Al hydroxides, calcium carbonate particles, or phosphate concentration (Kabata-Pendias, 2011).

Soils contamination with Pb can be associated with old mining areas, nonferric metal mining, the metal process industry, battery manufactory sites, sludge farmlands, urban gardens, and roadside soils (by deposition by automobiles through exhausting gases such as halide salts), and waste lime application (Kabata-Pendias, 2011). In addition, after settling on the ground through precipitation or as particulate matter, it is absorbed by soil particles, contaminating the soil and groundwater sources used for drinking and entering the food chain. It has been shown that Pb accumulates in plants and animals that live and feed on contaminated areas (Yousef et al. 1992).

The contamination of European soils by Pb is depicted in Figure 1.4, showing it varies from 1.63 to 151.12 mg.kg^{-1}, higher in some regions in Italy, Germany, England, and France (Tóth et al., 2016).

Pb does not have an essential role in plant metabolism, although if it is necessary for plant growth, the concentration should be lower than 2 µg/kg of dry biomass (Broyer et al.,1972; Kabata-Pendias, 2011).

The presence of a high level of Pb in plant tissues can affect some metabolic processes such as photosynthesis, mitosis, and water absorption, inhibit enzyme activities, and seed germination (Ayangbenro et al., 2017). Even though the symptoms of Pb contamination are not truly clear, some cereals present dark green leaves, wilting of older leaves, stunted foliage, and short brown roots (Kabata-Pendias, 2011). In general, the rate of leaf accumulation is higher in leaf vegetables. Also, root vegetables can accumulate a moderate Pb level (Alexander et al., 2006).

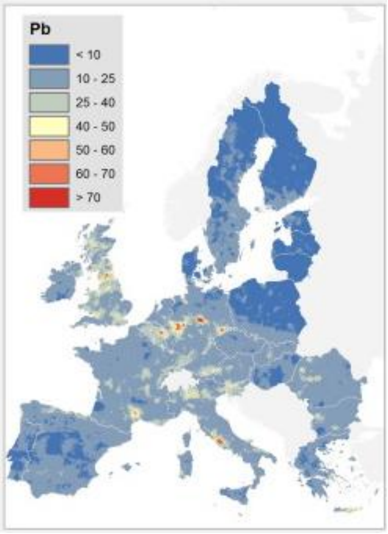

Figure 1.4. Pb-contaminated soils in Europe (Tóth et al., 2016)

Once in the food chain, it can cause health problems for humans. For example, human exposure to Pb contamination can be associated with neurological and reproductive hazardous effects, affecting the fetus's development in pregnant women (Lippert et al., 2020). In addition, high levels of exposure can cause toxic biochemical effects in humans, which include neurological damage, decreased intelligence quotient (IQ), anemia, muscle and joint pain, memory loss, decreased concentration, nervous disorders, problems with hemoglobin synthesis, issues on the kidneys, anorexia, chronic nephropathy, damage to neurons, high blood pressure, hyperactivity, insomnia, learning deficits, reduced fertility, renal system damage, a risk factor for Alzheimer's disease, gastrointestinal tract, infertility, increased blood pressure and chronic headache, leading in some extreme cases to death (Ayangbenro et al., 2017; U.S. Department of Health and Human Services, 2012).

1.3.1.4 Cadmium (Cd)

Cd is a heavy metal with atomic number 46 that belongs to group XII and the 5th period of the periodic table. The density of this metal is 8.650 $g.cm^{-3}$, with a melting and boiling point of 321.22 and 767°C, respectively. It is an essential component for life in low concentrations, but it is incredibly toxic in high concentrations. Its average content in the Earth's crust is 0.1 mg/kg, but it is rare in a pure form, mostly found in minerals, and commonly recovered from Zn ores (Kabata-Pendias, 2011).

Some Cd reserves are associated with Zn reserves, representing 0.03% of Zn ores (USGS, 2021). China is the major Cd miner, accounting for over one-third of the world's production in 2020. Despite reducing the mining activity from 2019 to 2020, the Republic of Korea remains the second biggest Cd producer. Japan, Canada, Kazakhstan, Mexico, and The Netherlands deserve the highlight. With Peru and Russia, these countries were responsible for 90% of all Cd mining globally in 2020. The world major's Cd production can be found in Table 1.11.

Table 1.11. World's cadmium production (USGS, 2021)

Country	Production (Thousand metric tons)	
	2019	2020
Canada	1,803	1,800
China	8,200	8,200
Japan	2,000	1,800
Kazakhstan	1,500	1,500
Republic of Korea	4,400	3,000
Mexico	1,395	1,300
Netherlands	1,100	1,100
Peru	772	700
Russia	900	900
Other countries	2,320	2,300
Total World	24,400	23,000

The primary sources of Cd exposure are Zn and Pb refineries, Ni/Cd batteries disposal of industrial wastes contaminated with Cd, electronic products, fertilizers, pesticides, mining processes, plastics, oil refining, welding, metal smelting, and refining, fossil fuel burning, application of phosphate fertilizers, and sewage sludge (Ayangbenro et al., 2017; Hussain et al., 2020; Li et al., 2019). The risk associated with human exposure to Cd puts it in seventh place on Substance Priority List - ATSDR in 2019.

The soils with a higher average concentration of Cd contamination are found in Ireland, in the north of Spain, and Slovenia, with the highest concentration at 3.17 and the lowest at 0.02 mg.kg^{-1} (Tóth et al., 2016). However, the contamination of Cd in surface soils can reach extreme levels, contaminating sites close to mining areas (e.g., 468 mg.kg^{-1} in Great Britain), on sites located close to metal processing industries (e.g., 1781 mg.kg^{-1} in Belgium), some urban gardens due to deposition of particles (e.g., 100 mg.kg^{-1} in the United States), or in farmlands due irrigation, in sludge or fertilized sites, reaching levels as 107mg.kg^{-1} in Poland (Kabata-Pendias, 2011).

The Cd-contaminated areas in Europe can be seen in Figure 1.5.

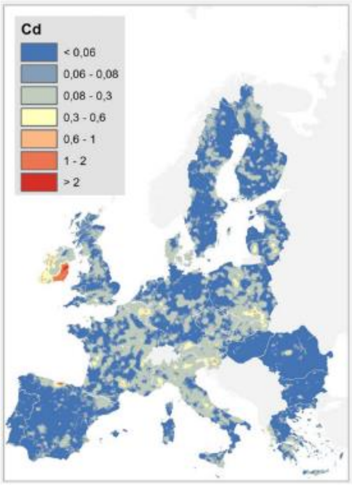

Figure 1.5. Cd-contaminated soils in Europe (Gergely Tóth et al. 2016)

Several factors can influence Cd absorption by plants, but pH is the primary factor that defines the total and the relative Cd absorption from the soil. Besides the soil pH, P, N, and Cl levels also contribute to Cd's bioavailability, increasing plant uptake (Kabata-Pendias, 2011).

In plants, Cd can negatively affect plants' growth and development, reducing the roots' length and damaging leaves. Cd presence can also have dangerous consequences in chlorophyll biosynthesis, reducing the photosynthetic capacity. In addition, the uptake of several elements can compromise the plants' nutrition (Ca, Mg, P, K, Zn, Mn, and B). Reactive oxygen species (ROS) and protein formation can also suffer significant changes (Tran et al., 2013).

To avoid the hazardous effects of Cd, plants have defense mechanisms that can follow two paths: avoidance or tolerance. In avoiding paths, the uptake of Cd is limited, while in the tolerance path, Cd can be accumulated, stored, and immobilized (Pál et al., 2006).

Cd is extremely dangerous for humans, causing severe problems such as bone disease, coughing, emphysema, headache, hypertension, kidney diseases, lung and prostate cancer, lymphocytosis, microcytic hypochromic anemia, testicular cancer atrophy, and vomiting (Ayangbenro et al., 2017; Bernhoft, 2013).

1.3.1.5 Nickel (Ni)

Ni is a heavy metal with atomic number 28, classified as a transition metal. The density of this metal is 8.912 g.cm^{-3}, and the melting and boiling points are 1455 °C and 2730 °C, respectively. It is a malleable, ductile, and abundant material, classified as number 58 in the substance priority list, capable of conducting heat and electricity, and presents ferromagnetic properties (ATSDR, 2019; Los Alamos National Security, 2021).

Table 1.12 shows that annual Ni production is around 2,500,000 thousand metric tons, where Indonesia is the most significant Ni producer, responsible for 30% of the world's production in 2020. Therefore, the Ni extraction by this country is justified as Indonesia's enormous Ni reserves, followed by Australia, Brazil, Russia, Cuba, and the Philippines. The Philippines is the second-biggest producer, followed by Russia, New Caledonia, Australia, Canada, and China. Brazil and Cuba also stand in a highlighted place. For these two countries, even though the production in 2020 was only 73,000 and 49,000 thousand metric tons, respectively, their reserves are 16,000,000 and 5,500,000 thousand metric tons, placing them have considerable potential Ni exploration market (USGS, 2021).

Table 1.12. World's nickel production (USGS, 2021)

Country	Production		Reserves
		(Thousand metric tons)	
	2019	2020	
United States	13,500	16,000	100,000
Australia	159,000	170,000	20,000,000
Brazil	60,600	73,000	16,000,000
Canada	181,000	150,000	2,800,000
China	120,000	120,000	2,800,000
Cuba	49,200	49,000	5,500,000
Dominican Republic	56,900	47,000	NA
Indonesia	853,000	760,000	21,000,000
New Caledonia	208,000	200,000	NA
Philippines	323,000	320,000	4,800,000
Russia	279,000	280,000	6,900,000
Other countries	310,000	290,000	14,000,000
World total (rounded)	2,610,000	2,500,000	94,000,000

Ni is widely used as a raw material in the metallurgical and electro-plating sectors, as a catalyst in the chemical and food industry, and as a component of electrical batteries (Salt et al., 2020; Shahzad et al., 2018). In addition, Ni is used in desalinization plants for steel production, coin manufacture, and color glass (Los Alamos National Security, 2021).

Despite the natural presence of Ni in nature, some human activities can accelerate and unbalance the Ni concentration in the environment. Some examples of anthropogenic Ni deposition in the environment include wastes from fossil fuel power plants, mining and smelting processes, emissions from the transport sector, industrial and urban wastes, and steel and cement industries (Alloway, 2013; Shahzad et al., 2018).

The Ni soil contamination map in Europe is depicted in Figure 1.6, where it is possible to see that Greece has the most severe Ni contamination problems, with a vast part of its territory containing Ni levels above the EU regulation limits, which are 110 mg.kg^{-1}. The Northwest of Italy also presents Ni-contaminated sites, with concentrations higher than 200 mg/kg^{-1}. Canada, Japan, China, and South Africa are non-European countries that also show high levels of Ni in soil (Kabata-Pendias, 2011; Tóth et al., 2016).

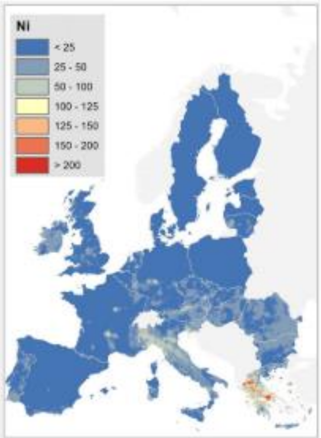

Figure 1.6. Ni-contaminated soils in Europe (Tóth et al., 2016)

Although the presence of Ni in the soil can occur naturally, anthropogenic activities are responsible for changing the equilibrium (Kabata-Pendias, 2011). The dynamic of Ni in soils is significantly influenced by pH and EH, the presence of Fe, Mn, SO4^{-2}, and the dissolved organic carbon content (Rinklebe et al., 2017).

In plants, Ni is an essential micronutrient, being part of the nitrogen cycle in the plant through its presence in some enzymes such as urease and hydrogenase (Shahzad et al., 2018). The absence of Ni in the soil prevents the plant from completing its growth cycle (Brown et al., 1987), although the excess of Ni can lead to several hazardous effects on the plant in morphological, physiological, and biochemical aspects (Shahzad et al., 2018). In some cereals, the excess Ni can be noticed by some plants characteristics, such as interveinal chlorosis in new leaves, grey-green leaves, and brown and stunted roots (Kabata-Pendias, 2011), and it can be responsible for yield reductions of 20% for some plants (Manicol et al.,1985).

Ni can enter humans by dermatologic, respiratory, and gastrointestinal paths. In the dermatologic path, absorption of Ni particles occurs through the skin. The respiratory path consists of inhaling tiny particles that can be retained and accumulated in the lungs, while the gastrointestinal route occurs by ingesting food and beverages containing the heavy metal (Buxton et al., 2019). Its presence in human metabolism can cause cardiovascular diseases, dermatitis, dizziness, reduction of respiratory capacity, headache, kidney diseases, and lung and nasal cancer (Ayangbenro et al., 2017; Prueitt et al., 2020).

1.3.1.6 Copper (Cu)

Cu is a heavy metal that has been mined for 5,000 years. It has the atomic number 29, and the melting and boiling points are 1084.6 °C and 2562 °C. It is malleable, ductile, and a good heat and electricity conductor (Los Alamos National Security, 2021).

As observed in Table 1.13, the major Cu miners are the South American countries Chile and Peru. Despite their colossal Cu production, also associated with the highest reserves of this metal, China leads in the Cu refining process, with the amount of Cu refined higher than all the other countries on the list combined. Although its enormous capacity for refinery processes, Cu Chinese reserves are not as expressive as the other countries on the list. In this topic, Australia and Russia presented expressive reserves of this heavy metal, increasing their share in the market.

Cu industrial applications justify the existing market. Copper metal is mostly used to produce pipes and electrical cables, which are also essential to the construction and transport sectors. Cu is also used in the form of sulfates, as in the agricultural field, as part of fungicides, algicides, and plant nutrition (Van-zwieten et al., 2004). Other uses include petroleum refineries, wood preservatives, water treatment, and jewelry (Barceloux, 1999).

Table 1.13. World's copper production (USGS, 2021)

Country	Mine		Refinery		Reserves
	(Thousand metric tons)				
	2019	2020	2019	2020	
United States	1,260	1,200	1,030	910	48,000
Australia	934	870	426	380	88,000
Canada	573	570	281	290	9,000
Chile	5,790	5,700	2,270	2,400	200,000
China	1,680	1,700	9,780	9,800	26,000
Congo (Kinshasa)	1,290	1,300	1,080	1,100	19,000
Germany	--	--	632	670	2,000
Japan	--	--	1,500	1,600	--
Kazakhstan	562	580	512	540	20,000
Republic of Korea	--	--	665	680	--
Mexico	715	690	477	470	53,000
Peru	2,460	2,200	308	330	92,000
Poland	399	400	566	550	32,000
Russia	801	850	1,050	1,060	61,000
Zambia	797	830	262	360	21,000
Other countries	3,100	3,300	3,640	3,500	200,000
World total (rounded)	20,400	20,000	24,500	25,000	870,000

As expected, this heavy metal produces contaminated residues that can contaminate all the environmental compartments, such as water, soil, and atmosphere. Figure 1.7 depicts the Cu concentration in European soils.

Although the area contaminated by Cu is not expressive compared to the area of the EU, it can be seen in some places of Italy and southern France with Cu levels in the soil higher than 100 ppm.

In plants, Cu is, at the same time, an essential and toxic element, depending on its level. It functions as a cofactor for metalloproteins, but when in excess, it inhibits the plant's growth and harms some critical cellular processes (Yruela, 2005).

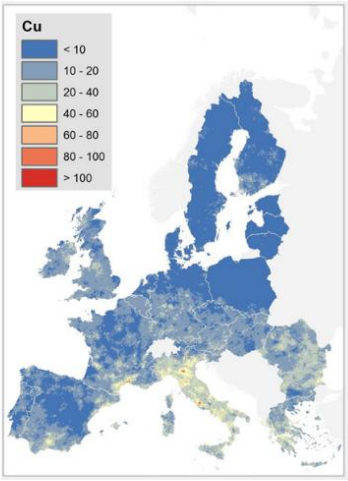

Figure 1.7. Cu-contaminated soils in Europe (Tóth et al., 2016)

To maintain the needed Cu levels, plants have developed mechanisms to regulate their homeostasis according to changes in the environmental conditions in which they are cultivated (Pilon, 2011; Yruela, 2005, 2009).

The essential role of Cu in humans was first assumed in 1928, being vital for metalloproteins and as a component in gene expression, and this metal deficiency can cause anemia, neutropenia, and abnormalities (Uauy et al., 1998). However, despite the essential functions, the excess of Cu can also cause toxic effects on humans, contributing to neurodegenerative diseases, such as Alzheimer's, arteriosclerosis, and diabetes mellitus (Brewer 2010). Remediation of contaminated soils

1.3.2 Contaminated soils remediation

Considering that contaminated lands are a global problem, the remediation of sites is necessary. The techniques for soil remediation can be grouped according to different criteria. Among the most common, separated methods by *ex-situ* or *in-situ*, or by the technological path (physical, chemical, or biological) are the most common (Corbin, 1991; Pavel et al., 2008).

Regarding *ex-situ* techniques, the methods can be grouped into physical, chemical, and thermal strategies. The *ex-situ* physical method consists of landfilling to remove the contaminated soil from the site and dispose of it in a safe landfill. Despite being a well-established technique, this technique could cost up to $500 to treat 1 ton of contaminated soil (U.S. Environmental Agency and U.S. Air Force, 1994).

The chemical methods are soil washing, which consists of washing the soil with chemical solutions, adapting the soil's acidity to decrease the availability of heavy metals, and solidification, which consists of the microencapsulation of the waste in the soil (Liu et al., 2018).

Vitrification is a thermal *ex-situ* technique that consists of incinerating organic matter and melting the mineral. After incineration, the soil is cooled in a fast process. This way, the mineral part of the soil remains in a glassy form that is no longer bioavailable (Mallampati et al., 2015).

In situ techniques for soil remediation are done in the contaminated site and divided into four groups according to the technological path: physical, electrical, chemical, and biological.

Surface capping and encapsulation are physical methods to remediate contaminated soils. The first consists of isolating the contaminated site and protecting the contaminants from being leached into underground water, while the latter consists of designing physical barriers to prevent the water from being in contact with the contaminants (Liu et al., 2018).

Electrokinetic extraction removes heavy metals from the soil by electrical adsorption, applying low-intensity direct current (DC) through electrodes in the ground (Virkutyte et al., 2002). Although this technique works well in the laboratory, the scale-up can present some technical difficulties, such as the soil condition, which needs to be acidified, reducing the environmental appeal of this technique. Another limitation is the time: The application can take weeks to years to complete remediation, a period when the soil will remain unproductive (Virkutyte et al., 2002).

In-situ chemical techniques consist of soil flushing, which involves soil washing, using solvents that can be recovered and reused in the same process, and immobilization, a technique very similar to solidification but applied in the contaminated site (Pavel et al., 2008).

Although some chemical and physical methods for soil remediation, such as ion-extraction, soil washing, ultrafiltration, or landfilling, can be efficient for site decontamination, they are not environmentally responsible (e.g., landfilling is not a treatment as it only transfers the contaminated soil to another site) or economically viable. On the other hand, biological methods usually do not interfere with the site's ecosystem, being more environmentally friendly and cheaper when compared to other techniques (Barbosa et al., 2015).

Among all the biological techniques, phytoremediation is a promising environmentally friendly technique that receives more daily attention. Phytoremediation is the utilization of plants to remove heavy metals from the soil. This utilization is sometimes used with bioremediation to remediate contaminated soils and wastewater (Pulford et al., 2003).

1.3.3 Phytoremediation

Phytoremediation is a versatile method used with different pollutants in high levels of single or multi-contamination to promote soil or wastewater decontamination (Cristaldi et al., 2017). The recent development in this field can be attributed to improvements in the biotechnology field, with new methods to promote the stabilization or volatilization of the contaminants (Agnello et al., 2016).

This technique can be divided into different groups, depending on the biochemical mechanisms that the plant uses to remediate. Figure 1.8 shows the different paths in phytoremediation and how they work. They are phytoextraction, phytostabilization, phytodegradation, phytostimulation, and phytovolatilization.

Plants can promote phytoextraction in different ways, like phytoaccumulation, phytosequestration, or photoabsorption. In this process, the plant's root system encourages the uptake of the contaminants, which can remain accumulated in the root system or be translocated to the aerial part of the plant (Nesler et al., 2017).

Phytoextraction is the process that allows heavy metal to be extracted from the soil. It can be designed for different contaminated soils due to the particularities of each rapid crop growth, the potential for metal accumulation, extensive root system, high tolerance to contaminants, broad geographic distribution, well adapted to prevailing climatic conditions, and repulsion to herbivores to avoid food chain contamination (Kumar et al., 1995; Do Nascimento et al., 2006).

Phytovolatilization is the plant uptake by organic compounds, followed by the translocation of pollutants and subsequent release of those VOCs from the stem/trunk and leaf surfaces. Indirectly, phytovolatilization is due to the increased flow of volatile contaminants from the soil or subsurface water due to the activity of the plant root (Limmer et al., 2016).

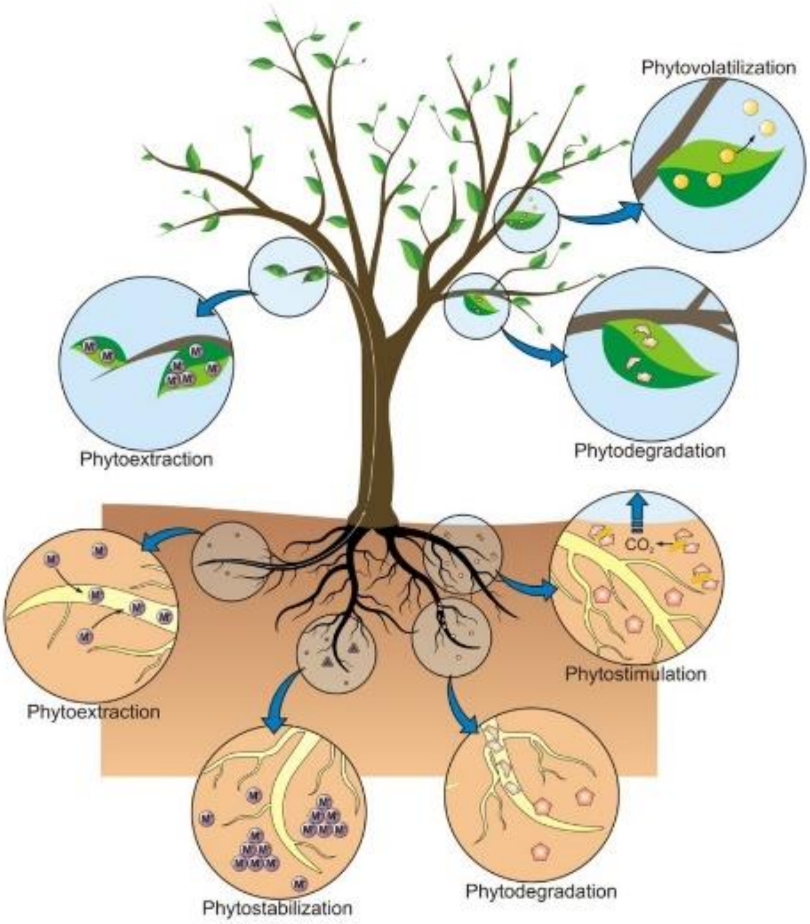

Figure 1.8. Mechanisms of phytoremediation (Costa. et al., 2014)

Phytostabilisation/phytoimmobilization is the transformation of toxic compounds into non-toxic/less poisonous forms fixed in the soil, thereby reducing their bioavailability in the environment (Ali et al., 2013). Immobilization of heavy metals in soils using plants can be achieved through metal sorption by roots, precipitation, complexation, or metal valence change/reduction in the rhizosphere (Ali et al., 2013). Rhizosphere bioremediation or rhizodegradation is enhanced biodegradation of pollutants by root-associated bacteria and fungi. Rhizosphere bioremediation is efficient within specific plant species (Ali et al., 2013) due to increased microbial metabolic rates. Plants supply the carbon sources required to stimulate the associated metabolic pathways to enhance the degradation of heavy metal contaminants within the soil.

Phytofiltration is also a phytoremediation mechanism and refers to the approach of using a plant's biomass for contaminants removal, like toxic metals, from polluted water by utilizing plant roots (rhizofiltration), seedlings (blastofiltration), or excised plant shoots (caulofiltration) (Limmer et al., 2016).

1.3.4 Promising crops for phytoremediation

Considering the further utilization of contaminated biomass, industrial crops become an excellent alternative for phytoremediation, besides the ability to extract, sequester, and detoxify non-volatile yields shown and the possibility of producing bioproducts using the phytoremediation biomass (Barbosa et al., 2015). Another aspect of the association between industrial crops and phytoremediation has been environmental friendly, decontaminating the soil and designing specific hyper tolerant and hyperaccumulator plants for each contaminant (Clemens, 2001). This condition can be improved with the association of microorganisms (Wang et al., 2019).

Some experiments designed to understand the potential of perennial crops to remediate heavy metal contaminated environments can be found in Table 1.14. Perennial crops are being studied for heavy metals phytoremediation under pots and hydroponics experiments to determine their potential to remove or immobilize specific heavy metals or a particular combination of heavy metals. They are also being studied under heavy metals multicontaminated sites in a diverse range of concentrations. In both cases, these crops present a good response:

Table 1.14. Phytoremediation of heavy metals using perennial crops

Crop	Contaminants	Experimental design	Concentration	Unit	Reference
Miscanthus sp.	Zn, Cr, Pb	Pot experiment	450-900, 300-600, 450-900	$mg.kg^{-1}$	(Barbosa et al., 2015)
	Zn, Cd, Pb	Field experiment	315-778, 4.4-12.7, 146-607	$mg.kg^{-1}$	(Pavel et al., 2014)
	Zn, Cd, Pb	Pot experiment	54.6-1116.0, 0.3-16.0, 37.3-898.6	$mg.kg^{-1}$	(Suhail et al., 2017)
	Cr	Hydroponic	50 – 1000	$\mu g.L^{-1}$	(Sharmin et al., 2012)
	Zn, Cd, Pb, Ni	Pot experiment	130.0, 1.23, 40.01, 9.58 (highest amount)	$mg.kg^{-1}$	(Ociepa-kubicka et al., 2016)
Saccharum spontaneum	Cd	Pot experiment	5 – 25	$mg.kg^{-1}$	(Kavitha et al., 2014)
	Zn, Cu, Pb	Field experiment	40, 35, 32	$mg.kg^{-1}$	(Datta et al., 2017)
Panicum virgatum	Pb, Cd	Pot experiment	4-12, 2-10	$mg.kg^{-1}$	(Arora et al., 2016)
	Cr	Pot experiment	131.25- 600	$mg.kg^{-1}$	(Li et al., 2011)
	Cd	Pot experiment	1 – 10	$mg.kg^{-1}$	(Sun et al., 2018)
	Cd, Pb	Hydroponic	9.45-110.46, 195.4-1204.6	$\mu g.L^{-1}$	(Guo et al., 2019)
	Cd, Cr, Zn	Hydroponic	0.4 – 20, 10 – 40, 7.5 - 450	µM	(Chen et al., 2012)
Agropyron elongatum	Cd	Hydroponic	100-300	$\mu g.L^{-1}$	(Fard et al., 2016)
	Pb, Cd, Zn	Field experiment	404.5, 16.7, 1977.5	$mg.kg^{-1}$	(Żurek et al., 2013)

(Continuation)

Crop	Contaminants	Experimental design	Concentration	Unit	Reference
Arundo donax	Zn, Cr, Pb	Pot experiment	450-900, 300-600, 450-900	mg.kg^{-1}	(Barbosa et al., 2015)
	Ni	Hydroponic	10-100	µg.L^{-1}	(Atma et al., 2017)
	Cd, Pb	Field experiment	2.3 – 525, 33.8-57194	mg.kg^{-1}	(Liu et al., 2019)
	Cr	Hydroponic	50-900	µg.L^{-1}	(Kausar et al. 2012)
	Cd, Ni	Pot experiment	973.8, 2543.3	mg.kg^{-1}	(Papazoglou et al., 2007)
	Zn, Cr	Pot experiment	250 – 2000, 125 – 1000	mg.kg^{-1}	
	Cu	Hydroponic	1 – 26.8	µg.L^{-1}	(Li et al., 2014)
	Cd, Cr, Cu, Ni, Pb	Hydroponic	0.2-1, 0.025-02, 1-2, 0.1-0.5, 1	µM	(Alshaal et al., 2014)
					(Cano-Ruiz et al., 2020)
Phalaris arundinacea	Zn, Pb, Cu, Ni, Cd, Cr,	Field experiment	9.93-224, 2.31 – 44.8, 2.71 – 42.5, 1.75 – 34.7, 0.03 – 2.11, 6.50 – 63.8	mg.kg^{-1}	(Klink, 2014)

1.3.4.1 Phytoremediation by *Arundo donax*

The versatility of *Arundo donax* allows its cultivation in soils with different stress conditions (Eid et al., 2016), such as salinity, pH, organic matter, and nitrogen content, or heavy metal contaminations in various concentrations, and availabilities (Papazoglou et al., 2005, 2007).

Considering its mechanisms to resist, tolerate, thrive on, and remediate toxic metalliferous soil, *Arundo donax* could be considered a suitable metalliferous pioneer plant to use in the phytoremediation of heavy metal-contaminated soils (Pilu et al., 2013).

In a previous remediation study, the biomass of giant reed seedlings in red mud and a mud-soil mixture (control) increased by 40.4% and 47.2% over time, respectively, and the concentrations of available Cd, Pb, Co, Ni, and Fe in the soil all concurrently decreased (Alshaal et al., 2014). Pb and Zn EDTA extractable soil fractions were also reduced by giant reed in a 2-year open-air experiment aimed at assessing the giant reed potential for phytoextraction and soil fertility restoration, confirming the ability of this crop to grow on contaminated soils (Fiorentino et al., 2017). Compost addition gives the highest biomass production and, consequently, the highest metal uptakes of giant reed (Fagnano et al., 2015). Similarly, a study in Lisbon (Barbosa et al., 2015) tested the adaptability and phytoremediation capacity of giant reed and Miscanthus spp. on contaminated soils (under the exposure of 450 and 900 mg kg^{-1} of Zn and Pb; 300 and 600 mg kg^{-1} of Cr), showing their suitability for phytoextraction and accumulation. In particular, the results confirm that bioaccumulation occurs mainly in the hypogeal part (i.e., rhizomes and roots), especially for Pb and Cr, while Zn is easily transported and accumulated in the aerial fractions. The application of soil amendments, including acetic acid, citric acid, and ethylenediaminetetraacetic acid (EDTA), also showed an improvement in the growth and phytoremediation potential of giant reed (Yang et al., 2012).

1.3.4.2 Phytoremediation by *Panicum virgatum*

The tolerance of switchgrass to stressful conditions may also be an up-and-coming crop associated with phytoremediation studies: several experiments analyze switchgrass yield and biomass quality under the pot, hydroponically, or in field experiments.

To understand switchgrass phytoextraction potential, Chen et al. designed a hydroponic experiment using three different heavy metals in different concentrations: Zn (7.5 – 450 µM), Cr (10 – 40 µM), and Cd (0.4 – 20 µM). The results showed that switchgrass is suitable for phytoextraction, classified as an accumulator plant of zinc, chromium, and cadmium (Chen et al., 2012).

In another experiment, the response of switchgrass under zinc, cadmium, lead, cobalt, and nickel was studied by Shrestha et. al.. The results showed that the crop tolerance for the heavy metals was high and could be improved by adding organic material to the soil (Shrestha et al., 2019).

Reed et al. tested four switchgrass varieties in different cadmium trials. After the harvest, it was observed that despite the accumulation of cadmium in the plants suggesting that switchgrass may not be suitable for a Cd phytoextraction process, the yield of the crop was compromised by the increase of the contaminant concentration in the soil, which may cause a decrease in the economic viability of the remediation treatment (Reed et al., 2008).

In another experiment, Reed et al. also studied the dependence of the soil pH on the plants' interactions with cadmium. It was observed that the yield decreased by 95% for higher concentrations of cadmium, and in lower pH soils, the accumulation of the metal in the plant was higher (Reed et al., 2006).

Arora et al. also study switchgrass behavior under Cd and Pb stress, testing microorganisms' utilization to help in the remediation process. The author concluded that despite switchgrass having excellent potential for accumulating heavy metals, this accumulation occurs in the belowground part, an obstacle to removing the contamination from the soil (Arora et al. 2016).

An experiment studying switchgrass in Cd and Pb contaminated soil was also made by Guo et al., which concluded not just that switchgrass is suitable for Cd and Pb phytoremediation processes but that Pb and the soil pH has a synergistic effect regarding the accumulation of cadmium. However, Cd and Pb harm the bioconcentration factor of cadmium in the biomass (Guo et al., 2019).

Switchgrass was also studied in Cr-contaminated soils by Li et al., which observed that switchgrass is tolerant to moderate concentrations of chromium contamination, being an excellent alternative to remediate Cr-contaminated soils, suffering a low reduction in productivity and being able to accumulate a significant amount of the contaminant (Li et al., 2011).

2

METHODOLOGY

2.1 Experimental design

The experiment in this work was designed to study the cultivation of two perennial crops (*Arundo donax* and *Panicum virgatum*) in soils contaminated with different heavy metals (zinc, lead, chromium, copper, cadmium, and nickel).

The experiment aims to understand how these heavy metals affect each mentioned crop's productivity, height, and composition. On the other hand, the study will also allow us to understand the phytoremediation potential of these crops and how this potential can be used to produce an alternative source of sustainable feedstock for the industry while decontaminating polluted soils. Also, the adequacy of those crops grown in contaminated soils for energy will be evaluated.

The experiment consists of 78 pots, 39 for *Arundo donax* and 39 for *Panicum virgatum*. The pots' diameter were 30 centimeters filled with 12kg of sifted soil. The trials were made in triplicate, and the pot disposal can be seen in Figure 2.1.

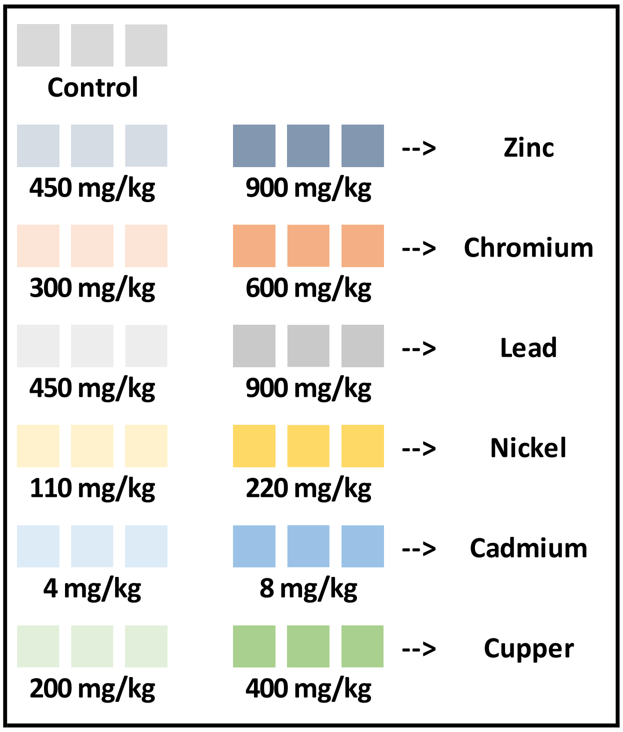

Figure 2.1. Design of the experimental trials

Each trial received just one contamination. The concentrations of the contaminants were chosen according to the limits established by Portuguese legislation regarding the heavy metal concentrations in the soil. The trials were made considering the limitations for alkaline soils and twice the limit. The limits established by the Portuguese legislation can be seen in Table 2.1.

Table 2.1. Design of the experimental trials

Contamination (mg.kg-1)	$pH \leq 5.5$	$5.5 \leq pH \leq 7$	$pH \geq 7$
Copper	50	100	200
Lead	50	300	450
Nickel	30	75	110
Cadmium	1	3	4
Zinck	150	300	450
Chromium	50	200	300

The soil was artificially contaminated by mixing contaminated sludges and salt solutions with the control soil to ensure a high bioavailability of the metals to the plants. Lead trials were contaminated with a mixture of industrial sludge containing 14% of Pb (dry weight basis) provided by "Sociedade Portuguesa do Acumulador Tudor", a battery manufacturing company located near Lisbon, in Castanheira do Ribatejo, and a salt solution of lead nitrate ($Pb(NO_3)_2$). Nickel contamination was performed by mixing an industrial sludge containing 36% Ni (dry weight basis), supplied by Centro para a Valorização de Resíduos, CVR, an association dedicated to providing solutions to waste recovery, and a salt solution of nickel sulfate ($NiSO_4.6H_2O$). Cadmium trials were contaminated with a solid residue rich in Cd (14%, dry weight basis), obtained through a recovery process from Ni-Cd batteries (supplied by Instituto Politécnico de Portalegre, IPP), mixed with a salt solution of cadmium nitrate ($Cd(NO_3)_2.4H_2O$). Chromium contamination was performed by mixing chromium (III) chloride ($CrCl_3.6H_2O$) and potassium dichromate (K_2CrO_4)(equimolar quantities) with an industrial sludge from AUSTRA-CTIC association, located in Alcanena, with Cr (8 $g.kg^{-1}$, dry weight basis), and other metals. Copper trials were contaminated with an electrolytic sludge rich in Cu (54%, dry weight basis), provided by the CIRVER-SISAV, a waste treatment company placed in Chamusca, mixed with a salt solution of copper (II) chloride ($CuCl_2.2H_2O$) and copper sulfate ($CuSO_4$). Zinc trials were contaminated with an industrial sludge rich in Zn (3.5%, dry weight basis), also provided by CIRVER-SISAV, mixed with a salt solution of zinc chloride ($ZnCl_2$) in Table 2.2.

Table 2.2. Heavy metals source and concentration for each trial

Contamination	Salt	Industrial Sludge	Lower Concentration ($mg.kg^{-1}$)	Higher concentration ($mg.kg^{-1}$)
Copper	$CuCl_2.2H_2O$ CuSO4	54% Cu (dry weight)	200	400
Lead	$Pb(NO_3)_2$	14% Pb (dry weight)	450	900
Nickel	$NiSO_4.6H_2O$	36% Ni (dry weight)	110	220
Cadmium	$Cd(NO_3)_2.4H_2O$	14% Cd (dry weight)	4	8
Zinc	$ZnCl_2$	3.5% Zn (dry weight)	450	900
Chromium	$CrCl_3.6H_2O$ K_2CrO_4	0.8% Cr (dry weight)	300	600

2.2 Soil analysis

The chemical characterization of the soil will be made to understand the initial conditions of the soil that was applied in the control trial. The analysis of the soil can be seen in Table 2.3.

Table 2.3. Soils analysis and methodologies

Analysis	Unit	Methodology	Reference
Moisture	%	Weight loss after drying the biomass at 105°C ± 2°C (4h).	(NP 84, 1965)
pH	Sorensen Scale	Determination carried out by potentiometry with a glass electrode. The soil sample is mixed with distilled water in a 1/2.5 ratio.	(Baize, 2000)
Conductivity	$\mu S.cm^{-1}$	For this determination, a conductivity meter was used. The soil sample is mixed with distilled water in a 1/2.5 ratio.	(Baize, 2000)
Organic Carbon	$mg\ C.\ kg^{-1}$	Walkley-Black method.	(Walkley and Black., 1934)
Cation exchange capacity (CEC)	$cmol.kg^{-1}$	Determination by Chapman's method at pH 7 with 1M NH4OAc	(Ross et al., 2011)
Total Nitrogen	% dw	Kjeldahl Method: Mineralization using HNO_3 and H_2SO_4, distillation, and titulation of the digested sample with HCl 0.02N.	(Watts et al., 1996)
Total Phosphorus	% dw	Digestion using HNO_3 and H_2SO_4. Phosphate's determination on the digested solution	(Watts et al., 1996)
Extractable Phosphate	$mg.kg^{-1}$	Extraction using $NaHCO_3$ 0.5M, in the ratio L/S 200. Determination of the phosphates in the filtrated extract through molecular absorption spectrophotometry, through the formation of a colored complex with a solution of ammonium molybdate in the presence of ascorbic acid and potassium tartrate and antimony	(Olsen et al. 1954; Watanabe et al., 1965)

(Continuation)

Analysis	Unit	Methodology	Reference
Zn	mg.kg^{-1}	Total metals: *Aqua-regia*: Digestion with *aqua-regia* according to ISO 11466. Determination of metals, in the digested, by atomic absorption spectrophotometry.	(ISO 11466, 1995)
Cu	mg. kg^{-1}		
Cd	mg. kg^{-1}		
Ca	g. kg^{-1}		
Pb	mg. kg^{-1}		
Mg	g. kg^{-1}	The bioavailable fraction of Zn, Pb, Cd, Cr, Cu, and Ni in the soils was quantified in the extracts obtained from the soils with 0.05 M EDTA at pH 7.5	(Iqbal et al., 2013;
Ni	mg. kg^{-1}		
K	g. kg^{-1}		
Na	g. kg^{-1}		
Cr	mg. kg^{-1}		

2.3 Biomass analysis

The evaluation of the biomass was made by biometric and Physic-chemical characterization. Table 2.4 shows the biometric measurements while Table 2.5 present the physical-chemical analysis of the biomass.

Table 2.4. Biometric analysis of the biomass (Costa 2014)

Parameter	methodology
Height	Using a measuring tape
Leaves Area	Determination using optical scanning (Li-3100C Area Meter, LI-COR Biosciences)
Weight	Mass determination

Table 2.5. Physico-chemical analysis of biomass

Parameter	Analytic methodology	Reference
Fiber composition	Hemicellulose, cellulose, and lignin were determined following a sequential extraction with neutral and acid detergents, treatment with 72% sulfuric acid, and incineration at 550ºC of the final residue.	(Goering et al., 1970)
Volatile material	The difference between the biomass weight and the residue obtained after the calcination of the biomass at 900 ± 20°C for 7 minutes.	(Chamberlain et al., 1974)
Ash content	The residue obtained after the incineration of the biomass was obtained in the carbonization at a temperature of 550 ± 50 ºC for 3 hours—an adaptation of the ASTM D3172-13.	Adapted: (ASTM E1755 - (Reapproved 2015), 2015)
Fixed carbon	. The fixed carbon was determined by subtracting the total dry biomass's volatile material and ash percentual.	(Chamberlain et al., 1974)
High heat value	The high heat value will be calculated using Yin's relation, which uses the fixed carbon and the volatile material to estimate the HHV	(Yin., 2011)
Total Nitrogen	Method Kjeldahl: Mineralization using H_2SO_4, distillation, and titulation of the digested sample with HCl 0.02N.	(Watts et al., 1996)
Total Phosphorus	Digestion using HNO_3 e H_2SO_4. Phosphate's determination of the digested solution by molecular absorption spectrophotometry, through the formation of a colored complex with an explanation of ammonium molybdate, in the presence of ascorbic acid and potassium and antimony tartrate.	(Watts et al., 1996)
Zn, Cu, Na, K, Ca, Mg, Fe, Mn, Al, Pb, Cr, Ni, Cd	Dry mineralization of the samples (incineration at 550 ± 50 ºC) and ashes dissolution using nitric acid. Heavy metals determination through AAS (atomic absorption spectroscopy).	(Vandecasteele et al., 1993)

2.4 Percolated water analysis

Percolated waters were collected just before the 2nd harvest, and their contaminant content (Zn, Pb, Cu, Cd, Cr, or Ni) was determined on acidified and filtered samples by atomic absorption spectrometry.

2.5 Mathematical formulas

The high heating value (HHV) expressed in MJ.kg^{-1} dry weight (d.w.) was calculated, taking into consideration both the volatile matter (VM) and the fixed carbon (FC), as given in Equation 1 (Yin., 2011):

$$\mathrm{HHV\ (MJ.kg\text{-}1,\ d.w.)} = 0.1905 \times [\mathrm{VM,\ \%\ w/w\ d.w.}] + 0.2521 \times [\mathrm{FC,\ \%\ w/w\ d.w.}]$$

Equation 1

To analyze the fouling/slagging propensity of the biomass ashes given the ash composition, an alkali index can be calculated based on the composition of the biomasses. The sum of sodium and potassium oxides, which melt at low temperature (Na_2O+K_2O), expressed in kg/GJ, can be used to calculate the fouling and the slagging probability to occur (Equation 2) (Vassilev et al. 2010).

$$\mathrm{AI} = (\mathrm{Na_2O} + \mathrm{K_2O})\ \mathrm{kg/GJ}$$

Equation 2

Using biomass characterization to determine plants' phytoremediation potential, different parameters can be calculated.

The tolerance index (TI) evaluates the crop tolerance to heavy metal contamination (Equation 3) (Kumar et al., 2008; Yadav et al., 2009).

$$\mathrm{TI} = \frac{\text{dry biomass weight of contaminated plants; } \mathrm{g.m^{-2}}}{\text{dry biomass weight of control plants; } \mathrm{g.m^{-2}}}$$

Equation 3

The modified accumulation index (mAI) will be used to determine the plant's capability to uptake and accumulate a contaminant in more significant amounts than it usually does (Equation 4) (Barbosa et al., 2015).

$$\mathrm{mAI} = \frac{\text{metal accumulation in the contaminated plants; } \mathrm{mg.m^{-2}}}{\text{metal accumulation in control plants; } \mathrm{mg.m^{-2}}}$$

Equation 4

The modified bioconcentration factor (mBCF) evaluates heavy metal concentration in the different biomass fractions compared with the metal concentration bioavailable to plants Equation 5 (Barbosa et al., 2015).

$$\mathrm{mBCF} = \frac{\text{metal concentration in the plant fraction; mg.kg}^{-1}}{\text{bioavailable metal concentration in the soil; mg.kg}^{-1}}$$

Equation 5

Instead of using the soil's total metal concentration, the use of the bioavailable content (to the plants) in the soil represents more realistically the ability of the plants to extract and concentrate the metals, helping to decontaminate the soil (Barbafieri et al., 2011; Barbosa et al., 2015)

The modified bioaccumulation factor (mBAF) is calculated to assess the plants' capability to take away the contaminants from the soil Equation 6 (Barbosa et al., 2015)

$$\mathrm{mBAF}(\%) = \frac{\text{metal accumulation in the plant fraction; mg.m}^{-2}}{\text{bioavailable metal content in the soil; mg.m}^{-2}} \times 100$$

Equation 6

The transfer of metals from the belowground biomass to the harvestable aerial fractions, measured through the translocation factor (TF) and the modified translocation factor (mTF) Equation 7 and Equation 8, respectively), can be used to determine the potential application of the crops in phytoextraction treatments (Barbosa et al. 2015).

$$\mathrm{TF} = \frac{\text{metal concentration in the aboveground plant fraction; mg.kg}^{-1}}{\text{metal concentration in the belowground plant fraction; mg.kg}^{-1}}$$

Equation 7

$$\mathrm{mTF} = \frac{\text{metal accumulation in the aboveground plant fraction; mg.m}^{-2}}{\text{metal accumulation in the belowground plant fraction; mg.}^{-2}}$$

Equation 8

2.6 Environmental and Socio-Economic Impact Assessment of the production of giant reed and switchgrass in heavy metals contaminated soils

This study assumed that switchgrass and giant reed were used as solid fuels (biomass harvested, crushed, dried, and pelletized and then transported to be used in combined heat and power plants). In the case of giant reed, it was assumed that only stems were used as solid fuel. Leaves must be left in the field as they produce large amounts of ash, contributing to field fertilization and carbon sequestration. To estimate the environmental and socio-economic impact on the production of both crops in heavy metals contaminated soils, results obtained in the current work were the basis for the assessment.

According to the methodology developed and applied by Schmidt and collaborators (Schmidt et al., 2015) and Fernando et al. (Fernando et al., 2018), the study focused on several categories for the environmental impact assessment. First, energy savings were calculated by subtracting the energy input from the potential energy produced by the biomass's combustion being produced. Second, energy balance results obtained from Schmidt and collaborators (Schmidt et al., 2015) were used to estimate energy savings. Third, the reduction of greenhouse gas emissions was calculated based on the same work. Finally, the socioeconomic analysis was based on the work of Khanna et al. (2008) and Soldatos et al. (2009).

2.7 Statistical analysis

Results obtained in this study were statistically interpreted using one-way ANOVA (analysis of variance) followed by the Tuckey test to find means that are significantly different from each other (IBM SPSS Statistics version 23, IBM, USA). The results obtained from the triplicate analysis were presented as the mean ± standard deviation. The propagation of the deviation obtained in contaminated plants and control plants was used to calculate the uncertainty of the TI and mAI results.

3

RESULTS AND DISCUSSION

3.1 Soil characterization

The uncontaminated soil's physical and chemical properties are presented in Table 3.1. The soil presents low levels of organic matter and essential chemical elements, such as N or P. Also, it has an alkaline pH with low initial levels of the studied contaminants, Cd, Ni, and Cr. Due to the soil's high pH, the high CEC value may indicate a likelihood of overestimating exchangeable bases by extracting nonexchangeable Ca and Mg from carbonate solids.

Table 3.1. Characterization of Control soil. DW - Dry weight

Parameters	
pH	8.4 ± 0.2
Electrical conductivity (dS m^{-1})	0.289 ± 0.026
CEC (cmol(+)kg^{-1}, DW)	42 ± 3
Total organic carbon (g C kg^{-1}, DW)	1.48 ± 0.14
Total nitrogen (g N kg^{-1}, DW)	0.96 ± 0.22
Total phosphorus (g P kg^{-1}, DW)	0.72 ± 0.01
Available phosphorus (mg P kg^{-1}, DW)	216 ± 10
Total potassium (g K kg^{-1}, DW)	2.15 ± 0.01
Total calcium (g Ca kg^{-1}, DW)	20 ± 6
Total sodium (g Na kg^{-1}, DW)	8 ± 2
Total magnesium (g Mg kg^{-1}, DW)	5.30 ± 0.08
Total zinc (mg Zn kg^{-1}, DW)	50 ± 7
Total chromium (mg Cr kg^{-1}, DW)	21 ± 2
Total lead (mg Pb kg^{-1}, DW)	26 ± 5
Total cadmium (mg Cd kg^{-1}, DW)	0.26 ± 0.04
Total nickel (mg Ni kg^{-1}, DW)	18 ± 4
Total copper (mg Cu kg^{-1}, DW)	7.1 ± 0.4

The heavy metals levels of contaminated soils, built from these initial properties, are presented in Table 3.2, showing the total and bioavailable content of Cd, Ni, and Cr in the control and artificial soils. The extraction with 0.05 M EDTA was used to determine the contaminants' bioavailable amount. Results obtained at the beginning of the experiment show the bioavailable fractions of Cd, Ni, and Cr were, respectively, 76–87–100, 32–76–75, and 6–19–22 % of the total element content in control and contaminated soils (low and high contamination). Accordingly, these percentages reflect the concentration of contaminants available for interaction with biological systems.

Table 3.2. Control and artificial soil characterization.

The main element of contamination	Parameters	Soil Type		
		Control	Low	High
Zn	Total zinc (mg Zn kg^{-1}, d.w.)	50 ± 7	465 ± 18	923 ± 45
	Bioavailable zinc (mg Zn kg^{-1}, d.w.)	12.3 ± 0.9	359 ± 16	718 ± 83
Cr	Total chromium (mg Cr kg^{-1}, d.w.)	21 ± 2	345 ± 56	663 ± 82
	Bioavailable chromium (mg Cr kg^{-1}, d.w.)	1.308 ± 0.002	67.0 ± 2.2	146 ± 13
Pb	Total lead (mg Pb kg^{-1}, d.w.)	26 ± 3	465 ± 18	923 ± 45
	Bioavailable lead (mg Pb kg^{-1}, d.w.)	13 ± 5	416 ± 143	805 ± 270
Cd	Total cadmium (mg Cd kg^{-1}, d.w.)	1.0 ± 0.4	4.8 ± 0.9	9.2 ± 1.4
	Bioavailable cadmium (mg Cd kg^{-1}, d.w.)	0.76 ± 0.05	4.2 ± 0.5	9.9 ± 0.6
Ni	Total nickel (mg Ni kg^{-1}, d.w.)	18 ± 4	118 ± 18	242 ± 32
	Bioavailable nickel (mg Ni kg^{-1}, d.w.)	5.8 ± 0.4	90 ± 6.2	182 ± 3
Cu	Total copper (mg Cu kg^{-1}, d.w.)	7.1 ± 0.4	221 ± 35	396 ± 28
	Bioavailable copper (mg Cu kg^{-1}, d.w.)	6.8 ± 0.3	192 ± 69	316 ± 3

d.w. - dry weight

Table 3.3. Percentage of bioavailable heavy metals

	Low	High
Zn	77%	78%
Cr	19%	22%
Pb	89%	87%
Cd	87%	100%
Ni	76%	75%
Cu	87%	80%

All the studied heavy metals, except for Cr, showed a high ratio bioavailable/total concentration, indicating that these metals (Zn, Pb, Cd, Ni, and Cu) are highly mobilized from the soil. On the other hand, Chromium was less mobilized in the soils, confirming what was described in the literature (Kabata-Pendias 2011).

3.2 Biomass growth and productivity

3.2.1 Biometric parameters

Due to the difference between the structures of *Arundo donax* and *Panicum virgatum*, the biometric parameters analyzed were not the same for both plants. For *Arundo donax,* the parameters were: the number of shoots, length of the highest plant, number of leaves per pot, number of leaves of the highest plant, leaves' area per pot, and leaves area of the highest plant. On the other hand, for *Panicum virgatum*, only the number of tillers and the length of the highest plant were analyzed. Furthermore, due to the thinness of *Panicum virgatum* leaves, once the plant is dry, it becomes difficult to measure the leaves area, as separate the leaves from the rest of the plant. In this way, aiming to keep the best possible measurements, these values were not considered.

3.2.1.1 Giant reed's biometric parameters

The average number of shoots per pot is presented in Figure 3.1. The number of shoots per pot in contaminated trials was statistically the same as the Control in the first year, apart from Cu_{400} (p = 0.0132), which statistically had a higher development of shoots than the Control. In the second year, the number of tillers decreased in Pb_{900} ($p = 2.2e^{-16}$), Cd_8 ($p = 2.2e^{-16}$), Cu_{200} (p = 0.01613), and both Ni trials, Ni_{110} ($p = 2.2e^{-16}$) and Ni_{220} (p = 0.01613). Thus, Control and Zn_{450} present an increase in the number of shoots per pot from the first to the second year. The results obtained for Cd did not follow the trends in literature, which indicates that when exposed to Cd contamination, giant reed reduction in the number of shoots is damage-related with Cd concentration. The study considered that Cd could affect photosynthetic pigments, interfering with chlorophyll synthesis and, in this way, reducing the absorption of nutrients (Sabeen et al. 2013b). In this way, the plant may maximize its survival chance by reducing the number of shoots while increasing the nutrients for the developed ones.

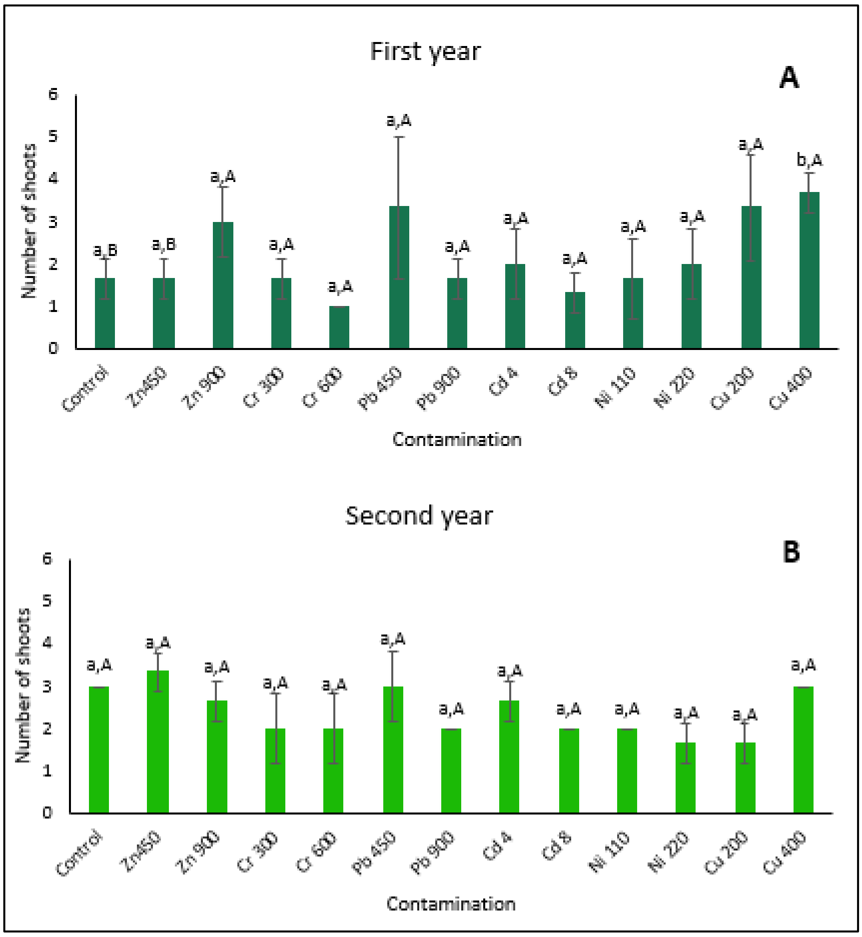

Figure 3.1. Giant reed shoots per pot in the first (A) and second (B) growing year.
Different lower-case letters indicate statistical significance ($p < 0.05$) among treatments per year. Different capital letters indicate statistical significance ($p < 0.05$) between first and second year results for identical treatments. Zn_{450} and Zn_{900}, 450 and 900 mg Zn kg^{-1}. Cr_{300} and Cr_{600}, 300 and 600 mg Cr kg^{-1}. Pb_{450} and Pb_{900}, 450 and 900 mg Pb kg^{-1}. Cd_4 and Cd_8, 4 and 8 mg Cd kg^{-1}. Ni_{110} and Ni_{220}, 110 and 220 mg Ni kg^{-1}. Cu_{200} and Cu_{400}, 200 and 400 mg Cu kg^{-1}.

The length of the highest plant is observed in Figure 3.2. In crops like *Arundo donax*, the study of the size is related to evaluating all the meristematic activity, which occurs in the region between the nodes in the plant, which is a region that contains mature cells in the base and young cells in the top (Barbosa and Fernando 2014). The sum of the meristematic area's height constitutes the plant's length. In the first growing cycle, only Ni_{220} ($p = 0.03943$) and Cu_{200} ($p = 0.02631$) had heights lower than the Control. During the second growing cycle, however, Pb_{900} ($p = 0.02864$) was the only trial that indicated hazardous effects from contamination regarding plant length. In the second year, Zn_{900}, Pb_{450}, Pb_{900}, Cd_4, Cd_8, and Ni_{110} reached lower heights when compared to the first growing cycle. Despite the length differences between years, the capacity of giant reed to keep developing regarding length was also observed in the literature.

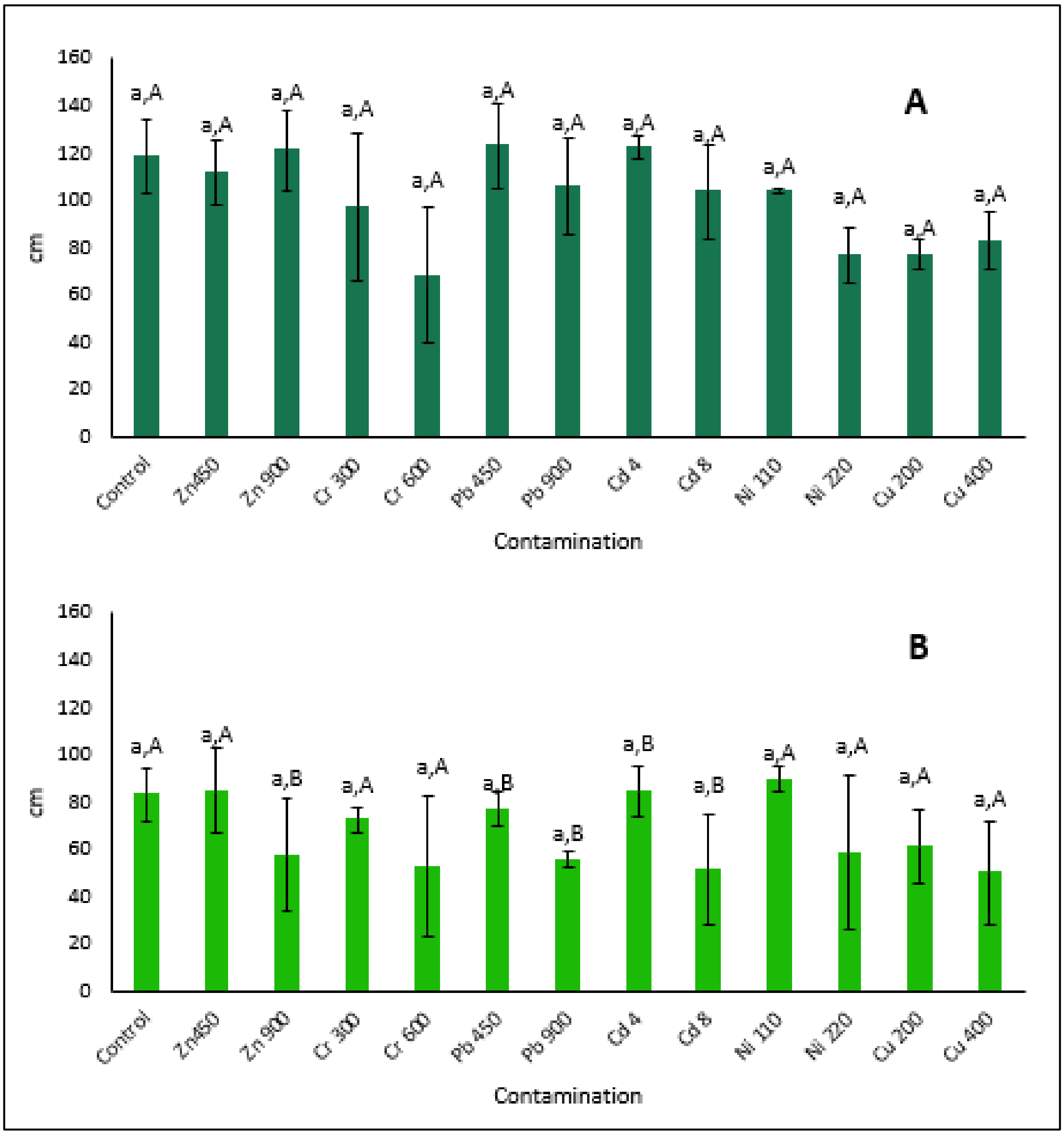

Figure 3.2. Giant reeds' height (highest plant) in the first (A) and second (B) growing year.
Different lower-case letters indicate statistical significance (*p* < 0.05) among treatments per year. Different capital letters indicate statistical significance (*p* < 0.05) between first and second year results for identical treatments. Zn_{450} and Zn_{900}, 450 and 900 mg Zn kg^{-1}. Cr_{300} and Cr_{600}, 300 and 600 mg Cr kg^{-1}. Pb_{450} and Pb_{900}, 450 and 900 mg Pb kg^{-1}. Cd_4 and Cd_8, 4 and 8 mg Cd kg^{-1}. Ni_{110} and Ni_{220}, 110 and 220 mg Ni kg^{-1}. Cu_{200} and Cu_{400}, 200 and 400 mg Cu kg^{-1}.

The number of leaves per pot and the number of leaves in the highest plant can be seen respectively in Figure 3.3 and Figure 3.4, while the leaves per pot and in the highest plant are observed in Figure 3.5 and Figure 3.6, respectively.

The number of leaves per pot in the first year had only two treatments with a considerable difference from Control, Cr_{300} ($p = 0.0438$), showing a lower number of leaves, and Cu_{400} ($p = 0.009655$), which had developed more leaves per pot than Control. In the second growing cycle, the number of leaves decreases in different contaminated trials. In both chromium trials, Cr_{300} ($p = 0.004529$) and Cr_{600} ($p = 0.002007$) developed significantly fewer leaves than the Control. The highest concentrations trials Pb_{900} ($p = 0.003387$) and Cd_8 ($p = 0.01669$). The unexpected behavior occurred in Cu trials, with Cu200 (p = 0.00547) being affected in the number of leaves, while the higher concentration trial

Cu400 did not suffer any significant alterations. Several high-concentration trials decreased its leaves from the first to the second year, including Cr_{600}, Pb_{900}, Cd_8, and Cu_{400}. Cu_{200} also suffered a reduction in the number of leaves being even more affected than the highest concentration of copper trial Cu_8. Papazoglou et al. also indicated that its length is not affected when exposed to Cd and Ni giant reed (Papazoglou et al. 2005).

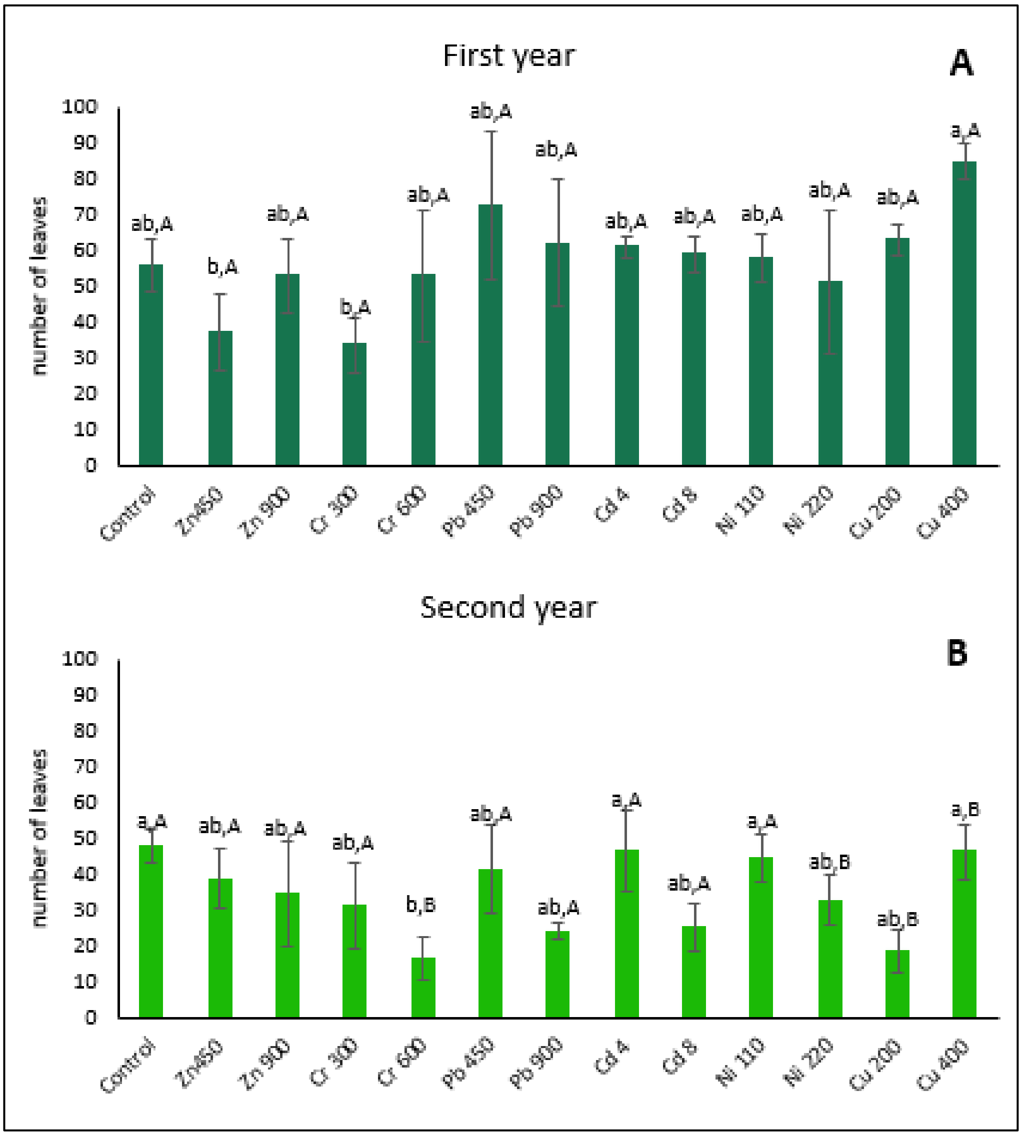

Figure 3.3. Giant reed number of leaves per pot in the first (A) and second (B) growing year.
Different lower-case letters indicate statistical significance ($p < 0.05$) among treatments per year. Different capital letters indicate statistical significance ($p < 0.05$) between first and second year results for identical treatments. Zn_{450} and Zn_{900}, 450 and 900 mg Zn kg^{-1}. Cr_{300} and Cr_{600}, 300 and 600 mg Cr kg^{-1}. Pb_{450} and Pb_{900}, 450 and 900 mg Pb kg^{-1}. Cd_4 and Cd_8, 4 and 8 mg Cd kg^{-1}. Ni_{110} and Ni_{220}, 110 and 220 mg Ni kg^{-1}. Cu_{200} and Cu_{400}, 200 and 400 mg Cu kg^{-1}.

The number of leaves in the highest plant was also affected by some contaminants. In the first growing cycle Zn_{450} (p = 0.01316), Cr_{300} (p = 0.001121), Cr_{600} (p = 0.00504), Pb_{900} (p = 0.01152), Cd_4 (p = 0.04344), Cd_8 (p = 0.02711), Ni_{110} (p = 0.04366), and Cu_{200} (p = 0.001921) produced statistically less leaves than the Control. However, the second growth cycle in most trials, relating the number of shoots and leaves productivity with the number of leaves in the highest plant, indicate that the plant directs most of its nutrients to the highest plant. The number of leaves was only affected in chromium trials, Cr_{300} ($p = 0.02028$) and Cr_{600} ($p = 0.00565$), and the highest concentration of lead Pb_{900} ($p = 0.005087$). There was no significant difference between the number of leaves from the first to the second growing cycle in any trial.

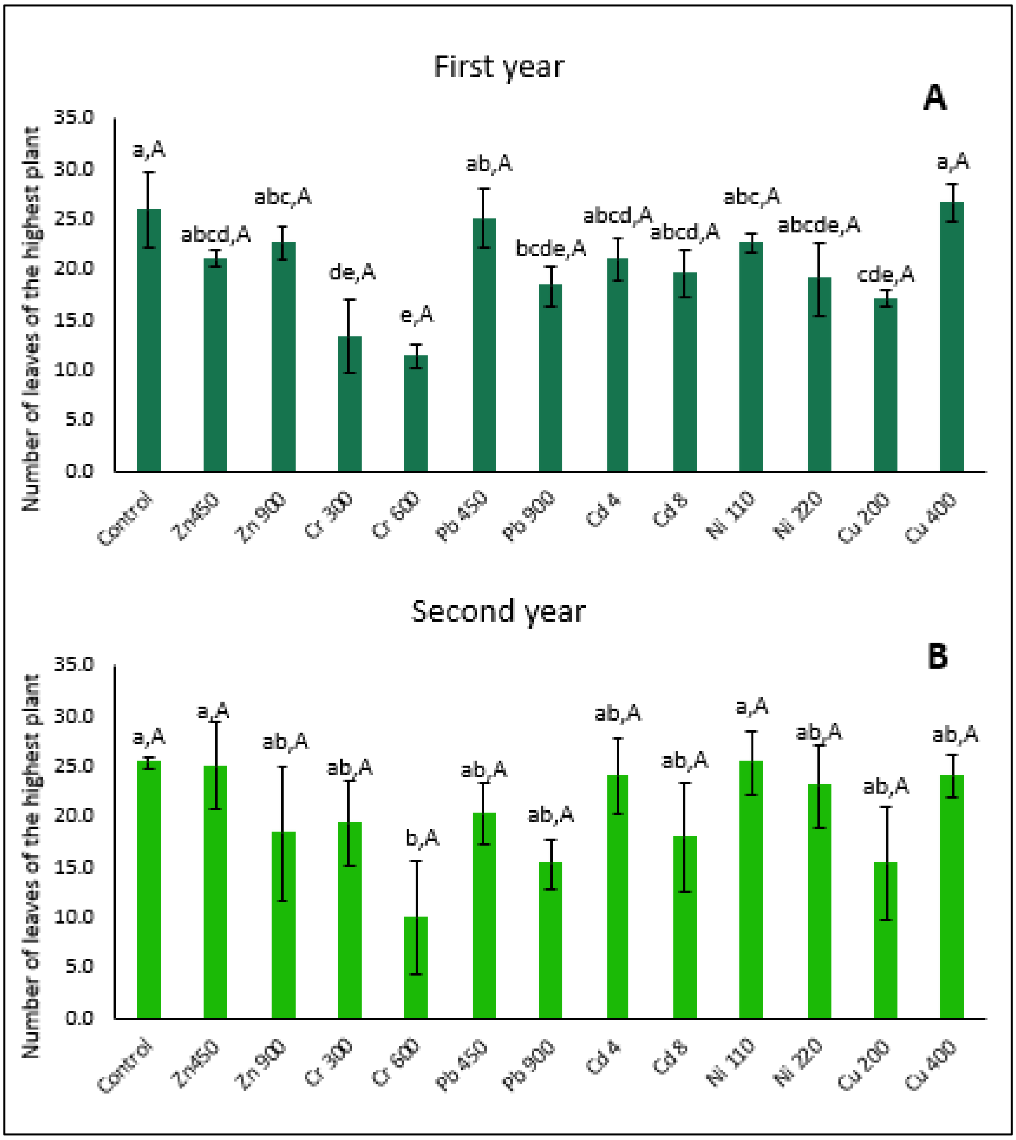

Figure 3.4. Giant reed number of leaves of the highest plant in the first (A) and second (B) growing year.
Different lower-case letters indicate statistical significance ($p < 0.05$) among treatments per year. Different capital letters indicate statistical significance ($p < 0.05$) between first and second year results for identical treatments. Zn_{450} and Zn_{900}, 450 and 900 mg Zn kg^{-1}. Cr_{300} and Cr_{600}, 300 and 600 mg Cr kg^{-1}. Pb_{450} and Pb_{900}, 450 and 900 mg Pb kg^{-1}. Cd_4 and Cd_8, 4 and 8 mg Cd kg^{-1}. Ni_{110} and Ni_{220}, 110 and 220 mg Ni kg^{-1}. Cu_{200} and Cu_{400}, 200 and 400 mg Cu kg^{-1}.

In the leaves area per pot, during the first growing cycle, only Cr_{300} ($p = 0.02748$) showed significant differences to Control. In the second year, however, the increase in the Control leaves area was not followed at the same proportion. In this way, all the trials had a lower leaves area than the Control. However, the biggest difference between Control and contaminated trials is in Cr_{300}, Cr_{600}, Cd_8, and

Cu_{200}. Besides Control, Zn_{450} also develops a higher leaf area in the second growing cycle. However, still lower than the Control.

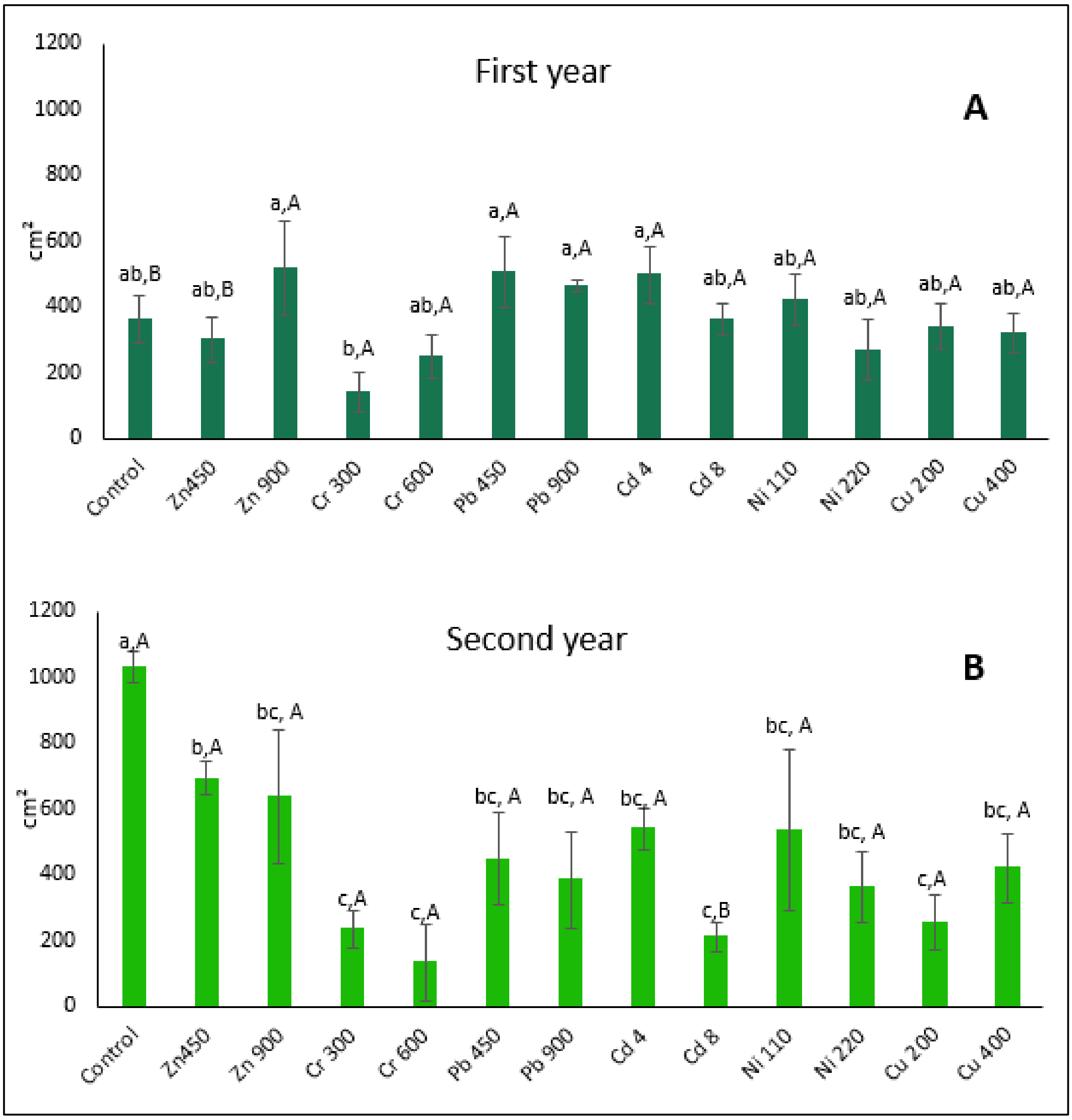

Figure 3.5. Giant reed leaves' area per pot in the first (A) and second (B) growing year.
Different lower-case letters indicate statistical significance ($p < 0.05$) among treatments per year. Different capital letters indicate statistical significance ($p < 0.05$) between first and second year results for identical treatments. Zn_{450} and Zn_{900}, 450 and 900 mg Zn kg^{-1}. Cr_{300} and Cr_{600}, 300 and 600 mg Cr kg^{-1}. Pb_{450} and Pb_{900}, 450 and 900 mg Pb kg^{-1}. Cd_4 and Cd_8, 4 and 8 mg Cd kg^{-1}. Ni_{110} and Ni_{220}, 110 and 220 mg Ni kg^{-1}. Cu_{200} and Cu_{400}, 200 and 400 mg Cu kg^{-1}.

The leaf area in the highest plant followed a similar behavior. In the first growing cycle, no significant effects were observed. However, the increase in Controls leaves an area not followed by several contaminated trials in the second year. Only Zn_{450} and nickel trials, Ni_{110} and Ni_{220}, followed the increase observed in Control. All other contaminated trials had a smaller leaf area, the most affected Cr_{600} and Cd_8. From the first to second year, Control, Zn_{450}, and Cr_{300} suffered significant increases in leaves area. $Cd_{8,}$ however, showed a different trend, being more damage affected in the second year.

The effects of Zn in leaves are observed in different plants such as barley (Kherbani, Abdi, and Lounici 2015), maize (Islam et al. 2014), wheat (Paunov et al. 2018), and tomato (Cherif et al., 2011), for both the reduction of leaf area and a number of leaves. Once submitted to a saturated environment, the plant stores the excess Zn in the leaves, causing modification in these organs, chlorosis, and necrosis, affecting the leaf morphology and development (Todeschini et al., 2011). On the other hand, in a study using *Jatropha curcas L.*, it was observed that the leaf growth and development and the elongation of the roots decreased as much as the Pb concentration in the soil increased (Shu et al., 2012). The presence of Pb in the soil also provoked a reduction in leaves, stems, and roots observed in *Elsholtzia argyi* (Islam et al., 2008), where not only the changes in productivity but also in leaf morphology were noticed. When exposed to Ni contamination, Giant reeds' productivity was also studied by Atma et al. (Atma et al., 2017a), who exposed giant reed shoots to irrigation using a Ni-contaminated solution (10, 50, 100 $mg.L^{-1}$). It was noticed that the concentration of the heavy metal in the irrigation solution affected the crops' yield due to the effects mostly observed in the leaves. The reason could be the high accumulation of Ni in this fraction of the plant, as described in the literature (Kabata-Pendias, 2011).

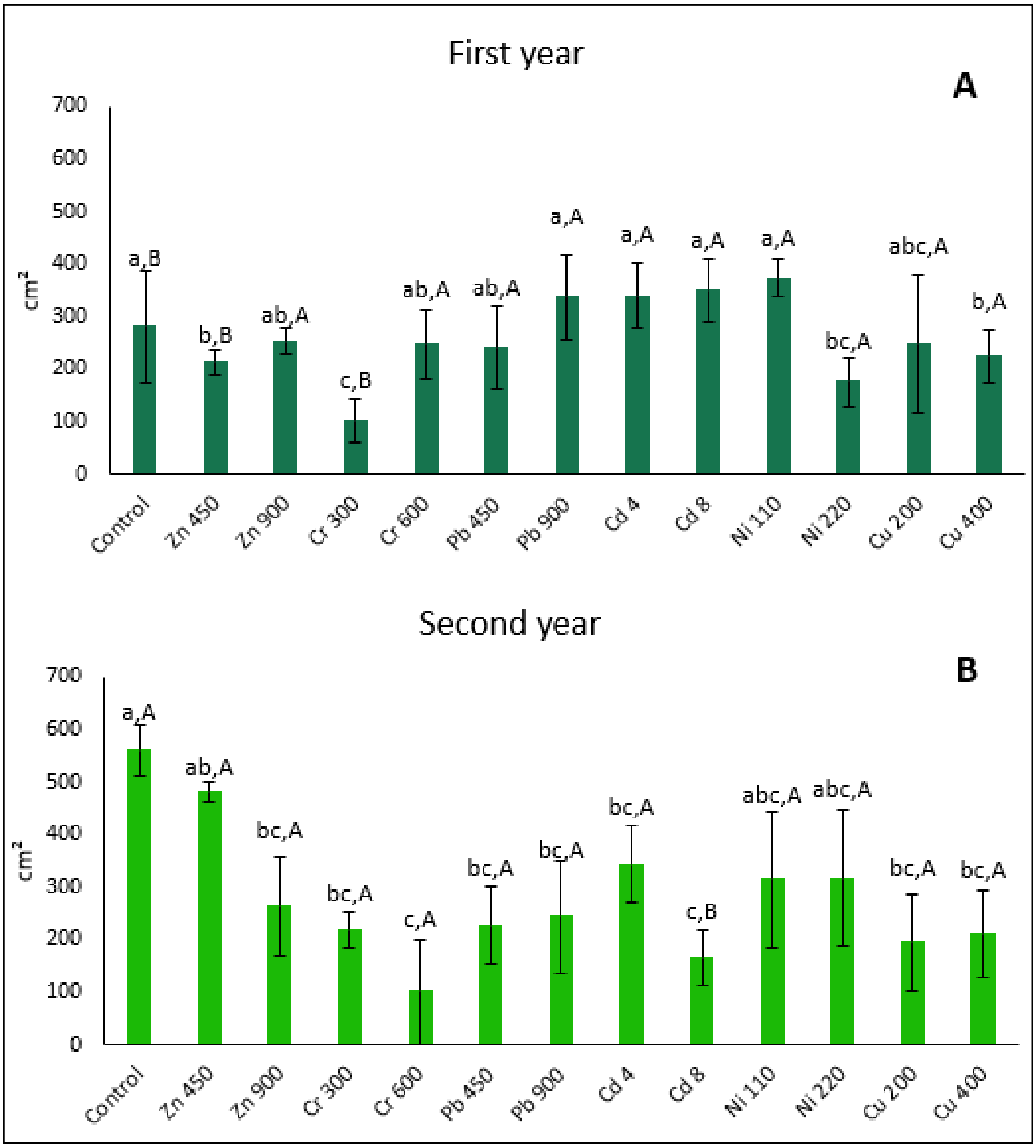

Figure 3.6. Giant reed leaves' area of the highest plant in the first (A) and second (B) growing year.
Different lower-case letters indicate statistical significance ($p < 0.05$) among treatments per year. Different capital letters indicate statistical significance ($p < 0.05$) between first and second year results for identical treatments. Zn_{450} and Zn_{900}, 450 and 900 mg Zn kg^{-1}. Cr_{300} and Cr_{600}, 300 and 600 mg Cr kg^{-1}. Pb_{450} and Pb_{900}, 450 and 900 mg Pb kg^{-1}. Cd_4 and Cd_8, 4 and 8 mg Cd kg^{-1}. Ni_{110} and Ni_{220}, 110 and 220 mg Ni kg^{-1}. Cu_{200} and Cu_{400}, 200 and 400 mg Cu kg^{-1}.

3.2.1.2 Switchgrass' biometric parameters

The number of tillers per pot in Figure 3.7, as the length of the highest plant in Figure 3.8, presented a similar behavior toward productivity. However, only Zn_{900} did not demonstrate significance in the highest plant length from the first to the second year of the experiment. Apart from Cr trials, all the other treatments increased the length of the highest plant. However, the effect caused by the number of tillers only increased in Cd4 and Ni, and Cu trials. The higher development of switchgrass in the second year can be seen in the increase of the tillers number for Cd_4, Ni, and Cu trials and the increase in the length of the highest plant for all trials. Despite this increase, it is possible to observe the higher concentrations of Cd and Ni, as both trials of Cu showed a reduction trend in the number of tillers compared to the Control. This effect could be related to a survival strategy of the plant to try to minimize the effects of the contaminants in the crop development. This behavior can be reaffirmed by the length of the highest plant, which suffered no effect, indicating that the length of the tillers maintained its size despite the lower number.

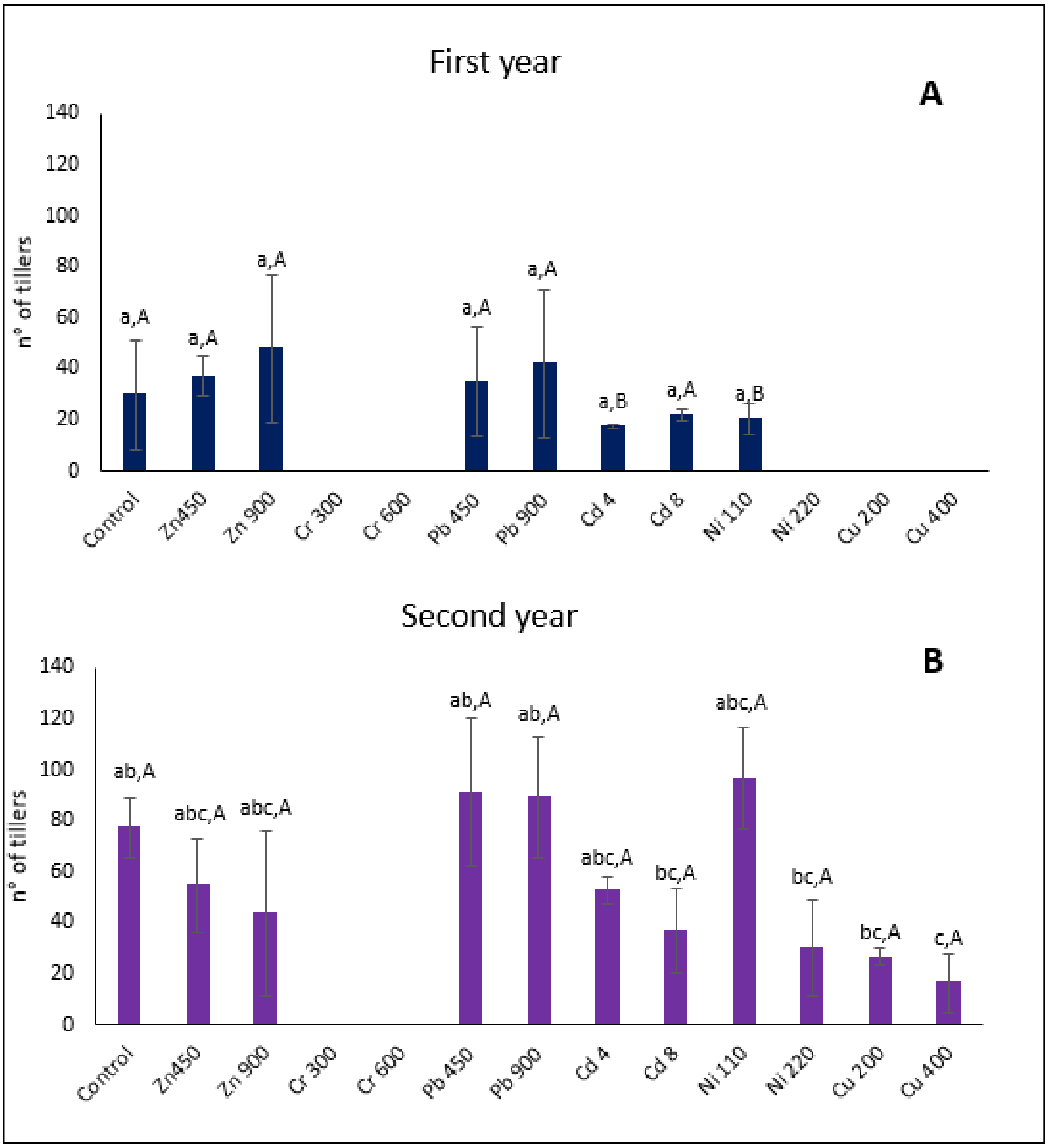

Figure 3.7. Switchgrass number of tillers in the first (A) and second (B) growing year.
Different lower-case letters indicate statistical significance ($p < 0.05$) among treatments per year. Different capital letters indicate statistical significance ($p < 0.05$) between first and second year results for identical treatments. Zn_{450} and Zn_{900}, 450 and 900 mg Zn kg^{-1}. Cr_{300} and Cr_{600}, 300 and 600 mg Cr kg^{-1}. Pb_{450} and Pb_{900}, 450 and 900 mg Pb kg^{-1}. Cd_{4} and Cd_{8}, 4 and 8 mg Cd kg^{-1}. Ni_{110} and Ni_{220}, 110 and 220 mg Ni kg^{-1}. Cu_{200} and Cu_{400}, 200 and 400 mg Cu kg^{-1}.

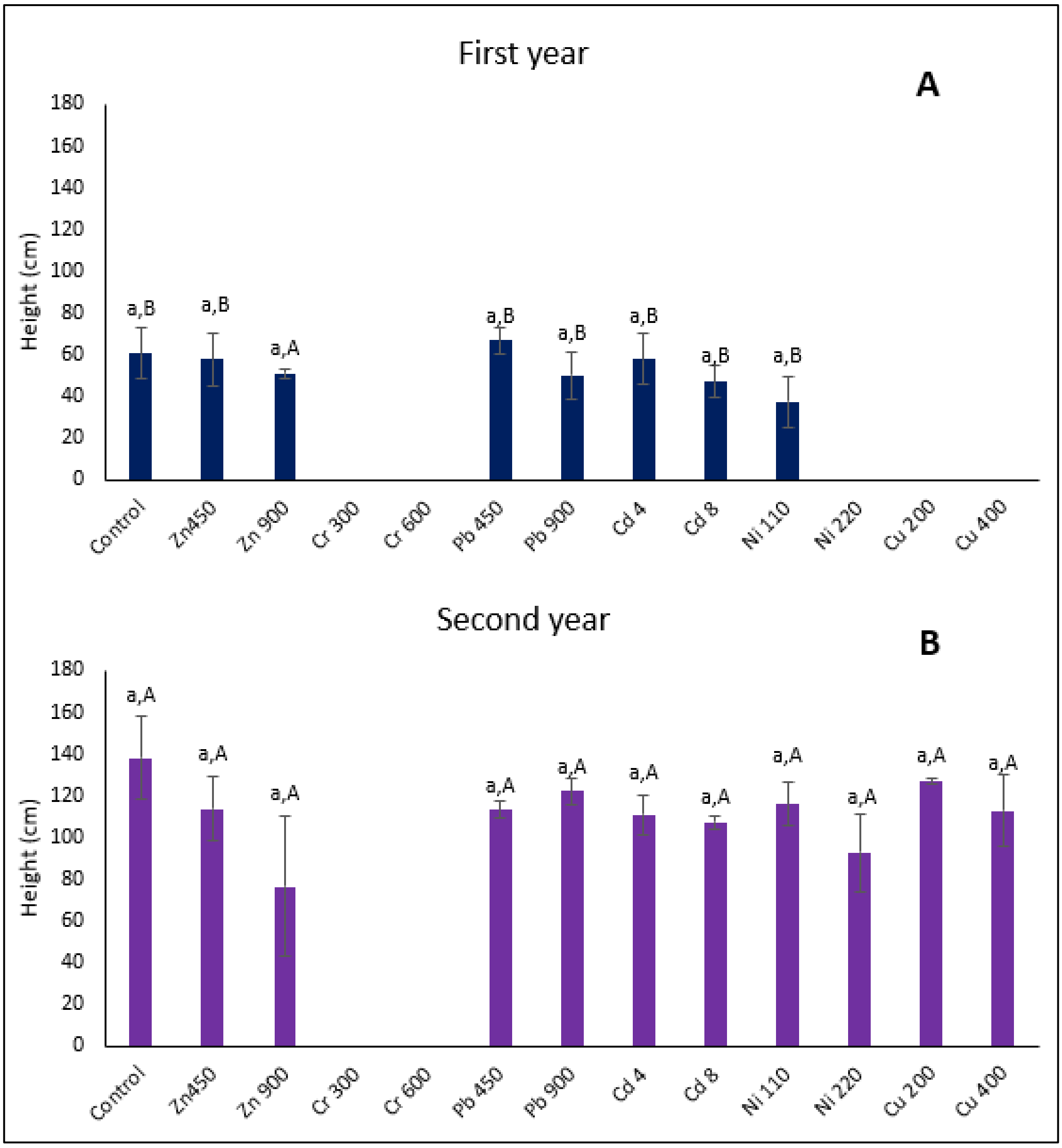

Figure 3.8. Switchgrass length of highest tiller in the first (A) and second (B) growing year.
Different lower-case letters indicate statistical significance ($p < 0.05$) among treatments per year. Different capital letters indicate statistical significance ($p < 0.05$) between first and second year results for identical treatments. Zn_{450} and Zn_{900}, 450 and 900 mg Zn kg^{-1}. Cr_{300} and Cr_{600}, 300 and 600 mg Cr kg^{-1}. Pb_{450} and Pb_{900}, 450 and 900 mg Pb kg^{-1}. Cd_4 and Cd_8, 4 and 8 mg Cd kg^{-1}. Ni_{110} and Ni_{220}, 110 and 220 mg Ni kg^{-1}. Cu_{200} and Cu_{400}, 200 and 400 mg Cu kg^{-1}.

3.2.2 Productivity

3.2.2.1 Giant reed's productivity

Giant reed's stems and leaves productivity are observed in Figure 3.9 and Figure 3.10, respectively, for both growing cycles. Giant reed's aerial productivity can be observed in Figure 3.11

Regarding the stems, in the first-year trials suffer significant effects Ni_{220} (p = 0.007855) and both Cu_{200} (p = 0.1187) and Cu_{400} (p = 0.02878). In the second year of experiment, Zn_{450}, Zn_{900}, Pb_{450}, Ni_{110}, and Ni_{220} presented a similar productivity than Control, however Pb_{900} (p = 0.01586), and both Cr_{300} (p = 0.01472) and Cr_{600} (p = 0.007225), suffer a decrease in stems productivity. Cd trials indicate that the effects of this heavy metal in giant reed stem yield depends on its concentration in the soil. It was observed that for $Cd_{4,}$ no significant effect in terms of stem productivity was noticed. However, when Cd concentration increased in Cd_8 (p = 0.01558), the giant reed decreased the yield compared to Control. Cu trials also had a yield lower than the Control in the second year in Cu_{200} (p = 0.03971) and Cu_{400} (p = 0.04025).

Still, regarding stem productivity was possible to observe that compared to the first growing cycle, Zn_{900}, Pb_{450}, Pb_{900}, and Cd_4 presented a significative reduction in stems productivity. The higher susceptibility of giant reed to Cr exposure was also detected in the experiment of Barbosa et al., where despite not being affected at the total concentration of 300 mg.kg^{-1}, the aerial productivity of the crop decreased when it was exposed to 600 mg.kg^{-1}. The tolerance of giant reeds to Zn and Pb was also noticed in the experiment, reinforcing the results obtained in this work (Barbosa et al., 2015). The high impact of Cr in giant reed productivity could be related to the incapability of plants to uptake nutrients in Cr heavy metals contaminated soils once this heavy metal can form insoluble complexes and affect the absorption of several micro and macronutrients like iron (Fe), magnesium (Mg), phosphorus (P), calcium (Ca), manganese (Mn), sulfur (S), copper (Cu), zinc (Zn), potassium (K), and nitrogen (N) (Kabata-Pendias, 2011; Osu, 2019; Sharma et al., 2019).

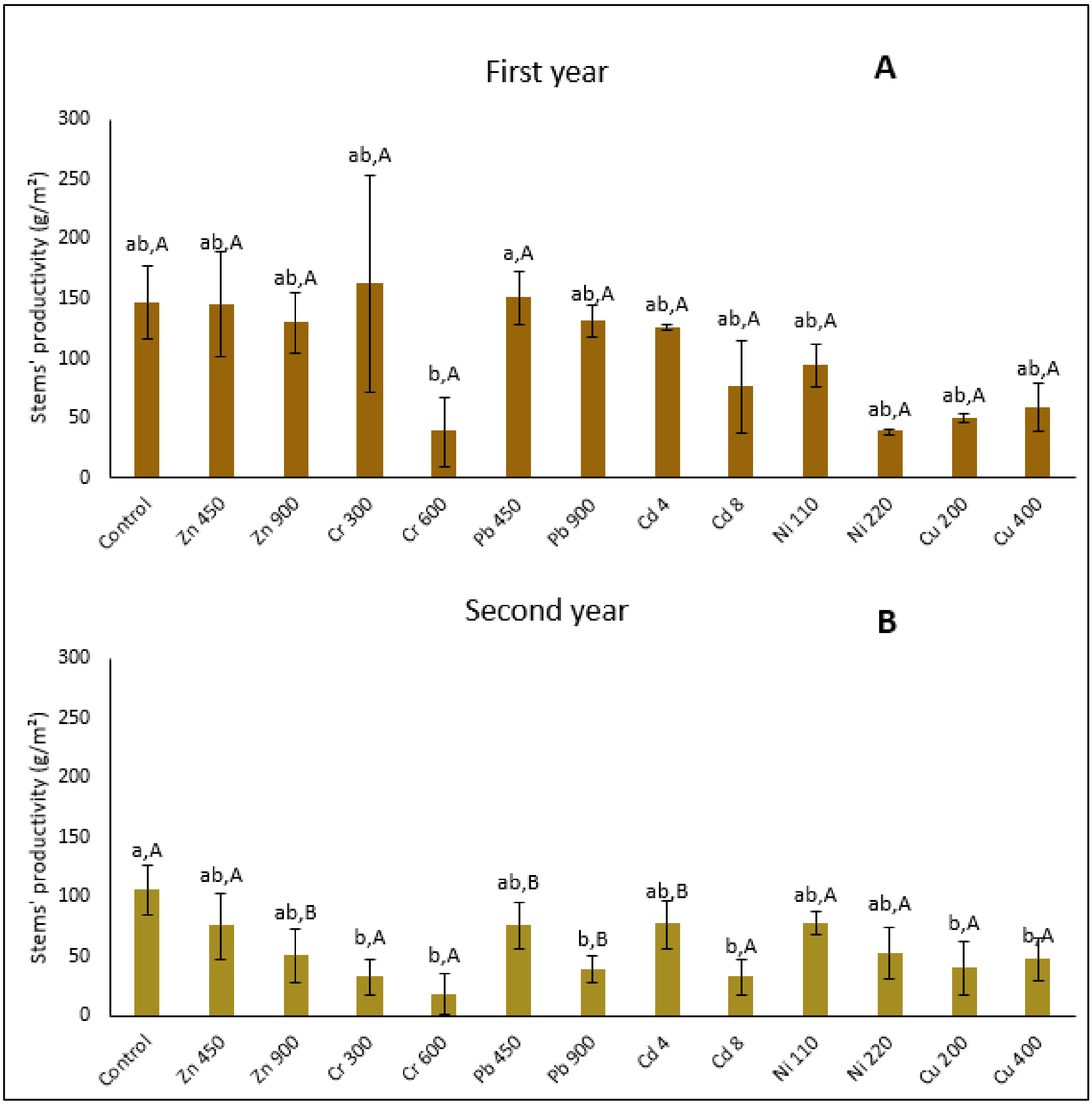

Figure 3.9. Giant reed stems' productivity in the first (A) and second (B) growing years.
Different lower-case letters indicate statistical significance ($p < 0.05$) among treatments per year. Different capital letters indicate statistical significance ($p < 0.05$) between first and second year results for identical treatments. 450 and 900 mg Zn kg^{-1}. Cr_{300} and Cr_{600}, 300 and 600 mg Cr kg^{-1}. Pb_{450} and Pb_{900}, 450 and 900 mg Pb kg^{-1}. Cd_{4} and Cd_{8}, 4 and 8 mg Cd kg^{-1}. Ni_{110} and Ni_{220}, 110 and 220 mg Ni kg^{-1}. Cu_{200} and Cu_{400}, 200 and 400 mg Cu kg^{-1}.Zn_{450} and Zn_{900},

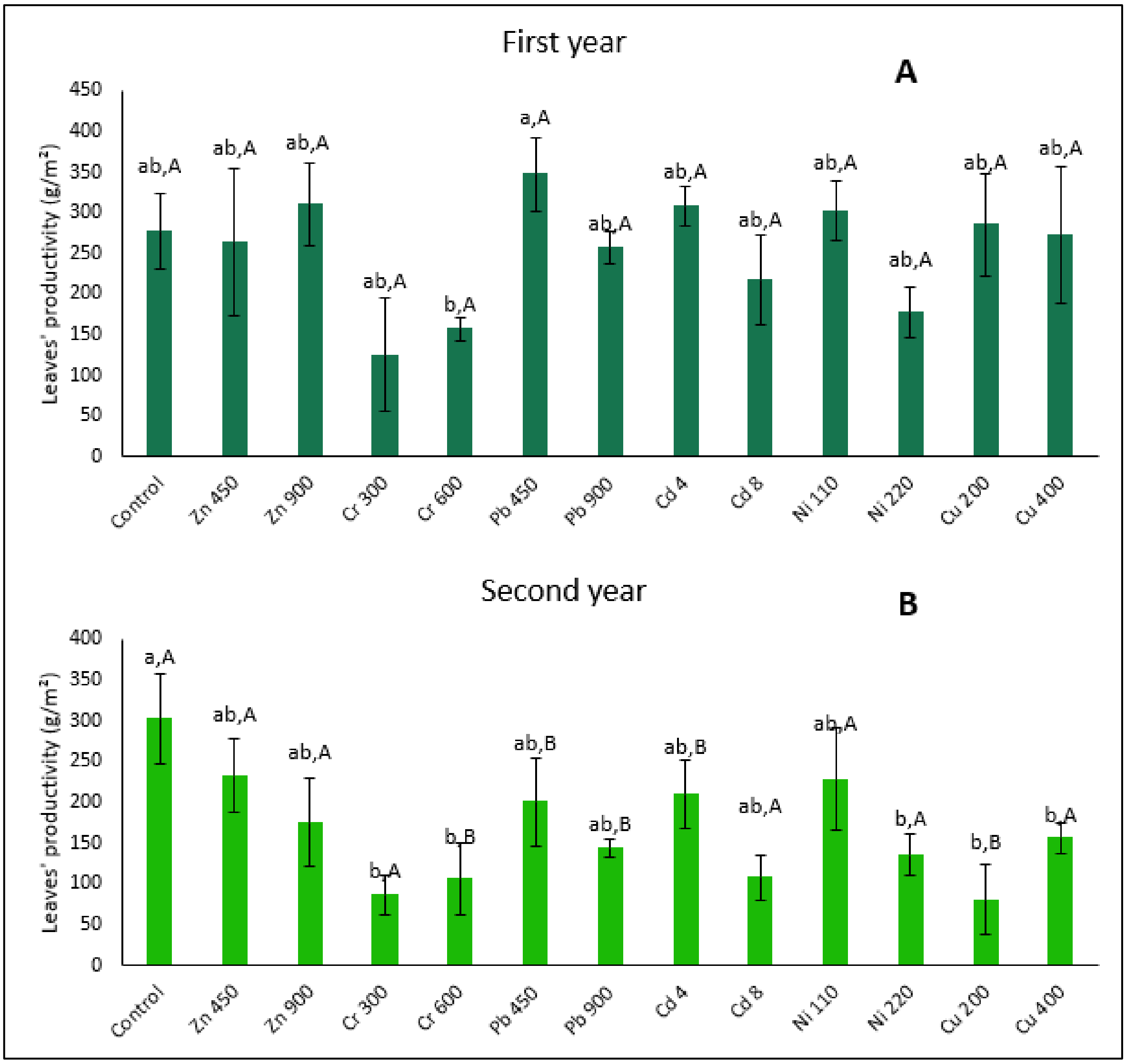

Figure 3.10. Giant reed leaves' productivity in the first (A) and second (B) growing years.
Different lower-case letters indicate statistical significance ($p < 0.05$) among treatments per year. Different capital letters indicate statistical significance ($p < 0.05$) between first and second year results for identical treatments. Zn$_{450}$ and Zn$_{900}$, 450 and 900 mg Zn kg^{-1}. Cr$_{300}$ and Cr$_{600}$, 300 and 600 mg Cr kg^{-1}. Pb$_{450}$ and Pb$_{900}$, 450 and 900 mg Pb kg^{-1}. Cd$_{4}$ and Cd$_{8}$, 4 and 8 mg Cd kg^{-1}. Ni$_{110}$ and Ni$_{220}$, 110 and 220 mg Ni kg^{-1}. Cu$_{200}$ and Cu$_{400}$, 200 and 400 mg Cu kg^{-1}.

During the first growing cycle, giant reed leaves' productivity did not suffer any significative changes in Zn$_{450}$, Zn$_{900}$, Cr$_{300}$, Pb$_{450}$, Pb$_{900}$, Cd$_{4}$, Cd$_{8}$, Ni$_{110}$, Ni$_{220}$, Cu$_{200}$, and Cu$_{400}$. The only trial that showed significant differences in leaf productivity compared with Control was Cr$_{600}$ ($p = 0.02483$). The effect of the heavy metals in giant reed is better observed during the second growing cycle, when the root system is better established, absorbing higher amounts of nutrients and increasing the productivity of the crop (El Bassam, 2010). The second growing cycle, Zn$_{450}$, Zn$_{900}$, Pb$_{450}$, Pb$_{900}$, Cd$_{4}$, Cd$_{8}$, and Ni$_{110}$, had not shown any significant changes in leaf productivity. However, Cr$_{300}$ ($p = 0.006837$) and Cr$_{600}$ ($p = 0.006428$) suffered a significant reduction in leaf biomass productivity. Ni$_{220}$ ($p = 0.01734$), Cu$_{200}$ ($p = 0.0107$), and Cu$_{400}$ ($p = 0.02325$) also showed statistical differences when compared to Control. Additionally, several trials in the second year reduced productivity compared to the first year. Cr$_{600}$, Pb$_{450}$, Pb$_{900}$, Cd$_{4}$, and Cu$_{200}$ showed a lower leaf yield.

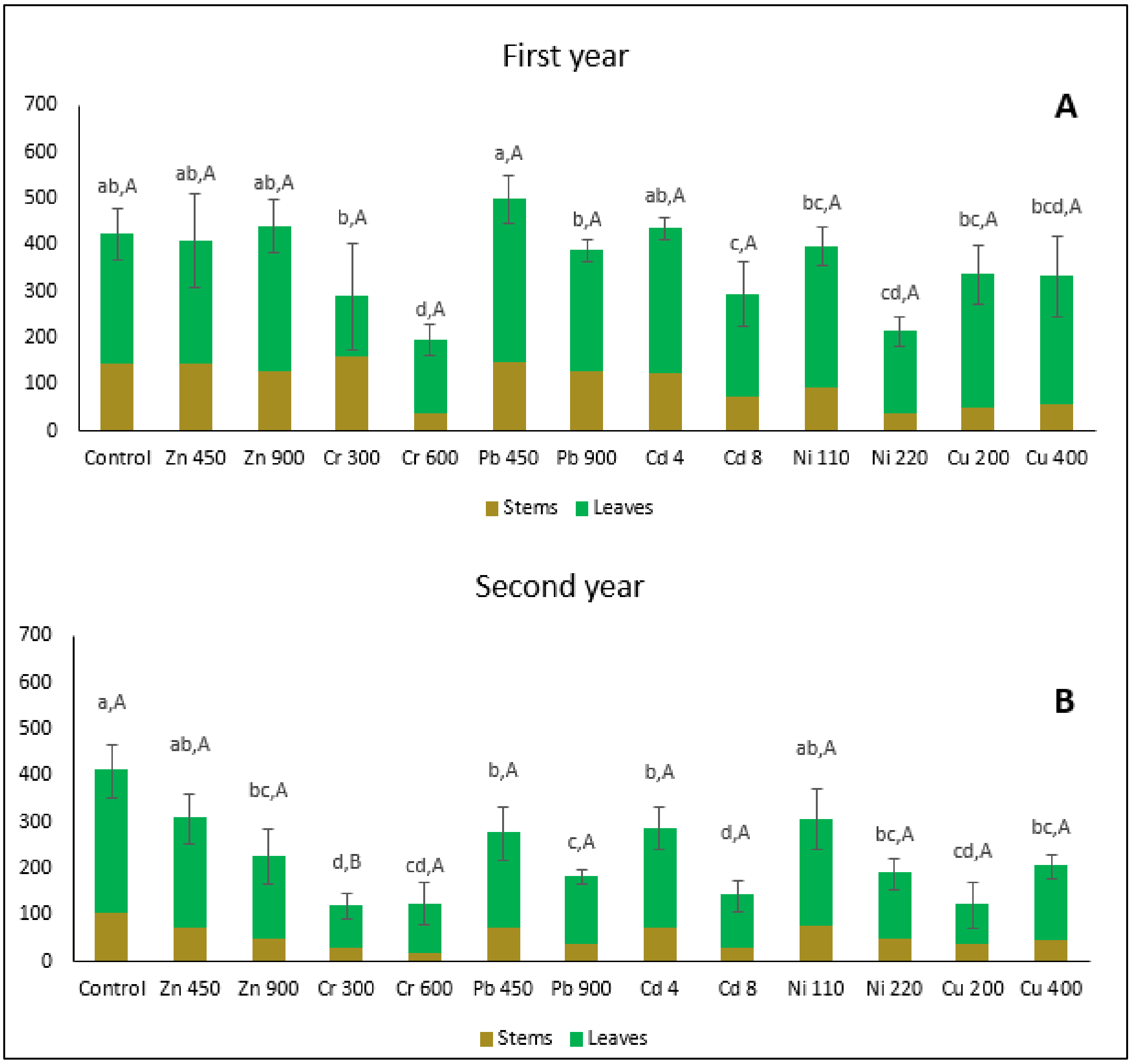

Figure 3.11. Giant reed aerial productivity in the first (A) and second (B) growing year.
Different lower-case letters indicate statistical significance (p < 0.05) among treatments per year. Different capital letters indicate statistical significance (p < 0.05) between first and second year results for identical treatments. Zn_{450} and Zn_{900}, 450 and 900 mg Zn kg^{-1}. Cr_{300} and Cr_{600}, 300 and 600 mg Cr kg^{-1}. Pb_{450} and Pb_{900}, 450 and 900 mg Pb kg^{-1}. Cd_{4} and Cd_{8}, 4 and 8 mg Cd kg^{-1}. Ni_{110} and Ni_{220}, 110 and 220 mg Ni kg^{-1}. Cu_{200} and Cu_{400}, 200 and 400 mg Cu kg^{-1}.

The leaves are the major fraction of *Arundo donax* aerial biomass, and when compared to the biometric parameters, it was observed that the leaf's productivity was mostly affected in terms of area, reducing the photosynthetic capacity of the plant, and as a consequence, reducing the thickness of stems, compromising the crops yield. However, despite leaves representing a higher part of switchgrass aerial productivity in the first year, the stems were the most affected part by Zn, Cr, and Ni. It can be observed due to the increase of the ratio of leaves/stem with the concentration of the pollutant in trials contaminated with Zn, Cr, Cd, and Ni, while in Pb and Cu contaminated trials, the increase of the contamination affected more the yield of the leaves than the yield of stems. In the second year, however, the ratio leaves/stem suffered no significant alterations in Cr trials, while Pb increased with the heavy metal concentration, indicating that the steams were more affected than the leaves. When the aerial biomass is compared with the root system, it is observed that the ratio of aboveground/belowground biomass for the control indicates that the aerial biomass has productivity around 3.5 times higher than the root system. When it comes to the contaminated trials, this ratio falls, having the highest ratio value in Cd trials. However, more due to the reduction in the root system than an indication of high tolerance of *Arundo donax* to Cd-contaminated soils. In some trials, such as Cr_{600}, the ratio is lower than 1, indicating that the productivity of the root system is higher than the aerial fraction of biomass. This can be related to the root system did not develop, indicating that the crop has no tolerance in terms of root system development for heavy metals contaminated soils, or/and the defense mechanism of the plant could be the accumulation of these heavy metals, that could be done in the roots, in this way compromising the plant nutrient and water uptake and in this way reducing the aerial biomass yield, or accumulating the metal in the aerial fraction, reducing the development of stems and leaves.

The productivity of belowground biomass took into consideration the roots and rhizomes of giant reed after the second growing cycle, as shown in Figure 3.12. It is observed that from all belowground biomass collected, Cd_8 was the trial most affected by the contamination causing a reduction in the yield. Cr_{300} and Ni_{220}appered to suffer a slight decrease in productivity while Zn_{900}, Cr_{600}, Pb_{450}, Cd_4, and Ni_{110} had productivity values similar to Control. On the other hand, Zn_{450}, Cu_{200}, and Cu_{400} presented an increase in the yield.

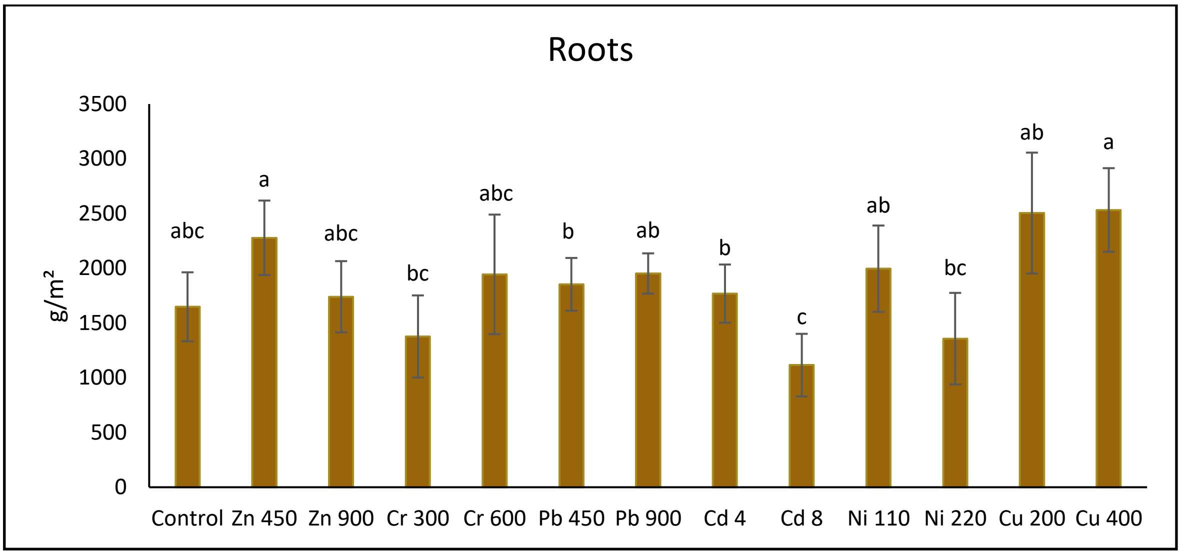

Figure 3.12. Giant reed belowground productivity in the second year.
Different lower-case letters indicate statistical significance ($p < 0.05$) among treatments. Zn_{450} and Zn_{900}, 450 and 900 mg Zn kg^{-1}. Cr_{300} and Cr_{600}, 300 and 600 mg Cr kg^{-1}. Pb_{450} and Pb_{900}, 450 and 900 mg Pb kg^{-1}. Cd_4 and Cd_8, 4 and 8 mg Cd kg^{-1}. Ni_{110} and Ni_{220}, 110 and 220 mg Ni kg^{-1}. Cu_{200} and Cu_{400}, 200 and 400 mg Cu kg^{-1}.

The maintenance of the root system productivity is not common in soils containing high levels of Zn (Kaur et al., 2021); however, this, and even the enlargement of the root system, could be a response by the plant trying to adapt to the stressed environment. Still, regarding root system, the low effect of Cr in giant reeds' root system is consistent with the literature; although in similar concentrations, the increase o perennial crops' roots was already observed under similar Cr concentrations (Li et al., 2014).

3.2.2.2 Switchgrass' productivity

Due to its capacity to produce high amounts of biomass (Arora et al., 2016; Guo et al., 2019; Li et al., 2011)., switchgrass becomes an alternative to be cultivated in heavy metal contaminated soils (Balsamo et al., 2015; Lucia, 2008).

The behavior of switchgrass in the first year after sowing is described in the literature as a year of adaptation and development of the root system, where switchgrass reaches only 33% of its productivity potential. (El Bassam, 2010). This experiment also observed this pattern, having a low crop development and, consequently, low productivity in the first year, as shown in Figure 3.13.

Concerning switchgrass yield in contaminated trials, the yield was inversely proportional to the concentration of the heavy metals in the soil. This decreasing trend was better noticed in the second year, with the better development of the aerial fraction of switchgrass in trials containing Zn, Pb, Cd, Ni, and Cu.

The aerial biomass productivity in the second year increased, as expected in all trials, except in Zn_{450} and $Zn_{900,}$ which did not suffer any alteration. The aerial productivity of switchgrass was observed to be a consequence of the root system's development, as shown in Figure 3.14 since the trials with higher aerial productivity also had higher belowground productivity (the exception was Zn_{900}). The increase in switchgrass' aerial biomass in the second year is a behavior already reported in several studies (Alexopoulou et al., 2008; El Bassam, 2010; McLaughlin et al., 2005) and can be related to the development of the root system. In the first year, a small root system limits the amount of absorbed nutrients, making it harder for the plant to develop the aerial part. However, as the first growing cycle finishes, the root system continues developing, making the translocation of nutrients and water for the shoots abundant, and in this way, increasing the aerial biomass production.

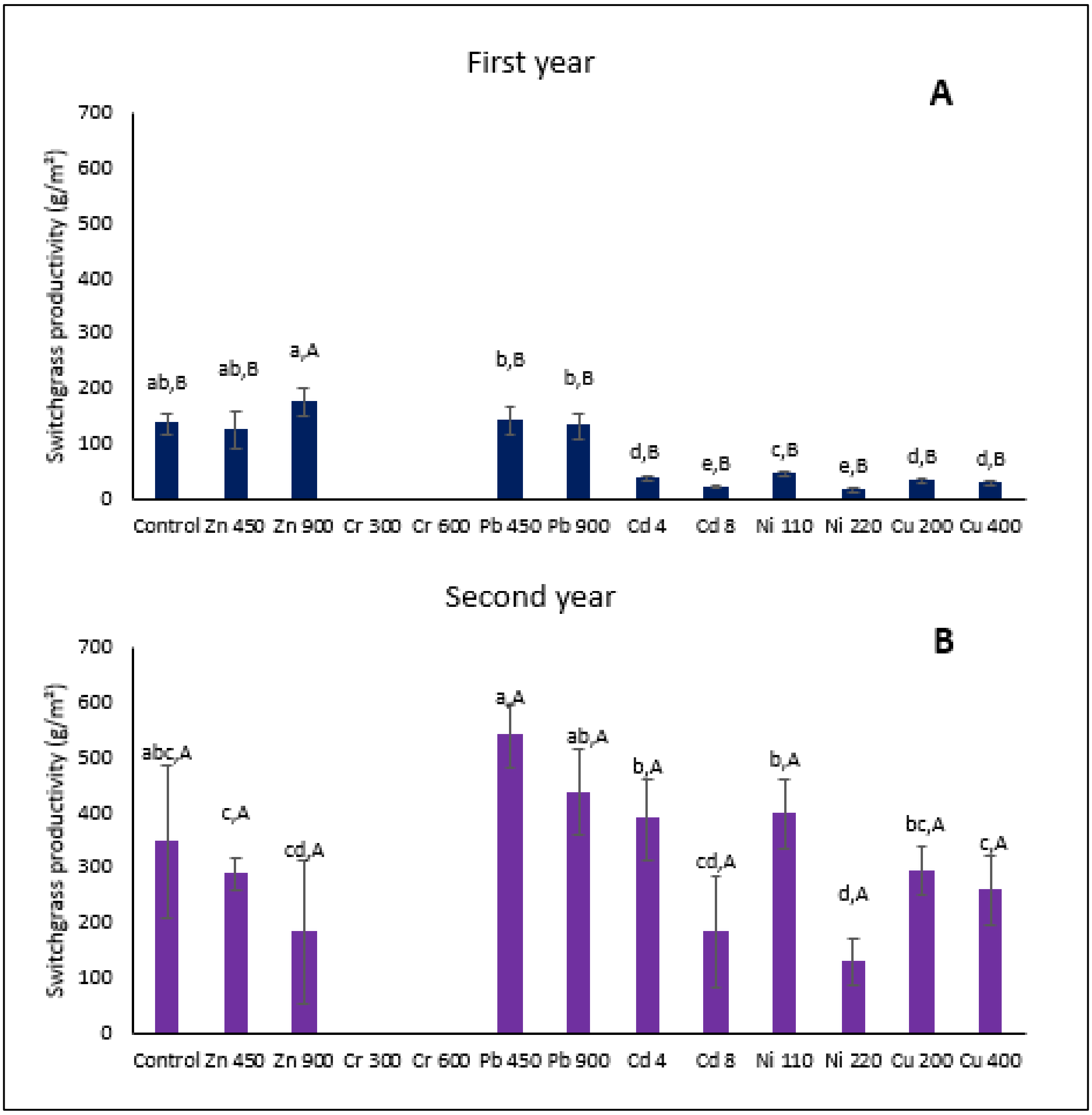

Figure 3.13. Switchgrass aboveground productivity in the first (A) and second (B) growing year.
Different lower-case letters indicate statistical significance ($p < 0.05$) among treatments per year. Different capital letters indicate statistical significance ($p < 0.05$) between first and second year results for identical treatments. Zn_{450} and Zn_{900}, 450 and 900 mg Zn kg^{-1}. Cr_{300} and Cr_{600}, 300 and 600 mg Cr kg^{-1}. Pb_{450} and Pb_{900}, 450 and 900 mg Pb kg^{-1}. Cd_4 and Cd_8, 4 and 8 mg Cd kg^{-1}. Ni_{110} and Ni_{220}, 110 and 220 mg Ni kg^{-1}. Cu_{200} and Cu_{400}, 200 and 400 mg Cu kg^{-1}.

In Cr trials, the germination of switchgrass was inhibited. In the first year of the experiment, despite some seeds germinating, the toxicity of Cr did not allow the development of the plants. In the second year, sowing was repeated, but the same problem was observed. Despite no evidence of Cr's role in plant metabolism, it can affect plants differently (Kabata-Pendias, 2011). Usually, Cr bind in the root's cell walls. Its toxicity is linked to its oxidation state, and for some plants, even in small concentrations (1 or 2 mg/kg biomass), it can affect the plant's development (Kabata-Pendias, 2011). There are different ways that Cr can affect the plant, by reducing the yield or even inhibiting the seed's germination (Shanker et al., 2005). Switchgrass had already been tested in Cr contaminated soils by Li et al. under concentrations of 131.25, 162.5, 225, 350, and 600 mg.kg^{-1} (Li et al., 2011). The crop accumulated Cr in both aboveground and belowground biomass. However, the productivity decreased for 350 and 600 mg.kg^{-1}. Despite having promising results, the short exposure time (40 days) and the bioavailability of the contaminant (which was not specified) could have camouflaged the tolerance of the crop to Cr.

Despite statistically not harming switchgrass, the Cd trials observed from the aerial biomass, belowground biomass, and the number of tillers could lead to a trend that Indicated the toxicity of Cd for this crop. Arora also tested switchgrass in Cd-contaminated soils. Switchgrass was exposed to soils contaminated in concentrations of 2,4,6,8, and 10mg.kg.$^{-1}$ for 20 weeks (Arora et al., 2016). reducing almost 65% in switchgrass yield for the higher contamination level. The reduction in switchgrass yield was also noticed in the first year of this work, where in both concentrations, 4mg/kg and 8mg/kg presented a reduction higher than 50% and 75%, respectively, while the reduction in Arora for the same contamination was lower (around 15% for 4mg/kg and 50% for 8 mg/kg). Chen et al. (Chen et al. 2011) used a higher range of Cd with concentrations up to 60 mg.kg^{-1}, leading to a drastic reduction in grass productivity, which was observed in the higher level of contamination, with 63% losses. In this experiment, Chen and collaborators contaminated two different soils with cadmium salts in concentrations of 20 mg/kg and 60 mg/kg, also comparing with the influence of chellant agents (control, CA, and EDTA) for 110 days. The analysis of the soil's fractions observed that despite the concentration of heavy metals in the soil were high, Cd was present in different forms: carbonate, iron manganese-bound, organic-bound, residual, and exchangeable fractions, each fraction with different mobility levels that can affect the development of the crop.

Nickel affected switchgrass very hard in the first year. This heavy metal can be found in different oxidation states; however, the most toxic is Ni^{2+}. Plants usually take nickel and transport it to leaves and stems, where it can be stored. Although some hypertolerant and hyperaccumulator plants, like *Berkheya coddi*, can reach levels of 18,000 mg/kg in their biomass, other Ni sensitive plants, such as oats, can be affected by concentrations from 24-308 mg/kg of metal in their biomass (Kabata-Pendias, 2011). Ni can lead to several hazardous effects on the plant in a morphological, physiological, and biochemical aspects (Shahzad et al., 2018). In some cereals, the excess Ni can be noticed by plant characteristics, such as interveinal chlorosis in new leaves, grey-green leaves, and brown and stunted roots (Kabata-Pendias, 2011). Experiments of switchgrass in Ni-contaminated soils were not found, but it is possible to see through other perennial crops the trend of nickel accumulate trials to accumulate in the aboveground biomass, as found in the literature (Kabata-Pendias, 2011).

Similar to Ni trials in the first year, Cu also inhibited switchgrass development. The toxic effect of Cu in switchgrass is already documented in the literature. To study the effect of Cu in switchgrass, Juang et al.(Juang et al., 2011) designed a hydroponic experiment using concentrations of 0.5, 1.0, 2.0, and 5.0 µg/mL Cu. The study showed a critical reduction in crop growth, with losses of up to 80% of the yield.

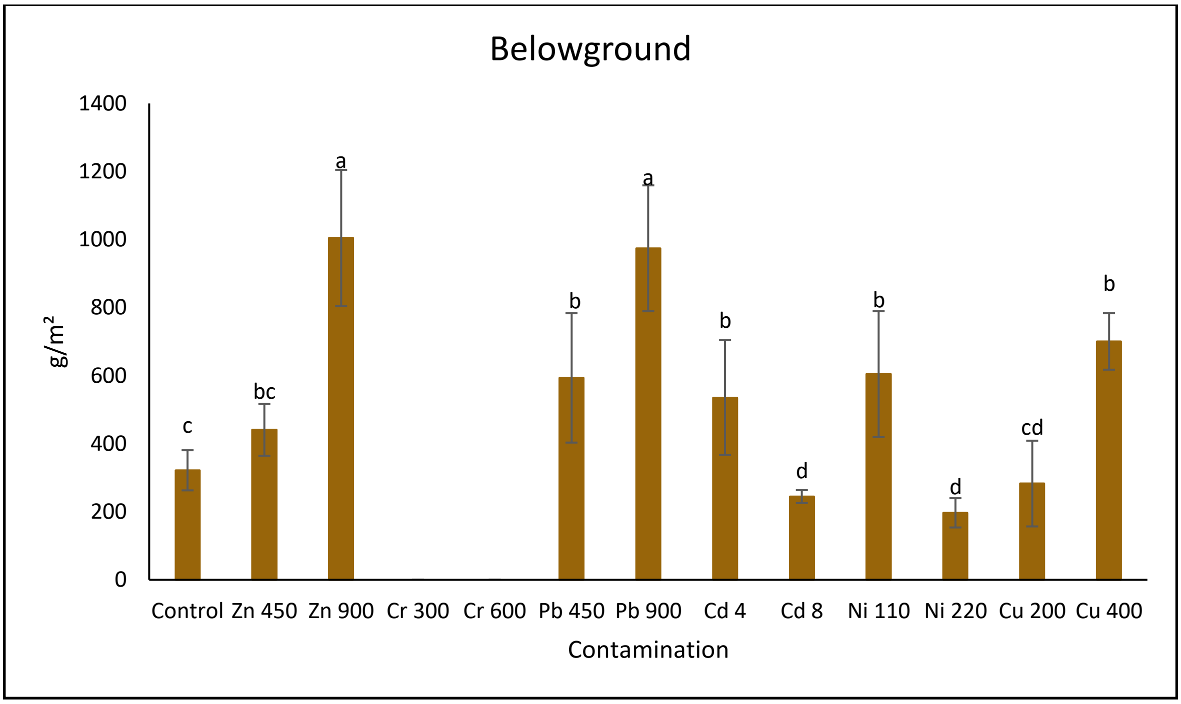

Figure 3.14. Switchgrass belowground productivity in the second year.
Different lower-case letters indicate statistical significance ($p < 0.05$) among treatments. Zn_{450} and Zn_{900}, 450 and 900 mg Zn kg^{-1}. Cr_{300} and Cr_{600}, 300 and 600 mg Cr kg^{-1}. Pb_{450} and Pb_{900}, 450 and 900 mg Pb kg^{-1}. Cd_4 and Cd_8, 4 and 8 mg Cd kg^{-1}. Ni_{110} and Ni_{220}, 110 and 220 mg Ni kg^{-1}. Cu_{200} and Cu_{400}, 200 and 400 mg Cu kg^{-1}.

The ratio of aerial aboveground/belowground productivity for Control is slightly higher than 1, indicating that aboveground switchgrass yield is slightly higher than below ground. On the contrary, in contaminated trials, the results indicated that the productivity in belowground biomass is higher than aboveground biomass, being 2.2 times higher for Pb_{900}, and 2.7 times higher for Cu_{400}, indicating that due to the contamination, the root system nutrients and water uptake capability are compromised and, the plant increase its root system to compensate this effect, reducing the potential of the aerial biomass growth the absorbed nutrients and water were not exclusively used for the development of the aerial biomass but also in the increase of the root system.

3.2.2.3 Productivity: Giant reed *vs.* switchgrass

A general comparison between giant reed (stem and leaves) and switchgrass productivity can be seen in Figure 3.15. It is possible to observe that giant reed adapted better during the first year of the experiment. In all trials, giant reed productivity overcomes switchgrass. In the second year, switchgrass recovery had similar productivity to giant reed in Control, Zn, Cd, and Ni trials. Switchgrass did not produce any biomass in the Cr trial, while giant reed, despite the significant decrease in productivity, was able to produce biomass. In Pb trials, however, switchgrass yield was twice that of giant reed, making this crop more suitable for cultivating in Pb-contaminated soil. Cu contaminated soils presented unusual behavior, having switchgrass had a higher yield in Cu_{200} while the productivity of both crops was the same in Cu_{400}.

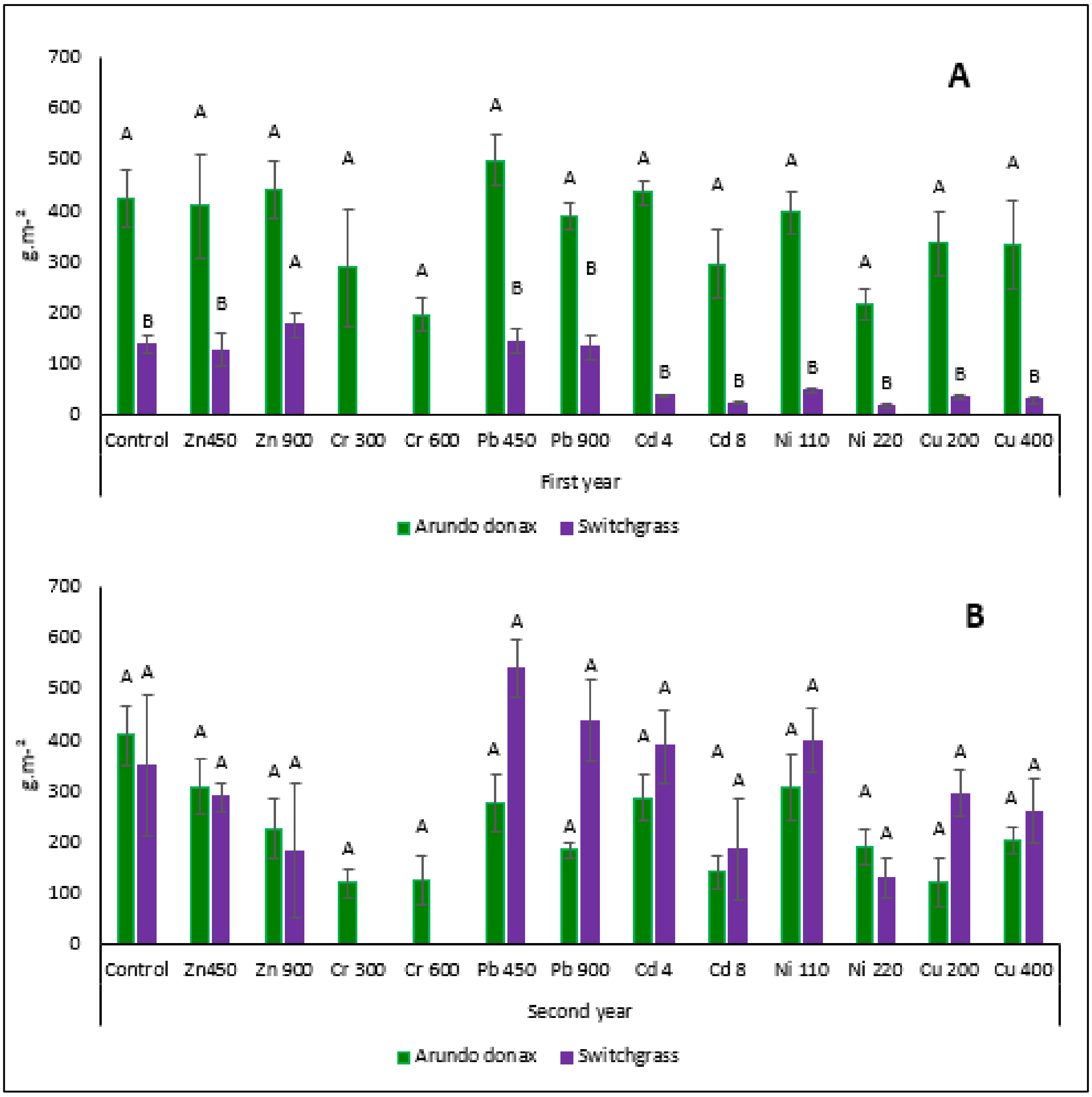

Figure 3.15. Productivity: giant reed (stems and leaves) vs. switchgrass in the first (A) and second (B) growing years. Different capital letters indicate statistical significance ($p < 0.05$) between the first and second years' results for identical treatments. Zn_{450} and Zn_{900}, 450 and 900 mg Zn kg^{-1}. Cr_{300} and Cr_{600}, 300 and 600 mg Cr kg^{-1}. Pb_{450} and Pb_{900}, 450 and 900 mg Pb kg^{-1}. Cd_{4} and Cd_{8}, 4 and 8 mg Cd kg^{-1}. Ni_{110} and Ni_{220}, 110 and 220 mg Ni kg^{-1}. Cu_{200} and Cu_{400}, 200 and 400 mg Cu kg^{-1}.

3.2.2.4 Tolerance index

The tolerance index of both giant reed and switchgrass is observed in Table 3.4. Used to Indicate how tolerant the plant Is to stressful conditions, in this case, heavy metal contaminated soils. This Index shows the reduction in plant growth due to the contaminant. The crop can be classified into four different categories according to its TI value: high tolerance: TI ≥ 0.75; moderate tolerance: 0.50 ≤ TI < 0.75; low tolerance: 0.25 ≤ TI < 0.50; critical tolerance: TI < 0.25.

The TI was calculated considering the second growing cycle of the biomass, considering it to be more representative of the full productivity potential (El Bassam, 2010). It was observed that the giant reed's aerial fraction has a high tolerance to Zn-contaminated soils. Pb_{450}, Cd_4, and Ni_{110} are also tolerant to contamination. A moderate tolerance for giant reed's aerial biomass is seen in Pb_{900}, Cd_8, Ni_{220}, and both Cu trials, Cu_{200} and Cu_{400}. The low tolerance for the aerial part is observed only in Cr trials, Cr_{300} and Cr_{600}.

Switchgrass also showed high tolerance to Zn, Pb, and Cu. For the low concentrations of Cd and Ni, switchgrass showed high tolerance. However, when the concentration of Cd increases in Cd_8 and Ni to $Ni_{220,}$ the tolerance goes from high to moderate.

Table 3.4. Tolerance index

	Giant reed	Switchgrass
Zn_{450}	0.8 ± 0.2	0.82 ± 0.29
Zn_{900}	0.6 ± 0.2	0.78 ± 0.3
Cr_{300}	0.3 ± 0.1	-
Cr_{600}	0.3 ± 0.1	-
Pb_{450}	0.7 ± 0.2	1.55 ± 0.55
Pb_{900}	0.4 ± 0.1	1.26 ± 0.48
Cd_4	0.7 ± 0.2	1.11 ± 0.42
Cd_8	0.4 ± 0.1	0.53 ± 0.31
Ni_{110}	0.8 ± 0.2	1.14 ± 0.43
Ni_{220}	0.5 ± 01	0.37 ± 0.16
Cu_{200}	0.4 ± 0.2	0.84 ± 0.31
Cu_{400}	0.5 ± 0.1	0.74 ± 0.3

Different lower-case letters indicate statistical significance ($p < 0.05$) among treatments per year. Different capital letters indicate statistical significance ($p < 0.05$) between first and second year results for identical treatments. Zn_{450} and Zn_{900}, 450 and 900 mg Zn kg^{-1}. Cr_{300} and Cr_{600}, 300 and 600 mg Cr kg^{-1}. Pb_{450} and Pb_{900}, 450 and 900 mg Pb kg^{-1}. Cd_4 and Cd_8, 4 and 8 mg Cd kg^{-1}. Ni_{110} and Ni_{220}, 110 and 220 mg Ni kg^{-1}. Cu_{200} and Cu_{400}, 200 and 400 mg Cu kg^{-1}

The tolerance of giant reed and switchgrass under Zn450 and Zn900 contamination is confirmed by literature, similar to the TI for miscanthus (Barbosa et al., 2015). A study using giant reed in Ni and Cd contaminated soils showed a trend to a TI close to 1, implying the high tolerance of giant reed to this heavy metal. However, despite appears promising, the bioavailable heavy metals in the experiment are lower than In this experiment (Papazoglou et al., 2005). The experiment realized by Arora showed a moderate tolerance of switchgrass to Pb and Cd with a tolerance index around 0.57 and 0.5 in the highest analyzed scenarios (Arora et al., 2016). When exposed to Cr, switchgrass experience a different scenario than observed in this work. With a TI higher than 0.75 for concentrations until 350 mg.kg^{-1}, however, when this concentration increases to 600 mg.kg$^{-1,}$ the TI falls to 0.33, demonstrating that the plant has a low tolerance to Cr in this concentration. Although switchgrass exposure to Cr trends are different from this work, the bioavailable Cr is not specified (Li et al., 2011).

3.3 Biomass composition and energetic potential

The biomass composition and energetic potential were analyzed to evaluate the possibilities for the contaminated biomass application for bioenergy, bioproducts, and biofuels. Despite the design of the experiment only allowing pot utilization, variations in the studied parameters in different treatments can indicate the effect of contamination in biomass composition, affecting the potential utilization in biorefinery processes. To evaluate this potential, some parameters were analyzed. For the first year, nitrogen (N), phosphorus (P), ash content, and other macroelements (Ca, Mg, Na, K, Fe, and Mn) were analyzed. In the second year, along with the same parameters, the immediate analysis was also made for both crops, indicating the volatile matter, the fixed carbon, and the high heating value (HHV), to evaluate how the presence of heavy metals in soil affects the potentiality of both crops to energy. In addition, in this second year, the composition of both biomasses in terms of cellulose, hemicellulose, and lignin was also addressed to evaluate the effects of the presence of heavy metals in the soil on the fiber composition.

3.3.1 Immediate analysis and high heating value

The biomass ash content is essential for mainly two reasons, to determine if the presence of the heavy metals in the soil increases the ash content, indicating that the heavy metals could be absorbed by the crops, enhancing the remediation of the site, and the amount of generated residues when this contaminated biomass are submitted to thermochemical processes. Furthermore, the immediate analysis, present for the second year, helps to understand the thermochemical potential of the biomass, such as the potential biochar production indicated by the fixed carbon amount and the thermochemical potential indicated by the high heating value.

In the present work, the immediate analysis of giant reed’s for the first year and the second year can be observed in Table 3.5 and Table 3.6, while for switchgrass, the values will be presented in Table 3.7 and Table 3.8. The contents in terms of P, K, Ca, Mg, Na, and other elements are also important to evaluate the potential of biomass to be used in thermochemical processes. Indeed, they affect fly ash emissions, deposit formation, and ash handling/utilization/disposal. The values for these elements varied among the different biomasses. This chapter does not evaluate the heavy metals (Zn, Cr, Pb, Cd, Ni, and Cu), which will be presented and discussed in Chapter 3.4.

Table 3.5. Giant reed's ash content in the first growing cycle

	Giant reed - First year												
	Control	**Zn_{450}**	**Zn_{900}**	**Cr_{300}**	**Cr_{600}**	**Pb_{450}**	**Pb_{900}**	**Cd_4**	**Cd_8**	**Ni_{110}**	**Ni_{220}**	**Cu_{200}**	**Cu_{400}**
Leaves													
Ash (% dw)	9.58 ± 0.40 e,A	9.06 ± 0.25 e,A	11.66 ± 0.01 cd,A	12.38 ± 0.38 bcd,A	15.61 ± 0.31 b,A	10.60 ± 0.15 de,A	11.02 ± 0.40 cde,A	12.85 ± 0.01 bc,A	11.80 ± 0.49 cd,A	12.26 ± 0.72 bcd,A	11.89 ± 0.19 cd,A	11.84 ± 0.22 cd,A	14.10 ± 0.46 ab,A
Stems													
Ash (% dw)	5.42 ± 0.11 c,A	5.98 ± 0.03 c,A	5.73 ± 0.05 c,A	7.35 ± 0.38 bc,A	6.16 ± 0.46 a,A	5.87 ± 0.13 c,A	6.00 ± 0.03 bc,A	6.21 ± 0.30 c,A	8.01 ± 1.35 bc,A	6.15 ± 0.62 b,A	7.43 ± 1.48 c,A	5.87 ± 0.16 c,A	5.79 ± 0.03 c,A

Different lower-case letters indicate statistical significance ($p < 0.05$) among treatments per year. Different capital letters indicate statistical significance ($p < 0.05$) between first and second year results for identical treatments. Zn_{450} and Zn_{900}, 450 and 900 mg Zn kg^{-1}. Cr_{300} and Cr_{600}, 300 and 600 mg Cr kg^{-1}. Pb_{450} and Pb_{900}, 450 and 900 mg Pb kg^{-1}. Cd_4 and Cd_8, 4 and 8 mg Cd kg^{-1}. Ni_{110} and Ni_{220}, 110 and 220 mg Ni kg^{-1}. Cu_{200} and Cu_{400}, 200 and 400 mg Cu kg^{-1}

Table 3.6. Giant reed's immediate analysis and HHV in the second growing cycle

	Giant reed - Second year												
	Control	**Zn$_{450}$**	**Zn$_{900}$**	**Cr$_{300}$**	**Cr$_{600}$**	**Pb$_{450}$**	**Pb$_{900}$**	**Cd$_{4}$**	**Cd$_{8}$**	**Ni$_{110}$**	**Ni$_{220}$**	**Cu$_{200}$**	**Cu$_{400}$**
Leaves													
Volatile Matter (% dw)	76.6 ± 0.7 ab	76.6 ± 1.0 ab	75.4 ± 0.5 c	74.2 ± 0.3 d	74.7 ± 0.3 d	74.8 ± 0.3 cd	79.3 ± 0.4 a	74.9 ± 0.2 cd	73.3 ± 0.4 e	76.6 ± 0.4 b	77.6 ± 1.1 ab	79.8 ± 1.9 a	78.5 ± 0.1 ab
Ash (% dw)	9.17 ± 0.40 bc,A	9.09 ± 1.02 c,A	10.04 ± 0.80 abc,A	10.97 ± 0.57 abc,B	11.61 ± 0.60 a,B	10.33 ± 0.79 b,A	9.38 ± 0.88 abc,A	10.39 ± 0.15 abc,B	11.06 ± 0.20 abc,A	8.68 ± 0.41 bc,B	9.40 ± 0.94 c,A	9.37 ± 1.22 abc,A	9.52 ± 1.02 abc,B
Fixed Carbon (% dw)	14.5 ± 0.7 ab	15.2 ± 0.8 ab	15.1 ± 0.7 ab	15.3 ± 0.1 a	14.1 ± 0.1 c	15.5 ± 0.3 a	11.9 ± 0.4 d	14.8 ± 0.1 b	15.7 ± 0.6 a	15.1 ± 0.3 a	13.5 ± 1.1 bc	11.3 ± 0.6 d	12.8 ± 0.2 c
HHV (MJ.Kg-1)	18.3 ± 1.1 a	18.2 ± 2.0 a	18.2 ± 1.3 a	18.0 ± 0.0 a	17.8 ± 0.1 a	18.1 ± 1.1 b	18.1 ± 1.3 a	18.0 ± 0.0 b	17.9 ± 0.1 a	18.4 ± 0.0 a	18.2 ± 0.1 a	18.0 ± 3.1 a	18.2 ± 1.1 a
Stems													
Volatile Matter (% dw)	80.5 ± 0.0 b	78.4 ± 0.4 d	78.6 ± 1.8 bcde	77.1 ± 0.0 b	74.2 ± 0.2 b	76.9 ± 0.7 b	76.0 ± 1.5 de	75.9 ± 0.4 d	74.6 ± 0.2 e	81.4 ± 0.2 a	79.8 ± 0.2 c	77.0 ± 0.1 e	77.7 ± 1.7 cd
Ash (% dw)	5.39 ± 1.31 b,A	6.97 ± 0.67 ab,A	6.59 ± 1.19 ab,A	7.35 ± 0.38 ab,B	6.16 ± 0.46 ab,B	7.44 ± 0.87 ab,A	6.94 ± 0.70 ab,A	6.21 ± 0.30 ab,A	8.01 ± 1.35 a,A	6.15 ± 0.62 ab,A	7.43 ± 1.48 ab,A	7.59 ± 1.33 ab,A	6.53 ± 0.42 ab,A
Fixed Carbon (% dw)	15.1 ± 0.6 a	14.6 ± 0.4 f	15.1 ± 0.4 e	15.8 ± 0.3 d	19.4 ± 0.1 a	15.3 ± 0.2 e	16.4 ± 1.6 cde	18.2 ± 0.3 b	18.3 ± 0.1 b	12.7 ± 0.2 h	13.7 ± 0.4 g	16.5 ± 0.0 c	16.0 ± 1.3 cd
HHV (MJ.Kg-1)	19.1 ± 1.3 a	18.6 ± 1.1 a	18.8 ± 3.0 a	18.7 ± 0.1 a	19.0 ± 0.1 a	18.5 ± 1.6 a	18.6 ± 2.2 a	19.0 ± 0.0 a	18.8 ± 0.0 a	18.7 ± 0.1 a	18.7 ± 0.1 a	18.8 ± 1.4 a	18.8 ± 2.1 a
Rhizomes and Roots													
Ash (% dw)	17.77 ± 1.45 d	37.02 ± 17.11 ab	53.81 ± 4.69 a	50.76 ± 4.51 a	53.98 ± 7.15 a	40.53 ± 10.39 a	36.51 ± 1.31 b	40.42 ± 3.77 b	54.67 ± 17.60 a	31.70 ± 3.08 c	54.11 ± 1.90 a	42.07 ± 1.29 b	32.47 ± 0.97 c

Different lower-case letters indicate statistical significance (*p* < 0.05) among treatments per year. Different capital letters indicate statistical significance (*p* < 0.05) between first and second year results for identical treatments. Zn$_{450}$ and Zn$_{900}$, 450 and 900 mg Zn kg^{-1}. Cr$_{300}$ and Cr$_{600}$, 300 and 600 mg Cr kg^{-1}. Pb$_{450}$ and Pb$_{900}$, 450 and 900 mg Pb kg^{-1}. Cd$_{4}$ and Cd$_{8}$, 4 and 8 mg Cd kg^{-1}. Ni$_{110}$ and Ni$_{220}$, 110 and 220 mg Ni kg^{-1}. Cu$_{200}$ and Cu$_{400}$, 200 and 400 mg Cu kg^{-1}

In the first year, giant reeds ash content in giant reed leaves was affected in Zn_{900} ($p = 0.03447$), both Cr_{300} and Cr_{600} ($p = 0.03604$ and $p = 0.00688$), Cd_4 ($p = 0.01436$), Ni_{220} ($p = 0.03428$), and both Cu trials, Cu_{200} and Cu_{400} ($p = 0.03786$ and $p = 0.01758$), while in the stems Zn_{450} ($p = 0.04176$), Cr_{600} ($p = 0.00054$), Pb_{900} ($p = 0.03869$), and Ni_{110} ($p = 0.00792$). At the end of the second year, the leaves were affected in Cr and Cd trials ($p = 0.0021$ and $p = 0005$ for Cr_{300} and Cr_{600}, and $p = 0.0012$ and $p = 0.0001$ for Cd_4 and Cd_8, respectively), while the stems were also affected by the first level of Pb contamination, Pb_{450} ($p = 0.039$). From the first to the second year, Cr_{300} and Cr_{600} ($p = 0.04478$ and $p = 0.001178$) also demonstrate an Increase In ash content. Cd_4, Ni_{110}, and Cu_{400} are also influenced by the establishment of the crop in the pot, suffering alterations between growing cycles.

The stems, however, were less affected in the first year, having suffered alterations only in Zn_{450}, Cr_{600}, Pb_{900}, and Ni_{110} ($p = 0.0418$, $p = 0.0005$, $p = 0.0387$, and $p = 0.0079$, respectively). In the second year, Cr_{300} ($p = 0.0279$), Pb_{450} ($p = 0.04$), and Cd_8 ($p = 0.0314$) were affected in terms of ash content. Despite small variations between years, Cr trials were the only ones statistically affected from the first to the second year ($p = 0.0376$ and $p = 0.0001$ for Cr_{300}, and Cr_{600}, respectively).

The roots ash content value was considerably higher than the Control, which could have occurred due to heavy metals or contaminants in the sample. Cr and Cu trials, as Zn_{900} ($p = 0.0181$), Pb_{900} ($p = 0.0108$), Cd_4 ($p = 0.0304$), and Ni_{220} ($p = 0.0043$) were also affected.

Table 3.7. Switchgrass ash content considering the first growing cycle

Switchgrass – First year													
	Control	**Zn_{450}**	**Zn_{900}**	**Cr_{300}**	**Cr_{600}**	**Pb_{450}**	**Pb_{900}**	**Cd_4**	**Cd_8**	**Ni_{110}**	**Ni_{220}**	**Cu_{200}**	**Cu_{400}**
Aerial Biomass													
Ash (% dw)	12.83 ± 3.07b	12.40 ± 2.48 b	11.14 ± 0.53 b	-	-	11.63 ± 2.69 b	12.55 ± 2.78 b	13.69 ± 3.32 b	21.19 ± 3.07 a	14.54 ± 3.32 ab	-	-	-

Different lower-case letters indicate statistical significance (*p* < 0.05) among treatments per year. Different capital letters indicate statistical significance (*p* < 0.05) between first and second year results for identical treatments. Zn_{450} and Zn_{900}, 450 and 900 mg Zn kg^{-1}. Cr_{300} and Cr_{600}, 300 and 600 mg Cr kg^{-1}. Pb_{450} and Pb_{900}, 450 and 900 mg Pb kg^{-1}. Cd_4 and Cd_8, 4 and 8 mg Cd kg^{-1}. Ni_{110} and Ni_{220}, 110 and 220 mg Ni kg^{-1}. Cu_{200} and Cu_{400}, 200 and 400 mg Cu kg^{-1}

Table 3.8. Switchgrass immediate analysis and HHV in the second growing cycle

Switchgrass – Second year													
	Control	**Zn_{450}**	**Zn_{900}**	**Cr_{300}**	**Cr_{600}**	**Pb_{450}**	**Pb_{900}**	**Cd_4**	**Cd_8**	**Ni_{110}**	**Ni_{220}**	**Cu_{200}**	**Cu_{400}**
Aerial Biomass													
Volatile Matter (% dw)	78.7 ± 0.5 abc	77.3 ± 0.6 abcd	76.2 ± 0.5 bcd	-	-	79.7 ± 0.7 a	77.6 ± 0.3 a	75.9 ± 0.1 abc	74.5 ± 0.1 cde	73.0 ± 0.1 de	78.8 ± 1.2 e	79.0 ± 0.4 abc	79.0 ± 0.2 ab
Ash (% dw)	8.32 ± 0.33 a	8.39 ± 0.37 a	8.87 ± 0.34 a	-	-	7.26 ± 0.31 a	7.37 ± 0.08 a	7.47 ± 0.22 a	7.19 ± 1.98 a	8.12 ± 0.20 a	9.33 ± 3.22 a	6.58 ± 0.29 a	6.90 ± 0.58 a
Fixed Carbon (% dw)	13.2 ± 0.4 bc	14.7 ± 0.8 abc	15.0 ± 0.4 abc	-	-	13.3 ± 0.5 a	15.1 ± 0.3 bc	16.8 ± 0.1 abc	17.2 ± 0.2 ab	18.8 ± 0.1 ab	10.8 ± 2.4 a	14.6 ± 0.2 c	14.6 ± 0.1 abc
HHV (MJ.Kg-1)	18.3 ± 0.8 a	18.4 ± 1.0 a	18.3 ± 0.8 a	-	-	18.5 ± 1.0 a	18.6 ± 0.4 a	18.7 ± 0.0 a	18.5 ± 0.0 a	18.6 ± 0.0 a	17.7 ± 0.8 a	18.7 ± 0.7 a	18.7 ± 0.8 a
Underground Biomass													
Ash (% dw)	9.80 ± 0.05 cde	11.50 ± 0.43 c	11.13 ± 0.01cd	-	-	9.55 ± 0.01 de	15.17 ± 0.37 b	10.50 ± 0.25 cd	17.60 ± 0.00 a	15.78 ± 0.19 ab	8.34 ± 0.01 e	10.81±0.01 cd	11.66±0.01 c

Different lower-case letters indicate statistical significance (*p* < 0.05) among treatments per year. Zn_{450} and Zn_{900}, 450 and 900 mg Zn kg^{-1}. Cr_{300} and Cr_{600}, 300 and 600 mg Cr kg^{-1}. Pb_{450} and Pb_{900}, 450 and 900 mg Pb kg^{-1}. Cd_4 and Cd_8, 4 and 8 mg Cd kg^{-1}. Ni_{110} and Ni_{220}, 110 and 220 mg Ni kg^{-1}. Cu_{200} and Cu_{400}, 200 and 400 mg Cu kg^{-1}

In the first year of the switchgrass, only Cd_8 ($p = 0.01607$). In the second year, however, Pb contaminated trials, Pb_{450} ($p = 0.003522$) and Pb_{900} ($p = 0.001451$), Cu trials, Cu_{200} ($p = 0.000225$) and ($p = 0.005599$), and the lower concentration of Cd, Cd_4 ($p = 0.000553$) suffered alterations in the aboveground biomass. In belowground biomass, the trials there were affected were Zn_{900}, Pb_{450}, Pb_{900}, Cd_8, Ni_{110}, Ni_{220}, and Cu_{200} ($p = 0.001334$, $p = 0.04111$, $p = 0.004728$, $p = 0.00003931$, $p = 0.001056$, $p = 0.001145$, $p = 0.002336$, respectively).

Giant reed ash contents vary from the stems, 3.56% to 8.7%, and the leaves, 8.9% to 18.1% (Dragoni et al., 2015; Krička et al., 2017; Riggi et al., 2019). The biggest gap occurs when the ash is in different biomass parts. A huge difference is observed among leaves, stems, and roots, indicating that the higher ash contents are higher in roots and leaves than in stems. This could be justified by the accumulation of heavy metal in these parts of the plants, Increasing the amount of Inorganic material In this fraction of the plant (Kabata-Pendias, 2011). The ash content In switchgrass varies from 2.1% to 10.51%, depending on the experiment's conditions (Hu et al., 2010; Iqbal et al., 2015; Liu et al., 2011; Mani et al., 2004; Maret, 2016). The results obtained in this experiment are supported by the values observed in the literature. The further utilization of these crops in energy conversion is strongly related to the ash content in biomass. The limits for ash content In thermochemical conversion processes can limit the utilization of the crops since most of these technologies can only work with ash content lower than 5% (S2 Biom, 2016). In combustion processes, an alternative with a higher limit of ash content appears for fixed bed reactors, up to 10% (S2 Biom, 2016). Some gasification processes also have a higher limit when a fluidized or updraft fixed bed is used. Another problem related to the high ash content Is the amount of residues, which Is not only higher but also contaminated, Increasing the cost of the conversion processes.

In giant reed, fixed carbon and volatile materials did not suffer any alterations in the leaves. However, the high heat value changes In Cr_{600} ($p = 0.0322$) and Cd_4 ($p = 0.018$). The Cr and Cd contaminations in stems were affected at all levels, while Pb, Ni, and Cu were affected only in the lowest contamination. In switchgrass, the changes in fixed carbon and volatile material are insignificant for most trials, despite Cd4 for fixed carbon (p = 0.006055), Cd_8, and Ni_{110} for volatile material ($p = 0.01667$ and $p = 0.009149$, respectively). The HHV did not change in any contamination for giant reed; however, in switchgrass, Pb, Cd, and Cu trials were affected in terms of HHV. The other affected trials are Zn_{900} ($p = 0.0327$) and Ni_{110} ($p = 0.0002552$). Despite some changes regarding the contaminants for both giant reed and switchgrass, the measured values are according to the literature (Basso et al., 2005; Hu et al., 2010; Jeguirim et al., 2009; Lemus et al., 2008; Oginni et al., 2019; Saikia et al., 2015; Wright, 2014; Yang et al., 2020).

Regarding the leaves of *Arundo donax*, it was observed that, in general, after the establishment of the crop, only Cd, Cr, and Pb_{450} provoked a raise in ash content. Regarding the stems for the same growing cycle, Cr and Cd_8 also increased the biomass ash content. In general, leaves presented a higher ash content than stems, contributing to the reduction of the HHV. Despite this, the HHV was not affected by the contamination either in stems or in leaves. On the other hand, while in leaves, the nitrogen content was not affected by the contaminants, in stems, nitrogen content increased for almost all studied contaminants.

In terms of biomass composition, ash content and HHV are necessary to determine the potential utilization of biomass in thermochemical processes. The literature related values for switchgrass ash content and HHV indicates that this crop has the potential for biofuels and bioenergy production (Hu et al., 2010). In this experiment, variations in *Arundo donax* and *Panicum virgatum* HHV were not detected when compared to literature (in the range 16.4–21.9 $MJ.kg^{-1}$ for *Panicum virgatum* (Lemus et al., 2008; Yan et al., 2010) and in the range 17.2 and 19.0 $MJ.kg^{-1}$ for *Arundo donax* (Oginni et al., 2019; Saikia et al., 2015)). Since switchgrass ash and giant reed (stems), ash content suffered low variations, utilizing these biomasses in thermochemical conversion processes becomes an option. However, this option is not valid for giant reed leaves, with higher ash content and reduced HHV compared to stems. Therefore, they do not become feasible for thermochemical conversion. The ash contents observed in switchgrass aerial fraction and giant reed stems are also aligned with the values described in the literature (for giant reed stems, in the range of 4.8–7.4% (Fernando et al., 2016; Yang et al., 2020), and 3.9–8.2% for switchgrass (Ogden et al., 2010; Tang et al., 2020)). Despite the values in these experiments follow literature, they were unexpected since the experiments were designed in pots (having a lower crop development), while in the literature, the biomass came from field experiments.

3.3.2 Nitrogen content

The analysis of parameters is essential to determine the further utilization of the contaminated biomass. The nitrogen content indicates the potential for the crop to have NOx residues when submitted to thermochemical processes, indicating the impact of these conversion processes on GHG emissions. Determining phosphorus is important to balance its availability for the next year. Since this element is essential to plant development, determining the plants' uptake is essential to determine its application as fertilizer for the subsequent year.

Giant reed and switchgrass N content are presented in Table 3.10 and Table 3.11, respectively.

Table 3.9. Giant reed's N content

	Giant reed - First year		**Giant reed - Second year**		
	Leaves	**Stems**	**Leaves**	**Stems**	**Rhizomes and Roots**
			N (% dw)		
Control	2.37 ± 0.01 ab,A	0.79 ± 0.02 bcd,A	2.47 ± 0.77 a,A	0.77 ± 0.05 de,A	0.90 ± 0.12 b
Zn$_{450}$	2.30 ± 0.08 ab,A	0.68 ± 0.01 bcd,A	1.67 ± 0.44 ab,A	1.57 ± 0.39 bc,A	0.80 ± 0.09 b
Zn$_{900}$	2.26 ± 0.02 ab,A	0.66 ± 0.02 cd,B	1.07 ± 0.12 b,A	0.70 ± 0.05 e,A	0.80 ± 0.17 b
Cr$_{300}$	1.43 ± 0.02 b,A	0.60 ± 0.07 d,A	1.42 ± 0.00 ab,A	1.02 ± 0.00 cde,A	1.23 ± 0.00 ab
Cr$_{600}$	1.66 ± 0.03 ab,A	1.71 ± 0.3 a,A	1.67 ± 0.00 ab,A	1.69 ± 0.00 b,A	1.06 ± 0.00 ab
Pb$_{450}$	1.55 ± 0.12 b,A	0.76 ± 0.04 bcd,A	1.89 ± 0.58 ab,A	0.92 ± 0.05 de,A	0.83 ± 0.02 b
Pb$_{900}$	1.99 ± 0.11 ab,A	0.73 ± 0.04 bcd,A	1.58 ± 0.30 ab,A	1.17 ± 0.29 cde,A	0.89 ± 0.07 b
Cd$_{4}$	2.03 ± 0.42 ab,A	0.78 ± 0.04 bcd,A	1.58 ± 0.00 ab,A	1.71 ± 0.00 b,A	1.56 ± 0.00 a
Cd$_{8}$	1.79 ± 0.18 ab,A	0.82 ± 0.13 bcd,A	1.97 ± 0.00 ab,B	2.31 ± 0.00 a,A	1.10 ± 0.00 ab
Ni$_{110}$	1.83 ± 0.04 ab,A	0.83 ± 0.00 bcd,A	1.51 ± 0.20 ab,A	1.30 ± 0.35 cd,A	1.08 ± 0.70 ab
Ni$_{220}$	2.13 ± 0.14 ab,A	0.98 ± 0.06 bc,A	1.65 ± 0.20 ab,A	1.21 ± 0.45 cde,A	0.90 ± 0.07 b
Cu$_{200}$	2.23 ± 0.16 ab,A	1.00 ± 0.08 b,A	1.71 ± 0.19 ab,A	1.07 ± 0.58 de,A	0.77 ± 0.01 b
Cu$_{400}$	2.54 ± 0.25 a,A	0.92 ± 0.02 bcd,A	1.49 ± 0.55 ab,A	1.21 ± 0.35 cde,A	0.93 ± 0.01 b

Different lower-case letters indicate statistical significance ($p < 0.05$) among treatments per year. Different capital letters indicate statistical significance ($p < 0.05$) between first and second year results for identical treatments. Zn$_{450}$ and Zn$_{900}$, 450 and 900 mg Zn kg^{-1}. Cr$_{300}$ and Cr$_{600}$, 300 and 600 mg Cr kg^{-1}. Pb$_{450}$ and Pb$_{900}$, 450 and 900 mg Pb kg^{-1}. Cd$_{4}$ and Cd$_{8}$, 4 and 8 mg Cd kg^{-1}. Ni$_{110}$ and Ni$_{220}$, 110 and 220 mg Ni kg^{-1}. Cu$_{200}$ and Cu$_{400}$, 200 and 400 mg Cu kg^{-1}

Table 3.10. Switchgrass' N content

	Switchgrass – First year	**Switchgrass – Second year**	
	Aerial Biomass	**Aerial Biomass**	**Underground Biomass**
		N (% dw)	
Control	1.6 ± 0.1 a	0.71 ± 0.01 b	1.29 ± 0.37 c
Zn$_{450}$	1.6 ± 0.2 a	0.74 ± 0.01 b	1.75 ± 0.01 b
Zn$_{900}$	1.6 ± 0.2 a	0.76 ± 0.05 b	1.23 ± 0.20 b
Cr$_{300}$	-	-	-
Cr$_{600}$	-	-	-
Pb$_{450}$	1.7 ± 0.2 a	0.70 ± 0.01 b	1.57 ± 0.06 c
Pb$_{900}$	1.7 ± 0.2 a	0.54 ± 0.02 b	1.65 ± 0.39 b
Cd$_{4}$	1.5 ± 0.3 a	1.23 ± 0.00 a	1.63 ± 0.00 b
Cd$_{8}$	1.5 ± 0.3 a	1.11 ± 0.00 a	3.12 ± 0.00 a
Ni$_{110}$	1.6 ± 0.2 a	0.78 ± 0.33 b	1.71 ± 0.48 b
Ni$_{220}$	-	0.89 ± 0.08 b	1.84 ± 0.10 b
Cu$_{200}$	-	0.70 ± 0.02 b	1.26 ± 0.18 c
Cu$_{400}$	-	0.73 ± 0.26 b	1.18 ± 0.54 bc

Different lower-case letters indicate statistical significance ($p < 0.05$) among treatments per year. Zn$_{450}$ and Zn$_{900}$, 450 and 900 mg Zn kg^{-1}. Cr$_{300}$ and Cr$_{600}$, 300 and 600 mg Cr kg^{-1}. Pb$_{450}$ and Pb$_{900}$, 450 and 900 mg Pb kg^{-1}. Cd$_{4}$ and Cd$_{8}$, 4 and 8 mg Cd kg^{-1}. Ni$_{110}$ and Ni$_{220}$, 110 and 220 mg Ni kg^{-1}. Cu$_{200}$ and Cu$_{400}$, 200 and 400 mg Cu kg^{-1}

Regarding the first growing cycle, the N content in giant reed, presented in was affected in the leaves In Cr trials ($p = 0.0005$ and $p = 0.0022$ for Cr$_{300}$ and Cr$_{600}$ respectively), Zn$_{900}$ ($p = 0.0345$), and Pb$_{450}$ (p =

0.0219). Considering the stems, only Zn_{450} ($p = 0.0283$), Cr_{600} ($p = 0.0015$), and Cu_{400} ($p = 0.0299$) were affected. In the second growing cycle, it was possible to observe that only the stems of Cd_8 ($p = 0.0382$) trials were affected. However, from the first to the second year, the N content suffers statistical changes only in leaves of the Zn_{900} trial ($P = 0.0098$). Nitrogen content in the leaves was higher than in the stems for all contaminants. The nitrogen level in the roots was not affected by the contaminants in the ground.

Nitrogen is an essential nutrient for plants due to its amino groups, which constitute proteins and play a crucial role in plant metabolism (Wahocho et al., 2016). In addition, nitrogen content Is also linked to NO_x emissions, which can cause environmental problems like ground-level ozone, acid rain, degradation of forests, and an increase in the greenhouse effect (Srivastava et al., 2005). The nitrogen content also can limit biomass utilization in energy conversion processes. The most tolerable process through the thermochemical path is combustion. Some combustion processes work with nitrogen contents up to 15% of nitrogen. The limit decreases to 2.5% for pyrolysis and 1% for gasification (S2 Biom, 2016). Considering the thermochemical conversion processes, the studied switchgrass and giant reed trials are suitable for combustion and pyrolysis processes. However, switchgrass and giant reed leaves are suitable only for circulating fluidized bed reactors for gasification.

The increase in nitrogen levels witnessed in switchgrass and giant reed stems due to the heavy metals may represent an obstacle to using contaminated biomass in thermochemical processes. The gases generated in these processes are directly related to human and environmental problems (Casagrande et al., 2020), and an increment in the emissions due to contamination may represent a limitation for their use in pyrolysis, gasification, and combustion plants. However, despite the increase in switchgrass and giant reed stems' N content, their biomass can be used in pyrolysis and combustion processes since the maximum N content in these processes should be 2.5% (pyrolysis) and up to 3% (domestic stoves or pellet burners for heat) or up to 15% (fixed bed combustion) (Gomes et al., 2018; S2 Biom, 2016). For gasification, however, some processes–e.g., bubbling fluidized beds and dual fluidized beds–limit the N content of the feedstock to 1% (Gomes et al. 2018; S2 Biom 2016), which will limit the use of giant reed stems harvested from contaminated soils (except for giant reed from Cr_{300} pots) and the switchgrass collected from Cd_8 pots. Processes such as circulating fluidized beds for CHP (combined heat and power, gas engine) and circulating fluidized beds for syngas production have a higher limit of 2% in N content, and those processes can be applied to the biomass harvested from contaminated soils that exceed the limit of 1% N. Giant reed N content observed in this experiment agrees with literature that reports values from 0.3 to 1.5% (Basso et al., 2005; Saikia et al., 2015; Vernersson et al., 2002). Only biomass stems from Cd pots showed a higher value than the range presented. This relation between Cd and N may result from the strong synergic interaction between these two elements due to the formation of very stable complexes between proteins and Cd. Cd has high electronegativity values and can bond easily with protein sulfur (Kabata-Pendias, 2011). Therefore, the presence of Cd in the soil can stimulate the uptake and mobilization of N in the plant. For switchgrass, the values observed in this experiment are also in line with what is observed in the literature, with values ranging from 0.35 to 0.88% (Ogden et al., 2010; Tang et al., 2020). Interestingly, the same relation between Cd and N was also observed with switchgrass in Cd-contaminated pots, where switchgrass biomass presented a higher N content than the values presented in literature and control pots.

3.3.3 Other macroelements

The contents in terms of P, K, Ca, Mg, Na, and other elements are also important to evaluate the potential of biomass to be used in thermochemical processes. Indeed, they affect fly ash emissions, deposit formation, and ash handling/utilization/disposal. The values for these elements varied among the different biomasses. This chapter does not evaluate the heavy metals (Zn, Cr, Pb, Cd, Ni, and Cu), which will be presented and discussed in Chapter 3.4 Phosphorus content for both giant reed and switchgrass are presented in Table 3.11 and Table 3.12, respectively.

Table 3.11. Giant reed's P content

	Giant reed - First year		**Giant reed - Second year**		
	Leaves	**Stems**	**Leaves**	**Stems**	**Rhizomes and Roots**
			P (% dw)		
Control	0.129 ± 0.005 b,A	0.052 ± 0.005 b,A	0.199 ± 0.000 b,A	0.089 ± 0.011 b,A	0.178 ± 0.012 a
Zn_{450}	0.108 ± 0.000 b,A	0.049 ± 0.000 b,A	0.108 ± 0.015 d,A	0.206 ± 0.077 a,A	0.092 ± 0.033 c
Zn_{900}	0.121 ± 0.017 a,A	0.053 ± 0.008 b,A	0.131 ± 0.026 c,A	0.065 ± 0.004 b,A	0.072 ± 0.058 c
Cr_{300}	0.110 ± 0.109 abc,A	0.105 ± 0.013 b,A	0.259 ± 0.000 b,A	0.048 ± 0.021 b,A	0.080 ± 0.010 c
Cr_{600}	0.208 ± 0.030 a,A	0.078 ± 0.009 b,A	0.369 ± 0.057 ab,A	0.087 ± 0.014 b,A	0.134 ± 0.003 b
Pb_{450}	0.085 ± 0.004 c,A	0.063 ± 0.002 b,A	0.112 ± 0.004 c,B	0.063 ± 0.004 b,A	0.093 ± 0.008 c
Pb_{900}	0.144 ± 0.031 b,A	0.058 ± 0.001 b,A	0.108 ± 0.009 c,A	0.083 ± 0.005 b,A	0.093 ± 0.013 c
Cd_4	0.059 ± 0.001 d,B	0.068 ± 0.010 b,A	0.459 ± 0.176 a,A	0.194 ± 0.067 a,A	0.076 ± 0.023 c
Cd_8	0.051 ± 0.005 d,A	0.052 ± 0.002 b,A	0.218 ± 0.042 b,A	0.092 ± 0.025 b,A	0.123 ± 0.077 abc
Ni_{110}	0.145 ± 0.046 b,A	0.063 ± 0.002 b,A	0.227 ± 0.080 bc,A	0.062 ± 0.030 bc,A	0.133 ± 0.060 abc
Ni_{220}	0.138 ± 0.047 b,A	0.061 ± 0.010 b,A	0.337 ± 0.054 ab,A	0.058 ± 0.016 bc,A	0.061 ± 0.019 c
Cu_{200}	0.197 ± 0.011 b,A	0.076 ± 0.002 b,B	0.113 ± 0.007 d,B	0.215 ± 0.010 a,A	0.113 ± 0.002 b
Cu_{400}	0.127 ± 0.019 b,A	0.337 ± 0.006 a,A	0.207 ± 0.032 bc,A	0.050 ± 0.007 c,B	0.089 ± 0.007 c

Different lower-case letters indicate statistical significance ($p < 0.05$) among treatments per year. Different capital letters indicate statistical significance ($p < 0.05$) between first and second year results for identical treatments. Zn_{450} and Zn_{900}, 450 and 900 mg Zn kg^{-1}. Cr_{300} and Cr_{600}, 300 and 600 mg Cr kg^{-1}. Pb_{450} and Pb_{900}, 450 and 900 mg Pb kg^{-1}. Cd_4 and Cd_8, 4 and 8 mg Cd kg^{-1}. Ni_{110} and Ni_{220}, 110 and 220 mg Ni kg^{-1}. Cu_{200} and Cu_{400}, 200 and 400 mg Cu kg^{-1}

In giant reed, phosphorus suffered a decrease in the first year, being more affected in giant reed leaves, Zn$_{450}$ (p = 0.0463), Pb$_{450}$ (p = 0.017), Cd$_4$ (p = 0.0046), Cd$_8$ (p = 0.0076), and Cu$_{200}$ (p = 0.0301) than in stems, Cu$_{400}$ (p = 0.0007). In the second year, only giant reed’s belowground biomass was affected in terms of P uptake. On the other hand, switchgrass was not affected by the contaminants. Phosphorus also increased in leaves in Cd$_4$, Cr$_{600}$, and Ni$_{200}$ trials and Cd$_4$ and Cu$_{200}$ regarding the stems. In general, phosphorus content was higher in leaves than in stems. Regarding K and Ca, a trend indicating the increase of these two elements in contaminated trials was observed, while Mg was not affected by the contaminant's presence, and Na decreased its concentration in both stems and leaves. Magnesium and sodium content was also higher in leaves than in stems, while potassium did not present any clear pattern between leaves and stems.

Table 3.12. Switchgrass' P content

	Switchgrass – First year	**Switchgrass – Second year**	
	Aerial Biomass	**Aerial Biomass**	**Underground Biomass**
		P (% dw)	
Control	0.146 ± 0.006 b	0.052 ± 0.006 b	0.350 ± 0.228 a
Zn$_{450}$	0.150 ± 0.010 b	0.042 ± 0.001 b	0.085 ± 0.029 a
Zn$_{900}$	0.191 ± 0.010 a	0.039 ± 0.001 b	0.469 ± 0.112 a
Cr$_{300}$	-	-	-
Cr$_{600}$	-	-	-
Pb$_{450}$	0.182 ± 0.010 a	0.042 ± 0.000 b	0.079 ± 0.000 a
Pb$_{900}$	0.139 ± 0.015 bc	0.045 ± 0.002 b	0.099 ± 0.040 a
Cd$_4$	0.119 ± 0.006 c	0.342 ± 0.109 a	0.536 ± 0.132 a
Cd$_8$	0.127 ± 0.007 c	0.180 ± 0.038 a	0.850 ± 0.498 a
Ni$_{110}$	0.167 ± 0.008 b	0.192 ± 0.038 a	0.455 ± 0.071 a
Ni$_{220}$	-	0.344 ± 0.163 a	0.129 ± 0.004 a
Cu$_{200}$	-	0.033 ± 0.002 b	0.078 ± 0.022 a
Cu$_{400}$	-	0.053 ± 0.008 b	0.046 ± 0.008 a

Different lower-case letters indicate statistical significance ($p < 0.05$) among treatments per year. Zn$_{450}$ and Zn$_{900}$, 450 and 900 mg Zn kg^{-1}. Cr$_{300}$ and Cr$_{600}$, 300 and 600 mg Cr kg^{-1}. Pb$_{450}$ and Pb$_{900}$, 450 and 900 mg Pb kg^{-1}. Cd$_4$ and Cd$_8$, 4 and 8 mg Cd kg^{-1}. Ni$_{110}$ and Ni$_{220}$, 110 and 220 mg Ni kg^{-1}. Cu$_{200}$ and Cu$_{400}$, 200 and 400 mg Cu kg^{-1}

Potassium accumulation for giant reed and switchgrass can be seen in Table 3.13 and Table 3.14, respectively. In the first year, the aerial fraction of both giant reed and switchgrass accumulated similar amounts of K. In giant reed, this accumulation was equally divided by leaves and stems. In the second year, switchgrass K absorption was affected in the aerial part in Pb contamination and the lowest Cu contamination trial, while considering the aboveground part, Ni contamination, and the highest level of Cd trial. For giant reed, there is no statistical difference in K absorption regarding the heavy metal’s presence in soil. Alharby and collaborators (Alharby et al., 2022) noticed in an experiment using quinoa that synergetic treatment using K and Si increased the plant's tolerance to Cd and Pb, reducing the oxidative effects caused by these heavy metals. However, an antagonist behavior regarding Pb and Cd, and K absorption indicates that these elements' uptake mechanisms are the same, and these two heavy metals compete with K regarding planting uptake.

Table 3.13. Giant reed's K content

	Giant reed - First year		**Giant reed - Second year**		
	Leaves	**Stems**	**Leaves**	**Stems**	**Rhizomes and Roots**
	K (g.kg^{-1},dw)				
Control	13.9 ± 0.9a	16.8 ± 1.3a	13.9 ± 0.9a	14.1 ± 2.6ab	5.9 ± 1.5ab
Zn_{450}	14.8± 1.9a	15.2 ± 1.8a	14.8 ± 1.9a	19.4 ± 2.5ab	4.3 ± 1.7b
Zn_{900}	14.6 ± 1.4a	15.2 ± 1.6a	14.6 ± 1.4a	19.7 ± 3.0ab	6.7 ± 2.0ab
Cr_{300}	16.1 ± 2.1a	17.3 ± 1.9a	16.1 ± 2.1a	16.4 ± 0.8ab	6.7 ± 1.6ab
Cr_{600}	15.8 ± 2.3a	17.5 ± 2.1a	15.8 ± 2.3a	19.6 ± 1.8a	4.5 ± 1.6ab
Pb_{450}	15.4 ± 2.1a	15.8 ± 1.9a	15.4 ± 2.1a	18.8 ± 0.7a	9.6 ± 1.5a
Pb_{900}	16.2 ± 2.2a	16.2 ± 2.2a	16.2 ± 2.2a	18.4 ± 0.7ab	7.5 ± 0.9ab
Cd_4	15.2 ± 1.8a	15.4 ± 1.7a	15.2 ± 1.8a	15.8 ± 0.9ab	8.8 ± 2.6ab
Cd_8	14.2 ± 1.7a	15.8 ± 1.8a	14.2 ±1.7a	15.2 ± 0.8b	5.1 ± 2.6ab
Ni_{110}	16.5 ± 1.9a	17.2 ± 1.9a	16.5 ± 1.9a	14.3 ± 2.3b	6.8 ± 0.9ab
Ni_{220}	15.4 ± 1.8a	16.9 ± 1.9a	15.4 ± 1.8a	15.8 ± 1.5a	5.6 ± 0.9ab
Cu_{200}	16.2 ± 1.6a	17.5 ± 1.5a	16.2 ± 1.6a	18.1 ± 2.2ab	6.2 ± 1.3b
Cu_{400}	15.8 ± 1.6a	17.2 ± 1.3a	15.8 ± 1.6a	15.0 ± 2.2ab	4.4 ± 1.3b

Different lower-case letters indicate statistical significance ($p < 0.05$) among treatments per year. Zn_{450} and Zn_{900}, 450 and 900 mg Zn kg^{-1}. Cr_{300} and Cr_{600}, 300 and 600 mg Cr kg^{-1}. Pb_{450} and Pb_{900}, 450 and 900 mg Pb kg^{-1}. Cd_4 and Cd_8, 4 and 8 mg Cd kg^{-1}. Ni_{110} and Ni_{220}, 110 and 220 mg Ni kg^{-1}. Cu_{200} and Cu_{400}, 200 and 400 mg Cu kg^{-1}

Table 3.14. Switchgrass' K content

	Switchgrass – First year	**Switchgrass – Second year**	
	Aerial Biomass	**Aerial Biomass**	**Underground Biomass**
		K ($g.kg^{-1}$,dw)	
Control	33 ± 1a	11.6 ±0.9ab	12.3 ± 3.7a
Zn_{450}	27 ± 4ab	9.0 ± 1.0bcd	11.5 ± 2.3a
Zn_{900}	25 ± 3b	10.8 ± 2.1abcd	11.8 ± 1.7a
Cr_{300}	-	-	-
Cr_{600}	-	-	-
Pb_{450}	31 ± 5ab	9.3 ± 0.8 bcd	9.9 ± 0.1a
Pb_{900}	30 ± 5ab	7.6 ± 2.1d	9.2 ± 0.8ab
Cd_4	28 ± 4ab	10.9 ± 0.9abc	10.5 ± 1.0a
Cd_8	28 ± 3ab	10.8 ± 1.2abcd	8.5 ± 2.3abc
Ni_{110}	36 ± 5a	11.5 ± 0.7ab	6.5 ± 0.3c
Ni_{220}		11.3 ± 0.9ab	7.2 ± 1.3bc
Cu_{200}	-	8.5 ± 2.7cd	10.6 ± 2.3ab
Cu_{400}	-	14.7 ± 3.1a	11.1 ± 2.4a

Different lower-case letters indicate statistical significance ($p < 0.05$) among treatments per year. Zn_{450} and Zn_{900}, 450 and 900 mg Zn kg^{-1}. Cr_{300} and Cr_{600}, 300 and 600 mg Cr kg^{-1}. Pb_{450} and Pb_{900}, 450 and 900 mg Pb kg^{-1}. Cd_4 and Cd_8, 4 and 8 mg Cd kg^{-1}. Ni_{110} and Ni_{220}, 110 and 220 mg Ni kg^{-1}. Cu_{200} and Cu_{400}, 200 and 400 mg Cu kg^{-1}.

Sodium accumulation is presented in Table 3.15 for giant reed and Table 3.16 for switchgrass. Sodium absorption was higher in giant reed than in switchgrass. In giant reed, the accumulation in stems was higher than in the leaves. In the second year, the accumulation of Na in the leaves had no significant alterations, while it decreased in Cr and Cd trials regarding the stems. However, sodium uptake in roots and rhizomes was not affected by the heavy metal's presence. In switchgrass, however, an increase in Na uptake in Cu trials was observed, while no significative alterations were noticed in roots and rhizomes. Despite indicating an antagonism behavior between Na and Cd, Na and Cr, and a synergetic behavior between Na and Cu, no literature confirming or disclosing these hypotheses was found.

Table 3.15. Giant reed's Na content

	Giant reed - First year		Giant reed - Second year		
	Leaves	**Stems**	**Leaves**	**Stems**	**Rhizomes and Roots**
	Na (g.kg^{-1},dw)				
Control	0.78 ± 0.06a	1.1 ± 0.2a	0.78 ± 0.06a	1.8 ± 0.3ab	1.8 ± 0.5ab
Zn$_{450}$	0.72 ± 0.12a	1.4 ± 0.2a	0.72 ± 0.12a	1.2 ± 0.2bc	1.4 ± 0.1b
Zn$_{900}$	0.74 ± 0.14a	1.3 ± 0.2a	0.74 ± 0.14a	1.2 ± 0.2bc	1.2 ± 0.0b
Cr$_{300}$	0.83 ± 0.17a	1.4 ± 0.3a	0.83 ± 0.17a	0.80 ± 0.23c	2.2 ± 0.3a
Cr$_{600}$	0.91 ± 0.19a	1.6 ± 0.3a	0.91 ± 0.19a	0.77 ± 0.24c	2.6 ± 0.3a
Pb$_{450}$	0.76 ± 0.13a	1.0 ± 0.3a	0.76 ± 0.13a	1.3 ± 0.4abc	1.3 ± 0.1b
Pb$_{900}$	0.75 ± 0.15a	1.1 ± 0.2a	0.75 ± 0.15a	1.7 ± 0.1a	1.4 ± 0.2
Cd$_{4}$	0.82 ± 0.18a	1.5 ± 0.2a	0.82± 0.18a	0.75 ± 0.16c	2.0 ± 0.2a
Cd$_{8}$	0.94 ± 0.25a	1.6 ± 0.3a	0.94 ± 0.25a	0.87 ± 0.19c	2.0 ± 0.2a
Ni$_{110}$	0.79 ± 0.19a	1.4 ± 0.3a	0.79 ± 0.19a	1.2 ± 0.4abc	2.3 ± 0.3a
Ni$_{220}$	0.84 ±0.21a	1.3 ± 0.3a	0.84 ± 0.21a	1.3 ± 0.3abc	1.8 ± 0.3ab
Cu$_{200}$	0.85 ± 0.21a	1.4 ± 0.2a	0.85 ± 0.21a	1.6 ± 0.2ab	1.1 ± 0.5b
Cu$_{400}$	0.93 ± 0.22a	1.5 ± 0.3a	0.93 ± 0.22a	1.8 ± 0.2a	1.8 ± 0.5ab

Different lower-case letters indicate statistical significance ($p < 0.05$) among treatments per year. Zn$_{450}$ and Zn$_{900}$, 450 and 900 mg Zn kg^{-1}. Cr$_{300}$ and Cr$_{600}$, 300 and 600 mg Cr kg^{-1}. Pb$_{450}$ and Pb$_{900}$, 450 and 900 mg Pb kg^{-1}. Cd$_{4}$ and Cd$_{8}$, 4 and 8 mg Cd kg^{-1}. Ni$_{110}$ and Ni$_{220}$, 110 and 220 mg Ni kg^{-1}. Cu$_{200}$ and Cu$_{400}$, 200 and 400 mg Cu kg^{-1}.

Table 3.16. Switchgrass' Na content

	Switchgrass – First year	Switchgrass – Second year	
	Aerial Biomass	**Aerial Biomass**	**Underground Biomass**
	Na (g.kg^{-1},dw)		
Control	1.4 ± 0.1a	1.09 ± 0.05b	2.7 ± 1.3ab
Zn$_{450}$	1.3 ± 0.2a	0.65 ± 0.10c	2.6 ± 1.1ab
Zn$_{900}$	1.4 ± 0.2a	0.78 ± 0.51bc	2.6 ± 0.9ab
Cr$_{300}$	-	-	-
Cr$_{600}$	-	-	-
Pb$_{450}$	1.3 ± 0.2a	0.72 ± 0.28bc	3.5 ± 1.0a
Pb$_{900}$	1.4 ± 0.3a	1.28 ± 0.5abc	1.7 ± 0.7ab
Cd$_{4}$	1.4 ± 0.2a	0.99 ± 0.09b	2.1 ± 0.4ab
Cd$_{8}$	1.5 ± 0.3a	1.18 ± 1.08b	3.0 ± 1.0ab
Ni$_{110}$	1.2 ± 0.2a	0.99 ± 0.07b	1.9 ± 0.6ab
Ni$_{220}$	1.4 ± 0.3a	1.13 ± 0.08b	3.0 ± 1.0ab
Cu$_{200}$	-	1.70 ± 0.27a	1.7 ± 0.4b
Cu$_{400}$	-	2.24 ± 0.50a	2.5 ± 1.3ab

Different lower-case letters indicate statistical significance ($p < 0.05$) among treatments per year. Zn$_{450}$ and Zn$_{900}$, 450 and 900 mg Zn kg^{-1}. Cr$_{300}$ and Cr$_{600}$, 300 and 600 mg Cr kg^{-1}. Pb$_{450}$ and Pb$_{900}$, 450 and 900 mg Pb kg^{-1}. Cd$_{4}$ and Cd$_{8}$, 4 and 8 mg Cd kg^{-1}. Ni$_{110}$ and Ni$_{220}$, 110 and 220 mg Ni kg^{-1}. Cu$_{200}$ and Cu$_{400}$, 200 and 400 mg Cu kg^{-1}.

Magnesium accumulation for giant reed is observed in Table 3.17. It is possible to see that in the aerial part of the first year, stems and leaves accumulated similar amounts of Mg. The accumulation of Mg for switchgrass is observed in Table 3.18. Switchgrass' aerial fraction in the first year accumulated half of the amount accumulated for giant reed. In the second year, however, with the root system better established, the Mg uptake showed some differences for switchgrass and giant reed trials. For giant reed, the aerial uptake improved in the leaves for Zn, Pb, and Cu, in the lower treatments and for Cd in the high treatment.

Regarding roots and rhizomes, Zn trials were affected in the lowest concentration, with a lower uptake of heavy metals. For the switchgrass aerial part, Mg content was negatively affected by Zn and Cu in the lower concentration and Pb in the highest concentration, while for roots and rhizomes, Zn in the lowest concentration was the only trial significantly affected by the heavy metal's presence. A behavior was noticed in the lower Zn concentrations, in which switchgrass and giant reed were affected. The reason could be related to the saturation of the root system with the heavy metal, reducing the Mg uptake content. However, the effect in leaves was already observed in the literature (Shivay et al., 2016), indicating a synergetic behavior between Zn and Mg.

Table 3.17. Giant reed's Mg content

	Giant reed - First year		**Giant reed - Second year**		
	Leaves	**Stems**	**Leaves**	**Stems**	**Rhizomes and Roots**
			Mg (g.kg^{-1},dw)		
Control	5.8 ± 0.2a	5.4 ± 0.6a	4.0 ± 0.8b	3.0 ± 0.2bc	4.9 ± 1.4ab
Zn_{450}	6.1 ± 1.2a	5.8 ± 1.3a	6.9 ± 1.3a	3.7 ± 0.6ab	2.0± 1.6c
Zn_{900}	5.6 ± 0.9a	6.1 ± 1.0a	5.1 ± 1.7ab	3.5 ± 1.0 abcd	4.3 ± 1.7abc
Cr_{300}	5.6 ± 1.0a	6.4 ± 0.9a	4.7 ± 1.3ab	3.8 ± 1.3abcd	3.5 ± 1.2abc
Cr_{600}	6.3 ± 0.9a	6.4 ± 1.2a	6.5 ± 1.7ab	4.3 ± 0.9a	2.1 ± 1.1bc
Pb_{450}	5.9 ± 0.7a	5.6 ± 0.8a	6.9 ± 1.1a	3.0 ± 0.9abcd	2.7 ± 1.7abc
Pb_{900}	5.8 ± 0.9a	5.5 ± 0.7a	6.6 ± 1.8ab	1.8 ± 0.7d	6.0 ± 2.4ac
Cd_4	6.2 ± 0.8a	6.1 ± 0.7a	5.2 ± 1.2ab	3.3 ± 0.7abc	4.2 ± 0.1a
Cd_8	6.2 ± 1.2a	6.6 ± 0.8a	6.5 ± 1.3a	4.4 ± 1.2ab	4.3 ± 0.2a
Ni_{110}	6.1 ± 0.9a	6.3 ± 0.8a	4.9 ± 1.5ab	2.7 ± 0.8abcd	3.4 ± 1.7abc
Ni_{220}	6.5 ± 0.9a	6.7 ± 0.9a	7.5 ± 1.8a	3.2 ± 0.7abcd	5.1 ± 1.2abc
Cu_{200}	6.4 ± 0.9a	6.3 ± 1.2a	5.6 ± 0.3a	2.2 ± 0.6cd	4.3 ± 0.4a
Cu_{400}	6.6 ± 0.8a	6.5 ± 0.9a	6.0 ± 1.6ab	3.1 ± 0.7abcd	3.8 ± 0.7abc

Different lower-case letters indicate statistical significance ($p < 0.05$) among treatments per year. Zn_{450} and Zn_{900}, 450 and 900 mg Zn kg^{-1}. Cr_{300} and Cr_{600}, 300 and 600 mg Cr kg^{-1}. Pb_{450} and Pb_{900}, 450 and 900 mg Pb kg^{-1}. Cd_4 and Cd_8, 4 and 8 mg Cd kg^{-1}. Ni_{110} and Ni_{220}, 110 and 220 mg Ni kg^{-1}. Cu_{200} and Cu_{400}, 200 and 400 mg Cu kg^{-1}

Table 3.18. Switchgrass' Mg content

	Switchgrass – First year	**Switchgrass – Second year**	
	Aerial Biomass	**Aerial Biomass**	**Underground Biomass**
		Mg (g.kg^{-1},dw)	
Control	6.4 ± 0.6a	10.6 ± 1.0a	6.5 ± 0.6ab
Zn$_{450}$	6.3 ± 1.2a	7.6 ± 1.0cde	7.1 ± 1.3ab
Zn$_{900}$	7.4 ± 1.0a	9.6 ± 1.0abc	7.0 ± 0.6a
Cr$_{300}$	-	-	-
Cr$_{600}$	-	-	-
Pb$_{450}$	6.8 ± 1.3a	8.7 ± 0.5bcd	4.5 ± 0.6cd
Pb$_{900}$	7.3 ± 1.3a	8.0 ± 0.4c	4.3 ± 0.5cd
Cd$_{4}$	7.8 ± 1.4a	9.3 ± 0.9abcd	5.3 ± 0.8bc
Cd$_{8}$	8.1 ± 1.5a	8.8 ± 1.2abcd	3.8 ± 1.3cd
Ni$_{110}$	8.1 ± 1.6a	9.5 ± 0.7ab	4.8 ± 0.7cd
Ni$_{220}$	8.3 ± 1.7a	8.4 ± 1.4abcde	3.3 ± 1.0d
Cu$_{200}$	-	5.8 ± 1.4e	5.4 ± 0.8bc
Cu$_{400}$	-	8.6 ± 1.7 abcde	3.9 ± 1.1 cd

Different lower-case letters indicate statistical significance ($p < 0.05$) among treatments per year. Zn$_{450}$ and Zn$_{900}$, 450 and 900 mg Zn kg^{-1}. Cr$_{300}$ and Cr$_{600}$, 300 and 600 mg Cr kg^{-1}. Pb$_{450}$ and Pb$_{900}$, 450 and 900 mg Pb kg^{-1}. Cd$_{4}$ and Cd$_{8}$, 4 and 8 mg Cd kg^{-1}. Ni$_{110}$ and Ni$_{220}$, 110 and 220 mg Ni kg^{-1}. Cu$_{200}$ and Cu$_{400}$, 200 and 400 mg Cu kg^{-1}

Calcium absorption is presented for giant reed in Table 3.19 and switchgrass in Table 3.20. The accumulation in the aboveground fraction for the first growing cycle was higher in giant reed than in switchgrass. In giant reed, leaves accumulated a higher amount of Ca than stems. In the second year, giant reed leaves and roots and rhizomes Ca uptake was not significantly affected by the presence of the contaminants. However, an increase in Ca absorption was observed in stems with the highest levels of Cu and Pb. For switchgrass, however, regarding the aerial biomass, calcium concentration increased in the highest level of Zn while decreased for Cu trials. Regarding the belowground biomass, the most noticeable effect occurred in Cd trials, where an increase in Ca uptake occurred, suggesting a synergetic behavior between Cd and Ca. Opposite behavior considering what was observed in the literature, suggests that the carriers mechanisms of Cd and Na ions are the same, causing competition between the heave metals that should decrease the Ca absorption (Kabata-Pendias, 2011).

Table 3.19. Giant reed's Ca content

	Giant reed - First year		**Giant reed - Second year**		
	Leaves	**Stems**	**Leaves**	**Stems**	**Rhizomes and Roots**
	Ca (g.kg^{-1},dw)				
Control	8.9 ± 0.5a	5.7 ± 0.7a	8.9 ± 0.5a	2.3 ±0.1b	11 ± 5abc
Zn_{450}	8.6 ± 1.2a	5.3 ± 0.8a	8.6 ± 1.2a	1.8 ± 0.4b	8.5 ± 2.1c
Zn_{900}	8.1 ± 0.8a	5.4 ± 0.7a	8.1 ± 0.8a	2.3 ± 0.4ab	10 ± 4abc
Cr_{300}	8.5 ± 1.3a	6.2 ± 0.9a	8.5 ± 1.3a	2.4 ± 0.2ab	10 ± 2bc
Cr_{600}	9.6 ± 1.9a	6.3 ± 1.2a	9.6 ± 1.9a	1.9 ± 0.2b	7 ± 2c
Pb_{450}	8.6 ± 0.9a	5.3 ± 0.5a	8.6 ± 0.9a	2.4 ± 0.3ab	12 ± 4abc
Pb_{900}	8.8 ± 0.8a	5.3 ± 0.5a	8.8 ± 0.8a	2.8 ± 0.2a	18 ± 4a
Cd_4	8.2 ± 0.8a	5.5 ± 0.2a	8.2 ± 0.8a	2.6 ± 0.8ab	12 ± 4abc
Cd_8	9.4 ± 0.9a	5.6 ± 0.4a	9.4 ± 0.9a	2.0 ± 0.7ab	17 ± 5ab
Ni_{110}	9.3 ± 1.2a	5.8 ± 0.5a	9.3 ± 1.2a	2.6 ± 0.5ab	11 ± 5abc
Ni_{220}	9.5 ± 1.4a	6.0 ± 0.8a	9.5 ± 1.4a	2.4 ± 0.5ab	10 ± 4abc
Cu_{200}	8.4 ± 1.2a	6.2 ± 0.7a	8.4 ± 1.2a	2.4 ± 0.5ab	18 ± 3a
Cu_{400}	9.2 ± 1.1a	6.3 ± 1.2a	9.2 ± 1.1a	3.2 ± 0.5a	17 ± 2a

Different lower-case letters indicate statistical significance ($p < 0.05$) among treatments per year. Zn_{450} and Zn_{900}, 450 and 900 mg Zn kg^{-1}. Cr_{300} and Cr_{600}, 300 and 600 mg Cr kg^{-1}. Pb_{450} and Pb_{900}, 450 and 900 mg Pb kg^{-1}. Cd_4 and Cd_8, 4 and 8 mg Cd kg^{-1}. Ni_{110} and Ni_{220}, 110 and 220 mg Ni kg^{-1}. Cu_{200} and Cu_{400}, 200 and 400 mg Cu kg^{-1}

Table 3.20. Switchgrass' Ca content

	Switchgrass – First year	**Switchgrass – Second year**	
	Aerial Biomass	**Aerial Biomass**	**Underground Biomass**
	Ca (g.kg^{-1},dw)		
Control	12 ± 1a	11 ± 2bcd	9.0 ± 0.2c
Zn_{450}	11 ± 2a	16 ± 3ab	10 ± 2bcd
Zn_{900}	12 ± 2a	21 ± 5a	12 ± 2b
Cr_{300}	-	-	-
Cr_{600}	-	-	-
Pb_{450}	12 ± 2a	18 ± 5ab	7.1 ± 0.5d
Pb_{900}	13 ± 2a	12 ± 3bcd	13 ± 2b
Cd_4	14 ± 2a	12 ± 3bcd	14 ± 3b
Cd_8	15 ± 3a	14 ± 3abc	21 ± 3a
Ni_{110}	13 ± 2a	12 ± 2bcd	9.3 ± 2.1bcd
Ni_{220}	15 ± 3a	9 ± 2cde	6.0 ± 2.5d
Cu_{200}	-	6.6 ± 0.8e	11 ± 2bc
Cu_{400}	-	8.1 ± 2.1de	12 ± 3bc

Different lower-case letters indicate statistical significance ($p < 0.05$) among treatments per year. Zn_{450} and Zn_{900}, 450 and 900 mg Zn kg^{-1}. Cr_{300} and Cr_{600}, 300 and 600 mg Cr kg^{-1}. Pb_{450} and Pb_{900}, 450 and 900 mg Pb kg^{-1}. Cd_4 and Cd_8, 4 and 8 mg Cd kg^{-1}. Ni_{110} and Ni_{220}, 110 and 220 mg Ni kg^{-1}. Cu_{200} and Cu_{400}, 200 and 400 mg Cu kg^{-1}

Manganese uptake is present in Table 3.21 and Table 3.22 for giant reed and switchgrass, respectively. The presence of contamination in the soil did not affect the Mn absorption for both crops. However, the absorption of manganese was higher in switchgrass than in the giant reed's aerial part. The uptake of Mn by giant reed aerial fraction showed that the accumulation was two to three times higher in leaves than in stems. In the second year, switchgrass and giant reed aboveground and belowground biomass showed not to have Mn absorption affected by the presence of the contaminants in the soil.

Table 3.21. Giant reed's Mn content

	Giant reed - First year		**Giant reed - Second year**		
	Leaves	**Stems**	**Leaves**	**Stems**	**Rhizomes and Roots**
	Mn ($g.kg^{-1}$,dw)				
Control	21 ± 1a	7.4 ± 1.5a	13.4 ± 0.7a	2.7 ± 0.6a	11.6 ± 2.1a
Zn_{450}	21 ± 2a	8.1 ± 1.8a	13.9 ± 1.7a	3.1 ± 1.8a	11.5 ± 2.6a
Zn_{900}	21 ± 2a	6.9 ± 1.8a	13.4 ± 1.5a	2.9 ± 1.8a	12.1 ± 2.3a
Cr_{300}	21 ± 2a	8.3 ± 1.6a	15.1 ± 1.8a	4.9 ± 2.1a	15.1 ± 2.6a
Cr_{600}	22 ± 3a	7.9 ± 1.8a	16.1 ± 2.4a	4.3 ± 2.5a	14.9 ± 3.1a
Pb_{450}	19 ± 2a	8.3 ± 1.6a	13.2 ± 1.1a	3.3 ± 1.2a	13.1 ± 2.6a
Pb_{900}	21 ± 2a	8.4 ± 1.8a	13.1 ± 1.5a	3.5 ± 1.6a	11.5 ± 2.6a
Cd_4	19 ± 2a	8.2 ± 1.7a	14.6 ± 1.2a	4.2 ± 1.5a	12.6 ± 2.5a
Cd_8	21 ± 2a	6.8 ± 2.2a	14.8 ± 1.3a	4.8 ± 2.1a	13.1 ± 2.6a
Ni_{110}	20 ± 2a	7.2 ± 1.6a	15.2 ± 1.6a	4.2 ± 1.8a	12.8 ± 2.6a
Ni_{220}	21 ± 2a	7.8 ± 1.9a	15.3 ± 1.6a	4.8 ± 1.8a	13.5 ± 3.1a
Cu_{200}	22 ± 2a	7.2 ± 1.8a	15.3 ± 1.4a	4.2 ± 1.7a	13.2 ± 2.5a
Cu_{400}	25 ± 4a	7.6 ± 1.8a	16.1 ± 1.7a	3.6 ± 1.5a	14.1 ±2.6a

Different lower-case letters indicate statistical significance ($p < 0.05$) among treatments per year. Zn_{450} and Zn_{900}, 450 and 900 mg Zn kg^{-1}. Cr_{300} and Cr_{600}, 300 and 600 mg Cr kg^{-1}. Pb_{450} and Pb_{900}, 450 and 900 mg Pb kg^{-1}. Cd_4 and Cd_8, 4 and 8 mg Cd kg^{-1}. Ni_{110} and Ni_{220}, 110 and 220 mg Ni kg^{-1}. Cu_{200} and Cu_{400}, 200 and 400 mg Cu kg^{-1}

Table 3.22. Switchgrass' Mn content

	Switchgrass – First year	**Switchgrass – Second year**	
	Aerial Biomass	**Aerial Biomass**	**Underground Biomass**
	Mn (g.kg^{-1},dw)		
Control	40 ± 7a	10.1 ± 0.2a	10.1 ± 0.4a
Zn$_{450}$	45 ± 6a	10.3 ± 0.8a	9.8 ± 1.2a
Zn$_{900}$	45 ± 6a	9.7 ± 0.5a	9.1 ± 1.3a
Cr$_{300}$	-	-	-
Cr$_{600}$	-	-	-
Pb$_{450}$	45 ± 7a	10.5 ± 1.1a	10.9 ± 0.9a
Pb$_{900}$	44 ± 8a	10.8 ± 1.4a	10.5 ± 0.9a
Cd$_{4}$	47 ± 7a	12.5 ± 2.3a	10.6 ± 1.2a
Cd$_{8}$	41 ± 7a	11.5 ± 1.1a	11.5 ± 1.3a
Ni$_{110}$	49 ± 7a	11.2 ± 0.7a	10.7 ± 0.7a
Ni$_{220}$	42 ± 6a	12.1 ± 0.5a	10.8 ± 0.9a
Cu$_{200}$	-	12.4 ± 1.7a	10.8 ± 0.8a
Cu$_{400}$	-	11.9 ± 1.6a	10.8 ± 0.9a

Different lower-case letters indicate statistical significance ($p < 0.05$) among treatments per year. Zn$_{450}$ and Zn$_{900}$, 450 and 900 mg Zn kg^{-1}. Cr$_{300}$ and Cr$_{600}$, 300 and 600 mg Cr kg^{-1}. Pb$_{450}$ and Pb$_{900}$, 450 and 900 mg Pb kg^{-1}. Cd$_{4}$ and Cd$_{8}$, 4 and 8 mg Cd kg^{-1}. Ni$_{110}$ and Ni$_{220}$, 110 and 220 mg Ni kg^{-1}. Cu$_{200}$ and Cu$_{400}$, 200 and 400 mg Cu kg^{-1}

In the first year of both giant reed and switchgrass, the contamination did not affect the Fe absorption, as can be observed in Table 3.23 and Table 3.24 for giant reed and switchgrass, respectively. It was possible to observe also that Fe uptake was higher in the aerial fraction of switchgrass than in the aerial fraction of giant reed. Regarding the aboveground biomass of giant reed, iron uptake was higher in the leaves than in the stems. In the second year, iron uptake had not suffered any significant changes indicating a relation between the heavy metals presence and soil for both switchgrass and giant reed.

Table 3.23. Giant reed's Fe content

	Giant reed - First year		**Giant reed - Second year**		
	Leaves	**Stems**	**Leaves**	**Stems**	**Rhizomes and Roots**
	Fe (g.kg^{-1},dw)				
Control	125 ± 6a	31 ± 5a	171 ± 36a	9.5 ± 6.3a	1417 ± 135a
Zn$_{450}$	128 ± 18a	32 ± 7a	184 ± 28a	9.5 ± 4.6a	1402 ± 121a
Zn$_{900}$	131 ± 18a	34 ± 9a	165 ± 38a	10.1 ± 3.1a	1456 ± 126a
Cr$_{300}$	143 ± 18a	41 ± 9a	201 ± 48a	14.7 ± 4.2a	1621 ± 154a
Cr$_{600}$	143 ± 18a	38 ± 8a	193 ± 3.5a	15.2 ± 4.5a	1608 ± 161a
Pb$_{450}$	128 ± 18a	31 ± 8a	194 ± 25a	9.4 ± 3.8a	1396 ± 123a
Pb$_{900}$	142 ± 16a	36 ± 8a	168 ± 32a	10.8 ± 2.2a	1456 ± 145a
Cd$_{4}$	143 ± 12a	33 ± 7a	183 ± 22a	11.5 ± 3.6a	1524 ± 142a
Cd$_{8}$	115 ± 26a	38 ± 8a	151 ± 36a	15.1 ± 5.2a	1546 ± 156a
Ni$_{110}$	139 ±15a	35 ± 6a	193 ± 26a	12.4 ± 2.6a	1602 ± 151a
Ni$_{220}$	125 ± 17a	32 ± 7a	152 ± 37a	14.3 ± 4.3a	1615 ± 163a
Cu$_{200}$	143 ± 17a	41 ± 7a	201 ± 47a	12.2 ± 2.6a	1564 ± 151a
Cu$_{400}$	148 ± 18a	44 ± 8a	198 ± 36a	13.5 ± 4.6a	1584 ± 146a

Different lower-case letters indicate statistical significance ($p < 0.05$) among treatments per year. Zn$_{450}$ and Zn$_{900}$, 450 and 900 mg Zn kg^{-1}. Cr$_{300}$ and Cr$_{600}$, 300 and 600 mg Cr kg^{-1}. Pb$_{450}$ and Pb$_{900}$, 450 and 900 mg Pb kg^{-1}. Cd$_{4}$ and Cd$_{8}$, 4 and 8 mg Cd kg^{-1}. Ni$_{110}$ and Ni$_{220}$, 110 and 220 mg Ni kg^{-1}. Cu$_{200}$ and Cu$_{400}$, 200 and 400 mg Cu kg^{-1}

Table 3.24. Switchgrass' Fe content

	Switchgrass – First year	**Switchgrass – Second year**	
	Aerial Biomass	**Aerial Biomass**	**Underground Biomass**
	Fe (g.kg^{-1},dw)		
Control	270 ± 22a	161 ± 4a	859 ± 42a
Zn$_{450}$	262 ± 32a	162 ± 37a	951 ± 95a
Zn$_{900}$	258 ± 48a	198 ± 44a	964 ± 125a
Cr$_{300}$	-	-	-
Cr$_{600}$	-	-	-
Pb$_{450}$	263 ± 31a	223 ± 58a	991 ± 102a
Pb$_{900}$	265 ± 38a	184 ± 27a	901 ± 45a
Cd$_{4}$	256 ± 35a	205 ± 42a	913 ± 62a
Cd$_{8}$	222 ± 45a	192 ± 38a	954 ± 73a
Ni$_{110}$	288 ± 28a	204 ± 48a	915 ± 54a
Ni$_{220}$	294 ± 42a	194 ± 32a	870 ± 48a
Cu$_{200}$	-	174 ± 42a	870 ± 24a
Cu$_{400}$	-	175 ± 35a	881 ± 56a

Different lower-case letters indicate statistical significance ($p < 0.05$) among treatments per year. Zn$_{450}$ and Zn$_{900}$, 450 and 900 mg Zn kg^{-1}. Cr$_{300}$ and Cr$_{600}$, 300 and 600 mg Cr kg^{-1}. Pb$_{450}$ and Pb$_{900}$, 450 and 900 mg Pb kg^{-1}. Cd$_{4}$ and Cd$_{8}$, 4 and 8 mg Cd kg^{-1}. Ni$_{110}$ and Ni$_{220}$, 110 and 220 mg Ni kg^{-1}. Cu$_{200}$ and Cu$_{400}$, 200 and 400 mg Cu kg^{-1}

For switchgrass, according to the work of Monti et al. (Monti et al. 2008), the amount of those elements in terms of g.kg^{-1} are in the following ranges, 0.25–0.77 (P), 1.50–3.56 (K), 1.10–8.20 (Ca), 1.02–2.71 (Mg), and 0.32–0.87 (Na). Compared to these results, the biomass we collected from the pot essays presented, for K, Ca, Mg, and Na, had higher values, and for P, similar values. For giant reed, the same work of Monti et al. (Monti et al. 2008) reported the following contents of those elements in stems: 0.32 (P), 5.61 (K), 0.97 (Ca), 1.03 (Mg) and 0.13 (Na), in g.kg^{-1}. As mentioned for switchgrass, the contents reported in the stems of giant reed harvested from the current pot essay showed consistently higher values than those reported by Monti et al. in their study (Monti et al., 2008). The higher concentration of elements reported in the current work compared to the literature may be derived from the fact that the biomass was harvested from pots and not from the field. Therefore, biomass production is lower, and those macro-elements may be more concentrated. The differences observed may have also resulted from the soil type, the stage of growth, the type of plant tissue, the environment, cultural practices, etc. (Monti et al., 2008; Ogden et al., 2010)

Considering the elements K and Na, a high amount the biomass is linked to the damage of the equipment, such as pipes and furnaces, due to corrosion processes. Potassium and sodium also remain in the ash, decreasing its melt point and provoking its volatilization (Niu et al., 2016). This can damage the combustion chambers (through sintering or slag formation), provoking a decrease in its lifetime and compromising the availability of the plant (Bierdermann et al., 2005). The study's results indicate that contamination did not affect the K content of switchgrass and giant reed stems (except for stems of giant reed obtained in Cr_{600} pots). The trend of increased K content with significant contamination in Cd pots (all levels of contamination) and Cr300 pots was observed regarding giant reed leaves. In terms of Na content, the contamination reduced the amount in giant reed stems and leaves; in switchgrass, the contents were similar to control. Table 3.25 presents the alkali index calculated for both crops in control and contaminated pots.

Table 3.25. Alkali index ($Na_2O + K_2O$, kg/GJ) of switchgrass and giant reed stems in control and contaminated pots.

	Giant reed stems	**Switchgrass**
Control	0.8 ± 0.2	0.8 ± 0.1
Zn_{450}	1.4 ± 0.1	0.7 ± 0.0
Zn_{900}	1.4 ± 0.2	0.8 ± 0.1
Cr_{300}	1.1 ± 0.2	-
Cr_{600}	1.3 ± 0.1	-
Pb_{450}	1.4 ± 0.1	0.7 ± 0.0
Pb_{900}	1.4 ± 0.2	0.6 ± 0.0
Cd_4	1.1 ± 0.1	0.8 ± 0.1
Cd_8	1.0 ± 0.1	0.8 ± 0.1
Ni_{110}	1.0 ± 0.2	0.8 ± 0.1
Ni_{220}	1.1 ± 0.1	0.8 ± 0.1
Cu_{200}	1.4 ± 0.1	0.7 ± 0.0
Cu_{400}	1.4 ± 0.2	1.2 ± 0.0

.Zn_{450} and Zn_{900}, 450 and 900 mg Zn kg^{-1}. Cr_{300} and Cr_{600}, 300 and 600 mg Cr kg^{-1}. Pb_{450} and Pb_{900}, 450 and 900 mg Pb kg^{-1}. Cd_4 and Cd_8, 4 and 8 mg Cd kg^{-1}. Ni_{110} and Ni_{220}, 110 and 220 mg Ni kg^{-1}. Cu_{200} and Cu_{400}, 200 and 400 mg Cu kg^{-1}

To determine the propensity of fuel to slagging and fouling, Tortosa Masiá and collaborators (Masiá et al., 2007) presented an alkali index (AI) that relates the amount of Na and K in the biomass per unit of energy with the probability of slagging and fouling formation through the thermochemical conversion of biomass. According to the authors, the AI can be classified in terms of indication for slagging and fouling: $0.17 < AI < 0.34$—probable; $AI > 0.34$, slagging and fouling is virtually certain to occur. According to these, the values of the alkali index presented in Table 3.25 indicate that biomass from pots, either giant reed or switchgrass, have a high probability of causing slagging and fouling once results are higher than 0.34–even the biomass from control pots. These results are in line with what is considered a problem when perennial grasses are exploited through thermochemical processes, especially due to the high potassium content present in those biomasses, which is significantly higher than what is observed in woody biomass. Vassilev et al. (Vassilev et al. 2013) indicate a mean of 10.75 of K_2O (% weight to total ash) in wood and woody biomass and a mean of 26.65 of K_2O (% weight to total ash) in herbaceous and agricultural biomass. Both the occurrence of slagging and fouling a challenge to the thermochemical conversion of biomass since the ash particles melt and accumulate in the walls of furnaces and pipes, reducing the temperature inside the furnace and the oxidation of the fuel, increasing the emission of CO (Hrdlička et al., 2016; Lachman et al., 2021). Both crops showed a lower fuel quality when compared to coal or pine chips, with AI values of 0.04 and 0.17, respectively (Masiá et al., 2007). However, they have a similar AI index to olive residues (1.14) (Masiá et al.,2007). This indicates that to proceed with the thermochemical valorization of these biomasses, and some treatment must be applied, especially to reduce the fouling formation trend (linked with K and Na contents). Comparing the data obtained for control and contaminated pots, it is interesting not to report differences in AI for switchgrass. In the case of giant reed stems, AI increased due to contamination, but this increment was more problematic in Cr_{600} pots.

3.3.4 Fiber composition

The fiber content results indicate that for both giant reed and switchgrass, shown in Table 3.26, the hemicellulose content is the highest fraction, followed by cellulose and lignin. This trend in the fiber composition of biomass was extensively reported in the literature for both giant reed (Fiore et al., 2014; Giudicianni et al., 2014; Krička et al., 2017; Oginni et al., 2019; Saikia et al., 2015) and switchgrass (Brummer et al., 2002; Hu et al., 2010; Imam et al., 2012; Lemus et al., 2008; Yan et al., 2010).

In giant reed, the cellulose and lignin content are higher than in switchgrass; however, the hemicellulose content is lower. There are no indications of the contaminant's effect on biomass's fiber composition since the results obtained in Control are statistically the same in the contamination trials. The high value of fiber content indicates an opportunity for biomass valorization in biorefinery processes. All three fractions presented appealing values for their separation and transformation in bioproducts. There were no significant variances in the hemicelluloses, celluloses, lignin, and fiber content regarding the contaminated soils, for both crops. A significant difference was observed between *Arundo donax* and *Panicum virgatum* in lignin content, increasing the fiber content of this crop.

Table 3.26. The fiber content of contaminated biomass

		Hemicelluloses	Cellulose	Lignin	Total Fiber
Giant reed	Control	31.5 ± 1.4	25.6 ± 5.1	20.0 ± 1.7	77.1 ± 2.0
	Zn_{450}	32.6 ± 1.5	22.0 ± 0.0	21.1 ± 2.2	75.7 ± 0.7
	Zn_{900}	29.4 ± 3.7	23.5 ± 0.1	22.3 ± 0.3	75.1 ± 3.3
	Cr_{300}	30.4 ± 1.3	19.5 ± 2.3	22.5 ± 1.4	72.3 ± 0.5
	Cr_{600}	30.7 ± 1.1	22.5 ± 1.3	21.9 ± 1.4	75.1 ± 1.1
	Pb_{450}	28.4 ± 1.4	26.0 ± 3.1	19.3 ± 3.6	73.8 ± 1.3
	Pb_{900}	31.8 ± 3.6	21.2 1 2.5	19.5 ± 1.7	72.5 ± 0.6
	Cd_4	29.7 ± 1.0	27.8 ± 4.5	21.2 ± 3.1	78.7 ± 2.5
	Cd_8	30.3 ± 4.7	26.5 ± 1.7	20.8 ± 2.7	76.7 ± 1.7
	Ni_{110}	30.1 ± 0.7	26.3 ± 0.7	19.5 ± 1.9	75.9 ± 0.5
	Ni_{220}	30.3 ± 2.0	25.1 ± 3.0	19.1 ± 0.4	74.5 ± 1.3
	Cu_{200}	32.2 ± 3.2	22.1 ± 4.1	21.9 ± 2.8	76.2 ± 2.0
	Cu_{400}	31.3 ± 2.5	20.7 ± 2.1	20.7 ± 0.2	72.7 ± 0.3
Switchgrass	Control	34.6 ± 0.7	22.3 ± 0.1	15.2 ± 1.4	72.1 ± 2.0
	Zn_{450}	33.2 ± 3.7	21.7 ± 4.2	13.5 ± 0.5	68.4 ± 1.0
	Zn_{900}	32.7 ± 1.7	23.3 ± 0.2	12.3 ± 1.4	68.3 ± 0.6
	Cr_{300}	-	-	-	-
	Cr_{600}	-	-	-	-
	Pb_{450}	35.5 ± 4.2	24.7 ± 1.4	12.4 ± 1.4	72.6 ± 4.2
	Pb_{900}	32.8 ± 1.1	25.4 ± 1.0	12.5 ± 0.4	70.6 ± 0.5
	Cd_4	35.6 ± 6.9	23.5 ± 1.6	14.9 ± 0.5	74.0 ± 5.7
	Cd_8	34.0 ± 1.8	25.2 ± 1.0	14.7 ± 0.5	73.8 ± 1.3
	Ni_{110}	30.0 ± 2.1	22.9 ± 4.8	18.1 ± 3.5	71.0 ± 0.8
	Ni_{220}	33.4 ± 1.4	23.2 ± 5.5	19.8 ± 7.0	76.3 ± 0.1
	Cu_{200}	32.4 ± 2.1	21.5 ± 0.6	16.3 ± 2.0	70.2 ± 3.5
	Cu_{400}	33.7 ± 1.2	20.2 ± 0.5	19.5 ± 0.7	73.4 ± 0.0

.Zn_{450} and Zn_{900}, 450 and 900 mg Zn kg^{-1}. Cr_{300} and Cr_{600}, 300 and 600 mg Cr kg^{-1}. Pb_{450} and Pb_{900}, 450 and 900 mg Pb kg^{-1}. Cd_4 and Cd_8, 4 and 8 mg Cd kg^{-1}. Ni_{110} and Ni_{220}, 110 and 220 mg Ni kg^{-1}. Cu_{200} and Cu_{400}, 200 and 400 mg Cu kg^{-1}

3.4 Phytoremediation potential

3.4.1 Heavy metals content and uptake

The giant reed's heavy metals uptake can be observed in Table 3.27. Under Zn contamination, all plant fractions Increased the heavy metal accumulation. For $Zn_{450,}$ the accumulation in leaves was 6.5 times higher than in control, while In $Zn_{900,}$ the accumulation Increased by thirteen times. In stems, the accumulation In Zn_{450} was twice the control, while In Zn_{900}, It was three times higher. In Cr trials, the highest accumulation occurred in roots, eight times higher in Cr_{300} and twenty-five times higher for Cr_{600}.

Table 3.27. Giant reed heavy metals accumulation in biomass fractions

Element Analyzed	Treatment	The concentration of heavy metal in the structure of the plant ($mg.kg^{-1}$)				
		First year		Second year		
		Leaves	Stems	Leaves	Stems	Roots
Zinc	Control	90 ± 28b	67 ± 17c	19 ± 3c	38 ± 4c	25 ± 2c
	Zn_{450}	199 ± 84b	128 ± 17b	129 ± 16b	99 ± 1b	156 ± 42b
	Zn_{900}	354 ± 60a	244 ± 17c	269 ± 46a	123 ± 1a	366 ± 15a
Chromium	Control	3.63 ± 1.38c	4.25 ± 0.28c	20 ± 6b	0.6 ± 0.04c	29 ± 3c
	Cr_{300}	339 ± 240b	236 ± 24a	28 ± 7b	1.9 ± 0.1b	245 ± 70b
	Cr_{600}	917 ± 119a	125 ± 14b	92 ± 11a	4.6 ± 1.5a	744 ± 94a
Lead	Control	11.5 ± 6.3b	9.5 ± 2.3b	23 ± 7a	54 ± 8a	54 ± 17c
	Pb_{450}	13.5 ± 1.7b	12.4 ± 3.0ab	25 ± 5a	62 ± 9a	219 ± 29b
	Pb_{900}	25.9 ± 6.1a	16.0 ± 0.6a	28 ± 8a	69 ± 16a	359 ± 39a
Cadmium	Control	0.71 ± 0.12b	0.39 ± 0.07b	1.0 ± 0.3b	0.3 ± 0.2c	0.61 ± 0.04c
	Cd_4	1.38 ± 0.18a	0.61 ± 0.29b	3.1 ± 0.7a	0.9 ± 0.2b	1.9 ± 0.1b
	Cd_8	3.58 ± 2.09a	1.39 ± 0.03a	4.3 ± 0.7a	4.4 ± 0.6a	4.6 ± 1.5a
Nickel	Control	4.2 ± 0.5c	5.98 ± 0.40c	96 ± 14a	5.5 ± 0.0c	41 ± 7b
	Ni_{110}	6.9 ± 0.6b	8.08 ± 0.54b	77 ± 10a	9.6 ± 0.1b	54 ± 6b
	Ni_{220}	32.1 ± 8.9a	12.9 ± 0.8a	90 ± 16a	15.1 ± 0.1a	134 ± 10a
Copper	Control	7.5 ± 4.9c	4.9 ± 0.7c	21 ± 5a	12 ± 2c	7.6 ± 11c
	Cu_{200}	116 ± 13b	9.5 ± 0.4b	18 ± 6a	38 ± 8b	128 ± 33a
	Cu_{400}	274 ± 85a	20.5 ± 3.2a	19 ± 4a	66 ± 14a	63 ± 8b

In Pb trials, the aboveground biomass accumulation was not affected by the contamination at any level. On the other hand, the belowground biomass increased its Pb accumulation by four times In Pb450 and by six times for Pb_{900}.

Giant reed cultivated In Cd increased their Cd levels in plant fraction. For Cd_4 trials, the accumulation on leaves, stems, and roots were three times higher than in control, accumulating more in leaves, followed by roots and stems. On the other hand, Cd_8 presented a similar accumulation in all three fractions.

Nickel in the Control was higher in leaves, double the concentration in roots, and fifteen times more than in stems. The increase of Ni in trial pots increased the accumulation in stems and roots.

In Cu trials, only stems and roots were affected had suffered any effect regarding the accumulation of the heavy metal. However, while in stems, the amount of heavy metal in the structure Increased, in the roots, the increase in Cu concentration decreased its accumulation.

The results observed In giant reed Zn and Pb uptake followed the literature, observed In an experiment using the same concentrations as In this work designed by Barbosa et al. (Barbosa et al., 2015), where the aerial biomass uptake was higher only In Zn trials, while, for belowground biomass, both Zn and Pb Increased the uptake.

Cr accumulation in giant reed was also studied by Barbosa et al., that for the same concentrations, also observed an increase in Cr In all structures, being higher in belowground biomass. The Increase, however, was 90% lower than detected in this work, following the lower Cr bioavailability in Barbosa's paper (Barbosa et al., 2015). In a hydroponic Cr experiment, the Cr accumulation was higher in leaves and stems; however, the studied contaminations were lower than in this work (Kausar et al., 2012).

Giant reed In Cd soils was studied by Sabeen et al. Showing that in both hydroponic and pot trials, Cd accumulation was higher in the roots. On the other hand, regarding the aerial biomass, the results were the opposite of this experiment, having higher accumulation in the stems when compared to leaves (Sabeen et al., 2013).

The accumulation of Ni In giant reed was also observed in this work. Nickel accumulation In giant reed's aerial biomass Is related to Ni's bioavailability in soil, and Ni's higher accumulation occurs In giant reeds' belowground biomass (Atma et al., 2017).

The switchgrass heavy metals accumulation is observed in Table 3.28. The Zn accumulation in aboveground biomass increased with the Zn concentration in the soil. However, in the belowground biomass, Zn_{450} accumulation was higher than Zn_{900}. Lead accumulation was only significant in the belowground part, where for $Zn_{450,}$ the uptake was eighteen times higher, while for Pb_{900} was more than four hundred times higher.

Table 3.28. Switchgrass accumulation in biomass fractions

Element Analyzed	Treatment	The concentration of heavy metal in the structure of the plant (mg.kg^{-1})		
		First year	Second year	
		Aerial Biomass	Aerial Biomass	Belowground Biomass
Zinc	Control	57 ± 4b	39 ± 1c	29 ± 1c
	Zn$_{450}$	241 ± 31a	180 ± 4b	223 ± 2a
	Zn$_{900}$	293 ± 30a	231 ± 10a	191 ± 0b
Chromium	Control	2.70 ± 1.22	16.2 ± 1.9	8.8 ± 3.0
	Cr$_{300}$	-	-	-
	Cr$_{600}$	-	-	-
Lead	Control	4.4 ± 3.0c	15 ± 5a	6.6 ± 2.2c
	Pb$_{450}$	93 ± 39b	25 ± 5a	111 ± 0b
	Pb$_{900}$	264 ± 67a	28 ± 6a	2534 ± 562a
Cadmium	Control	1.04 ± 0.13c	0.33 ± 0.1c	1.98 ± 0.02c
	Cd$_4$	3.16 ± 0.19b	1.09 ± 0.14b	5.0 ± 0.6b
	Cd$_8$	38.0 ± 4.3a	1.33 ± 0.01a	10.6 ± 1.2a
Nickel	Control	4.5 ± 0.4b	8.0 ± 2.0b	21 ± 3b
	Ni$_{110}$	474 ± 74a	12.4 ± 3.8ab	53 ± 3a
	Ni$_{220}$	-	14.7 ± 1.1a	58 ± 2a
Copper	Control	24.5 ± 4.1	14 ± 3 b	30 ± 7c
	Cu$_{200}$	-	25 ± 4a	77 ± 2a
	Cu$_{400}$	-	24 ± 2a	53 ± 2b

Cadmium also increased its concentration in biomass with the increase of soil contamination. In aboveground biomass, the Cd$_4$ concentration was three times higher, while in Cd$_8$, it was eight times the concentration in control. In belowground biomass, Cd concentration in Cd$_4$ was 2.5 times higher, while five times higher for Cd$_8$.

For Ni, the Ni concentration in Ni110 for the aboveground biomass was 50% higher and almost twice the Ni in Control in Ni$_{220}$. In belowground biomass, the Ni concentration increased with the presence of the heavy metal but showed not to be dependent on its concentration. In Cu trials, the aboveground biomass increased with the contaminant in the soil. However, the increase in the contaminant level has not increased the amount of Cu in switchgrass aerial biomass. In the belowground biomass, the Cu concentration in contaminated trials was higher than in Control, but the amount of heavy metal was higher in Cu$_{200}$ than in Cu$_{400}$.

Despite the potential of this crop, switchgrass is not present in many phytoremediation studies, being not found in studies of switchgrass under Ni, Cu, and Zn contamination, regarding the heavy metal uptake. Switchgrass under Pb concentration was studied by Arora et al. observing that the Increase In Pb levels did not affect the amount of heavy metals In aerial biomass, even when treatments were used to Increase the bioavailability of Pb (Arora et al., 2016).

Cadmium uptake of switchgrass was studied by Reed et al. under different pH levels and concentrations higher than in this work. A relation was observed between the concentration of Cd In soil and the uptake of Cd. It was also observed that the uptake of Cd Increased with the decrease in pH. For all contamination levels, the uptake In roots was higher than In the aerial part, a similar behavior to this experiment (Reed et al., 2002).

For giant reed, from the first to the second year, the concentration decreased in Zn_{450} and Zn_{900} stems, in leaves and stems of Cr trials, and the leaves of Cu trials. On the other hand, stems of Pb, Cd, and Ni trials suffered an increase, and the leaves in Ni trials, Pb_{450}, and Cd_4 suffered an increase in the contamination level. The development of the root system also increased the contaminants storage capability in the belowground biomass, reducing the translocation for the aerial fraction of the plant, an effect observed in Cr trials. On the other hand, the increase of the root system also increased the uptake of the heavy metal, and for some metals, such as Cd and Ni trials, this also improved the accumulation of heavy metals in the aerial part.

Considering switchgrass, Zn, Pb, Cd, and Ni (Ni_{110}) suffered a reduction in the accumulation of heavy metals in the aerial part from the first to the second year. However, the development of the belowground biomass suggests that the heavy metal accumulated mostly in this plant’s fraction, showing that switchgrass may not be the best option for phytoextraction processes.

The zinc accumulation potential can be observed in Figure 3.16 for aerial biomass, roots, and rhizomes. The zinc accumulation potential of switchgrass was around 50% higher than giant reed for the aerial biomass. However, the accumulation potential in the belowground biomass is higher than in the aerial part. In the belowground biomass, the accumulation potential of giant reed was around three times higher than switchgrass.

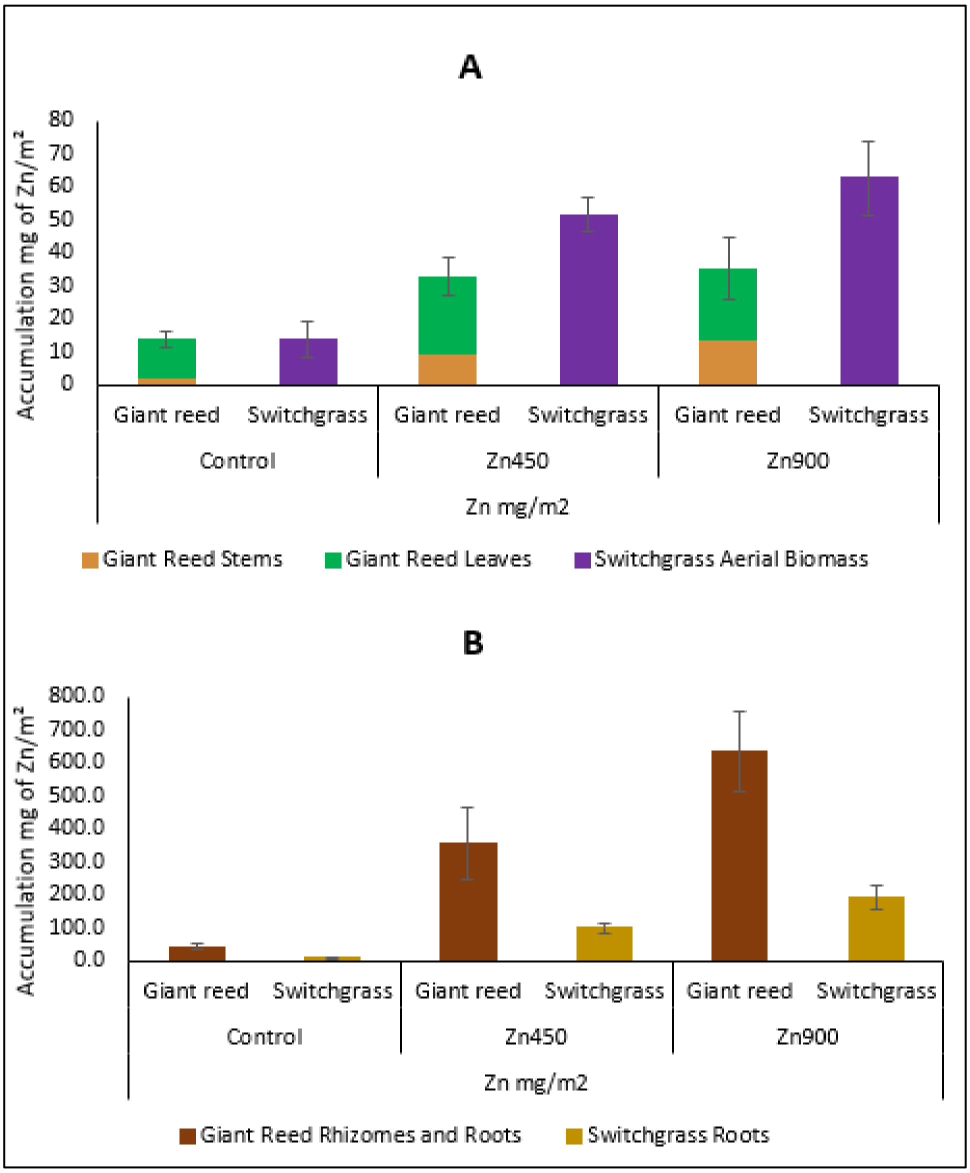

Figure 3.16. Zinc accumulation in giant reed and switchgrass aboveground fraction (A) and a belowground fraction (B).

Chromium accumulation potential for giant reed and switchgrass Figure 3.17. Considering giant reeds' aerial biomass, the accumulation potential increased with increased Cr in the soil. In the belowground biomass, the accumulation potential also increased with the contamination. The accumulation of belowground biomass was higher than in the aerial biomass, being 10,000% higher than in the aerial biomass, showing that the Cd extraction potential through giant reed phytoextraction potential is not viable.

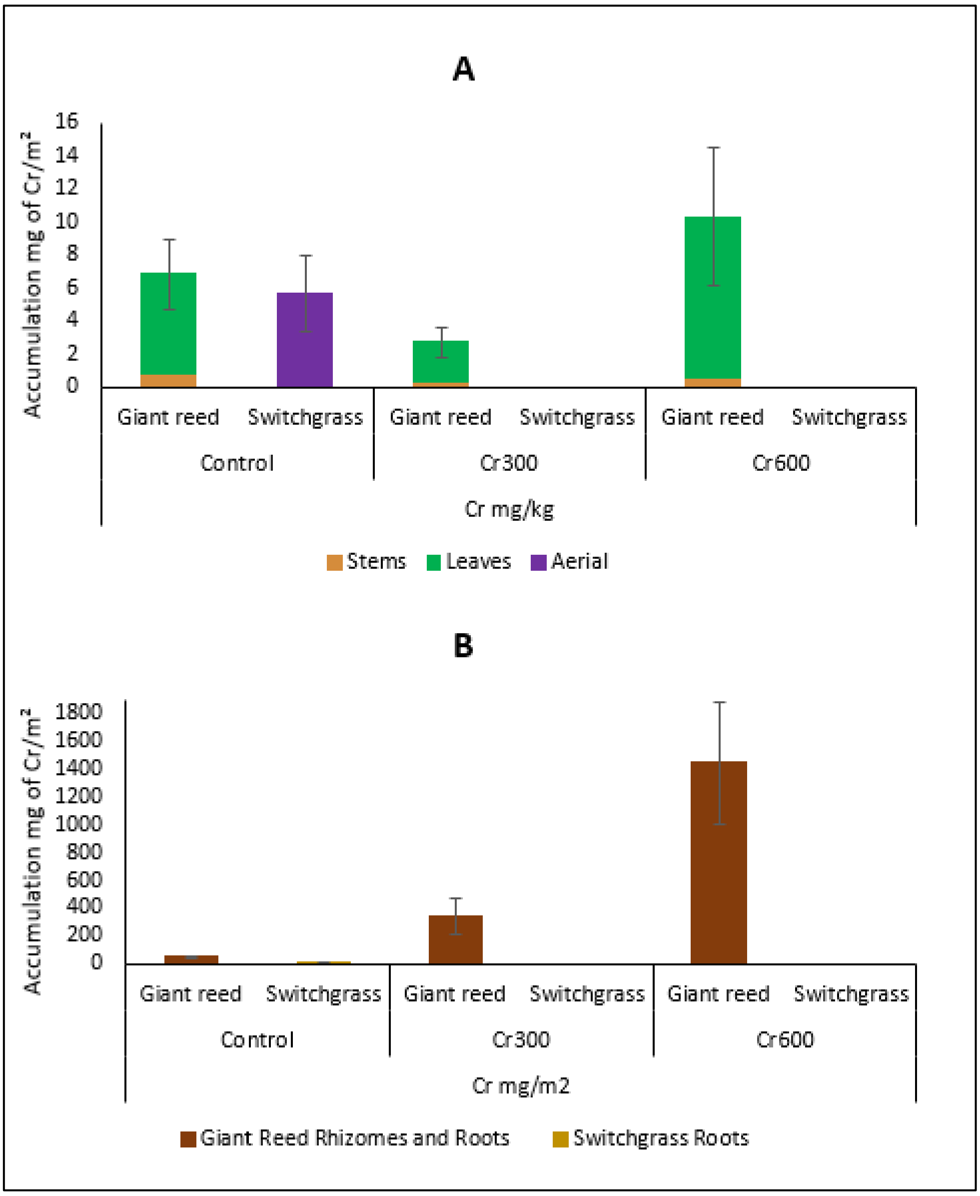

Figure 3.17. Chromium accumulation in giant reed and switchgrass aboveground fraction (A) and a belowground fraction (B).

Regarding the Pb accumulation potential, observed in Figure 3.18, the aerial biomass potential of giant reed and switchgrass are similar and were not affected by the lead concentration in the soil. However, the Pb accumulation potential in the belowground biomass was higher for switchgrass than in giant reed. Therefore, the Pb accumulation potential in the belowground biomass was higher than in the aerial biomass, and for switchgrass, it was affected by the concentration of the contaminant.

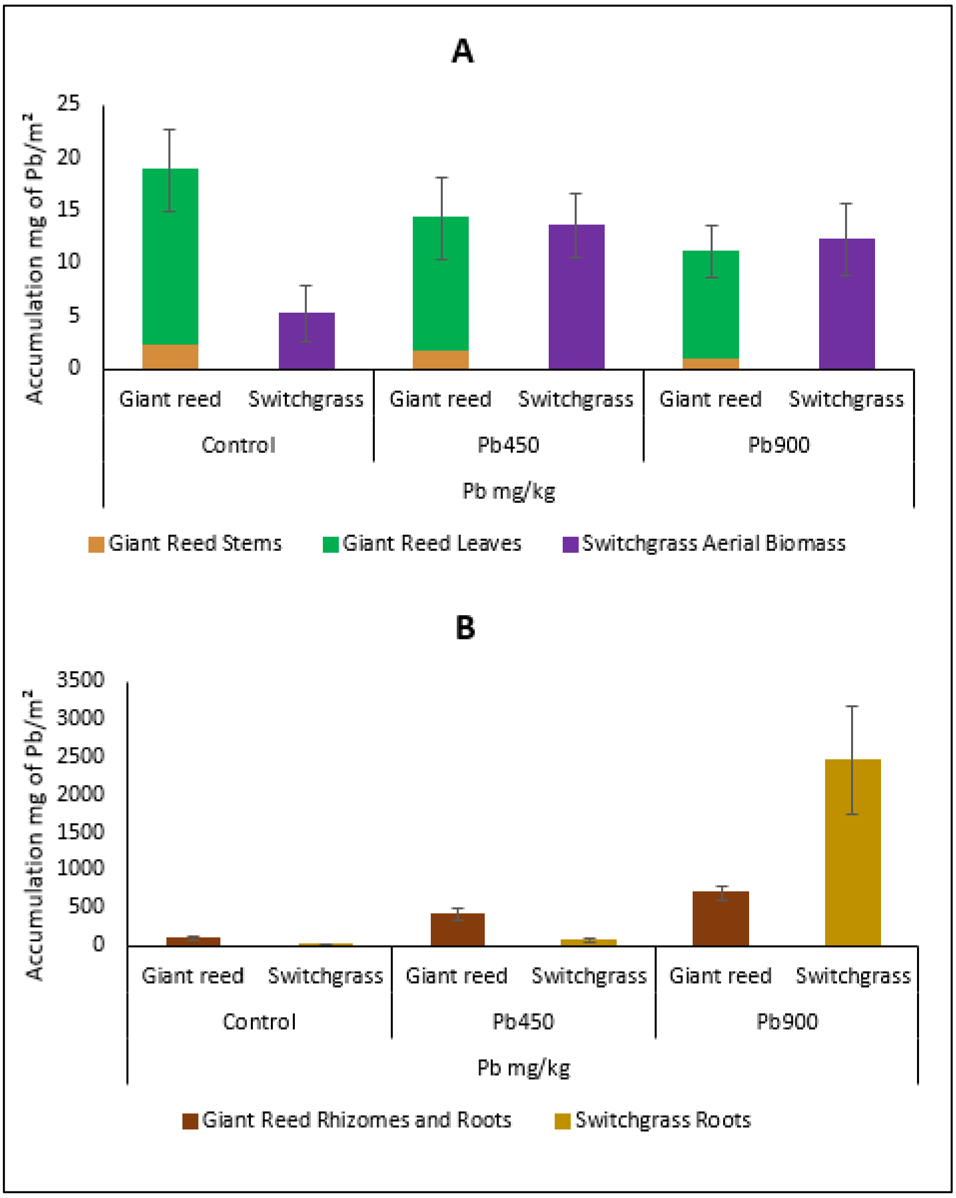

Figure 3.18. Lead accumulation in giant reed and switchgrass aboveground fraction (A) and a belowground fraction (B).

The accumulation potential of cadmium is presented in Figure 3.19. Considering the aerial biomass in the lower concentration, the giant reed was not affected by the contamination but by the concentration of heavy metal in the soil. When the heavy metal concentration in soil increased, giant reed accumulation potential also increased. However, for switchgrass, the accumulation potential was not affected. Regarding the belowground biomass, the accumulation potential increased with soil contamination, but the contaminant concentration was not affected. Therefore, the belowground accumulation potential for switchgrass and giant reed was similar for both crops.

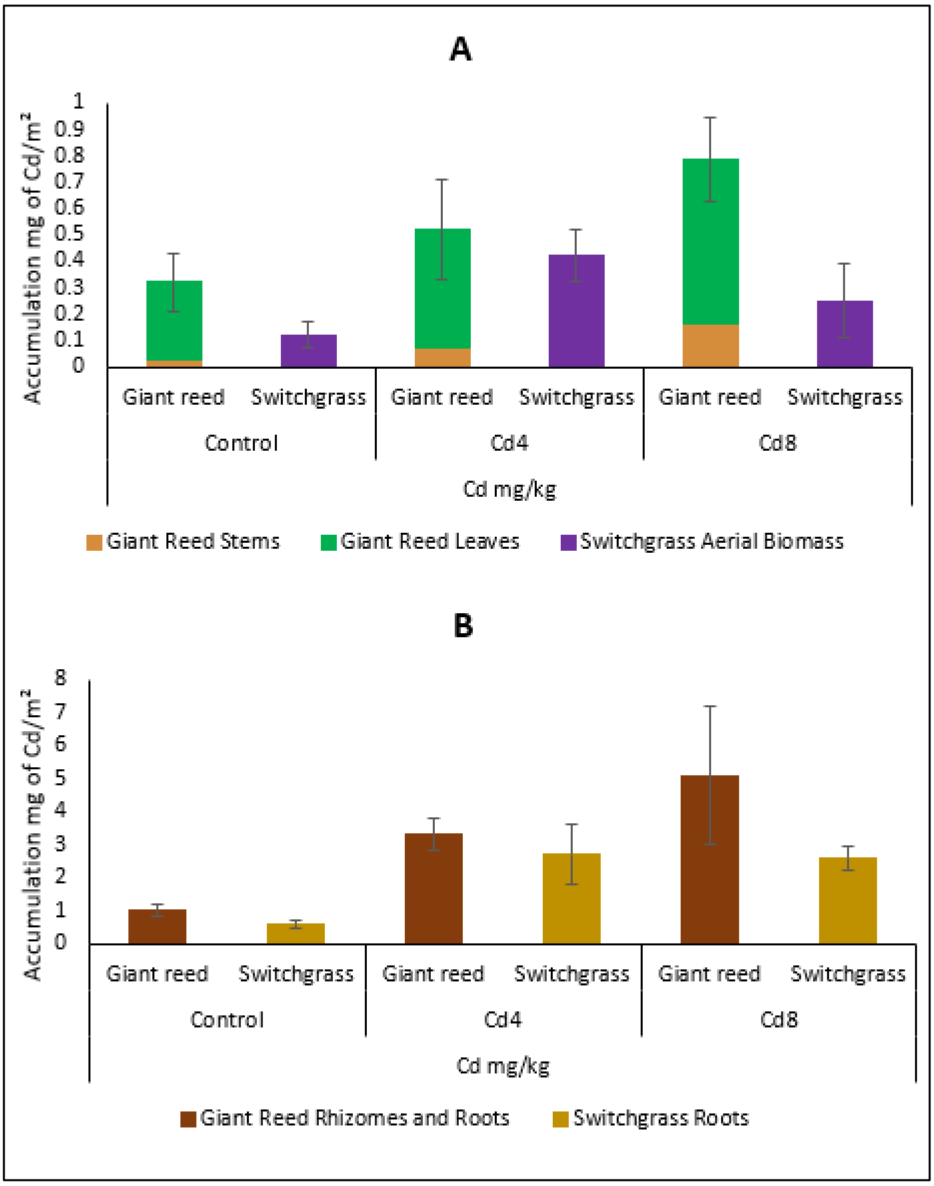

Figure 3.19. Cadmium accumulation in giant reed and switchgrass aboveground fraction (A) and a belowground fraction (B).

Nickel accumulation potential is presented in Figure 3.20. It is possible to observe that giant reed nickel accumulation potential is higher than switchgrass in both aboveground and belowground biomass. For switchgrass, however, Ni contamination had not affected biomass accumulation in any concentration. The accumulation potential for the belowground biomass was higher than in the aerial biomass for both biomasses and in all tested trials.

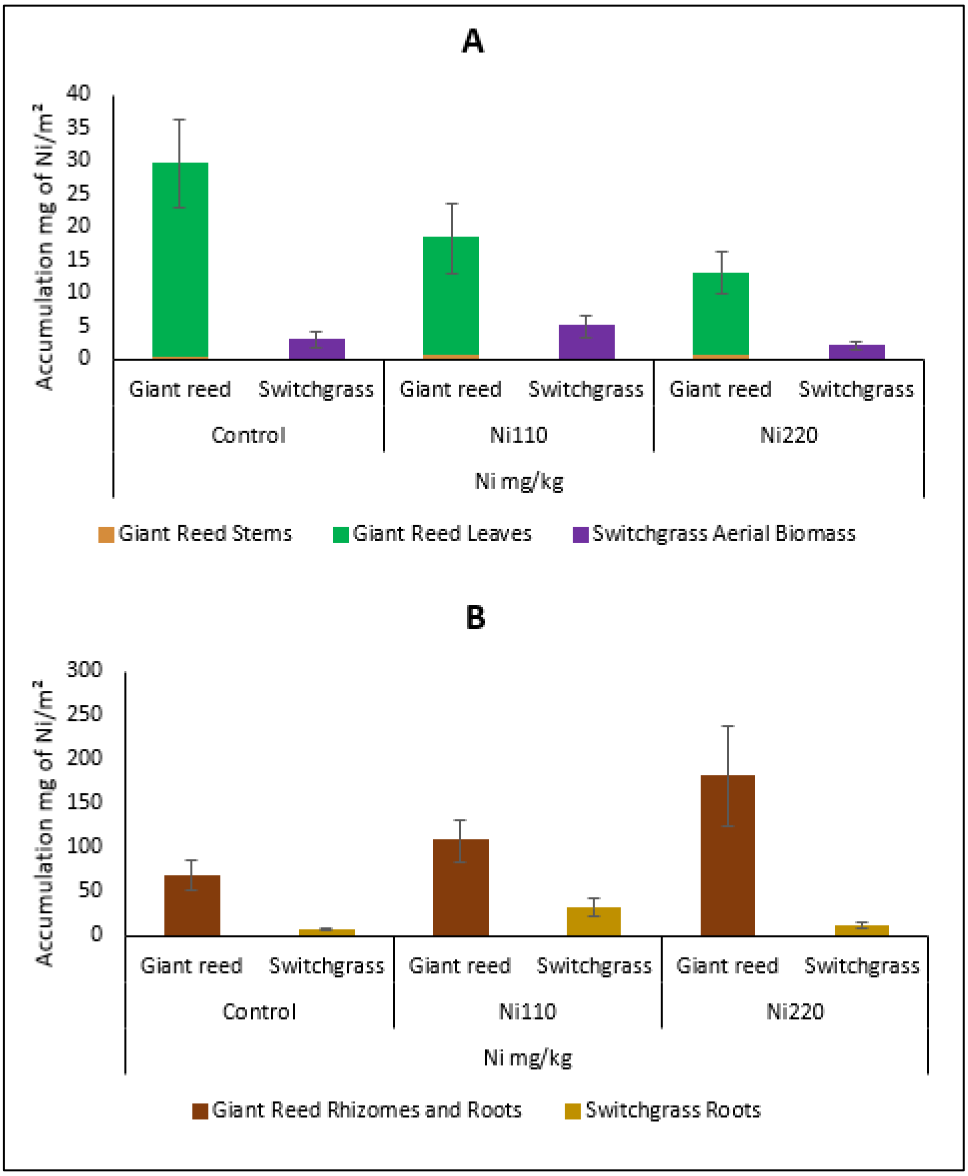

Figure 3.20. Nickel accumulation in giant reed and switchgrass aboveground fraction (A) and a belowground fraction (B).

Copper accumulation for aerial biomass was not affected in the low concentration trials for giant reed or switchgrass. Figure 3.21 shows the Cu accumulation potential for giant reed and switchgrass. However, while switchgrass was not affected by the increase in contamination level in the high concentration trial, giant reed showed a higher accumulation potential in the aerial biomass. In addition, the accumulation potential in the belowground biomass was higher than in the aerial biomass for both crops in all Cu trials. However, while Cu did not affect the accumulation potential in belowground biomass for switchgrass, for giant reed, this potential increased in all Cu tested trials.

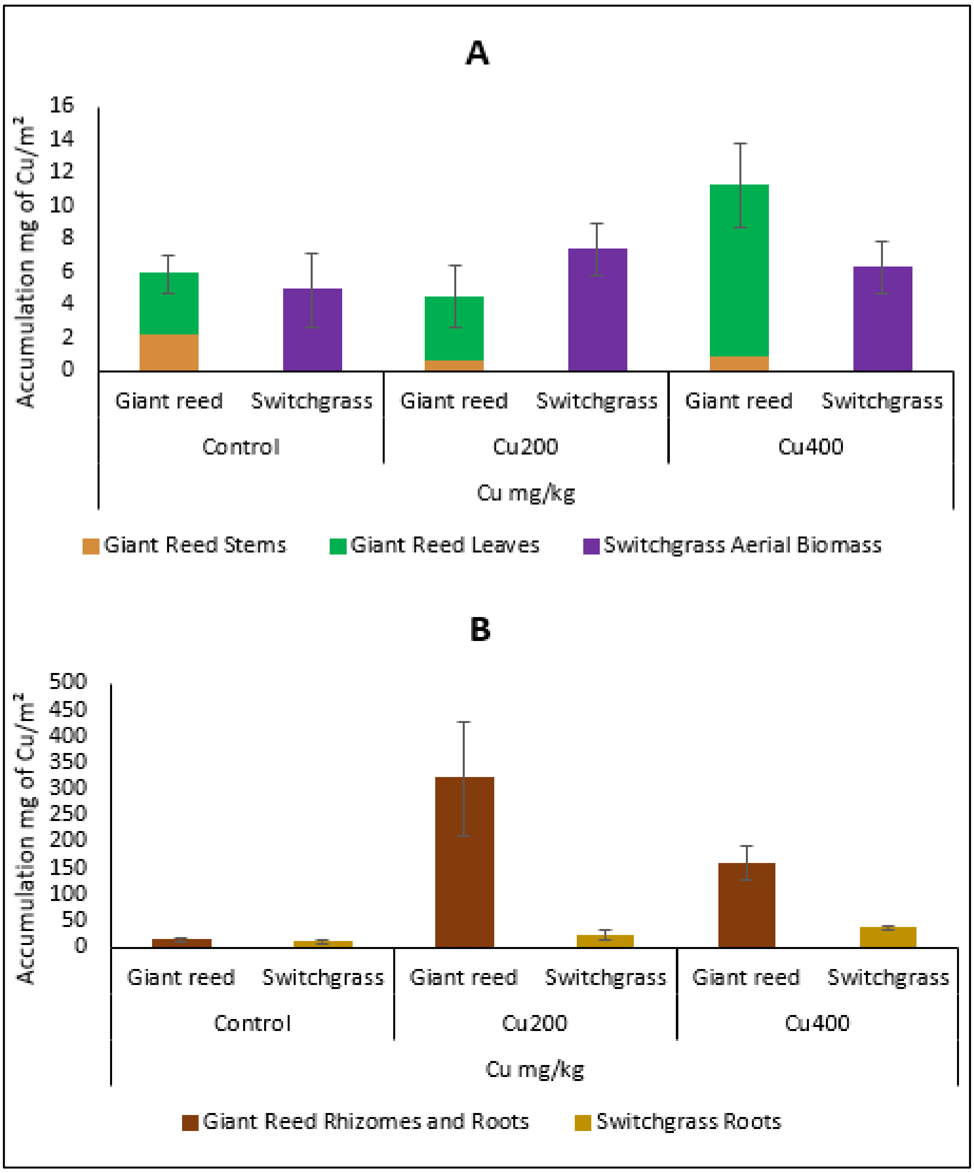

Figure 3.21. Copper accumulation in giant reed and switchgrass aboveground fraction (A) and a belowground fraction (B).

According to Lueng et al. (Leung et al., 2007), the accumulation profile of the plants when cultivated in soils contaminated with metals can classify the crop as excluders, accumulators, and indicators. Excluders are plants in which the variation of the contaminant’s concentration in the soil does not affect the concentration of the same contaminant in the shoots. Accumulators, in which the storage of the contaminants are made in aboveground biomass. Indicators can be described as plants in which the concentration of heavy metal in the aerial fraction increases proportionally with the increase of the contaminants in the soil. According to this classification, the giant reed can be considered an excluder plant in soils contaminated with Cr, Pb, and Ni. Cu, Cd, and Ni giant reed act as an indicator increasing the concentration of heavy metal in the aboveground biomass as the concentration of the heavy metal in the soil increases. Switchgrass, on the other hand, acts as an indicator in Zn, Pb, and Cd contaminated soils. In Ni and Cu trials, switchgrass act as an excluder, limiting the accumulation of these heavy metals to a critical point without any changes regarding the soil contamination levels.

3.4.2 mAI, mBCF, mBAF, TF, and mTF

Phytoremediation potential is related to the capability of the plant to absorb and accumulate contaminants in its organs, remediating the contaminated soil (Fernando, 2005). The remediation occurs through releasing enzymes and exudates or stimulating microorganisms in the soil-roots interface (Fernando, 2005; Jin et al., 2015). The phytoextraction is analyzed considering various points: (a) The influence of the contaminant in plants growth that TI indicates; (b) The capability of the crop to absorb and store the pollutants when exposed to a higher amount than the usual, showed by the mAI; (c) the capability of the crops to accumulate the contaminants, presented by mBCF and mBAF; (d) the potential of the plant to restore the transfer the contaminants accumulated in the belowground biomass to the aerial part of the plant, indicated by the TF and mTF. The Indexes for switchgrass and giant reed are presented in Table 3.29 and Table 3.30, respectively.

The plant’s capability to uptake heavy metals in a higher amount than usual is indicated in the mAI. It is observed that Zn and Cu are easier absorbed by giant reed, while Pb shows to be a difficult contaminant to be uptake by this crop. The mAI indicates that switchgrass can absorb all the studied heavy metals in an amount higher than usual (mAI>1). The mAI value also Indicated that the capacity of both giant reed and switchgrass to accumulate Cd Is high compared to all the other heavy metals.

The index representing the plant fraction in which the plant accumulates the contaminants Is the mBCF. It can be measured considering the aboveground and belowground biomass or each different structure (i.e., rhizomes, roots, stems, leaves, seeds). In this experiment, it was observed using the mBCF that Zn accumulation occurs mainly in giant reed's root system and stems. Pb accumulates more in the root system while Cu, on the other hand, has higher accumulation in the aerial part. Cd and Cr are mostly in belowground biomass, while Ni is in a similar ratio in belowground biomass and leaves. For switchgrass, the concentration in the belowground biomass was higher for Pb, Ni, Cd, and Cu. For Zn, while Zn_{450} had a higher concentration in belowground biomass, Zn_{900} accumulated more in the aerial part.

The mBAF Is used to evaluate the potential of the plant to remediate the soils from heavy metal contamination showed that for both giant reed and switchgrass, the belowground biomass promotes the remediation at a higher level than the aerial part for all studied heavy metals, indicating that the major potential for these crops is the phytostabilization of the heavy metals in soil. However, for giant reed, when considering only the aerial biomass, Pb contamination appears as a challenge to be remediated.

TF and mTF also indicate the plants' capability to accumulate heavy metals in the aerial part. Among the studied contaminants, Pb appeared to be difficult to be accumulated in the aerial part, difficulty the extraction of this heavy metal from the soil (TF_{Pb}, and $mTF_{Pb} << 1$). Among all the studied trials, Cu trials containing giant reed were the only ones with a TF higher than 1, indicating that the main accumulation occurred in the aerial part. This indicates that the plant has a potential for phytoextraction, which means that the heavy metal would b removed from the soil, reducing the total concentration of Cu In the soil, considering all the oxidate states.

Table 3.29. Switchgrass indexes table

Structure	Trial	mAI			mBCF			mBAF (%)			TF			mTF		
Aboveground	Zn$_{450}$	3.8	±	1.36	0.5	±	0.02	0.085	±	0.009	0.81	±	0.02	0.53	±	0.102
	Zn$_{900}$	4.6	±	1.8	0.32	±	0.04	0.051	±	0.011	1.21	±	0.05	0.33	±	0.087
	Cr$_{300}$	-		-	-		-	-		-	-		-	-		-
	Cr$_{600}$	-		-	-		-	-		-	-		-	-		-
	Pb$_{450}$	2.58	±	1.36	0.06	±	0.02	0.019	±	0.008	0.23	±	0.05	0.21	±	0.08
	Pb$_{900}$	2.34	±	1.29	0.03	±	0.01	0.009	±	0.004	0.01	±	0	0.01	±	0.002
	Cd$_{4}$	3.67	±	1.85	0.26	±	0.05	0.059	±	0.014	0.22	±	0.04	0.16	±	0.06
	Cd$_{8}$	2.14	±	1.42	0.13	±	0.01	0.015	±	0.007	0.13	±	0.02	0.09	±	0.05
	Ni$_{110}$	1.77	±	0.96	0.14	±	0.04	0.032	±	0.011	0.23	±	0.07	0.15	±	0.07
	Ni$_{220}$	0.68	±	0.35	0.08	±	0.01	0.006	±	0.002	0.25	±	0.02	0.17	±	0.06
	Cu$_{200}$	1.5	±	0.69	0.13	±	0.05	0.023	±	0.009	0.33	±	0.05	0.34	±	0.166
	Cu$_{400}$	1.28	±	0.59	0.08	±	0.01	0.012	±	0.003	0.45	±	0.04	0.17	±	0.043
Belowground	Zn$_{450}$	10.53	±	2.68	0.62	±	0.03	0.161	±	0.029						
	Zn$_{900}$	20.56	±	5.61	0.27	±	0.03	0.157	±	0.036						
	Cr$_{300}$	-		-	-		-	-		-						
	Cr$_{600}$	-		-	-		-	-		-						
	Pb$_{450}$	30.97	±	15.4	0.27	±	0.09	0.093	±	0.044						
	Pb$_{900}$	1161.36	±	556.91	3.15	±	1.27	1.806	±	0.803						
	Cd$_{4}$	4.2	±	1.61	1.19	±	0.2	0.376	±	0.134						
	Cd$_{8}$	4.07	±	0.93	1.07	±	0.14	0.155	±	0.023						
	Ni$_{110}$	4.73	±	1.84	0.59	±	0.05	0.21	±	0.067						
	Ni$_{220}$	1.69	±	0.54	0.32	±	0.01	0.037	±	0.008						
	Cu$_{200}$	2.26	±	1.21	0.4	±	0.14	0.067	±	0.038						
	Cu$_{400}$	3.85	±	1.24	0.17	±	0.01	0.069	±	0.009						

Table 3.30. Giant reed indexes table

Structure	Trial	mAI	mBCF	mBAF (%)	TF (Aboveground/Belowground)	mTF (Aboveground/Belowground)
Stems	Zn_{450}	4.8 ± 1.99	0.359 ± 0.047	0.016 ± 0.005	0.68	0.09
	Zn_{900}	6.81 ± 3.31	0.375 ± 0.077	0.011 ± 0.005	0.43	0.06
	Cr_{300}	0.38 ± 0.2	0.14 ± 0.02	0.0026 ± 0.0011	0.15	0.01
	Cr_{600}	0.69 ± 0.35	0.11 ± 0.01	0.0022 ± 0.0009	0.38	0.03
	Pb_{450}	0.77 ± 0.35	0.06 ± 0.024	0.003 ± 0.001	0.24	0.04
	Pb_{900}	0.46 ± 0.24	0.035 ± 0.015	0.001 ± 0	0.17	0.02
	Cd_{4}	2.31 ± 1.73	0.22 ± 0.06	0.0098 ± 0.0034	0.32	0.05
	Cd_{8}	5.19 ± 3.79	0.44 ± 0.06	0.0093 ± 0.0027	0.46	0.06
	Ni_{110}	1.32 ± 0.27	0.11 ± 0.01	0.005 ± 0.0007	0.77	0.12
	Ni_{220}	1.38 ± 0.55	0.08 ± 0	0.0026 ± 0.0009	0.93	0.13
	Cu_{200}	2.65 ± 1.28	0.813 ± 0.365	0.022 ± 0.012	1.56	0.09
	Cu_{400}	6.63 ± 2.55	1.158 ± 0.049	0.033 ± 0.011	5.72	0.46
Leaves	Zn_{450}	2 ± 0.51	0.276 ± 0.013	0.038 ± 0.007		
	Zn_{900}	1.87 ± 0.62	0.171 ± 0.02	0.018 ± 0.005		
	Cr_{300}	0.39 ± 0.18	0.42 ± 0.1	0.021 ± 0.0073		
	Cr_{600}	1.59 ± 0.79	0.63 ± 0.09	0.0394 ± 0.0153		
	Pb_{450}	0.76 ± 0.27	0.149 ± 0.056	0.018 ± 0.008		
	Pb_{900}	0.61 ± 0.2	0.086 ± 0.035	0.007 ± 0.003		
	Cd_{4}	2.19 ± 0.97	0.73 ± 0.18	0.0908 ± 0.0271		
	Cd_{8}	1.58 ± 0.73	0.43 ± 0.07	0.0277 ± 0.0086		
	Ni_{110}	0.6 ± 0.21	0.86 ± 0.13	0.1154 ± 0.0322		

(Continuation)

Structure	Trial	mAI	mBCF	mBAF (%)	TF (Aboveground/Belowground)	mTF (Aboveground/Belowground)
Roots	Ni_{220}	0.42 ± 0.13	0.49 ± 0.09	0.0395 ± 0.0094		
	Cu_{200}	1.33 ± 0.75	1.141 ± 0.437	0.067 ± 0.038		
	Cu_{400}	3.42 ± 1.46	1.136 ± 0.124	0.104 ± 0.025		
	Zn_{450}	8.63 ± 3.2	0.435 ± 0.119	0.583 ± 0.181		
	Zn_{900}	15.46 ± 4.36	0.51 ± 0.063	0.522 ± 0.117		
	Cr_{300}	1.09 ± 0.4	2.3 ± 0.25	1.8694 ± 0.5465		
	Cr_{600}	1.89 ± 0.7	1.3 ± 0.17	1.4927 ± 0.4625		
	Pb_{450}	4.56 ± 1.88	0.526 ± 0.194	0.575 ± 0.224		
	Pb_{900}	7.87 ± 3.11	0.446 ± 0.157	0.513 ± 0.187		
	Cd_{4}	1.36 ± 0.44	1.85 ± 0.35	1.9298 ± 0.4621		
	Cd_{8}	1.04 ± 0.37	0.95 ± 0.14	0.6268 ± 0.1675		
	Ni_{110}	1.16 ± 0.45	0.86 ± 0.18	1.0146 ± 0.2926		
	Ni_{220}	0.76 ± 0.35	0.41 ± 0.09	0.3263 ± 0.1231		
	Cu_{200}	25.59 ± 38.36	0.667 ± 0.295	0.983 ± 0.486		
	Cu_{400}	12.74 ± 18.76	0.199 ± 0.025	0.297 ± 0.059		

3.5 Percolated waters

Another important value is the fraction of the contaminants in the soil that can be leaching, and contaminating belowground water, presented in Table 3.31. It is possible to see that the leached increases with heavy metal in soil for Cr, Ni, and Cd. Another important factor is related to the crop. The giant reed trials suffered more with leaching processes than switchgrass trials for both concentrations of the studied contaminants. The values obtained in Cd and Ni do not exceed the limits values for wastewater (0.2 $mg.L^{-1}$ Cd and 2.0 $mg.L^{-1}$ Ni). However, in the trials of the highest concentration of Cr, the values exceed the wastewater limit (2.0 $mg.L^{-1}$ Cr)(Ministério do Meio Ambiente, 1998). The study showed that giant reed and switchgrass contribute to preventing Cd and Ni leaching, while Cr leaching prevention depends on the metal concentration in the soil. The switchgrass leach process did not exceed the limits for either copper or lead, considering both the low and the high concentrations of the contaminant in the pot. For giant reed, however, the percolated water can exceed the maximum allowed limits in giant reed's trials with a high concentration of Pb. It was also observed that switchgrass was more efficient for Zn, Pb, and Cu trials than giant reed to prevent the leaching of the contaminants.

Table 3.31. Heavy metal concentration in percolated water

The main element of contamination	**Crop**	**Heavy metal in percolated water (mg/L)**			**Limit of heavy metals in wastewater** (Ministério do Meio Ambiente 1998)
		Control	Low	High	
Zn	Switchgrass	0.020 ± 0.008	0.151 ± 0.045	0.136 ± 0.026	--
	Giant reed	0.028 ± 0.008	0.380 ± 0.056	0.697 ± 0.112	
Pb	Switchgrass	0.181 ± 0.036	0.330 ± 0.062	0.491 ± 0.112	1.0 mg/L Pb
	Giant reed	0.563 ± 0.065	0.674 ± 0.122	0.939 ± 0.125	
Cr	Switchgrass	0.418 ± 0.015	1.008 ± 0.101	0.632 ± 0.156	2.0 mg/L Cr
	Giant reed	0.399 ± 0.015	1.034 ± 0.056	4.344 ± 0.225	
Cd	Switchgrass	0.048 ± 0.007	0.059 ± 0.012	0.076 ± 0.012	0.2 mg/L Cd
	Giant reed	0.031 ± 0.008	0.111 ± 0.012	0.105 ± 0.015	
Ni	Switchgrass	0.036 ± 0.007	0.128 ± 0.032	0.082 ± 0.016	2.0 mg/L Ni
	Giant reed	0.048 ± 0.008	0.105 ± 0.018	0.488 ± 0.062	
Cu	Switchgrass	0.121 ± 0.023	0.142 ± 0.033	0.109 ± 0.029	1.0 mg/L Cu
	Giant reed	0.074 ± 0.014	0.208 ± 0.032	0.238 ± 0.027	

Different lower-case letters indicate statistical significance ($p < 0.05$) among treatments per year. Zn_{450} and Zn_{900}, 450 and 900 mg Zn kg^{-1}. Cr_{300} and Cr_{600}, 300 and 600 mg Cr kg^{-1}. Pb_{450} and Pb_{900}, 450 and 900 mg Pb kg^{-1}. Cd_4 and Cd_8, 4 and 8 mg Cd kg^{-1}. Ni_{110} and Ni_{220}, 110 and 220 mg Ni kg^{-1}. Cu_{200} and Cu_{400}, 200 and 400 mg Cu kg^{-1}

3.6 Environmental and Socio-Economic Impact Assessment of the Switchgrass and Giant reed Production in Heavy Metals Contaminated Soils

The energy balance estimated for switchgrass production and use in heavy metal contaminated soils is presented in Figure 3.22.

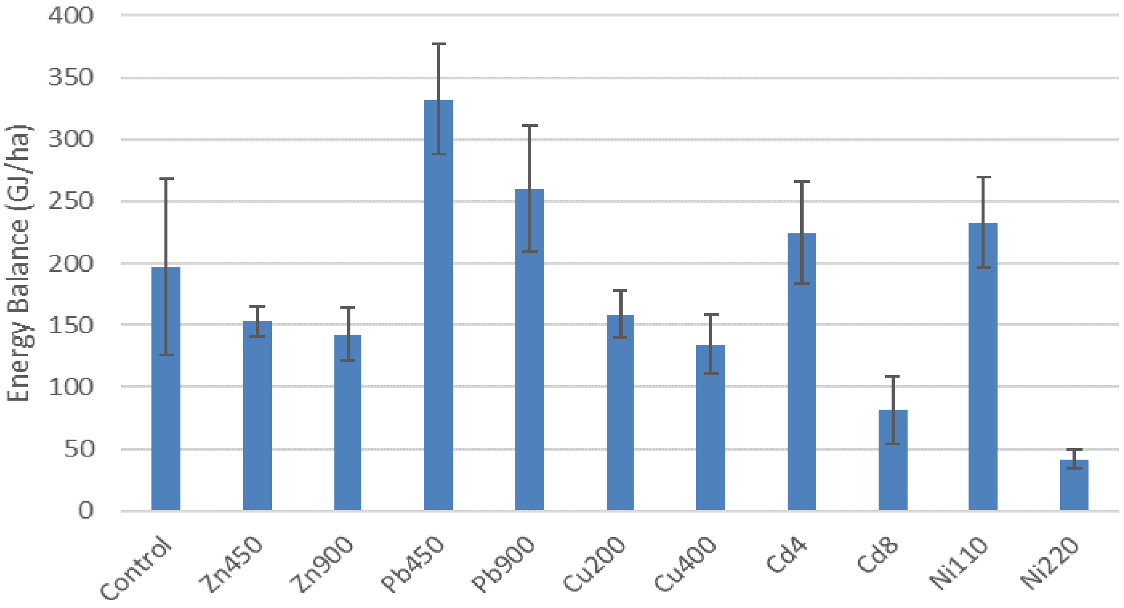

Figure 3.22. Estimated energy balance (GJ/ha) for switchgrass production and use.
Zn_{450} and Zn_{900}, 450 and 900 mg Zn kg^{-1}. Cr_{300} and Cr_{600}, 300 and 600 mg Cr kg^{-1}. Pb_{450} and Pb_{900}, 450 and 900 mg Pb kg^{-1}. Cd_4 and Cd_8, 4 and 8 mg Cd kg^{-1}. Ni_{110} and Ni_{220}, 110 and 220 mg Ni kg^{-1}, Cu_{200} and Cu_{400}, 200 and 400 mg Cr kg^{-1}.

It is possible to observe that the energy balance depends on the yields obtained. Where contamination affected most of the yields (Cd_8, Ni_{220}), the energy balance was also affected but still showed a positive value. In the remaining contaminations, the energy balance was similar to or even higher than control soils. In the analysis of switchgrass, the estimates in Cr soils were disregarded due to no yields in those pots. This balance highlights that the energy potential of the crop was not affected by the contaminants studied (in the applied concentrations), except for Cd_8 and Ni_{220}. According to the scenario presented, the Control showed an energy balance of 197 GJ/ha, Zn and Cu pots (low and high contamination) showed lower energy balance than the control, but without significant differences to control, and Pb (low and high contamination), Cd_4, and Ni_{110} showed higher energy balance than the control (Pb_{450} showed a significantly higher balance compared to control). In the contaminated soils, the energy balance follows the yield pattern, being lower when yields are lower and higher when yields are higher. Therefore, it is possible to see that in all the contaminated soils (except in Cr), the production and use of switchgrass for bioenergy through combustion will allow for saving a significant amount of fossil energy.

The energy balance estimated for giant reed production and use in heavy metal contaminated soils is presented in Figure 3.22.

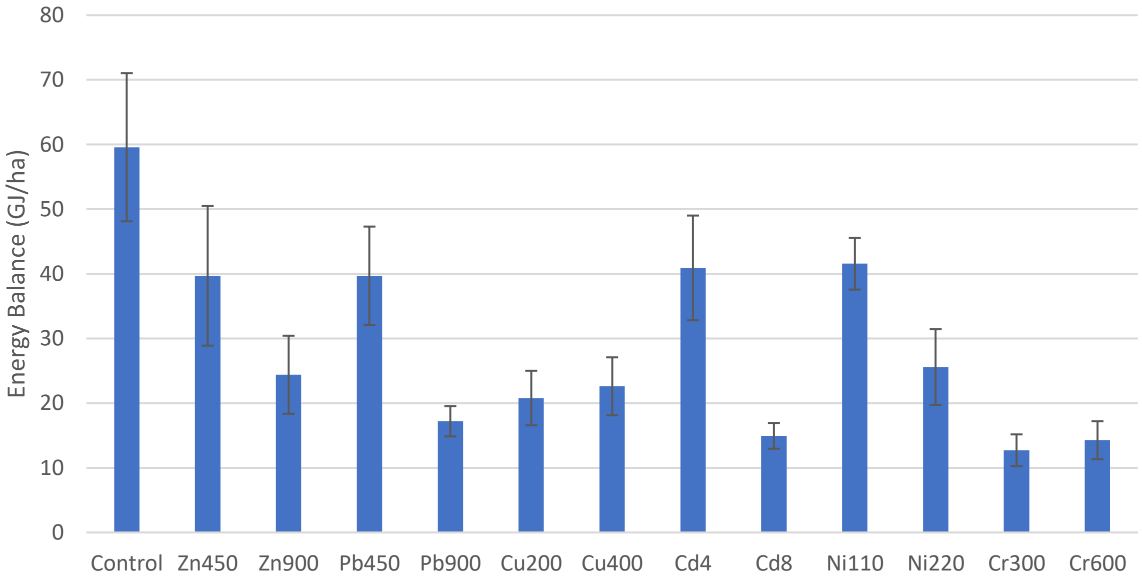

Figure. 3.23. Estimated energy balance (GJ/ha) for giant reed production and use.
Zn_{450} and Zn_{900}, 450 and 900 mg Zn kg^{-1}. Cr_{300} and Cr_{600}, 300 and 600 mg Cr kg^{-1}. Pb_{450} and Pb_{900}, 450 and 900 mg Pb kg^{-1}. Cd_4 and Cd_8, 4 and 8 mg Cd kg^{-1}. Ni_{110} and Ni_{220}, 110 and 220 mg Ni kg^{-1}, Cu_{200} and Cu_{400}, 200 and 400 mg Cr kg^{-1}.

As seen with switchgrass, in the contaminated soils, the energy balance follows the yield pattern, being lower when yields are lower and higher when yields are higher. As yields of giant reed in the 2nd year were significantly lower than yields of switchgrass, the energy balance observed was significantly lower, and a lower amount of fossil energy can be saved by using giant reed to produce energy. Nevertheless, the balance was always positive, thus indicating that even with lower yields, the output was always higher than the input. Control shows an energy balance of 60 GJ/ha. Zn_{450}, Pb_{450}, Cd_{4}, and Ni_{110} showed lower energy balance than the control but without significant differences from to control. The remaining pots showed a significantly lower amount of energy saved from biomass to bioenergy.

Figure. 3.23 presents the potential reduction of greenhouse gases emissions (GHG) due to switchgrass production and use. According to the results estimated, the use of switchgrass as solid fuel is relevant to reduce GHG emissions, even when biomass is obtained from heavy metal contaminated soils (in this case, the estimates in Cr soils were disregarded). Furthermore, the experiment showed that the contamination does not significantly impact the amount of saved GHG, except when the yields were significantly lower than in control soils, namely in Cd_8 and Ni_{220} pots.

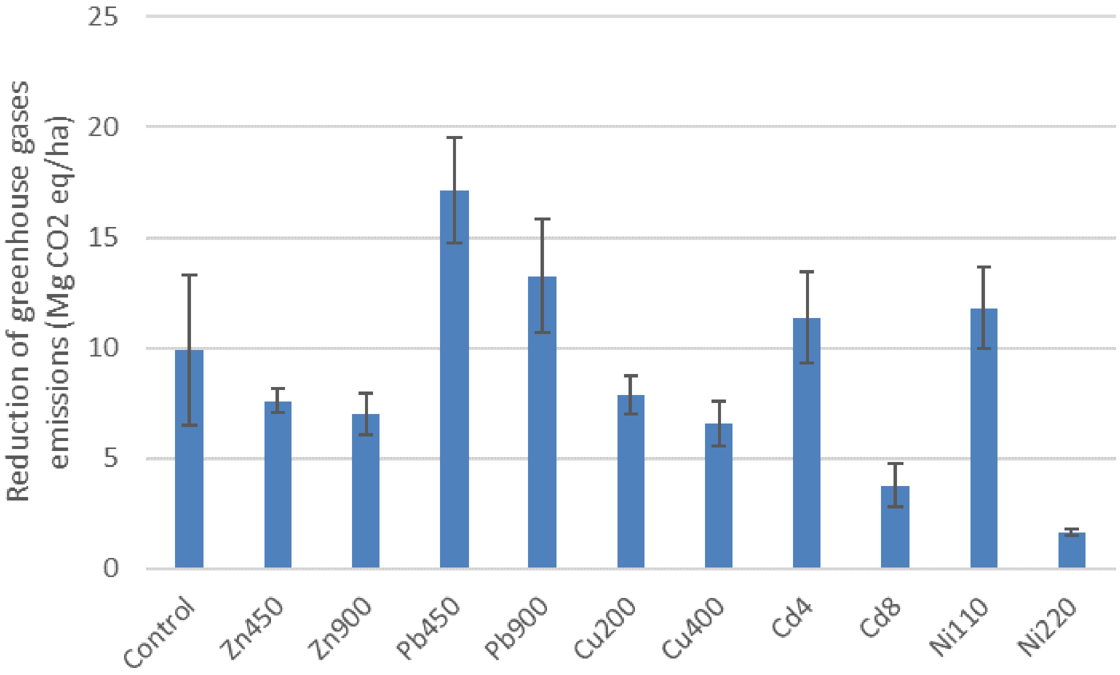

Figure. 3.24. Estimated reduction of greenhouse gases emissions (Mg CO2 eq/ha) due to switchgrass production and use as solid fuel.
Zn_{450} and Zn_{900}, 450 and 900 mg Zn kg^{-1}. Cr_{300} and Cr_{600}, 300 and 600 mg Cr kg^{-1}. Pb_{450} and Pb_{900}, 450 and 900 mg Pb kg^{-1}. Cd_4 and Cd_8, 4 and 8 mg Cd kg^{-1}. Ni_{110} and Ni_{220}, 110 and 220 mg Ni kg^{-1}, Cu_{200} and Cu_{400}, 200 and 400 mg Cr kg^{-1}.

Figure. 3.23 presents the potential reduction of greenhouse gases emissions (GHG) due to giant reed production and use. According to the results estimated, the use of giant reed as solid fuel presents a lower relevance to reducing GHG emissions than switchgrass because yields were also significantly lower. Nevertheless, the savings are positive or almost null even when biomass is obtained from heavy metal contaminated soils. The experiment showed that the contamination significantly affected the amount of saved GHG, especially when the yields were significantly lower than in control soils, namely in Cd_8 and all the Cr-contaminated pots. The reduction in yields also affected negatively the energy balance and the greenhouse gasses emission in the work of Schmidt et al. (Schmidt et al., 2015), which tested switchgrass in marginal soils of Europe.

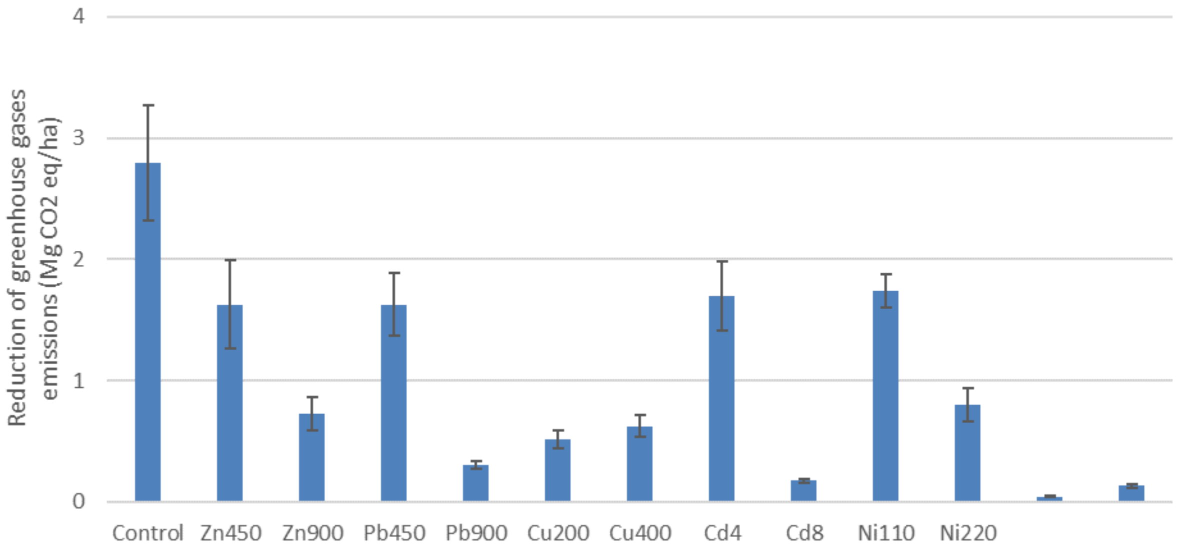

Figure. 3.25. Estimated reduction of greenhouse gases emissions (Mg CO2 eq/ha) due to switchgrass production and use as solid fuel.
Zn_{450} and Zn_{900}, 450 and 900 mg Zn kg^{-1}. Cr_{300} and Cr_{600}, 300 and 600 mg Cr kg^{-1}. Pb_{450} and Pb_{900}, 450 and 900 mg Pb kg^{-1}. Cd_4 and Cd_8, 4 and 8 mg Cd kg^{-1}. Ni_{110} and Ni_{220}, 110 and 220 mg Ni kg^{-1}, Cu_{200} and Cu_{400}, 200 and 400 mg Cr kg^{-1}.

A comparison of the land use of switchgrass in heavy metals contaminated soils, with control, is presented in Figure. 3.26.

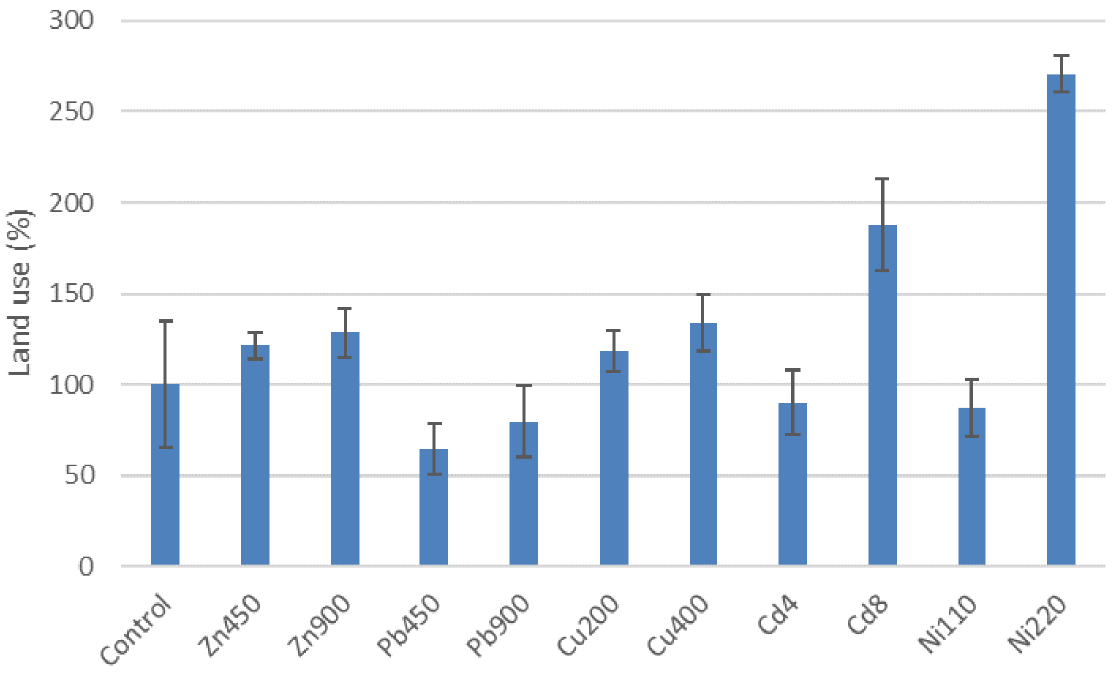

Figure. 3.26. Land use of switchgrass in heavy metals contaminated soils compared with a control (%).
Zn_{450} and Zn_{900}, 450 and 900 mg Zn kg^{-1}. Cr_{300} and Cr_{600}, 300 and 600 mg Cr kg^{-1}. Pb_{450} and Pb_{900}, 450 and 900 mg Pb kg^{-1}. Cd_4 and Cd_8, 4 and 8 mg Cd kg^{-1}. Ni_{110} and Ni_{220}, 110 and 220 mg Ni kg^{-1}, Cu_{200} and Cu_{400}, 200 and 400 mg Cr kg^{-1}.
.

It is possible to observe that the soil contamination did not significantly affect the area needed for switchgrass cultivation (in this case, the estimates in Cr soils were disregarded). Less area was needed when yields were higher than control (case of Pb soils and Cd_4 and Ni_{110} soils), and a higher area was needed to produce the same quantity of biomass for bioenergy when yields were lower (case of Zn and Cu pots), but differences to control soils were not significant. Nevertheless, in Cd_8 and Ni_{220} pots, the land area needed to produce the same amount of energy was significantly higher due to the significantly lower yields.

A comparison of the land use of giant reed in heavy metals contaminated soils, with control, is presented in Figure. 3.26.

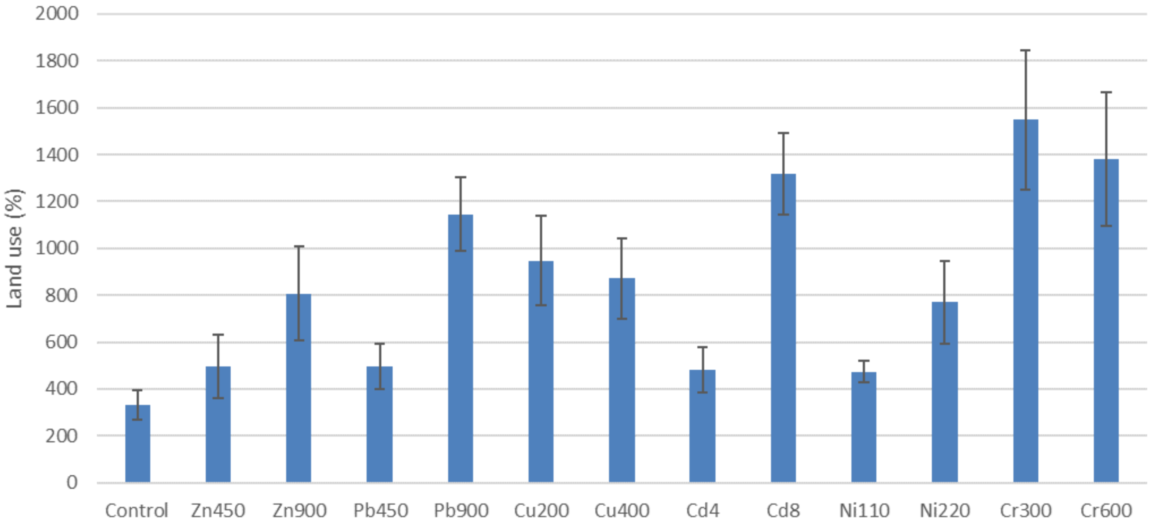

Figure. 3.27. Land use of giant reed in heavy metals contaminated soils compared with the amount of land needed to produce 200 GJ/ha with switchgrass (%).
Zn_{450} and Zn_{900}, 450 and 900 mg Zn kg^{-1}. Cr_{300} and Cr_{600}, 300 and 600 mg Cr kg^{-1}. Pb_{450} and Pb_{900}, 450 and 900 mg Pb kg^{-1}. Cd_4 and Cd_8, 4 and 8 mg Cd kg^{-1}. Ni_{110} and Ni_{220}, 110 and 220 mg Ni kg^{-1}, Cu_{200} and Cu_{400}, 200 and 400 mg Cr kg^{-1}.

In this case, It is possible to observe that as giant reed yields were significantly lower than switchgrass, the amount of land needed to produce *circa* 200 GJ/ha was three times more than the land needed by switchgrass. In this case, due to the significant effect of the contamination in the yields, the amount of land needed increased significantly even compared with the control soils of giant reed, namely in Zn_{900}, Pb_{900}, all the Cu-contaminated pots, Ni_{220}, Cd_8, and all the Cr contaminated pots. This may cause some constraints on using contaminated land to produce biomass for energy. The higher the land needed, the higher the need for long transportation distances, which can also affect the energy balances and the amount of GHG emissions saved. The same sort of pattern was also observed in the work of Boléo et al. (2013). In this work, lower yields of Miscanthus were obtained in Zn-contaminated soils; therefore, a higher land area was needed to obtain the same amount of energy material.

This result indicates that switchgrass is a good option as an energy crop cultivated in soils contaminated with heavy metals, but giant reed is less promising. In fact, for both crops, using contaminated land to produce biomass for biobased materials, biofuels, heat, and power can avoid problems derived from land use competition for food and feed. Also, considering that the land is degraded, this change in land use may be beneficial for the environment and landowners due to the possible restoration of the functions and services of the soil ecosystem. The possibility of producing biomass in degraded soil will cover wildlife and enrich the landscape, namely aesthetics or structural heterogeneity, thus contributing to the landscape and biological diversity. Moreover, perennial grasses like switchgrass and giant reed require reduced agrochemicals and soil tillage use. These plants have high aerial and extended belowground biomass, which increases soil organic matter content and litter deposition, and their presence in the soil contributes to reducing the erosion risk. These conditions favor the occurrence of soil fauna and microfauna, especially decomposers, contributing to biodiversity.

Moreover, a late harvest, e.g., the end of January, may provide a shelter for small mammals and birds and a site for invertebrates during wintering. In addition, switchgrass fields gain heterogeneity if planted in smaller plots instead of as a wide landscape monoculture. For example, the case of postmining land, where the remediation areas are not contiguous (Barbosa et al., 2018; Dauber et al., 2012; Fernando et al., 2018).

In terms of economic and social aspects, is the production of switchgrass and a giant reed in contaminated soils of relevance?

The breakeven delivered price for switchgrass production, resulting from the operating costs at the farm gate plus transportation, is presented in Figure. 3.28. Production of switchgrass in heavy metals contaminated soils significantly increases the breakeven delivered price of the biomass when yields are lower than in non-contaminated soils. In control, the breakeven price is about 20€/Mg biomass. The breakeven delivery price for switchgrass in Zn and Cu-contaminated soils showed an increase of around 25%, while for switchgrass in the lead, Ni_{110}, and Cd_4 contaminated soils, the value is similar or even lower (as in the case of Pb soils). Therefore, the increase in the cost makes switchgrass from zinc and copper contaminated soils a less viable alternative. On the other hand, lead, Ni_{110}, and Cd_4 already represent a viable alternative to biomass production, making this an up-and-coming option for Pb, low Ni, and low Cd-contaminated soil landowners. However, the breakeven delivered price associated with Cd_8 and Ni_{220} soils shows to be prohibitive once the price was doubled and three times more than in control soils. The cost of switchgrass for bioenergy, even in the Control, is noticeably higher than the cost of fossil-based energy (coal energy-equivalent biomass price, 15 €/Mg) (Khanna et al., 2008) and is not yet economically attractive. Nevertheless, if grants and subsidies are credited to the production and conversion of biomass to energy and if supplementary compensation for CO_2 abatement or other benefits is attributed, then this result can be minimized. On the other hand, if the cost of contaminated land remediation is included, switchgrass production in contaminated land may become economically sustainable.

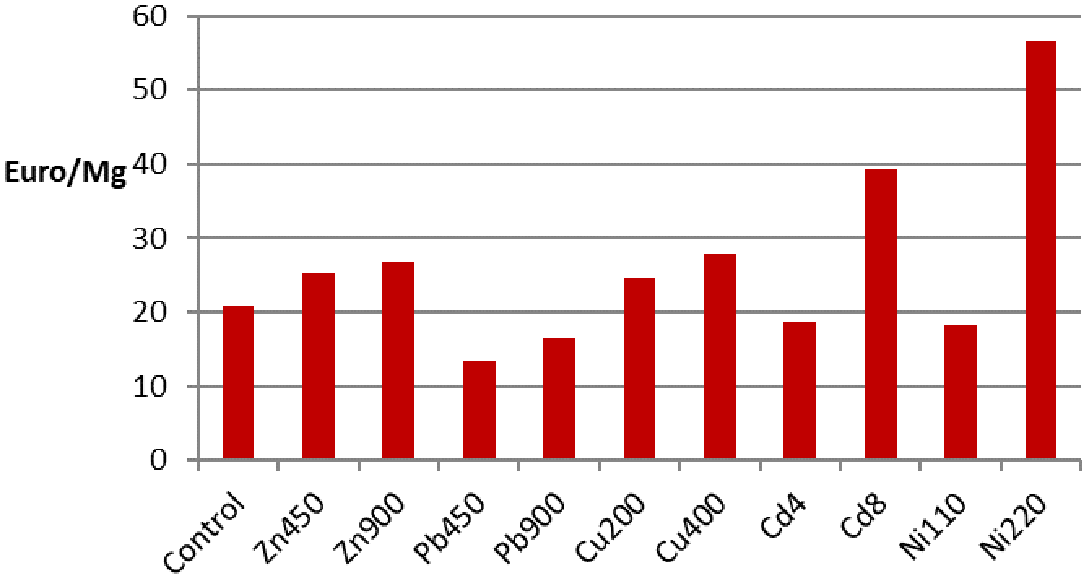

Figure. 3.28. Breakeven delivered the switchgrass production and transportation price (€/Mg).
Zn_{450} and Zn_{900}, 450 and 900 mg Zn kg^{-1}. Cr_{300} and Cr_{600}, 300 and 600 mg Cr kg^{-1}. Pb_{450} and Pb_{900}, 450 and 900 mg Pb kg^{-1}. Cd_4 and Cd_8, 4 and 8 mg Cd kg^{-1}. Ni_{110} and Ni_{220}, 110 and 220 mg Ni kg^{-1}, Cu_{200} and Cu_{400}, 200 and 400 mg Cr kg^{-1}.

The breakeven delivered price for giant reed production, resulting from the operating costs at the farm gate plus transportation, is presented in Figure. 3.28. Production of giant reed in heavy metals contaminated soils significantly increases the breakeven delivered price of the biomass when yields are lower than in non-contaminated soils, especially in Zn_{900}, Pb_{900}, all the Cu-contaminated pots, Ni_{220}, $Cd_{8,}$ and all the Cr contaminated pots. In control, the breakeven price is about 60€/Mg biomass, which is also prohibited compared with the results for switchgrass. Even in the Control, the cost of giant reed for bioenergy is noticeably higher than that of fossil-based energy (coal energy-equivalent biomass price, 15 €/Mg) (Khanna et al., 2008) and is not yet economically attractive.

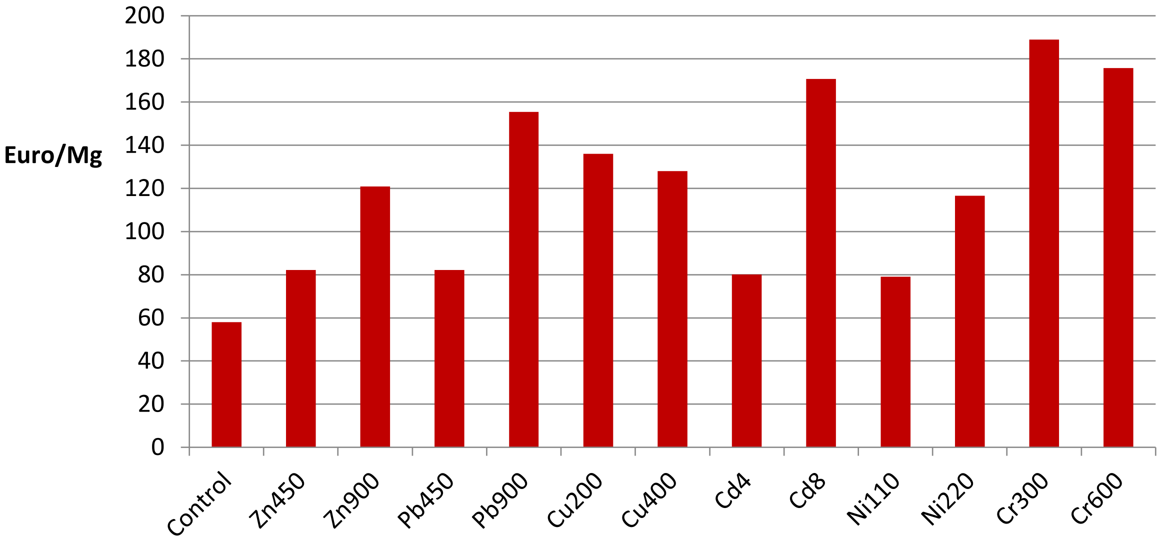

Figure. 3.29. Breakeven delivered the price (€/Mg) of the giant reed production and transportation.
Zn_{450} and Zn_{900}, 450 and 900 mg Zn kg^{-1}. Cr_{300} and Cr_{600}, 300 and 600 mg Cr kg^{-1}. Pb_{450} and Pb_{900}, 450 and 900 mg Pb kg^{-1}. Cd_4 and Cd_8, 4 and 8 mg Cd kg^{-1}. Ni_{110} and Ni_{220}, 110 and 220 mg Ni kg^{-1}, Cu_{200} and Cu_{400}, 200 and 400 mg Cr kg^{-1}.

Production of these perennial grasses in contaminated soils can provide human communities with many social benefits. The use of degraded land for energy crop production still comprises much debate and is not always socially accepted, but simultaneously this approach involves new opportunities, especially with a non-food crop that may have an economical income. Production of both crops on heavy metals contaminated soils contributes to reducing the contamination of these soils, reestablishing its ecosystem function and services, thus reducing environmental and human exposure to pollutants. Also, the production of switchgrass in heavy metals contaminated soils reduces human exposure to the effects health of GHG emissions and the environmental exposure to these emissions. Switchgrass and giant reed production and use also positively improve employment creation in small and medium-sized enterprises and rural areas, especially in less productive areas due to contamination. It also contributes positively to more balanced rural development and prevents rural exodus. Labor requirements per hectare for the production, in the farm, of switchgrass and giant reed is 9 hours per hectare per year (Fernando et al., 2009). Cultivation in contaminated soils does not represent an increase in agricultural activities by comparison with control, and so does not lead to an increase in employment; however, if we compare it with permanently degraded land (0-2 hours per hectare per year) (Fernando et al., 2009), then there is a considerably higher increase in employment.

4

CONCLUSIONS

Results demonstrate the potential of giant reed and switchgrass in the phytoremediation process. Despite the decrease in productivity due to the contamination of the pots, giant reed showed a great accumulation potential for heavy metals, especially in the roots, evidencing its potential for phytoremediation through the stabilization of the contaminants. This behavior was also observed in switchgrass for Cd and Ni contamination, while this crop was unsuitable for Cr-contaminated soils. Switchgrass also presented great potential for heavy metals phytoextraction due to the plant's accumulation in the aerial part.

Chromium was the most toxic heavy metal studied in this experiment, inhibiting the growth of switchgrass and reducing the yield of giant reed, in this last one, accumulating in the belowground fraction and, in this way, reducing the nutrients uptake due to the competition for the absorption mechanism.

On the other hand, despite being a micronutrient for plants, it was observed that when submitted to elevated Zn concentration in soil, the yield of the crops reduced. In this way, the ideal zinc concentration in soil for energy crop cultivation can be a promising subject of study.

The behavior of switchgrass indicates that as a response to the contaminants' saturation of the root system, switchgrass compensates for increasing the belowground biomass to maintain its productivity. This scenario was well observed in the second year for Zn_{900} and Pb_{900}. Compared to giant reed, this study showed the importance of designing phytoremediation experiments using perennial crops considering several growing cycles since the yield for switchgrass considerably increased in the second year due to the development of the root system, which allowed a higher nutrient absorption capacity. In contrast, giant reed, which was planted with better-established rhizomes, had a higher development in the first year.

Giant reed and switchgrass showed to maintain their properties regarding the thermochemical potential and the fiber content, despite heavy metals in the soil. This shows that these crops' utilization for heat and electricity production for thermochemical conversion processes, as their utilization for lignocellulosic products, remains viable regarding their cultivation in heavy metal contaminated soils.

However, the effect of these contaminants in the equipment can be harmful, creating costs in the long term scenario. Furthermore, the increase of ash content associated with heavy metals in soils can increase the amount of the residue, which must be treated before being discarded. Therefore, utilizing these biomasses in conversion processes that recover these heavy metals can eliminate the residual treatment problem and generate a commercial sub-product. However, the viability of these processes needs to be better developed.

Regarding phytoremediation potential, despite this technique creating a renewable and sustainable feedstock, the amount of cultivation cycles needed to decontaminate the soils and extract the contaminants through the harvest of the aerial fraction of the biomass limits this technique's viability.

Despite the environmental advantages of phytoremediation, heavy metal uptake occurs at a lower rate than the ideal to promote the decontamination of the soil in a reasonable time. Also, the tendency of biomass to accumulate a higher amount of heavy metals in the belowground fraction makes it difficult the extraction these contaminants from the soil.

However, considering the ecological and social advantages of marginal land utilization, the possibility of replacing fossil feedstock for biomass must be considered the decisive factor when deciding to cultivate industrial crops in marginal lands. Valorizing the contaminated biomass is vital to make phytoremediation processes economically feasible. This way, the valorization of giant reed and switchgrass contaminated biomasses through thermochemical conversion processes was challenging for pyrolysis and gasification due to the ash content value. The nitrogen content of biomass is the central challenge to its application in combustion processes due to the related emissions. However, more studies regarding the interactions between the contaminants and the equipment used in the conversion processes are necessary to evaluate energy production through contaminated biomass.

One of the biggest constraints for developing the bioenergy sector is optimizing and balancing its economic, environmental, and social sustainability. The phytoremediation of heavy metals contaminated soils with switchgrass and giant reed is a good option compared to abandoning heavy metals contaminated soil or traditional physical and chemical remediation techniques. Both from an environmental standpoint, with the restoration of the degraded soil ecosystem and its services, or from an economic standpoint, either from the bioenergy revenues to land owners or from the lower cost of phytoremediation compared to the traditional remediation processes. Overall results advocate that the production of switchgrass and a giant reed in heavy metals contaminated soils have positive characteristics and others that are less positive over the production of those crops in non-contaminated soils. The productivity loss in contaminated soils reduces the greenhouse and energy savings, but it may enrich those soils' biological and landscape diversity and the quality of waters and soils. Moreover, in some contaminated soils, results are promising compared with results obtained in non-contaminated soils, making both crops an alternative to being produced and used as solid fuel, helping to tackle the greenhouse effect and decontaminating at the same time that generates energy and profits for the owners of the heavy metals contaminated soils. Overall results suggest that switchgrass production in heavy metals contaminated soils is more promising than giant reed, considering the environmental and economic aspects of its production and use.

The future steps of this work could better explore the energetic potential of the contaminated biomass, analyze the effect of contamination in biofuels and bioenergy production, try to understand how the heavy metals affect microorganisms in fermentation and anaerobic digestion processes if there are limits in the heavy metals concentration that can inhibit the biological processes. Regarding the thermochemical processes, despite a few studies already touching on this subject, evaluating the long term influence of each heavy metal in gasifiers, combustion chambers, and pyrolysis reactors could reveal small points to improve. The valorization of the contaminated residues should also be evaluated to promote a circular economy.

5 REFERENCES

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