Department of Agricultural Science

University of Naples Federico II

Ph.D. Thesis in Sustainable Agricultural And Forestry Systems And Food Security

Linda Carrino

# Phytomanagement of contaminated and marginal lands with *Ricinus communis* L.

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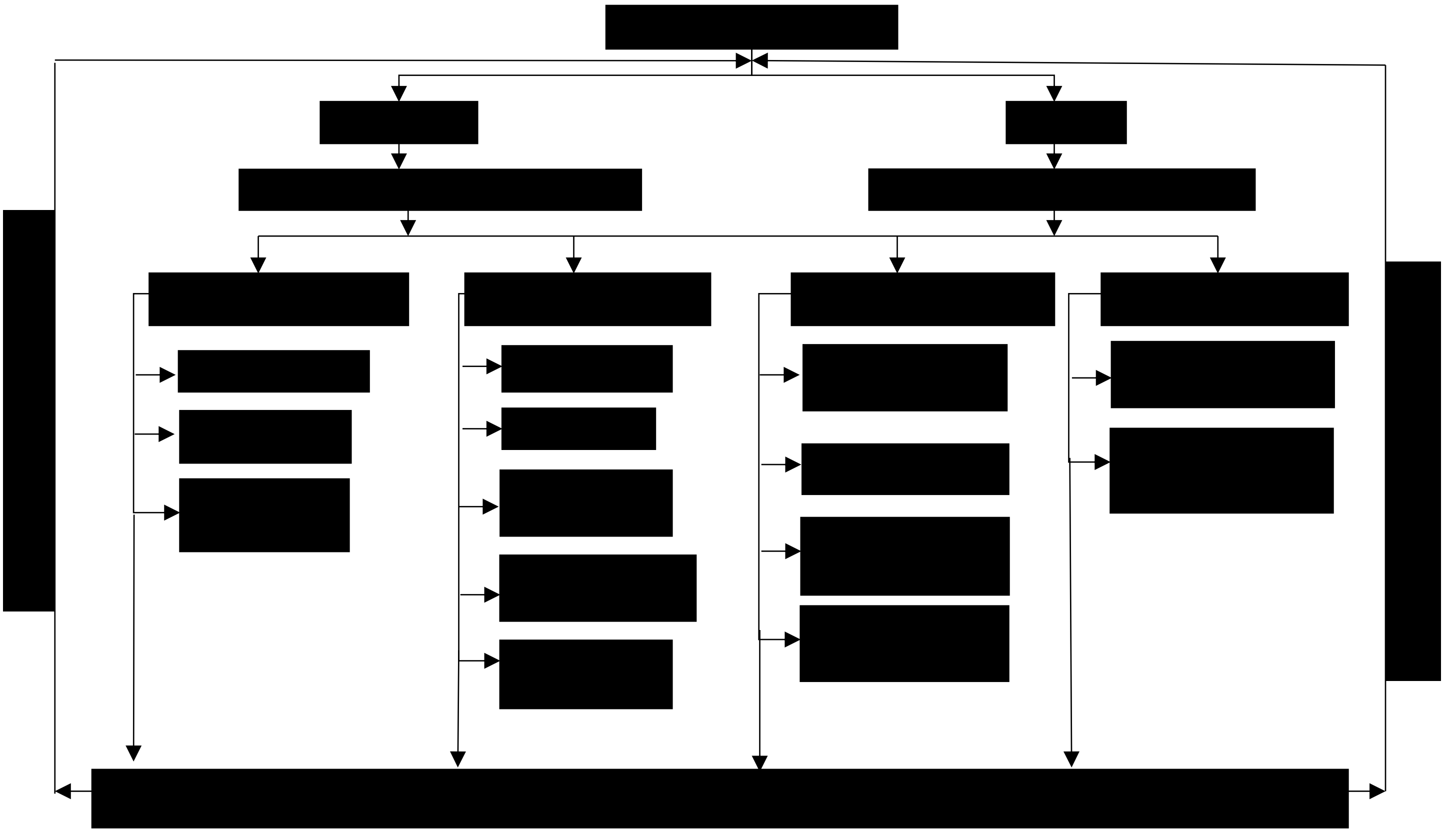

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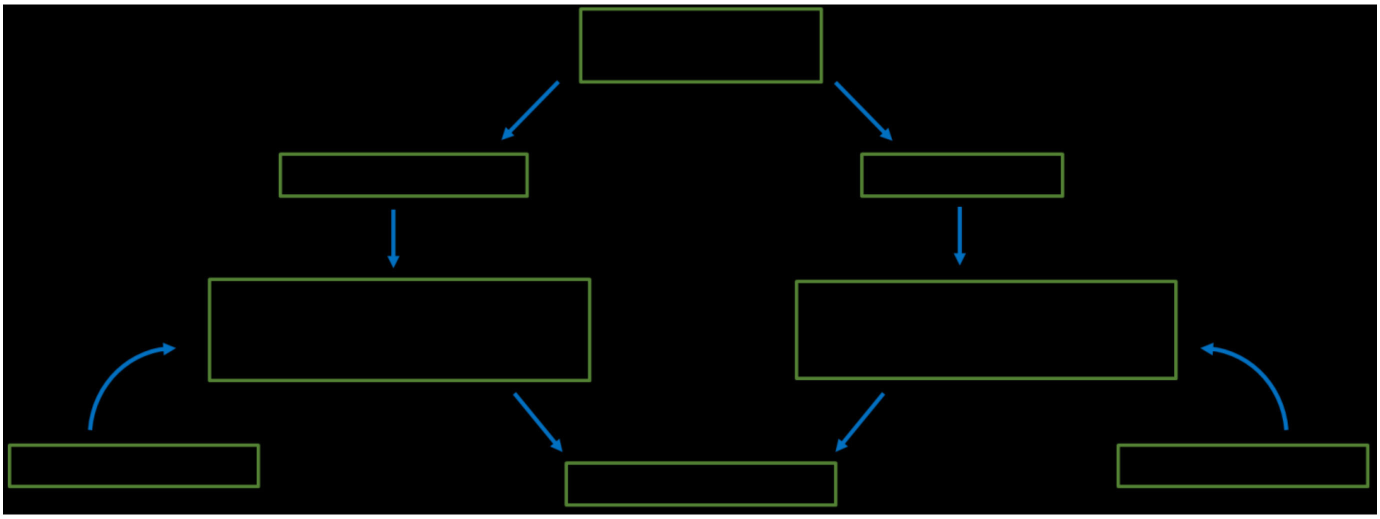

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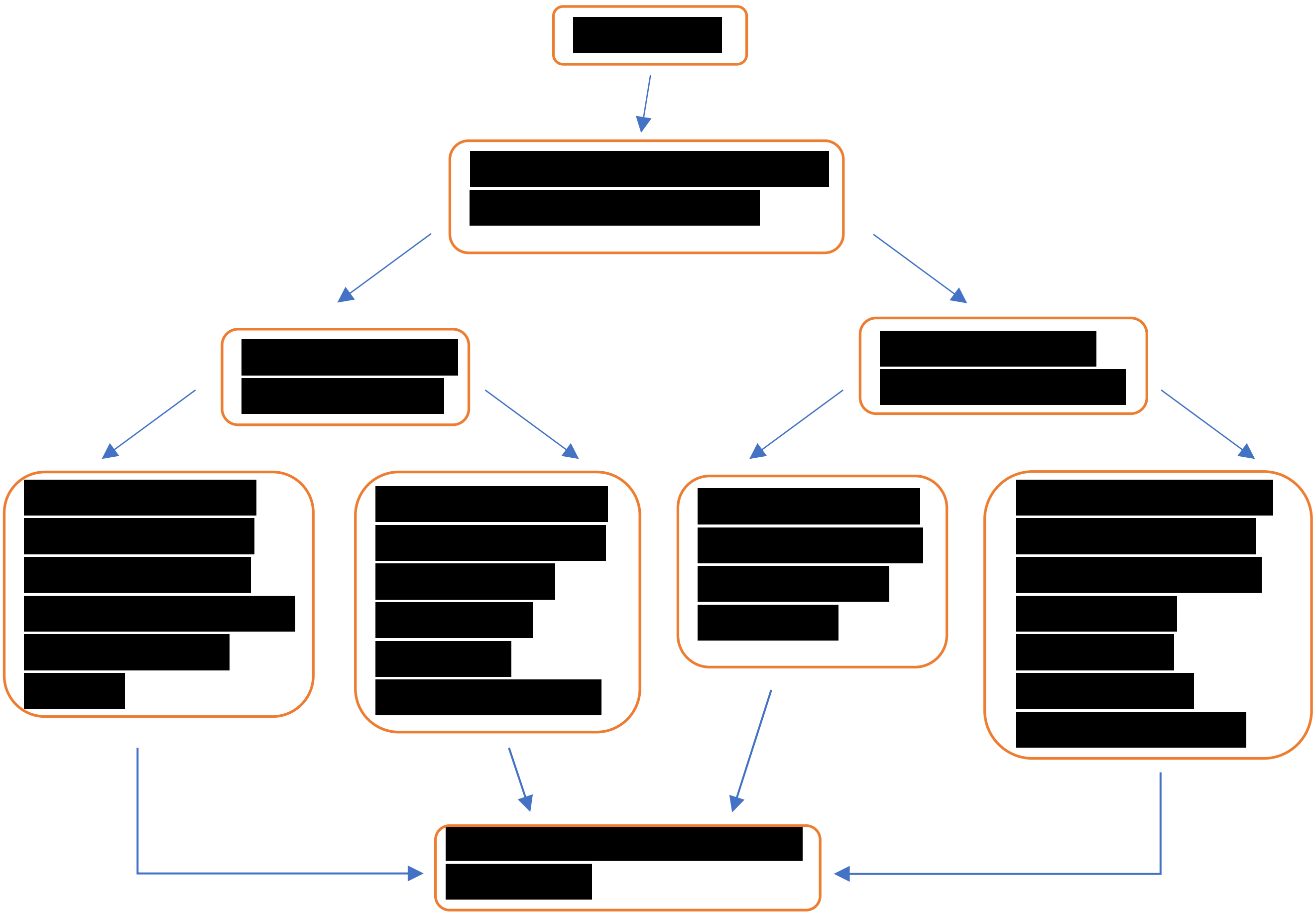


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# Chapter 2

# Biofuel production with castor bean: a win-win strategy for marginal land

agronomy

*Review*

## Biofuel Production with Castor Bean: A Win–Win Strategy for Marginal Land

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# 2. Biofuel production with castor bean: a win-win strategy for marginal land

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**Abstract:** The urgency to reduce resource depletion and waste production drives through to an economy based on renewable resources. Biofuels, for instance, are a great green alternative to fossil fuels, but they currently derive from edible vegetable oils such as soybean, palm and sunflower. Concerns have been raised about the social-economic implication and ecological impacts of biodiesel production. Cultivating new lands as biodiesel feedstock rather than food supply, with the consequent increase in food prices, leads to so-called Indirect Land Use Change (ILUC). Establishing bioenergy crops with phytoremediation ability on contaminated soils offers multiple benefits such as improving soil properties and ecosystem services, decreasing soil erosion, and diminishing the dispersion of potentially toxic elements (PTEs) into the environment. Castor bean is an unpalatable, high biomass plant, and has been widely demonstrated to possess phytoremediation capability for several PTEs. Castor can grow on marginal lands not suitable for food crops, has multiple uses as a raw material, and is already used in biodiesel production. These characteristics make it perfect for sustainable biodiesel production. Linking biofuel production with environmental remediation can be considered a win-win strategy.

## 2.1. Introduction

The increasing industrialization, which follows the “take-make-dispose” plan, has led to the depletion of non-renewable resources, producing waste, and causing environmental impacts due to air, soil and water contamination (Fagnano, 2018). Currently, there is an increased use of renewables (e.g., biofuels) to replace the over-reliance on fossil fuels, to reduce resource consumption and waste production (Poščić et al., 2019). The most popular biodiesels are mainly produced from edible crops such as soybean, rapeseed, palm, mustard and sunflower (Chatzakis et al., 2011). However, some concerns have recently been raised about the socio-economic implications and ecological impacts of biofuel production (Bentivoglio & Rasetti, 2015). To be sustainable, biofuels should not affect the quality, quantity and use of water or soil, with unacceptable social consequences (Lora et al., 2011). Consequently, a biofuel feedstock has to reduce the Indirect Land Use Change, (e.g., the emission of more carbon dioxide as a consequence of the cultivation of new land in response to biofuel demand) which causes a subsequent deficit in food supply and increases in food prices (Malins et al., 2014).

It is well known that many areas of the world are contaminated. Taking as an example, the European Union has estimated the existence of around 2.8 million sites where land contamination exists or is taking place (Pérez & Rodríguez Eugenio, 2018). Hence, linking the production of renewable energy with phytoremediation may be considered a winning strategy to avoid land competition with traditional food crops, protecting human health by remediating land contamination, and mitigating the energy crisis and climate change (Bauddh et al., 2017; Kiran & Prasad, 2017). In particular, the establishment of bioenergy crops with phytoremediation potential on soil contaminated by potentially toxic elements (PTEs) may offer multiple environmental benefits, such as improving soil properties and ecosystem services, decreasing soil erosion, and diminishing the mobility of PTEs through their adsorption and accumulation in roots or their precipitation within the root zone (Fiorentino et al., 2017). Phytoremediation involves the use of plants for the restoration of polluted environments being an *in situ*, solar-powered alternative to conventional remediation procedure, with a very high public acceptance (Fagnano & Fiorentino, 2018). Fast-growing perennial crops with high tolerance to biotic and abiotic stress are able to lower soil available PTEs (phytoextraction), reducing their mobility/bioavailability (phytostabilization), being considered the best option for phytoremediation programs (Fiorentino et al., 2017). Besides this, while remediating a contaminated site, the plant biomass can be used for green fine chemistry, bioplastic, and renewable energy and can be considered an integral part of a sustainable economy (Pošćić et al., 2019). However, uncertainties have been raised about the safe use of contaminated plant biomass for energy conversion. According to numerous studies, different thermal conversion methods, especially pyrolysis, are exploited to convert metal contaminated biomass after phytoremediation (Giudicianni et al., 2017; Grottola et al., 2019). Pyrolysis greatly reduces the weight and volume of the biomass, meaning easier disposal, while concentrating the PTEs in the char/ash fraction which can eventually undertake additional treatments or metal extraction before discarding (Liu et al., 2012). The most contaminated plant part, or the metal-enriched slags generated from energy conversion, can be removed according to heavy metal safe disposal (Dastyar et al., 2019).

Taking this in mind, castor bean (CB), an unpalatable, fast-growing plant with high biomass production, has been widely demonstrated to have phytoremediation potential for several PTEs (Table 2.1), as well as a high tolerance to salt and drought stress (Babita et al., 2010; Bauddh &

Singh, 2012a; Dieter Jeschke & Wolf, 1988; Pinheiro et al., 2008; Sausen & Rosa, 2010). In this review, we evaluated the potential of using castor bean for phytoremediation programs linked to biofuel and by-product production.

**Table 2.1.** Studies made on Castor bean (*Ricinus communis)* phytoremediation capability for PTEs.

| **Contaminants** | **Aims of the Research** | **Reference** | **Genotype** |
|---|---|---|---|
| As | Phytoremediation potential of CB and *H. annus* | (Melo et al., 2012) | cv. Guarany |
| As, B, Cu, Fe, Mn, Zn | Phytoremediation potential | (Pandey, 2013) | Not specified |
| As, Cd, Pb | Phytoremediation potential co-planting CB with *P. vitatta* with chitosan addition | (Yang et al., 2017) | Not specified |
| As, Cd, Pb, Zn | Phytoremediation potential of CB and *Z. mays* with chelates | (Silva et al., 2017) | Not specified |
| B, Cd, Cr, Cu, Fe, Mn, Ni, Pb, Zn | Effects of organic matter addition | (Abreu et al., 2012) | Not specified |
| Ba | Phytoremediation potential of CB, *B. juncea* and *H. annus* | (Coscione & Berton, 2009) | Not specified |
| Cd | Cd accumulation and drought stress | (Shi et al., 2015) | Cv. Zibi 5 |
| Cd | Phytoremediation potential | (Ye et al., 2018) | JX-22, ZB-9 |
| Cd | Phytoremediation potential | (Zhang et al., 2014) | Zibo 5 and Zibo 8 |
| Cd | Phytoremediation potential | (Bauddh et al., 2016) | Cv. Kalpi |
| Cd | Phytoremediation potential of CB and *B. juncea* | (Bauddh & Singh, 2012a) | Cv. Kalpi |
| Cd | Phytoremediation potential of CB and *B. juncea* + salinity and drought stress | (Bauddh & Singh, 2012b) | Cv. Kalpi |
| Cd | Phytoremediation potential of CB and *B. juncea* + Organic and Inorganic amendments | (Bauddh & Singh, 2015) | Cv. Kalpi |
| Cd, Cu, Mn, Ni, Pb, Zn | Crude oil and bioproducts | (González-Chávez et al., 2015) | Plants established naturally on contaminated site |
| Cd, Cu, Mn, Pb, Zn | Phytoremediation potential | (Ruiz Olivares et al., 2013) | |
| Cd, Cu, Ni, Pb, Zn | Phytoremediation potential of fly ash disposal site | (Pandey, 2013) | |

## 2.2. Botanical aspects and ecological characteristics

### 2.2.1 Botanical aspects

Castor bean (*Ricinus communis* L.) is a tropical plant with C3 metabolism of the *Euphorbiaceae* family (Figure 2.1) (Anjani, 2012), with numerous wild and semi-wild types that differ genotypically and phenotypically (McKeon, 2016). Castor bean can be 1.5-2.4 m high in a temperate climate, or as tall as a moderate-sized tree in tropical and sub-tropical areas (10-13m) (Anjani, 2012; Falasca et al., 2012). In Ethiopia, where is thought to be originated, plant size varies from a perennial tree or shrub to a small annual (Alemaw et al., 2013; McKeon, 2016). Leaves are palmate with 5 to 11 lobes and alternate; are often dark glossy green, but the color can vary from light green to dark red (Anjani, 2014; Milani & Nòbrega, 2013). The fruit is a spiny, greenish to reddish-purple capsule with 3 locules containing one oval, shiny, and highly poisonous brownish seed with marble-gray marks and a light brown caruncle (Milani & Nòbrega, 2013; Salihu et al., 2014); at maturity, the capsules are dried and may have dehiscence, depending on the genotype (Vallejos et al., 2011). Some castor varieties can produce capsules with rudimentary spines, others soft, flexible, and non-irritant spiny capsules, and others spiny irritant capsules (Salihu et al., 2014). The seeds of castor bean grow inside capsules on raceme that develops progressively over the life of the plant. Seeds, exposed to different environmental conditions, end in an inhomogeneous maturity, with different developmental stages among the raceme and their order (Koutroubas et al., 1999; Vallejos et al., 2011). The seeds can differ in color, size, external markings, weight, and shape between cultivars (Anjani, 2014; Velasco et al., 2015; M. L. Wang et al., 2010; Ming Li Wang et al., 2011), but being on average of an oval form. The number of capsules per raceme depends on the number of female flowers on it. Male flowers are yellowish green with creamy stamens, while female flowers lie in undeveloped spiny capsules with prominent red stigmas. Castor plants can be "normal monoecious" with pistillate flowers on the upper part of the raceme and staminate flowers on the lower part, or "interspersed monoecious" with pistillate and staminate flowers interspersed along the entire raceme axis (Koutroubas et al., 1999; Milani & Nòbrega, 2013). Rarely, castor inflorescence can terminate with a hermaphrodite flower that regularly drops off before capsule setting (Anjani, 2012). Female and male flower proportion on the raceme can vary within and among genotypes (Milani & Nòbrega, 2013), and is extensively influenced by the environment. Racemes can have different shapes (conical, cylindrical, or oval) with different capsule arrangements, which can be compact, semi-compact, or loose (Salihu et al., 2014). According to the order of manifestation the racemes are called primary, secondary and tertiary, and their numbers increase geometrically with the number of branches (Vallejos et al., 2011). The castor stem is round, sometimes covered with a waxy bloom, and it may be green, reddish, or purple (Salihu et al., 2014). The dark purple stem and the sulfur-yellow colors are occasional (Anjani, 2014).

**Figure 2.1**. *Ricinus communis* L. (CB).

### 2.2.2 Ecological niche

Castor bean can grow well in a wide range of ecosystems, from temperate to tropical desert, to wet forests (Gómez et al., 2016), in a range of 250-4250 mm annual precipitation (Anastasi et al., 2014; Falasca et al., 2012), and in a wide range (4.5-8.3) of soil pH (Anjani, 2014). Considered a wasteland colonizer plant, it's easy to find it on landfills, railway tracks, roadsides, etc. Castor cultivation spreads to 40°N and 40°S latitudes, but some cultivars have been found at 52°N in Russia (Milani & Nòbrega, 2013). It can grow from sea level to more than 2000 m above sea level (Anjani, 2012), but the optimal altitude is 300–1800 m a.s.l. (Milani & Nòbrega, 2013).

## 2.3. Tolerance to abiotic stress

### 2.3.1 Drought resistance

Castor bean is well known to be tolerant to two main abiotic stresses: salinity and drought, making its cultivation possible in marginal lands that are not suitable for food crops (Bauddh & Singh, 2012a). The deep taproot and the extensive root system enable CB plants to uptake water from deep soil layers, surviving in dry conditions under which other crops would be severely inhibited. Osmotic adjustment (OA), the active accumulation of solutes in response to water deficit, has been reported to be a drought adaptation mechanism in several crop plants. OA helps maintain turgor, providing a more efficient extraction of water from the soil (Maggio et al., 2005). Osmotic adjustment capacity can vary greatly among CB genotypes, however CB plants under water deficit accumulate proline (+ 12 %), total soluble sugars (+ 61 %), total free amino acids (+17 %), and potassium (+ 2.8 %), indicating that sugars are the main contributors for osmotic adjustment in CB leaves. This is in contrast with other crops in which potassium has been found to contribute the most (Fagnano & Fiorentino, 2018). Also, prompt stomatal closure seems to be linked to drought resistance in CB plants, resulting in reduced photosynthesis (- 59 %) and minimal water loss by transpiration (- 96 %), while maintaining high net $CO_2$ fixation rates (Sausen & Rosa, 2010). Water deficiency leads to reduced leaf area and fewer leaves, roots, and shoots biomass and reduced height, with shoot elongation being affected very early after irrigation suspension (Sausen & Rosa, 2010; Shi et al., 2015; W. R. Silva et al., 2017). This early growth response and the reduced size attained by water-stressed plants may contribute to plant survival, reducing the plant's water requirements (Sausen & Rosa, 2010). Seed yield is significantly decreased by water stress mainly in the primary racemes since the reduction is less pronounced in secondary and compensated in higher-order racemes (Lakshmamma et al., 2017). Water deficiency stimulates CB plants to increase wax deposition, contributing to the maintenance of relative water content, since wax is an efficient obstacle against leaf transpiration (Silva et al., 2020). Leaf expansion is detectable 30 min after re-watering, showing that after 2 days of no expansion there is still potential to develop (Schurr et al., 2000), and after 7 days of re-watering, proline and total soluble sugars accumulation decrease, though remaining higher than control plants. Drought stress increases K, Ca, and Na contents in CB plants as the drought severity intensifies and decreases Fe, Cu, Zn, and Mg contents according to genotype (Tadayyon et al., 2018). Castor drought resistance makes its cultivation possible without irrigation, thus reducing its costs.

### 2.3.2 Salt resistance

In addition to drought, land salinization represents an important environmental constraint that reduces crop growth and yield (Pinheiro et al., 2008). Castor bean can grow on marginal lands, which are mostly located in arid and semi-arid regions where soil salinity is too high for most common food crops (Anjani et al., 2014; Sun et al., 2013). Castor bean salt tolerance seems to be related to its roots' marked ability to limit $Na^+$ uptake, being selective in $K^+$ uptake, excluding it from leaf blades and maintaining relatively high $K^+$ concentrations in leaves (Jeschke & Wolf, 1988). Besides, potassium is selectively translocated to young shoots, retaining $Na^+$ and $Cl^-$ in older tissue. The stem and petiolar tissue can remove $Na^+$ from the xylem and phloem (Jeschke & Wolf, 1988). Castor bean cotyledons are less affected by saline stress than true leaves, enabling seedling survival in salty soils (Wang et al., 2019). After 59 days under 30 mM NaCl, corresponding to 2 g NaCl kg$^{-1}$ soil, Pinheiro et al. (2008) observed a recovery of leaf water potential, suggesting an ability of CB seedling to acclimatize to high salt conditions. The potential photosynthetic activity is augmented by salt stimulation, as reflected by the increased $F_v/F_0$ ratio, a very sensitive indicator of the potential photosynthetic activity, in CB plants grown under 100 mM L$^{-1}$ (Li et al., 2010). A certain level of NaCl stimulation may promotes CB growth as suggested by the increase of chlorophylls in seedlings (Li et al., 2010). Salt stress effects on chlorophyll a and chlorophyll b contents can be seen only after 59 days (Pinheiro et al., 2008). The salt tolerance of CB can be indicated by the maintenance of cellular integrity, as indicated by leaf electrolyte leakage, high photorespiratory activity and nitrate assimilation (Neto et al., 2014). The salinity threshold for seed emergence was identified by Zhou et al. (2010) at 7.1 dS m$^{-1}$, but in some cultivars the emergence index can even increase at 10.3 dS m$^{-1}$ (Sun et al., 2013; Zhou et al., 2010). Serious plasma membrane lipid peroxidation may not occur, as indicated by the non-significant increase in malondialdehyde at 200 mM L$^{-1}$, and the proline increase in response to salt stress (Li et al., 2011). The effects of saline irrigation water on the oil content of the racemes are small and more pronounced in primary than in secondary racemes (Nobre et al., 2012). Castor bean growth parameters are affected by salt stress (Pinheiro et al., 2008; Sun et al., 2013), but the sum of the distinct responses to salinity appears to be quite a successful strategy, well-organized in the whole plant allowing survival and reproduction even under adverse conditions of excessive external $Na^+$ and $Cl^-$ (Jeschke & Wolf, 1988). The deep-rooted perennial CB can be used to ameliorate seashore saline soils increasing the soil porosity and thus facilitating the transfer of salts into deeper soil layers and improving soil organic matter content. Furthermore, CB plants positively influence microbial community activity and biodiversity, increasing functional bacteria such as halophilic, phosphate-solubilizing, potassium-solubilizing, cellulose decomposing, ammonifying and nitrogen-fixing bacteria, thus enhancing soil nutrient availability, and improving soil structure (Wu et al., 2012). The application of nitrogen fertilizers such as monoammonium phosphate plus urea has been shown to increase root biomass and stem diameter on CB cv. BRS Energia, reducing the effect of salinity on CB growth (Nobre et al., 2013). Finally, arbuscular mycorrhizal fungi stimulate CB growth alleviating salt stress, increasing the aboveground biomass, phosphorus, carotenoid and chlorophyll, soluble protein and proline content while decreasing malondialdehyde (MDA) (Zhang et al., 2014; Zhang et al., 2018).

## 2.4 Agronomic features

### 2.4.1 Growth requirements

Castor bean requires temperatures between 20 and 26°C (Severino et al., 2012); shoots die at temperatures below -1°C and adult plants at -3°C (Anjani, 2014). Castor bean requires a frost-free period of 140–180 days, and at least 140 days with a mean temperature between 20° and 27°C for satisfactory yields (Anjani, 2012; Falasca et al., 2012; McKeon, 2016) (Table 2.2). Castor grows in all kinds of soils but prefers well-drained moisture retentive soil like sandy loam (Salihu et al., 2014). Castor cultivation necessitates fertile, well-aerated soils with a pH of 6 – 7.3, and rainfall of 600 – 700 mm for optimum yield (Salihu et al., 2014). Is a long-day plant, but is adaptable to a wide range of photoperiods even if with reduced (Salihu et al., 2014). The optimal relative air humidity range falls between 30 and 60% (Anjani, 2014), with low relative humidity in the growth phase to obtain maximum productivity; humid and cloudy days, despite the temperature, can be reflected in lower seed yield (Severino et al., 2012).

**Table 2.2.** Average seed and oil yield of castor bean in different countries under different treatments. n.s., not specified.

| Country | Site | Seed yield t ha$^{-1}$ | Oil yield t ha$^{-1}$ | Genotype | Treatment | Reference |
|---|---|---|---|---|---|---|
| Ethiopia | Rift Valley | 1.2-1.4 | 0.6-0.7 | Hiruy | Planting density | (Alemaw et al., 2013) |
| Greece | Aliartos | 3.0-3.8 | n.s. | Kaima 93, C-853, C-855, C-856, C-864, C-1002, C-1008 | Genotype evaluation (year 2014) | (Alexopoulou et al., 2015) |
| Italy | Cadriano | 0.7-4.0 | n.s. | C-855, C-856, C-857, C-864, C-1008 | Genotype evaluation (year 2014) | (Alexopoulou et al., 2015) |
| Italy | Ragusa | 0.7-7.3 | 0.3-3.3 | Local 1, Local 2, Brazil, Tunisia | Autumnal sowings | (Anastasi et al., 2014) |
| Mexico | Texcoco | 2.6-5.2 | n.s. | Krishna, Rincon | Optimal soil moisture | (Buendía-Tamariz et al., 2019) |
| Colombia | Cordoba | 0.8-1.2 | 0.3-0.6 | Monteira, Cienaga de Oro, Los Cordobas, BRS Nordestina | Planting density | (Cabrales et al., 2011) |
| USA | Florida, Citra | 0.7-1.3 | 0.3-0.6 | Birminghan, Hale | Plant growth regulator and harvest aid | (Campbell et al., 2014) |
| USA | Florida, Jay | 0.7-1.2 | 0.3-0.6 | Birminghan, Hale | Plant growth regulator and harvest aid | (Campbell et al., 2014) |
| Italy | Sardinia | 1.4-2.5 | n.s. | Hazera 22, ISCIOR 101 | Irrigation | (Laureti & Marras, 1995) |
| USA | Texas | 0.2-2.7 | n.s. | BRS Nordestina | Irrigation | (Severino & Auld, 2013) |
| Brazil | Carnaubais | 0.1-1.2 | n.s. | BRS Nordestina | Fertilization | (Severino et al., 2006c) |
| Pakistan | Bahawalpur | 1.2-2.4 | n.s. | DS-30 | Fertilization | (Yousaf et al., 2018) |

Castor bean has a slow and cold-sensitive germination (Severino et al., 2012). Seeds (Figure 2.2) may have a dormancy period of several months, depending on variety, while others can germinate from freshly harvested seeds without any treatment (Severino & Auld, 2013). The base temperature for CB seed emergence was found to be 15 ˚C, optimum at 31°C and maximum at 35-36°C, requiring 464 degree-days after pollination to reach physiological maturity (Anjani, 2014; Severino & Auld, 2014; Severino et al., 2006a).

### 2.4.2 Planting density

Plant arrangement is a simple low-cost technology that can affect yield (Anjani, 2012; Soratto et al., 2012), ranging from 4200 plants ha$^{-1}$ for tall cultivars to 70,000 plants ha$^{-1}$ for dwarf varieties (Zhou et al., 2010). CB plants compensate for a low population density by producing a higher number of racemes (Alves et al., 2015; Souza-Schlick et al., 2014) which, however, do not increase the seed yield considering the reduced number of plants per hectare (Oliveira et al., 2017). A lower plant population increases basal stem diameter and survival rate (Severino et al., 2006; Soratto et al., 2012; Souza-Schlick et al., 2014). Seed number, a highly hereditable characteristic, is hardly influenced by environmental or exogenous factors (Soratto et al., 2012). The raceme size is slightly influenced by plant density (Soratto et al., 2012; Souza-Schlick et al., 2014). In all the aforementioned studies oil content, oil yield, or oil quality were not influenced by plant density (Cabrales et al., 2011).

### 2.4.3 Irrigation

Castor bean is very sensitive to root hypoxia caused by soil flooding: irreversible damage occurs after just 3 days of flooding (Severino et al., 2006). The deep taproots and extensive root systems enable the plant to uptake water from deep soil layers and allow seed production with little or no irrigation. Obviously, despite the adaptability of CB to drought, the greatest yields are obtained with irrigation. There is almost a linear increase in seed yield with irrigation nearly doubling when additional water is supplied (Koutroubas et al., 2000; Laureti & Marras, 1995). In Brazil, a rainfed (376 mm) CB field produced 1774 kg $ha^{-1}$ of seeds, +24 % with supplementary irrigation (1099 mm), and +139 % with 1662 mm of water supplied (Souza et al., 2007). Castor bean plants' response in seed yield to water treatments differs between cultivars, but most of the variation can be explained by the number of racemes, followed by seeds per raceme and seed weight (Laureti & Marras, 1995; Severino & Auld, 2013b). The seed yield increase in irrigated CB fields is small compared with that of other common crops cultivated in the same area, suggesting that is more suitable for low-input, arid environments (Buendía-Tamariz et al., 2019; Laureti & Marras, 1995; Neves et al., 2013). Castor bean can grow well also with wastewater irrigation (Anjani, 2014; Chatzakis et al., 2011; Yadav & Anjani, 2017). Wastewater is an alternative water source being recently exploited to irrigate biofuel crops without depleting the already scarce water resources. A study by Tsoutsos et al., (2013) investigate the use of wastewater on the quality of castor bean oil and biodiesel production. Oil samples derived from wastewater irrigation provided a lower concentration of free fatty acids and a slight reduction in viscosity. According to Abbas et al., (2015), irrigation with wastewater resulted in higher fresh and dry weights of castor roots, shoots, leaves, and seeds ($g^{-1}$ plant) than the ones irrigated with freshwater, due to nutritive elements contents such as N, P, and K.

### 2.4.4 Fertilization

CB can doubtless grow on agriculturally marginal lands, but obviously, it benefits considerably from the addition of fertilizer. For example, nitrogen applications can increase seed yield by 114 % compared to unfertilized plants (Oliveira et al., 2017; Severino et al., 2006b). Organic fertilization can increase productivity by 458 kg $ha^{-1}$, mineral fertilization by 824 kg $ha^{-1}$, and the combination of organic fertilization and mineral by 1,009 kg $ha^{-1}$. Mineral fertilization with N, P, and K, with the addition of organic material, contributed to an increase in productivity of 184 kg $ha^{-1}$ (Severino et al., 2006c). Unfertilized plants produced 46 % less fruit compared to well-fertilized ones, with a 50 % decrease in fruit dry weight (Reddy & Matcha, 2010). However, CB plants selected to grow at a certain nutrient level have adapted to produce the maximum at that level (Severino et al., 2006b); when cultivated in very fertile soils, tend to produce large vegetative mass at the expense of seed production. The oil content in seeds seems to increase only in response to P and was not influenced by other nutrients (Severino et al., 2006b). Among the organic fertilizers, poultry manure seemed to be more effective (Omotehinse & Igboanugo, 2019).

**Figure 2.2.** *Ricinus communis* L. seeds.

## 2.5. Castor bean products

Castor bean has been used for a very long time, and is one of the oldest commercial products (Nahar & Pan, 2015), known in the traditional medicine of the ancient Mediterranean and Asian cultures (Polito et al., 2019), being still used in traditional medicine worldwide (e.g., Chinese and

Ayurveda)(Anjani, 2012; Polito et al., 2019). Long before “biobased” became a catchphrase, CB oil-derived products were used for centuries (e.g., in ancient Egypt lamps) (Anjani, 2012; Copley et al., 2005). Nowadays, CB oil has more than 700 industrial uses, and its global demand is increasing steadily by 3–5 % per year (Zhou et al., 2020). It’s a well-recognized commodity with a well-established market, costing 2-3 times more than soybean oil being cultivated only in a few countries (Anjani, 2012). Castor bean oil consists mainly of ricinoleic acid (85-90 %), a hydroxylated fatty acid with one double bond, and some unique properties. Castor has an oil close to a technical grade of purity, a rare natural phenomenon (Anjani, 2012; Bateni & Karimi, 2016). Is more versatile than other vegetable oils and it is extensively used in a variety of industries, such as cosmetic and pharmaceutical, in paint, varnish and lacquer production (Borg et al., 2009; Ogunniyi, 2006). Because of its high viscosity, it’s used as a lubricant in two-stroke engines, neat or blended, reducing smoke emissions by up to 50–70 % (Lemos et al., 2016; Singh, 2011). It is a polyol that can readily form polymers making polyurethanes that find applications in adhesives and coatings, electrical insulators, semi-rigid foams used in thermal insulation (Cardoso et al., 2012) and it was also suggested as a possible candidate biomaterial for wound dressings (Uscátegui et al., 2019) and as a graft for bone defect treatments (Sousa et al., 2018). The so-called Turkey red oil, produced by CB oil sulphation is widely used in textile industries in dyeing and in finishing cotton and linen (Mubofu, 2016). The CB oil obtained mechanically by pressing results in CB cake, while CB meal derives from CB oil production through solvents. CB cake is a good organic fertilizer, containing about 5.5 % nitrogen, 1.8-1.9 % phosphorus and 1.1 % potassium (Lima et al., 2011; Shrirame et al., 2011). It can be applied in moist soil 3 weeks before sowing the crops allowing for toxicants degradation (Gupta et al., 2004). It has been used as a substrate for tomato seedlings and as fertilizer for onion production (Lopes et al., 2011; Mello et al., 2018). CB cake has also shown great potential for biogas production and is found to be a very interesting feedstock for the production of pyrolysis bio-oil (Bateni et al., 2014; Kalogiannis et al., 2016). According to Gonzalez-Chavez et al. (2019), castor cake derived from plants naturally established on polluted mine tailings can be utilized as organic fertilizer due to the lower levels (e.g., Pb in cake: 2.6-8.8 mg $kg^{-1}$) of metal contamination allowed by EU regulations (e.g., maximum limit values of Pb in organic fertilizer 120 mg $kg^{-1}$ of dry matter) (EU, 2019).

Castor bean meal may contain up to 55.8 % crude protein and can be used as a protein source for animal feedstock (Nicory et al., 2015). Due to its ricin content, CB meal use necessitates caution. Different types of seed processing can reduce or eliminate this toxin (Akande et al., 2016; McKeon et al., 2013). For instance, it can be detoxified with calcium oxide replacing up to 50 % of soybean meal in the lambs’ diet (Nicory et al., 2015) and reducing the production costs in a beef cattle grazing system (De Matos et al., 2018). Furthermore, up to 15 % non-detoxified CB meals can be used in goat feed ( Silva et al., 2015). Castor bean can also be considered an eco-friendly and economic alternative to synthetic insecticidal agents (e.g., against *Spodoptpera frugiperda, S. littoralis, Musca domestica* and *Phlebotomus duboscqi*, the Leishmania vector) (Bakr et al., 2015; Rossi et al., 2012; Samuel et al., 2016; A. Singh, 2016). Leaf extracts have also shown antimicrobial potential and antifungal activity (Carolina et al., 2019; Naz & Bano, 2012; Shazia et al., 2016). Castor bean leaves are used, especially in India and Africa (Sharma et al., 2020; Umer et al., 2016), as food for *Samia cynthia*, a moth used to produce silk, and in Italy the use of senescent leaves for eri-silkworm artificial diet has provided a promising opportunity for valorizing residual biomass to good use after biorefinery (Zanetti et al., 2017). Moreover, the reactive surface of CB leaf powder has been studied as a green adsorbent for the removal of heavy metals from natural river water (Martins et al., 2013). In the eastern part of Nigeria, CB seeds are used as a food seasoning called Ogiri and CB can be used in honey production (Ogunniyi, 2006; Severino et al., 2012).

### 2.5.1 Castor biodiesel

Recently, castor bean biodiesel is receiving great attention (Keera et al., 2018). Biodiesel is the alcoholic ester of vegetable oils obtained by transesterification. It presents many advantages over fuel, e.g., non-toxicity, biodegradability, renewability, and the decline of most exhaust emissions. For instance, the presence of oxygen in biodiesel makes it burn cleaner, and its higher viscosity cancels the need for added sulfur compounds in diesel, reducing $SO_2$ emissions (McKeon, 2016; Osorio-González et al., 2020). Biodiesel production begins with vegetable oil extraction from the seeds, generally carried out with mechanical pressing, solvent extraction, or a combination of both technologies (Osorio-González et al., 2020). Lately, supercritical fluids, ultrasound, and microwave are the newest technologies developed for oil extraction (Osorio-González et al., 2020). After oil extraction, some refining steps are carried out to improve biodiesel quality, such as filtration or discoloration (Osorio-González et al., 2020). Subsequently, biodiesel is obtained through the transformation of triglycerides into fatty acids (FA), which can be performed with ethanol (resulting in FAEEs) or methanol (FAMEs), in the presence of catalysts that can be chemical (alkali or acid catalysts) or biological (enzymes) (Issariyakul & Dalai, 2014). Afterward, separation by centrifugation or decantation is performed to decrease the impurities and recover all products (biodiesel, solvent, and glycerol) (Osorio-González et al., 2020). The Fatty acid composition of the feedstock, its property, and the production process employed, are the parameters that mainly affect biodiesel quality (Sajjadi et al., 2016). The biodiesel obtained, used alone or blended with petrodiesel, has to conform to specific standards, like ASTM D6751 or EN 14214 (Ismail et al., 2014; Osorio-González et al., 2020). Some important biodiesel properties that need to conform to standards are kinetic viscosity, cetane number, cloud and pour point, and flashpoint.

Castor oil is mainly composed of ricinoleic acid (85-90%). CB has a very high percentage of seed oil content (40-55 %), higher than other normally used oil crops such as soybean (15-20 %), sunflower (25-35 %), or rapeseed (38-46 %), with a cultivation cost reduced by up to 50 % compared to rapeseed (Table 2.3) (Keera et al., 2018). Castor oil can be used in diesel engines with few modifications (Bello et al., 2020; Scholz & da Silva, 2008), lowering the level of pollutants, carcinogens, and greenhouse gasses (McKeon, 2016; Osorio-González et al., 2020). According to Anjani (2014), about 79782 t of $CO_2$ emission can be saved if 10% of total castor seed oil produced is transesterified into biodiesel. The world average castor seed production is 1.1 Mg $ha^{-1}$, corresponding to 460 kg of castor oil with a seed oil content of 47% and oil yield of 90%, but a higher yield can be obtained, indicating promising oil productivity (Bateni & Karimi, 2016; Scholz & da Silva, 2008). Castor oil FAMEs present an unacceptably high value of kinematic viscosity (which influences characteristics such as the amount of fuel that drips in the injection pump (Issariyakul & Dalai, 2014)) and low cetane number (that quantifies the time between injection and ignition of the fuel (McKeon, 2016)) that do not allow it to achieve the standard specifications (Berman et al., 2011; Sajjadi et al., 2016; Scholz & da Silva, 2008). Blending castor biodiesel with diesel is nowadays the only way to use it in the current diesel engine without complicating engine performance, and to meet all the required specifications (Berman et al., 2011; Scholz & da Silva, 2008). Castor biodiesels' high viscosity could improve diesel lubricity when blended, at a concentration of 2 g $kg^{-1}$, while rapeseed needs to be added at a concentration above 7.5 g $kg^{-1}$ to achieve the equivalent effect (Severino et al., 2012). Castor biodiesel presents a cetane number (43.7) lower than diesel CN (51). Nevertheless, the B5 blend gave a CN of 50.6 (Keera et al., 2018). Moreover, castor biodiesel also presents a high cloud and pour point (which monitors the flow proprieties at low temperatures (Issariyakul & Dalai, 2014), making it suitable for extreme winter temperatures, alone and blended (Berman et al., 2011; Keera et al., 2018). Castor biodiesel requires a negligible amount of catalysts to give a high biodiesel yield, reducing the production costs on a large scale (Berman et al., 2011; McKeon, 2016). Furthermore, castor biodiesel can be obtained at low temperatures (Bateni & Karimi, 2016; Da Silva et al., 2013): for instance, Keera et al. (2018) produced castor biodiesel through alkaline transesterification, with biodiesel yield obtained at 30°C similar to those obtained at 60°C. It is highly soluble in alcohol, due to the presence of hydroxyl groups, with great advantage during transesterification (Da Silva et al., 2013; Demirbas et al., 2016;

Osorio-González et al., 2020; Severino et al., 2012). A study by Bateni & Karimi (2016) demonstrated that the whole castor plant may be used in biodiesel production, with transesterification performed with ethanol obtained by saccharification and fermentation of plant residues: 1 kg of castor plant produced 149 g of biodiesel and 30.1 g of ethanol. Meneghetti et al. (2006) studied a comparison of ethanolysis versus methanolysis on commercial castor oil, obtaining similar yields but a shorter reaction time for methanolysis. All the above-mentioned studies indicate that castor bean is a great feedstock for biodiesel production. Some mathematical experimental designs and methodologies, such as Response Surface Methodology (Da Silva et al., 2013; Sánchez et al., 2015) or Taguchi approach (Karmakar et al., 2018; Ramezani et al., 2010), can improve and optimize castor oil transesterification. New technological innovations, new diesel engines, and mathematical model applications could greatly increase castor biodiesel production and utilization. According to Amouri et al. (2017), who studied the impact of castor biodiesel production on global warming, energy return-on-energy investment (EROEI), and ecosystem and human health, castor biodiesel showed a positive carbon balance, equivalent to the reduction of climate-change emission, and an EROEI of 2.60. The above-mentioned positive impacts of castor biodiesel can also be improved by reducing its ILUC: according to Gonzalez-Chavez et al. (2019), oil produced by *Ricinus* shrubs grown on metal-polluted sites presents low levels of contamination (e.g., Cd: 0-1.26 mg/L; Pb: 0-2.2 mg/L) and could be used as raw material.

**Table 2.3.** Comparison between the most common biodiesel feedstocks

| Feedstock | Seed oil content | Advantages | Disadvantages | References |
|---|---|---|---|---|
| Castor oil | 45-55% | Non-edible, High flash point. Hight pour and cloud POINT (useful in winter condition), Can grow on marginal and PTEs contaminated soils, Miscible in alchool, Easy to transesterificate, | Low cetane number, high viscosity, Ricin content | (Barbosa et al., 2010; Bello et al., 2020; Scholz & da Silva 2008) |
| Soybean | 15-20% | Low viscosity, high thermal stability | High production cost, edible, high acid value | (Qiu et al., 2011; Uyumaz et al., 2018) |
| Sunflower | 25-35% | Low viscosity | Edible, high acid value, long-term cultivation unsustainable | (Balat et al., 2011; Demirbas et al., 2007) |
| Palm | 18-40% | Cheap feedstock, high flashpoint | High cloud point, edible, long-term cultivation unsustainable | (Balat et al., 2011; Mekhilef et al., 2011) |
| Mustard | 28-32% | High cetane number, Cheap feedstock, can grow on soils contaminated with PTEs | High viscosity, Lower heating value, high cloud point | (Alam et al., 2013; Sanjid et al., 2014) |
| Rapeseed | 38-46% | High flash point and low cloud point | Effective power and torque decrease at all engine loads, increase NOx emissions up to 15% in most of the experiments | (Qiu et al., 2011; Rashid et al., 2008; Aldhaidhawi et al., 2017) |

### 2.6. Phytoremediation potential

Castor bean has a great potential for phytoremediation programs because it is a high biomass fast-growing plant, unpalatable to stock, and a potential phytoaccumulator of several PTEs, such as As, Cd, Pb, and Zn (Abreu et al., 2012; Amouri et al., 2017; Bauddh & Singh, 2012a; Huang et al., 2011). Being a perennial plant, its vegetation cover can immobilize PTEs in the rhizosphere, reducing their wind dispersion, and thus interrupting the exposure pathways. In addition, it has a massive root growth, which can reduce PTEs leaching into the water (Fagnano & Fiorentino, 2018b; Visconti et al., 2019), and makes it capable of adsorbing a high number of contaminants (Palanivel et al., 2020; Rehn et al., 2019). Castor bean low translocation factor demonstrates that it's highly suitable for phytostabilization of heavy metals and metalloids (Bauddh et al., 2015; Palanivel et al., 2020; Silva et al., 2017). The phytoremediation potential of castor plants is of primary importance, given the increasing number of PTEs contaminated soil. According to Dastyar et al. ( 2019), one-third of world resources are contaminated, mainly by heavy metals, although the real rate could be higher.

Copper accumulation, originating from the long-term use of Cu-based fungicides, carries an environmental risk of progressive increase of Cu in agricultural soils (Fagnano et al., 2020). Lead, considered one of the most hazardous PTE, can have a geological origin in soils or be released into the environment by smelting, battery recycling and mining (Fagnano & Fiorentino, 2018b). Cadmium, by contrast, is less common in soils but exposure even to low concentrations can cause serious human health problems, with carcinogenic effects, cell injury and endocrine destruction ( Huang et al., 2011).

Copper toxicity results in growth cessation in plants, chlorosis and necrosis symptoms and interference with many biological processes (e.g. cellular respiration) (Huang et al., 2018). Castor bean plants exhibit a well-documented copper phytostabilization aptitude. Copper contaminated soil seems to increase the biomass production of CB plants (Andreazza et al., 2013; Palanivel et al., 2020), without significant phytotoxic symptoms except for chlorosis in few leaves, indicating its Cu-tolerant capacity (Palanivel et al., 2020; Zhou et al., 2020). Copper concentration in roots greatly exceeds the concentration in other tissue (Huang et al., 2018; Ren et al., 2017). Depending on soil type, CB is able to remove 5900 g $ha^{-1}$ of Cu in Inceptisol and 3052 g $ha^{-1}$ in Mollisol, with root copper concentration 90 times higher than leaves and stems (Andreazza et al., 2013). Castor bean plants exhibit a bioconcentration factor (BCF, a ratio of element concentration in the plant shoots to element concentration in soil (Visconti et al., 2018, 2019) and translocation factor (TF, the ratio of element concentration in shoots and roots (Duri et al., 2020; Visconti et al., 2020) lesser than 1, indicating that CB is not a Cu accumulator plant and is well suited for phytostabilization due its low metal transfer rate (Andreazza et al., 2013; Napoli et al., 2019; Palanivel et al., 2020). Copper accumulation in CB seems to be directly related to phosphorous content in soils (Palanivel et al., 2020). Accordingly, phosphorous fertilization at 300 mg P $kg^{-1}$ increased Cu root concentration by 68 % while decreasing malondialdehyde (MDA) content (Huang et al., 2018). Sulfur application decreases copper accumulation in roots by 30 %, by reducing Cu bioavailability in soils (Ren et al., 2017). Conversely, nitrogen fertilization increases Cu roots content, while restricting Cu transport from the underground part to the aboveground, thus reducing the translocation factor (Zhou et al., 2020).

Cadmium soil contamination events have been increasing progressively over the last few years, because of the excessive use of chemical fertilizers and pesticides, mining, smelting and industrial wastewater irrigation (Bauddh et al., 2016). Almost 5.6 – 38× $10^6$ kg $year^{-1}$ of Cd released into the environment is anthropogenic, e.g., by metallurgic works, wastes from the cement industry, municipal waste, sewage sludge, mining, and metal processing: Cd production worldwide in 2015 estimated at 24,900 metric tons (Khan et al., 2017). CB has a strong ability for Cd accumulation in roots (Bauddh et al., 2016; Bauddh & Singh, 2012a). Eight months old CB plants grown in soil characterized by a total Cd concentration of 17.50 mg Cd $kg^{-1}$ showed no morphological differences with the controls, with only a 5% decrease in the number of capsules and seeds per plant (Bauddh et al., 2016). After the harvest, in the same study, Bauddh et al. (2016) observed a reduction of about 27 % in soil cadmium. Cadmium tolerance and accumulation are dependent on the cultivar (Ye et al., 2018; Zhang et al., 2014). Compared with a well-known Cd hyperaccumulator, *Brassica juncea*, CB accumulates 17 times higher Cd in roots, appearing more suitable for longer-term soil remediation in single sowing, thus reducing operational costs roots (Bauddh et al., 2016; Bauddh & Singh, 2012a). Synthetic chelates, such as ethylenediaminetetraacetic acid (EDTA) or ethylenediamine disuccinic acid (EDDS) can increase the plant's ability to uptake cadmium (Fiorentino et al., 2018). EDDS was shown to be the most suitable chelate for the phytoremediation of Cd in soil (Zhang et al., 2016). Besides chelates, the application of water-soluble chitosan also enhances Cd uptake (Yang et al., 2017) Moreover, crop co-planting with *Medicago sativa* can increase the cumulative amount of cadmium by 1.14 times (Xiong et al., 2018). Under the saline condition, Cd translocation from soil to CB roots is enhanced due to salt induced Cd mobilization in soil and Cl-Cd complex formation that increase Cd accumulation in plants. On the other hand, drought reduces it (Bauddh & Singh, 2012a). Application of bio-stimulants, such as *Bacillus subtilis* and *Azotobacter chrocoocum*, and inorganic fertilizer (e.g., urea, diammonium phosphate) enhanced Cd accumulation, improved tolerance mechanism and decreased MDA content (Bauddh & Singh, 2015). Spent mushroom substrate (SMS) applied as an organic amendment increased plant Cd uptake and the total amount of Cd accumulation in CB by 28–152 % (Cheng et al., 2018).

Lead accumulation in soils, and subsequently in plant tissue can reduce biomass and photosynthetic activity, root and shoot elongation, and increase the generation of reactive oxygen species (ROS) (Pal et al., 2013). Castor bean was able to accumulate high amounts of Pb in roots, tolerating above phytotoxic levels of Pb without any symptom of toxicity (Pal et al., 2013; Romeiro et al., 2006). Fungi are well known for their ability to detoxify potentially toxic elements through precipitation or valence transformation, and by passive and active uptake (Fiorentino et al., 2018). According to this, arbuscular mycorrhizal fungi treatments significantly influenced rhizosphere soil pH, Pb bioavailability and CB shoots Pb concentration (González-Chávez et al., 2019). Amendments, such as biochar or rice husk ash, can be used in mitigating Pb toxicity, improving plant growth and decreasing Pb accumulation in roots by up to 59% by immobilizing Pb (Kiran & Prasad, 2019). Among the chelates, citric acid can remove 17 times more Pb than untreated plants (Silva et al., 2017) and improves photosynthesis and plant growth (Mallhi et al., 2019). EDTA is the most effective for Pb phytoextraction, but due to environmental persistence is not the best option for field use (Zhang et al., 2016). Castor beans seem to defend itself against lead toxicity by increasing its production of proline and carotenoids, and by upregulation of ABC transporter transcript which are likely responsible for Pb detoxification in roots (Pal et al., 2013). According to Costa-Souza, CB growth was not affected by lead (2012).

Among the other PTEs, CB also emerged as a Zn phytostabilizer (González-Chávez et al., 2015; Olivares et al., 2013; Palanivel et al., 2020) and for being As and Ba tolerant (Coscione & Berton, 2009; Melo et al., 2012). Only B and Mn were translocated more intensely into the shoots, showing a TF greater than 1 (Abreu et al., 2012; Olivares et al., 2013; Palanivel et al., 2020). Furthermore, CB has also been used for phytostabilization and revegetation of fly ash disposal, derived from coal-fired power plants, showing a BCF in roots greater than 1, and a TF lower than 1 (Pandey, 2013). Castor bean can also provide other benefits such as carbon sequestration and an esthetically pleasant landscape (Pandey, 2013).

Organochlorine pesticides such as dichlorodipheno-xytrichloroethane (DDT) and organic pollutants are widely known for their toxicity, persistence in the environment and bio solubility in fatty tissue (Rissato et al., 2015). Accumulation of organic pollutants in plant roots can be the result of two processes: 1) uptake and translocation, for pollutants with low hydrophobicity, and 2) adsorption in root tissue (Rissato et al., 2015). However, nowadays, it is well known that the presence of arbuscular mycorrhizal fungi, which increase the contact surface and interact with roots and rhizosphere, can modify the bioavailability of organic contaminants and enhance plant adsorption (Rissato et al., 2015). Moreover, microbe-assisted phytoremediation, i.e., rhizoremediation, the degradation of pollutants in the rhizosphere, is affected by root characteristics and exudate compounds, which influence soil properties and organic pollutant mobilization (Wang et al., 2013). Studies have proven that CB can be used in the phytoremediation of soils contaminated with these kinds of pollutants (Huang et al., 2011; Rissato et al., 2015). For example, co-planting CB with *Sedum alfredii* enhances the degradation of pyrene and anthracene, two polycyclic aromatic hydrocarbons (PAHs) (Wang et al., 2013). Moreover, CB plants grown on soil contaminated with mineral oil can remove up to 81 % of soil hydrocarbons, manifesting visual toxic effects only after 45 days of treatment (Rehn et al., 2019). Remediation of soils contaminated by organic pollutants with CB is a potential biotechnological approach with the side effects of erosion control, site restoration, carbon sequestration, and feedstock production for biofuel (Rissato et al., 2015).

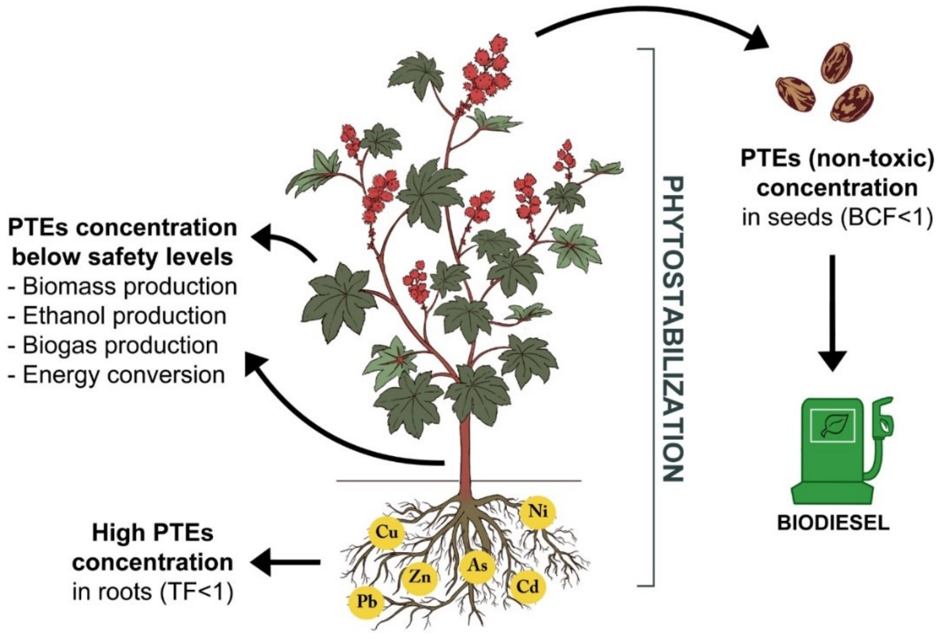


**Figure 2.3.** Castor bean phytostabilization scheme for biodiesel production. TF is Translocation Factor. BCF is Bio Concentration Factor. PTEs are Potentially Toxic Elements.

## 2.7. Conclusions

The multiple uses of CB oil clearly show that it is one of the most promising sources of renewable raw materials for many industries. Being a non-edible plant, its use as an energy source does not compete with food production, and unlike other industrial plants, CB can grow on marginal and PTE-polluted lands not suitable for food crops. It can survive in conditions under which other crops would be severely damaged, allowing seed production with little or no irrigation. CB fast growth and high biomass production can reduce the time required for phytoremediation programs, which is considered the real weakness of phytoextraction/phytostabilization. Furthermore, it has a higher oil yield potential, compared to other bioenergy crops.

According to the life-cycle analysis of the whole production system, CB cultivation has a major impact on the environment. Thus, exploiting metal-contaminated lands for bioenergy production might decrease CB cultivation impacts, reduce ILUC of biodiesel production, and convert contaminated soils into fully utilized and productive sites (Fig. 2.3). Moreover, oil produced from CB plants grown in PTE-polluted mine tailings had higher linoleic acid content, which enriches fuel properties (ignition quality, cloud point), and non-toxic concentrations of Cd, Pb, Zn, Ni, Mn. Besides this, CB plant residues of biodiesel production could be used in biogas and ethanol production, when the PTEs concentration allows it. Among thermal conversion methods, pyrolysis reduces the weight and volume of the contaminated biomass while concentrating the PTEs in the char/ ash fraction, which can be removed according to heavy metal safe disposal.

Despite its high adaptability to a different climate, CB oil is produced mainly in India, China, and Brazil, but one-quarter of its transformation is done by the EU oleochemical industry, which is completely dependent on imports. Using CB in different countries such as Europe to remediate contaminated sites and produce biofuel and by-products could result in a great opportunity for the environment and a bio-based economy, leading to new job creation and opportunities. Moreover, the biodiesel produced from CB grown on marginal lands and contaminated soils would be able to eliminate the Indirect Land Use Change making the production of biofuels truly sustainable.

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# Chapter 3

# 3. *Ricinus communis* cultivation on marginal lands

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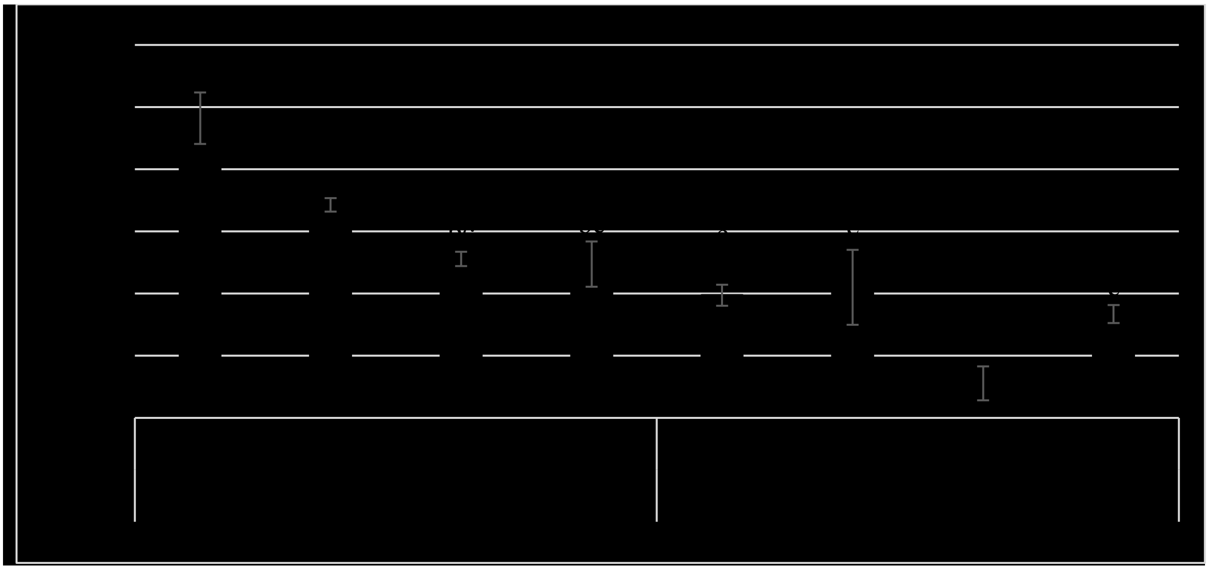

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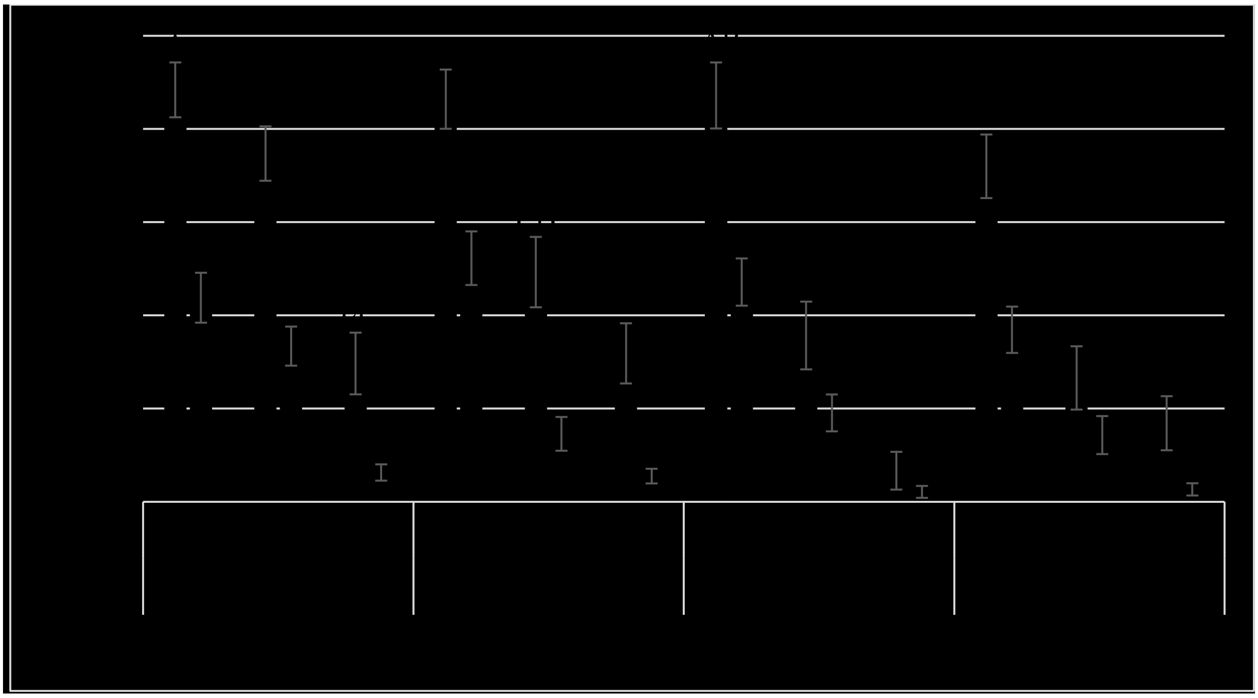

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# Chapter 4

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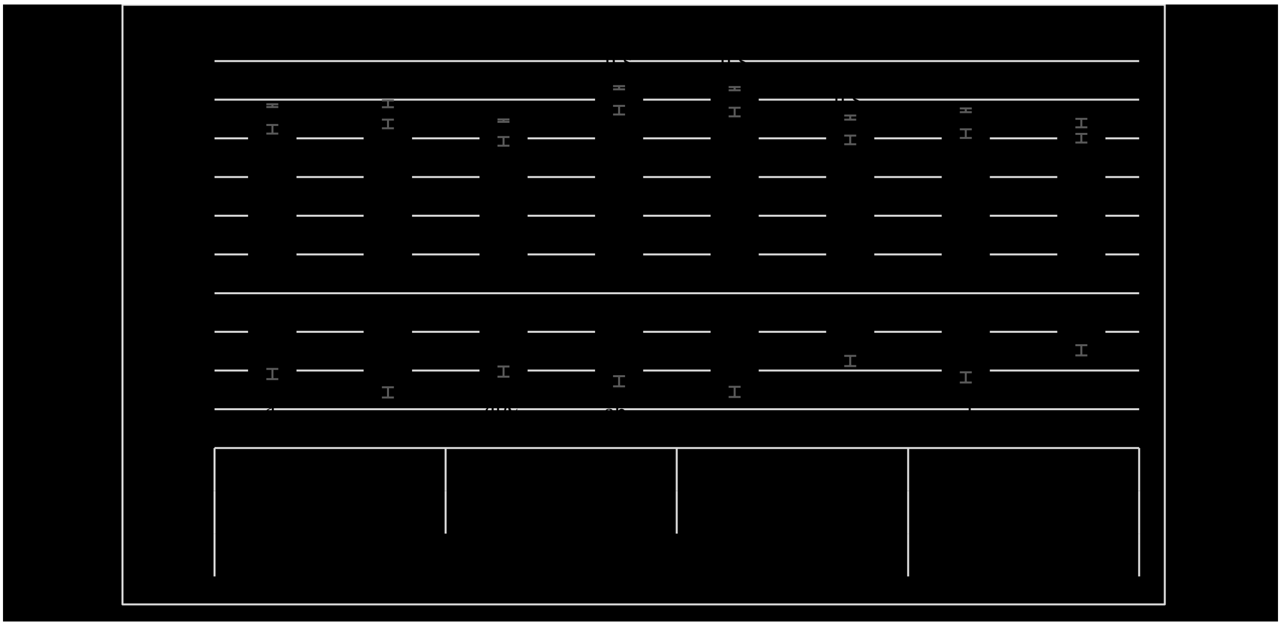

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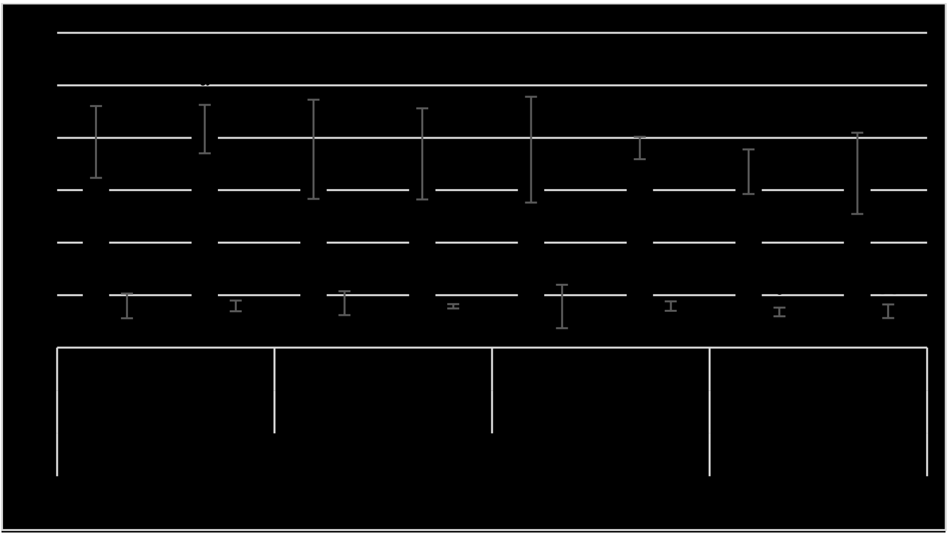

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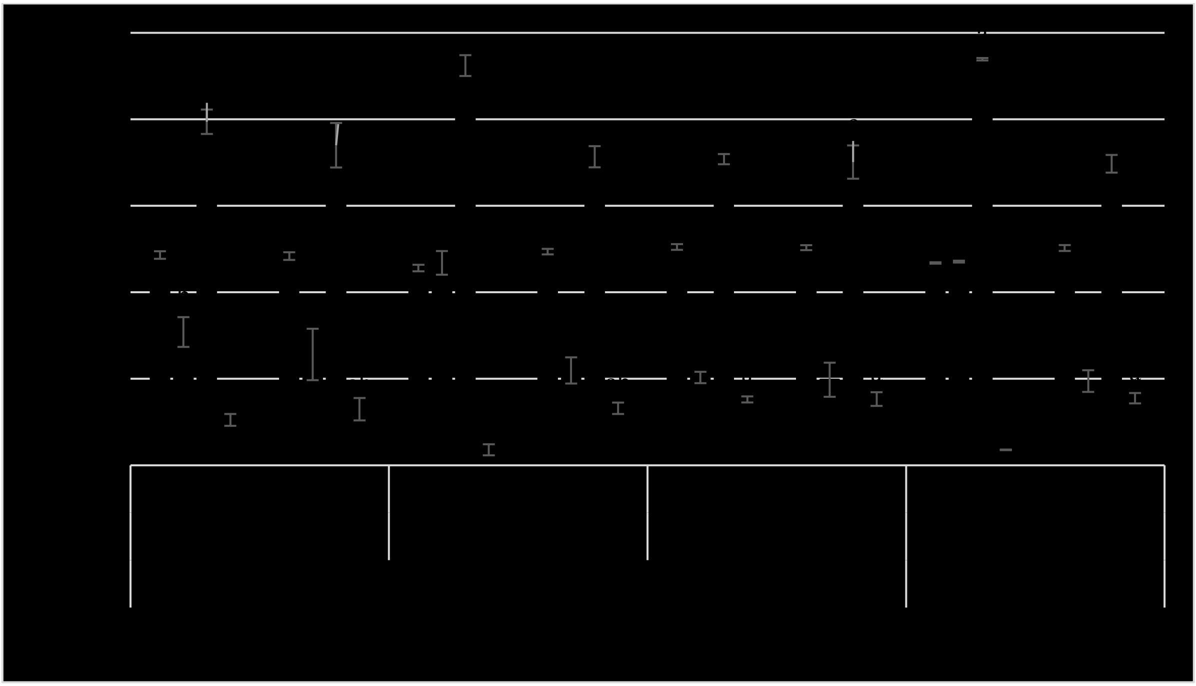

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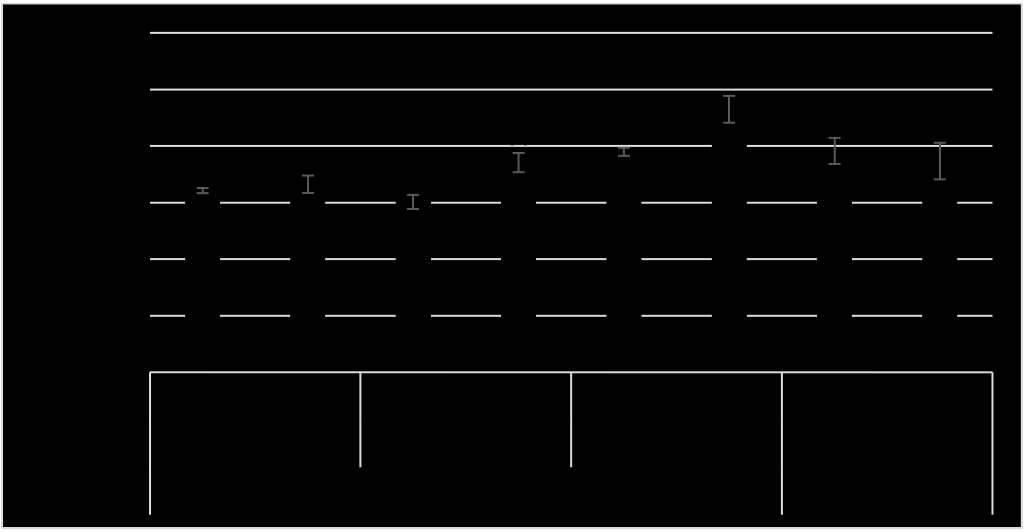

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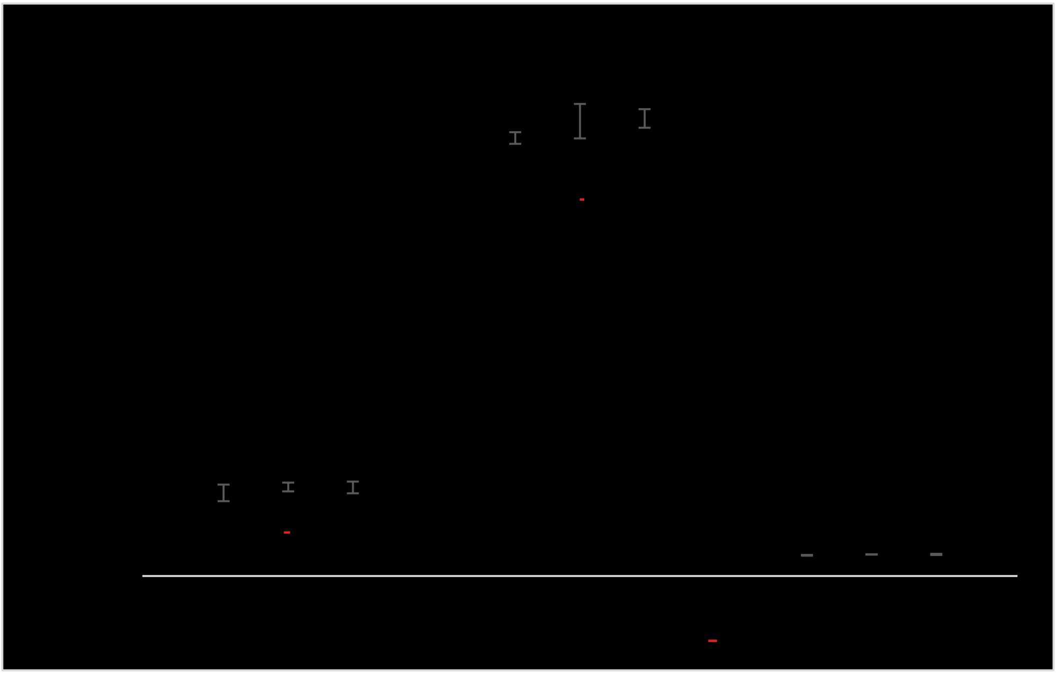

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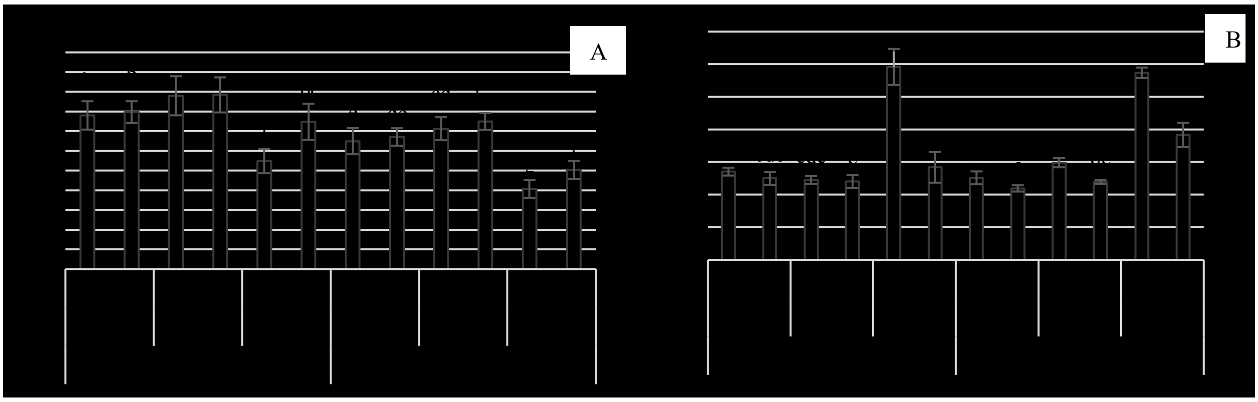


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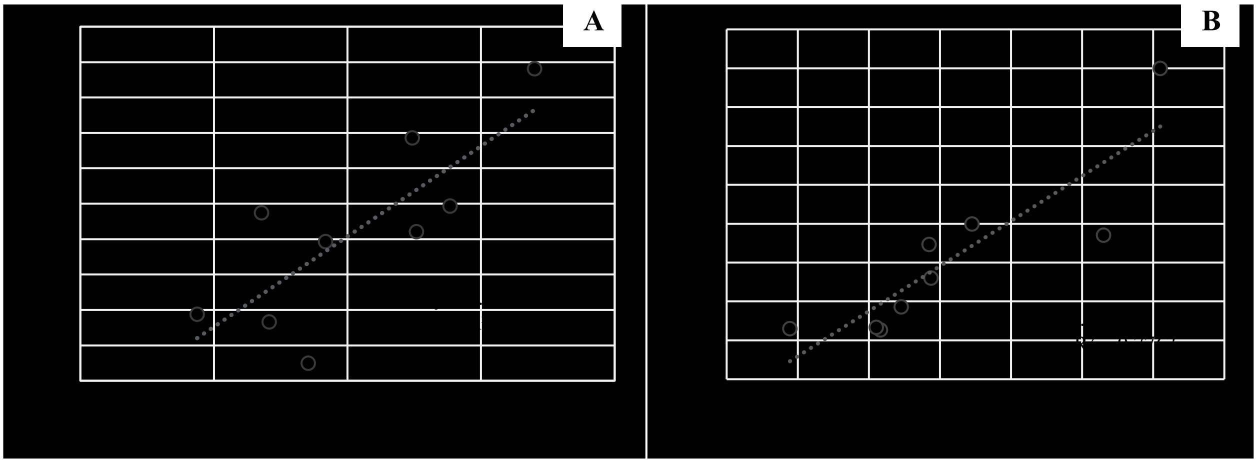


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# Chapter 5

# 5.Conclusions and future perspectives

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