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Phytoremediation of soil contaminated with Zn using Canola(Brassica napus L)

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ABSTRACT

Soil contamination by heavy metals is one of the major environmental problems in the world. In such environments, especial species of plants have the ability to grow, adaptation and uptake the heavy metals. Although the use of Brassica familyplants heavy metals phytoremediation is well known, there is little information about the impact of *Brassica napus L* increasing concentrations of heavy metals on parameters. A three-month study was carried to evaluate: the capacity of Canola *Brassica napus L* plants to phytoextract Zn from artificially polluted soil and the study the effect of increasing Zinc doses on the parameters of canola *Brassica napus L* (stem height, root length, leaf number, and dry matter yield). The findings show that Canola is a Zinc hyperaccumulator and high doses of it do not affect Canola growth. The statistical study after three months (12 weeks) of experimentation revealed a significant positive correlation between increasing Zinc doses and the four studied parameters.This study reveals that Canola plants were highly tolerant to metal pollution Zn and capable of reaching high biomass values in the presence of Zn.

1. Introduction

Heavy metals are among the most toxic contaminants frequently released in the environment. Their presence in the soil may lead to harmful effects on both the ecosystem and human health (Wang et al., 2003; Chemlal et al., 2014; Girard et al., 2005; Demim et al., 2013a; Kadouche et al., 2012; Demim et al., 2014; Cheballah et al., 2015). Long-term use of fertilizers and pesticides is a factor of pollution in agricultural soil. The industrial activity military, mining and urban activities that contributes to contamination of soil by heavy metals (Edgar et al., 2014). In contrast with air pollution, which affects both urban and rural areas where industries are established, soil pollution affects firstly the countryside. It is primarily a consequence of the extension of some modern agricultural technologies, which has to produce increasing quantities of food, while the cultivable land area available per inhabitant is continuously diminishing because of population growth and urban sprawl, industrialization, and other non-agricultural uses of land. Farmers try to reach this goal through the intensification of farming practices, which increasingly disrupts the energy flow and the cycles of matter in the agro-ecosystem (Ramade, 1982; Deviron, 1991). Like for organic pollutants, the spread of trace elements in the environment is due either to natural process or human activity (Bourrellier and Berthelin, 1998). Zinc is an essential trace element for the mineral nutrition of plants, but at high doses it becomes toxic for them and behaves like a heavy metal (Baize, 1997; Demim et al., 2013b; Demim et al., 2013a; Tang et al., 1998; Delorme et al., 2001; Tremel-Scheub and Feix, 2005; Pereira and Sonnet, 2007; Fässler et al., 2010; Marchiol et al., 2004). Even though some heavy metals are essential to life (Zinc, for example, is involved in protein synthesis: it is said to activate RNA polymerase), it is an essential element in the plant metabolism. Zinc is the activator of anhydrous carbonic enzyme, which is an essential enzyme for the utilization of carbonic acid. Zinc acts as a cofactor for alcohol dehydrogenize and various other enzymes (Heller, 1984; Horst, 1998). The toxicological impact of heavy metals depends on their chemical formula, their concentration in the environmental context, and the possibil-

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ity of their introduction in the “life cycle”. By contrast with organic pollution, which is biodegradable, heavy metals (e.g. zinc, copper, lead, cadmium) and metalloids (e.g. selenium, arsenic) persist in the environment and inevitably accumulate. They may migrate to surface or ground water or get into the food chain through plants and end up in animals and eventually humans. Anthropogenic presence of Zinc in the environment results from three groups of activities: mining and industrial sources, agricultural spreading, and other activities such as road traffic, which releases Zinc in the environment, and/or garbage incineration dust (Pichard et al., 2005). In the environment, Zinc is mainly found in the oxidation state of +2 (often in ZnS form). But several other ionic forms may be found in soil (Kabata-Pendias and Pendias, 2001). Trace elements of natural origin, such as Zinc, are found in the earth’s crust mainly as sulphide (blende),quite uniformly present in bedrocks (magmatic, metamorphic, and sedimentary rocks), and incidentally in other forms such as smithsonite $ZnCO_3$, or hemimorphite ($Zn_4Si_2O_7$ $(OH)_{2.}$ H_2O) or hydrozincite (Zn_5 $(OH)_6(CO_3)_2$)à (Pichard et al., 2005). Recently, many industrialised countries have engaged in the management of soils and sites polluted by metal elements by determining the evolution, tolerance, and accumulation of metals in wild vegetation, which is a new method of bioremediation (Raskin et al., 1997; Baker and Brooks, 1998; Lasat, 2000; McGrath et al., 2001; Wei et al., 2008). The utilization of plants to transport and concentrate metals from the soil into the harvestable parts of roots and biomass, appears a promising, cost-effective technology for the remediation of metal polluted soils(Kumar et al.,1995; Rufus et al., 1997; Salt et al., 1998; Pilon-Smits, 2005; Audet and Charest, 2007; Fässler et al., 2010; Schwartz et al., 2001). So the phytoremediation is a promising cleanup technology for contaminated soils, groundwater, and wastewater that is both low-tech and low-cost (Schwartz et al., 1999; Jiyeon et al., 2012; Mudassar et al., 2014). Plants used in phytoremediation include: sunflower, Brassica plant, barley, and various cruciferous plants. They are capable of accumulating some concentration of a given metal in recoverable parts (Zhao et al., 2003; Duquéne et al., 2009; Koopmans et al., 2008; Ben Ghnaya et al., 2009). Canola (*Brassica napus L*) is an annual herbaceous plant belonging to the Brassica family, which includes several accumulator species (Purakayastha et al., 2008). Several studies have reported phytotoxicity of Zinc on the morphological parameters of plants (Munzuroglu and Geckil, 2002; El-Ghamery et al., 2003), and Zn toxicity can induce decreased growth (El-Ghamery et al., 2003; Gisbert et al., 2006).

The aim of the present study was to investigate the effects of Zinc on growth on canola plants (*Brassica napus* L). The question that arises is the following: is Canola an excellent candidate for phytoextraction Zn?

2. Materials and methods

2.1. Soil characterization and experimental design

The study of the impact of increasing concentrations of Zinc on canola was based on a test conducted in vegetation pots of 250 g, in a greenhouse at the experimental station of the National Higher School of Agronomy of El-Harrach (ENSA).The experimental station of ENSA is situated in the Hassan Badi plateau, in the municipality of Oued Smar, *daira* (sub-prefecture) of El-Harrach, *wilaya* (prefecture) of Algiers. The School is located at an altitude of 48 m with a North West and South East orientation at 3°8′ of East longitude and 36°43′ of North latitude. The experimentation required the use of plastic pots of 9 cm in diameter and 9 cm of height, with a capacity of 250 g, containing soil brought from the experimental station of ENSA. The soil was taken from the topsoil at a depth of 0–25 cm. The soil was mixed with mould (1/3 mould, 2/3 soil).), we added moult it because the soil is poor in element nutritive, moult it does not modify the chemical composition of the soil, contrary to the mineral fertilization which is likely to modify the Zn concentration of the soil, knowing that we worked on a caused pollution i.e. a pollution artificial. The plant species is canola *(Brassica napus L.)*.The weight of 1000 seeds is 4.68 g and germinative capacity is 70%. Canola is an annual herbaceous plant that belongs to the Brassica family, which includes several species that accumulate heavy metals, hence the choice of canola in this study. The processing performed on canola is increasing mg Kg^{-1} concentrations of Zinc in mg Kg^{-1}, we used Zinc sulphate heptahydrate ($ZnSO_4$,7H_2O). Zinc concentrations (mg Kg^{-1}) used in this study and their correspondence in mg of Zn/250 g of soil and the quantity of the product $ZnSO_4$,7H_2O used for each treatment of Zinc, are represented in Table 1.To weigh the product $ZnSO_4$,7H_2O, and also to measure the dry vegetative masse, we used a precision balance of the brand LAC114 (110 g/0.1 mg). The oven used to dry the vegetative mass is of the brand Fisher Bioblock Scientific Venticell. After calculating the needed quantity of $ZnSO_4$,7H_2O for each pot of Zinc concentration, and to avoid any possible difficulty that may occur in case of loss of solution, we took a security margin by multiplying the values obtained by 13 instead of 12, which is the number of pots for each zinc concentration. Then, we dissolved each of the quantities of $ZnSO_{4,}$7H_2O mentioned above corresponding to the required concentrations in 1768 mL of distilled water. This amount of distilled water (1768 mL) corresponds to the retention capacity of the soil in the 13 pots multiplied by 2: to ensure the required concentration. We saturated each pot by twice the retention capacity, which is 68 mL. Therefore, the irrigation of 13 pots required the solution of (68 × 2) x 13 volumes, which is 1768 mL. The analyses were performed on a sample collected after homogenization, soil sample was air dried, and passed through a 2 mm sieve, according to standards (Clement, 2003). Total calcareous ($CaCo_3$ t) (%) was determined with the volumetric method with the calcimeter of Bernard. Electric conductivity (EC) in dS m^{-1}, was determined with Conductimeter (1/5 w/v). Soil pH was determined in deionised water (1/2.5 w/v). Phosphorus (P) in mg kg^{-1} of soil was determined with OLSEN Method, Organic carbon (Corg) in% was determined with Anne Method, Organic matter (OM) in%, OM% = Corg% x 1.72. Total Nitrogen (NT) in% was determined with Kjedhal. Method cation capacity of exchange (CEC) in Cmol+ kg^{-1} of soil was determined with Method of percolation with the ammonium acetate1N. Particle size distribution was determined by the Pipette Robinson. The soil was a Silty Clay, a pH of 7.85, an organic matter (OM) content of 2.61%, a calcareous content of 6.75%, a total nitrogen (N) content of 2%, a C/N ratio of 1.32, a phosphorus (P) content of 59.79 mg kg^{-1}, and an electrical conductivity of 0.68 dS m^{-1}, The cation exchange capacity (CEC) content of 19.84 $Cmol^{+}$1 kg $^{-1}$. The calcareous soil has a slightly alkaline pH.The pH value of soil cover samples ranges from 6.1 to 7.3 and the pH conditions were favorable for the plants to grow, it is not saline. The results show that the clay-humic complex is dominated by Ca^{++}. The cation exchange capacity (CEC) of the soil indicates that it has average fertility. The soil had high organic matter content because it was blended with mould. The C/N ratio indicates that organic matter was well decomposed. The soil sample was moderately rich in P. The interpretation of soil analysis was made in accordance with standards (AFNOR, 1994). The aim of germination test is to determine the germinative capacity of the seeds; we collected 10 healthy seeds from different parts of the seed bag and put them in a Petri dish whose bottom is first covered with a layer of cotton. The seeds were then covered with another thin cotton layer, and the whole was humidified with distilled water without excess. After one week, we counted the number of germinated seeds (N). Germinative Capacity (GC) is expressed as a percentage (%) and given as: GC = 100.N/10. It is 70%.The experimentation was conducted in a plastic greenhouse