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# Transfer heavy metal from soil treated to colza

Najla Lasoued<sup>1</sup>; Essaid Bilal<sup>2</sup>; Saloua Rejeb<sup>1</sup>; Issam Guenole-Bilal<sup>3</sup>; Nejib Rejeb<sup>1</sup>; Frédéric Gallice<sup>2</sup>; Fernando Machado de Mello<sup>4</sup>

## RESUMO

Foram testados impactos de metais pesados nas lamas de estações de tratamento de águas residuais urbanas e industriais, tentando compreender a sua influência sobre o crescimento e bioacumulação na colza. A colza foi escolhida pelas suas características específicas. A colza é uma planta da família *Brassica napus* e é uma excelente bio-acumuladora de metais pesados. Os valores médios encontrados no solo, em ordem de importância, são: Fe >> Mn > Zn > Pb > Cu > Ni > Co > Cd. Os teores de metais pesados na lama são muito elevados e excedem os valores europeus autorizados para este tipo de utilização. Os efeitos da contribuição das lamas são manifestados por um aumento significativo dos metais pesados da planta inteira resultando numa variação da razão entre a parte aérea e das raízes da planta, sendo que esta proporção tende a aumentar com a dose de lama trazida no solo. As raízes das plantas apresentam altos níveis de Zn no solo, mesmo sem tratamento do solo. O conteúdo de Ni, Pb e Zn, em comparação com Cu e Co, são maiores nas raízes de colza.

Palavras-chave: Lama, metais pesados, colza, bioacumuladores, águas residuais.

## ABSTRACT

The impact of heavy metals were tested in sludge from urban and industrial wastewater treatment plants, trying to understand their influence on colza growth and their bioaccumulation. We chose the colza to their specific characteristics. The colza is a plant of the family *Brassica Napus*, is an excellent bio-accumulator of heavy metals. The mean levels found in the soil are organized in the following order: Fe >> Mn > Zn > Pb > Cu > Ni > Co > Cd. The contents of heavy metals in the sludge are very high and exceed European values allowed for that type of use. The effects of the contribution of sludge are manifested by a significant increase in the heavy metals of the colza, these results in a variation of the ratio between the aerial part and roots of the plant; this ratio tends to increase with the dose of mud brought in soil. The roots of plants show high levels of Zn even on the ground of untreated soil. The contents of Ni, Pb and Zn, compared to Cu and Co, are higher in the roots of colza.

Keywords: Mud, heavy metals, colza, bio-accumulator, wastewater.

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<sup>1</sup> Laboratoire de recherche Gestion des Risques Environnementaux, Institut National de Recherche en Génie Rural Eaux et Forêts (INRGREF), Tunis. (lassoued\_najla@yahoo.fr),

<sup>2</sup> Ecole Nationale Supérieure des Mines, Geosciences & Environment Department, CNRS:UMR5600, F-42023 Saint Etienne, France (bilalessaid@gmail.com),

<sup>3</sup> Département de Physiologie, Université de Lausanne-CHUV, Lausanne, Suisse, (bilal.issam.1698@gmail.com)

<sup>4</sup> Universidade Federal Rural do Rio de Janeiro (UFRRJ), BR 465, Km 7 Seropédica-RJ. Tel 21 37873673 (fermamll@ufrj.br)

## 1. INTRODUCTION

Colza is primarily grown for its oil, accounted for 12% of the oil consumption in the world, behind palm oil (27%) and soybeans (24%). In Europe, its markets are divided between food and non-food outlets, in particular biofuels. Hence the importance of studying the effects of additions of sewage sludge on the development of colza and transfer of heavy metals from soil to colza. The main objective of this study is to evaluate effect of urban and industrial sewage sludge containing heavy metals especially lead and chromium. These sewage sludge are made at different doses (5, 25, 50 and 100 t/ha). We are therefore interested in the growth and absorption of heavy metals by rapeseed Colza and follow the fate of the latter in the ground to prevent pollution events and toxicity.

## 2. MATERIALS AND METHODS

The experimental protocol was installed in the field of the Agricultural Experiment Station of Oued Souhil - Nabeul, situated about 60 kilometers from Tunis and belonging to the National Institute for Research in Rural Engineering Water and Forest.

The urban mud used in this study is taken from the wastewater treatment plant in Korba with a treatment system at low load activated sludge followed by maturation. The sludge from this station underwent a stabilization in aerobic followed by a drying on beds. The dry sludge is removed from the drying bed.

The mud is from the industrial wastewater treatment plant in Bou Argoub which hosts two industrial zones, companies in the Refrigeration and Brasserie Company of Tunis (SFBT) specialized in the food industry, and Assad specialized in the electrical industry. Sludge from this station underwent a stabilization in aerobic followed by drying on beds. This sludge is loaded with heavy metals especially lead and chromium.

The plant material used in this experiment is the rapeseed (*Brassica napus*) is an annual plant with yellow flowers of the family *Brassicaceae*. The colza is chosen for their ability to accumulate metals and it is widely cultivated for the production of food oil diesel fuel (biodiesel). That is, with sunflower and olive, one of the three main sources of edible vegetable oil.

The test device is installed on two juxtaposed plots reserved for each crop (wheat or rapeseed). For each type of sludge, four doses (5, 25, 50 and 100 t / ha) were brought into play and compared to control soil without any addition.

Field work began in September 2010 with the spreading of sludge realized September 20, 2010. They were manually dug into the ground. Before application, the sludge was analyzed.

The soil was sampled twice, first before the application of sludge and the second time after harvest. The samples were taken between the lines by auger at four depths (0-10, 10-20, 20-40 and 40-60 cm).

In the laboratory, soil samples were dried in open air and sieved to 2mm or 0.2mm depending on the type of analysis required. Soil testing is in progress. The main parameters are determined particle size, total calcium, conductivity, carbon, organic matter, total nitrogen and determination of heavy metals. For the particle size we used the method of the International pipette Robinson, it is primarily the destruction of soil organic matter using  $H_2O_2$  and dispersion of clays by sodium hexametaphosphate. Clays and silts are measured in the suspension of land following the decay time that depends on particle diameter (NF X 31-107). The settling velocities of particles can be calculated by the formula of Stokes.

For plants, we performed the semi rapeseed (50 seeds /m<sup>2</sup>) December 29, 2010 and semi wheat (350 seeds /m<sup>2</sup>) January 5, 2011. The rapeseed harvest was performed after the formation of slices May 25; 2011. We weighed the aerial part and the root. The same work was done with wheat June 9, 2011. The samples were subsequently dried and crushed ore to determine the mix of metals in different parts of the plant.

The Mud and soil samples were analyzed by XRF (X-Ray Fluorescence) and ICP-AES (Inductive Coupled Plasma Atomic Emission Spectrometry Activa–Horiba Jobin Yvon Spectrometer) in the Geosciences and environment Department of Ecole Nationale Supérieure des Mines de Saint Etienne. The soil pH was measured using a 1:2 soil to water ratio. Metal

accumulation in plant tissues was evaluated from the most abundant species. Plant samples were thoroughly washed in tap water and rinsed three times with distilled water. Samples were then separated into leaves, stems and roots, dried at 40 °C to constant weight and grounded and sieved at 2 mm. Digestion of plant samples was performed using hot nitric concentrated acid, according to Zarcinas et al. (1987), Petrescu and Bilal (2006, 2007), Secu et al. (2008) and Lăcătușu et al. (2009). The plant extracts were analysed by ICP-AES.

### 3. RESULTS

Sand is the most representative size fraction in this soil which is a sandy loam soil texture. The analytical results show that the soil of the plot used is characterized by an alkaline pH, conductivity ranging from 1.06 to 1.52 mmho/cm resulting low salinity, soil saturation is between 30.4 and 31.8 ml/100g.

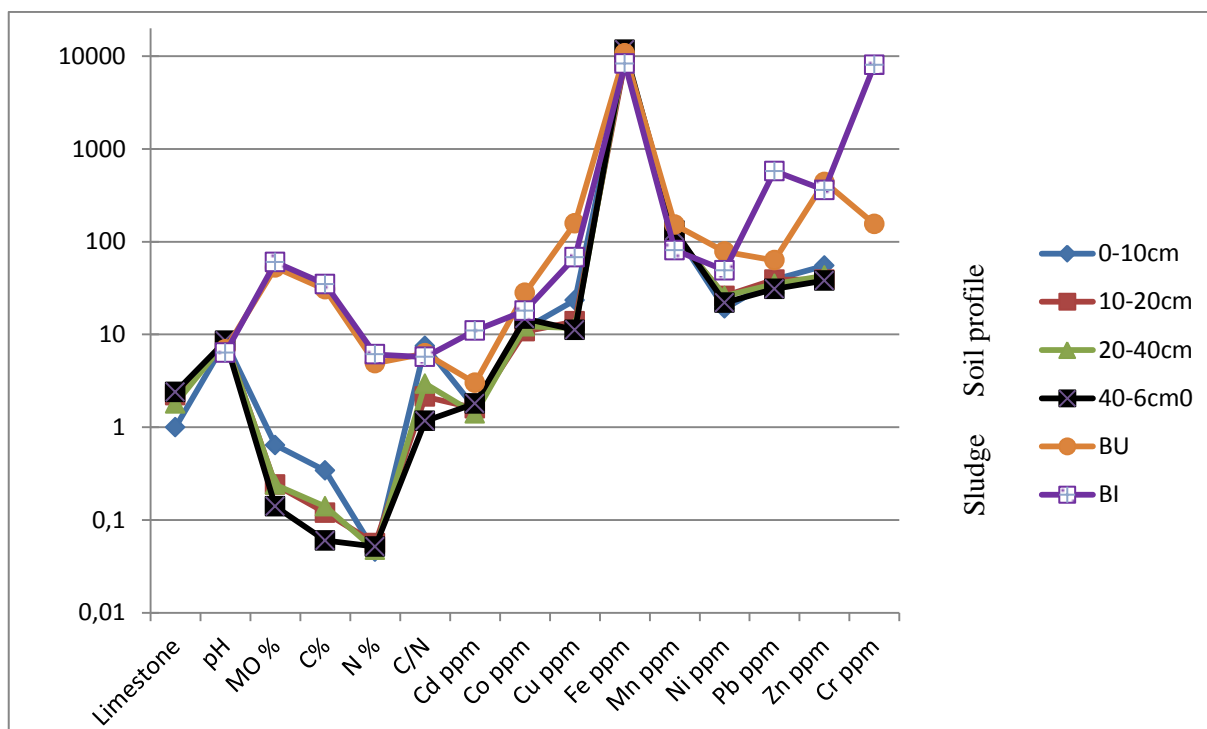


Figure 1. Chemical composition of different horizons of the soil profile four depths (0-10, 10-20, 20-40 and 40-60 cm) and sludge (Urban sludge (UB) and Industrial sludge (BI)). MO: organic matter.

Limestone is totally inherited components of soil, possibly modified by repeated and massive supply of amendments basic. The analysis of the limestone is needed to refine the characterization of soil constituents. The percentages of limestone (**FIGURE 1**) in the different horizons are less than 5% so it is a non-calcareous soil, with very low organic matter content. Contents of total nitrogen are relatively low. The C/N ratio is widely used to characterize and classify types of organic matter in soil. This ratio C/N is about 7 at the first horizon (0-10cm) indicates that organic matter will be quickly mineralized. Concerning trace elements, iron is the most representative. The main levels found in the soil are organized in the following order: Fe >> Mn > Zn > Pb > Cu > Ni > Co > Cd main concentrations of heavy metals introduced by the sludge. The factor and the degree of contamination allow evaluation of soil contamination. The calculation of the contamination factor (CF) is calculated by dividing the concentration of each metal in the soil concentration in unpolluted soil (Muller, 1969; Hakanson, 1980; Rashed, 2010). The values of CF (Bu) of most metals are less than 1 except for Cu and Zn from 25t/ha treatment (**FIGURE 2**), soil contamination is low. In contrast, the heavy metals CF (BI) are greater than 1, except for Cd (**FIGURE 3**). The contamination is very high for Cr and Pb and moderate for Co, Ni and Zn.

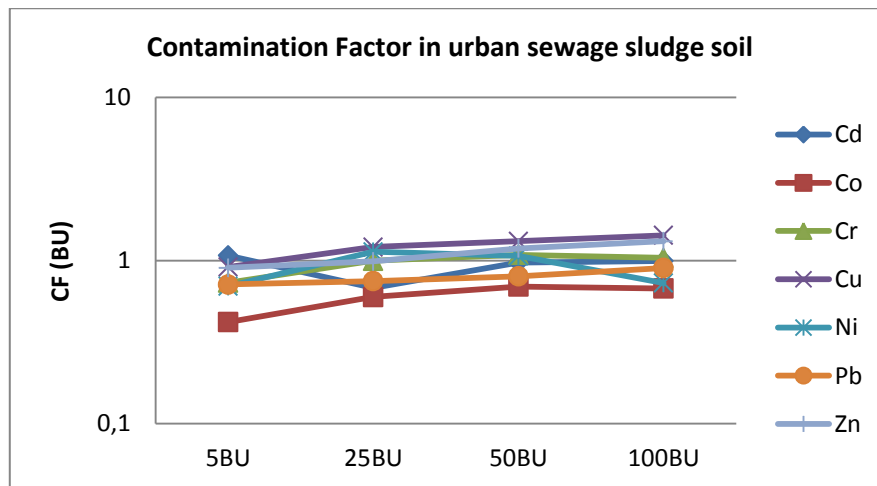


Figure 2. Influence of different doses (5, 25, 50 and 100 t/ha) of urban sewage sludge treatment on contamination factor (CF).

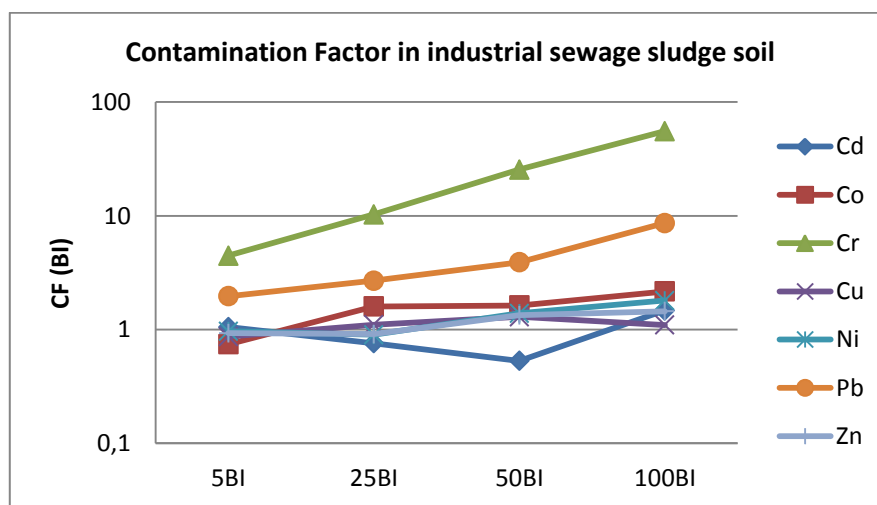


Figure 3. Influence of different doses (5, 25, 50 and 100 t/ha) of industrial sewage sludge treatment on contamination factor (CF).

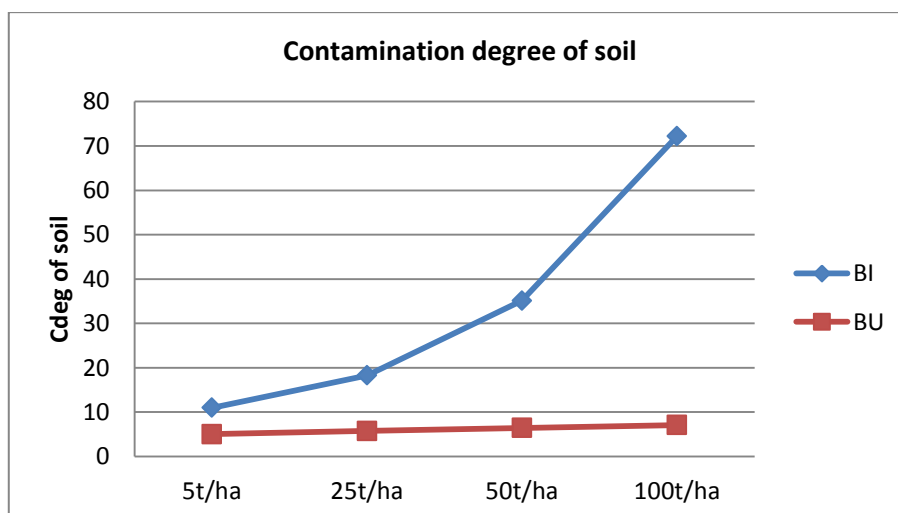


Figure 4. Influence of different doses (5, 25, 50 and 100 t/ha) of urban (BU) and industrial (BI) sewage sludge treatment on contamination degree of soil (Cdeg).

The sum of all factors of contamination for all elements examined is the degree of contamination (Cdeg) of the environment. According to a classification by Hakanson, 1980 and Loska et al., 2003, the Cdeg (Bu) is less than 8 (**FIGURE 4**) corresponds to low level contamination and the Cdeg (BI) is moderate of soil treated about 5t/ha, considerable for 25t/ha and very high degree of contamination for 50t/ha and 100t/ha.

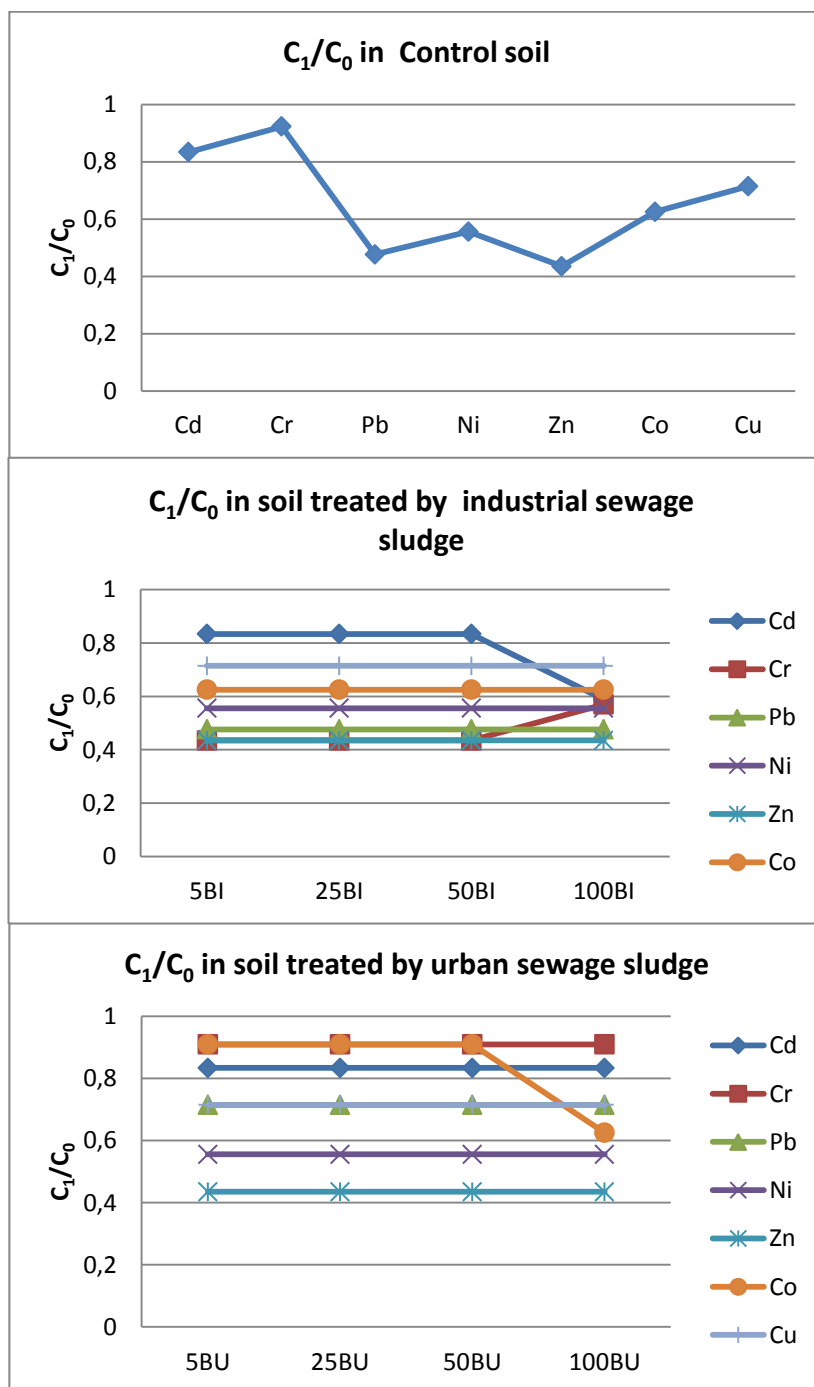


Figure 5. Heavy metals behavior in control soil and treated soils before and after the harvest of crops.

Treated soils and control soil were analyzed before and after the harvest of crops. We note that in the control soil levels of Pb, Ni and Zn were reduced by half, and the Co and Cu contents from 35 to 30 % and 17% for Cd and 10% for Cr (**FIGURE 5**). In soils treated with

sewage sludge, we observed in soils treated with industrial sludge lower metal contents of 57% for Zn 53% for Pb, 45% for Ni, 38% Co, 28% for Cu and 17% for Cd. The levels of these metals remain constant regardless of the treatment except for contents of 45% Cr and 43% for Cd treated soil 100t/ha. For soils treated with urban sewage sludge (**FIGURE 5**), we have the same tendency for soils treated with sewage sludge industry, the levels of some metals remain constant is that the sludge spreading rate. Though with some differences due to the nature of the sludge, 57% loss for Zn, 45% for Ni, 29% for Pb, 17% for Cd and 10% for Co, Cu and Cr, with however, a decrease of 38% Co contents for soils treated about 100t/ha.

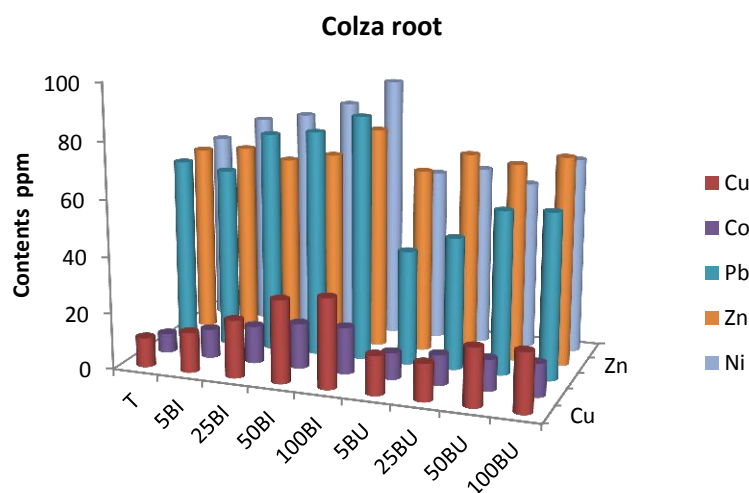


Figure 6: Distribution of heavy metals in the root of colza in soils with addition (5t/ha, 25t/ha, 50t/ha and 100t/ha) of urban (BU) and industrial (BI) sludge and control soil (T).

The Pb concentrations in roots and seed of colza ranged respectively from 65 to 86 ppm and 53 to 71 ppm (**FIGURE 6**) from industrial sludge treatment and 40 to 59 ppm and 46 to 67 ppm from urban sludge treatment. The Ni (68 to 93 ppm and 74 to 174 ppm) and Zn (67 to 79 ppm and 71 to 97 ppm) are high concentration in roots and seed colza from industrial sludge. The Ni and Zn concentrations from soil treated with urban sludge are ranged from 65 to 70 ppm in root and 71 to 86 ppm. The bioconcentration factor (BCF) can be used to estimate the potential of a plant for phytoremediation. The ability of a plant to accumulate metals from soil can be estimated using the bioconcentration factor (BCF) is defined as the ratio of metal concentrations in the roots compared to those in the soil (Dowdy et al. 1997; Yoon et al. 2006; Gupta & Sinha 2008). Species that showed BCF values lower than 1 are not suitable for phytoextraction. The ability to move the metals from root to shoot, is measured using the FT (translocation factor), which is defined as the ratio of metal concentrations in shoots compared to those in roots (Yoon et al. 2006. Gupta and Sinha, 2007). According to McFarlane et al. (2007), FT is defined as the ratio of metal concentrations in leaves compared to those in the roots.

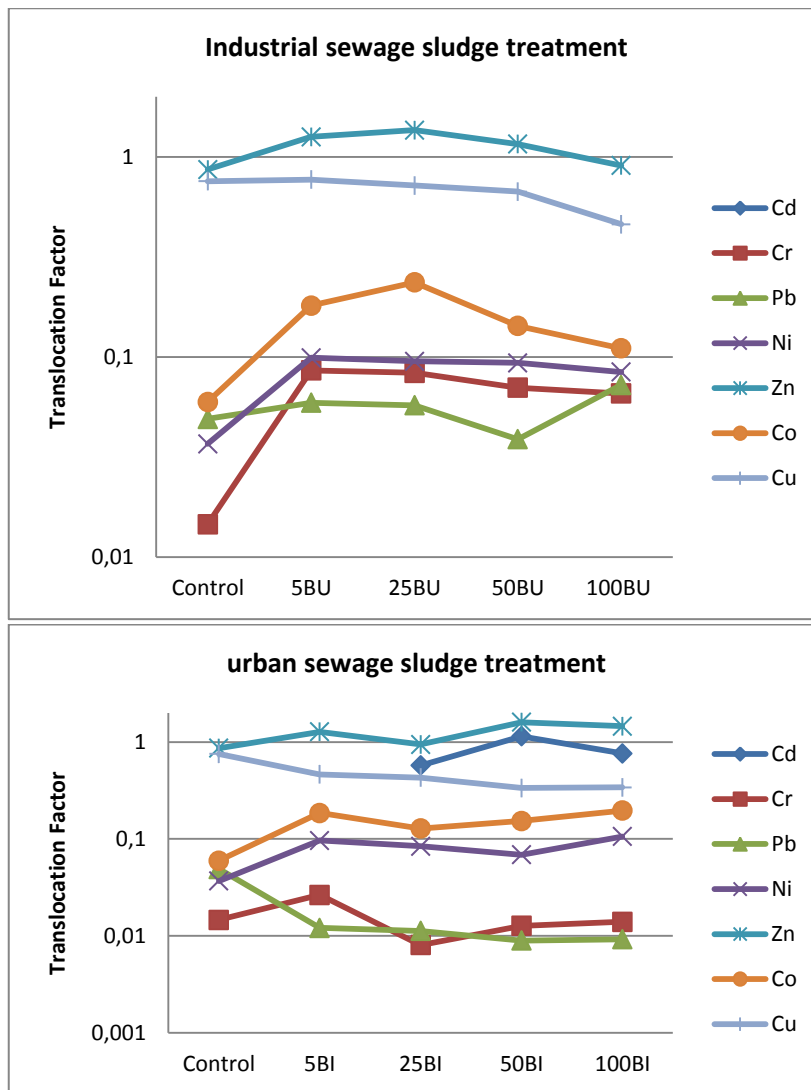


Figure 7: The canola translocation factor (TF) in soil with the contribution different doses of industrial and urban sewage sludge.

The ability to move the metals from root to shoot, is measured using the TF (translocation factor or transfer factor), which is defined as the ratio of the concentrations of metals in the shoots by compared to those in the roots (Yoon et al., 2006; Gupta and Sinha, 2007). According to McFarlane et al. (2007), TF is defined as the ratio of metal concentrations in leaves compared with those in the roots.  $TF > 1$  indicates the preferential distribution of metals in roots. Colza Zn accumulates preferentially in the roots ( $TF > 1$ ), while it appears to have the ability to transfer to its aerial parts ( $TF < 1$ ) Co, Ni, Cr, Pb and to a lesser extent Cu. Deng et al. (2004) also found that Zn, Cu and Cd accumulated, were mainly distributed at the root parts compared to aerial parts. Translocation Factors weaker were examined in the case of accumulation of Cr, Ni and Pb (**FIGURE 7**). This result highlights the ability of colza to transfer the metals of these roots to its aerial parts. Fe and Mn were significantly decreased in the stems, roots, siliques and leaves by the addition of sewage sludge. However, this decrease was significantly marked with the contribution of urban sludge in the siliques and leaves colza.



#### 4. DISCUSSION AND CONCLUSION

Metal concentrations in plants vary with plant species (Secu et al., 2008, Petrescu and Bilal, 2007 and Lacatusu et al., 2009). Plant uptake of heavy metals from soil occurs either passively with the mass flow of water into the roots, or through active transport, crosses the plasma membrane of root epidermal cells.

The mean metal contents found in the soil are organized in the following order: Fe >> Mn > Zn > Pb > Cu > Ni > Co > Cd, main concentrations of heavy metals were introduced by the sludge. The contamination factor (CF) of most metals in urban sludge soil is less than 1 except for Cu and Zn from 25t/ha treatment, the contamination of soil treated by urban sludge is low comparatively with soil treated by industrial sludge where CF (BI) is greater than 1, except for Cd. In this soil, the degree of contamination is very higher from 25 ha/t and levels observed for Cr and Pb is high and moderate for Co, Ni and Zn. After the harvest of crops, the control soil contents of Pb, Ni and Zn are reduced by half, and the Co and Cu contents from 35 to 30 % and 17% for Cd and 10% for Cr. In soils treated with sewage sludge, we observed in soils treated with industrial sludge lower metal contents of 57% for Zn 53% for Pb, 45% for Ni, 38% Co, 28% for Cu and 17% for Cd. The levels of these metals remain constant regardless of the treatment except for contents of 45% Cr and 43% for Cd treated soil 100t/ha.

In soils treated with industrial sludge, the metal concentrations decreased by 57% for Zn, 53% for Pb, 45% for Ni, 38% Co, 28% for Cu and 17% for Cd. The level of these metals remains constant regardless of the treatment, except for 100t/ha treated soils. We have a super saturation of the plant from a threshold beyond which the colza cannot assimilate metals. We have a threshold effect that occurs to 100t/ha where some metals such as Co, Cr and Cd react differently. The result of this study highlights the ability of colza to transfer the metals of these roots to its aerial parts. The Zn, Cd and more or less Cu accumulates preferentially in the colza roots, while it appears to have the ability to transfer to its aerial parts Co, Ni, Cr, Pb. Deng et al. (2004) also found that Zn, Cu and Cd accumulated, were mainly distributed at the root parts compared to aerial parts. Weaker translocation factors were observed in the case of accumulation of Cr, Ni and Pb, this result highlights the ability of colza to transfer these metals of these roots to its aerial parts. According to Deng et al. (2006), this phenomenon could be explained by the anatomical characteristics of the plant. Unlike Ni and Zn, which play an essential role in plant nutrition and enzymatic processes, Cd is a highly toxic metal that can affect the growth and metabolism of plants.

According to Raskin and Ensley (2000) cadmium uptake by plants can be explained by the existence of competition between Cd and Zn mainly because of their close chemistries. In addition, studies conducted by Clemens (2006) and Verbruggen et al. (2009) indicate that Cd uptake by plants seems to occur through ion channels and transporters Zn molecules. These observations are consistent with the strong correlation obtained in our study between the concentrations of Cd and Zn measured in the colza roots (**FIGURE 7**). The Pb, Ni and Zn concentrations are very high in roots and seed of colza from soil treated. The seed colza are used for product oil, we don't know what the reaction of the human body of the colza oil from this treated soil with sewage sludge.

The stress generated by heavy metals in industrial sludge spreading, produces in colza cells programmed death. Several authors (Gilchrist, 1998; Larsen, 1994 White, 1996; Wyllie et al. (1980) observed this type of behavior in tobacco and mammals. Heavy metals inhibit the absorption of nutrients Ca, Mg, Mn, Zn, Cu and Fe and cause phenomena that we have observed in TEM cells colza. Srivastava and Jaiswal (1990); Breckle (1991); Cataldo et al., (1983); Costa and Morel (1994); Ouariti et al. (1997); Burzynski and Grabowski (1984) and Sandalio et al. (2001) showed the close relationship between metals heavy, the nutrient deficiency and structural aberrations in colza cells.

We must complete these observations in a larger study in cell biology and biochemistry to better understand the phenomenon of colza cell autophagy and its relations with the spreading of industrial sludge rich in heavy metals. These transformations will have a significant impact on the colza oil produced by this type of culture and therefore an impact on the human body.

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