American Journal of Applied Sciences

Original Research Paper

Eucalyptus Gomphocephala: Morphological Characterization and Seed Germination under Influence of Contaminated Soil

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Article history
Received: 09-02-2015
Revised: 30-01-2017
Accepted: 31-03-2017

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Abstract: To study the impact of soil pollution on plant growth, our work focused on soil polluted by heavy metals. This soil was taken from the municipal landfill of Jendouba (North of Tunisia). We have undertaken to study the morphological behavior of a forest specie "*Eucalyptus gomphocephala*". Two treatments are chosen (substrate containing 100% and 50% of soil taken from municipal solid waste of Jendouba) compared to a control (pilot soil). Morphological Parameters (stem height, root length, leaf area, the ratio of the stem/root and biomass) was measured. The plants grown on substrates based on an S1 (100% soil taken from municipal waste) and S2 (50%), exhibit morphological appreciable contribution to the control soil.

Keywords: Municipal Solid Waste, Substrate, Stem Height, Root Length, Leaf Area, Biomass

Introduction

Urban environment does not offer to the trees an environment which corresponds to their requirements of life; every day, the tree undergoes disturbances which generate a stress deteriorating its growth and threatens its survival. In fact, the urban environment is a special medium where the soil always does not have very favorable characteristics for the right growth of plants (compaction, water balance deficit, etc...) (Garrec, 1989; Bory, 2000). Therefore, many authors tackled these problems of growth, Kramer (1958) affirm that a tree, even placed under the greatest environmental conditions does not grow all the year. For a very large number of plant species, too high levels of available copper in the soil are harmful to development, or even toxic. In this case, the growth having reduced and more particularly the roots, which having thickened with less branched rootlets abnormally color dark (Loué, 1993). For Cadmium, the rates of absorption are variable and depend on the plant species (Kim *et al*., 1988), the cadmium concentration in the soil, as well as other factors influencing the cadmium bioavailability, particularly the pH of the soil.

Nowadays, the problem of waste has become increasingly alarmed. Indeed, the solid waste production follows the population growth and the socioeconomic development. It increases and takes significant proportions in the developed countries or those in the process of development and their elimination became a problem of increasing concern, as the waste sector has a direct effect on the health and the population quality of life and in general the environment quality. Therefore, it is a management, controlling problem of the increasingly large quantity of waste. Indeed, waste represents a threat to the human health, the environment and mainly the soil contamination and the groundwater with toxic substances (heavy metals, solvents and pesticides), the biogas emission in ambient air are potential sources of environmental contamination and exposure for people living near sites waste (Brisham, 1986). The high concentrations of heavy metals in the soil have a selective effect on some populations of plants. The result is a low species diversity in different trophic levels (Ernst *et al*., 2004; Arriagada *et al*., 2006). Indeed, heavy metals may remain in the soil for a long time. Thus, the tolerance is defined by the plants or micro-organisms ability to live and support the raised concentrations of heavy metals in the soil (Dietz *et al*., 1999; Arriagada *et al*., 2006).

The aim of our study was to determine the impact of soil polluted by heavy metals on the growth of *Eucalyptus gomphocephala* (seed germination and morphological parameters).

Materials and Methods

Setting in Culture

Plantings having made in perforated polyethylene bags, to avoid the irrigation water stagnation, 20 cm depth and 12 cm in diameter, filled with 3 types of substrates: S1 soil from municipal solid waste of

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Jendouba, S2 50% soil from municipal solid waste of Jendouba + 50% control soil and S3 control soil (Table 1). So we have for one specie three substrates.

The bags implementation approved in the nursery at a rate of 90 bags/treatment/specie: (30 bags/species/repetition).

Analyses of substrates realized in the laboratory of the Sylvo-Pastoral Institute of Tabarka (Tunisia).

Plant Material

The wood species used for this study was *Eucalyptus gomphocephala*.

Presentation of the Species Used

The genus Eucalyptus is originating in Australia (Kelly, 1989). It was introduced in Algeria, Tunisia and in Morocco and plays a leading role in the existing forests enrichment and/or the open areas reforestation (FAO, 1954).

Among the most common plants in the world, the Eucalyptus was affirmed as the most plastic group (Marion and Poupon, 1974), because of its rapid growth, particularly on poor soil nutrients (Cossalter *et al*., 1999; Specht, 1996) and of their resistance to drought, moisture and fire (Daya, 2006).

In fact, it develops in a variety of climates which go from the tropical to the Mediterranean climate and in savanna (Charas, 1971).

In addition, Eucalyptus plantations have an agro-economic confident interest. Indeed With a very fast growth, a great homogeneity, strong productivity, an excellent fiber and a wood of high density, the eucalyptus became the leafy tree more planted in the world (90 countries) (Kirch *et al*., 2011; Vance *et al*., 2014). they represent important sources of various productions as sawing, wood of mine, posts, pulp paper, charcoal, essential oils, honey and tannin production, etc (Jacoby, 1979; Boudy, 1950; Bang, 2013).

Table 1. Summary of the compared chemical parameters of different soil types used for the culture

Substrate	S1	S2	S3
PH	7.23	7.520	8.23
Conductivity CE (memos/cm)	2.85	1.553	0.145
Organic Matter MO%	10.00	8.000	5.00
Total Organic Carbone COT%	5.77	4.630	2.89
Nitrogen N (%)	3.78	2.100	0.98
Ratio C/N	2.43	2.200	3.24
Olsen Phosphorus P (mg/Kg)	10.00	3.000	0.85
Potassium P (mg/Kg)	1500.00	800.000	300.00
Calcium Ca (%)	4.00	2.450	1.50
Zinc Zn (mg/Kg)	150.60	45.000	15.00
Manganese Mn (mg/Kg)	1479.00	600.000	270.00
Cobalt Co (mg/Kg)	1.85	0.990	0.99
Magnesium Mg (mg/Kg)	52.80	50.000	126.00
Copper (mg/Kg)	311.00	40.000	30.00

The culture of different species of eucalyptus with these advantages caused a strong demand of paste of eucalyptus for paper but also for the manufacturing of furniture. Recently, renewable energy sources have had a growing impact in the European Union. This increase was due also to lignocellulosic biomass production deriving from agricultural activity and Eucalyptus is one of the biomass champions (Sgroi *et al*., 2015). This Biomass can be used to power plants that generate electricity or heat.

Moreover, the plantations of Eucalyptus, developed outside forests, reduced the pressure on the natural forests and reinforce their double interest. Widely adopted by large international forestry companies, wood Eucalyptus became in shortly, the green gold of the paper industry.

Determination of Morphological Parameters of Plants Seed Germination

Germination is a critical phase of the development cycle because it conditions the seedlings installation (Naouar, 1999). We determined the rate of germination of the studied species when we noted a homogeneous seedling emergence.

Study of the Growth and Development of Eucalyptus Gomphocephala on Different Substrates

The Growth of *Eucalyptus Gomphocephala* (E.G) introduced in nursery on different substrates: S1 100% (soil from municipal solid waste of Jendouba), S2 50% (soil from municipal solid waste of Jendouba) + 50% soil control, S3 100% control soil (soil pilot), was estimated by the collar diameter, the height of the stem, the total root length, the ratio height stem length root, the ratio biomass stem biomass root. We selected 5 plants randomly, due to the low percentage of germination.

Measurement of Leaf Area

Leaf area determined using Mesurim software and scanned images. It was measured on a sample of 10 leaves taken randomly in the middle of the plant (median sheets). The value estimated in mm^2.

Collar Diameter

It corresponds to the diameter of principal stem, at the contact zone between the aerial part and the root part. It is important as the height of the stem factor, but it is admitted that it is better to predict the potential for survival and growth plant (Mexal and Landis, 1990).

Height of the Stem

The height of the stem measured starting from the collar to the base of the bud terminal (Thompson, 1985).

Root Architecture

The Root length considered as a very important morphological index of a plant which enables us to predict the future of the plant and to test the effect of the substrate on the architecture of the root system (Mahdi *et al*., 2001).

The root length of the seedlings was estimated by a grid of 1 cm *1 cm by counting the number of the intersections of the root with the position of each square of 1 cm *1 cm. The roots of each plant fixed on the grid to help with counting. Once counting finished, the root will be cut off so that it does not recounted.

The total number of intersections for each order converted into length according the relation modified of Tennant (1976).

Root length = (11/14) * Number of intersections * the grid unit so for a grid of 1(1 cm, we have.

Root length = 0.7857 * Numbers of intersections (cm).

Ratio Aerial Root Biomass

The use of the ratio BA/BR (based on dry weight) is one of the most morphological criteria used. It is considered as a balance measurement between the perspiring surface and the absorbent surface of a plant. According to Russel (1977) a value of 1, 4 corresponds to better balanced seedlings. However, its use is limited because of its variation depending on the size of the plant (Ledit *et al*., 1970).

Statistical Analysis

The statistical analyzes were performed using the statistiXL software.

Results

Seed Germination

The germination rate is shown in Table 2.

The germination rates are variable from one treatment to another. For the studied species we recorded low rates, the maximum recorded for plants taken from contaminated soils which are of the order of 47% and decrease in the control soil (8%).

This mortality is probably due to the long summer drought, the issue is heightened by the spontaneous vegetation competition (Hadri and Tschinkel, 1976).

Leaf Area

Figure 2 shows the variation of leaf area in *Eucalyptus gomphocephala* according to the substrates, which was in the range of 1000 to 3500 mm^2.

The results of the analysis of the leaf area variance of the *Eucalyptus gomphocephala* in different substrates (S1, S2 and S3) prove that substrate factor exerts a highly significant effect (p<0,001).

The results of the variance analysis of the plant studied in different substrates show that growth in height of the stem exerts significant variation by substrates (p<0,001).

The results about the length of the stem at *Eucalyptus gomphocephala* illustrated in the Fig. 1 and 3.

Fig. 1. Seedlings of *Eucalyptus gomphocephala* (A corresponds to plants introduced in Substrate S1, B in S2 et C in S3)

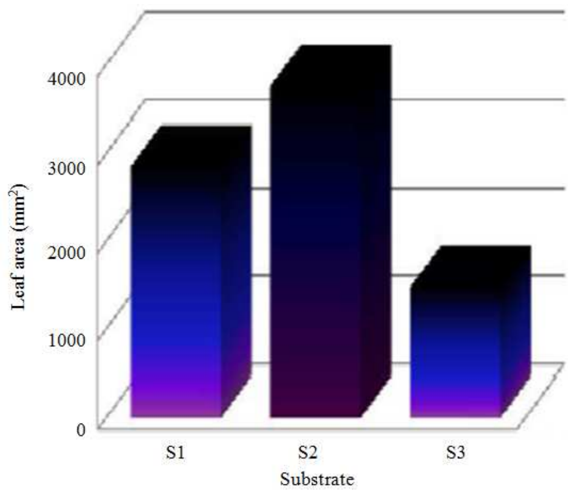

Fig. 2. Variation of leaf area in *Eucalyptus gomphocephala* according to the substrates

Table 2. The rate of germination after 60 days of the date of sowing

Germination rate (%)	Treatment	Specie E.G
	S1	47.0
	S2	15.5
	S3	8.0

The highest value recorded in plants introduced in a soil having undergone a deposit of household waste and it is around 103 cm and decreases considerably in plants taken from a control soil (64 cm).

Collar Diameter

The results of the variance analysis of the studied plant in different substrates show that the substrate factor exerts a significant effect on the mean diameter of the collar ($p<0,05$). With *Eucalyptus gomphocephala* collar diameter increases while passing from control substrate (2, 5 mm) and reached 4 mm in the two other substrates (Fig. 4).

Ratio Height Diameter

The results of the analysis of the variance show that the substrate factor is non-significant and has no effect on the ratio height diameter of the stem (Fig. 5).

Root Length

The variance analysis of root growth showed that the substrate factor exerts a non significant effect on the growth in total root length. The length of the root is often employed by various authors as an index of stress and toxicity (Kelly *et al*., 1979). Figure 6 shows the variation of the root length in *Eucalyptus gomphocephala*, the highest average value of the total length of roots is 400 cm recorded in the substrate S2.

Ratio Height Stems/Root Length

For *Eucalyptus gomphocephala* introduced in different substrates, the analysis of the variance shows that the substrate factor does not exert any significant effect (Fig. 7).

Ratio Stem, Root Biomass

The analysis of variance shows that the substrate exert a significant effect ($p<0.05$) on the ratio A/R (Aerial Biomass/Root Biomass). This ratio varies between 1, 5 and 3 for Eucalyptus for different substrates and reaches its maximum for the soil of the landfill (Fig. 8).

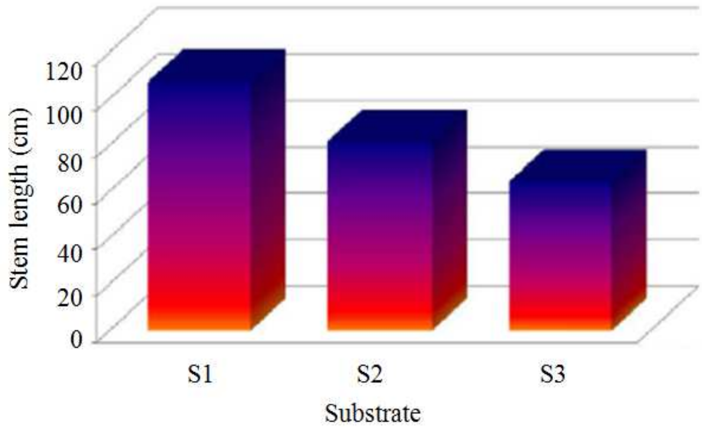

Fig. 3. Variation of the stem length in *Eucalyptus gomphocephala*

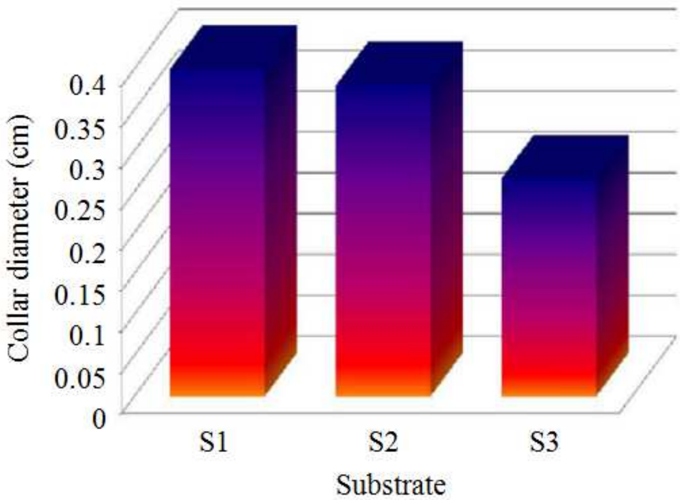

Fig. 4. Variation of collar diameter in *Eucalyptus gomphocephala*

DOI: 10.3844/ajassp.2017.623.631

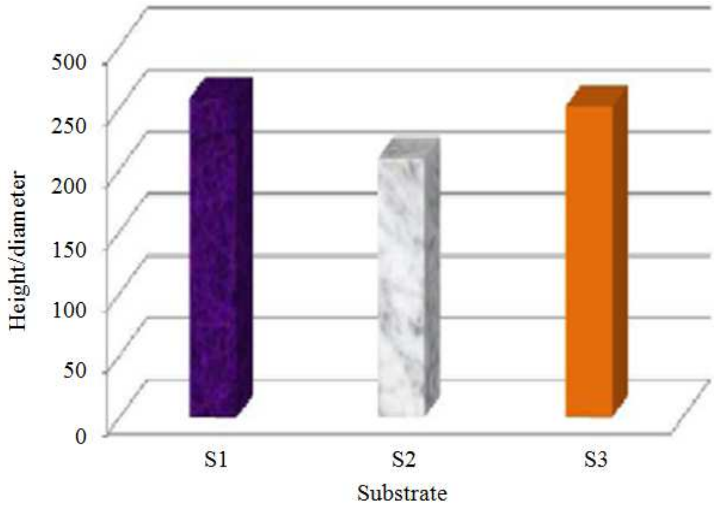

Fig. 5. Variety of H/D ratio in *Eucalyptus gomphocephala* in different

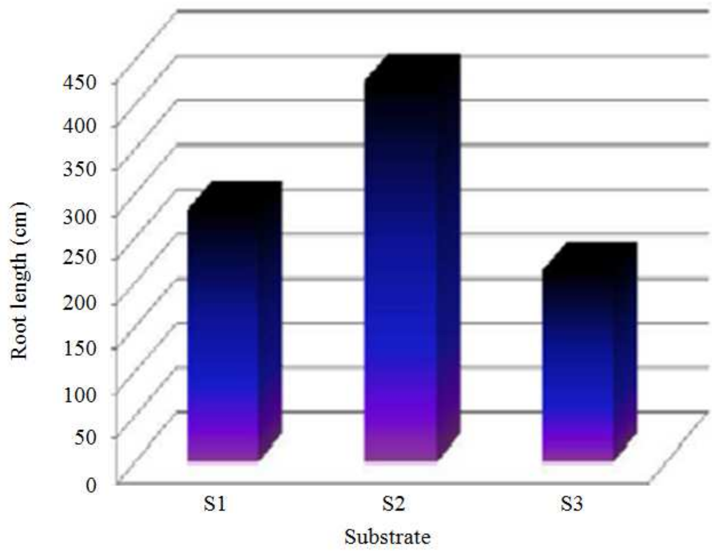

Fig. 6. Variation of the root length in *Eucalyptus gomphocephala*

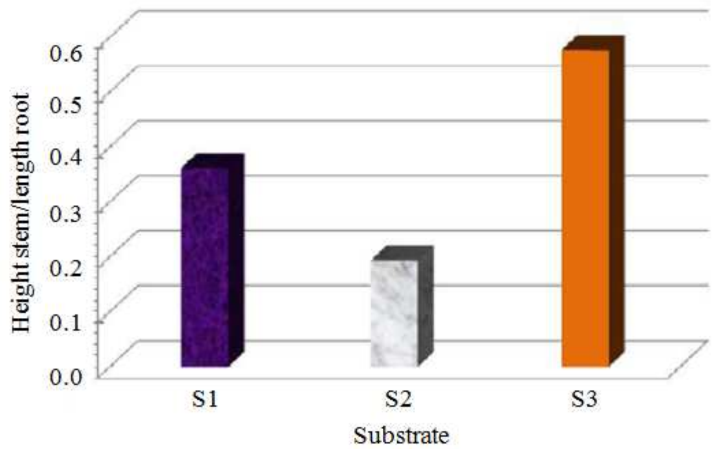

Fig. 7. Variation of the height stem, length Root ratio in *Eucalyptus gomphocephala* in different substrates

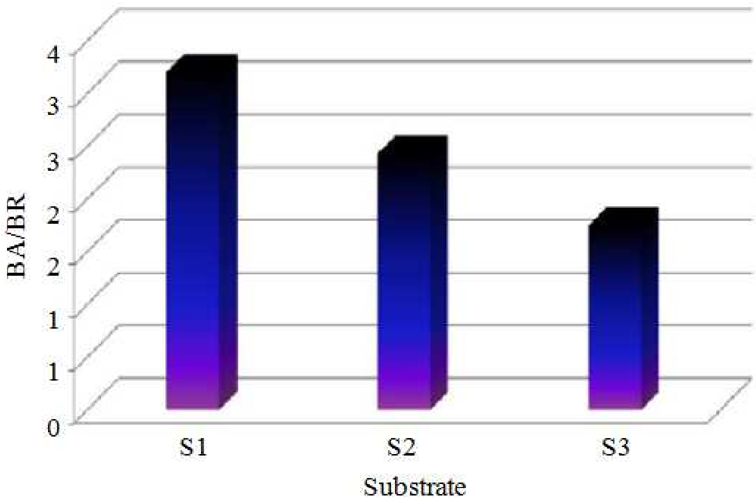

Fig. 8. Variation of BA/BR ratio in *Eucalyptus gomphocephala* according to the substrates

Discussion

The leaf is the seat of the photosynthetic activity and the area could be considered as a stress tolerance index (Benterrouche, 2007).

The Low values of leaf area recorded in pilot substrate are due probably to a deficiency in nutritive elements.

A large amount of study has shown that abiotic, hydrous (Mefti *et al*., 1998) and salt stress (García-Legaz *et al*., 1993), or toxicity of heavy metals (Cadmium) (Thripathi and Tripathi, 1999) affects negatively the leaf surface. This explains well the decrease in the value of the leaf area recorded in contaminated soil.

The highest value of the length of the stem at *Eucalyptus gomphocephala* observed in plants introduced in a soil having undergone a deposit of household waste. According to Paliwal *et al*. (1998), the rise in the growth can be attributed to the toxic effects lack, such as low levels of heavy metals.

In urban waste, when the metal content does not exceed the limits recommended for agricultural use, they became a source of organic matter for the soil. However, toxicity and possible soil pollution by heavy metals observed only in the long term (Terce, 2001).

On the contrary, Kozlowski (1997) affirms that salinity reduces stem growth by the initiation suppression and the leaf expansion and the leaf abscission acceleration (Kozlowski, 1997).

In our case, the length of the stem evolves in a way inversely proportional to the Sodium content in the soil. This is possibly due to the experiment duration or a stress adaptive strategy developed by the plant.

These results also affirm that Eucalyptus is one of the fastest growing forest species with less demanding to the environment (Nguyen-The and Melun, 2003).

For the collar diameter, the Increase in the growth of the collected plants in soils coming from landfills is probably due to the important load of organic matter, macro and micro nutrients, especially total nitrogen available (Paliwal *et al*., 1998).

The ratio height diameter is a key indicator to test the effect of the competition for the light according to Jobidon (2000) and in that sense it reflects the growth potential of seedlings in a plantation. The results of the analysis of the variance demonstrate that the substrate factor has no effect on the ratio height diameter of the stem.

The root system growth in relation to soil conditions helped to show up changes in the root system architecture in different structures of the soil (Szota *et al*., 2007). We observed a decrease in root length for the *Eucalyptus gomphocephala* taken from a contaminated soil. This root length decrease was also found by Kelly *et al.* (1979). They found that the root elongation is negatively affected following the increasing soil levels of cadmium. According to Epron *et al*. (1999), who worked on oak, found that the moderate and high salt treatments deteriorate strongly the root elongation. In fact, they explained the reduction by salinity to the cells extension inhibition following the swelling reduction (Epron *et al*., 1999). In *Acacia cyanophylla*, Benterrouche (2007) noted, a root length increase, following a high intake of mud (substrate containing 80% mud), but this increase is not significant (Benterrouche, 2007). Similar results were found by Kleiner *et al*. (1992) in several species of oak due to hydrous stress.

The ratio, height stem S/R length root on various substrates is lower than 1.

Brower (1983) reports that the growth of aerial and root systems follows a strict harmony and the relationship between these two systems is predictable on a great variety of environments. However, this ratio variation is sometimes common. According to Ferchichi (1990), a soil rich and well supplied with water can boost the growth of the aerial system.

The results of analyzes of different morphological parameters studied for *Eucalyptus Gomphocephala* show that the effect of the substrate has a significant effect ($p<0,05$) on the average value of leaf area, stem length, the length of the root and the ratio BA/BR.

But, no significant effect had observed for the diameter and the ratio rod length diameter rod.

The ratio A/R considered an index of the equilibrium between the surface of perspiration (foliage) and the absorption surface of a plant (roots) (Lamhamdi *et al*., 1997). Since the seedlings having a root mass equal to that of the stems must have a good balance roots/stems, it must also have a good root architecture without deformation (Salahadine, 2005). A low ratio indicates that the roots are abundant compared to the leaf biomass and in this case plant can endure and survive in drought conditions after planting. On the other hand, a high ratio means that the roots are not abundant and that this type of plant will be more sensitive to hydrous stress, particularly in the semi-arid zones or in areas where evaporative demand is important (Lamhamdi *et al*., 1997).

The weight considered as a fundamental criterion for measuring plant growth. Indeed, in the research of the best extraction reagent, the Agrochemicals will use as a criterion, the element content in the plant, or the dry material produced (Coïc and Coppenet, 1989). The ratio of biomass BA/BR tested as a marker of stress by many authors (Kaufman, 1977).

According to Kelly *et al*. (1979), the root biomass reduction it would have obvious implications as for the air production and the survival of the seedling, these observations raise the impact of the heavy metal issues on forest regeneration in areas where heavy metals were introduced. For many authors, the BA/BS ratio tested as a marker of stress and toxicity indicating the air part sensitivity by contributing to the root part (Paliwal *et al*., 1998). These same authors demonstrated that the root growth is less sensitive to various types of stress than the stem growth. Xiong and Zhu (2002), found a decrease in this ratio, which follows a hydrous and osmotic stress indicating a strong root development and a growth in root length better than the growth in the length of the aerial parts. Similarly, Thripathi and Tripathi (1999) found the same result as a response to a metal stress. According to Parent and Devreotes (1999), nitrogen excess supports stem elongation at the root development and maturation which can lead to inadequate supply out of water and minerals. This ratio expresses the tree plasticity and its resistance to salt (Duke, 1983). According to Coïc and Coppenet (1989), the substrate coming from landfill is a substrate where it has a better production, thus this substrate assigned to the normal zone. Indeed, when an element is present, in as similable form, in very important quantity, the plant enriched in element production without the increasing of the dry matter (this is about luxury consumption). But, beyond a certain rate, the culture yield decline and plant death can occur in the case extremes (Salahadine, 2005).

Conclusion

The soil is the first link in the food chain; it plays an important role in restoring the biological quality of disturbed ecosystems for a long-term. It is a complex and multifunctional environment. Indeed, soil is the support of the human development and the most terrestrial ecosystems. It was the subject of a large number of researches.

Although the study of soil pollution by heavy metals, were recent, as it has been for a long time characterized by the phenomenon of self-purification. This study attempted to explain the consequences of the dump of a municipal landfill on the soil and on the plants due to the heavy metal's presence and if there is a possibility for organic waste valorization in the compost production.

Acknowledgement

The researchers would like to express their gratitude to sylvopastoral Institute of Tabarka (Lab of Earth Sciences and Environment) for their financial support and ICIME 2014 jury member for their help in the publication of this article.

Author's Contributions

Ouled seghaier Wided: Participated in experiments, data analysis and contributed to the writing of the manuscript.

Abbes Chaabane and Hasnaoui Brahim: Conceptualize and design the experiment, interpretation of the data and review and proof-read the manuscript.

Ethics

This article is original and contains unpublished material. All of the authors have read and approved the manuscript and no ethical issues involved.

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