THE PHYSIOLOGICAL AND CELLULAR STUDY OF TRITICUM TURGIDUM GROWN IN AMENDED SOIL WITH SEWAGE SLUDGE (URBAN AND INDUSTRIAL) IN OPEN FIELDS UNDER SEMI-ARID CLIMATE CONDITIONS

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Abstract: A field experiment was conducted to demonstrate the beneficial and negative aspects of sewage sludge application on wheat and to determine the way of heavy metals in the sludge-soil-plant system. Two types of sewage sludge (urban and industrial) were applied at different rates (5, 25, 50 and 100 t dry matter (DM) per ha). Results showed that growth and yield have been improved by sewage sludge use. However, sewage sludge applications modified the content of heavy metals in plant tissues especially for industrial sewage sludge and high rates where significant accumulations including Co, Cr and Pb, were detected mainly in the roots. This has been coupled by an alteration of the root cellular ultra-structure and the presence of heavy metal deposition as electron-dense granules and crystals.

Keywords: Urban and Industrial sewage sludge, Heavy Metals, cellular ultra-structure, Durum wheat.

1. INTRODUCTION

The increase in the volume of sludge generated by the treatment of water, linked to both demographic growth, urban expansion, and the expansion of industrial activities represent increasingly severe constraints for their storage and disposal. The use of sewage sludge in agriculture is an interesting alternative that is becoming more widespread. The disposal of sludge improves fertility and soil physical properties (Smith, 1992; Wolejko et al., 2014; Wu et al., 2012; Sharma et al., 2017; Bozkurt et al., 2020). However, the presence of several potentially harmful contaminants such as heavy metals, commonly found in sludge, limit their application. In order to avoid any problems of pollution and organic and inorganic contamination of natural resources, the application must be monitored and well controlled (McBride, 2003). Heavy metals such as Cd, Cu, Ni, Pb and Zn are commonly found in urban and industrial sludge (Dudka et al, 1996). In soil, these elements are often adsorbed by the organic material in an exchangeable or strongly attached to the status of complex shape by carbonates, Fe and Mg oxides and primary or secondary minerals (Adriano, 1986; Ross, 1994; Alonso et al., 2002). Plants absorb most of the nutrients from the soil solution and it is often assumed that the dissolved metals are readily available to organisms (Barbier, 1984) where the risk of contamination of the food chain (Pergent & Pergent-Martini 1999). Determining the content of the trace metals may provide useful information on the bioavailability and toxicity of these elements (Knight et al., 1998).

Besides the effects on the chemical composition of the plant, the trace metals, once absorbed, can cause anatomical and structural changes (Kupper, 2000; Vitioria, 2004; Bansal et al., 2014).

In this study, we examined the influence of sewage sludge on the growth and accumulation of trace metals in the plant and on the ultra-structure root of durum wheat (Triticum turgidum).

2. MATERIALS AND METHODS

2.1. Characteristics of Soil and Sewage Sludge

The experiment was conducted at the experimental station of Oued Souhil Nabeul located northeast of Tunisia, 60 km from Tunis. The soil is sandy plot, at basic pH (8.36). It is poor in organic matter (0.64%) and the carbon content is 0.34%. The levels of heavy metals are low, which makes it suitable for use in agriculture.

Urban sludge comes from the wastewater treatment plant of Korba. This sludge undergoes aerobic stabilization followed by drying on beds. The pH is approximately 6.7, slightly acidic. Urban sludge is rich in organic matter (66.62%) with high levels of nitrogen compounds (5.2%) and phosphorus contents (3.24%). The C/N ratio of between 8 and 10 indicates a good mineralization of the organic material. The levels of Zn, Cu, Pb and Ni and their sum are below the Tunisian standards (Table 1).

Table 1. Chemical contents (mg/kg) of Industrial sewage sludge, Urban sewage sludge and Tunisian standard values.

Contents	Industrial sludge	Urban sludge	Tunisian standard
pH	6.3	6.7	
MO %	57.9	66.6	
N %	4.3	5.2	
C %	31.9	39.0	
C/N	7.5	7.4	
Fe_2O_3 %	1.02	1.88	
MnO %	0.03	0.02	
MgO %	0.8	1.22	
CaO %	8.51	13.4	
P_2O_5 %	2.18	3.24	
Cd mg/kg	11	3	20
Co mg/kg	18	28	
Cu mg/kg	68	158	1000
Fe mg/kg	8300	10700	
Mn mg/kg	81	152	
Ni mg/kg	49	78	200
Pb mg/kg	577	63	800
Zn mg/kg	360	440	2000
Cr mg/kg	8030	155	500

The industrial sludge used is from the Bou Argoub wastewater treatment plant that hosts the industrial areas of the region. The sludge from this station has undergone aerobic stabilization followed by drying on beds. This sludge is rich (Table 1) in organic material (57.93%), N (4.3%) and P (2.0%). The Cr (11387 mg/kg), Pb (2.9 mg/kg) and Cd (163 mg/kg) concentrations are very high and exceeded the limits of the NT-106 Tunisian standard values.

2.2. Plant material

The experiment was conducted on using "Karim" durum wheat range. This is an herbaceous plant, which belongs to the monocot genus Triticum of the grass family. Moreover, the cultivation of durum wheat is generally associated with semi-arid with an average annual rainfall of about 300-400 mm (Srivastava, 1984; Daaloul, 1988). North African (Tunisia, Algeria, Morocco and Libya) culture of durum wheat extends over an area of about 305 million hectares.

The trial, conducted in randomized complete block, includes nine treatments, replicated four times. Two types of sludge (urban and industrial) and four doses of sludge (5, 25, 50 and 100 t/ha) are involved and compared with a control soil without any addition. In all, we have thirty-six basic plots of 10 m² each to application, the sludge was removed and was subjected to chemical analysis. The seeding was realized in December in line, with a density of 350 grains/m² and harvested in June.

2.3. Methods of analysis

Granulometric Laser Mastersizer 2000 carried out the particle size of the soil. Organic carbon was determined by the method of Kalra & Maynard (1991). The total nitrogen was determined by the Kjeldahl method. The samples of soil and sludge were digested with a mixture of HCl/HNO_3 (McGrath & Cunliffe, 1985) and the total concentrations were determined by emission spectroscopy in plasma torch (ICP-OES) using a HORIBA Jobin Yvon device type.

The different parts of the plant were dried at 80°C to constant weight and then ground to a fine powder using a porcelain mortar to prevent metal contamination. Digestion is done at high temperature (70°C) with aqua regia.

Histology is a term that refers to the study of the microscopic anatomy of tissues and cells. Proper histological sample preparation for transmission microscopy is essential for obtaining quality results from tissue samples. These samples were set at 4°C with a solution of 20.5% glutaraldehyde, pH maintained at 7.4 with a solution of sodium cacodylate (0.1M). The samples were then washed with sodium cacodylate buffer (0.1M) and post-fixed in a solution of 1% osmium tetroxide in veronal buffered (0.1M) (Sabatini et al., 1963). After several washes in distilled water, the samples were dehydrated with a graded ethanol series of increasing concentrations going from 30% to 100%. The final inclusions were made from a mixture of resin (Spurr, 1969). Only the sections with interference colors are gray or silver (thickness of 600 to 900A° (1A°=0.1nm)) were collected and deposited on a copper grid with 3 mm diameter. The ultrathin sections were mixed by the use of an alcoholic solution of uranyl acetate and 7% by 1% lead citrate (Reynolds, 1963). Observations were made using a Hitatchi H800 electron microscope.

The data was subjected to analysis of variance. The comparison of means at 5% level of significance was performed by the Newman-Keuls test using the Statistica 7 software.

As total, heavy metal quantity of sludge is poor indicator of metal availability for Triticum Turgidum plant uptake; accumulation factors were calculated based on metal availability and its uptake by a particular plant. A calculation for biological concentration factor (BCF) was as in equation 1, biological accumulation factor (BAF) as in equation 2, and transfer factor (TF) as equation 3.

BCF = metal content (mg/kg) in root / metal content (mg/kg) in sludge (1)

BAF = mean metal content (mg/kg) in shoot (root+straw+spike) / metal content (mg/kg) in sludge (2)

TF = mean metal Content (mg/kg) in shoot (root+straw+spike) / metal content (mg/kg) in root (3)

3. RESULTS

3.1. Effect of sludge on the growth of durum wheat

The incorporation of sludge into the soil has a positive influence on the growth and production of wheat. Indeed, there was an increase in production of the straw and corn for all treatments compared to the control sludge (Fig. 1). This is due to the wealth of sludge organic matter, nitrogen and trace elements. This increase is more pronounced with urban sludge. The application of sewage sludge treatment caused a significant increase in the total weight of grain produced per m² (Table 2). The number of spike and grain per m² is based on increasing doses and this regardless of the type of sludge. For the dose 100t/ha of sludge, the increase was 53 and 28 spikes for urban sludge and industrial sludge respectively. However, the average thousand grain weight (TGW) decreases. The increase in grain production is observed thanks to the increase of grain number and not to an increase in seed weight.

3.2. Determination of heavy metals mobility.

Whatever the type and dose of sludge, heavy metals have transfer factor (TF) less than 1. Heavy metals preferably store in the roots of wheat. BCF factor does not exceed 1 for all metals except Ni. Wheat has low BAC factors less than 1, which means a limited capacity of the accumulation of heavy metals.

3.3. Accumulation of trace metals in different parts of durum wheat

The analysis of the results showed that Co is virtually absents in the different parts of durum wheat no matter what dose or type of sludge added. The mean levels of Co of the aerial parts swing between 0.3 mg/kg and 0.1 mg/kg, while the contents in the roots are twice as high (Fig. 2). For both types of sludge, after applied doses, it resulted in an increase of the levels of Co in the roots, the aerial part and the glumes versus the control plant. Over the seeds, a significant increase was noted from 5t/ha for industrial sludge. However, for the urban sludge, only few traces of Co are detected in seed. The Cr contents increase only in roots (Fig. 2), essentially with the industrial sludge treatment with maximum level 50 ± 2.26 mg/kg with the highest dose of industrial sludge (100t/ha). The levels of Cr in wheat treated with urban sludge are similar to those of the soil control and no significant accumulation was recorded with increasing doses.

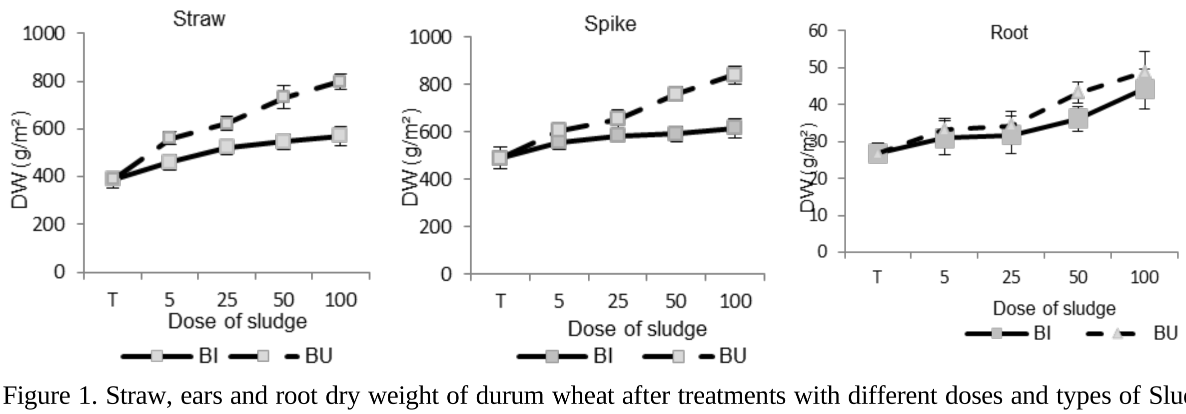

Figure 1. Straw, ears and root dry weight of durum wheat after treatments with different doses and types of Sludge. Each value represents the average of four separate repeats. T: soil control, BI: Industrial sewage sludge, BU: Urban sewage sludge.

Table 2. Indicator of metal availability for Triticum Turgidum wheat. Transfer Factor (TF), Biological Concentration Factor (BCF), and Biological Accumulation Factor (BAF). BI: Industrial sewage sludge, BU: Urban sewage sludge, T: soil control and four doses for each type of sludge (5, 25, 50 and 100 t/ha).

		T	5BI	25BI	50BI	100BI	5BU	25BU	50BU	100BU
	TF	0	0	0	0	0	0	0	0	0
Cd	BCF	0	0	0	0	0	0	0	0	0
	BAF	0	0	0	0	0	0	0	0	0
	TF	0.2	0.2	0.1	0.1	0.0	0.2	0.3	0.2	0.4
Cr	BCF	0.7	0.2	0.4	0.2	0.1	0.5	0.5	0.2	0.1
	BAF	0.12	0.03	0.04	0.02	0.00	0.10	0.12	0.04	0.04
	TF	0.1	0.8	0.9	0.4	0.2	0.6	0.4	0.9	0.9
Pb	BCF	0.0	0.0	0.0	0.1	0.1	0.0	0.0	0.0	0.0
	BAF	0.00	0.03	0.06	0.02	0.02	0.03	0.02	0.03	0.03
	TF	0.84	0.97	0.87	0.65	0.86	0.90	0.89	0.95	0.87
Ni	BCF	0.1	0.7	1.2	1.8	1.9	1.3	1.8	1.8	1.5
	BAF	0.12	1.04	1.04	1.16	1.66	1.13	1.57	1.94	1.55
	TF	0.70	0.56	0.65	0.80	0.73	0.76	0.71	0.82	0.59
Zn	BCF	0.9	0.7	0.6	0.5	0.4	0.8	0.7	0.6	0.4
	BAF	0.76	1.31	0.96	0.57	0.56	1.03	1.01	0.68	0.69
	TF	0.4	0.3	0.3	0.6	0.5	0.4	0.4	0.4	0.4
Co	BCF	0.0	0.3	0.3	0.3	0.3	1.4	0.3	0.2	0.3
	BAF	0.01	0.11	0.10	0.15	0.13	0.58	0.12	0.07	0.16
	TF	0.4	0.4	0.4	0.4	0.4	0.3	0.4	0.4	0.4
Cu	BCF	0.9	0.6	0.7	0.9	0.4	1.2	0.8	1.0	0.5
	BAF	0.77	0.22	0.29	0.36	0.15	0.40	0.33	0.37	0.21

The Cu contents are not modified in the aerial parts and wheat's glumes. However, for the roots, the levels of Cu increase with the addition of 5t/ha of sludge then remain constant. Over the seeds, the increase is more visible in the industrial sludge. The Pb contents is highly in the roots with doses 50t/ha and 100t/ha of the industrial sludge (Fig. 3) and in the seeds for both sludge with 50t/ha and 100t/ha doses treatments.

The Ni content is increased in the roots with the intake of 100t/ha industrial sludge. The same result is obtained with the aerial parts and seeds. No effects have been reported with other treatments. For Zn, no significant difference was recorded with the input of two types of sludge (Fig. 4).

The Fe contents decrease in the roots for the two types of sludge. This decrease is recorded from the dose of 5t/ha for industrial sludge with declines of 32%. For urban sludge, the Fe decrease was observed with the dose of 25t/ha at 24% (Table 3). As for Mn, there was a significant increase of this element when the dose is 5t/ha for industrial sludge and 25t/ha for urban sludge. The Ca contents undergo a significant increase from 5t/ha and 50 t/ha dose for industrial sludge and urban sludge. No effect was noted for Mg. Moreover, the levels of the Fe, Ca, Mg and Mn in the aerial parts and seeds remain constant regardless of the type or dose of sludge applied.

3.4. Ultra-structural observations

The various cuts made at the root of wheat show two distinct zones. Cortical area formed by the rhizoderme, cortical parenchyma and endoderm and a central zone containing the conducting tissue and marrow (fig. 5a). With the addition of 100 t/ha of industrial sludge (Fig. 5b and 5c) changes in the root structure appeared. We see a deformation of the cortex, which sometimes ends up disappearing. This causes an asymmetry and disruption at the root level. The deformations are by distance or destruction of parenchymal cells. In the central cylinder, we denote the gaps apparition the center, which may be formed following the degeneration of some vessels of the xylem. Following the hypertrophy of the middle lamella, we note the presence of intercellular spaces (Fig. 6c). Parenchymal cells have a thin plasma membrane, a dense cytoplasm, a nucleus in the center with one or two nucleoli with compaction and marginalization of nuclear chromatin and the cell walls are unaltered Eigen spaces. Changes of the structure in the roots of wheat appeared when we added 100 t/ha of industrial sludge (Fig. 6 and 7). Among the most dramatic changes, we noted that there was a

Table 3. Yield component in the Triticum Turgidium wheat. BI: Industrial sewage sludge, BU: Urban sewage sludge, T: soil control. Values of the same line with the same letter are not significantly different at $P < 0.05$ (Newman-Keuls test).

	Spike/m²	Number of Seeds /m²	Thousand grain weight (TGW) in grams	Total weight of grains (g/m²)
T	339a	8273a	41.7a	351.7a
5BI	3345a	11160b	36.8a	412.1ab
25BI	321a	11776bc	39.5a	462.9ab
50BI	370b	12786c	34.4a	431.8ab
100BI	368b	12526c	34.5a	430.8b
5BU	339a	9610b	40.5a	393.4a
25BU	355a	10696c	37.5ab	401.6a
50BU	406b	11810c	33.8b	392.5a
100BU	392b	12558d	32.6b	401.2a

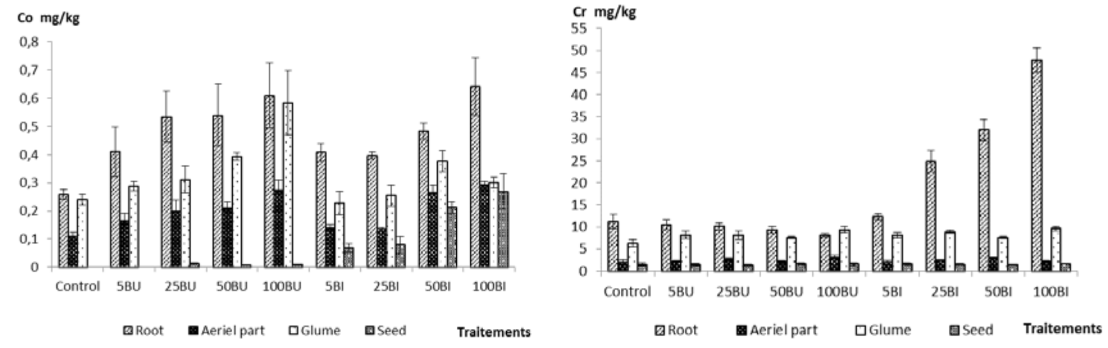

Figure 2. Variation of the accumulation of Co and Cr in different parts of durum wheat grown in the presence of increasing doses of urban and industrial sewage sludge.

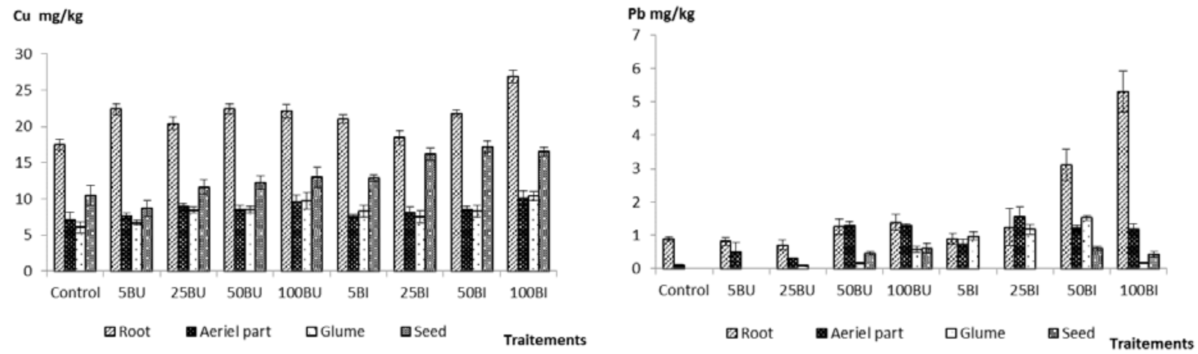

Figure 3. Variation of the accumulation of Cu and Pb in different parts of durum wheat grown in the presence of increasing doses of urban and industrial sewage sludge.

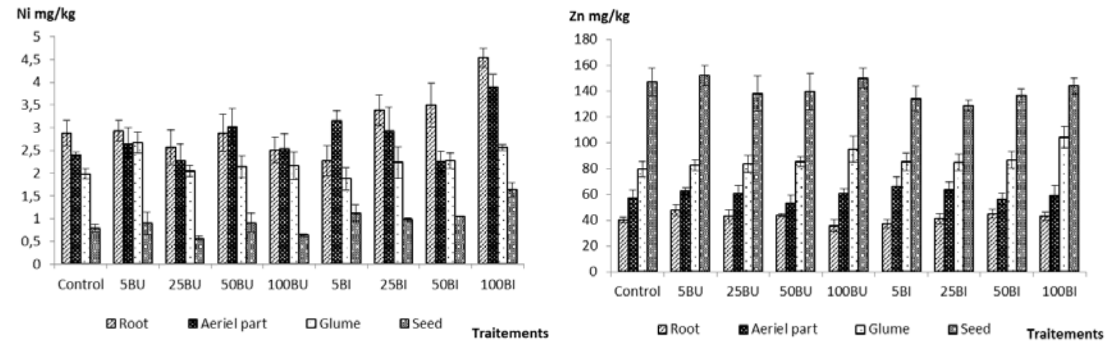

Figure 4. Variation of the accumulation of Ni and Zn (mg/kg contents) in different parts of durum wheat grown in different treatments (BU, BI) urban and industrial sewage sludge.

condensation of chromatin, which tended to be tacked onto the nuclear casing. The second morphological manifestations are situated at the wall where there has been a separation of the plasma membrane, to a plasmolysis and condensation of the cytoplasm. Deposition of heavy metals in the form of granules and dense electron crystals were observed next to vacuoles. We could also observe a defect in the structure of mitochondria.

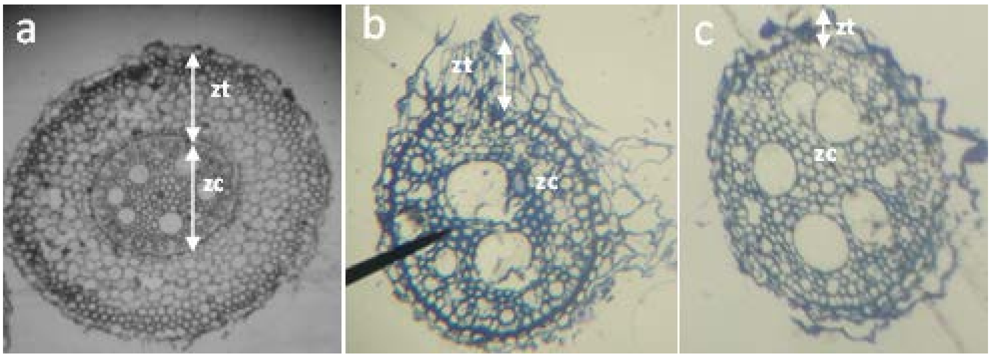

Figure 5. Cross-section of durum wheat root (magnification x 40). (a) Cortical zone (zt) and a central zone (zc) reduced the control wheat. (b and c) root crop with input of 100t/ha industrial sewage sludge showing deformations at the cortical zone.

Table 4. Effect of treatment (urban and industrial sewage sludge) on the composition of the Fe, Ca, Mg and Mn in different parts of durum wheat. Values of the same line with the same letter are not significantly different at P <0.05 (Newman-Keuls test).

	Root								
	T	5BU	25BU	50BU	100BU	5BI	25BI	50BI	100BI
Fe	634.3a	562a	482.3b	490.3b	466.3b	431.3b	452b	467b	408b
Mn	44.5a	53.5a	56.5a	56.5a	58.25b	55a	56a	62.75b	62.97b
Mg	49.75a	67.25a	66a	67.5a	67.75a	69a	51.75a	65.67a	63.33a
Ca	258.5a	278.25a	278.75a	296.25b	295.5b	348.5b	327.67b	395.5b	387.61b
	Aerial part								
	T	5BU	25BU	50BU	100BU	5BI	25BI	50BI	100BI
Fe	135.5a	148a	141.1a	154.5a	144a	123.5a	127.7a	134.5a	136.8a
Mn	18.5a	21.58a	23.5a	26.25a	20.5a	13.25a	16.75a	18.53a	20.75a
Mg	62a	50.5a	55.17a	65.75a	66.25a	62.5a	58.25a	51.25a	62.06a
Ca	170a	165.75a	167.69a	200.25a	219a	172a	151a	160a	168.44a
	Seed								
	T	5BU	25BU	50BU	100BU	5BI	25BI	50BI	100BI
Fe	44.5a	50.5a	49.5a	47.3a	45a	54a	52.3a	53.3a	57.8a
Mn	24.5a	28.5a	28.75a	29.25a	30.25a	21.75a	21.75a	22.25a	25.75a
Mg	46.75a	51.33a	47.75a	54a	52.75a	55.5a	59.25a	59.25a	54.33a
Ca	18a	18.75a	21.5a	22.25a	28.5a	15.25a	16.25a	21a	17a

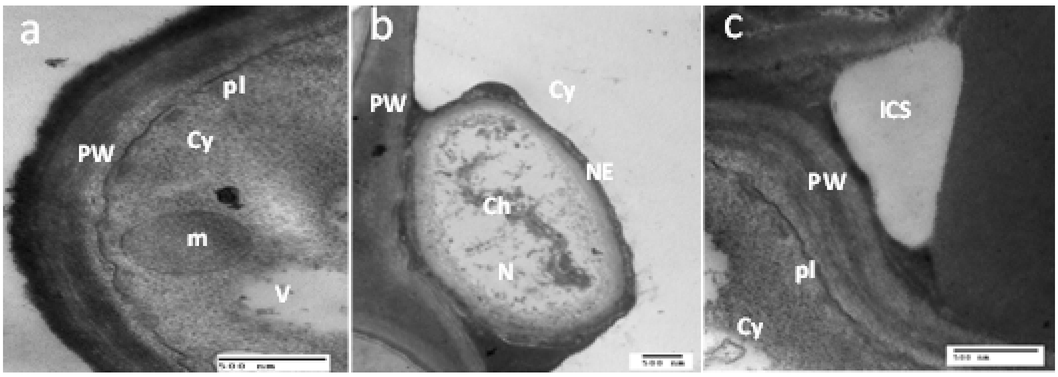

Figure 6. The micrographs produced by the transmission electron microscope showing the ultrastructure of root cortical cells (Triticumturgidum L.). a and b: cells root of control durum wheat and c: root cells of durum wheat with input of 100t / ha industrial sewage sludge, ICS: Intercellular spaces, NE: Nuclear envelope, C: cytoplasm, Ch: Chromatin, N: Nucleus, V: Vacuole, PW: Pectocellulose wall and pl: Plasmalemma.

The latter took a very distinctive appearance with swelling and loss peaks and their grouping into a pole. A matrix of the hernia and a rupture of the outer membrane were clearly observed. The cortical cells of the roots of control plants have a typical structure and ultrastructure. Parenchymal cells have a thin plasma membrane, a dense cytoplasm, mitochondria with a dense matrix (Fig. 6a), a core located with one or two nucleoli with a compaction and marginalization of the nuclear chromatin. The cell walls appear unaltered. Following gelation of the middle lamella, we observe the presence of intercellular spaces (Fig. 6c).

Among the most dramatic ultrastructural changes occurred with the application of sludge in most cortical cells, there is a chromatin condensation (Fig. 7a), which tends to append to the nuclear envelope, which is usually expanded by comparison with the control plants. Side events were observed at the cell wall where we witness a detachment of the plasma membrane (Fig. 7b) to a plasmolysis and condensation of the cytoplasm. We also noted the presence of small vesicles containing electron-dense granules formed by the plasma membrane. Pelleted metal deposition and electron dense crystals gradually accumulate in the vacuole and the cytoplasm. It is important to note vacuolization of the cytoplasm and the appearance of autophagosome (Fig. 7e and 7f), which is a type of cell death observed strongly morphological and whose main characteristic is the double or containing multi-membrane of electron-dense material.

If we compare the composition of total fatty acids for the two companions (Table 4), we note during the second application a considerable increase in oleic acid at 50t/ha dose. This acid went from 61.28% (soil control) to 73.33% (50t/ha bi treatment), an increase of 12%. The content of linoleic and linolenic acids in the oil undergoes a greater decrease under the cumulative effect. The highest dose of industrial sludge (100t/ha) resulted in a 52.64% decrease in linoleic acid and 39.39% in linolenic acid. These changes in fatty acid composition do not affect the nutritional quality of the oil. The fatty acid composition of total lipids, shows an increase (Fig. 2) in the percentage of mono-unsaturated fatty acids (oleic acid) at the expense of poly-unsaturated fatty acids) in the presence of sludge industrial. The increase of the acid at the expense of linoleic and linolenic acids can be explained by an alteration induced by the metal.

Regarding the action of sludge on the trace metal content of rapeseed, we noticed that the response of the plant differs according to the type of sludge spread, the amount of sludge added and Organelles can be seen in these structures as well as in the lysosome or vacuole with which the autophagosome merged. The dark spots in the autophagosome match the material from degradation in progress or already completed.

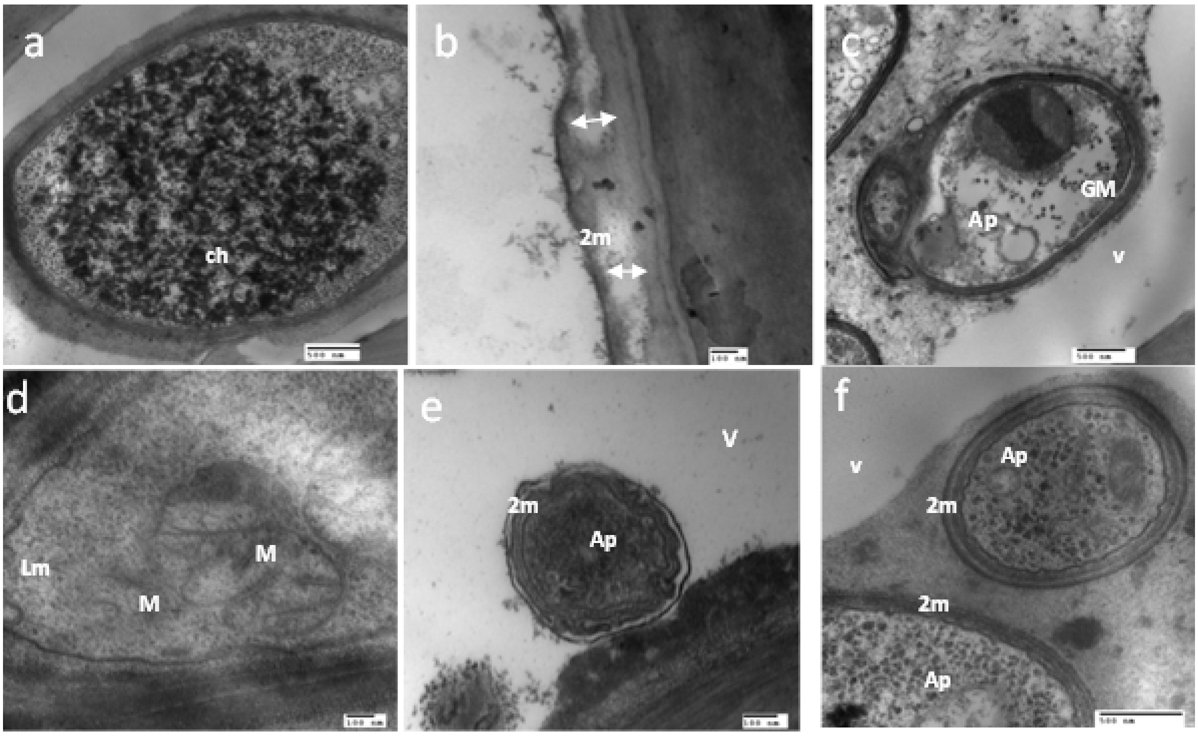

Figure 7. The micrographs produced by the transmission electron microscope showing the toxic effects of heavy metals brought by 100t/ha industrial sewage sludge on the ultrastructure of root cortical cells durum. En: nuclear envelope, Mc: cytoplasm membrane, C: cytoplasm, Ch: Chromatin, N: nucleus, V: Vacuole, M: mitochondria, Lm: lysosome membrane, 2m: double membrane of autophagosome (Ap).

We could also observe an irregularity in the structure of mitochondria with swelling and loss crests (Fig. 7d) and their grouping into a pole. Similarly, we have seen much of the matrix hernia and rupture of the outer membrane. Thus, acute exposure to high levels of antioxidants, particularly the presence of Ca, promotes permeabilization of the mitochondria, the uncoupling of oxidative phosphorylation with drastic consequences for energy production and contributes to cell death by channels of necrosis and / or apoptosis (Aguilar et al., 2005).

4. DISCUSSION AND CONCLUSION

Depending on the applied dose, the sewage sludge can maintain soil fertility and cover, in part or in whole, the crop needs in N, P, Mg, and Ca (Menbraham et al., 2003; Ponnier et al., 1965; Dissonnais et al., 1996; Lassoued et al., 2014). The use of sewage sludge appears as an attractive alternative to increase production (Rejeb et al., 2003; Menbraham et al., 2003; Metahri et al., 2009) and enrich the soil with P and organic matter (Fartinez et al., 2003).

In this study, the contribution of increasing doses of sludge from 5t/ha up to 100t/ha acted favorably on the production of durum wheat. The number of grains per square meter is the highest number obtained with the dose of 100t/ha where it reaches 12558 grains/m². Similar results were found by Dridi et al., (2000) who have observed a positive effect of the application of sewage sludge on the dry solids' production of durum wheat.

Most metals brought by industrial sewage sludge remains trapped in the roots of wheat, which is an advantage point in terms of ecotoxicological view, since the transfer of metals to the aerial parts is an undesirable property since accumulated heavy metals can enter in the food chain through herbivores.

In roots, we found an increase in Ca related to increased doses of sludge, unlike Fe for which we recorded a decrease. Cd is also not absorbed by the roots even in the presence of high doses of industrial sludge. Studies in pots (Anderson & Christensen, 1988; Jarvis et al., 1976) have shown that the increase of Ca in the medium leads to a decrease in the removal of Cd, suggesting competition between Ca and Cd at the root surface. Similarly, competition between Cd and Zn is often observed (Haghiri 1973; Jarvis et al., 1976; Bingham et al., 1979). This competition would result in a decrease in the influx of Cd in the presence of Zn (Costa & Morel, 1994). An inhibitory effect of Mn overlooked the Cd has also been shown in nutrient solution (Jarvis et al., 1976). According to Cataldo et al., (1983), Costa & Morel (1994), the essential divalent cations such as Ca, Mn, Zn are competitors with regard to the toxic metals such as Cd and increasing their concentrations in the solution decreases the absorption of toxic metals.

The presence of Cr in large quantities in industrial sludge of Bou Argoub could explain the observed accumulation in the roots of wheat grown in the presence of sludge compared to the control plants and compared with urban sludge element. Cr is considered toxic to plants in its oxidation states Cr (III) and Cr (VI) (Mortvelt & Giordano, 1975; Bartlett et al., 1979), the form of the Cr (VI) is considered the most toxic that to say up to 10-100 times more (Katz, & Salem, 1994; Kotas & Stasicka, 2000). Symptoms of Cr toxicity have often been described for plants growing on a nutrient solution enriched with Cr or soil amended with sludge (Pratt, 1966; Foroughi et al., 1976; Joshi et al., 1999).

Wheat shows its ability to immobilize heavy metal absorption and accumulation in roots, and by adsorption or precipitation on the roots in the rhizosphere. The addition of urban sludge (pH 6.5) and industrial sludge (pH 6.33) necessarily involves a change in soil control pH (pH 8.36 before the addition of sludge) and therefore a change in the root zone. This area is characterized by intense biological activity and can modify the physico-chemical conditions of the soil. The assimilation of metals in the roots is done by redox or by enzymatic complexation according to the level of stress of the plant due to the presence of heavy metals. The bioavailability of certain metals such as Cd is very sensitive to changes of soil pH around the roots.

The changes in the chemical composition of the particular wheat roots has been accompanied by an alteration of the cellular ultra-structure root and the presence of heavy metal deposition in the form of granules and electron dense crystals. The same alterations in the ultra-structure have been often recounted by several authors. However, these experiments were usually held on a single metal and not on the combined effect of several metals. Thus, the toxicity of Cr across the cell was observed by several authors (Sayato et al., 1980; Yamamoto & Wada, 1981; Chatterjee & Chatterjee; 2000, Han et al., 2004). Once in the cell, Cr is found in the cytosol and in high dose can cause several ultra-structural changes. These changes are characterized by deposits of electron dense material in the cell walls, inlays surrounded by membranes within vacuoles, disintegration of organelles and high vacuolization in the cytoplasm. According to Liu & Kottke (2003), the highest amounts of Cr accumulate mainly in cell walls and in the vacuoles of the fourth or fifth cortical layer.

According to Dauda et al., (2009), the increase of Cd in the medium induces several functional and ultra-structural changes and causes senescence effects of transgenic cotton cultivars. For Liu & Kottke (2004) and Aravind & Prasad (2005), the accumulation of Cd within the tissue may cause an increase in the number of vacuoles and their enlargement, an increase of the nucleoli, and condensation of cytoplasm, chromatin, a reduction of mitochondrial cristae, plasmolysis, disruption of the structure and chloroplast disruption of nuclear casing, plasma membrane and the mitochondrial membranes. A decrease in the mitotic index, pyknosis (shrinking nucleus), chromosomal aberrations, impaired synthesis of RNA and a slowdown of ribonuclease have been observed on various crops growing in contaminated environments by Cd (Liu & Kottke, 2003). Although it is an essential trace element, excess of Cu also has a cytotoxic effect, which is manifested by a decrease in the mitotic index of root meristem cells (Yildiz et al., 2009). This item binds predominantly in the roots of plants (Brun et al., 2001; Sela et al., 1988.), especially at cell walls (Sela et al., 1988). Cd and Cu can be genotoxic and cause visible chromosomal aberrations during mitosis (Souguir et al., 2008; Yildiz et al., 2009). It was also reported that the increase in the concentration of Pb in cells leads to a series of ultra-structural changes (Wierzbicka, 1987; Islam, 2008). According to Sharma & Dubey (2005), even in small amounts, Pb produces a negative effect on the physiology of the plant. These transformations are more or less similar to those of Cr and Cd. For Pb, the major part accumulated in the roots, is localized in the insoluble fraction of the cell wall and nucleus. This accumulation is generally accompanied by a strong cytoplasmic vacuolization, deposits of electron dense granules in the cytoplasmic and mitochondrial membranes, swelling and loss of mitochondrial crests, vacuolation endoplasmic reticulum and dictyosomes (Wusheng & Donghua, 2010).

All these findings are consistent with the ultra-structural changes that were observed in the roots of wheat grown in the presence of high doses of industrial sludge. This could be attributed to the combined trace metals accumulated in the plant and effects including Cr and Pb brought in large quantities by industrial sludge.

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