Evaluation of the accumulation of some heavy metals in the red beetroot (Beta vulgaris L.) grown on two different soils and irrigated by wastewater from the SETP Bouznika (Morocco)

D. Ez-zarhouny*, E.M. Hbaiz, M. Lebkiri, S. Elanza, A. Lebkiri, E.H.Rifi, N. Habbadi

Abstract — The present work aims at evaluating the metal contamination of the red beet (Beta vulgaris L.), irrigated by wastewater treated by wheat straw of Bouznika STEP (Morocco) and cultured on two different soils, one of Maâmora and the other of Bouznika. The results of the ICP-AES analysis showed that the increasing intake of raw sewage causes a significant accumulation of metals in different organs of the plant. However, irrigation with treated wastewater (EUT) allowed a reduction of the concentrations of various metals especially those of lead, nickel and chromium. So, with 100% of treated wastewater, the concentration of lead rose from 5,88ppm to 3,1ppm in the soil of Bouznika.The statistical study by principal component analysis (PCA) revealed the existence of correlation between wastewater intake and the metallic levels in the plant's organs.

Keywords: treated Wastewater, straw, soil, beetroot, heavy metals, STEP, PCA, ICP - AES.

1 Introduction

The are as of the two cities of Bouznika and Kenitra (Morocco) are favorable areas for intensive agriculture by their regular and abundant rainfall (> 600 ml / year). Therefore, the high costs associated with the use of fertilizers, have led some farmers to use wastewater in irrigation, especially those used for vegetable crops. The wastewater used in domestic and industrial irrigation is rich in organic matter and nutrients (P, K, N ...) [1]. However, they contain undesirable chemical elements which can have high levels of heavy metals such as (Cd, Cu, Cr, Ni, Pb, Zn,..) [2,3]. These elements accumulate in the soil and according to the biogeochemical conditions by various phenomena (adsorption, absorption...) [4]. The metals conveyed by the wastewater are absorbed by the [5,6] plants, and may possibly contaminate them.

In this work, we are interested in the beetroot plant cultured in two different soils irrigated firstly by increasing inputs of wastewater (0%, 25%, 50%, 75% , and100%) and secondly by the wastewater treated by the wheat straw (50% and 100%). At first, we carried out a preliminary analysis of soil and water of irrigation. The metal concentrations in the different organs of the plant (leaf and hypocotyl) were determined by the method of Inductively Coupled Plasma-/ Atomic- Emission Spectrometry (ICP-AES).

In a second step, we applied a statistical method to all the obtained results called the principal component analysis (PCA) in order to highlight the presence or absence of a correlation between variables.

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2 MATERIALS AND METHODS

2-1 MATERIAL

2-1-1 Irrigation water

Three types of water are used in this test: The First is drinking water from the city of Kenitra, the Second is the waste water from the treating station (WWTP) of raw wastewater from the city of Bouznika (Morocco) and the third is wastewater treated by wheat straw.

The collection of raw wastewater from the (WWTP) was performed at one go in April 2014. The physicochemical analysis of the water that has a dual domestic and industrial wastewater shows that these are characterized by a slightly acidic pH (6.82) and rich in nutrients [7].

The preparation of the wastewater sample intended for analysis in the ICP-AES is carried out as follows: a sample of 10 ml of wastewater, without prior calcination, was taken up in 10 ml of 50% hydrofluoric acid and dried again in a Teflon beaker in a sand bath. The obtained residue is supplemented with distilled water till 20ml.

Table 1 shows the content of the metallic elements studied in raw wastewater of the Bouznika EPST with the admitted standards.

Table 1: wastewater content of the Bouznika EPST of heavy metals and the standards recommended in ppm

Metals	Cd	Cr	Cu	Ni	Pb	Zn
Wastewater metal content of the STEP (ppm)	0,01	0,07	14,7	9	38,5	86
Maximal recommended content (ppm) [8]	0,01	1	2	2	5	2

The obtained results show high concentrations for Cu, Ni, Pb and Zn. These values are superior to standard values recognized by the United Nations for Food and Agriculture [8].

2-1-2 Wastewater Treatment by wheat straw

The presence of ligands [9] in the wheat straw gives it a high possibility of substituting metal ions in thewastewater.
Wheat straw was crushed and sieved, then washed with ethanol and finally dried in an oven. The extraction of metal cations by straw is performed by a mass matrix m = 1kg priorly introduced into a wastewater tank 50litre .Thus the obtained treated wastewaters were re-used in the irrigation of beetroot. The results were compared with those of raw wastewater.

2-1-3 Ground

The used soil samples come from two different regions, one of Kenitra in the Gharb region and the other one of Bouznika in the plain of Chaouia. These samples were taken in April 2014 from a depth of 0 to 30 centimeters.
The analysis of the different physicochemical parameters of both soils showed that they have different textures. Indeed, the soil of Maamora has a sandy texture of a loose structure, while the soil of Bouznika is isohumic, hydromorphic, calcimanesic and has a silty clay texture [7].
Table 2 shows the concentrations of heavy metals in ppm in Maâmora and Bouznika soil with the admitted standards [10].

Table 2: Concentration of metals in ppm in the two soils of Maâmoraand Bouznika

Métal	Cd	Cr	Cu	Ni	Pb	Zn
Maâmora Soil	0,3	6,3	2,8	33	31	76
Bouznika Soil	0,3	8,4	10	36	32	79
NFU44041 AFNOR norm(1985) [10]	2	150	100	50	100	300

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The results of the analysis show that the contents of heavy metals (Cd, Cr, Cu, Ni, Pb and Zn) of Bouznika soil are higher than those of Maâmora but remain below the acceptable standards. This can be explained by the difference in physicochemical properties of each soil (texture, clay percentage, pH, cation exchange capacity (CEC), organic matter content, etc.).

2-1-4 Vegetable material

To study the effect of wastewater on the bioaccumulation of heavy metals in market garden plants, we realized a culture of: red beetroot (Beta vulgaris L), whose hypocotyl grows in the ground.

2-2 Methods

2-2-1 Culture Conditions

Disinfected and dried red beet seeds, were germinated in pots disinfected by chlorine bleach at 10% and containing Maâmora and Bouznika soil prepared beforehand.
The irrigation is performed with increasing percentages in waste water (0%, 25%, 50%, 75% and 100%) and with the wastewater inputs (50% and100%) treated by wheat straw.

2-2-2 Sampling

After 15 weeks of cultivation, harvesting was performed. The plants are collected in dry weather with their roots, hypocotyls and leaves. They are freed from coarse earth elements adherents before being separately packaged in sealed plastic bags and pre-rinsed with distilled water and stored at 4 ° C [11 - 13].

2-2-3 Sample preparation and analysis

The collected plants are washed extensively with distilled water (to remove fine soil particles remaining glued). The different parts of the plant (leaves and hypocotyl) are separated and dried in an oven at a temperature of 75 ° C for 72 hours [14].
The resulting sample is crushed using a grinder so as to obtain a fine powder [14].
The mineralization of the samples is performed in a dry way by which a quantity of 1 g of each samples are placed in a muffle oven and heated gradually to 500 ° C and thus maintained for 4 hours. The organic material is combusted. The residue consists of the mineral ash after the dissolution, by aqua regia according to the method of Tauzin J & C Fair (1986)[15].
The dosage is performed by ICP-AES method (Inductively Coupled Plasma-/ Atomic- Emission Spectrometry). The used emission spectrometer is made available to us by CNRST (National Center of Scientific and Technical Research) which is Jobin Yvon Ultima type 2.

3 Results and Discussion

3-1 Effect of wastewater on the bioaccumulation of heavy metals in the organs of the beet grown on both Maâmora (SM) and Bouznika (SB) soils.

In this part, we studied the evolution of the transfer of heavy metals in the organs of the beet, grown on two different soils and irrigated by increasing concentrations of raw wastewater.
The quantities of metals in the two bodies of the beet, irrigated by increasing concentrations of wastewater are reported in Table 3 (**see below**).
Analysis of the various results illustrated in Table 3 shows that the metal bioaccumulation varies with the body of the plant, the nature of the element and the wastewater concentration in both soils.
Cadmium (Cd): The figure 1 represents the contents of the CD in the organs of red beetroot irrigated with increasing percentages of waste water.

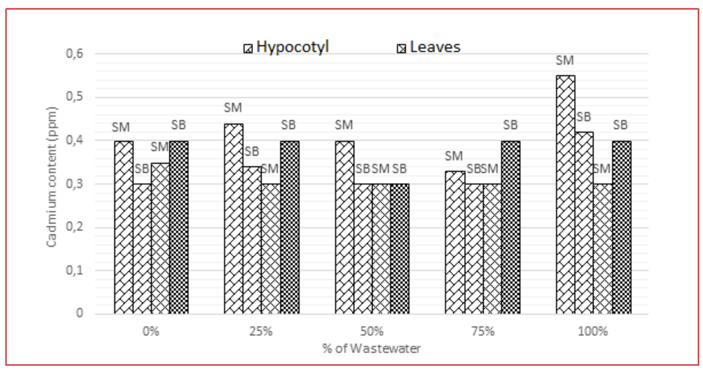

Fig 1: Content of cadmium in different organs of beetroot on two floors (Maâmora and Bouznika) versus % of wastewater.

The graph shows the presence of cadmium in the two bodies of beetroot but at low concentrations. For both soils irrigated with growing percentages of wastewater, the content of this metal in the different organs of the plant remains almost constant. The highest values of bioaccumulated Cadmium are obtained in the hypocotyl with 100% of waste water 0,42 ppm in Bouznika soil and 0,55ppm in Maâmora soil. These values remain below the toxic threshold set at 2ppm. [16].

In addition, the contents of cadmium in the hypocotyl of the crop in Maâmora soil are superior to those recorded in Bouznika soil. In contrast, the values of Cd in the leaves of the plant grown in Bouznika soil are superior to those recorded in Maâmora soil.

Chrome (Cr): The figure2 represents the contents of Cr in the organs of the irrigated beetroot with increasing percentages of waste water.

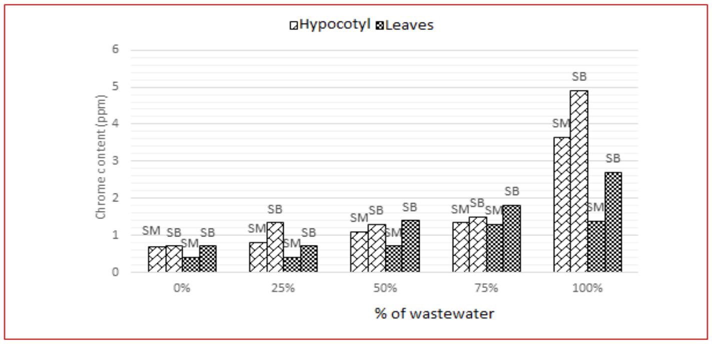

Fig2: chromium content in different organs of beetroot in two soils (Maâmora and Bouznika) according to % of wastewater.

The above graph shows that chromium is accumulated in different organs of the plant. It is noted that the values obtained for the Bouznika soil are higher than those found in the Maâmora soil. This can be attributed to the difference in texture of the two soils. The content of the studied metal elements increases linearly with the addition of waste water for both soils. Maxima are recorded in the hypocotyl in which 3,65ppm and 4,91ppm are respectively present in Maâmora and Bouznika soils for pots completely irrigated by raw waste water. We notice that this element accumulates in underground parts of the plant including the hypocotyl where there was a peak of 4,91ppm which slightly exceeds the threshold of toxicity (4ppm) [16]. Previous studies on pepper [7] and cucumber [17] showed that chromium has mobility towards the roots of the plant.

Copper (Cu): The figure 3 represents the contents of Cu in the organs of beetroot irrigated with increasing percentages of waste water.

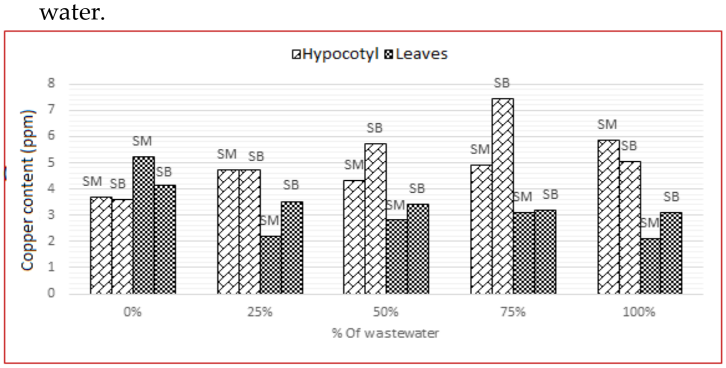

Fig3: Copper content in different organs of beetroot on two soils (Maâmora (Bouznika) according to % of wastewater.

Analysis of the results shows the bioaccumulation of copper at both organs, it varies according tothe increasing contribution of waste water irrigating the two soils. The obtained values for the hypocotyl pass from 3,7ppm to 5,87ppm in Maâmora soil and 3,6ppm to 7,44ppm in Bouznika soil. Thus the contents stored in the leaves are smaller in both soils. It should be noticed that the accumulation of copper is made preferentially in the underground part (the hypocotyl) where the diffusion of this metal is easy in both soils .This result can be explained by the abundance of copper in soil and wastewater

Nickel: The figure 4 represents the contents of Ni in the organs of beetroot irrigated with increasing percentages of wastewater obtaining any security clearances.

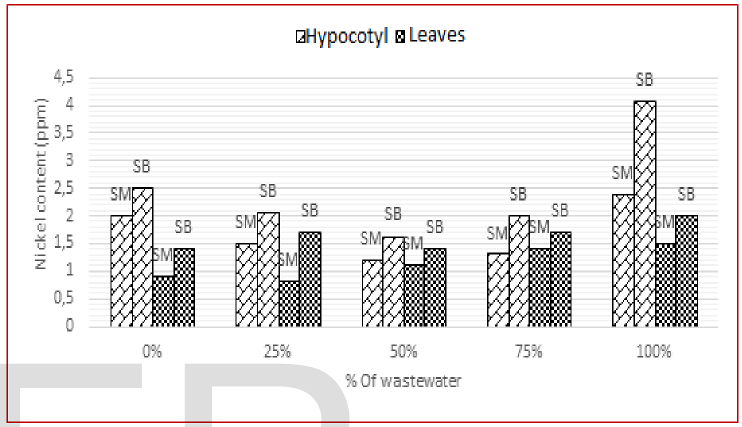

Fig4: Nickel content in different organs of beetroot on two floors (Maâmora Bouznika) versus% of wastewater**.**

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Concerning its bioaccumulation, the results show that this element is concentrated in the underground part of the plant and the increase of the wastewater fraction increases the nickel content in the different organs. We also notice that the contents of Ni in the hypocotyls and leaves of the plant cultivated in the soil of Bouznika are higher than those obtained in the soil of Maâmora. However, these levels remain below the toxicity limits [16].

Lead: The figure 5 represents the contents of Pb in the organs of beetroot irrigated with increasing percentages of wastewater.

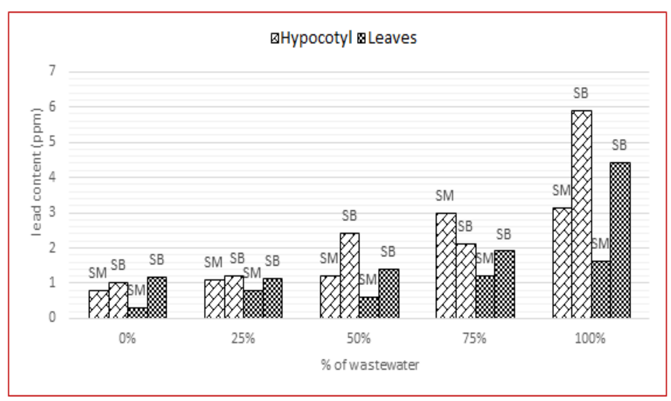

Fig 5: Content of Lead in different organs of beetroot in the two soils (Maâmora / Bouznika) according to % of wastewater.

The data of the graph show that the accumulation of lead in the two bodies is influenced by the waste water concentration. The

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maximum levels for lead are recorded in the hypocotyl of the irrigated plant with 100% of wastewater in both soils. They are of the order of 5,88ppm for Bouznika soil and 3.12 ppm for Maâmora soil. Thus, bioaccumulation is also maximal in leaves with an irrigation of 100% of waste water. The recorded values in the leaves in Bouznika and Maâmora soils are respectively 4,4ppm and 1.6 ppm. We also notice that the contents of this metal dosed by ICP in the two organs of the plant cultivated in the soil of Bouznika are greater than their analogues in the soil of Maâmora except for the irrigated sample with 75% of wastewater. We find that the plant is not contaminated by this metal during this culture.

Zinc: figure 6 represents Zn levels in the bodies of beetroot irrigated with increasing percentages of waste water.

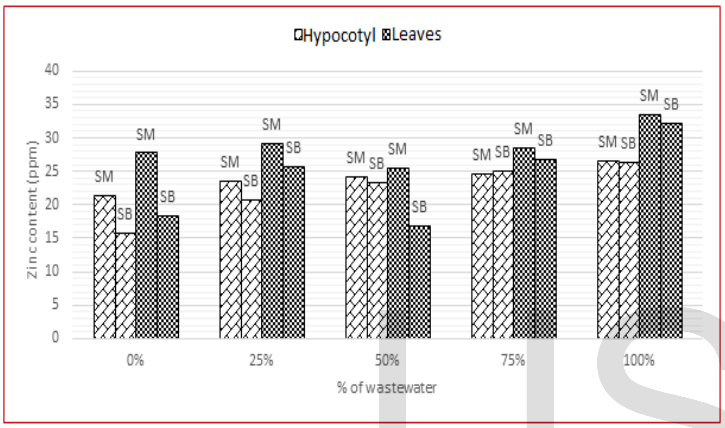

Fig 6: Zinc content in different organs of beetroot in two soils (Maâmora Bouznika) according to % of wastewater.

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The accumulation of zinc increases according to the increasing concentration of waste water in both soils. The maximum levels for hypocotyl were reached at 26.41 ppm for a culture amended by 100% of wastewater in Bouznika soil and 26.6 ppm in Maâmora soil with a contribution of 100% of wastewater.We also notice that the mobility of zinc is higher in the bodies of the beet grown in the soil of Maâmora where we recorded the highest levels in comparison with the one obtained in the soil of Bouznika. This result is confirmed by previous works on cucumber [17].

3-2 Effects of wastewater treatment by wheat straw on bioaccumulation of metallic elements

The quantities of metals in both plant organs irrigated by increasing concentrations of raw wastewater and treated wastewater grown in Bouznika soils are reported in Table 4.

Table 4: metal content in both organs of beet depending on the concentration of EU and EUT grown in Bouznika soil

SB	%EU EUT	Hypocotyl EU	Hypocotyl EUT	Leaf EU	Leaf EUT
Cd	50%	0,3	0,29	0,3	0,3
	100%	0,42	0,3	0,4	0,35
Cr	50%	1,3	0,6	1,4	0,2
	100%	4,91	2,2	2,7	0,4
Cu	50%	5,7	5,5	3,4	2,5
	100%	5,02	4,9	3,1	2,5
Ni	50%	1,6	1,27	1,4	0,8
	100%	4,06	2,9	2,5	1,8
Pb	50%	2,4	1,07	1,4	1
	100%	5,88	3,1	4,4	1,3
Zn	50%	23,3	22,8	16,8	5,2
	100%	26,41	19,8	32,2	16,5

The quantities of metals in both organs of beetroot, irrigated by increasing concentrations of waste water and treated waste water grown in Maâmora soil, are reported in Table 5.

Table 5: metal content in both organs of beet depending on the concentration of EU and EUT grown in Maâmora soil

SM	%EU EUT	Hypocotyl EU	Hypocotyl EUT	Leaf EU	Leaf EUT
Cd	50%	0,4	0,35	0,3	0,28
	100%	0,55	0,49	0,3	0,3
Cr	50%	1,1	1,02	0,7	0,4
	100%	3,65	1,75	1,37	0,53
Cu	50%	4,3	3,64	2,8	2,6
	100%	5,87	4,95	2,1	1,4
Ni	50%	1,2	1,2	1,1	1
	100%	2,38	2,08	1,5	0,9
Pb	50%	1,2	1,15	0,6	0,5
	100%	3,12	2,05	1,6	1,4
Zn	50%	24,1	23,78	25,5	18,7
	100%	26,6	25,78	33,4	25,6

The concentrations of the various studied metals all suffered a decrease in concentration after the treatment of wastewater by the wheat straw in the plant. Indeed, in the soil of Bouznika, the concentrations of various metals decrease especially those of chromium and lead. After the treatment, the chromium presents the most important change, for example at 100% in the hypocotyl, it goes from 4,91ppm (EU) to 2,2ppm (EUT) and also for the lead in hypocotyl at 50% rising from 2, 4ppm (EU) to1, 07ppm (EUT).
Similarly, the studied contents of metals in Maâmora soil decrease as in the case of 100% irrigation (EUT), the concentrations of Chrome fall to 53%. They rise from 1,37 ppm to 0,53 ppm in leaves and from 3,65ppm to 1,75 ppm in the hypocotyl.

3-3 Statistical analysis of results

All the collected data was analyzed by PCR, a method which has allowed us to develop 3 factorial axes explaining the studied phenomenon. Thus, one must interpret the meaning of each physicochemical axis.

3-3-1factor axes C1, C2 and C3

As shown in Table 6, the axis C1 constitutes 63.636% of the

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explained inertia, the axis C2 represents 22.406% and the axis C3 9.718% for a total of 95.759% of the available inertia. The latter value is high enough to explain the studied phenomenon.

Table 6: The total variance explained

component	proper Initial values			sums extracted from square loads	
	Total	% of the variance	% Cumulative	Total	% of the variance
1	3,818	63,636	63,636	3,818	63,636
2	1,344	22,406	86,042	1,344	22,406
3	0,583	9,718	95,759		
4	0,155	2,588	98,347		
5	0,072	1,195	99,542		
6	0,027	0,458	100,000		

Table 7: Percentage of different variables in the construction of two main axes C1 and C2

Variable		Cr	Ni	Zn	Pb	Cd	Cu
Component	1	0,963	0,913	0,721	0,699	0,127	0,318
	2	- 0,106	0,269	0,631	0,695	-0,895	0,712

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3-3-2 Interpretation of factorial plans C1xC2.

The projection of the variables and measurements are shown respectively in Figures 7 and 8.

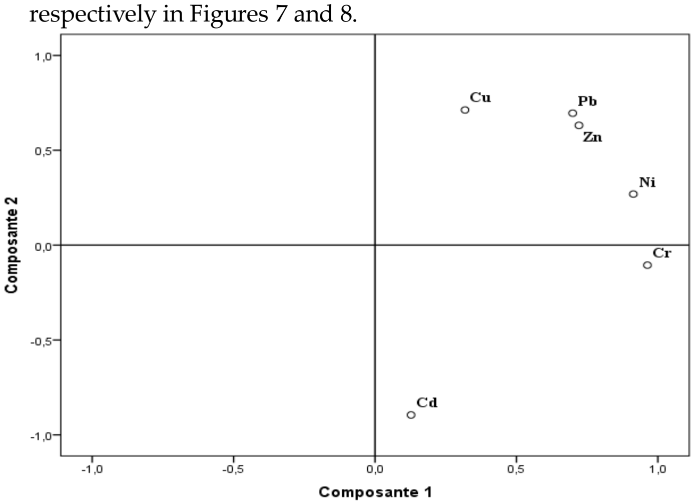

Figure 7: Projection of variables on the factorial plan C1xC2

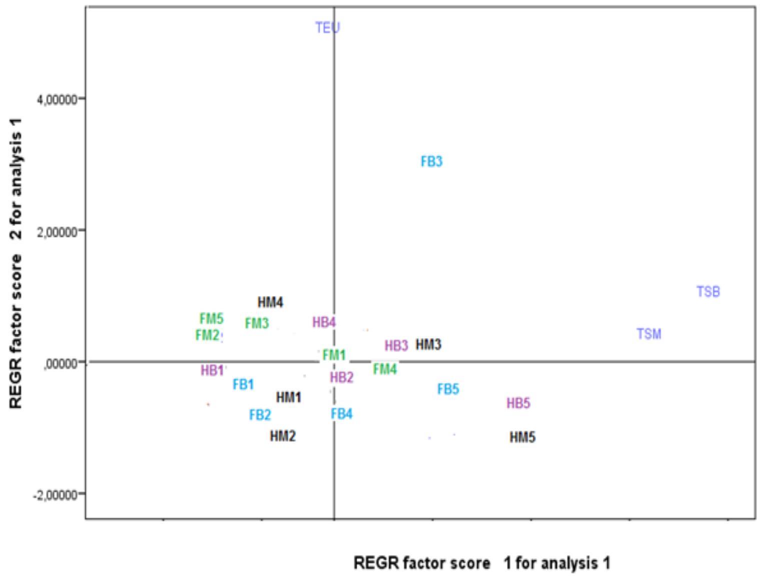

Figure 8: Projection of measurements on the factorial plan C1xC2

Figures 7 and 8, show that the variables and axes are not well represented in all circles of correlations. There are two types of variables:

- Variables that contribute significantly to the formation of the C1 axis, are: Cr, Ni, Pb and Zn.
- Cadmium and cooper are the variables that contribute to the formation of the axis C2
-The axis C3 is not correlated to any variable.

Taking into account the contribution of each variable considered, we deduced the following:

C1: an increasing gradient of Cr, Ni, Pb and Zn.

C 2: a decreasing gradient of Cd and an increasing gradient of Cu.

Case of Maâmora soil

The concentrations in hypocotyl of Cr, Ni, Pb and Zn are more increased in HM3 (50%, Inc) and HM5 (100%, Inc) than in HM1, HM2 and HM4 which respectively correspond to the results of irrigation with 0%, 25% and 75% of waste water. The contents of these elements in remain weak at the level of leaves. It is important in the case of FM4 which corresponds to the irrigation with 75% of wastewater. For Cd and Cu, their accumulation in leaves is not influenced by the percentage of wastewater. But in the hypocotyls, the concentration of Cd is higher and that of Cu.

Case of Bouznika soil:

The concentration of 4 elements (Cr, Ni, Pb and Zn) increase with the increasing of raw wastewater in the plant organs.

The concentration of Cd and Cu remain moderate in the hypocotyl regardless of the percentage of wastewater. By contrast, they are slightly lower in the leaves except for the case of irrigation with 50% of wastewater where the accumulation of Cu is high.

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4 Conclusion

In this work, we studied the effect of raw and treated wastewater of a treatment wastewater station (Bouznika - Morocco) on the bioaccumulation of some traces of metals (Cd, Cr, Cu, Ni, Pb and Zn) in the hypocotyl and the leaves of red beet grown in two soils of different textures and composition (Bouznika and Maâmora soils). The results of spectroscopic analysis (ICP-AES) have revealed:

-the presence of heavy metals in the raw wastewater and in the two soils studied.

-The irrigation of beetroot by the wastewater leads to a bioaccumulation of metals in their bodies without exceeding the recommended standards, except for chrome in which there was a spike of 4,91 ppm in the hypocotyl which slightly exceeds the toxicity threshold (4ppm).

- The irrigation by the water which is treated by wheat straw reduces the accumulation of different metals studied in the hypocotyl and leaves knowing that the content of chromium in the hypocotyl goes from 4,91ppm when the plant is irrigated by dose of 100% of wastewater to 2,2 ppm in the irrigation by the wastewater treated by wheat straw, hence the importance of a pretreatment before the use of wastewater.

-The treatment of results by PCA reveals the existence of correlation between the percentage of waste water of irrigation and the accumulation of metallic elements in plant organs, influencing the mobility of these elements.

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Table 3: Effect of wastewater on the accumulation of heavy metals (in ppm) in the cultivated beet in both Maâmora and Bouznika soils

		HYPOCOTYL				LEAF					
	E%	**0%**	**25%**	**50%**	**75%**	**100%**	**0%**	**25%**	**50%**	**75%**	**100%**
Cd	**SM**	0,4	0,44	0,4	0,33	0,55	0,35	0,3	0,3	0,3	0,3
	SB	0,3	0,4	0,3	0,3	0,42	0,4	0,4	0,3	0,4	0,4
Cr	**SM**	0,7	0,8	1,1	1,33	3,65	0,4	0,4	0,7	1,3	1,37
	SB	0,7	1,33	1,3	1,5	4,91	0,7	0,7	1,4	1,8	2,7
Cu	**SM**	3,7	4,74	4,3	4,9	5,87	5,2	2,2	2,8	3,1	2,1
	SB	3,6	4,7	5,7	7,44	5,02	4,11	3,5	3,4	3,2	3,1
Ni	**SM**	2	1,5	1,2	1,3	2,38	0,9	0,8	1,1	1,4	1,5
	SB	2,5	2,07	1,6	2	4,06	1,41	1,7	1,4	1,7	2,5
Pb	**SM**	0,8	1,1	1,2	2,96	3,12	0,3	0,8	0,6	1,2	1,6
	SB	1	1,2	2,4	2,11	5,88	1,17	0,9	1,4	1,9	4,4
Zn	**SM**	21,3	23,5	24,1	24,6	26,6	27,7	29,1	25,5	28,5	33,4
	SB	15,7	20,7	23,3	25	26,41	18,35	25,7	26,8	26,8	32,2

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