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THE BIOACCUMULATION OF TRACE ELEMENTS IN SUGAR BEET (BETA VULGARIS) AND SOIL

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Abstract

This study assessed the bioaccumulation of metallic elements in sugar beet which is irrigated by the surface waters of Beht and Sebou rivers. The study focused on four supports*: the bark, the pulp, molasses (raw sugar) and the leaves. Seven metal elements (Cd, Co, Cr, Cu, Ni, Pb and Zn) were analyzed by spectrometry emission coupled plasma (ICP). The obtained results showed that the levels of Cd in irrigating water exceeds the thresholds set by Moroccan standards. The concentrations of Cd and Cr in soil (0-15, 15-30cm) are higher than those set by the AFNOR and the contents of Cd, Cr, Cu and Ni in* the supports of the beet *exceed normal levels which are established by Kabata-Pendias and Mukherjee.* Multivariate analysis (*PCA) of* average values of heavy metal says that the increase of variable induces the growth of the other, that is to say, *there is* a positive interaction between the elements for the physicochemical conditions of irrigation waters and agricultural soil collected. *The obvious bioaccumulation of metallic elements which are identified in sugar beet are normally caused by the use of water surfaces of Beht and Sebou as sources of irrigation.*

***Keyword**: Morocco; Sebou and Beht River; Metallic elements; Irrigation water; Sugar beet; Soils.*

Introduction

The cultivation of sugar beet is mainly localized in the irrigated areas of Lokkos, Doukkala, Tadla, Moulouya and Gharb. The latter, located northwest of Morocco, has about 30% of sugar beet surfaces and provides 25.5% of the whole production which is 763.712 tons. However, in recent years, a reduction was observed in yields. The latter went from 49.5 tons per hectare in 1999 to 45.5 tons in 2002 while the area under cultivation increased from 13 679 to 16 963 hectare.

Among the water resources which are threatened is the Sebou basin and Beht (rushes of the left bank of the Sebou river). Sebou is the most important river (watershed) in Morocco which measures 6.6.109m^3/year. It is a source of irrigation water supplying the national agricultural area of nearly 267.600 hectares and containing 30% of the national total resources that are currently and highly threatened [1]. Beht is a course of sustainable and relatively powerful water; it originates from the Middle Atlas and its average annual inflows are of the order of 410 million m^3 [2].

The two basins are given a load of metal elements exceeding 140 tons/year originating mainly from artisanal industries estimated at no less than 2000 units [3]; therefore, any

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pollution drained along this stream is transferred to the soil through irrigation water and subsequently in cultures and finally to man. The purpose of this study is to evaluate the average contents of seven metal elements (Cd, Co, Cr, Cu, Ni, Pb and Zn) in sugar beet (leaves, bark, pulp and molasses) grown in soils irrigated by surface water Sebou and Beht.

Experimental part

Study site

The two rural communes (Mograne and Sidi Allal Tazi) develop various cultures, mainly based on sugar crops, forage crops and cereals [4]. The samples (water, soil and crops) were collected from four areas located at 27km (rural commune of Mograne) and 44km (rural commune of Sidi Allal Tazi) of the province of Kenitra. The sampling points are located on figure 1.

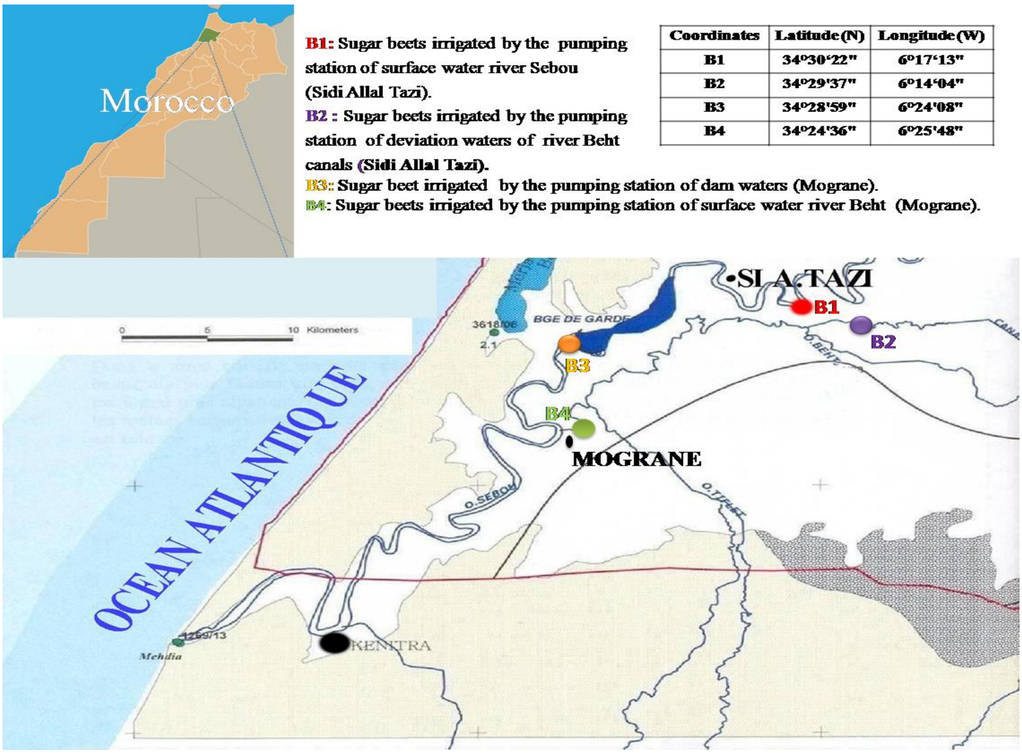

Fig. 1. Location of study sites on Sebou and Beht rivers

Soil sampling

The floor is collected through five sampling points with the aid of a helical auger at two different depths (0 - 15 and 15 - 30cm). The same soil of the two different depths is mixed, put into a plastic bag and then transported to the laboratory. The soil was dried in the open air and sieved to 2mm to remove unwanted materials such as rocks and stones.

Collection of cultures

The collected sugar beet (five points on average in a zone) of each sampling zone (B_1, B_2, B_3 and B_4) was put into a clean plastic bag and labeled to be conveyed to the laboratory where they are washed and rinsed with tap water. The supports of the same kind belonging to a similar area were mixed and subdivided to obtain four test samples from each sampling point.

Preparation of molasses (raw sugar) and beet pulp

After washing the sugar beet, its pieces were colected from each of them to be peeled and grated in cossette. The samples consisting of 140g of these mixed cosettes were placed in a saucepan with 200mL of distilled water and a few drops of hydrochloric acid. The mixture was brought to gentle boiling for 15 minutes then filtered to obtain the sweet juice of beet (filtrate or molasses) and the filtered solid (beet pulp). The concentration of the juice was performed by carrying on the gentle boiling again until a thick syrup (saturated sugar solution). The start of the crystallization was done by adding a pinch of powdered sugar while maintaining the heating [5].

Ground mineralization

An amount of 0.5 to 1.0g of soil was calcined in a muffle furnace at 450°C for 2 hours. Each sample, previously ground, was placed in a Teflon beaker and added to 10mL of hydrofluoric acid (HF) to 50% to be re-dried on a sand bath to dryness. The obtained residue is taken up by a hot mixture of hydrochloric and nitric acid (7.5 and 2.5mL) in order to be concentrated until its complete dissolution. The suspension obtained after the decantation is transferred to a 50mL vial. It is then calibrated with distilled water and then homogenized. In the presence of refractory compounds and/or high concentrations of silica, a residue will always remain. The silica can be eliminated by repeating the first step for a second time (HF-HCl) [6].

Mineralization of water and molasses (raw sugar)

A sample of 10mL of water (the mineralization of molasses is the same as that of water by dissolving between 4 and 6g of molasses in 10mL of distilled water), without any prior calcination, has been taken up by 10mL of hydrofluoric acid (HF) to 50% and dried again in a Teflon beaker in a sand bath. Dissolving the obtained residue is affected by the addition of 7.5mL of hydrochloric acid and 2.5mL of pure nitric acid. The beaker is covered with a watch glass and placed on a hotplate until disappearance of red vapors synonymous with a complete mineralization. The resulting solution is made up to 10mL with distilled water. Whites of mineralization were conducted jointly [6].

Mineralization of plant material

A test sample of 1 to 2g of vegetable, which are dried at 70°C for 48 hours and crushed, was calcined in a muffle oven at 450°C for 4 hours. The obtained ash is mineralized by aqua regia (HNO_3 25% and 75% HCl) then reduced to dryness on a sand bath until the complete discoloration of the solution. The obtained residue is redissolved in 10mL of HCl (5%), then filtered to 0.45 microns, before diluted with HCl (5%) to the final volume of 20mL [6].

The analyzes of the physico-chemical parameters of soil and irrigation water were conducted in the laboratory of the regional office of agricultural development of Gharb and the determination of metal fractions (Chromium, Cobalt, Copper, Zinc, Lead, Cadmium and Nickel) were read in ICP-MS (Ultima 2) at the National Center of Scientific and Technical Research (Rabat) [7-10].

The metal concentrations of the conversion formula from mg/l to mg/kg for solid materials is given as follows:

$$C_{ech}\ (mg/kg) = C_{ech}\ (mg/L) \times V_{minéralisation}\ (L)/\text{dry mass of the test portion (kg)} \quad (1)$$

with: C_{ech} (mg/kg): The final concentration of metal in mg/kg; C_{ech} (mg/L): The final concentration of metal in mg/L; $V_{minéralisation}$: The volume of the sample after the mineralization in liters.

Dry test sample mass: The mass of the sample dried before calcination.

Results and discussions

Analysis of irrigation water

The results of the pH measures and contents of the obtained metal fractions are shown in Table 1.

Table 1. pH and metallic characteristics (mg/L) of irrigation water

Parameters	B_1	B_2	B_3	B_4	Verages	Foroccan standards for irrigation
pH	7.95	7.22	7.91	7.76	7.71	6.5-8.5
Cd	0.042	0.037	0.058	0.049	0.042	0.01
Co	0.034	0.032	0.066	0.076	0.052	0.5
Cr	0.218	0.232	0.411	0.698	0.389	1
Cu	0.117	0.088	0.211	0.184	0.150	2
Ni	0.132	0.088	0.214	0.323	0.189	2
Pb	0.296	0.175	0.438	0.389	0.324	5
Zn	0.556	0.119	0.311	0.410	0.349	2

Soil analysis

Table 2 shows the physicochemical characteristics of the metal and solid supports of soil.

Table 2. The physico-chemical and metalic characteristics (mg/kg) of soil from different study areas

Parameters	B_1	B_2	B_3	B_4	Averages	Standards	
pH	8.07	8.52	8.23	8.21	8.25	-	
Clay (%)	40.26	48.2	48.68	41.18	44.58	-	
Fine silt (%)	31.02	27.95	28.73	35.64	30.83	-	
Coarse silt (%)	16.46	16.98	25.03	22.26	20.18	-	
Fine sand (%)	12.89	4.55	0.72	0.90	4.76	-	
Coarse sand (%)	1.93	0.81	0.41	0.63	0.94	-	
CEC	30.5	35.5	35.5	32.5	33.5	-	
O.M rate (%)	1.25	2.10	2.10	1.67	1.78	-	
						AFNOR	**Agricultural soils**
Depth (0-15):							
Cd	10.76	7.87	8.9	7.2	9.31	2	1-300
Co	20.12	17.4	27.2	13.5	19.55	30	20-50
Cr	450	321.2	366.85	391.7	382.43	150	50-200
Cu	40.3	24.4	42.25	48.5	38.86	100	60-150
Ni	42.79	33.77	46.65	38.05	40.31	50	20-60
Pb	16.39	20.87	36.25	36.55	27.51	100	20-300
Zn	114.4	86.93	133.3	91.7	106.58	300	1-300
Depth (15-30):							
Cd	9.34	9.2	8 .55	8.15	8.89	2	1-300
Co	21.93	20.61	29.25	18.1	22.47	30	20-50
Cr	604.3	523	190.3	364.35	420.48	150	50-200
Cu	47.7	46.74	46.7	44.8	46.48	100	60-150
Ni	43.6	46.25	45.5	37.25	43.15	50	20-60
Pb	13.95	9.66	34.65	20.25	19.62	100	20-300
Zn	130.2	103.64	104.78	108.6	111.80	300	1-300

The study areas are characterized by a basic pH soil, low organic matter content, a high cationic exchange capacity (CEC) and a grain size of silty clay with variations of clay percentages of a sampling point to another. The results of the analyzes show that metalic contents of Cd and Cr in soil (0-15 and 15-30cm) exceeds the thresholds set by AFNOR, which is explained by the excess in urban wastes (industrial, craft activities), the agricultural activities, the chemical forms ofmetalinthe soil matrix, and the physico-chemical characteristics of the soil.

Analysis of sugar beet

The results of the metal fractions which are contained in the different samples of the harvested beet through the studied surfaces were summarized in table 3.

The transformation of sugar beet generally causes a reduction of the levels of certain trace elements [10]. In fact, the purification of sugar juice of the sugar beet (molasses) by lime and carbonate in the laboratory has been neglected because this operation is likely to result in reducing the metal contents which will hinder the distinction of the modes of transfer of the latter (sequestration at the root wall, diffusion into the cells of the chair of sugar beet migration and the leaves) of different metals based on their distribution in % in sugar beet. Therefore, some studies showed that the industrial white sugar contains between 0 and 20% of the metallic elements contained in the raw sugar [11].

Table 3. Metal levels (mg/kg) in the supports of sugar beet

Media	B_1	B_2	B_3	B_4	Averages	Kabata et Pendias* (2007) [9]	N.E.U**
Pulp :							
Cd	0.583	0.719	0.423	0.492	0.554	0.005–0.04	1
Co	0.177	0.222	0.232	0.198	0.207	0.005–0.27	-
Cr	0.750	1.417	0.465	0.869	0.875	0.01–0.41	-
Cu	4.760	6.719	7.047	6.020	6.136	3.00–8.00	-
Ni	2.802	4.952	4.878	3.120	3.938	0.06–1.30	-
Pb	2.135	8.846	3.227	2.010	4.054	0.20–2.40	10
Zn	11.510	16.285	11.481	15.424	13.67	1.20–27.00	-
Leaf :							
Cd	0.581	0.584	0.494	0.564	0.555	0.005–0.04	1
Co	0.295	0.303	0.272	0.245	0.270	0.005–0.27	-
Cr	1.438	0.820	0.454	0.907	0.904	0.01–0.41	-
Cu	4.867	4.946	7.111	7.926	6.212	3.00–8.00	-
Ni	4.479	3.091	3.636	4.159	3.841	0.06–1.30	-
Pb	2.897	3.946	3.353	2.159	3.088	0.20–2.40	10
Zn	19.234	14.041	14.535	9.791	14.400	1.20–27.00	-
Bark:							
Cd	0.600	0.708	0.533	0.655	0.624	0.005–0.04	1
Co	0.442	0.458	0.586	0.655	0.535	0.005–0.27	-
Cr	2.157	2.052	1.586	3.408	2.300	0.01–0.41	-
Cu	4.747	5.875	8.693	8.032	6.836	3.00–8.00	-
Ni	4.010	2.968	6.733	4.494	4.551	0.06–1.30	-
Pb	3.715	3.864	3.28	4.365	3.806	0.20–2.40	10
Zn	15.789	15.947	10.8	17.096	14.908	1.20–27.00	-
Molasses:							
Cd	0.540	0.300	0.316	0.370	0.381	0.005–0.04	1
Co	0.410	0.175	0.241	0.090	0.229	0.005–0.27	-
Cr	2.750	2.916	2.141	2.180	2.496	0.01–0.41	-
Cu	2.420	3.058	2.333	1.960	2.442	3.00–8.00	-
Ni	2.310	2.541	1.491	1.780	2.030	0.06–1.30	-
Pb	2.450	2.010	2.000	1.180	1.910	0.20–2.40	10
Zn	4.700	8.625	3.641	3.420	5.096	1.20–27.00	-

(*): Average levels of the metallic elements in agricultural crops sown in normal soil.
(**): European standards of foods intended for consumption by livestock.

Our study showed that the levels of trace elements in commercial pulp and white sugar are higher than those found by Von Steinle, 1977. Table 4 shows these results.

The results of the metal fractions, which are contained in the various media sugar beet (Table 2) and collected through the studied surfaces, were converted to% in order to understand the mobility of heavy metals of the root zone (pulp + molasses + bark) to the aerial part (leaves).

Copper

For the studied sugar beet, it is found that the roots hold most of the trace elements previously taken (62 to 90%), which explains that the contents are often higher than those in the aerial parts as shown in figure 2 (10 to 38%). This fluctuation of the concentrations depends on the nature of the metal. In fact, the transition from one organ to another is done through transporters that regulate the migration of the element and the chemical mechanisms (complexation with organic acids, sugars, phenols and peptides, precipitation) that facilitate the movement or reduce it [12]. A portion of the trace elements remains immobilized in the root cells and another part flows from cell to another in order to move to another part of the plant [13].

Table 4. Levels of trace elements in industrial pulp and white sugar in mg/kg

Trace elements	Cd	Co	Cr	Cu	Ni	Pb	Zn
Commercial Pulp	0.1	0.3	4.05	17.51	1.21	1.62	8.3
White sugar	0.1	0.1	1.5	0.6	0.8	1.6	1.8
Pressed pulp (Steinle Von, 1977) [10]	0.08	-	-	-	-	1.24	-
White sugar (Steinle Von, 1977) [10]	0.003-0.004	0.05-0.08	0.001-0.05	0.1-2.4	0.04-0.08	0.09-0.1	0.001-0.2

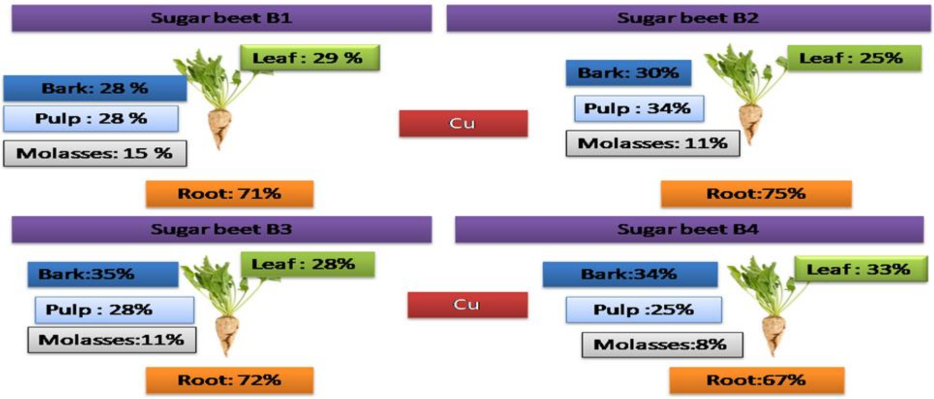

Fig. 2. Percentage distribution of copper in sugar beet

The distribution (in%) between the roots and the leaves for trace elements analyzed in this study confirms that for a silty clay soil, the aggregate number of metals is observed at the root zone.

Cadmium

Cadmium is not an essential nutrient and can have toxic effects at low concentrations. Under normal conditions, plants absorb only small amounts of soil's Cd. The ratio between Cd and Zn in plant tissue is considered to be biologically significant [14].

Regarding the current study, the Cd contents in the pulp (0.554), leaves (0.555), bark (0.624) and molasses (0.381) from sugar beet have exceeded the normal level set by *Kabata and Pendias* (0.005 to 0.04mg/kg)**.** The circulation of Cd in the sugar beet begins from the wall (shell) to the root cells then it is trapped by organic acids or other molecule, with limited mobility to the aerial part.

Cobalt

Cobalt is also an important micro-nutrient for some bacteria fixing nitrogen such as *Rhizobium*, therefore it is essential for many vegetables [14].

In this study, the average concentrations of Co in the different supports of the sugarbeet does not exceedthe normal level fixed by *Kabata and Pendias* (0.005 to 0.27mg/kg) (Table 3) with the except of the bark. Up to 50% of the content remains immobilized in the walls of the root cell.

Chromium

Although both forms of Cr (Cr(III) and Cr(VI)) may be toxic to plants and animals, the toxicity of Cr(III) occurs at higher concentrations and this shape is in fact a nutrient essential to humans and other animals. On the other hand, the Cr(VI) is toxic at much lower concentrations and tends to be more mobile and more bioavailable than Cr(III) in the environment [9, 14].

In this study, the contents of Cr (total) in the pulp (0.875), leaves (0.904), bark (2.300) and molasses (2.496) of sugar beets are higher than the maximum concentration of Cr typically associated with plants which are grown in a normal unaffected soil (0.1 to 0.5mg/kg) but below the toxic level (5mg/kg) for plants which are reported by *Kabata-Pendias and Mukherjee* (Table 3). Given the current conditions of the study area, the Cr is likely to be present mainly in the form $Cr(OH)_3$ which is usually inaccessible to plants.

Lead

Lead is a toxic substance known for animals and plants and it is very persistent in the environment. As Cd, it is not an essential nutrient for plants that can accumulate Pb soil and atmosphere, especially in highly industrialized areas. The plants which are grown under non-contaminated sites have relatively low levels of metals including Pb [9]. The average concentrations of Pb in food plants which are cultivated in several countries vary between 0.2 and 2.4mg/kg.

The measured average concentration of Pb in the different samples like pulp (4.054), leaves (3.088) and bark (3.806) of sugar beet are beyond normal values given by *Kabata-Pendias and Mukherjee* (0.2 to 2.4 mg/kg) with the except of the molasses (Table 3). The soil in the study area is slightly alkaline and has a clayish structure, therefore the bioavailability of Pb to the plants may be limited. The Pb shows the same behavior of Cd, that is to say, a complexation with the molecules of the root that reflects the low transfer to the leaves.

The metallic elements of Cu, Ni and Zn, which are not toxic to humans and animals, record the greatest mobility to the aerial part. These metal cations are transported under the following chemical forms [15]:

- The Zn as chelated form of organic acids.
- The Cu as complex form of amino acids.
- The Ni as a complex form Ni-peptide.

Copper

Copper is an essential element as it is involved in a number of physiological processes. In excess, the absorbed copper may be considered as a toxic element which leads to the inhibition of growth. The excess of this metal can also damage the cell membrane and suppression of enzymatic activities [16]. For most crops, the critical level for copper toxicity in the leaves is above 20-30mg/kg. Copper concentrations in maize samples showed significant variability, on the contrary, in mint it tends to be similar in all the samples [9]. The bioavailability of copper is significantly reduced when the pH exceeds 7. The increase in the pH of a soil solution, which can be accomplished by liming, can lead to the formation of hydrolysis products that have different affinities to the exchanging sites. In soils which are characterized by pH> 7 and high concentration of $CaCO_3$, copper hydrolyzed species can be more important for the absorption of metals [9, 14].

In this study, the mean levels of copper in the pulp (6.136) samples, leaves (6.212), and bark (6.836) of sugar beet are above the tolerable level (5mg/kg) for the cultivated plants, but they do not exceed the toxic levels (20mg/kg) (Table 3). In the collected soils, the pH values were above 7 and the concentrations of $CaCO_3$ were high. The hydrolyzed species of copper, such as $CuOH^+$, $Cu(OH)_4^{2-}$, $Cu(OH)_3^-$ and $Cu(CO_3)_2^{2-}$, may be more favorable for the plant absorption. The interactions between Ca and Cu may affect the bioavailability of copper [9].

Nickel

Since nickel is ubiquitous in the environment, it is a normal constituent of plant tissues. Many natural plant species accumulate relatively high levels of Ni in their tissues; although, as for many traces element, the concentrations in various plant tissues are affected by soil properties and the form in which the Ni is. *D.C. Adriano, J.T. Weber* [17] showed that the concentrations of nickel in the field of natural vegetation and crops vary from 0.05 to 5mg/kg. The average concentrations of Ni in food plants cultivated in various countries are reported to vary from 0.06 to 1.3mg/kg.

The average of Ni concentrations measured in this study in the different supports of sugar beet parts, like pulp (3.938), leaves (3.841), bark (4.551) and molasses (2.030) exceeds the normal values given by *Kabata-Pendias and Mukherjee* (0.06 to 1.3mg/kg) (Table 3).

Organic matter and its state of degradation are key to the transfer of soil-plant nickel, because the cation exchange capacity is an important factor in the chemistry of nickel.

Zinc

Zinc is an essential nutrient for plants assuming some essential metabolic functions including the molecular structure of enzymes. Although the concentrations of Zn in plants vary, their concentrations in some food and forage plants are fairly stable. The range of mean concentrations of zinc in food plants is from 1.2 to 27mg/kg. The tolerable daily intake of Zn for humans was fixed by WHO/EU (in 1983) between 0.30 and 1.0mg/kg (body weight). In plant tissues, zinc concentration is higher in the roots, followed by leaves, branches, and finally the main trunk or stem [9].

In this study, the average levels of zinc in samples of sugar beet (pulp (13.67), leaves (14.40), bark (14.903) and molasses (5.096)) are in the range given by *Kabata-Pendias and Mukherjee* (1.2 to 27mg/kg). The proportion of zinc complex in the soil solution increases with pH (5 to 90%). Removable zinc decreases with increasing the pH. Zinc also makes insoluble complexes with organic materials [18, 19]. The minimum zinc concentration in solution is between pH = 7 and pH = 8. The Zn concentrations in the different samples of the beets are higher relative to other elements (Fig. 3) which may have been due to a combination of factors including the interaction with P.

Statistical analysis

The examination of metal contents was conducted by the Principal Component Analysis (PCA) and this method was carried out through SPSS statistics 20 software.

Proper Values

It can be seen from the graphical representation of the propre values (Fig. 3) which indicate the amount of information represented by each axis; that axis 1 (93%) and 2 (4%) contain 97% of the variables, hence their adoption for the representation of results.

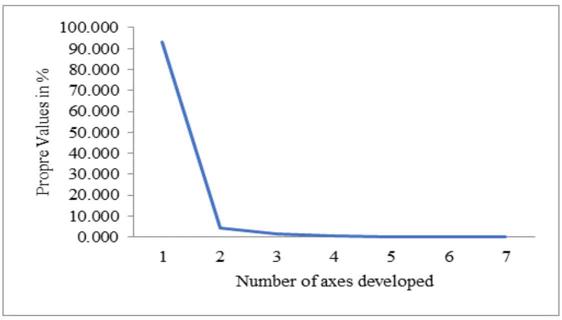

Fig. 3. Change in values along the axes.

Correlations of metal elements

The factorial map after rotation (Fig. 4) has identified the type of correlation between metal concentrations measured in this study in order to assimilate mathematically the influence of changes in variables on their interactions.

All metal parts are highly correlated on axis 1 in contrast to the axis 2 which shows some low correlations (<0.5). So, this observation shows that the increase of a variable induces the growth of the other, that is to say there is a positive interaction between the elements for physico-chemical conditions of irrigation waters and agricultural soils collected from fields of sugar beet at the Gharb area (rural municipalities: Mograne and Sidi Allal Tazi).

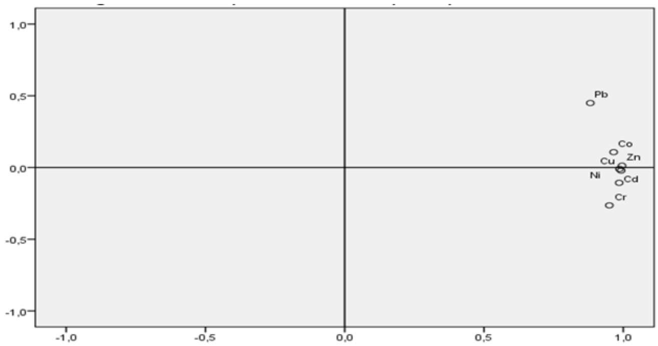

Fig. 4. Diagram of components in space after rotation (Component diagram in space after rotation)

Conclusions

In this study, we studied the bioaccumulation of metallic elements in sugar crops (beet) irrigated by waters of pumping stations of Sebou and Beht rivers. The obtained results showed that the levels of Cd of irrigation water exceed the thresholds set by Moroccan standards, the concentrations of Cd and Cr in soil (0-15, 15-30cm) are higher than those set by the AFNOR and contents of Cd, Cr, Cu and Ni in the supports of the beet exceed normal levels established by *Kabata-Pendias and Mukherjee.* Multivariate analysis (PCA) of average values of heavy metal says that the increase of variable induces the growth of the other, that is tosay, there is a positive interaction between the elements for the physico-chemical conditions of irrigation waters and agricultural soil collected. The high absorption of certain trace elements by the beet tissue may be associated with the chemical forms of metal in the soil matrix, with the physico-chemical characteristics of the latter and to then to the nature of the plant species themselves. The contents of metal elements in the roots (62 to 90%) are higher than those in the aerial parts (10 to 38%). Thanks to its high content of pectin, sugar beet is able to trap divalent ions (trace elements) in the following order of specificity: $Zn^{2+} >> Cu^{2+} >> Ni^{2+} \sim Pb^{2+} > Cr^{2+} > Cd^{2+} \sim Co^{2+}$.

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