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THE EFFECT OF HEAVY METALS ON PLANT COMMUNITIES DISTRIBUTION IN AN ABANDONEND MINING AREA (NORTHEAST-ALGERIA)

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Abstract: We investigated the correlation between vascular flora and edaphic parameters [pH (water), total nitrogen (TN), organic matter (OM), carbon/nitrogen (C/N), cation exchange capacity (CEC), cadmium (Cd), lead (Pb), zinc (Zn) and copper (Cu)] in the area of a former lead and zinc mine (Northeast-Algeria). The floristic and soil data were the subject of one-way variance analysis and canonical correspondence analysis (CCA). These statistical analyses highlighted five plant groupings. A grouping bound to cadmium and copper, a grouping connected to zinc, a grouping linked to lead and two other groupings seem independent from heavy metal contents. The results show a strong bioindicative value of identified plant communities on metallic pollution of the studied mining zone.

Key-words. plant communities, soil, heavy metals, mining area, canonical correspondence analysis.

1. INTRODUCTION

The presence of heavy metals in soils allows the installation only of a limited number of species supporting their toxicity (Antonovics, 1971). The toxicity of heavy metals operates a very elaborate selection by elimination of numerous species which under other conditions would grow in these places (Ernst et al., 1992). According to Remon et al., (2009) the presence of toxic heavy metals, if it does not still constitute the dominant factor of the determinism of the plant communities, still represents a limiting factor which forces the plant to adapt. However, the potential effects of toxic matter on the organism are often difficult to verify on the ground (Klok & Kraak, 2008). Under field conditions, the big variety of physical and chemical factors of the soils and biotic processes jointly determines the presence and abundance of the species (Ter Braak, 1987). Therefore, the absence of species of contaminated sites does not reflect inevitably the exclusion owed in toxicity caused by heavy metals (Chapman et al., 2002). This implies that a simultaneous analysis of all relevant environmental factors is necessary at the same time to distinguish the potential effects of the contamination of the ground from those of other environmental factors and to place the effects of metal contamination in a realistic perspective (Van Gestel, 2008; Loos et al., 2010).

Many studies have been undertaken in various countries (Pichtel & Anderson, 1997; Cobb et al., 2000; Stoltz & Greger, 2002; Borũvka et al., 2005; Dazy et al., 2009) including Algeria (Rached-Mosbah & Gardou, 1988; Rached-Mosbah et al., 1992; Bentellis et al., 2014) on the contamination of soils and plants resulting from mining activity. However, most of these studies contain only lists of "plant of interest" for accumulation of analysis in aerial or underground parts. Only a few studies have examined the relationship between plant communities and heavy metal content in soil (Rached-Mosbah & Gardou, 1988; Bentellis et al., 2014). The abandoned mining area of lead and zinc is situated in the northeast of Algeria, the exploitation of which definitively stopped in 1984 (Boukhalfa, 2007). Past mining operations have left up major mining waste that can be at the origin of the contamination by heavy metals of the surrounding region. To our knowledge, no study has yet been conducted on such a possibility of an impact on the vegetation in place.

The objective of this study was to determine the potential impacts of metal contamination on the original vegetation that grows on the mining area and around the land and to identify a bioindicative value of the plant communities based on the statistical analysis of floristic and soil data.

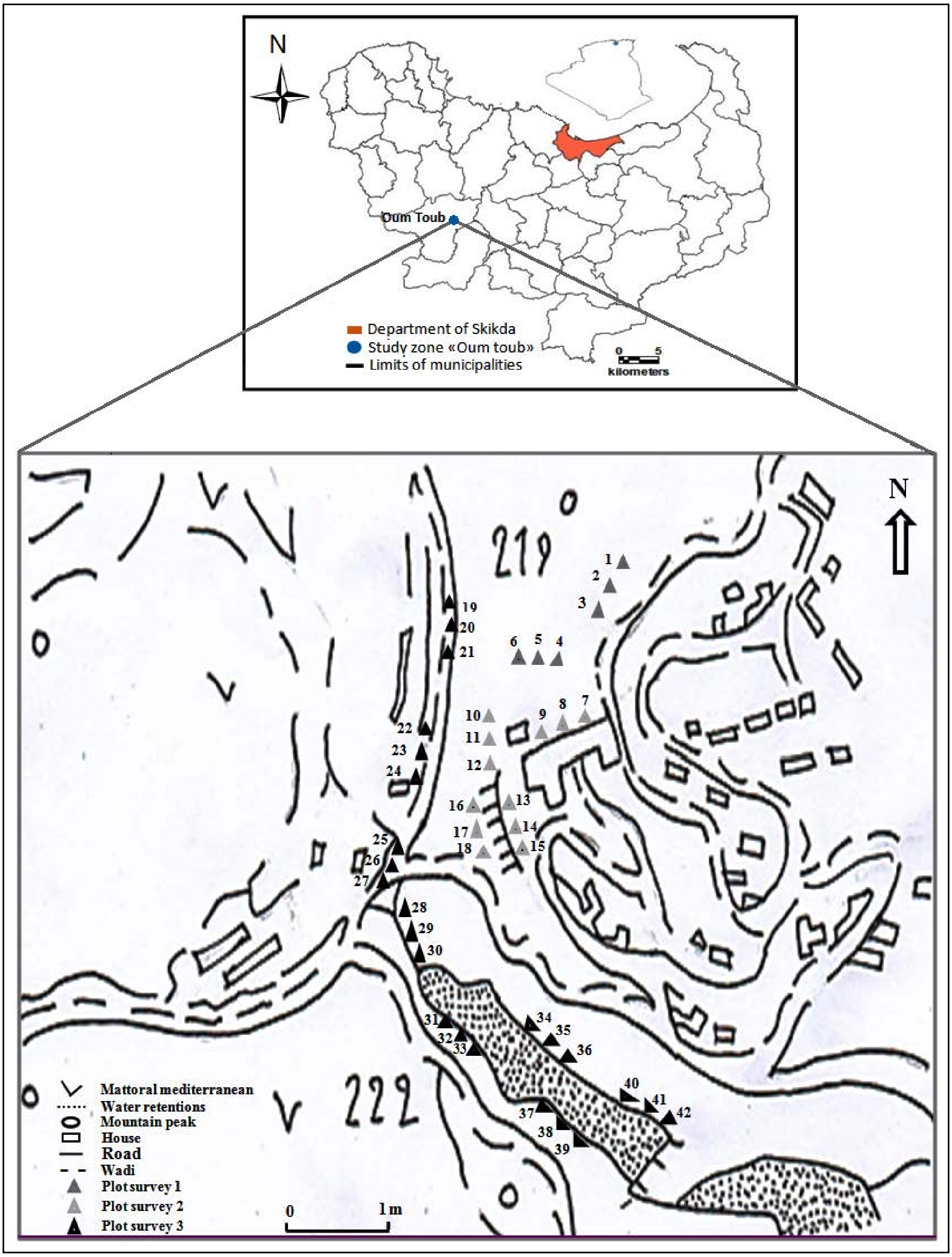

Figure. 1. Location map of the floral statements (source: extract of the topographic map of Ain-Kechera (Algeria). Scale 1/25000 (BOUYAHMED modified).

2. MATERIALS AND METHODS

2.1. Study site

The study area is located in Skikda region, north-eastern Algeria (36°41'12", 6°34'29") (Fig. 1). The relief of the study area is hilly. It is formed by mounts with heights varying between 200 and 500 m. The slope varies between 2 and 25%. It is very strong in the northern land and northeast and low in the south and southwest valleys. The study area has a very dense river network including three permanent wadis (Essouk Wadi, Fessa Wadi, and Malouh Wadi) which all open in the dam "Kenitra" that feeds the Skikda region.

The climate is Mediterranean located in the sub-humid bioclimatic mild winter. The annual average temperature is 19°C with an accumulation of annual pluviometry of 742,35 mm and the annual average humidity is estimated at 68,8%. The vegetation is degraded forest of cork oaks.

2.2. Vegetation sampling method

The study site is divided into three stations including 42 samples (quadrats 1m² each were selected) (Fig.1). i) so as to be located upstream of the mine. Plot1 (R1-R6), ii), on the two-major mining waste. Plot 2 (R7 to R18) and iii) along the edges of the river closest to the mine (R19 to R42) (Fig. 1). In each quadrant, all ecological parameters (exposure, slope, soil cover, and the index abundance-dominance) were recorded. The abundance-dominance was estimated according to the scale given by Braun blanquet (1964). The determination of plant species was conducted in the laboratory of Biotechnology Research Center (C.R.Bt) with the new flora of Algeria (Quézel & Santa, 1963) and flora of North Africa (Maire, 1987).

2.3. Soil analysis

Samples of bare soil and rhizosphere were systematically collected in each quadrant at a depth of less than 20 cm. Soil samples were dried in the open air and at ambient temperature and manually ground in a porcelain mortar and sieved to 2 mm. Soil pH was determined with a glass electrode in a mixture of 1 suspended in water and 2.5 soil (W/V). With a pH meter calibrated with three buffer solutions (pH = 4, 7 and 11). The organic material was analyzed by dichromate oxidation and titration with ferrous ammonium sulfate (Walkley & Black, 1974). The cation exchange capacity (CEC) was measured by titration method with H_2SO_4, using ammonium oxalate in the presence of calcium carbonate (Pansu & Gautheyrou, 2006). Total nitrogen was analyzed by the Kjeldhal method and titration according to the procedure described by Pansu & Gautheyrou (2006).

All the physical and chemical analyses were made in environmental testing laboratory of the C.R.Bt. All the procedures used were in full conformity with the standard French Afnor NF X 31-151(Afnor, 1994). The digestion of the samples of ground (100mg) was made in a speedwave microwave oven with a mixture of strong acids (HNO_3/HCL) in a ratio of 65/30. The determination of heavy metals (Cd, Pb, Zn and Cu) has been carried out by ICP-MS Agilent 7700x at the environmental analysis laboratory of C.R.Bt.

2.4. Data processing

A canonical correspondence analysis (CCA) (Benzeckri, 1973; Leberton et al, 1988) was applied to the floristic data and the physical and chemical soil variables [OM, TN, C/N, pH, CEC, Cd, Zn, Pb and Cu] to highlight the relationship between vegetation and soil parameters. For this, the stat Excel version 9.1 software was used introducing locations and such observations as plant species and edaphic variables as variables.

The results of physical and chemical soil parameters were evaluated by the LSD (least significant difference) test ($P <0.05$) using one-way Anova (Statistica Version 5.1). A Pearson correlation test was also applied to highlight the relationship between the physical and chemical soil variables.

3. RESULTS

3.1. Site characterisation based on edaphic variables

Table 1 summarizes the main physical and chemical characteristics of soil including heavy metal concentrations of 3 studied plots. The most obvious characteristic of these soils is the presence of the relatively high contents of heavy metals. The heavy metal content of the soils studied significantly exceeds the levels of the French standard Afnor NF X 31-*151*(Afnor, 1994). The levels of heavy metals vary largely from one statement to the other on the same station because of the variation of the topographic factors of the site (height, slope, outstrips to the source).

The heavy metal content recorded for [Pb-Zn-Cu] in mining waste greatly [P2] exceeds those of other sites [P1, P3] it's not applicable for cadmium [Cd]. The levels of heavy metals in the river borders [P3] are above the upper allowable values and soil of the plot [P1] have metal content in excess of the levels of the lower permissible limit of the French standard.

Processing data by the LSD test (least significant difference) using one-way Anova analysis revealed a significant difference (P <0.05) of Cd and CEC content between plot1 and the other plots [2 and 3]. No significant differences were recorded between plot1and plot2 for organic matter and nitrogen contents; it is the same for the Cu content. Significant differences were observed between the three plots for the parameter (C/N). A significant difference was also observed between plot3 and the other plots [P1 and P2] for pH. A significant difference was observed between mining waste [P2] and plots [P1 and P3] for Zn and Pb (Table 1).

The correlation coefficients computed between the physical and chemical parameters are presented in tables 2 and 3 respectively. The analysis of the results revealed a strong correlation between Cd and Cu with (r = 0,93) and between these two elements and the organic matter respectively with (r = 0,91 and r = 0.88). A strong correlation was observed between C/N and Pb with (r = 0.91). No significant correlation was observed between the metal contents with CEC and pH (Tables 2 and 3).

The correlations were observed between Zn and Cu and between Cd and Pb respectively with (r = 0,94) and (r = 0,78). The total nitrogen C/N, the CEC and pH have strong correlations with Cd respectively with (r = 0.79, r = 0.68 and r = 0.97). No correlation was recorded between edaphic parameters [OM, C/N, TN, CEC and pH] with Zn and Cu. A correlation was observed between Cd and Zn with (r = 0.62) and the edaphic parameters studied have no significant correlation with these two elements. Whereas, a strong correlation was observed between Cu and pH with (r = 0,75) and between the lead and C/N with (r = 0.68) (Tables 2 and 3).

3.2. Vegetation

The sampling of the vegetation present in the contaminated site in the northeast of Algeria allowed us to record 94 species; most of these botanical species were herbaceous annual or long-lived belonging mainly to the families of *Asteraceae* and *Poaceae*. The studied flora is characterized by therophytes with ruderal affinity, being considered the ultimate stadium of degraded ecosystems (Raunkiaer, 1934) or, stress tolerant adapted to drastic habitat with limited resources and often low productivity seeds (Grime, 1977). Among all surveyed taxa, six species were selected potentially bioindicative of the metal pollution (*Rumex bucephalophorus, Lamarckia aurea*, *Trifolium compestre, Cistus monspeliensis, Calicotome spinosa and Verbascum sinuatum*).

According to Damien et al., (2004) and Deram et al., (2007) all the sites where these species are known to grow in the Mediterranean are located in severely degraded ecosystems such that the ends of the roads, in proximity to factories disused processing ore non-ferrous metal or correspond to waste which often contains large amounts of trace metals. These species were not recorded in Algeria as metalophytes.

3.3. Species groupings based on edaphic variables

Axis 1 of CCA opposed to the positive side of soil chemical parameters such as [OM, C/N, CEC, Cd, Cu, Pb and Zn] to [TN and pH] from her part on side negative. This shows an increasing chemical gradient of the metallic pollution of the studied ground by going from the negative side to the positive side of this axis (Fig.2). Plant species that have strong contributions to this axis of its positive side are: *Paranchia argentea*, *Phalaris brachystachys*, *chrysanthemum myconis*, *Lamarckia aurea*, *Raphanus raphanustrum*, *Calicotome spinosa*, *Cistus monspeliensis*, *Trifolium compestre and Rumex bucephalophorus*.

These species are also opposed to the most taxa contributing to the axis of its negative side: *Hypparhenia hirta, Rumex pulcher, Trifolium maritimum, Lamium amplexicaule, Senchus tenerrimus, Lolium perenne and Verbascum sinuatum.*

Table1. Mean values standard deviation of the physical and chemical parameters at the studied sites

Site	**OM**	**TN**	**C/N**	**CEC**	**pH**	**Cd**	**Zn**	**Cu**	**Pb**
	mean± S.D.	mean± S.D.	mean± S.D.	mean± S.D.	mean± S.D.	mean± S.D.	mean± S.D.	mean± S.D.	mean± S.D.
P1	9.8± 5.07a	0.71± 0.58a	10.5± 4.5b	18.25± 2.44a	5.63± 0.92b	107.11± 105.92a	8748.81± 8486.25ab	1182.83± 897.85a	4907.63± 2405.85b
P2	9.3± 2.83a	0.66± 0.24a	19.41± 7.51a	10.45± 7.55b	5.28± 1.21b	51.66± 34.10b	1311.86± 9384.61a	1364.87± 860.17a	1804.67± 8884.29a
P3	5.58± 2.51b	0.32± 0.38b	5.23± 1.75c	11.22± 3.67b	5.98± 0.82a	32.22± 14.40b	8211.86± 138.62b	369.41± 138.62b	7103.44± 7479.36b

Values in each row followed by the same letter do not differ significantly at (P<0.05) by the test LSD [(a, b, c and ab) Homogeneous groups]; Mean. Mean values S.D. standard deviation.OM: organic matter; TN; total nitrogen; C/N: carbon/nitrogen ratio; CEC: cation exchange capacity; pH: pH in water; Cd: cadmium; Pb: lead; Zn: zinc and Cu: copper. Trace element concentrations are given in mg/kg.

These are related to soil rich in nitrogen and live on grounds with pH close to neutrality. This group of species seems to avoid the high concentrations of heavy metals (Fig.2).

Table 2. Correlation coefficients between metal concentrations in soil

SITE	Cadmium (Cd)	Zinc (Zn)	Copper (Cu)	Lead (Pb)
P1				
Ca	1.00			
Zn	0.66	1.00		
Cu	0.93*	0.60	1.00	
Pb	0.87	0,73	0.67	1.00
P2				
Cd	1.00			
Zn	-0.12	1.00		
Cu	-0.32	0.94*	1.00	
Pb	0.78*	0.03	-0.07	1.00
P3				
Cd	1.00			
Zn	0.62*	1.00		
Cu	0.36	0.11	1.00	
Pb	0.27	0.21	0.17	1.00

Table 3. Correlation coefficients between heavy metals and edaphic variables

SITE	MO	N	C/N	CEC	pH
P1					
Cd	0.91*	0.83*	0.75	0.22	-0.59
Zn	0.72	-0.69	0.74	-0.16	-0.52
Cu	0.88*	-0.79	0.51	0.37	0.34
Pb	0.83*	-0.77	0.91*	-0.19	0.81
P2					
Cd	ns	0.74*	0.79*	0.68*	0.97*
Zn	ns	-0.02	0.13	0.34	0.15
Cu	ns	-0.06	0.01	0.25	-0.31
Pb	ns	0.57	0.62	0.84*	0.86*
P3					
Cd	0.10	-0.08	0.30	-0.12	0.48*
Zn	-0.08	-0.36	0.44*	-0.17	-0.34
Cu	0.53*	0.34	0.05	0.08	0.75*
Pb	-0.12	-0.33	0.68*	0.13	0.35

Ns: not significant, significant*; OM: organic matter; C/N: report on the carbon nitrogen; TN total nitrogen; CEC, cation exchange capacity; pH, water pH. Cd: cadmium, Cu: copper, Pb: lead and Zn: zinc

The axis 2 of CCA, the median values of edaphic variables [OM, TN, pH and CEC] are positively correlated to this axis. Whereas, the ratio [C/N] is negatively correlated to the same axis, it also for the zinc and the lead. Moreover, the most contributory plant species to the axis of its positive side are: *Raphanus raphanustrum*, *Lagarus ovatus, phalaris brachystachys, Paranchia argentea and Lamium amplexcaule.* These are positively correlated with [CEC, pH and OM] and oppose the species that have strong contributions to this same axis of its negative side. *Inula viscosa, Rumex bucephalophorus, Coronopus didymus, Silene niceensis and Cistus monspeliensis*. This group of species seems tolerant to very high concentrations of Pb and Zn (Fig.2).

Five plant groupings were identified according to distribution of physical and chemical variables of soil (Fig.2). The groups [I, II, III and IV] oppose the group V along the axis of CCA (Fig.2). This shows an increasing chemical gradient of heavy metal from the negative side to the positive side. According to axis 2 of the CCA groups [I, II and V] are opposed to groups [III and IV]. This shows that groups [III and IV] contain ruderals supporting the high toxicity of Zn and Pb (Fig.2). Groups I and II consist of plants that occur on acid soils rich in organic matter. In group II, the pH slightly increased. Groups III and IV are characterized by plants growing on soils that are acidic to very acidic, with very low nitrogen content. Group V is characterized by a flora that occurs in soils with pH close to neutral and rich in organic nitrogen. In addition, the values of soil variables such as the CEC and OM decreased from group I to group IV. pH and TN increases from the negative side to the positive side of axis 2.

Based on the concentrations of trace elements, in groups [I and V] the soil is considered less polluted, species coexist within these groups seem to escape high levels of heavy metals especially zinc and lead. Group II is considered polluted and intermediate concentrations are above the levels of the standard Afnor NF X 31-*151*(Afnor, 1994) especially for Cd and Cu. Plant species of this group grow in soils rich in organic matter and tolerate high levels of cadmium and copper. Developing plants on soils with concentrations of trace metals (Zn-Pb) well above the French Afnor standard (Afnor, 1994) are in groups [III and IV]. The plants in the latter group seem to favor high levels of zinc and lead and grow in poorer soils in organic matter and poor in nitrogen. However, when a group of species is correlated with a group of variables, it does not mean that each species is correlated with all variables simultaneously. Thus, if some variables are correlated, it is sufficient that one of the species is correlated with one of the variables for the other species of the group to also appear. Cd and Cu are very dependent on the level of organic matter in the soil; thus, plants correlated with cadmium and copper will also appear with the organic matter without actually being (Bentellis et al., 2014) (Fig.2).

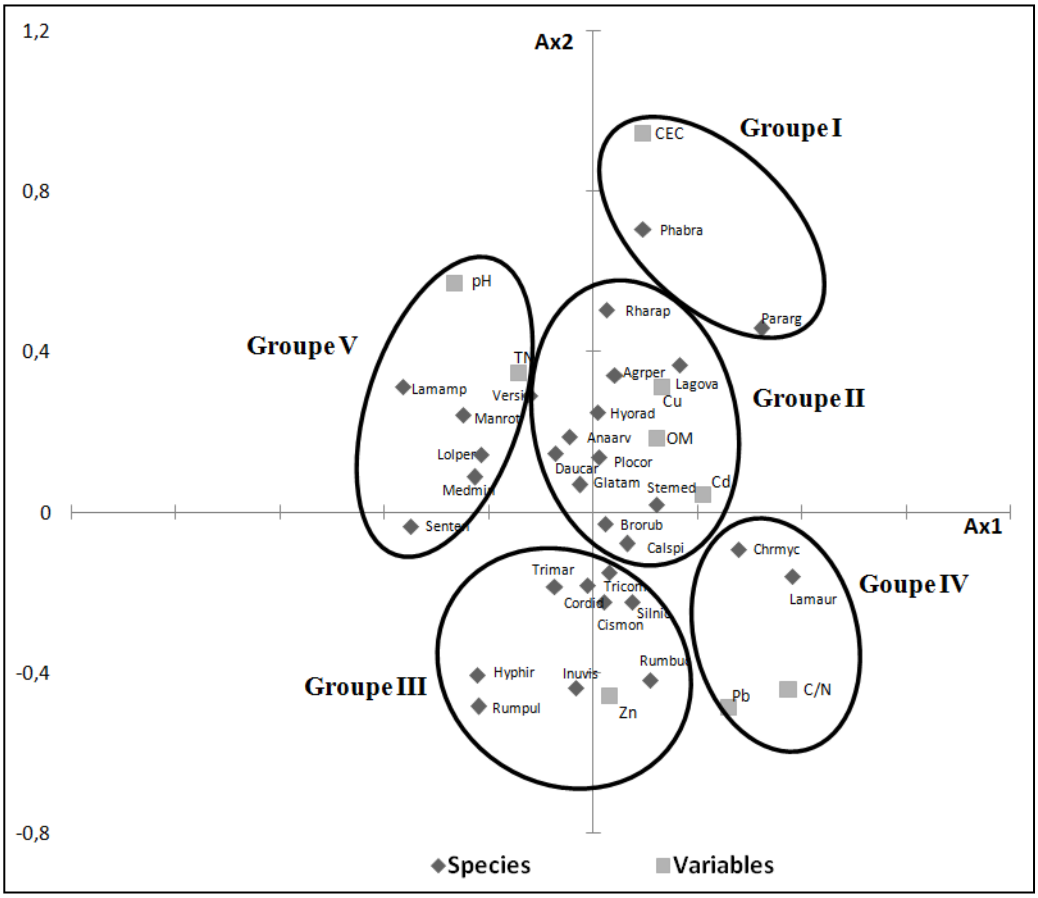

Figure 2. Factorial map applied to the floristic and edaphic variable data conducted by an Analysis Corresponding Canonic

4. DISCUSSION

Table 4 shows the composition of the flora characterizing the five groups statistically identified by CCA. We can deduce that each group combines plants specifically adapted to soil characteristics allowing us to develop a good tool to diagnose sites that are polluted by heavy metals. In all five groups, the vascular plants present various stages of evolution, depending on the concentration of heavy metals in the soil. Out of all the specific population studied, woody species are scarce and only represented by *Calicotome spinosa* and *Cistus monspeliensis*. According to Ernst et al., (1992) the growth of woody species in degraded soils is inhibited, leading to the development of plant communities that are purely herbaceous or weak shrubs, while the biennial herbs such as, *Phalaris brachystachys*, *Verbascum sinuatum*, *Lamium amplexcaule* and *Mentha rutendofolia* (Groups I and V) are dominant in less polluted soils. These species are classified by Grime (1977) as acidophyl-subneutrophil that are little or not related to human impact and especially observed on sandy soils. In contaminated soil, group II is regarded as an intermediate step with the presence of woody species (*Calicotome spinosa*) and some herbaceous biennial (*Raphanus raphanustrum*, *Daucus carrota*, *Bromus rubens*, *Galactites tamantosa* and *Hyoserus radiata*). However, almost all plants observed in highly polluted soils [Groups III and IV] are herbaceous annuals with ruderal affinity (*Rumex bucephalophorus*, *Trifolium compestre*, *Coronopus didymus*, *Hypparhenia hirta*, *Lamarckia aurea* and *Chrysanthemum myconis*). The species observed in these groups [III and IV] are generally prevalent in colonized anthropogenic sites and regularly in degraded environments (Grime, 1977).

The data processing of physical and chemical soil parameters including heavy metals by one-way Anova revealed that the variables such as soil, pH and CEC were important factors for low contaminated soil justifying the presence of two groups of plants [I and V]. Moreover, the pH is a factor that controls the solubility and / or retention of heavy metals in the soil and according to Li & Thornton (2001) pluralities of metals are relatively more mobile at acidic pH and in oxidizing conditions. Plants observed in these groups (*Phalaris brachystachys*, *Paranchia argentea*, *Lamium amplexicaule, Mentha rentudofolia* and *Sonchus tenerrimus*) seem to escape high levels of heavy metals. In Finland, according to Salemaa et al., (2001) heavy metals submitted vegetation of a mining site to a strong selection pressure, species that are sensitive to heavy metals have disappeared and competitive interactions between species may have been modified by heavy metals.

The whole of group II is considered polluted, high levels of cadmium and copper were observed, but also regarded as an intermediate group where ruderals and / or stress-tolerant species are observed, such as, *Agropyrum rupens, Bromus rubens, Anagallis arvensis, galactites tamantosa* and *Hyoserus radiata.* Depending on the presence of the species along the gradient of pollution, annual and biennial plants were more resistant than each other, while the phanerophytic plants were the most sensitive taxa. According to the classification established by Grime (1977) these species are typical habitat where there are moderate intensities of stress and disruption, and the life of these species may be less than 12 months (Table 4).

On the other hand, most species observed in groups [III and IV] were annual or biennial plants, nitrophilous and thermophiles that colonize substrates that are regularly disturbed by man (Grime, 1979). Trace elements (Zn-Pb) were the major factors for these groups, but also the low levels of organic matter and nutrients in these soils are a major limiting factor for the development of the vegetation. These two plant groupings are strongly correlated to the contamination by heavy metals especially (Zn-Pb).

Table 4. Classification of plant species based on edaphic variables and levels of heavy metals using canonical correspondence analysis (CCA).

Grouping plants	Code	Life form	Strategy Grime	Physical and chemical soil variables
Group 1				
Phalaris brachystachys	*Phabra*	hemicryptophytes	CS	CEC
Paranchia argentea	*pararg*	therophytes	CR	
Group 2				
Raphanus raphanustrum	*Raprap*	hemicryptophytes	CSR	MO,
Lagarus ovatus	*Lagova*	hemicryptophytes	SR	Cu-Cd
Agropyrum rupens	*Agrper*	hemicryptophytes	R	
Hyoserus radiata	*Hyorad*	therophytes	RS	
Anagallis arvensis	*Anaarv*	therophytes	SC	
Plantago coronopus	*Placor*	therophytes	R	
Daucus carrota	*Daucar*	hemicryptophytes	RC	
Galactites tamantosa	*Glatam*	hemicryptophytes	R	
Stellaria media	*Stemed*	therophytes	R	
Bromus rubens	*Brorub*	therophytes	R	
Calicotome spinosa	*Calspi*	phanerophytes	CR	
Group 3				
Trifolium maritimum	*Trimar*	therophytes	R	Zn
Coronopus didymus	*Cordid*	therophytes	R	
Trifolium compestre	*Tricom*	therophytes	R	
Silene niceensis	*Silnic*	therophytes	CR	
Cistus monspeliensis	*Cismon*	Chamephytes	CR	
Rumex bucephalophorus	*Rumbuc*	Therophytes	R	
Inula viscosa	*Inuvis*	Chamaephytes	CR	
Hypparhenia hirta	*Hiphir*	hemicryptophytes	R	
Rumex pulcher	*Rumpul*	therophytes	RC	
Group 4				
Chrysanthemum myconis	*Chrmyc*	therophytes	R	C/N,
Lamarckia aurea	*lamaur*	therophytes	R	Pb
Group 5				
Verbascum sinuatum	*Versin*	hemicryptophytes	R	pH,
Lolium perenne	*Lolper*	therophytes	R	TN
Senchus tenerrimus	*Sonten*	therophytes	R	
Mentha rutendofolia	*Menrut*	geophytes	S	
Lamium amplexcaule	*Lomamp*	geophytes	S	
Medicago minima	*medmin*	therophytes	RS	

Among the plants that coexist within these groups *(Trifolium compestre*, *Lamarckia aurea* and *Rumex bucephalophorus)* are highly developed in abundance-dominance at mining soils that are very polluted by heavy metals, especially Zn and Pb. They were highly correlated with axis 1 (axis of chemical gradient of trace metals). Generally, in Europe, plants of the same type are considered typical of coal mines containing important concentrations of Pb (Deram et al, 2007 and Remon et al., 2009). In France, these plants are most commonly found in disturbed secondary sites such as railways, increasingly on bare mineral soil with a relatively low pH and low humus (Damien et al., 2004). In India, a list of species of plants accumulating heavy metals has been established and plants of the same botanical family as those found by Mani et al., (2012) appear in groups 3 and 4 and are known to grow on anthropogenic habitats including wells and brownfield sites. They are increasingly found in the most polluted sites (Damien et al., 2004; Deram et al., 2007).

5. CONCLUSION

Heavy metals are discriminating factors for identifying a specific flora of polluted soil. Other parameters such as edaphic [OM, pH, CEC, TN and C/N] cannot be excluded and may be important for some groups. A bioindicative value has been identified for some species that grow in soils polluted by heavy metals northeast-Algeria (*Calicotome spinosa*, *Cistus monspeliensis, Lamarckia aurea, Verbascum sinuatum, Rumex bucephalophorus* and *Trifolium compestre*).

The mere presence of one or all of these five species groups identified in the mine site could indicate metal pollution in the studied site, but it could also be probably due to a decline in competitiveness of the other species of plants caused by stress-induced contamination by heavy metals. Finally, as suggested by Damien et al., (2004) the systematic description of plants with respect to soil composition is a prerequisite for studying the physiological tolerance and speciation of heavy metals.

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