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Review Article

TOXICOLOGICAL STUDY OF *VERNONIA AMYGDALINA* ON A SOIL CONTAMINATED WITH DIFFERENT CONCENTRATIONS OF LEAD AND CADMIUM AND ITS INTERACTION IN THE TRANSFER OF ESSENTIAL TRACE MINERALS

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Abstract

Vernonia amygdalina is a vegetable widely used in nutrition and phytotherapy. Its toxicological quality poses real concerns for its use. Greenhouse cultivation is carried out with twelve batches of six vegetative vases in order to evaluate the soil-plant transfer. Atomic Absorption Spectrophotometry analysis allowed an investigation of the lead, cadmium and bivalent iron, magnesium, calcium, manganese, zinc and copper contents of the different plant organs. Lead and cadmium concentrations vary according to the concentration of the two metals in the crop soil. The lowest concentrations of lead and cadmium are found in the leaves, that are the most consumed part of the plant. In the leaves, cadmium (0.937-33.004ppm); lead (0.945-31.701ppm). Lead concentration is positively correlated with the trace elements iron ($r = 0.465$), manganese ($r = 0.342$), magnesium ($r = 0.349$); there is a weak correlation between cadmium and trace elements in leaves. In the stem, cadmium (0.145-96.680ppm); lead (0.050-288.364ppm). Lead and cadmium correlate respectively with zinc ($r = 0.552$; $r = 0.547$), manganese ($r = 0.741$; $r = 0.708$); iron ($r = 0.432$; $r = 0.452$). There is a strong negative correlation between cadmium-calcium $r = -0.341$. At root level cadmium (0.572-237.043); lead (2.594-151.358ppm). The following correlations can be observed: cadmium-copper $r = 0.648$; cadmium-zinc $r = -0.379$; cadmium-iron $r = -0.412$; lead-magnesium $r = 0.369$; lead-calcium $r = 0.410$. This study permits to orient the cultivation of this vegetable in order to guarantee its virtues and protect the consumers.

Keywords: Vernonia amygdalina, Lead, cadmium.

INTRODUCTION

Environmental pollution has become one of the major problems conditioning the future of our civilization (Gana and Toba, 2015). Household waste in the heavily populated areas of large cities has caused an accumulation of trace metal elements (TME) in the ecosystem (soil, air, water) (Topanou *et al.,* 2011 and Yémadje *et al.,* 2015). A part from foliar exposure, plants absorb all the elements they need for the growth from the soil. Toxic metals present in soils are also taken into account but do not play any physiological role in the plant. The trace elements: iron, zinc, copper, manganese are bivalent heavy metals (Malayeri, 1995) that participate in the nutrition of plants, animals and humans, as well as the macroelements calcium and magnesium. Trace elements are not dangerous and are necessary for life. Only the quantity and speciation make them toxic. At very low concentrations in the living tissues of plants and animals, they play important roles in metabolic reactions, coordinate the structure and stability of enzymes and proteins (Burdin, 2014). They can create metal-protein bonds and are capable of modifying the tertiary structure of the protein (Malayeri, 1995). The availability of trace elements in the organs of a plant strengthens its nutritional and therapeutic quality in relation to their role in biological reactions. At high concentrations of the toxic metals lead and cadmium in the soil, the plant absorbs these metals in preference to the essential elements iron, zinc, copper, calcium, magnesium and manganese (Malayeri, 1995).

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These metallic trace elements thus become competitors for the essential elements that the plant needs (Remon, 2006 and Wallace *et al.,* 2008). *Vernonia amygdalina* is a vegetable used for nutrition and treatment of various pathologies (Tilahum *et al.,* 2007; Ajibesin *et al.,* 2008). Cultivated in polluted areas such as market gardening sites and built-up areas, it is contaminated with the toxic metals lead and cadmium, as are most market garden produce (Deguenon *et al.,* 2020; Oguh *et al.,* 2019; Tomaszewska-sowa *et al.,* 2018). Leaves and roots are the plant's organs of exchange with its external environment. All organs of *Vernonia amygdalina* are edible for feeding and for treating pathologies. The general objective of this study is to research the toxicological nature of this plant which will allow us to situate its growing in relation to areas polluted by lead and cadmium, to limit the consumption of the preferred storage organ of these two metals and then the existing correlations between them and the essential elements during the soil-plant transfer.

MATERIALS AND METHODS

In order to determine the organ in which *Vernonia amygdalina* bio-accumulates lead and cadmium, we had disposed of twelve batches of six 3 kg vegetative vases. The soil in the vases is contaminated differently according to Table 1. The concentrations of 5.06ppm for Pb+1.53ppm for Cd are those found in water samples in 2009 during Koumolou's work at the Godomey market gardening sites in the commune of Abomey-Calavi in Benin. This value is taken as a reference for preparing the solutions in arithmetic sequence.

Table 1. Contamination of irrigation water with the toxic metals lead and cadmium

V	V1	V2	V3	V4	V5	V6	V7	V8	V9	V10	V11	V12
C	C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	C11	C12

Legend : V= vegetation vase; C= concentration of Pb and Cd;C1=5.06ppm Pb+1.53ppm Cd;C2=10.12ppmPb+3.06ppmCd;C3=20.24ppm+6.12ppmCd; C4=40.48ppmPb+12.24ppmCd;C5=80.96ppmPb+24.48ppmCd;C6=161.92ppmPb+48.96ppmCd;C7=323.84ppmPb+97.92ppm;Cd;C8=647.68ppmPb+195.84ppmCd;C9=1295.36ppmPb+391.68ppmCd;C10=2590.72ppmPb+783.36ppmCd;C11=5181.44ppmPb+1566.72ppmCd;C12=10362.88ppmPb+3133.44ppm Cd.

The plants are treated with the prepared solutions after 4 weeks. Each plant receives 300mL of the prepared solution divided in three times at a ratio of 100mL per day after ten weeks of cultivation, all organs of the plant are harvested, root, stem and leaves. The leaves are washed, dried in an oven at 100°C for 12 hours. The stems and the roots are washed and dried in an oven at 120°C for 24 hours. The three organs of the plant, that is the leaves, stem and root, are then reduced to powder in a porcelain mortar and stored in aluminium foil for analysis by Atomic Absorption Spectrophotometry (AAS).

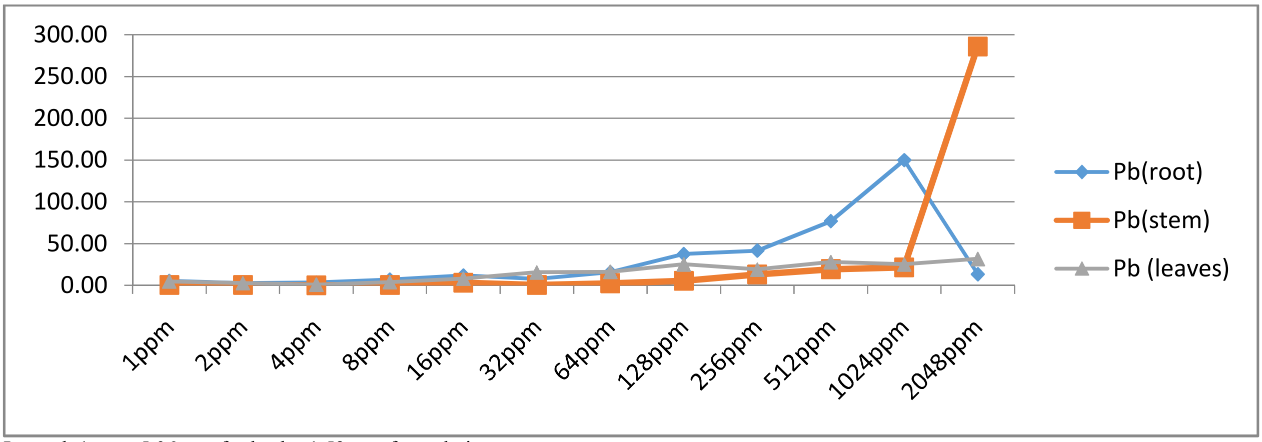
Statistical analysis

The XLSTAT 2019 software has allowed us to perform a descriptive analysis of the data. The Pearson correlation coefficient allowed us to show the correlation between the different metals and the macroelements contained in the leaves, stem and root of the plant.

RESULTS

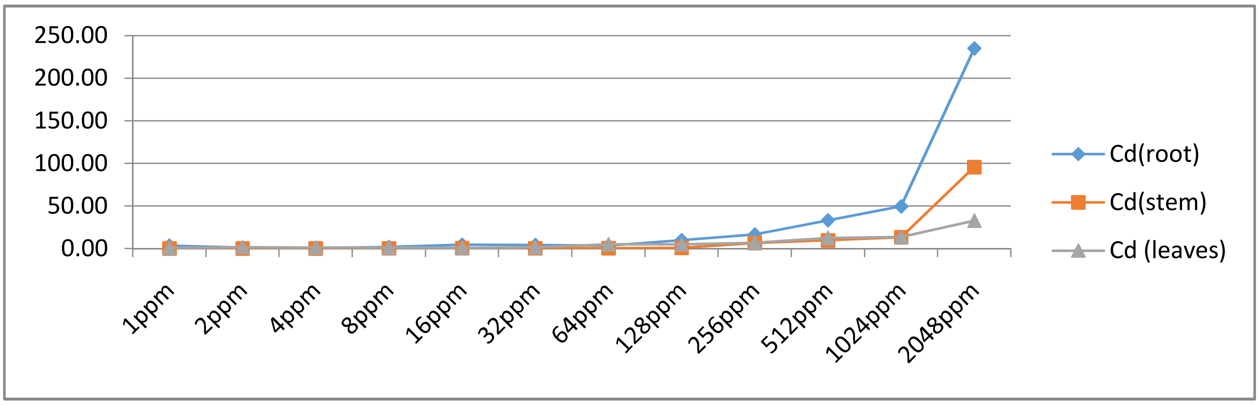
The variation in the concentration of the growing medium did not influence the appearance of the plant during the period of the culture which is ten weeks. At a concentration higher than 3.454g for lead and 1.044g for cadmium per kilogram of soil, the plants are burned (Figure 1). Therefore, the acceptability threshold of the plant for both metals for this type of soil is 3.454g for lead and 1.044g for cadmium per kilogram of soil. The applied concentrations can be found in mining areas where both metals are used. This is a model experiment to estimate different concentrations of lead and cadmium in the environment.

Figure 1. The plants of *Vernonia amygdalina* burnt



Legend: 1ppm=5.06ppm for lead + 1.53ppm for cadmium

Figure 2. Variation in lead accumulation in plant organs



Legend: 1ppm=5.06ppm for lead + 1.53ppm for cadmium

Figure 3. Variation in cadmium accumulation in plant organs

Table 2. Descriptive statistics of observations (leaves)

Variable	Observations	Obs. missing data	Obs. without missing data	Minimum	Maximum	Average	Ecart-type
Cd (leaf)	36	0	36	0,937	33,004	7,052	8,94
Pb (leaf)	36	0	36	0,945	31,701	15,102	10,52
Cu (leaf)	36	0	36	2,05	31,42	8,835	7,841
Zn (leaf)	36	0	36	12,17	91,68	23,014	20,933
Mn (leaf)	36	0	36	19,84	161,7	38,975	37,64
Fe (leaf)	36	0	36	89,42	1729,71	367,146	428,554
Mg (leaf)	36	0	36	167,64	1209,34	292,411	280,789
Ca (leaf)	36	0	36	6751,39	100503,73	24214,928	23802,814

Table 3. Correlation matrix (Pearson) for leaves

Variables	Cd (leaf)	Pb (leaf)	Cu (leaf)	Zn (leaf)	Mn (leaf)	Fe (leaf)	Mg (leaf)	Ca (leaf)
Cd (leaf)	1							
Pb (leaf)	0,772	1						
Cu (leaf)	0,065	0,146	1					
Zn (leaf)	0,109	0,313	0,877	1				
Mn (leaf)	0,14	0,342	0,874	0,999	1			
Fe (leaf)	0,161	0,465	0,79	0,97	0,973	1		
Mg (leaf)	0,14	0,349	0,871	0,999	0,999	0,976	1	
Ca (leaf)	0,181	0,426	0,868	0,982	0,987	0,975	0,988	1

Values in bold are different from 0 at significance level alpha=0.05

Table 4. Descriptive Statistics of observations (stem)

Variables	Observations	Obs. missing data	Obs. without missing data	Minimum	Maximum	Average	Standard deviation
Cd (stem)	36	0	36	0,145	96,680	10,760	26,340
Pb (stem)	36	0	36	0,050	288,364	29,311	78,701
Cu (stem)	36	0	36	0,820	8,990	3,474	2,522
Zn (stem)	36	0	36	12,610	1351,000	51,124	222,838
Mn (stem)	36	0	36	7,250	25,000	13,855	4,458
Fe (stem)	36	0	36	22,760	61,740	44,376	13,271
Mg (stem)	36	0	36	148,000	196,530	163,974	12,459
Ca (stem)	36	0	36	3355,670	13441,760	8955,417	3310,373

Table 5. Correlation matrix (Pearson) for stem

Variable	Cd (stem)	Pb (stem)	Cu (stem)	Zn (stem)	Mn (stem)	Fe (stem)	Mg (stem)	Ca (stem)
Cd (stem)	1							
Pb (stem)	0,997	1						
Cu (stem)	-0,080	-0,069	1					
Zn (stem)	0,547	0,552	-0,030	1				
Mn (stem)	0,708	0,741	0,077	0,429	1			
Fe (stem)	0,452	0,432	-0,322	0,219	0,395	1		
Mg (stem)	0,100	0,094	-0,393	0,033	0,204	0,566	1	
Ca (stem)	-0,341	-0,355	-0,083	-0,215	-0,093	0,176	0,504	1

Values in bold are different from 0 at significance level alpha=0.05

There is a small variation in lead concentration in the three plant organs from 1ppm to 64ppm. A peak is observed at 1024ppm for the roots followed by a fall at 2048ppm. At the leaves a slight variation is observed until 2048ppm. Lead concentration peaked at 2048ppm. The plant *Vernonia amygdalina* bioaccumulates lead in the leaves, root and stem in ascending order (Figure 2). A small variation in cadmium concentration is observed from 1ppm to 64ppm. An increase in the concentration of cadmium in all three organs is noted from 128ppm to 2048ppm with one peak. The plant bioaccumulates cadmium in leaves, stems and roots in an increasing way (Figure 3). The plant bioaccumulates lead and cadmium differently in its organs. The small proportion is observed in the leaves. The toxic metals lead and cadmium are bivalent chemical elements. The presence of these two chemical elements in soil influences the adsorption of copper (Cu), zinc (Zn), manganese (Mn), iron (Fe) and magnesium (Mg). The descriptive statistics of the observations of the concentration of the different metals in the leaves show us the minimum, maximum, mean and standard deviation values (Table 2). The Pearson correlation matrix shows that there is a strong correlation between the concentration of lead and cadmium in leaves. The correlation is weak and almost zero between the concentrations of Cd, Cu, Zn, Mn, Fe, Ca and Mg. There is a positive correlation between the concentration of Pb, Fe, Mg, Mn, Ca. There is a strong positive correlation between the concentrations of Cu, Zn, Mn, Fe, Mg, Ca. Among the two toxic metals lead and cadmium, only lead positively influences the adsorption of Fe, Mg, Mn and Ca (Table 3). The descriptive statistics of the observations of the concentration of the different metals in the stem show us the minimum, maximum, mean and standard deviation values (Table 4). The Pearson correlation matrix shows that there is a strong positive correlation between the concentrations of Cd, Zn, Mn, Fe. There is a strong negative correlation between Cd and Ca. Pb concentration is strongly correlated with Zn, Mn, Fe. It is negatively and strongly correlated with that of Ca. A positive correlation links Zn and Mn; Mn and Fe; Fe and Mg; Mg and Ca. Cu and Mg are negatively correlated. The concentrations of the two toxic metals Pb and Cd positively influence the concentrations of Zn, Mn, Fe. The influence is negative for Ca in the stem (Table 5). The concentration's observation descriptive statistics of the different metals in the root show us the minimum, maximum, mean and standard deviation values (Table 6).

Table 6. Descriptive statistics of observations (root)

Variables	Observations	Obs. missing data	Obs. without missing data	Minimum	Maximum	Average	Standard deviation
Cd (root)	36	0	36	0,572	237,043	30,377	64,165
Pb (root)	36	0	36	2,594	151,358	31,007	42,074
Cu (root)	36	0	36	2,250	8,010	4,238	1,775
Zn (root)	36	0	36	7,000	26,860	13,406	4,655
Mn (root)	36	0	36	10,840	37,320	22,381	5,668
Fe (root)	36	0	36	103,360	1010,260	472,499	244,686
Mg (root)	36	0	36	127,680	331,120	162,710	51,808
Ca (root)	36	0	36	5576,860	13177,720	6932,419	2042,234

Table 7. Correlation matrix (Pearson) for root

Variables	Cd (root)	Pb (root)	Cu (root)	Zn (root)	Mn (root)	Fe (root)	Mg (root)	Ca (root)
Cd (root)	1							
Pb (root)	0,096	1						
Cu (root)	0,648	-0,013	1					
Zn (root)	-0,379	0,043	0,238	1				
Mn (root)	0,043	-0,205	0,645	0,833	1			
Fe (root)	-0,412	0,136	0,252	0,858	0,648	1		
Mg (root)	-0,089	0,369	0,344	0,897	0,756	0,704	1	
Ca (root)	-0,063	0,410	0,365	0,857	0,718	0,750	0,951	1

Values in bold are different from 0 at significance level alpha=0.05

The Pearson correlation matrix shows a weak correlation between Pb and Cd. The correlation is strong and positive between Cd and Cu. The concentrations of Cd, Zn and Fe are negatively and strongly correlated. There is a correlation between Pb, Mg and Ca. Among others there is a positive correlation between Cu, Mg, Ca; between Zn, Mg, Fe, Mg, Ca. All in all, Cd and Pb influence the essential metals in the root of *Vernonia amygdalina* differently. Cd influences Cu positively and Zn and Fe negatively. On the other hand Pb has a positive influence on Mg and Ca in the root (Table 7). The correlation between the toxic metals Pb, Cd and trace elements varies from organ to organ of the *Vernonia amygdalina*. All organs of the plant are consumable, but the leaves are more stressed than the stem and root. Therefore the data from the leaves will be used for a more detailed discussion.

DISCUSSION

Vernonia amygdalina bioaccumulates lead and cadmium in the leaves, stem and root. Concentrations of these metals vary from organ to organ. *Vernonia amygdalina* bioaccumulates lead in the stem while cadmium is more accumulated in the roots compared to other organs. These results are confirmed by those of (Kleckerová and Dočekalová, 2014) who showed during their work, that *Taraxacum officinale* of the Asteraceae family bioaccumulates Cd in these roots compared to the leaves when it is found in an environment contaminated by toxic metals. Work by (Cecchi, 2008) showed that Pb is strongly immobilized in the root cell wall as Pb pyrophosphate. After absorption at the root level, the metals undergo translocation to the other organs. This phenomen on depends on the metal and the species of the plant. Several proteins are involved in this transport mechanism. Cadmium after absorption is transported in free form to air organs in tomatoes (Remon, 2006). Translocation of the metal to aerial parts of the plant is important in some species, such as lettuce and spinach (Tremel-Schaub and Feix, 2005; Patra *et al.,* 2004)). This is the case of *Vernonia amygdalina* in the present study. The concentration of lead and cadmium increases in all three organs but is higher in the roots compared to the other organs. The transfer phenomenon is therefore progressively from the roots to the leaves via the stems. Concentrations in the leaves increase with age. Those are the reasons why concentrations of toxic metals in older leaves are higher than in younger leaves (Remon, 2006). The equilibrium observed between the concentrations of lead and cadmium in the leaf and root organs of *Vernonia amygdalina* in relation to the concentrations of the metals in the soil allows us to classify it in the category of indicator plants for these two toxic metals. Some heavy metals, namely Fe, Zn, Cu, Mn, are trace elements that at low doses are involved at the level of molecular processes in anabolic and catabolic reactions (Rengel, 1999) as well as the macroelements Ca and Mg (Burdin, 2014). They are bivalent cations and are in competition with the toxic metals lead and cadmium. In the sheets our study shows that there is a strong positive correlation between Pb, Mg, Fe, Ca, Mn and very weak with Cd, Mg, Fe, Ca and Mn. The presence of lead in the medium promotes the bioavailability of the metals Mg, Fe, Ca, Mn. In the stem the concentrations of Pb, Cd, Zn, Mn, Fe are strongly positively correlated. The correlation is negative between Pb, Cd, Ca. The presence of these two metals in the medium does not favour the absorption of Ca towards the stems. In the roots, Cd and Cu are highly correlated while lead is correlated with Mg and Ca. The relationships between the two toxic metals and trace elements vary from one organ to another in the vegetable. All parts of the plant are edible and for treating various pathologies. The leaves are the most used and are the parts exploited in this study. The existence of the strong correlation between lead and the minerals Mn, Fe, Mg, Ca is beneficial for the vegetable in the appreciation of its quality in nutritional elements. The low correlation existing in the leaves between Cd, Mg, Fe, Ca and Mn depletes the leaves in these trace elements and even the nutritional quality of the leaves. These results are confirmed by those of (Liénard and Colinet, 2018) who showed that there is a weak relationship between Cd and Zn in barley grains from contaminated areas in Belgium. The presence of one of them does not condition the presence of the second. (Dauguet *et al.,* 2011) showed that maize grains bioacumulate less toxic metals than sunflower grains. In maize grains, a variability in Pb and Zn concentration is observed according to the nature of the soil. For asafe consumption, maize grains are preferable to sunflower grains. In the same way the leaves of *Vernonia amygdalina* are better indicated compared to the other organs stem and root. (Sanita di Toppi and Gabbrielli, 1999; Greger,

1999) have shown that Cd is a competitor of Ca at the level of plant roots. Both metals use the same membrane calcium channels. This competition is strong at the stem level and weak in the roots of *Vernonia amygdalina*. The variability in the correlation of toxic metals and essential elements in the organs of the vegetable makes it necessary to observe the plant in different types of soil.

Conclusion

Vernonia amygdalina bioaccumulates lead and cadmium in the root, stem and leaves depending on the concentration of these two metals in the growing soil. There is a positive correlation between lead and essentials elements Fe, Mn, Mg, Ca in the leaves. This is not the case for cadmium. It is negatively correlated with most essential elements in the plant root. Its presence in very high quantities does not guarantee the growth of the plant but its death by burning. Monitoring must be done for the cultivation of the vegetable on soils contaminated by this toxic metal. It is therefore the cause of the blight observed on vegetables. A study is necessary to observe the behaviour of the vegetable with respect to this metal alone and in different types of soil. The correlation between the two metals is strongly positve in the leaves and stem. This does not call into question the presence of the two metals in the soil for the experiment. The existing positive correlation between lead and essential metals does not exclude the monitoring of lead in soil in order to limit its contamination because both are toxic metals that can be transmitted to humans through the food chain. Mg, Fe, Ca, Mn.

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