Nutritional, therapeutic and toxicological risk assessment of *Vernonia amygdalina* cultivated with and without phytosanitary products in two communes in southern Benin

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Abstract: The use of plant protection products raises real concerns for consumers regarding the nutritional and therapeutic quality of *Vernonia amygdalina* and its contamination with metallic trace elements. The aim of this study is to evaluate and compare the nutritional value, phytotherapeutic value, lead and cadmium contamination of the leaves of *Vernonia amygdalina* grown with and without plant protection products. A semi-structured interview allowed us to identify the plant protection products used on the experimental sites. Atomic absorption spectrophotometry was used to determine the mineral elements and toxic metals. The colorimetric and precipitation dosage reveal the chemical elements. The plant protection products used are from the family of pyrethrinoids, neonicotinoids, insecticides, fungicides, fertilizers. We note the presence of alkaloids, catechic tannins, gallic tannins, flavonoids, leuco-anthocyans, quinonic derivatives, steroids, saponosides, reducing compound, mucilages, C-heterosides, O-heterosides.The content of copper, magnesium, iron, calcium, potassium, manganese, zinc, sodium, phosphorus, carbohydrates, proteins, polyphenols, flavonoids, tannins, antioxidants are not significantly different ($p \leq 0.05$). The lead content is not significantly different, while the cadmium content is significantly different: site 1 greenhouse Pb (0.13 ±0.04); Cd: (0.01± 00) real site 1 Pb: (0.17 ±0.03); Cd (0.03 ±00); site 2 greenhouse Pb: (0.15 ±0.11); Cd (0.01± 00) real site 2 Pb: (0.17± 00) ; Cd (0.03± 00).The phytosanitary products do not modify the nutritional and phytotherapeutic value of *Vernonia amygdalina*. The risk of lead contamination is not related to the use of these plant protection products, which is not the case for cadmium.

Keywords: protection products, *Vernonia amygdalina,* lead, cadmium.

INTRODUCTION

Vernonia amygdalina is a nutritional and phytotherapeutic plant. It is a member of the asteraceae family. It is known as bitter leaf in English-speaking countries such as Nigeria; in tropical Africa, South Africa (Afolayan *and al.,* 2006), West Africa such as Cameroon, Ghana, Nigeria and much appreciated for its medicinal value. It is used against infections caused by helminths, protozoa, bacteria (Ibrahim, 2009) and very rich in phytochemical elements such as saponins, alkaloids, terpenes, steroids, flavonoids, phenolic acids, lignans, xanthones, anthraquinones, edotites and sesquiterpenes (Udochukwu *and al.*, 2015).The aqueous extract of *Vernonia amygdalina* reduces metabolism and increases the bioavailability of Nifedipine, a conventional drug used in the treatment of diabetes and hypertension (Owolabi *and al.,* 2013).It is therefore considered as a food supplement (Sodamade, 2013). From the wild, it is domesticated for its nutritional and therapeutic value in urban areas and on market gardening sites, where it is supplied to the populations of large cities. Planted in polluted areas, they absorb toxic metals. Toxic metals are of natural origin such as wind erosion, volcanic activities, sea spray, forest fires. They are of anthropogenic origin such as fossil fuel combustion, use of agricultural inputs, household and industrial waste incineration, poor plastics management have led to an increase in their concentrations in environmental compartments (Lamprea 2009, El Hachimi *and al.,* 2014). All consumables are contaminated with metal trace metals. These metals are toxic because they play no physiological role in human and animal organisms. Lead and cadmium are very harmful to health. Lead is found to be toxic to the nervous system, kidneys (Magali, 2007). It accumulates in the liver, kidneys, brain, heart. It blocks several enzymes necessary for hemoglobin synthesis. Cadmium inhibits thiol enzymes and their affinity for hydroxyl and carboxyl. It accumulates in the kidneys, pancreas, thyroid and liver (Zielonka *and al.,* 2009; Godt *and al.,* 2006). As consumers are informed of the presence and danger associated with exposure to toxic metals, they prefer to focus on crops without plant protection products, which would increase the risk of contamination of fruit and vegetables via soil, air and water. Not only is there a fear linked to the contamination of consumable crops treated with plant protection products with metallic trace elements, but there is also a doubt about the nutritional and phytotherapeutic value of these culinary and medicinal vegetables such as *V. amygdalina*.

The objective of this study is to evaluate and compare the levels of nutritional and therapeutic elements in the leaves of *V. amygdalina* grown with and without plant protection products and their contamination with lead and cadmium.

MATERIALS AND METHODS

The identification of the tonsil *Vernonia amygdalina* leaves was carried out at the Benin National Herbarium under number AA6730 /HNB. A real and controlled growing procedure was carried out on two market gardening sites. After harvesting, the leaves are packed in plastic bags for laboratory analysis. A semi-structured interview allowed us to identify the plant protection products used at both sites. The leaves are divided into two batches in the laboratory. One batch for spectrophotometric analyses; the second batch for phytochemistry screening and quantification of chemical compounds. Phytochemical screening

Phytochemical screening was performed using the standard method based on colouring and precipitation reactions as described by Amoussou *and al.,* 2019.

The determination of mineral elements and the extraction of metals is carried out using the Atomic Absorption Spectrophotometer (AAS) in the Waste Management and Treatment Laboratory of the University of Lomé using the method described by Koumolou, 2014.

This methodology allowed us to obtain results that were statistically analyzed.

Statistical analysis of the results

The results obtained are entered in Excel. This allowed us to obtain the mean values, the standard deviation. The Kruskal-Wallis ANNOVA, the multiple comparison of the average ranks of all groups, allowed us to analyze the results obtained for $p \leq 0.05$.

RESULTS AND DISCUSSION

We have listed the phytosanitary products that are frequently used on both sites and by most market gardeners growing *V. amygdalina*. The nature, trade name, active ingredient, and chemical composition of the plant protection products used are listed in Table 1.

The plant protection products used at the two study sites (Table 1) are of chemical origin in addition to chicken droppings, which are of organic origin. From these results, trace metal elements are not mentioned as elements forming part of the chemical composition of these plant protection products. Boland *and al.,* 2004 showed that inorganic chemicals in plant protection products include sulphide, lead arsenate, copper-lime mixtures, borax and chlorates, and mercury compounds. Pan., 2010 showed that the high amount of cadmium in soils is due to the excessive use of agricultural fertilizers.

Comparative evaluation of the nutritional and therapeutic elements content of *V. amygdalina* with and without treatment with plant protection products.

Phytochemical screening of *Vernonia amygdalina* leaves grown with and without the addition of plant protection products is carried out and presented inTable 2.

These chemical elements contained in the aqueous extract of *Vernonia amygdalina* allow it to have a very powerful phytotherapeutic potential (Adebayo *and al.,* 2014; Iroanya *and al.,* 2014; Adetutu and Olorunnisola, 2013). Plant protection products did not affect the nature of the chemical elements contained in the leaves of *Vernonia amygdalina*. The determination of nutritional and therapeutic element contents makes it possible to know the contribution of phytosanitary products in each type of leaf.

Content of phytotherapy elements in the leaves of *Vernonia amygdalina* with and without the addition of phytosanitary products. The results are shown in Table 3.

The results show that there is no statistically significant difference between the contents on the same site for leaves treated and untreated by plant protection products except lipids. Plant protection products had no effect on the nutritional content of the treated leaves. Atangwho *and al.,* 2009 confirmed that the leaves of *Vernonia amygdalina* contain proteins, vitamins, mineral elements and very rich in phytochemical elements. Its richness in its various elements gives it its effectiveness in the treatment of various pathologies (Ly *and al.,* 2015). Their beneficial effects and their role in modulating the risk of high-prevalence diseases are difficult to demonstrate due to the high variability of their bioactive structures and actions. Estimating the polyphenol content of foods is complex due to their variability in foods and prepared dishes; (Tresserra-Rimbau *and al.,* 2018). Like polyphenols, flavonoids are able to modulate the activity of certain enzymes, to modify the behaviour of several cellular systems.

As for tannins, they are involved in many biological activities revealed by various research studies (Biaye, 2002). Carbohydrates have an energetic role (40 to 50%) of the calories provided by human food are carbohydrates. Lipids represent about 20% of the body's weight, they are a mobilizable energy reserve. Low lipid content is observed in both types of leaves at both sites.

The therapeutic actions of the chemical elements contained in the leaves of *Vernonia amygdalina* are reinforced by the presence of mineral elements. At low doses these mineral elements act in anabolic and catabolic reactions in the body. Mineral element content in the leaves of *Vernonia amygdalina* with and without the addition of plant protection products are presented in Table 4.

The predominant minerals in both types of leaves are Mg, Ca, Na and K, the minority minerals are Cu, Mn, Zn, Fe and P. The supply of phytosanitary products did not modify the levels of these minerals in the leaves grown in real conditions under treatment. There is no significant difference in magnesium and potassium content at site 1 between the leaves of the greenhouse and real medium, while there is a significant difference for these two major minerals at site 2 for the two culture media.

There is a significant difference in iron content between the two leaves at site 1 while at site 2 no significant difference is noted. The variation in the content of Mg, K and Fe in the leaves on the sites may be due to the nature of the inputs of plant protection products that make these minerals bioavailable on one soil compared to another.

These results are confirmed by those of Sodamade, 2013. The use of plant protection products for intensive production reduces the density of microorganisms and makes the soil less fertile (Terras, 2013). This excessive use of plant protection products has consequences for vegetables via the soil. They increase the soil's content of metallic trace elements and make them bioavailable for consumable fruits and vegetables.

Evaluation of toxic metals lead and cadmium in the leaves of *Vernonia amygdalina* grown with and without the use of plant protection products.

The analysis with Atomic Absorption Spectrophotometry of the leaves of *Vernonia amygdalina* gives us the results of Table 5.

The lead content does not differ statistically significantly on sites treated with and without plant protection products $p=0.44$. There is a statistically significant difference in cadmium content at sites 1 and 2 between leaves grown with and without crop protection products $p=0.032$. This result allows us to say that there is no lead input from the plant protection products used at these two selected sites. This is contrary to the work carried out by Schneider *and al.,* 2016a; Schneider *and al.,* 2016b; showed that lead bioavailability is strictly related to soil pH and organic matter content. Taking these results into account, we can say that the intake of plant protection products has contributed to changing the soil pH, the pH at which lead is bioavailable.This is why we did not notice any difference in lead content in the two types of *Vernonia amygdalina* leaves.

The cadmium content differs significantly in the leaves of *Vernonia amygdalina* grown with and without plant protection products. The levels obtained are low in leaves grown under glass without plant protection products compared to those of the real environment grown with plant protection products (Table 5). Plant protection products have not only increased the content of cadmium in the soil but also its mobility by increasing the pH of the medium to 6. KOUAME *and al.,* 2006 showed that cadmium is bioavailable at pH=6. The supply of plant protection products therefore promotes the bioavailability of cadmium in relation to lead at market gardening sites by modifying the pH of the soil and adding mineral elements.

CONCLUSION

The elements provided by the plant protection products do not alter the therapeutic quality of the leaves of Vernonia amygdalina. Nor do they affect the nutrient content. Crop protection products increase and make cadmium bioavailable compared to lead. In order to eliminate or limit the factors contributing to the mobility and availability of trace metals Pb and Cd for the benefit of edible fruit and vegetables, users must participate in their reasonable and controlled use.

REFERENCES

Adebayo OL, James A, Kasim SB and Jagri OP. 2014. Leaf Extracts of *Vernonia amygdalina* Del. from Northern Ghana Contain Bioactive Agents that Inhibit the Growth of Some Beta-Lactamase Producing Bacteria *in Vitro*. *British Journal of Pharmaceutical Research* 4, 192-202.

Adetutu A and Olubukola SO. 2013. Hepatoprotective Potential of Some Local Medicinal Plants against 2-Acetylaminoflourene-Induced Damage in Rat. Journal of Toxicology. 5p

Afolayan AJ, Grierson DS, Eraso P. 2006. Bioactive sesquiterpene lactones from the leaves of *vernonia amygdalina*. *J Ethnopharmacol*. 106, 117-120.

Amoussou FB, Badoussi ME, Houndji S, Odjegnide S, Gbenou J, Kayode P*.* 2019. Essai de Production d'extrait Bioactif et Bioaromatisant à Partir des Racines de *Mondia whitei (Hook f.) Skeels (*Apocynaceae*)* d'écologie Béninoise European Scientific Journal, 15(21), 339-360.

Atangwho IJ, Ebong PE, Eyong EU, Williams IO, Eteng MU and Egbung GE. 2009. Comparative chemical composition of leaves of some antidiabetic medicinal plants: *Azadirachta indica, Vernonia amygdalina* and *Gongronema latifolium.* African Journal of Biotechnology. 8(18), 4685-4689.

Biaye M. 2002. Actions pharmacologiques des tanins. Thèse de doctorat unique en pharmacie. Université Cheikh Anta Diop de Dakar. 44p

Boland J, Koomen I, Jeude JVL, Oudejans J. 2004. Les pesticides : composition, utilisation et risques. Editors: Jeroen Boland, Irene Koomen Fondation Agromisa, Wageningen. 90, 1-9.

El Hachimi ML, Fekhaoui M, Abidi AE, Rhoujatti A. 2014. Contamination des sols par les métaux lourds à partir de mines abandonnées : le cas des mines Aouli-Mibladen-Zeïda au Maroc. Cah Agric 23, 213-9.

Godt J, Scheidig F, Grosse-Siestrup C, Esche V, Brandenburg P, Reich A and Groneberg DA. 2006. The toxicity of cadmium and resulting hazards for human health. Journal of Occupational Medicine and Toxicology, 1-22

Ibrahim TA, Lola A, Adetuyi FO and Jude-Ojei B. 2009. Assessment of the antibacterial activity of *Vernonia amygdalina* and *Ocimum gratissimum* leaves on selected food borne pathogens. Int. *J. Third World Med.* 8(2), 23-24.

Iroanya OO, Adebesin OA, Okpuzor J. 2014. Evaluation of the Hepato and Nephro-protective effect of a Polyherbal Mixture using Wistar Albino rats. Journal of clinical and diagnostic research. 8(6), 15-21.

Kouame IK, Gone DL, Savane I, Kouassi EA, Koffi K, Goula BTA et Diallo M. 2006. Mobilité relative des métaux lourds issus de la décharge d'Akouédo et risque de contamination de la nappe du Continental Terminal (Abidjan - Côte d'Ivoire) *Afrique Science*. 02(1), 39 – 56.

Koumolou L. 2014. Evaluation du risque d'exposition aux mélanges de métaux lourds dans les produits maraichers et essai de réduction des effets toxiques par la cure de Spiruline. Thèse de doctorat unique de l'Université de Lomé. 193p.

Lamprea K. 2009.Caractérisation et origine des métaux traces, hydrocarbures aromatiques polycycliques et pesticides transportés par les retombées atmosphériques et les eaux de ruissellement dans les bassins versants séparatifs péri-urbains. Ingénierie de l'environnement. Ecole Centrale de Nantes (ECN), 296p.

Ly C, Yockell-Lelièvre J, Ferraro ZM, Arnason JT, Ferrier J, Gruslin A. 2015.The effects of dietary polyphenols on reproductive health and early development. *Human Reproduction Update*, 21(2), 228-248.

Magali S. 2007. Spéciation du cadmium, du plomb et du zinc dans les poussières d'émissions atmosphériques d'origine sidérurgique - Approche de l'impact toxicologique des poussières. Sciences de la Terre. Université de droit, d'économie et des sciences - Aix-Marseille III, 334p

Owolabi MA, Adeniji EA, Oribayo OO and Akindehin OE. 2013. Effects of V*ernonia amygdalina* Aqueous Leaf Extract on the Pharmacokinetics of Nifedipine in Rabbits. Journal of Pharmacognosy and Phytochemistry 2 (1), 55-65.

Pan J, Plant JA, Voulvoulis N, Oates CJ, Ihlenfeld C. 2010. Cadmium levels in Europe: implications for human health. Journal of environmental geochemistry and health, 32 (1), 1–12.

Schneider AR, Morvan X, Saby NPA, Cances B, Ponthieu M, Gommeaux M, Marin B. 2016. Multivariate spatial analyses of the distribution and origin of trace and major elements in soils surrounding a secondary lead smelter" Environmental Science and Pollution Research » 23(15), 15164-15174.

Schneider RA, Cances B, Ponthieu M, Sobanska S, Benedetti FM, Pourret O, Conreux A, Calandra I, Martinet B, Morvan X, Gommeaux M, Marin B. 2016. Lead distribution in soils impacted by a secondary lead smelter: experimental and modelling approaches. Science of the Total Environment. 568, 155-163.

Sodamade A. 2013. Proximate Analysis, Mineral Content, Amino Acid Composition and Functional Properties of *Vernonia amygdalina* Vegetable Leaf Protein Concentrates. Greener Journal of Agricultural Sciences. 3(3), 204-210.

Terras Franck. 2013. Les nutriments de nos plantes. www. Lesateliersenherbe. Com. Consulté le 19/112019. 13h00

Tresserra-Rimbau A, Lamuela-Raventos RM, Moreno JJ. 2018. Polyphenols, food and pharma. Current knowledge and directions for future research. Biochemical pharmacology. 156, 186-195.

Udochukwu U, Omeje FI, Uloma IS, Oseiwe F D. 2015. Phytochemical analysis of *Vernonia amygdalina* and *Ocimum gratissimum* extracts and their antibacterial activity on some drug resistant bacteria. American Journal of Research Communication, 3(5), 225-235.

Ly C, Yockell-Lelièvre J, Ferraro ZM, Arnason JT, Ferrier J, Gruslin A. 2015.The effects of dietary polyphenols on reproductive health and early development. *Human Reproduction Update*, 21(2), 228-248.

Magali S. 2007. Spéciation du cadmium, du plomb et du zinc dans les poussières d'émissions atmosphériques d'origine sidérurgique - Approche de l'impact toxicologique des poussières. Sciences de la Terre. Université de droit, d'économie et des sciences - Aix-Marseille III, 334p

Owolabi MA, Adeniji EA, Oribayo OO and Akindehin OE. 2013. Effects of V*ernonia amygdalina* Aqueous Leaf Extract on the Pharmacokinetics of Nifedipine

in Rabbits. Journal of Pharmacognosy and Phytochemistry 2 (1), 55-65.

Pan J, Plant JA, Voulvoulis N, Oates CJ, Ihlenfeld C. 2010. Cadmium levels in Europe: implications for human health. Journal of environmental geochemistry and health, 32 (1), 1–12.

Schneider AR, Morvan X, Saby NPA, Cances B, Ponthieu M, Gommeaux M, Marin B. 2016. Multivariate spatial analyses of the distribution and origin of trace and major elements in soils surrounding a secondary lead smelter" Environmental Science and Pollution Research » 23(15), 15164-15174.

Schneider RA, Cances B, Ponthieu M, Sobanska S, Benedetti FM, Pourret O, Conreux A, Calandra I, Martinet B, Morvan X, Gommeaux M, Marin B. 2016. Lead distribution in soils impacted by a secondary lead smelter: experimental and modelling approaches. Science of the Total Environment. 568, 155-163.

Sodamade A. 2013. Proximate Analysis, Mineral Content, Amino Acid Composition and Functional Properties of *Vernonia amygdalina* Vegetable Leaf Protein Concentrates. Greener Journal of Agricultural Sciences. 3(3), 204-210.

Terras Franck. 2013. Les nutriments de nos plantes. www. Lesateliersenherbe. Com. Consulté le 19/112019. 13h00

Tresserra-Rimbau A, Lamuela-Raventos RM, Moreno JJ. 2018. Polyphenols, food and pharma. Current knowledge and directions for future research. Biochemical pharmacology. 156, 186-195.

Udochukwu U, Omeje FI, Uloma IS, Oseiwe F D. 2015. Phytochemical analysis of *Vernonia amygdalina* and *Ocimum gratissimum* extracts and their antibacterial activity on some drug resistant bacteria. American Journal of Research Communication, 3(5), 225-235.

Tables :

Table I: Nature, trade name, active ingredient and composition of plant protection products used on the sites.

Nature of the plant protection product	Trade name	Active ingredient	Chemical composition
Pesticides (pyrethrinoid)	Lambda super 2.5 EC	Lambda-cyhalothrin	$C_{23}H_{19}ClF_3NO_3$
Fungicides	Mancozeb	Dithiocarbamique	$C_8H_{12}MnN_4S_8Zn$
Fertilizers	Havest More	NPK	N(30),P_2O_5(10),K2O (10),B(0.02),Co(0.002),Cu (0.05),Mn(0.05),Mo (0.005), Zn (0.1)
Fungicides	Topsect-M	Thiophanate-methyl	$C_{12}H_{14}N_4O_4S_2$
Pesticides (Pyrethrinoid + Neonicotinoid)	Plant Master	Lambda-cyhalothrin15+acetamipride20	$C_{23}H_{19}ClF_3NO_3 + C_{10}H_{11}ClN_4$
Pesticides Pyrethrinoid+ Neonicotinoid	K-Optimal	Lambda-cyhalotrin 15g/l et l'Acetamipride 20g/l.	$C_{23}H_{19}ClF_3NO_3 + C_{10}H_{11}$ClN4
Insecticides	Attack	Emamectin benzoate	$C_{56}H_{81}NO_{15}$
Fertilizers	Urea	Nitrogen	CH_4N_2O

Table II: Phytochemical screening of leaves.

Chemical groups	Site 1 greenhouse	Site 1 Real	Site 2 greenhouse	Site 2 Real
Alkaloids	+	+	+	+
Catechic tannins,	+	+	+	+
Gallic tannins	+	+	+	+
Flavonoids	Flavon	Flavon	Flavon	Flavon
Leuco-anthocyans	+	+	+	+
Quinonic derivatives	+	+	+	+
Terpenoids	-	-	-	-
Steroids	+	+	+	+
Cardenolides,	-	-	-	-
Saponosides	+	+	+	+
Cyanogenic derivatives	-	-	-	-
Reducing compound	+	+	+	+
Mucilages	+	+	+	+
Free anthracene derivatives	-	-	-	-
C-heterosides	+	+	+	+
O-heterosides	+	+	+	+

Legend: + = presence of the desired element; - = absence of the desired element; greenhouse = controlled environment without supply of plant protection products; real = environment in which vegetables are treated with plant protection products.

Table III: Nutrient content, therapeutic elements in the leaves of *V. amygdalina* with and without phytosanitary products.

Samples	Glucides	Proteines	Lipides	Polyphenols	Flavonoïdes	Tanins	Antioxydants
Site 1greenhouse	354,842 ±1,953**a**	37,435 ±0,764 **a**	0,580 ±0,032 **b**	335,957 ±5,002 **a**	236,263 ±15,331 **a**	8,499 ±0,002 **a**	1,461 ±0,158 **a**
Site 1 real	230,891 ±1,380**a**	69,284 ±1,109 **a**	2,572 ±0,027 **a**	320,775 ±0,514 **a**	213,031 ±6,655 **a**	8,890 ±0,261 **a**	0.702 ±0.166 **a**
Site 2greenhouse	170,469 ±0,596**b**	78,911 ±0,704 **b**	0,733 ±0,021 **b**	309,245 ±11,085 **b**	273,109 ±3,410 **b**	9,546 ±0,147 **b**	0,491 ±0,043 **b**
Site 2real	238,154 ±0,567 **b**	77,504 ±0,455 **b**	1,380 ±0,003 **b**	326,380 ±1,734 **b**	236,357 ±12,278 **b**	10,985 ±0,708 **b**	0,717 ±0,082 **b**

Legend: The numbers with the letters a for site 1 and b for site 2 for a metal are not significantly different (p≤0.05).

Table IV: Mineral element content in the leaves of *Vernonia amygdalina* with and without the addition of plant protection products

Samples	Cu en mg/kg	Mg en mg/kg	Ca en mg/kg	Mn en mg/kg	Zn en mg/kg	Fe en mg/kg	Na en mg/kg	K en mg/kg	P en mg/kg
Site1greenhouse	2,96 ±0,006**a**	3371 ±16,093 **a**	8846,88 ±17,80 **a**	26,440 ±0,097 **a**	14,597 ±0,350 **a**	36,585 ±0,263 **a**	142,533 ±32,197 **a**	10913,889 ±38,491 **a**	0,333 ±0,027 **a**
Site1Real	1,684± 0,021**a**	3729,44±68,664 **a**	8183,778 ±605,6 **a**	34,521 ±0,025 **a**	13,464 ±0,626 **a**	104,217 ±0,136 **b**	516,344 ±22,682 **a**	15215,77 ±379,85 **a**	0,070 ±0,001 **a**
Site2grenhouse	3,250 ±0,283 **b**	5193,667 ±115,335 **b**	10451,66 ±773,5 **b**	55,660 ±2,122 **b**	12,311 ±0,577 **b**	44,184 ±2,387 **b**	138,878 ±39,808 **b**	15720,11 ±301,99 **a**	0,118 ±0,009 **b**
Site 2 Real	7,955 ±0,231 **b**	3131,889 ±186,066 **a**	7967,556 ±95,09 **b**	66,030 ±0,786 **b**	9,493 ±0,108 **b**	53,053 ±2,363 **b**	252,400 ±65,587 **b**	9556,555 ±525,02 **b**	1,105 ±0,093 **b**

Legend: The numbers with the letters a for site 1 and b for site 2 for a metal are not significantly different (p≤0.05). Copper=copper, Mg=magnesium; Ca=calcium; Mn=manganese; Zn=zinc; Fe=iron; Na=sodium; K= potassium; P= phosphorus

Table V: Lead and cadmium content of *Vernonia* leaves at greenhouse and actual sites.

Heavy metals		Site 1 greenhouse	Site 1 Real	Site 2 greenhouse	Site 2 Real
Pb (ppm)	Maximum	0.162	0.193	0.243	0.171
	Minimum	0.087	0.141	0.038	0.104
	Average	0.126 **a**	0.172 **a**	0.154 **a**	0.148 **a**
	standard deviation	±0.038	±0.027	±0.105	±0.039
Cd (ppm)	Maximum	0.011	0.029	0.013	0.032
	Minimum	0.01	0.024	0.004	0.027
	Average	0.011**b**	0.026 **c**	0.01 **b**	0.029 **c**
	Standard deviation	±0.0006	±0.0029	±0.0046	±0.0028

Legend: Numbers that have the same letters on the same site for a metal are not significantly different.