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Original Research Article

Establishment and bioavailability of lead and cadmium in soils and *Vernonia amygdalina* at market gardening sites in Southern Benin

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Abstract

Vegetables from market gardening sites are contaminated with lead and cadmium. Agricultural inputs such as fertilizer and contaminate from the environment are suspected of making these toxic metals bioavailable. The research aims to establish the parameter that makes lead and cadmium bioavailable in soils for *Vernonia amygdalina* at market gardening sites in Southern Benin. Different agricultureral inputs were used on market gardening sites in southern Benin on *Vernonia amygdalina* crops in a controlled environment. The impact of the inputs on plant growth by measuring stem length and leaf biomass was evaluated. The levels of lead and cadmium in leaves was determined using Atomic Absorption Spectrophotometry. Fisher's analysis of variance, Dunnett's test showed that only the mixing of inputs had a positive impact on plant growth (significance p=0.05). Using plants that received no inputs as a reference, all other parameters contributed to an increase in both toxic metals. For Cd (chicken droppings < urea <pesticides <contaminated water <mixture), and Pb (pesticides <urea <chicken droppings < contaminated water <mixture). It would be very difficult to raise awareness against the use of agricultural inputs because of their positive impact on agricultural yield. The research concludes that lead and cadmium are bioavailable in soils for *Vernonia amygdalina* at market gardening sites.

Keyword: Bioavailability, Cadmium, Lead, *Vernonia amygdalina*

INTRODUCTION

Toxic metal contamination of crops on market garden sites is a concern for consumers and many researchers (Agueh *et al.,* 2015 ; Yehouenou *et al.,* 2010 ; Koumolou *et al.,* 2014). On this basis, several studies are being conducted to find out the true source of these contaminants among the inputs used and irrigation water. Some researches have shown that agricultural inputs are the sources of external inputs of toxic metals (Fangnon *et al.,* 2012 ; Rico and Rosa, 2000), others claim that contaminated irrigation water is the true source of this contamination (Koumolou *et al.,* 2013). In addition, El Gharmali et al., 2010 ; Harge 1997 showed that the metal can be present in the soil without being made available to the plant. And this depends on the speciation of the metal, the chemical parameters of the soil (granulometry, pH, solubility, organic matter). Trace metal elements are multiple in nature. Others are trace elements such as copper, zinc, iron which participate in anabolism and catabolism reactions at low doses and toxic at high doses (Brigo, 1993). Some such as lead and cadmium are highly toxic and are not biodegradable. Cd accumulates in particular in the kidneys and liver (El Idrissi, 2009). The

Table 1. The different treatments applied to plants in vegetative vases

Vegetable vases	V1	V2	V3	V4	V5	V6
Inputs	A	B	C	D	E	F

Legend: V= Vegetation silt; A= Soil + site water; B= Soil + 300mL site water containing 5.06ppm lead and 1.53ppm cadmium; C= Soil + site water + chicken liverwort (organic fertilizer); D= Soil + site water + chemical fertilizer (urea); E= Soil + site water + pesticides; F= Soil + treatment in V2, V3, V4, V5.

intestine absorbs 4-7% of a single dose of Cd ingested in humans (Kitamura *et al.,* 1970; Rahola *et al.,* 1971). About 10% of ingested Pb is absorbed by the human body in adults (USEPA, 1986). In the blood, more than 95% is mobilized by erythrocytes and then distributed to all soft organs (El Idrissi, 2009). It accumulates in the liver, kidneys, brain, heart and only about 3% remains in the plasma (Moore *et al.,* 1977). The presence of these two toxic metals in our daily consumable fruits and vegetables poses a real public health problem. Awareness of market gardeners would be handicapped enough if the source of this contamination is not well specified. *Vernonia amygdalina* is a vegetable used in nutrition and phytotherapy. Populations in large cities get their supplies in the towns and on market garden sites. This plant bioaccumulates lead and cadmium on market gardening sites (Deguenon *et al.,* 2020). In order to limit the risks of toxic metal contamination of *Vernonia amygdalina* in particular and of all market garden products in general on market gardening sites, we need to determine among agricultural inputs and contaminated irrigation water which makes toxic metals bioavailable and then the impact of these external soil components on plant growth.

MATERIALS AND METHODS

Soil from a market gardening site is collected for the cultivation of *Vernonia amygdalina* under glass to eliminate any foliar contamination. Eighteen 3kg growing pots are set up for the crop. Each vase received 3kg of soil. The vases are numbered and randomized. The soil contained in the vases is sprinkled with 100mL of water from the experimental site before the seedlings are planted and their roots are rinsed with water beforehand. Each plant receives 100mL of groundwater per day for 4 weeks prior to treatment. Plant treatment procedure (table 1)

Plants are harvested 6 weeks after treatment. The fresh leaves are washed with deionised water and dried in an oven at 100°C for 12 hours. The dry leaves are weighed to determine the biomass according to the inputs. They are then reduced to powder using a porcelain mortar and stored in aluminium foil for analysis with a spectrophotometer. The length of the stems is measured to assess plant growth.

Statistical analysis of results

The statistical software STATICA and XLSTAT 2019 allowed us to perform the statistical analysis of the results. The simultaneous comparison of several averages including the analysis of Fisher's variance (ANOVA) allowed us to appreciate the different contributions on the stem length and the dry matter mass of the leaves of Vernonia amygdalina. The descriptive statistics of the variables are made as well as the Pearson's correlation coefficient for lead and cadmium concentrations contained in the leaves. The significance level is set at 0.05.

RESULTS

Analysis of stem length as a function of inputs.

The length of the rods is measured with a length measuring instrument. The analysis of these results allowed us to determine the impact of the application of phytosanitary products on the length of *Vernonia amygdalina* stems. Figure 1

The results show that the nature of the input significantly influences the length of the stems. Plants treated with irrigation water containing lead and cadmium were shorter than the controls. On the other hand, in increasing order, pesticides, chicken droppings and urea had a positive impact on stem length. Multiple comparison of means and post-hoc analysis of differences (Dunnett's test; stem length variable) shows that the mean stem lengths of plants subjected to water containing lead 5.06ppm + 1.53ppm cadmium, chicken droppings, pesticides are not significantly different from those of control plants (which did not receive any treatment, $p>0,05$; with a mean length of 28.33). On the other hand, those of the plants subjected to urea and mixing (Pb + Cd, chicken manure, urea, pesticides) are significantly different from those of the control plants (which did not undergo any treatment, $p <0.05$; with a mean length of 28.33) see Table 2 of the p values.

Analysis of dry leaf mass (biomass) as a function of inputs.

Biomass results allowed us to deduce the influence of

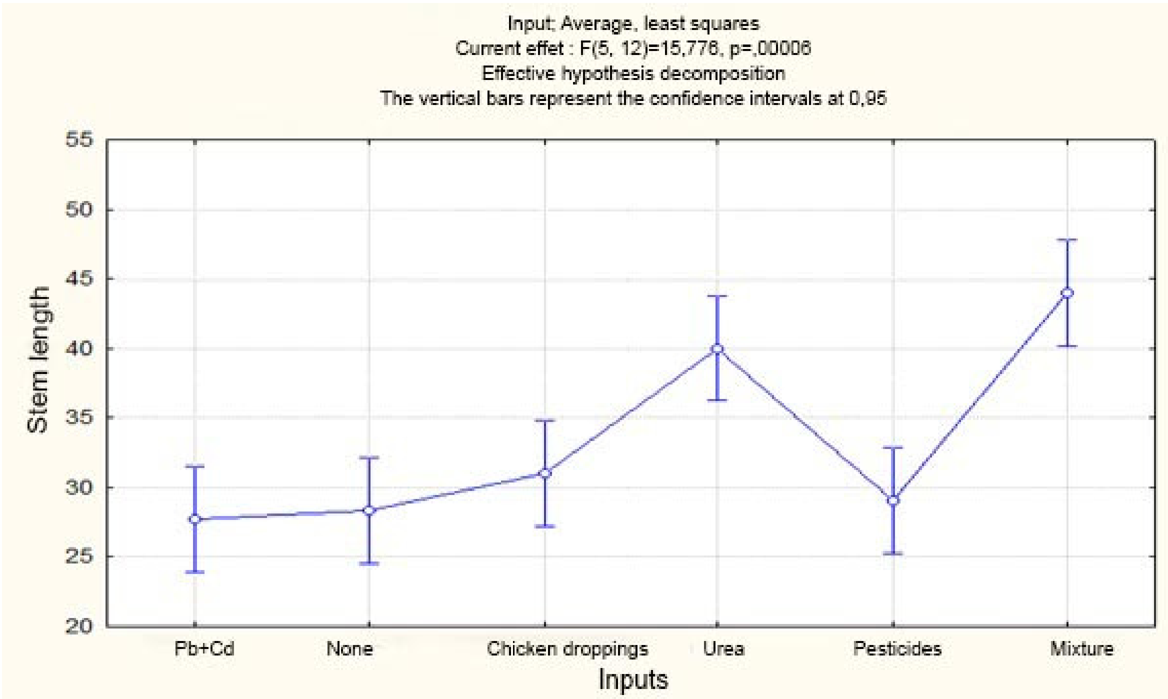

Figure 1. Fisher's analysis of variance for stem length as a function of inputs

Table 2. The p-value showing differences in stem length.

Inputs	p
Pb+Cd	0,999
Chicken droppings	0,723
Urea	0,002
Pesticides	0,999
Mixture	0,0001

Legend: Pb=lead; Cd=cadmium

inputs on dry leaf mass. Fisher's analysis of variance shows that the nature of the inputs significantly influences the biomass ($p<0.05$) see figure 2.

Plants subjected to watering containing lead and cadmium have a lower biomass than untreated control plants. In ascending order pesticides, chicken manure, urea and then mixing positively influenced biomass compared to control plants. Post-hoc analysis of the differences (Dunnett's test; variable: dry matter mass) shows that apart from the mixture there is no statistically significant difference compared to the controls (Table 3). Inputs therefore do not influence biomass when used in isolation. They then have a synergistic action on plant biomass.

Evaluation of the concentration of lead and cadmium in *Vernonia amygdalina* leaves.

The analysis at the SAA allowed us to evaluate the lead and cadmium concentrations contained in the leaves of *Vernonia amygdalina* treated by the different inputs. Table 6

The theoretical average is 0.18 for lead and 0.12 for cadmium with a significance level of 5%.The null hypothesis (H0): the difference between the averages is 0.The alternative hypothesis (Ha): the difference between the averages is different from 0.The calculated p-value is less than alpha=0.05 (significance level). The null hypothesis is therefore rejected. The lead and cadmium concentration in leaves is statistically different depending on the inputs. The figure below allows us to quantify the lead and cadmium concentration of the different intakes.

If we take the lead concentration found in leaves with no intake as a reference, we can see that each intake has a positive impact. In ascending order, pesticides and urea come first, chicken droppings, contaminated water and then mixing. There is a synergistic action of the different inputs in the contamination of the leaves. Contaminated water presents more risk in the availability of lead for *Vernonia amygdalina* plants. Figure 4

The concentration of cadmium brought by the various

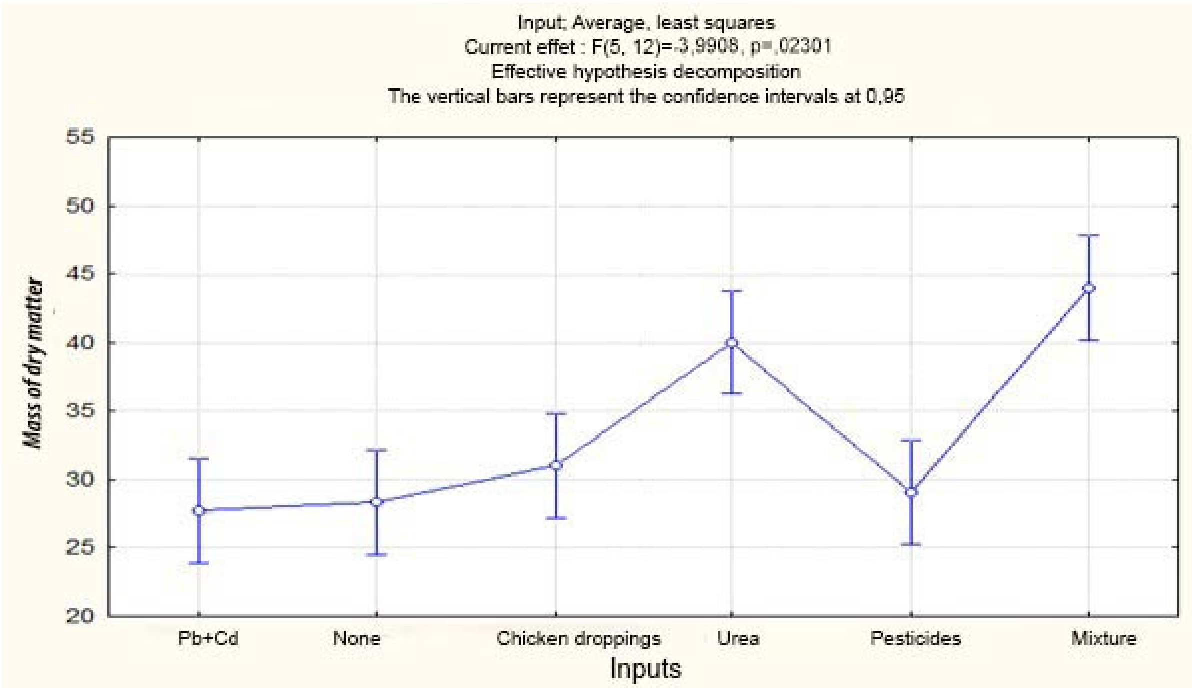

Figure 2. Analysis of variance of Fisher dry leaf mass versus input

Table 3. The value of p showing the differences between the masses of dry matter

Inputs	p
Pb+Cd	0,858
Chicken droppings	0,316
Urea	0,178
Pesticides	0,926
Mixture	0,045

Table 4. Descriptive statistics of observations (Pb)

Variable	Observations	Obs.with missing data	Obs. without missing data	Minimum	Maximum	Average	Ecart-type
Pb	5	0	5	1,205	3,405	2,007	1,011

Table 5. Descriptive statistics of observations (Cd)

Variable	Observations	Obs. with missing data	Obs. without missing data	Minimum	Maximum	Average	Ecart-type
Cd	5	0	5	0,270	0,657	0,459	0,163

Table 6. z-test for a sample/bilateral test for Pb and Cd concentrations

Difference	1,827
z (Value observed)	4,040
|z| (critical Value)	1,960
p-value (bilateral)	< 0,0001
alpha	0,05

Difference	0,339
z (Value observed)	4,638
|z| (critical Value)	1,960
p-value (bilateral)	< 0,0001
alpha	0,05

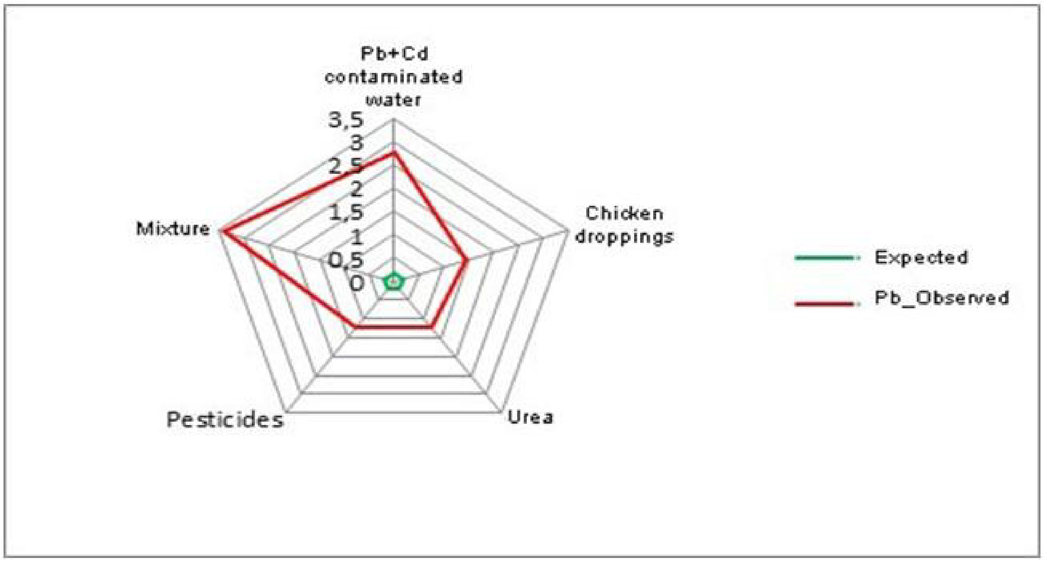

Figure 3. Concentration of lead in sheets according to intake

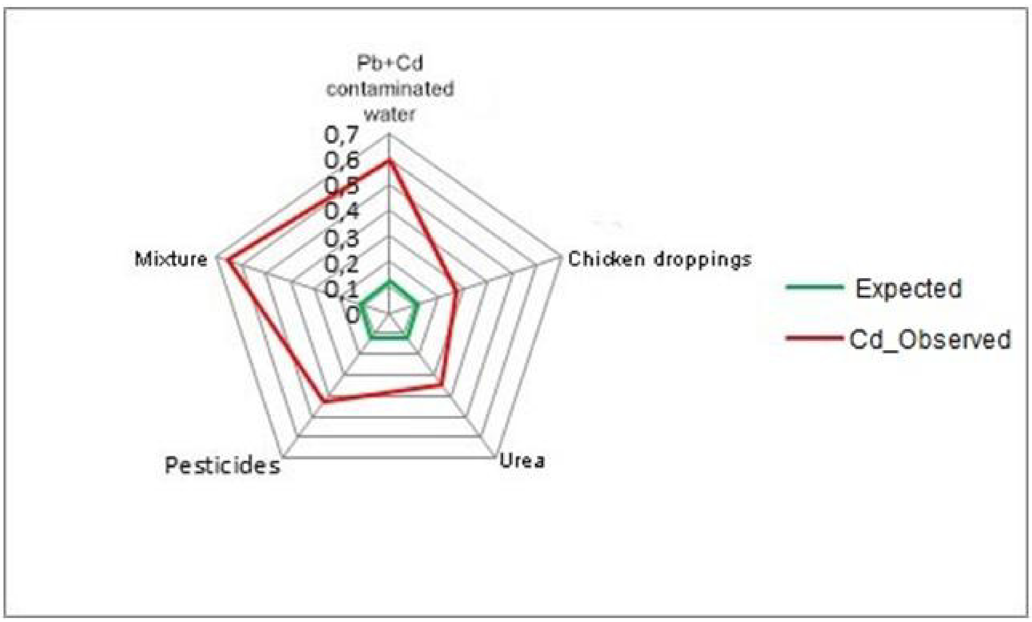

Figure 4. Cadmium concentration in leaves according to intakes

inputs is in increasing order chicken droppings, urea, pesticides, contaminated water and then the mixture. The different inputs have a synergistic effect in relation to the cadmium concentration. Apart from the chicken droppings, all other inputs make cadmium more bioavailable in the soil.

DISCUSSION

The results of the impact of inputs on plant growth, stem specifically length and biomass, showed that only mixture had a statistically significant positive influence. This shows the interest of market gardeners in the use of a whole mixture of agricultural inputs. The synergistic effect of the products is favoured to increase yield in a very short time. In this process of yield improvement, ignorance is observed with regard to toxic metal contamination. At low doses below the standard, the danger to the consumer can be neglected. Chronic exposure to these metals is the most important element to be taken into account. For this reason, all traces of these elements must be taken into conside-ration. All inputs have contributed to an increase in the concentration of lead and cadmium in leaves. Contaminated irrigation water significantly increased the concentration of lead and cadmium. But it is not the only source of toxic metal contamination. This result is confirmed by the work of Sharma *et al.,* 2016, who observed high concentrations in vegetable crops watered with groundwater. Acidifying fertilizers such as nitrogen in the form of ammonium cause an increase in the transfer of toxic metals to the plant. Also sulphur inputs as a fungicide lead to soil acidification and increased transfer of trace elements to plants (Loué, 1986; Wu, 1989). Lead and cadmium contamination of *Vernonia amygdalina* leaves is observed with the application of urea. In an attempt to bypass the chemical treatment, knowing that this causes the toxic trace metals to be made available to the plants, some producers are turning to the use of composts which are also dangerous in terms of their composition. Thus the anthropic contributions in the soils by industry, phosphate fertilizers, the spreading of waste make the Cd biodisposable towards the plants. (Tremel-Schaub A and Feix I, 2005). Chicken manure is a very privileged organic fertilizer in so-called "organic" crops. This fertilizer contains a non-negligible quantity of toxic metals lead and cadmium. Work by Dougnon *et al.,* in 2014 showed that chicken droppings are very rich in lead and that anaerobic digestion reduces it to one tenth. This reduction observed in an anaerobic environment does not exclude a follow-up of the use of the product on different types of soil before its extension proposed by the author on a large scale. Acidic soils may contradict the satisfactory results observed, as lead is bioavailable at acidic pH like many metals (Schneider *et al.,* in 2016). The presence of nitrogen compound in chicken droppings makes the environment very acidic. Anaerobic digestion has shifted the pH above the mobility pH of lead through the reduction of the nitrogenous material. Vigilance is required for the author's proposal. Lead is a non-biodegradable metal. When it is present in a medium it can be chelated and therefore not bioavailable and not disappear. Chicken droppings also contain a significant quantity of cadmium, which is observed through the leaves of the vegetable, as well as urea and pesticides. The metal may be absent in the input but its composition contributes to the mobility of the metal in the soil. The input can act by modifying the physical and chemical parameters of the soil or directly on the metal through the phenomenon of speciation. The salts brought during fertilization by industrial activities have an important part in the assimilation of toxic metals by plants.

CONCLUSION

The positive impact of the mixture of agricultural inputs on plant growth factors justifies their use by market gardeners. Vegetables resulting from the mixture of inputs are contaminated with lead and cadmium. Taken alone, agricultural inputs also contributed significantly to the contamination of the vegetable Vernonia amygdalina by the two toxic metals. Chicken droppings used as organic inputs for so-called organic crops are not exempted from lead and cadmium contamination sources. The difficulty persists as to which can be proposed for disposal in order to guarantee the toxicological quality of the plant. A moderate use of these inputs is required by users. Irrigation water previously contaminated by the two toxic metals is the source of contamination. The total suppression of the use of agricultural inputs would meet a lot of resistance. The only parameter that can be reliably controlled is the substitution of contaminated surface irrigation water and well water by deep well water. Similarly, a probable limit to the contamination of fruit and vegetables produced on market gardening sites depends on the choice of soil, the characteristics of the plant and the control of the use of acidifying inputs for the crop.

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