Phytoextraction of Lead and Zinc in Garages in Southern Benin using *Lantana camara*

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Abstract The ability of *Lantana camara* to accumulate lead (Pb) and zinc (Zn) was studied in a continuous phytoremediation experiment in the soils of four garages in southern Benin. The soil collected in each garage using a manual auger at a depth of 30cm by the diagonal method was characterized. The cultivation of about twenty *L. camara* seedlings from nursery was conducted for each garage on a 2m x 1.5m spacing. The experiment was carried out over 12 weeks from April to June 2018. Sampling was collected from each garage every week during the first month, then every two weeks during the second month and then a last sampling was done during the third month. The levels of lead and zinc in soils and dry biomasses of plants were measured using the dithizone and Zincon methods, respectively. The results of these analyses showed an increase in the content of lead and zinc in dry biomasses with the wastes. The concentrations of lead and zinc in the soil range from 10.31 mg/kg to 11.45 mg/kg and from 40.72mg/Kg to 193.03mg/Kg. The high accumulation capacity of *L. camara* was recorded in the first eight weeks for lead and varied and increased over the twelve weeks for Zinc. In this continuous phytoremediation experiment, *Lantana camara* absorbed up to 68.32 mg/Kg of lead and 7662.67 mg/Kg of zinc.

Keywords *Lantana camara*, car garage, concentration, heavy metals, phytoremediation

Introduction

Phytoremediation is an alternative technique for extracting heavy metals from contaminated soils. It is an ecological and biological technique very effective when compared to chemical and physical treatments that are environmentally friendly and affect the properties of soils, destroy biodiversity and can make the soil useless for agriculture [1]. Various plants are used in phytoremediation The metal tolerance of plants varies from one to the other; for example, Indian mustard (*Brassica juncea*) is effective in accumulating Cd, Cr, Cu, Ni, Pb and Zn [2] while sunflower (*Helianthus annuus*) [2] and wild sage (*Lantana camara)* [3], effectively removes lead.

According to the work carried out by various authors in the literature, [4,5], it appears that *L. Camara* has a very important phytoremedial action. This was confirmed by heavy accumulations of lead, cadmium, nickel and chromium obtained in the literature, with better removal of lead [6]. Otherwise, *in vitro* phytoremediation experience of soils contaminated with heavy metals with two plants (*Datura inoxia* and *lantana camara*)

demonstrated a strong performance of *L. camara* in the accumulation of metals compared to *Datura inoxia* [7]. [8] reported that the bioaccumulation factor of *L. camara* in Nickel is greater than one, which makes this plant, a nickel accumulator compared to *Polygonum glabrum*. Recently the work of [9] showed that the *L. camara* is a Cadmium hyperaccumulator with a high tolerance in this metal , an effective coordination of photosynthesis and a strong reactivity.

Moreover, it emerges from the literature that this plant can develop under extreme conditions [5,10], and is very resistant to the climatic conditions of hot countries, supporting long periods of drought in full sun or heavy rain [11]; in addition, the plants of *L. Camara* require little maintenance, spread easily and even are desired for landscaping [12]. *L. camara* known in southern India [13], Malaysia, Zimbabwe and South Africa [14,16] as an invasive plant, has advantages in many fields: in agriculture as an antifungal [17] and bio pesticide [18]; [19]; *L. camara* acts on the property of the soil to promote rice cultivation [20]; in medicine this plant is used very often as an antibiotic [21], it is a natural antioxidant for therapeutic use [22]. In addition, the weeds *Eichhornia crassipes* and the alga *Microcystis aeruginosa* can be inhibited by the dead leaves of *L. camara* [23,24]. Its use in the environment is also very important and contributes to the Sustainable Development Goals. The literature reports its effectiveness in soil remediation [5,6,25], as well air pollution control [20].

In light of the literature on the use of *Lantana Camara* in phytoremediation, the effectiveness of this species in the rehabilitation of soil contaminated with sewage oils has not yet been studied. To this end, the purpose of this study is to evaluate the effectiveness of *L. camara* in restoration of soil contaminated with used waste oils.

Materials and Methods
Nursery Setting
Hundred cuttings of *L camara* of about 20cm in length were planted in nursery at the Centre Technologique Pratique pour l'Eau et Assainissement (CTPEA) of the National Water Institute at the University of Abomey – Calavi. These nurseries were watered twice a day with 200mL of tap water.

Photo: L. camara nursery

Soil Collection and Seeding of *L camara*
**Collection of Soil**
Four garages were identified for soil sample collection. These garages are located in three municipalities (two in Abomey -Calavi, one in Cotonou and one in Sémé-Podji)
Soil was collected in each of these garages at oil change sites. The collection was done using a manual auger 30 cm deep by the diagonal method. At each garage, soils collected at the diagonal points and at the centre of 30 cm depth were mixed. A representative sample of this mixture was collected for laboratory analysis. The discharge time of waste oil was 11 months, 5 years and 10 years.

Table 1: Geographic Coordinates of Sampling Sites

Sampling Sites	Geographical Coordinates	
	Longitude	Latitude
Soil under plants (UAC)	002°20'19.4	06 2446.7
Garage 1 (calavi)	002°20'39.2	06 2517.1
Garage2 (Godomey)	002°20'24.3	06 2315.2
Garage3 (Cotonou)	002°22'10.5	06 2243.9
Garage4 (Agblangandan)	002°2936.9	06 2223.9
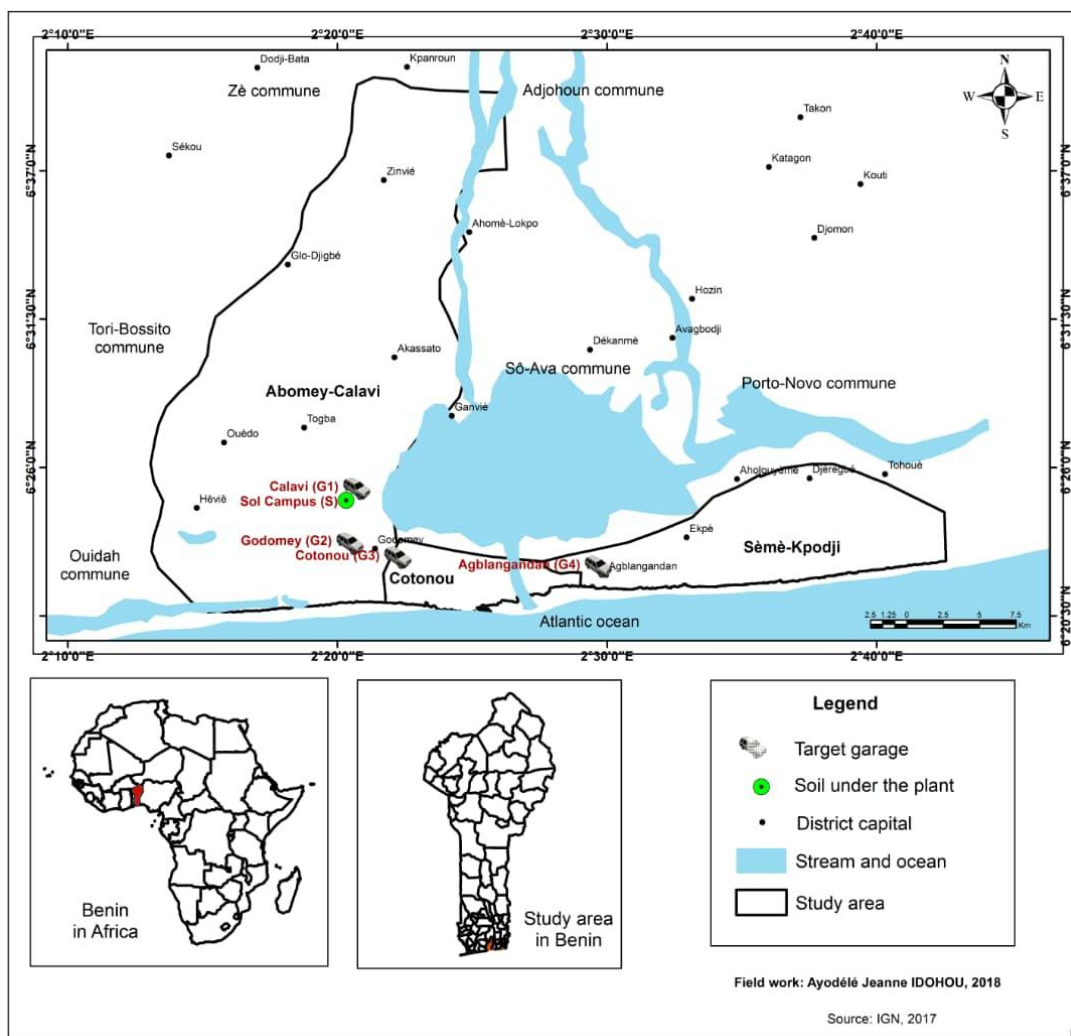
Seeding of *L camara*

About 20 plants are sown in an area of 1.25 * 1.25 m^2 per garage just after the soil was collected.

The plants were watered with 200mL of tap water twice a day (morning and evening). One plant was harvested per garage each week during the first month, every two weeks during the second month and once in the third month.

Characterics of Soil Samples

Soils collected in car garages and stored in polyethylene bags were characterized in the laboratory. Six soil samples were characterized: four samples collected from car garages, one sample of the manure used and one soil sample collected from soil where *Lantana camara* were growing

The samples were subjected to two following treatments [26].

- A first treatment in which soil samples were dried at about 70°C in crucibles that were thoroughly washed, then ground and sieved using 2 μm sieve
- For the second treatment, a sample of 0.1 to 0.5 g (depending on the appearance in terms of the abundance of metals in the sample) of each sample is dried and ground in 100ml digesdahl flask to which 4 mL of concentrated sulphuric acid was added. The mixture was then heated up to 440°C in mineralizer and allowed to carbonize for three to five minutes. 30% oxygenated water was added with a capillary funnel, then fractions of 5mL were added until the mixture became quite clear. After the evaporation of oxygenated water, the flask was removed from the mineralizer. And its content was supplemented with distilled water up to 100mL. The parameters evaluated were pH_{water}, pH_{KCl}, electrical conductivity, organic matter, zinc and lead from soils, the metal parameters mentioned above were only determined from plant biomass.

pH (Electrometric Method)

It is determined in the soil solution in a 1 / 2.5 ratio by direct reading on the pH meter.

50 mL of distilled water was added to 20 g of soil sieved in 2 mm mesh. The solution was stirred for 15 min using magnetic stirrer. Then left to rest for 30 min. after which the pH of solution was measured.

The pH of KCl was determined after adding 3.72g of KCl to the previous solution and following stirring for 5 min. (We carefully washed equipment between each measurement).

Where, $pH = pH_{KCl} - pH_{H2O}$ reveals the potential acidity or exchange acidity

Electrical Conductivity

The conductivity values were determined using the conductivity meter, immediately after measuring the pH_{H2O}.

The organic matter of soils was determined by the oven method according to the formula

% MO = $(P_2 - P_3) * 100 / P_1$

Where:

P_1 is the weight of initial soil plus crucible placed in the oven at 550 °C for 2 hours,

P_2 the weight of the soil after drying in the oven at 105 °C for 24 hours,

P3 the weight of the soil after drying in the oven at 550 °C for 24 hours

Zinc Content

A precise volume (25mL) of the solution was neutralized with the addition of 5N NaOH with pH value varying between 4 and 5 and twice the initial volume was added. Readings of various dilutions from spectrophotometer were compared to the mineralized and neutralized sample with Zincover reagent without cyclohexanone. The detection limit for Zinc was 0.01mg/L.

The zinc content was given by the following formula:

Zn (mg/kg) = (A x 5000)*factor/ (B x C) where

A is the value read on the device in mg/L; B: the weight engaged for mineralization; and C is the volume of neutralized solution of the sample for analysis.

Lead Content

The method used to assess the lead content of the samples was that of Dithizone. This method used the Dithiver reagent, which is a stable form of dithizone powder. Lead ions in basic solution react with dithizone to form a pink to red lead-dithizonate complex, which was extracted by chloroform. The principle is based on differential solubility

The procedure is as follows:

Distilled water was added to 20mL of the mineralized products up to 250ml then poured into a 500mL lab round glass bottle. To this citrate buffer for heavy metals was added. The round glass bottle was tightly closed then stirred to dissolve the reagent. At the same time, 30mL of chloroform was measured in a graduated test tube with the

addition of Dithiver reagent. The capped test tubes was stirred up several times to allow mixing of the solution. In addition, 20mL of 50% sodium hydroxide and 0.1 g of potassium cyanide were added to the lab round glass bottle. After vigorous agitation for 15min, the cap was removed to allow the contents to rest for one minute. Dithiver's solution was then added to the lab round glass bottle. The lab round glass bottle was then capped, inverted and its valve was open to release the pressure. The lab round glass bottle was stirred once or twice after closing the valve

Finally, after a vigorous stirring of 60 minutes and one minute of rest, a small piece of cotton was inserted into the flow tube of the round glass bottle in order to slowly extract the lower layer (the Chloroform complexed) in a clean, dry colorimetric flask, this was the prepared sample. The white constituted only the chloroform in another colorimetric flask. The reading of the molecular absorption was done through spectrophotometer.

Lead content in ppm is given by the expression

$$\boldsymbol{Pb}\left(\frac{\boldsymbol{mg}}{\boldsymbol{l}}\right) \boldsymbol{ou}\,(\frac{\boldsymbol{mg}}{\boldsymbol{kg}}) = \frac{\boldsymbol{A}\ \ X25}{\boldsymbol{B}\ \ X\ \ \boldsymbol{C}}$$

With:

A: Value read on the instrument in µg/L.

B: Mineralized volume in mL.

C: Sample volume to be read in mL

Characterization of Plants

24 samples of the macrophyte collected during the 12 weeks of experiments in garages were characterized: 15 samples during the first four weeks, 6 samples for the next 4 weeks and 3 samples for the last 4 weeks. For each sample after drying at 70°C to a constant mass [27], all parts of the plant (leaves, stem and roots) are were grinded and a mass of about 0,5 g was mineralized [28].

The evaluation of the zinc and lead content followed the same procedure as above [26].

Results and Discussion

Characterization of Soils

Table 2: Characterization of soils

Parameters	G1	G2	G3	G4	T	S	Average	Standard deviation
pHeau	7.5	6.1	7.6	7.1	7.6	6.5	7.07	±0.63
pHKCl	7	5.6	7.2	6.7	6.9	6.2	6.6	±0.6
Ec	31	28	79.5	26.7	28.7	32.8	37.78	±20.55
Orgnique Material (%OM)	3.9	3.8	6.5	3.8	11.1	1.3	5.06	±3.38

pH is very important in soil dynamics. It has an influence on the bioavailability of nutrients, biological activity and structural stability. Compared to the work of [29] , the pH values of the soils studied were : Weakly acidic (6-6.5) for G2; Neutral (6.5-7.3) for S and G4 and weakly basic (7.3-7.8) in the case of G1, G3 and T.

The conductivity of a soil or sediment was a measure of the quantity of ions present in the soil or sediment and which could be found in solution in water. For solids, the conductivity can be reported in mS / cm or in salinity (S = A / 1000 with, S the Salinity expressed in mS / cm, A the Conductivity expressed in µm / cm and 1000 the correction factor between mS / cm and µm / cm) [30]. The values obtained in the analysis of the electrical conductivity of the soils studied compared to the standards [29], show that soils G1, G2, G4 and T were highly saline (Ec between 16dS / m and 32 dS / m) while the soils S and G3 were very highly saline (Ec greater than 32 dS / m)

Soil organic matter is an important indicator for assessing the level of degradation of soil quality. Indeed organic matter contributes to the stability of the soil, to the increase of the water retention capacity of the soil, to the fixation of mineral elements and the substrate for soil microorganisms [29].

The organic matter contents of the soils studied compared to standards [29] show that soils G1, G2, and G4 were rich in OM (between 3% and 6%); sample S was poor in organic matter which is obvious since it is the soil under the plant, the majority of the organic matter in the soil is used by the plant for its growth and the branches of the shrub prevent possible photosynthesis. For G3, the organic matter content was more or less greater than 6%. The soil under this garage is very rich and should allow the development of plants. The OM content of sample T was well above 6%, which is normal since it is this soil that supplied the nutrients necessary for the growth of seedlings in the nurseries.

Lead and Zinc Content in Garage Soils

The physicochemical characterization of the soils at the experimental sites revealed lead contents of between 10.31mg / Kg and 11.45mg / Kg then values of zinc concentrations between 40.72mg / Kg and 193.03mg / kg. The highest values in Lead (11.45mg / Kg) and zinc (193.03mg / Kg) were from site-G3 in the heart of the city of Cotonou. The water table in these surroundings was less than 1m above soil surface.
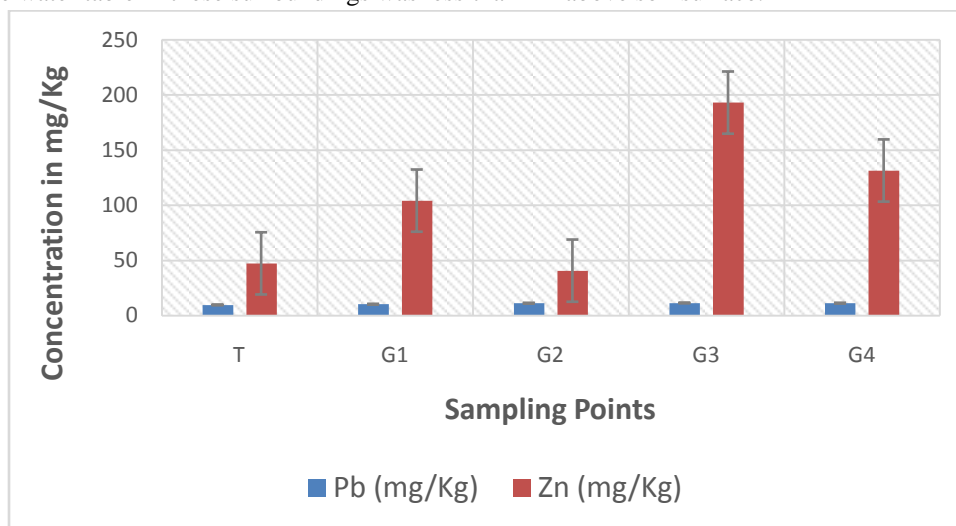

Figure 1: Lead and Zinc content of soils at experimental sites

The four garages have almost the same lead concentrations. The values were around 11mg / Kg. The lowest concentration (10.31 mg / Kg) was observed in the garage1 located in Calavi and the highest value (11.45 mg / Kg) was observed in the garage 3 located in Cotonou. These soil concentration values under the garages compared to those of the soil where *L camara* grew (9.68 mg / Kg), do not show a significant difference ; however, they should not be overlooked given the danger posed by the presence of lead in the environment. As a result, the sites under study are not too polluted in Lead. The lead concentrations found remain below 50 mg / Kg (value of the lead concentration in uncontaminated soils) [6]. Regarding Zinc, the concentrations vary from one garage to another. The highest value (193.03 mg / Kg) was found in garage 3; and the lowest concentration (40.72 mg / Kg) was observed in garage 2. The variability of the Zinc concentrations in the garages studied can be explained by the diversity of additives used in the new oil change poured on the ground. These additives are used in the composition of waste oils. Indeed these oils consist of heavy metals, Lead, Zinc, copper etc and additives, most of which are based of Zinc [31,32]. These metals (Zinc, Copper and Lead) are important because they can be toxic to plants, animals including humans [33]. Furthermore, these zinc contents can come from vehicle engines as well as from anthropogenic activities. The values of the metals studied, however, remain below the regulatory limit for the soil (300 mg / Kg for Lead and 300 mg / Kg for Zinc) [34]. However, if these metals are lixiviated in the soil, they can pollute the groundwater reserves, in particular in areas with a ground water level, and these lead and zinc metals can form complexes with the organic matter of the soil.

Zinc and Lead Plants Contents

Lead and Zinc Concentrations at the Various Sites

L camara has a good capacity for resistance and conditioning of air pollution and its use is recommended as a biomonitor [20]. For an element to be assimilated by a plant, it must be in the soil solution and bioavailable [35]. The passage of metals into solution takes place under the influence of various factors including pH, organic matter.
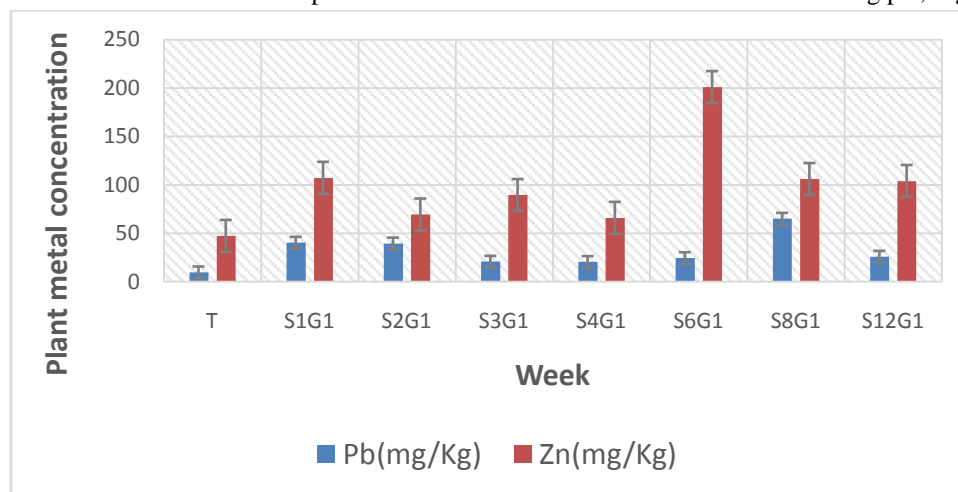

Figure 2: Lead and Zinc content of garage 1

The *L camara* accumulates lead and Zinc metals over the entire experimental period. The highest values in lead and in Zinc are obtained in the eighth week and the sixth week, respectively. The bioavailability of lead and zinc metals could be explained by the weakly basic nature of the soil (pH = 7.5) in this garage. This bioavailability of metals in this soil is favored by organic matter (%MO = 3.9). This result is in agreement with [35]: the more the soil is organic, the lesser it contains metals, including cadmium which will tend to go into solution. Here we have the Lead whose reactivity is similar to cadmium.
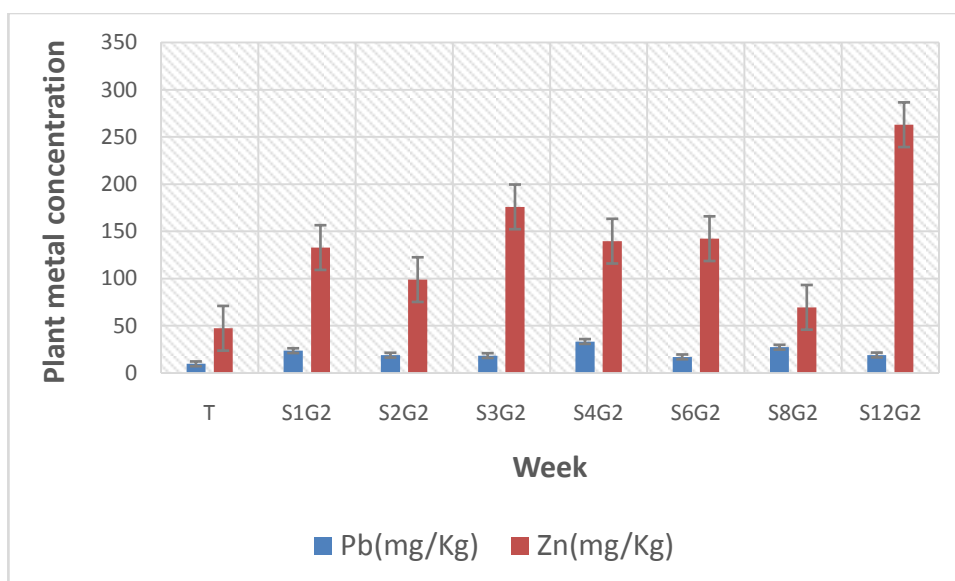

Figure 3: Lead and Zinc content of garage 2

The highest values in lead and in Zinc are obtained respectively the fourth week and the twelfth week
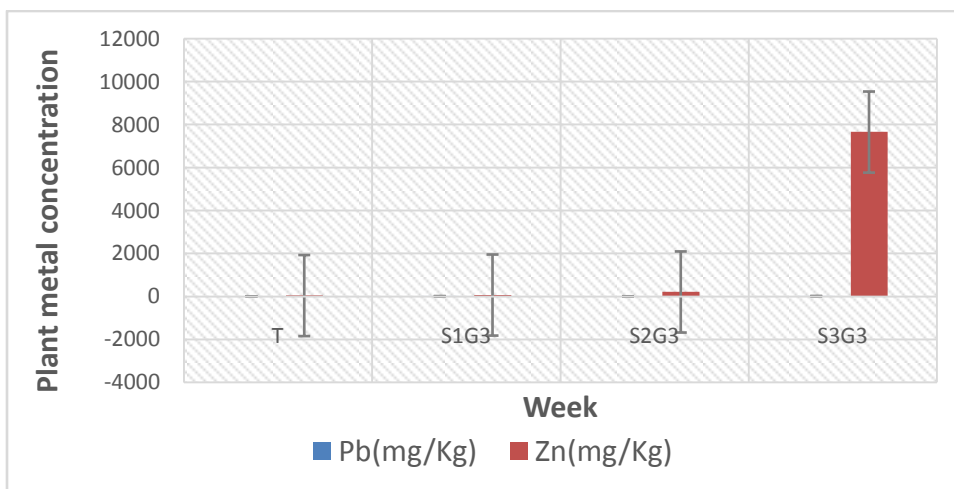
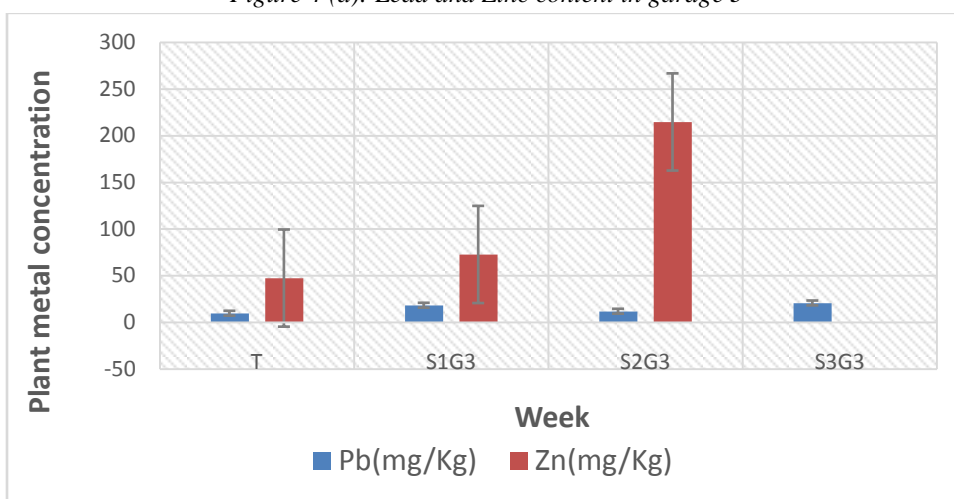*Figure 4 (a): Lead and Zinc content in garage 3*

Figure 4 (b): Lead and Zinc content of garage 3

Through the two graphs above, the highest values for lead and Zinc were obtained in the third week. An increase in the accumulation of Zinc.
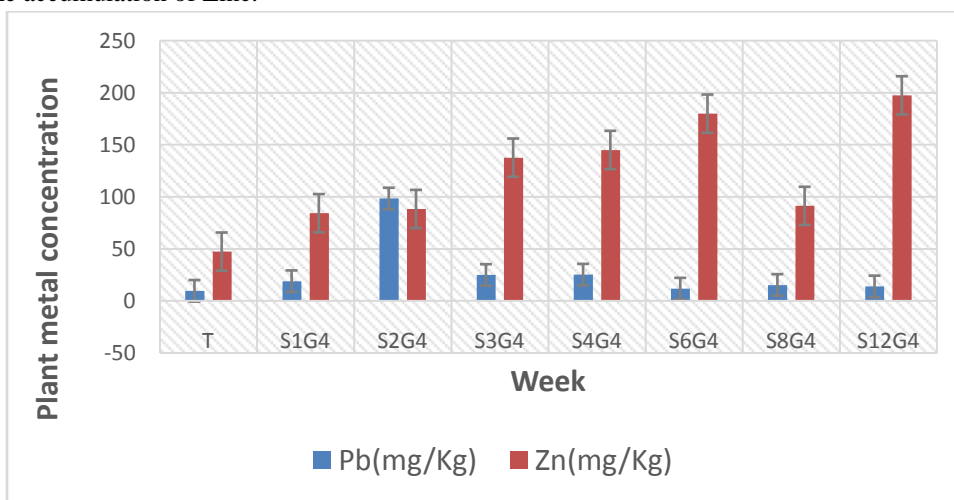

Figure 5: Lead and Zinc content of garage 4

The highest values for lead and Zinc were obtained respectively on the twelfth week and the second week. In addition, there is an increase in the accumulation of Zinc compared to the accumulation of lead.

Compared to the Zinc concentration of the control plant, we found that the Zinc concentrations of all the plants are greater than 47.31 mg / Kg, proof that *Lantana camara* really accumulates Zinc. The lowest absorption (22.09 mg / Kg) was observed in the garage 1 (Abomey calavi) in the second week. The high absorptions were recorded on the one hand in garage 2 (Abomey calavi) at the twelfth week (215.46 mg / Kg) and on the other hand in garage 3 (Cotonou) at the third week when the plant up took 7662.67 mg / Kg. The high accumulation of zinc in three-week intervals by *L. camara* indicates the effectiveness of this plant in the short term. This confirms the results of [5]. In addition, this value is clearly high. It not only exceeds the Zinc concentration in the soil in the garage (193.03 mg / Kg) but also the regulatory limit (300 mg / Kg) in Zinc concentration in the environment [34,36]. However, the plants could not survive on this site. The zinc concentrations extracted by macrophytes after twelve weeks in the experimental sites indicate the presence of zinc materials in this soil. In general, it should be noted that apart from the initial ground, there is the presence of certain Zinc materials due to the backfilling in garages. Watering and soil solution would have facilitated absorption of excess zinc into the soil by macrophytes. In addition, due to the performance of *L camara* in conditioning air pollution [20], the leaves of *L camara* could retain Zinc particles in suspension; this hypothesis can be verified. The case of garage 3 located in Cotonou in the heart of the city seems worrying, given that the water table is less than 1.5m from the ground. The Bioconcentration Factor (BCF) which indicates the ratio between the concentration of the compound studied in the medium and the concentration in the organism is greater than 1. This study shows that in addition, Chromium [4], Lead [6], cadmium [9], nickel [8], *L camara* is a hyperaccumulator of zinc. This calls for caution in its use as a biopesticide to fight against corn weevils[18] as well as in the fight against toxigenic molds isolated from peanuts in post-harvest [17]. A physico-chemical characterization study of the extracts of *L camara* used in biochemistry as a biopesticide is necessary.

Compared to the Lead value of the control plant, it is observed that all the plants have higher or lower concentrations. This is evidence of the accumulation of lead in the tissues of *L camara*. This result is in agreement with the results of [5] and [6]. The lowest absorptions 2.12 mg / Kg and 2.09 mg / Kg were observed respectively in the second week in garage 3 and in the sixth week in garage 4. While the highest concentrations accumulated by *L camara* 58.65 mg / Kg and 55.32 mg / Kg are observed respectively in the second week in the garage 4 and in the eighth week in the garage 1. These different absorptions were made without any amendment. The results are lower than those of [5] (262.2mg / Kg of Lead in the leaves and 88.4mg / Kg in the shoots). The lead concentrations absorbed compared to those of the soil of the garages at the start (10.31 mg / Kg, 11.25 mg / Kg ; 11.45 mg / Kg; 11.28 mg / Kg) show a low absorption of lead from plants and the presence of certain lead materials whose absorption by plants would be facilitated by soil solution. This is certainly what justifies the high lead concentrations (exceeding those of the starting soil) at certain sampling points. The Bioconcentration Factor (BFC) is also greater than 1.

Generally speaking, in situ phytoextraction experimentation with *L camara* has shown that this plant accumulates metals. This is in agreement with [5-6]. In addition, this study has shown that *L Camara* is a Zinc hyperaccumulator. The accumulation of zinc increases over time and that of lead is optimal in the first 8 weeks.

Conclusion

The Phytoremediation experiment with *L camara* in four garages in the South of Benin has shown that this plant accumulates Zinc as well as lead. The absorption of lead is optimal in the first eight weeks and that of Zinc increases over time.

The *L camara* can be suggested to extract the metals from used oil service sites, thus contributing to the restoration of soils at garages.

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