Journal Homepage: -www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH (IJAR)

Article DOI:10.21474/IJAR01/20058
DOI URL: http://dx.doi.org/10.21474/IJAR01/20058

ISSN NO. 2320-5407

RESEARCH ARTICLE

EFFECT OF DIFFERENT TYPES OF BIOCHAR ON HEAVY METAL BIOACCUMULATION BY LETTUCE AND HUMAN FOOD POISONING RISK

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Manuscript Info

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Manuscript History
Received: 13 October 2024
Final Accepted: 16 November 2024
Published: December 2024

Key words:-
Heavy Metals, Bioaccumulation, Biochar, Contamination.

Abstract

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The objective of this study was to evaluate the impact of biochar application on the reduction of heavy metal contamination in soils and their bioaccumulation in lettuce. The study was carried out in Bobo-Dioulasso, Burkina Faso. Lettuce was grown in pots containing contaminated soil amended with three different types of biochar at three different doses. At maturity, the lettuce was harvested and the concentration of heavy metals in various organs was determined. The results showed that in terms of bioaccumulation in lettuce, the roots showed the highest accumulation of heavy metals, especially copper and zinc, with peak concentrations of 55.72 mg/kg for copper and 193.13 mg/kg for zinc. Cadmium was not detected in any part of the plant. The modelling results indicate that biochar application can effectively reduce the heavy metal hazard quotient, particularly in children and adolescents. To illustrate, a dose of 1 t/ha of chemically activated biochar was observed to reduce the hazard quotient for copper, lead and zinc in children by 0.06, 0.23 and 0.13 respectively. Significant reductions were also observed in adolescents and adults, with a maximum reduction of 0.39 for lead in adolescents. The chemically activated biotankswere found to be the most effective in reducing the above risk, followed by the physically activated biotanks. The study highlights the effectiveness of biochar, particularly in the context of reducing heavy metal contamination of crops and the associated risk of food poisoning, especially in vulnerable populations such as children.

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Introduction:-

Heavy metal contamination of soils poses a growing threat to food security and human health, particularly through vegetable crops. Toxic elements such as cadmium (Cd), lead (Pb) and mercury (Hg) can accumulate in plants, especially in leafy vegetables such as lettuce, and thus enter the human food chain, increasing the risk of poisoning (Zhuang et al., 2009). In this context, there is an urgent need to find ways to reduce the bioavailability of these metals in soils in order to limit their uptake by plants. Soil decontamination methods such as phytoremediation and soil washing have been used, but they are often costly and require considerable time to achieve measurable results

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(Ghosh and Singh, 2005). In addition, innovative approaches, including the use of biochar as a soil amendment, are increasingly being explored for their ability to limit the bioavailability of heavy metals. Biochar, produced by the pyrolysis of organic matter, has unique properties to adsorb contaminants and improve soil quality (Beesley et al., 2011).

In this study, we investigate the effect of different types of biochar on reducing the bioaccumulation of heavy metals in lettuce to assess the risk of food poisoning to consumers. Our main objective is to determine which type of biochar best limits the uptake of heavy metals in plants. Specifically, we want to (i) characterise the physico-chemical properties of the biochars tested, (ii) compare their adsorption capacities for heavy metals and (iii) evaluate the impact of each biochar on reducing the risk of food poisoning to consumers. The results obtained will allow us to popularise the type of biochar effective against food poisoning among consumers. They will also be used to advise growers to develop techniques to reduce contamination of vegetables.

Materials and Methods:-

Location of the study area:-

The study was conducted in the city of Bobo-Dioulasso, located 365 km southwest of Ouagadougou (INSD, 2012) (Figure 1). Bobo-Dioulasso is located in the Sudano-Guinean climate zone (Fontès and Guinko, 1995). The rainfall curve in Bobo-Dioulasso over the last ten years shows a very variable rainfall with a minimum of 681.7 mm in 2017 and a maximum of 1370.2 mm in 2019. However, it is poorly distributed in time and space. Temperatures ranged from 28.01 °C in 2013 to 28.43 °C in 2016. According to the National Soil Bureau (BUNASOLS, 2015), the different soil types found in Bobo-Dioulasso are raw mineral soils, ferralitic soils, tropical ferruginous soils and hydromorphic soils. However, the tropical ferruginous soils are the most dominant. They have a fairly permeablekaolinitic sandy-clay texture (Sanou, 2017).

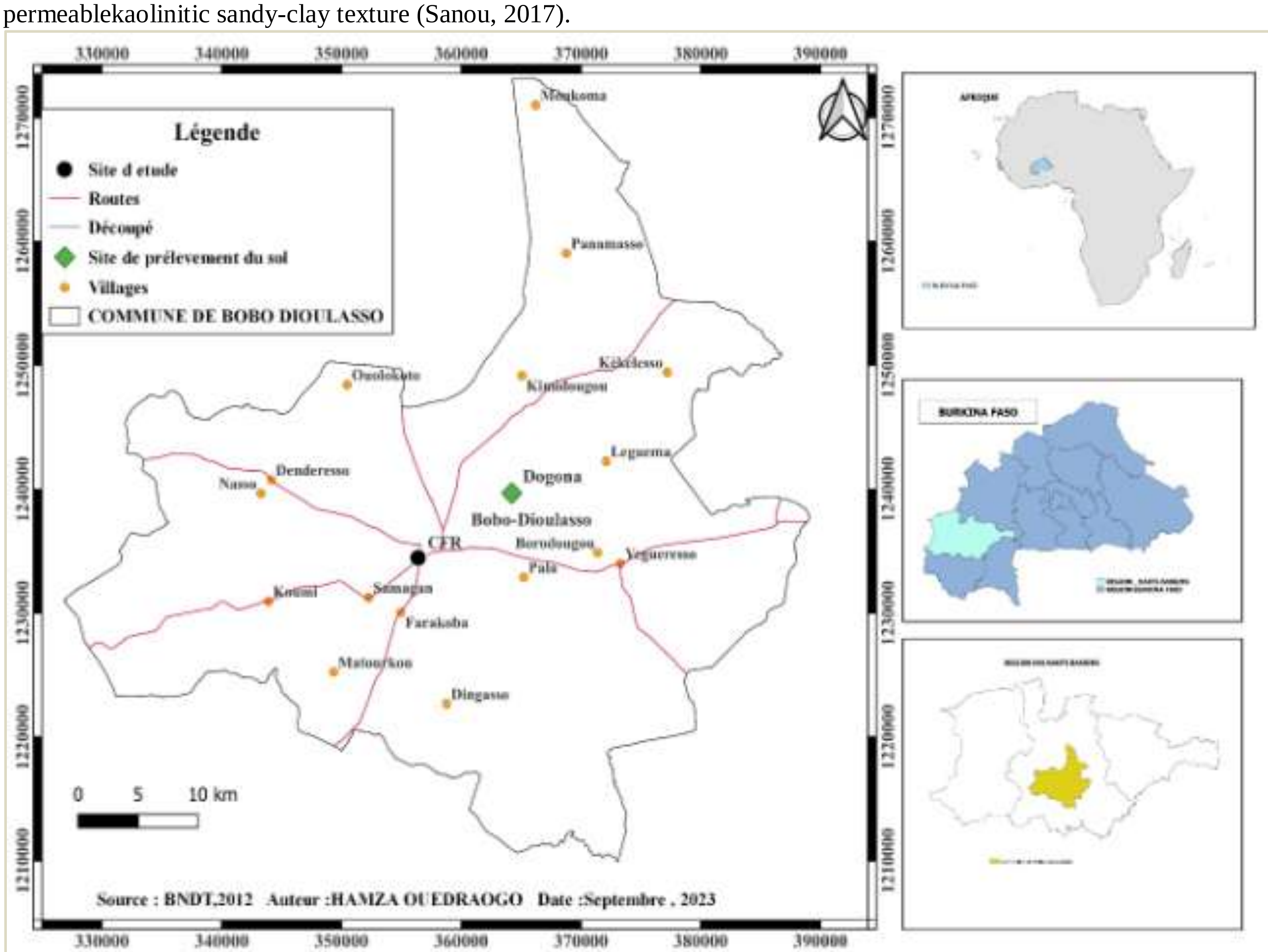


Figure 1:- Location map of the study site.

Study material
The study material consists of three elements: contaminated and uncontaminated soil, lettuce plants and biochar.

The contaminated soil samples were collected in the industrial zone of Bobo Dioulasso, where high levels of heavy metal contamination have been found in the surrounding vegetable gardens. They will then be analysed to determine the baseline levels of heavy metals such as lead (Pb), cadmium (Cd), copper (Cu) and zinc (Zn) in order to establish a baseline for assessing the effectiveness of the biochar.

The uncontaminated soil comes from the Dogona horticultural area, which is very rich in organic matter because it comes from landfills. These samples are used because of their high heavy metal content.

Lettuce plants (Lactuca sativa) are good study materials and bioindicators in case of food poisoning. Their bioaccumulative property makes it possible to assess the transfer of heavy metals in the food chain.

The biochar used was produced from cotton stalks. It has been physically activated at 500 °C and chemically activated with calcium chloride to increase its adsorption capacity. Several types of biochar, activated and non-activated, were applied to soil samples in laboratory culture pots. Each type of biochar was characterised according to its physico-chemical properties, such as pH, specific surface area and metal adsorption capacity, in order to optimise its contaminant retention potential.

Experimental device
The study was designed as a factorial block design with seven different treatments, each replicated three times. Two main factors were investigated: the type of biochar and the doses applied. The types of biochar used included: physically activated biochar (PAB), chemically activated biochar (CAB) and non-activated biochar (NAB). Two dose levels were maintained: 2.5 and 5 t.ha-1. A control treatment without biochar was also included. A total of 21 plots were established, distributed among the three blocks. Each plot contained four pots, giving a total of 84 pots for the whole study. The treatments applied were T0D0 for the control without biochar; T1D1 for 2.5 t.ha-1 of chemically activated biochar (CAB); T1D2 for 5 t.ha-1 of chemically activated biochar (CAB); T2D1 for 2.5 t. ha-1 of physically activated biochar (PAB); T2D2 for 5 t.ha-1 of physically activated biochar (PAB); T3D1 for 2.5 t.ha-1 of non-activated biochar (NAB); and finally T3D2 for 5 t.ha-1 of non-activated biochar (NAB).

Lettuce cultivation
Lettuce (Lactuca sativa) plants were grown in amended and unamended (control) soils. After 45 days of growth, they were harvested for analysis to determine the levels of accumulated heavy metals. These data allow the calculation of heavy metal transfer coefficients in plants and to perform correlations and linear regressions to evaluate the effect of biochar on metal bioavailability.

Assessment of the risk of food poisoning
The risk of food poisoning was assessed by comparing the heavy metal concentrations obtained with the standards set by the health authorities. Risk assessment models were used to estimate the potential human intake of heavy metals from the consumption of lettuce grown on the amended soils. The analysis makes it possible to determine whether the use of biochar helps to reduce bioaccumulation sufficiently to minimise risks to human health.

Biocharcharacterisation

pH at zero charge
The pH at the point of zero charge (pHPCN) is the pH at which the biochar is electrically neutral in solution. This measurement is essential to understand how the surface charge of the biochar affects the adsorption mechanism. We determined it using the method of Lopez-Ramon et al. (1999). Solutions of 0.1 $mol.L^{-1}$ sodium chloride (NaCl) were prepared at pH 2 to 10, adjusted using aqueous solutions of NaOH or HCl at 0.1 M. Then 0.1 g of dry biocharwas mixed with 20 mL of each solution in sealed vials. The mixture was stirred at room temperature for 72 hours. The solutions were then filtered to measure the pH again. A curve representing the final pH as a function of the initial pH was plotted and its intersection with the bisector indicates the pH at the point of zero charge.

Iodine index

The iodine value is a measure of the microporosity of adsorbent materials, assessed by their ability to adsorb iodine molecules. The standard method of Girgis and El-Hendawywas used for this determination. In an Erlenmeyer flask, 10 ml of a 0.1 N iodine solution was titrated with a 0.1 N sodium thiosulphate solution, adding two drops of a 1% starch solution as an indicator, until a colourless solution was obtained. Then 0.05 g of biocharwas added to an Erlenmeyer flask containing 15 mL of 0.1 N iodine solution and the mixture was stirred for 4 minutes before being filtered. A volume of 10 mL of the filtrate was titrated with a standard sodium thiosulfate solution, again using the starch indicator. The iodine index was then calculated using the following formula.

$$\mathbf{I_d} = \frac{(\mathbf{V_b} - \mathbf{V_S}) \times \mathbf{N}\text{x}\mathbf{126.9}\text{x}\left(\frac{\mathbf{15}}{\mathbf{10}}\right)}{\mathbf{m_{CA}}}$$

Where: Id: the iodine index in mg/g; Vb: the volume (ml) of sodium thiosulphate ($Na_2S_2O_3$) poured during the blank test (without activated charcoal); Vs: the volume (ml) of sodium thiosulphate (Na2S2O3) poured during the test with activated charcoal; N: the normality of the sodium thiosulphate solution (mol/L); 126.9: the atomic mass of iodine (g/mol); mCA: the mass of activated charcoal (g).

Specific surface

The specific surface area of biochar was determined using the methylene blue adsorption method. This method consists of measuring the adsorption of methylene blue at different concentrations using a constant mass of adsorbent. This allows the maximum adsorption capacity to be evaluated according to the Langmuir isotherm. The specific surface is calculated using the following relationship.

$$\mathbf{S_{BM}} = \frac{\mathbf{Q_0} \quad \mathbf{S} \quad \mathbf{N_A}}{\mathbf{M_{BM}}}$$

Where, SBM: specific surface area determined using BM as adsorbate (m²g-1); Qm: maximum adsorption capacity (mg g-1); S: area occupied by one BM molecule (175 Å2); NA: Avogadro's number; MBM: molar mass of BM (319.853g/mol).

Heavy metal contamination assessment

Heavy metal contamination (Cu, Pb, Cd and Zn) was assessed by flame atomic absorption spectrometry, preceded by wet mineralisation of the samples (AFNOR NF ISO 11-460).

Data statistical processing

The data obtained were entered and processed using Excel, and statistical analysis was performed using R software version 4.3. The data were normalised using the Shapiro-Wilk test and homogeneity of variances using the Levene test. The normal data were then subjected to ANOVA at the 5% significance level. When the conditions for parametric tests were not met, the Kruskal-Wallis test was used. For groups showing significant differences, the means were compared using the Tukey's honest significant difference (HSD) test for parametric analyses and the Dunn's test for non-parametric analyses. Relationships between heavy metal concentrations and other soil physicochemical parameters were determined using the Pearson correlation test. Simple linear regressions were used to quantify the relationship between heavy metal concentrations and the risk of food poisoning.

Results:-

Heavy metal levels in soils

The concentrations of heavy metals in initial (SI) and post-harvest (SAR) soils are presented in Table 1. In initial soils, the concentrations of heavy metals are 12.96 mg/kg, 214.34 mg/kg, 86.72 mg/kg and 371.79 mg/kg, respectively, for Cd, Cu, Pb and Zn. It is evident that these contents are all higher than those found in post-harvest soils. Laboratory analyses did not reveal any cadmium value for SAR except for the control treatment, which shows a value of 1.03 mg/kg of soil.

For Cu, analysis of variance at the 5% threshold revealed a significant difference between treatments ($P<0.001$). The maximum content recorded was 190.53 mg/kg of soil, which was obtained in the T0D0 treatment, while the minimum content, recorded in the T1D2 treatment, was 139.82 mg/kg of soil. The results of the analysis of variance on the lead contents indicated significant differences ($P < 0.001$). These ranged from 79.72 mg/kg (T0D0) to 61.93

mg/kg (T1D2). With respect to zinc, the T0D0 treatment exhibited the highest content of 321.79 mg/kg of soil, while the T1D2 treatment demonstrated the lowest content of 152.21 mg/kg of soil. In general, heavy metal contents are higher in soils without biochar input, followed by soils receiving 2.5 t/ha and 5 t/ha of biochar. Furthermore, it was observed that soils treated with chemically activated biochar exhibited minimal contamination post-harvest, in comparison to soils treated with physically activated biochar and non-activated biochar. With respect to the order of abundance of metallic elements in the soil, zinc is the most concentrated, followed by copper, lead and cadmium.

Table 1:- Cd, Cu, Pb and Zn contents in soil.

Treatments		Cu-ppm	Zn-ppm	Pb-ppm	Cd-ppm
Control	**T0 D0**	187.53±0.93[a]	321.79±5.58[a]	86.72±3.02[a]	1.3±0.8
BAC	**T1 D1**	121.35±2.71[ab]	231.14±2.87[c]	52.49±4.01[b]	0
	T1 D2	84.82±11.50[c]	152.21±3.51[d]	28.49±3.35[c]	0
BAP	**T2 D1**	142.51±2.58[a]	264.29±5.56[ab]	67.93±3.39[ab]	0
	T2 D2	126.42±10.30[abc]	212.96±6.70[b]	58.14±2.11[b]	0
BNA	**T3 D1**	169.48±3.03[a]	286.63±4.84[ab]	73.11±3.06[ab]	0
	T3 D2	144.54±2.91[b]	236.47±6.67[c]	59.67±2.46[b]	0

Comparative effects of pHPCN and pH-H_2O on the adsorption of heavy metals

As demonstrated in Figure 1, a comparison is made between the pH values at the point of zero charge (pHPCN) of three types of biochar (BNA, BAC, and BAP), along with the minimum and maximum pH values of water in which they are used. The pHPCN, which denotes the point at which the biochar surface is electrically neutral, is 7.78 for BNA, 7.01 for BAC, and 8.86 for BAP.

The pH water values (6.38 minimum and 6.9 maximum) are lower than the pHPCN of all the biochars studied. This finding indicates that at these pH values, the surface of the biochars is predominantly positively charged. Consequently, these biochars exhibit a heightened capacity for the adsorption of negative ions (anions) present in water, and a diminished capacity for positive ions (cations), such as certain heavy metals.

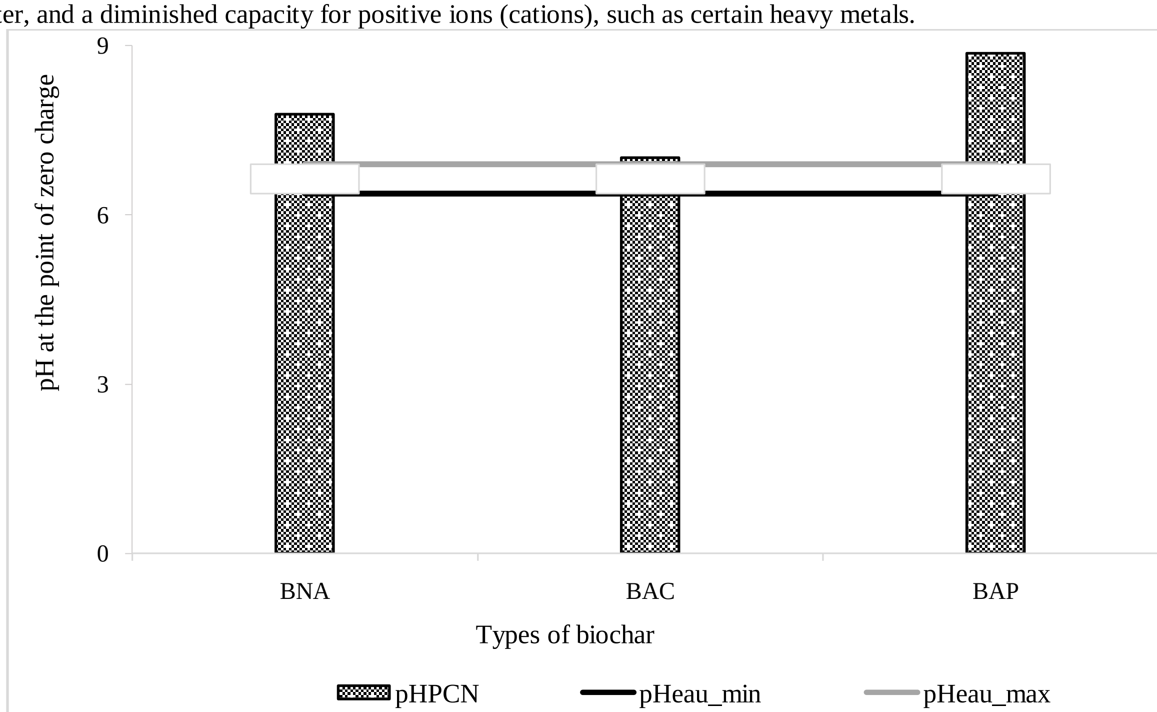


Figure 1:-pHPCN and pH-H_2O of different types of biochar.

Iodine (Id) and methylene blue specific surface area (MBS) indices for three types of biochars

As illustrated in Figure 2, the iodine value (Id) and methylene blue specific surface area (MBS) values for three distinct types of biocharsare presented: BAC, BAP, and BNA.

The iodine value (Id) is observed to be highest for BAP biochar, with a value of 1877 mg/g, followed by BAC at 1618 mg/g, and finally BNA with a value of 945 mg/g. This finding indicates that BAP biochar exhibits a high adsorption capacity for micropores, a property that is advantageous for the removal of certain contaminants. Conversely, the methylene blue specific surface area (MBS), which quantifies the adsorption capacity of mesopores, is maximal for BAC (1285 m²/g), followed by BAP (1013 m²/g), and is minimal for BNA (875 m²/g).

These observations suggest that BAC, with its enhanced methylene blue specific surface area, may be more effective in adsorbing larger molecules. Conversely, BAP, with its elevated iodine number, might be more efficient in adsorbing smaller molecules or ions.

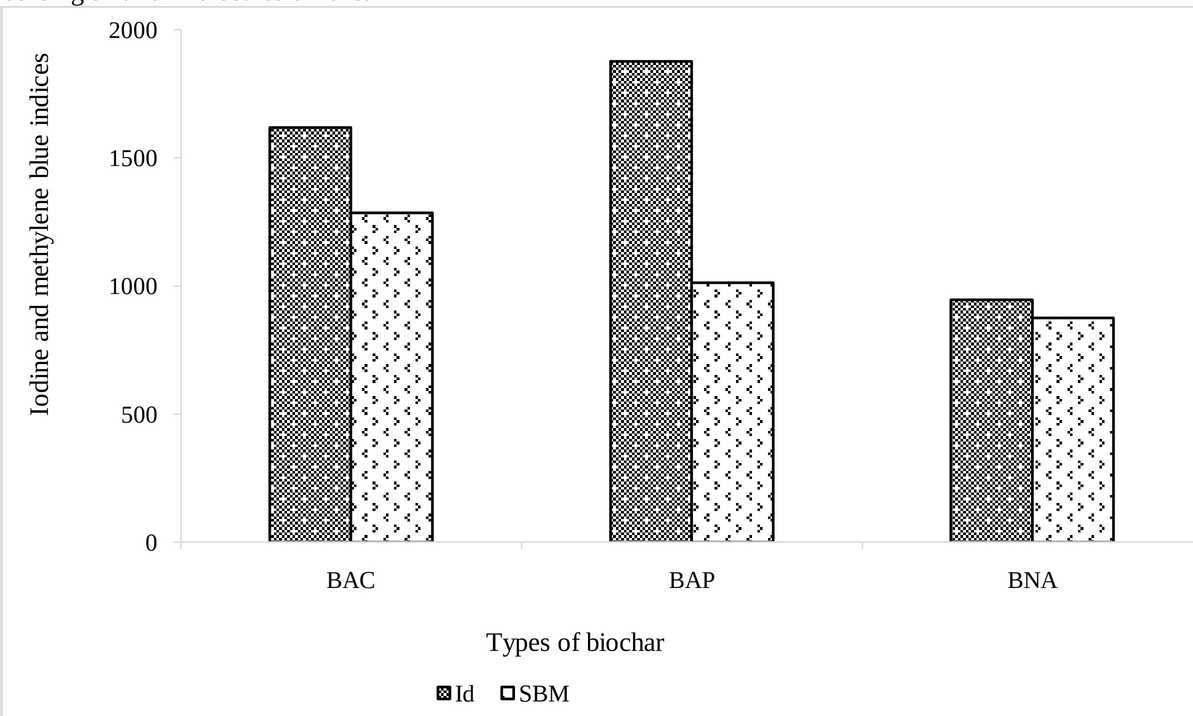


Figure 2:-Id and SBM of BAC, BAP and BNA biochars.

Heavy metal contents in different parts of lettuce

As demonstrated in Figure 3, there is a considerable variance in the concentrations of heavy metals (Cd, Cu, Pb, Zn) among the various parts of the lettuce (leaf, stem, root) contingent on the distinct treatments applied. For cadmium (Cd), the concentrations remain zero in all parts of the plant, regardless of the combination of treatment and doses applied.

For copper (Cu), the concentrations range from 11.22 to 29.82 mg/kg in leaves, from 22.24 to 43.45 mg/kg in stems, and from 18.98 to 55.72 mg/kg in roots. The highest concentrations of copper are observed in the roots of plants treated with high doses of biochar and amendment, especially for treatments T3D0 and T3D2, with values reaching up to 55.72 mg/kg in the roots. This finding indicates that copper accumulation is predominantly occurring in the root system.

Concentrations of lead (Pb) ranged from 5.53 to 19.12 mg/kg in leaves, from 0 to 13.27 mg/kg in stems, and from 15.02 to 28.93 mg/kg in roots. The highest values were observed in the roots of the T0D0 and T3D0 treatments, with concentrations of 28.93 mg/kg and 28.48 mg/kg, respectively, suggesting that lead was also highly accumulated in the roots. Zinc (Zn) exhibited higher concentrations compared to the other metals, with values ranging from 51.63 to 86.51 mg/kg in leaves, 64.39 to 110.87 mg/kg in stems, and 95.96 to 193.13 mg/kg in roots. The highest zinc concentrations were found in the roots of plants treated with T0D0, T2D0 and T3D0, reaching up to 193.13 mg/kg, which confirms a high accumulation of the element in the roots.

In summary, the roots are the part of the lettuce that accumulates the most heavy metals, especially copper, lead and zinc. The translocation of heavy metals to the leaves and stems of the plant is limited. The findings further demonstrate that biochar application influences the accumulation of metals in various plant parts.

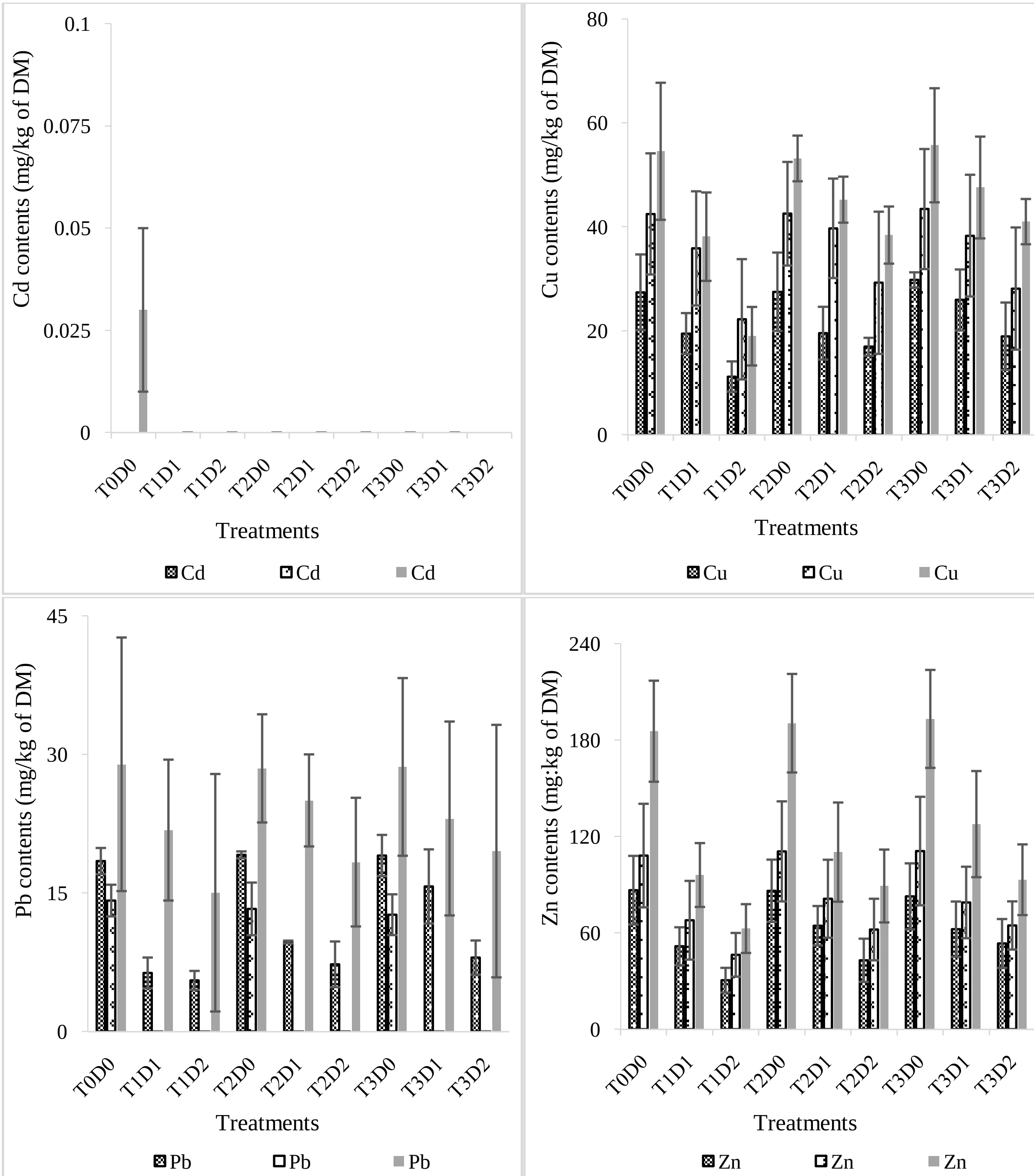


Figure 3:- Heavy metal contents in different parts of the plant depending on biochar treatments.

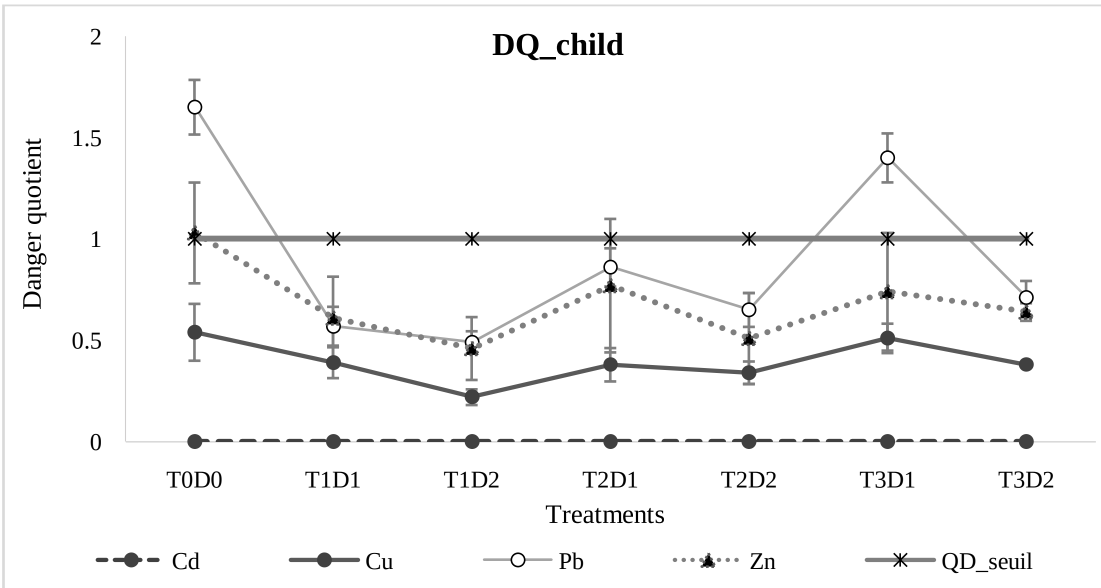
Variation in the danger quotient according to age groups

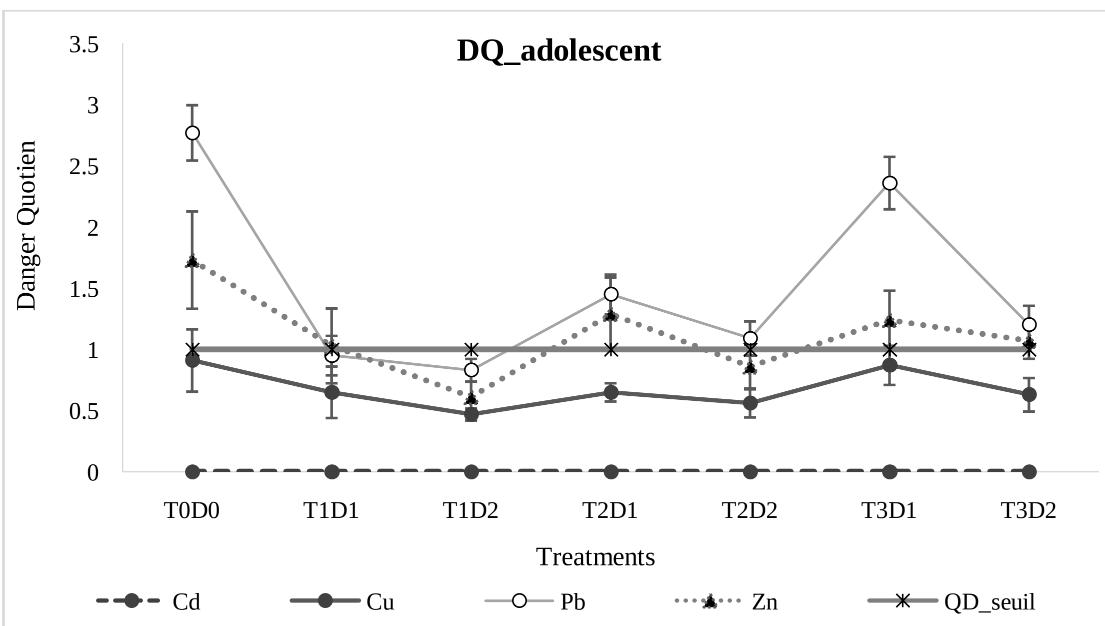
The variations in the hazard quotients (HQ) of heavy metals (Cd, Cu, Pb, Zn) according to age groups (children, adolescents and adults) and the different treatments are shown in Figure 4. For cadmium, the concentrations remain zero in all age groups and for all treatments, indicating an absence of Cd absorption under these experimental conditions.

With regard to copper, the highest concentrations were observed in adolescents, particularly in the T0D0 treatment group, where the HQ was equal to 0.91 mg/kg. In children, the concentrations range from 0.22 to 0.54, while in adults, the range is from 0.54 to 0.87. The T3D1 and T3D2 treatments show a slightly higher risk of copper in adults, with 0.82 and 0.6, respectively, but remain below the maximum values observed in adolescents.

Concentrations of lead exhibit a similar trend, with the highest observed levels also being recorded in adolescents and adults. In adolescents, the T0D0 treatment recorded a value of 2.77, while in adults it reached 2.64 for the same treatment. In children, the QD values for lead remain generally lower, ranging from 0.49 to 1.65 depending on the treatment, with the maximum value obtained under T0D0.

Conversely, the T0D0 treatment exhibited the highest zinc concentrations across all age groups. These varied between 0.46 and 1.03 in children, 0.61 and 1.73 in adolescents, and 0.58 and 1.65 in adults. The results indicate that adolescents and adults exhibit higher levels of accumulation for copper, lead and zinc compared to children, particularly in the absence of an additional dose (T0D0).





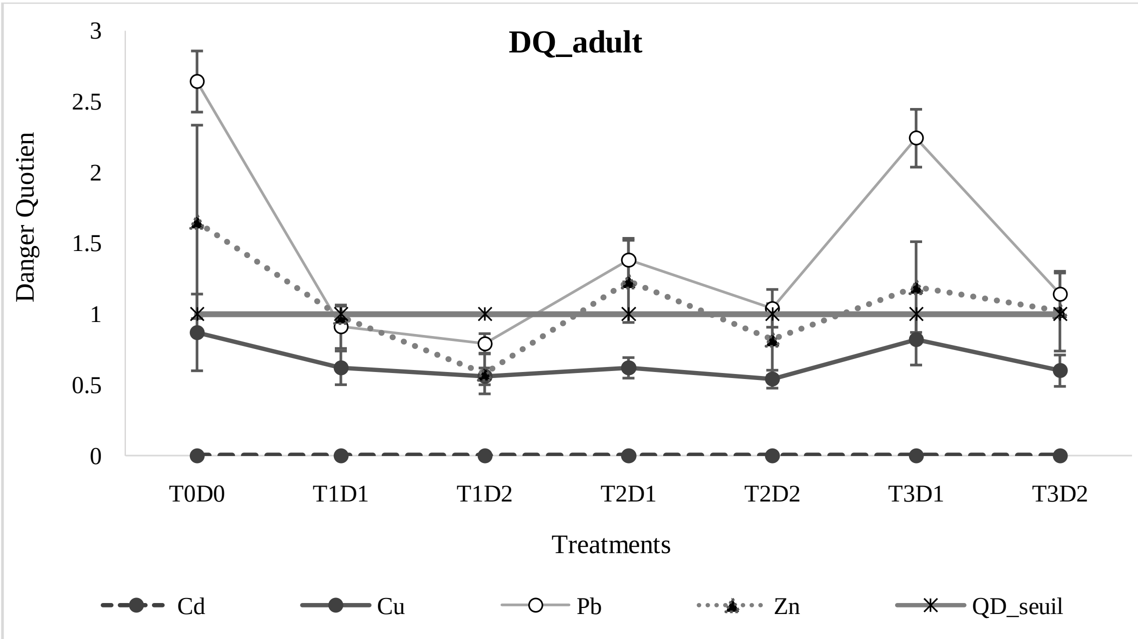


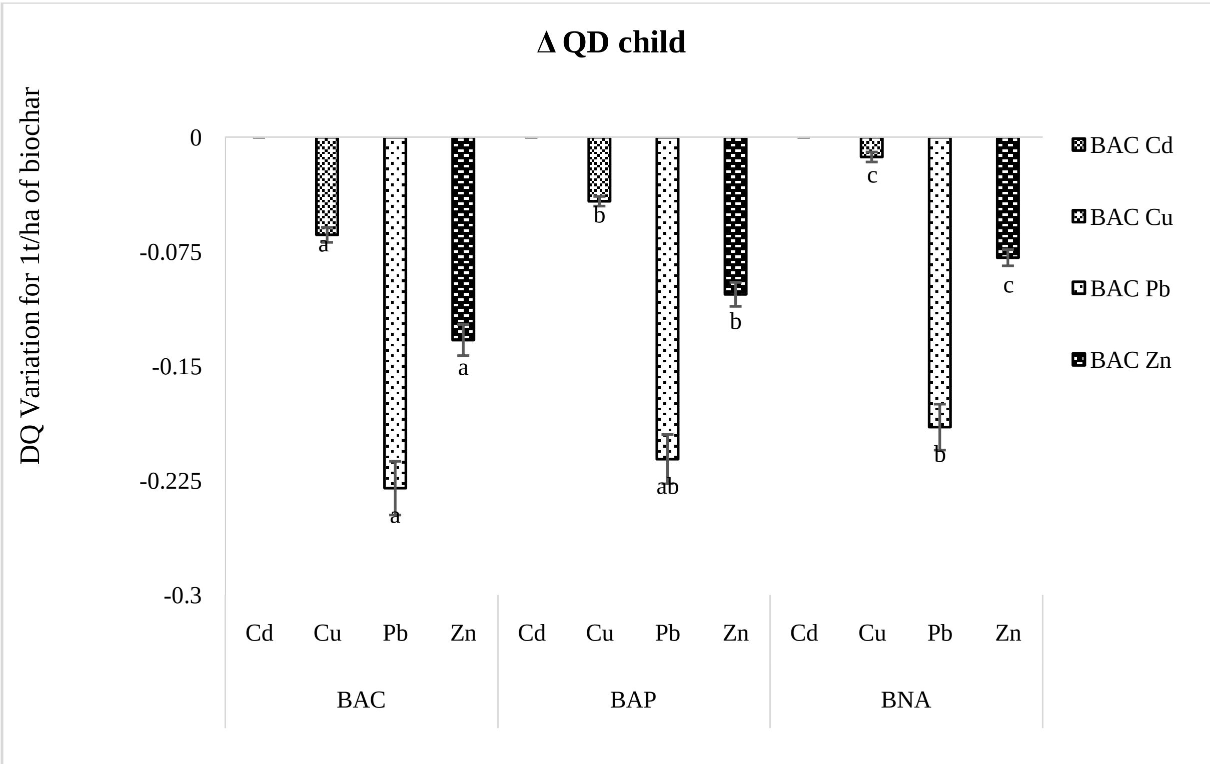
Figure 4:- Hazard quotient associated with consumption of contaminated lettuce leaves.

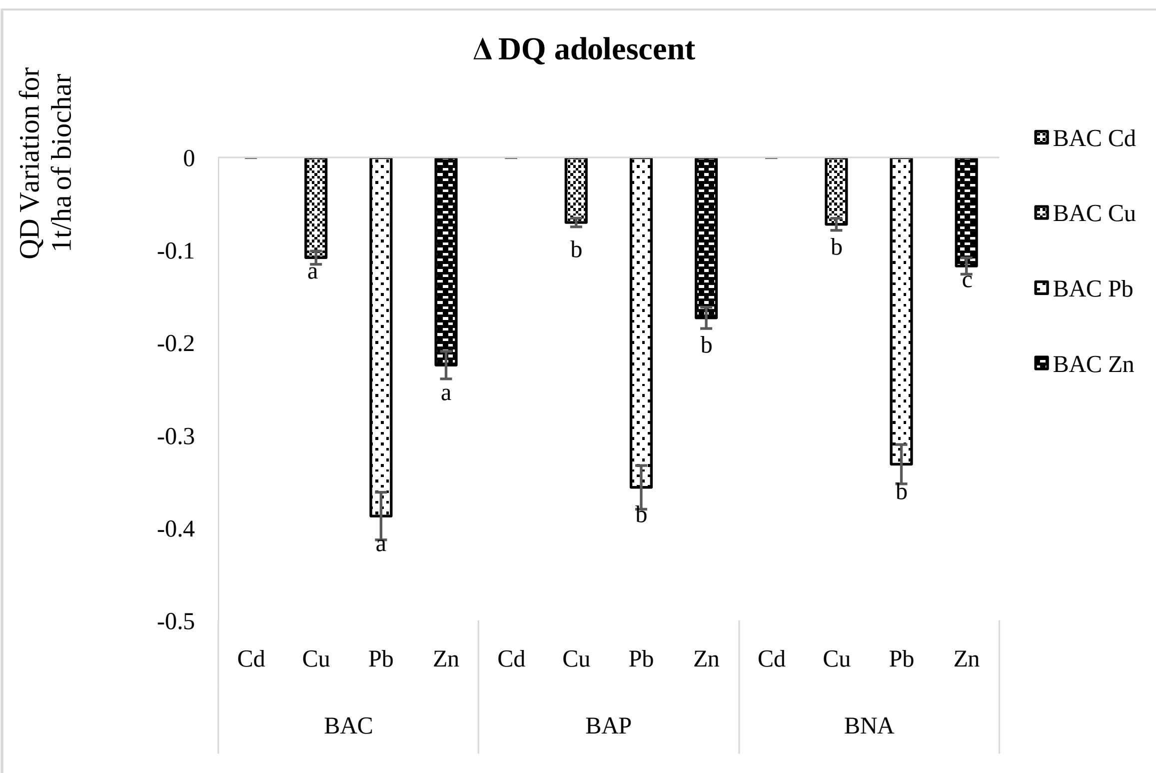
Modeling the risks of poisoning linked to the consumption of lettuce leaves

The variation in the hazard quotient for heavy metals for 1 t/ha of each type of biochar is illustrated in Figure 5. For cadmium, no variation in the quotient was observed across generations. Modelling of the hazard quotient data as a function of the biochar amendment doses showed that the dose of 1 t/ha induced a different effect on the hazard quotient for heavy metals (Cu, Pb and Zn) depending on the type of biochar and the generation considered.

Specifically, for chemically activatedbiochar, an increase in the amendment dose of 1 t/ha led to a reduction in the risk of copper, lead and zinc poisoning in children by 0.06, 0.23 and 0.13, respectively. Conversely, for physically activatedbiochar, an augmentation in the amendment dose of 1 t/ha has been shown to reduce the risk of copper, lead and zinc poisoning in children by 0.04, 0.21 and 0.1, respectively. Conversely, for non-activated biochar, the risk reduction in children is 0.01 for copper, 0.19 for lead, and 0.08 for zinc. In a similar manner, an increase of 1 t/ha in the dose of chemically activated biochar led to a reduction in the hazard quotient of 0.11 for copper, 0.39 for lead and 0.22 for zinc, as revealed by the results of statistical analysis conducted on adolescents. For physically activated biochar, the reductions were 0.07 for copper, 0.36 for lead and 0.17 for zinc. The use of non-activated biochar resulted in a 0.07 reduction for copper, 0.33 for lead, and 0.12 for zinc.

For adults, increasing the dose of 1 t/ha of chemically activated biochar reduced the hazard quotient for copper by 0.1; for lead, the reduction was 0.37 and 0.21 for zinc. The findings for physically activated biochar yielded reductions of 0.07 for copper, 0.34 for lead, and 0.16 for zinc. The reductions observed for non-activated biochar were 0.07 for copper, 0.32 for lead, and 0.11 for zinc. The most efficacious method of reducing the risk of poisoning was found to be the chemical activation of biochar, followed by the physical activation of biochar and, finally, non-activated biochar.


Δ QD child
DQ Variation for 1t/ha of biochar
0
-0.075
-0.15
-0.225
-0.3
a
a
a
b
ab
b
c
b
c
Cd
Cu
Pb
Zn
BAC
BAP
BNA
BAC Cd
BAC Cu
BAC Pb
BAC Zn


Δ DQ adolescent
QD Variation for 1t/ha of biochar
0
-0.1
-0.2
-0.3
-0.4
-0.5
a
a
a
b
b
b
b
b
c
Cd
Cu
Pb
Zn
BAC
BAP
BNA
BAC Cd
BAC Cu
BAC Pb
BAC Zn

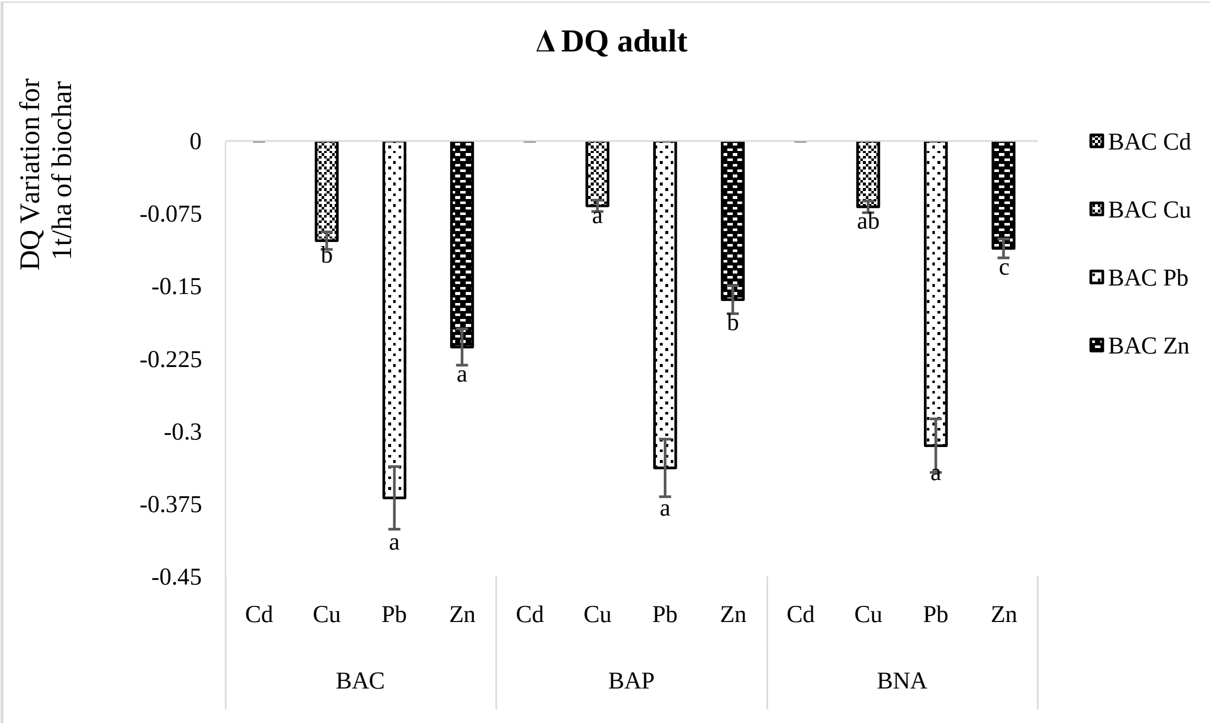


Figure 5:-Variation of the hazard quotient for 1t/ha of biochar.

Effects of Id and SBM on DQ and BCF

As demonstrated in Figure 6, an increase in the Id and SBM indices has a significant effect on the values of hazard quotients (HQs) and bioaccumulation coefficients (BCFs). In children, an increase of one unit in the Id_HQ index results in a decrease of 1.27.10-4 and 2.80.10-4 in the Qs of copper and lead, respectively, while zinc increases by 1.37.10-4. A parallel trend is exhibited by the SBM_HQ index, which, upon a one-unit increase, results in a heightened increase in the Qs for lead and zinc, reaching 1.21.10-3 and 4.52.10-4, respectively. For adolescents, a one-unit increase in the Id_QD index also results in a slight decrease in the QDs for copper and lead, with values of 1.70.10-4 and 4.65.10-4, respectively, while zinc increases by 2.24.10-4. A one-unit increase in the SBM_QD index results in a more pronounced increase in the QDs for lead and zinc, reaching 2.03.10-3 and 7.66.10-4, respectively.

In adults, a one-unit increase in the Id_QD index results in a decrease in the QDs for copper and lead by 1.54.10-4 and 4.41.10-4, respectively, while zinc increases by 2.08.10-4. A one-unit increase in the SBM_QD index results in a larger increase in the QDs for lead and zinc, reaching 1.93.10-3 and 7.39.10-4, respectively.

For BCFs, a one-unit increase in the Id_BCF index results in slight changes, with a decrease of 4.70.10-6 for cadmium and an increase of 6.36.10-6 for zinc. Conversely, a one-unit increase in the SBM_BCF index results in a decrease of 9.52.10-5 for lead and 1.27.10-4 for zinc.

These findings indicate that the Id and SBM indices exert a more substantial influence on the hazard quotients for lead and zinc across the three age groups, while their effect on BCFs remains predominantly negligible but consistent across groups.

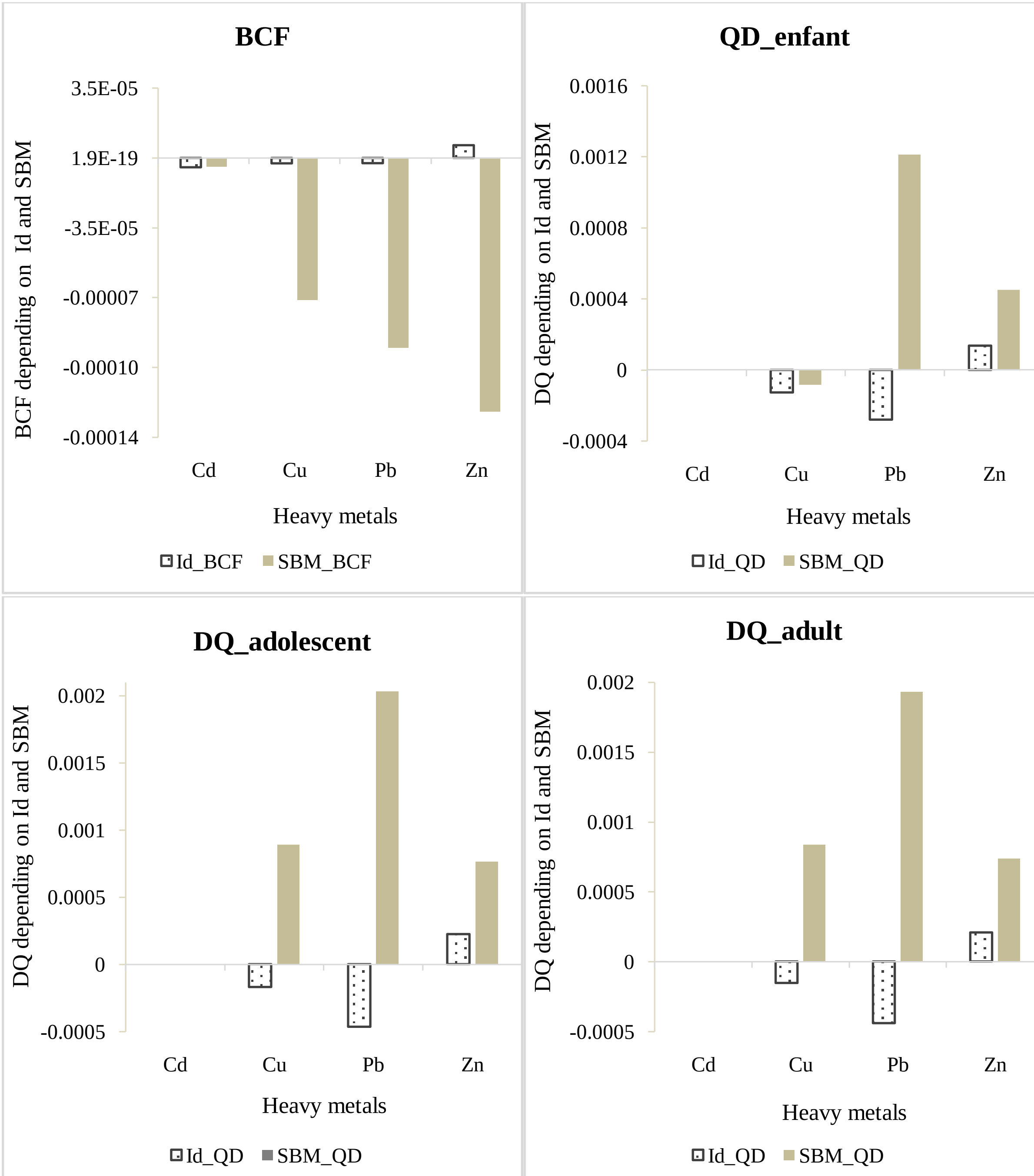


Figure 6:- Impacts of Id and SBM indices on DQ and BCF.

Discussion:-

The presence of heavy metals (Cu, Pb and Zn) in the various organs of lettuce exhibits variation both between organs and according to the treatments applied. The roots were found to be the most contaminated, followed by the stems and leaves in that order. This suggests that lettuce accumulates heavy metals preferentially at the level of its roots. These results are consistent with those reported by Cherra and Djeddou (2018), who demonstrated that lettuce exhibits a preference for stabilising toxic elements that pose a threat to its metabolism in the roots, with a view to minimising the associated risks. To achieve this, lettuce employs cellular compartments where metals are sequestered in specific organelles, such as vacuoles, or in certain structures, such as trichomes (Clemens, 2006). This ability of lettuce to sequester heavy metals in its roots affords it the capacity to thrive in contaminated environments without exhibiting any symptoms of poisoning in its leaves. However, translocations to the aerial parts are not zero, as evidenced by the copper, lead and zinc contents above the toxicity threshold for human consumption (Clemens, 2006). The potential for the translocation of these toxic elements to the aerial parts could be exacerbated by the competition between heavy metals and nutrients in lettuce. As posited by Sanita di Toppi and Gabbrielli (1999) and Greger (1999), certain heavy metals, such as Cd, have been shown to utilise the same transport mechanisms as plant nutrients, resulting in their distribution to all organs.

The impact of diverse treatments on the accumulation of heavy metals by lettuce was also examined. Specifically, lettuces cultivated in biochar-free treatments exhibited elevated concentrations of heavy metals in comparison to those in biochar-amended treatments, irrespective of the type of biocharutilized.The findings of this study are consistent with the hypothesis that biochars, when employed as a soil amendment, can immobilize heavy metals present in the soil solution, thereby reducing their bioavailability to lettuce. Research by Mukherjee et al. (2011) and Karami et al. (2011) had previously demonstrated that biochar stabilised heavy metals in the soil, thereby rendering them less available to the plant.

However, the accumulation of heavy metals by lettuce exhibited variation depending on the type of biochar and the dose applied. A comparison of the heavy metal contents in plant tissues revealed lower bioaccumulation in treatments that received chemically activated biochar compared to treatments amended with physically activated biochar and non-activated biochar. This discrepancy can be attributed to the superior capacity of chemically activated biochar in adsorbing heavy metals present in the soil solution, as compared to physically activated biochar, which also exhibited higher efficacy than non-activated biochar.

According to Tseng and Tseng (2005), chemical activation changes the chemical composition of the biochar surface and renders it more reactive with cation ions. Due to its properties, physically activated biochar is able to capture more heavy metals and thus reduce their content in lettuce. The former possesses a greater number of pores, yet its surface is not as reactive as that of the latter (Tseng and Tseng, 2005). This could be a contributing factor to its reduced performance in adsorbing metal ions in the soil solution, but not less than that of non-activated biochar, which has poorly exposed pores.

However, given that pHwater is lower than pHPCN, it can be deduced that the adsorption of metal cations should not be possible. Theoretically, the adsorption of negative ions should be favoured. Nevertheless, there are several reasons why biochar can still adsorb heavy metals under such conditions. Indeed, in some cases, biochar can form hydroxides or other insoluble complexes capable of retaining metal ions (Li et al., 2019). This capacity is not contingent upon the biochar surface charge, but rather on the chemistry of the heavy metal in solution (Li et al., 2019). As asserted by Kamari et al. (2011), metals such as lead, copper, and zinc have the potential to precipitate as metal hydroxides when the local pH around the surface becomes slightly basic. This phenomenon can be influenced by surface reactions. The porous nature of biochar, characterised by micropores and mesopores, facilitates the trapping of metal ions through physical interactions and Van der Waals forces. As Bolan et al. (2011) further demonstrate, this process is not impeded by the overall surface charge, and the metal ions are retained within the biochar pores, resulting in a high degree of contact between the ions and the adsorption sites.

The biochar dose administered exerts a significant influence on the bioaccumulation of heavy metals. It is hypothesised that the higher the dose, the greater the quantity of heavy metals trapped in the pores of the biochars, and the fewer free metal ions the lettuce has to absorb.

Consequently, it can be deduced that the various treatments may have contributed, albeit indirectly, to the observed variation in the risk of poisoning. Indeed, the hazard quotients associated with the consumption of lettuce leaves contaminated with cadmium and copper are less than 1, indicating that there is no risk of poisoning from these heavy metals. For lead and zinc, only lettuce from treatments T1D1 and T1D2 could be consumed without risk. Conversely, treatments T0D0, T2D1, T3D1 and T3D2 exhibited hazard quotients greater than 1, suggesting a probable risk of lead and zinc poisoning.

The toxic effects of lead (lead poisoning) have been demonstrated to manifest through disorders of psychomotor and intellectual development (Gorbel et al., 2002), renal failure (Garnier, 2005), atrophy of the sexual organs followed by a reduction in fertility and risks of abortion for pregnant women (Gorbel et al., 2002; Garnier, 2005). In the case of zinc, the risks are reported to be stomach cramps, nausea and vomiting (Pichard, 2005). In the most severe cases

(QD ≥ 1.5), the consequences may include sideroblastic anaemia with neutropenia, a reduction in hematocrit and ferritin, a decrease in the level of HDL (High Density Lipoprotein) and an alteration of immune and inflammatory responses (Pichard, 2005). However, modelling the variation in hazard quotient across biochar types showed that chemically activated biochar is most effective in reducing the risk of heavy metal poisoning, followed by physically activated biochar and then non-activated biochar.

Conclusion:-

Soil contamination by heavy metals, including cadmium, copper, lead and zinc, poses a significant threat to food safety and human health, particularly with regard to bioaccumulation in lettuce crops. In this context, the objective of this study was to determine the effectiveness of different types of biochar as soil amendments to reduce the bioavailability of heavy metals and thus limit their uptake by lettuce plants, in order to minimize the risks of food poisoning in consumers.

The findings indicated that the adsorption of heavy metals in soil is influenced by the physicochemical characteristics of biochar, including porosity, specific surface area, and activation methods. The doses administered also have a significant impact on the translocation and QD of heavy metals. It was observed that chemically and physically activated biochars exhibited higher specific surface area and adsorption capacity in comparison to non-activated biochar. Consequently, these activated biochars exhibited a substantial reduction in bioaccumulation of heavy metals in lettuce. These findings underscore the potential of biochar to diminish the bioavailability of these metals to plants, thereby mitigating their movement into the food chain.

The purpose of this study is to demonstrate the efficacy of biochar as a sustainable solution for the decontamination of soils polluted by heavy metals. As a natural method, biochar offers an alternative to costly and often time-consuming phytoremediation or soil washing techniques. This study offers novel insights into the potential of biochar for sustainable agriculture, particularly in the context of vegetable production with a view to eliminating the risk of poisoning.

The research will explore the effectiveness of biochar in soils of various compositions and contamination, as well as optimising the production and activation of biochar to increase its performance. Furthermore, conducting long-term studies to assess the impact of repeated biochar application on soils, crops and overall food security would be a relevant next step.

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