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Oil-Based Drill Cuttings Influence on Soybeans (*Glycine max* (L.) Merr.) Growth (Oil-Based Drill Cuttings Influence on Soybeans Growth)

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ABSTRACT

The spill of petroleum drilling wastes and fluids on crop lands would produce negative effects on crops growth and development of and soil properties. In this context, oil-based drill cuttings concentrations (2, 4, 6, 8 y 10%) effect on vegetative characters of soybean (Glycine max (L.) Merr.) were evaluated. Moreover, heavy metal (Ba, Ni, Cr, Cd, Pb, Zn and Fe) concentration changes in the mixtures and their influence on plant shoot heavy metal detection were determined. Stem diameter did not show changes in 2-10% combinations, throughout the six weeks of growing; in contrast, plant height exhibited drastic reduction at week five and six with 8 and 10% doses, and leaves number showed adverse effect at week six with the mixtures of 8 and 10%. Addition of oil-based drill cuttings to savannah soil increased Ba, Cr and Zn concentrations in the mixtures but they did not overcome the stabled limits or the concentrations found in oil-based drill cuttings. Cd, Ni y Pb concentrations, steady as traces throughout drill cuttings additions (2-10%), increasing only Ba concentration, and Cr, Zn and Fe concentrations were similar. Shoot Ni, Cd y Pb concentrations maintain similar values to control (savanna soil) and soil-oil based drill cuttings mixtures (2-10%). Shoot Ba, Zn and Fe increased joined to their increment in the combinations. Accordingly to bio-accumulation coefficients (BAC), soybeans can be considered as a Fe-low accumulator (BAC=0.02-0.05), Ba-moderate accumulator (BAC=0.5-0.6) and Zn-hyper-accumulator (BAC=4.3-6.2).

Keywords: *Glycine max*, heavy metals, savannah soil, drill cuttings, Venezuela.

INTRODUCTION

Drilling fluids are used in the oil industry during well drilling and construction. Specifically, drilling fluids are used for cooling, wall stabilization, pore sealing and pressure control of the oil well, lubrication of the pipes and as a means of accumulation and drag of the excavated rock or drill cuttings [26]. There are three main parts in the composition of drilling fluids: a liquid base (water, oil, or emulsion), a solid part (including both the soluble material that gives thixotropic characteristics and high density insoluble material for weight), and additional chemical substances that added directly or in solutions, to control desired characteristics [5].

Drill cuttings, muds and other polluting wastes are generated at the beginning of the oil well drilling process. Drill cuttings are composed of a mixture of rock, radioactive substances, hydrocarbons and other potential pollutants [11]. After the well drilling is completed, the wastes are often spilled on agricultural lands, where they cause changes in the soil physical, chemical and microbiological properties. Adequately addressing the environmental impact of this practice is a main priority of the oil industry, both at national and international level. This disposal practice has led to the establishment of world regulations for their application to agricultural lands [27,49].

Untreated drill cuttings may contain from 10 to 15% of total hydrocarbons, as well as heavy metals, radioisotopes and chlorides that can raise the electrical conductivity of the soils [20,37]. The United States Environmental Protection Agency has incorporated 16 hydrocarbons from oil-based drill fluids or cuttings in the priority list of pollutants that are potentially harmful and may be carcinogenic and mutagenic, These include: naphthalene, acenaphthylene, acenaphthene, anthracene, phenanthrene, fluorene, pyrene, benzo [a] anthracene, fluoranthene, chrysene, dibenzo [a,h] anthracene, benzo [b] fluoranthene, benzo [k] fluoranthene, benzo [a] pyrene, benzo [g,h,i] perylene and indeno [1,2,3-cd] pyrene [21,29;37]. Heavy metals detected in drill waste include cadmium, lead, mercury, copper, chromium, cobalt, iron, manganese, molybdenum, antimony, barium, silver, thallium, titanium, tin, zinc, chromium and vanadium, as well as the semi-metal selenium and the non-metal strontium [38]. The radioactive isotopes Radio 266[9] and Radio 228[10] have been detected in the formation water as result of the decomposition of uranium and thorium present in the oil-producing formation rocks [42]. There are many studies that show the negative impacts of drill cuttings on plant growth and development and on soil properties [15,16,35]; however, there are discrepancies between studies, explained by variations in the content of heavy metals in the drill cutting types and rates applied to the soil by investigators [8].

Venezuela, a founding member of the Organization of Petroleum Exporting Countries (OPEC), is one of world's major oil producers. The largest reserve of heavy oil is located in the eastern part of the country, which is also an area with high agricultural activity. Thus, there is high interest in knowing the effects of oil production and transport on crop growth and productivity as well as potential ways for remediation of impacted soils, not only from the ecological point of view but also to preserve agricultural productivity [33].

The purpose of this study is to assess the impact of different concentrations of oil-based drill cuttings on growth and development of soybean and its relationship with the concentration of heavy metals in soil/oil-based drill cuttings combinations and plant shoot.

MATERIALS AND METHODS

The experiment was carried out in a greenhouse (Universidad de Oriente, Campus "Juanico", Maturín: Longitude 10° 44' N and Latitude 63 ° 23' W, 90 masl, Monagas state, Venezuela), under a randomized block design with four repetitions, with savannah soil classified as Oxicplinthustulf, from the Experimental Station of Savannah of (Universidad de Oriente, Campus "Jusepín", Monagas), at inclusion rates of 0, 2, 4, 6, 8 and 10 percent of oil-based drill cuttings (PRO-AMBIENTE Co., Venezuela), for a total of six treatments. Both, savannah soil and oil-based drill cuttings were analyzed for fertility, microelements, salinity and heavy metals. In addition, heavy metal concentrations in the mixtures soil-drill cuttings before planting and plant shoot at 47 days after planting were determined. Analyses were performed in the Laboratory of Agricultural and Environmental Services of the Universidad de Oriente (LABSEA, UDO).

Soybean seeds var. Samba (GROSOSCA, Monagas state, Venezuela) were disinfected with chlorine solution (10%) for 3 minutes, then washed seven times with sterile distilled water, and left soaking for one hour in the final wash water. Three seeds were planted in each polyethylene bag (capacity 3 kg) loaded with the corresponding treatment mix of soil and oil-based drill cuttings. The seeds were inoculated just before planting with *Bradyrhizobium japonicum* (BIOAGRO 10), which was prepared and incorporated on seeds in accordance with the manufacturer's recommendations. Fertilizer was applied at the time of planting and consisted of 95.2 mg of $(NH_4)_2SO_4$, 93.1 mg of KH_2PO_4 and 20.9 mg of KCl per bag. Irrigation was carried out every 3 days (300-400 ml of water per bag) to cover plant requirements. Weed control was performed manually.

Plants were evaluated weekly for height, stem diameter and number of leaves starting on the second week after planting and up to the sixth week (beginning of flowering). Variance analysis for experimental design, correlation analysis and averages comparison were executed using the Least Significant Difference ($p \leq 0.05$) [2].

RESULTS AND DISCUSSION

Physico-chemical characteristics of substrates

Savannah soil (control) had a sandy-silty texture with low organic matter content and characterized by a slightly acidic pH, low phosphorus and potassium concentrations, medium levels of calcium and magnesium, trace amount of aluminum and low effective cation exchange capacity (ECEC). The microelements zinc, copper and manganese were found below the values of reference, except iron. Heavy metal content was traces. Analysis of salinity showed values for electrical conductivity (EC) and sodium absorption ratio (SAR) below normal levels (Table 1).

Table 1. Analysis of microelements, fertility, salinity and heavy metals carried out on savannah soil and oil-based drill cuttings, prior to planting.

			Fertility^{a}			
Identification	unit	Reference values			Determination	
		Low	Medium	High	Soil^{b}	OBDC^{c}
pH					5,2	8,90
P	mg kg^{-1}	<11	>11-30	>30	1,97	2,98
Ca	me 100g^{-1} de suelo	<0,50	>0,50-1	>1	0,88	0,91
Mg		<0,25	>0,25-0,65	>0,65	0,43	0,30
K		<0,13	>0,13-0,26	>0,26	0,02	0,02
Al		<0,25	>0,25-0,50	>0,50	Trazas	Trazas
H					0,13	0,08
CICE					1,46	1,31
Al saturation	%				0,00	0,00
Organic matter		<1,5	>1,5-3,0	>3,0	0,70	2,56
Texture						
Clay	%				7,2	17,2
Class					Sandy-silty	Silty- sandy
			Microelements^{a}			
Zn	mg kg^{-1}	<1,5	>1,5-2,5	>2,5	0,88	2,88
Cu		<0,8	>0,8-1,2	>1,2	Traces	0,16
Mn		>1,5	>1,5-2,5	>2,5	0,6	7,22
Fe		<7,0	>7,0-11,0	>11,0	20,40	97,60
			Salinity^{a}			
		Soil^{b}			OBDC^{c}	
		Normal levels^{a}	Determination		Límit^{a}	Determination
Sulphates	me L^{-1}	>5,2	0,106			5,37
Carbonates			Trazas			0,342
Bicarbonate		<5,0	0,108			5,94
Chloride		<10,0	Traces		<2.50,00	4,39
Ca		>2,5	0,255			0,565
Mg		>3,0	0,175			0,07
K		>1,0	0,136			0,187
Na			0,061			19,17
SAR		>15,0	0,13		<8,0	34,23
EC					<3,5	1,85
			Heavy metals^{a}			
		Soil^{b}			OBDC^{c}	
	mg kg^{-1}	Determination			Límit^{a}	Determination
Pb		Traces			150,0	13,0
Ni		Traces				35,5
Ba		Traces			20.000,00	395,5
Cd		Traces			8,0	Traces
Cr		Traces			300,0	16,0
Zn		Traces			300,0	108,5
Fe		4.415,0			23.405,0	38.225,0

[a] Laboratory of Agricultural and Environmental Services (LABSEA), Universidad de Oriente, Núcleo de Monagas, Maturín. [b] Savannah soil Oxicplinthustulf type. [c] Oil-based drill cuttings.

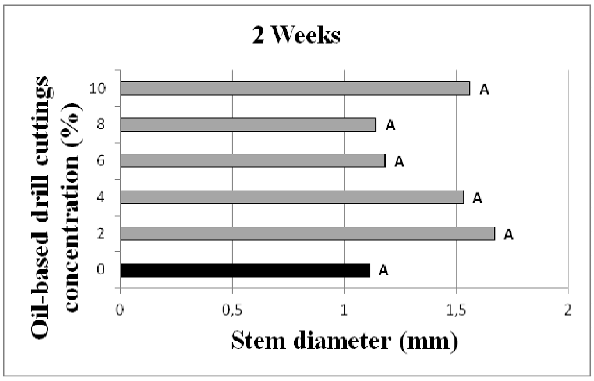
Oil-based drill cuttings had a silty-sandy texture, very alkaline pH, low concentrations of phosphorus and potassium, and medium levels of calcium and magnesium, and medium organic matter content. With the exception of copper, the microelements zinc, manganese and iron were present in higher concentrations higher than the values of reference and much higher than in the Savannah soil. Heavy metals were found in concentrations well above those found in the Savannah soil, with the exception of cadmium. Salinity analysis revealed an EC (1.85^{-1}mmhoscm) within the limit (<3,5) and SAR (34,23 mg kg^{-1}) well over the authorized maximum (<8) (Table 1). In accordance with the United States

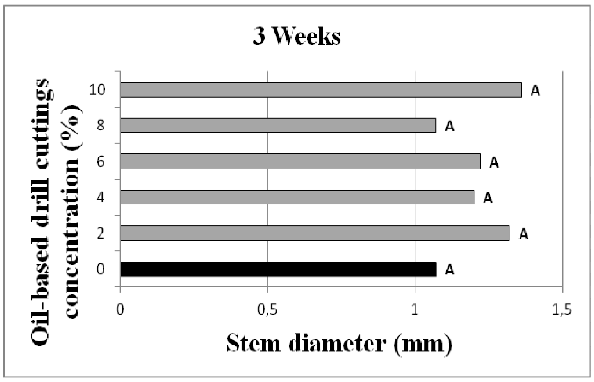
Department of Agriculture [48] and Guerrero-Alves [17], the following characteristics SAR>15, EC<4,0, pH>8.5 and interchangeable Na > 15, characterize the oil-based drill cuttings as a sodic soil.

It is clear that the microelements, except copper, were found in higher concentrations in the oil-based drill cuttings than in savannah soil, which suggests that it could be used as fertilizer through their incorporation into the soil at low concentrations and frequent monitoring through physico-chemical analysis; however, the pH could negatively affect the nutrients absorption by plants. Drill cuttings specifically have a very alkaline pH (8.90). This high pH could affect the availability of microelements by influencing fixation, or predominance of non-available and interchangeable forms as previously observed in studies on the availability of copper, iron, manganese and zinc [36,43].

Evaluation of vegetative characters of soybean

Variance analysis for soybean plants stem diameter showed no statistically significant differences among the different inclusion rates of oil-based drill cuttings at two to six weeks after planting (F=1,49, 0,35, 0,23, 0,23 and 0,38, respectively) (Figure 1). In contrast, plant height showed significant differences between treatments at fifth and sixth weeks (F=3,16 and 3,22, respectively) (Figure 2). A stunting effect was observed for the inclusion rates of 8 and 10%, with an observed height reduction of 40% at the fifth week and 35% at the sixth week.





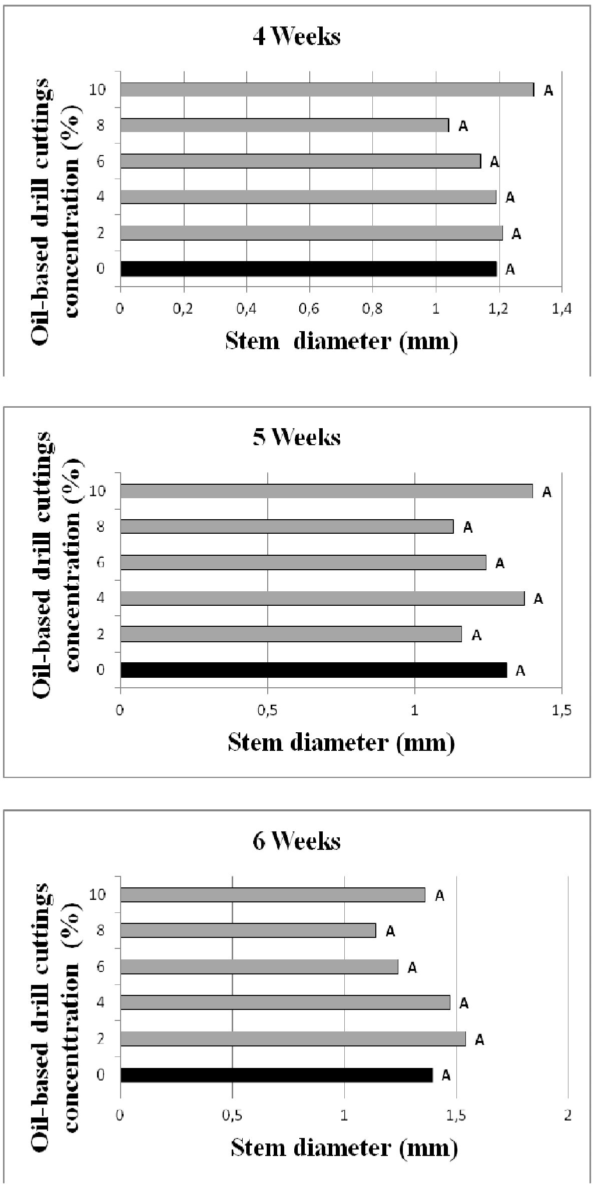
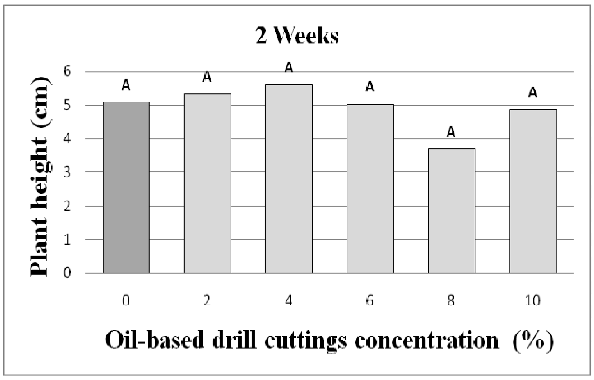


Figure 1. Stem diameter of soybean plants grown at six concentrations (0 - 10%) of oil-based drill cuttings at two, three, four, five and six weeks of growing. Different letters indicate statistically different means (LSD test, p≤0.05).



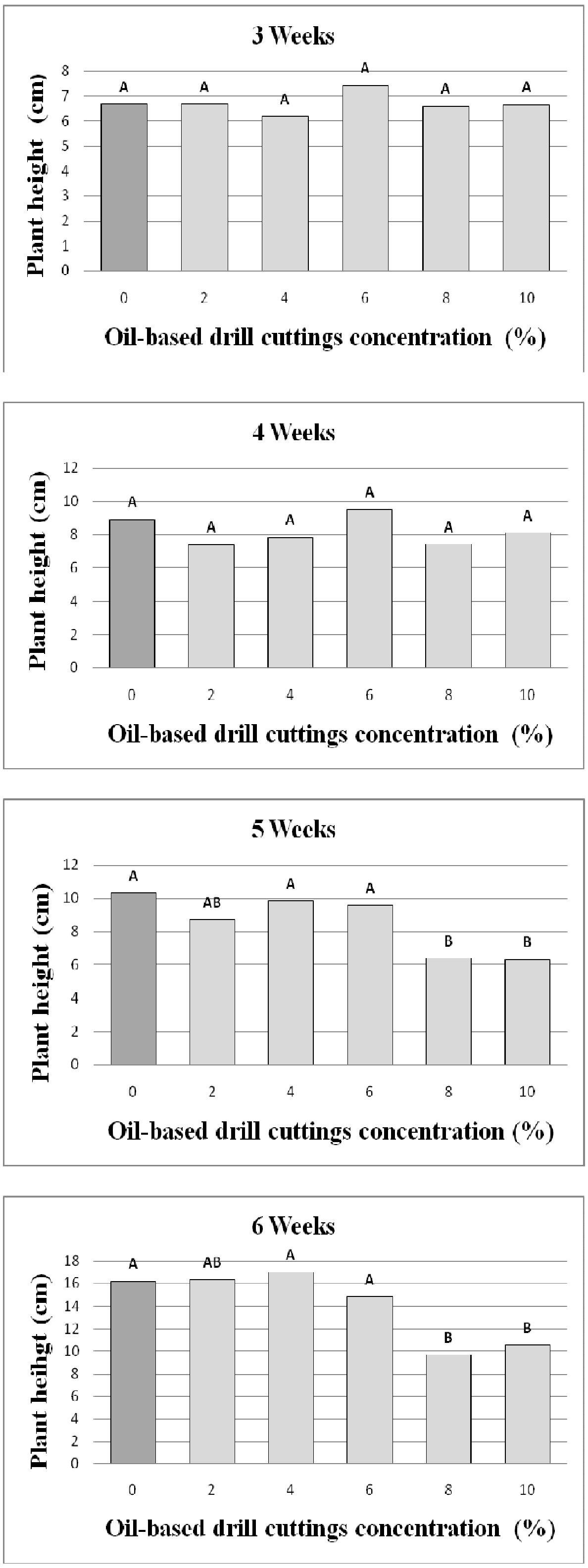
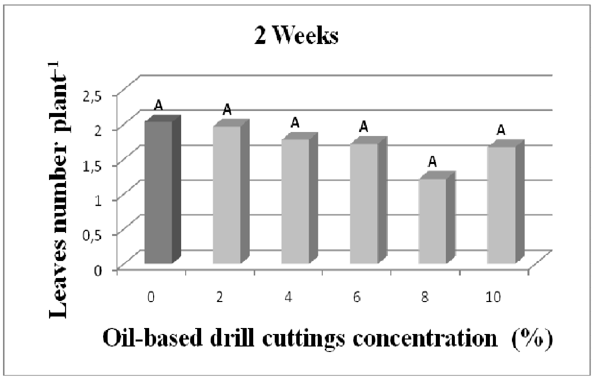
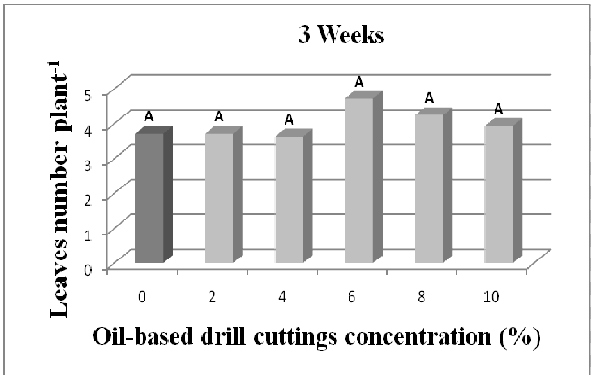
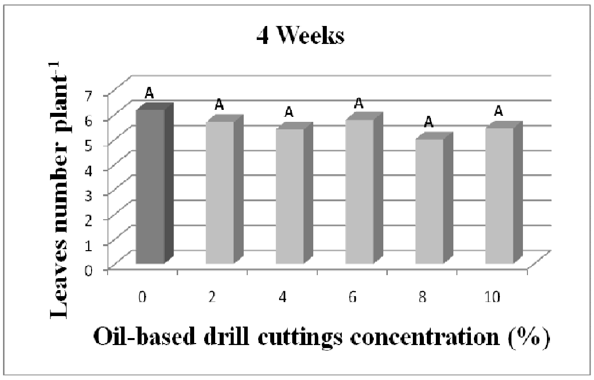


Figure 2. Height of soybean plants grown for six weeks in various concentrations (0-10%) of oil-based drill cuttings. Different letters indicate statistically different means (LSD test, p≤0.05).

Similar to plant height, the number of leaves was affected by both inclusion rates of 8 and 10% at the sixth week (F=3.45); 10.8 leaves were observed in control plants, while 5.5 and 5.7 leaves were detected for 8% and 10%, respectively (Figure 3).







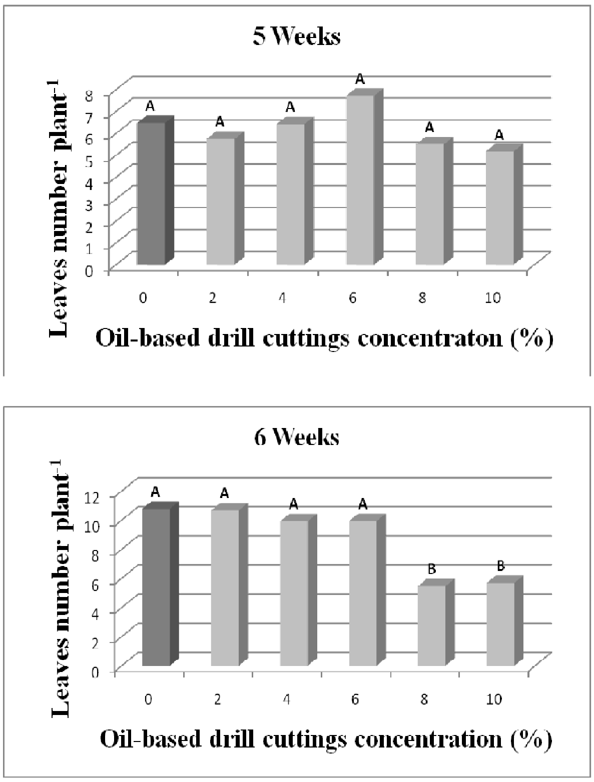


Figure 3. Leaves number of soybeans plants cultivated for 2, 3, 4, 5 and 6 weeks in savannah soil mixed with different concentrations of oil- based drill cuttings (0-10%). Different letters indicate statistically different means (LSD test, $p \leq 0.05$).

Drill cuttings inclusion rates of 8 and 10% negatively affected the growth of soybeans, shown by the reduction of height and the number of leaves. On one hand, this could be attributed to reduced availability of micronutrients caused by the high pH in the drill cuttings. Well known are the detrimental effects of alkaline pH (pH>7) on availability and absorption of the macroelements phosphorus, calcium, magnesium and potassium, and the microelements zinc, copper, manganese and iron, and its impact on plant growth. In this context, it has been reported that the availability of phosphorus is strongly tied to soil pH; the maximum solubility and availability occurs at pH 6.5, declining as it increases [19], such as occurs with calcium, magnesium and potassium, whose range of appropriate pH is reported between 6 and 6.5 [10,41].

Phosphorus deficiency leads to stunted plant growth, mainly observed as a decrease in shoot growth, and triggered deep changes in plant development and biochemistry, as well as at gene expression level [44,45]. In the case of symbiosis in legumes, there has been observed an adverse effect on nodule development and functioning [6,40]. For its part, calcium is a crucial regulator on plant growth and development whose deficiency results in poor radical development, leaf split and necrosis, among other disorders associated with the stabilization of the residues of pectin in the wall, the increased permeability of plasma membrane and the adverse effects of the enzyme polyphenol oxidase and cell wall damages [3,18,50]. Moreover, potassium deficiency can induce growth retardation and downgrading of fruit quality, as result of the unfavorable effect on photosynthesis, enzyme activation, assimilation and transport of nutrients, cell turgor and ion homeostasis [22,24,34]. Likewise, magnesium deficiency affects the synthesis of chlorophyll, such as being a component of the chlorophyll molecule, the processes of phosphorylation, dephosphorylation and hydrolysis of several compounds because it acts as a cofactor. Also, it can disrupt the aggregation of ribosomal subunits since it operates as a bridge; the ultimate consequence of these effects results in the reduction of growth and the modification of the root/stem relationship [13].

Coupled with deficiencies of macronutrients resulting from the alkalinity of the 8 and 10% inclusion rate treatments (pHs 7.90 and 8.03, respectively), a negative effect of alkaline pH on availability and absorption of microelements may occur. In this regard, Broadley [12] and Martínez *et al*. [32] indicate that zinc deficiency is the most common among the micronutrients deficiencies in soils of temperate and tropical regions. Incipient zinc deficiency produces reduction of protein and starch synthesis and in cases of severe zinc deficiency, death of radical tip "dieback", interveinal chlorosis, reddish or bronze spots and similar response to auxin deficiency, such as internodes shortening, epinasty and leaf size twisting and reduction may occur. Meanwhile, copper deficiency produces general growth depression with reduction in leaves size and number, and mature flower number, thus with lower productivity given by the lowest number of fruits and seeds produced. In addition, deterioration occurs in seed quality due to reduction in protein and carbohydrate (sugars and starch) concentrations and decrease in leaves chlorophyll a and b concentrations [9,28]. In addition, manganese is essential in respiratory and nitrogen metabolism, where a deficiency inhibit the activities of the enzymes nitrate reductase, glutamine synthetase and glutamic-oxaloaceto transaminase, with consequences in the synthesis of chlorophyll and soluble proteins. Manganese deficiency is characterized by chlorotic and necrotic areas between veins of young leaves [16,20]. The detrimental effects described above, may be associated to or in addition to others effects due to iron deficiency. Iron is essential in chlorophyll synthesis and electrons transport in photosynthesis and respiration. At the mitochondrial level, it is essential for the synthesis of the heme group and the Fe-S complex of proteins. Characteristic of its deficiency is the development of chlorotic or white areas between the veins of young leaves, which finally develop in necrotic areas [4,7,30]. However, total iron concentration in leaves did not correlate to iron deficiency.

In addition to the above effects on plant mineral nutrition, there is the possibility of adverse effects produced by the probable sodicity at the concentrations of 8 and 10%. As it was quoted earlier, oil-based drill cuttings, can be catalogued as a sodic soil (pH: 8.90, SAR: 34,23, EC: 1.85 and Na:19,17), according to Guerrero-Alves [17] and USDA [48] which *per se* brings negative consequences on plant growth, development and productivity. In context, Alhajhoj [1] observed an increase of sodicity on soil of Saudi Arabia by the addition of oil sludge and its consequent negative effects on the growth of *Vinca rosea*. The sodicity may negatively affect root penetration as well as water and air movement in soil. In addition, Na^+ can displace Ca from link points on the outside surface of root cells plasma membrane, so inducing not only calcium deficiency, but also radical cells lengthened in radial form and non-axial form, leading to the formation of thickened roots [39].

Heavy metal concentrations in savannah soil and savannah soil/oil-based drill cuttings mixtures

Values of cadmium, nickel and lead were unchanged (traces) all over the various inclusion rates (2-10%) of oil-based drill cuttings to savannah soil only barium concentrations increased in all inclusion rates (2-10%). Chromium, zinc and iron remained similar. It should be noted that by mixing savannah soil and oil-based drill cuttings there was an increase of barium, chromium, zinc and iron with regard to the concentrations found in soil, but in any case they exceeded the permissible limits (Table 2).

Table 2. Heavy metal concentrations in savannah soil (SS), oil-based drill cuttings (OBDC) and SS/OBDC mixtures.

Element (mg/kg)	Limit (mg/kg)	Soil[a]	OBDC[b]	SS/OBDC mixtures (%)[c]				
				2	4	6	8	10
Ba	20.000	T[d]	395,5	68,0	228,0	300,0	309,5	310,0
Ni	T	T	35,5	T	T	T	T	T
Cr	300	T	16,0	7,5	9,5	9,5	8,5	7,5
Cd	8	T	T	T	T	T	T	T
Pb	150	T	13,0	T	T	T	T	T
Zn	300	T	108,5	16,5	20,5	18,5	23,0	23,0
Fe	23.405,0	4.415,0	38.225,0	3.560,0	3.890,0	4.780,0	4.220,0	4.305,0

[a] Savanah soil, [b] Oil-based drill cuttings, [c]savannah soil/oil-based drill cuttings mixtures, [d] Traces.

Heavy metal concentrations in shoots

Shoot chromium concentration remained similar (traces) to that found in savannah soil, so that the increase that occurred in mixtures (2-10%) with the addition of **oil-based drill cuttings** might not be available for absorption; likewise, cadmium, nickel and lead did not show changes in the concentrations (traces), maintaining values similar to those found in control soil (savannah soil) and in the mixtures with **oil-based drill-cuttings**. The rise of barium and zinc concentrations (68 to 310 mg kg^{-1} and 16.5 to 23.0 mg kg^{-1}, respectively) in mixtures (2 to 10%) was reflected in shoot barium and zinc concentrations, which indicates their absorption by the plant (Tables 2 and 3). The majority of plants have small amounts of barium (4 to 50 mg kg^{-1}) in their tissues, thus higher concentrations could become toxic [13]. Suwa *et al.* [47] found foliar soybean concentrations of barium ranging from 75 to 450 mg kg^{-1}, which resulted in reduced growth, oxidative stress and inhibition of photosynthetic activity (phytotoxic effect) by closing the stomata and disturbing carbon metabolism. Zinc is an essential micronutrient when it is present in soil at trace levels, but it has been found to become phytotoxic at concentrations ranging from 100 to 500 mg kg^{-1} [23]. Kim and McBride [25] found a reduction in soybean biomass as a result of zinc's phytotoxic effect. In this study, zinc concentrations above 100 mg kg^{-1} were found at inclusion rates of 6, 8 and 10% (110.0, 143.0 and 142.0 mg kg^{-1}, respectively) which could lead to the observation of phytotoxic effects. Kabata-Pendias and Pendias [23] identified zinc concentrations from 100 to 500 mg kg^{-1}as toxic limits. The occurrence of Zn and Ba in oil-based drilling cuttings is attributed to the zinc carbonate and barite ($BaSO_4$) present in the drilling fluid [26].

Table 3. Heavy metal concentrations in foliage of soybeans grown in savannah soil (SS), oil-based drill cuttings (OBDC) and SS/OBDC mixtures.

Element (mg/kg)	Limit (mg/kg)	Soil[a]	OBDC[b]	SS/OBDC mixtures (%)[c]					
				0	2	4	6	8	10
Ba	20.000	T[d]	395,5	45,0	134,0	142,0	148,0	169,0	185,0
Ni	T	T	35,5	T	T	T	T	T	T
Cr	300	T	16,0	T	T	T	T	T	T
Cd	8	T	T	T	T	T	T	T	T
Pb	150	T	13,0	T	T	T	T	T	T
Zn	300	T	108,5	T	85,0	88,0	110,0	143,0	142,0
Fe	23.405,0	4.415,0	38.225,0	116,0	109,0	93,0	138,0	178,0	200,0

[a] Savanah soil, [b] Oil-based drill cuttings, [c] savannah soil/oil-based drill cuttings mixtures, [d] Traces.

Bio-accumulation coefficients

Bio-accumulation coefficient (BAC) has received different names in the literature [e.g., see 29, 34]. It was calculated as the ratio of shoot metal concentration (BACsh) to soil metal concentration (BACso), on dry weight basis. In accordance with Malik *et al*. [31], BAC = BACsh/BACso (mg kg^{-1}). Sekabira *et al*. [46] consider the bio-accumulation coefficient as the rate of metal cumulative efficiency. On the basis of the categorization proposed by these authors: < 0.01 non accumulator plants, 0.01-0.1 low accumulator plants, >0.1-1 moderate accumulator plants and >1-10 high accumulators or hyper- accumulator plants, soybeans can be regarded as Fe-non accumulator plant (BAC = 0.02-0.05), Ba-moderate accumulator plant (CBA = 0.5-0.6), except for the lowest level of oil-based drill cuttings (2%) and Zn-hyper- accumulator plant (CBA = 4.3-6.2) (Table 4).

The Phytoremediation is a promising method that allows the cleaning of contaminated soils with toxic metals by use of hyper- accumulator plants [14,31]; in this category this variety of soybean could serve for decontamination of zinc contaminated soils. Similarly, Kisic *et al*. [26] typified soybean as a Zn-moderately to highly accumulating plant, with higher content in aboveground biomass than in seeds.

Table 4. Heavy metals (Fe, Ba and Fe) bio-accumulation coefficients in soybeans aerial biomass.

SS-OBDC mixtures[a] (%)	BAC[b]		
	Fe	Ba	Zn
2	0,03	2,0	5,2
4	0,02	0,6	4,3
6	0,03	0,5	5,9
8	0,04	0,5	6,2
10	0,05	0,6	6,2

[a] Savannah soil/oil-based drill cuttings mixtures, [b] Bio-accumulation coefficients.

CONCLUSION

Among the measured vegetative parameters, stem diameter did not show (statistically significant differences) changes at all inclusion rate treatments throughout the six weeks of measured growth., However, plant height reduction was observed at weeks five and six for the 8 and 10% inclusion rate treatments, while a reduction in leaf number was observed at week six for both 8 and10% inclusion rate treatments.

All the savannah soil/oil-based drill cuttings mixtures showed an increase in the Ba, Cr and Zn concentrations, in comparison with the control soil (savannah soil at 0% inclusion rate), but the concentrations did not exceed permissible limits nor the concentrations found in oil-based drill cuttings. Cd, Ni and Pb concentrations remained in traces through all the inclusion rates of oil-based drill cuttings, increasing only for Ba; and the Cr, Zn and Fe concentrations remained similar.

Shoot Ni, Cd and Pb concentrations did not show changes, thus maintaining similar values (traces) to those found in control (savannah soil) and soil/oil-based drill cuttings mixtures (2-10%). Shoot Ba, Zn and Fe increased together with their increase in soil/oil-based drill cuttings mixtures. There was Cr presence in the mixtures; remaining as traces in all combinations.

According to bio-accumulation coefficients (BAC), soybean can be considered as a Fe-low accumulator plant (BAC = 0.02-0.05), Ba-moderate accumulator plant (CBA = 0.5-0.6) and Zn-hyper-accumulator plant (BAC = 4.3-6.2).

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