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EFFECT OF HEAVY METAL CONTAMINATION ON MICROBIAL POPULATION, ORGANIC CARBON AND NITROGEN MINERALIZATION IN SOIL PLANTED TO LEGUMES

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ABSTRACT

This study was carried out to investigate the effects of heavy metal contamination on the microbial population, organic carbon and nitrogen mineralization in the soil planted to legumes. Two heavy metals (*Zinc and lead)* were used at 150 mg kg $^{-1}$ and 200 mg kg^{-1} concentrations respectively. Four legumes (*Mucuna, Cowpea, Soyabean and Centrosema)* were planted in pots filled with 4 kg of 2 mm sieved soil. The experiment was a 3 x 5 factorial, arranged in a Completely Randomized Design with three replications. The soil reaction (pH), organic carbon, nitrate nitrogen (NO_3-N), ammonium nitrogen (NH_4-N) and microbial count were determined at 4 and 8 weeks after contamination (WAC). The soil pH ranged from 6.49 to 7.11 and 6.42 to 7.02, organic carbon ranged from 14.3 to 27 g kg^{-1} and 10.5 to 20.0 g kg-^{1} at 4 and 8 WAC, respectively. Nitrate nitrogen ranged from 140 to 159.7 mg kg^{-1} and 12.0 to 101.7 mg kg^{-1}, NH_4-N also ranged from 31.7 – 980 mg kg^{-1} and 17.7 to 80.7 mg kg^{-1} at 4 and 8 WAC, respectively. Contamination with heavy metal increased the soil pH, organic carbon content, and nitrogen mineralization in some pots while reduction occurred in others. Microbial population was found to increase significantly with contamination at 8 WAC. The organic carbon content was increased by about 40 % and 42% at 4 and 8 WAC, respectively. The increase in nitrate nitrogen mineralization was about 80% and 52 % at 4 and 8WAC, respectively. Microbial population increased by about 57 % and 62 % at 4 and 8 WAC, respectively.

Key words: mineralization; contamination; heavy metals.

INTRODUCTION

Elevated amount of heavy contaminants resulting from industrialization and urbanization is a serious environmental concern. Besides, the metal of lithogenic origins, resulting from different human activities (mining, metallurgy, combustion of fossil, energy sources, solid waste or sewage sludge disposal, animal effluents and fertilizer inputs for agriculture) are deposited in soils. Heavy metals in soils exist in various physicochemical forms both in the solid and solution phase. They are associated with several fractions in soil solution as free metal ions and soluble metal complexes; adsorbed on

inorganic soil constituents at ion exchange sites; bound to soil organic matter; precipitated such as oxides, hydroxides and carbonates; and embedded in structure of the silicate minerals (Tessier *et al*., 1979). Heavy metals are toxic to most organisms when present in high concentration. They affect growth, morphology and metabolism of microorganism in soils. They cause protein denaturation or the destruction of the integrity of all membranes. They generally exert an inhibitory action on soil microorganism by displacing essential metal ions, blocking essential functional groups, or by modifying the active conformation of biological molecules. Consequently, the activities of micro-organisms would be reduced (Amlinger and Boltzmann, 1994). This study examined the effects of zinc and lead on the population of microbes, organic carbon and nitrogen mineralization in soil planted to legumes.

MATERIALS AND METHODS

Surface soil samples (0-15cm) of an alfisol (Iwo series) were collected at the University of Agriculture, Abeokuta, Nigeria. The bulk sample soil was air dried and sieved (2mm screen) to remove roots and stones before analysis as described by Page *et al.* (1982). The analysis of initial soil sample gave the following chemical composition: pH (H_20) 6.59, 27.5 g kg^{-1} of organic matter, 49 mg kg^{-1} of ammonium nitrogen, 63 mg kg^{-1} of nitrate nitrogen, 6.04 mg kg^{-1} available P, 4.23 cmol kg^{-1} of Calcium 0.51 cmol kg^{-1} of Mg, 0.53 cmol kg^{-1} of Na and 0.51 cmol kg^{-1} of K (ammonium acetate extractable). Distribution of soil particle size includes 87.4% sand, 6.8% silt and 5.8% clay.

Four Kilogram (4 kg) aliquots of sieved (2mm) soil were weighed into polythene bags. Seeds of *Mucuna*, cowpea, soybean and *Centrosema*, were grown in the pots and moistened with water. Stock solutions of zinc and lead nitrate were applied to the soil four weeks after planting (WAP). The experiment was a 3 x 5 factorial arranged in Complete Randomized Design. The factors involved were two heavy metals (Zn and Pb) plus the control (no metal), and the four legumes and the control (No plant).

Soil samples were collected from each pot at 4 and 8 weeks after contamination (WAC) with heavy metals. The pH of the sample was determined potentiometrically in 1:2 soil to water suspension. Organic carbon was determined using chromic acid digestion method. NO^{-}_3-N and NH_4-N were determined by steam distillation techniques after extraction with 2N KCl. Microbial count of the soil samples was carried out using serial dilution method. Data were subjected to analysis of variance using SAS User Guide. Means were separated using Duncan's Multiple Range Test.

RESULTS

Effect of heavy metals on soil pH

Contamination of the soil with Zn and Pb resulted in significant increase and decrease in the pH levels of the unplanted soil (in the control) at 4 and 8 WAP respectively (Table 1).

A significant increase in pH was observed generally between the planted and unplanted soils at 4 and 8 WAC in the control pots (no metal contaminant). At 8 WAC, *Centrosema* planted pot was significantly lower in pH than other planted pots (*Mucuna*, cowpea and soybean) which were not different in pH. At 4 and 8 WAC, Pb contaminated pots were consistently higher in pH values than Zn contaminated pots except for *Mucuna* pots at 8 WAC. The highest pH value was observed in *Centrosema* pot at 8 WAC.

Table 1: Effect of Zn and Pb Contamination on Soil pH

	4 WAC			8 WAC		
Legumes	**Uncontaminated**	**Zn**	**Pb**	**Uncontaminated**	**Zn**	**Pb**
Unplanted	6.49d	6.67b	6.70c	6.56c	6.42b	6.50c
Mucuna	7.02a	6.61bc	6.64c	6.84a	6.87a	6.63b
Cowpea	6.65c	6.54c	7.11a	6.89a	6.84a	6.95a
Soyabean	6.83b	6.83a	7.03a	6.87a	6.85a	6.98a
Centrosema	7.05a	6.88a	6.88b	6.75b	6.92a	7.02a

Means with the same letter in columns are not significantly (P<0.05) different

Effect of heavy metals on organic carbon

The mean organic carbon (OC) varied considerably among samples (Table 2). Generally, contamination of the soil with Zn and Pb resulted in a significant increase in organic carbon content of soils when compared with the control (no contaminant). This increase was generally higher with Zn contamination than Pb contamination. Among the Zn contaminated pots, soil from cowpea pots had the highest organic carbon at 4 WAC, followed by the control (unplanted pot), mucuna, centrosema and soybean in the decreasing order.

In the Pb contaminated pots, cowpea also had the highest OC followed by mucuna, soybean, control and centrosema in that order. Significant difference in OC was also observed among the different planted pots with no metal contamination. Soil from cowpea pot had the highest organic carbon followed by soil from centrosema, soyabean and mucuna pots in that order. The least OC was recorded by the unplanted pot.

At 8 WAC, contamination with Zn and Pb resulted in significant increase in the organic carbon content only in soils from the unplanted and centrosema pots. A general decrease was observed in others with soil from soyabean pot having the lowest organic carbon.

Table 2: Effect of Zn and Pb Contamination on Organic Carbon (g kg^{-1})

	4 WAC			8 WAC		
Legumes	**Uncontaminated**	**Zn**	**Pb**	**Uncontaminated**	**Zn**	**Pb**
Unplanted	14.3e	22.4b	19.6d	11.5d	18.0b	19.2a
Mucuna	18.1d	21.7c	21.3c	20.0a	19.5a	13.5b
Cowpea	26.5a	27.0a	22.5a	15.5b	15.5c	14.0b
Soyabean	18.5c	17.5e	21.0b	14.5c	11.5e	13.0c
Centrosema	20.5b	20.2d	18.7e	10.5e	13.9d	14.0b

Means with the same letter in columns are not significantly (P<0.05) different

Effect of heavy metal on nitrogen mineralization

Ammonium nitrogen (NH_4-N)

Heavy metal contamination had significant effect on nitrogen mineralization in the soil (Table 3). At 4 WAC, contamination of the soil with Pb resulted in a significant increase in NH_4-N content in soils from mucuna pot, unplanted pot and centrosema pot. However, a significant decrease was observed in cowpea and soyabean pots. On the other hand, Zn contamination only resulted in significant increase in NH_4-N in centrosema planted pot when compared with soil without metal contamination.

Significant differences were also observed in NH_4-N content of soil without heavy metal contamination. Ammonium nitrogen was highest in *Mucuna* pot (84.0 mg kg^{-1}) and least in *Centrosema* pot (34.7 mg kg^{-1}). NH_4-N concentration was in the order: *Mucuna* pot >cowpea>soyabean pot>unplanted pot>*Centrosema* pot. Cowpea and soyabean pots were not significantly different in NH_4-N content. At 8 WAC, contamination with Pb varied considerably among the pots in NH_4- N content. The increase followed the order: unplanted pot > *Centrosema* pot > soyabean > *Mucuna* > cowpea. *Centrosema* and unplanted pots were similar in NH_4-N content. Zinc contamination significantly affected ammonium content among the pots. The increase in NH_4-N content was in the order: *Centrosema* pot > *Mucuna* > soyabean > unplanted pot > cowpea. However, when compared with contaminated pots, a general decrease was observed. Similar trend was observed in Pb contamination when compared with uncontaminated pots with the exception of unplanted pot where a significant increase in NH_4-N was observed (60.3 % increase).

Table 3: Effect of Zn and Pb Contamination on NH_4-N (mg kg^{-1})

	4 WAC			8 WAC		
Legumes	**Uncontaminated**	**Zn**	**Pb**	**Uncontaminated**	**Zn**	**Pb**
Unplanted	59.7bc	31.7d	80.7b	32.0c	31.7cd	80.7a
Mucuna	84.0a	84.0a	98.8a	73.7a	52.7ab	31.7cd
Cowpea	73.7ab	74.0ab	60.7c	73.7a	17.7d	24.7d
Soyabean	66.7b	60.3bc	47.3cd	49.3b	45.7bc	45.7bc
Centrosema	34.7d	51.0c	53.3cd	56.0b	66.7a	66.7a

Means with the same letter in columns are not significantly ($P<0.05$) different

Nitrate nitrogen (NO_3-N)

Contamination with Zn and Pb significantly affected NO_3-N in the various pots planted to legumes (Table 4). At 4 WAC, Zn contaminated soils revealed that NO_3-N was highest in *Mucuna* pot and least in unplanted pot. This followed the order: *Mucuna* pot> cowpea> *Centrosema* pot> soyabean pot> unplanted pot. On the other hand, Pb contamination showed that *Centrosema* pot was highest in NO_3-N and least in soyabean pot. This also followed the order: *Centrosema* pot>unplanted pot > *Mucuna* > cowpea > soyabean.

At 8 WAC, Zn and Pb contamination resulted in a general decrease in NO_3-N when compared with 4 WAC. Similar trend was also observed among uncontaminated pots. Zinc and lead contamination also differed in their effects on NO_3-N among the various legume pots at 8 WAC. Zinc contamination revealed that *Centrosema* pot had the highest NO_3-N while cowpea pot has the least and it followed a decreasing order: *Centrosema* pot > soyabean > *Mucuna* pot > unplanted pot > cowpea.

Lead, on the other hand, followed the order: *Mucuna* pot > unplanted pot > *Centrosema* > soyabean > cowpea. However, among the uncontaminated pots, NO_3-N content also differed significantly following a decreasing order: *Mucuna* pot > *Centrosema* pot>cowpea pot>unplanted pot > soyabean.

Among the uncontaminated pots, soyabean pot was highest in NO_3-N and the unplanted pot was the least. Zinc and lead contamination resulted generally in a significant increase in NO_3-N among the various pots compared with the control (no contaminant), except only in soyabean pot where a decrease in NO_3-N was observed. Similar trend was observed at 8 WAC with the exception of cowpea pot, where a decrease in NO_3-N was observed.

Table 4: Effect of Zn and Pb Contamination on NO_3-N (mg kg^{-1})

	4 WAC			8 WAC		
Legumes	**Uncontaminated**	**Zn**	**Pb**	**Uncontaminated**	**Zn**	**Pb**
Unplanted	14.0c	45.7d	150.7a	52.7bc	59.7b	77.0b
Mucuna	87.7a	150.7a	126.0b	80.7a	69.7ab	101.7a
Cowpea	94.7a	145.3a	77.0c	66.7ab	42.0c	45.7c
Soyabean	101.7a	66.7c	59.7d	43.7c	73.7ab	45.7c
Centrosema	52.7b	108.7b	159.7a	73.7a	84.0a	73.7b

Means with the same letter in columns are not significantly (P<0.05) different

Effect of Zn and Pb contamination of microbial population in soil

Microbial population varied considerably with respect to heavy metal contamination among pots (Table 5). Contamination of soil at 8 WAP resulted generally in a significant increase in the number of microbes in various pots when compared with the control (uncontaminated pots). The increase was slightly higher in lead than Zn contaminated pots, although differences were not significant among various legume pots. At 4 WAC, *Mucuna* pot was consistently higher in microbial population than other legume planted pots with respect to zinc and Pb contamination.

Table 5: Effect of Zn and Pb Contamination on microbial population (x 10^4 cfu ml^{-1})

	4 WAC			8 WAC		
Legumes	**Uncontaminated**	**Zn**	**Pb**	**Uncontaminated**	**Zn**	**Pb**
Unplanted	124a	110abc	102c	61a	144a	160ab
Mucuna	135a	149a	162a	71a	146a	148ab
Cowpea	140a	84c	117bc	88a	160a	124b
Soyabean	128a	110abc	102c	90a	163a	156ab
Centrosema	64b	97bc	112c	75a	128a	176a

Means with the same letter in columns are not significantly (P<0.05) different.

DISCUSSION

The results showed that it was not possible to quantify the effect of heavy metal contamination on soil pH with confidence. Although Zn and Pb caused increase in pH and organic carbon in some cases, there were also cases of decrease in pH levels of soil and organic carbon content in the soil. This is similar to the observation made by some authors (Doelman and Haanstra, 1984; Hiroki, 1992).

Heavy metal contaminations had more effect on NH_4-N compared with NO_3-N. This was evident by a greater decrease in NH_4-N content in soil than in NO_3-N. This may be due to the fact that most crops take up nitrogen in form of NO_3-N. Contamination of the soil did not adversely reduce population of the microbes especially at 8WAC. This suggests that some soil microorganisms may tolerate heavy metal toxicity. There were a number of reports of increased abundance of metal tolerance with severe soil pollution found a higher level of metal tolerance in *Pseudomonas* isolated from soil around industrial sites compared with isolate taken from uncontaminated agricultural soils.

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