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Changes in Bacterial Density, CO_2 Evolution and Enzyme Activities in Poultry Dung Amended Soil

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ABSTRACT

The utilization of cattle and poultry manure as organic fertilizer improves soil productivity, but arsenic contaminated poultry dung may interfere in soil metabolism and soil fertility. The study was conducted to assess the effects of poultry dung as well as arsenic contamination on soil properties in 1%, 3% and 5% poultry dung amended soil and 1, 5 and 10 ppm sodium arsenite contaminated soil. pH and conductivity were found to be increasing with increase in poultry dung in soil. Other chemical parameters like nitrate, phosphate and organic carbon were found higher in poultry dung amended soil than that of arsenic contaminated soil. Soil bacteria, CO_2 evolution and enzymatic activities like amylase, invertase and dehydrogenase were also found higher in poultry dung amended soil suggesting the effectiveness of poultry dung in enhancing soil productivity, even if it was contaminated by As through feed additive.

Keywords: Soil; Poultry Dung; Arsenic; Soil Enzyme; CO_2 Evolution; Bacteria

1. Introduction

The increasing demand of chicken meat has prompted more poultry farming with consequent effects on increased utilization of organic wastes (e.g. chicken dung manure) as fertilizers. Organic wastes contain varying amounts of water, mineral nutrients, organic matter [1,2]. While the use of organic wastes as manure has been in practice for centuries world-wide [3,4], there still exists a need to assess the potential impacts of poultry manure on soil chemical properties and crop yield and in particular evaluating the critical application levels. Moreover, the need and utilization of poultry manure has overtaken the use of other animal manure (e.g. pig manure, kraal manure) because of its high content of nitrogen, phosphorus and potassium [5,6]. Escalating prices of inorganic fertilizers due to the increase in the fuel prices has also prompted the use of poultry manure [7]. Similarly, organic wastes are also being advocated for by different environmental organizations world-wide to preserve the sustainability of agricultural systems. Recent studies have shown a host of nutrient management practices undertaken by small scale African farmers [8]. While the relative adoption rates between organic and mineral nutrients vary by location, the incidence of organic practices is often more than the use of mineral fertilizers. Increase in nitrogen levels from 40% - 60% and 17% - 38% with respect to control for Norfolk sandy soils and Cecil sandy loam soils, respectively following application of manure has been reported [9]. In addition, application of poultry manure to soil enhances concentration of water soluble salts. Plants absorb plant nutrients in the form of soluble salts, but excessive accumulation of soluble salts (or soil salinity) suppresses plant growth.

Roxarsone is added to poultry feed at the rate of 22.7 to 45.4 grams per ton, or 0.0025 to 0.005 per cent as feed additive [10]. Most of the roxarsone passes through the birds and is excreted unchanged. Each broiler excretes about 150 milligrams of roxarsone during the 42-day growth period in which it is administered [11]. Chemical and microbial reactions readily transform roxarsone into inorganic forms of arsenic. These inorganic forms are then subject to a variety of chemical and biological reactions in the soil. Soil mineralogy, soil moisture, soil pH, and microbial reactions all determine arsenic mobility and its toxicity.

2. Materials and Methods

The physico-chemical analysis like pH, conductivity, organic carbon, nitrate, phosphate and arsenic content of soil samples were done following standard methods. pH and conductivity were measured using digital pH meter and conductivity meter with automatic temperature compensation and calibrated with calibration solutions [12]. Organic Carbon was determined by Walkey-Black titration method [13], Nitrate was estimated by phenol disul-

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phonic acid method [14] and phosphate by stannous chloride method [15]. Estimation of arsenic was done by silver diethyldithiocarbamate method [16].

Amylase and invertase activities in soil were measured employing Sorenson's buffer (0.06 M) with 1% soluble starch for amylase activity and 5% soluble starch for invertase activity [17]. The dehydrogenase activity was estimated by 2,3,5-tetrazolium chloride reduction technique [18]. The CO_2 evolution from soil was measured by alkali absorption method [19,20]. Dilution plate technique was employed for the enumeration of soil bacteria [21].

Laboratory experiment was conducted taking arsenic contaminated soil and poultry dung amended soil to study the soil parameters. Arsenic contaminated soil (1 ppm, 5 ppm and 10 ppm), poultry dung amended soil (1%, 3% and 5%) and control with only soil (3 replicates) were selected. The soil biochemical parameters were measured on 0^{th}, 15^{th}, 30^{th}, 45^{th} and 60^{th} day.

3. Results and Discussion

3.1. Physico-Chemical Characteristics

pH: The pH of soil samples was found to be alkaline in nature. pH in arsenic contaminated soil varied from 8.22 ± 0.31 (0^{th} day) to 8.26 ± 0.29 (60^{th} day) (F_1 = 470.846*, F_2 = 7.384**, $p < 0.05$), where as pH of poultry dung amended soil varied from 8.42 ± 0.35 (0^{th} day) and 8.5 ± 0.31 (60^{th} day) (F_1 = 309.611*, F_2 = 7.567**, $p < 0.05$). However, the pH of control sample was found to be less than that of arsenic contaminated and poultry dung amended soils (**Table 1**). The pH levels were expected to rise with the addition of the poultry dung due to release of ammonia from the decomposing manure.

Conductivity: Conductivity was found to be increasing with increase in arsenic concentration and poultry dung in soil. In arsenic contaminated soils, conductivity ranged from 49.64 ± 2.74 µS/cm on 0^{th} day to 52.54 ± 1.34 µS/cm on 60^{th} day (F_1 = 3.607**, F_2 = 9.393**, $p < 0.05$) where as it was highest (280.7 ± 121.77 µS/cm) on 60^{th} day and lowest (277.46 ± 121.21 µS/cm) on 0^{th} day (F_1 = 113703.2*, F_2 = 40.573**, $p < 0.05$) in poultry dung amended soil. Like pH, conductivity of control was found less than that of arsenic contaminated and poultry dung amended soils (**Table 1**). Soil EC is the indication of the salinity status of the soil. The high EC in poultry dung is attributable to higher salt levels of N and P nutrients which are proportionally high.

Organic carbon: The organic carbon content in poultry dung amended soils showed an increase with 43.4 ± 1.04 mg/g on 0^{th} day to 47.26 ± 0.6 mg/g on 60^{th} day (F_1 = 33.24*, F_2 = 0.342**, $p < 0.05$). But in case of arsenic contaminated soil it was found reverse as 32.3 ± 4.15 mg/g on 0^{th} day to 31.2 ± 4.68 mg/g on 60th day (F_1 = 58.17*, F_2 = 28.532*, $p < 0.05$) which is less than that of control (**Table 1**).

Nitrate: The nitrate content in arsenic contaminated soil was lower than control and was found to be 0.211 ± 0.022 mg/g on 0^{th} day which gradually decreased with increase in days to 0.129 ± 0.058 mg/g on 60^{th} day (F_1 = 5.344**, F_2 = 16.361*, $p < 0.05$). Poultry dung amended soil showed increased nitrate content with increase in days as 0.271 ± 0.022 mg/g on 0^{th} day to 0.298 ± 0.012 mg/g on 60^{th} day (F_1 = 3.877**, F_2 = 0.277**, $p < 0.05$) (**Table 1**).

Phosphate: The phosphate content in arsenic contaminated soil was found to be much lower than that of control and poultry dung amended soil. In control the phosphate content was found to be 4.16 mg/g on 0^{th} day and 4.39 mg/g on 60^{th} day. Both Arsenic contaminated and poultry dung amended soil showed decreased phosphate content in soil with increase in days *i.e.* 2.95 ± 0.15

Table 1. pH, EC, OC, nitrate and phosphate content of arsenic contaminated and poultry dung amended soil.

parameters↓	days↓	control	Arsenic contaminated soil				poultry dung amended soil			
			1 ppm As	5 ppm As	10 ppm As	Avg.	1% PD	3% PD	5% PD	Avg.
pH*	0	7.82	7.92	8.2	8.54	8.22 ± 0.31	8.02	8.56	8.69	8.42 ± 0.35
	60	7.85	8	8.21	8.58	8.26 ± 0.29	8.15	8.59	8.76	8.5 ± 0.31
EC*	0	49.46	48.09	48.03	52.81	49.64 ± 2.74	143.1	310.7	378.6	277.46 ± 121.21
	60	51.52	51.07	52.85	53.71	52.54 ± 1.34	145.8	313.8	382.5	280.7 ± 121.77
Organic Carbon**	0	35.2	34.8	34.6	27.5	32.3 ± 4.15	42.9	42.7	44.6	43.4 ± 1.04
	60	36.8	33.6	34.2	25.8	31.2 ± 4.68	46.7	47.2	47.9	47.26 ± 0.6
Nitrate*	0	0.241	0.236	0.205	0.193	0.211 ± 0.022	0.258	0.259	0.297	0.271 ± 0.022
	60	0.198	0.195	0.113	0.081	0.129 ± 0.058	0.288	0.295	0.312	0.298 ± 0.012
Phosphate*	0	4.16	3.12	2.93	2.81	2.95 ± 0.15	4.86	4.89	4.92	4.89 ± 0.03
	60	4.39	2.28	2.18	2.14	2.2 ± 0.07	3.66	3.38	3.31	3.45 ± 0.18

*Significant; **Not significant (Two-way ANOVA, $p < 0.05$).

mg/g to 2.2 ± 0.07mg/g (F_1 = 11.839*, F_2 = 4.178**, $p < 0.05$) and 4.89 ± 0.03 mg/g to 3.45 ± 0.18 mg/g (F_1 = 0.037**, F_2 = 5.746**, $p < 0.05$) respectively (**Table 1**).

Mitchell and Donald reported that repeated application of poultry litter can increase the soil carbon content along with nitrogen and phosphorus [22]. With addition of poultry dung, there was increased phosphate content [2, 23] where in case of arsenic contaminated soil, the organic carbon, nitrate and phosphate content was found lower than control which may be due to their utilization by microbial components in soil.

3.2. Soil Bacteria

The total soil bacterial (×10^3 CFU/g soil) population was found to be higher in poultry dung amended soil than that of control and As contaminated soil. In arsenic contaminated soil, it was found decreasing with increasing days *i.e.* 20 ± 2 on 0^{th} day to 13 ± 2 on 60^{th} day. Whereas, control and poultry dung amended soil showed increased soil bacterial density with increasing days *i.e.* 24 on 0^{th} day to 31 on 60^{th} day and 36 ± 4 on 0^{th} day to 40 ± 2 on 60^{th} day respectively (**Figure 1**).

Many authors have reported toxic effects of various metals on microorganisms and found heavy metals as known inhibitors of bacterial population [24-30]. They are of the view that microorganisms are the first group of soil organisms to be affected because of their ubiquity, abundance, shape and small size and consequently extremely large surface area to volume ratio. It is known that sufficient metal exposure would normally results in immediate death of cells due to changes in their viability or competitive ability.In case of Poultry dung contaminated with As, there was no detrimental effect on soil bacteria.

3.3. CO_2 Evolution

The rate of CO_2 evolution ($g/m^2/hr$) was found to be higher in poultry dung amended soils than the control and arsenic contaminated soils (**Figure 2**). Control soil showed CO_2 evolution of 0.083 on 0^{th} day and 0.095 on 60^{th} day. Arsenic contaminated soil showed decreased CO_2 evolution of 0.053 ± 0.014 on 0^{th} day to 0.031 ± 0.009 on 60^{th} day. Whereas poultry dung amended soil showed an increased CO_2 evolution of 0.226 ± 0.038 on 0^{th} day to 0.341 ± 0.018 on 60^{th} day. Such significant increase may be due to the fact that the poultry dung amendments in soil provide the nutritive elements for mineralization by micro flora [31-33].

3.4. Soil Enzymes

Amylase Activity: Amylase activity (mg glucose/g soil/24 hr) varied from 0.689 on 0^{th} day to 0.744 on 60^{th} day in control and 0.723 ± 0.077 on 0^{th} day to 0.786 ± 0.043 on 60^{th} day in poultry dung amended soil. Unlike control and poultry dung amended soil, arsenic contaminated soil showed a decreased amylase activity with increase in days *i.e.* 0.577 ± 0.095 on 0^{th} day to 0.383 ± 0.118 on 60^{th} day (**Figure 3**).

Invertase Activity: Invertase activity (mg glucose/g soil/24 hr) also showed the same trend as that of amylase activity. It varied from 1.595 on 0^{th} day to 1.662 on 60^{th} day in control and 0.812 ± 0.482 on 0^{th} day to 2.108 ± 0.091 on 60^{th} day in poultry dung amended soil. Unlike control and poultry dung amended soil, arsenic contaminated soil showed a decreased invertase activity with increase in days *i.e.* 0.812 ± 0.482 on 0^{th} day to 0.717 ± 0.453 on 60^{th} day (**Figure 4**).

Dehydrogenase Activity: Like amylase and invertase activity, dehydrogenase activity (µg triphenyl formazan /g soil/24 hr) showed an increased value in respect to days with 2.436 on 0^{th} day to 2.603 on 60^{th} day in control and 2.562 ± 0.472 on 0^{th} day to 2.885 ± 0.226 on 60^{th} day in poultry dung amended soil. Arsenic contaminated soil

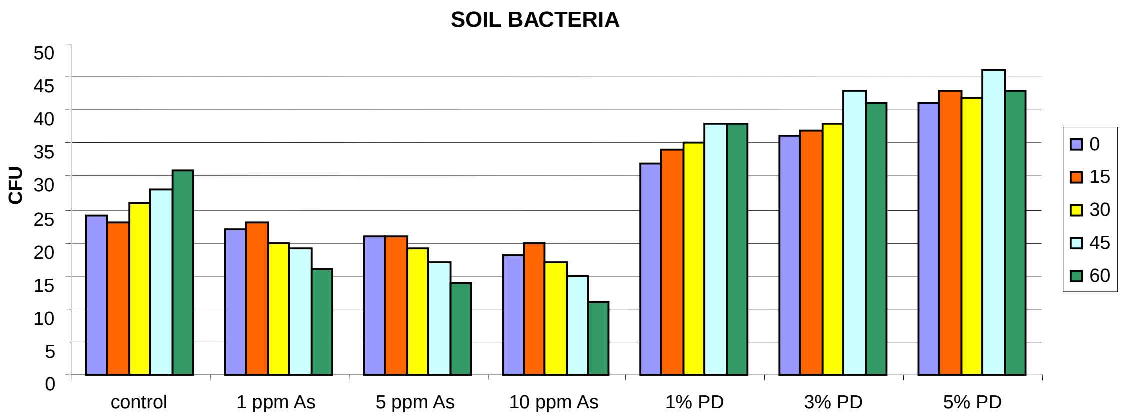

Figure 1. Soil bacteria in contaminated soil and poultry dung amended soil. Arsenic contaminated soil: (F_1 = 9.723*, F_2 = 0.871, $p < 0.05$); Poultry dung amended soil: (F_1 = 141.99*, F_2 = 13.471*, $p < 0.05$); *Significant; **Not Significant (Two-way ANOVA).**

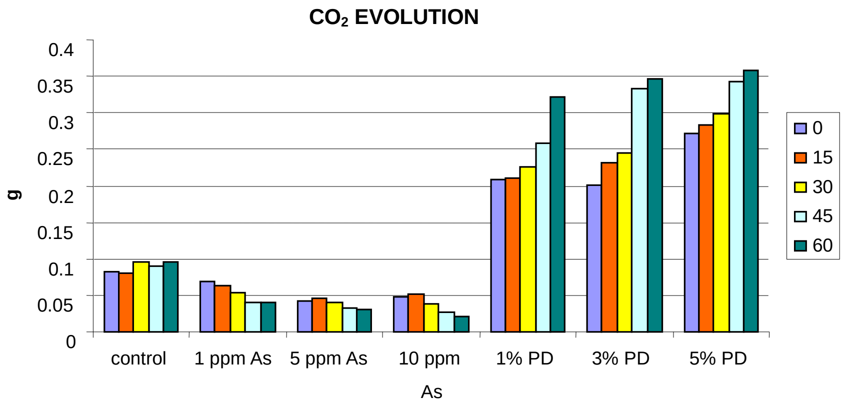

Figure 2. CO_2 evolution in arsenic contaminated soil and poultry dung amended soil. Arsenic contaminated soil: $F_1 = 13.108^*$, $F_2 = 3.36^*$, $p < 0.05$); Poultry dung amended soil: ($F_1 = 48.797^*$, $F_2 = 4.83^*$, $p < 0.05$); *Significant (Two-way ANOVA).

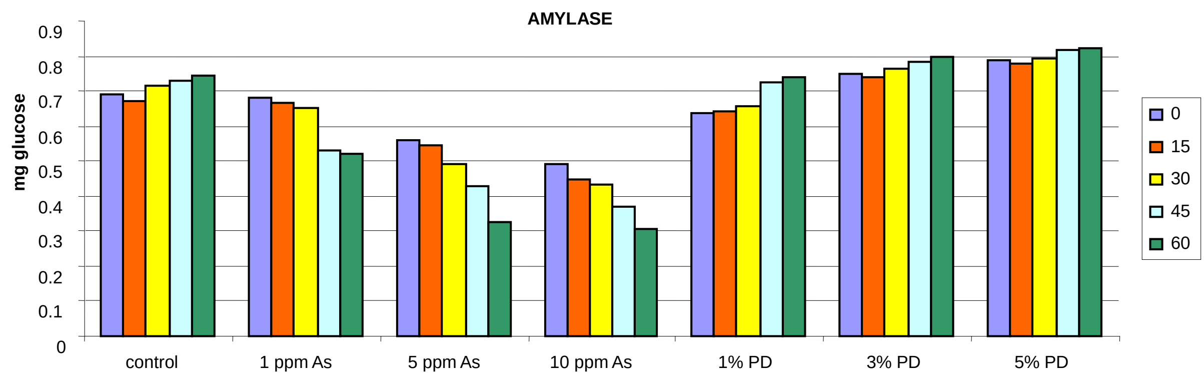

Figure 3. Amylase activity in arsenic contaminated soil and poultry dung amended soil. Arsenic contaminated soil: ($F_1 = 28.042^*$, $F_2 = 3.535^*$, $p < 0.05$); Poultry dung amended soil: ($F_1 = 69.542^*$, $F_2 = 17.899^*$, $p < 0.05$); *Significant (Two-way ANOVA).

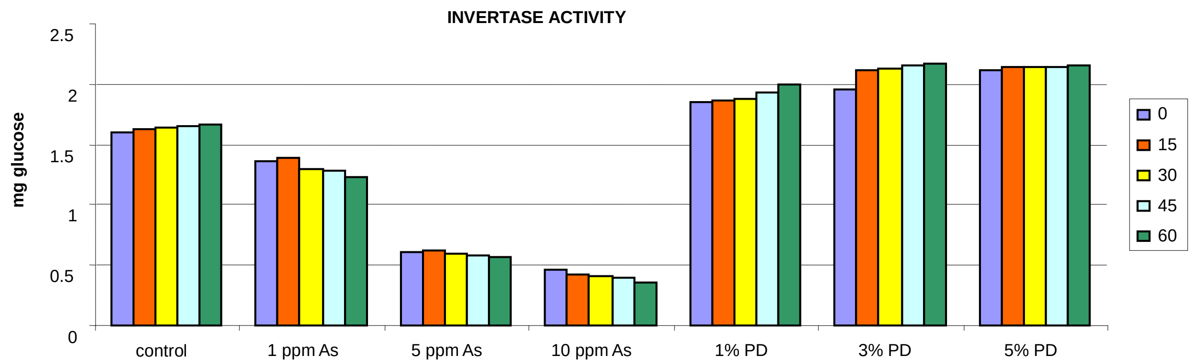

Figure 4. Invertase Activity in arsenic contaminated soil and poultry dung amended soil. Arsenic contaminated soil: ($F_1 = 1069.054^*$, $F_2 = 1.587^{**}$, $p < 0.05$); Poultry dung amended soil: ($F_1 = 199.081^*$, $F_2 = 5.577^*$, $p < 0.05$); *Significant; **Not Significant (Two-way ANOVA).

showed decreased enzyme activity with increase in days with 1.891 ± 0.219 on 0^{th} day to 1.619 ± 0.42 on 60^{th} day (**Figure 5**).

4. Conclusion

In poultry dung amended soils, bacterial density, CO_2 evolution and the enzymatic activities significantly increased ($P < 0.05$) with increase in dung amendments indicative of favourable soil condition. It is known that sufficient metal exposure would normally results in immediate death of cells due to changes in their viability or competitive ability [29] which resulted in decreased

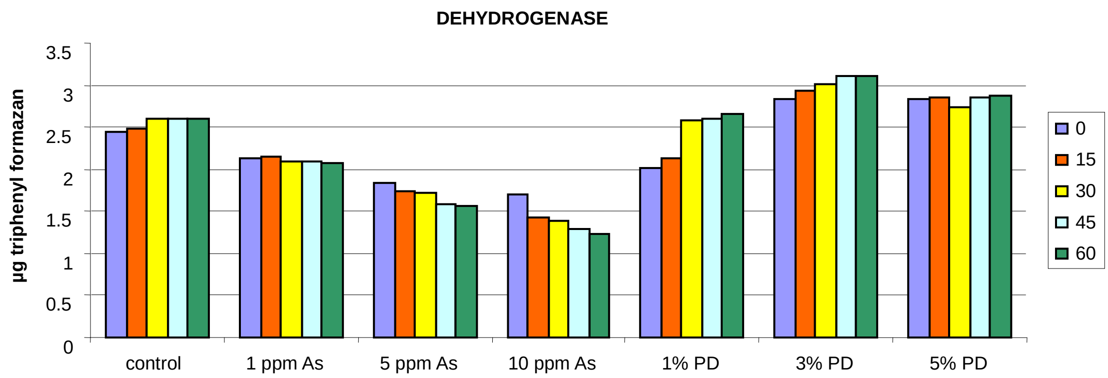

Figure 5. Dehydrogenase activity in arsenic contaminated soil and poultry dung amended soil. Arsenic contaminated soil: (F_1 = 97.384*, F_2 = 1.252, $p < 0.05$); Poultry dung amended soil: (F_1 = 20.97*, F_2 = 3.394*, $p < 0.05$); *Significant; **Not Significant (Two-way ANOVA).**

microbial population along with lower CO_2 evolution in arsenic contaminated soil. The reduction in the level of activities of enzymes in arsenic contaminated soils may be due to 1) masking of active groups 2) by protein demodulation 3) by other effects on enzyme configuration 4) the decreased level of contribution from microorganisms and v) failure of the resistant organisms to elaborate the enzymes [34]. Therefore, poultry dung amendment favoured the soil fertility status.

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