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Release of soluble phosphorus through biodegradation of poultry litter by *Bacillus*

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Abstract

The mineral content of two types of poultry litter; that is from meat producing chickens (broilers) and from egg laying chickens (layers) was investigated. The egg poultry litter was superior to meat poultry litter in the mineral composition, and its mineral content (% DW) was P 3.5, K 3, Ca 9, N 2.4, Mg 0.5, and Fe 1.2 in addition to moderate levels of Mn, Zn, Cu and Mo. The litter content of the toxic heavy metals was small; ranging from 2.6 ppm (mg kg-1) for Cd and 7.4 ppm for Pb to relatively high levels of As (36.3 ppm).

The microbial content of egg poultry litter was dense and amounted to 2×106 Colony Forming Unit (CFU) per g dry litter, distributed among twenty four bacterial isolates and seven isolates of actinomycetes; and the bacterial isolates were sorted into twenty-one Gram positive endospore-forming rods and three Gram negative rods. Each of the twenty four bacterial isolates were incubated with sterile egg poultry litter and the release of inorganic phosphorus (Pi) was monitored over time for a period of 20 days. Out of the 24 bacterial isolates three exhibited outstanding ability to release Pi from litter; and from these three isolates, one was selected for further cultural, microscopic and physiological investigations, and was identified as Bacillus subtilis. Upon incubation of Bacillus subtilis with litter the yield of Pi increased progressively with time from an initial value of 0.86 mg Pi g-1 litter to 2.17 mg Pi g-1 litter by the fifteenth day, beyond which it leveled off up to the twentieth day.

Keywords: Arsenic, Bacillus subtilis, biodegradation, phosphorus, poultry litter.

Introduction

Phosphorus is the second key plant nutrient after nitrogen; it is essential for many physiological activities such as cell division, photosynthesis, root proliferation, utilization of carbohydrates and energy metabolism in general. Soil P is primarily found in the organic form and the concentration of soluble P in soil is usually very low (Marschner, 1995). The overall P use efficiency of plants is low because of the formation of insoluble salts with soil minerals, particularly aluminum, iron and calcium; which necessitates frequent application of soluble P_i to the soil. Nevertheless, with excessive application of P fertilizers, leaching of P_i from the surface soil into ground water would

result in eutrophication of aquatic systems (Del Campillo *et al*., 1999).

The use of animal manures as bio-fertilizers seems a tempting practice in recent agriculture. Among the commonly used organic wastes for soil fertilization is poultry litter. Poultry litter consists primarily of poultry manure, mixed with the original bedding material, feathers, and spilled feed. Monogastric animals such as poultry and swine are not as efficient at utilizing organic P (phytin) in the feed as are cattle. Furthermore, the diets of these animals are often supplemented by mineral P additions. Therefore, poultry litter is particularly rich in P which amounts to about 9.8–27.1 g kg^{-1}. In addition, the litter contains appreciable amounts of nitrogen in the form of undigested proteins and uric acid, carbohydrates in the form of cellulose, starch and soluble sugars, lipids and minerals including Ca, K, Mg, S and micronutrients **(**Kelleher *et al*., 2002; Pote *et al*., 2003). Since most of the elements contained in poultry litter are in the organic form, they are not readily available to the plant after immediate application.

The advantage of litter over the traditional chemical fertilizers is that litter acts both as a fertilizer and also as a soil conditioner; for it furnishes the soil with organic matter. Organic matter improves soil structure and increases the holding capacity of the soil for water and nutrients. Consequently, organic fertilizers provide the plant with a moderate steady level of balanced nutrient supply which ensures consistent growth and development; this in contrast to the application of chemical fertilizers where there is mostly a sudden increase in the level of one nutrient, which disrupts the nutrient balance within the soil and lead to luxury consumption by the plant or even toxicity.

Application of poultry litter increases P accumulation in surface soils, and the magnitude of increase depends on application rates and frequencies (Liechty *et al*., 2009). Currently, the main purpose of managing soil phosphorus is to optimize crop production, and meanwhile to minimize P loss from soils. Recently, phosphate solubilizing microorganisms have attracted considerable attention as soil inocula to improve plant growth and yield (Goldstein *et al*., 1999). Plant growth-promoting bacteria (PGPB) are soil and rhizosphere bacteria that can benefit plant growth by different mechanisms (Glick, 1995); among which is their P-solubilization ability. Soil microorganisms are effective in releasing P from inorganic and organic pools of soil P through solubilization and mineralization (Hilda and Fraga, 1999). Solubilization of mineral phosphate by phosphorus solubilizing bacteria (PSB) is generally mediated by the release of low molecular weight organic acids (Goldstein *et al.*, 1999; Gyaneshwar *et al*., 2002), while the release of Pi from organic P compounds is mediated by production of phosphatase enzymes; and species of *Pseudomonas, Bacillus, Rhizobium* and *Enterobacter* are the most efficient in this respect (Whitelaw, 2000). In addition, these bacteria also produce other bioactive materials such as vitamins and the growth promoting hormones: auxins and gibberellins (He *et al*., 2002). Application of both phosphorus solubilizing bacteria (PSB) and plant growth-promoting bacteria (PGPB) increased P uptake efficiency by 50% and also increased the contents of nitrogen, potassium, calcium and iron in the plant leaves and fruits (Yazdani *et al*., 2009). Poultry litter has usually a large number of inhabiting microorganisms. The average density of viable microorganisms in poultry pine-sawdust litter was $6{\cdot}3 \times 10^7$/g dry material, 1-6% of which was aerobic heterotrophic bacteria and the density of acidophilic bacteria, aerobic spore-forming bacteria, actinomycetes and fungi were $4{\cdot}8\times10^4$, $8{\cdot}1\times10^4$, $5{\cdot}2\times10^4$ and $8{\cdot}9\times10^4$ colony forming unit (CFU) per gram respectively (Nodar *et al*., 1990).

The present work aims to evaluate the mineral content of two kinds of poultry litter from Damietta Province, and to investigate the potentiality of the inhabiting microflora to release P_i from litter. This ultimately aims at screening and identification of the most efficient bacterial species for further use of poultry litter as a biofertilizer for crop plants.

Materials and methods

Chemicals

Chemicals used in this work were obtained from Sigma (Sigma Chemical Co. Poole, Dorest, England) unless otherwise stated

Collection of Poultry litter samples

Poultry litter samples used in this work were collected from poultry farms in the Damietta province, Egypt. Two types of litter were collected; that is from meat producing chickens (broilers) and egg laying chickens (layers).

Determination of water holding capacity and pH of litter

Water holding capacity and pH of litter was determined according to Allen *et al*. (1986).

Extraction of the total and soluble minerals of litter

For determination of the total mineral content of litter, dried poultry litter was ground into a fine powder prior to digestion by the sulfuric acid/hydrogen peroxide method of Allen *et al*. (1986). Digestion mixture was prepared by mixing 0.42 g selenium and 14 g lithium sulfate hydrated in a one-liter flask. To the mixture, 350 ml H_2O_2 (100 volume) and 420 ml conc. H_2SO_4 were added slowly, in order, with cooling. Five ml of the digestion mixture were added to 50-100 mg of litter in a Kjeldhal digestion flask. The digestion flasks were heated gradually until the white fumes were cut off. After cooling the extract was made up to 50 ml in a volumetric flask. For determination of the soluble mineral fraction of litter, a known weight (10 g) of the dry litter was added to 150 ml water in a 250 ml conical flasks and the mixture was shaken using a Lab–Line Model No.3521 orbital shaker for an hour. The mixture was filtered and the content of minerals was determined in the clear extract.

Determination of mineral composition of litter

Phosphorus content was determined in the clear extract spectrophotometrically according to the method of John (1970). Nitrogen content was determined by the direct Nesslerization method of APHA (1992). The contents of K, Na and Ca were determined by using a PFP-7 flame photometer and those of Cd, Cu, Zn, Hg, Fe, As, Mo, Mg, Mn, Pb by a Pye-Unicam SP 90 atomic absorption spectrophotometer.

Isolation and counting of bacteria and actinomycetes from native poultry litter

The dilution plate technique described by **Johnson *et al*. (1959)** was used for isolation of bacteria and actinomycetes. Under aseptic conditions 10 g of poultry litter were transferred into a conical flask containing 90 ml of sterile water and the mixture was shaken for 20 minutes using a Lab–Line Model No.3521 orbital shaker. Serial dilutions of 10^{-1} to 10^{-6} were made from the supernatant. One ml of each dilution was transferred aseptically into sterile petri dishes and 20 ml portions of nutrient agar for bacteria and starch–nitrate agar for actinomycetes were added with gentle mixing. After solidification, the dishes were incubated at 30°C for 24-48 hours for bacteria and 7 days for actinomycetes. The plates were examined and the colonies were counted and characterized. The screened most active isolated of bacteria was identified using growth characteristics and various physiological and biochemical activities according to Logan and Vos (2009) and The Procaryotes (1991).

Morphology of the isolated bacterial colonies

Bacterial colonies resulting from growth on the nutrient agar medium at 30°C for 2 days were examined for the following characteristics: shape, transparency, margin, consistency, color and pigmentation. Gram reaction and endospore formation were also examined in cell suspension.

Physiological and biochemical tests

In addition to the morphological characteristics, several biochemical and physiological tests were carried out to identify the selected, most active bacterial isolate in release of Pi from litter. These tests included: (a) the ability to degrade: starch, gelatin, milk, urea, egg yolk (lecithinase production), tyrosine, citrate, propionate, phenylalanine, hydrogen peroxide (catalase test); (b) the ability to produce: nitrite (nitrate reduction), H_2S, indole, dihydroxyacetone, acid and gases from glucose, acetylmethylcarbinol from glucose (Voges-Proskauer test), acid from: D-glucose, L-arabinose, D-xylose and D-mannitol and (c) growth at different concentrations of NaCl, different temperatures and at pH of 5.7.

Incubation of litter with bacteria

Sterilized litter portions of equal weight were brought to 80% of water holding capacity and inoculated with equal number of cells of the different isolates and incubated at 30°C and P_i released from incubated poultry litter was determined in the clear water extract at frequent intervals using the procedure of John (1970).

Results

The total phosphorus content was 50% higher in the egg poultry litter than in the meat poultry litter

(Fig.1). Therefore, egg poultry litter was used for the detailed subsequent investigation. The egg poultry litter lot used in the present work was characterized with a moderate pH (7.97), fairly high water holding capacity (124%) and appreciable contents of plant nutrients, particularly P (Table 1); and the content of nutrients (% DW basis) was: P 3.5, K 3, Ca 9, N 2.4, Mg 0.5 , Fe 1.2, in addition to moderate content of the micronutrients Mn, Zn , Cu and Mo. The litter content of toxic heavy metals was low; ranging from 2.6 ppm (mg kg^{-1}) for Cd and 7.4 ppm for Pb to 36.3 ppm for As. The soluble fraction of the mineral content of litter was in general low and ranged from (% of total content) 29 for Mg to 11-17 for Cd, Na and Cu and to extremely low values (0.15% for As and Ca and 0.01% for Fe).

Investigation of the microflora inhabiting fresh egg poultry litter returned a dense population of microorganisms **(**2×10^6 CFU g^{-1} DW), which was distributed among 24 bacterial isolates and seven isolates of actinomycetes. The 24 bacterial isolates were examined microscopically with regard to Gram reaction and spore formation. These isolates were sorted into the following three categories: 14 isolates of Gram positive spore former solitary rods with central oval spores, seven isolates of Gram positive spore former chains of rods with central oval spores and three Gram negative rods (Fig. 2).

In addition, each of the 24 bacterial isolates was incubated with sterile egg poultry litter and the release of P_i was monitored at time intervals for a period of 20 days. The time course of P_i release exhibited different patterns in the different isolates (Fig.3). Three isolates (8, 9 and 24) exhibited outstanding activity to release P_i from the litter compared to the other isolates. These isolates were subjected to further investigation and isolate number 8 was chosen for identification since it showed a progressive increase in P release with time up to 15 days. The activity of the screened bacterial isolate in release of Pi from litter was markedly diminished with sequential culturing. The initial maximum activity of isolate 8 was as high as 6.8 mg P_i g^{-1} litter (Fig.3); this was reduced to 2.23 mg P_i g^{-1} litter in the second and third cultures (Figs.4 and 5). In addition, the time course of release of P_i from litter varied with subsequent culturing. In the first culture, P_i release by isolates 8, 9 and 24 exhibited a common pattern; a transient reduction on the tenth day, followed by a sharp rise by the fifteenth day and finally a reduction by the twentieth day of incubation (Fig. 3). In the second culture the time course of P_i release differed among isolates but was of lesser magnitude compared to the first culture (Fig.4). In the third culture of isolate 8, P_i release increased progressively with elapse of time reaching a maximum of 2.2 mg g^{-1} litter by day 15 (Fig. 5).

The most efficient bacterial isolate in release of P_i (isolate 8) exhibited the following characteristics: the colony was opaque, of irregular shape, soft consistency, yellowish white color, no pigmentation and entire margin. Microscopic examination revealed Gram positive spore forming rods, with central spore (Table 2). The physiological characteristics were categorized into degradative activities, assimilation of certain resources, production of specific compounds and growth in specific environments. The selected isolate exhibited ability to degrade casein, egg yolk, starch, citrate, H_2O_2 and urea but failed to degrade gelatin, tyrosine, propionate and phenylalanine. It had the ability to produce dihydroxyacetone, acetylmethylcarbinol and nitrite (nitrate reduction), but failed to produce H_2S, indole and acid from D-glucose, L-arabinose, D-xylose and D-mannitol. The organism was a strict aerobe, salt sensitive and grew optimally at 30˚C and pH 5.7. These characteristics evaluates this isolate to be *Bacillus subtilis* according to Bergey`s Manual of Systematic Bacteriology.

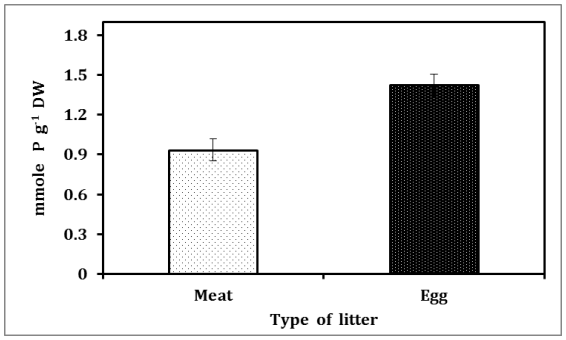

Fig. 1. Phosphorus content of egg and meat poultry litters. Each value is the mean of three replicates ± SE.

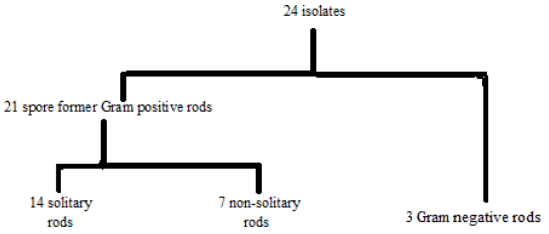

Fig. 2. Classification of the 24 bacterial isolates obtained from the poultry litter sample according to shape of cells, Gram reaction and endospore formation.

Table 1. Composition of the poultry litter lot used in the present work. Each value is the mean of three replicates ± SE. CFU is the number of colony forming units (viable cells), WHC is the water holding capacity. ND = not determined

Microbial density (CFU g^{-1} DW)	2×10^6	
W H C (% DW)	124	
pH	7.97	
Element	Total % DW	Soluble % DW	% soluble
N	2.413 ± 0.083	0.145 ± 0.015	6.02
P	3.508 ± 0.122	0.086 ± 0.001	2.45
K	3.065 ± 0.105	0.381 ± 0.040	12.4
Na	3.760 ± 0.128	0.414 ± 0.042	11.0
Ca	9.205 ± 0.322	0.014 ± 0.002	0.15
Mg	0.488 ± 0.016	0.143 ± 0.0156	29.2
Fe	1.249 ± 0.016	0.000125 ± 0.000	0.01
	mg kg^{-1} DW	mg kg^{-1} DW	
Mn	311.2 ± 8.10	18.0 ± 0.981	5.78
Zn	300.0 ± 7.798	ND	
Cu	52.53 ± 1.122	5.851 ± 0.467	11.1
Mo	76.80 ± 1.717	4.952 ± 0.443	6.45
As	36.31 ± 0.743	0.060 ± 0.005	0.16
Pb	7.418 ± 0.116	0.450 ± 0.053	6.03
Cd	2.612 ± 1.496	0.452 ± 0.044	17.4
Hg	ND	0.075 ± 0.008	

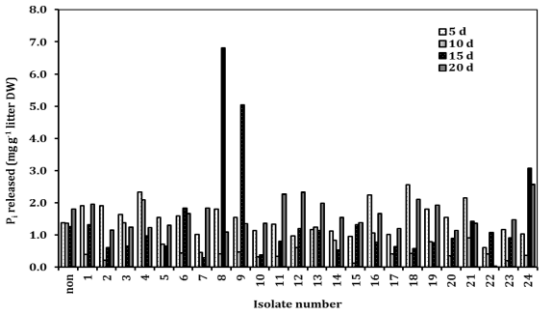

Fig. 3. Time course of release of inorganic phosphorus (P_i) from poultry litter by the action of 24 bacterial isolates.

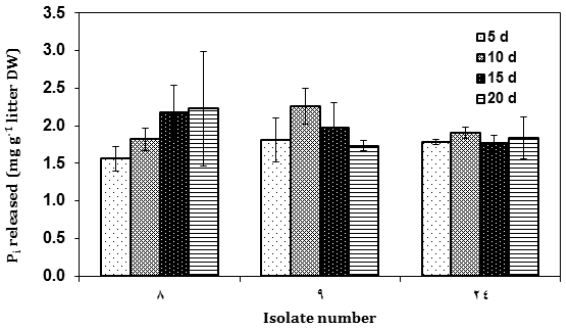

Fig. 4. Time course of release of inorganic phosphorus from poultry litter by the action of three selected bacterial isolates (isolates 8, 9 and 24). Each value is the mean of 2 replicates ± SE.

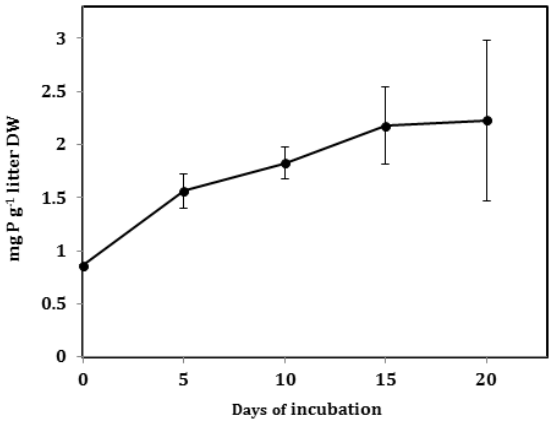

Fig. 5. Time course of release of inorganic P from poultry litter incubated with the selected isolate of bacteria (isolate 8). Each value is the mean of 3 replicates ± SE.

Table 2. Colonial, microscopic and physiological and biochemical characteristics of the selected most active bacterial isolate in release of P_i from litter (isolate 8).

Character	Grade	Character	Grade
Macroscopic (colonial)		Production of:	
Shape	Irregular	Nitrite (nitrate reduction)	+
Consistency	Soft	H_2S	-
Color	Yellowish white	Indole	-
Transparency	Opaque	Dihydroxyacetone	+
Pigmentation	Non	Gas from glucose	-
Margin	Circle	Acetylmethylcarbinol from glucose (Voges Proskauer test)	+
Microscopic		Acid from carbohydrate:	
Gram reaction	Gram +ve	D- glucose	-
Spore forming	Spore former	L- arabinose	-
Physiological and biochemical		D- xylose	-
Degradation (utilization) of:		D- mannitol	-
Casein	+	Growth at:	
Starch	+	0% NaCl	+++++
Gelatin	-	2% NaCl	++++
Urea	+	5% NaCl	+++
Proteolysis of egg yolk	+	7% NaCl	++

Tyrosine	-	10% NaCl	+
Citrate	+	pH 5.7	+
Propionate	-	5°C	-
Phenyalanine	-	30°C	+++
H_2O_2 (Catalase test)	+	50°C	-

Discussion

The amount of litter produced by the poultry farms is huge and represent an environmental problem; every broiler produces 1.46 - 2.67 kg of waste over its life span (Nachman *et al*., 2005). Nevertheless, these problematic wastes can be exploited, after composting by microorganisms, for several uses, among which is the addition to the soil as a bifertilizer. The high water holding capacity (124 %) of the litter, its moderate pH (7.97) and its high content of plant nutrients particularly P (up to 3.5%) along with the fairly low content of toxic heavy metals evaluate the litter for use as a safe, economic and efficient biofertilizer and also as a soil conditioner, particularly for the dry low-in-organic matter sandy soils. Organic wastes are characterized with high water holding capacity, a feature of critical value for improving physicochemical properties of the soil in favor for plant growth. Thurow *et al*. (1987) reported water holding capacity of up to 300% for litter from different plant origins, where the magnitude differed according to plant species and litter texture. The P content of the egg poultry litter lot used in the present work (3.5%) is higher than that reported by Kelleher *et al*. (2002) and Pote *et al*. (2003) which ranged from 0.98 to 2.71%. This high P content justifies the use of litter as an efficient biofertilizer, particularly on the basis of plant P demand. An additional benefit of biofertilizers is their ability to enrich the soil with micro- and macro-nutrients via nitrogen fixation, phosphate and potassium solubilization or mineralization, release of plant growth regulating substances, production of antibiotics and biodegradation of organic matter in the soil (Sinha *et al*., 2014).

Poultry litter normally contains arsenic; which arises primarily from implementation of organoarsenic compounds in the treatment of poultry, which is a common practice in poultry farms worldwide. Organoarsenic compounds such as roxarsone are used as a feed additive for control of fungal diseases and for weight gain improvement in poultry farms in the United States (Jones, 2007).There are no regulatory standards for trace elements concentrations in poultry litter; only municipal biosolids have regulatory limits for trace metals and these are 20, 750, and 1400 mg kg^{-1} for As, Cu, and Zn respectively in soil amended with biosolids (Miller and Miller, 2000). These standards are often used as a reference for the other land-applied wastes (Ashjaei *et al*., 2011). Thus, the level of As encountered in the used litter lot (36.3 mg kg^{-1}) can be considered moderate and do not seem to impose a serious environmental risk, for it slightly exceeded the allowable limits. Furthermore, the situation seems less severe in the light of the fact that a negligible fraction of the arsenic content of litter (0.16%) is in the soluble from. In this respect, Han *et al*. (2004) reported that about 47% of As in poultry litter occur in the hazardous, water-soluble pool. Likewise, the litter content of Cu and Zn (52 and 300 mg kg^{-1} respectively) are by far below the above mentioned regulatory limits and the soluble fraction of Cu amounted to only 16% of the total Cu in the litter.

In addition to its high content of plant nutrients, egg poultry litter is also rich in microorganisms which occur in a great variety and high density. Incubation of the native litter at appropriate conditions yielded a dense population of inhabiting microorganisms (2×10^6 CFU g^{-1} DW) which was distinguished into 24 different bacterial isolates, mostly spore former Gram positive rods, and seven isolates of actinomycetes. This seems reasonable, hence poultry litter, by virtue of its high organic matter content and consequently its high water holding capacity, in addition to the moderate pH can host a dense and diverse population of microorganisms, particularly bacteria. The average density of viable microorganisms in poultry pine-sawdust litter was $6{\cdot}3 \times 10^7$/g dry matter; with small proportion of aerobic heterotrophic bacteria and the majority being acidophilic bacteria, aerobic spore-forming bacteria, actinomycetes and fungi which approached $4{\cdot}8 \times 10^4$, $8{\cdot}1 \times 10^4$, $5{\cdot}2 \times 10^4$ and $8{\cdot}9 \times 10^4$ CFU/g dry litter respectively (Nodar *et al*., 1990). This population of microorganisms is crucial for soil fertility and the integrity and efficient performance of the root system. Living roots can support even a more dense population of microorganisms which might approach up to 10^{11} microbial cells per gram of root in the rhizosphere (Egamberdieva *et al*., 2008). The rhizosphere microflora in general,

includes a vast array of naturally occurring microorganisms which benefit the soil ecosystem through improving the soil physicochemical properties, plant growth and development and crop productivity (Singh *et al*., 2011; Sahoo *et al*., 2014).

The present results suggest that sequential culturing of the isolated bacteria affect both efficiency and time course of release of P_i from litter. The activity of bacteria in P_i release generally diminished with subsequent culturing. In addition, whereas in the earlier culture, P_i release increased with the progress of incubation time reaching a maximum at specific time, post which it declined; in the latter culture the increase in P_i releasing activity was progressive, reaching a limit at the end of incubation period but without subsequent decline. This might point to fluctuation in the relative efficiency of release and of consumption of P_i by bacteria in the successive cultures. It seems that during the early periods of incubation with litter the activity of P_i release exceeds that of its fixation by bacteria; but this balance was shifted in favor of the latter activity with the progress of time. The cultural, microscopic, biochemical, and physiological investigations of the selected most efficient bacterial isolate justifies its identification as *Bacillus subtilis* according to Logan and Vos (2009) and The Procaryotes (1991). In this respect, Pindi and Satyanarayana (2012) reported that among the active organisms in release of P_i are *Pseudomonas*, *Bacillus* and *Micrococcus* and that a key advantage of these bacteria is to assimilate phosphorus for their own use; which in turn becomes available for plant uptake. Furthermore, a phosphate-solubilizing bacterial strain NII-0909 of *Micrococcus* sp. has multiple roles including phosphate solubilization and siderophore production (Dastager *et al*., 2010).

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الملخص العربي

عنوان البحث: تحرير الفوسفور الذائب من خلال التحلل الحيوى لمخلفات الدواجن باستخدام بكتيريا Bacillus subtilis

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تم فى هذه الدراسة تقييم المحتوى المعدنى لعينتين من مخلفات مزارع الدواجن: إحداهما مخلفات الدجاج البياض والأخرى مخلفات دجاج اللحم. اتضح ان مخلفات الدجاج البياض كانت ذات محتوى معدنى أعلى من مخلفات دجاج اللحم فقد وصل تركيز العناصر فيها (كنسبة مئوية من الوزن الجاف) إلى: ٣,٥ للفوسفور ، ٣ للبوتاسيوم ، ٩ للكالسيوم ، ٢,٤ للنيتروجين ، ٠,٥ للمغنسيوم ، ١,٢ للحديد ، هذا عدا محتوى معتدل من المغذيات الصغرى: المنجنيز والزنك والنحاس والموليبدينوم ومحتوى ضئيل من العناصر الثقيلة تفاوت ما بين ٢,٦ ملجم/كجم من الكادميوم و ٧,٤ ملجم /كجم من الرصاص ألى ٣٦,٣ ملجم/كجم من الزرنيخ. تميزت مخلفات الدجاج البياض أيضا بمحتوى غزير من الكائنات الدقيقة بلغ وحدة ميكروبية /جم توزع على ٢٤ عزلة بكتيرية مختلفة بالاضافة الى سبع عزلات من الاكتينوميسيتات. وتم تصنيف العزلات البكتيرية الى ٢١ عزلة من البكتيريا العصوية المتجرثمة موجبة الجرام و ثلاث عزلات من البكتيريا العصوية سالبة الجرام. تم تحضين كل من العزلات البكتيرية مع المخلفات المعقمة وتقدير كمية الفوسفور

الغير عضوى المنطلق مع الزمن لفترة امتدت الى عشرين يوما. ومن بين العزلات الأربع والعشرين تم انتقاء ثلاث عزلات كانت اكثرهم قدرة على تحرير الفوسفور من المخلفات ومن بين هذه الثلاث الأخيرة تم انتقاء واحدة خضعت للفحص التفصيلى من حيث الصفات المزرعية والميكروسكوبية والفسيولوجية حيث تم تعريفها على أنها Bacillus subtilis**. وبتحضين هذه البكتيريا الأخيرة مع المخلفات وتتبع الفوسفور المنطلق مع الزمن وجدت زيادة مطردة فى تركيز الفوسفور الغير عضوى من قيمة مبدئية هى ٠,٨٦ ملجم/جم حتى ٢,١٧ ملجم/جم فى اليوم الخامس عشر من التحضين ثم ثبت المستوى بعد ذلك حتى اليوم العشرين.**