



Cultivation of beans (*Phaseolus vulgaris* L.) in limed or unlimed wastewater sludge, vermicompost or inorganic amended soil

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ABSTRACT

Wastewater sludge can be vermicomposted to increase its nutrient content and reduce pathogens. The effect of vermicompost wastewater sludge on soil characteristics and growth of bean plants (*Phaseolus vulgaris* L.) was compared with that of inorganic fertilizer, untreated sludge or sludge treated with lime to pH 12. Plants cultivated in vermicompost amended soil developed best while those amended with inorganic fertilizer least. Bean plants grown in the unlimed amended soil had the highest total N content of 3.97 g kg⁻¹, but only 6 active and 8 total nodules. Plants cultivated in unamended soil and added with inorganic fertilizer had >20 active and total nodules, but ≤2.12 g N kg⁻¹ dry plant. It was found that limed and unlimed sludge stimulated development of bean plants to a larger extent than those cultivated in inorganic fertilized soil or unamended soil in the greenhouse, but less than those cultivated in vermicompost amended soil.

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1. Introduction

Treatment of wastewater has increased dramatically in Mexico in recent years due to new legislations. Contamination of surface water and aquifers has reduced concordly, but large amounts of biosolids are being generated. In Mexico, most of the treated waste water is from households so the resulting biosolid is rich in organic material and nutrients, low in toxic compounds and heavy metals, but often contains large amounts of pathogens, such as eggs of helminthes and fecal coliforms. The wastewater sludge could easily be used as fertilizer in agricultural practices, to vegetate soil or restore soil fertility if concentrations of pathogens could be reduced (Krogmann et al., 1997). Different techniques have been applied to reduce pathogens in biosolid, such as pasteurization, irradiation or applying lime (Eriksen et al., 1996; Ahmed and Sorensen, 1997; Capizzi-Banas and Schwartzbrod, 2001), but lately, wastewater sludge has been composted or vermicomposted to reduce pathogens while maintaining the nutrient content of the biosolid (Bernal et al., 1998; Maboeta and van Rensburg, 2003). Each of different mentioned techniques to reduce pathogen in wastewater sludge has advantages and disadvantages. Lime is routinely

applied to biosolid to increase pH to 12, which is cheap, and most micro-organisms are killed, but not to the same extent as after irradiation or vermicomposting (Franco-Hernández et al., 2003). Eriksen et al. (1996) therefore recommended to store the biosolid at pH 12 for up to three months sharply increasing the costs. Additionally, liming generates a product high in pH that when added to soil might affect its biological functioning and inhibit plant growth. Irradiation is very effective in reducing pathogens, but too expensive to apply routinely (Capizzi-Banas and Schwartzbrod, 2001). Vermicomposting is also very effective in reducing pathogens and the vermicompost is rich in nutrients and organic material, and although the process is faster than composting it can still take up to two months (Ndegwa and Thompson, 2001). An important factor that will determine the usefulness of the technique applied, apart from the effectiveness and costs to reduce pathogens, will be the effect the generated product has on plant growth and soil characteristics (Atiyeh et al., 2000). If the generated product, i.e. limed biosolid or vermicompost, has no negative effects on soil and plant development, then it could be used as an alternative for inorganic fertilizer.

Reciclagua (Sistema Ecológico de Regeneración de Aguas Residuales Industriales, S.A. de C.V.) in the state of Mexico provides service to 160 companies within the Toluca industrial corridor and the Lerma industrial park and treats 0.4 m³ s⁻¹ wastewater all year round since 1982 (Reciclagua, personal communication). The generated biosolid is rich in organic material, of excellent quality

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considering its heavy metal content, but contains large amounts of pathogens, especially eggs of helminthes (Franco-Hernández et al., 2003). Therefore, the biosolid is incinerated which might pollute the air and at a high cost to the company.

Bean (*Phaseolus vulgaris* L.) was selected to study the effect of limed biosolid and vermicompost on plant development and soil characteristics, because it is often the only source of proteins for poorer people in Mexico. Beans with a protein content of 24–26%, originates from the Americas, but is now cultivated throughout the world. In Mexico, 800,000 ha are cultivated yearly with yields of more than 200 kg ha⁻¹. Although bean is a leguminose and through its symbioses with *Rhizobium* an N₂ fixator, it is often fertilized (Nadal-Moyano et al., 2004).

In this study, unlimed and limed biosolid, vermicompost derived from the same biosolid and inorganic fertilizer were added to soil cultivated with bean (*P. vulgaris* L.). The amount of inorganic N added to soil was similar in each treatment. An untreated soil served as control. Plant development, nodule formation, and plant and soil characteristics were monitored in the greenhouse for 60 days and that over three crop cycles. The objective of this study was to investigate the effect of application of vermicompost, limed biosolid, unlimed biosolid and inorganic fertilizer on bean development and soil characteristics.

2. Materials and methods

2.1. Sampling site, collection and characteristics of soil

The sampling site is located near the ex-convent of Acolman in the State of Mexico, Mexico (N.L. 19°38' W.L. 98°55'). Its average altitude is 2250 m above sea level and characterized by a sub-humid temperate climate with a mean annual temperature of 14.9°C and average annual precipitation of 624 mm mainly from June to August (<http://www.inegi.gob.mx>). Details of the experimental site can be found in Betancur-Galvis et al. (2006). Briefly, the soil is clayey with pH 6.3 and electrolytic conductivity (EC) 0.8 dS m⁻¹ and an organic carbon (C) content of 19 g C kg⁻¹ soil. The area is mainly cultivated with maize and that for >20 years, receiving a minimum amount of inorganic fertilizer without being irrigated (<http://www.inegi.gob.mx>). Soil was sampled at random by augering the 0–15 cm top-layer of three approximately 0.5 ha plots. The soil from each plot was pooled and as such three soil samples were obtained.

2.2. Biosolid and vermicompost

Wastewater sludge was obtained from Reciclagua as described by Franco-Hernández et al. (2003). Briefly, Reciclagua treats wastewater from different companies. Ninety percent of the wastewater is of industrial alimentary origin, mainly from yeast and coffee industries, and the rest from households. The wastewater from each company has to comply with the following guidelines: biological oxygen demand <1000 mg l⁻¹, lipids <150 mg l⁻¹ and phenol <1 mg l⁻¹. The collected wastewater is digested aerobically in a reactor. The biosolid obtained after the addition of a flocculant is passed through a belt filter to reduce water content. Heavy metal concentrations in the biosolid are low (Franco-Hernández et al., 2003) making these biosolid of excellent quality (USEPA, 1994), as were concentrations of toxic organic compounds (Reciclagua, personal communication). The biosolid can be classified as a class “B” biosolid (Franco-Hernández et al., 2003) considering its pathogen content (USEPA, 1994). The biosolid was mixed with Ca(OH)₂ to pH 12.

Eisenia fetida was cultivated in the same biosolid. The vermicompost with the best stability and maturity and a weight loss of

18% was obtained with 1800 g biosolid, no straw and 800 g manure at 70% water content. More details of the vermicomposting can be found in Contreras-Ramos et al. (2005).

2.3. Cultivation of bean plants in the greenhouse

Forty-five sub-samples of 3.25 kg untreated soil from each plot were added to polyvinyl chloride (PVC) tubes (length 50 cm and Ø 16 cm) filled at the bottom with 7 cm of gravel topped up with 3 cm sand. Treated 3.25 kg soil was then added on the top of the untreated 3.25 kg soil already added to the PVC tubes. As such, a layer of 15 cm treated soil was placed on top of 15 cm untreated soil. Five treatments, considered the CONTROL, BIOSOLID, LIMED BIOSOLID, VERMICOMPOST and FERTILIZER treatments, were applied to nine sub-samples of 3.25 kg soil. Details of the different treatments can be found in Table 1. A 20 g sub-sample of treated soil was taken and characterized for inorganic N, pH, EC and extractable phosphorus (P).

Bean seeds were placed on wet cotton for 20 days and one bean plantlet was planted in each soil column. The PVC tubes were placed in a greenhouse for 60 days. During the first experiment that started the 10th of September 2005 and lasted until the 9th of December 2005, 500 ml water was added to each column every seven days. The amount of chlorophyll, area to foliate, number of flowers, days of flourish, days of fruitful in the leaves was monitored. The amount of chlorophyll was measured with a Minolta SPDA Chlorophyll meter.

Thirty, and 60 days after transplantation, three PVC tubes were selected at random from each treatment. The entire soil column was removed from the PVC tube and the 0–15 cm and a 15–30 cm layer sampled taken care not to damage the root structure. The roots were separated from the shoots, the amount of active and inactive nodules were determined and the root and shoot length measured. Roots and shoots were air-dried, weighted and analyzed for total N and P. The whole experiment was repeated twice. In the second experiment that run from the 21st of December 2005 to 21st of March 2006 and the third running from 11th of April to 10th of July 2006, the same amount of water was added every seven days.

2.4. Chemical and microbiological analyses

Details of the techniques used to characterize the soil can be found in Franco-Hernández et al. (2003). The wastewater sludge was analyzed for total and faecal coliform, *Salmonella* sp., *Shigella* sp. and for eggs of helminthes (USEPA, Appendix F, G, I, 1999). *Salmonella* and *Shigella* were determined by serial dilution. A sub-sample of 10 g wastewater sludge was added to 90 ml 1% peptone solution under sterile conditions and 10⁻¹, 10⁻² and 10⁻³ dilutions were made with sterile 0.8% sodium chloride (NaCl) solution. A 100 µl aliquot was plated on two selective media *Salmonella*–*Shigella* agar and sulfite–bismuth agar. The second medium is highly specific for *Salmonella*. The colonies were identified by form and color (USEPA, Appendix G, 1999). Rose-bengal agar amended with 0.1-mg streptomycin–sulphate ml⁻¹ was used to enumerate fungi. The plates were inoculated with 100–µl wastewater sludge suspension and three plates per suspension were kept at 25 °C for 3–7 days.

The USEPA method (USEPA, Appendix I, 1999) was used to concentrate, detect, and enumerate *Ascaris* ova and to determine their viability. Samples were mixed with buffered water (0.1 M phosphate buffer pH 7.2) containing a surfactant (Triton X-100, 0.1%) and large particles were removed by a second screening on a small mesh size screen. The solids were allowed to precipitate and the supernatant was decanted. The sediment was subjected to a density gradient centrifugation using magnesium sulfate (specific gravity 1.2) at 2000 × rpm for 5 min. Small particles were removed by a sec-

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