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Recycling Organic Waste for Enhancing Soil Urease and Invertase Activity

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Abstract

Recycling animal manure could be explored in agricultural production for growing vegetable crops to reduce dependence on inorganic fertilizers. Arugula (Eruca sative) and mustard (Brassica juncea) were grown in a randomized complete block design (RCBD) under four soil management practices: 1) control (no-mulch untreated soil); 2) sewage sludge; 3) horse manure; and 4) chicken manure. Sewage sludge compost elevated soil urease and invertase activities indicating increased soil microbial activities. Total soil enzyme activities were significantly ($P < 0.05$) greater in sewage sludge amended soil compared to no-mulch native soil. It could be concluded that sewage sludge and chicken manure increased soil fertility and the activities of soil urease and invertase could be used as an indicator of soil biological activity after addition of soil amendments. This investigation revealed that soil incorporated with sewage sludge or horse manure promoted biomass production of arugula and mustard by 26 and 21%, respectively compared to no-mulch bare soil. Future trends in agricultural production should make a good use of natural resources to reduce dependence on synthetic fertilizers.

Keywords: Sewage sludge; Chicken manure; Horse manure; Soil urease; Invertase activity

Introduction

The use of animal manure as organic fertilizer has important properties that cannot be gained from synthetic inorganic fertilizers. Animal manures increase soil organic matter, improve soil physical structure, enhance soil fungal and bacterial activity, reduce eutrophication (excess N and P in natural water resources), provide low-cost adsorbents that binds with agricultural contaminants and prevent natural water contamination by pesticides and inorganic fertilizers [1], and hence, reducing the impact of xenobiotics on surface and groundwater quality. Over the last 50 years, the amount of N and P pollution entering our nation's waters has escalated dramatically. Thirty percent of U.S. streams have high levels of N and P contamination and drinking water violations due to nitrates and phosphates (eutrophication) that have been doubled in the last eight years [2] due to over application of inorganic fertilizers. There is a need for affordable sources of organic fertilizers for use as alternative to inorganic synthetic fertilizers. Municipal sewage sludge (SS), a by-product of sewage treatment plants, is currently applied to some agricultural soils as an alternative to conventional inorganic fertilizers. Microorganisms in SS and other animal manure, facilitate the release of the three main nutrients, carbon, nitrogen, and phosphorus, through recycling from organic matter. Bacteria, fungi, protozoa, and algae present in animal manure release various enzymes, such as ureases, invertases, dehydrogenases, cellulases, amylases, and phosphatase that are primary means of degrading xenobiotics in soil systems, mineralization of organic compounds, and release of nutrients for plant uptake. Soil enzymes are sensitive indicators to environmental stress caused by soil pollution [3]. Accordingly, soil enzymatic measurements could be explored as indicators of soil health and biological processes when animal manure is used as fertilizer.

Urease (urea amidohydrolase, EC 3.5.1.5) is the enzyme that catalyzes the hydrolysis of urea to CO_2 and NH_{4+} ions by acting on C-N non-peptide bonds in linear amides. It is an important enzyme in soil that mediates the conversion of organic nitrogen to inorganic nitrogen by hydrolysis of urea to ammonia. The presence of urease and invertase (β-D-fructofuranosidase) in soil allows the release of carbon and nitrogen from complex forms of organic matter for the growth and multiplication of soil microorganisms. Microbial biomass, metabolism, and enzymes are often measured to provide immediate information about small changes in soils [4]. Carbon (C) is a key factor governing soil microorganism growth [5]. Monitoring invertase activity in soil could provide information about soil's long-term productivity [6]. Invertase (β-D- fructofuranoside fructohydrolase, EC 3.2.1.26) [7], the enzyme that catalyzes the hydrolysis of sucrose to glucose and fructose, is widely distributed in bacteria, plants, animals, microorganisms, and soils [8]. The activity of this enzyme in soils is important because its substrate, sucrose, is one of the most abundant soluble sugars in plants. Invertase in soil facilitates the breakdown of complex forms of plant litter in soils [9] allowing nutrients to recycle again and again. The positive aspects of using SS compost may outweigh its negative aspects (such as presence of trace-elements) due to its ability to increase soil organic matter content [10] and crop yield [11,12]. Despite their relatively low amounts, soil microorganisms play a significant role in keeping and recycling C, N, and P in soil.

Recycling animal manure would reduce dependence on inorganic fertilizers and provides an alternative source of inorganic fertilizers for improving soil structure and nutrient status [13].

The literature review and the use of organic amendments has been clearly demonstrated [14,15]. The U.S. Environmental Protection Agency (USEPA) allows the safe use of biosolids for growing vegetable crops to reduce the needs for waste disposal sites. Sewage sludge contains humic substances and macro- and micro-nutrients important for plant growth and the use of biosolids as soil conditioners to enhance soil physical, chemical, and microbial conditions might also

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enhance soil bioremediation [16,17]. Agricultural uses of SS was successful in growing many field crops and production of vegetables [11,12]. Sewage sludge improved soil physical properties, nutrient and water holding capacity, total pore space, aggregate stability, soil erosion, and decreased soil density [18]. In addition, organic waste increases soil organic matter such as humic acid (HA) and fulvic acid (FA) [19], which improves soil aeration and moisture retention [20,21]. Animal manure enhances soil biological activity and fertility, nutrients status and growth of several groups of microorganisms, such as bacteria, fungi, and actinomycetes [22-26].

The objectives of this study were to: 1) assess the impact of mixing native agricultural soil with municipal SS, chicken manure (CM), horse manure (HM), and yard waste (YW) on biomass production of arugula and mustard plants and 2) monitor the activities of the soil enzymes that hydrolyze urea (urease) and sucrose (invertase), after the addition of animal manure and yard waste to native agricultural soil.

Materials and Methods

A trial was conducted in arugula (Eruca sative) and mustard (Brassica juncea) field. Plants were grown in 30' × 144' beds of freshly tilled soil at the University of Kentucky South Farm (Lexington, KY). Each bed, measuring 12' × 30' was divided into three replicates in a randomized complete block design (RCBD) with four soil treatments. The entire study area contained 24 experimental plots (2 crops × 3 replicates × 4 treatments). The treatments were 1) sewage sludge (SS) amended with soil, 2) chicken manure (CM) amended with soil, 3) horse manure (HM) amended with soil, and 4) no-mulch bare soil used for comparison purposes. Animal manures were applied to native agricultural soil to achieve a concentration of 5% N in each plot, except in no-mulch bare soil (control treatment). SS used in this study was purchased from the Metropolitan Sewer District, Louisville, KY, whereas, chicken manure was obtained from the Department of Animal and Food Sciences, University of Kentucky, Lexington, Kentucky, and horse manure was obtained from Kentucky horse park (Lexington, Kentucky). Whereas, yard waste was obtained from Con Robinson Inc. (Lexington, KY).

Arugula and mustard were grown according to Kentucky agricultural guidelines [27] and no inorganic fertilizers were applied. Soil samples (n = 3) were collected from the soil rhizosphere (a zone of increased microbial and enzyme activity where soil and root make contact) of growing arugula and mustard plants to a depth of 15 cm using a plastic linear tubes tube (Clements Associates, Newton, IA) of 2.5 cm i.d. for maintenance of sample integrity. Soil samples were air-dried, passed through a 2 mm sieve, and kept in plastic bags at 4°C up to 24 h before use.

Urease activity was determined using five g of soil, placed in 50 mL volumetric flasks and 10 mL of 0.1 M phosphate buffer (pH 6.7). The flasks were kept in a water bath at 30°C for 1 h to allow the soil temperature to equilibrate as described by Tabatabi and Bremner [28]. Concentrations of NH_{4+} ions were determined by the selective electrode method [29]. A series of standard solutions of NH_4Cl covering the concentrations of 0.1-100 μg NH_4-N mL-1 of water was used for calibration. Urease activity was expressed as mg NH4-N released per g dried soil during the 1 h incubation at 37°C [13]. Whereas, invertase activity in soil was assessed using the method described by Balasubramanian et al. [30]. A standard calibration curve was obtained with each group of samples using analytical grade glucose in the range of 10-50 μg mL-1 glucose standards

Plant weights, root and shoot weights obtained from each of the soil four treatments were recorded. Biomass data, activities of urease and invertase were compared using analysis of variance (SAS Institute [31]) and Duncan's multiple range test for mean comparisons.

Results and Discussion

Average weights of arugula and mustard plants (0.4 and 0.5 kg plant-1, respectively) grown in soil amended with SS were significantly greater (P< 0.05) compared to the other three treatments. Results also revealed that HM was a good choice for growing arugula and mustard plants, because it produced the 2nd great biomass production after SS, whereas, the biomass produced from CM was similar to the no-mulch bare soil. Soil amended with SS increased biomass production in arugula and mustard by 26 and 21%, respectively compared to no-mulch (NM) bare soil (Table 1). Figure 1 revealed that SS increased soil urease activity. Urease is an enzyme that depends on Ni for its activity [32]. Accordingly, Ni might be the cause of elevated urease activity. This increase could be also due to the presence of urea, the substrate of the enzyme urease. SS obtained from municipal plants contains great amounts of enzymatic substrates [33].

Figure 1: Arugula and mustard plants grown in soil amended with sewage sludge (white arrows) compared to other soil treatments at the University of Kentucky South Farm (Fayette County, KY).

Soil	Root Weight, g	Shoot Weight, g	Plant Weight, g
Arugula	74.24	290.95 a	365.20 a
HM	54.00	247.21 b	301.21 b
CM	41.64	235.60 c	277.23 c
NM	30.08	240.45 c	270.54 c
Mustard	61.43	426.60 a	488.00 a
HM	67.21	393.10 b	460.30 b
CM	75.87	307.40 d	383.30 c
NM	44.24	341.90 c	386.20 c

Table 1: Mean weights of arugula and mustard plants grown under four soil management practices. Statistical comparisons were carried out among soil amendments for each parameter tested.

In similar studies, SS used in growing vegetable crops including broccoli contained 1.2 μg Ni g-1 dry soil. However, broccoli plants

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grown in SS mixed soil showed normal growth under field conditions without any apparent symptoms of Ni toxicity or deficiency [34,35]. In addition, soil urease activity in the rhizosphere of arugula and mustard plants was increased after the incorporation of SS, CM, and HM compared to no-mulch bare soil (Figure 2), which might be due to increased soil microbial activities. Similarly, SS increased soil invertase activity compared to other treatments in mustard (Figure 3). Whereas, HM enhanced soil invertase activity in arugula soil compared to CM and NM arugula treatments. Regardless of the crop type, overall invertase activities were significantly greater in soils amended with SS and HM compared to CM and NM treatments (Figure 4).

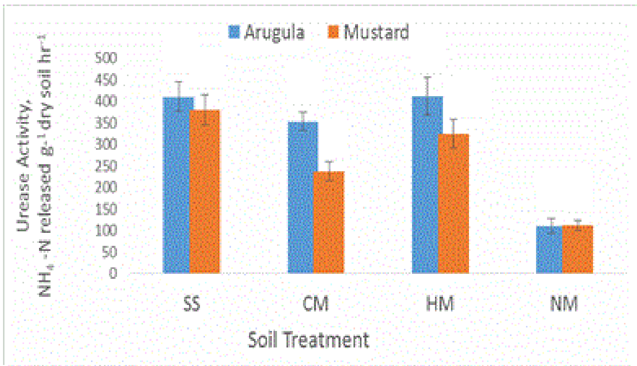

Figure 2: Urease activity, expressed as mg NH4-N released g-1 dry soil hr-1, in the rhizosphere of agrugula and mustard grown under four soil management practices. Statistical comparisons were carried out among four soil management practices. Bars accompanied by the same letter are not significantly different (P > 0.05) using Duncan's multiple range test.

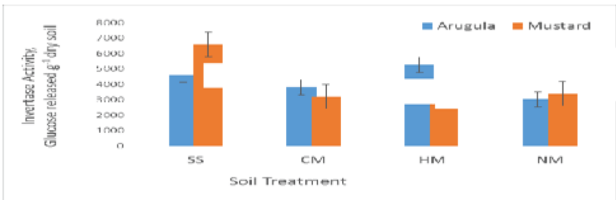

Figure 3: Invertase activity, expressed as mg glucose released g-1 dry soil, in the rhizosphere of agrugula and mustard grown under four soil management practices. Statistical comparisons were carried out among four soil management practices. Bars accompanied by the same letter are not significantly different (P > 0.05) using Duncan's multiple range test.

Conclusion

Soil is a vital natural resource that is non-renewable on a human time-scale. There is a little information on microbial response to recycled animal wastes such as sewage sludge (SS), chicken manure (CM), and horse manure (HM) added to soils. Some soil chemical (e.g. organic matter) or soil physical (e.g. texture) properties change too slowly or are little affected by human activities. Biological properties of soils, such as soil microorganisms and enzymatic activities, offer good potential as indicators of soil health since they can rapidly respond to external conditions. Soil enzymes secreted by soil microorganisms promote many soil processes such as, the synthesis of humic substances, the breading-down (mineralization) of organic matter, and the degradation of soil xenobiotics. Soil amendments contain high levels of nutrients important for plant growth. However, soil amendments may also contain harmful materials such as heavy metals, organic contaminants, pharmaceuticals, and personal care products that impact soil microorganisms and reduce their enzyme secretions and activities. Low enzymatic activity in soil can be related to decreased enzyme synthesis, inhibition of the enzyme due to metal(s) contamination that masks active enzyme groups, or inhibition of microbial population. Results of this investigation revealed a significant increase in soil urease and invertase activities in the rhizosphere of arugula and mustard plants after the incorporation of SS, CM, and HM compared to no-mulch bare soil, which is a direct indication of increased soil microbial activities.

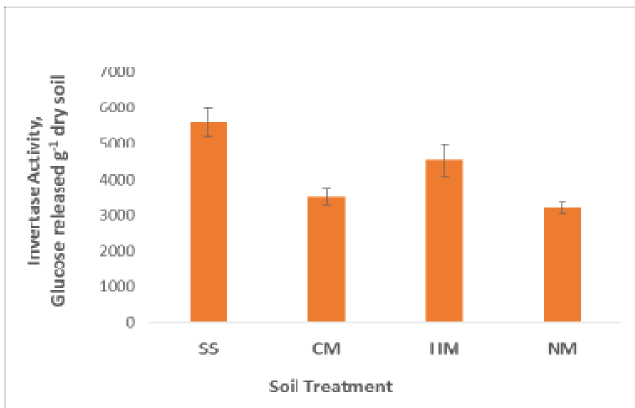

Figure 4: Overll urease (upper graph) and invertase (lower graph) activities in the rhizosphere of agrugula and mustard grown under four soil management practices. Statistical comparisons were carried out among four soil management practices. Bars accompanied by the same letter are not significantly different (P > 0.05) using Duncan's multiple range test.

This was also observed in urease activities. Soils amended with SS, CM, and HM enhanced urease activity compared to the NM treatment. These results confirmed that application of organic manure to agricultural soils could greatly increase soil enzymatic activities, nutrient uptake, and biomass production. Significant municipal SS and CM generation will be available in increasing quantities due to increased municipal SS composting facilities and the rapid growth in the poultry industry. Recycling these waste materials for use as a low-cost organic fertilizer has a positive effect on the growth and yield of vegetable crops. The presence of organic matter in recycled biosolids often improves soil physical and chemical properties and promotes soil biological activities. Composts improves the physical properties of soils by increasing nutrient and water holding capacity, total pore space, aggregate stability, erosion resistance, temperature insulation, and decreasing apparent soil density [11,12]. Microbial diversity is affected by biosolids additions. A study in Poland found that biosolids added to soil increased colony- forming units of fungal and bacterial communities [36-39]. Accordingly, recycling SS and CM manures provide a useful organic fertilizer that could be used as an alternative to synthetic fertilizers.

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