WILSON, JOHN ROBERT

PLANT GROWTH REGULATOR IN MUNICIPAL WASTEWATER

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PLANT GROWTH REGULATOR IN MUNICIPAL WASTEWATER

by

John Robert Wilson

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This thesis has been approved on the date shown below:

A. D. Day — 12-15-82

A. D. DAY
Professor of Plant Sciences

Date

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ABSTRACT

Three experiments were conducted, one each with barley (*Hordeum vulgare* L.), Bermudagrass (*Cynodon dactylon* L.), and tomato (*Lycopersicum esculentum* Mill.). In the barley and Bermudagrass experiments, an extract from municipal wastewater was given to the plants through the irrigation water. In the tomato experiment, the extract was administered with a soaking treatment. The barley and Bermudagrass were grown under greenhouse conditions until ready to harvest. After harvest, the plant growth and yield means for the barley and Bermudagrass were compared by the Student-Newman-Keuls' Test to determine different populations between treatments. In the tomato experiment, the roots of cuttings were excised and their dry weight means compared by the Least Significant Difference Test. The results indicated that barley and Bermudagrass utilized the nutrients in wastewater equally as well as the nutrients applied in chemical form. Interpretation of the tomato data suggests that the extract from municipal wastewater may have enhanced root initiation and growth.

INTRODUCTION

Throughout history, man has used human waste as a nutritional supplement to improve the growth of crop plants. In the last century, the first steps were taken to establish land application of municipal waste as a viable source of fertilizer. Because of inadequate waste disposal techniques these efforts failed. With increasingly expensive chemical fertilizers, today's agronomists and waste disposal experts are again investigating the feasibility of land application of municipal waste. Treated municipal wastewater from modern sewage processing plants is a potential source of irrigation water and plant nutrients for the commercial production of field crop plants.

LITERATURE REVIEW

Human waste has long been used as an agricultural amendment. Prior to the development of inexpensive chemical fertilizers, some of the scientists of the nineteenth century devoted themselves to perhaps the largest recycling campaign in history (Hamilin, 1980). Ambitious sewage farms were started in Europe to provide for the agricultural use of sewage; however, most of these farms failed because of an inadequate understanding of sewage decomposition. Methods of modern sewage treatment were not known until the beginning of the twentieth century and by this time there was abundant hydroelectric power which resulted in the production of economical nitrate fertilizers. The idea of using the land as a method to treat human waste did not begin to gain acceptance again until the recent advent of strict anti-pollution laws and the end of the era of cheap energy. Currently, investigators are endeavoring to solve problems associated with land application of both the solid (sludge) and liquid (wastewater) components of sewage. In addition, efforts are also being made to understand the nature of sewage and how to use this valuable resource to its maximum possible advantage.

The use of municipal wastewater for irrigation has the potential to produce higher yields when compared with the use of well water alone for irrigation. Feigin et al. (1978) found an increase in seed weight simultaneous with a reduction in lint percentage with cotton. Day et al. (1981) found cotton grown with a pump water-wastewater mixture produced more seed cotton and seed with a higher seed weight than did cotton irrigated with only pump water. Additionally, they found that cotton grown with the mixture produced a higher total number of seeds than did cotton grown with pump water alone. They concluded that a plant growth constituent may have been present in the wastewater and may have contributed to the increased seed size and number of seeds. Reynolds et al. (1979) reported that when alfalfa was grown on an effluent treated site the alfalfa had a greater fresh and dry weight than did the control. They also noticed when alfalfa was grown on a treated site it experienced a greater growth and more rapid recovery rate after harvesting. Day et al. (1979) observed that on large fields from 1970 through 1977 barley which had been irrigated with a pump water and wastewater mixture had taller plants and higher grain yields than barley irrigated with pump water alone. In addition, it was reported that the barley grown with the pump water and wastewater combination produced greater amounts of vegetative growth as measured by plant height and straw yield. Day, Taher, and Katterman (1975) stated that when wheat was grown with wastewater it contained more total protein than when it was grown with well water plus suggested amounts of nitrogen, phosphorus, and potassium. Sanai and Shayegan (1980) noted that when vegetation was irrigated with wastewater it produced higher yields than did vegetation irrigated with fresh water. This held true even when equivalent amounts of fertilizers were applied in the fresh water. Working with six different species of Christmas trees, Cooley (1980) found that regular applications of sewage effluent during the growing season increased the growth and survival of all of the tested species, except Douglas fir (*Pseudotsuga menziesii* Mirb.). However, Deghi, Ewel, and Mitsch (1980) observed that after two years of application, annual litter fall in a cypress (*Taxedium distichum* L.) swamp did not show any increase that could be attributable to sewage effluent addition. Marten, Larson, and Clapp (1980) stated that when maize (*Zea mays* L.) fodder was irrigated with municipal wastewater it produced from 17 to 36% more dry matter and from 23 to 41% more digestible dry matter per hectare than did reed canarygrass. However, the differences grew progressively smaller as wastewater application increased from 0 to 10 cm/week. They also noted that the canarygrass yielded from 30 to 52% more crude protein per hectare than did maize. These differences grew progressively greater as the wastewater application increased.

The primary reason suggested for the increases in growth in the preceding discussion of wastewater application to crops is one of greater available nutrients. However, Alemu (1976) found an extract in municipal wastewater which increased total chlorophyll retention in wheat leaf tissue. He concluded that the high yields of high protein plant products obtained from plants grown with wastewater may have resulted from cytokinin-like growth substances present in treated municipal wastewater. Katterman and Day (1980) isolated a ureido adenosine chromaphore from municipal wastewater which exhibited a moderate cytokinin-like activity.

While this study involved a chemical found in municipal wastewater, a detailed examination of current research is necessary to more fully understand the problems involved with the application of municipal waste to crops. To provide the investigator with sufficient background information so that municipal waste may be used more efficiently, other pertinent work is also reviewed.

Hyde (1976) studied the long-term environmental effects of agricultural utilization of wastewater and concluded that sludge rates of 9 to 71 mt/ha improved forage production and quality. Dodolina (1971) reported that irrigation with sewage had a beneficial effect on the yield of perennial grasses. The grasses developed better root systems, and the irrigation increased the humus content of the A_1 and A_2 soil horizons by 0.2%. Touchton and Boswell (1975) studied the effects of sludge as a greenhouse soil amendment and its effects on soil chemical constituents. It was noted that the HCl-extractable soil elemental concentrations increased with the application of sludge for the soil studied. However, these increases were influenced by soil type. Magdoff and Amadon (1980) found that corn (Zea mays L.) when grown on sludge-treated Hadley sandy loam soil out-yielded corn grown with nitrogen fertilizer. However, they felt that the greater response was probably caused by factors other than nitrogen contribution. In other tests they noticed that hay grown on Hadley sandy loam soil responded more to the application of sludge than to the application of nitrogen. Milne and Graveland (1972) observed statistically significant increases in barley (Hordeum vulgare L.) grain yields for their first crop on all soils tested, with increasing amounts of sludge up to 101 metric tons/ha. Their second crop showed no significant response from sludge application on two of the three soils tested. Wells and Whitton (1977) reported that tomatoes (Lycopersicum esculentum Mill.) grown with sludge grew larger and faster than did tomatoes grown with the control. Spotswood and Raymer (1973) noted that when digested sludge was mixed with soil the rate of oxidation of the sludge organic matter was high enough for greater dosage rates than usual. It was also suggested that the high organic content of the soil was the major reason for its continued fertility. Korcak (1980) studied apple (Malus pumila Mill.) seedlings and how they were affected by the addition of semi-solid sludge. He reported that leaf dry weights were significantly increased by fluidized bed material applications when compared to the control. He also noted that soil electrical conductivity was significantly increased with increasing fluidized bed material applications.

Much research has been performed regarding the effects of municipal waste on indigenous pathogens and also on soil organisms. Dunigan and Dick (1980) stated that their results indicate that there is a seasonal effect which will determine the losses of fecal coliform bacteria in surface runoff waters. Brown, Jones, and Donnelly (1980) also stated that there was a seasonal effect on fecal coliform. They concluded that the survival of fecal coliform on grass treated with sludge was more dependent on the climate than on the crass species or on the fraction of solids in the sludge. Also, they observed that rainfall reduced coliform populations only slightly and that rainy humid weather resulted in increases in populations and prolonged their survival. In addition, they reported that coliphage were rapidly exterminated from vegetative surfaces when the plants receiving sludge treatments were allowed to dry and that simulated rainfall was effective in removing the majority of the residual coliphage from the plants. Orchard (1979) investigated the effect of sewage in Nocardia and found that the application of dried sludge on a pasture resulted in an increase in populations of Nocardia asteroidies of 100 times over 14 months. Although greater than the control, the numbers of Nocardia isolated from wet sludge plots showed little increase over the same time period. Nocardia asteroidies is an opportunistic pathogen of man and animals. Orchard (1980) reported that the populations of Nocardia and Micromonospora did not increase when wet sludge, which contained less organic matter than dry sludge, was added to the soil. However, he found that the population of Thermeactinomyces species increased in the wet sludge plots as opposed to the dried sludge plots. Lemeshkina and Rodionova (1976) noted that in Siberia a 5-day interval between irrigations provided adequate sewage purification in the soil which created favorable conditions for biochemical processes. Tate and Terry (1980) found that the increased microbial activity in sewage amended plots on Pahokee muck soil resulted from augmented soil moisture and not the added nutrients of the sewage. They also stated that while the survival of coliforms was limited in Pahokee muck, viable coliforms had penetrated throughout the soil profile. Beard and Montgomery (1981) noted that when effluent was discharged to an area not saturated with effluent the removal of bacteria from the effluent was more efficient than where effluent was discharged to a saturated zone. Working with the earthworm Eisenia feotida, Hartenstein, Neuhauser, and Narahara (1981) suggested that there may be relatively little variation in sludge quality at a particular plant with respect to the growth potential of *E. foetida*. Hartenstein and Hartenstein (1981) concluded that much of the enhanced decomposition rate of sludge due to earthworms must be attributed to stimulation of the microbial heterotrophic decomposition process. In addition, they noted that *E. foetida* may be employed to reduce sludge accumulations and odors at wastewater treatment plants. Uiga and Crites (1980) compared the health factors associated with the activated sludge and slow-rate land treatment methods of wastewater purification. They reported that the use of wastewater for irrigation of food crops may have a greater risk than irrigation of non-food crops, since the transmission cycle may be shorter and positive removals of pathogens by soil may be shortened through crop harvest. They also noted that when current rates of wastewater application are assessed, a safety factor of 10^8-10^{13} is attained over the last reported incidence of disease transmittal on food crops during the early 1900's. The health hazard to site workers from municipal wastewater constituents was no greater than to the general public. They concluded that land treatment systems had fewer risks because smaller quantities of sludge were generated and dispersed over a large land area.

Recently, considerable research has been undertaken to determine the relationships between applied sewage and uptake of the heavy metals present in the sewage by plants, particularly cadmium, zinc, and copper. Gaynor and Halstead (1976) found that sludge applications to a clay and sandy loam soil increased DTPA-extractable cadmium, lead, and zinc. Dudas and Pawluk (1975) working with lettuce (Lactuca sativa L.) reported that the lettuce grown on a soil amended with sewage sludge at a rate of 160 metric tons/ha had a lower content of cadmium, zinc, and strontium than did lettuce grown on the same soil amended at a rate of 80 metric tons/ ha. They reasoned the difference might have been due in part to poor root development on the part of the plants grown with the higher rate. Singh (1981) suggested that cadmium accumulation in plants affects growth mechanisms in plants. He found that the dry matter weights of lettuce were greater at a sewage sludge cadmium level of 2.5 ppm than were the dry matter weights of lettuce grown with a sludge cadmium level of 5.0 ppm. Application of increased cadmium tended to increase total zinc uptake in plants. He also noted that the cadmium content of plants grown in sludge-treated soils was about one-fifth of the cadmium concentration in plants grown in inorganic treatments. Hinesly et al. (1976) studied the assimilation of zinc and cadmium from sludge amended soils in soybeans (Glycine max Merrill) and stated that there were no indications that the absorption and translocation of relatively large amounts of zinc and cadmium into leaf, petiole, and seeds caused any phytotoxic condition. They reported that the magnitude of annual sludge applications was a more important determinant of amounts of cadmium and zinc in plant tissues than were amounts of the sludge-borne elements accumulated in the soil from sludge applications in previous years. They concluded that the organic constituents of sludge provide very little protection to plants against zinc and cadmium concentrations in the soil. Soon, Bates, and Moyer (1980) investigated the heavy metal content of plants grown on sludge amended soil. Working with corn and bromegrass (*Bromus* sp.) they found that the copper concentration in bromegrass increased as the rate of sludge application increased. They also noted an increase cadmium concentration in bromegrass, that they believed was the result of a nitrogen nutrition effect from high rates of sludge application. They also observed that sludge did not have any effect on chromium or lead concentration in either corn or bromegrass. Keefer et al. (1979) reported that cadmium, chromium, nickel, and lead were absorbed only in small amounts by corn grown in sludge amended soil. They observed that heavy metals added through sewage sludges did accumulate in soils and continual sludge application could create undesirably high soil metal content. Bingham, Page, and Ganje (1976) stated that increasing the soil cadmium level to 640 μg Cd/g resulted in a yield decrement of 30% for flood-grown rice (*Oryza sativa* L.) while non-flood rice did not survive treatments greater than

160 µg Cd/g. They reasoned that the mechanism by which cadmium was rendered unavailable to rice grown under flood condition was probably a result of precipitation in the form of CdS. Bingham, Page, and Strong (1980) working with rice found that the uptake of Cd was essentially a linear function of the cadmium addition rate and soil pH, particularly at low addition rates of nickel. Also, they concluded that the toxicity values for heavy metals differ according to pH, metal, addition rate of the metal, and crop species. Mascianica and Barker (1979) suggested that concentrations of zinc in radish (Raphanus sativus L.) in response to varied zinc levels and changes in pH in soil are similar whether the metal carrier is a sludge-borne organic chelate or EDTA. Vlamis et al. (1978) found that barley grown on sludge amended soil exhibited no differences due to type or rate of sludge application with regards to nickel, cobalt, chromium, and cadmium content. Lewin and Deckett (1980) reviewed the current literature in the area of soil heavy metals. They concluded that in sludges heavy metals are held in several forms or pools, some of which are potentially unstable in soils and will release heavy metals into the soil solution, and some of which are very resistant. From the different forms of heavy metals, there is no single available fraction from which the plant may draw the element. These pools of elements tend to be mobilized by microbial and root activity and tend to be immobilized by

crystalization and other physical processes. Sterritt and Lester (1980) studied forty different sludges in England, and stated that zinc, copper, and nickel would most frequently present the greatest hazards to agriculture. David and Williams (1979) found that for both peas (Pisum sativum L.) and ryegrass (Lolium sp.) the levels of potassium, zinc, cadmium, and nickel were elevated in plants grown on sewage treated soil, while the manganese and iron levels were lower. They concluded that changes in soil properties as a result of sewage treatment, particularly an increase in pH and organic matter content, together with natural fixation reactions combined to ensure that toxic levels were not reached. Maclean and Dekker (1978) investigated the uptake of metals in corn and lettuce when grown on sludge amended soils. They observed that on corn, sewage sludge eliminated the toxic effect of copper, reduced the toxic effect of nickel and reduced the concentrations of zinc and nickel in the plants. They also noted that lettuce grown in a loam soil was more susceptible than corn to the toxic effects of the metals and to zinc when grown in a clay soil. Schauer, Wright, and Pelchat (1980) reported that in edible tissues, cadmium was greatest in lettuce followed by carrots (Dancus carota L.), tomatoes, and radishes. The copper concentrations were found to be greatest in lettuce shoots and tomatoes. Concentrations of nickel were highest in carrots, followed by radishes, lettuce, and tomatoes. The zinc concentrations which accumulated in the highest quantities, were found to be highest in lettuce, followed by radishes, carrots, and tomatoes. Shaeffer et al. (1979) found that when soil temperature was increased there were no consistent effects on copper, nickel, lead, or cadmium in corn tissue. No residual effects of soil temperature were observed on metal concentrations in legumes or small grains. They also noted that concentrations of zinc, copper, nickel, and cadmium were increased in corn, legume and small grain tissue by sludge treatments. Hyde et al. (1979) found that sludge application on corn had no effect on the concentration of cadmium, copper, nickel and zinc in corn grain. Ritter and Eastburn (1978) noticed no harmful effects on the growth of corn and soybeans from trace elements native to sludge when applied at high rates on a short term basis. Williams (1975) stated that at soil pH values below 6.5 the toxic effects of zinc, copper, nickel, and chromium were serious. Williams et al. (1980) studied the movement of heavy metals in the soil profile and reported that only zinc and lead moved as far into the soil profile as 10 cm and cadmium, chromium, copper, and mercury moved only as deep as 5 cm. Naylor and Loehr (1981) reviewed the literature and concluded high-quality sludges applied as a nitrogen fertilizer could be applied as much as 200 years before reaching the maximum cumulative cadmium loading allowable for acid soils. The

United States federal guidelines note that even though the maximum cumulative cadmium loading may eventually be reached, the soil would remain suitable for production of food crops, including fruits and vegetables. Zwarich and Mills (1979) investigated the effects of heavy metals, from sludge, on crops. They arrived at the following conclusions: Chromium: in growth chamber experiments, the element appears to be excluded by or unavailable to plants. Lead: Sludge added some to the soil, but growth chamber experiments suggest it is unavailable to plants. Copper: Sludge treatments had very little effect on wheat (*triticum aestivum* L.) straw or kernels; however, with alfalfa (*Medicago sativa* L.) and bromegrass mixture (forage), elevated levels were found. Zinc: Levels in wheat kernels from the growth chamber showed a linear increase with increasing rates of added sludge; levels in a forage crop had a statistically significant increase. Cadmium: The uptake of cadmium by wheat kernels was approximately a linear function of sludge concentration. The addition of high rates of sludge to alkaline soils resulted in a three-to-sixfold increase in cadmium concentration. In growth chamber experiments with the forage mixture it was observed that the cadmium concentration increased with increasing amounts of sewage sludge.

Because municipal waste is a potential source of nitrogen, research has been conducted with the nitrogen in sludge and its relationship to the plant and soil. Stewart et al. (1975) reported that the total nitrogen concentration in corn grain was increased slightly by application of sludge greater than 1.25 m T/ha. Approximately 30% of the applied NH_4^+ in their experiment was lost due to volatilization one week after application. The apparent recovery of nitrogen in the sludge was 19%. About 3-12% of the total nitrogen applied as sewage sludge was removed by the corn. However, they did not find any increase in yields of corn grain and stover by sludge application in excess of 1.25 mT/ha. Premi and Cornfield (1969) noted that mineralization of native soil organic nitrogen was increased by 14 and 17 ppm where 0.25 and 0.5 ml of sludge were added to tubes holding 10 grams of soil, respectively. Immobilization of nitrogen occurred only where 2 ml of the sludge was added. The effect was temporary, accounting for 35% of the added NH_4-N after two weeks and 12% after eight weeks. They suggested that the presence of trace elements, perhaps copper or zinc, may have resulted in the stimulating of the two lower levels of sludge on the mineralization of native soil organic nitrogen. Dunigan and Dick (1980) observed slightly lower total soluble-nitrogen runoff losses with applied sewage sludge when compared with commercial fertilizer. This, they reasoned, was a result because 82% of the sewage sludge-nitrogen was in the organic form while all of the fertilizer-nitrogen was in the NH_4 form. English, Miller, and Koelliker (1980) noted that ammonia concentration was apparently not affected by the organic strength of the sludge. Hsieh, Douglas, and Matto (1981a) studied the decomposition patterns of different sewage sludges in the soils. They stated that activated sludge had appreciably higher contents of carbon and nitrogen than did digested sludge, but the carbon/nitrogen ratios of the two sludges were similar. They observed that the factors of temperature, time of incubation, rate of sludge application, type of sewage sludge, and interaction among these factors were significant at the 1% level. Hsieh, Douglas, and Matto (1981b) investigated nitrogen transformations in soil and reported that in a digested sludge system no significant increase in total inorganic nitrogen was observed during the first four weeks. After four weeks, the total inorganic nitrogen content increased rapidly at application rates of 8 and 12%. In an activated sludge system, the level of inorganic nitrogen increased within two weeks of incubation. After two weeks, the level of inorganic nitrogen declined as the sludge application rate increased to 12%. They also noted that denitrification seemed more important at 0.06 bars of moisture than at 0.33 bars.

One of the primary concerns in municipal wastewater disposal or reuse is the concentration of nitrogen in the effluent and its effects upon plants. Parkin and McCarty (1981) studied the sources of nitrogen in effluents. They estimated that under optimal conditions 20 to 40% of the effluent soluble organic nitrogen is produced biologically during activated sludge treatment and the remainder comes from residual organics from the wastewater. Kipnis et al. (1979) investigated the uptake of effluent nitrogen in Rhodes grass (Chloris gayana Kunth). They found that nitrogen originating from effluents could be utilized efficiently even when applied to soils possessing a low rate of nitrification. The nitrogen recovery of the plants was 73.9% of the total nitrogen input. When they increased the frequency of irrigation they found enhanced nitrogen uptake by the plants. Quin (1979) observed that effluent treated areas had a higher pH, higher total nitrogen, organic carbon, available phosphorus, and exchangeable cadmium and sodium levels, and lower levels of calcium and magnesium. Also, pasture production with effluent treated land was 50% higher than that of nonirrigated land. Brar, Miller, and Logan (1978) reported that organic carbon, total nitrogen, and soil carbon/nitrogen ratios were higher in the surface 7.5-cm layer of wastewater treated sites and that at lower depths there were no differences between control and treated sites. The denitrification potentials for all their locations were low and did not demonstrate any consistent relationship with wastewater treatment. They noted that available organic carbon is the limiting factor for denitrification. They disagreed with the assumption that increased denitrification in soils receiving wastewater is a result of enhanced anaerobic microsites. Feigin et al. (1978) stated that on cotton (Gossypium hirsutum L.) no nitrogen fertilizer was needed to obtain high yields in the effluent irrigated plots. They concluded that with proper management effluent-irrigated fields may result in better yields using less fertilizer. Lund et al. (1981) observed that the averages of nitrogen balances developed attributed 32, 60, and 9% of the nitrogen applied in the effluent to crop removal, leaching, and gaseous losses, respectively. Vaisman et al. (1981) found that when Rhodesgrass was grown on sand dune soil the grass effectively removed all of the nitrogen applied in the wastewater. Palazzo (1981) studied the nitrogen concentrations in three consecutive harvests of orchardgrass (Dactylis glomerata L.) after having been irrigated with primary treated wastewater. He noted that the nitrogen concentrations were greatest during the first and second harvests. This demonstrated that on a Hartland silt loam, in a cold environment, higher application rates would be possible during the first harvest period. Chen and Patrick (1981) studied nitrogen uptake in ryegrass. They observed that the amounts of N^{15} in ryegrass at various distances on 4% slope indicated that nitrogen removal was nonlinearly related to slope distance. The adsorption and retention of NH_4^+-nitrogen in the soil matrix accounted for approximately 70-90% of the NH_4^+-nitrogen applied. Their study indicated that the overland flow system of wastewater disposal is capable of removing 80-90% of the added NH_4^+ nitrogen in wastewater. Feigin, Feigenbaum, and Limon (1981) reported that the availability of nitrogen from treated sewage effluents is probably somewhat lower than that of fertilizer-nitrogen incorporated into the soil before seeding or planting. Lo and Clayton (1978) suggested that crops should be incorporated into nitrogen removal systems for greater efficiency. Green, Alexander, and Leggett (1981) investigated the formation of nitrosomine under conditions resembling land treatment of wastewater. Their results confirmed the likelihood of nitrosomine formation under conditions resembling the land application of municipal wastewater.

In comparison to nitrogen, less work has been done on phosphorus. However, there has been some concerning phosphorus and soil interactions. Tate and Terry (1980) noted that an organic soil effectively removed orthophosphate from wastewater effluent and that the rate of application had no effect on the orthophosphate content of the soil drainage waters. Palazzo (1981) stated that with three harvest periods for orchardgrass, the phosphorus concentrations decreased during the first period and increased during the second period. Iskandar and Syers (1980) reported that wastewater addition caused a substantial decrease in the phosphorus sorption capacity of the surface soil. Holford and Patrick (1979) found that if a soil was moderately chemically reduced, the addition of relatively small quantities of phosphorus in wastewater will bring the residual P concentration of the soil solution to unacceptably high levels. They added that the ability of the soil to decrease the phosphorus concentration of wastewater to levels below that required for significant biological activity may be relatively short-lived. Hill and Sawhney (1981) stated that the renovation of wastewater over long periods of time reduced the phosphorus sorption capacity of a soil, while periodic resting regenerated sorption sites and increased the potential for additional phosphorus sorption. Their data also showed that most of the phosphorus retained is transformed into unavailable forms. They suggested that by moving along preferred pathways, in heterogeneous soil, phosphorus would reach the ground water before all sorption sites were fully saturated. Vaisman et al. (1981) observed that on a sand-dune soil there was a negligible addition of phosphorus to the ground water. Ryden and Pratt (1980) reviewed the literature on phosphorus removal from wastewater applied to soil. They stated that the mechanisms for phosphorus mobility and for maintaining low phosphorus concentrations in the soil-solution appeared to involve a sorption reaction at soil surfaces with a hydrous oxide structure. If forage crops were harvested from land irrigated with wastewater they suggested that with a phosphorus application rate of 200 to 300 kg phosphorus/ha/yr, phosphorus removal may be as much as 30 to 40%. They concluded that the soil may represent an appreciable sink for phosphorus in wastewaters applied to land areas.

Heavy metals tend to be concentrated more in the sludge than in the effluent; however, a paper by Sidle, Hook, and Kardos (1977) suggested that heavy metals may also be a concern in effluent. Working with plots planted in corn and reed canarygrass (Phalaris arundinacea L.) which had been irrigated with wastewater, they measured the distribution of heavy metals in the soil profile. Their data indicated that extractable copper and zinc accumulated substantially while cadmium levels increased to a lesser extent at the depth of 0 to 30 cm in the soil used for the reed canarygrass plot. In the area planted with corn, copper was the only metal which accumulated at a significant rate over time in the 0- to 30-cm depth. There was no evidence that heavy metals moved from the 0- to 30-cm depth. They found no accumulation trends with time for lead, nickel or cobalt.

Some work has been done on the effects of sewage sludge on the growth of plants. Sikara et al. (1980) found a linear relationship between yield and the addition of sewage sludge compost amendment for Evesboro and Fauquier soils. They noted that the mineralization of compost organic nitrogen was the limiting factor in grass yield.

Kirkham (1980), who grew wheat in soil columns to determine the effect on plant growth when sludge was applied in a layer below the surface of the soil, stated that sludge placed at the top of the columns resulted in more dry matter production of wheat than sludge placed at a 18- to 20-cm depth. His conclusion was that if metal concentrations in the sludge were low, placing the sludge on the surface rather than injecting it would have the effect of increasing yield. McIlveen and Cole (1977) reported that the lodging resistance of corn increased as the amount of sludge applied increased. However, no differences were detected on populations, leaves per plant, and plant morphology. Sjogren (1977) noted that the addition of sludge did not increase crop yields to the same extent as fertilizer alone. He cited three possible explanations: (1) application rates based on available nitrogen (NH_4-N) were too low, (2) the initial application of sludge reduced plant growth due to lowered oxyben levels, and (3) metal ion deficiency, especially potassium. Gouin, Link, and Kundt (1978) observed that soils amended with 113 or 225 tons/ha of sludge produced more red maple (*Acer rubrum* L.) seedlings which had longer stems than seedlings receiving no compost or compost of 450 tons/ha. They also noted that additional potassium as a supplement may be necessary for optimum plant growth.

The objective of this research was to study the effects of municipal wastewater extract on the growth of barley, Bermudagrass (*Cynodon dactylon*, L.), and tomato.

MATERIALS AND METHODS

Barley Experiment

In a Randomized Complete Block experimental design with four replications, 20-cm clay pots were filled with equal amounts of sterilized soil. The original soil was a Cave series loamy, mixed, thermic, shallow, typic paleorthid which had been modified in texture to 60.4% sand, 23.1% silt, and 7.5% clay. Seven seeds of 'Harlan II' barley were planted in each pot. On the ninth day after planting the plants were thinned to the four most uniform plants in each pot. On the 11th day after planting, the treatments with wastewater extract were initiated.

All wastewater was obtained at approximately the same time in the afternoon from the Roger Road Sewage Treatment Plant, Tucson, Arizona. The wastewater was stored in a closed plastic container for about seven days before being renewed with a fresh supply. The container was wrapped with aluminum foil to decrease the growth of algae.

Concentrations of extract of 1, 2, and 3 mg/l were used in this study. The reason for the specific values was that they were thought best for producing distinct growth reactions (Katterman and Day, 1980).

The specific derivation of the extract, tentatively identified as a N-6 Ureido Adenosine Chromaphore, from wastewater was published previously (Katterman and Day, 1980). After the compound was removed from wastewater, the extract was stored as a powder under dry conditions until time of use. When the extract was stored in a liquid state refrigeration was needed to prevent putrefaction. The extract was dissolved in regular well irrigation water and applied to the pots as an irrigation treatment. The following listing illustrates the type of treatment each pot received:

Treatment Number	Type of Treatment
1	well water alone
2	well water plus extract (2.0 mg/l)
3	well water plus recommended NPK for barley
4	well water plus NPK equal to NPK in wastewater
5	wastewater alone
6	well water plus NPK plus extract, 1 mg/l
7	well water plus NPK plus extract, 2 mg/l
8	well water plus NPK plus extract, 3 mg/l

In order to compensate for the nutrients present in the wastewater experimental treatments 4, 6, 7, and 8 were amended with 25 mg/l of urea and 31 mg/l of potassium phosphate monobasic. The addition of urea and potassium phosphate monobasic to well water resulted in an amended well water which contained 16, 7, and 9 mg/l of nitrogen, phosphorus, and potassium, respectively.

The barley was grown to the mature seed stage under greenhouse conditions. At the hard-dough stage of seed development watering was discontinued and the plants were allowed to dry out. At maturity, the plants were harvested and the following data were recorded: plant height, heads/pot, seeds/head, seed weight, above-ground weight, below-ground weight, and grain yield. The standard analysis of variance was applied to all data and the Student-Newman-Keuls' test was used to compare treatment means (Steel and Torrie, 1960).

Bermudagrass Experiment

In a Randomized Complete Block experimental design with four replications, 20-cm clay pots were filled with equal amounts of soil (60.2% sand, 19.5% silt, 20.3% clay). Into each pot 4-inch (10.16 cm) plugs of Coastcross Bermudagrass were planted. All plugs were obtained from the Maricopa County Extension Office, Phoenix, Arizona. All wastewater was obtained at approximately the same time in the afternoon from the Roger Road Sewage Treatment Plant, Tucson, Arizona. Methods of application, storage, and renewal periods were the same as in the barley experiment.

Concentrations of extract equal to those in the barley study were used. Identical types of treatments were received by each pot of bermudagrass as were received by the pots of barley. In order to compensate for the nutrients present in the wastewater experimental treatments 4, 6, 7, and 8 were amended with ammonium nitrate (68.6 mg/l), and potassium phosphate monobasic (318 mg/l). Treatment number three was amended with ammonium nitrate (1.05 g/l), potassium phosphate monobasic (0.396 g/l), and potassium sulfate (0.300 g/l). Treatment number three was given this solution after every harvest. The bermudagrass was grown until half of the plants in the 32 pots were heading. At this time all plants were clipped to within 5 cm of the soil surface and the following data were obtained: plant height, number of stems, number of stolons, stolon length, and above-ground dry weight. The initial cutting was discarded to better equilibrate future harvests. The standard analysis of variance was applied to all data and the Student-Newman-Keuls' test was used to compare treatment means (Steel and Torrie, 1960).

Tomato Experiment

An experiment was conducted to determine the effects of wastewater extract on the rooting of tomato (CV Royalflush-17153) cuttings. Ten centimeter cuttings were taken by cutting horizontally across the terminal growth. A total of 24 cuttings were used in a four replication Randomized Complete Block experimental design. All cuttings were planted in a 50% vermiculite/commercial potting soil using Speedling trays. Two rows and every other position of the Speedling tray were used. The plants were then positioned so that each cutting was within 2.5 cm of being equidistant from a misting head. Prior to planting, leaves were removed and the cuttings were subjected to the following treatments:

Treatment Number	Treatment	Comments
1	Wastewater extract	Same extract as was used in the barley and Bermudagrass studies in a saturated solution (= 25.25 OD units)
2	Distilled H_2O	
3	Pure extract	Purified extract of the ureido compounds from crude powder (eluted from chromatograph paper)
4	Cytokinen	Concentration equal to 0.05 mg/l
5	Cytokinen	Concentration equal to 5 mg/l

Treatment Number	Treatment	Comments
6	Extract of chromatograph paper	Equal to treatment number three except without the presence of the extract

The cuttings were treated by placing them vertically in a solution of their respective treatments for one hour. The soil was then moistened to field capacity and the cuttings planted. During the study, well water was applied by a mister at a rate of 15 seconds every half hour. After two weeks the cuttings were removed, the roots excised, and root fresh weight measured. Later the dry weight of the roots was determined. The standard analysis of variance was applied to all data and the Least Significant Difference test together with a square root transformation was used to compare treatment means (Little and Hills, 1975).

RESULTS AND DISCUSSION

Barley Experiment

Barley grown with well water alone and with well water plus 2 mg/l of wastewater extract produced fewer heads per pot and less above-ground plant material than did any other treatments (Table 1). There were no significant differences between irrigation and fertilizer treatments in average plant height, number of seeds per head, seed weight, below-ground weight, and grain yield (Table 1). These data indicate that barley utilized the fertilizer nutrients equally effectively in both well water and commercial fertilizer and in municipal wastewater in the production of plant growth and grain yield (Table 1). The foregoing data also show that the addition of wastewater extract to the irrigation and fertilizer treatments used in this experiment did not significantly improve the growth and yield of barley from the growth and yield obtained without the addition of wastewater extract. However, this experiment indicates that in semiarid areas where normal well water is in short supply for irrigation municipal wastewater may be utilized effectively as a source of irrigation water and plant nutrients in the commercial production of barley grain.

Table 1. Average plant height, number of heads per unit area, number of seeds per head, seed weight, above-ground dry weight, below-ground dry weight, and grain yield per unit area for forage and grain from barley grown with eight irrigation and fertilizer treatments in the greenhouse at Tucson, Arizona in 1980-81.

Irrigation and fertilizer treatments	Plant height (cm)	Heads/ pot (no.)	Seeds/ head (no.)	Seed weight (mg/seed)	Above-ground weight (g/pot)	Below-ground weight (g/pot)	Grain yield (g/pot)
1. Well water alone	48 a	9 bcd	9 a	40 a	23 b	14 a	2.8 a
2. Well water + extract (2 mg/l)	47 a	7 cd	8 a	40 a	18 b	16 a	2.2 a
3. Well water + NPK recommended for barley	48 a	14 a	7 a	35 a	45 a	25 a	3.6 a
4. Well water + NPK equal to NPK in wastewater	50 a	13 ab	7 a	38 a	37 a	24 a	3.4 a
5. Wastewater alone	47 a	13 ab	11 a	33 a	45 a	25 a	5.4 a
6. Well water + NPK + extract at 1 mg/l (low)	48 a	12 abc	9 a	35 a	41 a	22 a	3.5 a
7. Well water + NPK + extract at 2 mg/l (medium)	49 a	13 ab	10 a	38 a	34 a	21 a	4.6 a
8. Well water + NPK + extract at 3 mg/l (high)	47 a	14 a	7 a	33 a	39 a	24 a	3.5 a

Table 1.--Continued

Irrigation and fertilizer treatments	Plant height (cm)	Heads/ pot (no.)	Seeds/ head (no.)	Seed weight (mg/seed)	Above-ground weight (g/pot)	Below-ground weight (g/pot)	Grain yield (g/pot)
Coefficient of Variability	4%	16%	43%	13%	15%	27%	43%
Significance of Differences:							
Between Treatments	ns	**	ns	ns	**	ns	ns

Means followed by the same letter are not different at the 5% level of significance using the Student-Newman-Keuls' Test.

** = Significant at the 1% level. ns = not significant at 5% level.

Bermudagrass Experiment

There were three harvests for which data were collected for Coastcross Bermudagrass grown under greenhouse conditions. Five growth characteristics were measured: (1) number of stems per pot, (2) number of stolons per pot, (3) stem height in cm, (4) stolon length in cm, and (5) above-ground dry weight in g/pot. After these characteristics were compared by the Student-Newman-Keuls' statistical test, only one growth characteristic, the above-ground dry weight, was found to be significantly different (Tables 2, 3, 4) for the entire experiment.

In the data for the first harvest there was a trend for treatments 1, 2, and 3 to be significantly different from treatments 4 through 8. The trend became a distinct separation in harvests 2 and 3. The separation of the two populations of above-ground dry weight probably was not due to treatment effects, but to nutrient effects. Treatment numbers 1 and 2 received no supplemental nutrients and treatment number 3 received the recommended NPK in a single application after each harvest. The application of the nutrients in treatment number 3 was not as efficient as the constant application in treatments 4 through 8. This would tend to remove treatment 3 from the population consisting of 4 through 8 and bring it closer to the population comprised of treatments 1 and 2. By referring to Table 4 it can be seen that treatments 1, 2, and 3 again formed a separate

Table 2. Average number of stems per pot, stolons per pot, stem height, stolon length, and total above-ground dry weight per pot from Coastcross Bermudagrass grown with eight irrigation and fertilizer treatments in the greenhouse at Tucson, Arizona in 1981 (first harvest).

Irrigation and fertilizer treatments	Stems per pot	Stolons per pot	Stem height	Stolon length	Above-ground dry weight
	(no.)	(no.)	(cm)	(cm)	(g/pot)
1. Well water alone	63 a	17 a	50 a	78 a	24.1 b
2. Well water + extract (2 mg/l)	60 a	21 a	57 a	90 a	29.2 ab
3. Well water + NPK recommended for Bermudagrass	69 a	29 a	56 a	99 a	37.5 a
4. Well water + NPK equal to NPK in wastewater	64 a	36 a	46 a	85 a	34.9 a
5. Wastewater alone	59 a	33 a	54 a	90 a	38.9 a
6. Well water + NPK + extract at 1 mg/l (low)	51 a	28 a	57 a	116 a	36.1 a
7. Well water + NPK + extract at 2 mg/l (medium)	62 a	25 a	51 a	86 a	37.7 a
8. Well water + NPK + extract at 3 mg/l (high)	67 a	33 a	49 a	94 a	33.5 a
Coefficient of Variability	24%	34%	15%	23%	12%
Significance of Differences: Between treatments	ns	ns	ns	ns	**

Means followed by the same letter are not significantly different at the 5% level of significance using the Student-Newman-Keuls' Test.

** = Significant at the 1% level. ns = not significant at 5% level.

Table 3. Average number of stems per pot, stolons per pot, stem height, stolon length, and total above-ground dry weight per pot from Coastcross Bermudagrass grown with eight irrigation and fertilizer treatments in the greenhouse at Tucson, Arizona in 1981 (second harvest).

Irrigation and fertilizer treatments	Stems per pot	Stolons per pot	Stem height	Stolon length	Above-ground dry weight
	(no.)	(no.)	(cm)	(cm)	(g/pot)
1. Well water alone	66 a	9 a	46 a	125 a	18.4 b
2. Well water + extract (2 mg/l)	69 a	5 a	39 a	56 a	13.1 b
3. Well water + NPK recommended for Bermudagrass	77 a	5 a	42 a	77 a	14.6 b
4. Well water + NPK equal to NPK in wastewater	80 a	14 a	48 a	148 a	26.1 a
5. Wastewater alone	91 a	13 a	41 a	126 a	27.1 a
6. Well water + NPK + extract at 1 mg/l (low)	71 a	12 a	41 a	128 a	25.7 a
7. Well water + NPK + extract at 2 mg/l (medium)	84 a	14 a	38 a	138 a	28.0 a
8. Well water + NPK + extract at 3 mg/l (high)	90 a	14 a	49 a	122 a	26.1 a
Coefficient of Variability	16%	39%	20%	32%	3%
Significance of Differences: Between treatments	ns	ns	ns	ns	**

Means followed by the same letter are not different at the 5% level of significance using the Student-Newman-Keuls' Test.

** = Significant at the 1% level. ns = not significant at 5% level.

Table 4. Average number of stems per pot, stolons per pot, stem height, stolon length, and total above-ground dry weight per pot from Coastcross Bermudagrass grown with eight irrigation and fertilizer treatments in the greenhouse at Tucson, Arizona in 1982 (third harvest).

Irrigation and fertilizer treatments	Stems per pot	Stolons per pot	Stem height	Stolon length	Above-ground dry weight
	(no.)	(no.)	(cm)	(cm)	(g/pot)
1. Well water alone	69 a	5 b	27 a	115 a	11.4 c
2. Well water + extract at 2 ml/l	62 a	6 b	30 a	162 a	11.0 c
3. Well water + NPK recommended for Bermudagrass	80 a	9 b	29 a	218 a	24.8 c
4. Well water + NPK equal to NPK in wastewater	99 a	17 a	29 a	156 a	36.3 ab
5. Wastewater alone	97 a	15 a	32 a	183 a	31.3 b
6. Well water + NPK + extract at 1 mg/l (low)	90 a	17 a	30 a	149 a	38.8 a
7. Well water + NPK + extract at 2 mg/l (medium)	98 a	20 a	30 a	136 a	33.6 ab
8. Well water + NPK + extract at 3 mg/l (high)	96 a	15 a	33 a	174 a	34.9 ab
Coefficient of Variability	11%	35%	18%	28%	15%
Significance of Difference: Between treatments	ns	**	ns	ns	**

Means followed by the same letter are not different at the 5% level of significance using the Student-Newman-Keuls' Test.

** = significant at the 1% level. ns = not significant at the 5% level.

population for the stolons per pot. This should be viewed with some skepticism not only for the nutrient reason stated above, but also because of the 35% coefficient of variability.

As a result of the lack of a significant difference between treatment 5, wastewater, and the other treatments it may be inferred that for the majority of the experiment wastewater produced as much growth as well water plus recommended amounts of NPK. This may be of use where it is desirable to use municipal wastewater as irrigation water for commercial agriculture.

It can be concluded that the addition of the extract from municipal wastewater did not change the growth patterns or forage yield from Bermudagrass. While the addition of the extract did not influence growth in a positive manner, neither did it act negatively. Further experimentation will be necessary to ascertain the exact effects of the extract upon plant growth.

Tomato Experiment

Tomatoes grown with a pre-planting treatment consisting of a one-hour imbibition period in a solution of crude powder, purified extract, or chromatogram paper extract produced more root tissue, as measured by dry weight, than did all other treatments. There were no significant differences between those cuttings which were given a pre-planting treatment of distilled water, 0.05 mg/l cytokinen, or 5.0 mg/l cytokinen (Table 5). These data indicate that the crude extract powder when compared with distilled water enhanced root initiation and growth. This may be of use where root stimulation is needed. Interpretation of the data also suggests that the effects of the crude extract powder may not be as "cytokinenlike" (Alemu, 1976) as originally supposed. Further experimentation is necessary to ascertain the exact effects of the compound on the growth of plants. Also of interest, is the fact that there was no significant difference between the crude powder and the purified extract from chromatogram paper as well as the extract of chromatogram paper. From this information it can tentatively be suggested that there may be a chemical in the extraction process or in the chromatogram paper which is a root stimulator.

Table 5. Average dry weight of roots excised from tomato plants grown with six treatments in the greenhouse at Tucson, Arizona in 1982.

Treatment number	Pre-planting treatment	Root dry weight	Average dry weight of roots (transformed data)
		(grams)	(grams)
1	crude powder	0.0091	.714 a
2	distilled water	0.0036	.708 b
3	pure extract	0.0198	.722 a
4	cytokinen (0.06 mg/l)	0.0127	.709 b
5	cytokinen (5.0 mg/l)	0.0000	.707 b
6	chromatogram paper extract	0.0266	.726 a
Coefficient of Variability		1.4%	1.4%
Significance of Differences: Between treatments			*

Means followed by the same letter are not different at the 5% level of significance using the Least Significant Difference Test together with a square root transformation.

* = significant at the 5% level.

SUMMARY

In both the barley and Bermudagrass experiments the addition of an extract from municipal wastewater failed to produce any increase in plant growth and yield.

Interpretation of the growth parameters for the barley experiment indicated that barley utilized the plant nutrients in well water plus recommended levels of NPK and the nutrients in municipal wastewater equally effectively in the production of plant growth and grain yield. Analysis of the data from the Bermudagrass experiment suggests that, with the possible exception of the above-ground dry weight, the plants utilized the nutrients in the wastewater equally as well as the nutrients in amended well water. Additional experiments may be necessary to further define what effects, if any, the municipal wastewater extract has on the growth of plants. The fact that nutrients from wastewater are used as effectively as nutrients from chemical fertilizer may be of use in semiarid areas where wastewater is to be used as a source of irrigation water and plant nutrients in commercial agriculture.

In the tomato experiment, the data obtained by weighing dry roots indicated that there may be a root stimulator present in the crude and pure wastewater extract that may increase plant growth. This information may be useful where the enhancement of root growth is required.

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